DNDi

La mejor ciencia
para los más desatendidos

FIND

Diagnóstico para todos

FINAL REPORT

MEETING ON DIAGNOSTIC EVALUATION AND ECONOMIC IMPACT ANALYSIS OF NEW DIAGNOSTIC METHODS FOR CHAGAS DISEASE

Report of Meeting on diagnostic evaluation and economic impact analysis of new diagnostic methods for Chagas disease

BACKGROUND

Between 6 and 7 million people worldwide, mostly in Latin America, are estimated to be infected with *Trypanosoma cruzi*, the parasite that causes Chagas disease (CD) and 70 million are at risk of the disease globally. Every year, over 10,000 CD related deaths are reported, and the estimated burden of the disease exceeds USD 690 million in healthcare costs and USD 8 billion in annual economic losses [1]. CD is mainly a chronic silent condition, and a substantial number of CD cases are missed. Fewer than 10% of people chronically infected with *T. cruzi* are diagnosed and only about 1% receive etiological treatment [2,3].

Barriers that limit access to healthcare for people affected by CD include a diagnosis process that is often cumbersome, time-consuming and costly, the limited availability of tools and materials at primary health centers and the lack of integration of diagnosis into maternal and child health policies and practices. *T. cruzi* infection is curable if treatment is initiated soon after infection. In chronically infected patients, antiparasitic treatment can prevent mother-to-child transmission and potentially prevent or curb disease progression.

Although there are new point-of-care (POC) diagnostic methods (serological and molecular) commercially available, and under development, that could simplify the diagnosis, these tests are not widely used. According to the Pan-American Health Organization (PAHO) and the national guidelines for diagnosis of CD in endemic countries, serological rapid diagnostic tests (RDTs) are indicated only for screening or research purposes. Molecular diagnostic methods, such as Real-Time PCR assays, have been incorporated in a few endemic countries, such as Argentina and Chile.

As part of the transition from disease control to complete elimination, the World Health Organization (WHO) has suggested priority actions in its 2030 road map targets for Neglected Tropical Diseases (NTD) [4]. The recommendations for CD aim to streamline and modernize diagnostic methods, focusing on the development and evaluation of POC diagnostic tests. Independent evidence regarding the performance and economic impact of new CD diagnostic tests is currently being generated by several institutions.

To simplify and bring up to date the diagnosis for CD, and to support countries towards the elimination of mother-to-child transmission, there is a need to create generic study protocols and establish harmonized standards and procedures, to guide the development and evaluation of new cost-effective diagnostic tools.

SCOPE

To further discuss these topics, a scientific meeting was convened and sponsored by FIND and DNDi, with the technical support of PAHO and co-organized by INGEBI-CONICET. The meeting took place on May 6th and 7th, 2024, in Buenos Aires, Argentina, bringing together 44 invited experts and 17 observers. The participants included researchers mainly from Latin America, as well as from the USA, Spain and Switzerland, alongside technical health authorities from the endemic countries, and representatives from PAHO (List of invited experts, **annex 1**). **The main goal of the meeting was to achieve consensus among the invited experts on three key components of interest:**

1. **A Generic Research Protocol**: To evaluate RDTs for CD, ensuring the implementation of high-quality comparable studies in the Americas, generating conclusions with greater recommendation strength.
2. **Key Product Characteristics, standards, and quality controls of molecular tests for the early diagnosis of *T. cruzi* infection**: To assess and guide the development and use of these tests.

3. **Evidence on Cost-Effectiveness and Economic Impact**: To agree on the necessary evidence to facilitate the integration of new diagnostic methods into the health systems in Latin America.

SUMMARY

Before the meeting (early April) the selected documentation was sent to the invited experts. This included: *(i)* the Generic research protocol for the evaluation of RDTs, developed by PAHO; and *(ii)* a survey to gather feedback from the invited experts prior to the meeting. The majority of the experts (57%, 25/44) sent their feedback by the end of April.

On May 6th and 7th 2024, an in-person meeting was held in Buenos Aires, to achieve consensus on the three components of interest. The agenda included: (1) a summary presentation on the Generic Research Protocol for the evaluation of RDTs, developed by PAHO; (2) a summary presentation on the Survey Results Report, (3) additional guests' presentations to further debate the three components of interest, and (4) on the second day, the invited experts were divided in three subgroups (12-18 experts each), to achieve consensus on each of the three components of interest.

- **Group 1** achieved consensus on critical aspects and recommendations for modifying or including some sections in the Generic Research Protocol for the evaluation of RDTs. Key points included specifying that the protocol is intended for clinical performance evaluation of individual RDTs on the field, defining performance metrics (point estimates and margin of error of diagnostic sensitivity and specificity) for selecting investigational products and test acceptance, including reference test method(s), providing guidance about sample size estimations, test interpretation, result reporting, and incorporating a cost-effectiveness sub-study.

- **Group 2** reached consensus on the key product characteristics, controls and standards for molecular tests for early diagnosis. Discussions focused on diagnostic sensitivity and specificity, analytical sensitivity, reference methods for field validation studies, clinical specimen type and preparation, multiplexed formats, diagnostic algorithms combining methods, and quality assurance issues. Topics identified for further discussion include developing protocols for manufacturing quality control panels and standards at the regional level, and determining the most suitable biological material for quality control panels and standards.

- **Group 3** discussed about the evidence on cost-effectiveness and economic impact necessary to facilitate the integration of new POC diagnostic methods for Chagas disease. This group reviewed various models presented on the first day, including the new model and app developed by FIND, considering costs and assumptions, and requirements for changes as suggested by experts from the health technology assessment agencies in the Latin American region. Additionally, this group deliberated on questions concerning the cost-effectiveness sub-study that needed to be incorporated into the Generic Research Protocol for the evaluation of RDTs and committed to continuing their work virtually after this meeting to finalize this the annex in collaboration with PAHO and FIND.

DAY 1. EXPERTS PRESENTATIONS AND DISCUSSION IN PLENARY

The first day of the meeting took place at Mundo Sano Foundation headquarters, featuring presentations and discussions in plenary sessions (6th of May from 9am to 6pm). Prior to the meeting, experts were invited to provide their input via a survey. For each question, experts indicated whether they agreed (*fully* or *mostly agree*) or disagreed (*neither agree or disagree, somewhat disagree,* or *disagree*), providing comments if they disagreed. The majority of the expert submitted their input in April, prior to the meeting (57%, 25/44). The key insights from this survey were presented on the first day which helped to identify the topics prioritized for further discussion over the two days (aggregated

results available in Survey Results Report, **annex 2**). The detailed agenda and the presentation slides are available in **annex 3**.

1. A Generic Research Protocol to evaluate RDTs for CD, ensuring the implementation of high-quality comparable studies in the Americas, generating conclusions with greater recommendation strength

- Presentation from Freddy Perez (PAHO) and Maria Isabel Jercic (Instituto Nacional de Salud Publica de Chile).
    - A summary of the Generic Protocol, developed by PAHO and INS Chile, was presented, including the development process, the 23 sections of the document, and the feedback from the experts received prior to the meeting. The discussions over the following two days were very important to develop it further.
    - The presentation began with a scoping review of RDT evidence, limited to *T. cruzi* in humans, studies with reported sensitivity/specificity, from 1990, onward in Spanish and English. A total of 247 articles were identified of which 30 were included after review by four experts. Results from the literature search identified 41 tests; 25 of which are commercially available. Reported sensitivity ranged from 90.1–100% (94.6% mean), and specificity 90.1–100% (mean 94.6%). Key characteristics of RDT selection were discussed along with presentation of internal quality control and proficiency testing for RDT readers.
    - Key insights from the feedback provided by experts on the Generic Protocol prior to the meeting were also presented.

- Presentations from Laura Bohorquez (FIND): Key insights from the survey results from the experts (prior to the meeting) about the Generic protocol.
    - The majority of experts agreed with the considerations outlined in all questions (>50% *fully* and *mostly agree*).
        - For the following topics, less than 20% of experts expressed neutrality (*neither agree* or *disagree*) or disagreement (*somewhat disagree,* and *disagree*):
            - **Testing Algorithms and Usage Scenarios**: It was suggested that in hard-to-reach populations, testing algorithms should include the combination of two RDTs (with a tie-breaker) that meets acceptance criteria (combined sensitivity / specificity and PPV/NPV at a given prevalence) as recommended by PAHO.
            - **Guidance for Researchers:** The protocol should provide guidance on *(i)* statistical considerations for estimating the minimum sample size, according to the acceptance performance criteria (point estimates and margin of error); *(ii)* ensuring the appropriate execution, interpretation and documentation of RDT results; and *(iii)* identifying the most cost-effective testing methods or algorithms that provide the best value for money in terms of costs and patient outcomes.
        - Although the majority of experts agreed with the following statements (>50% *fully* and *mostly agree*) there was a considerable proportion (20-32%) that *neither agree or disagree, somewhat disagree,* and *disagree*:
            - The investigational products including RDTs, should incorporate different antigenic principles.
            - There should be consensus on a single reference test method.
            - A single serological external quality assurance (EQA) panel should be established, with easy access for researchers (e.g. for assessing appropriate RDTs for chronic *T. cruzi* infection should be the WHO International Standard 1st WHO anti-*Trypanosoma cruzi* I and II Antibody Reference Panel NIBSC code: 11/219).

- Presentations by Laura Bohorquez (FIND), Berra Erkosar (FIND), and Andres Caicedo (DNDi): about statistical considerations and recommendations for the Generic Protocol.
    - A summary of independent laboratory performance evaluations of up to 11 RDTs conducted, using autochthonous samples and the reference test method in Argentina, Bolivia, Colombia and Brazil. The results showed that 25%, 40%, 45% and 0% of the RDTs available in those countries, respectively, would comply with the selection performance criteria proposed in the Generic Protocol (92% Se and 95% Sp [5,6,7,8] (published 2023-2024 by FIND and partner institutions).
    - Considerations for both retrospective (laboratory) and prospective (field) verification were summarized, highlighting the need to clearly distinguish these two stages in the Generic Protocol. The differences between them include sample handling, timing, processing conditions, total costs and results reporting, which vary significantly between laboratory and field settings.
    - It was recommended that the protocol should provide specific details in the following sections : *i)* Reference Test Method: Define the reference test method, potentially including a panel of reference tests; *ii)* Inclusion Criteria for Samples/Patients: Outline the criteria for selecting samples and patients; *iii)* Sample Size Estimations: Provide guidance on estimating sample sizes, especially if experts agree on the acceptance performance criteria; *iv)* Usability Assessment of RDTs: Include a guide on how to assess the usability of RDTs; *v)* Certified External Panels: Offer guidance on accessing certified external panels, such as those provided by WHO. The purpose of including 20% low-reactivity samples in the protocol was unclear, as the objective of this Generic Protocol is to evaluate clinical performance, not analytical performance. Additionally, there was uncertainty regarding the inclusion of patients who had already been treated with antiparasitic drugs.

- Questions and discussion between the audience and panellists:
    - The audience expressed concerns about whether the protocol is focused more on RDTs for screening or diagnostics. If the protocol is intended for RDTs used in diagnosis, it should recommend the use of RDTs with different antigens to improve diagnostic accuracy.
    - Both types of studies (RDTs for screening and diagnosis) were included in the PAHO scoping review. However, more evidence is needed for diagnostic RDTs, and this protocol aims to support the development and implementation of studies to generate this evidence.
    - It was noted that laboratory serological tests are not perfect, none of them are used as stand-alone tests but as a composite (at least 2 tests). The goal is to generate further evidence on RDTs performance to recommend their use as part of diagnostic algorithms.
    - Learning from the experience with HIV, it was emphasized that the community must become more comfortable with the use of RDTs for diagnosis, not just for screening purposes. In HIV diagnosis, a second confirmatory step is always required, and a PPV of 99% is now the standard use in HIV testing. As its prevalence has decreased, a three-test approach has been adopted. The group need to decide whether one, two or three tests are sufficient for CD.
    - Concluded to use samples from untreated patients for test evaluation.
    - The audience raised concern about the presence of very low-reactive samples in some regions, such as Central America and Mexico. It was reiterated that this Generic Protocol is intended for the evaluation of clinical performance of RDTs, not analytical performance, which would require a different protocol in the future. It was also emphasized that the protocol should not demand more from RDTs than what it is required from serological laboratory-based tests. Thus, the protocol

should clearly distinguish between pre-clinical (laboratory) evaluation, potentially as a first stage, and field evaluation.

- In some countries more evidence on RDT performance has been generated using autochthonous populations (e.g. Bolivia). The pre-selection of RDTs should especially consider performance demonstrated in autochthonous populations. To achieve consensus tomorrow about the values of both selection performance and acceptance performance parameters.

2. Key Product Characteristics, standards, and quality controls of molecular tests for the early diagnosis of *T. cruzi* infection: To assess and guide the development and use of these tests

- Presentations from Alejandro Schijman (CONICET), Otacilio Moreira (Fiocruz), Elena Ivanova (FIND), and Marcelo Rodriguez (FIND and ANLIS), state of the art of molecular diagnostic methods for detection of *T. cruzi* infection (including LAMP and RT-PCR), other molecular diagnostic tools that could be adapted for CD, controls and standards for molecular methods.
  - A summary of LAMP methodology for detection of *T. cruzi* infection was presented. First feasibility and analytical performance study results were highlighted, emphasizing the need to standardise rapid DNA extraction methods designed for POC detection. Two methods were discussed: (1) a repositioned 3D printer to rapid DNA extractor, and (2) an ultrarapid DNA extraction method (PURE). The first LAMP field evaluation conducted in Gran Chaco Bolivia. involved *T. cruzi* infected mothers and their neonates. Testing was done using microscopy, LAMP and qPCR at delivery, at 2 months and 9 months. A total of 224 neonates were included. LAMP specificity compared to qPCR was 98.6% at birth and 98.2% at 2 months of age [9].
  - The use of qPCR in CD was summarized including the consensus on PCR and qPCR established in Buenos Aires 2008 and 2011, as well as the Target Product Profiles for CD developed in 2015 and 2020. Four commercially available qPCR kits were also compared: Realstar, VIASURE, Wiener, Kit BioMol. This comparison showed that two of the kits display sensitivity, specificity, PPV and NPV of 100% (95%CI lower bound above 96.4%). It was mentioned that the majority of transmission in Brazil is now oral, so the priority in this country is to detect it using good performing molecular methods.
  - The presentation outlined the key requirements for molecular diagnostic tools to make a transformational impact, particularly in primary healthcare facilities. The Covid-19 pandemic spurred innovation, bringing molecular testing closer to patients from near POC (basic lab) to true POC (portable/battery operate) or even instrument free POC. Pipeline of development– 161 in total (10 true POC, 3 instrument-free, 10 supported by FIND). Trade-offs are unavoidable. Key requirements and technical distinctions: PCR assay & reader (high power needed, higher costs). Isothermal assay & reader (less sensitive, multiplexing capacity limited). Isothermal single-use platforms (higher cost per test, environmental impact). Novel methodologies (early-stage technology, limited clinical data currently). Summarised isothermal amplification techniques, amplification-free methodologies. Key challenges in leveraging true POC technologies from Covid-19 to CD: 1. Sample compatibility (whole blood/urine), 2. Clinical performance, 3. Limited menu (additional financing and incentives are required to accelerate menu expansion).
  - The presentation also summarised main issues and challenges surrounding tools for analytic control and diagnostic methods: considering type of controls - Panels for verification, validation, performance, regional versus

international/national/local - Calibration curves - Strong and weak positive controls - Amplification controls. Highlighted the importance of the dimensions of analysis: diagnostic lab -> ref lab -> production lab -> WHO (international) lab. The need to reduce bias was also emphasized, with a focus on using calibration curves and interlaboratory studies to ensure accuracy and reliability. To increase precision, it is necessary to include amplification curves, as well as strong and weak positive controls. A major concern in the development and evaluation of molecular diagnostic methods for CD is the lack of international reference standards, which prevents the comparison of parasitic loads between laboratories using international units or parasite equivalents measures.

- Presentations from Alejandro Schijman (CONICET), Maria Jesus Pinazo (DNDi) and Costança Britto (Fiocruz), about key insights form the survey results from the experts (prior to the meeting) about the Generic protocol.
    - The majority of experts agree with the considerations stated in all questions (>50% *fully* and *mostly agree*).
        - Besides, in the following topics, the proportion of the experts that *neither agree or disagree, somewhat disagree,* and *disagree* was low (<15%):
            - Intended use • Target operator • Target use setting • Target analyte • Reference method • Analytical specificity • Strain specificity • Quantitation • Training needs • Specimen type • Processing steps / transfer volume • Time sample-to-results • Data analysis • Internal quality control • External quality control • Power requirements / connectivity / result capture • Operating conditions • Diagnostic sensitivity (POC platforms) • Scale of manufacture (POC platforms).
        - In the following topics, although the majority of experts agree with the statements (>50% *fully* and *mostly agree*) there was a considerable proportion (>15%) who *neither agree or disagree, somewhat disagree,* and *disagree*:
            - Diagnostic specificity • Analytical sensitivity • Time stability of reagents • Quality assurance • Specimen capacity (POC platforms) • Instrument integration (POC platforms) • Diagnostic sensitivity (RT-PCR) • Instrument price (thermocyclers).
    - Topics to be discussed on day 2 to achieve consensus were presented: diagnostic sensitivity and specificity, reference test methods, analytical sensitivity (including confidence intervals and LoD), specimen type and specimen preparation, design of multiplex molecular methods, considering different settings and use cases (EMTCT-plus initiative, field surveys of acute febrile illnesses), potential cost-benefit, diagnostic algorithms of combined methods and quality assurance.

- Questions and discussion between the audience and panellists:
    - While there is a TPP for CD, published in 2015, it is based on evidence available up to 2011. Therefore, there is a need for consensus on the general characteristics of diagnostic tools considering current needs and new technologies. Although LAMP is available, it has not yet been fine-tuned for all settings of CD. These developments can take up to 10 years, so guidance is needed for developers on what the ideal diagnostic tools would be or what needs optimization. It is also important to compare methods in terms of cost-effectiveness such as qPCR versus LAMP, considering not only costs but also other factors. Multiplex is ideal, but it involves trade-offs with costs. The discussion highlighted the need to recommend what it would be most relevant for CD diagnosis.
    - Samples with heparin can be used for but not for PCR. This distinction needs to be clarified, as it depends on the extraction method employed. Additionally, validation studies are required depending on the type of samples. For example, both whole

blood and guanidine treated samples may be necessary to achieve high sensitivity. A direct comparison of LAMP versus qPCR in different samples – such as blood, guanidine, EDTA- is proposed. We aim for POC extraction, ideally compatible with both LAMP and qPCR, but we are limited by the need for portable equipment such as a 3D printer. In field studies comparing LAMP and qPCR, it is important not to use the same samples for both methods. Validated Standard Operating Procedures (SOPs) provided with the kit manual must be followed strictly; deviations are not permissible during research. Thus, comparing the two methods involves more than just amplification; it includes the entire process, meaning that using the same sample types for both LAMP and qPCR is not appropriate. Point-of-care (POC) means different things in Latin America, so a clear definition is needed. Additionally, caution is advised with LAMP, as it is currently produced by a single Japanese manufacturer, raising concerns about the sustainability of supply for Latin America.

- For congenital diagnosis, specific parameters need to be compared differently than to other situations, such as oral infections or CD reactivation. Syphilis has been frequently mentioned in relation to multiplex methods. Learning from HIV, for asymptomatic patients, two PCR tests are required. The process is more complex than just running a PCR, factors such as cycle thresholds (CTs) for different clinical groups need to be considered in the case of CD. Implementing PCR for congenital diagnosis has been challenging. There is a reiterated need to differentiate between clinical and analytical sensitivity and specificity.

3. Evidence on Cost-Effectiveness and Economic Impact: To agree on the necessary evidence to facilitate the integration of new diagnostic methods into the health systems in Latin America

- Presentations by Sarah Girdwood and Kyra Grantz (FIND):
  - Diagnostic Pathway and Laboratory Serology Issues. The presenters outlined the diagnostic pathway and discussed issues related to laboratory serology. RDTs can simplify the diagnosis process and are likely to cost the same as a laboratory serology. However, RDTs reduce visit costs for patients and the healthcare system. To determine the most cost-effective algorithm, it is necessary to balance the trade-off between loss to follow up (and access) and test performance (and cost) across different settings. A simplified example with preliminary costs data collected in Argentina compared different algorithms: (1) The standard of care testing algorithm based on RDTs is as efficient as laboratory serology in identifying positive cases but more efficient when visit costs are included. (2) Serial versus parallel test algorithms (same cohort) based on RDTs identify slightly fewer positive cases but are more efficient (cheaper per patient identified) due to fewer tests being performed.
  - Demonstrated Chagas Diagnostic Algorithms application: <https://finddx.shinyapps.io/chagaspathway/>. Online, interactive tool and steps demonstrated: (1) Select algorithm structure (e.g. number of scenarios to model – parallel versus serial etc.). (2) Enter test parameters (tests type, sensitivity/specificity, complexity level where tests performed, tests costs). (3) Adjust optional settings (e.g. per visit fixed costs to health system and patient, loss to follow up, prevalence, linkage to treatment and treatment effect). (4) Generate and download HTML results report (total costs, cases linked to treatment, DALYs – plots of PPV, NPV, cost per disease, prevalence). Shared feedback survey: <https://forms.gle/h584XtkKmsATiCUf7>.

- Questions and discussion between the audience and panellists:
  - Tool could be even more interesting adding variables related to cost savings with RDTs. While it is important to keep the tool as simple as possible, the application

already considers changes in access with RDTs (cost/time to seek care and access diagnostics). It is known that access can be increased by 30% with RDTs, for example. Users can manually set the range of costs and DALYs or exclude DALYs for a simpler model. On average, DALYs are associated with untreated Chagas, the app allows for adjustment of these DALYs depending on population.

- Presentations by Rafael Herazo (DNDi), Yerly Magnolia Useche (Fiocruz CUIDA Chagas), Elisa Sicuri (ISGlobal), and Santiago Hasdeu (redArets Argentina), with comments from the audience after these presentations:
    - Analysis of patients-incurred costs by using data from Colombia's health system (2023), where 99% of the population is covered, but only 41% are satisfied with the availability of medical attention. Herazo's work considered patient perspectives and showed the impact of receiving care in primary healthcare facility: 4-fold reduction in travel time, a 5-fold reduction in transport expenses, 5.5-fold reduction in food and housing expenses, and a 2-fold reduction in income losses. Primary care level attention reduces costs related to health interventions, out of pocket expenses, and lost income.
    - Health Economics Analysis Plan of the CUIDA Chagas protocols were presented. Cost-effectiveness of RDT algorithms for CD diagnosis in Brazil, Bolivia and Colombia, chronic CD in adults and children at primary healthcare centers. The ongoing economic evaluation considers study perspectives, timing of analyses, discounting for costs and benefits, cost-effectiveness thresholds, healthcare resources costs, analysis of QALYs, cost-utility analysis, sensitivity & subgroup analysis, and model simulation. Expected results include RDT versus standard testing algorithms in terms of diagnosis opportunity and treatment coverage.
    - IS Global work compared three models: (i) 2 ELISA, (ii) 2 RDT, (iii) RDT + ELISA. The model structure consisted of a decision tree and a Markov model. The total cost was very similar across three approaches, as were the total QALYs. The RDT model had lower sensitivity, higher testing costs (due to the need for confirmatory tests), and a lower proportion of treated individuals (assuming equal probability of linkage to care). The mixed strategy (RDT + ELISA) was more cost-effective than the RDT alone up to an RDT sensitivity of 90%. For sensitivity >90%, the RDT strategy was more cost-effective than the mixed strategy. The RDT strategy weakly dominates the mixed strategy with prevalence below 5% and the dominance increased with lower prevalence (e.g.1%).
    - RedArets (Argentine Public Network for Health Technology Assessment) presented the key elements of budget impact and cost-effectiveness analyses that influenced policy changes in Argentina. They provided examples from HPV and TB and discussed lessons learnings that could be applied by the CD community. The HPV budget impact analysis showed a $9billion budget impact in the first year followed by savings over five years. In discussing the cost-effectiveness study of GeneXpert for TB, the importance of considering the 'hidden' costs beneath the iceberg (maintenance, lifetime cycle, spare parts, software updates, human resources) was emphasized. They also stressed, the importance of incorporating social indirect costs (medical care, work absenteeism, days of limited activity, transportation). The economic evaluation considered regional differences across countries such as Argentina, Peru, Paraguay, Malawi, Tunisia and Uganda, highlighting the variability in epidemiology, health system, clinical practice, heterogeneity in costs, difference in payment capacity and willingness to pay threshold.

DAY 2. CONSENSUS ACHIEVED AND CONCLUSIONS

On the second day the meeting was held at NH City Hotel from 8:30 to 11:00 am. Three working groups of 12-18 experts (including two moderators to guide the discussion and a rapporteur). Each group focused on one of the three components of the meeting.

GROUP 1. A Generic Research Protocol to evaluate RDTs for CD, ensuring the implementation of high-quality comparable studies in the Americas, generating conclusions with greater recommendation strength

This Group achieved consensus on critical aspects and recommendations for sections of the Generic protocol that should be modified or included:

- **Overall Structure / Sections:**
    - Specify that the protocol is intended for the clinical performance evaluation of individual RDTs in the field compared to the reference standard method. It is not for the evaluation of analytical performance or clinical performance in the laboratory setting. Alternatively, the generic protocol should clearly separate the three different sections, purposes and methods 1. analytical performance evaluation; 2. clinical performance evaluation in the laboratory / controlled environment; and 3. clinical performance evaluation in field / at the point-of-care).
    - As analytical performance evaluation is not the primary objective of this Generic protocol, it should not recommend selecting samples with different antibodies levels, or including 20% of low antibody levels.
    - Include sections in the Generic protocol on the reference test method(s), guidance on sample size estimations, test interpretation and result reporting, and a cost-effectiveness sub-study.

- **Clinical performance:**
    - Recommend selecting investigational products (RDTs), that displayed sensitivity of 92% and specificity of 90%, with values obtained within 5% full-width margin of error (95% confidence interval +/- 2.5%), ideally. If possible, prioritizing primarily studies that have used autochthonous populations (in the country of interest), and secondarily, independent performance evaluations.
        - The suggested performance in the protocol (92% sensitivity and 95% specificity, referred to the lower bound of the margin of error) were values set according to the performance obtained in the scoping review conducted by PAHO (sensitivity ranged from 90.1%-100%, with an average of 94%; and specificity ranged from 95.5%-100%, with an average of 98.5%). It is likely to be largely based on the performance evaluations published by manufacturers, that usually do not specify the sample populations (regions/countries) used.
    - For test acceptance (ultimately used for sample sizing estimations), recommend that the performance be set at **92% as the lower bound of sensitivity, and 95% as the lower bound of specificity.** Values obtained for the given RDT(s) within 5% full-width margin of error (95% confidence interval +/- 2.5%). If possible, prioritizing studies that have used autochthonous populations (in the country of interest), and secondarily, independent performance evaluations.

- **Reference test method:**
    - The clinical performance of the investigational products (RDTs) should be compared against validated methods (the gold standard for chronic infection), following the recommendations of the PAHO diagnostics guidelines (2018), i.e. using as reference test method the agreement of at least two serological tests (including ELISA, IHA and IIF).
    - Employ region-specific reference methods with high sensitivity and specificity values. The experts agreed that this recommendation will ensure comparability of studies (or comparability of the performance of a given RDT) between countries across the region.

- **Sample size estimations:**
    - Include a general guidance on sample size estimations, adding the reference to the book that has been widely cited on diagnostic evaluations (Zhou, X. H., McClish, D. K., & Obuchowski, N. A. (2009). *Statistical methods in diagnostic medicine*. John Wiley & Sons), to calculate the number of confirmed positive/negatives by the reference test method, needed to estimate expected

sensitivity/specificity with the given confidence interval. If researchers would like to adjust the parameters, they can use the shinyapp developed by FIND, publicly available <https://finddx.shinyapps.io/SampleSize/>.

In brief, the formula is as follows:

$$n = \frac{[(z_{\alpha/2} + z_\beta)\sqrt{V(\hat{\theta})}]^2}{(L)^2},$$

Where *Za/2* is the upper *a*/2 percentile of a standard normal distribution, *Zβ* is the upper *β* percentile of a standard normal distribution where 1- *β* is the desired power, and L is the desired width of one-half of the CI.

And the formula which links the n to be screened to statistical power, based on the prevalence:

$$\frac{(N_{total} \times Prev_p) - n}{\sqrt{N_{total} \times Prev_p \times (1 - Prev_p)}} = z_\beta,$$

Where Prevp is the prevalence, and n is the n from the calculation above.

Table 1. Sample size estimations suggested, for measuring a sensitivity / specificity of 95% or 98% of the index tests with 80% power and 5% significance level, with an error margin of 2.5% on one side of the confidence interval.

Sensitivity / Specificity	Error Margin (half width CI)	Disease Prevalence	n Positives / n Negatives	N Total to Screen
95%	2.50%	5%	597	12338
98%	2.50%	5%	247	5188
95%	2.50%	10%	597	6164
98%	2.50%	10%	247	2590
95%	2.50%	15%	597	4106
98%	2.50%	15%	247	1725
95%	2.50%	20%	597	3076
98%	2.50%	20%	247	1292

- In terms of precision, it is advised to work with a maximum width of the 95% confidence interval of 5% (+/- 2.5%). Also, if researchers prefer to reduce the sample size, they should first consider decreasing the power (usually 90 to 80%). If reducing power to 80% is not sufficient, widening the CI can be considered.
- State that estimations should be reviewed by a statistician who tailors to the specific study objectives.

- **Methodology:**
  - Include guidance about test interpretation and result reporting. As the Generic protocol recommends following the manufacturer's instructions for investigating products, the "indeterminate" test classification for RDTs should be removed. Results should be classified as positive/reactive, negative/non-reactive, or invalid as per instructions of manufacturers.
  - It is recommended to conduct field validation studies using smartphone/tablet applications for standardized photo results recording.

- **Cost-effectiveness sub-study:**
  - Include a sub-study section in the Generic protocol on guidance that allows the researchers to identify the most efficient test methods (or test algorithms), and that provides the greatest value for money assessing costs and patient outcomes.
  - The **Group 3** of experts on this meeting will develop such a section to be incorporated in the Generic protocol. In general, researchers can use and adjust the parameters in the shinyapp developed by FIND, which is publicly available <https://finddx.shinyapps.io/chagaspathway/>, and can also provide further feedback to adjust it in <https://forms.gle/h584XtkKmsATiCUf7>.
- **Diagnostic Algorithms:**
  - Consensus was achieved on that the Generic protocol is for evaluating individual RDTs, and several sections of the protocol (including the algorithm included in workflow) must be

developed further to clarify this. Given that PAHO has recently recommended RDTs for screening purposes, but requires more evidence to recommend RDTs for diagnosis (or RDTs as one of the accepted serological tests in the guidelines for diagnosis of chronic infection along with ELISA, IHA and IIF), this protocol will support development and implementation of studies to generate this evidence.

- A specific protocol must be developed if the researchers aim to validate an algorithm based on RDTs. In such a case, it is recommended to include only evaluated tests. A non-inferiority approach can be also used to compare composite tests (in that case, sample size estimations and acceptance performance would be different than the recommendations proposed to be included in this Generic protocol). Experts would need to agree on both the non-inferiority margin and the expected difference between two tests or two algorithms.

- **Future perspectives for the Generic Research Protocol of RDTs:**
  - PAHO representatives announced that the Generic protocol would incorporate the suggestions of the consensus achieved and the conclusions. PAHO will publish the protocol in English and Spanish, in the following weeks acknowledging the meeting sponsored by FIND and DNDi in collaboration with CONICET. Additionally, three sites will be selected to implement the protocol.
  - PAHO is in the process of updating the diagnostic guidelines within two years and it is expected that more evidence is generated on RDTs to support their inclusion as one of the, accepted serological tests in the guidelines for diagnosis of chronic infection, alongside ELISA, IHA and IIF.
  - Additional comments and recommendations from individual experts included:
    - It is necessary to generate more evidence to reach a consensus on a validation protocol for RDT algorithm implementation.
    - It is recommended to include more than one RDT reader for RDT interpretation in the protocol and provide general guidance on assessing the ease-of-use of the investigational products (usability sub-study).
    - It is recommended that the Informed Consent is translated to other local languages (for indigenous populations) and adapted especially to illiterate people, minors and children. This recommendation will be reviewed and considered by the bioethics group (PAHO ERC).
    - The development of Artificial Intelligence applications for supporting results registration, reading and interpretation of RDTs is recommended.
    - Experts from Central America and Mexico raised the need to develop a Generic protocol to evaluate the analytical performance of RDTs, specifying the required, percentage of low antibody levels according to the analysis plan.

GROUP 2. Key Product Characteristics, standards, and quality controls of molecular tests for the early diagnosis of *T. cruzi* infection: To assess and guide the development and use of these tests

This Group achieved consensus on the following key product characteristics for molecular tests for diagnosis of *T. cruzi* infection, and recommendations for researchers and test developers:

- **Clinical performance:**
  - Minimum lower bond of sensitivity is 90%, with an ideal range between 95%-100% and specificity is 98%-100%, in both parameters including the margin of error (95% CI).
  - Experts request PAHO to perform a systematic review to redefine the sensitivity and specificity ranges based on current technologies and eco-epidemiological contexts, considering sample size and regional epidemiological conditions.

- **Reference Test Methods (in Field Validations):**
  - Use available field-validated molecular diagnostic kits as comparator tests (reference test method).
  - Conduct validation studies in multicenter trials using local clinical samples.

- Define the gold standard based on the clinical-epidemiological context: parasitological and serological methods for congenital cases, direct parasitological methods for other ones.

- **WHO-validated molecular diagnostic test validation panel:**
  - Experts request WHO and PAHO to provide calibrators, international reference standards, and positive controls for research purposes.

- **Analytical Sensitivity:**
  - Use validated sequences for quantifying copy numbers in multicenter studies.
  - The minimum acceptable value is 10 parasite genome equivalents per sample, with an ideal value of 1 parasite equivalent within a confidence interval of 1 log (1-9.9).

- **Specimen Type and Preparation:**
  - Ideal samples are anticoagulated whole blood or dried blood spots, compatible with DNA extraction kits.
  - Use stabilizing solutions like Guanidine Hydrochloride 6M, EDTA 0.2 M, pH 8.00 (GE) for transporting fluid samples, following national guidelines based on clinical-epidemiological conditions for sample collection and DNA extraction.
  - WHO/PAHO-driven validation programs should generate evidence on multiple sample collections or extractions.

- **Multiplex Formats:**
  - Develop and validate multiplex molecular methods, including internal amplification controls for POC molecular tests assays (including LAMP).
  - Diagnosing CD in a multi-pathogen context is unnecessary due to insufficient clinical-epidemiological evidence of co-morbidity e.g. with pathogens causing the diseases included in the EMTCT-Plus initiative (HIV, syphilis and HepB).

- **Diagnostic Algorithms of Combined Methods:**
  - Use validated methodologies (LAMP or qPCR) based on clinical-epidemiological scenarios and it is recommended to generate more evidence to validate these methodologies, focusing especially on acute oral transmission outbreaks of *T. cruzi*.
  - It is not advised combining molecular diagnostic methods in algorithms as there is not sufficient experimental evidence.

- **Quality Assurance:**
  - Perform quality assurance processes when instruments, reagent batches, or operators change.
  - Follow strictly the instructions of manufacturers with operational controls included in the commercial kits.
  - Evaluate proficiency testing panels before implementing new molecular assays, with at least one panel per year, ideally two.
  - Experts request PAHO and WHO to promote the production of suitable biological materials for external quality control panels, developed regionally with appropriate materials for each clinical-epidemiological context.

- **Future perspectives for molecular tests for diagnosis of *T. cruzi* infection:**
  - Develop protocols for manufacturing quality control panels at the regional level.
  - Advocate with PAHO and WHO to produce suitable and accessible biological materials for panels (e.g., reference laboratory strains, regional circulating isolates, DTUs).
  - There was no consensus achieved on expected specificities values in field studies, suggesting the need for more research to determine minimal specificities values.

GROUP 3. Evidence on Cost-Effectiveness and Economic Impact: To agree on the necessary evidence to facilitate the integration of new diagnostic methods into the health systems in Latin America

This Group discussed the different models, assumptions and learnings from the presentations on this component the previous day, and set the recommendations for the economic impact evaluations of diagnostic tests for CD.

- A cost-effectiveness analysis can be conducted to compare (1) the current standard of care for diagnosing chronic *T. cruzi* infection to, (2) new algorithms that incorporate new testing technologies such as rapid diagnostic tests (RDTs) adopted at lower levels of the healthcare system (the intervention). This is an annex to the main study, which will evaluate the performance of the new algorithms incorporating these new test technologies in Country/Setting X.
- There could be four components on this study: (A) Estimating the potential impact in terms of effectiveness of CD diagnostic care cascade in relation to the standard of care and intervention scenario, (B) estimating the costs associated with the different testing algorithms, (C) evaluating the cost-effectiveness of the different testing algorithms, and (D) performing a Budget Impact Analysis (BIA) to assess the financial impact of adopting a new algorithm.
- The approach can incorporate the direct benefits on the diagnostic pathway for CD. The Chagas Diagnostic Cascade Model Framework can be created using the FIND Chagas Diagnostic Algorithm application (https://finddx.shinyapps.io/chagaspathway/), developed in collaboration with DNDi, or alternative decision-tree models. Models representing the current standard of care within Setting X should be compared to models representing the diagnostic algorithms incorporating new testing technologies to estimate changes in overall diagnostic accuracy and costs. Required parameters for CD diagnostic cascade model were discussed, and the addition the Cost analysis with a excel tool that reflects both the patient- and provider-perspective.
- Costs can be assigned to resource outputs (number of tests by type and location of testing, and number of individual visits before diagnosis by location) from the key outcomes of the different diagnostic algorithms. Effectiveness outcomes, such as the number of true positive and true negative cases, as well as the number of positive individuals linked to further care/treatment will be used to calculate the cost per correct diagnosis and the cost per positive case linked to further care and treatment for the different algorithms. The costs and the outcomes for each algorithm can then be used to calculate the incremental cost-effectiveness ratios (ICER) for each diagnostic algorithm.
- A one-way sensitivity analysis can be conducted on key parameters that significantly influence which algorithm is considered more cost-effective. A budget impact analysis will be added, it aims to assess the financial implications of implementing the new diagnostic approach (the intervention) compared to the standard of care. This analysis entails determining the total cost of testing the care-seeking population under both scenarios: using the standard diagnostic procedure and employing the intervention algorithm.

- **Future perspectives for evidence on cost-effectiveness and economic impact:**
  - The members of this subgroup committed to continuing their work virtually after this meeting to finalize the cost-effectiveness sub-study that needed to be incorporated into the Generic Research Protocol for the evaluation of RDTs, in collaboration with PAHO and FIND (**annex 4**).
  - There was not a consensus achieved on where the protocol (incorporating the annex on cost-effectiveness) would be carried out. Given that the variability of the economic evidence between countries can be high, the selection of the sites shall consider different countries and settings,

with variable lower levels of the healthcare system (where the intervention “new RDT algorithms” would be incorporated).

In the end of the second day a representative of each of the three Groups presented in plenary the conclusions and further steps.

ACKNOWLEDGEMENTS

FIND, on behalf of DNDi, CONICET and PAHO, thanks to the participating experts for their engagement and insightful contributions. We thank specially to Mundo Sano Foundation for providing the warm reception of the event in its premises. We are optimistic that the consensus we have reached will guide the development, optimization and implementation of cost-effective diagnostic tests, ultimately increasing access to prompt diagnosis of the most affected undeserved communities. We hope that our conclusions will support countries in their efforts to combat the disease and achieve control and elimination targets by 2030.

ANNEXES

Annex 1. List of attendees

Annex 2. Survey Results Report (prior to the meeting)

Annex 3. Agenda and Presentation slides

Annex 4. Annex to Generic Protocol: Cost-effectiveness sub-study (version 06 June 2024)

REFERENCES

[1] Lee BY, Bacon KM, Bottazzi ME, Hotez PJ. Global economic burden of Chagas disease: A computational simulation model. Lancet Infect Dis. 2013 Apr;13(4):342–8.

[2] Chaves GC, Arrieche MAS, Rode J, Mechali D, Reis PO, Alves RV, et al. Estimación de la demanda de medicamentos antichagásicos: Una contribución para el acceso en América Latina. Rev Panam Salud Publica/Pan Am J Public Heal. 2017 Jun 8;41:e45–e45.

[3] Cucunubá, Z. M., Manne-Goehler, J. M., Díaz, D., Nouvellet, P., Bernal, O., Marchiol, A., Basáñez, M.-G., & Conteh, L. (2017). How universal is coverage and access to diagnosis and treatment for Chagas disease in Colombia? A health systems analysis. *Social Science & Medicine (1982)*, *175*, 187–198.

[4] World Health Organization. Ending the neglect to attain the sustainable development goals: a road map for neglected tropical diseases 2021–2030. Geneva, CH: WHO, 2020.

[5] Rivero R, Santini MS, Lopez-Albizu C, Rodriguez M, Calbosa A, Oliveto D, Esteva M, Bisio M, Bohorquez LC. Comparative evaluation of four rapid diagnostic tests that detect human Trypanosoma cruzi-specific antibodies to support diagnosis of Chagas Disease in urban population of Argentina PLoS Negl Trop Dis. 2024;18(3):e0011997 (PMID: 38489395).

[6] Lopez R, García A, Aruni JJ, Balboa V, Rodríguez A, Erkosar B, Kamoun A, Rodriguez M, Fortun E, Bohorquez LC. Comparative evaluation of lateral flow assays to diagnose chronic Trypanosoma cruzi infection in Bolivia. PLoS Negl Trop Dis. 2024;18(3):e0012016 (PMID: 38437237).

[7] Marchiol A, Florez AC, Caicedo A, Segura M, Bautista J, Ayala MS, Herazo H, Forsyth C, Bohorquez LC. Laboratory evaluation of eleven rapid diagnostic tests for serological diagnosis of Chagas disease in Colombia. PLoS Negl Trop Dis. 2023;17(8):e0011547 (PMID: 37607214).

[8] Iturra JAD, Leony LM, Medeiros FAC, Souza Filho JAd, Siriano LdR, Tavares SBdN, Luquetti AO, Belo VS, Sousa ASd and
Santos FLN. A multicenter comparative study of the performance of four rapid immunochromatographic tests for the detection of anti-Trypanosoma cruzi antibodies in Brazil. Front. Med. 2023; 10:1031455. doi: 10.3389/fmed.2023.1031455 (PMID: 36936214).

[9] Rojas Panozo L, Rivera Nina S, Wehrendt DP, Casellas A, Pinto L, Mendez S, Kuo CW, Lozano DF, Ortiz L, Pinazo MJ, Picado A, Sanz S, Abril M, Gascon J, Wong S, Schijman AG, Torrico F, Alonso-Padilla J. Evaluation and validation of a PrintrLab-based LAMP assay to identify Trypanosoma cruzi in newborns in Bolivia: a proof-of-concept study. Lancet Microbe. 2024; S2666-5247(24)00110-1. doi: 10.1016/S2666-5247(24)00110-1. Epub ahead of print (PMID: 38971173).

Annex 1. List of attendees

Meeting on diagnostic evaluation and economic impact analysis of new diagnostic methods for Chagas disease

Day 1 - 6th May 2024 at Mundo Sano Foundation headquarters, Buenos Aires

Name	Affiliation (country)
Laura Bohorquez # *	FIND (Colombia)
Shaukat Khan #	FIND (Switzerland)
Kyra Grantz #	FIND (Switzerland)
Sarah Girdwood #	FIND (Switzerland)
Marcelo Rodriguez	FIND (Argentina)
Alejandro Schijman # *	INGEBI-CONICET (Argentina)
Arturo Muñoz	INGEBI-CONICET (Argentina)
Maria Jesus Pinazo *	DNDi (Brazil)
Natalie El Kheir	DNDi (Brazil)
Andres Caicedo #	DNDi (Brazil)
Rafael Herazo #	DNDi (Brazil)
Colin Forsyth *	DNDi (USA)
Freddy Pérez *	PAHO (USA)
Hector Coto	PAHO (USA)
María Isabel Jercic #	INS (Chile)
Belkisyolé Alarcón de Noya	Intituto de Medicina Tropical-UCV (Venezuela)
Alejandro Hasslocher	Fiocruz (Brazil)
Andrea García Balderrama	INLASA (Bolivia)
Antonieta Rojas de Arias	CEDIC (Paraguay)
Constança Britto *	Fiocruz (Brazil)
Franciana Rosa Da Silva	CUIDA Chagas (Brazil)
Fred Luciano Neves Santos	Fiocruz (Brazil)
Igor Almeida	UTEP (USA)
Julio Alonso Padilla *	ISGlobal (Spain)
Karina Egüez Soliz	Servicio Departamental de Salud - Ministry of Health (Bolivia)
Karla Lange	Universidad de San Carlos de Guatemala (Guatemala)
Laura Lamfre	redArets (Argentina)
Lizeth Rojas Panozo	CEADES (Bolivia)
Oscar Noya	Intituto de Medicina Tropical-UCV (Venezuela)
Otacilio Moreira #	Fiocruz (Brazil)
Santiago Hasdeu #	redArets (Argentina)
Vidalia Lesmo	SENEPA (Paraguay)

Yerly Magnolia Useche #	CUIDA Chagas (Brazil)
Zulma Cucunuba	Universidad Javeriana (Colombia)
Bertha Espinoza	IIBO-UNAM (Mexico)
Montserrat López Serafín	INDRE (Mexico)
Fernan Aguero	IIBio-UNSAM-CONICET (Argentina)
Jaime Altcheh	Hospital De Niños Ricardo Gutierrez (Argentina)
Marcelo Abril	Fundación Mundo Sano (Argentina)
Margarita Bisio *	INP Fatala Chaben (Argentina)
Nasim Iusef	Ministerio De Salud Provincia De Buenos Aires (Argentina)
Silvia Longhi	INGEBI-CONICET (Argentina)
Juan Carlos Ramirez	Hospital De Niños Ricardo Gutierrez (Argentina)
Rocío Rivero	INP Fatala Chaben (Argentina)
Observers	
Berra Erkosar #	*FIND (Switzerland - virtual)*
Elena Ivanova #	*FIND (Switzerland - virtual)*
Elisa Sicuri #	*ISGLOBAL (Spain - virtual)*
Ana Pereiro	*Fundación Mundo Sano (Argentina)*
Ivan Scandale	*DNDi (Brazil)*
Eric Chatelain	*DNDi (Brazil)*
Stéphane Hugonnet	*DNDi (Brazil)*
Sergio Sosa Estani	*DNDi (Brazil)*
Rosa Maldonado	*UTEP TEXAS (USA)*
Eric Dumontiel	*TULANE UNIVERSITY (USA)*
Gabriela Levy	*IIBio-UNSAM-CONICET (Argentina)*
Cecilia Saux	*UNPaz (Argentina)*
Jessica Rodríguez Durán	*INGEBI-CONICET (Argentina)*
Diego Mendicino	*Universidad Nacional Del Litoral (Argentina)*
Gustavo Landfried	*FIND (Argentina)*
Emmaria Danesi	*ANLIS Malbrán. Ministerio de Salud (Argentina)*
Constanza Lopez Albizu	*INP Fatala Chaben (Argentina)*

Speakers, * Moderators


Presentation of the
generic study protocol to
evaluate CD RDTs,
developed by PAHO
Dr. Freddy Pérez
Dra. María Isabel Jercic

Day 2 - 7th May 2024 at NH Hotel, Buenos Aires (subgroups discussions)

GROUP 1. CD RDTs

Discussion Group on the Generic research protocol for the evaluation of Rapid Diagnostic Tests (RDTs) for Chagas disease, to ensure high quality studies in the Americas.

Name	Affiliation (country)
Laura Bohorquez *	FIND (Colombia)
Andres Caicedo	DNDi (Brazil)
Rafael Herazo	DNDi (Brazil)
Freddy Pérez	PAHO (USA)
Hector Coto	PAHO (USA)
María Isabel Jercic *	INS (Chile)
Alejandro Hasslocher	Fiocruz (Brazil)
Andrea García Balderrama	INLASA (Bolivia)
Franciana Rosa Da Silva	CUIDA Chagas (Brazil)
Fred Luciano Neves Santos	Fiocruz (Brazil)
Karina Egüez Soliz	Servicio Departamental de Salud - Ministry of Health (Bolivia)
Karla Lange	Universidad de San Carlos de Guatemala (Guatemala)
Oscar Noya	Intituto de Medicina Tropical-UCV (Venezuela)
Vidalia Lesmo	SENEPA (Paraguay)
Bertha Espinoza	IIBO-UNAM (Mexico)
Fernan Aguero	IIBio-UNSAM-CONICET (Argentina)
Jaime Altcheh	Hospital De Niños Ricardo Gutierrez (Argentina)
Silvia Longhi #	INGEBI-CONICET (Argentina)
Rocío Rivero	INP Fatala Chaben (Argentina)
Observers	
Eric Dumontiel	TULANE UNIVERSITY (USA)
Diego Mendicino	Universidad Nacional Del Litoral (Argentina)
Emmaria Danesi	ANLIS Malbrán. Ministerio de Salud (Argentina)

* Moderators # Relator

GROUP 2. CD MOLECULAR DIAGNOSTICS

Discussion Group on Key product characteristics, standardized methods and quality controls, to assess and to guide the development and use of molecular tests for early diagnosis of *T. cruzi* infection.

Name	Affiliation (country)
Marcelo Rodriguez	FIND (Argentina)
Alejandro Schijman *	INGEBI-CONICET (Argentina)
Arturo Muñoz #	INGEBI-CONICET (Argentina)

Maria Jesus Pinazo	DNDi (Brazil)
Colin Forsyth	DNDi (USA)
Belkisyolé Alarcón de Noya	Intituto de Medicina Tropical-UCV (Venezuela)
Antonieta Rojas de Arias	CEDIC (Paraguay)
Constança Britto	Fiocruz (Brazil)
Igor Almeida	UTEP (USA)
Julio Alonso Padilla	ISGlobal (Spain)
Lizeth Rojas Panozo	CEADES (Bolivia)
Otacilio Moreira *	Fiocruz (Brazil)
Montserrat López Serafín	INDRE (Mexico)
Margarita Bisio	INP Fatala Chaben (Argentina)
Juan Carlos Ramirez	Hospital De Niños Ricardo Gutierrez (Argentina)
Observers	
Rosa Maldonado	*UTEP TEXAS (USA)*
Constanza Lopez Albizu	*INP Fatala Chaben (Argentina)*
Juan David Ramirez	*Universidad del Rosario (Colombia)*

* Moderators # Relator

GROUP 3. ECONOMIC IMPACT

Discussion Group on the evidence on cost-effectiveness and economic impact necessary to be generated in order to facilitate the integration of new diagnostic methods into the health systems in Latin America.

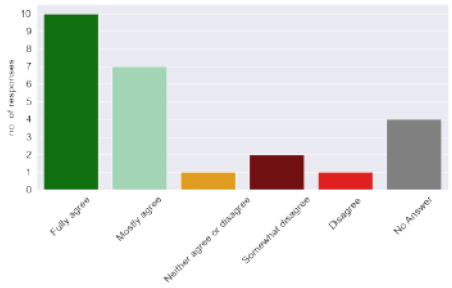
Name	Affiliation (country)
Shaukat Khan *	FIND (Switzerland)
Kyra Grantz #	FIND (Switzerland)
Sarah Girdwood *	FIND (Switzerland)
Natalie El Kheir #	DNDi (Brazil)
Freddy Pérez	PAHO (USA)
Laura Lamfre	redArets (Argentina)
Santiago Hasdeu	redArets (Argentina)
Yerly Magnolia Useche	CUIDA Chagas (Brazil)
Zulma Cucunuba	Universidad Javeriana (Colombia)
Nasim Iusef	Ministerio De Salud Provincia De Buenos Aires (Argentina)
Observers	
Gabriela Levy	*IIBio-UNSAM-CONICET (Argentina)*
Cecilia Saux	*UNPaz (Argentina)*

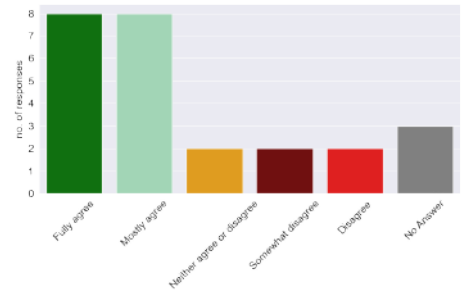
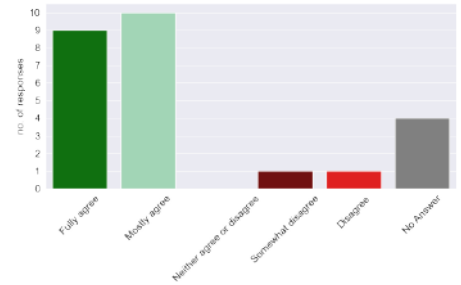
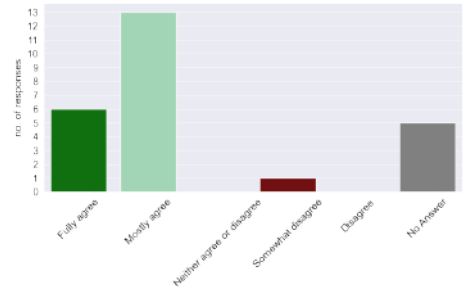
* Moderators # Relator

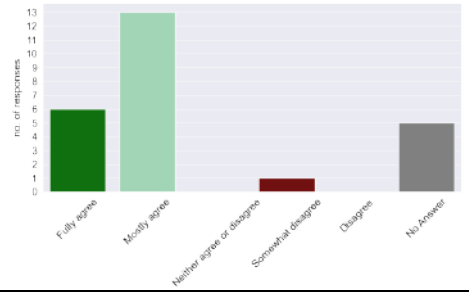
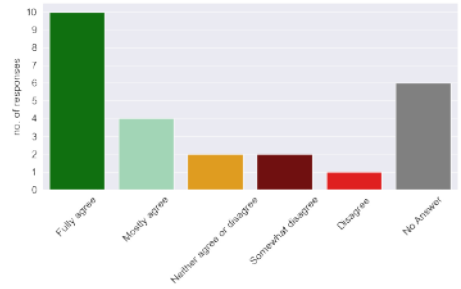
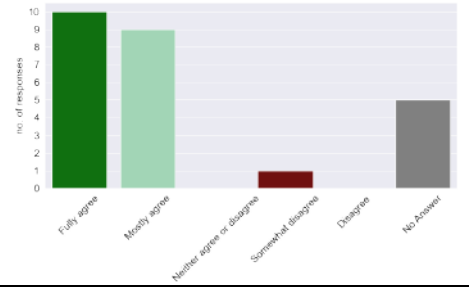
Annex 3. Survey Results Report (prior to the meeting)

1. Generic research protocol for selecting and assessing appropriate RDTs for chronic *T. cruzi* infection, developed by PAHO

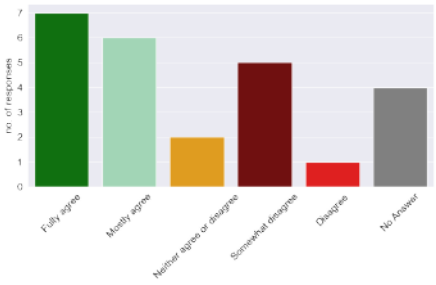
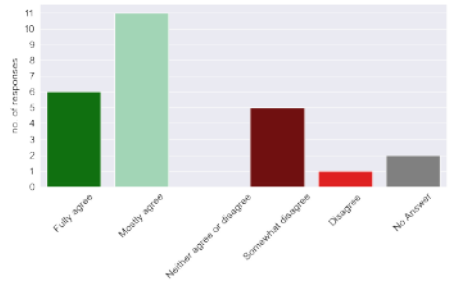
i. Do you agree with including further the following prioritization of testing algorithms and test usage scenarios in the generic protocol?

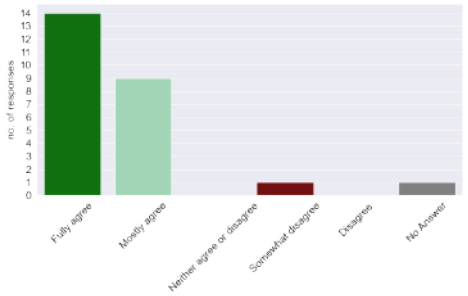
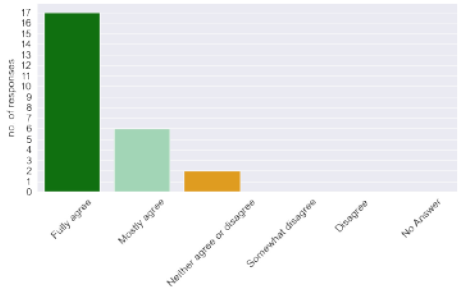
	Assign your score (from 1-5) or NA **1.** Disagree **2.** Somewhat disagree **3.** Neither agree or disagree **4.** Mostly agree **5.** Fully agree **NA (no answer).** I prefer not to answer / do not have the expertise	**If your level of agreement was 3 or less, please provide an explanation**
Additional test usage and testing algorithms		
In hard-to-reach populations Screeening combining 2 RDTs for chronic infection plus a laboratory-based confirmatory test With the acceptance criteria of combined performance recommended by PAHO / WHO (values TBD)		- The added value of a laboratory test such as ELISA, IFA or HAI to a sample that already has two positive/negative RDTs is questionable/unknown. The use of the confirmatory test in this setting would result in "disagreements" between the 2 initial RDTs or if there are still doubts about the performance of the combined tests. - The results of the systematic review in the appendix appear to indicate that the techniques have low sensitivity and high specificity. Therefore, an algorithm based solely on the use of RDTs could result in missed opportunities to detect infection. If new technological developments allow for RDTs with high sensitivity and low specificity, algorithms that permit confirmation only of positive results would make more sense. In such cases, these algorithms would be used in situations representing the sole diagnostic opportunity. - If the RDTs were used for populations with difficult access, the sending of the sample or the difficult transfer of the patient to a laboratory of medium / high complexity makes the use of rapid tests lose the meaning. - In hard to reach populations it is going to be difficult to run lab-based confirmatory test.

In hard-to-reach populations Diagnostic algorithms based combining 2 RDTs plus a third tie-breaker RDT With the acceptance criteria of combined performance recommended by PAHO / WHO (values TBD)		- I believe that we still do not know the performance of all the PDRs, in order to break the tie with one of them. Although it could be possible, if this third test had a very good Se and Sp. - I prefer to use a laboratory-based confirmatory test, with a different principle, instead a tie-breaker RDT. - It is too expensive. - I recommend that confirmatory test should be a traditional technique (Ex: ELISA, Hemagglutination, Lytic antibodies). - It is necessary to evaluate different algorithms with combinations of tests before suggesting one for diagnosis. - Until the process of validation and verification in each country ensures the accuracy of each test, and algorithm in each country.
The acceptance criteria of combined clinical performance should consider the following parameters:		
Minimum combined accuracy (i.e. combined Se % and combined Sp % with values TBD)		- Combined accuracy is not an estimation parameter described in international guidelines. - A high sensitivity is necessary to ensure the accuracy of the test. It will be used mainly as a screening test.
Minimum combined PPV % (values TBD) (at a prevalence of <5% or less) (at a prevalence of 5-10%) (at a prevalence of >10%)		- There is limited epidemiological information available to us that would allow us to determine the prevalence in many of the intervention areas.

Minimum combined NPV % (values TBD) (at a prevalence of <5% or less) (at a prevalence of 5-10%) (at a prevalence of >10%)		- There is limited epidemiological information available to us that would allow us to determine the prevalence in many of the intervention areas.
That the tests (including RDTs) have different antigenic principles. (If it is not declared by the manufacturers it is possible to assess the shared common false reactivity of the tests)		- The manufacturer must declare the composition of the product and its performance in different regions taking into account the variability of the parasite. - Due to patent issues, commercial developments do not disclose the antigens used. I am not familiar with what "the shared common false reactivity of the tests" refers to. In my opinion, tests using the same methodological principle should be used as long as they use different antigenic preparations (lysates vs. recombinants). - I absolutely agree with that the antigenic sources are different, this guarantees greater sensitivity when the RDTs are implemented as an algorithm, but if the manufacturers do not inform the antigenic source, it is not enough to evaluate the Analytical Specificity carried out in depth as in the Immunoassay Interference guidelines. by Endogenous Antibodies; Approved Guideline. CLSI document I/LA30-A (ISBN 1-56238-658-1). Even though two IVDs or RDTs have the same antigenic source, the problem with this is that the sensitivity will be lower when the composite standard is used. It has been shown that the specificity of the RDTs is very good. - The RDT should always provide the Ag.
Identify the most efficient algorithm or the one that provides the greatest value for money using the costs and the outcomes for each algorithm. E.g. the incremental cost-effectiveness ratios (ICER) for each diagnostic algorithm, which compares the additional cost of one algorithm relative to the next least costly algorithm.		- Sometimes more than the direct cost of the test are other parameters to be considered a long time, as confidence of the population with false positive or negative cases.
Further comments: None		

ii. Do you agree that the should be needed to include the following considerations for assessing appropriate RDTs for chronic *T. cruzi* infection in the generic protocol?

	Assign your score (from 1-5) or NA **1.** Disagree **2.** Somewhat disagree **3.** Neither agree or disagree **4.** Mostly agree **NA (no answer).** I prefer not to answer / do not have the expertise	**If your level of agreement was 3 or less, please provide an explanation**
There should be a unique serological external quality assurance (EQA) panel; and a facilitated way to be obtained by researchers (e.g. for assessing appropriate RDTs for chronic *T. cruzi* infection should be the WHO International Standard 1st WHO anti-*Trypanosoma cruzi* I and II Antibody Reference Panel NIBSC code: 11/219)		- I think that it should not be a single panel. It is clear that it is advisable to use a quality assurance panel, but being only one, it could bring inconveniences of availability and marketing. The only available panel only has two verified samples, for DTU I and II. A much more varied panel could be suggested, from different patients, with different titers and covering various aspects of the disease. It should be made easier for researchers. - If there is an excellent and validated reference test at a local place, I would select it. - CD is a complex matter. A good external quality assurance panel already validated locally with a reference test should be done in each reference center. - The panels are very expensive for Latin America and this may hinder the implementation of the trial. - Limited access to panels and logistical and administrative challenges with imports can restrict the conduct of studies in the region. - Antibody Reference Panel NIBSC code: 11/216 include anti-Trypanosoma cruzi antibodies TcII and TcI. - The panels for the evaluation of antibodies for cd must be autochtonous panels or regional panels. there should not be a single eqa panel since eqa is a continuous process and 1 panel is just a photo. Reference centers must be trained to make autochtonous panels as the industry does, and that researchers are provided with the same. - WHO international Standards harboring anti - T.cruzi IV Antibodies should be added, given it can be useful in improving sero-diagnosis of patients from some oral outbreaks.
There should be consensus on a unique reference test method		- I think that would be ideal, however, I think that the reference standard used in each study should be the one used in the country and the one recommended by the National Institutes of Health. What could be added to the protocol is a guide and a small section where the reference standards are suggested. - References must consider geographic regions and specifications in the circulating parasite. - Several methods available already validated could be used. - There should be consensus so that RDTs and new technologies can be incorporated as possible reference tests with flexibility and openness, but above all evaluating the new tests as part of the composite standard and not with a lower hierarchy of the tests in use, and in prospectively. - Due to the differences in results between North, Central and South America, it would be better that there is a consensus method by region. - Consideration should be given to the varied epidemiological scenarios of T. cruzi infection, aiming to establish consensus on reference tests that are most suitable for specific scenarios.

There should be guidance on statistical considerations to estimate the minimum sample size, according to the acceptance performance criteria values achieved by consensus *(sample size estimation tool developed by FIND is available for non-expert statisticians https://finddx.shinyapps.io/SampleSize/)*		- It is more appropriate to have a statistical professional on the team.
There should be consensus on how to ensure the appropriate execution, interpretation and registration of results once the use of RDTs scale up to real scenario conditions		- The protocol is generic, so trying to cover in detail the recommendations on the RDT tests that are chosen by each region would make the protocol very complex and impractical. - I think it is not strong enough in the context of external quality assurance, if we can count on WHO or better documented approaches evidence based.
Further comments: None		

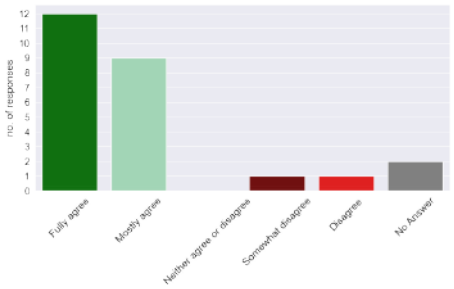
2. Performance and operational characteristics, standardized methods and quality controls to assess and to guide future use of molecular tests for CD

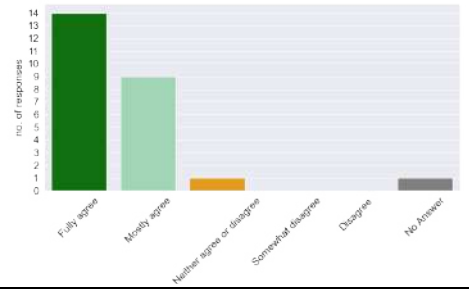
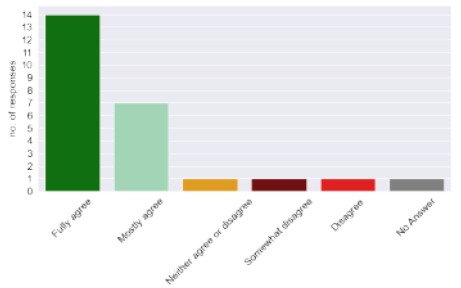
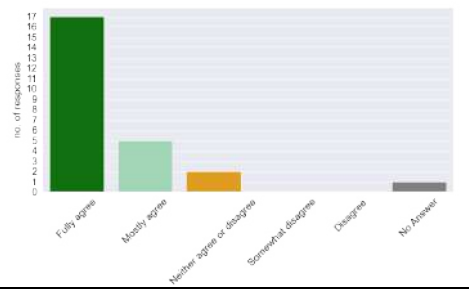
Table 1. Molecular laboratory methods for diagnosis of *T. cruzi* infection

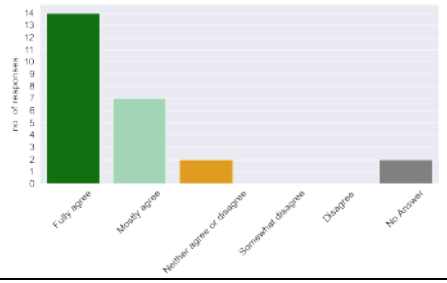
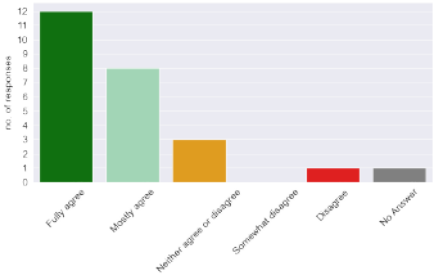
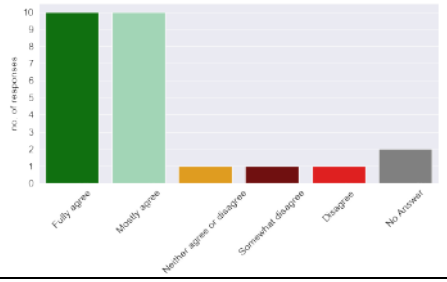
Assay	Required equipment and reagents	Required personnel	Assay duration (From DNA extraction to results)	Cost per sample (USD) Excluding labor	Positive and negative amplification controls	Limitations	Appropriate setting for use	Ref.
Real time PCR	**Equipment**: Incubator, Microcentrifuge	Trained staff	<6h	13-20	- Parasite DNA sample with known genotype	compartmentalized rooms for PCR (see Annex)	- National reference laboratory	PHS, 2017, Q4E guide

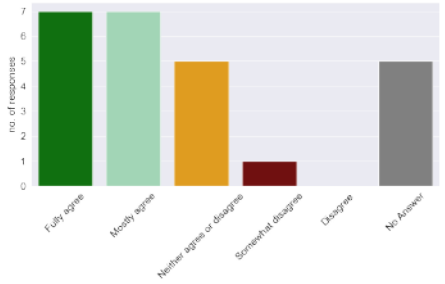
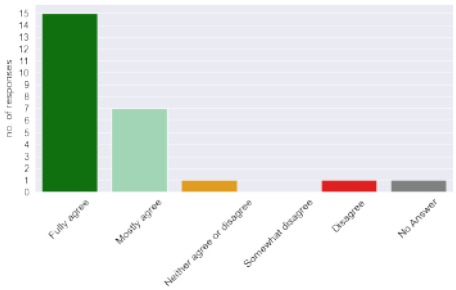
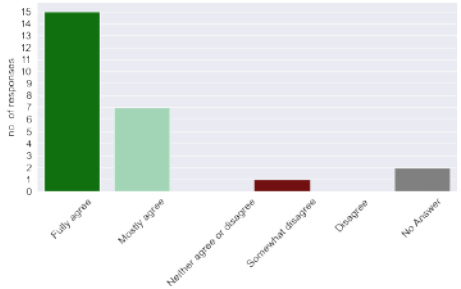
	DNA clean working Hood, Thermocycler, Computer **Reagents:** DNA extraction reagents PCR reagents Oligonucleotide & probes				Strong positive: 10 fg DNA Weak positive 1 fg DNA - Sample without DNA template	There is no consensus regarding the DTU or using synthetic DNA for positive control	- Research laboratory	
Loop mediated isothermal amplification (LAMP)	**Equipment:** Incubator, Microcentrifuge Hood **Reagents:** DNA extraction reagents LAMP reagents	Staff with minimum training	<4h	5-10	- Parasite DNA sample with known genotype - Sample without DNA template	- only qualitative - Low throughput> There is no consensus regarding the DTU or using synthetic DNA for positive control	- Second Level Hospital, Maternity, Field laboratory	Wehrendt et al, 2021 Longhi et al, 2023

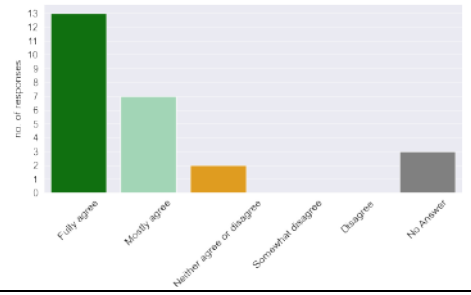
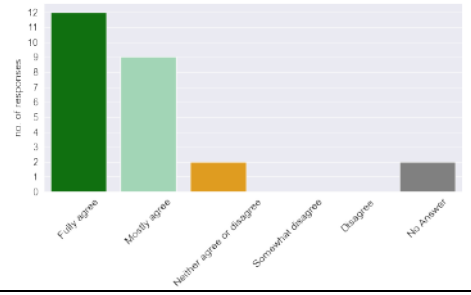
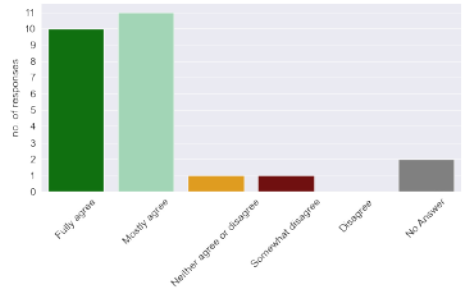
i. Do you agree with the suggested "priority features for point of care molecular testing methods (incl. LAMP) for *T. cruzi* infection" below?

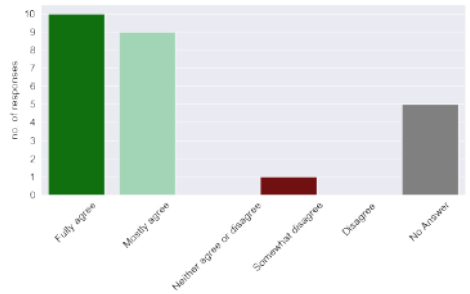
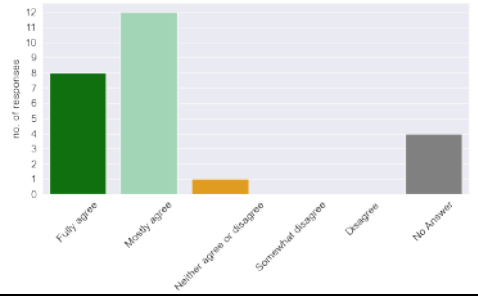
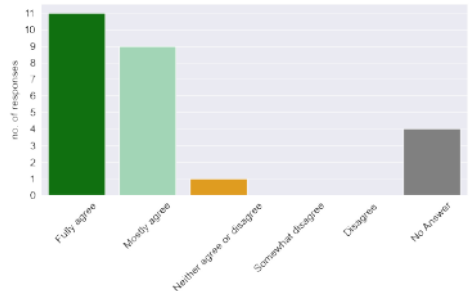
Feature	**Minimum**	**Ideal**	**Assign your score (from 1-5) or NA** **1.** Disagree **2.** Somewhat disagree **3.** Neither agree or disagree **4.** Mostly agree **5.** Fully agree **NA (no answer).** I prefer not to answer / do not have the expertise	**If your level of agreement was 3 or less, please provide an explanation**
SCOPE				
Goal of the test. Intended use	• Point of care diagnosis for patients in the acute phase (associated with congenital, vector, oral, transplant, or transfusion transmission or infection reactivation) (See Annex 1)	• Point of care diagnosis for patients in the acute phase (associated with congenital, vector, oral, transplant, or transfusion transmission or infection reactivation) • Diagnosis for asymptomatic or symptomatic patients in the chronic phase Assessment of response to antiparasitic treatment in the chronic phase		- There is no evidence or recommendation for the use of molecular tests in the diagnosis of chronic phase. - These molecular tests are not for chronically ill patients.

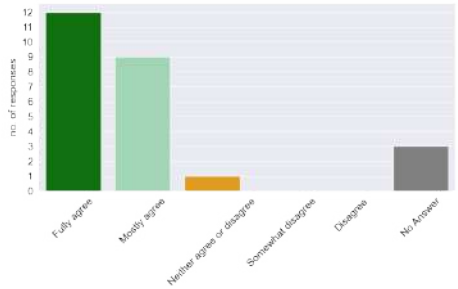
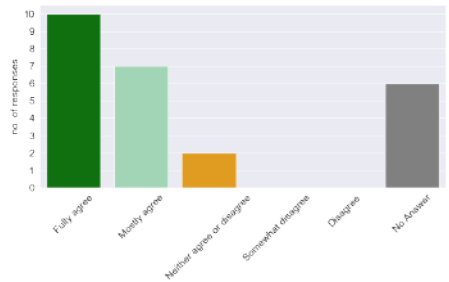
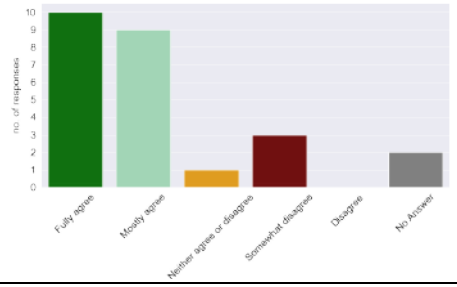
Target operator of the test	Laboratory Technician or Biochemist	Laboratory Technician or Biochemist		- Laboratory technician could be defined more specifically, looking for professional adequacy
Lowest setting for implementation. Target use setting	Low complexity - Second Level Hospital	Rural or field laboratory		- A level II hospital does not have the appropriate conditions to perform molecular tests, most are specialized hospitals or laboratories. - I understand that the device is not fully available for field use, there is still a DNA purification step that is done in the lab. - What is the definition and variety of complexity of Rural Laboratories? Even so I believe that if the Technician was trained and his expertise evaluated, it could implement Lamp.
Target analyte to be detected	*T.cruzi* DNA	*T.cruzi* DNA		- It could include multiplex pathogens that have similar clinical manifestations in those use cases. - It would be interesting to have a LAMP design that includes an internal control of sample integrity.
PERFORMANCE CHARACTERISTICS				

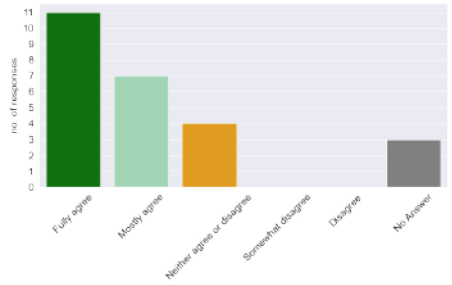
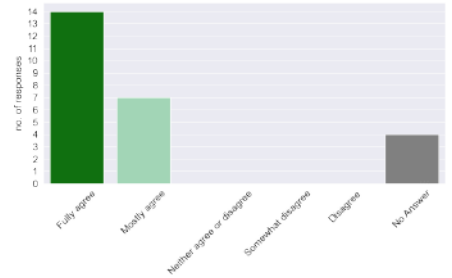
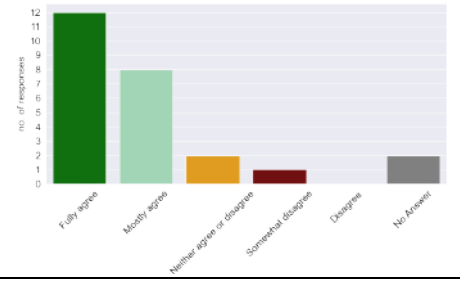
Diagnostic sensitivity	≥95% (point estimate). More than any microscopy test and similar than that of real time PCR	≥98% (point estimate). More than any microscopy test and similar than that of rtPCR		- Maybe the minimun S we are asking in too high for several epidemiological settings. Agree with the ideal - We need to conceptualize what an interval means for the diagnostic parameters, 95% confidence interval, the estimation of diagnostic parameters has associated errors and these are reflected in a 95% CI, then, in the case of sensitivity, 95% should be the lower limit of the 95% CI, and 98% the lower limit of the 95% CI.
Diagnostic specificity	Equivalent to microscopy tests and rtPCR, higher than ELISA			- The comparison is not clear. - The reference standard should be the one proposed by PAHO 2018. - I don't understand this point, compared to what Elisa? compared against what microscopic method and with what operator? The ideal would be to set a "high" specificity of 95? or 98? or a range with those values when the diagnostic specificity is estimated against an uninfected subpopulation. - In terms of specificity for molecular biology techniques, achieving a value of 100% is feasible; therefore, this should be the ideal reference value.
Reference test method / algorithm to evaluate clinical sensitivity/specificity	Any microscopic assay for early diagnosis Complete algorithm for Congenital CD Serological diagnosis for Chronic CD			- There could be a consensus on the reference test method to allow comparability between studies. - The reference standard must follow PAHO 2018 guidelines. - The reference method must be performed according to evidence-based guidelines from PAHO 2018, based on GRADE methodology.

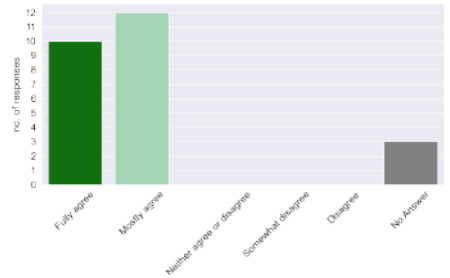
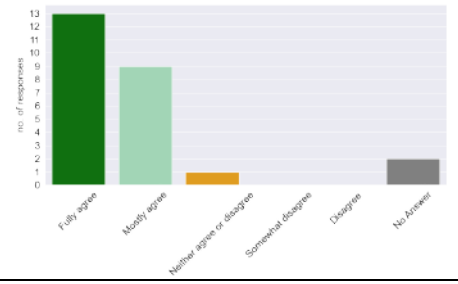
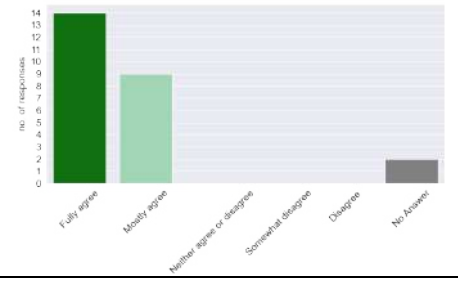
Analytical sensitivity	1 eq. parasite per mL (eq. par./mL) fluid blood / 20 eq. par/mL DBS	0.1 - 0.5 eq. par./mL		- It should be in accordance with the reference method. - Regarding which essay? - Same comment than in case of S: maybe 1-3 parasites for the minimum. - Ideal: it should be standardized to a number of copies of the target gene so that results between different laboratories can be compared. - Also the LOD and its estimate are associated with an error and this is reflected in the width of the 95% confidence interval. I suggest that the width of the CI in a given estimate should be less than 1 log. Whatever the value of the LoD, the error of its estimate should not be greater than 1 log. - The units expressed under the ideal condition should be equivalent to parasites per mL (Eq.Par/mL).
Analytical specificity	No cross-reactivity with *Trypanosoma* species or *Leishmania* spp., or other pathogens present in the blood			- There may be a serological cross-reaction with other pathogens. - If cross-reactivity is described in the IFU, the healthcare team can perform clinical interventions considering this information.
Strain specificity - Inclusivity	Single universal test detecting all DTUs			- If limitations for detecting a specific DTUs are described in the IFU, the healthcare team can perform clinical interventions taking these limitations into account.

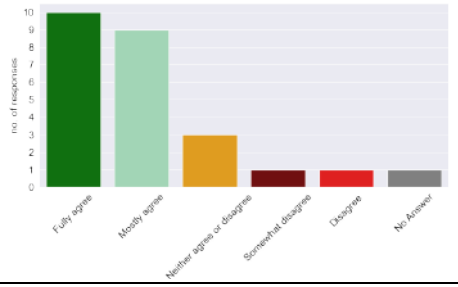
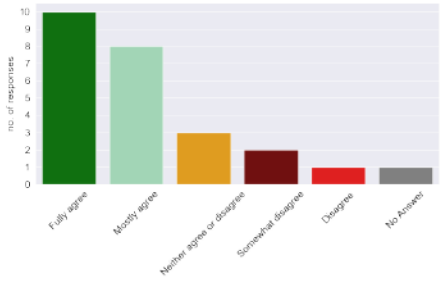
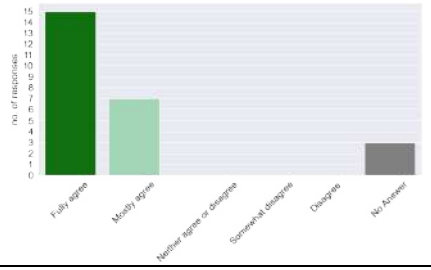
Multiplex detection	No	Yes, including internal amplification control		- If multiplex is ideal, there should be an indication of which other pathogens to be included. - Minimum including internal amplification control, even in singleplex. Ideal: internal control in multiplex.
Quantitation	No	Yes		- "Semiquantitavie/quantitative" to be included as "ideal" and qualitative as "minimum". - LAMP is not a quantitative technique. For point of care diagnostic use, it would be enough to render a qualitative result.
OPERATIONAL CHARACTERISTICS				
Training needs. Time dedicated to training session for end users	DNA clean working station or bench. Heater device 5 days training	DNA clean working station or bench. Heater device 2 days training		- Minimum 2 days training; Ideal: 5 days training/ Minimum: DNA clean working station (with UV light) or bench; Ideal: DNA clean working station (with UV light). - I would include training for test reading.

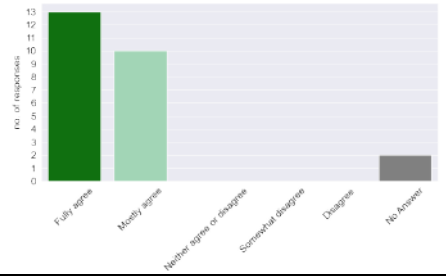
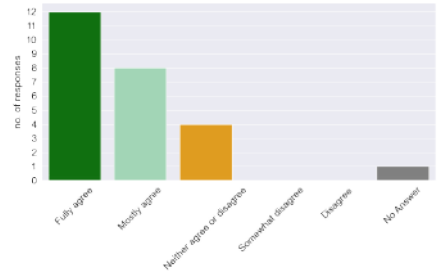
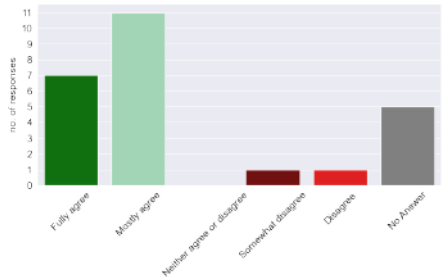
Specimen type	Anticoagulated whole blood (fluid blood): up to 500 µL / Filter paper dried blood spot (DBS): up to 125 µL	Anticoagulated whole blood: 30 µL / Filter paper 3-6 mm DBS punch		- Ideal: direct sample testing on the detection device.
Specimen prep (total steps)	Rapid DNA extraction, a single replicate			- Depending on the scenario, more DNA extractions can be necessary. For example: in oral outbreaks, depending on the time after infection, when the first blood sample was obtained, the parasitic load can be low, similar to a chronic patient. Thus, more DNA extractions from the same specimen could increase the sensitivity. I recommend a second DNA extraction if the first one have a negative result.
Critical processing of steps to be considered	No centrifugation needed No pipetting needed during DNA extraction			- Critical: No centrifugation required (only).

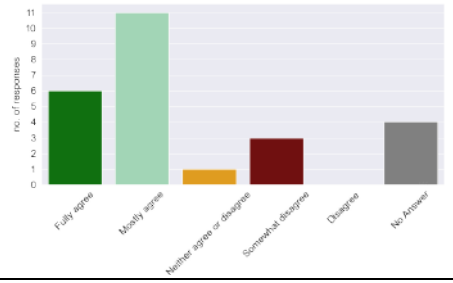
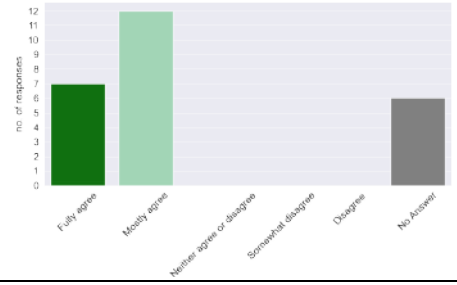
Need for operator to transfer a precise volume of sample	Yes	No		- If a robot is available, prioritize its use.
Time from collected (blood) sample to result	5 h 40-50 min (only amplification)	2 h 40 min (only amplification)		- In case of minimum: is it realistic? - In certain settings, LAMP result could be achieved in one hour (15-20 min DNA extraction and 40 min of amplification)
Specimen capacity + throughput	8-well strip	96- well plate		- Ideal: 1 well strip. - The ideal parameter should be a 96-well plate. - Thinking of scalability and cost-benefit of the tests, it might be necessary to consider a higher throughput. Maybe up to 12 samples per run. - The capacity to process should be adapted to the needs of the laboratory or health care place in charge of performing the assay, which in turn may depend on the epidemiological setting (oral outbreaks, early diagnosis of a newborn to a seropositive mother, etc).

Instrumentation integration	Simple reading device	Instrument-free visual (naked eye)		- In weak positive results, a naked eye can be inaccurate and operator-dependent. The use of a simple device is ideal to me, especially if the device can read the result using an AI algorithm (submit result to an app that could use AI to interpret the result). - The quantitative result is more relevant than a qualitative result. - Visualizing with naked eye is highly operator-dependent. This methodology coupled with a cost-effective equipment for fluorescence visualization would increase the analytical sensitivity, especially in patients with low parasite loads. - The instrumentation integration should fit the REASSURED criteria.
Power requirements	110 /220 W	No electricity needed / Portable batteries / Solar energy		
Data analysis	No	Yes		- If the technique is visual and allows for positive and negative internal control, it is not necessary to have an application for data analysis. - Minimum: Yes; Ideal: No. The ideal is to have a device that gives the final result with accuracy, without the need of an operator analysis. - It will be necessary to analyze the data for future decision making.

Connectivity	No	Yes (REASSURED)		
Result capture, documentation, data display	No	Yes		- Minimum: Yes; Ideal: Yes, with a device with AI algorithm to interpret the results.
Operating temperature / humidity / altitude	Room temperature			

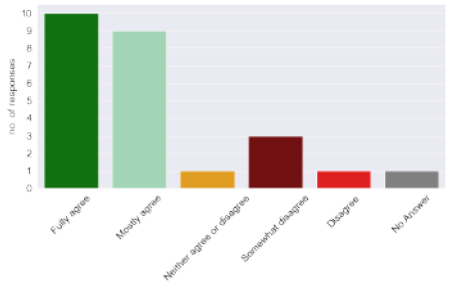
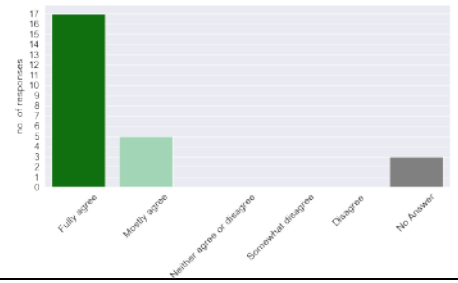
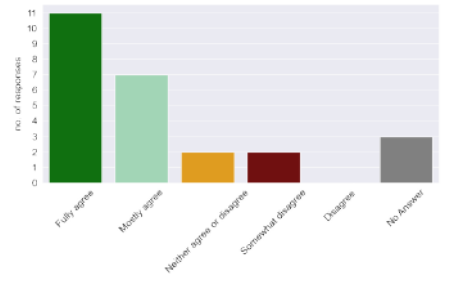
Reagent kit storage	-20C	Room temperature		- The insert has the ability to give information for the storage of the commercial kit, some reagents degrade or precipitate if they reach freezing temperature. - It could not be done in the field if it has to be at -20. - Minimun at 4ºC. - Minimun: 4°C also.
Reagent kit stability	6 months	18 months		- It will depend on the batch and the quality and stability of reagents during use. - The minimum should be 12 Months due to the low use required in some places. - Minimum at list 12 months. - The minimum stability of reagents should be at least 6 months, considering that POC centers do not have the same patient volume as health centers located in more urbanized areas. - Taking into account that distribution and supply will pose a major challenge in some areas, perhaps a shelf life of 12 month should be the minimum desirable. - Reagent stability should last for at least 12 months.
Internal process quality control	Positive control included in kits, non-template control plus negative DNA extraction control			

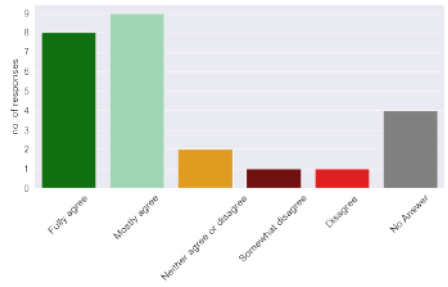
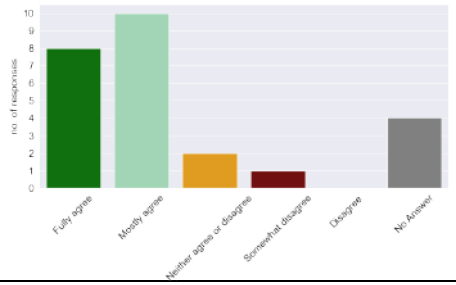
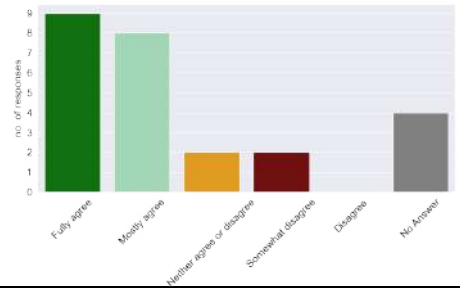
External Controls	Use of third-party panels of samples (see Annex III).	International certified third-party panels of samples (See Annex III). Prospective Field Studies with blind samples.		
Quality assurance	Proficiency testing panels evaluated before starting implementation of a new assay in the laboratory, and every two years thereafter.	Proficiency testing panels evaluated every year.		- Quality control programs that ensure long-term quality and allow for laboratory quality management should be available, and for new operators. - Minimun: Proficiency testing panels evaluated before starting implementation of a new assay in the laboratory, every two years thereafter, or upon any change of operator in the working group. - Quality assurance should be done also on each situation involving change of instruments, kit batch or operators.
PRICING				
Maximum price for individual test (reagent costs only; at scale; ex-works)	5-10 USD	5 USD		- For field work should be cheaper. - It depends on each country, the import and export of reagents leads to the payment of taxes and customs clearance, causing variability in the cost.

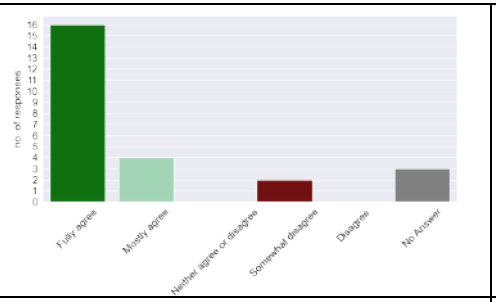
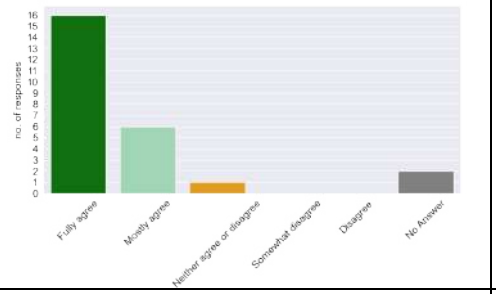
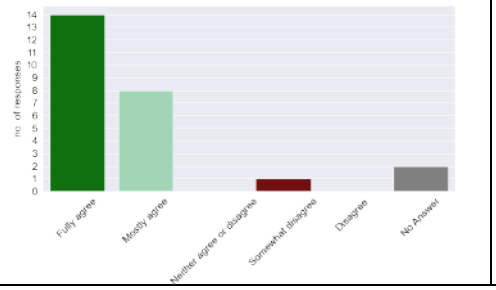
Maximum price for instrumentation	10 000 USD	1 500 USD		- Minimum cost could be for other POC molecular tests <1000 US $; and ideal < 200 US $ or instrument-free. - Lower price. - The minimum should not exceed 5,000 USD. - That price range for minimum to ideal seems unrealistic for the cost of a rtPCR thermocycler (minimum scenario perhaps closer to 30K USD), or to purchase a LF160 for LAMP (ideal scenario closer to 2.5K USD).
Expected scale of manufacture	**values TBD**	**values TBD**		
Further comments: None				

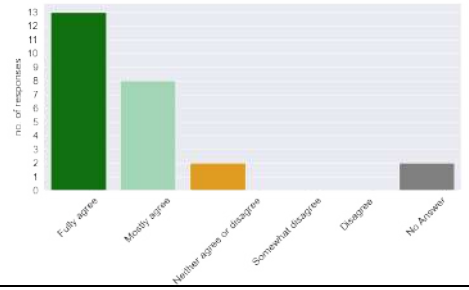
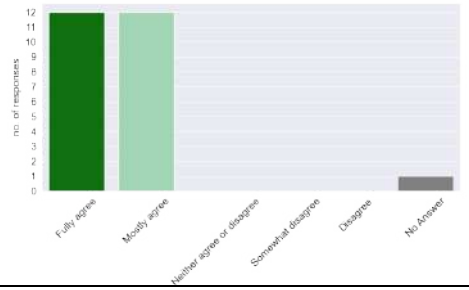
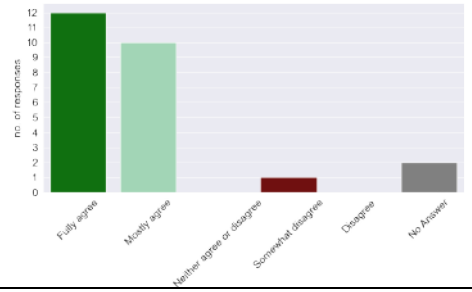
ii. Do you agree with the suggested "priority features for **Real Time PCR tests** for *T. cruzi* infection" below?

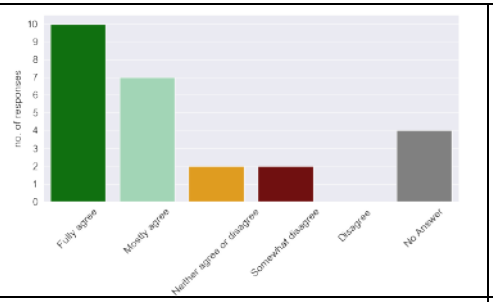
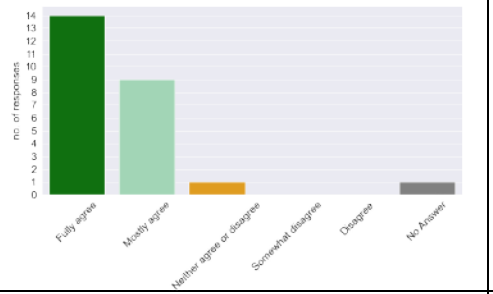
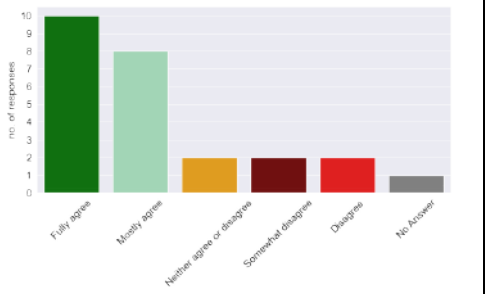
Feature	**Minimum**	**Ideal**	**Assign your score (from 1-5) or NA** **1.** Disagree **2.** Somewhat disagree **3.** Neither agree or disagree **4.** Mostly agree **5.** Fully agree **NA (no answer).** I prefer not to answer / do not have the expertise	**If your level of agreement was 3 or less, please provide an explanation**
SCOPE				

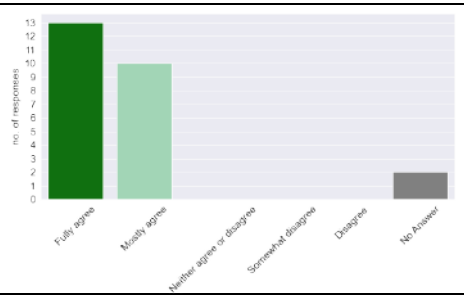
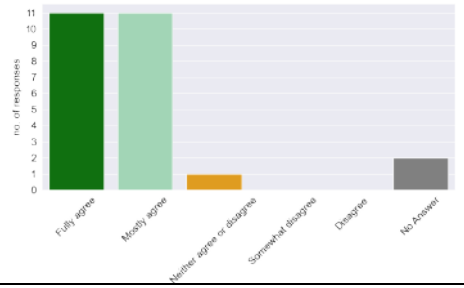
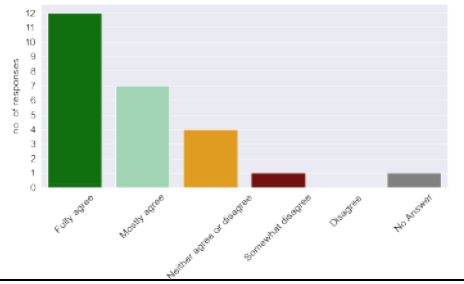
Goal of the test. Intended use	• Diagnosis for patients in the acute phase (associated with congenital, vector, oral, transplant, or transfusion transmission or infection reactivation) (See Annex 1)	• Diagnosis for patients in the acute phase (associated with congenital, vector, oral, transplant, or transfusion transmission or infection reactivation) • Diagnosis for asymptomatic or symptomatic patients in the chronic phase • Assessment of response to antiparasitic treatment in the chronic phase		- There is no evidence or recommendation for the use of molecular tests in the diagnosis of chronic phase. - In the acute phase there is a risk of antibody cross-replication, in the case of mother and child at birth. - It should be only for: Diagnosis for patients in the acute phase and assessment of response to antiparasitic treatment in the chronic phase. - The implementation of real-time PCR in rural area laboratories is very difficult in countries such as Bolivia, due to the high costs and the great needs existing in the diagnosis of congenital Chagas. The budgets allocated to health are low and municipal governments would not be willing to assume this responsibility. - Diagnosis at the chronic stage should be done based on serological assays. At present PCR is not sensitive enough in chronic infection.
Target analyte to be detected	*T.cruzi* DNA plus internal amplification control			
PERFORMANCE				
Diagnostic sensitivity	92% with margin error of maximum +/- 5%	95% with margin error of maximum +/- 5%		- The minimum diagnostic sensitivity should not be below 95%. - Diagnostic sensitivity should be considered in relation to the clinical study group. - Assessment of the Diagnostic Accuracy of Laboratory Tests Using a 95% CI of +/-, 2.5%, 200 positive samples and 200 negative samples. CLSI document EP24-A2 (ISBN 1-56238-778-2). User Protocol for Evaluation of Qualitative Test Performance CLSI establish a minimum of 50 reactive and 50 non-reactive patient samples, in this way the diagnostic parameters are estimated with an error or 95% CI of +/- 8.5%.

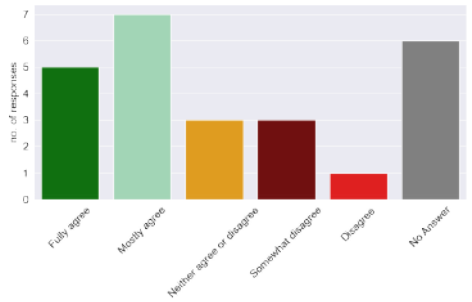
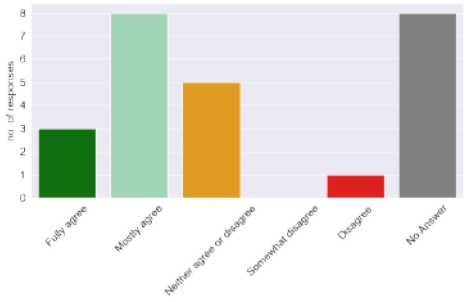
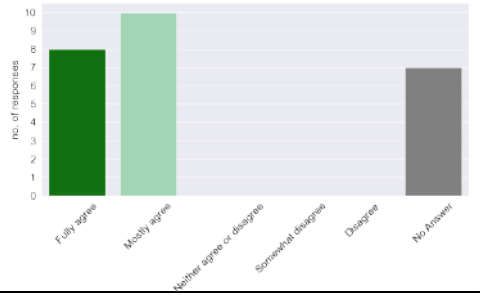
				- Diagnostic sensitivity depends on the epidemiological / clinical scenario of T.cruzi infection. The mentioned values are expected for acute infections.
Diagnostic specificity	Equivalent to microscopy tests, higher than ELISA	Equivalent to microscopy tests, higher than ELISA		- ELISA detection is higher in performance and results than a microscopic test. - In acute cases, the specificity of the assay is higher than that of ELISA. - I don't understand this point, compared to what Elisa? compared against what microscopic method and with what operator? The ideal would be to set a "high" specificity of 95? or 98? or a range with those values when the diagnostic specificity is estimated against an uninfected subpopulation - In terms of specificity for molecular biology techniques, achieving a value of 100% is feasible; therefore, this should be the ideal reference value.
Reference test method / algorithm to evaluate clinical sensitivity/specificity	Any microscopic assay for early diagnosis Complete algorithm for Congenital CD Serological diagnosis for Chronic CD			- There should be consensus on the reference test method that could simplify comparability between studies. - The first point is not clear, it should be more precise. - The reference method must be performed according to evidence-based guidelines from PAHO 2018, based on GRADE methodology
Analytical sensitivity	1 eq. parasite per mL (e.q par/mL)	0.1 - 0.5 eq. par/mL		- It is not in line with the sensitivity stated above. - Ideal: it should be standardized to a number of copies of the target gene so that results between different laboratories can be compared. - Also, associated with an error (width of the 95% confidence interval). I suggest that the width of the CI is less than 1 log. Whatever the value of the LoD, the error of its estimate should not be greater than 1 log. - The units expressed under the ideal condition should be: equivalent to parasites per mL.

Analytical specificity	Do not detect other *Trypanosoma* species or *Leishmania* spp., or other pathogens present in the blood			- If cross-reactivity is described in the IFU, the healthcare team can perform clinical interventions considering this information. - I think it is important that the price of the technology is not a specific impediment to the testing strategy and that resources be used efficiently so that the impact of the cost of diagnosis is relatively low compared to the resources allocated to treatments for the people who tested positive.
Strain specificity - inclusivity	Single universal test detecting all DTUs	Single universal test detecting all DTUs		- If limitations for detecting a specific DTUs are described in the IFU, the healthcare team can perform clinical interventions taking these limitations into account.
Multiplex detection	Yes (*T.cruzi* target plus internal amplification control)	Yes (*T.cruzi* target plus internal amplification control) - In the context of ETMI plus: add multiplex detection of the other pathogens - In the context of epidemiological service (e.g. similar febrile illnesses)		- It is not recommended that a multifunction test be attempted. Better to focus on one that is for the diagnosis of T cruzi in the right way.

Quantitation	No	Yes		- "Semiquantitative" to be included. - It is not clear what method is being referred to.
OPERATIONAL CHARACTERISTICS				
Training needs. Time dedicated to training session for end users	Compartmentalized PCR laboratory (see PHS, 2017 Q4E guide) or Annex 2 One week			
Specimen type	Anticoagulated blood with stabilizing agent (e.g. Guanidine Hydrochloride EDTA)	Anticoagulated blood without stabilizing agent (GE) Dried blood spots		- Ideal: Anticoagulated blood without stabilizing agent (GE) Dried blood spots.

Specimen prep (total steps)	Column based DNA extraction commercial kit			- Dried blood spots. - Column based DNA extraction commercial kit or magnetic bead based automated device. - A method not widely used, more efficient is the magnetic pearl method. - In case of using automatic or semiautomatic DNA extractor devices, DNA is obtained using kits based on magnetic beads, commercially available.
Time from collected (blood) sample to result	Two working day	One working days		- It should be 1 day.
Reagent kit stability	6 months	18 months		- It depends on the time in which it will be imported or exported, since it will be measurable from its laboratory use. - The minimum must be 1 year. - Minimum at least 12 months. - The minimum stability of reagents should be at least 6 months, considering that POC centers do not have the same patient volume as health centers located in more urbanized areas. - Longer minimum shelf life would be advisable if possible. - Reagent stability should be at minimum for one year.

Internal process quality control	Positive control included in kits, non-template control plus negative DNA extraction control.	Positive controls (weak and strong) included in kits, non-template control plus negative DNA extraction control.		
External Controls	Use of third-party panels of samples (see Annex III).	International certified third-party panels of samples (See Annex III). Prospective Field Studies with blind samples.		- I agree with the use of honeycombs but they should be affordable for the countries of the southern cone.
Quality assurance	Proficiency testing panels evaluated before starting implementation of a new assay in the laboratory, and every two years thereafter.	Proficiency testing panels evaluated every year.		- Ideal but not feasible in many countries. - Quality control programs that ensure long-term quality and allow for laboratory quality management should be available, and for new operators. - Proficiency testing panels should be evaluated before starting the implementation of a new assay in the laboratory, every two years thereafter, or upon any change of operators in the work group. - EQC should be also implemented when reagent batch, instruments or operators change.
PRICING				

Maximum price for individual test (reagent costs only; at scale; ex-works)	20	15		- Ideal 15 USD. - Very expensive for developing countries. - It is expensive for developing countries. - I think it is important that the price of the technology is not a specific impediment to the testing strategy and that resources be used efficiently so that the impact of the cost of diagnosis is relatively low compared to the resources allocated to treatments for the people who tested positive. - I don´t have enough information. - In the context of countries such as Bolivia, the cost per test is very high, considering that children born to mothers positive for CD correspond to more than 15% of pregnant women. - A minimum cost of 30-40 USD would seem closer to current situation.
Maximum price for instrumentation	40000 USD two channels thermocycler	25000 USD two channels thermocycler		- Ideal: 25.000 USD - More accessible. - I think it is important that the price of the technology is not a specific impediment to the testing strategy and that resources be used efficiently so that the impact of the cost of diagnosis is relatively low compared to the resources allocated to treatments for the people who tested positive. - I don´t have enough information. - Very high costs to implement, especially in rural areas. - Minimum: 30000 USD two channels, Ideal: 15000 USD two channels.
Expected scale of manufacture	values TBD	values TBD		
Further comments: None				

ANNEXES

ANNEX I.

Acute Chagas disease occurs after a short incubation time (5– 15 days on average, longer for cases of transmission by blood transfusion) and can last for 2 months.
Infection may occur by vectorial transmission when T. cruzi parasites enter the body via a skin break caused by a bug bite, by skin breaching after scratching the bite site, or via mucosal entry (e.g., oral transmission through contaminated food).
Vector-independent transmission routes include: **congenital infection**; **blood transfusion; cell, blood, or tissue transplantation; and needle sharing**. Infection can also occur accidentally after the manipulation of infected triatomines and/or infected animals or laboratory samples.
Immunocompromised patients with chronic T. cruzi infection are at risk of the disease being reactivated and then undergoing an acute presentation with a high mortality rate.
Immunocompromised patients due to organ transplantation include seronegative receptors that may have received organs from seropositive donors and acquire a ***T.cruzi*** **primary infection** ungergoing acute manifestations

ANNEX II.

COMPARTIMENTALIZED ROOMS FOR PCR (PHS, 2017, Q4E guide)
Physical Separation: The room is physically separated from other areas of the laboratory to prevent the entry of contaminants. It may have its own entrance and exit to control access.
Air Filtration: PCR containment rooms are equipped with high-efficiency particulate air (HEPA) filters and ventilation systems to maintain positive air pressure, preventing airborne contaminants from entering the room.
UV Sterilization: Some rooms may be equipped with UV lamps for sterilization between PCR runs, reducing the risk of cross-contamination.
Dedicated Equipment: Each PCR containment room is equipped with dedicated PCR machines (thermocyclers), microcentrifuges, pipettes, and other equipment to prevent the transfer of contaminants between samples.
Workstation Design: The layout of the room is designed to facilitate efficient workflow while minimizing the risk of contamination. Workstations may be arranged to ensure proper separation of pre- and post-PCR activities.
Personal Protective Equipment (PPE): Personnel working in PCR containment rooms must wear appropriate PPE, such as lab coats, gloves, and face masks, to minimize the introduction of contaminants.
Overall, compartmentalized rooms for PCR are essential for maintaining the integrity and accuracy of PCR-based experiments by providing a controlled environment that minimizes the risk of contamination.

ANNEX III.

Validation and verification of Molecular Assays for Licensing IVD molecular Kits:
It is crucial to understand the quality of available diagnostic reagents, their efficacy as a method, and the necessary requirements for their optimal implementation, with the aim of ensuring the quality of the results obtained.
Need for DNA Standards:
DNA standards are crucial for accurate molecular diagnosis, serving as reference materials for calibrating assays, assessing performance, and ensuring consistency across laboratories.
It's important to determine which standards to use and explore the possibility of certification by organizations like PAHO for collaborative centers to produce and provide these standards.
Determining Validation Cohort Sample Sizes:
Validation studies should establish appropriate sample sizes considering variations in molecular techniques and combinations with serological methods.
Consensus regarding minimum sample sizes for validating PCR and LAMP in different settings is necessary to ensure statistical robustness and generalizability.
External Quality Assurance:
External quality assurance programs are essential for maintaining accuracy and reliability in molecular diagnostic assays.
Participation in proficiency testing programs helps laboratories identify errors, maintain competency, and meet regulatory requirements, ensuring the quality of CD diagnostics.

Third party panels for validation and verification of Analytical methods:
During the validation and verification stages of analytical methods, the use of third-party panels is recommended. These panels typically include samples with low analyte levels. Within these panels, samples should be included for which the parasitic load has been measured, and from these, at least 20% of the samples to be processed should be chosen with values close to the detection limit of the test used in the panel characterization. Representative samples should also be included to ensure that the tests are capable of identifying infected patients without being affected by the geographic distribution of the different DTUs identified for T. cruzi.

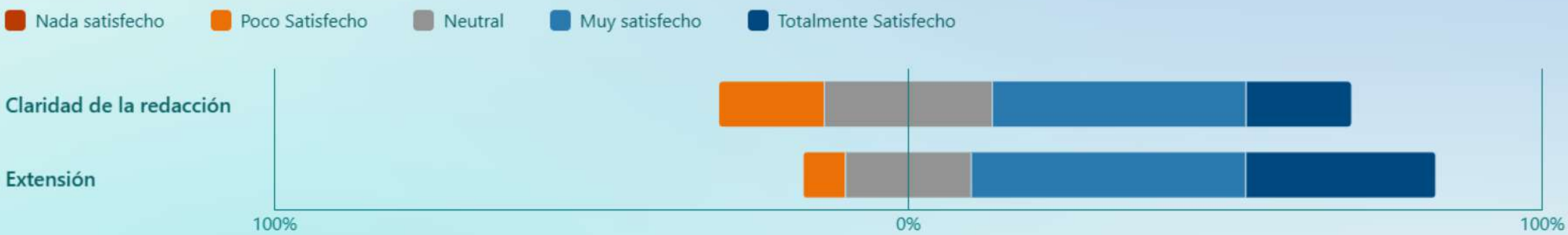
30 respuestas enviada

Nombre (opcional)

WordCloud | Todas las respuestas

30 respuestas enviada

Sobre el contenido en general del documento indique su grado de satisfacción

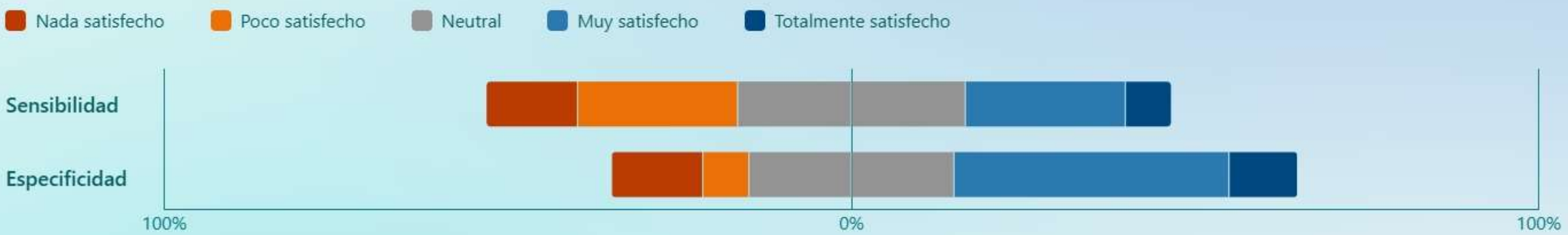


30 respuestas enviada

En la página 5, se indican conceptos que se incluirán como Glosario. Si considera que faltan, por favor indíquelos:

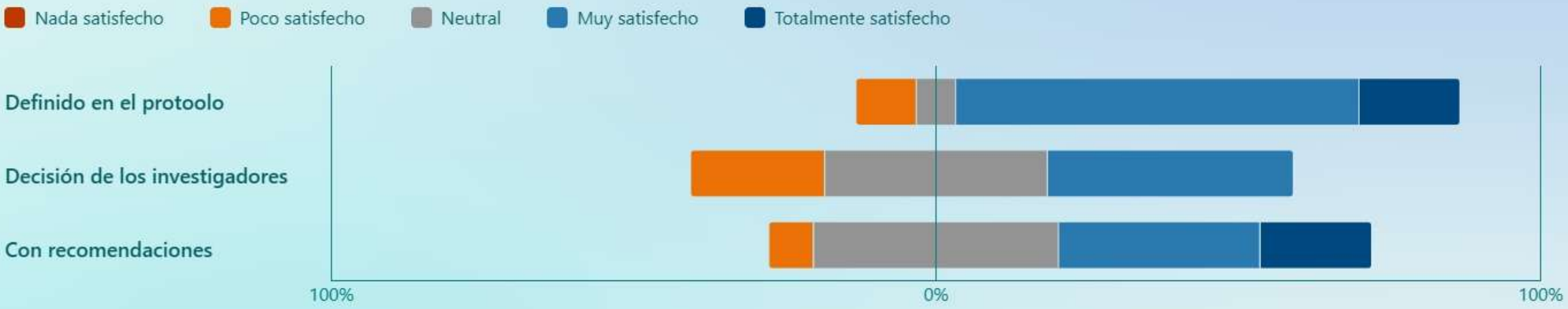
30 respuestas enviada

Sobre los criterios de selección de la prueba mencionado en la página 9, está de acuerdo con los valores de:



30 respuestas enviada

Sobre la selección de las muestras mencionada en la página 11 considera que debe quedar en el documento



30 respuestas enviada

Si no está de acuerdo con el porcentaje de SENSIBILIDAD indicado ¿Cuál sería su propuesta? Fundamente

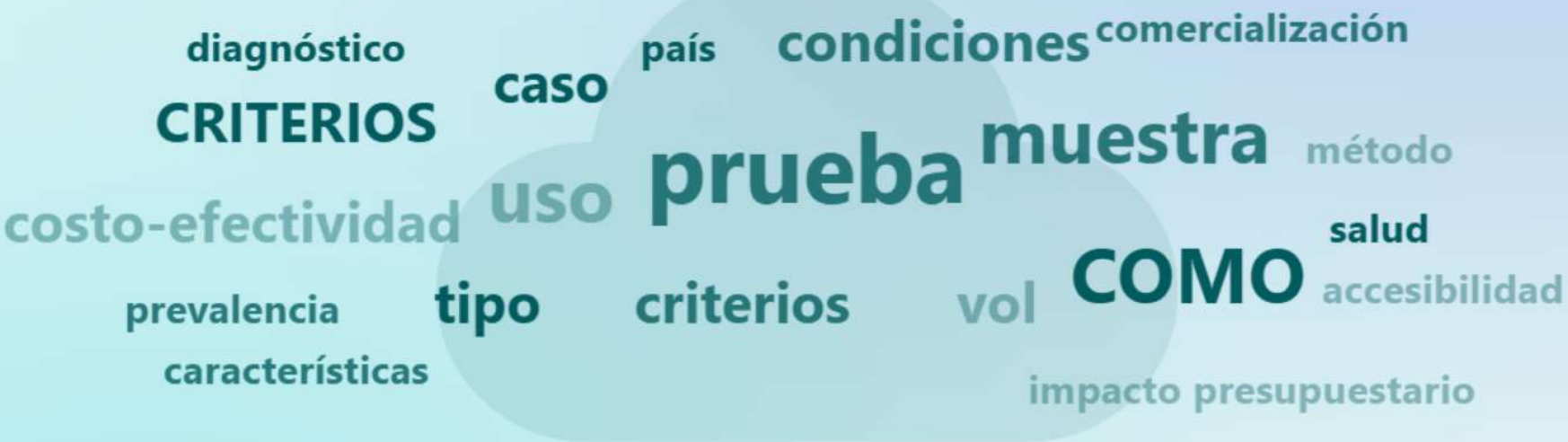
WordCloud | Todas las respuestas

30 respuestas enviada

Si no está de acuerdo con el porcentaje de ESPECIFICIDAD indicado ¿Cuál sería su propuesta? Fundamente

30 respuestas enviada

Indique si agregaría otro criterio para la selección de método



WordCloud | Todas las respuestas

30 respuestas enviada

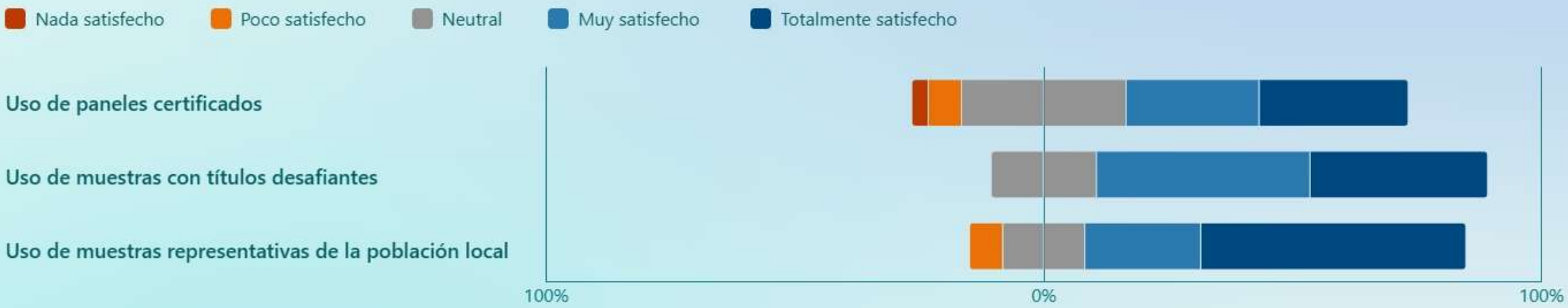
Con respecto a la Tabla 2 ubicada en la pagina 12, Ficha resumen de las características principales de la prueba seleccionada por favor indique si agregaría otro criterio:

30 respuestas enviada

Con respecto a la Tabla 2 ubicada en la pagina 12, Ficha resumen de las características principales de la prueba seleccionada por favor indique si agregaría otro criterio:

30 respuestas enviada

En relación al panel de muestras de tercera opinión para la verificación del método mencionado en la página 13, está de acuerdo con:



30 respuestas enviada

En relación con el consentimiento informado propuesto en la página 16, indique si agregaría algún punto no considerado

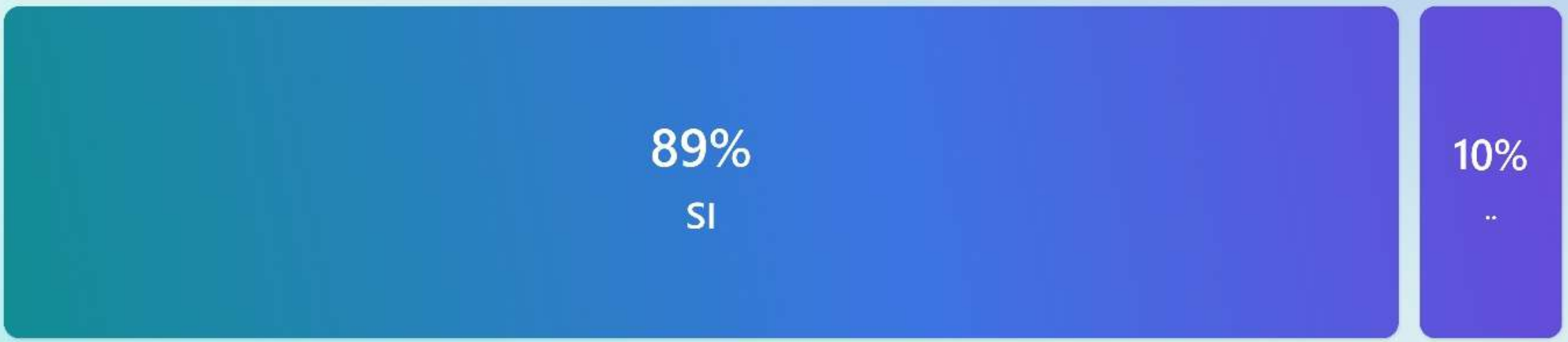
NO

SI

Si su respuesta fue SI indique su sugerencia

30 respuestas enviada

Considera que se deban incluir un anexo para el cálculo de tamaño de muestra



Rectángulos | Barra

30 respuestas enviada

Si su repuesta fue SI Tiene algún modelo que recomendaría para el cálculo de tamaño de muestra

30 respuestas enviada

En relación a la propuesta considera que se debería incluir una propuesta para estudio de costo efectividad

Rectángulos | Barra

30 respuestas enviada

Si su respuesta fue SI, tiene algun modelo que recomendaría incluir

WordCloud | Todas las respuestas

16 de 21

30 respuestas enviada

En la página 18 se muestra un Algoritmo General agregaría otra propuesta de algoritmo

Rectángulos | Barra

30 respuestas enviada

Si su repuesta fue SI, indique su propuesta:

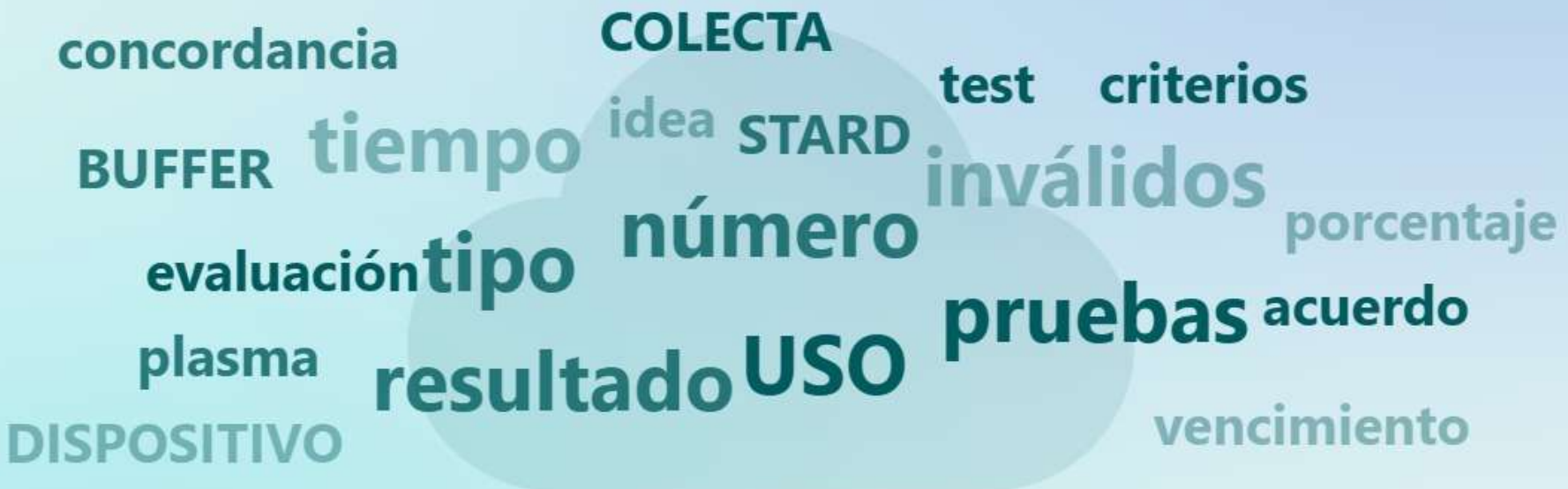
30 respuestas enviada

En que otros escenarios a parte de los mencionados en la pagina 19 se podría usar como guía el documento propuesto:

WordCloud | Todas las respuestas

30 respuestas enviada

Con respecto a la Tabla 4 Criterios para comparación de dos o más de pruebas que se encuentra en la página 19 indique que otros criterios se podrían incluir:



30 respuestas enviada

Si en su opinion, después de leer el documento completo, falta algún aspectos a considerar por favor indíquelo

WordCloud | Todas las respuestas

Annex 3. Agenda and presentation slides

WELCOME TO OUR

MEETING ON DIAGNOSTIC EVALUATION AND ECONOMIC IMPACT ANALYSIS OF NEW DIAGNOSTIC METHODS FOR CHAGAS DISEASE

6-7 MAY 2024
BUENOS AIRES,
ARGENTINA

ORGANIZED BY

FIND
Diagnóstico para todos

DNDi
La mejor ciencia para los más desatendidos

IN COOPERATION WITH

Organización Panamericana de la Salud

CO-ORGANIZED BY

INGEBI
CONICET

AGENDA

DAY 1 | MONDAY, 6 MAY

Auditorio Fundación Mundo Sano, Paraguay 1535

SCOPE

Our main goal is to achieve consensus among the invited experts, including the scientific community, technical health authorities, PAHO and WHO, about:

01.
Generic research protocol for selecting and assessing appropriate RDTs for chronic *T. cruzi* infection, to ensure high quality studies in the Americas" developed by PAHO.

02.
Performance and operational characteristics, standardized methods and quality controls to assess and to guide future use of molecular tests for diagnosis of *T. cruzi* infection.

03.
The evidence on cost-effectiveness and economic impact necessary to be generated in order to facilitate the integration of new diagnostic methods into the health systems of endemic countries.

ACTIVITIES

- A meeting in person of the invited experts is planned on 6 - 7 may 2024, in Buenos Aires, Argentina, convened and sponsored by FIND and DNDi, with the technical support of PAHO, co-organized by INGEBI-CONICET.

- The selected documentation that will be assessed by the invited experts and guiding questions to give their feedback will be sent by the end of March (before the meeting) and the experts will be requested to send virtually their feedback by the end of April, answering guiding questions.

- The key insights of the invited expert´s input, and the guests' presentations, will be discussed to achieve consensus during the meeting in person (6-7 May 2024).

AGENDA

DAY 1 | MONDAY, 6 MAY

Auditorio Fundación Mundo Sano, Paraguay 1535

8:30	REGISTRATION	
9:00	Opening / introduction	**Presented by** **Laura Bohorquez (FIND)** **Alejandro Schijman (INGEBI-CONICET)** **Maria Jesus Pinazo (DNDi)** **Hector Coto (PAHO)** **Marcelo Abril (FMS)**

EVALUATION OF CD RDTS		PRESENTED BY	MODERATED BY
9:30	Presentation of the generic study protocol to evaluate CD RDTs, developed by PAHO (sent to the invited experts in early April)	**María Isabel Jercic (Instituto Nacional de Salud Pública, Chile)** **Freddy Pérez (PAHO)**	**Julio Alonso Padilla (ISGlobal)** **Rafael Herazo (DNDi)**
9:50	Key insights on the input received from the invited experts in written (by April) about the documentation to evaluate CD RDTs	**Laura Bohorquez (FIND)** **Andrea Marchiol (DNDi)**	
10:10	**BREAK**		
10:40	Discussion of relevant components in the generic study protocol for the evaluation of CD RDTs	**Berra Erkosar (FIND)** **Andrés Caicedo (DNDi)** **Andrea Silvestre (CUIDA Chagas)**	**Laura Bohorquez (FIND)** **Julio Alonso Padilla (ISGlobal)**
11:10	Discussion in plenary about the generic study protocol to evaluate CD RDTs		

EVALUATION OF MOLECULAR DIAGNOSTIC TOOLS FOR CD

		PRESENTED BY	MODERATED BY
11:45	State of the art of LAMP as point of care diagnostic tool for CD	**Alejandro Schijman (INGEBI-CONICET)**	**Margarita Bisio (INP Fatala ANLIS)** **Colin Forsyth (DNDi)**
12:00	State of the art of qPCR for CD	**Otacilio Moreira (Fiocruz)**	
12:15	State of the art, other molecular point of care diagnostic tools that could be adapted for CD	**Elena Ivanova (FIND)** Pre-Recorded Video presentation	
12:30	Controls and standards	**Marcelo Rodriguez (FIND)**	
12:45	**LUNCH**		
14:15	Key insights on the input received from the invited experts in written (by April) about the guiding questions to prioritize the following parameters to evaluate the molecular methods for CD (focus on LAMP and qPCR).	**Alejandro Schijman (INGEBI-CONICET)** **María Jesús Pinazo (DNDi)** **Constança Britto (Fiocruz)**	**Margarita Bisio (INP Fatala ANLIS)** **Colin Forsyth (DNDi)**
14:35	Discussion in plenary about the evaluation of molecular methods for CD		

COST EFFECTIVENESS AND ECONOMIC IMPACT MODELLING OF NEW DIAGNOSTICS FOR CD

		PRESENTED BY	MODERATED BY
15:00	Optimizing CD Diagnosis: Comparing Algorithm Performance and Cost.	**Sarah Girdwood (FIND)**	**Shaukat Kahn (FIND)** **Freddy Pérez (PAHO)**
15:15	Diagnose More Cases, Spend Less: A User-Friendly Shiny App Model for Chagas Diagnosis.	**Kyra Grantz (FIND)**	
15:30	Economic impact linked to out of pocket expenses. The experience of Colombia.	**Rafael Herazo (DNDi)**	

COST EFFECTIVENESS AND ECONOMIC IMPACT MODELLING OF NEW DIAGNOSTICS FOR CD

15:45	Economic impact evidence/cost-effectiveness analyses evaluating the incorporation of new diagnostic methods for CD in the health systems of Brazil, Bolivia, Paraguay and Colombia.	**Yerly Magnolia Useche (Fiorcruz CUIDA Chagas)**	**Shaukat Kahn (FIND)** **Freddy Pérez (PAHO)**
16:00	Economic impact evidence / cost-effectiveness analyses evaluating the incorporation of new diagnostic methods for CD in the health systems.	**Elisa Sicuri (ISGlobal)** Pre-Recorded Video presentation	
16:15	**BREAK**		
16:45	Economic evaluation of new diagnostic methods.	**Santiago Hasdeu (redArets Argentina)**	**Shaukat Kahn (FIND)** **Nasim Iusef (MoH Argentina)**
17:05	Plenary deliberations after the afternoon presentations.		
17:35 - 18:30	**CLOSURE OF THE DAY AND TOAST**		**FIND, DNDi, PAHO, INGEBI-CONICET**

AGENDA

DAY 2 | TUESDAY, 7 MAY

NH Buenos Aires City Hotel, Bolívar 160 / Luis Alberto Room

		MODERATED BY
8:30	Intro about the dynamics to achieve consensus (guiding questions developed and shared previously with the invited experts)	**Laura Bohorquez (FIND)** **Alejandro Schijman (INGEBI-CONICET)**
8:40	Sub-groups discussions, each group will write their main insights / answers to each question (HANDS ON)	
10:15	Discussion in plenary about the sub-group conclusions (HANDS ON)	**Andrea Marchiol (DNDi)** **Freddy Pérez (PAHO)**
11:00	**CLOSURE WITH ALL INVITED GUESTS**	**María Jesús Pinazo (DNDi)** **Laura Bohorquez (FIND)** **Alejandro Schijman (INGEBI-CONICET)**
11:00	**BREAK**	

11:30-13:30	Invited guests could continue in the annual meeting of the Global Chagas Coalition (in the same venue)
15:00-18:00	Consolidation of information, preparation of final reports, and planning next steps (in the same venue)

PARTICIPANTS

Belkisyolé Alarcón de Noya
IMT-UCV

Antonieta Rojas de Arías
CEDIC, Paraguay

Franciana Rosa Silva
CUIDA Chagas

Julio Alonso Padilla
ISGlobal

Laura Lamfre
redArets

Andrea García Balderrama
INLASA

Claudia Herrera
TULANE

Fred Luciano Neves Santos
Fiocruz

Karina Eguez Solis
MoH Bolivia

Lizeth Rojas Panozo
CEADES

Andrea Silvestre
Fiocruz CUIDA Chagas

Elisa Sicuri
ISGLOBAL

Igor Almeida
UTEP

Karla Lanage
Guatemala

Yerly Magnolia Useche
Fiocruz CUIDA Chagas

María Isabel Jercic
INS, Chile

Santiago Hasdeu
redArets

Laura Bohorquez
FIND

Shaukat Khan
FIND

Alejandro Hasslocher
Fiocruz

Alejandro Schijman
INGEBI-CONICET

Juan Carlos Ramirez
Hosp. de Niños Dr. R. Gutiérrez

Margarita Bisio
INP FATALA ANLIS

Marcelo Rodriguez
FIND

Héctor Coto
PAHO

María Jesús Pinazo
DNDi

Ivan Scandale
DNDi

Andrea Caicedo
DNDi

Oscar Noya
IMT-UCV

Vidalia Lesmo
SENEPA

Berra Erkosar
FIND

Kyra Grantz
FIND

Bertha Espinoza
IIIBO-México

Jaime Altcheh
Hosp. de Niños Dr. R. Gutiérrez

Silvia Longhi
INGEBI-CONICET

Rocío Rivero
INP FATALA ANLIS

Nasim Iusef
MoH Argentina

Colin Forsyth
DNDi

Natalie EL Kheir
DNDi

Rafael Herazo
DNDi

Andrea Marchiol
DNDi

Otacilio Moreira
Fiocruz

Zulma Cucunuba
Universidad Javeriana

Sarah Girdwood
FIND

Constança Britto
Fiocruz

Eric Dumontiel
TULANE

Fernán Agüero
CONICET-UNSAM

Arturo Muñoz Calderón
INGEBI-CONICET

Marcelo Abril
Fundación Mundo Sano

Freddy Pérez
PAHO

Montserrat López Serafín
InDRE-México

Juan David Ramírez
Universidad del Rosario, Colombia

Presentation slides

Meeting on Diagnostic Evaluation and Economic Impact Analysis of New Diagnostic Methods for Chagas Disease

May 6-7, 2024. Buenos Aires, Argentina

List of Presentations

Title	Speakers
Presentation of the generic study protocol to evaluate CD RDTs, developed by PAHO	Freddy Pérez (PAHO) María Isabel Jercic (INS Chile)
Key insights on the input received from the invited experts in written about RDTs	Laura Bohorquez (FIND) Andrea Marchiol (DNDi)
Statistical Concerns and Recommendations for the Generic Protocol	Berra Erkosar (FIND) Laura Bohorquez (FIND)
Discussion of relevant components in the generic study protocol for the evaluation of CD RDTs	Andres Caicedo (DNDi)
State of the art of LAMP as point of care diagnostic tool for CD	Alejandro Schijman (INGEBI- CONICET)
State of the art of qPCR for CD	Otacilio Moreira (FIOCRUZ)
Molecular point-of-care diagnostic tools: state of the art	Elena Ivanova Reipold (FIND)
Control and standards	Marcelo Rodríguez (FIND)
Key insights on the input received from the invited experts in written about Molecular Methods	Alejandro Schijman (INGEBI- CONICET) María Jesús Pinazo (DNDi) Constança Britto (FIOCRUZ)
Optimizing CD Diagnosis: Comparing algorithm performance and cost	Sara Girwood (FIND)
Diagnose more cases, spend less: A user friendly shiny app model for Chagas diagnosis	Kyra Grantz (FIND)
Economic impact linked to out of pocket expenses. The experience of Colombia	Rafael Herazo (DNDi)
Cost-effectiveness analyses evaluating the incorporation of new diagnostic methods for CD in the health systems of Brazil, Bolivia, and Colombia	Yerly Magnolia Useche (CUIDA CHAGAS)
Cost-Effectiveness Analysis of Chagas disease RDT in a health facility setting	Elisa Sicuri (IS Global)
Economic evaluation of new diagnostic methods	Santiago Hasdeu (RedArets)

ORGANIZED BY

IN COOPERATION WITH

COORGANIZED BY

MEETING ON DIAGNOSTIC EVALUATION AND ECONOMIC IMPACT ANALYSIS OF NEW DIAGNOSTIC METHODS FOR CHAGAS DISEASE

6-7 MAY 2024 BUENOS AIRES, ARGENTINA

FIND Diagnóstico para todos | DNDi La mejor ciencia para los más desatendidos

ORGANIZED BY

Pan American Health Organization

IN COOPERATION WITH

INGEBI CONICET

COORGANIZED BY

Presentation of the generic study protocol to evaluate CD RDTs, developed by PAHO

Dr. Freddy Pérez

Dra. María Isabel Jercic

Protocolo Genérico

Es un conjunto de acciones, métodos, y la observancia de determinadas reglas convencionales, que constituye un procedimiento planificado y estructurado convencional, destinado a estandarizar un comportamiento, ya sea humano u artificial ante una situación específica.

Posterior a la reunión de Bahía, 2023 se evidenció la necesidad de contar con Protocolo Genérico dirigido al uso de las Pruebas Rápidas en el marco de la Enfermedad de Chagas Crónica

Objetivo del trabajo

- Desarrollar un protocolo genérico para asegurar la armonización y la investigación de alta calidad de estudios epidemiológicos prospectivos en la región para evaluar algoritmos basados en pruebas rápidas para la infección crónica por *Trypanosoma cruzi.*

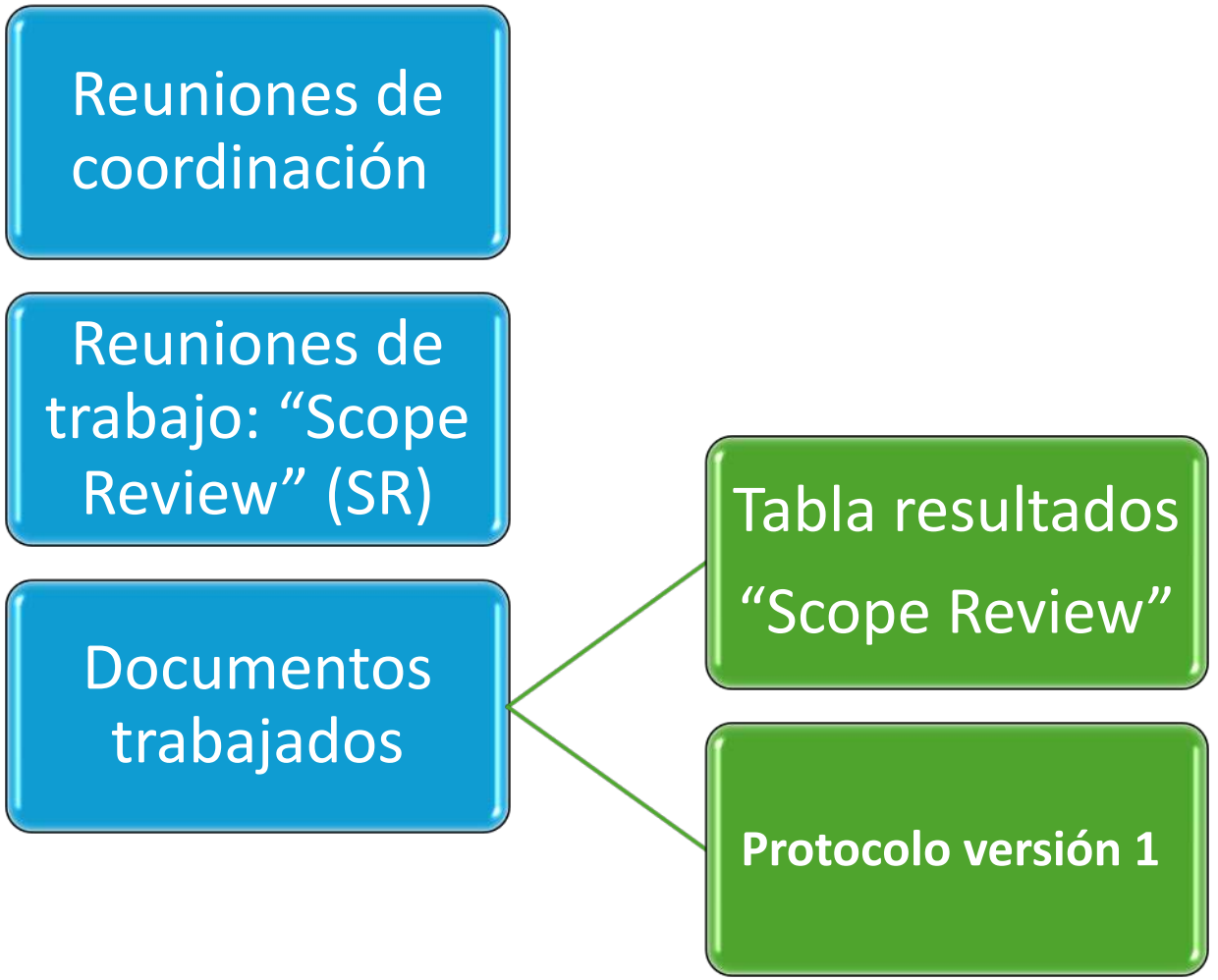
Alcance:

- El presente documento está dirigido a los investigadores y equipos de trabajo que requieran implementar el diagnóstico serológico, mediante la detección de anticuerpos específicos para la Infección por *Trypanosoma cruzi* crónica, incluyendo el uso de la metodología de formato rápido, Pruebas de Diagnóstico Rápido (RDT) basadas en inmunocromatografía.

Objetivo:

- Proporcionar orientación técnica estandarizada, relacionada con la selección y uso de un método que detecte anticuerpos específicos para *Trypanosoma cruzi* utilizando pruebas de diagnóstico rápidas con un enfoque para asegurar la calidad del diagnóstico para generar resultados técnicamente confiables y clínicamente útiles.
- Establecer un protocolo genérico que puede adaptarse a las realidades nacionales o locales en el contexto de implementación de algoritmos de diagnóstico de la infección por *Trypanosoma cruzi* crónica utilizando PDR.

Trabajo realizado



Plan de trabajo para elaboración del Protocolo

Revisión de alcance ‘Scope Review’

- Para la preparación de este protocolo se realizó una investigación bibliográfica basada en lo establecido como modelo de una revisión de alcance.

Tabla 1 *Criterios de revisión; inclusión y exclusión*

Criterios de inclusión	Criterios de exclusión
• **Test rápidos solo Infección por Trypanosoma cruzi o Trypanosoma cruzi** • **Estudios sobre diagnóstico** • **(expresa sensibilidad/especificidad eficacia de la prueba)** • **En humanos**	• Artículos que incluyan más de una enfermedad y/o tipo de prueba • Pacientes agudos • Trabajos en animales > preclínico • Que no sea un artículo original o revisión.
Límites: desde 1 de enero de 1990 hasta 6 de diciembre de 2024; Idioma inglés español.	

Búsqueda

- ***Tabla 2*** *Resultados de las expresione de búsqueda en las bases de datos utilizadas*

Base de datos	Expresión de búsqueda	Número de artículos seleccionados
PubMed	("Algorithms"[Mesh] OR "Algorithms"[tiab] OR "Rapid Diagnostic Tests"[Mesh] OR "Rapid Diagnostic Tests"[tiab]) AND ("Trypanosoma cruzi"[Mesh] OR "Trypanosoma cruzi"[Ti])	90
Scopus	TITLE-ABS-KEY ("Algorithms" OR "Rapid Diagnostic Tests") AND TITLE-ABS-KEY("Trypanosoma cruzi")	220
LILACS	(algoritmo) OR (algoritmos) OR (diagnóstico rápido) OR (test de diagnóstico rápido) AND (Trypanosoma cruzi)	13
	Luego de eliminar resultados duplicados	247

Pasos de la revisión

Profesionales con experiencia en Diagnóstico Enfermedad de Chagas revisaron los 247

- Calificaron pertinencia de inclusión "criterio de expertos"
- En caso de diferencia se recurrió a una tercera opinión.

Después de la revisión quedaron 32 trabajos seleccionados

Se trabajó propuesta de tabla de revisión

- Se incluyó Evaluación de Sesgo basado en Quadas 2

4 profesionales revisaron 8 artículos cada uno seleccionados al azar

30 trabajo pudieron ser evaluados

Principales resultados

Se pudieron identificar un total de 41 pruebas.

25 (61%) de ellas con posibilidad de obtenerlas comercialmente, pero con distribución diferenciadas en los diferentes países.

Existen publicaciones donde se presentan pruebas que solo fueron desarrolladas con fines de la investigación realizada que en total suman 2

De algunas no se conocía el destino u uso previsto o estaban aún en fase de validación

Sensibilidad	Límites 90,1% a 100 % con un Promedio de 94,6 % Moda de 92,5 %
Especificidad	Límites 90,1% a 100 % con un Promedio de 94,6 % Moda de 92,5 %

Tabla de contenido

Ficha de la prueba seleccionada

Una vez seleccionada la prueba a utilizar es recomendable dejar registros de sus características principales. Un ejemplo de ficha se muestra en la **Tabla 2**.

Tabla 2 Ficha resumen de las características principales de la prueba seleccionada

Nombre de la prueba	
Fabricante y distribuidor	
Sensibilidad	
Especificad	
UDTs evaluados	
Número de determinaciones	
Tipo de muestra	
Volumen de muestra	
Temperatura de trabajo	
Temperatura de almacenamiento	
Tiempo de lectura	
Autorización Sanitaria	
Fecha de la autorización	
Precio por determinación	
Tiempo de vencimiento	

Aseguramiento de la calidad de los resultados

- ***Tabla 3*** *Control de calidad interno. Porcentaje de concordancia en la interpretación de resultados*
- **Puntajes:**
- Si hay concordancia **10 puntos**
- No hay concordancia **0 puntos**

Fecha de la evaluación: ____/___/_____			
Identificación de las muestras	Lector 1 Nombre:	Lector 2 Nombre:	Concordancia (límite de aceptación 90%)
Muestra 1			
Muestra 2			
Muestra 3			
Muestra 4			
Muestra 5			
Muestra 6			
Muestra 7			
Muestra 8			
Muestra 9			
Muestra 10			

Evaluación de Pruebas

•Este protocolo puede ser usado para la evaluación de una o más pruebas rápidas.
•Como apoyo a la comparación de resultados entre las pruebas, la **Tabla 4** muestra criterios factibles de evaluar.

Tabla 4 Criterios para comparación de dos o más de pruebas

	Nombre del reactivo /Número de lote	Nombre del reactivo /Número de lote
N° de muestras Positivas		
N° de muestras Negativas		
Muestras Indeterminadas		
Muestras Repetidas		
Volumen de muestra		
Tiempo de lectura		
Rango temperatura trabajo		
Rango de humedad de trabajo		
Temperatura de almacenamiento		

Protocolo estándar

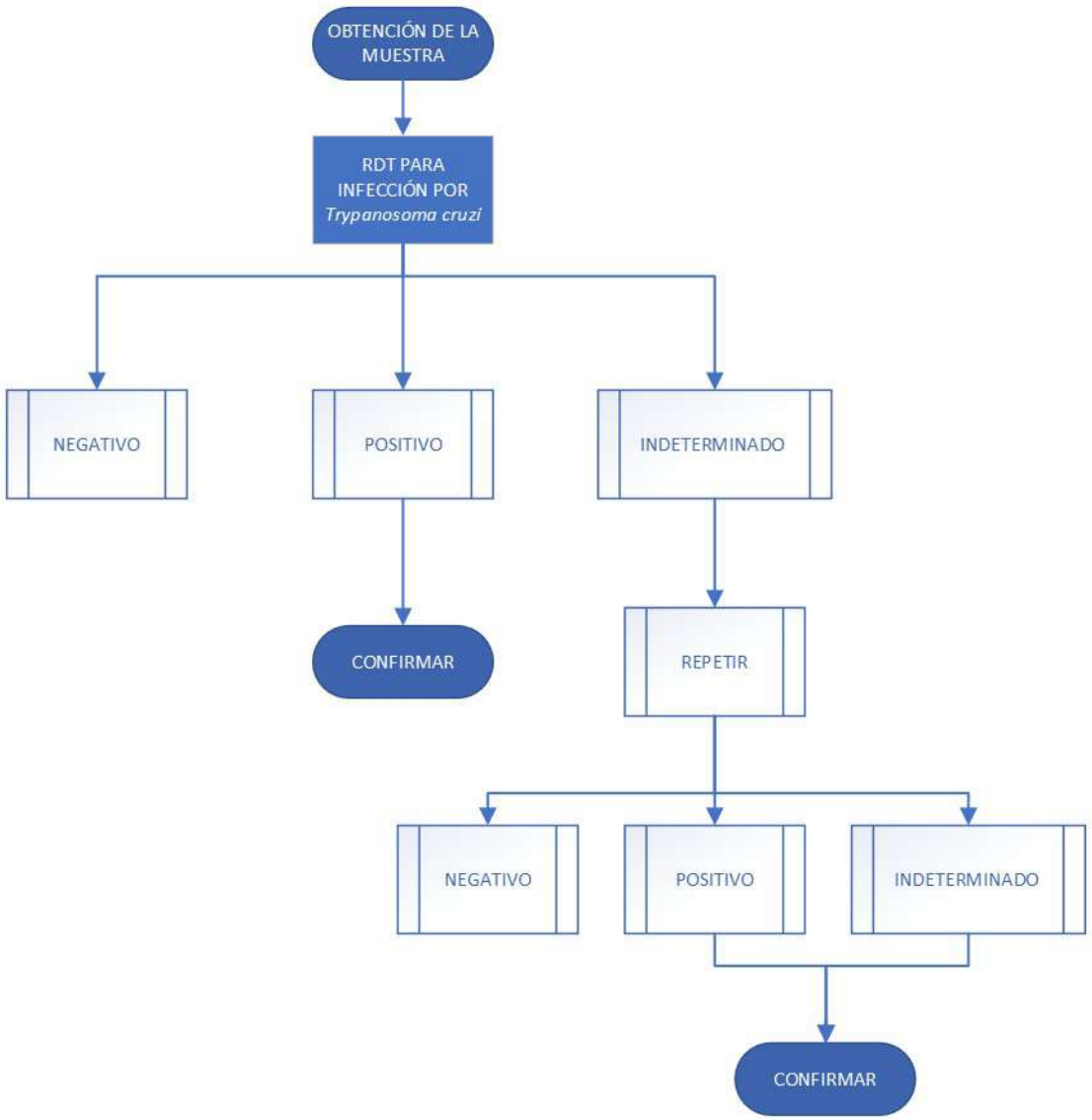
Se proponen los **puntos esenciales** que deben incluir todos los estudios que utilicen **pruebas rápidas** como método de diagnóstico para la detección de anticuerpos específicos contra antígenos de *Trypanosoma cruzi* **en la etapa crónica de la infección:**

1. **Nombre** de los investigadores e instituciones que participan en el estudio
2. **Alcance del objetivo general** para el cual fue previsto el estudio.
3. **Hipótesis**
4. **Objetivo General y Específicos:** debe explicitar el escenario de uso de la prueba según lo mencionado en este documento.
5. **Plan de Investigación**
6. **Cronograma de actividades**
7. **Responsabilidades**: Presentar en una lista o cuadro con las designaciones de personal específico para el protocolo y sus responsabilidades.
8. **Criterios de selección** de la(s) prueba(s) deben quedar registrados sensibilidad, especificidad, valor predictivo positivo y negativo declarados por el fabricante o que se hayan contemplado para su selección.
9. **Consideraciones** de uso según los **criterios vigentes de autorización** de él o los países en que se realizará el estudio.
10. **Ficha técnica de la prueba** a verificar Anotar el nombre completo del reactivo con su número de referencia, nombre del fabricante con dirección completa y el nombre del distribuidor con su dirección completa. La información debe tomarse de la documentación entregada en la caja y el inserto de la prueba.

11. **Informe de verificación** del método utilizando muestras con resultados conocidos en la misma matriz que considera el uso de la prueba, idealmente paneles de tercera opinión.
12. **Tamaño de la Muestra:** uso de herramientas estadísticas para que el número de muestras o personas incluidas en el estudio cumplan con los objetivos planteados.
13. **Requisitos preanalíticos** que corresponde a los criterios de selección de las muestras o sujetos. Este punto debe considerar inclusión y exclusión.
14. **Evaluación por comité de ética certificado** y propuesta de consentimiento informado.
15. Una vez que el comité de ética autorice la realización del estudio, los **responsables de este deben estar disponibles para explicar** a los participantes o sus tutores legales los alcances del trabajo y responder preguntas, ya sea en forma individual o en grupo.

16. **Aplicación de la(s) prueba(s)** siguiendo **estrictamente** lo indicado para su desarrollo por el fabricante.
17. **Uso de herramientas para registro de resultados** los cuales deben ser almacenados para su posterior revisión si fuera necesario para lo cual se sugiere el uso de fotografías.
18. **Implementación de un control interno** de la calidad que considere el punto crítico de comparación de lectura entre operadores si la prueba contempla lectura visual.
19. **Confirmación de los resultados** a través de la utilización de una segunda prueba de principio distinto a la seleccionada.
20. **Plan de Análisis y Gestión de Datos** que incluya análisis estadísticos de los resultados e interpretación.
21. **Estudio de costo efectividad del uso** de la(s) prueba(s) incluidas en el estudio.
22. **Limitaciones del estudio**
23. **Presentación de informe o publicación**

Algoritmo


OBTENCIÓN DE LA MUESTRA
RDT PARA INFECCIÓN POR *Trypanosoma cruzi*
NEGATIVO
POSITIVO
INDETERMINADO
CONFIRMAR
REPETIR
NEGATIVO
POSITIVO
INDETERMINADO
CONFIRMAR

Thank You.

Contact info:
perezf@paho.org
majercic@ispch.cl

ORGANIZED BY

IN COOPERATION WITH

COORGANIZED BY

ORGANIZED BY

IN COOPERATION WITH

COORGANIZED BY

MEETING ON DIAGNOSTIC EVALUATION AND ECONOMIC IMPACT ANALYSIS OF NEW DIAGNOSTIC METHODS FOR CHAGAS DISEASE

Key insights on the input received from the invited experts in written about RDTs

Laura Bohorquez, FIND

Andrea Marchiol, DNDi

6-7 MAY 2024 BUENOS AIRES, ARGENTINA

Key insights on the input received from the invited experts in written about RDTs

- 25 out of 46 experts = 55%

STOP

THINK

ACT

Key insights on the input received from the invited experts in written about RDTs

- 25 out of 46

  experts = 55%

STOP

0 questions

majority disagree

ACT

4 questions

disagree >20%

THINK

7 questions

disagree <20%

Key insights on the input received from the invited experts in written about RDTs

- 25 out of 46 experts = 55%

STOP ACT

0 questions

majority disagree

disagree <20%

THINK

4 questions

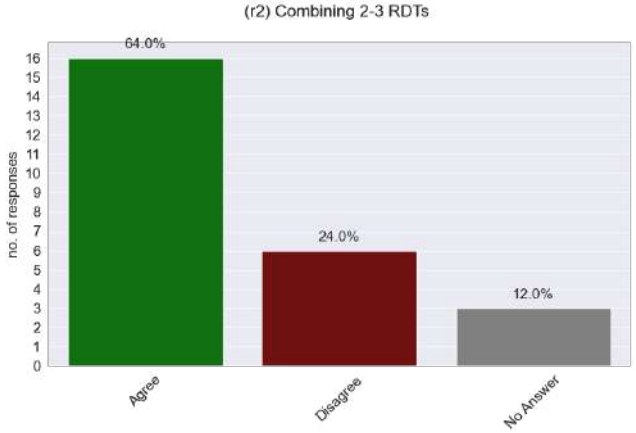
disagree >20%

7 questions

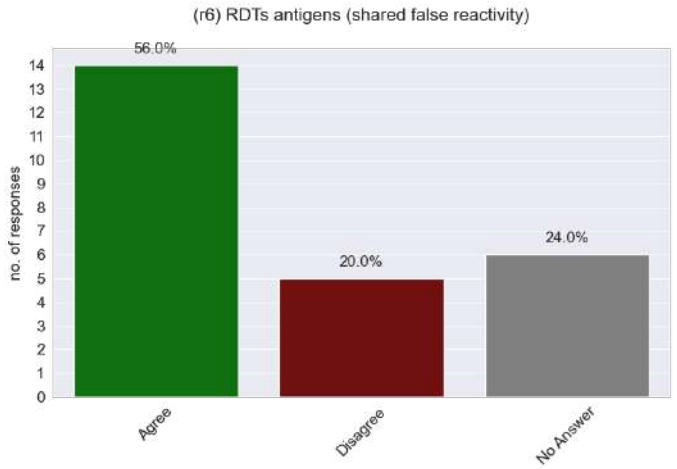
Key insights on the input received from the invited experts in written about RDTs

4 questions
disagree >20%

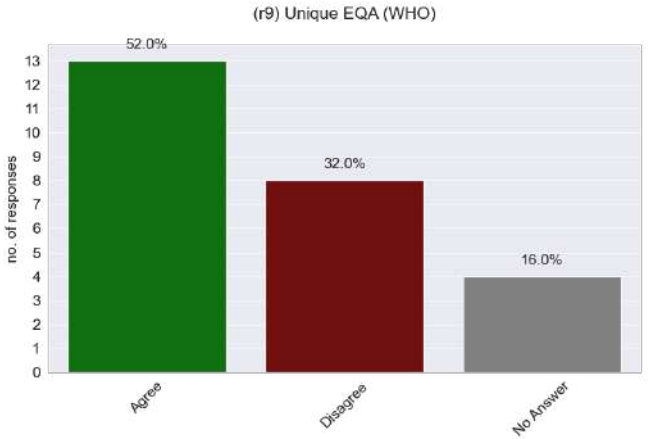
THINK



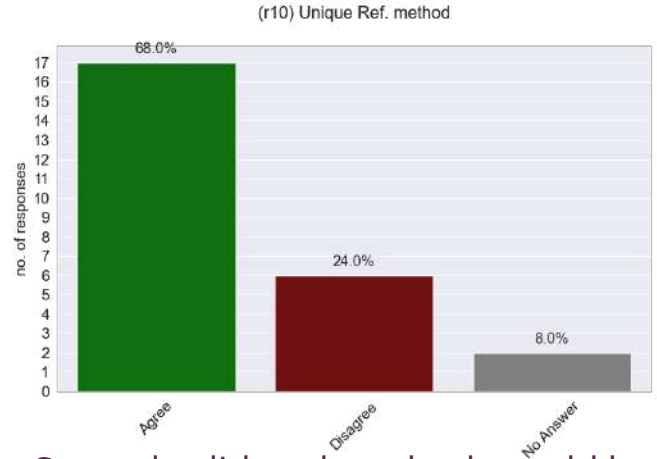
- To evaluate different algorithms combining tests for suggesting one for diagnosis



- When manufacturers do not declare antigens



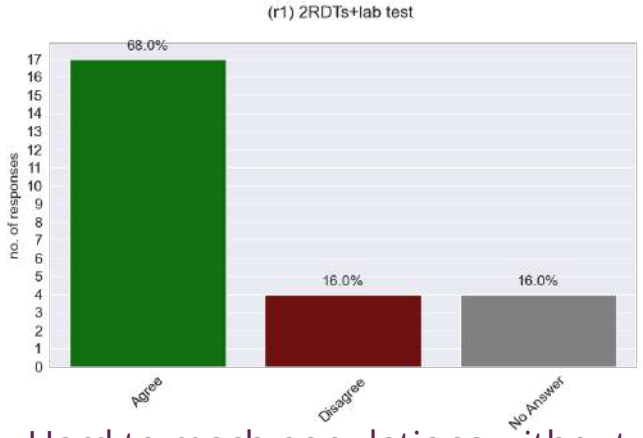
- Part of the diversity in the WHO standards (logistic. challenging)



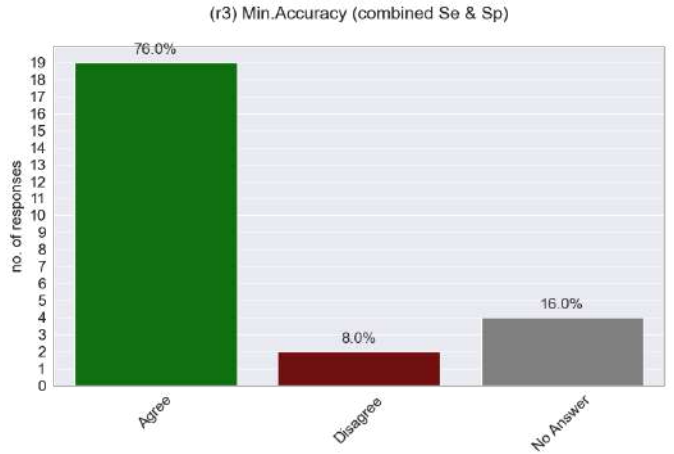
- Several validated methods could be used (incl. RDTs) as composite standard

Key insights on the input received from the invited experts in written about RDTs

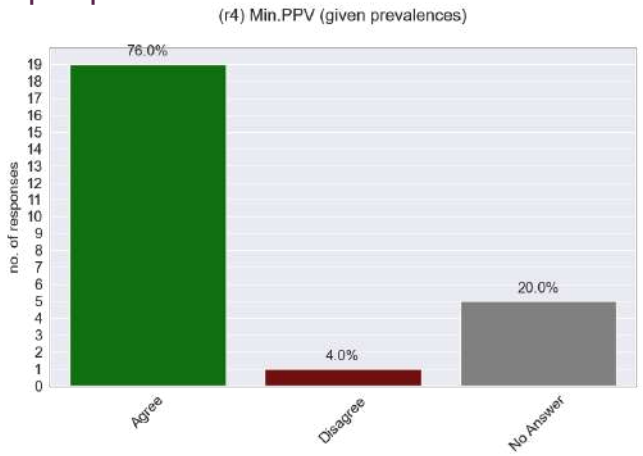
7 questions
disagree <20%



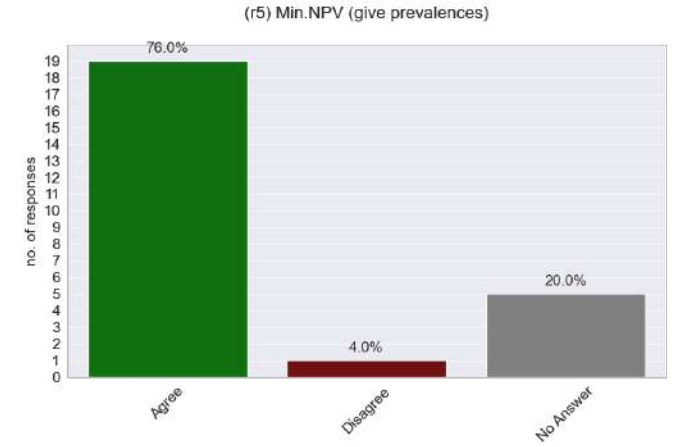
- Hard to reach populations without ref lab
- Advantage of using RDTs lose its purpose



- Min. combined Se and min. combined Sp



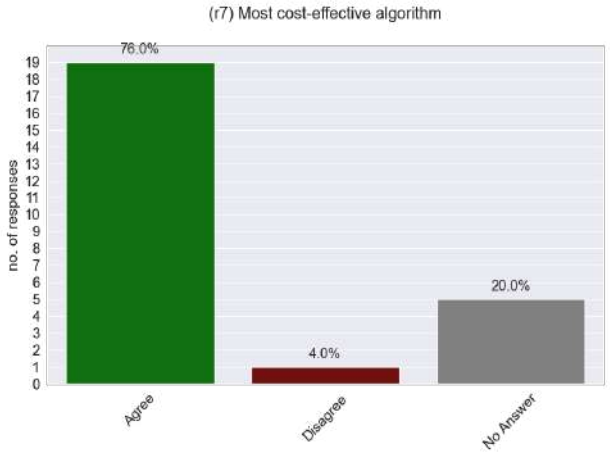
- Limited prevalence information in the intervention areas

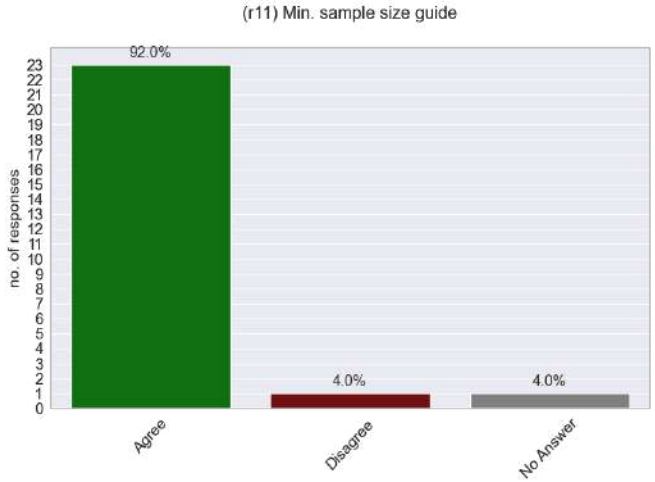


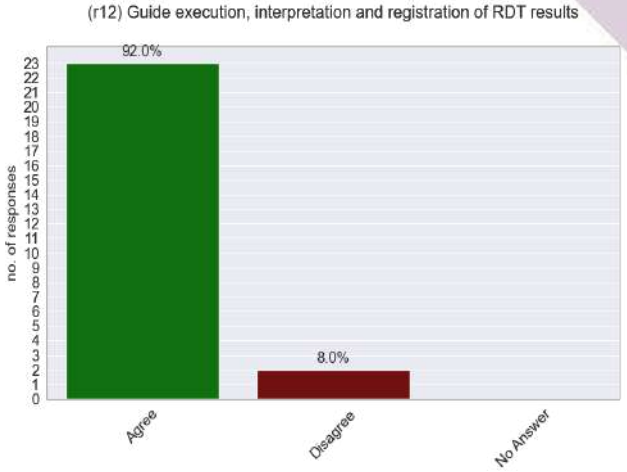
ACT

Key insights on the input received from the invited experts in written about RDTs

7 questions
disagree <20%







ACT

Key insights on the input received from the invited experts in written about RDTs

STOP

THINK

1. Recommend acceptance criteria:
   - That the tests (incl. RDTs) have different antigenic principles. (If not declared to assess shared false reactivity)
2. Recommend certified serological panel
3. Recommend Ref. tests

ACT

1. Include evaluation of additional test usage and testing algorithms based on RDTs
   1. screening 2 RDTs + confirmatory lab test
   2. combining 2-3 RDTs
2. Recommend acceptance criteria:
   - Min. combined Se and min. combined Sp
   - Min. PPV and NPV
     - at a prevalence of <5% or less
     - at a prevalence of 5-10%
     - at a prevalence of >10%
   - Guide to identify the most cost-effective algorithm
3. Guide estimation of min. sample size
4. Guide interpretation and registration of RDT results (real scenario conditions)

Thank You.

Contact info:

laura.bohorquez@finddx.org

amarchiol@dndi.org

ORGANIZED BY

IN COOPERATION WITH

COORGANIZED BY

ORGANIZED BY

IN COOPERATION WITH

COORGANIZED BY

MEETING ON DIAGNOSTIC EVALUATION AND ECONOMIC IMPACT ANALYSIS OF NEW DIAGNOSTIC METHODS FOR CHAGAS DISEASE

Berra Erkosar
Senior Biostatistician, FIND

Laura Bohorquez
Scientific Officer, FIND

6-7 MAY 2024 BUENOS AIRES, ARGENTINA

Statistical Concerns and Recommendations for the Generic Protocol

FIND

Recommendation to adjust in the protocol or add guidance about:

- Point estimates vs lower bond of the 95% CI?
- Criteria applied to manufacturer's IFU or independent evaluations?
- Autochthonous populations

Selection of Investigational Products

RDT with:

- 92% Sensitivity
- 95% Specificity

Selection of Investigational Products

Independent head-to-head comparison under controlled lab conditions

Sponsor	Countries	Commercially available RDTs													SE range	SE >92%	SP range	SP >95%	SE >92% & SP >95%
		Wiener Lab	Statpak Chembio	CTK biotech AdBio./ Aria	Bio-Manguinhos/ Fiocruz	SD Bioline Abbott	Artron Labs	Lemos Lab	Accu Biotech CO LTD	Human Dx Hexagon	Inbios Inc. cassette	Inbios Inc. strip	Xerion	Acro Biotech	Atlas Link Technology					
FIND	Argentina	■				■		■	■							92-100%	100% (4/4)	76-96%	25% (1/4)	25% (1/4)
	Bolivia	■	■	■	■	■		■	■				■	■	■	62-98%	50% (5/10)	78-100%	60% (6/10)	40% (4/10)
FIND & DNDi	Colombia	■	■	■	■	■	■	■		■	■	■	■			75-99%	54% (6/11)	71-100%	90% (10/11)	45% (5/11)
Fiocruz	Brazil	■		■	■	■										93-100%	100% (4/4)	78-92%	0% (0/4)	0% (0/4)

using autochthonous population samples and the ref. test method in each country

- Evidence about performance using autochthonous population

ARGENTINA *Rivero et.al. 2023 (PMID: 38489395)*
BOLIVIA *Lopez et.al. 2023 (PMID: 38437237)*
COLOMBIA *Marchiol et.al. 2023 (PMID: 37607214)*
BRAZIL *Iturra et.al. 2023 (PMID: 36936214)*

Reference Test Selection

- Each reference test has its own imperfect performance and there is no gold standard
- A composite reference is used to have high accuracy
- However, there is no recommendation for the reference standard that is used across different regions
- This creates a heterogeneity and jeopardizes the comparability of the results across different regions and reduces the possibility of doing pooled analyses, meta-analyses, etc.

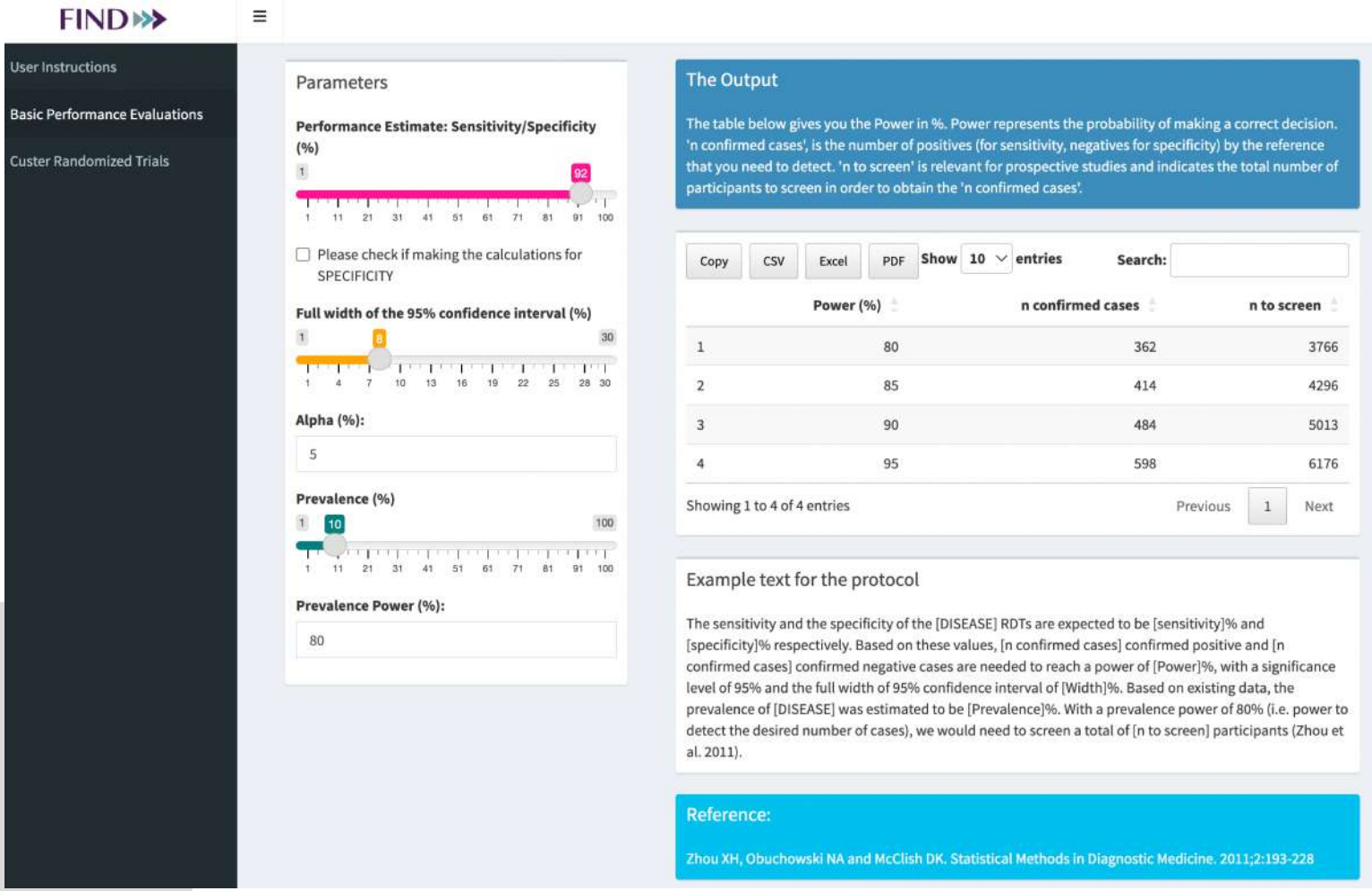
Recommendation to add in the protocol:

A panel of reference tests available in multiple countries would help increasing reproducibility

Sample Size Calculations

- Hypothesis:
  - **What should be the target point estimate? single test / composite**
  - **Precision**: what should be the maximum width of the confidence interval?

  In case of **non-inferiority**:
  - Non-inferiority margin
  - Expected Difference between two tests or two algorithms
- Power: 80% vs 90%



https://finddx.shinyapps.io/SampleSize/

Recommendation to add in the protocol a guide, however a statistician must verify for the purpose of each study

Sample Panel

- Certified external panel
- **The protocol recommends ≥20% of the samples with low reactivity (purpose and why 20%)**
- Representative samples

Summary of recommendations to achieve consensus

1. Lab-based tests are imperfect (composite)	
2. Selection of Investigational Products	• Se 92% and Sp 95% (Point estimate and margin of error) • Autochthonous populations
3. A panel of reference tests will increase reproducibility	• 5-6 ref. tests available in multiple countries
4. Sample size calculations	• Target point estimate, precision • Non-inferiority (comparing 2 tests or 2 algorithms)
5. Sample Panel	• Guidance on the certified external panel • Purpose of (20%) low reactivity samples

Berra Erkosar
FIND,
Senior biostatistician

Laura Bohorquez
FIND,
Scientific officer

Thank You.

Contact info:
berra.erkosar@finddx.org
laura.bohorquez@finddx.org

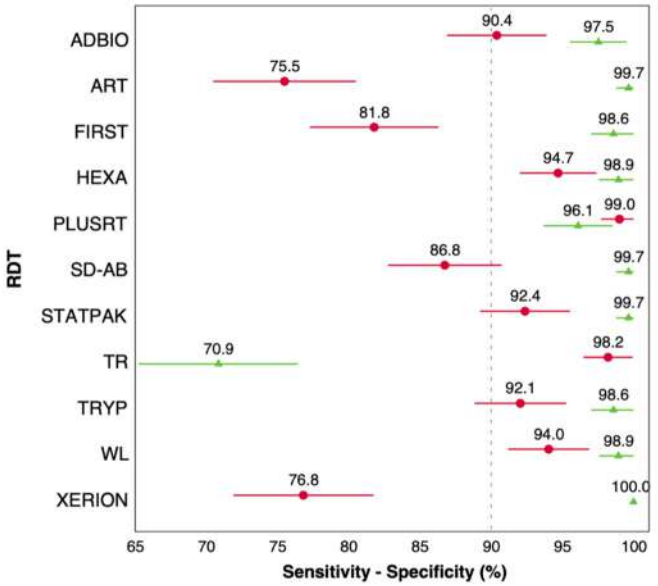
ORGANIZED BY

IN COOPERATION WITH

COORGANIZED BY

Independent head-to-head comparison of commercially available RDTs

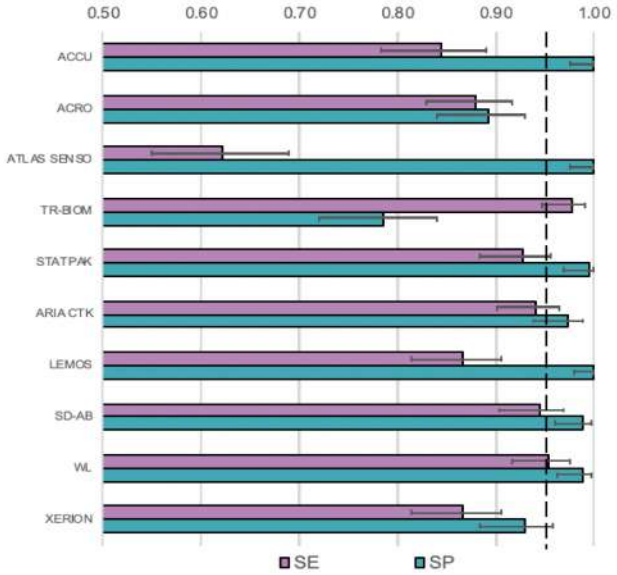
Colombia



SE range	SE >92%	SP range	SP >95%	SE >92% & SP >95%
75-99%	54% (6/11)	71-100%	90% (10/11)	45% (5/11)

Marchiol et.al. 2023 (PMID: 37607214)

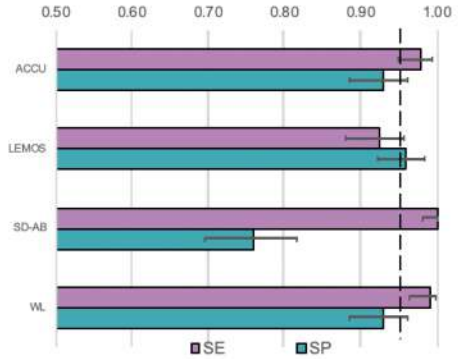
Bolivia



SE range	SE >92%	SP range	SP >95%	SE >92% & SP >95%
62-98%	50% (5/10)	78-100%	60% (6/10)	40% (4/10)

Lopez et.al. 2023 (PMID: 38437237)

Argentina

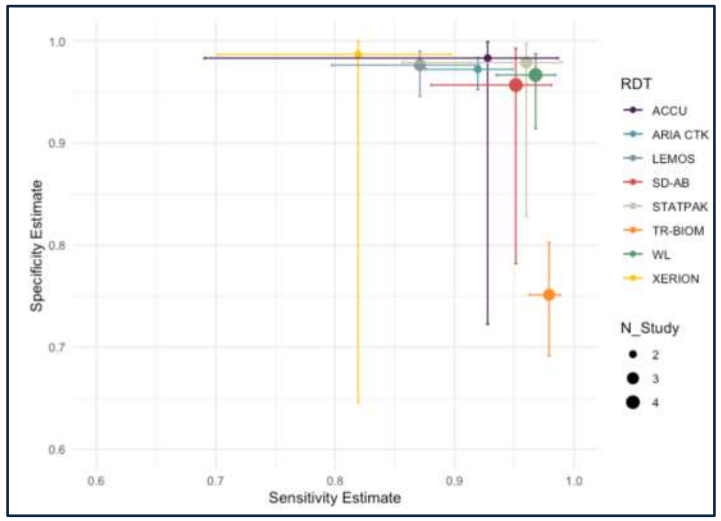


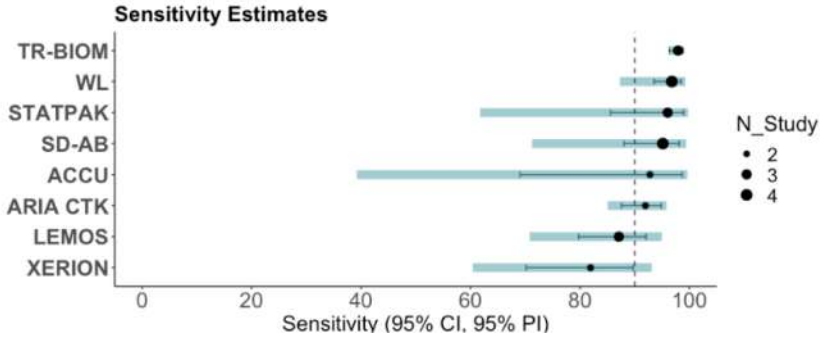
SE range	SE >92%	SP range	SP >95%	SE >92% & SP >95%
92-100%	100% (4/4)	76-96%	25% (1/4)	25% (1/4)

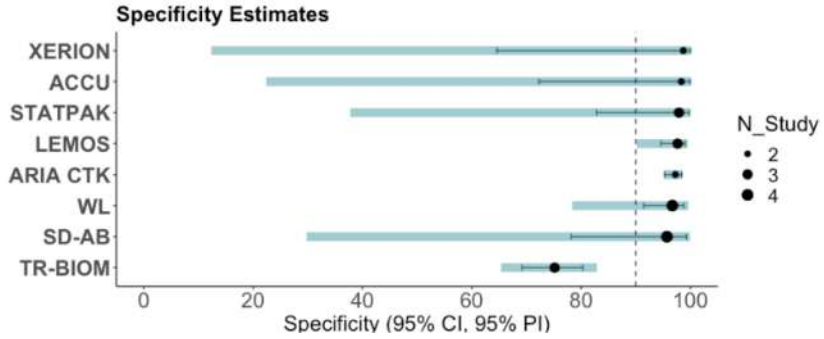
Rivero et.al. 2023 (PMID: 38489395)

Analysis Methodology

Meta-analysis







ARGENTINA	*Rivero et.al. 2023 (PMID: 38489395)*
BOLIVIA	*Lopez et.al. 2023 (PMID: 38437237)*
COLOMBIA	*Marchiol et.al. 2023 (PMID: 37607214)*
BRAZIL	*Iturra et.al. 2023 (PMID: 36936214)*

Analysis Methodology

- Performance estimate against composite reference – can lead to biased estimates
- Bayesian Latent Class Analysis

ORGANIZED BY

IN COOPERATION WITH

COORGANIZED BY

MEETING ON DIAGNOSTIC EVALUATION AND ECONOMIC IMPACT ANALYSIS OF NEW DIAGNOSTIC METHODS FOR CHAGAS DISEASE

Discussion of relevant components in the generic study protocol for the evaluation of CD RDTs

Andrés Caicedo
Access Program Chagas
DNDi

6-7 MAY 2024 BUENOS AIRES, ARGENTINA

Consideraciones
Verificación retrospectiva y prospectiva

Criterios	Retrospectivas	Prospectivos
Matriz utilizada/disponibilidad de la muestra	Sueros de biobancos de referencia	Muestra biológica tomada del paciente (sangre total/suero)
Tiempo de ejecución	Ejecutados rápidamente Emisión rápida de resultados	En función de la captación de pacientes Prevalencia meta, resultado previo desconocido
Condiciones de procesamiento	Condiciones controladas (temperatura/humedad)	Identificación de lugares adecuados y dentro de los rangos de los fabricantes
Costo total del ejercicio	Menor costo: insumos y operadores	Mayos costo: operadores en diferentes lugares, traslado de muestras, más tiempo contratación
Resultados	Soporte técnico para registro sanitario Exploración inicial del rendimiento Selección previa de mejor rendimiento	Mayor aporte en las recomendaciones nacionales Plasman mejor la realidad de uso

Detallar con mayor precisión criterios de selección de muestras

Detallar los criterios de inclusión relacionados con las muestras que ingresarán al estudio

- Pacientes **que representen todo el espectro de la enfermedad**, desde infecciones recientes hasta crónicos, con o sin daño órgano especifico.
- Muestras de suero con títulos **cercanos a los puntos de corte** de cada técnica de referencia.
- Procedencia **representativa del país** o del área a evaluar.
- Evidencia de **manipulación y almacenamiento** (incluyendo RDT).
- Estandarizar el **tamaño muestral** según criterios (prevalencia, recursos y tiempo disponibles).
- Especificar el **constructo** del “patrón de referencia” utilizado.

- ***¿Es posible incluir pacientes que hayan sido tratados con antiparasitario?***

Varios

Especificar la interpretación ”**Indeterminado**” en resultados

Control de Calidad
Participación de varios operadores, hoja de ruta ante discordancias entre operadores

Estandarizar el uso de **score**
Facilidad de lectura, fondo de la prueba, intensidad de las bandas, insumos adicionales

Estimular los **sistemas de recolección digital** de datos, disminución de errores de digitación, registro fotográfico

Disponibilidad herramienta de lectura **automatizada** de la RDT

Thank You.

Contact info:
Andrés Caicedo
acaicedo@extern.dndi.org
Access Program Chagas
DNDi

ORGANIZED BY

IN COOPERATION WITH

COORGANIZED BY

ORGANIZED BY

IN COOPERATION WITH

COORGANIZED BY

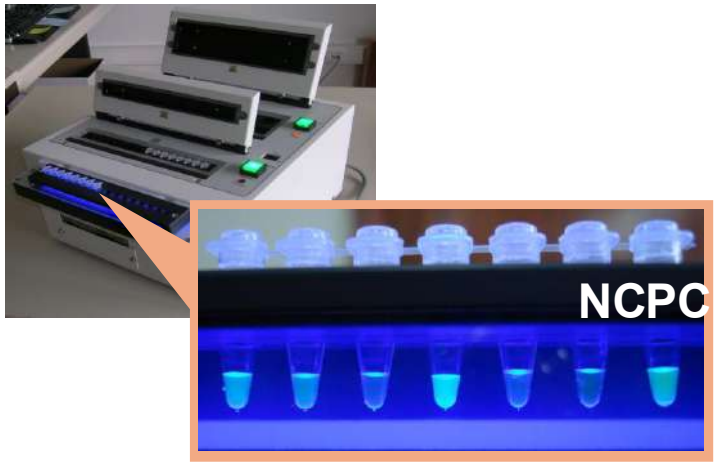
STATE OF THE ART OF LAMP AS POINT OF CARE TOOL FOR DIAGNOSIS OF CD

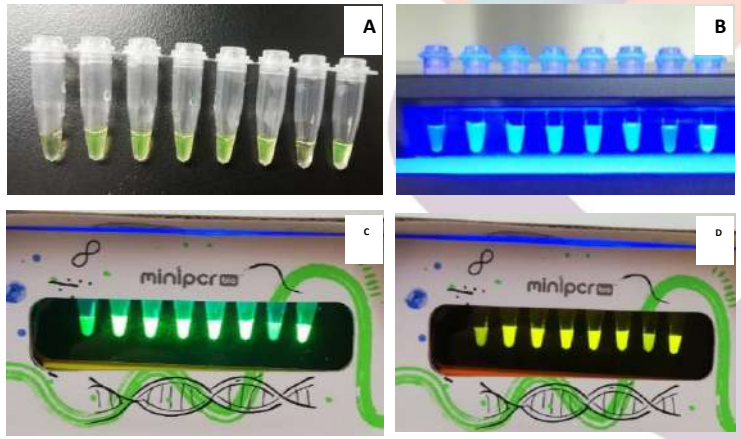
Schijman Alejandro

MEETING ON DIAGNOSTIC EVALUATION
AND ECONOMIC IMPACT ANALYSIS
OF NEW DIAGNOSTIC METHODS
FOR CHAGAS DISEASE

6-7 MAY 2024 BUENOS AIRES, ARGENTINA

LOOP MEDIATED ISOTHERMAL AMPLIFICATION - LAMP





LAMP

Community health worker | Health post | Microscopy centre | District hospital | Reference centre

EIKEN CHEMICAL CO.,LTD.

FIRST FEASIBILITY AND ANALYTICAL PERFORMANCE STUDIES

RESEARCH ARTICLE

Analytical sensitivity and specificity of a loop-mediated isothermal amplification (LAMP) kit prototype for detection of *Trypanosoma cruzi* DNA in human blood samples

Susana A. Besuschio[1], Mónica Llano Murcia[2], Alejandro F. Benatar[1], Severine Monnerat[3], Israel Cruz[3], Albert Picado[3], María de los Ángeles Curto[1], Yutaka Kubota[4], Diana P. Wehrendt[1], Paula Pavia[2], Yasuyoshi Mori[4], Concepción Puerta[2], Joseph M. Ndung'u[3], Alejandro G. Schijman[1]*

PLOS NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Trypanosoma cruzi loop-mediated isothermal amplification (*Trypanosoma cruzi* Loopamp) kit for detection of congenital, acute and Chagas disease reactivation

Susana A. Besuschio[1], Albert Picado[2], Arturo Muñoz-Calderón[1], Diana P Wehrendt[1], Marisa Fernández[3,4], Alejandro Benatar[1], Zoraida Diaz-Bello[5], Cecilia Irurtia[6], Israel Cruz[2,7], Joseph M Ndung'u[2], María L Cafferata[8], Graciela Montenegro[6], Sergio Sosa Estani[4], Raúl H. Lucero[9], Belkisyole Alarcón de Noya[5], Silvia A Longhi[1], Alejandro G Schijman[1]*

Diagn Microbiol Infect Dis. 2017 Sep;89(1):26-28. doi: 10.1016/j.diagmicrobio.2017.06.012. Epub 2017 Jun 19.

Rapid detection of Trypanosoma cruzi by colorimetric loop-mediated isothermal amplification (LAMP): A potential novel tool for the detection of congenital Chagas infection

Rocío Rivero [1], Margarita Bisio [2], Elsa Beatriz Velázquez [3], Mónica Inés Esteva [3], Karenina Scollo [3], Nicolás Leonel González [4], Jaime Altcheh [2], Andrés Mariano Ruiz [3]

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

PARASITOLOGY

Evaluation of the Performance of the Loopamp *Trypanosoma cruzi* Detection Kit for the Diagnosis of Chagas Disease in an Area Where It Is Not Endemic, Spain

María D. Flores-Chavez,[a,b] Alba Abras,[c,d] Cristina Ballart,[c,e] Ismael Ibáñez Perez,[a] Pilar Perez-Gordillo,[a] Montserrat Gállego,[c,e] Carmen Muñoz,[f,g] Zaira Moure,[h] Elena Sulleiro Igual,[h] Javier Nieto,[a] Emilia García Díez,[a] Israel Cruz,[i] Albert Picado[i]

NEED TO STANDARDIZE RAPID DNA EXTRACTION METHODS DESIGNED FOR POINT OF CARE DETECTION

(LABORATORIES LINKED TO SECOND LEVEL HEALTH CARE CENTERS OR MATERNITIES)

The Journal of Molecular Diagnostics, Vol. 23, No. 4, April 2021

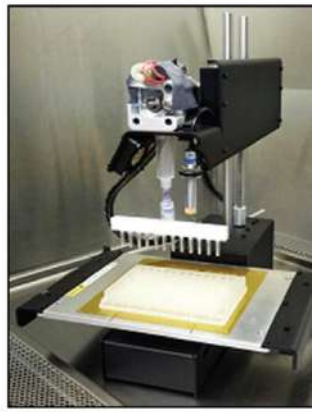
ELSEVIER

the Journal of Molecular Diagnostics
jmdjournal.org

TECHNICAL ADVANCE

Development and Evaluation of a Three-Dimensional Printer—Based DNA Extraction Method Coupled to Loop Mediated Isothermal Amplification for Point-of-Care Diagnosis of Congenital Chagas Disease in Endemic Regions

Diana P. Wehrendt,* Julio Alonso-Padilla,† Bo Liu,‡ Lizeth Rojas Panozo,§ Silvia Rivera Nina,§ Lilian Pinto,§ Daniel Lozano,§ Albert Picado,‖ Marcelo Abril,¶ Maria J. Pinazo,† Joaquim Gascon,† Faustino Torrico,§ Season Wong,‡ and Alejandro G. Schijman*



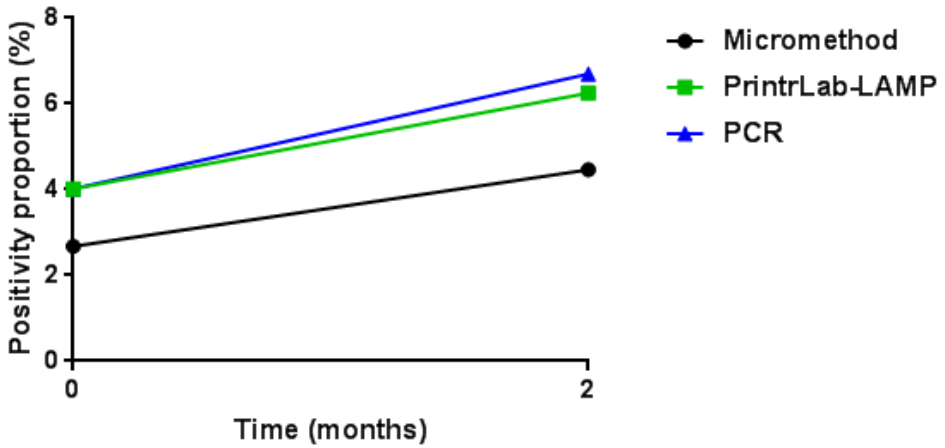
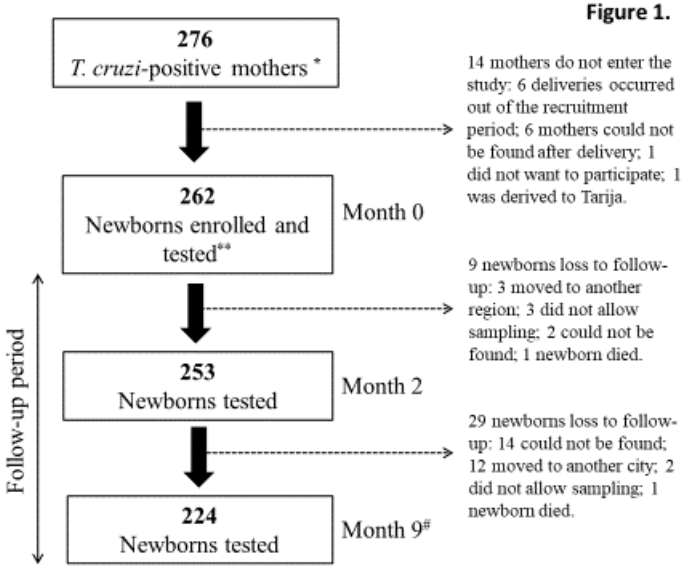


Figure 1.

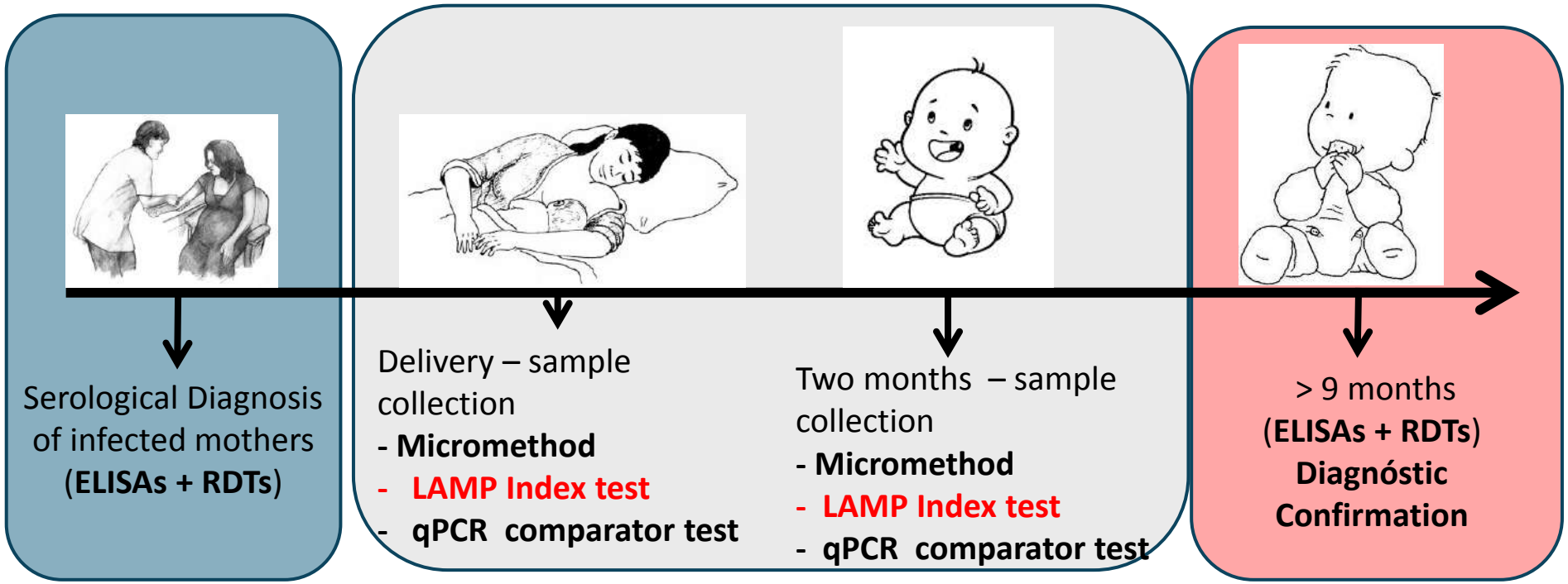


*Serological diagnosis based on the coincidence of two ELISA tests based on distinct antigen sets. Prevalence registered of 28.2% (278/986).
**Out of 278 mothers diagnosed, 259 entered the study; three of them delivered twins.
#Loss rate of the study was 14.5% by month 9: 38 newborns were loss to follow-up out of 262 initially enrolled at birth. *At month 0, besides those 9 newborns detailed as loss to follow-up, whole blood samples from 12 newborns that had micromethod result were not obtained. At month 2, whole blood samples from two more newborns who had micromethod results at that time point were not obtained either.*

	Sampling time	
	Month 0 (M0)	**Month 2 (M2)**
Agreement between tests (%)	96,9	99,1
Kappa (CI95%)	0.65 (0.41 - 0.89)	0.88 (0.73 - 1.00)
Discordant results	7 (3.1%)	2 (0.9%)

FIRST LAMP FIELD VALIDATION

- First Pilot Field Study: Mother-neonates recruitment in Hospitals of VILLA MONTES and YACUIBA localities at the Gran Chaco in Bolivia, financed by BID

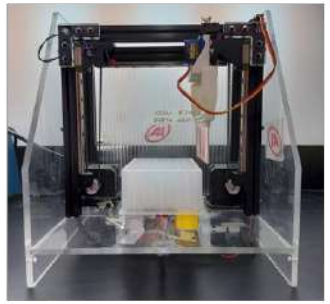


Preparación de la muestra

100 µL de sangre
+
proteinasa K
(fila A)

Placa de pozo
Profundo 2,2 mL

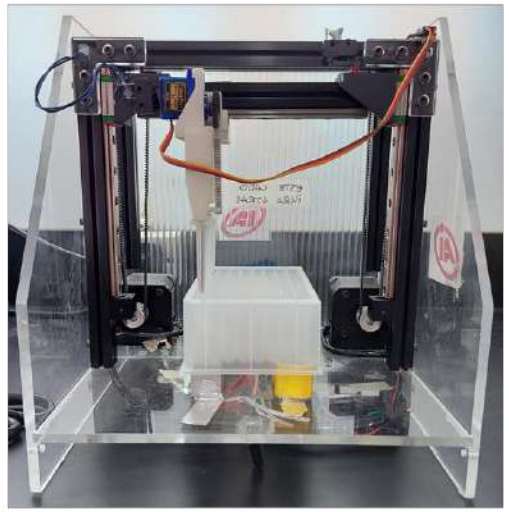
20 min a 65°C



Extracción de ADN PrintrLab

	1	2	3	4	5	6	7	8	9	10	11	12	
A													Buffer de unión + partículas magnéticas
B													
C													Solución de lavado I
D													Solución de lavado II
E													Solución de lavado II
F													Solución de lavado II
G													
H													Buffer de elución

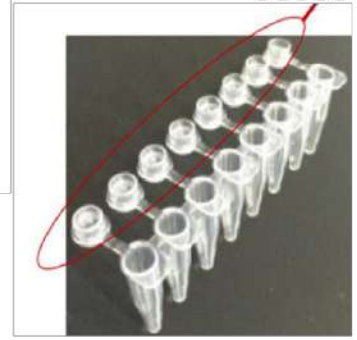
PrintrLab



Todos los pasos se realizan con agitación y captura lenta de las partículas magnéticas

Amplificación isotérmica LAMP

Reactivos secos

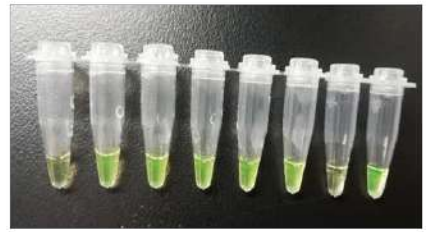


+
30 µL de ADN

Bloque térmico

40 min a 65°C

Detección



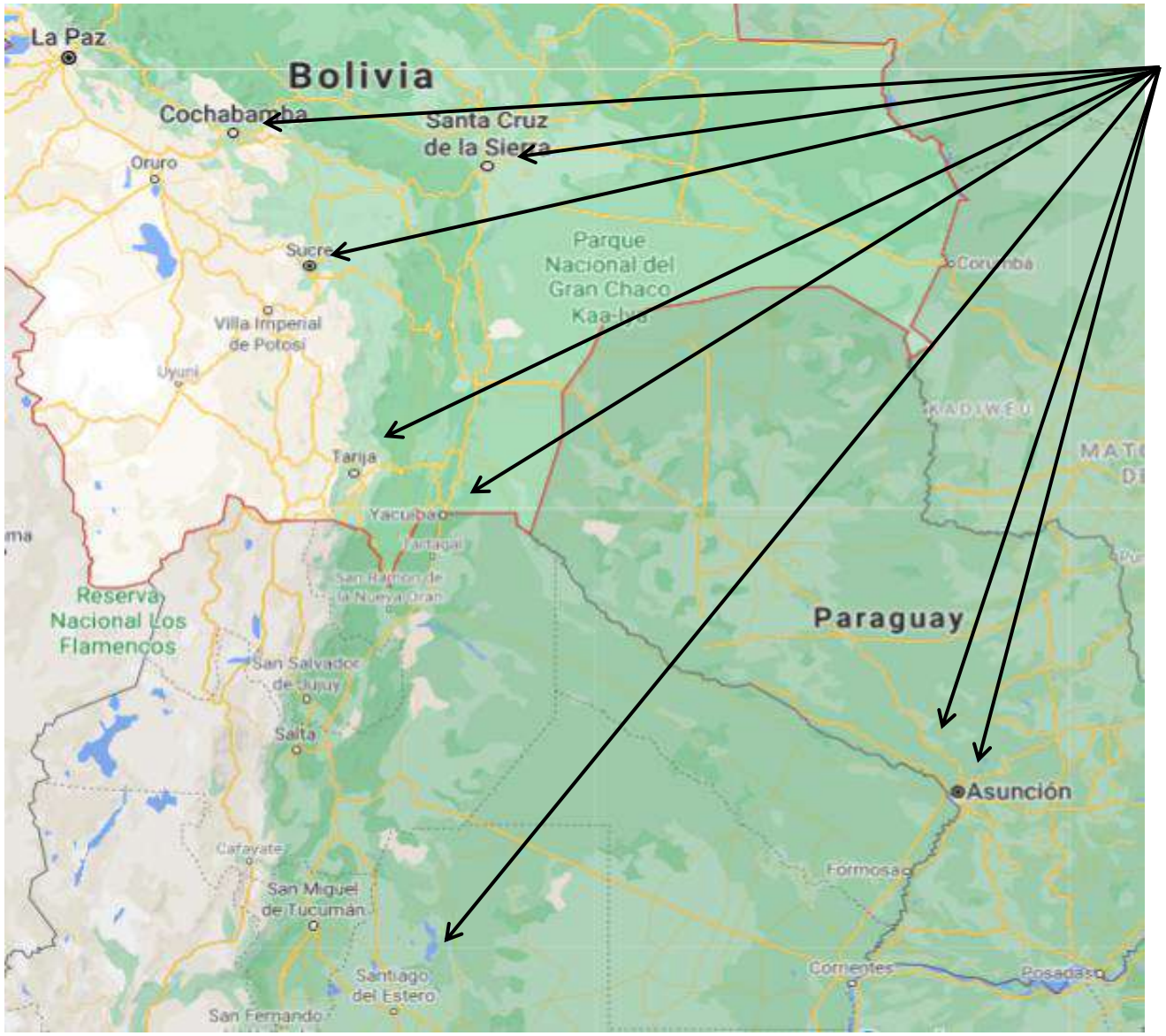
Ojo desnudo

Lámpara UV
P51™ Molecular
Fluorescence Viewer

PURE-LAMP

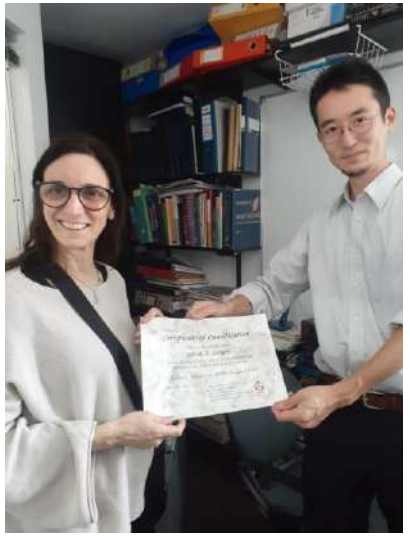
CHAGAS-LAMP GHIT 2020-203

TDR Chagas Project (LEG. ID 39002)



TRANSFERENCIA PURE-LAMP SOP
5 en Bolivia,
2 en Paraguay,
2 en Argentina.

DOS OPERADORES POR SITIO ENTRENAMIENTO CERTIFICADO POR EIKEN CHEMICAL



Preparación de la muestra

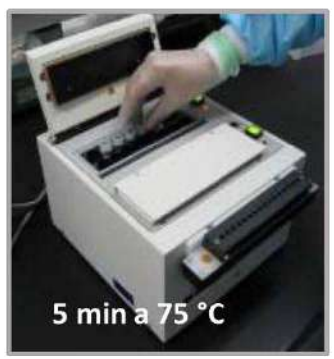
Extracción de ADN

Amplificación isotérmica

Detección

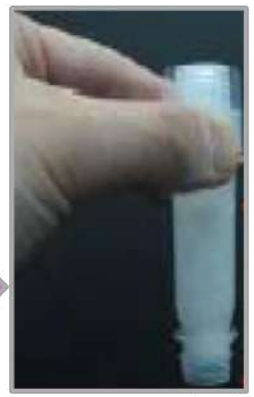
30 µL de 334 mM NaCl
+
30 µL de sangre en heparina
o 6mm FTA o 2 DBS W903

Heating tube



Adsorbent tube

Agitación vigorosa

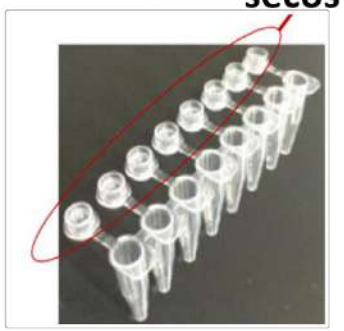


Injection cap

Elución del ADN

30 µL

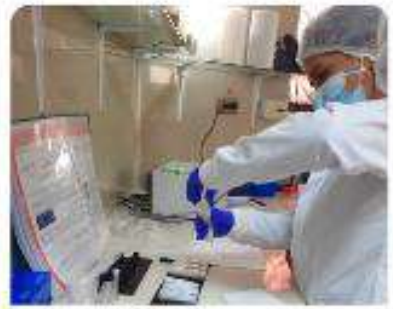
Reactivos secos



Ojo desnudo

Luz led incorporada en el *Loopamp*™ LF-160

Hosp. Ramón Carrillo, Stgo. del estero - Argentina, 27-04-22.
*La operadora del Hosp. La Banda se entrenó en este sitio



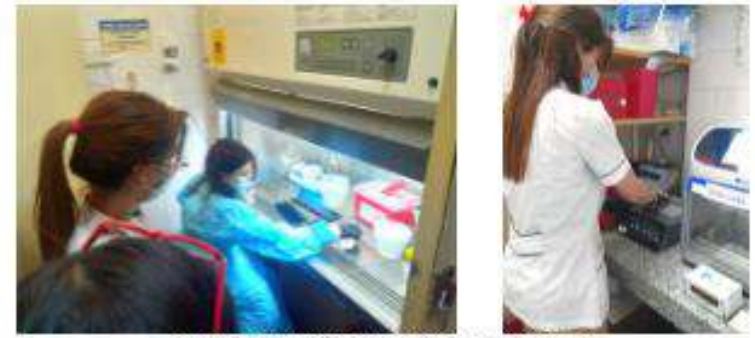
Hosp. México Sacaba- Bolivia
23-06-22

Hosp. Materno de Sucre- Bolivia
28-06-22

Hosp. Municipal Bajío del oriente, Sta. Cruz- Bolivia
18-07-22

Hosp. San Juan de Dios, Tarija - Bolivia
14-07-22

Hosp. Municipal Bajío del oriente, Sta. Cruz- Bolivia
18-07-22



Hosp. Donación Francisco Santojanni
26-12-22

Hosp. Dr, Rubén Zelaya,
Yacuiba- Bolivia 12-07-22

Hosp. San Pablo, Asunción-
Paraguay 17-10-22

Hosp. Regional de Villa Hayes - Paraguay
19-10-22

Hosp. de Tartagal "Juan Domingo Perón"
26-12-22

Title: Evaluation and validation of a PrintrLab-based LAMP assay to identify *Trypanosoma cruzi* in newborns in Bolivia: a proof-of-concept study.

Lizeth Rojas Panozo, Silvia Rivera Nina, Diana P. Wehrendt, Aina Casellas, Lilian Pinto, Susana Mendez, Chi-Wei Kuo, Daniel F. Lozano, Lourdes Ortiz, Maria-Jesus Pinazo, Albert Picado, Sergi Sanz, Marcelo Abril, Joaquim Gascon, Season Wong, Alejandro G. Schijman, Faustino Torrico, Julio Alonso-Padilla.

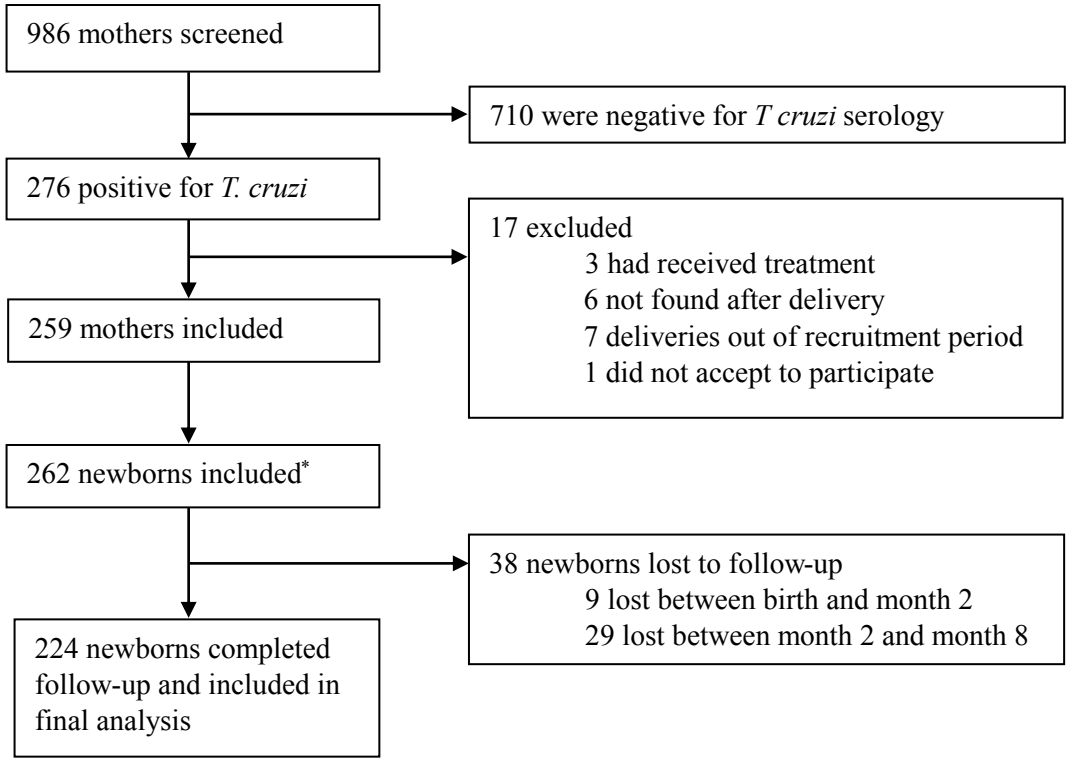


Figure 1: **Flow of participants.**
*Three mothers had twins.

Summary of positive cases by microscopy or any of the two molecular-based techniques (LAMP or PCR)

	At birth	At two months
Number of newborns positive for *Trypanosoma cruzi*		
Microscopy	6 (2·7%)	4* (1·8%)
PrintrLab–LAMP	**9 (4·0%)**	**8§ (3·6%)**
rtPCR	9 (4·0%)	10‡ (4·5%)
PrintrLab–LAMP accuracy#		
Increased detection of positivity *vs* microscopy	**9 *vs* 6 (50%)**	**8 *vs* 4 (100%)**
Specificity	**98·6% (0·86 - 1·13)**	**98·2% (0·86 - 1·12)**
rtPCR accuracy#		
Increased detection of positivity *vs* microscopy	9 *vs* 6 (50%)	10 *vs* 4 (150%)
Specificity	98·6% (0·86 - 1·13)	97·3% (0·85 - 1·11)
Agreement between PrintrLab-LAMP and rtPCRγ	0·77 (0·64 - 0·90)	0·88 (0·75 - 1·01)
Average parasite burden		
qPCR, mean Ct (SD)	21·7 (6·1)	21·1 (4·7)
Number of newborns treated	6	3

Performance of laboratories and/or operators in TP samples 50 and 20 par.Eq./Ml

Analysis of 14 laboratory technicians for all samples tested showed an ORA of 88.1% and κ = 0.718 (95% CI: 0.632 - 0.792).

Results retrieved for each laboratory.

Lab ID	Number of Operators	Number of Samples	κ	PNA	PPA	ORA
AS	1	12	1.000	100	100	100
VH	1	12	1.000	100	100	100
CB	1	12	1.000	100	100	100
SC	1	12	1.000	100	100	100
SU	2	24	1.000	100	100	100
TA	2	24	0.714	100	83.3	87.5
YA	2	24	0.714	100	83.3	87.5
SE	2	24	1.000	100	100	100
BA	1	12	1.000	100	100	100
TG	1	12	0.800	100	88.9	91.7
ALL CENTERS	**14**	**168**	**0.895**	**100**	**96.0**	**97.0**

Lab ID	Number of Operators	Number of Samples	κ	PNA	PPA	ORA
AS	1	12	0.800	100	88.9	91.7
VH	1	12	1.000	100	100	100
CB	1	12	1.000	100	100	100
SC	1	12	1.000	100	100	100
SU	2	24	0.412	50	88.9	79.2
TA	2	24	0.500	100	66.7	75.0
YA	2	24	0.455	83.4	72.2	75.0
SE	2	24	0.636	100	77.8	83.4
BA	1	12	0.636	100	77.8	83.3
TG	1	12	0	100	0.0	0.0
ALL CENTERS	**14**	**168**	**0.563**	**90.5**	**77.8**	**80.3**

.Results from the two operators of the same laboratory who showed differences in their performance.

Lab ID	Number of Operators	Number of Samples	κ	PNA	PPA	ORA
SU	SU-1	12	1.000	100	100	100
	SU-2	12	1.000	100	100	100
TA	TA-1	12	1.000	100	100	100
	TA-2	12	0.500	100	66.7	75.0

Lab ID	Number of Operators	Number of Samples	κ	PNA	PPA	ORA
SU	SU-1	12	-0.250	0.0	77.8	58.3
	SU-2	12	1.000	100	100	100
TA	TA-1	12	0.636	100	77.8	83.3
	TA-2	12	0.385	100	55.6	66.7
YA	YA-1	12	0.167	66.7	55.5	58.3
	YA-2	12	0.800	100	88.9	91.7
SE	SE-1	12	0.500	100	66.7	75.0
	SE-2	12	0.800	100	88.9	91.7

κ: Cohen's Kappa; NPA: negative percent agreement; PPA: positive percent agreement; ORA: overall rate agreement.

Thank You.

Contact info: schijmaningebi@gmail.com

ORGANIZED BY

IN COOPERATION WITH

COORGANIZED BY

ORGANIZED BY

IN COOPERATION WITH

COORGANIZED BY

Estado del arte de qPCR para diagnóstico y seguimiento de pacientes con la Enfermedad de Chagas

Otacilio Moreira
Fundação Oswaldo Cruz
Rio de Janeiro - Brasil

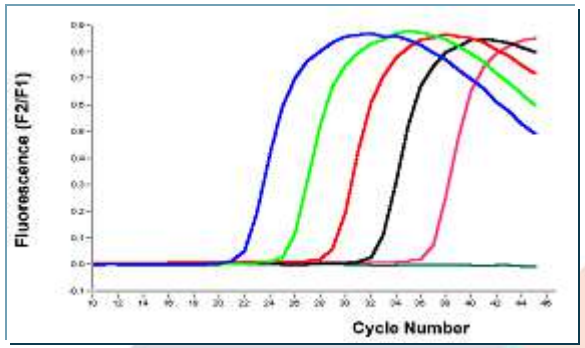
6-7 MAY 2024 BUENOS AIRES, ARGENTINA

PCR para diagnosticar la Enfermedad de Chagas

PCR convencional para el diagnóstico de infecciones crónicas y evaluación del tratamiento etiológico (Moser et al. 1989; Sturm et al. 1989; Avila et al. al. 1991; 1993; Britto et al. 1993; Wincker et al, 1994; Britto et al., 1995).

PCR en tiempo real para diagnóstico molecular y cuantificación de la carga parasitária (Cummings & Tarleton, 2003; Pirón et al., 2007; Duffy et al., 2009 y 2013; Moreira et al., 2013; Ramirez et al., 2015)

- ✓ **Especificidad mejorada**
- ✓ **Alta sensibilidad**



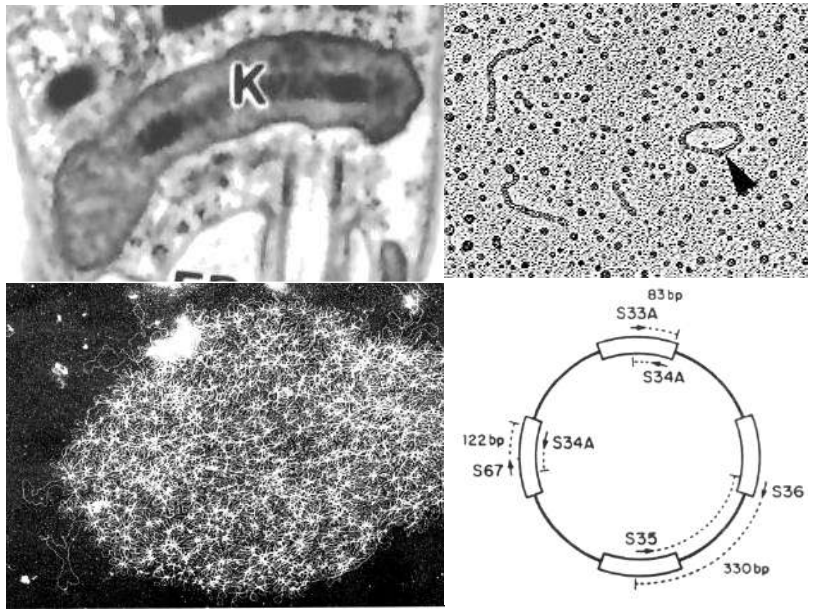
Uso de la PCR en Tiempo Real (qPCR):

- Diagnóstico molecular de recién nacidos (transmisión vertical)
- Seguimiento de pacientes inmunodeprimidos (trasplantes, Chagas/VIH)
- Pacientes agudos, en conjunto con pruebas parasitológicas (brotes orales y transmisión vectorial activa - vigilancia)
- Monitorización de la carga parasitaria en pacientes durante el tratamiento etiológico (marcador temprano de fracaso terapéutico y reactivación)
- Evaluación de la eficacia de nuevos fármacos candidatos o esquemas terapéuticos/fracaso terapéutico (investigación)

Blancos de la qPCR

ADN del cinetoplasto: minicírculo (kDNA)

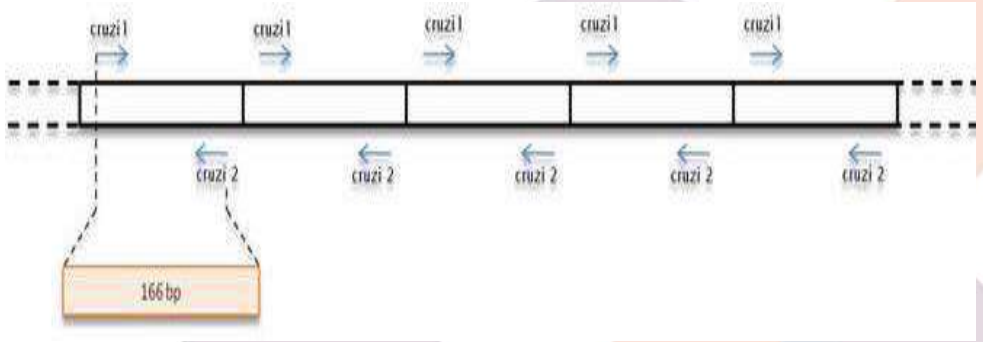
Sturm et al. *Mol. Biochem. Parasitol*. 33: 205-214, 1989



~ 10^4 minicírculos por red de kDNA

ADN nuclear satélite (satDNA)

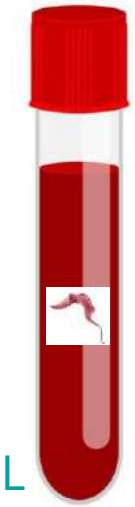
Moser et Al. *J Clinical Microb*, 27: 1477-1482, 1989



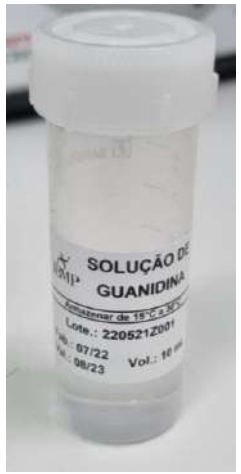
~ 120,000 copias/ Genoma de *T. cruzi*

Preservación de la sangre (importancia de la guanidina)

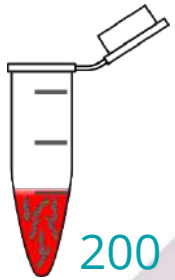
- Avila et al., 1993: Lisado de sangre (10 ml de sangre + 10 ml de guanidina/HCl -EDTA)
- Conservación de la sangre a temperatura ambiente.
- ADN extraído de 200 µL de lisado sanguíneo
- Detecta fragmentos de ADN incluso si la muestra de sangre de 10 ml contiene solo 1 parásito. Mayor sensibilidad



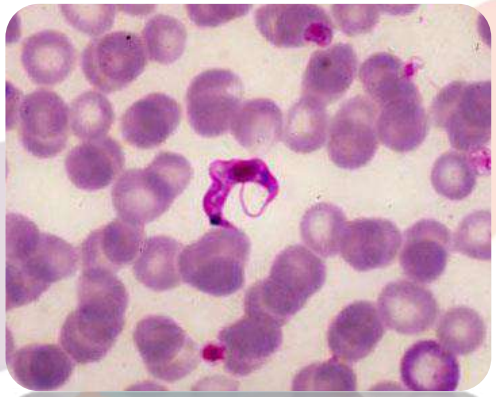
10mL



10mL

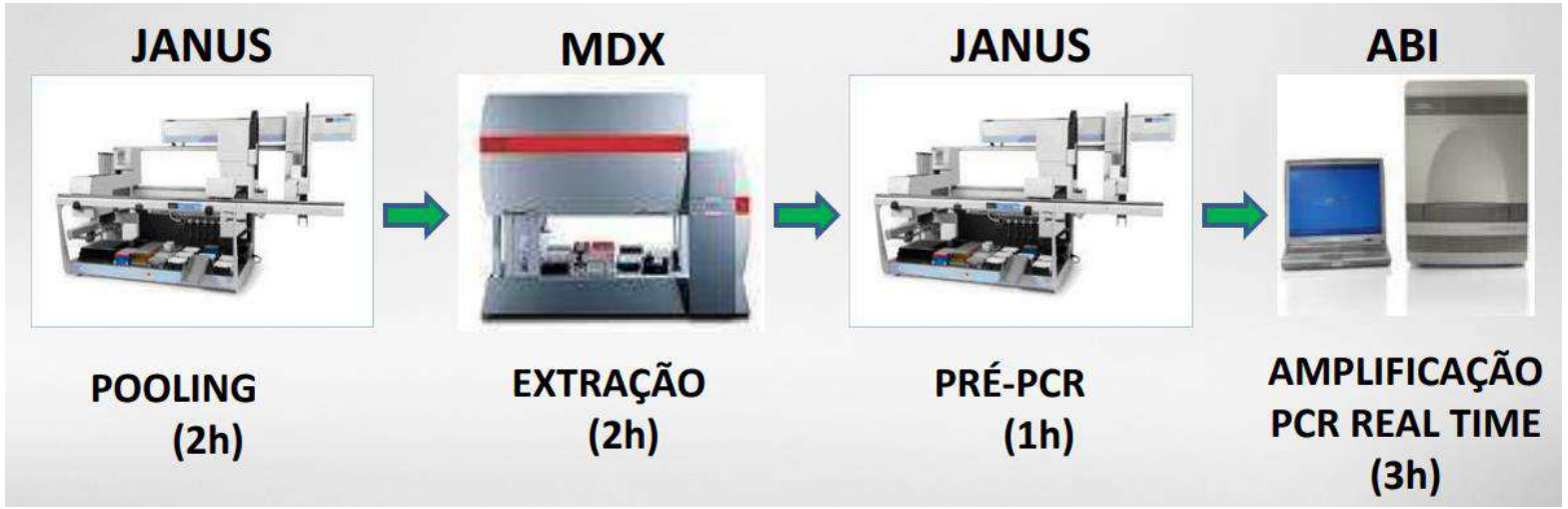


200 µL



Recolección de sangre en el campo ➡ Fácil transporte a los laboratorios centrales ➡ Diagnóstico rápido y sensible

Possibilidad de automatización



Kits NAT automatizados en laboratorios centrales de diagnóstico y bancos de sangre

Consensos de PCR y PCR en tiempo real

Primer taller de la PCR para el diagnóstico molecular de la enfermedad de Chagas (Buenos Aires, 2008)

OPEN ACCESS Freely available online

PLoS NEGLECTED TROPICAL DISEASES

International Study to Evaluate PCR Methods for Detection of *Trypanosoma cruzi* DNA in Blood Samples from Chagas Disease Patients

Alejandro G. Schijman[1]*, Margarita Bisio[1], Liliana Orellana[2], Mariela Sued[2], Tomás Duffy[1], Ana M. Mejia Jaramillo[3], Carolina Cura[1], Frederic Auter[4], Vincent Veron[5], Yvonne Qvarnstrom[6], Stijn Deborggraeve[7], Gisely Hijar[8], Inés Zulantay[9], Raúl Horacio Lucero[10], Elsa Velazquez[11], Tatiana Tellez[12], Zunilda Sanchez Leon[13], Lucia Galvão[14], Debbie Nolder[15], María Monje Rumi[16], José E. Levi[17], Juan D. Ramirez[18], Pilar Zorrilla[19], María Flores[20], Maria I. Jercic[21], Gladys Crisante[22], Néstor Añez[22], Ana M. De Castro[23], Clara I. Gonzalez[24], Karla Acosta Viana[25], Pedro Yachelini[26], Faustino Torrico[12], Carlos Robello[19], Patricio Diosque[16], Omar Triana Chavez[3], Christine Aznar[5], Graciela Russomando[13], Philippe Büscher[7], Azzedine Assal[4], Felipe Guhl[18], Sergio Sosa Estani[27], Alexandre DaSilva[6], Constança Britto[28], Alejandro Luquetti[29], Janis Ladzins[30]

Comparación de protocolos de PCR y qPCR (satDNA y kDNA). Recomendación de protocolo de PCR convencional (kDNA)

Consenso de PCR en tiempo real para cuantificar la carga parasitaria en pacientes con enfermedad de Chagas (Buenos Aires, 2011)

Analytical Validation of Quantitative Real-Time PCR Methods for Quantification of *Trypanosoma cruzi* DNA in Blood Samples from Chagas Disease Patients

CrossMark

the Journal of Molecular Diagnostics
jmd.amjpathol.org

Juan Carlos Ramírez,* Carolina Inés Cura,* Otacilio da Cruz Moreira,† Eliane Lages-Silva,‡ Natalia Juiz,* Elsa Velázquez,§ Juan David Ramírez,¶ Anahí Alberti,‖ Paula Pavia,** María Delmans Flores-Chávez,†† Arturo Muñoz-Calderón,‡‡ Deyanira Pérez-Morales,§§ José Santalla,¶¶ Paulo Marcos da Matta Guedes,‖‖ Julie Peneau,*** Paula Marcet,††† Carlos Padilla,‡‡‡ David Cruz-Robles,§§§ Edward Valencia,¶¶¶ Gladys Elena Crisante,‖‖‖ Gonzalo Greif,**** Inés Zulantay,†††† Jaime Alfredo Costales,‡‡‡‡ Miriam Alvarez-Martínez,§§§§ Norma Edith Martínez,¶¶¶¶ Rodrigo Villarroel,‖‖‖‖ Sandro Villarroel,***** Zunilda Sánchez,††††† Margarita Bisio,* Rudy Parrado,***** Lúcia Maria da Cunha Galvão,‖‖ Antonia Cláudia Jácome da Câmara,‖‖ Bertha Espinoza,§§ Belkisyole Alarcón de Noya,‡‡ Concepción Puerta,** Adelina Riarte,§ Patricio Diosque,‖ Sergio Sosa-Estani,§ Felipe Guhl,¶ Isabela Ribeiro,‡‡‡‡‡ Christine Aznar,*** Constança Britto,† Zaida Estela Yadón,§§§§§ and Alejandro G. Schijman*

Validación analítica y clínica de ensayos de qPCR para satDNA y kDNA. Recomendación de protocolo de PCR en tiempo real cuantitativa para satDNA

Target Product profiles (TPP) para la enfermedad de Chagas

Target Product Profile (TPP) for Chagas Disease Point-of-Care Diagnosis and Assessment of Response to Treatment (Porrás et al., 2015)

Target product profile for a test for the early assessment of treatment efficacy in Chagas disease patients: An expert consensus (Alonso-Padilla et al., 2020)

Necesidad de diagnóstico	Muestras	Numero de extracciones de ADN	Tipo de lectura
Transmisión congénita	Máximo 2 ml de sangre de cordón umbilical o periférico (1mL – TPP 2020). Ideal: orina	1 extracción/muestra	Cualitativa
Transmisión vectorial y oral	2 - 5 ml de sangre o suero. Ideal: orina o saliva	1 extracción/muestra	Cualitativa/cuantitativa
Reactivación da infección asociada a inmunosupresión y transmisión por transfusión de sangre	Sangre, líquido cefalorraquídeo, tejido de chagoma.	1 extracción/muestra	Cualitativa/cuantitativa
Pacientes infectados asintomáticos, individuos sintomáticos remitidos y donantes de sangre positivos.	Ideal: saliva, orina. Alternativa: sangre, plasma o suero	3 extracciones/muestra	Cualitativa
Respuesta terapéutica antiparasitaria (basada en la negativización persistente de la parasitemia o evaluación de la carga parasitaria reducida mediante métodos de biología molecular)	3 muestras (antes y después del tratamiento), sangre (máximo de 5 mL [adultos] y 2 mL [niños]); Ideal: orina	3 extracciones/muestra o 3 muestras con 1 extracción/muestra	Cualitativa/cuantitativa

PCR em tiempo real cuantitativa – curva padrón sintética

Estudios multicéntricos: la recomendación es hacer una curva padrón con la cepa prevalente en cada región del estudio ➡ diferencia en el número de copias satDNA/DTUs

ELSEVIER

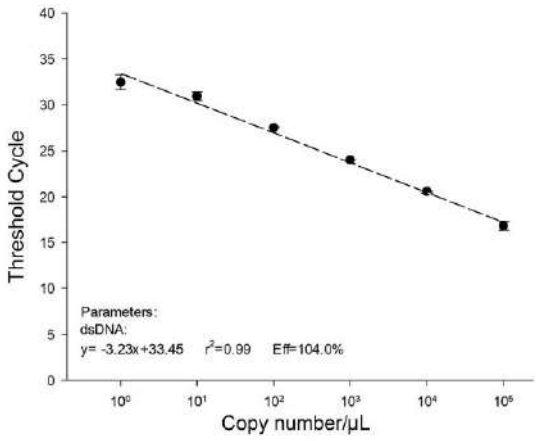
The Journal of Molecular Diagnostics

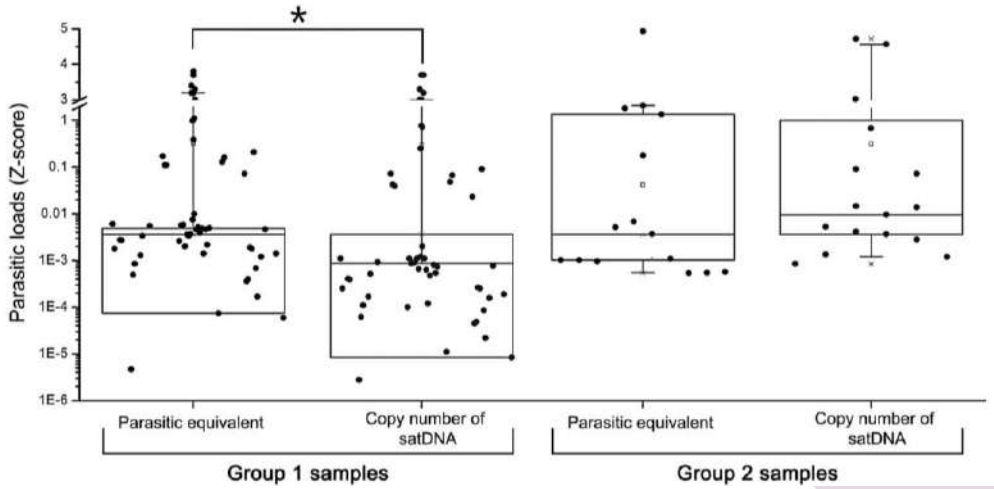
Available online 4 February 2021

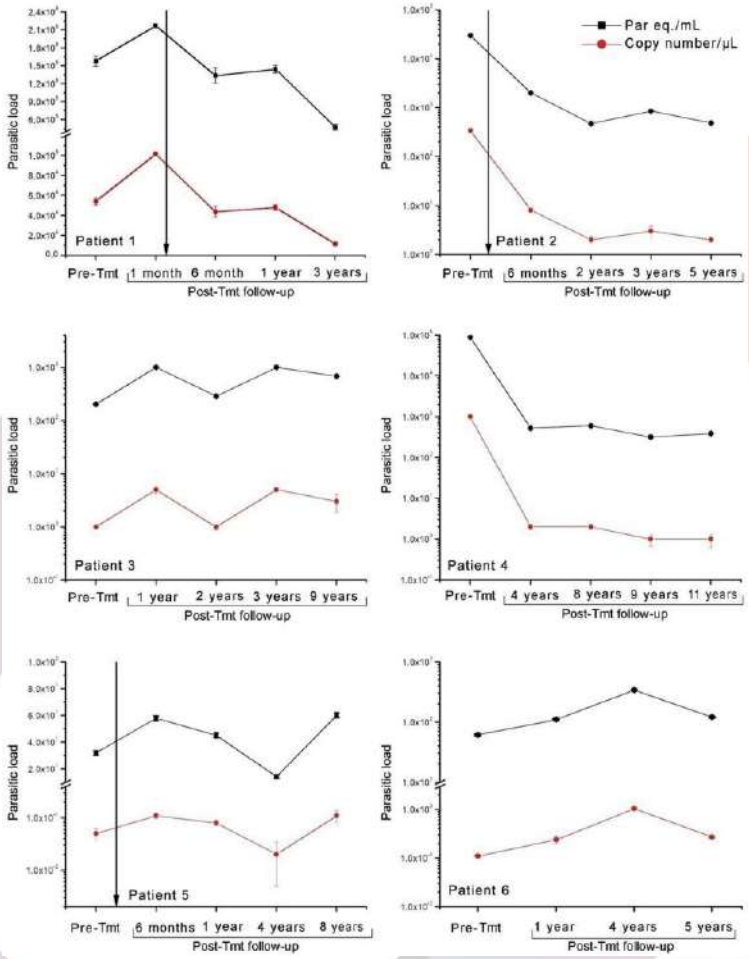
In Press, Journal Pre-proof

Towards the establishment of a single standard curve for quantification of *Trypanosoma cruzi* natural populations using a synthetic *satellite* unit DNA sequence

Arturo Muñoz-Calderón [1], Natalia Lins Silva-Gomess [2], Sofia Apodaca [1], Belkisyolé Alarcón de Noya [3], Zoraida Díaz-Bello [3], Leticia Rocha Quintino Souza [2], Alexandre Dias Tavares Costa [4], Constança Britto [2], Otacilio Cruz Moreira [2], Alejandro Gabriel Schijman [1]







Kits de PCR em tiempo real – Enfermedad de Chagas

RealStar Chagas PCR Kit 1.0
Altona Diagnostic, Alemania
Transporte: Hielo seco

VIASURE Trypanosoma cruzi Real Time PCR Detection Kit
CerTest, España
Transporte: Temparatura ambiente

T. Cruzi DNA Test
Wiener, Argentina
Transporte: 2-10 °C

Kit BioMol Chagas – IBMP (NAT Chagas)
IBMP, Brasil
Transporte: Hielo seco

Mercado de kits de PCR en tiempo real para Chagas

Produto	Fabricante	Apresentação (em reações)	Costo Kit (USD)	Reação	Registro na ANVISA
T. cruzi DNA qPCR Kit	Diagnostic Bioprobes	50	$ 556,00	$ 11,12	Não
RealCycler Chagas qPCR Kit	Progenie Molecular	30	$ 346,00	$ 11,53	Não
VIASURE *T. cruzi* qPCR Kit	CerTest BIOTEC	100	$ 450,00	$ 4,50	Não
T. cruzi DNA qPCR Test	Wiener Laboratorios	50	$ 750,00	$ 15,00	Não
RealStar Chagas PCR Kit	Altona Diagnostics	100	$ 1.600,00	$ 16,00	Não
T. cruzi DNA Advanced Kit	Genesig (Primerdesign)	150	$ 841,00	$ 5,61	Não
ViPrimePLUS *T. cruzi* qPCR Kit	Vivantis Technologies	150	$ 2.526,00	$ 16,84	Não
Kit BioMol Chagas (NAT Chagas)	**IBMP**	**96**	**$425,00**	**$ 4,42**	**Sim**
Loopamp Chagas (LAMP)	Eiken	48	$ 440,00	$ 9,16	Não

Instituto de Biologia Molecular do Paraná (IBMP)

Ejemplo de costo de transporte internacional:
69 kits NAT Chagas (6.624 reacciones)
IBMP (Paraná, Brasil) ➡ INGEBI (Buenos Aires, Argentina)
R$ 10.000,00 (US$ 1,923)

Estudio DiaChO (Diagnóstico de Chagas Oral)

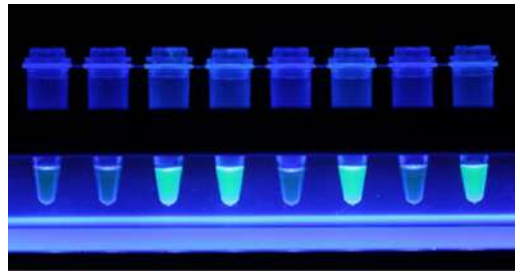
PROGRAMA
INOVA FIOCRUZ

Objetivo: Mejorar el diagnóstico de los pacientes involucrados en brotes orales de la enfermedad de Chagas, mediante a la validación comparativa de los kits Biomol Chagas IBMP (NAT Chagas) e Loopamp (LAMP Chagas) en muestras de pacientes agudos, crónicos recientes (pós-agudos) y alimentos involucrados en brotes orales de la enfermedad de Chagas em región Norte de Brasil.

Muestras de 200 pacientes (agudos y pós-agudos) y 100 muestras de açaí, de los estados de Pará, Amapá y Amazonas (Brasil). Evaluación de muestras de sangre periférica (GEB y heparina), sangre capilar en papel de filtro (Whatman Card) y açaí (en guanidina y papel de filtro).

Comparación de diferentes métodos (y cantidad) de extracción: columnas de sílica, beads-magnéticas (automatizado) y PURE (Eiken). Evaluación de los kits Biomol Chagas (NAT Chagas) e Loopamp (LAMP Chagas) en comparación a la qPCR in-house y métodos parasitológicos y serológicos.

Fiocruz (Otacilio Moreira y Constança Britto), INGEBI (Alejandro Schijman y Silvia Longhi), IEC/Pará (Lourdes Garcez), FMT-HVD (Graça Barbosa y Jorge Guerra) y LACEN/Amapá (Natália Castelo)



Thank You.

Contacto: Otacilio Moreira
Laboratório de Virologia e Parasitologia Molecular
Instituto Oswaldo Cruz/Fiocruz
E-mails: otacilio@ioc.fiocruz.br
otaciliocmoreira@gmail.com

ORGANIZED BY IN COOPERATION WITH COORGANIZED BY

MOLECULAR POINT-OF-CARE DIAGNOSTIC TOOLS: STATE OF THE ART

Elena Ivanova Reipold

Deputy Director, Technology Innovation, FIND

KEY REQUIREMENTS FOR MOLECULAR DIAGNOSTIC TOOLS TO MAKE A TRANSFORMATIONAL IMPACT

POINT-OF-CARE (POC)

Usable where people live and seek care
(incl. communities and primary care settings)

MULTI-PATHOGEN

Able to identify multiple diseases in one sample
(incl. outbreak-prone pathogens)

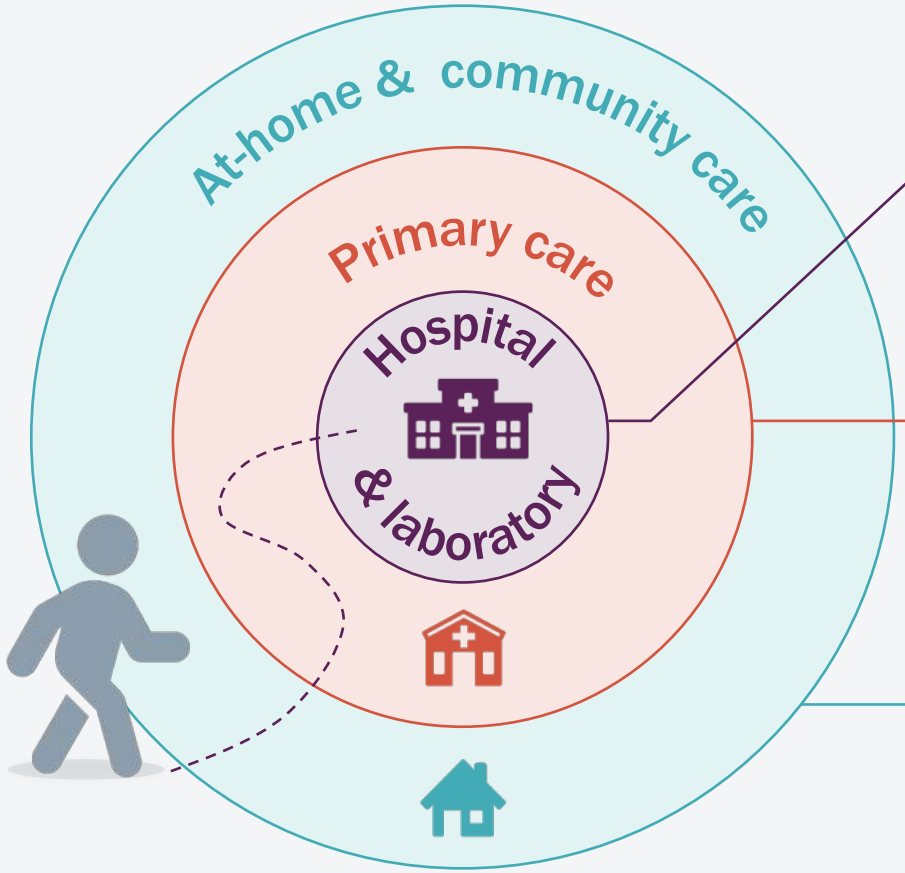
ACCURATE

Robust and highly sensitive results

AFFORDABLE

Pricing structures adapted to LMICs

MOLECULAR DIAGNOSTICS AT THE POINT OF CARE COULD FILL GAPS ACROSS DIFFERENT HEALTHCARE SETTINGS



Technologies suitable to testing infrastructures

Near POC
- Basic lab equipment requirements
- Benchtop/desktop units, mains power
- Semi-automated testing processes

True POC
- No laboratory equipment requirements
- Portable, battery-operated devices
- Kits including disposable sample materials
- Fully automated testing process

Instrument free POC
- No instrument or power requirements
- Kits including disposable sample materials
- Fully automated testing processes

Increasing accessibility

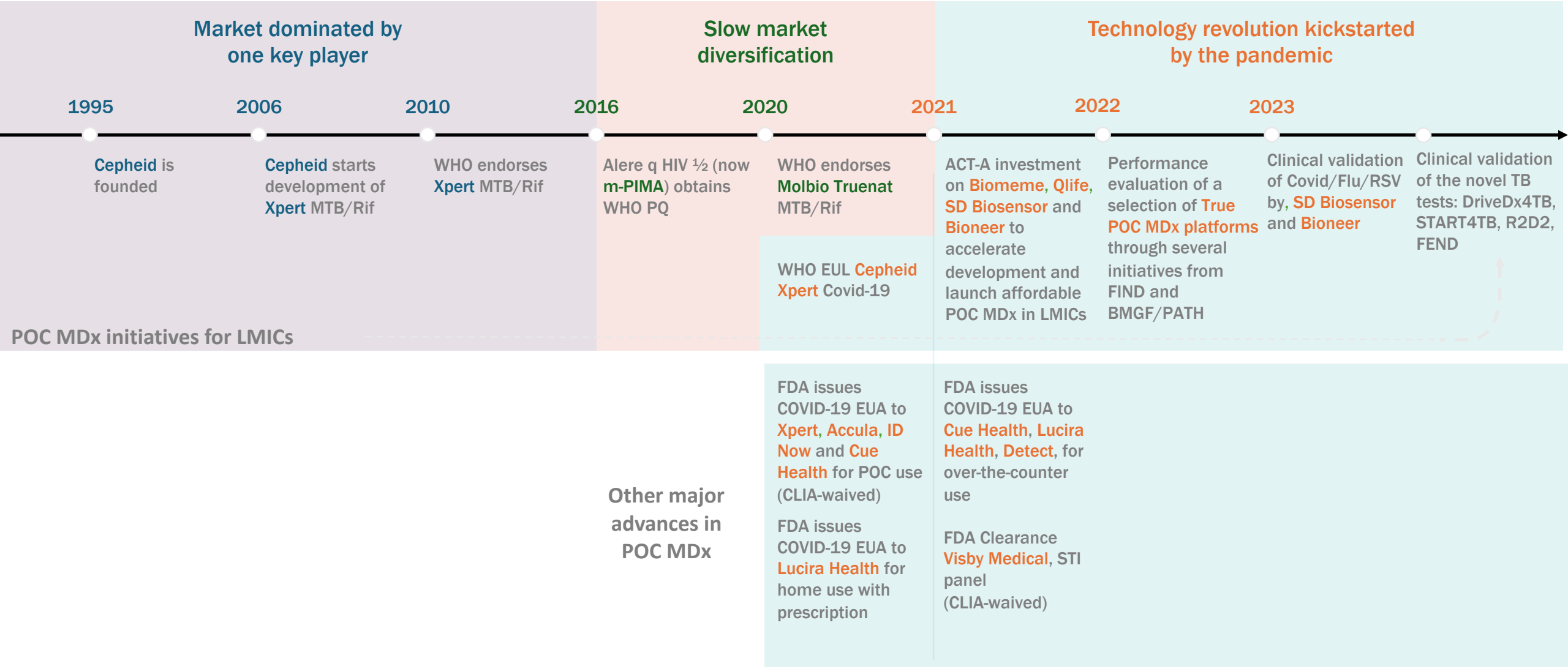
COVID-19 PANDEMIC SPURRED INNOVATION AND BROUGHT MOLECULAR TESTING CLOSE[illegible]NTS

AT HOME

PRIMARY CARE

HOSPITAL/LAB

MASSIVE MARKET ENTRY OF MOLECULAR POINT-OF-CARE DIAGNOSTICS IN THE POST-COVID ERA



Disclaimer: the companies, platforms and assays listed in this slide do not represent a comprehensive list of regulatory authorized/approved or commercialized tests

PIPELINE IN NUMBERS

Near POC

True POC

Instrument free POC

POCT MOLECULAR DIAGNOSTICS LANDSCAPE IN NUMBERS:

161
Total platforms in the landscape

75
New MDx POC launched in total post-COVID

10
True POC platforms launched

3
Instrument-free POC tests in the market

45%
Platforms based on isothermal amplification

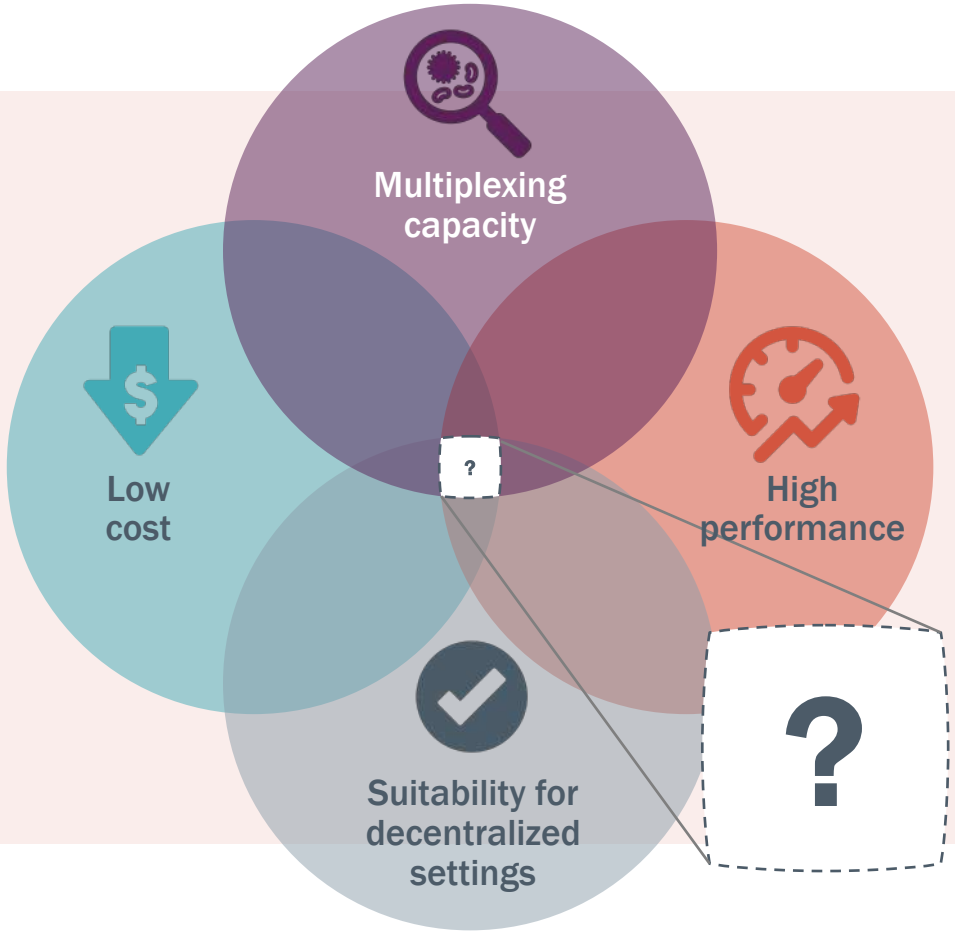
10
POC platforms supported by **FIND**

DESPITE CONTINIOUS TECHNOLOGY INNOVATION TRADE OFFS ARE UNAVOIDABLE

No ‘one size fits all’ solution, different platforms are addressing different use cases

FIND APPROACH

- **Diversify the multi-pathogen platforms** available in district hospitals and other **Level 2** facilities
- **Broaden access** to testing in locations that are convenient for patients (**Level 0 and 1**)



KEY REQUIREMENTS FOR NEW MOLECULAR TOOLS

	Minimal requirement	Optimal requirement
Target settings	Level 2	Level 0-1
Sample type compatibility	Swab, urine, plasma	Swab, urine, plasma, sputum, whole blood
Multiplexing	2-5	>5
Quantification	Qualitative	Semi-quantitative/quantitative
Maintenance	Infrequent maintenance/calibration conducted by minimally skilled operator	No maintenance/calibration
Result readout	Reader or mobile device	Visual reading
Cost (Instrument)	<1000 US $	< 200 US $ or instrument-free
Cost (test)	< 9 US $	< 5 US $

TECHNICAL DISTINCTIONS OF POINT OF CARE MOLECULAR PLATFORMS

PCR assay & reader	• Highest clinical sensitivity and specificity • Lowest analytical detection (LOD) • Multiplexing in a single chamber	• High power need for thermocycling • Higher cost for true POC
Isothermal assay & reader	• Portable, battery-operated • Lower cost and hardware simplicity • More robust to contamination	• Less sensitive (10,000-20,000 copies/mL) • Multiplexing capacity is limited • Sample prep limited beyond COVID
Isothermal single-use platforms (disposable)	• No maintenance • No need for long-term robustness • Battery operation for off-grid locations	• Higher cost per test • Environmental impact
Novel methodolgies	• Graphene/CMOS sensors may boost sensitivity and reduce need for amplification • CRISPR may improve isothermal assay specificity, resolves indeterminates	• Technologies at early stage • Limited clinical data

PCR-BASED TECHNOLOGIES: NEAR POC

Near POC

Hospitals / laboratories (L2)

Currently, the near-POC market in LMIC is dominated by Cepheid. However, more platforms are entering the market creating competitive environment

CEPHEID

SD BIOSENSOR

SANSURE

MOLBIO

PCR-BASED TECHNOLOGIES: TRUE POC

True POC

Primary care facilities (L1)

MagIC Bioscience

Type of detection: **Magnetic biosensors**
Multiplexing: **up to 64**

Early stage development

Co-Diagnostics

Type of detection: **Fluorescence**
Multiplexing: **4**

Late stage prototype

VISBY Medical

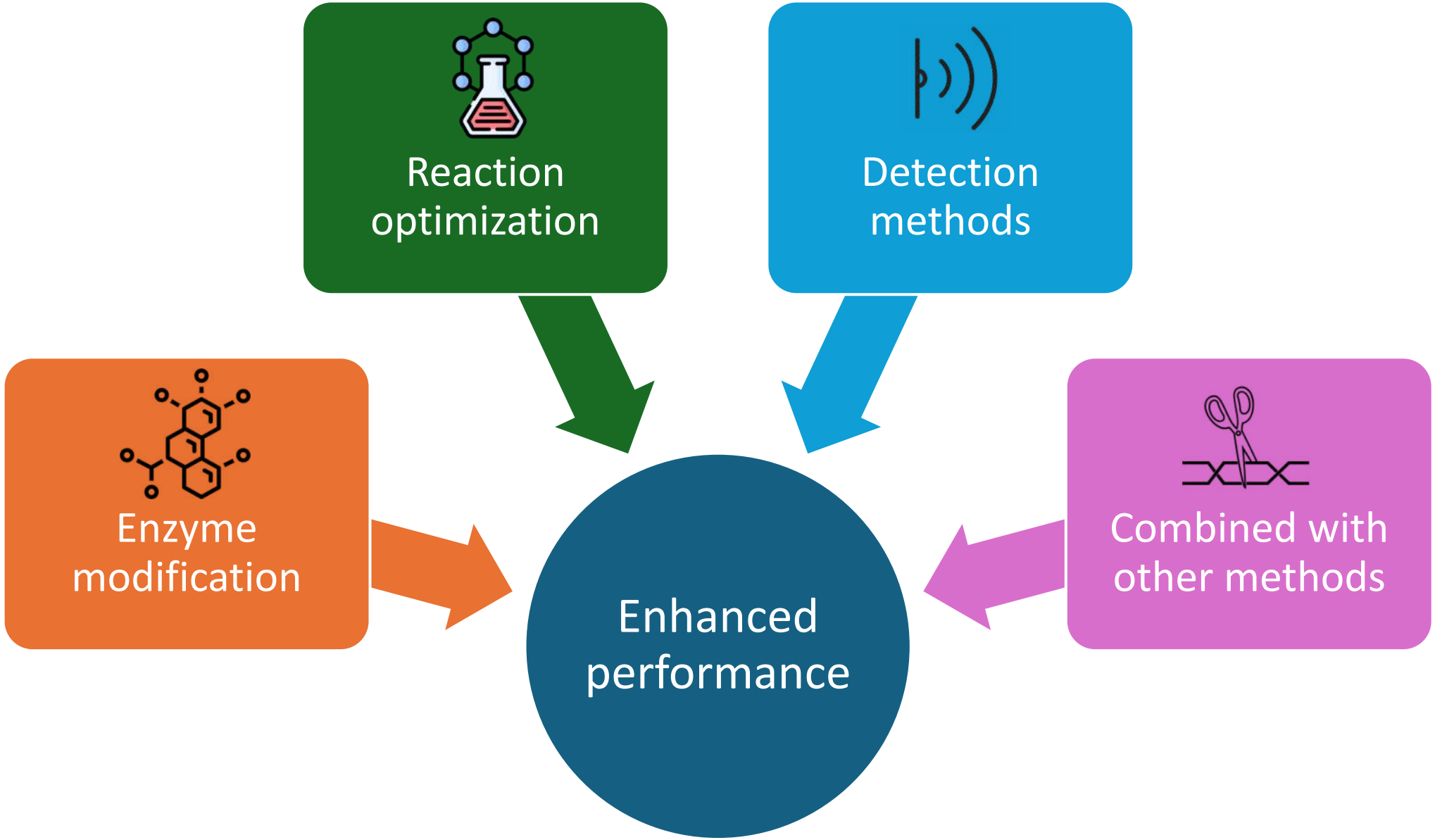
Type of detection: **Colorimetric**
Multiplexing: **4**

Commercially available
High costs

ISOTHERMAL AMPLIFICATION TECHNIQUES

METHOD	Template	Time	T°C	PRIMERS	ENZYMES	YEAR	FEATURES
Loop-mediated amplification (LAMP)	DNA	30-60 min	65°C	6-8	1	2000	Colorimetric detection, specific, primer design is complex, no multiplexing
Strand displacement amplification (SDA)	DNA	1-2h	37°C	4	2	1992	Power saving, sample prep required, non specific amplification, low efficiency for long target sequences
Recombinase polymerase amplification (RPA)	DNA	~30 min	37-42°C	2	2	2006	Power saving, simple primer design, quick, nucleic acid extraction required
Nucleic acid sequence based amplification (NASBA)	RNA	~2h	41°C	2	3	1991	Denaturation step required, only for short fragments (120-250bp)
Helicase-dependent amplification (HDA)	DNA	60-90 min	60-65°C	2	2	2004	Simple primer design, expensive enzymes, complex assay optimization

OPTIMIZATION OF ISOTHERMAL AMPLIFICATION TECHNIQUES



ENZYME MODIFICATION

True POC

Primary care facilities (L1)

Company: **PlusLife**
Type of amplification: **proprietary-RHAM**
Type of detection: **Fluorescence**
Multiplexing: **8**

- Low costs: assay cartridge 4-10 USD
- Good performance
- Extra sample preparation module

Platform commercially available (COVID-19 test)

REACTION OPTIMIZATION

True POC

Primary care facilities (L1)

Cue Health

Company: **USTAR**
Type of amplification: **isothermal-proprietary**
Type of detection: **Rapid**
Multiplexing: **2**

Company: **Detect**
Type of amplification: **LAMP**
Type of detection: **Optical**
Multiplexing: **8**

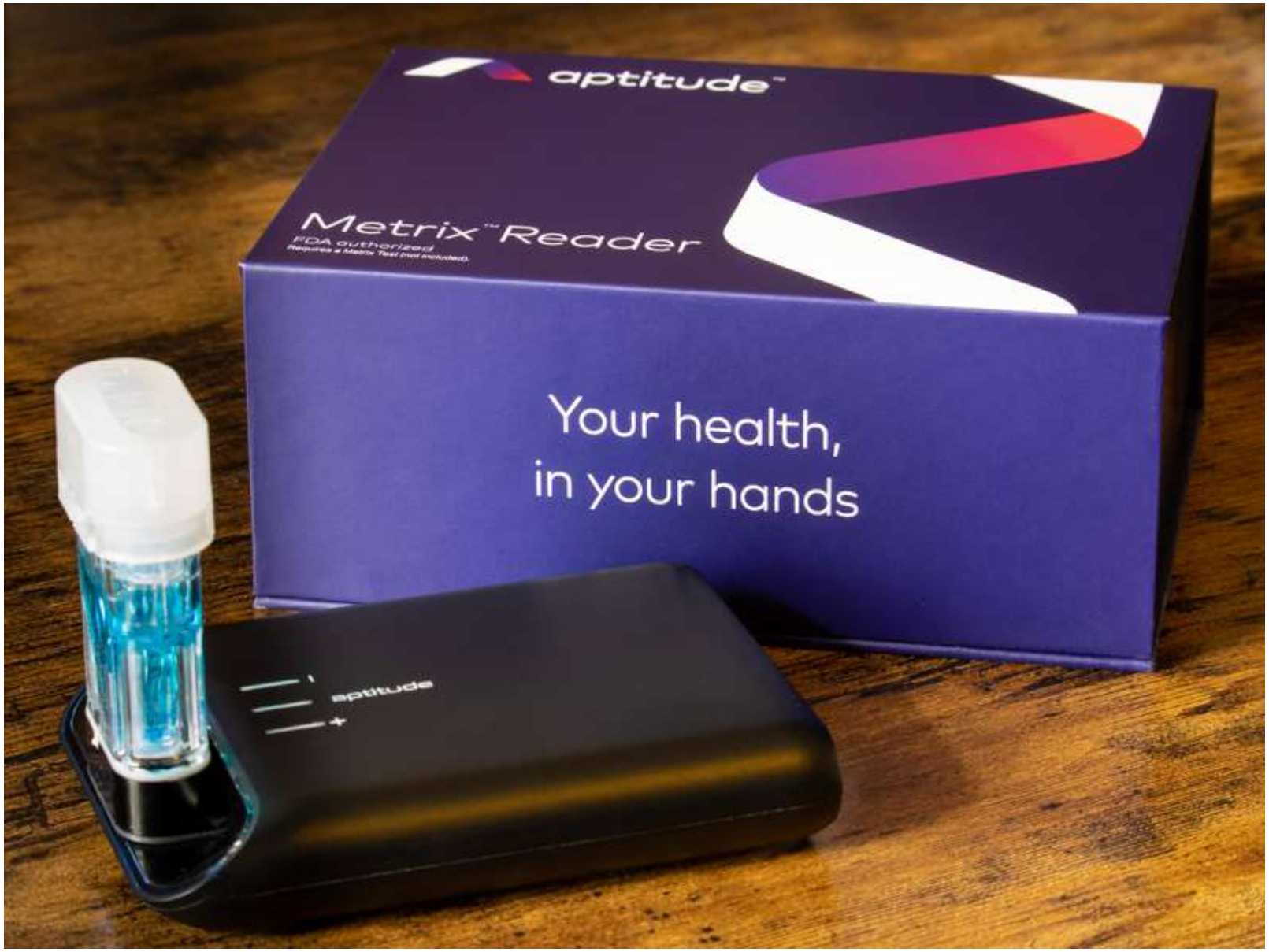
Lucira

REACTION OPTIMIZATION

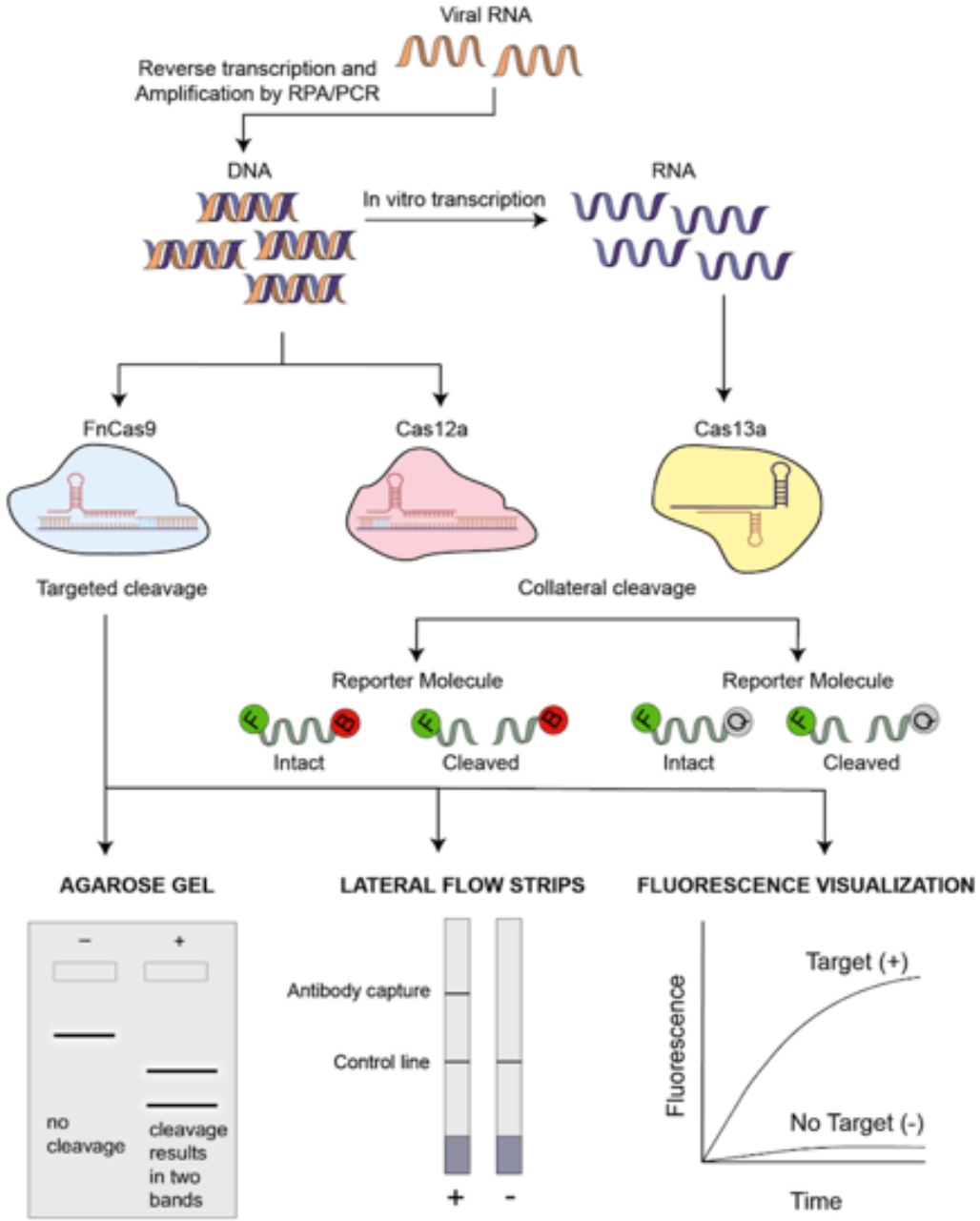
True POC

Primary care facilities (L1)

Company: **Aptitude Medical**
Type of amplification**: LAMP**
Type of detection: **Electrochemical**
Multiplexing**: 4**



APPLICATION OF CRISPR FOR DIAGNIOSTICS



Source: Mohammadi et al., CMBR 2922
https://www.cmbr-journal.com/article_154158.html

CRISPR-ENHANCED DIAGNOSTICS

True POC

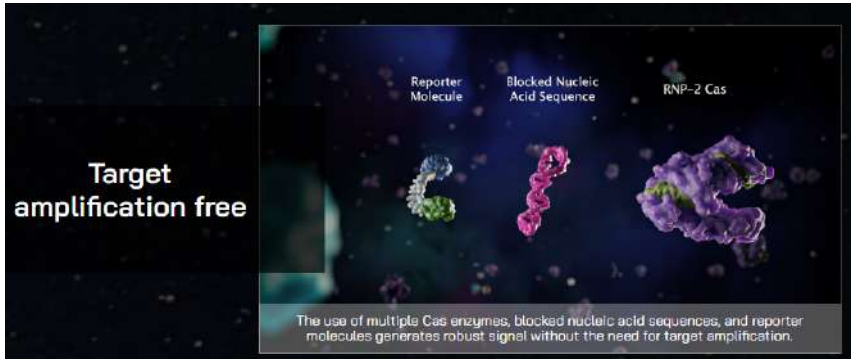
Primary care facilities (L1)

Company: **Sherlock**
Type of amplification: **LAMP plus CRIPR**
Type of detection: **Fluorescence**
Multiplexing: **2-4; more in future gen**

Low cost version in development

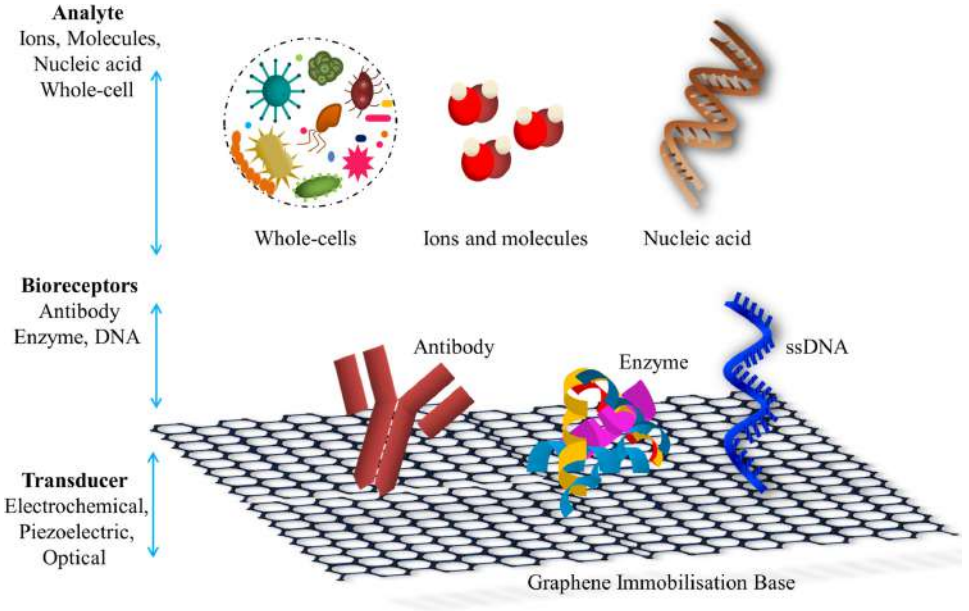
AMPLIFICATION-FREE METHODOLOGIES

CRISPR Cascade™, Vedabio



vedabio.com

Graphene sensors



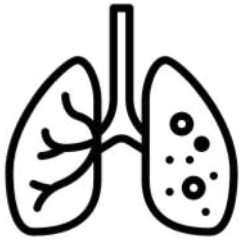
Shahdeo et al., Comprehensive Analytical Chemistry 2020

IdentifySensors

- Digital biosensors intend to rapidly detect multiple infections from saliva and other bodily fluids
- No enzymatic amplification or reagents
- Immediate results

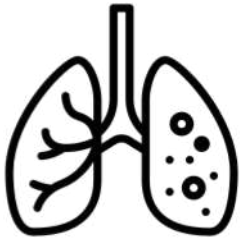
NEW POINT-OF-CARE MOLECULAR DIAGNOSTICS: TYPICAL PIPELINE

COVID/FLU/RSV

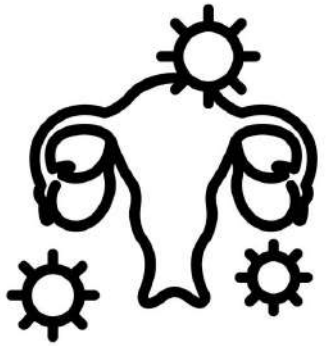


CT/NG/TV

TB
DR TB



HPV



KEY CHALLENGES IN LEVERAGING TRUE POC TECHNOLOGIES FROM COVID-19 TESTINF TO OTHER DISEASE DIAGNOSTICS

Sample compatibility: many platforms utilizes methodologies that are not compatible with complex sample matrices such as whole blood or urine. Separate sample preparation modules might be required

Clinical performance: analytical sensitivity of some true POC system may not be sufficient to meet minimal requirements in clinical performance

Limited menu: additional financing and incentives are required to accelerate menu expansion

Thank You.

Contact info: elena.ivanova@finddx.org

ORGANIZED BY

IN COOPERATION WITH

COORGANIZED BY

ORGANIZED BY

IN COOPERATION WITH

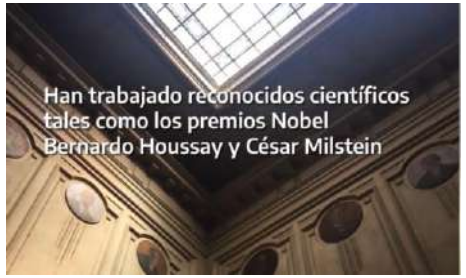
COORGANIZED BY

MEETING ON DIAGNOSTIC EVALUATION AND ECONOMIC IMPACT ANALYSIS OF NEW DIAGNOSTIC METHODS FOR CHAGAS DISEASE

Controles y Estándares

Msc.Marcelo Adrian Rodriguez
FIND-Depto De Parasitologia INEI
ANLIS “Carlos G.Malbran”

- Desarrollo y estandarización de IVD”in house”
- Evaluación Analítica y Diagnóstica
- Validación, Verificación y seguimiento de Desempeño
- Desarrollo e implementación de EQA
- Desarrollo Organización e implementación de Estudios Interlaboratorios
- Producción de Controles para serología y para Métodos Moleculares
- Producción y Calibración de Estándares Secundarios
- Producción de Paneles para Interlaboratorios y EQA
- Estudios de estabilidad



HERRAMIENTAS PARA CONTROL ANALITICO Y DIAGNOSTICO DE LOS MÉTODOS

PANEL DE VERIFICACIÓN

CONTROLES AMPLIFICACIÓN

PANELES PROFICIENCIA

PANEL DE VALIDACIÓN

PANELES DE PERFORMANCE

QUE TIPOS CONTROLES ?

PARA QUE LOS USO?

CONTROL POS ALTO Y DÉBIL

PANEL LOD

PANELES AUTÓCTONOS REGIONALES?

CURVAS DE CALIBRACIÓN

ESTANDARES PRIMARIOS

DIMENSIONES DE ANALISIS

controles y estándares

DIMENSIONES DE ANALISIS

LABORATORIO DE DIAGNOSTICO

LABORATORIO DE I+D

REDES DE LABORATORIO

LABORATORIO DE REFERENCIA NACIONAL

SUPRANACIONAL

LABORATORIOS I+D

LABORATORIOS PRODUCTORES

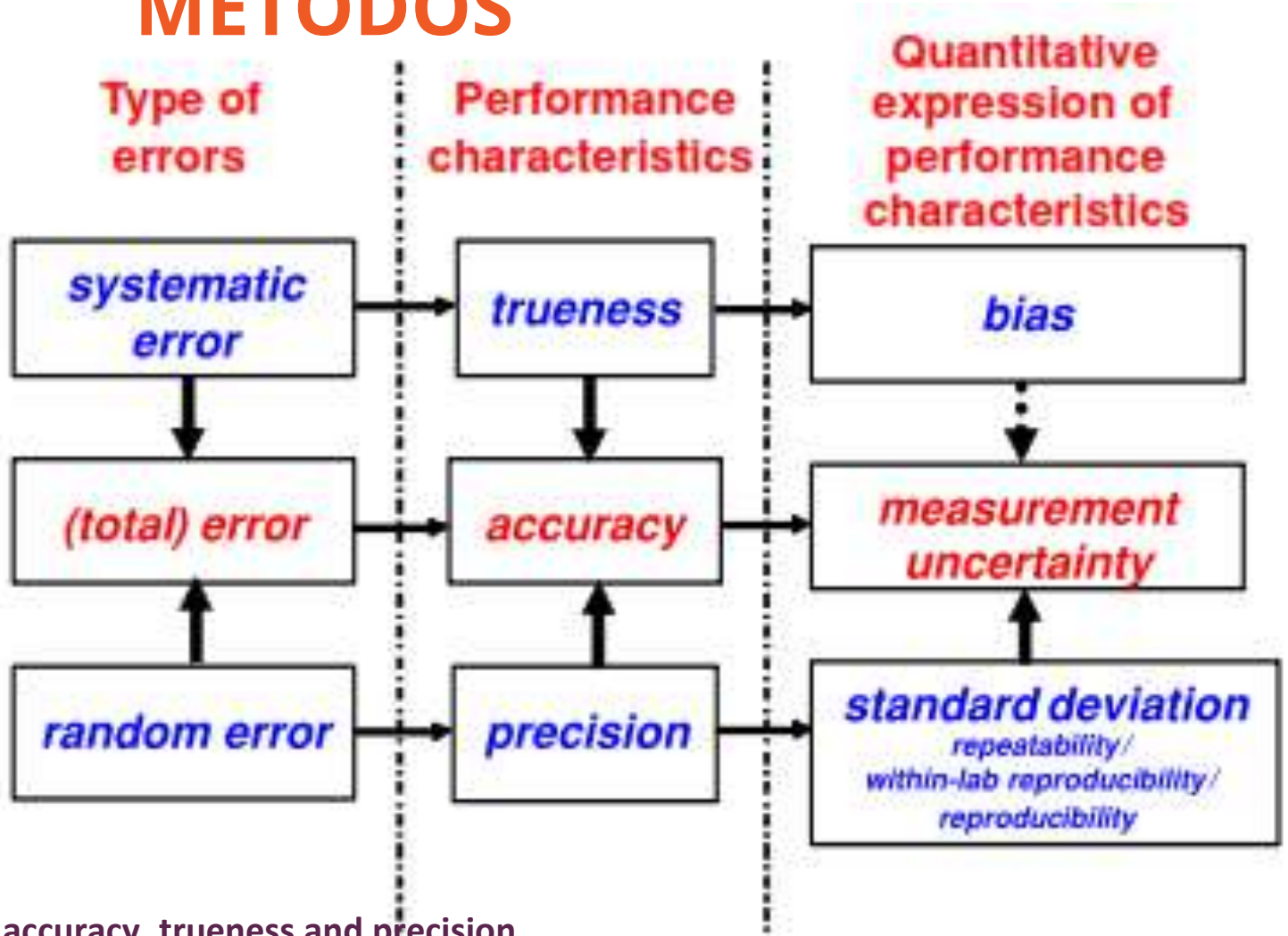
World Health Organization

LABORATORIO OMS NIBSC

NIBSC
Confidence in Biological Medicines

controles y estándares

ERROR ANALITICO DE LOS METODOS



Understanding the meaning of accuracy, trueness and precision
Accreditation and Quality Assurance ·
October 2007 DOI: 10.1007/s00769-006-0191-z

controles y estándares

BIAS

Media/Moda
Mediana
B% / UNIDADES DE CC

μ

rango/unidades del resultado
% / UNIDADES DE CC

PRECISIÓN

CV%/DS
% / UNIDADES DE CC

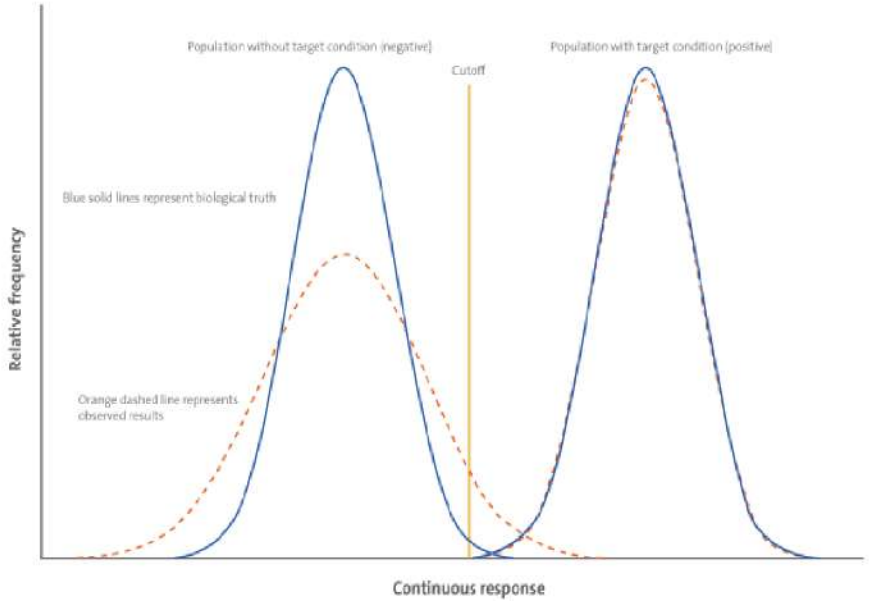
ESTANDARES 1-2RIOS
CURVAS DE CALIBRACIÓN
INTERLABORATORIOS
EQA

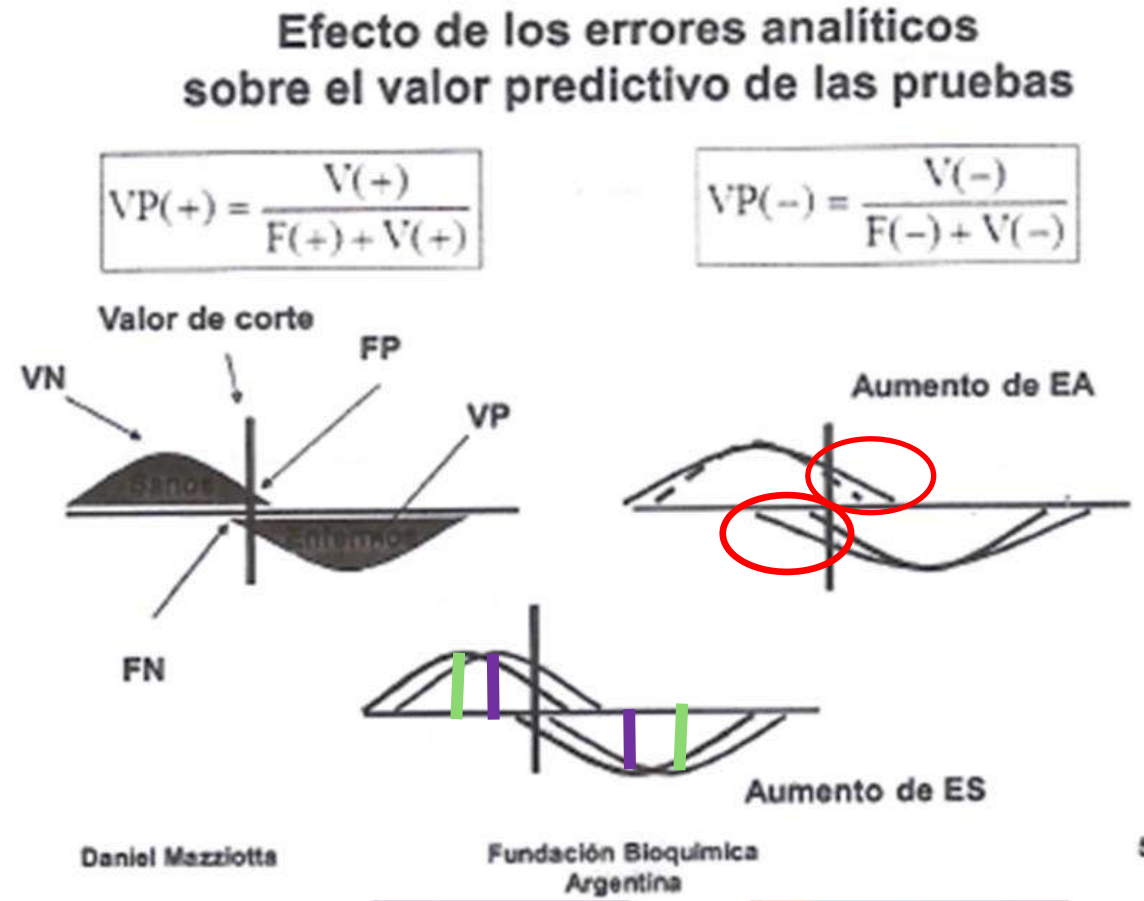
CONTROLES
AMPLIFICACIÓN
CONTROL POS
ALTO
Y DÉBIL

controles y estándares

Evaluación de métodos

controles y estándares





ERROR ANALITICO DE LOS MÉTODOS

Afecta los parámetros diagnósticos

RESULTADOS DE LOS PACIENTES

controles y estándares

DIMENSIONES DE ANALISIS

LABORATORIO DE DIAGNOSTICO

LABORATORIO DE I+D

REDES DE LABORATORIO

LABORATORIO DE REFERENCIA NACIONAL

SUPRANACIONAL

LABORATORIOS I+D

LABORATORIOS PRODUCTORES

World Health Organization

LABORATORIO OMS NIBSC

NIBSC
Confidence in Biological Medicines

DIMENSIONES DE ANALISIS

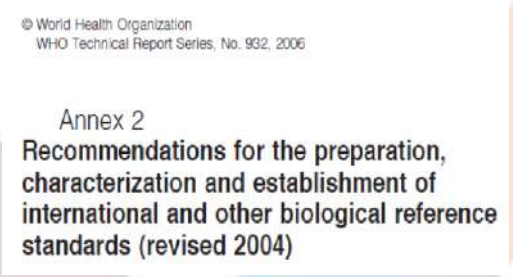
ISO 17034:2016(es)
Requisitos generales para la competencia de los productores de materiales de referencia
MRC

World Health Organization

LABORATORIO OMS NIBSC

NIBSC

Materiales y Métodos
Referencias Internacionales
Establecidos por concensos



DIMENSIONES DE ANALISIS

LABORATORIO OMS NIBSC

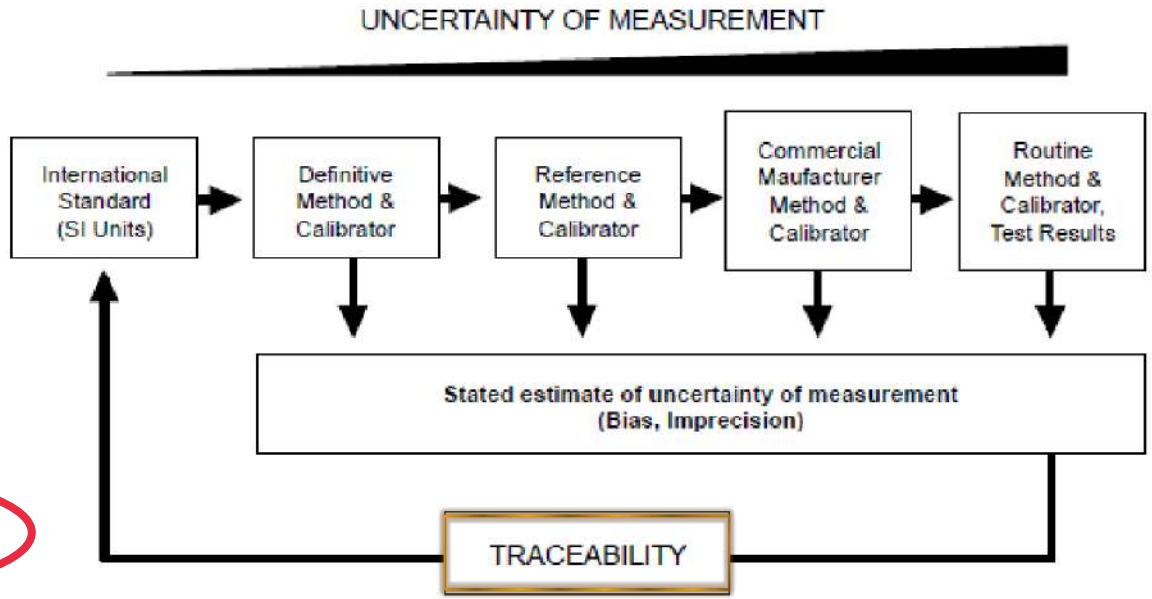
TRAZABILIDAD

Propiedad que permite transferir el valor verdadero un material y/o método de referencia a un procedimiento de rutina, a través de una cadena ininterrumpida de comparaciones que establecen una incertidumbre conocida

Valor Verdadero

INCERTIDUMBRE

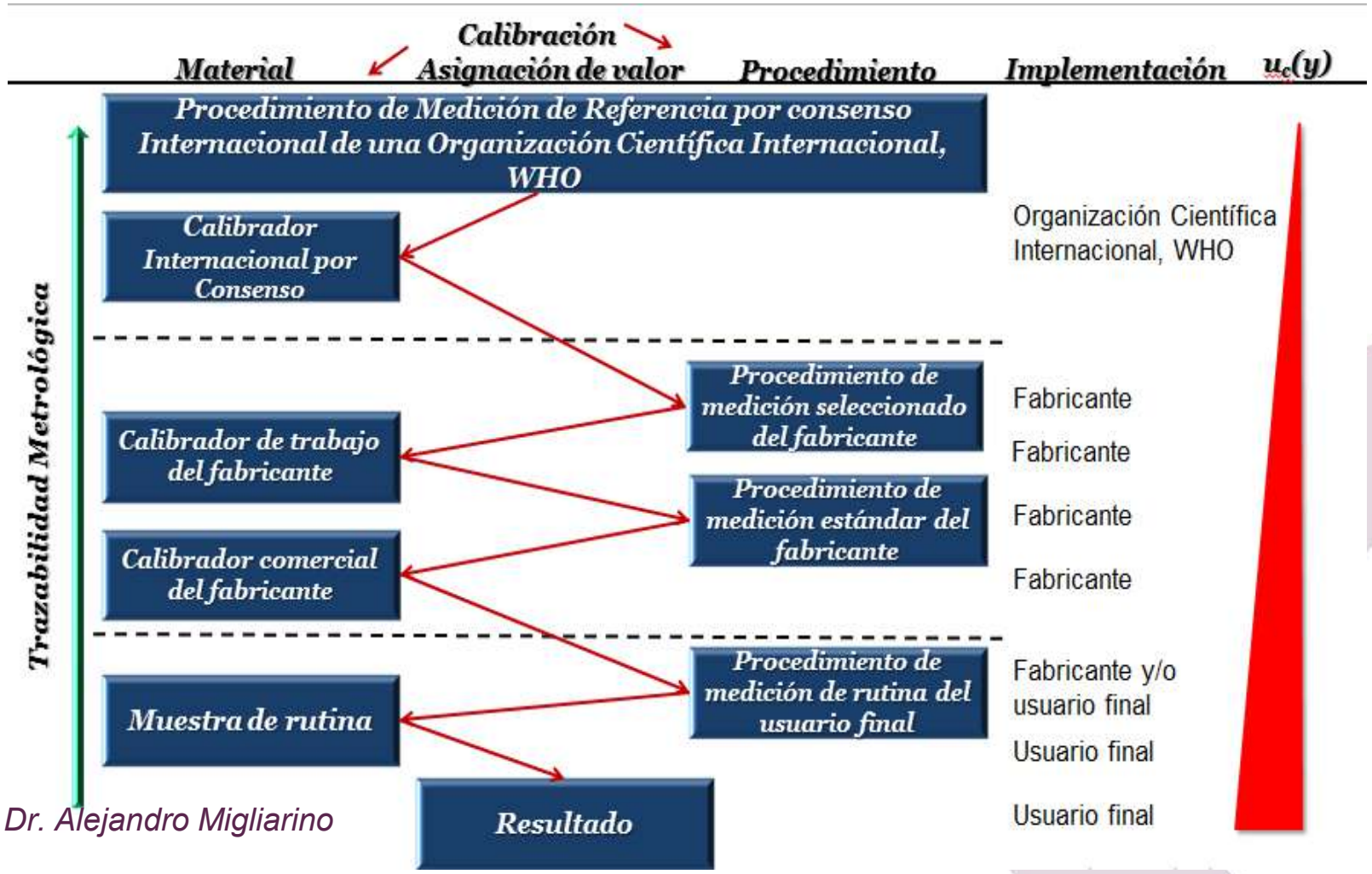
ISO 17511:2020



Valor observado

Clin Biochem Rev Vol 25 Suppl (ii) November 2004 G.H. White, I. Farrance

DIMENSIONES DE ANALISIS



Gentileza Dr. Alejandro Migliarino

World Health Organization

LABORATORIO OMS NIBSC

NIBSC

LABORATORIOS PRODUCTORES

LABORATORIO DE DIAGNOSTICO LABORATORIO DE I+D

DIMENSIONES DE

Si no hay referencia internacional

PRODUCTOR
IVD
ACME

Calibrador
ACME

PRODUCTOR
IVD
THE BEST

Calibrador
THE BEST

ATCC

Quantitative Genomic DNA from *Trypanosoma cruzi*

Organism: *Trypanosoma cruzi* Chagas
Derived from: *Trypanosoma cruzi* Tulahuen (ATCC 30266)

Genomic DNA from *Trypanosoma cruzi* strain SYLVIO-X10

Organism: *Trypanosoma cruzi* Chagas
Derived from: *Trypanosoma cruzi* SYLVIO-X10 (ATCC 50823)

Quantitative Synthetic *Trypanosoma cruzi* strain Y DNA

Organism: *Trypanosoma cruzi* Chagas
Genetic target: Preparation includes fragments from 18S rRNA, Kinetoplast minicircle, and Lathosterol oxidase (TcSC5D) regions, and a full-length satellite sequence.
Specification range: ≥ 1×10^5 to 1×10^6 copies/µL

CONMUTABILIDAD
ESTABILIDAD
HOMOGENEIDAD
INCERTIDUMBRE

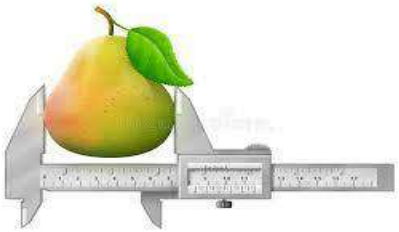
DIMENSIONES DE

Si no hay referencia internacional

PRODUCTOR
IVD
ACME

Calibrador ACME

50 paras
Equiv/ml



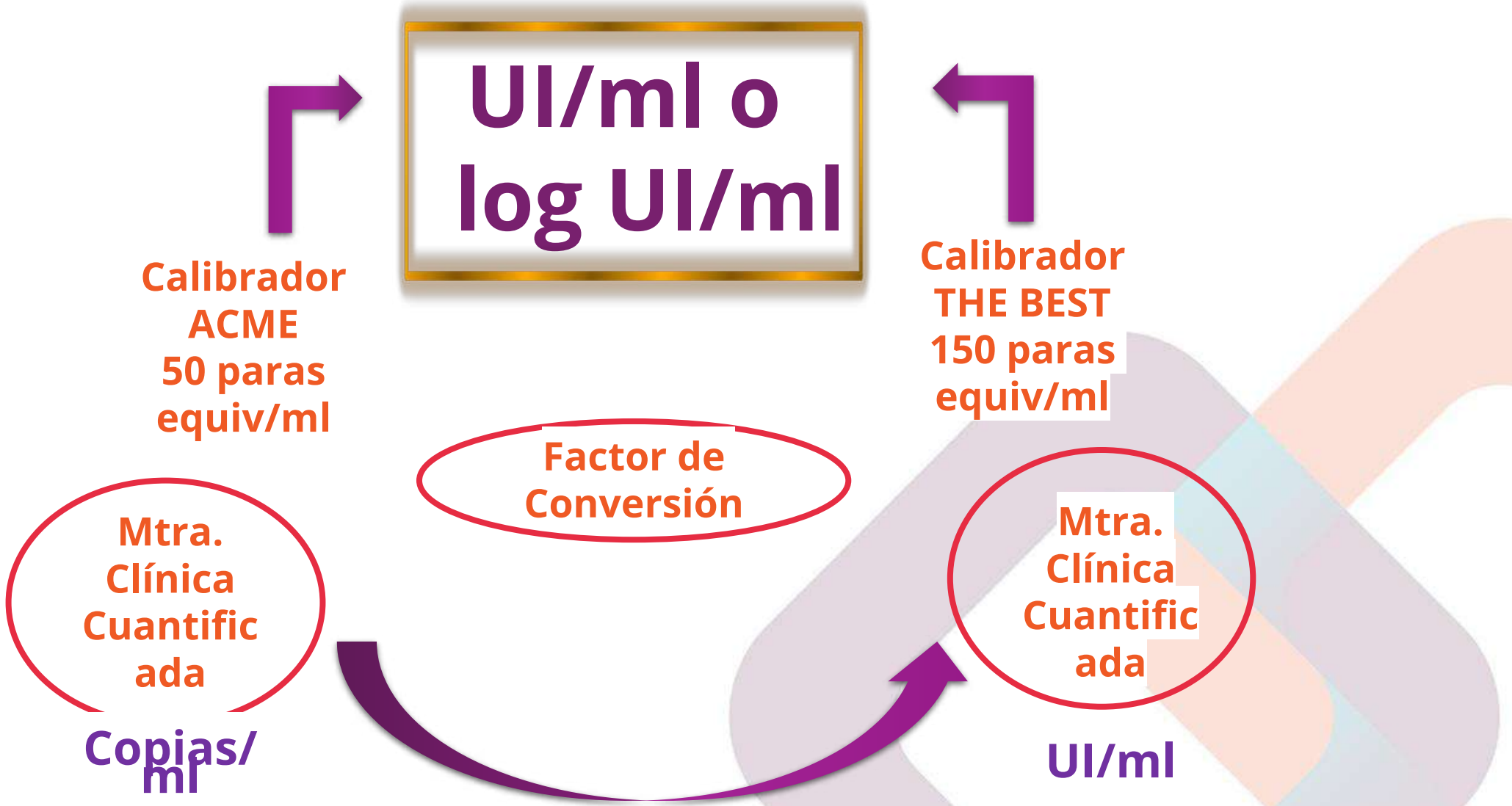
Los resultados no son comparables

PRODUCTOR
IVD
THE BEST

Calibrador
THE BEST

150 paras
Equiv/ml

DIMENSIONES DE
Si hay referencia internacional



DIMENSIONES DE

Si hay referencia internacional

PRODUCTOR IVD ACME

Calibrador ACME ~ 95 UI/ml

UI/ml o log UI/ml

PRODUCTOR IVD THE BEST

Calibrador THE BEST ~95 UI/ml

Los resultados son comparables

DIMENSIONES DE ANALISIS

NIBSC
A centre of the Health Protection Agency

Health Protection Agency

WHO International Standard
1st WHO International Standard for Human Papillomavirus (HPV) Type 16 DNA
NIBSC code: 06/202
Instructions for use
(Version 2.0, Dated 10/11/2010)

The 1st International Standard for HPV-16 DNA Nucleic Acid Amplification Techniques has been assigned a unitage of 5 x 10^6 International Units (IU) per ampoule.

NIBSC
Confidence in Biological Medicines

Medicines & Healthcare products Regulatory Agency

WHO International Standard
1st WHO International Standard for HHV-6B virus DNA
NIBSC code: 15/266
Instructions for use
(Version 3.0, Dated 23/11/2017)

This material has been assigned a concentration of 7.75 log10 International Units (IU) per vial when reconstituted in 1 mL of nuclease-

+/-0.24%.

NIBSC
Confidence in Biological Medicines

World Health Organization

Standardisation of Genome Amplification Techniques (SoGAT)

Trypanosoma cruzi

Responsible scientist – David Padley

Materials are currently being sourced for this study, if you have material that you would like to include please contact David directly at david.padley@nibsc.org

WHO reference material	WHO Collaborating Centre	Status
Aspergillus (1st WHO International Standard)	NIBSC	Seeking offers of candidate materials
Babesia (1st WHO International Standard)	CBER	-
Enterovirus (1st WHO International Standard)	NIBSC	Seeking offers of candidate materials
HIV, cell-associated (1st WHO International Standard)	NIBSC	Seeking offers of candidate materials
HIV-1 CRF (2nd WHO International Reference Panel)	CBER/NIBSC	Preparation of candidates
HSV-1/2 DNA (1st WHO International Standard)	NIBSC	Collaborative study completed. Further commutability assessment underway
Influenza A RNA (1st WHO International Standard)	NIBSC	Pilot study scheduled
Influenza B RNA (1st WHO International Standard)	NIBSC	Pilot study scheduled
Leishmania (1st WHO International Standard)	NIBSC	Seeking offers of candidate materials
Mycobacterium tuberculosis (1st WHO International Standard/panel)	NIBSC	Seeking participants for collaborative study
Plasmodium vivax (1st WHO International Standard)	NIBSC	Seeking offers of candidate materials
RSV RNA (1st WHO International Standard)	NIBSC	Pilot study scheduled
Trypanosoma cruzi (1st WHO International Standard)	NIBSC	Seeking offers of candidate materials
VZV DNA (1st WHO International Standard)	NIBSC	Candidates scheduled
WNV RNA (1st WHO International Standard)	NIBSC	Candidates being assessed in collaborative study

controles y

estandares

DIMENSIONES DE ANALISIS

SCIENCE
TECHNOLOGY
ENGINEERING
MATHEMATICS

LABORATORIO DE REFERENCIA NACIONAL REGIONAL

SUPRANACIONAL

LABORATORIOS I+D

OBJETIVOS

- Colaborar con la calidad de los métodos de los Laboratorios nacionales
- Evaluación de métodos del Mercado
- Asesoramientos a empresas de I+D locales

controles y estandares

SCIENCE TECHNOLOGY ENGINEERING MATHEMATICS

LABORATORIO DE REFERENCIA NACIONAL

SUPRANACIONAL

LABORATORIOS I+D

Quantitative Synthetic *Trypanosoma cruzi* strain Y DNA

Organism: *Trypanosoma cruzi* Chagas

Genetic target: Preparation includes fragments from 18S rRNA, Kinetoplast minicircle, and Lathosterol oxidase (TcSC5D) regions, and a full-length satellite sequence

Specification range: ≥ 1 x 10^5 to 1 x 10^6 copies/µL

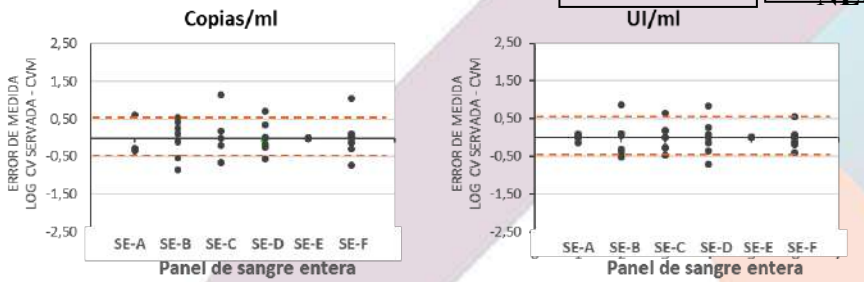
PANELES ARMONIZACION

Armonización Estudios Multicéntricos

HACIA LA ARMONIZACIÓN DE LAS PRUEBAS DE DETECCIÓN Y CUANTIFICACIÓN DEL VIRUS EPSTEIN-BARR: IMPACTO >30 centros DEL 1er. ESTÁNDAR INTERNACIONAL, A NIVEL NACIONAL E INTERNACIONAL

María Dolores Fellner
Servicio Virus Oncogénicos
Laboratorio Nacional y Regional (OPS/OMS)
HPV/EBV

2015	2017	2018	2019	2022	2023
3 Muestras 10^7 10^5 NEG	4 Muestras 10^7 10^5 10^3 NEG	5 Muestras 10^7 10^5 10^3 10^3 NEG	6 muestras 10^5 10^4 10^3 10^3 $5x10^2$ NEG	7 muestras 10^6 10^6 10^5 10^4 10^4 $5x10^3$ NEG	~~7 muestras~~ (en proceso)



Gentileza Dra. Dolores Felner

- **Variabilidad inter-labo**
- **Variabilidad intra-labo**

controles y

estandares

SCIENCE
TECHNOLOGY
ENGINEERING
MATHEMATICS

LABORATORIO DE REFERENCIA NACIONAL

SUPRANACIONAL

LABORATORIOS I+D

Evaluación de métodos de mercado

SEROLOGÍA

- ENTRE 5-10 MIEMBROS
- MUESTRAS CON RPX2/X3 CUT-OFF

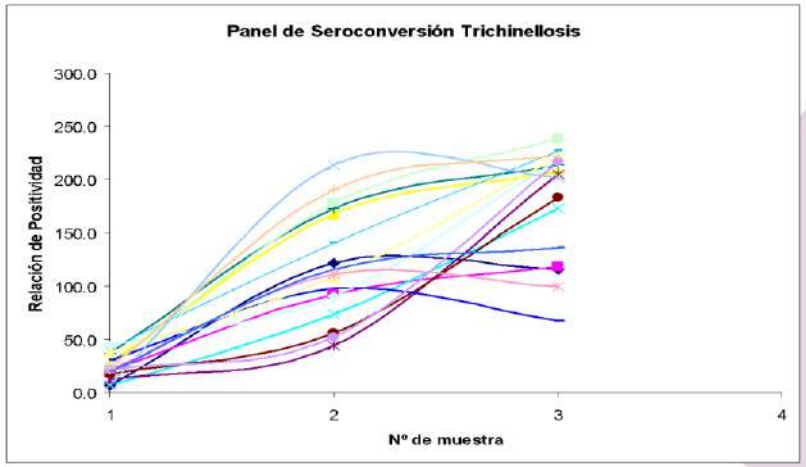
Q PCR CUALITATIVO

- CONTROL DE AMPLIFICACIÓN BAJO LOD X2/ LOD X3
- CONTROL DE VERACIDAD (CUANTIFICADO), CERCANO AL PUNTO DE CORTE
- MATERIALES DE EQA
- CONTROL NEGATIVO

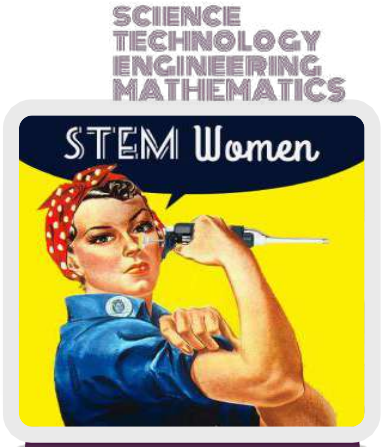
QPCR

- ENTRE 5-10 MIEMBROS
- LOD X2/ LOD X3
- ULOQ
- LLOQ
- MATERIALES DE EQA CUANTIFICADOS

PANELES SEROCONVERSION



controles y estándares



LABORATORIO DE REFERENCIA NACIONAL

SUPRANACIONAL LABORATORIOS I+D

PANELES DE VERIFICACIÓN PERFORMANCE

PANELES AUTÓCTONOS REGIONALES

OBJETIVO

Verificar la Performance Diagnóstica de kits del Mercado

CLSI EP12-ED3IG:2023 Verification of Performance of a Qualitative, Binary Output Examination Implementation Guide

Muestras	Réplicas	Carreras	Días	control de calidad	Revisión de resultados
Mínimo 10 positivos, 10 negativos	1 por muestra	1	1	Mínimo 2 niveles con cada carrera.	Sí

Verificar la Precision
Verificar la Estabilidad

Validar la Performance Diagnóstica de IVD “in house”

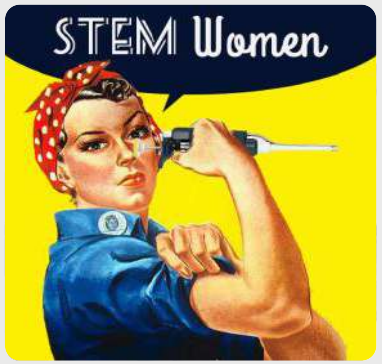
CLSI EP12 ED3:2023 Evaluation of Qualitative, Binary Output Examination Performance, 3rd Edition

controles y

estandares

DIMENSIONES DE ANALISIS

SCIENCE
TECHNOLOGY
ENGINEERING
MATHEMATICS



LABORATORIO DE REFERENCIA NACIONAL

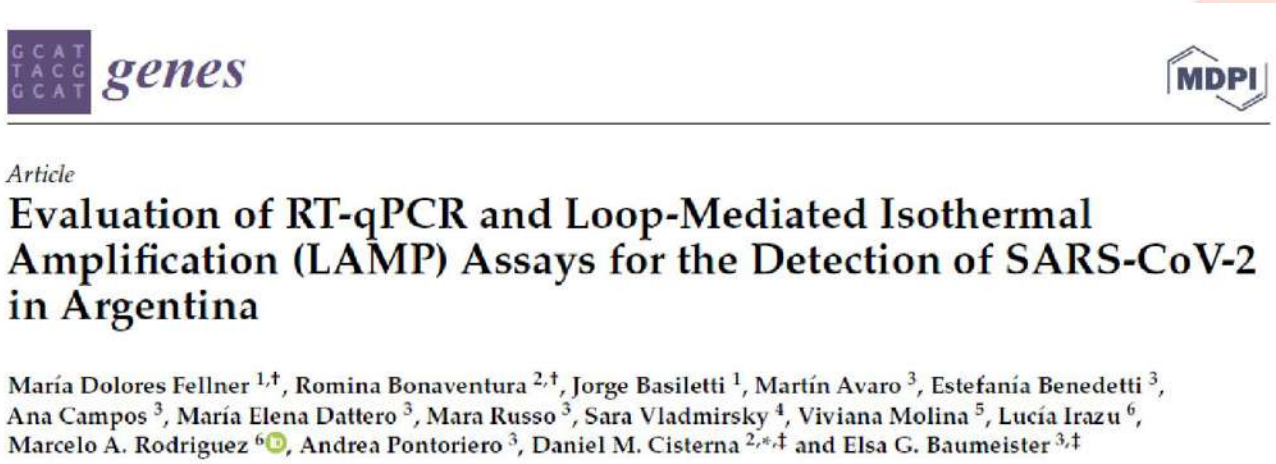
SUPRANACIONAL

LABORATORIOS I+D

Calibración o Producción De Estándares Secundarios

PAHO-WHO In-ternational Standard (SARS-like Wuhan ivRNA E, RdRp and N Genes; 1 × 108 copies/µL)

"SARS-CoV-2 Secondary Standard, RNA 002/20 batch, E, RdRp and N genes" (SARS-CoV-2 SStd)



genes

MDPI

Article

Evaluation of RT-qPCR and Loop-Mediated Isothermal Amplification (LAMP) Assays for the Detection of SARS-CoV-2 in Argentina

María Dolores Fellner [1,†], Romina Bonaventura [2,†], Jorge Basiletti [1], Martín Avaro [3], Estefanía Benedetti [3], Ana Campos [3], María Elena Dattero [3], Mara Russo [3], Sara Vladmirsky [4], Viviana Molina [5], Lucía Irazu [6], Marcelo A. Rodriguez [6], Andrea Pontoriero [3], Daniel M. Cisterna [2,*,‡] and Elsa G. Baumeister [3,‡]

Evaluación métodos SARS-CoV-2

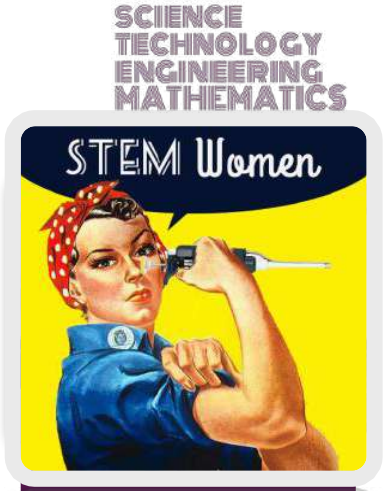
9 RT-qPCR

3 RT-Lamp

controles y

estandares

DIMENSIONES DE ANALISIS



LABORATORIO DE REFERENCIA NACIONAL

REGIONAL

SUPRANACIONAL LABORATORIOS I+D

Acreditacion ISO 15189:2022 Laboratorios clínicos. Requisitos para la calidad y la competencia.

Acreditacion ISO/IEC 17025:2017(es) Requisitos generales para la competencia de los laboratorios de ensayo y calibración

Acreditacion ISO/IEC 17043:2023(en) Conformity assessment — General requirements for the competence of proficiency testing providers

DIMENSIONES DE ANALISIS

LABORATORIO DE DIAGNOSTICO

LABORATORIO DE I+D

REDES DE LABORATORIO

OBJETIVOS

- **Desarrollar nuevos métodos, validarlos**
- **Verificar la Performance Analítica IVD**
- **Realizar el seguimiento de**
- **Desempeño del Diagnóstico Diagnóstica IVD**
  - Control de la Precision
  - Control de la Veracidad EQA
  - Verificar la performance por cambio de Lote de insumo critico

DIMENSIONES DE ANALISIS

Materia les

Controles amplificación /preisión

Para desarrollo y Validación

LABORATORIO DE DIAGNOSTICO

LABORATORIO DE I+D

REDES DE LABORATORIO

vircell MICROBIOLOGISTS

BIO-RAD

ACROMETRIX by life technologies

seracare

LGC CLINICAL DIAGNOSTICS

ATCC

ZeptoMetrix an antylia scientific company

DIMENSIONES DE ANALISIS

ESTIMACION DEL LOD

LABORATORIO DE DIAGNOSTICO

LABORATORIO DE I+D

REDES DE LABORATORIO

Tipo de Muestras

- Muestras de pacientes confirmadas
- Muestras suplementadas con el Mi no
- Muestras suplementadas con ADN target
- Líneas celulares
- Muestras con mas de 1 genotipo
- Muestras de CQE
- Otros materiales de referencia

- Como diluyente uso matriz Negativa para ese target

DIMENSIONES DE ANALISIS

LABORATORIO DE DIAGNOSTICO

LABORATORIO DE I+D

REDES DE LABORATORIO

Performance Characteristics of the COBAS AMPLICOR Hepatitis C Virus MONITOR Test, Version 2.0

Maria Erali, MS,[1] Edward R. Ashwood, MD,[1,2] and David R. Hillyard, MD[1,2]

Am J Clin Pathol 2000;114:180-187

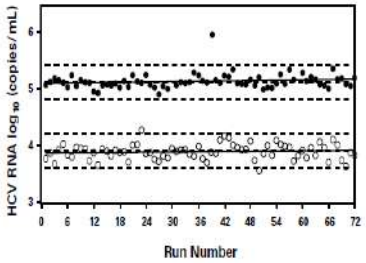
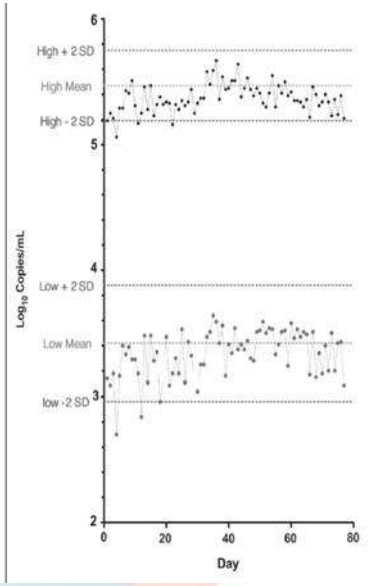


Figure 3 A Levey-Jennings plot of the high control (closed circle) and low control (open circle) for the COBAS AMPLICOR HCV MONITOR Test, version 2.0 (Roche Diagnostics, Indianapolis, IN). For the high control, n = 70 measured over 27 days by 3 operators on 3 instruments. For the low control, n = 72 measured over 27 days by 3 operators on 3 instruments. The mean $\log_{10}$ (copies per milliliter) for the high and low controls is 5.1 and 3.9, respectively. The dashed error bars are 2 SD units.

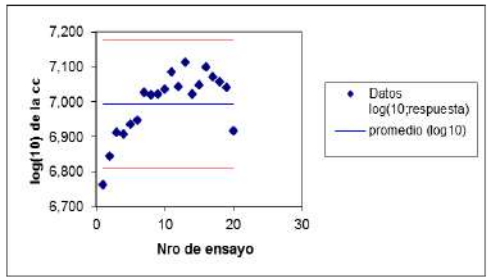
Vol. 33, No. 23 www.cmnewsletter.com December 1, 2011

Developing a Quality System for Quantitative Laboratory-Developed Tests

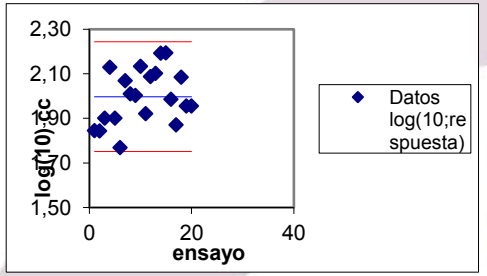
Thomas E. Grys, Ph.D., DABMM,[1,2] [1]Director of Microbiology, Department of Laboratory Medicine and Pathology, Mayo Clinic in Arizona, Phoenix, AZ, [2]Division of Clinical Microbiology, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota



Limite Superior Cuantificación ULOQ



Limite Inferior Cuantificación LLOQ



Controlo la precisión con la curva de calibración

DIMENSIONES DE ANALISIS

LABORATORIO DE DIAGNOSTICO

LABORATORIO DE I+D

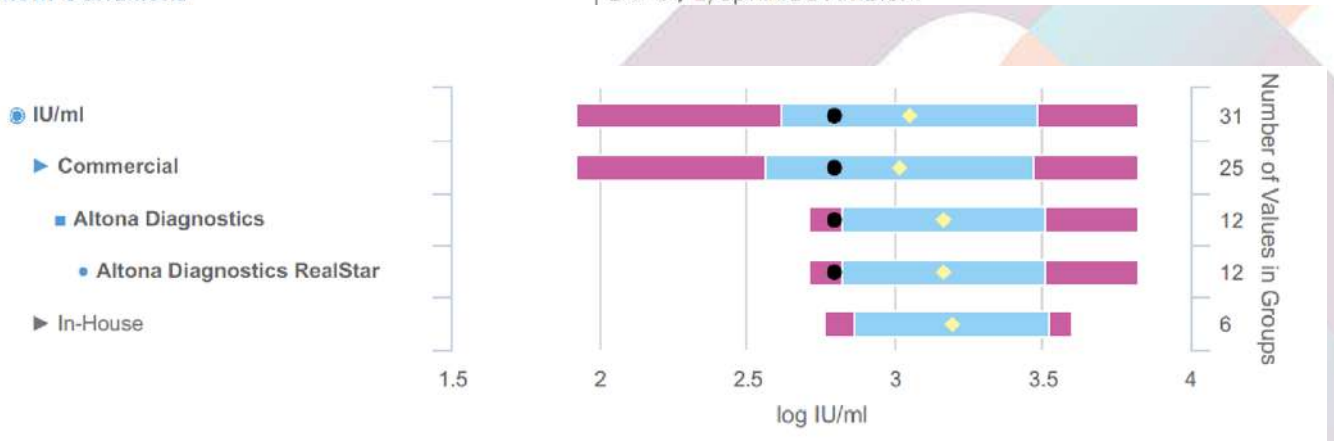
REDES DE LABORATORIO

Programas EQA

QCMD
Quality Control for Molecular Diagnostics

Feature	Available format(s)
Catalogue Number	QAP214217_1
Total Number of Challenges	1
Number of Panel Members	10
Distribution / Testing Period	Q4

Specifications	
Sample NA Target Source	Cultured and/or Clinical material
Matrix Panel Format	Whole Blood
Panel Member Target Range	Covering clinical range
Panel Member Sample Volume	Lyophilised
Panel Sample Pre-treatment Requirement	Reconstitution of lyophilised material
Panel Analysis type	Qualitative. Quantitative for information purposes only
Panel Testing	Evaluated by various molecular methodologies
Storage / Shipment Conditions	2-8°C / Lyophilised Ambient



DIMENSIONES DE ANALISIS

Conmutabilidad

El AND target en una matriz de una muestra clínica
Da la misma rta que la de una muestra suplementada ?

American Journal of Transplantation 2009; 9: 269–279
Wiley Periodicals Inc.

doi: 10.1111/j.1600-6143.2008.02514.x

Interlaboratory Comparison of Epstein-Barr Virus Viral Load Assays

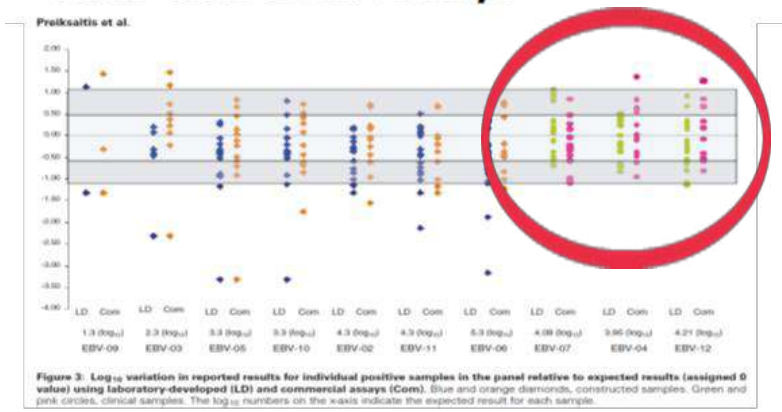
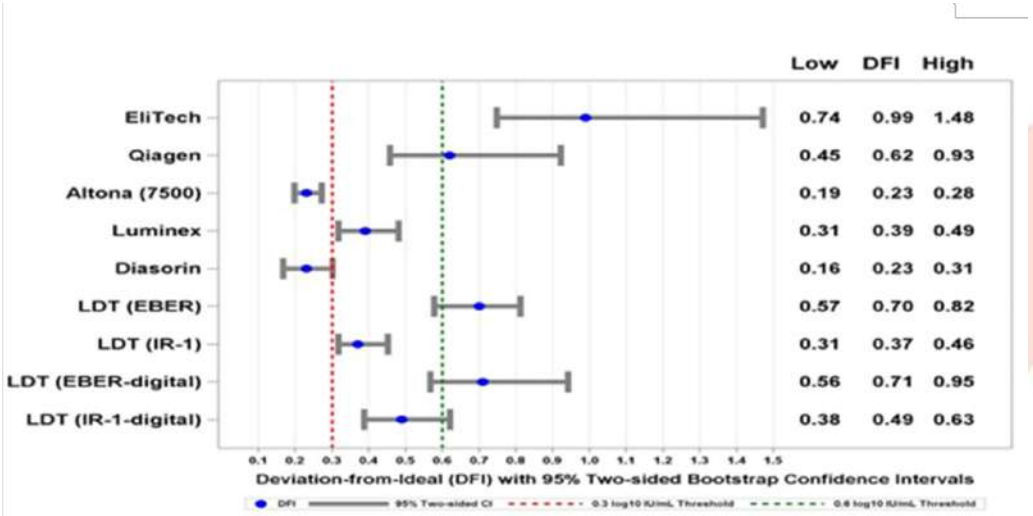


Figure 3: Log_{10} variation in reported results for individual positive samples in the panel relative to expected results (assigned 0 value) using laboratory-developed (LD) and commercial assays (Com). Blue and orange diamonds, constructed samples. Green and pink circles, clinical samples. The log_{10} numbers on the x-axis indicate the expected result for each sample.



Hayden y col. ; J Clin Microb, 2020

La falta de conmutabilidad afecta el BIAS Y LA PRECISION

CLSI EP39-ED1:2021 A Hierarchical Approach to Selecting Surrogate Samples for the Evaluation of *In Vitro* Medical Laboratory Tests, 1st Edition

Estabilidad

CONTROLES INTERNOS INMUNOENSAYOS

SeraCare LIFE SCIENCES

Long-Term Stability of Viral Markers in Plasma

PE Garrett, L Miller, B Anekella, MM Manak
SeraCare Life Sciences, Milford, MA

24th Clinical Virology Symposium
April 27 - 30, 2008
Daytona Beach, FL

Seroconversion panels for HIV, HBV and HCV that had been collected as early as 1981 and as recently as 1996 (18 panels in total) were evaluated by comparing the earliest test results available to test results generated on the same plasma in 2007. Antibodies to HIV and HCV, HBsAg, HIV and HCV RNA and HBV DNA were tested.

Results demonstrate that antibodies to HIV and HCV, and HBsAg, show no deterioration over more than 20 years even when stored in less than ideal conditions. These results, not unexpected, allowed us to propose 25 year dating for these plasma products.

‘minimally processed plasma’ (MPP)

Unidades de Plasma

⇩

Filtrado por 0.22 µm

⇩

Congelan y descongelan 5 veces

Uso de Biocidas

ProClin Y Kathon

DIMENSIONES DE ANALISIS

Falsos Negativos?
Falsos Positivos?
Sesgos de selección?
Muestreo?
Sensibilidad
IC95%??

Revisiones Sistematicas y Metaanalisis

Construcción de serotecas

ProClin, Kathon, Bronidox

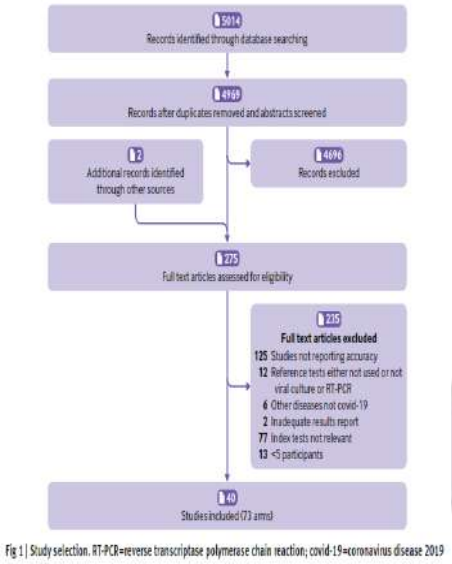


Fig 1 | Study selection. RT-PCR=reverse transcriptase polymerase chain reaction; covid-19=coronavirus disease 2019

Thank You.

Contact info: marcerodriguez2002@gmail.com
marcerodriguez@anlis.gob.ar

ORGANIZED BY IN COOPERATION WITH COORGANIZED BY

ORGANIZED BY

IN COOPERATION WITH

COORGANIZED BY

Key insights on the input received from the invited experts in written about Molecular Methods

MEETING ON DIAGNOSTIC EVALUATION AND ECONOMIC IMPACT ANALYSIS OF NEW DIAGNOSTIC METHODS FOR CHAGAS DISEASE

6-7 MAY 2024 BUENOS AIRES, ARGENTINA

Key insights on the input received from the invited experts in written about Molecular Methods

STOP

LAMP 0 questions

PCR 0 questions

Disagree > 50%

THINK

LAMP 8 questions

PCR 9 questions

Disagree >15%

ACT

LAMP 25 questions

PCR 12 questions

Disagree <15%

Key insights on the written input received from the invited experts shared about molecular methods LAMP & PCR

- Intended use
- Target Operator
- Target Use Setting
- Target Analyte
- Reference Method
- Analytical Specificity
- Strain Specificity
- Quantitation
- Training Needs
- Specimen Type
- Processing steps /Transfer Volumen
- Time- sample results
- Data Analysis
- Internal Quality Control
- External Quality Control
- **Power requirements /Connectivity / Result Capture**
- **Operating Conditions**
- **Diagnostic Sensitivity LAMP**
- **Manufacture Scale LAMP**

- Diagnostic Specificity
- Analytical Sensitivity
- Time stability of reagents
- Quality Assurance
- **Specimen capacity of LAMP**
- **Instrument integration LAMP**
- **Diagnostic Sensitivity of PCR**
- **Instrument Price PCR**

FIND Diagnóstico para todos

DNDi La mejor ciencia para los más desatendidos

ORGANIZED BY

Pan American Health Organization

IN COOPERATION WITH

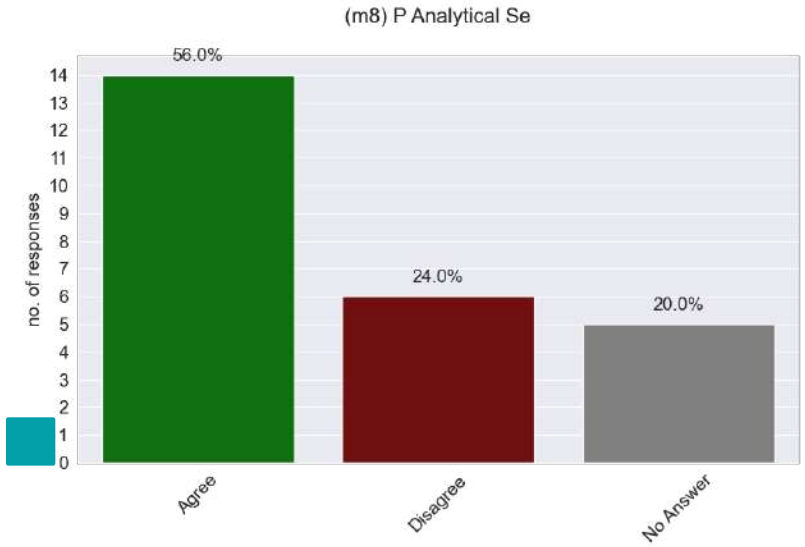
INGEBI CONICET

COORGANIZED BY

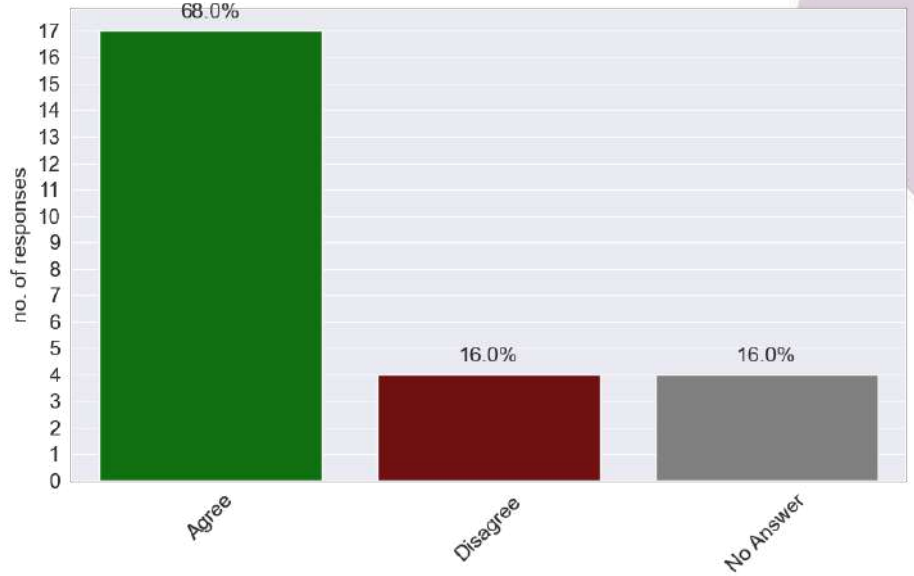
Comments in cases of > 15% disagreement

ANALYTICAL SENSITIVITY of MOLECULAR METHODS

PCR



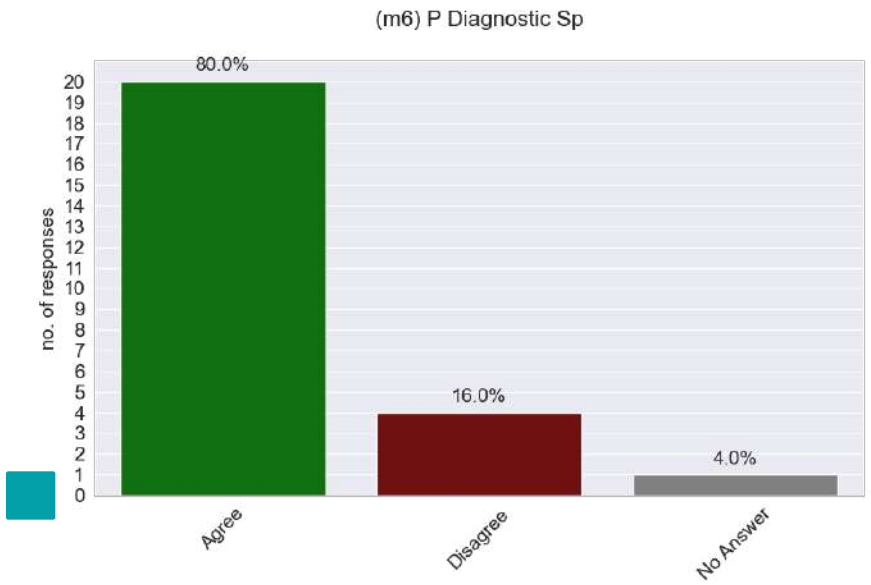
LAMP



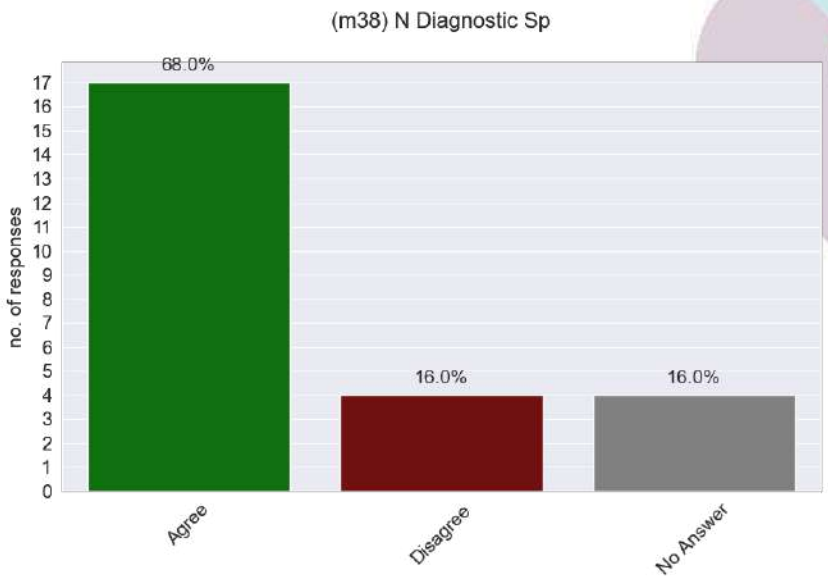
- Ideal: It should be standardized to a number of copies of the target gene so that results between different laboratories can be compared.
- Also the LoD and its estimate are associated with an error reflected in the WIDTH OF THE 95% CONFIDENCE INTERVAL Whatever the value of the LoD, the error of its estimate should not be greater than 1 log
- The units expressed under the ideal condition should be equivalent to parasites per mL (Eq.Par/mL)

DIAGNOSTIC SPECIFICITY SHARED BY LAMP and PCR

(m6) LAMP



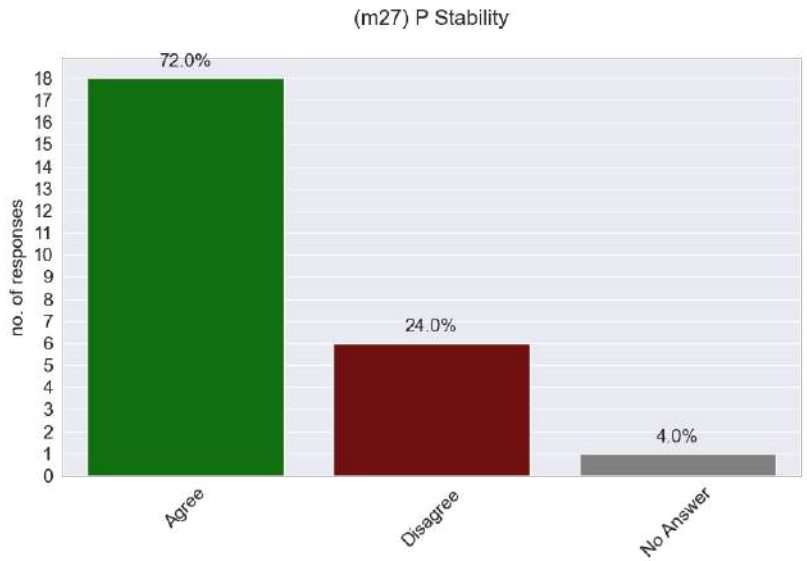
(m38) PCR



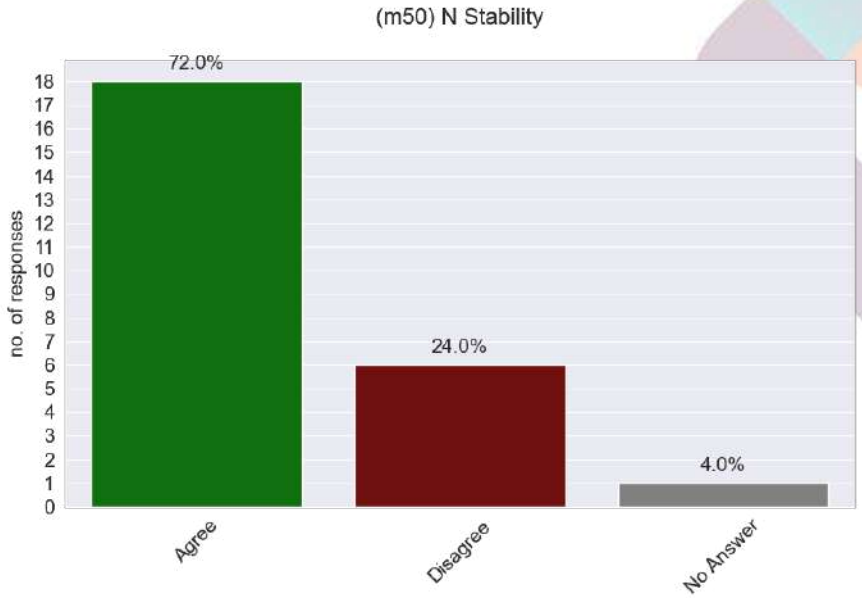
- The reference standard should be the one proposed **by OPS 2018**.
- *Diagnostic Sp compared to what Elisa? microscopic method and operator?*
- ***The ideal would be to set a "high" specificity of 95% or 98%? or a range between those values estimated against an uninfected subpopulation.***
- *In terms of specificity achieving a value of 100% is feasible; therefore, this should be the ideal reference value*.

STABILITY OF COMPONENTS

(m27) LAMP



(m50) PCR



(m27) LAMP P Stability

The minimum stability of reagents should be at least 6 months, considering that POC centers do not have the same patient volume as health centers located in more urbanized areas.

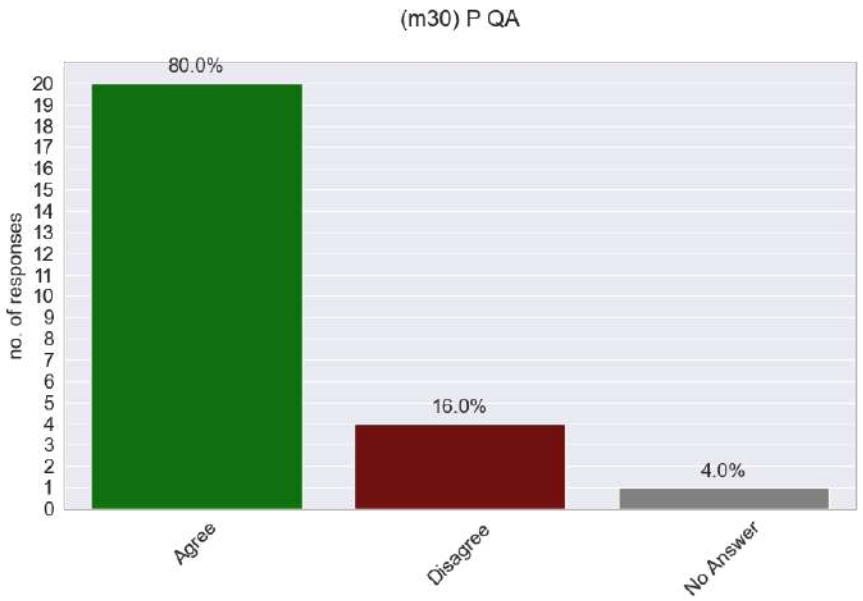
Taking into account that distribution and supply will pose a major challenge in some areas, as well as low use , a shelf life of 12 month should be the minimum desirable.

(m50) PCR Stability

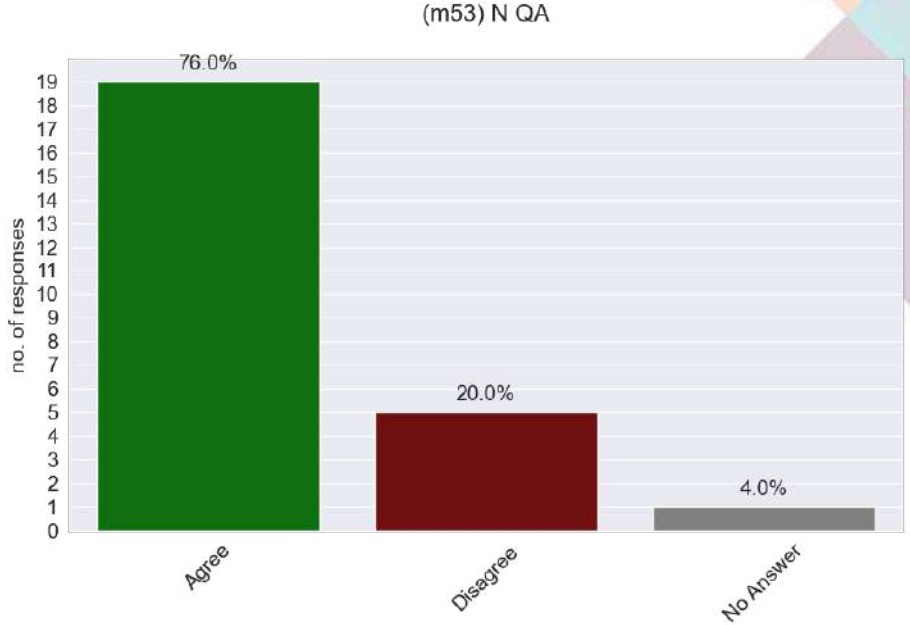
· Longer minimum shelf life would be advisable if possible.
· Reagent stability should be at minimum for 12 months .

QUALITY ASSURANCE

(m30) LAMP



(m53) PCR



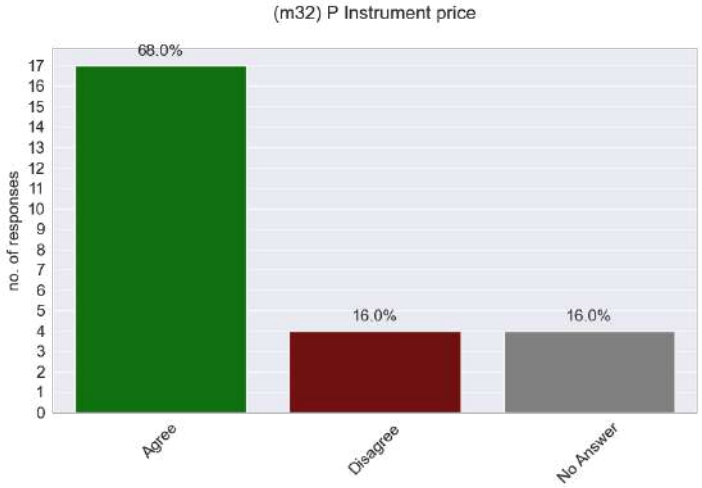
Common QA Comments for LAMP and PCR

· Minimun: Proficiency testing panels evaluated **before starting implementation of a new assay** in the laboratory, every two years thereafter

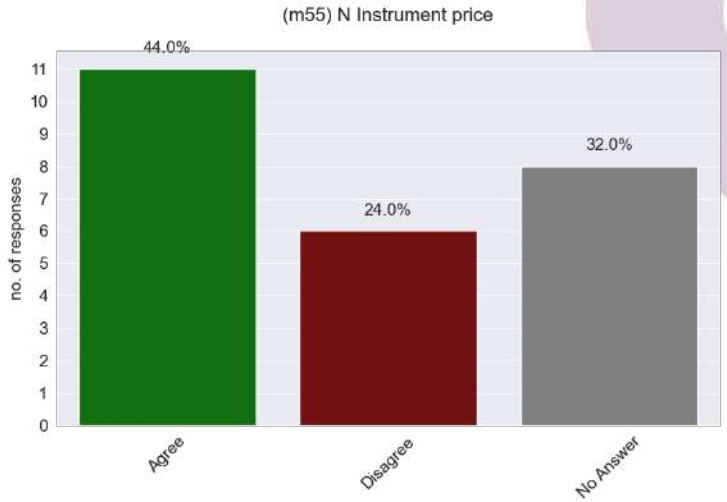
· Quality assurance should be done on each situation **involving change of instruments, kit batch or operators**.

INSTRUMENT PRICE

M 32 LAMP INSTRUMENT PRICE



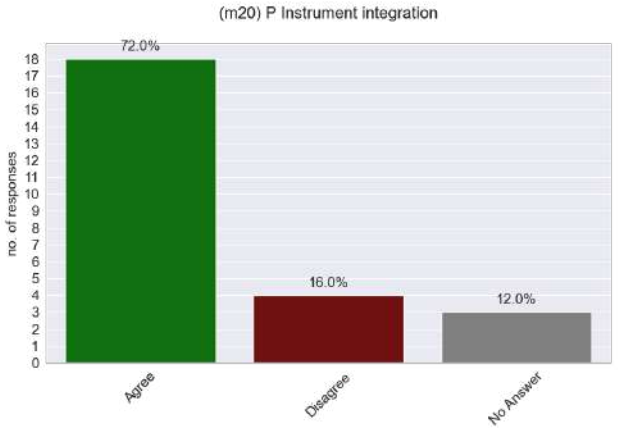
(m55) PCR N Instrument price

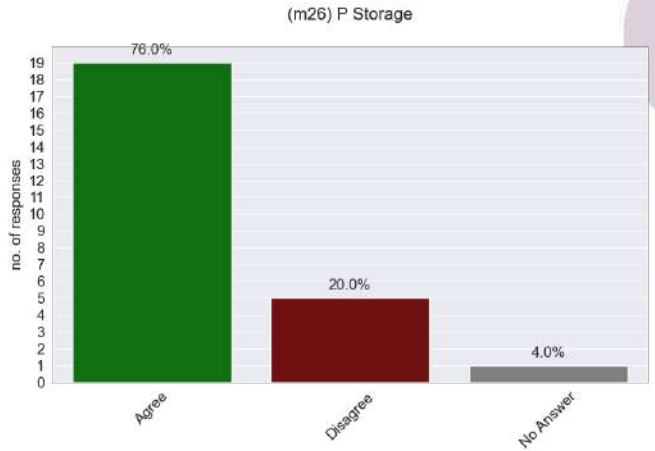


- Minimum cost for other POC molecular tests <1000 US
- ideal < 200 US $ or instrument-free
- The minimum should not exceed 5,000 USD.
- to purchase a LF160 heater for LAMP (ideal scenario closer to 2.5K USD).

- The price of the technology is not a specific impediment to the testing strategy and that resources be used efficiently so that the impact of the cost of diagnosis is relatively low compared to the resources allocated to treatment for the people who tested positive
- Minimum : 30000 USD, Ideal: 15000 USD two channels

ONLY FOR LAMP





(m20) P Instrument integration

In weak positive results, a naked eye can be inaccurate and operator-depending. The use of a simple device is ideal, specially if the device can read the result using an AI algorithm (photography to app in cell phone that could use AI to interpret the result).

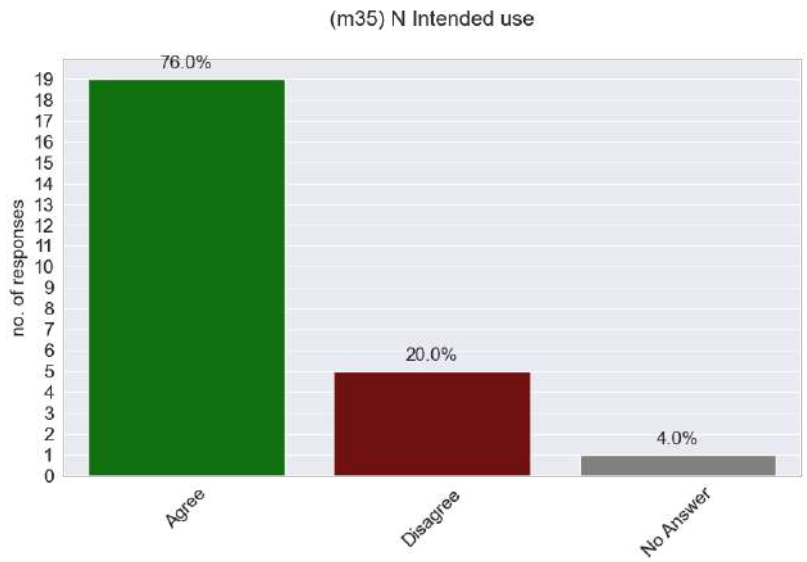
visualizing results with naked eye is highly operator-dependent. coupled with cost-effective equipment that allows for fluorescence visualization would increase analytical sensitivity, especially in patients with low parasite loads.

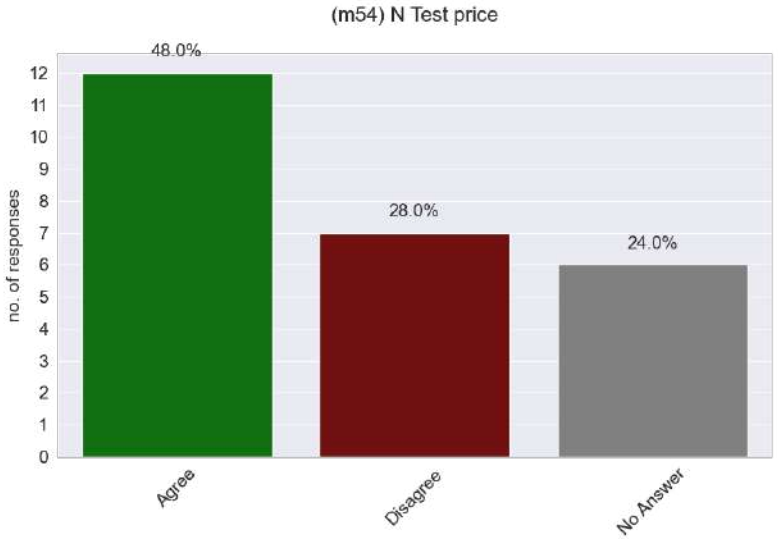
The instrumentation integration should fit REASSURED criteria

(m26) Storage

Temperatura ambiente.
el inserto con información para almacenamiento de kit comercial, algunos reactivos se degradan o precipitan si llegan a temperaturas de congelación
It could not be done in the field if it has to be at -20
Minimun at 4ºC

ONLY FOR PCR





(m35) Intended Use

Should only be for: Diagnosis for patients in the acute phase and assessment of response to antiparasitic treatment in the chronic phase
La implementación de PCR en tiempo real en laboratorios del área rural resulta muy difícil en países como Bolivia, altos costos y a las grandes necesidades existentes en el diagnóstico de Chagas congénito. Los presupuestos destinados a salud son bajos y los gobiernos municipales no estarían dispuestos a asumir esa responsabilidad.
Diagnosis at the chronic stage should be done based on serological assays.

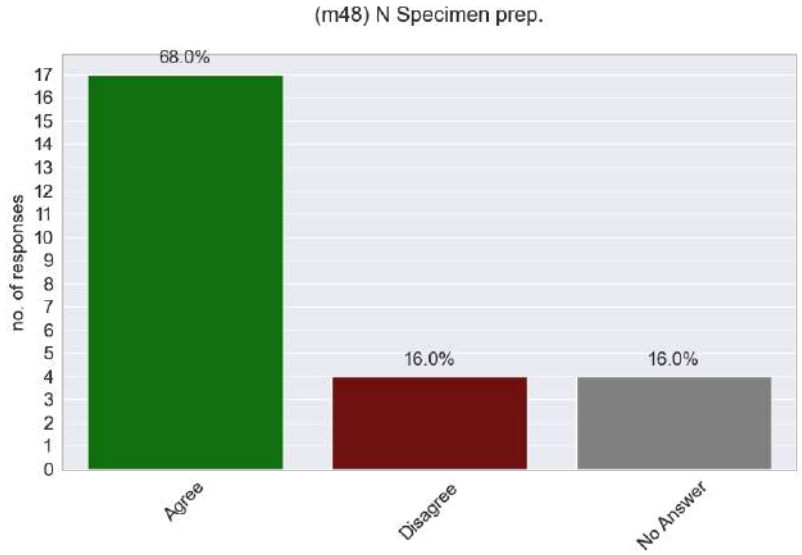
(m54) N Test price

-ideal 15 USD --minimum c30-40 USD closer to current situation. Very expensive for developing countries
-the price of the technology is not a specific impediment to the testing strategy and that resources be used efficiently so that the impact of the cost of diagnosis is relatively low compared to the resources allocated to treatments for the people who tested positive
-En el contexto de países como Bolivia, el costo por prueba es muy alto, considerando que los niños nacidos de madre positiva para Chagas corresponden a más del 15% de las gestantes.

ONLY FOR PCR

SPECIMEN PREPARATION

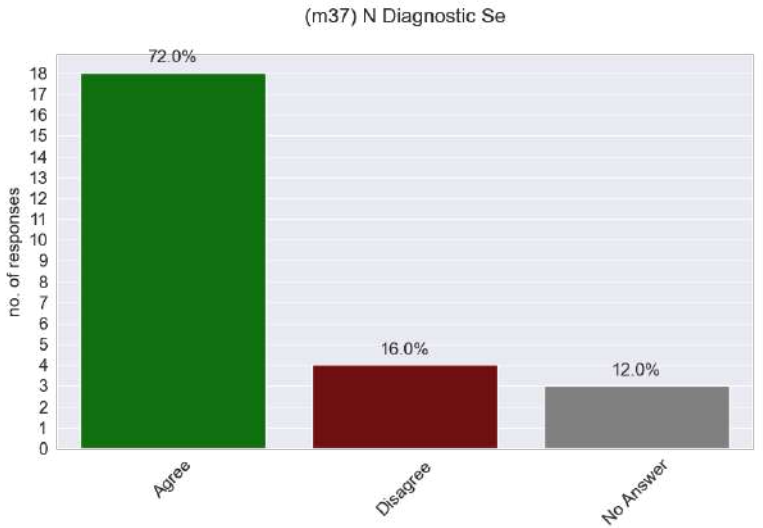
M48



Use of Dried blood spots - Column based DNA extraction commercial kit or magnetic bead based automated device As a reference test in molecular diagnostic laboratories, the should be considered, using automated DNA extraction devices, pippeting devices and commercial IVD qPCR kits. (Post Covid-19 pandemics reference diagnostic laboratories have Real Time PCR)

DIAGNOSTIC SENSITIVITY

m37



El minimo en sensibilidad diagnostica > 95%.
Diagnostic sensitivity should be considered in relation to the clinical study group.

To reach a 95% CI of +/-, 2.5%, 200 positive samples and 200 negative samples are needed. CLSI guide establish a minimum of 50 reactive and 50 non-reactive patient samples, foran error or 95% CI of +/-, 8.5%,

FIND Diagnóstico para todos
DNDi La mejor ciencia para los más desatendidos
ORGANIZED BY

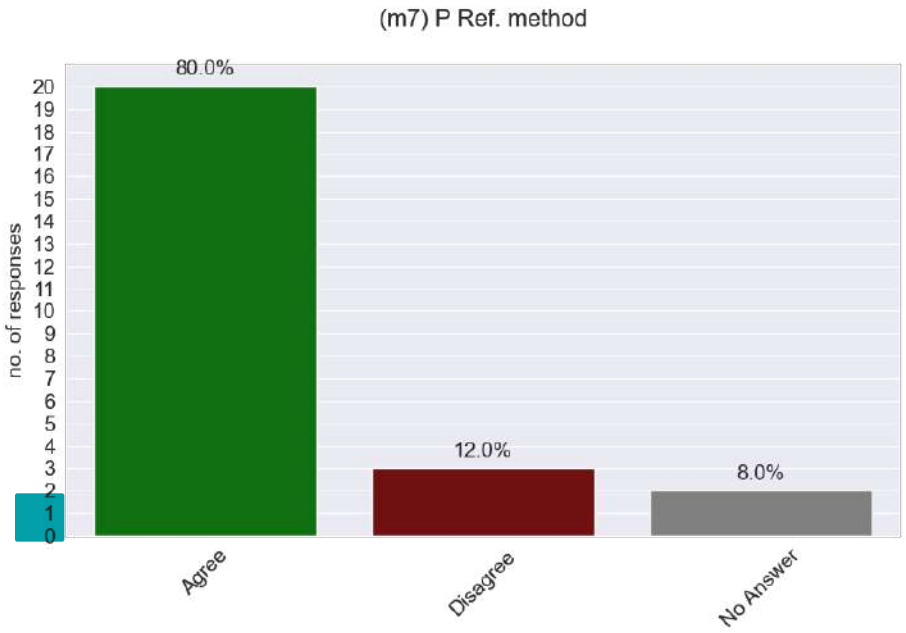
Pan American Health Organization
IN COOPERATION WITH

INGEBI CONICET
COORGANIZED BY

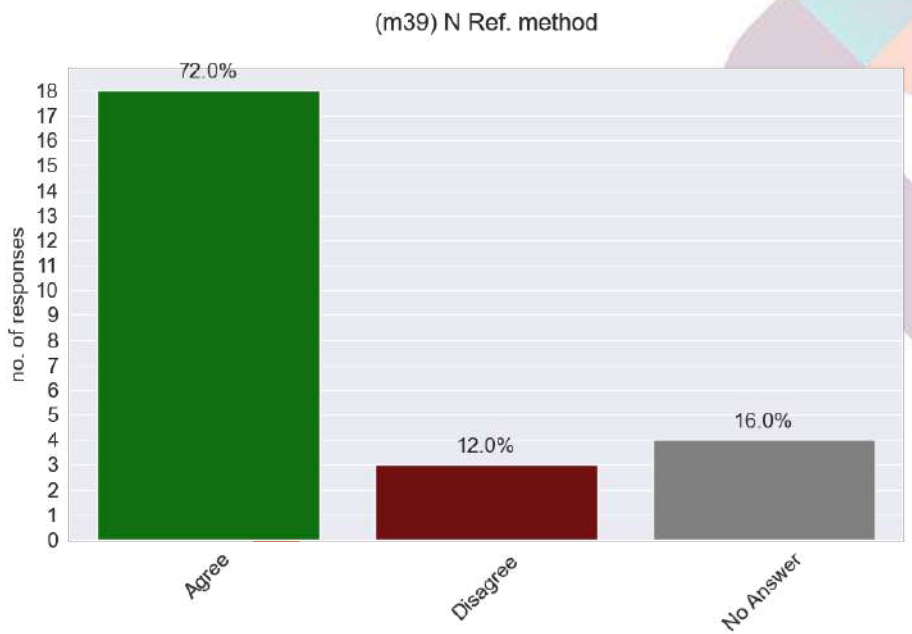
Comments in cases of 75% agreement

REFERENCE METHOD

(m7) LAMP



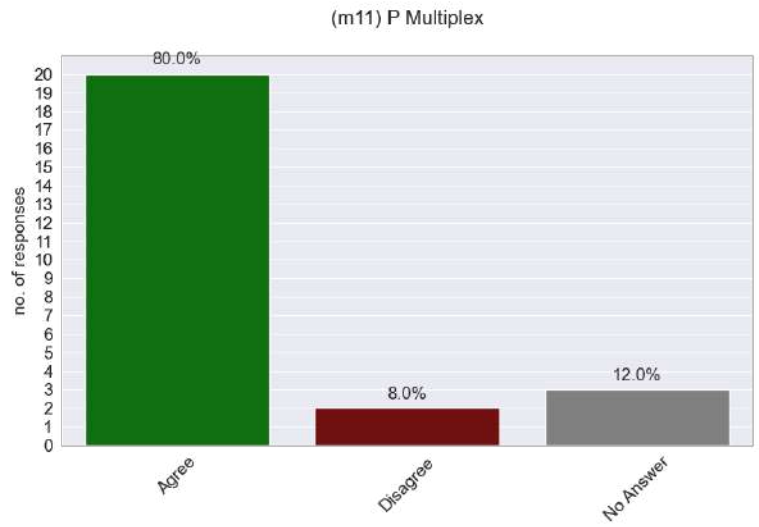
m39 PCR



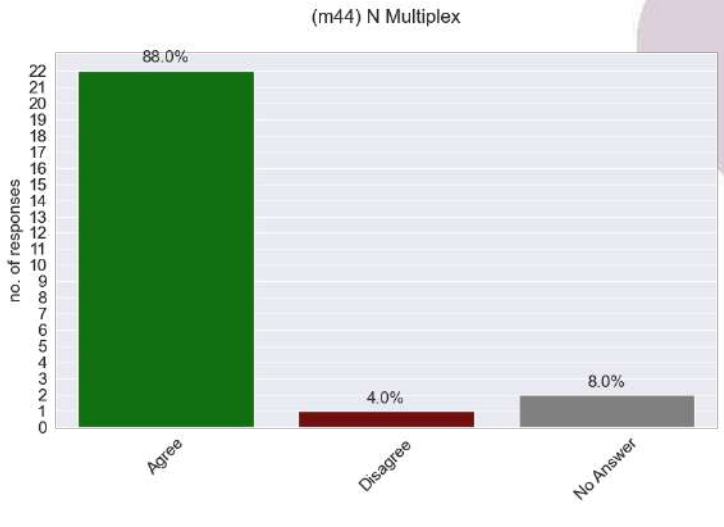
- *There should be consensus on the reference test method that could simplify comparability between studies*
- *The reference method must be performed according to evidence-based guidelines from PAHO 2018, based on GRADE methodology*

MULTIPLEX COMPONENTS

M11 LAMP



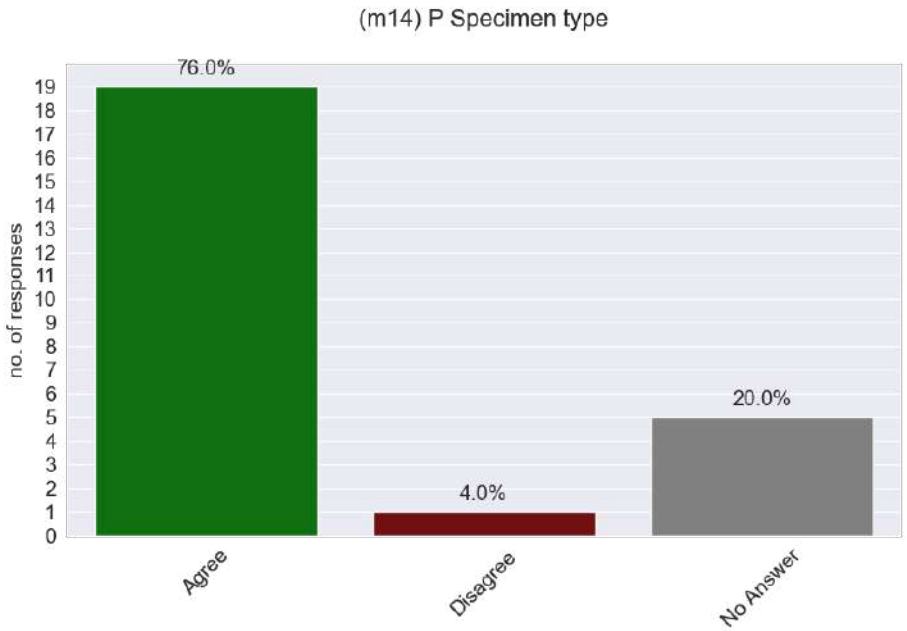
(m44) PCR



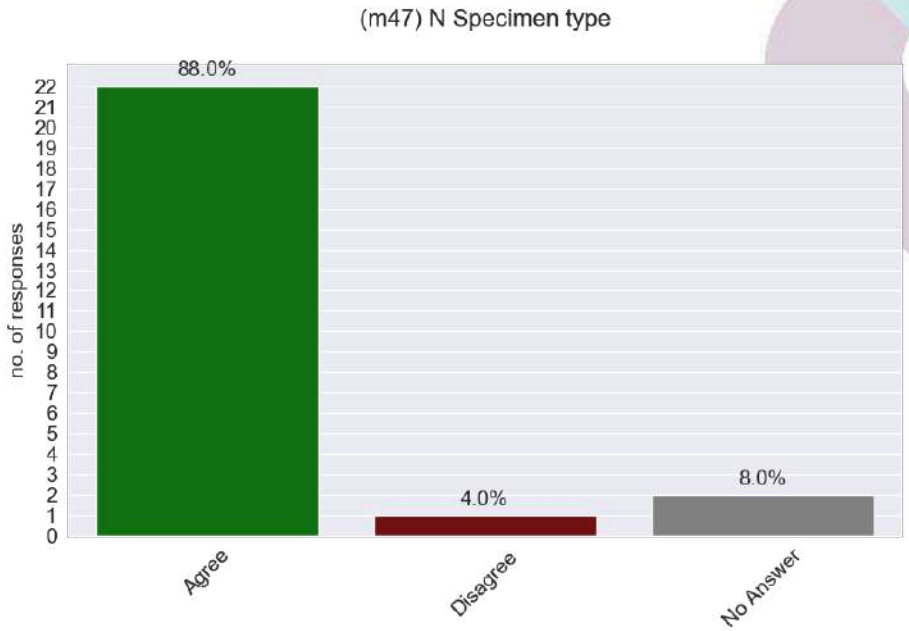
- If multiplex is ideal, there should be an indication of **which other pathogens to be included**
- Minimum including internal amplification control, even in singleplex.
- Ideal: internal control in multiplex
- It is not recommended that a multifunction test be attempted. Better to focus on one that is for the diagnosis of *T cruzi* in the right way

SPECIMEN TYPE

M14 LAMP



(m47) PCR

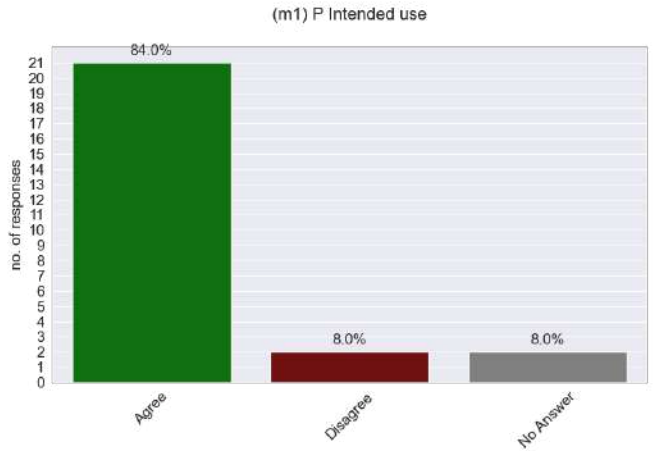


- Ideal: direct sample testing on the detection device

- Ideal: Anticoagulated blood without stabilizing agent (GE)
- Dried blood spots

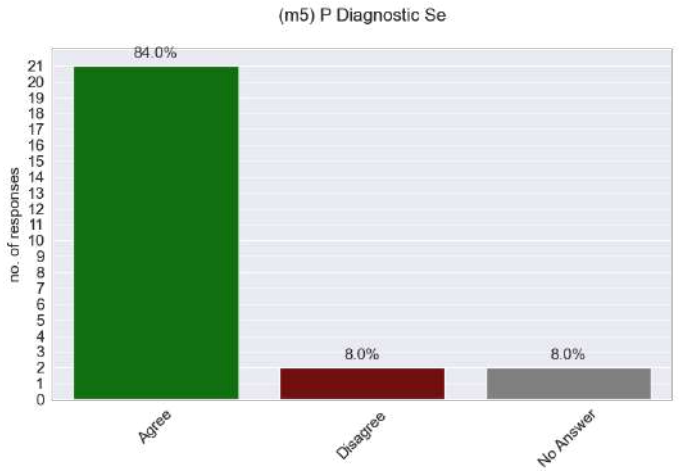
ONLY FOR LAMP

DIAGNOSTIC SENSITIVITY PREPARATION



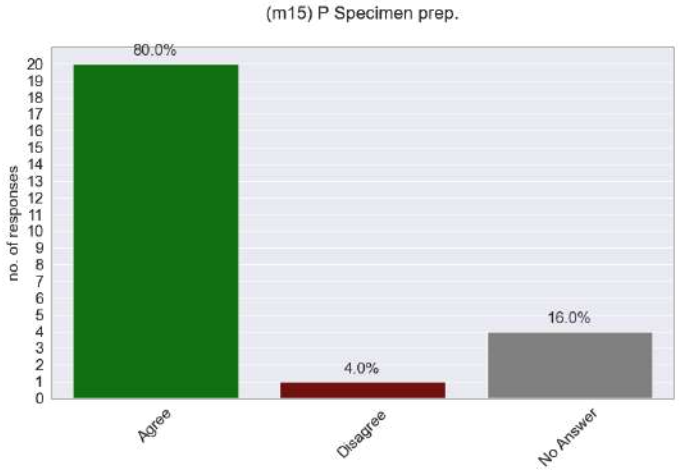
- In acute phase different scenarios and parasitic loads should be expected.
- **Thus, the algorithm for molecular diagnostic should include a 2d analysis in case of a neg result in the first, such as a 2d DNA extraction and repetition of the same or a 2d molecular test**
- **Proposed combination would be LAMP as the first test and qPCR and the confirmatory test, in the case of a negative result in LAMP.**

INTENDED USE



- 95% should be the lower limit of the 95% CI, and 98% the lower limit of the 95% CI
- Maybe the minimun S we are asking is too high for several epidemiological settings.

SPECIMEN



- In oral outbreaks, depending on the time after infection, when the first blood sample was obtained, the parasitic load can be low, similar to a chronic patient. Thus, more DNA extractions from the same specimen could increase the sensitivity. I recommend a second DNA extraction if the first one have a negative result.

ORGANIZED BY

IN COOPERATION WITH

COORGANIZED BY

NEXT STEPS

TOPICS FOR FURTHER DISCUSSION AND REPORT

POINTS ABOUT PCR AND LAMP THAT NEED TO BE ADDRESSED

DIAGNOSTIC SENSITIVITY and SPECIFICITY

-Which should be a consensus for minimum and ideal diagnostic sensitivity and specificity in the context of regional epidemiological settings ?

-Which would be an acceptable range of error ?

REFERENCE METHODS IN FIELD VALIDATIONS

- Which settings are covered in PAHO guidelines ?

-Available and field validated commercial molecular kits as comparator tests ?

-Gold standard and clinical diagnosis ?

ANALYTICAL SENSITIVITY of LAMP and PCR

-To standardize to number of copies of the target gene for comparison between laboratories?

-To generate panels of international standards to measure Se and Sp of molecular meethods

-To enable expressing values in International Units per ml of sample ?

- Which should be the CI 95% range ? at the LoD95% value ?

POINTS ABOUT PCR AND LAMP THAT NEED TO BE ADDRESSED

SPECIMEN TYPE and SPECIMEN PREPARATION FOR PCR

Ideal: Anticoagulated blood without stabilizing agent (GE); Dried blood spots?

Dependent on the scenario, more than one DNA extraction for the same specimen? A second DNA extraction if the first one results negative? Or a second sample withdrawn in a subsequent day / week / month ?

MULTIPLEX FORMATS

What can we do regarding developing and validation of multiplex molecular methods ?

In which settings (ETMI plus, field surveys of acute febrile illnesses) ?

Which other pathogens appart from T.cruzi and an internal amplification standard ?

Which would be the cost benefit of performing Multiplex Molecular Methods?

POINTS ABOUT PCR AND LAMP THAT NEED TO BE ADDRESSED

DIAGNOSTIC ALGORITHMS OF COMBINED METHODS

An algorithm for molecular diagnosis could include a second analysis in the case of a negative result in the first one ?

For example: a combination of LAMP as the first option and qPCR as confirmatory test, in the case of a negative result in LAMP ?

Which would be the cost-benefit of combined algorithm of molecular methods or even molecular combined with serological tests? In which settings ?

QUALITY ASSURANCE

Quality assurance should be done on each situation involving change of instruments, reagent batch or operators in each Laboratory performing molecular diagnosis?

Minimum condition: Proficiency testing panels evaluated before implementation of a new assay in the laboratory, and on which periodicity thereafter ?

Thank You.

Contact info: schijmaningebi@gmail.com
britto97@gmail.com
mpinazo@dndi.org.

ORGANIZED BY

IN COOPERATION WITH

COORGANIZED BY

ORGANIZED BY

IN COOPERATION WITH

COORGANIZED BY

Diagnostics: Comparing algorithm performance and cost

Sarah Girdwood
Impact Department, FIND

MEETING ON DIAGNOSTIC EVALUATION AND ECONOMIC IMPACT ANALYSIS OF NEW DIAGNOSTIC METHODS FOR CHAGAS DISEASE

6-7 MAY 2024 BUENOS AIRES, ARGENTINA

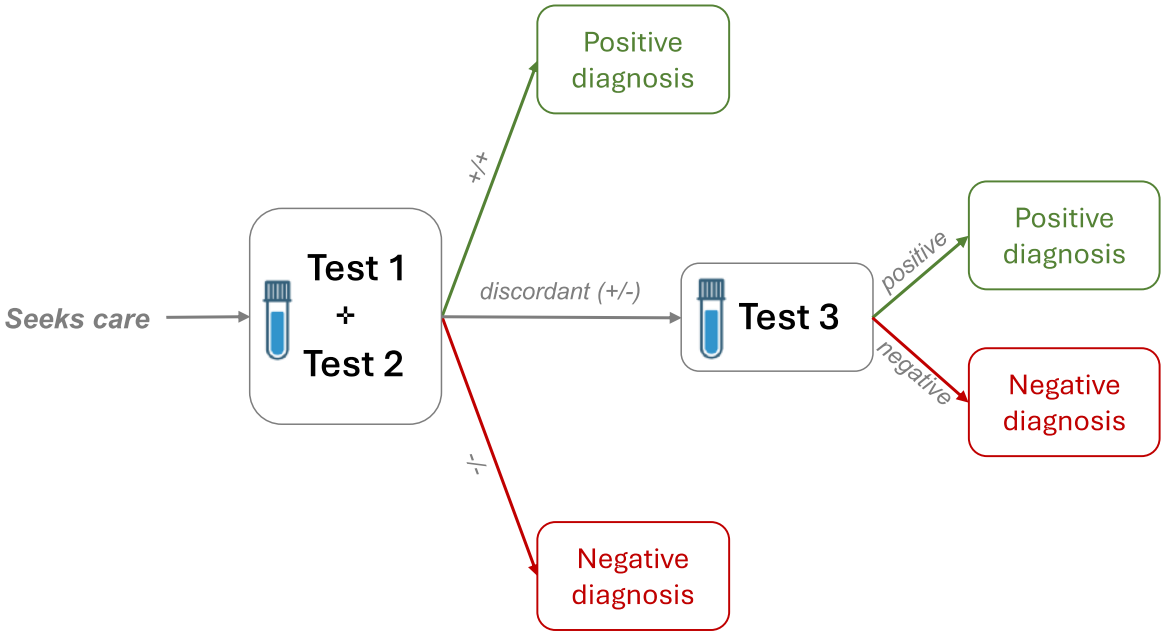
How do we diagnose Chagas disease?

Pan American Health Organization

World Health Organization
Regional Office for the Americas

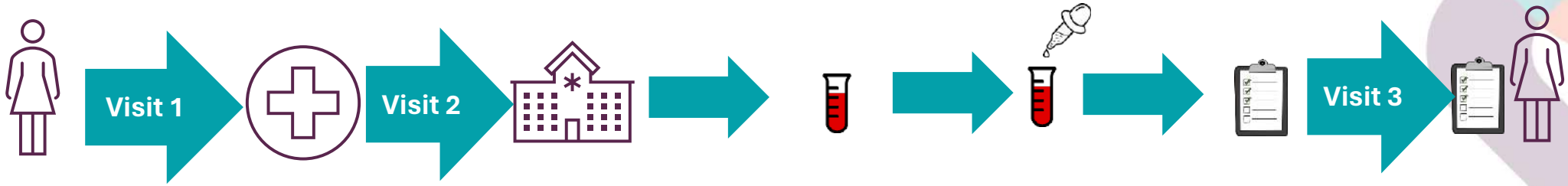
- Diagnostic **gold standard** of the agreement of **two serological tests.**
- A **third** tie-breaker serological test is required if the first two are **discordant**.
- Tests target **different antigens**.
- Most countries conduct the first two tests in **parallel**

Standard of care



GUIDELINES
for the Diagnosis and
Treatment of
Chagas Disease

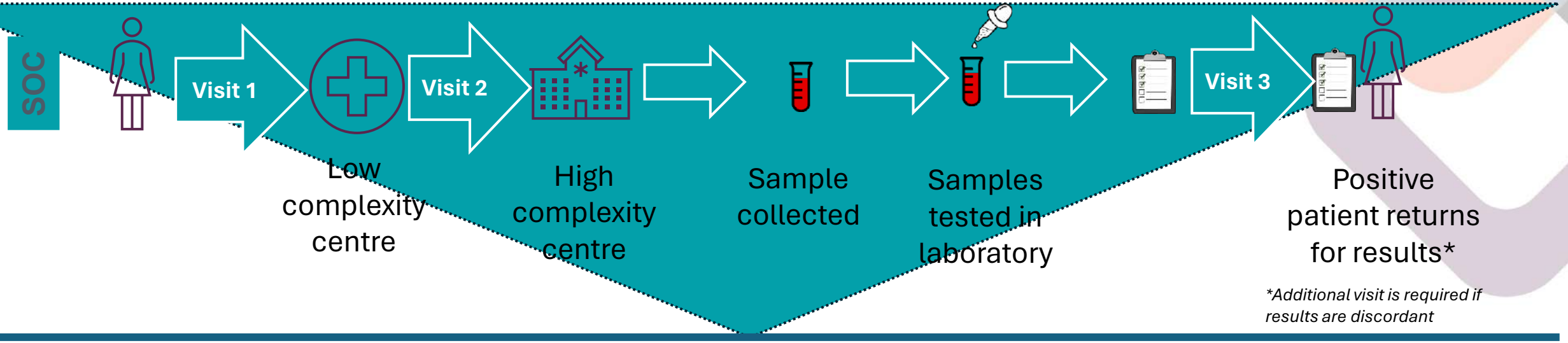
Current Standard of Care: from access to diagnosis

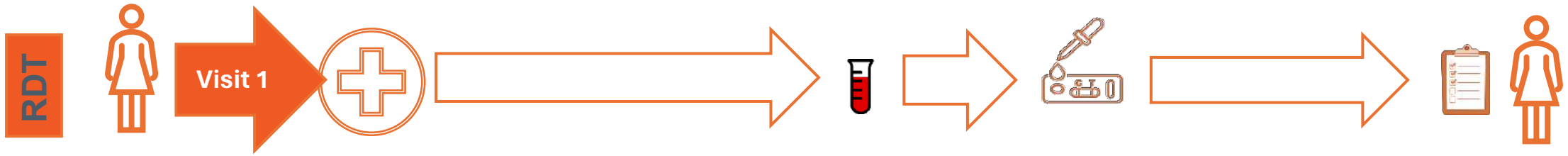


*Additional visit is required if results are discordant

- Lab-based testing is **complex** and **time-consuming.**
- Serology is **not suitable for point-of-care testing**
- **Majority** of patients first seek care at **low complexity centres** and have to be referred. (*Castillo-Requelme, 2008*)
- Requires **multiple visits** to the clinic and laboratory for diagnosis

Integrating new test technologies can simplify the diagnostic pathway



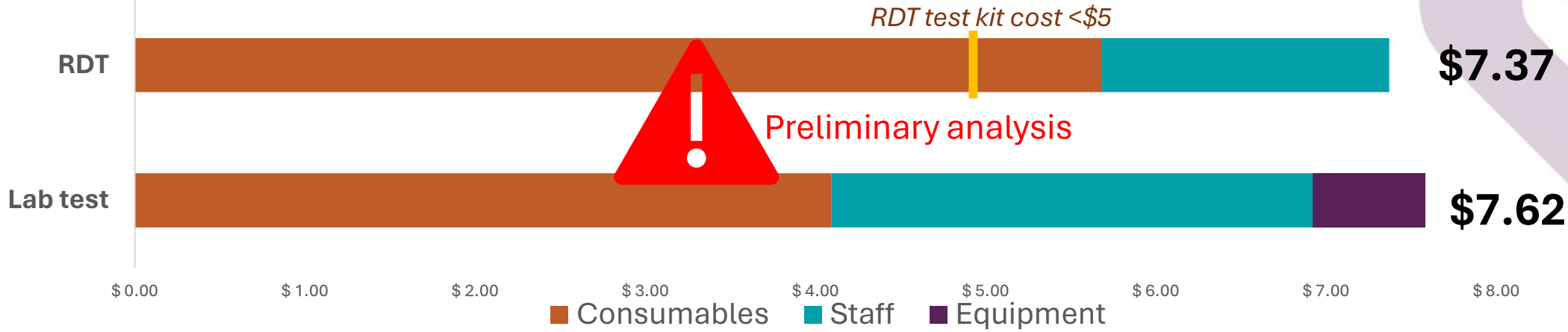


RDT-based algorithms allow for **point-of-care testing**

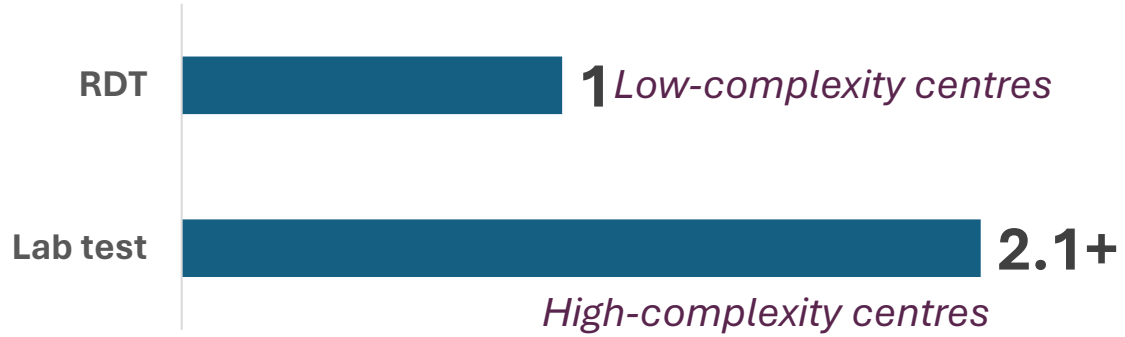
RDT-based algorithms decentralize testing and **reduce time to diagnosis** and **losses**

RDT is likely to cost the same as a serology test, but will reduce visit costs for patients and the healthcare system

Direct laboratory cost per test *(Argentina)*



Average number of visits to diagnosis



- A patient spends **5 times less** on transport, food & accommodation for care received at a low- vs. a high-complexity centre. (*Herazo, 2023*)
- Healthcare visits costs are **~30% less** expensive at a low- vs. a high-complexity centre for the provider. (*WHO CHOICES*)

Integrating new test technologies: evaluating the trade-offs

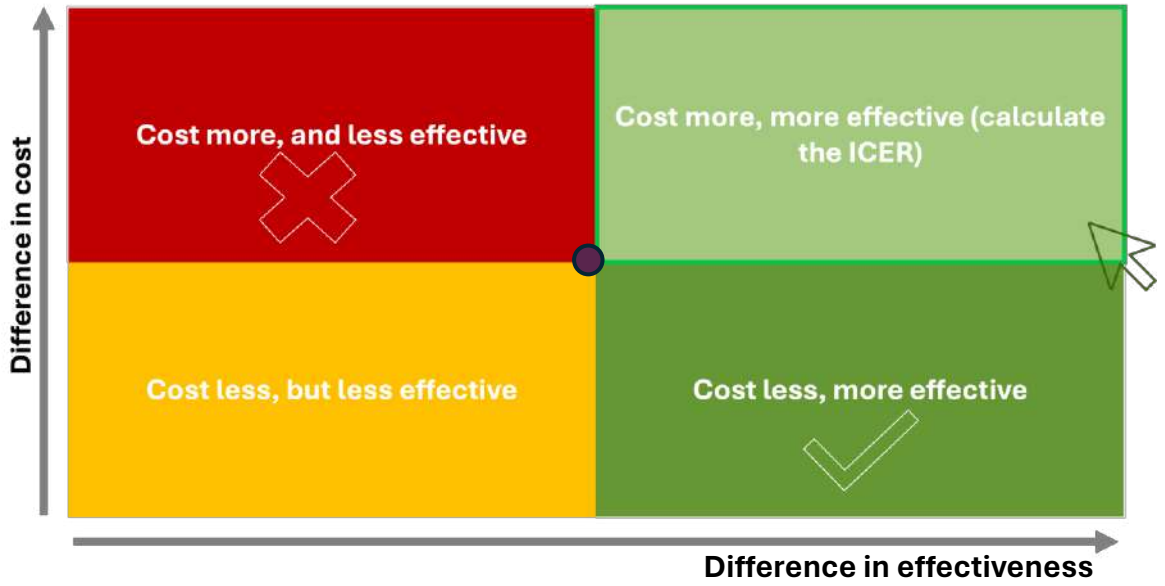
Loss to follow-up

Access

Cost

Performance

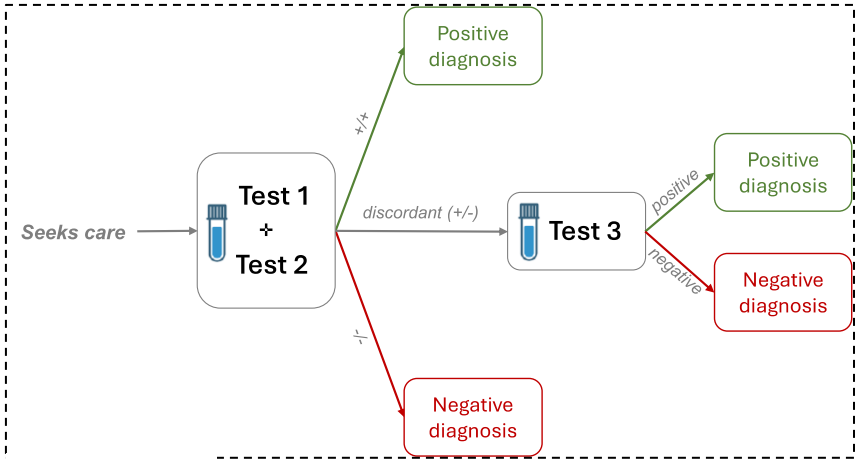
Economic evaluation can help us evaluate which is the most efficient in different settings



- The cost-effectiveness plane provides a visual framework for assessing the value of healthcare interventions.
- Understanding the position of interventions on the plane informs decision-making regarding healthcare spending and resource allocation.

Standard of Care testing using an RDT is as efficient as Serology at identifying a positive case but more efficient when visit costs are included

Parallel testing



Cohort 10,000. Prevalence 5%

Sensitivity: 97%
Specificity: 98%

$7.6/test

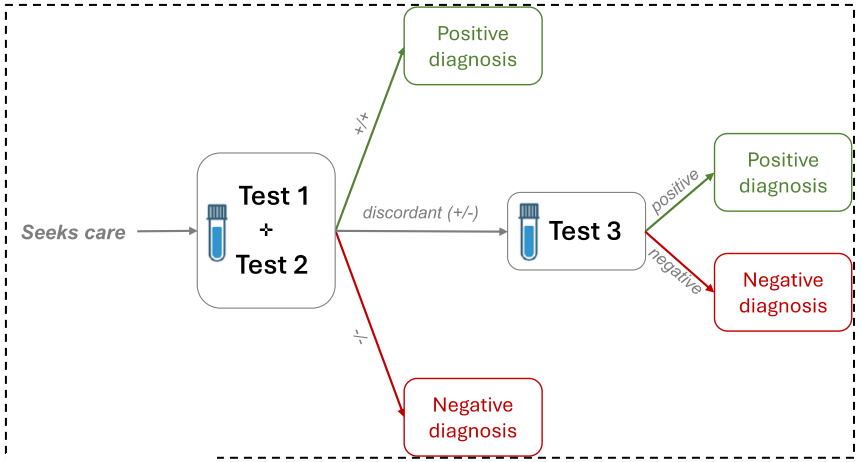
Sensitivity: 85%
Specificity: 95%

$7.4/test

	Serology	RDT
Total tests	20,402	21,030
Total cost	$155,459	$154,991
PPV	**98%**	**87%**
NPV	100%	100%
Proportion positives identified	99.7%	94%
Cost per positive identified	**$312**	**$330**

Standard of Care testing using an RDT is as efficient as Serology at identifying a positive case but more efficient when visit costs are included

Parallel testing



Cohort 10,000. Prevalence 5%

Sensitivity: 97%
Specificity: 98%

$7.6/test

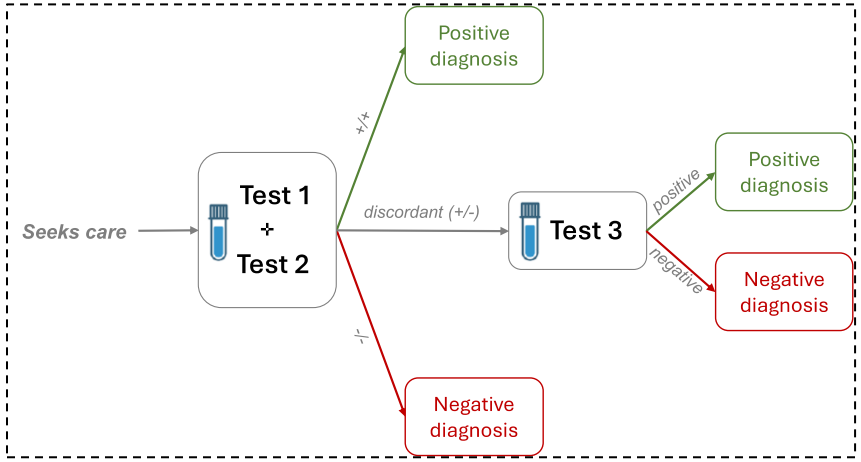
Sensitivity: 85%
Specificity: 95%

$7.4/test

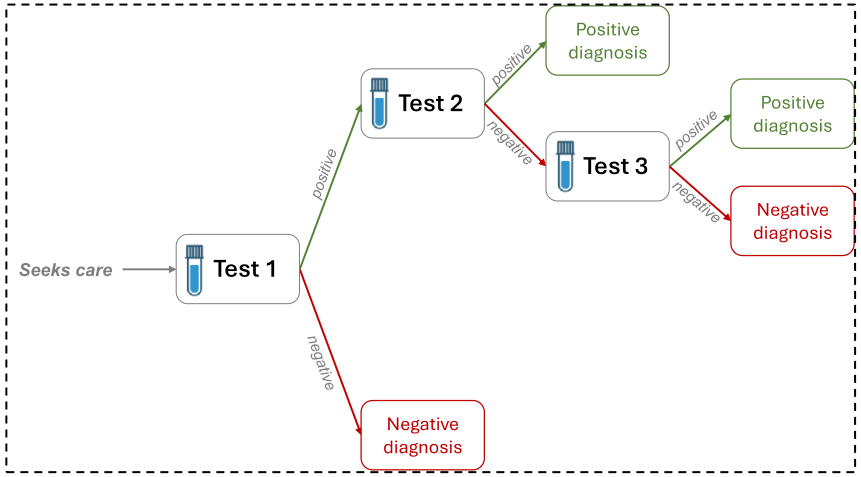
	Serology	RDT
Total tests	20,402	21,030
Total cost	$155,459	$154,991
PPV	**98%**	**87%**
NPV	100%	100%
Proportion positives identified	99.7%	94%
Cost per positive identified	**$312**	**$330**
Visit costs	**$133,417**	**$85,300**
Total cost per positive identified	**$579**	**$512**

Serial vs. parallel test algorithms using RDTs can impact the number of cases identified and the cost of diagnosing a patient

Parallel testing



Serial testing



	Parallel	Serial
Total tests	21,030	11,415
Total cost	$154,991	$84,129
PPV	**87%**	**90%**
NPV	100%	99%
Proportion positives identified	**94%**	**83%**
Cost per positive identified	**$330**	**$203**

Cohort 10,000. Prevalence 5%

Sensitivity: 85%
Specificity: 95%

$7.4/test

We can use RDTs to increase access whilst retaining current serology capacity at laboratories.

Parallel testing

Total serology tests conducted with serology Standard of Care algorithm (assuming cohort of **10,000**) = ~20,000

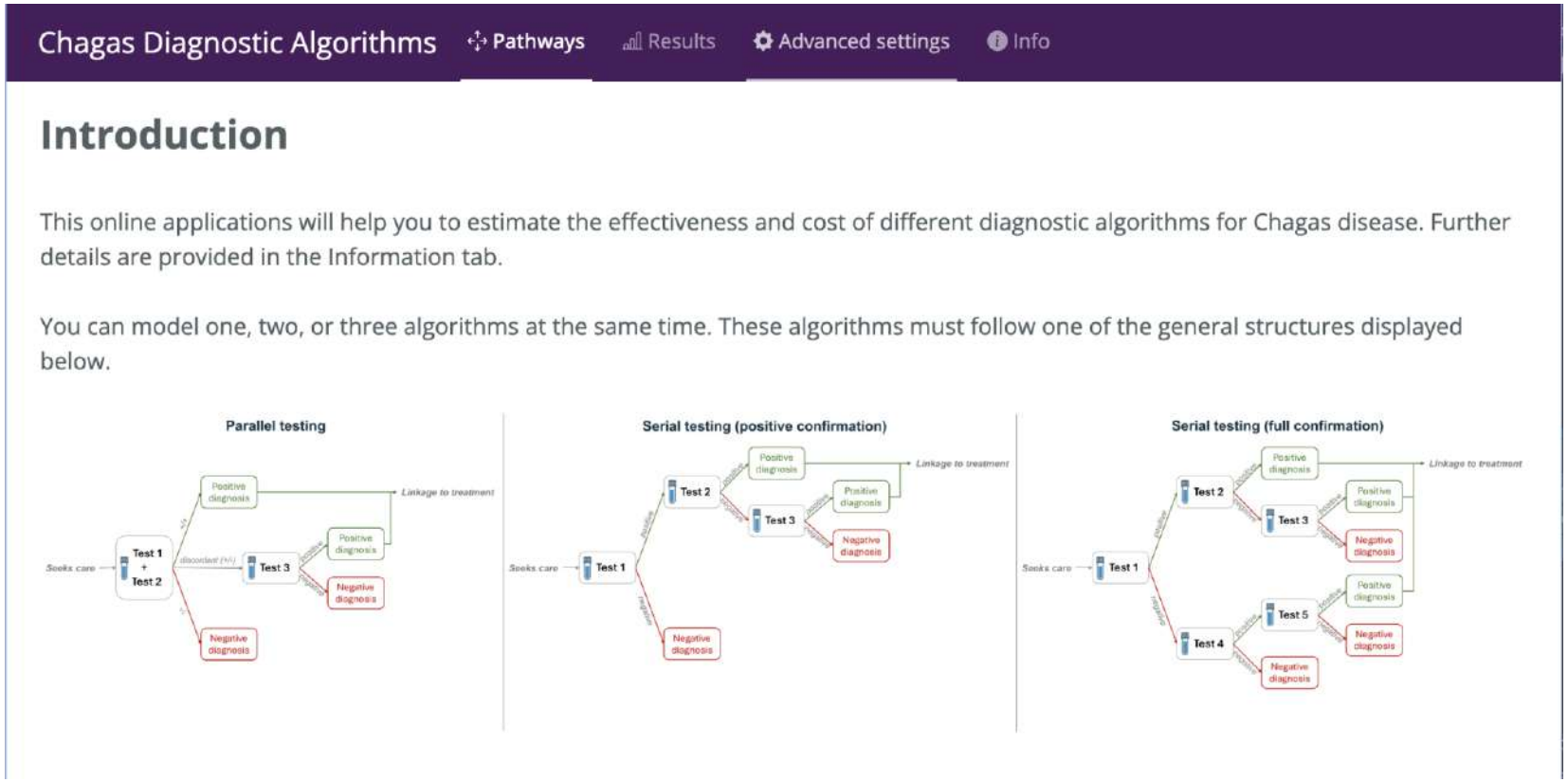
~10% require 3rd test (due to discordant results)

Can screen ~**200,000** population to ensure that ~20,000 serology tests will need to be conducted for discordant RDT tests

Access increases 20-fold

This is a simplified example with simplified assumptions

- This simple analysis does not consider an increase in **access** nor **lost to follow-up** which is likely to make RDTs even more cost-effective
- Next → the **Chagas Diagnostic Algorithm** application



Shaukat Khan
FIND,
Director,
Strategic Information Unit

Kyra Grantz
FIND,
Modeler, Epidemiologist

Sarah Girdwood
FIND,
Health economist

Thank You.

Contact info:
sarah.girdwood@finddx.org

ORGANIZED BY

IN COOPERATION WITH

COORGANIZED BY

ACKNOWLEDGEMENTS

The multicentric prospective study in Argentina is being conducted by our partners, CONICET, sponsored by the National Institute of Health, INP (National Institute of Parasitology), Fatala within ANLIS, with the support of FIND and DNDi

Thank You.

ORGANIZED BY

IN COOPERATION WITH

COORGANIZED BY

ORGANIZED BY

IN COOPERATION WITH

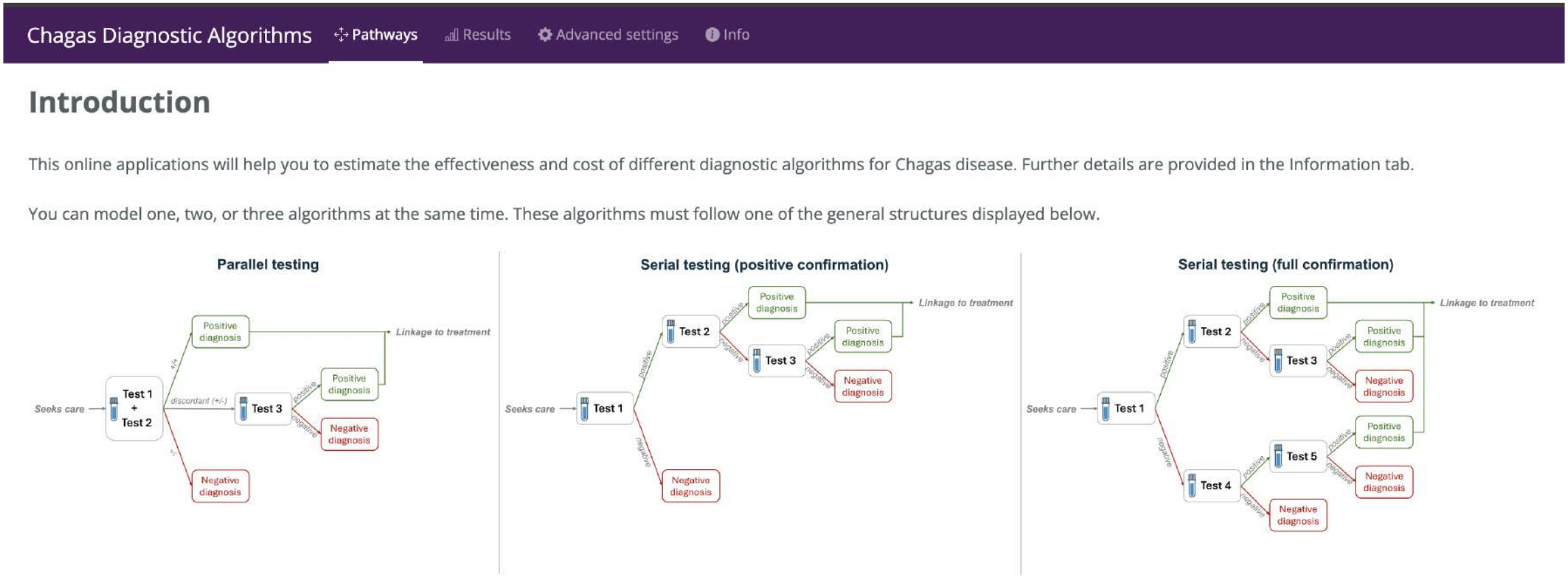
COORGANIZED BY

Diagnose More Cases, Spend Less: A User-Friendly Shiny App Model for Chagas Diagnosis

Kyra Grantz, PhD
Impact Department, FIND

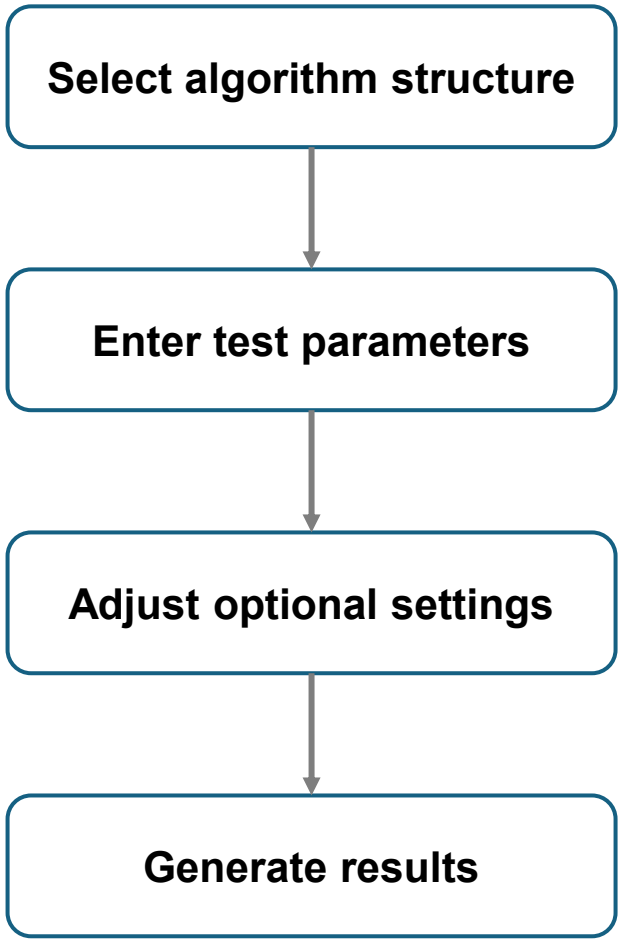
MEETING ON DIAGNOSTIC EVALUATION AND ECONOMIC IMPACT ANALYSIS OF NEW DIAGNOSTIC METHODS FOR CHAGAS DISEASE

6-7 MAY 2024 BUENOS AIRES, ARGENTINA



Chagas Diagnostic Algorithms application: online, interactive tool to compare performance and cost of diagnostic algorithms

Chagas Diagnostic Algorithms application



Chagas Diagnostic Algorithms application

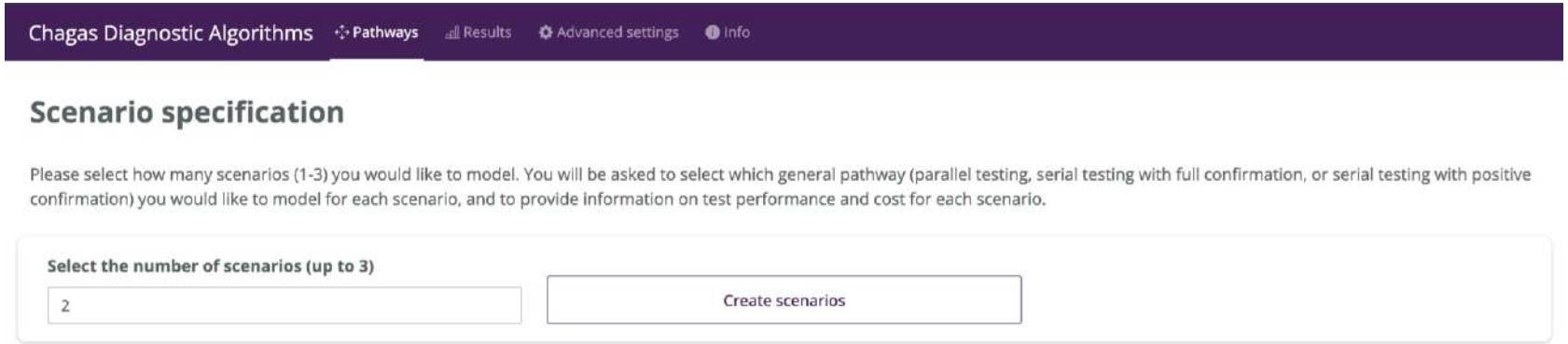
Select algorithm structure

Enter test parameters

Adjust optional settings

Generate results

1. Choose number of scenarios to model



Chagas Diagnostic Algorithms application

Select algorithm structure

Enter test parameters

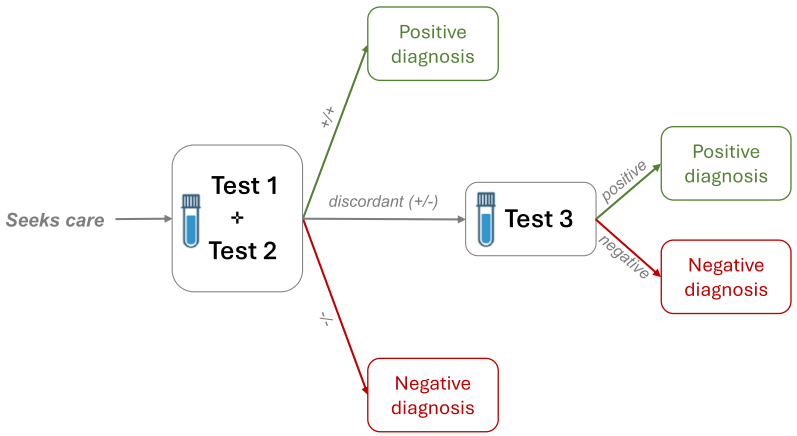
Adjust optional settings

Generate results

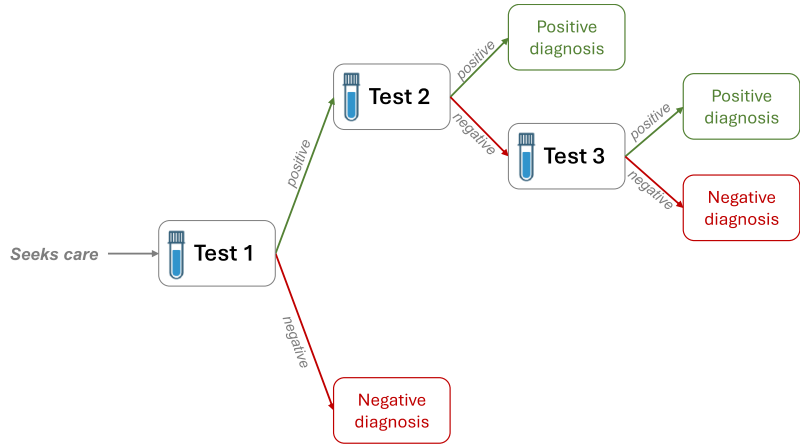
1. Choose number of scenarios to model

2. Select one of three possible generic structures:
   - Parallel testing
   - Serial testing with confirmation of positive results
   - Serial testing with confirmation of positive and negative results

Parallel testing



Serial testing



Chagas Diagnostic Algorithms application

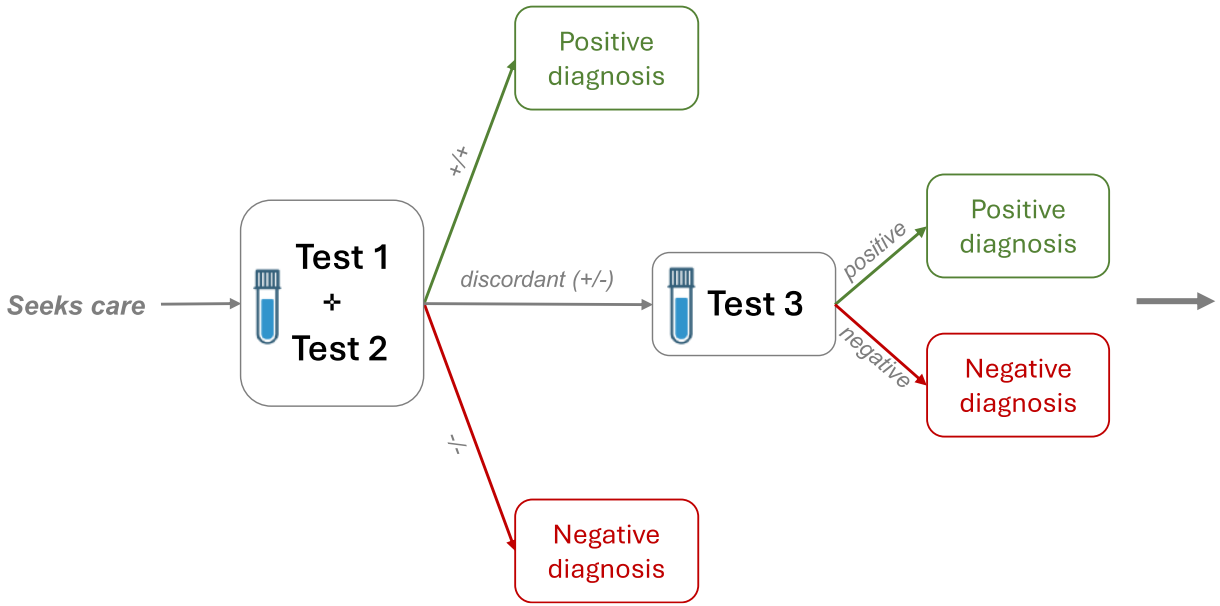
Select algorithm structure

Enter test parameters

Adjust optional settings

Generate results

1. Enter test information
   - Test type and name
   - Facility level at which test is conducted
   - Test sensitivity, specificity
   - Test cost



Enter data for Tests 1, 2, and 3

Scenario 1

Pathway type

(•) Parallel ( ) Serial (positive conf.) ( ) Serial (full conf.)

Test 1

Test type

( ) RDT (•) Laboratory-based

Label

Sero 1

Facility type

(•) High complexity ( ) Low complexity

Sensitivity (0-100%)

97

Specificity (0-100%)

98

Cost per test (USD)

7.62

Test 2

Test type

( ) RDT (•) Laboratory-based

Label

Sero 2

Facility type

(•) High complexity ( ) Low complexity

Sensitivity (0-100%)

97

Specificity (0-100%)

98

Cost per test (USD)

7.62

Test 3

Test type

( ) RDT (•) Laboratory-based

Label

Sero 2

Facility type

(•) High complexity ( ) Low complexity

Sensitivity (0-100%)

97

Specificity (0-100%)

98

Cost per test (USD)

7.62

Scenario 2

Pathway type

(•) Parallel ( ) Serial (positive conf.) ( ) Serial (full conf.)

Test 1

Test type

(•) RDT ( ) Laboratory-based

Label

RDT 1

Facility type

( ) High complexity (•) Low complexity

Sensitivity (0-100%)

85

Specificity (0-100%)

95

Cost per test (USD)

7.38

Test 2

Test type

(•) RDT ( ) Laboratory-based

Label

RDT 2

Facility type

( ) High complexity (•) Low complexity

Sensitivity (0-100%)

85

Specificity (0-100%)

95

Cost per test (USD)

7.38

Test 3

Test type

(•) RDT ( ) Laboratory-based

Label

RDT 3

Facility type

( ) High complexity (•) Low complexity

Sensitivity (0-100%)

85

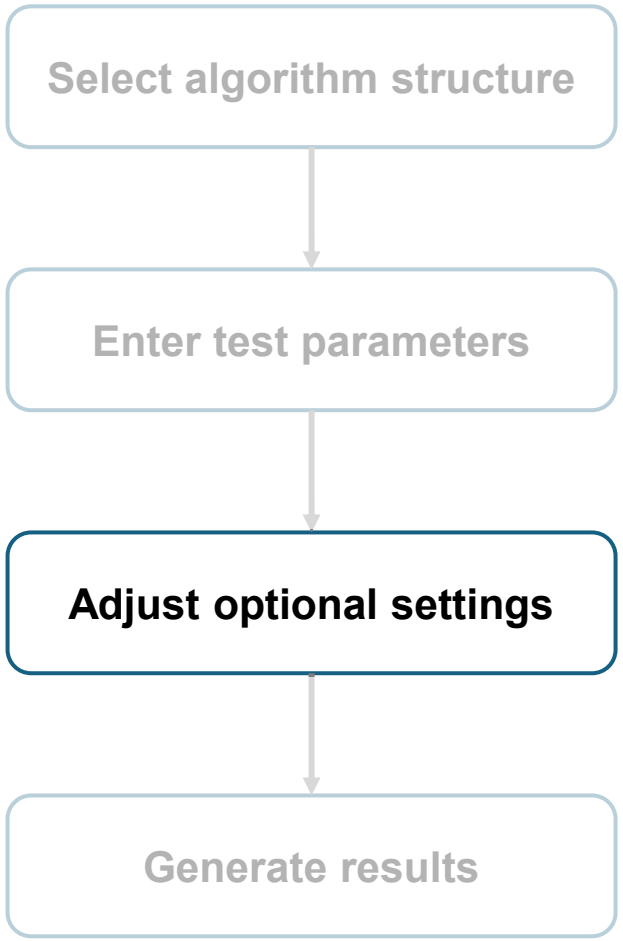
Specificity (0-100%)

95

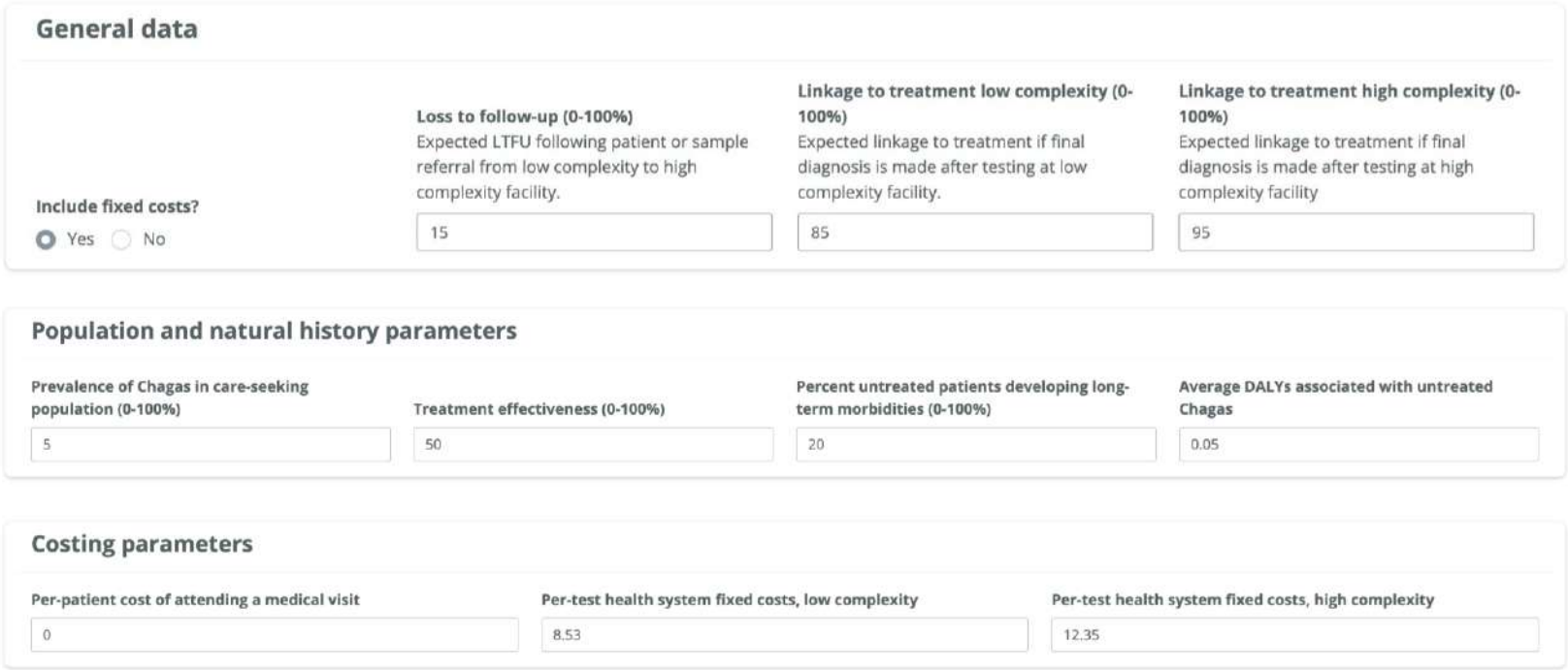
Cost per test (USD)

7.38

Chagas Diagnostic Algorithms application



1. Update parameters related to:
   - Per-visit fixed costs to health system
   - Per-visit fixed costs to patients
   - Loss to follow-up during referral
   - Chagas burden (prevalence, DALYs)
   - Linkage to treatment and treatment effectiveness



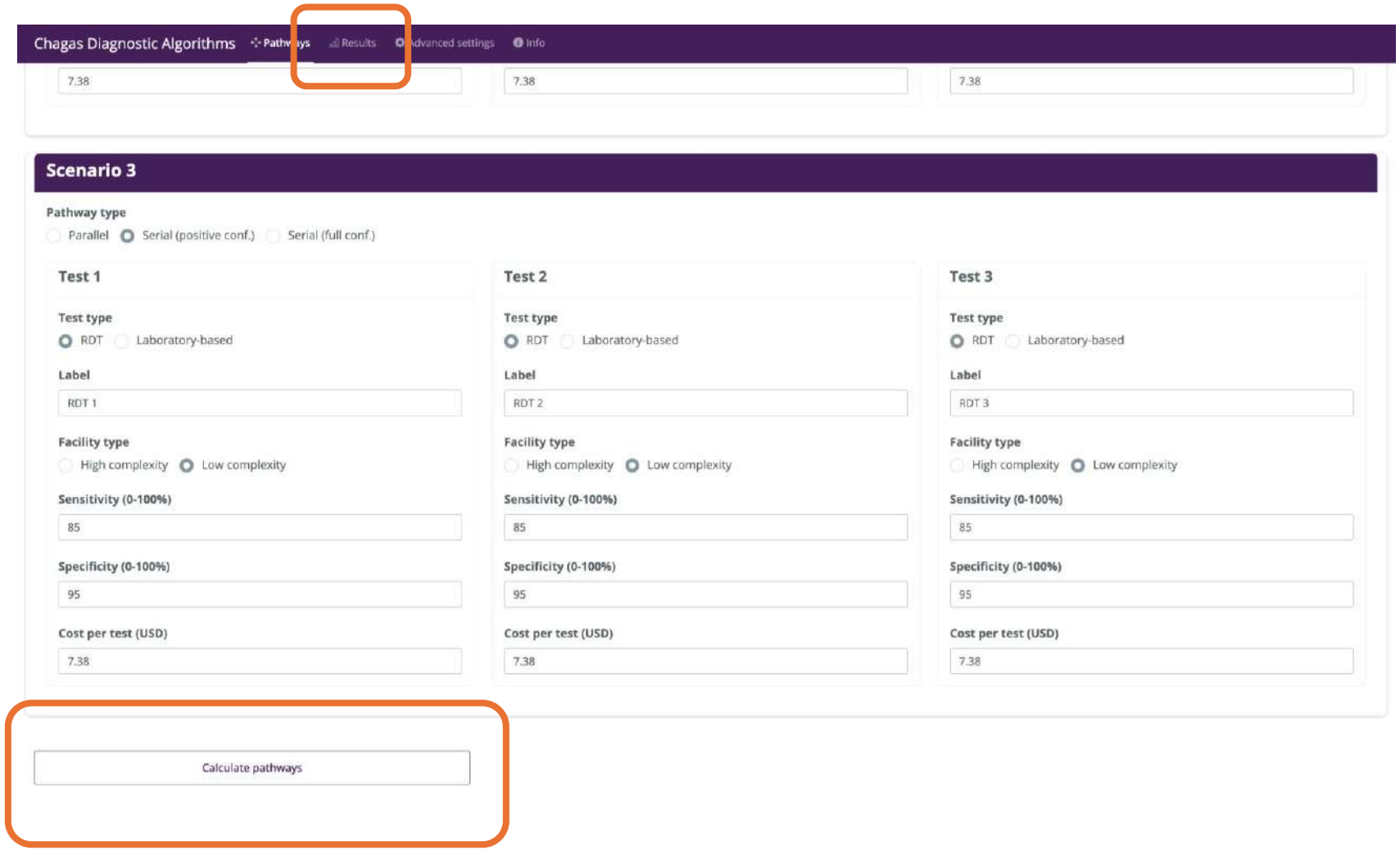
Chagas Diagnostic Algorithms application

Select algorithm structure

Enter test parameters

Adjust optional settings

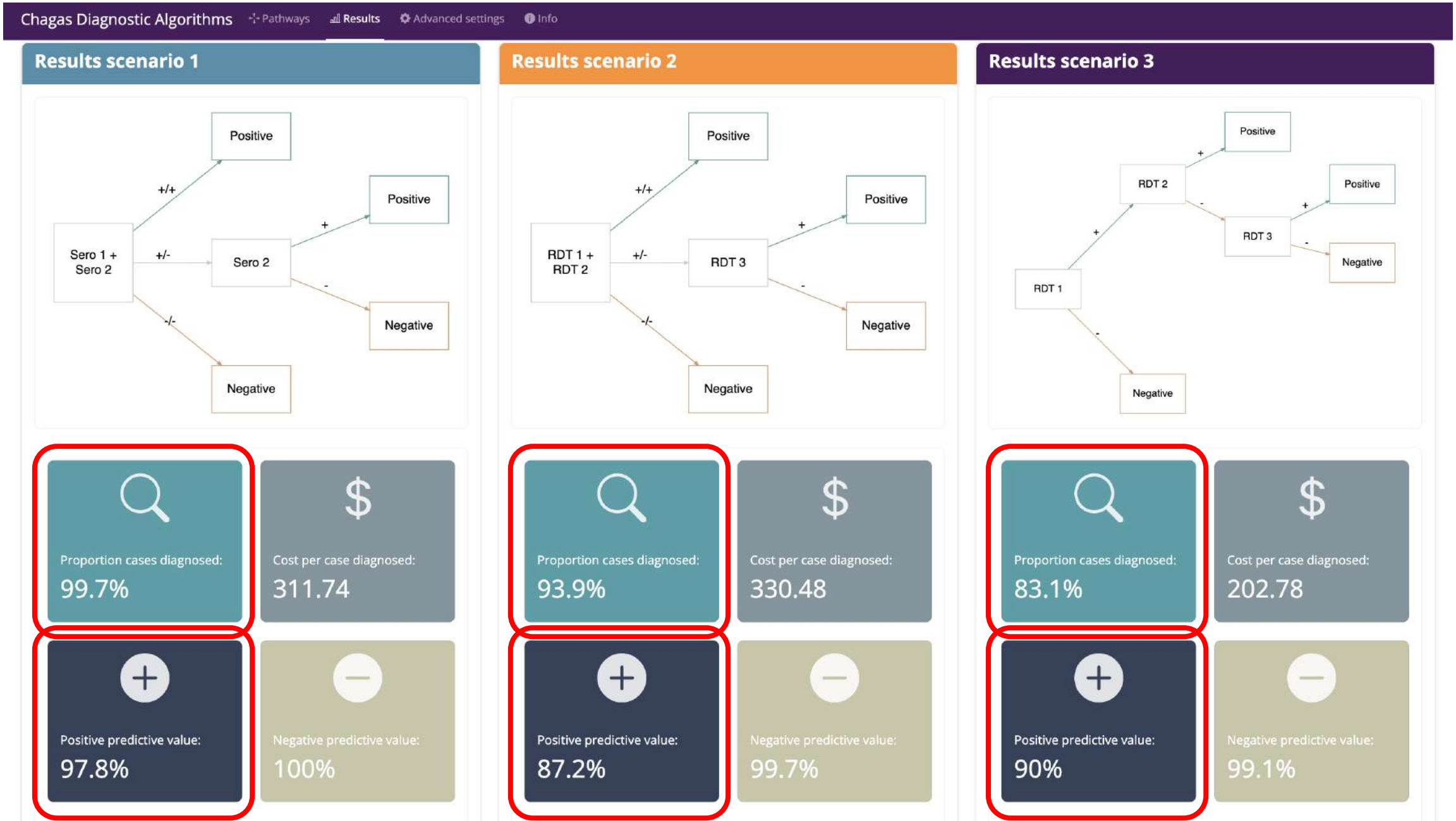
Generate results



SOC: parallel testing with sero. assays

parallel testing with RDTs

serial testing with RDTs

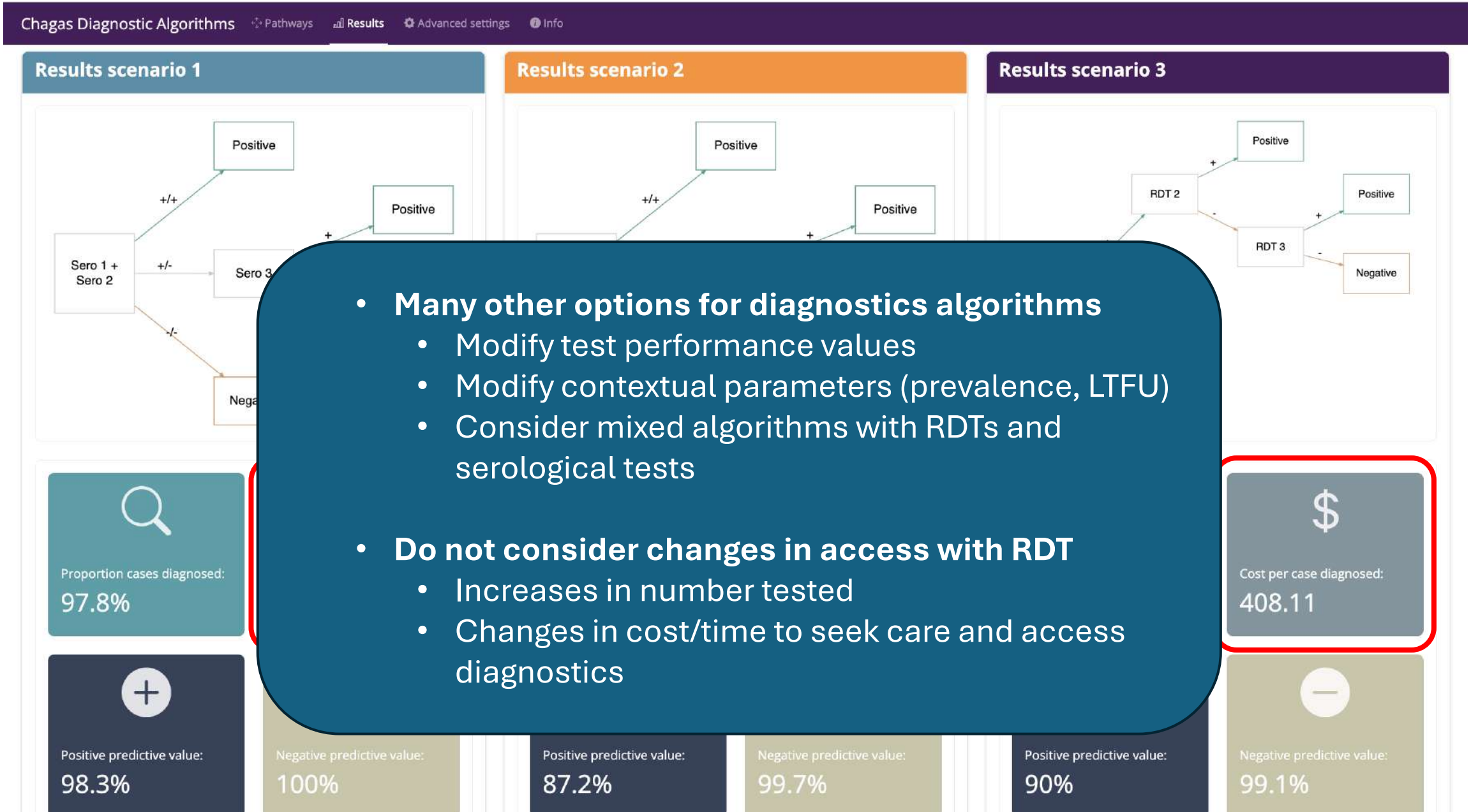


With loss to follow-up and fixed costs:

SOC: parallel testing with sero

parallel testing with RDTs

serial testing with RDTs



- **Many other options for diagnostics algorithms**
  - Modify test performance values
  - Modify contextual parameters (prevalence, LTFU)
  - Consider mixed algorithms with RDTs and serological tests

- **Do not consider changes in access with RDT**
  - Increases in number tested
  - Changes in cost/time to seek care and access diagnostics

Chagas Diagnostic Algorithms application

Select algorithm structure

↓

Enter test parameters

↓

Adjust optional settings

↓

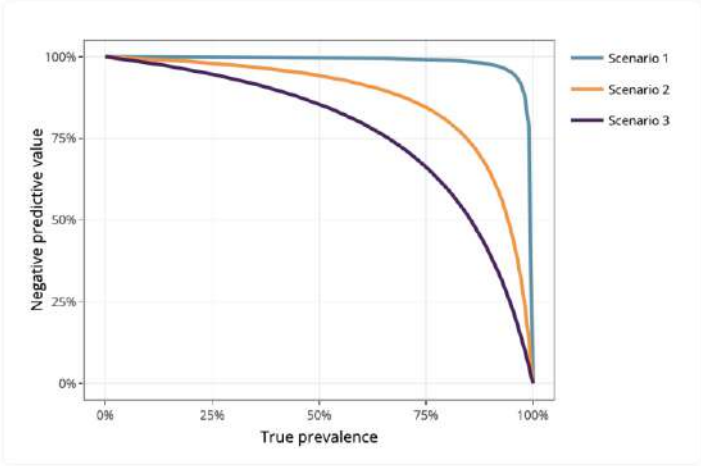
Generate results

Detailed results:

- Plots of PPV, NPV, cost-per-case by disease prevalence
- Detailed algorithm performance
- Total costs
- Cases linked to treatment
- DALYs averted through treatment

Downloadable report

- Downloadable, shareable HTML report of all model inputs and outputs



Algorithm performance			
N[1]	1,000	1,000	1,000
Number testing positive	50	54	46
Number true positive results	49	47	42
Number false positive results	1	7	5
Number testing negative	944	946	954
Number true negative results	943	943	945
Number false negative results	0	3	8
Number lost to follow-up	6	0	0
Percent all cases diagnosed	**98.9%**	93.9%	83.1%
Positive predictive value[2]	**98.0%**	87.2%	90.0%
Negative predictive value[3]	**100.0%**	99.7%	99.1%
Algorithm cost[4]			
Total cost	$28,271.53	$24,050.14	**$16,954.27**
Cost per case diagnosed	$571.79	$512.11	**$408.11**
Required visits per patient	1.03	1.00	1.00
Downstream outcomes[4]			
Number linked to treatment	48	46	39
Number true cases linked to treatment	47	40	35
Percent all cases linked to treatment	93.9%	79.8%	70.6%
DALYs averted through linkage to treatment	1.17	1.00	0.88

Chagas Diagnostic Algorithms application

https://finddx.shinyapps.io/chagaspathway/

Application guide:

- User manual describing model structure and instructions available in application

Feedback:

- Anonymous survey to provide feedback on application contents and usability
- https://forms.gle/h584XtkKmsATiCUf7

Survey:

ACKNOWLEDGEMENTS

The multicentric prospective study in Argentina is being conducted by our partners, CONICET, sponsored by the National Institute of Health, INP (National Institute of Parasitology), Fatala within ANLIS, with the support of FIND and DNDi

Thank You.

ORGANIZED BY

IN COOPERATION WITH

COORGANIZED BY

Shaukat Khan
Director,
Strategic Information Unit
FIND

Kyra Grantz
Modeler
FIND

Sarah Girdwood
Health Economist
FIND

Survey for feedback:

Contact info: Kyra.Grantz@finddx.org

App: https://finddx.shinyapps.io/chagaspathway/

ORGANIZED BY

IN COOPERATION WITH

COORGANIZED BY

FIND Diagnóstico para todos

DNDi La mejor ciencia para los más desatendidos

ORGANIZED BY

Pan American Health Organization

IN COOPERATION WITH

INGEBI CONICET

COORGANIZED BY

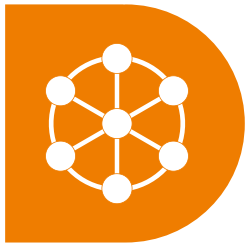
Análisis de los costos incurridos por los pacientes con enfermedad de Chagas: La experiencia en municipios endémicos de Colombia.

Analysis of the costs incurred by patients with Chagas disease: The experience in endemic municipalities in Colombia

6-7 MAY 2024 BUENOS AIRES, ARGENTINA

Concepto

Tradicionalmente se define como los pagos directos hecho por un individuo a los proveedores de atención médica en el momento de la prestación de un servicio, estos costos incluyen gastos médicos y no médicos.



Los gastos directos no médicos entre los cuales se incluyen el gasto de desplazamiento y alimentación, en conjunto con la pérdida de ingresos, son decisivos y pueden suponer una carga mayor para los hogares.

Un concepto ampliado es más útil y refleja mejor lo que ocurre en la práctica.

OIT, OPS.(1999). *El gasto de bolsillo en salud en América Latina y el Caribe: Razones de eficiencia para la extensión de la protección social en salud.* http://www.oitopsmexico99.org.pe
Hernández-Vásquez A, et al. (2020). *Análisis del gasto de bolsillo en medicamentos e insumos en Perú en 2007 y 2016.* /link.cgi/Medwave/Revisiones/Analisis/7833.act
Sauerborn R, et al. (1996). *Household strategies to cope with the economic costs of illness*. doi: 10.1016/0277-9536(95)00375-4.
McIntyre D, et al. (2005). *What are the economic consequences for households of illness and of paying for health care in low- and middle-income country contexts?* PMID: 16099574.
OMS/OPS. (2021). *Gastos directos de bolsillo en salud: la necesidad de un análisis de género*. https://doi.org/10.37774/9789275323540

Contexto

Sistema de salud en Colombia

- ✓ Basado en el aseguramiento
- ✓ Cobertura de 98.93% (2023)

Personas con un carné de salud

≠

? 41% satisfacción con la disponibilidad de atención médica de calidad (promedio de la OCDE 67%)

Casos notificados SIGIVILA (2022 – 2024 2p)

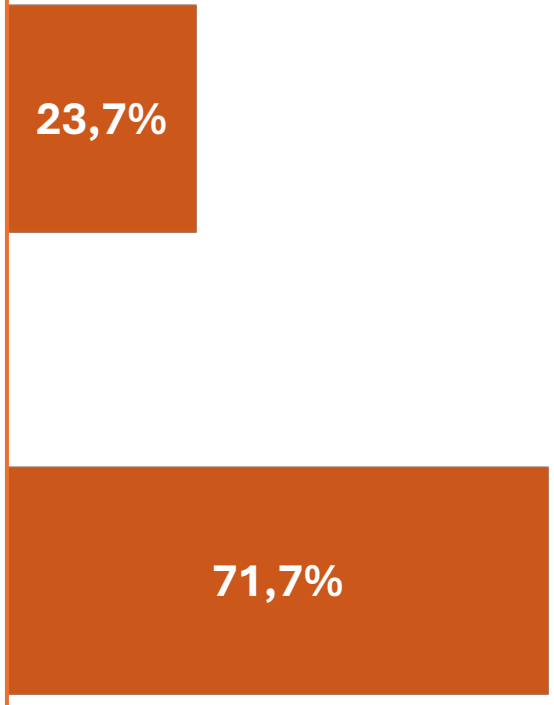


Imagen tomada de: https://www.larepublica.co/especiales/sistema-de-salud/el-abc-de-como-funciona-el-sistema-general-de-seguridad-social-en-salud-sgsss-3464091
Minsalud Colombia. (2023). *Boletín de aseguramiento en salud*. https://www.minsalud.gov.co/proteccionsocial/Regimensubsidiado/Paginas/coberturas-del-regimen-subsidiado.aspx
Instituto Nacional de Salud, Colombia. (2024). *Informe de evento y tableros de control: Chagas*. *https://www.ins.gov.co/buscador-eventos/Paginas/Info-Evento.aspx*
OECD. (2023). Access to care – Key indicators. https://www.oecd.org/colombia/health-at-a-glance-Colombia-EN.pdf

Contexto

OECD, 2023

- Gasto en salud US $1.640 dólares per cápita (78% a cargo del estado). GB equivale al 14% del gasto en salud.

Castillo-Riquelme, et al. 2008

- Estudio entomológico US $4,4 Fumigación US $27
- Costo tratamiento Chagas crónico US $46,4 – 7.981 / año
- Costo paciente de por vida, promedio US $11.619

Olivera M et al. 2021

- Costo nacional estimado de 13,1 MUSD (2017)
- 55.1% Costos directos
- 20.4% Costos directos no-médicos

OECD. (2023). Access to care – Key indicators. https://www.oecd.org/colombia/health-at-a-glance-Colombia-EN.pdf
Castillo-Riquelme M, et al. (2008). *The costs of preventing and treating chagas disease in Colombia.* doi: 10.1371/journal.pntd.0000336
Olivera M et al. (2021). *Economic costs of Chagas disease in Colombia in 2017: A social perspective*. https://doi.org/10.1016/j.ijid.2019.11.022

Acta Tropica 245 (2023) 106963

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Analysis of the costs incurred by patients with Chagas disease: The experience in endemic municipalities in Colombia

Rafael Herazo [a], Adriana Rey [a], Diogo Galvão [a], Manuel Medina Camargo [b], Nini Johana Cobos Pinzón [c], Alba Cecilia Rodriguez Sanchez [d], Andrea Marchiol [a,*], Maria Jesus Pinazo [a]

[a] DNDi, Drugs for Neglected Diseases Initiative-Latin America, Rua São José 70, Sala 60, Rio de Janeiro, RJ 20010-903, Brazil
[b] ETV Boyacá, Colombia
[c] ETV Boyacá, Soatá, Colombia
[d] Alcadía de Mogotes, Colombia

Perspectiva de los pacientes

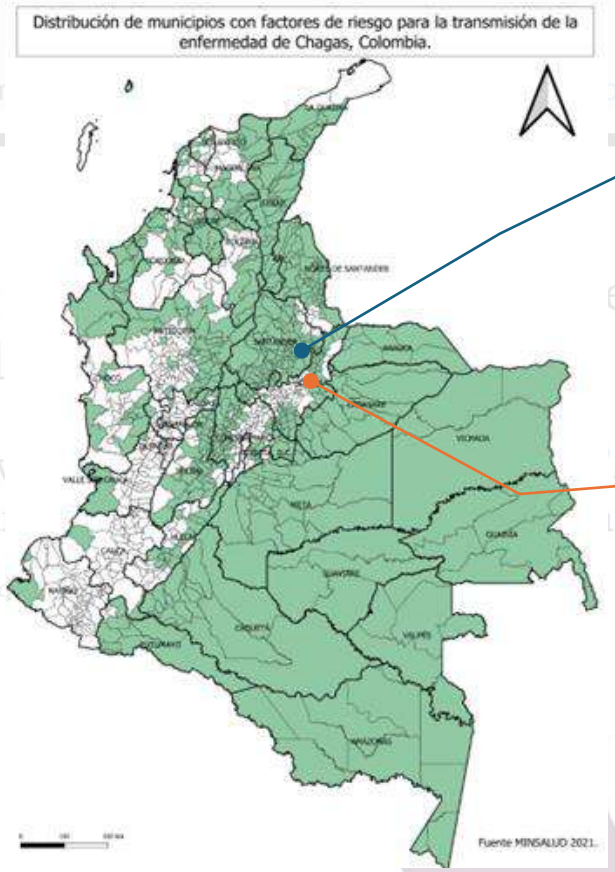
- ¿Cuál es el GB atribuible a la eCh en dos municipios endémicos de Colombia?
- ¿Cuál es la diferencia entre los GB entre pacientes atendidos en el nivel primario de atención y niveles de mayor complejidad?

Análisis transversal de costos

91

2019 - 2020

- ✓ Perfil socioeconómico de los pacientes
- ✓ GB (costos y el tiempo dedicado al transporte, alojamiento y alimentación)
- ✓ Pérdida de ingreso



Departamento: **Santander**
Municipio: **Mogotes**

Departamento: **Boyacá**
Municipio: **Soatá**

GB (gasto de bolsillo) = gastos médicos directos + no médicos

Resultados Perfil socioeconómico (n=91)

64.8% 35.2%

Residencia

56%

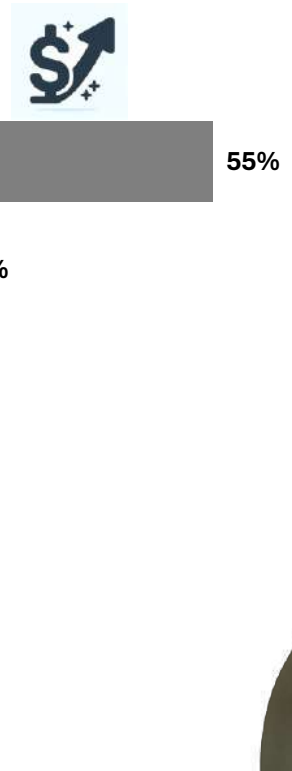
44%

Régimen de salud

Subsidiado 91%

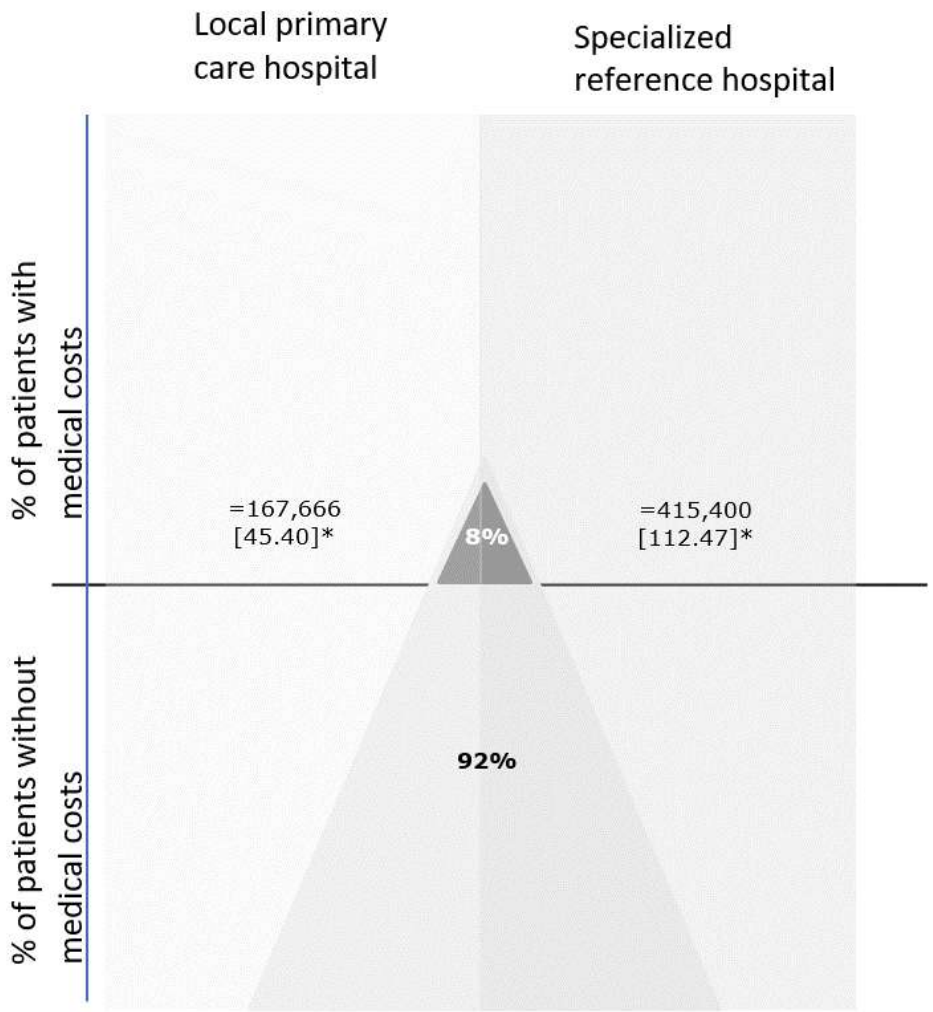
Contributivo 9%

Ingresos diarios



Salario diario legal, Colombia 2020 = US $7.92

Resultados GB y perdida de ingreso **por nivel de atención**



* COP: Colombian pesos [US$]. The values correspond to average of medical cost (paid per test/exams) per healthcare level.

Burden on patients and families	Primary healthcare level	Secondary or tertiary healthcare level	Impact of receiving care in primary healthcare
Travel time			
% patients impacted	58	67	4
Average time	<1 hour	>4 hours	Four-fold reduction in travel time
Transportation			
% patients impacted	60	83	5
Average spending*	12,986 [3.52]	68,453 [18.53]	Five-fold reduction in expenses
Food and housing expenses			
% patients impacted	65	94	5.5
Average spending*	10,626 [2.88]	57,991 [15.70]	Five-fold reduction in expenses
Income losses			
% patients impacted	46	51	2
Average spending*	22,982 [6.22]	45,400 [12.29]	Two-fold reduction in income losses

* COP: Colombian pesos [US$]

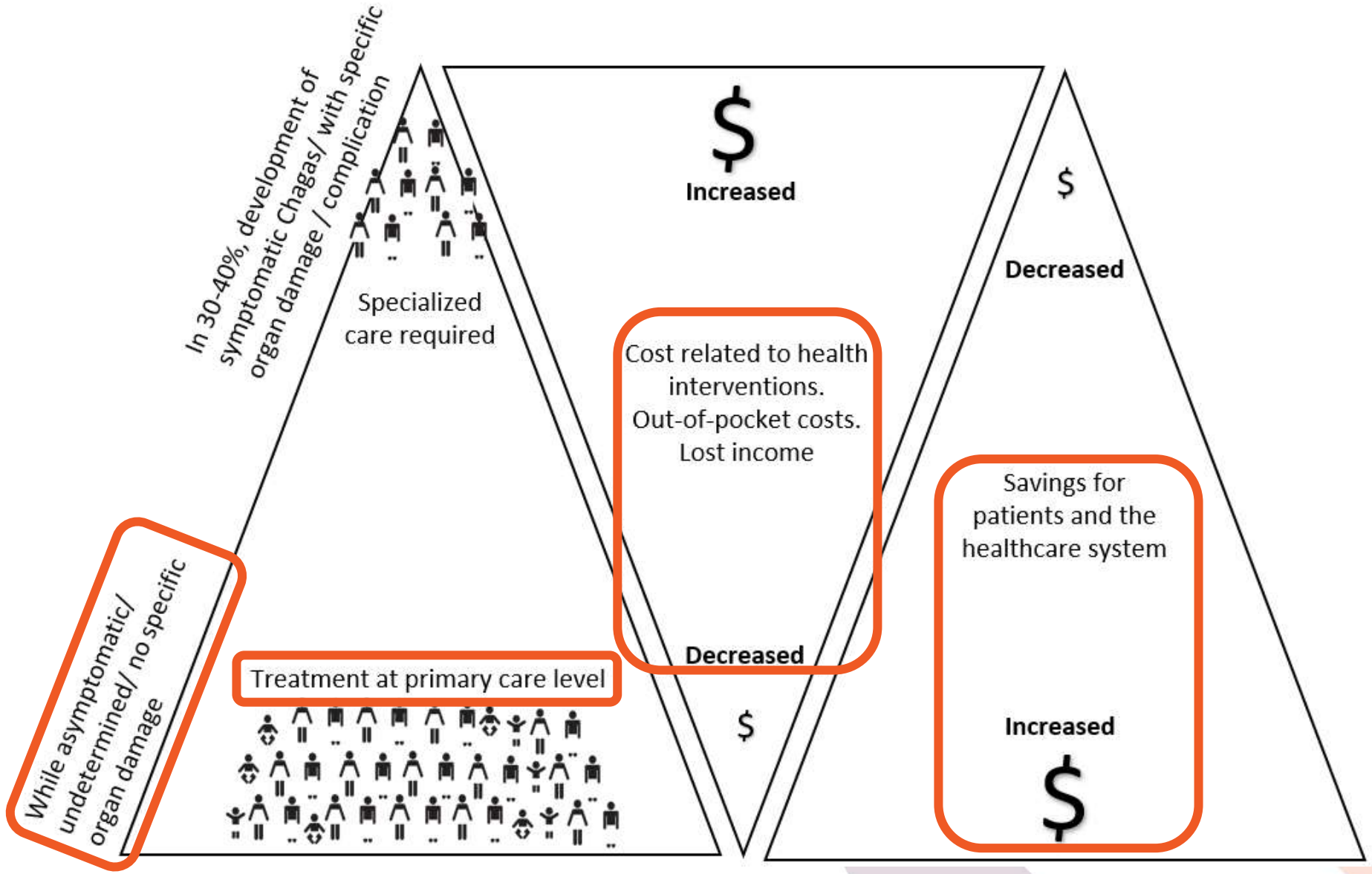
GB (gasto de bolsillo) = gastos médicos directos + no médicos

Para concluir

Algoritmo diagnóstico simplificado (basados en PDR)

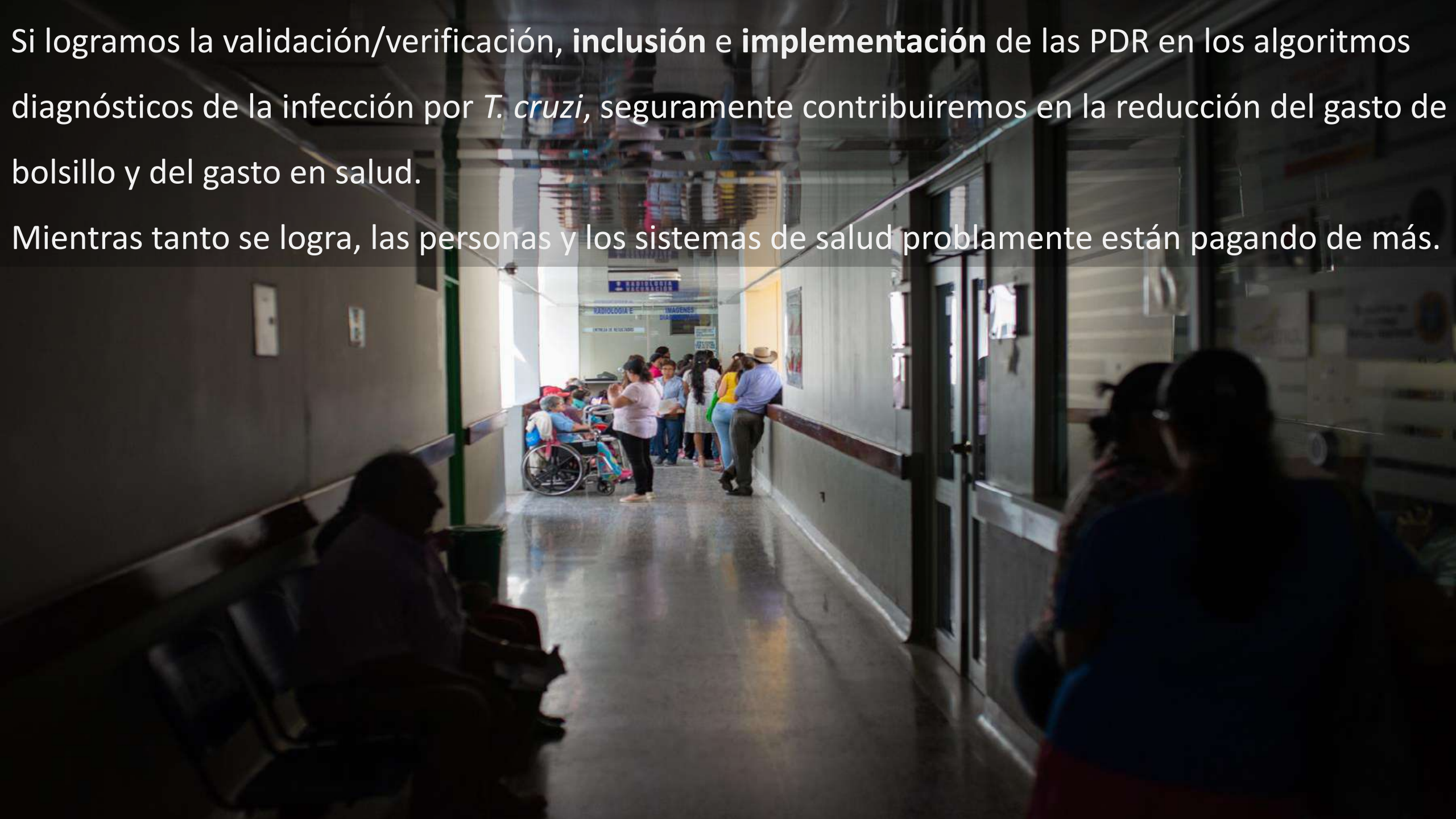
Es una necesita la implementación de una Ruta de atención centrada en el paciente (individual / comunitaria) con detección y tratamiento temprano

¡Desde todas las perspectivas se gana!



Si logramos la validación/verificación, **inclusión** e **implementación** de las PDR en los algoritmos diagnósticos de la infección por *T. cruzi*, seguramente contribuiremos en la reducción del gasto de bolsillo y del gasto en salud.

Mientras tanto se logra, las personas y los sistemas de salud problamente están pagando de más.



Thank You.

Contact info: rherazo@dndi.org

ORGANIZED BY

IN COOPERATION WITH

COORGANIZED BY

CUIDA CHAGAS

Cost-effectiveness analyses evaluating the incorporation of new diagnostic methods for CD in the health systems of Brazil, Bolivia, and Colombia

---

Yerly Magnolia Useche

Cost Effectiveness Team

Speaker

FIOCRUZ

INI Instituto Nacional de Infectologia Evandro Chagas

Unitaid SAVE LIVES FASTER

SUS

MINISTÉRIO DA SAÚDE

GOVERNO FEDERAL BRASIL UNIÃO E RECONSTRUÇÃO

CUIDA Chagas Protocols

Implementation

Validation of diagnostic algorithms based on Rapid Tests

Clinical Trial

Content

Health Economics Analysis Plan (HEAP)
Validation Protocol

Cost-effectiveness study of "Evaluation of algorithms based on rapid test to diagnose chronic infection of Trypanosoma cruzi in Brazil, Bolivia and Colombia"

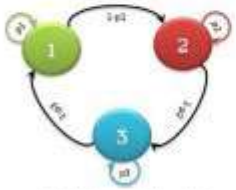
Contents of HEAP

Aim

Design

Data collection

Economic Evaluation



Model simulation

Reporting

1. Aim

Compare the cost-effectiveness of the use of algorithms based on RDTs versus the use of the standard algorithm to diagnose chronic CD in endemic countries.

2. Design

Acervo CUIDA Chagas

Design

Population:
Chronic adult and children

Primary Health Care Centers (PHCs)

3 or 4 regions / country
I:NI ➡ 200:200

Data Source

Primary: Project Forms

Secondary:
Literature
Goverment data bases

Acervo CUIDA

3. Data collection

Data collection

REDCap
Research Electronic Data Capture

FIOCRUZ

Descriptive statistical analysis of variables

Forms:

Healthcare resource use:

- Case Report
- Direct costs

Participants:

- Indirect costs
- EQ5D-3L

Data collection

1. Healthcare resource use in diagnosis:

Outcome of effectiveness:

% Correctly DC-diagnosed

% DC-missdiganosed individuals

Accuracy estimation:

Sensibility

Specificity

Each diagnostic algorithm / Country

General accuracy: meta-analysis method

Data collection

2. Unit costs: Microcosting

Protection

Disposal waste

Personal

DIRECT COSTS

Test application

Transportation
Refrigeration Samples

INDIRECT COSTS

Data collection

3. Health-related quality of Life (HRQOL):

EQ5D-3L application:

- Diagnosis time
- Posterior times: Implementation Protocol

4. Handling missing data (MD):

Missing data

MD Randomness
- MD completely at random (MCAR) → Record
- MD at random (MAR) → Multiple imputation

Association missingness and baseline values

4. Economic evaluation

Acervo CUIDA Chagas

Economic evaluation

1. Study perspectives:

Payer's perspective → Primary health system: Direct costs

Societal perspective → Indirect costs

2. Timing of analyses: Collecting data

Primary data:

Secondary data:

Economic evaluation

3. Discount rates for costs and benefits: 3 – 5%

WHO-CHOICE (Bertram, et al., 2021)
Brazilian Ministry of Health Economic Assessment Guidelines (Brasil, 2014).

4. Cost–effectiveness thresholds

Opportunity-cost-based cost-effectiveness thresholds (Ochalek, et al., 2018; Woods, et al., 2016).

Economic evaluation

5. Healthcare resources costs:

Analysis:

Mean (SD) {
- Item cost
- Participant: Σ individual costs
- Grouped: Intervention/Current protocol

Report:

Difference adjusted between means (95% CI)

Economic evaluation

6. Analysis and reporting of QALYs:

Qualys estimated differences from baseline in each CD state
Intervention/Current protocol
Bar charts

7. Cost utility analysis: Incremental cost and outcome

Decision trees coupled with Markov model microsimulation
ICER (Mean, 95%CI):

$$ICER = \frac{(C_1 - C_0)}{(E_1 - E_0)}$$

INMB = (E * WTP) – C
E:effectiveness; WTP:willingness-to-pay threshold; C:cost

Economic evaluation

8. Sensitivity and subgroup analysis:

Variation - model parameters ➡ Register of changes in outputs

Procedures
One-way
Multi-way
Extreme

➡

Probabilistic sensitivity analysis
PSA

➡

Cost-Effectiveness-Acceptability-Curves
CEAC

➡

Range of cost-effectiveness thresholds adjusted PPP (WB, 2021)

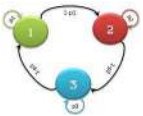
➡

Max. WTP (95% CI)

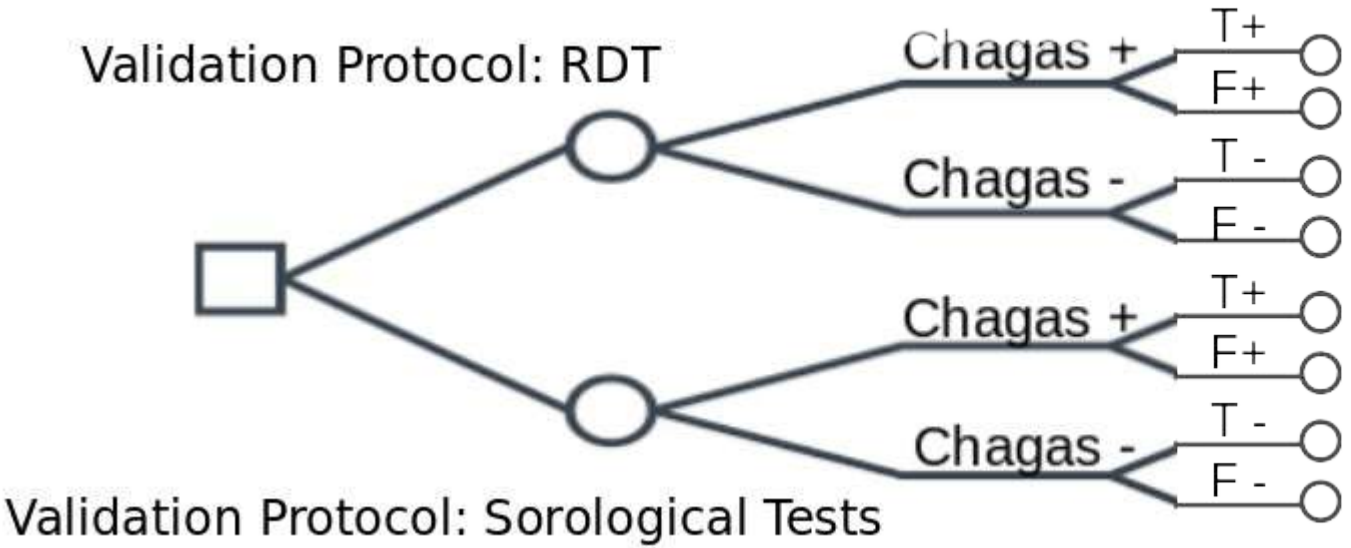
Acervo CUIDA Chagas

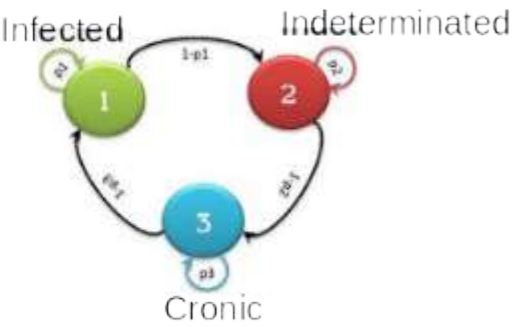
5. Model simulation

Model simulation



1. Model structure:

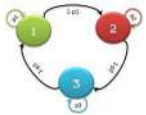




Adjusting Transition probabilities between CD states by age-sex

Multinomial logistic regression model

Model simulation



2. Expected results

High efficiency of the diagnosis of the chronic CD: RDTs vs Standard test algorithm

Opportunity of diagnosing: More people / Unit - time

Increase coverage: Diagnosis – Treatment

Reporting

Cheers statement (Husereau, 2022)

Search for reporting guidelines

Use your browser's Back button to return to your search results

Consolidated Health Economic Evaluation Reporting Standards 2022 (CHEERS 2022) Statement: Updated Reporting Guidance for Health Economic Evaluations

Reporting guideline provided for? (i.e. exactly what the authors state in the paper)	Economic evaluations of health interventions
	CHEERS 2022 checklist (PDF)
	Interactive CHEERS 2022 checklist to generate a completed Word or PDF checklist

References

Bertram MY, et al. 2021. Methods for the Economic Evaluation of Health Care Interventions for Priority Setting in the Health System: Na Update From WHO CHOICE. Int J Health Policy Manag. 2021 Nov 1;10(11):673-677.doi: 10.34172/ijhpm.2020.244.

Brasil. 2014. Ministério da Saúde. Secretaria de Ciência, Tecnologia e Insumos Estratégicos. Departamento de Ciência e Tecnologia. Diretrizes metodológicas: Diretriz de Avaliação Econômica / Ministério da Saúde, Secretaria de Ciência, Tecnologia e Insumos Estratégicos, Departamento de Ciência e Tecnologia. – 2. ed. – Brasília: Ministério da Saúde.

Husereau, D., et al. 2022. Consolidated Health Economic Evaluation Reporting Standards (CHEERS 2022) statement: updated reporting guidance for health economic Evaluations. BMC Med 20, 23. https://doi.org/10.1186/s12916-021-02204-0.

Ochalek J, et al., 2018. Estimating health opportunity costs in low-income and middle-income countries: a novel approach and evidence from cross-country data. BMJ Glob Health. Nov 5;3(6):e000964. doi: 10.1136/bmjgh-2018-000964.

Woods B, et al. 2016. Country-Level Cost-Effectiveness Thresholds: Initial Estimates and the Need for Further Research. Value Heal. Dec 1;19(8):929–35.

Thank You.

Contact info:

Yerly M. Useche
Cost effectiveness Team Member
Telefone: +55 21 972124092
Email: yerlyuseche@cuidachagas.org

ORGANIZED BY

IN COOPERATION WITH

COORGANIZED BY

Cost-Effectiveness Analysis of Chagas disease RDT in a health facility setting

Elisa Sicuri

Associate Professor
ISGlobal, LSE Health

Iris Lopes-Rafegas

Post-doctoral Fellow
ISGlobal

Model info

Strategies:

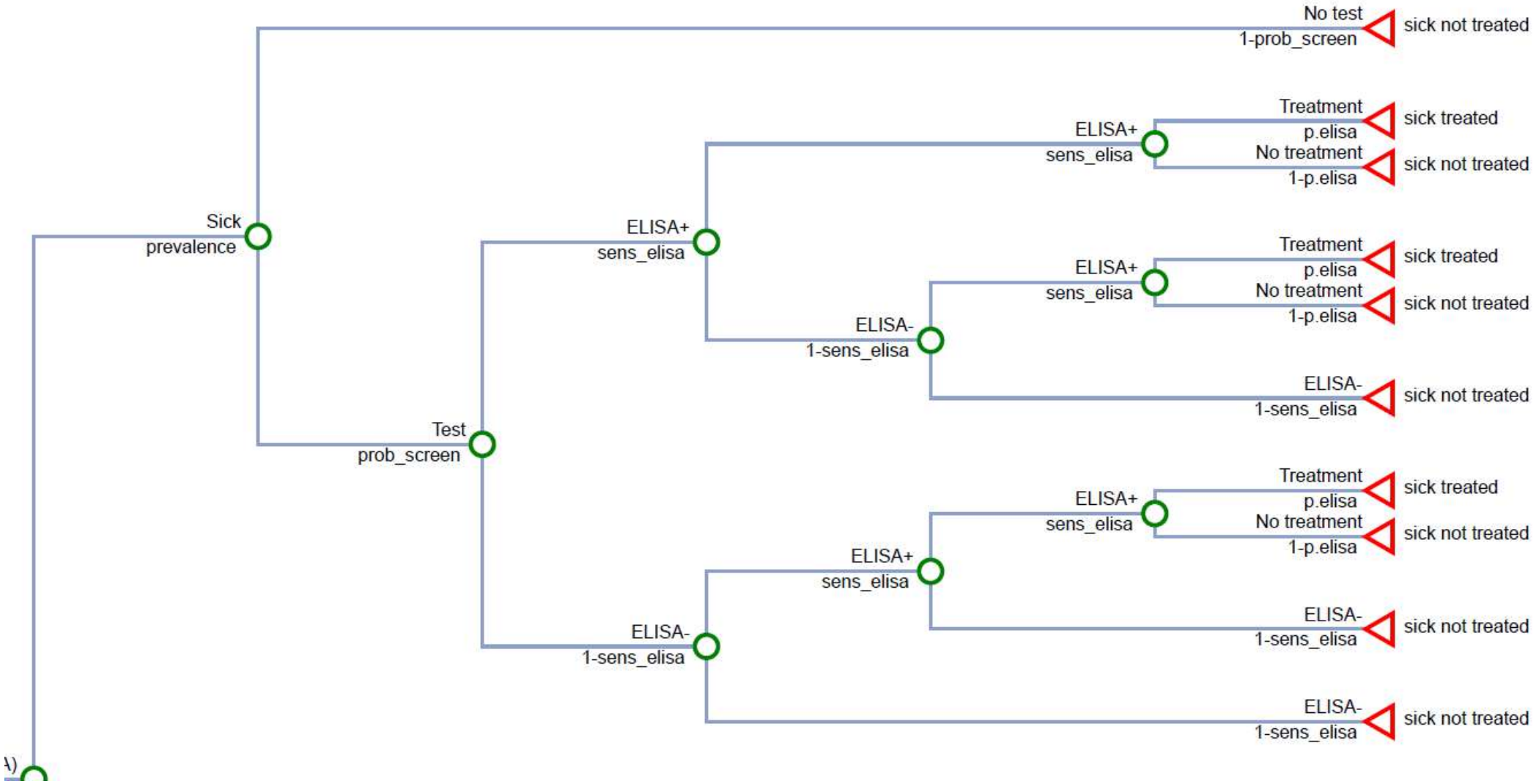
- ELISA - ELISA x2 (+1 ELISA)
- RDT - RDT x2 (+1 RDT)
- Mixed - RDT + ELISA (+1 ELISA)

Model structure:

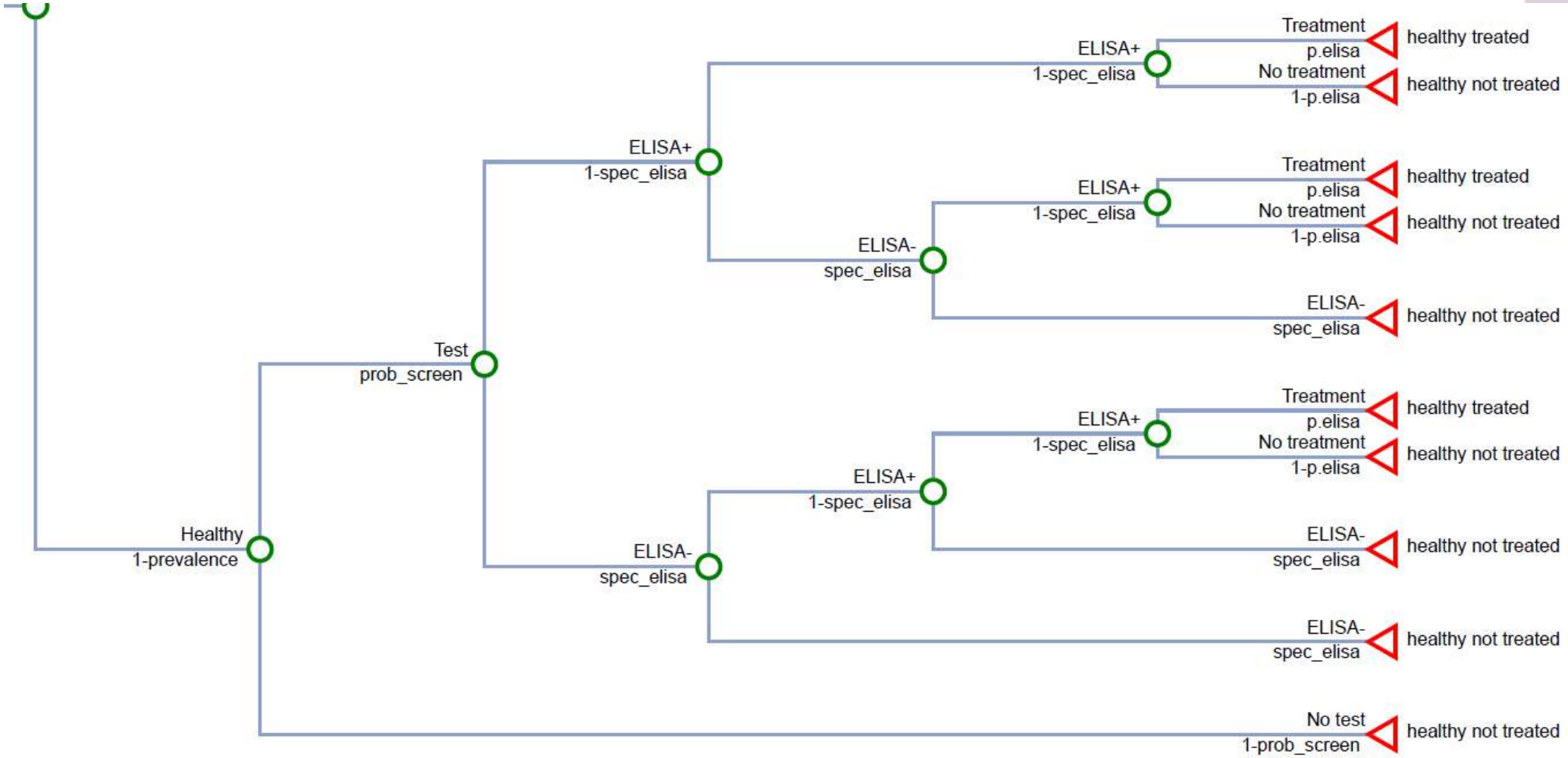
Decision tree + Markov model

Decision tree structure

ELISA, sick



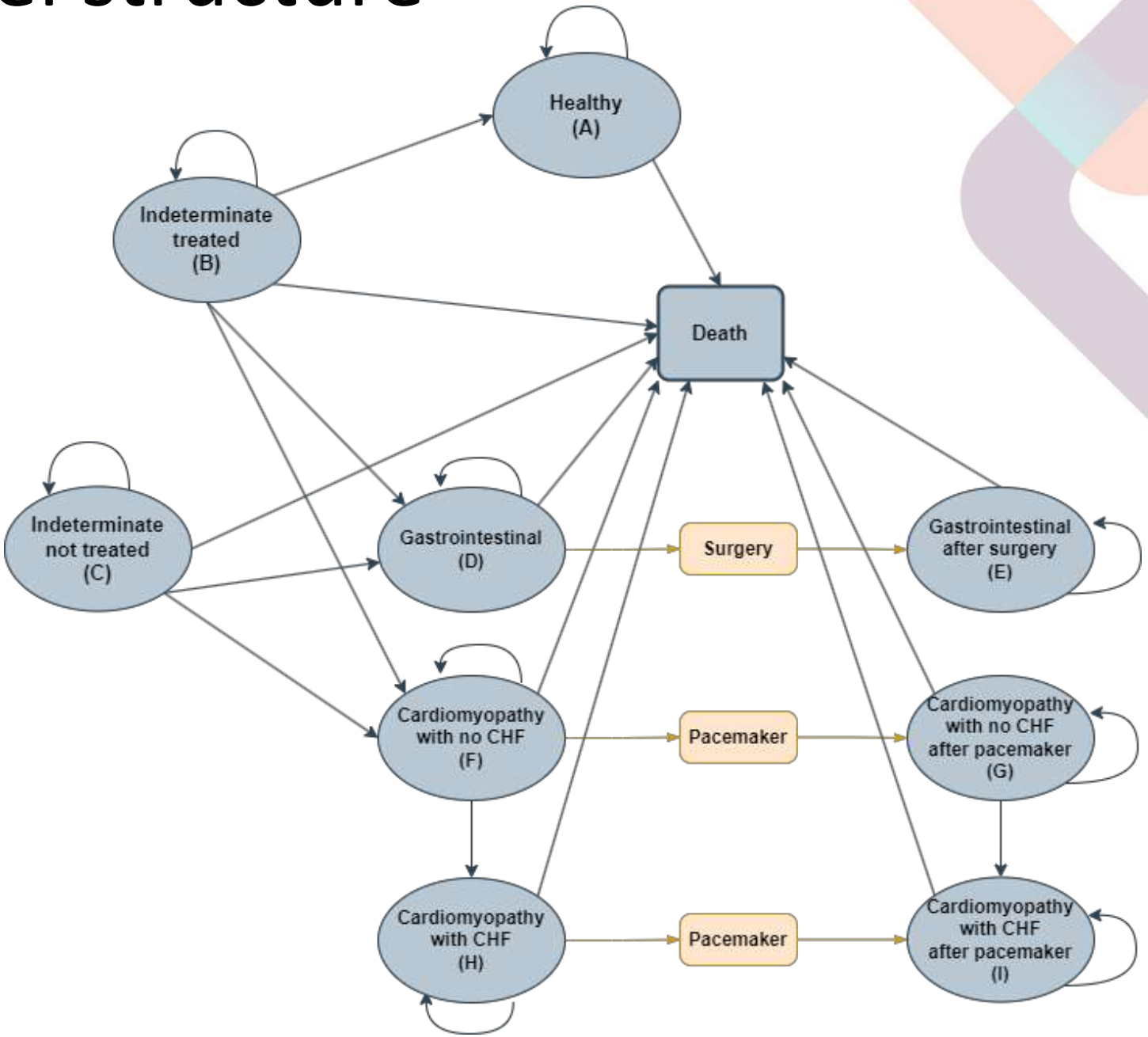
Decision tree structure
ELISA, healthy



Parameters decision tree

	Formula	Value	Senstivity Analysis
Prevalence	P(Sick)	0.2	At 1%, 5% and 10%
Probability Testing	P(Test)	0.07	Threshold analysis
Probability Treatment	P(Treat)	0.32	Threshold analysis
Sensitivity ELISA	P(ELISA + | Sick)	0.97	
Specificity ELISA	P(ELISA - | Healthy)	0.98	
Sensitivity RDT	P(RDT + | Sick)	0.875	Threshold analysis
Specificity RDT	P(RDT - | Healthy)	0.992	
Price ELISA		4.36	
Price RDT		6.5	Threshold analysis

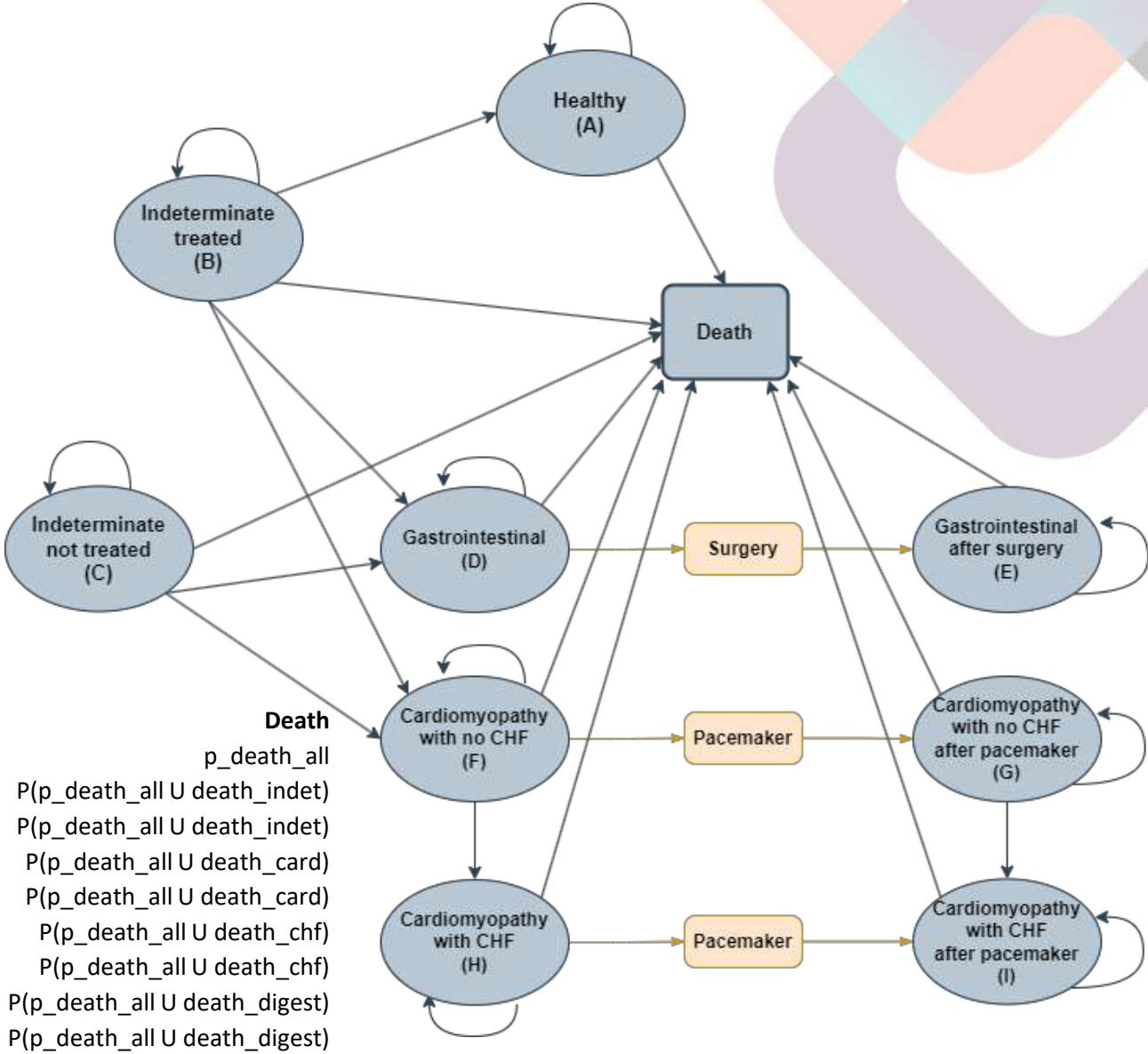
Markov model structure



Markov model structure

Initial Probabilities		Transition probabilities		Death probabilities	
Indeterminate	65%	p_incar	2.00%	death_indet	0.38%
Cardiomyopathy	20%	p_carchf	4.00%	death_card	4.39%
CHF	10%	p_indig	0.23%	death_chf	30.14%
Digestive	5%	p_pacemaker	2.00%	death_digest	2.45%
		p_surgery	1.00%	death_all	INE Bolivia

t / (t+1)	A	B	C	D	E	F	G	H	I	Death
A	C	0	0	0	0	0	0	0	0	p_death_all
B	0	C	0	p_incar	0	0	0	p_indig	0	P(p_death_all U death_indet)
C	0	0	C	p_incar	0	0	0	p_indig	0	P(p_death_all U death_indet)
D	0	0	0	C	0	p_carchf	0	0	0	P(p_death_all U death_card)
E	0	0	0	0	C	0	0	0	0	P(p_death_all U death_card)
F	0	0	0	0	0	C	0	0	0	P(p_death_all U death_chf)
G	0	0	0	0	0	0	C	0	0	P(p_death_all U death_chf)
H	0	0	0	0	0	0	0	C	0	P(p_death_all U death_digest)
I	0	0	0	0	0	0	0	0	C	P(p_death_all U death_digest)
Death	0	0	0	0	0	0	0	0	0	1



Parameters Markov Model

Model Inputs		Costs		QALYs	
Age	35	Healthy	50	Healthy	1
Cycles	60	Indeterminate	91.21	Indeterminate	0.96
Discount rate	0.03	Cardiomyopathy	775.26	Cardiomyopathy	0.77
Population	10000	CHF	2038.23	CHF	0.665
		Digestive	893.07	Digestive	0.8
		Pacemaker	822.13	Death	0
		Surgery	46.6		
		Drug	409.46		

Results Markov Model

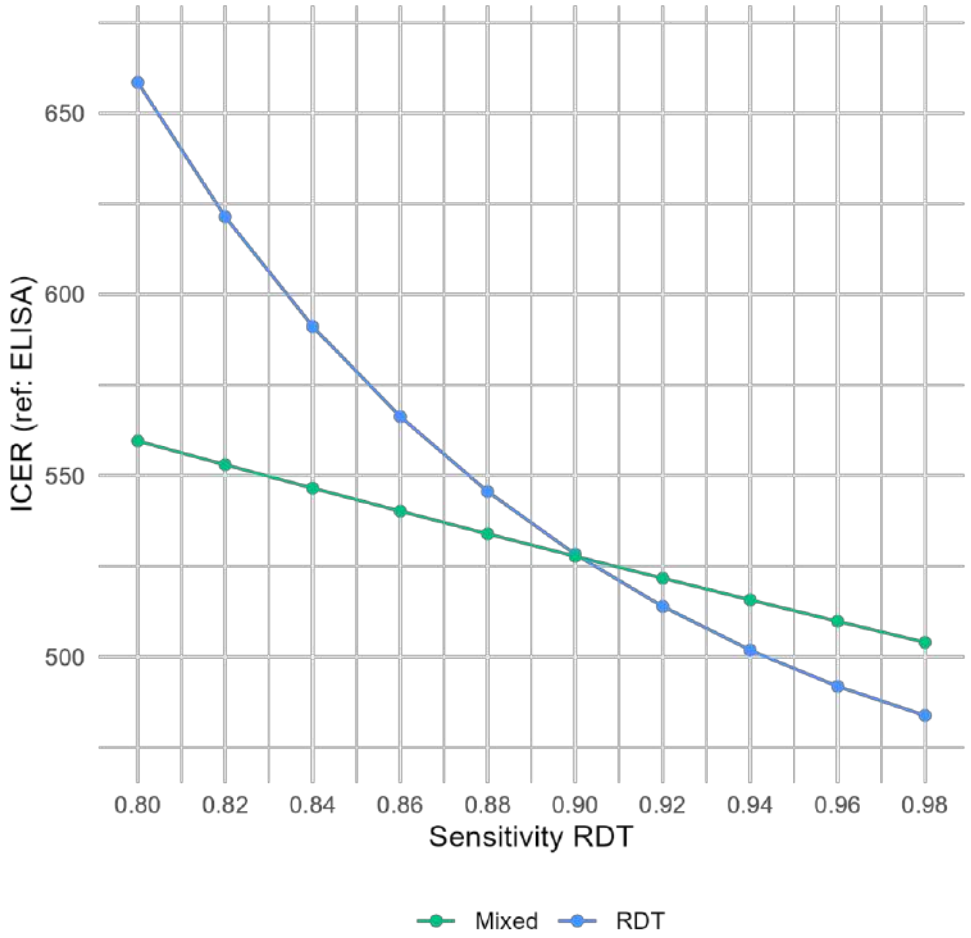
	ELISA	MIXED	RDT
Total Cost	**$2,136.06**	**$2,136.23**	**$2,136.39**
Testing Cost	$0.62350	$0.77595	$0.93568
Treatment Cost	$1.84	$1.82	$1.76
Disease Prog. Cost	$2,133.60	$2,133.63	$2,133.69
Total QALYs	**21.5054**	**21.5057**	**21.5060**

- RDT has lower sensitivity.
  - Higher testing costs (confirmatory test).
  - Lower proportion of treated individuals (under the assumption of equal probability of linkage to care). Fair assumption?

		ELISA	MIXED	RDT
Sick	Treat	2.23%	2.22%	2.14%
	No Treat	97.77%	97.78%	97.86%
		100.00%	100.00%	100.00%

Threshold analysis

Sensitivity RDT

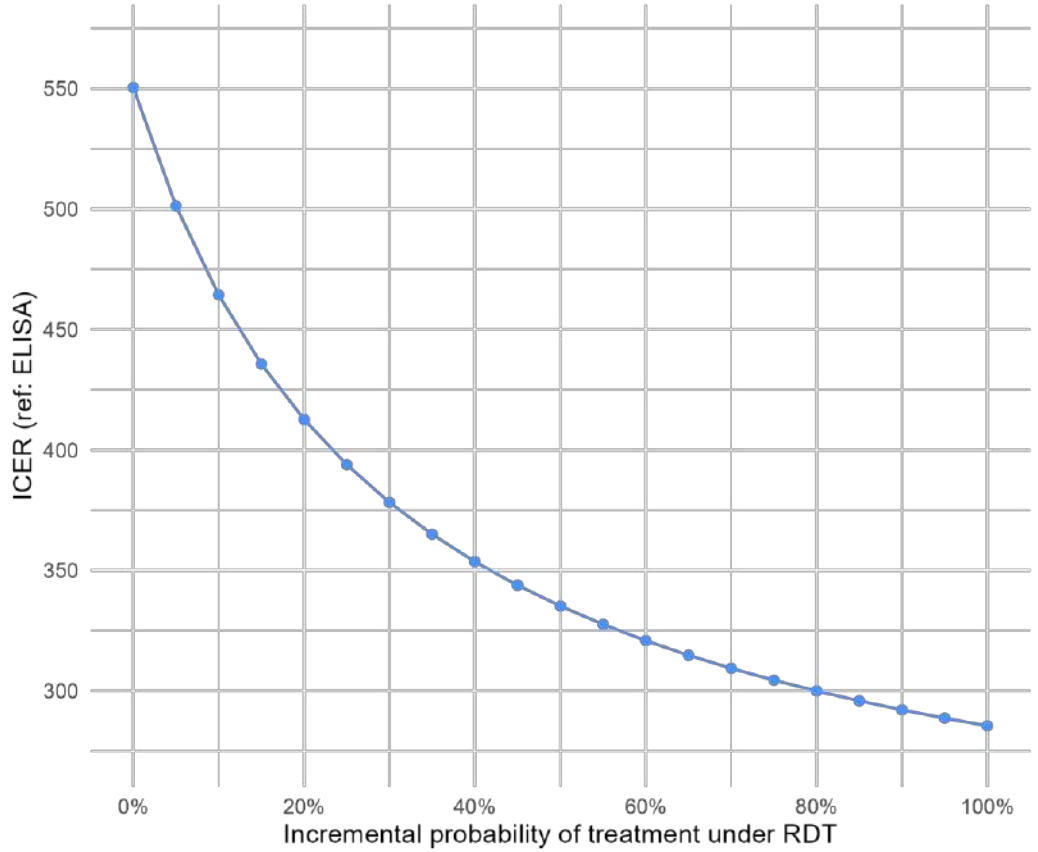


- Assumed sensitivity RDT at baseline model = 0.875
- Mixed strategy is more cost-effective than RDT up to an RDT sensitivity of 90%.
- For sensitivity >90%, RDT strategy is more cost effective than the Mixed strategy.

**Baseline scenario - prevalence = 20%*

Threshold analysis

Linkage to care RDT (incremental)

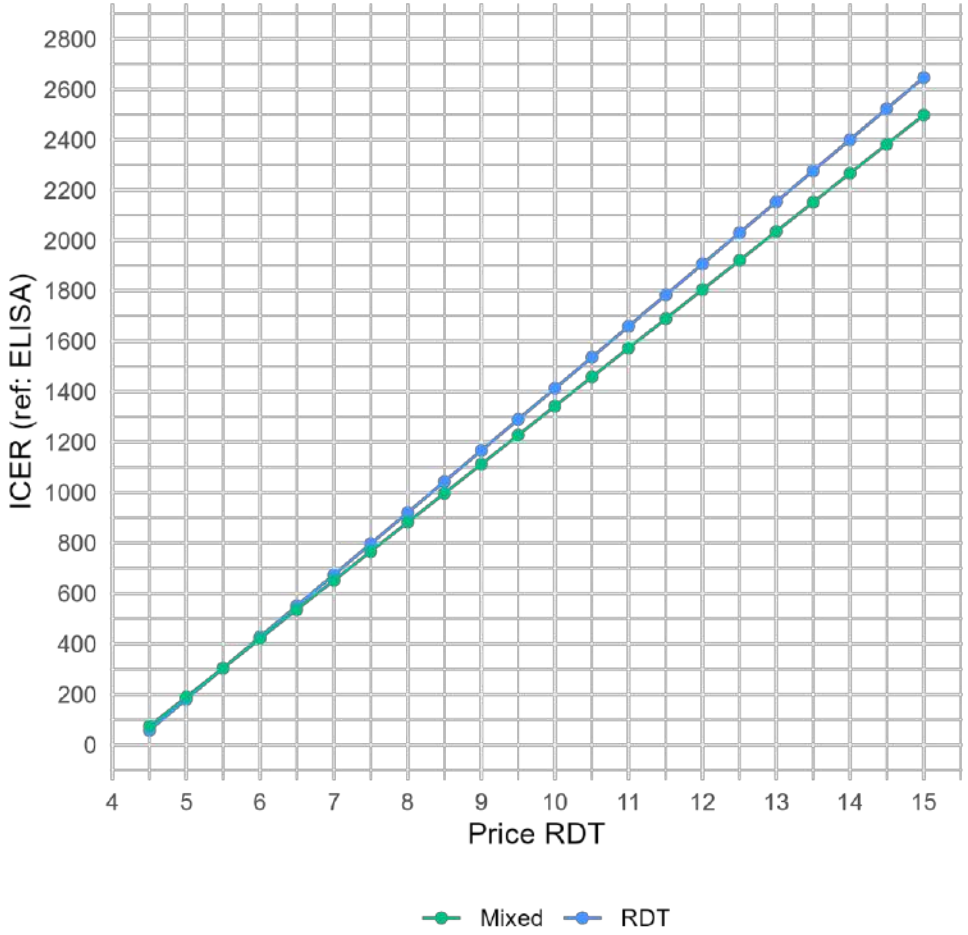


- In the baseline model, we assume prob. treatment under ELISA = prob. treatment under RDT
- Plot how ICER changes when the probability of treatment under RDT strategy increases by X% compared to ELISA
  - Driver: increased linkage to care.
- The higher the linkage to care of the RDT strategy vs. ELISA the more cost-effective the RDT strategy.

**Baseline scenario - prevalence = 20%*

Threshold analysis

Price RDT



- The mixed strategy is more cost-effective under prevalence 20%.
- Higher RDT unit prices make either strategy more costly compared to ELISA strategy.

**Baseline scenario - prevalence = 20%*

Scenario 1 – Prevalence at 10%

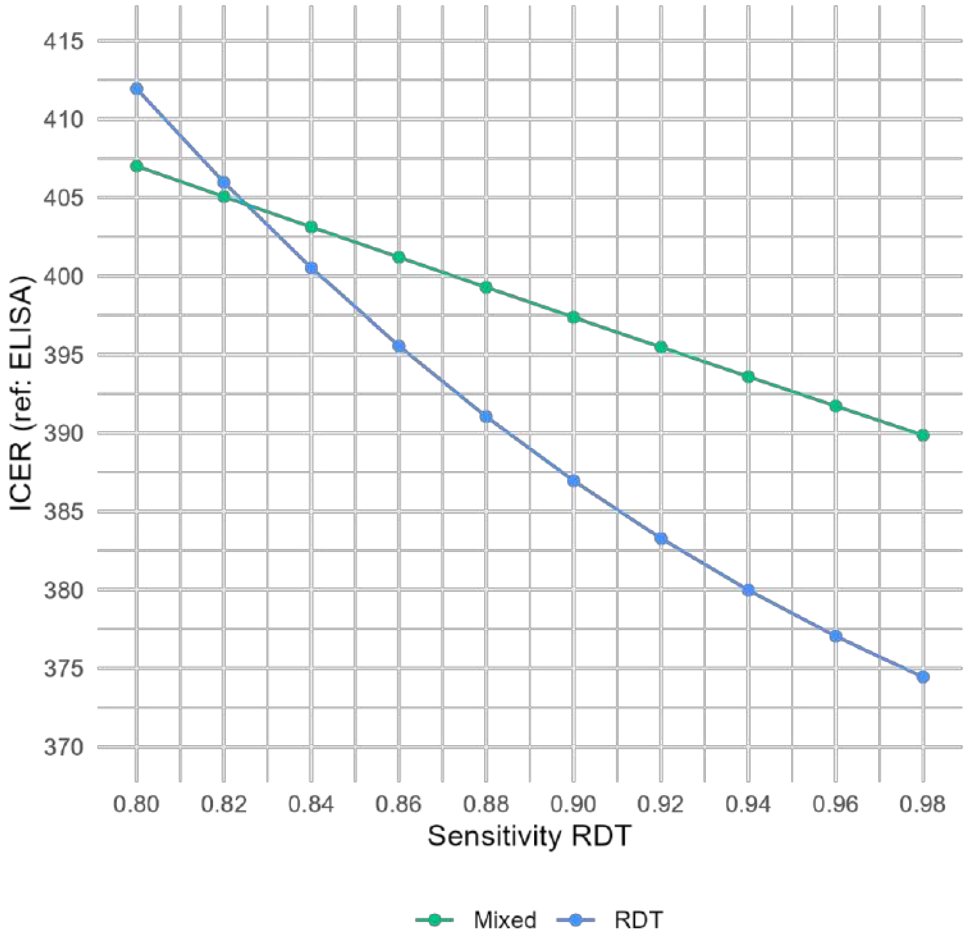
- Change in parameters:

	Formula	Value	Senstivity Analysis
Prevalence	P(Sick)	0.10	At 1%, 5% and 10%
Probability Testing	P(Test)	0.07	Threshold analysis
Probability Treatment	P(Treat)	0.32	Threshold analysis
Sensitivity ELISA	P(ELISA + | Sick)	0.97	
Specificity ELISA	P(ELISA - | Healthy)	0.98	
Sensitivity RDT	P(RDT + | Sick)	0.875	Threshold analysis
Specificity RDT	P(RDT - | Healthy)	0.992	
Price ELISA		4.36	
Price RDT		6.5	Threshold analysis

- Same decision tree and markov model structures.

Threshold analysis

Sensitivity RDT



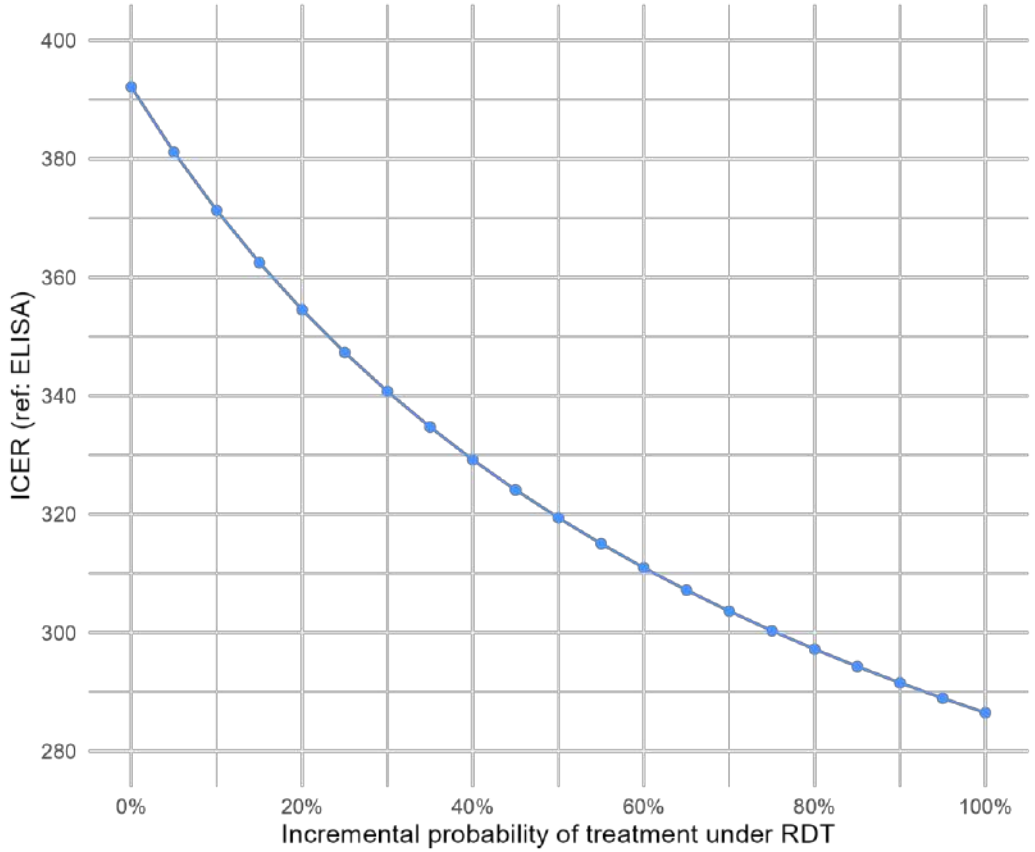
Under prevalence 10%,

- Mixed strategy is more cost-effective than RDT up to an RDT sensitivity of 82-83%.
- For sensitivity >83%, RDT strategy is more cost effective than the mixed strategy.

**Alternative scenario 1 - prevalence = 10%*

Threshold analysis

Linkage to care RDT (incremental)

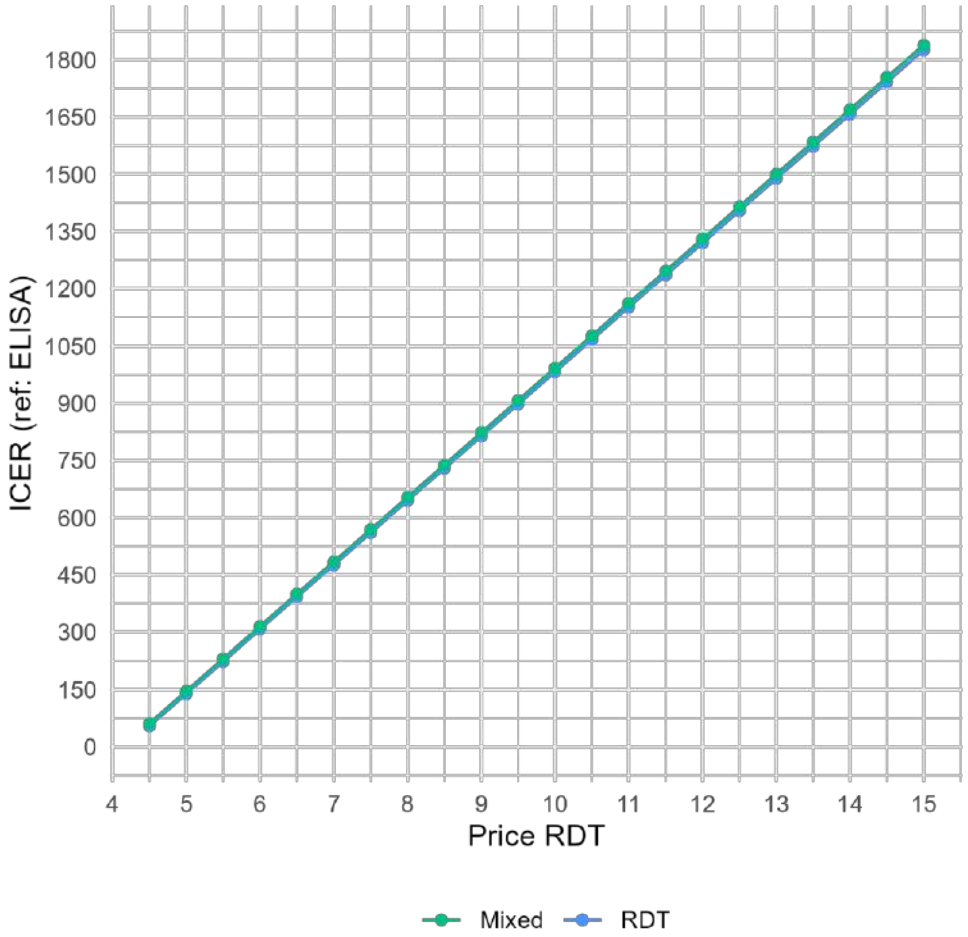


The higher the linkage to care of the RDT strategy vs. ELISA the more cost-effective the RDT strategy.

**Alternative scenario 1 - prevalence = 10%*

Threshold analysis

Price RDT



- The RDT strategy is (slightly) more cost-effective under prevalence 10%.
- Higher RDT unit prices make either strategy more costly compared to ELISA strategy.

**Alternative scenario 1 - prevalence = 10%*

Scenario 2 – Prevalence at 5%

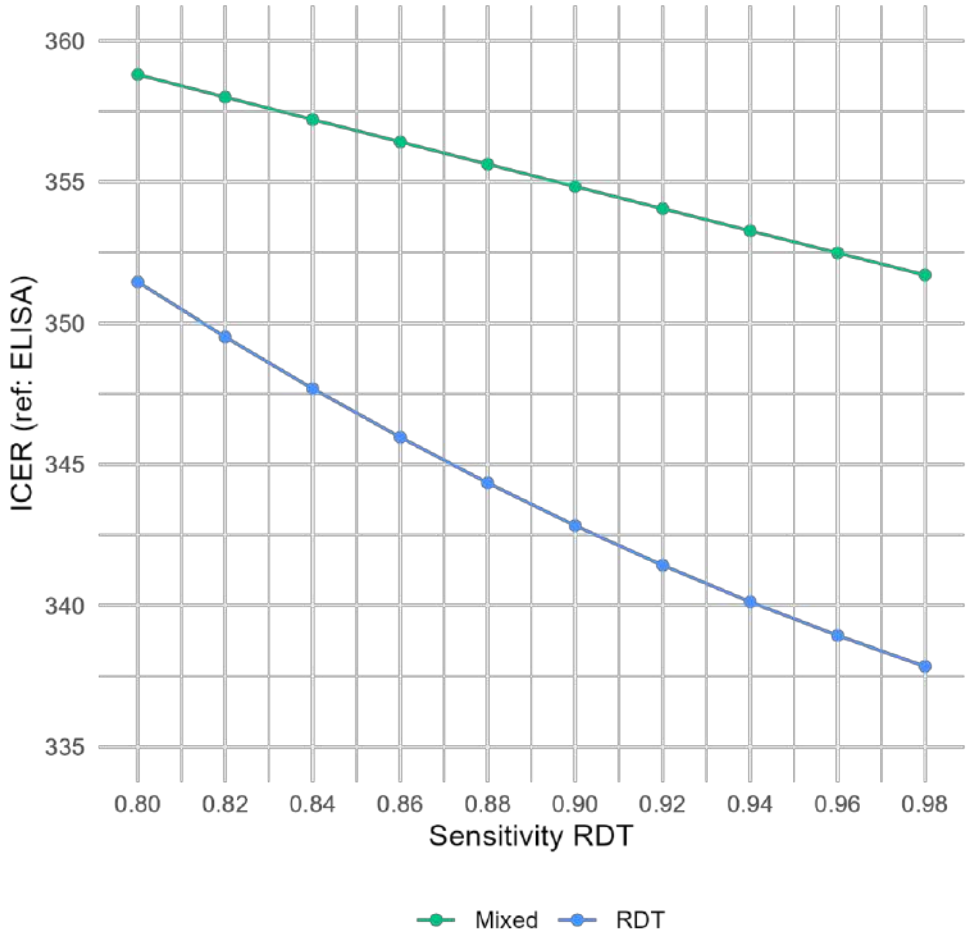
- Change in parameters:

	Formula	Value	Senstivity Analysis
Prevalence	P(Sick)	0.05	At 1%, 5% and 10%
Probability Testing	P(Test)	0.07	Threshold analysis
Probability Treatment	P(Treat)	0.32	Threshold analysis
Sensitivity ELISA	P(ELISA + | Sick)	0.97	
Specificity ELISA	P(ELISA - | Healthy)	0.98	
Sensitivity RDT	P(RDT + | Sick)	0.875	Threshold analysis
Specificity RDT	P(RDT - | Healthy)	0.992	
Price ELISA		4.36	
Price RDT		6.5	Threshold analysis

- Same decision tree and markov model structures.

Threshold analysis

Sensitivity RDT

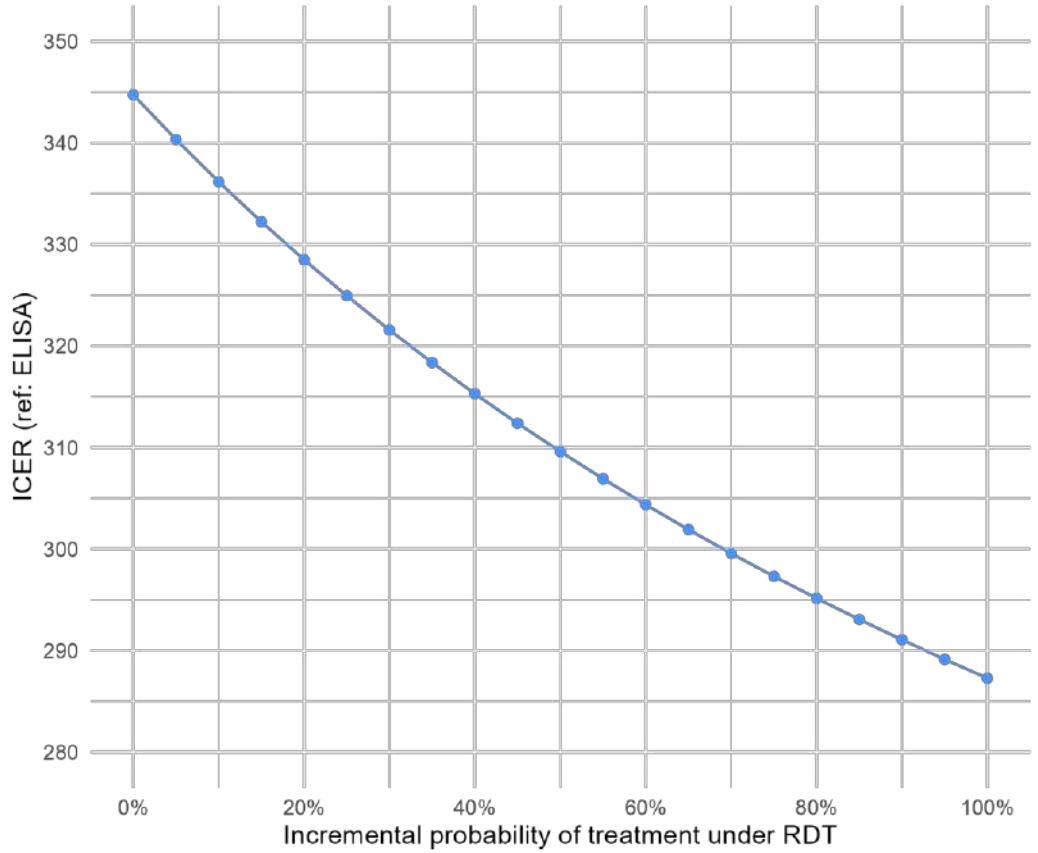


The RDT strategy weakly dominates the Mixed strategy under prevalence 5%.

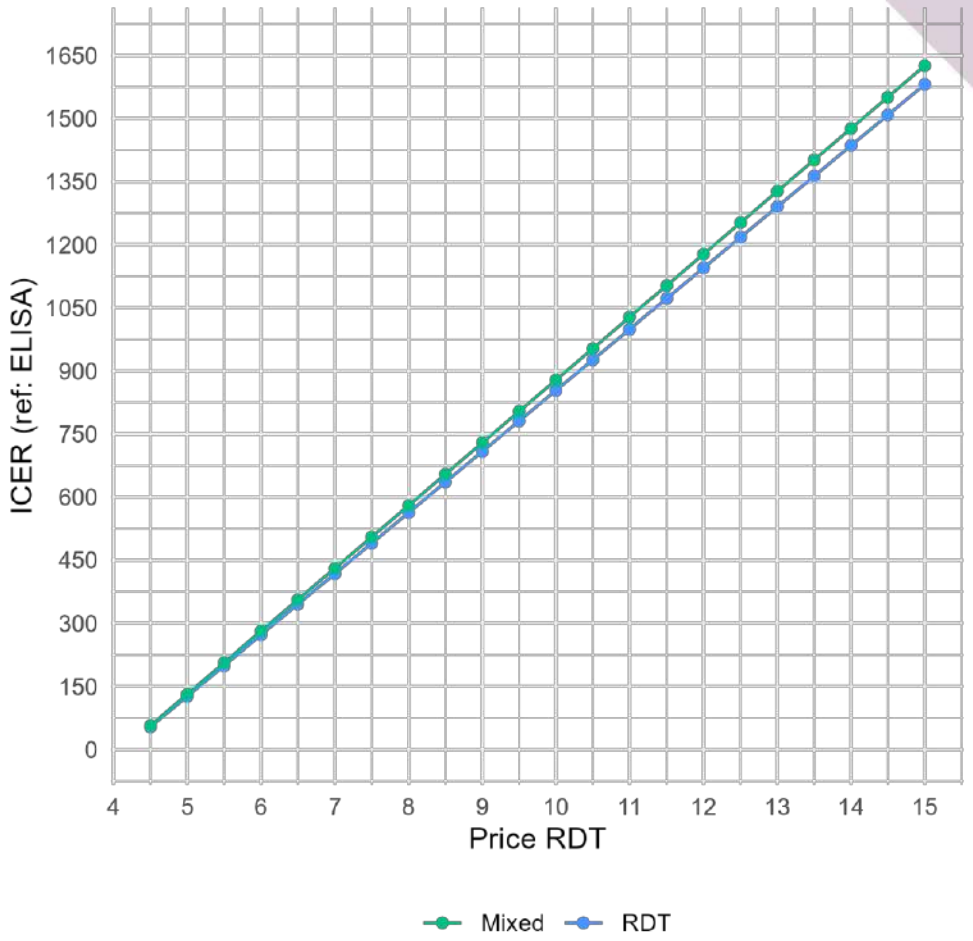
**Alternative scenario 2 - prevalence = 5%*

Threshold analysis

Linkage to care RDT (incremental)



Price RDT



**Alternative scenario 2 - prevalence = 5%*

Scenario 3 – Prevalence at 1%

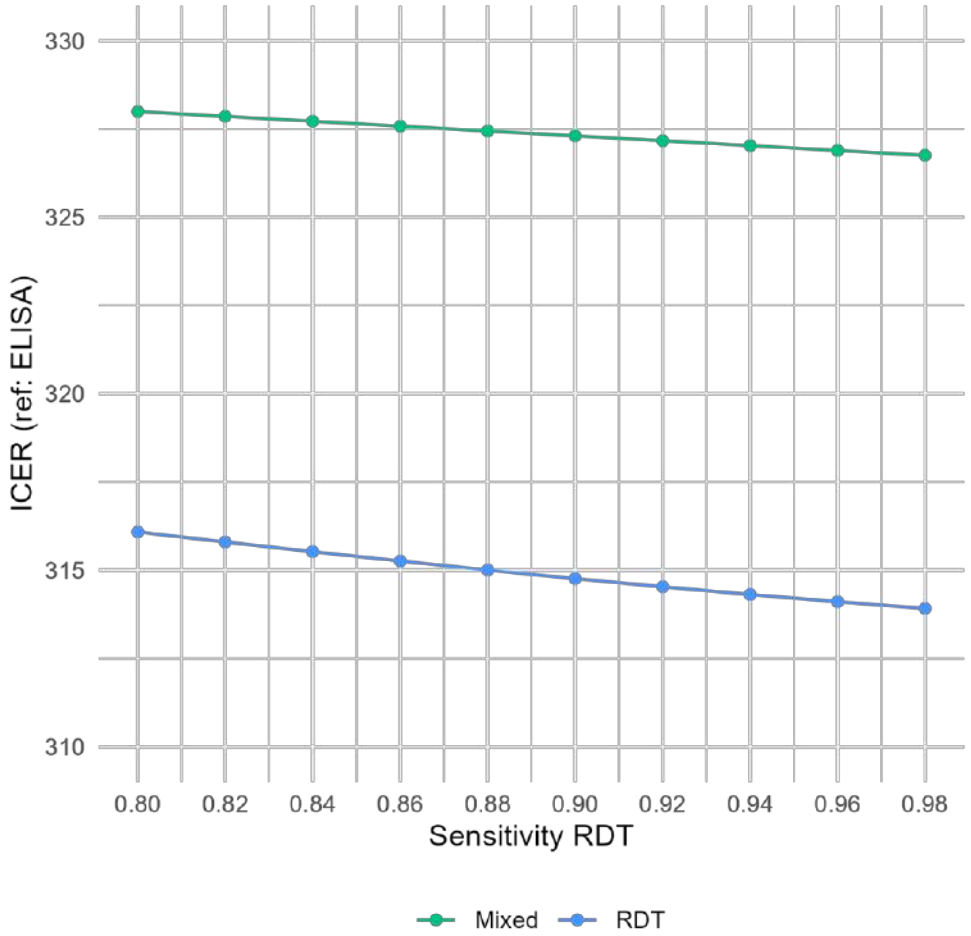
- Change in parameters:

	Formula	Value	Senstivity Analysis
Prevalence	P(Sick)	0.01	At 1%, 5% and 10%
Probability Testing	P(Test)	0.07	Threshold analysis
Probability Treatment	P(Treat)	0.32	Threshold analysis
Sensibility ELISA	P(ELISA + | Sick)	0.97	
Specificity ELISA	P(ELISA - | Healthy)	0.98	
Sensibility RDT	P(RDT + | Sick)	0.875	Threshold analysis
Specificity RDT	P(RDT - | Healthy)	0.992	
Price ELISA		4.36	
Price RDT		6.5	Threshold analysis

- Same decision tree and markov model structures.

Threshold analysis

Sensitivity RDT

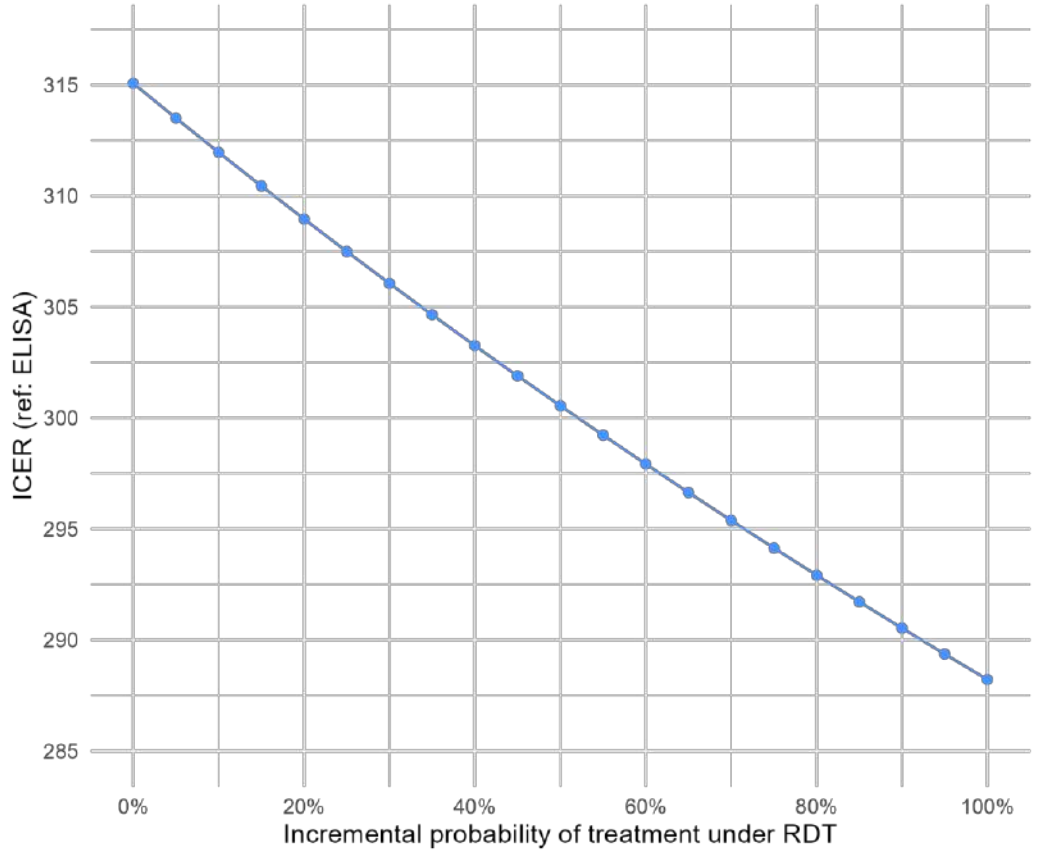


The RDT strategy extendedly dominates the Mixed strategy under prevalence 1%.

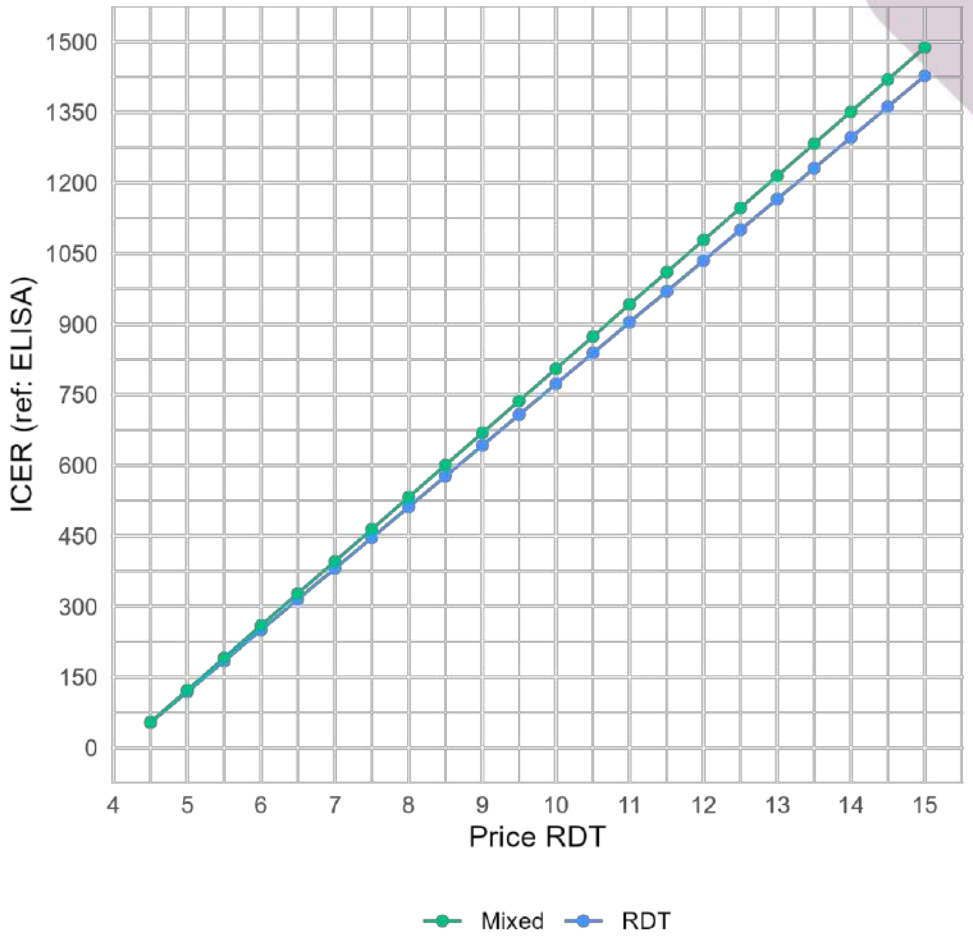
**Alternative scenario 3 - prevalence = 1%*

Threshold analysis

Linkage to care RDT (incremental)



Price RDT



**Alternative scenario 3 - prevalence = 1%*

Conclusion

- Under the current scenarios, we are conservative in the assumptions we make,
  - e.g. same probability of testing, same structural costs between the strategies.
- Focus is placed on the role of the RDT sensitivity and Price.
- At higher prevalence, RDT sensitivity has a subtantial role on the cost-effectiveness decision.
- The assumptions about incremental linkage to care are also crucial.
  - More research needed on the incremental linkage to care consequent to the use of RDTs at point of care.

Discussion

- What other scenarios should we consider?
  - e.g. other testing strategies?
  - e.g. some other clinical scenarios?
- Alternative assumptions on RDT vs ELISA?
  - Does RDT affect other parameters, e.g. does it improve the probability of testing, compared to ELISA?
- Uncertainty and lack of data on HRQoL associated with Chagas disease.
- Uncertainty of parameters to be investigated…

Thank You.

Contact info: elisa.sicuri@isglobal.org

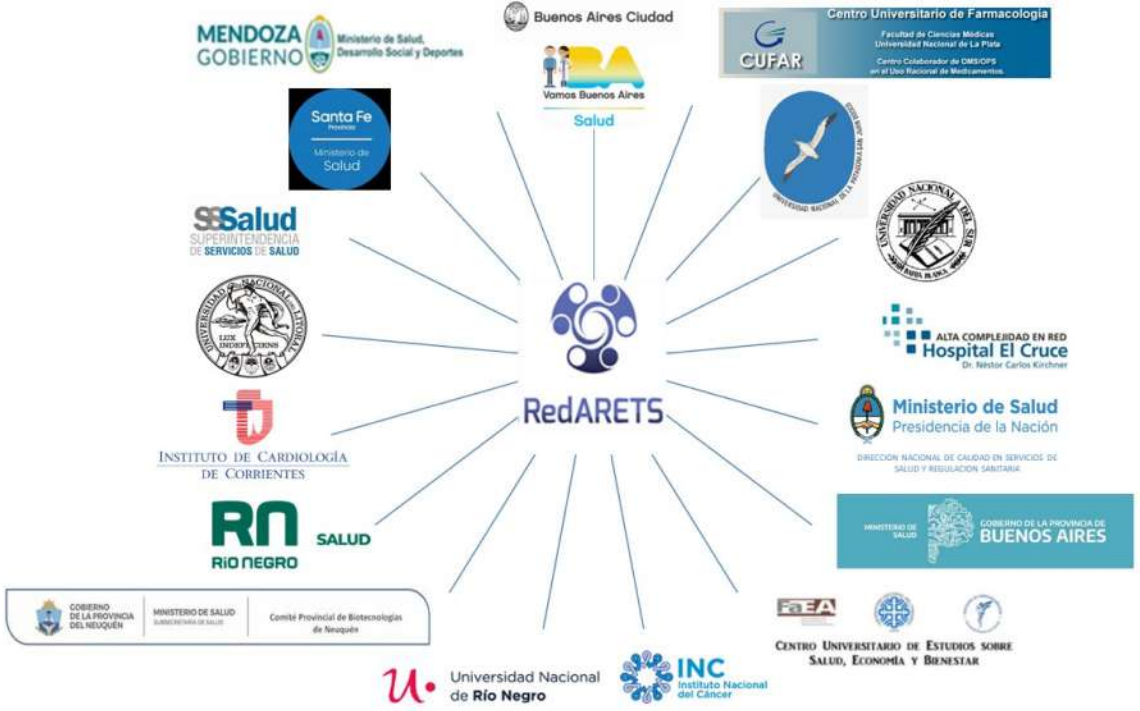
ORGANIZED BY

IN COOPERATION WITH

COORGANIZED BY

Economic evaluation of new diagnostic methods

- Describe some fundamental elements in the economic evaluations of new diagnostic methods, mainly:
  - Budget impact analysis
  - Cost-effectiveness studies
- Provide examples from evaluations carried out by our team
  - HPV – DNA test
  - Genexpert for Tuberculosis

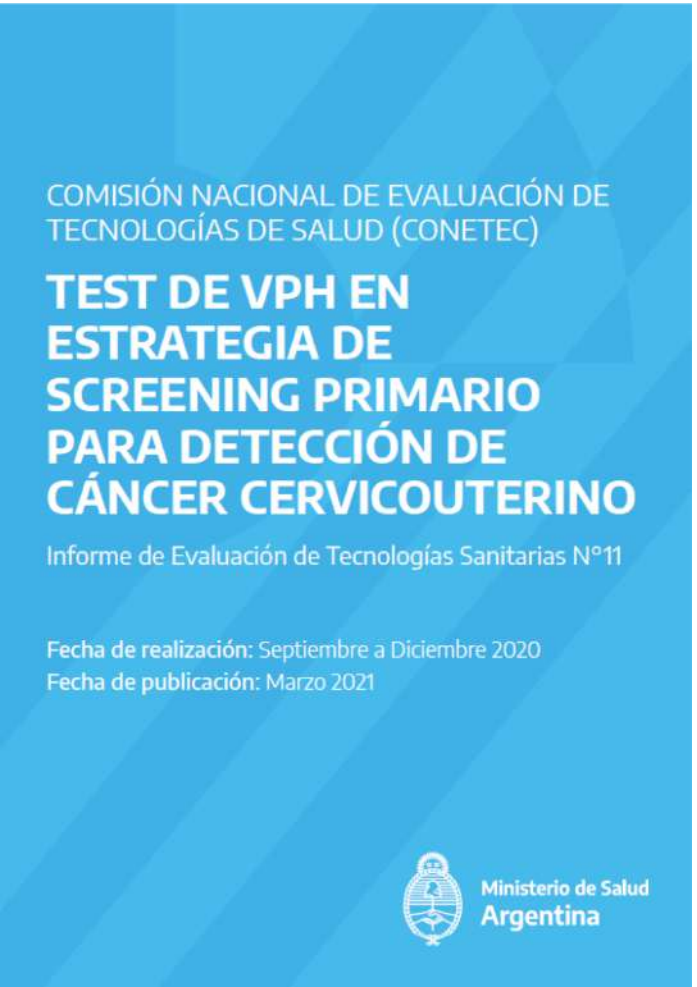


Santiago Hasdeu

Coordinador Ejecutivo RedArets
Red Argentina Pública de ETS

RedARETS

EXAMPLE ON BUDGET IMPACT ANALYSIS ON DIAGNOSTIC METHODS



Report for the National Commission on Health Technology Assessment - CONETEC-National Ministry of Health of Argentina

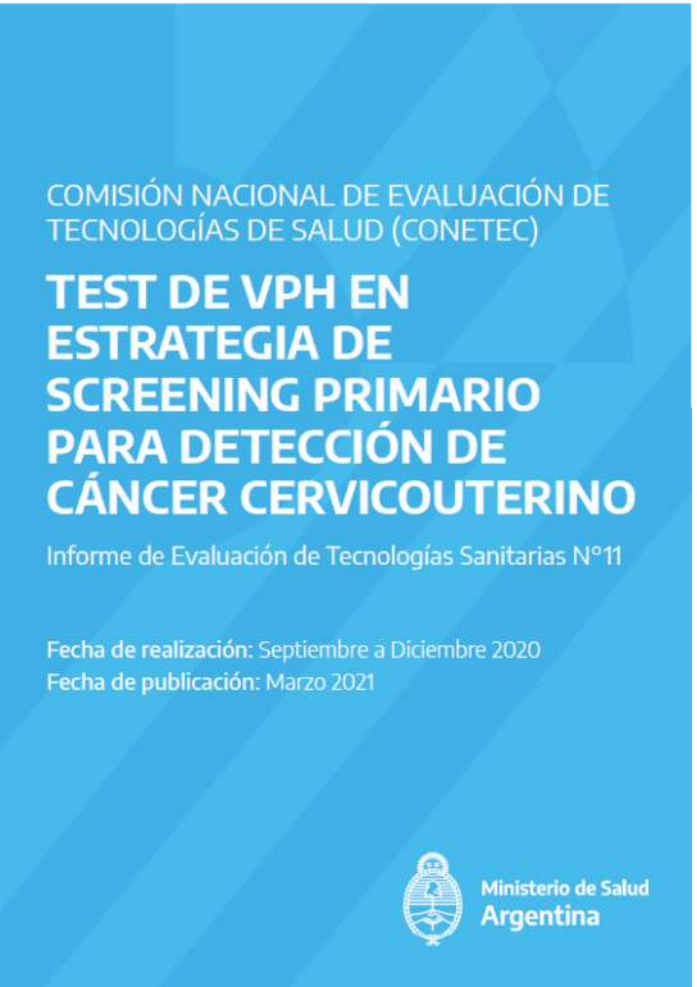
https://www.argentina.gob.ar/sites/default/files/informe-11-test-vph-marzo-2021.pdf

Authors

Santiago Hasdeu, Gabriela Luchetti, Julia Ismael, Laura Lamfre, Leandro Duarte

No conflicts of interests declared

Objective: Estimate the Budget Impact of implementing a cervical cancer screening strategy based on HPV test compared to the cytology based screening strategy in Argentina



Población	Mujeres de 30 a 64 años
Intervención	Test de VPH (toma dirigida y autotoma) c/5 años en tamizaje cáncer cervical
Comparador *	Test de PAP c/1-2 años en tamizaje de cáncer cervical
Desenlaces	Eficacia: desempeño diagnóstico (sensibilidad, especificidad, valor predictivo, etc), tasa de cáncer cervical, mortalidad por cáncer cervical. Impacto presupuestario: Impacto de ampliar la cobertura para la totalidad de las potenciales beneficiarias Equidad: Impacto en la equidad de la incorporación para el total de las potenciales beneficiarias de la tecnología
Diseño	Revisiones sistemáticas y meta-análisis, ensayos clínicos controlados aleatorizados, estudios observacionales, informes de evaluación de tecnologías, evaluaciones económicas, guías de práctica clínica, políticas de cobertura.

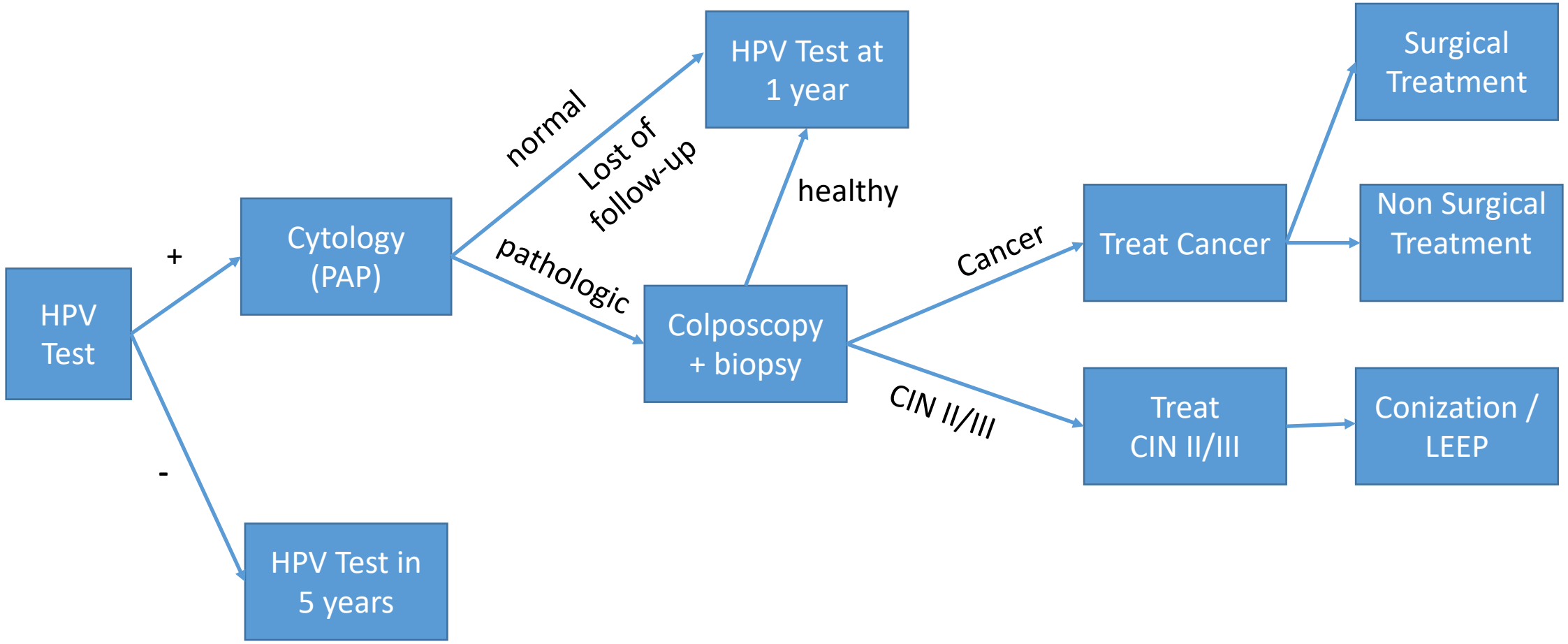
Población	Mujeres de 30 a 64 años
Intervención	Test de VPH (toma dirigida y autotoma) c/5 años en tamizaje cáncer cervical
Comparador *	Otros métodos de rastreo basados en test de VPH (toma dirigida y autotoma) c/5 años en tamizaje cáncer cervical
Desenlaces	Eficacia: desempeño diagnóstico (sensibilidad, especificidad), tasa de CCU, etc. Seguridad: eventos adversos graves asociados al método. Impacto organizacional: según factibilidad de realizar auto-toma, sencillez del método en sus distintas etapas (pre-analítica-analítica) Conveniencia económica: de tener resultados de eficacia comparables entre alternativas disponibles, estudios de costo-minimización
Diseño	Revisiones sistemáticas y meta-análisis, ensayos clínicos controlados aleatorizados, estudios observacionales, informes de evaluación de tecnologías, evaluaciones económicas, guías de práctica clínica, políticas de cobertura.

BUDGET IMPACT ANALYSIS

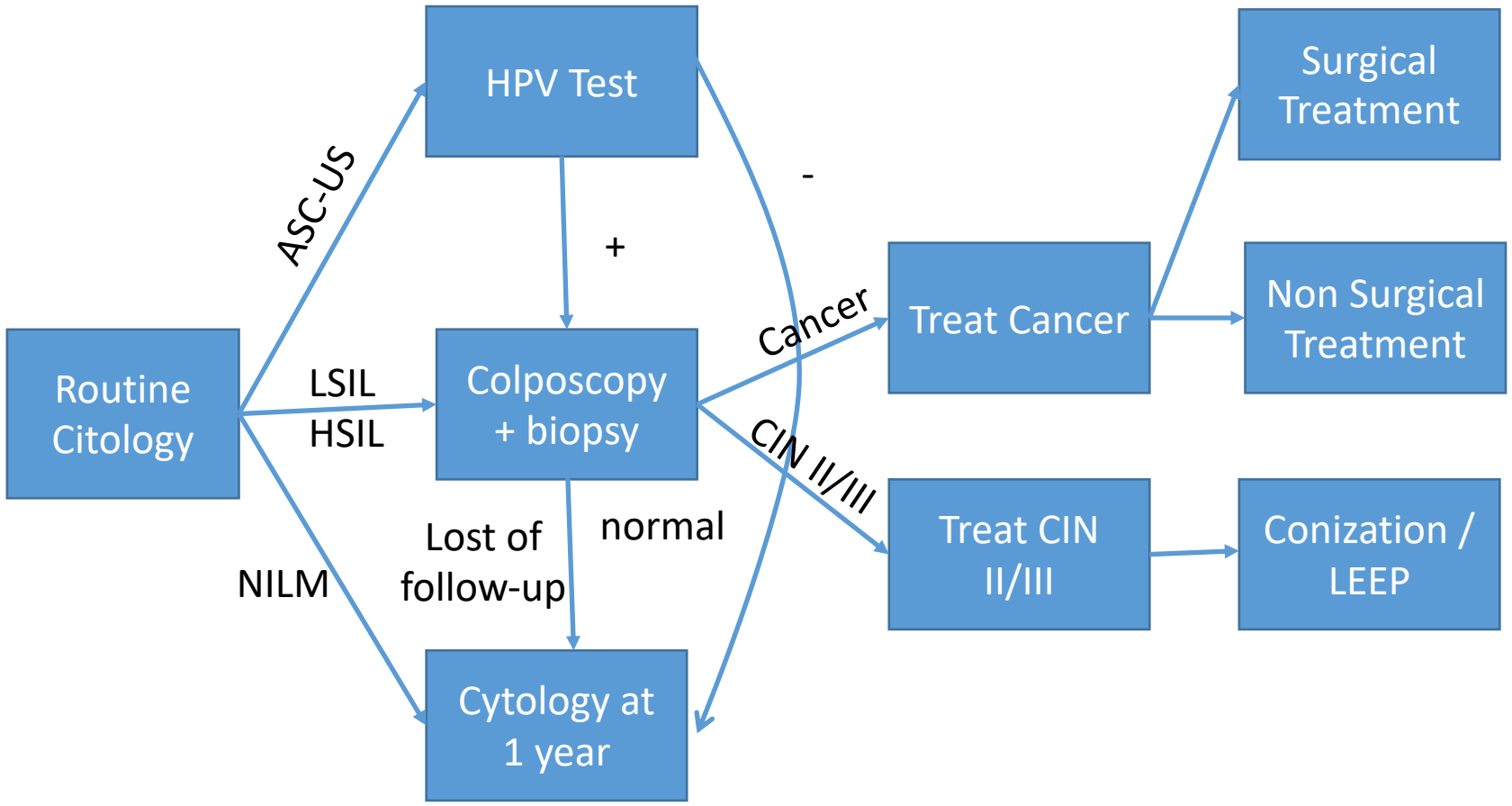
	Argentina
Women 30 to 64 years	9.457.976
% Public health exclusive coverage (ENFR2018)	30,5%
Women 30 to 64 years With Public health exclusive coverage	2.881.343
Excluded from screening (7%)	201.694
Women 30 to 64 years With Public health exclusive coverage candidates to participate in screening	2.679.649
Women with adherence to screening (71,6%)	1.918.629
Annual incidence of women with who turn 30 years of age, exclusive PH coverage and adherent to screening	71.599

- Population: Women 30 to 64 years old, healthy, public health coverage
- Intervention: Cervical cancer screening with HPV test
- Comparator: Cervical cancer screening with PAP (cytology)
- Perspective: National Public Health Sector
- Temporal Horizon: 5 YEARS
- Costs: Direct Costs associated to screening diagnostic and treatment of Cervical Cancer, based on microcosting technique.
- Source of information of costs: Health Benefits Nomenclator of Buenos Aires (Argentina) and information of health providers to novembre 2020

HPV based screening strategy:



Cytology (PAP) based screening strategy:



Unitary Costs

Item	Costs in Arg $	Item	Costs in Arg $
Costs screened woman HPV	$ 4.116	Post-surgical treatment follow-up 4 year	$ 5.396
Costs hpv test	$ 3.250	Post-surgical treatment follow-up 5 year	$ 5.396
Processing PAP	$ 188	Non-Surgical Treatment of Cervical Cancer	$ 2.899.361
Costs screened woman cytology	$ 1.054	Post non-surgical treatment follow-up 1 year	$ 7.234
Colposcopy/biopsy	$ 3.981	Post non-surgical treatment follow-up 2 year	$ 7.234
Treatment CIN II/III	$ 41.964	Post non-surgical treatment follow-up 3 year	$ 5.396
Surgical Treatment of Cervical Cancer	$ 206.721	Post non-surgical treatment follow-up 4 year	$ 5.396
Post-surgical treatment follow-up 1 year	$ 8.936	Post non-surgical treatment follow-up 5 year	$ 5.396
Post-surgical treatment follow-up 2 year	$ 8.936	2° line (1 year of follow-up, 6 Months of treatment)	$ 2.920.557
Post-surgical treatment follow-up 3 year	$ 5.396		

Probabilities:

Concept	Probability	Source	Cytology screening	Probability	Source
Adherence rate to HPV test	0,716	1	Adherence rate to HPV test	0,716	1
Non-Adherence to HPV test	0,284	1	Non-Adherence to HPV test	0,284	1
HPV+	0,118	2	ASCUS	0,053	4
Pathologic Cytology/HPV+	0,272	3	LSIL	0,019	4
Adherence to colposcopy	0,712	3	HSIL	0,004	4
Colposcopy to healthy in women with pathologic cytology	0,348	3	Healthy	0,924	4
Colposcopy to cancer in women with pathologic cytology	0,066	3	HPV+/ASCUS	0,253	5
Colposcopy to CIN II - III in women with pathologic cytology	0,586	3	HPV-/ASCUS	0,496	5
Surgical treatment Cervical cancer	0,525	3	Lost of follow-up with HPV	0,251	5
Non-Surgical treatment cancer	0,475	3			
Progress/Surgical treatm. of Cancer	0,090	3			
No progress/Surgical treatmCancer	0,910	3			
Progress/Non-surgical treatment of Cancer	0,610	3			
No progress/Non-surgical treatment of Cancer	0,390	3			

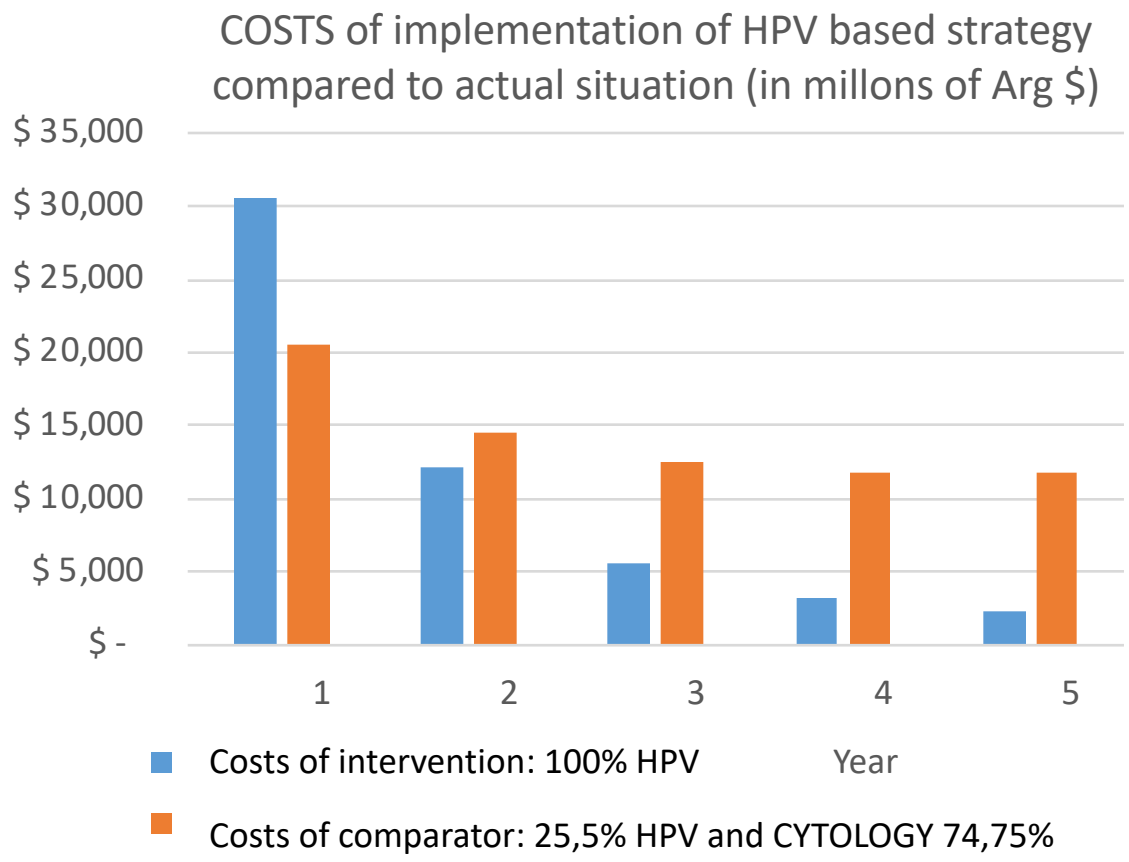
HPV based strategy:

COSTS – HPV Screening	YEAR 1	YEAR 2	YEAR 3	YEAR 4	YEAR 5
Costs initial HPV screening	$ 7.897.074.930	$ 3.145.091.158	$ 1.429.898.693	$ 810.812.994	$ 587.358.711
Costs pathologic cytology	$ 42.671.065	$ 16.994.190	$ 7.726.317	$ 4.381.148	$ 3.173.735
Costs Colposcopy/HPV+	$ 468.359.343	$ 186.528.916	$ 84.804.363	$ 48.087.658	$ 34.835.042
Costs treatment CIN II/III	$ 2.893.087.072	$ 1.152.201.625	$ 523.842.240	$ 297.040.691	$ 215.178.393
Costs Surgery Cancer	$ 842.704.001	$ 335.615.519	$ 152.585.781	$ 86.522.587	$ 62.677.579
Costs non-surgical Cancer	$ 10.693.670.099	$ 4.258.863.893	$ 1.936.269.445	$ 1.097.946.613	$ 795.360.350
Costs Progression Cancer/Surgical treatm.	$ 1.071.515.598	$ 426.742.087	$ 194.015.983	$ 110.015.262	$ 79.695.840
Costs Progression Cancer/non-Surgical	$ 6.570.828.457	$ 2.616.899.886	$ 1.189.759.386	$ 674.643.857	$ 488.716.818
Follow-up surgical cancer 1 year	$ 33.148.784	$ 13.201.843	$ 6.002.147	$ 3.403.471	$ 2.465.499
Follow-up Non-surgical cancer 1 year	$ 10.405.662	$ 4.144.162	$ 6.028.282	$ 1.068.376	$ 773.939
Follow-up surgical cancer 2 year		$ 33.148.784	$ 13.201.843	$ 6.002.147	$ 3.403.471
Follow-up Non-surgical cancer 2 year		$ 10.405.662	$ 4.144.162	$ 6.028.282	$ 1.068.376
Follow-up surgical cancer 3 year			$ 20.017.475	$ 7.972.165	$ 3.624.502
Follow-up Non-surgical cancer 3 year			$ 7.761.878	$ 3.091.248	$ 4.496.667
Follow-up surgical cancer 4 year				$ 20.017.475	$ 7.972.165
Follow-up Non-surgical cancer 4 year				$ 7.761.878	$ 3.091.248
Follow-up surgical cancer 5 year					$ 20.017.475
Follow-up Non-surgical cancer 5 year					$ 7.761.878

Cytology (PAP) based strategy:

COSTS - Tamizaje Citología	YEAR 1	YEAR 2	YEAR 3	YEAR 4	YEAR 5
Costs initial screening with cytology	$ 2.022.234.445	$ 2.063.325.559	$ 2.103.718.197	$ 2.143.424.233	$ 2.182.455.338
Costs HPV / ASCUS	$ 330.483.760	$ 337.199.077	$ 343.800.246	$ 350.289.207	$ 356.667.867
Costs Colposcopy	$ 278.115.606	$ 283.766.820	$ 289.321.974	$ 294.782.700	$ 300.150.604
Costs treatment CIN II/III	$ 2.893.087.072	$ 1.152.201.625	$ 523.842.240	$ 297.040.691	$ 215.178.393
Costs surgical cancer	$ 842.704.001	$ 335.615.519	$ 152.585.781	$ 86.522.587	$ 62.677.579
Costs Non-surgical cancer	$ 6.349.988.721	$ 6.479.018.323	$ 6.605.854.654	$ 6.730.534.997	$ 6.853.095.998
Costs Progression Cancer/surgical treatm.	$ 636.274.721	$ 649.203.606	$ 661.912.724	$ 674.405.809	$ 686.686.534
Costs Progression Cancer/non-surgical treatment	$ 3.901.811.651	$ 3.981.095.131	$ 4.059.030.935	$ 4.135.641.971	$ 4.210.950.757
Follow-up Cancer surgical 1 YEAR	$ 19.684.019	$ 20.083.991	$ 20.477.165	$ 20.863.655	$ 21.243.576
Follow-up Cancer non-surgical 1 YEAR	$ 6.178.967	$ 6.304.522	$ 12.732.464	$ 6.549.264	$ 6.668.524
Follow-up Cancer surgical 2 YEAR		$ 19.684.019	$ 20.083.991	$ 20.477.165	$ 20.863.655
Follow-up Cancer non-surgical 2 YEAR		$ 6.178.967	$ 6.304.522	$ 12.732.464	$ 6.549.264
Follow-up Cancer surgical 3 YEAR			$ 11.886.540	$ 12.128.071	$ 12.365.496
Follow-up Cancer non-surgical 3 YEAR			$ 4.609.067	$ 4.702.721	$ 9.497.505
Follow-up Cancer surgical 4 YEAR				$ 11.886.540	$ 12.128.071
Follow-up Cancer nn- surgical 4 YEAR				$ 4.609.067	$ 4.702.721
Follow-up Cancer surgical 5 YEAR					$ 11.886.540
Follow-up Cancer non-surgical 5 YEAR					$ 4.609.067

	Intervention: COSTS HPV 100%	Comparator: COSTS HPV 25,25% - CYTOLOGY 74,75%	BUDGET IMPACT ANALYSIS
YEAR 1	$ 30.523.465.012	$ 20.624.395.731	$ 9.899.069.281
YEAR 2	$ 12.199.837.725	$ 14.542.382.703	-$ 2.342.544.978
YEAR 3	$ 5.576.057.996	$ 12.483.034.619	-$ 6.906.976.622
YEAR 4	$ 3.184.795.852	$ 11.872.087.832	-$ 8.687.291.979
YEAR 5	$ 2.321.671.688	$ 11.782.559.276	-$ 9.460.887.588



Conclusions:

- In the first year of implementation of HPV test based screening strategy, compared to the cytology based strategy, there is a net budget impact of $ 9.899.069.281. In the following years the Budget impact is negative (savings).
- HPV test based screening is cost saving in a 5 year horizon period, compared to cytology based screening strategy
- It is important to know the budgetary impact of the incorporation of HPV, to take into account the potential opportunity cost when analyzing the incorporation of future health technologies.

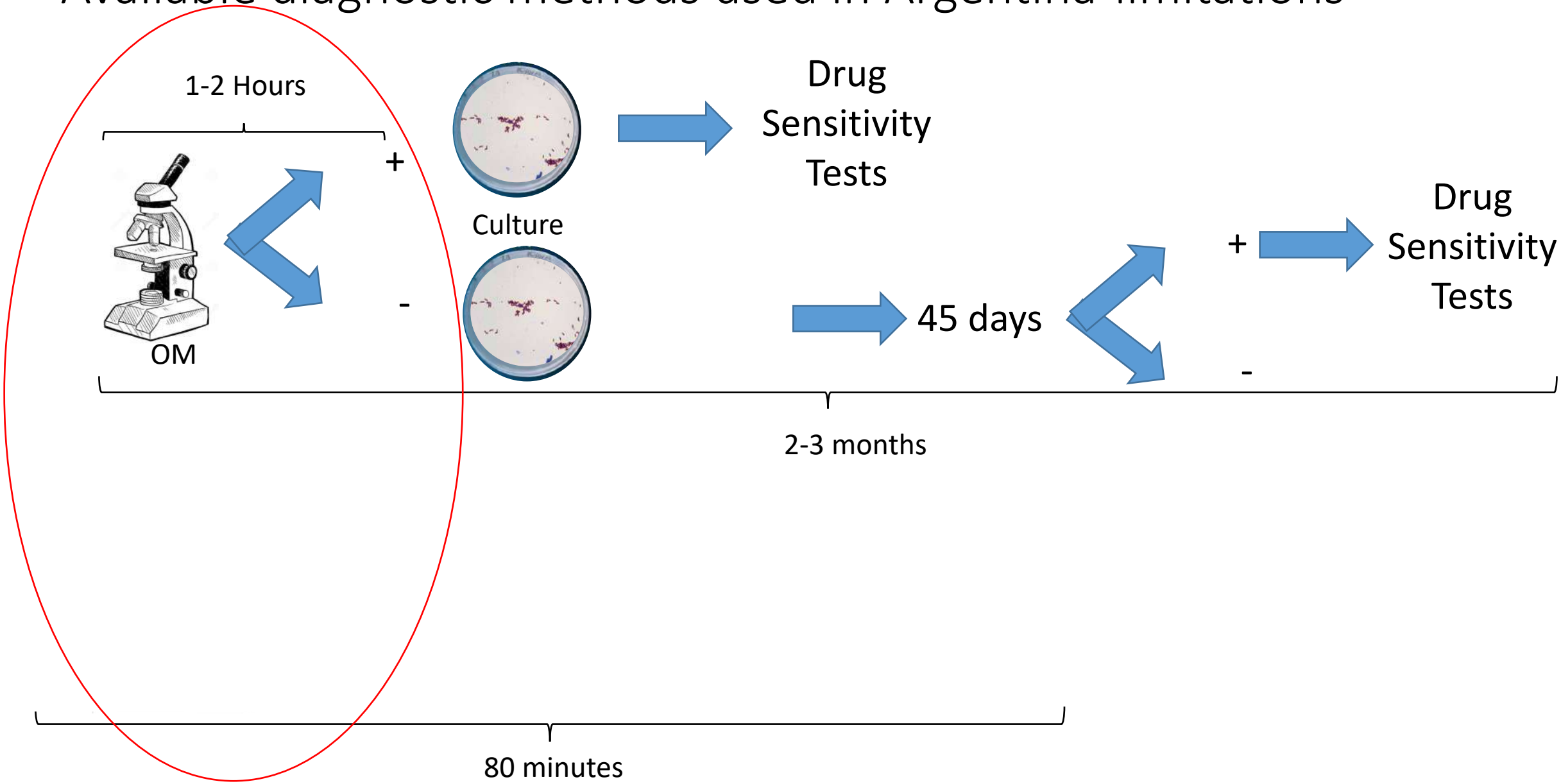
EXAMPLE ON COST-EFFECTIVENESS STUDY ON DIAGNOSTIC METHODS

Assessment of the cost-effectiveness of the GeneXpert technology for the diagnosis of Tuberculosis in selected African and Latinamerican countries

A-B

Tuberculosis:

Available diagnostic methods used in Argentina-limitations



Our model/s for Argentina will be developed in a flexible way, in order to capture differences among subnational regions and countries:

- Epidemiological differences
  - Incidence, Distribution on territory, clusters? Co-infection HIV, % drug resistence (MDR, XDR), mortality rate
- Differences in the health system organization
  - Diagnostic network, transport/derivation of samples, Human and technological resources, DOT, Clinical Guidelines for Dx and treatment, GenExpert already incorporated? If so, how many? Usage rate? Hours/day?
- Variability in clinical practice
  - Adherence to Clinical Practice Guidelines (%)?, Empiric treatments (%?), Only BK (?), % with culture?, % with drug-sensitivity testing?, DOT (%)? HIV testing at Dx?
- Heterogeinity in costs
  - Microbiologists salaries, other health care workers, costs of reactants and supplies for BK and cultures, Cost for GenExpert equipment and supplies
  - Costs of treatment: medicines (for sensitive and for drug resistant TB, radiology, hospitalizations, etc.)
  - Indirect costs: out of pocket expenses, transport, loss of productivity, costs of family care
- Differences in payment capacity and willingness to pay threshold
  - GDPpc of each country/región
  - Stablished threshold, opportunity cost, other priorities

A	
Population	**Adult patients with suspected pulmonary tuberculosis** **Pediatric patients with suspected pulmonary tuberculosis** **Adults living with HIV with suspected pulmonary tuberculosis** **Adult institutionalized people with suspected pulmonary tuberculosis** **Asymptomatic people** **Patients with suspected drug-resistant tuberculosis** **Patients with suspected extrapulmonary tuberculosis (CSF, pleural fluid, lymph nodes, etc.)** **Same populations but living in areas of high incidence and high drug resistance (CABA+Buenos Aires+Santa Fé)**
Intervention and comparators	Intervention: Xpert Ultra - Xpert MTB/Rif Comparators: BK, culture, Phenotipic drug sensitivity tests
Relevant end points	Efficacy: Mortality, TB cases detected, diagnostic performance values. Sequelae, Early diagnosis Safety: False positives associated with unnecessary treatments
Study designs	Systematic reviews and meta-analyses of randomized controlled clinical trials, cohort and cross-sectional studies; Health technology assessment reports, economic evaluations, clinical practice guidelines, coverage policies.
Exclusion Criteria	None

Previous cost-effectiveness studies on GenExpert

- A systematic review of cost-effectiveness studies on different TB screening strategies found no mention of the Xpert technology until 2010 (Nienhaus et al., 2011)
- A recently published systematic review of cost-effectiveness studies on different TB screening strategies (Hao et al., 2020) identified 21 full high-quality economic evaluations, including 7 cost-effectiveness and 14 cost-utility.
- This review found that most of the evaluations were carried out in high-TB burden settings, and that, although most conclude that Xpert is cost-effective, the cost differences compared to standard bacteriology are very high.

Previous cost-effectiveness studies on GenExpert

- Xpert didn´t show to be cost-effective in a study conducted for B&MGF in South Africa (Anna Vassall et al. Cost-effectiveness of Xpert MTB/RIF for tuberculosis diagnosis in South Africa: a real-world cost analysis and economic evaluation. Lancet Glob Health 2017; 5: e710–19)
- The results in cost-effectiveness studies of GenExpert were very sensitive to the modification of certain parameters (M. Pinto et al., 2016). Of all the studies identified, the one from the USA (Choi et al., 2013) and the one from Hong Kong (Li et al., 2018) are the only ones that represent countries with low to intermediate prevalence of TB, which find that the incorporation of Xpert would be cost-effective from the perspective of their healthcare systems.
- Á study suggested that Xpert was underused in Uganda and did not significantly increase the number of patients starting TB treatment. The authors conclude that more attention needs to be paid to the proper implementation of new diagnostic tests for TB if they are to have an impact on health outcomes (Hanrahan et al., 2016).

Cost-effectiveness studies:

Desirable consequences
- Health benefits
- Other beneficial impacts
- ***Savings***

Health (natural units eg: YLG, diagnosed patients, etc)

Utility (QALY, DALY)

Monetary savings

Undesirable consequences
- Adverse health effects
- Other harmful impacts
- ***Costs***

Human resources

Supplies

Complications

Equipment

Social costs

Cost-effectiveness:

BK+Culture+DST

Diagnostic
Cases (N°)
Costs ($)

Treatment & Follow-up
Cases (N°)
Costs ($)

Infections in contacts
Progression of disease in cases

Societal Costs ($)
Societal benefits

Cost/Effectiveness
$/Deaths avoided
$/YLG $/case Dx

Compare

GenExpert

Diagnostic
Cases (N°)
Costs ($)

Treatment & Follow-up
Cases (N°)
Costs ($)

Infections in contacts
Progression of disease in cases

Societal Costs ($)
Societal benefits

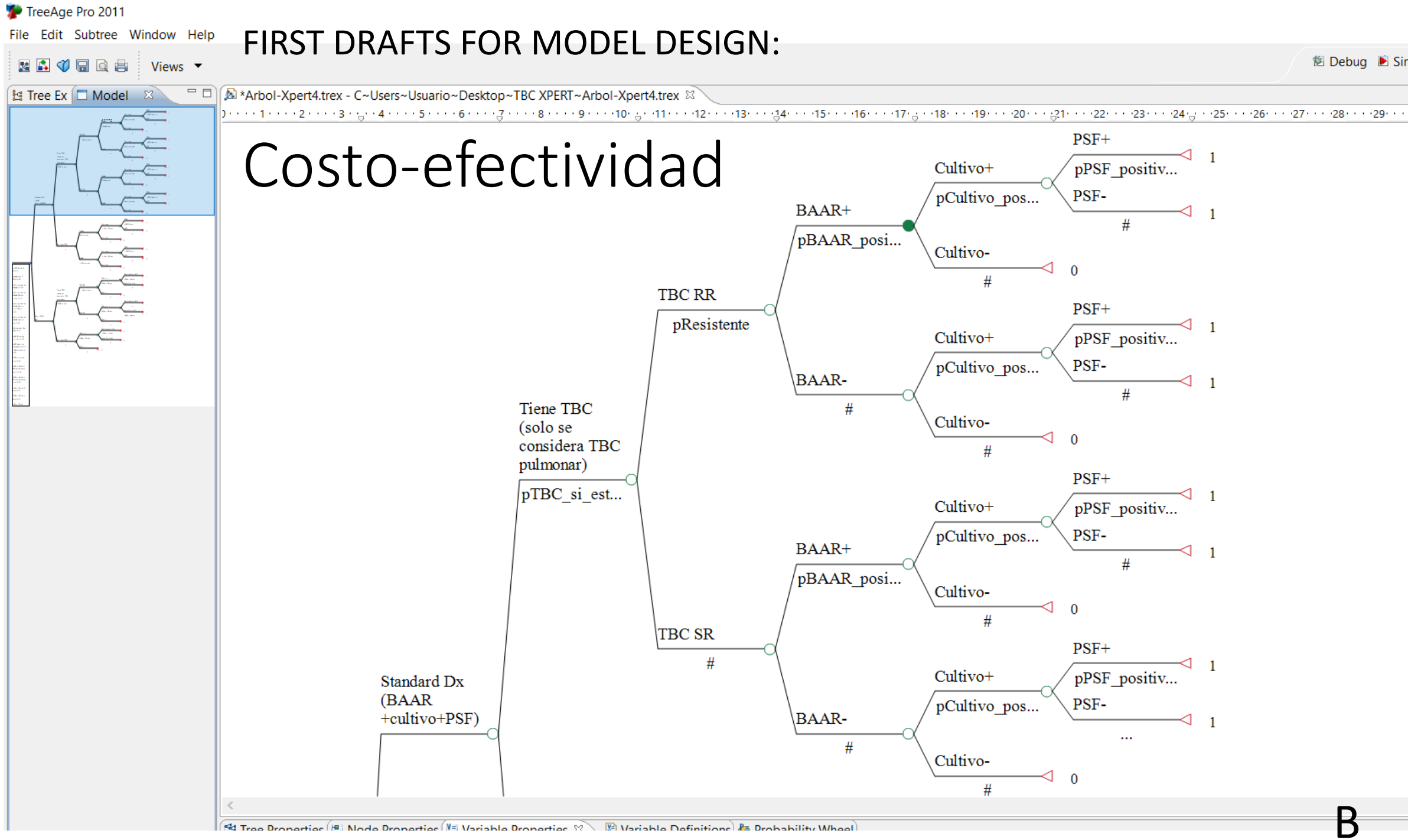
Cost/Effectiveness
$/Deaths avoided
$/YLG $/case Dx

¿How many GenExpert equipments are necessary?

	Annual working days	N° samples proccessed per day	N° samples proccessed per year
1° year	250	6	1500
2 ° year (…)	250	12	3000

A-B

FIRST DRAFTS FOR MODEL DESIGN:



B

Hidden costs:

TOTAL COST OWNERSHIP
Aquisition cost +
Operational costs +
Maintenance costs +
Training costs +
Replacement costs

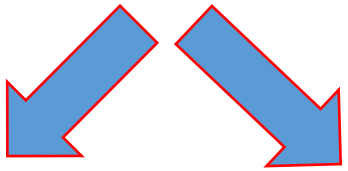
Aquisition cost

Initial training

Infrastructure/ installing

Maintenance
Lifetime cycle
Accessories / Supplies
Spare parts
Recycling
Training - Learning curve
Updates/Software
Substitution/Replace
Human Resources
Preoperative Evaluation
Surgery-Anesthesia
Rehabilitation

Treatment and follow-up costs per patient: two alternatives:



- **Micro-costing:**

<u>Advantages</u>

More detailed and accurate

Representative of the local reality

<u>Disadvantages</u>

laborious, takes time

Requires a large sample

Problems with subgroups (MDR and sensitive TB, mild TB and severe TB, child and adult, co-infection HIV, etc.)

- **Take an added value :**

<u>Advantages</u>

Faster

<u>Disadvantages</u>

May not be representative of local reality

B

B

Micro-costing

- Example of a study from Mexico, applied to patients who were admitted to a hospital due to Tuberculosis:

Tabla I. Determinación del costo de atención de la tuberculosis, 2002 (cifras dadas en pesos mexicanos).

Concepto	Frecuencia	Costo	Mínimo	Máximo
Biopsia	1	2,939	2,939	2,939
Citología de líquido pleural o ascitis	1	780	780	780
Pieza quirúrgica	1	5,139		5,139
Determinación anti SHB (hepatitis B)	1	229	229	229
Prueba de VIH	1	236	236	236
Electrocardiograma	1	180	180	180
Embolización de arterias bronquiales	1	27,908		27,908
Bilobectomía	1	10,897		10,897
Lobectomía	1	10,897		10,897
Neumonectomía	1	10,897		10,897
Nodulectomía (resección de nódulo pulmonar)	1	10,897		10,897
Segmentectomía	1	10,897		10,897
Cierre de pleurotomía abierta	1	4,671		4,671
Lavado de cavidad pleural y decorticación	1	10,897		10,897
Pleurotomía abierta (Eloesser)	1	4,671		4,671
Plastia de pared torácica	1	10,485		10,485
Toracoscopia	1	6,239		6,239
Biopsia de ganglio	1	2,323		2,323
Biopsia pleural	1	4,253		4,253
Toracocentesis	1	3,131		3,131
Broncoscopia diagnóstica	1	1,342	1,342	1,342
Broncoscopia terapéutica (por sangrado o extracción de cuerpo extraño)	1	1,812		1,812
Preconsulta de neumología	1	347	347	347
Consulta de neumología	1	347	347	347
Clínica de Tuberculosis	1	347	347	347
Espirometría c/broncodilatador (curva flujo/volumen con broncodilatador)	1	342	342	342
Espirometría simple (curva flujo/volumen simple)	1	264	264	264
Gasometría completa (en reposo y ejercicio)	1	326	326	326
Oximetría de pulso	1	64	64	64
Paquete habitación sala general	12-20 días promedio	1,883	22,594	37,657
Estudio bacteriológico de líquido pleural	1	1,036	1,036	1,036
Biopsia	1	539	539	539
Baciloscopia (bacilo de Koch)	5	336	1,682	1,682
Cultivo de micobacterias	2	814	1,629	3,258
Cultivo de expectoración	1	341	341	341
Pcr para tuberculosis y atípicos *(legionella*, micoplasma y *clamydia)*	1	571	571	571
Albúmina	1	44	44	44
Biometría hemática	3	57	171	513
Coproparasitoscópico (muestra única)	1	45	45	45
Examen general de orina (ego)	1	65	65	65
Líquido pleural (estudio físico-químico)	1	33	33	33
Pruebas de funcionamiento hepático	2	406	812	1,624
Pruebas de coagulación	1	134	134	134
Química sanguínea (glucosa, urea, creatinina y ácido úrico)	3	169	508	1,523
Adenosin desaminasa (ada)	1	35	35	35
Mantoux (ppd)	1	144	144	144
Tórax (ap o pa)	3	100	301	904
Tórax (lateral)	3	100	301	904
Tomografía helicoidal contrastada (1 región)	1	5,250	5,250	5,250
Paquete atención-médica urgente	20%	844		844
Paquete día-cama urgencias	20%	1,963		1,963
Totales		43,976	202,862	

Fuente: Unidad de Costos, INER. 2002.

Incorporate social indirect costs into the model:

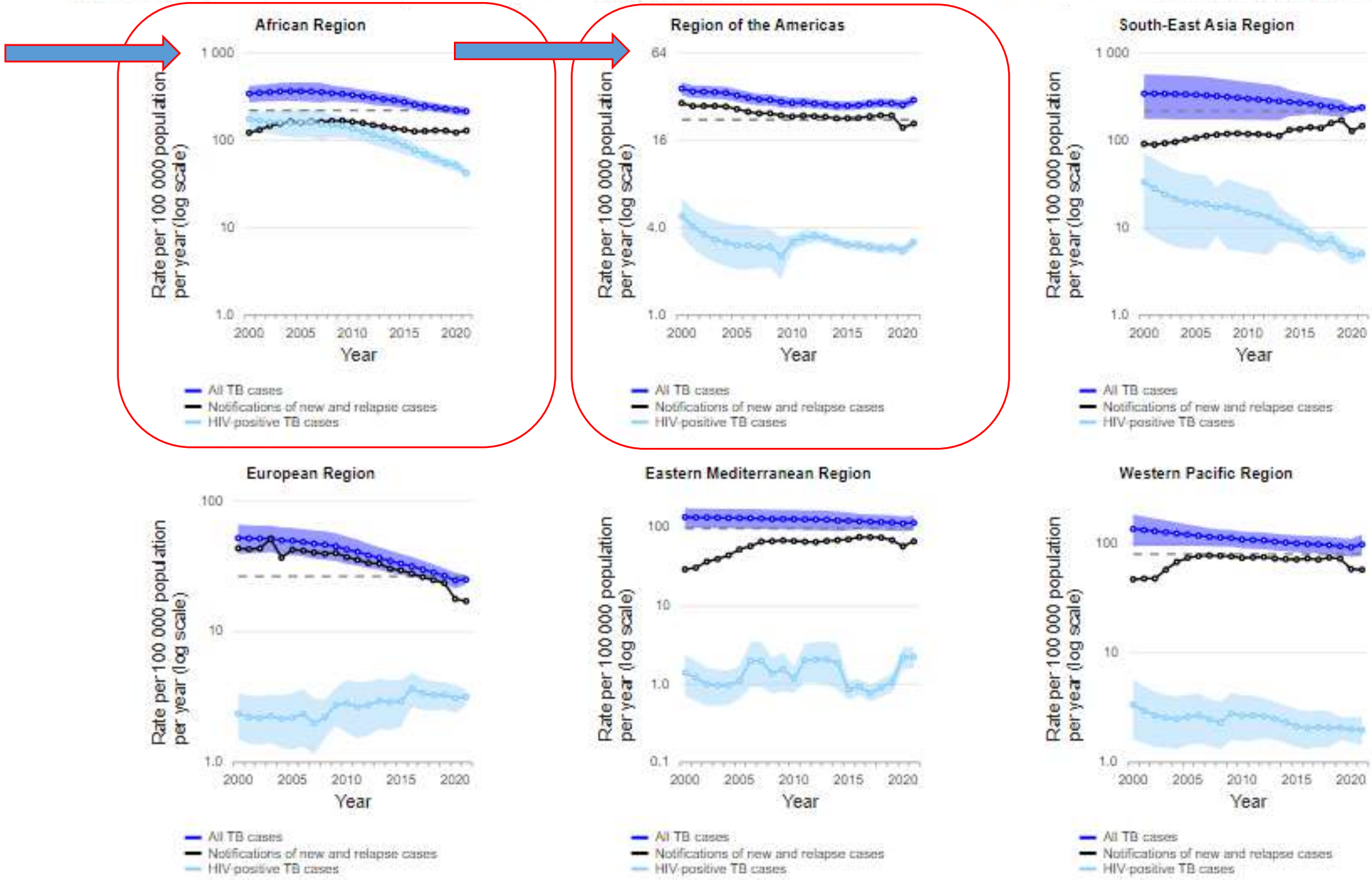
Medical care costs borne by the health system	39,12%
work absenteeism	22,94%
DAL (Days of Limited Activity) costs, transportation and intangibles	37,94%

Cusamano et al, Revista Argentina de Salud Pública

B

Fig. 2.1.7 Trends in estimated TB incidence rates by WHO region, 2000–2021

Total TB incidence rates are shown in blue and incidence rates of HIV-positive TB are shown in light blue. The black solid lines show notifications of new and relapse cases for comparison with estimates of the total incidence rate. Shaded areas represent uncertainty intervals. The horizontal dashed line shows the 2020 milestone of the End TB Strategy.



Differences among regions and differences among countries of the same region:

- Very important differences were noticed between the six participating countries of the study (Argentina, Peru and Paraguay, Malawi, Tunisia and Uganda)
- The differences in the incidence rate of TB in HIV positive patients between the country with the highest and the lowest rate is 35,5 times.
- The differences in the mortalilty rate of TB in HIV negative patients, between the country with the highest and the lowest rate is 12 times (Higher in American country compared to African country: 15 vs 1,25 per 100.000)
- The differences in the incidence rate of MDR/RR TB between the country with the highest and the lowest rate is 11,6 times
- The differences in the incidence rate of TB between the country with the highest and the lowest rate was 6,8 times
- The differences in the percentage of bacteriologically confirmed new cases of TB between the country with

Thank You.

Contact info: hasdeusanti@gmail.com

ORGANIZED BY

IN COOPERATION WITH

COORGANIZED BY

Annex 4. Annex to Generic Protocol: Cost-effectiveness sub-study (version 06 June 2024)

Annex to Generic Protocol for the evaluation of Rapid Diagnostic Tests (RDTs) for Chagas disease, to ensure high quality studies in the Americas: Cost-effectiveness sub-study

Overview

A cost-effectiveness analysis can be conducted to compare (1) the current standard of care for diagnosing chronic *T.cruzi* infection to, (2) new algorithms that incorporate new testing technologies such as rapid diagnostic tests (RDTs) adopted at lower levels of the healthcare system (the *intervention*). This is an annex to the main study, which will evaluate the performance of the new algorithms incorporating these new test technologies in Setting X .

Two main scenarios will be modelled:

1. ***Standard of care (the comparator)*:** This represents the current standard of care, or status quo, for diagnosing chronic *T.cruzi* infection in Setting X. The standard of care can be described, detailing the sample type, test technology, algorithm structure and the place of testing. For example, venous blood is collected from individuals accessing high complexity centres (secondary/tertiary centres), or samples are transported to high- complexity centres from low complexity centres (primary healthcare centres) for testing. Two laboratory-based serology IgG tests for *T. cruzi* are performed in parallel; a third test is performed in case of discordance.
2. ***Intervention*** (***the new algorithms incorporating new testing technologies*****):** This is the new proposed algorithms that include RDTs which can be used to decentralize the diagnosis of *T. cruzi* to lower levels of the healthcare system.

There are four components to this study: (A) Estimating the potential impact in terms of effectiveness of the Chagas disease (CD) diagnostic care cascade in relation to the standard of care and intervention scenario, (B) estimating the costs associated with the different testing algorithms, (C) evaluating the cost-effectiveness of the different testing algorithms, and (D) performing a Budget Impact Analysis (BIA) to assess the financial impact of adopting a new algorithm.

The approach described in this Annex is conservative in terms of determining the full benefit of the introduction of a new technology to diagnose chronic *T.cruzi* infection. It only incorporates the direct benefits on the diagnostic pathway for Chagas disease, however, there are potentially many additional benefits that are likely to arise that strengthen the primary healthcare system more broadly. These however are not quantified here. More complex analyses may be conducted that measure improvements in access

for those seeking care or conduct a distributional cost-effectiveness analysis that evaluates how the intervention improves health inequalities for different population sub-groups.

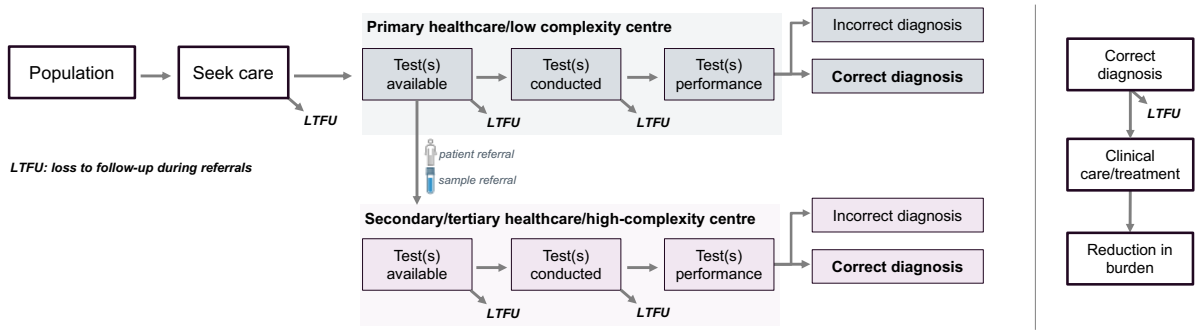
A. Chagas Diagnostic Cascade Model Framework

The performance and impact of the different diagnostic cascades may be directly estimated from evaluation or pilot studies that assess outcomes among patient groups tested under different algorithms. In these situations, studies should be designed to collect appropriate data to estimate the key outcomes listed below, ensuring the representativeness of the study population for the target population of interest.

Where it is not possible to directly measure the key outcomes of each diagnostic algorithm of interest, modelling can be a useful tool to estimate overall impact of diagnostic algorithms based on data from individual test performance. A model of the CD diagnostic care cascade in Setting X can be created using the FIND Chagas Diagnostic Algorithm application (https://finddx.shinyapps.io/chagaspathway/) developed with the support of DNDi, or alternative decision-tree models. Models representing the current standard of care within Setting X should be compared to models representing the diagnostic algorithms incorporating new testing technologies to estimate changes in overall diagnostic accuracy and costs.

An example of a generic diagnostic care cascade for standard of care and a new algorithm that incorporates RDTs at a lower level of care, is shown below.

Figure 2: The Chagas disease diagnostic cascade



These decision tree models should capture the diagnostic tests used, the performance (sensitivity/specificity) of these tests and the healthcare level at which patients seek care and at which samples are collected and tested. The patient and sample flow should be considered to identify touchpoints at which individuals or samples could be lost from the cascade and estimate the number of visits required to receive a final diagnosis. For both scenarios (standard of care and the intervention), an estimated number of individuals diagnosed correctly with *T.cruzi* infection (of those who truly have *T.cruzi* infection) is determined as well as the number of individuals who receive the positive test(s) who are successfully linked to further care/treatment.

The new intervention algorithms will then be compared to the standard of care against a number of key outcomes which seek to capture the potential for increased yield through better test performance or

increases in accessibility to testing and results, or a reduced proportion of individuals being lost during the diagnostic process with new diagnostic algorithms:

- The number of individuals receiving a correct/incorrect diagnosis (true positives/negatives, false positives/negatives)
- The number (and proportion) of positive individuals linked to further care/treatment
- The number of each test conducted
- The number of patient visits (by healthcare level) prior to diagnosis
- Number of individuals lost to follow-up prior to final diagnosis
- Additional: Disability-adjusted life-years (DALYs) averted through linkage to treatment

Parameters required for the Chagas Diagnostic Algorithm application to be estimated through the main Performance Evaluation Study, as well as possible sources for parameters that are not collected through evaluation studies, are described in Table 1. Note that parameter values should be customized to represent the specific context and population of interest.

Table 1. Required parameters for CD diagnostic cascade model.

Cost category	Detail
Population	Seroprevalence of *T.cruzi* infection in population[1] being tested as part of this study (prospective evaluation of RDTs for *Trypanosoma cruzi* infection in Setting X) Socio-demographic characteristics of those being offered the test: age, sex, urban/rural, access to healthcare, level of education (as aligned with main protocol and the sub-analyses stipulated there). *Source: surveillance data; recruitment participant data*
Test characteristics	Test assay sensitivity, specificity *Source: literature/manufacturer specifications, performance evaluation study*
Linkage to further care/treatment	Probability linked to further care/treatment following a positive test result. This can differ depending on the setting where the final diagnosis is made, the target population and the availability of treatment at this healthcare level. *Source: literature*
Loss to follow-up	Loss to follow-up in the diagnostic cascade occurs whenever a patient has to return for a subsequent visit prior to the final diagnosis confirmation or where a sample has to be transported to another facility for processing and testing. It is the percent of the population seeking care who are unlikely to return for an additional visit – alternatively, the probability that a sample is lost between collection and testing. *Source: literature, National surveillance system, as per defined standard definitions : for example – no contact for more than 30 days.*
Access	Estimated increase in the population who will be able to access testing (for example, due to decentralized RDT testing).

[1] The population is as defined in the main study protocol

	Source: literature, to be varied in a sensitivity analysis
Error rates	Proportion of test results that are expected to be invalid/indeterminate (and require a second test of that test type) *Source: performance evaluation study, manufacturer specifications*

Box 1: Note and guidance on the end-point of this analysis:

The end-point for this analysis is the confirmation of the final diagnosis and linkage to further care/treatment. The downstream impact of treatment is not included. Should the end-point include treatment of those diagnosed with *T.cruzi* infection, the following additional parameters/data are required and an additional model (e.g. Markov model) is necessary to evaluate the impact of treatment. For example, outputs on the number of cases linked to further care/treatment from the FIND Chagas Diagnostic Algorithm application can be used as inputs into a disease progression model or other to estimate the downstream impact.

Treatment effectiveness: Among patients in the study population with chronic infection who initiate treatment, the proportion that achieve cure by age group (sero-negativization, i.e. two non-reactive conventional serological assays). The probability of cure might be also predicted by a decreasing antibody titres for T. cruzi over time (e.g. after 12, 24, 48 months after treatment). Treatment effectiveness may also be measured as the proportion linked to a cardiological intervention for those with early silent cardiomyopathy.

Adverse events: Among those infected and treated, the proportion who will experience adverse events (by type of event) and the associated health burden (in average DALYs lost) of those events.

Vertical transmission: Among those infected girls and women of childbearing age (up to 44 years) who receive treatment, or among pregnant women if they constitute the target population, the lifetime risk of transplacental transmission of *T. cruzi* to future children that could be prevented with successful treatment.

Burden: Among patients in the study population with *T.cruzi* infection who do not receive any treatment, or who fail treatment, what are the average lifetime DALYs lost due to *T. cruzi* infection – i.e. the DALYs associated with untreated chronic infection (dependent on age and sex). This can include DALYs associated with delayed diagnosis and treatment of newborns if the target population for testing is pregnant women.

Costs: Healthcare costs associated with the care and treatment of those infected with *T.cruzi* infection.

B. Cost analysis

The fully loaded testing cost per individual tested for *T.cruzi* infection will be estimated for the intervention (the novel testing algorithms) and the standard of care. An ingredients-based approach will be used to identify and quantify all the inputs required to perform the respective test, as well as their estimated quantities and value. Costs will reflect both the patient- and provider-perspective. Costs will be estimated on a per test level, and on a per healthcare visit level for both the patient and the provider.

1. *Test-level*: This includes the cost of the test kit/reagents, equipment and consumables required to conduct a given test, cost to transport a sample, as well as the staff salary cost associated with time spent on sample collection, testing, and interpreting the test result by the relevant healthcare-

worker cadre at different levels of care. Additional costs relating to training, quality assurance etc, are detailed in Table 2 below and the accompanying workbook on data collection.

2. *Patient-level visit cost:* A patient-level visit cost will be assigned to the number of visits that it takes an individual to receive a diagnosis, by level of care accessed. This is the cost to the patient of visiting a healthcare centre and may include transportation, accommodation, food, and other non-medical out-of-pocket costs, as well as productivity loss costs associated with the time spent seeking care. The cost to the patient may differ depending on the level of the healthcare system that the patient accesses – for example, higher costs may be incurred to access high-complexity centres versus low-complexity centre. These costs may be sourced from the literature or estimated as part of a separate study (see cost workbook for additional details).
3. *Provider-level outpatient visit cost*: An outpatient visit cost is the overhead cost borne by the health care system associated with a patient visit. The cost is differentiated by the different levels of the healthcare system where the individual is seeking care and receiving testing: e.g. low complexity centres versus high complexity centres. The cost includes overhead costs such as utilities, infrastructure/space costs, staff overhead costs etc. and can be collected as part of the study or estimated using the literature.

Table 2 (and the cost collection workbook) details the key input cost categories: staff, test consumables, test equipment, overhead costs, and transport, and visit costs to the patient and the provider. The per-test costs include all costs related to specimen collection, sample processing and analysis, data management and result delivery. Resource use will be determined through interviews with individuals involved in the implementation of the performance evaluation study in Setting X. All costs will be reported in YEAR USD and converted using standard market rates from the local currency in Setting X. All capital costs will be annuitized and discounted using the discount rate most appropriate for Setting X.

Table 2: Detail on healthcare costs to be sourced (non-exhaustive).

Cost category	Detail
Per test costs (see workbook for further detail)	
Staff	Staff time and salary cost to collect sample, process sample and conduct test and report and record result
Test consumables	Sample collection consumables: e.g. needle, collection tube, cotton wool etc. Laboratory testing consumables: landed cost of test-kit/reagents in Setting X, rack, gloves, tips etc.
Test equipment	Sample storage, centrifuge, ELISA reader, plate washer, pipettes Not applicable for RDT; Equipment required for laboratory serology testing.
Sample transport	If applicable. Sample transportation costs from facility where sample collection occurs to the laboratory where the sample is tested. (Note: if the patient moves, and not the sample, the patient will incur the visit cost)
Training	Cost of training healthcare workers to conduct testing of *T. cruzi* chronic infection by test type (e.g. venue, staff time, accommodation, etc.)
Quality Assurance	External quality assurance cost per year by test type (proficiency testing, supervisory visits per year)
Result delivery and linkage to care	Any costs associated with result delivery and interpretation and linkage to care
Per visit costs	
Patient visit costs	This includes the costs borne by the patient to attend a healthcare visit – transportation, accommodation, food; as well as productivity loss costs associated with the time lost by attending healthcare visits. These costs may be estimated from the literature and the human capital approach using minimum wage data for Setting X may be used to calculated productivity losses.
Outpatient cost per visit	This is the overhead cost borne by the healthcare system associated with a patient visiting the level of care where the sample is collected, and the test performed. This cost includes overhead costs such as utilities, infrastructure/space costs, staff overhead costs etc. These costs could be collected as part of the study and allocated to a diagnostic visit, alternatively they can be estimated using the literature - for example, the WHO CHOICE (Choosing Interventions that are Cost-Effective) website provides unit costs for outpatient visits at different levels of care for a range of countries.

C. Cost-effectiveness analysis

Costs, as described above, will be assigned to resource outputs (number of tests by type and location of testing, and number of individual visits before diagnosis by location) from the key outcomes of the different diagnostic algorithms (the standard of care and the intervention). Effectiveness outcomes (as described above in section A) such as the number of true positive and true negative cases, as well as the number of positive individuals linked to further care/treatment will be used to calculate the **cost per correct diagnosis** and the **cost per positive case linked to further care and treatment** for the different algorithms. The costs and the outcomes for each algorithm can then be used to calculate the incremental cost-effectiveness ratios (ICER) for each diagnostic algorithm, which compares the additional cost of one algorithm relative to the next least costly algorithm, or the standard of care. The ICER will identify the most efficient algorithm or the one that provides the greatest value for money. This formula is depicted below for Outcome^{i} - correct diagnosis, or a positive case linked to further care and treatment.

$$ICER = \frac{Costs^{i}_{ITV} - Costs^{i}_{SOC}}{Outcome^{i}_{ITV} - Outcome^{i}_{SOC}}$$

The **time horizon** is from the point where a person first seeks care to linkage to further care and treatment. No downstream costs and outcomes of treatment are included since the primary objective of this analysis is to assess the performance of alternative diagnostic algorithms and the efficacy of treatment is uncertain and the probability of a false-positive result commencing treatment is low/uncertain. However, the time horizon can be extended to include treatment outcomes and costs in an extended analysis as described in Box 1.

Sensitivity analyses

A one-way sensitivity analysis can be conducted on key parameters that significantly influence which algorithm is considered more cost-effective.

- Firstly, variations in disease prevalence rates can be explored to understand how different prevalence levels impact the cost-effectiveness of each diagnostic algorithm.
- Secondly, the sensitivity analysis can assess the effect of fluctuations in the price of test kits, as this cost can substantially affect the overall cost of the diagnostic algorithm.
- Thirdly, variations in the rate of patient loss to follow-up between visits can be examined, considering its implications on total diagnostic yield.
- Fourth, variations in the diagnostic test performance can be varied.
- Lastly, the assumptions regarding the likely increase in access to testing through the decentralisation of testing can be explored to gauge the influence on diagnostic yield and costs.

If appropriate, a probabilistic sensitivity analysis can be conducted to demonstrate the uncertainty surrounding the cost-effectiveness results by incorporating probability distributions for the key parameters mentioned above and running multiple simulations to generate a distribution of ICERs.

By systematically varying these critical parameters, the sensitivity analysis provides insights into the robustness and reliability of the economic evaluation results, offering stakeholders a comprehensive

understanding of the potential impacts of uncertainties surrounding key factors in decision-making regarding diagnostic algorithm selection for diagnosing chronic *T.cruzi* infection.

D. Budget impact analysis

A BIA aims to assess the financial implications of implementing the new diagnostic approach (the intervention) compared to the standard of care. This analysis entails determining the total cost of testing the eligible care-seeking population under both scenarios: using the standard diagnostic procedure and employing the intervention algorithm. The BIA is calculated from the payer's perspective (depending on the setting, this is likely to be the government body who is responsible for public sector healthcare budget). The costs involved include those associated with determining the test and provider visit costs, as described above. It excludes the patient visit costs as the perspective is the healthcare funder. Care must be taken to only include *un*discounted costs. By comparing the total costs between the standard of care and the intervention algorithm, the BIA will ascertain the change in budget required for adopting the new diagnostic approach.

To conduct this analysis, the following information is required:

- Time horizon: A time horizon of 1- 3 years is recommended.
- Eligible population: It is necessary to estimate the *annual* care-seeking population in Setting X for which the payer is responsible (the population that is covered by the payer). For example, only those accessing care at public health facilities if the payer is a public health entity. Next, of those seeking care, estimate the eligible population for testing (those who meet the clinical criteria to be tested for CD; note this will differ by specific sub-populations, e.g. mandatory testing for pregnant women). If relevant, estimate the location (level of the health system) at which the eligible population first seeks care.
- Number of tests required per eligible individual. Using output from the cost-effectiveness model, determine the average number of tests required per person tested taking into account loss to follow-up, level of care at which testing occurs and the algorithm structure (for example, additional tests required if there is discordance).
- Uptake of the new intervention: To determine what proportion will receive the new intervention, it is important to estimate the uptake of the new intervention, whether this is likely to (1) completely replace the current standard of care (substitution), or, (2) there will be a combination of both the standard of care and the new intervention (for example, standard of care remains for those first seeking care at high complexity centres, and the intervention for those seeking care at low complexity centres); or, 3) the new intervention would be used at all sites where there is currently no standard of care testing or increase access to testing to the eligible population (expansion).

There are many dependencies associated with the BIA that rely on uncertain assumptions. A sensitivity analysis should explore these: for example, changes in exchange rates, inflation rates and the expected prices of the new testing technology over time, as well as the expected prices of the new testing technology may change depending on total volumes required for the different uptake scenarios. In addition, the

sensitivity analysis should explore the impact of different assumptions regarding uptake of the new intervention.

This assessment will provide stakeholders, such as healthcare providers, policymakers, and payers, with valuable insights into the financial impact of implementing the new algorithm, aiding in decision-making regarding resource allocation and healthcare budget planning.

ORGANIZED BY

FIND Diagnóstico para todos

DNDi La mejor ciencia para los más desatendidos

IN COOPERATION WITH

Organización Panamericana de la Salud

COORGANIZED BY

INGEBI CONICET

ACKNOWLEDGMENTS

Mundo Sano