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

Summary

Background Vertical transmission of *Trypanosoma cruzi* represents approximately 20% of new Chagas disease cases. Early detection and treatment for women of childbearing age and newborns is a public health priority, but the lack of a simple and reliable diagnostic test remains a major barrier. We aimed to evaluate the performance of a point-of-care loop-mediated isothermal amplification (LAMP) assay for the detection of *T cruzi*.

Methods In this proof-of-concept study, we coupled a low-cost 3D printer repurposed for sample preparation and amplification (PrintrLab) to the Eiken *T cruzi*-LAMP prototype to detect vertically transmitted *T cruzi*, which we compared with standardised PCR and with the gold-standard algorithm (microscopy at birth and 2 months and serological study several months later). We screened pregnant women from two hospitals in the Bolivian Gran Chaco province, and those who were seropositive for *T cruzi* were offered the opportunity for their newborns to be enrolled in the study. Newborns were tested by microscopy, LAMP, and PCR at birth and 2 months, and by serology at 8 months.

Findings Between April 23 and Nov 17, 2018, 986 mothers were screened, among whom 276 were seropositive for *T cruzi* (28·0% prevalence, 95% CI 25·6–31·2). In total, 224 infants born to 221 seropositive mothers completed 8 months of follow-up. Congenital transmission was detected in nine of the 224 newborns (4·0% prevalence, 1·9–7·5) by direct microscopy observation, and 14 more cases were diagnosed serologically (6·3%, 3·6–10·3), accounting for an overall vertical transmission rate of 10·3% (6·6–15·0; 23 of 224). All microscopy-positive newborns were positive by PrintrLab-LAMP and by PCR, while these techniques respectively detected four and five extra positive cases among the remaining 215 microscopy-negative newborns.

Interpretation The PrintrLab-LAMP yielded a higher sensitivity than microscopy-based analysis. Considering the simpler use and expected lower cost of LAMP compared with PCR, our findings encourage its evaluation in a larger study over a wider geographical area.

Funding Inter-American Development Bank.

Lancet Microbe 2024

Published Online
https://doi.org/10.1016/S2666-5247(24)00110-1

For the Spanish translation of the abstract see Online for appendix 1

Fundación Ciencias y Estudios Aplicados para el Desarrollo en Salud y Medio Ambiente (CEADES), Cochabamba, Bolivia (L Rojas Panozo MSc, S Rivera Nina BSc, L Pinto MSc, D F Lozano MD, F Torrico MD); **Laboratorio de Biología Molecular de La Enfermedad de Chagas, Instituto de Investigaciones en Ingeniería Genética y Biología Molecular "Dr. Héctor N. Torres" (INGEBI), Buenos Aires, Argentina** (D P Wehrendt PhD, A G Schijman PhD); **Barcelona Institute for Global Health (ISGlobal), Hospital Clinic – University of Barcelona, Barcelona, Spain** (A Casellas MSc, S Mendez MSc, J Gascon MD, S Sanz PhD, J Alonso-Padilla PhD); **AI Biosciences, College Station, TX, USA** (C-W Kuo PhD, S Wong PhD); **Universidad Mayor de San Simón, Cochabamba, Bolivia** (D F Lozano, F Torrico); **Plataforma de Chagas Tarija – Universidad Autónoma Juan Misael Saracho, Tarija, Bolivia** (L Ortiz MD); **Drugs for Neglected Diseases Initiative (DNDi), Rio de Janeiro, Brazil** (M-J Pinazo MD); **CIBER de Enfermedades Infecciosas (CIBERINFEC), Madrid, Spain** (M-J Pinazo, J Gascon, J Alonso-Padilla); **Foundation for Innovative Diagnostics (FIND), Geneva, Switzerland** (A Picado PhD); **Fundación Mundo Sano, Buenos Aires, Argentina** (M Abril BSc)

Introduction

Chagas disease, caused by the parasite *Trypanosoma cruzi*, is endemic to 21 countries in Latin America, where it exerts a greater burden than any other parasitic disease.[1] Large migratory flows between endemic and non-endemic regions have expanded the impact of Chagas disease over the past two decades.[2,3] Chagas disease is estimated to affect 6 million people worldwide,[4] but surveillance and reporting of infection and disease rates are underestimated.

T cruzi (order Kinetoplastida; family Trypanosomatidae) is transmitted by triatomine vectors (order Hemiptera; family Reduviidae).[4] Vector-mediated transmission by ingestion of parasite-contaminated food or drink leads to outbreaks of acute oral Chagas disease,[4] while vector-independent acquisition of the parasite can occur through transfusion of parasitised blood or blood-derived products, by organ transplantation from a *T cruzi*-positive donor, and via vertical transmission.[4,5]

According to the Pan American Health Organization (PAHO), an estimated 1·12 million women of childbearing age are positive for *T cruzi*, and around 9000 newborns positive for *T cruzi* are born each year, accounting for approximately 20% of all new cases.[5] Timely detection and treatment of women and newborns should become a priority for public health policies. In line with this, PAHO has called for active screening and treatment of women of childbearing age as an effective strategy for preventing vertical transmission of *T cruzi*.[5,6] However, despite efforts towards expanding and improving access to diagnosis and treatment, there are several barriers to tackling congenital

Correspondence to:
Dr Julio Alonso-Padilla, Barcelona Institute for Global Health (ISGlobal), Hospital Clinic – University of Barcelona, 08036 Barcelona, Spain
julio.a.padilla@isglobal.org

Research in context

Evidence before this study

We searched PubMed for studies published between database inception and Jan 1, 2023, using the search terms "Chagas disease", "*Trypanosoma cruzi*", and "LAMP" without language restrictions. We found 11 original research articles that evaluated the use of loop-mediated isothermal amplification (LAMP) as a diagnostic method for Chagas disease. In three of them, it was clinically evaluated against the gold-standard algorithm to detect vertically transmitted *Trypanosoma cruzi*. Eiken *T cruzi*-LAMP, which targets the parasite satellite DNA gene, showed a sensitivity range of 93–97% and a specificity range of 94–100%. Another LAMP development targeting the 18s rRNA gene, despite showing a 100% specificity, had a poorer sensitivity (69·2%).

Added value of this study

We have analysed the use of an innovative tool that combines a low-cost DNA extraction and amplification system (PrintrLab) and a high-quality, easy-to-use LAMP test, as an alternative method to circumvent current limitations towards timely detection of vertically transmitted *T cruzi*.

Implications of all the available evidence

The promising results obtained in this study encourage a larger evaluation of the PrintrLab–*T cruzi*-LAMP, which could eventually lead to an alternative molecular diagnostic test for detection of *T cruzi* in newborns shortly after birth. This would be of utmost importance considering that timely treatment of newborns is highly efficacious.

transmission. The lack of a sensitive and rapid diagnostic test means that many positive newborns are lost to follow-up, which prevents treatment at a stage in their lives when it is most effective.[6]

The current methodology to detect vertically transmitted *T cruzi* within the first months of life involves looking for the parasites in the newborns' peripheral blood by microscopy, following techniques that are operator-dependent and have a sensitivity below 50%.[7,8] Hence, a serological study must be performed several months later, once maternal *T cruzi* antibodies have waned.[6] The use of molecular diagnostics yields higher sensitivity than microscopy, providing timely diagnosis of vertically transmitted *T cruzi*.[7,8] It has been implemented in high-income non-endemic regions such as Catalonia (Spain),[7] and real-time PCR (rtPCR) products are commercially available.[9] However, despite the increased use of PCR after the COVID-19 pandemic, it is still very costly and requires highly trained personnel. Consequently, it is not used in many regions where the disease is endemic.

The easy-to-use loop-mediated isothermal amplification (LAMP) assay stands as a technological solution to those drawbacks. Eiken Chemical (Tokyo, Japan) pioneered this technology.[10] Its *T cruzi*-LAMP is an extensively evaluated prototype that works best with purified DNA as template.[11–14] To provide this type of sample at a fraction of the cost of current nucleic acids purification systems, AI Biosciences (College Station, TX, USA) developed a 3D-printer inspired low-cost device: the PrintrLab.[15] Its extraction protocol relies on magnetic beads kits to yield automated purification of nucleic acids. The PrintrLab also hosts the isothermal amplification step, providing a system for nucleic acids purification and amplification. Interestingly, a procedure to couple it to the *T cruzi*-LAMP has been described.[16]

The objective of this proof-of-concept study was to validate the use of the PrintrLab–*T cruzi*-LAMP (PrintrLab-LAMP; the index test) as a point-of-care test to detect *T cruzi* transmission in newborns shortly after birth.

Methods

Study design and participants

Enrolment of participants was offered upon information, education, and communication regarding the study purposes. Decision to participate was acknowledged by signature of the corresponding informed consent forms. We recruited pregnant women aged 16 years or older who attended the maternity wards of the Hospital Rubén Zelaya in Yacuiba and the Hospital Municipal of Villa Montes for their last routine checkup or delivery visit. *T cruzi*-seropositive mothers were offered an opportunity for their newborns to participate. Yacuiba and Villa Montes are the main urban centres of the Bolivian Gran Chaco province. Recruitment of participants took place between April and November, 2018. Having received antiparasitic treatment (benznidazole) for Chagas disease and a delivery date outside of the recruitment period were exclusion criteria.

To compare the performance of the PrintrLab-LAMP (and rtPCR) with that of the microscopy-based tests within the current algorithm, additional samples of whole blood in EDTA (edetic acid) were collected from newborns of *T cruzi*-seropositive mothers at birth (24–48 h after delivery) and between 1 month (30 days) and 3 months of age. These sampling times coincide with the analysis of the two microscopy-based tests (known as the micromethod) performed for the newborns; the micromethod involves centrifugation of blood (four heparinised capillary tubes per newborn) followed by examination for the presence of swimming trypomastigotes in the interphase layer.[17] Additionally, serum samples were collected from children at 8 months of age for the serological study. Self-reported presence of triatomine vectors in the household was collected to consider vector transmission events.

PrintrLab-LAMP performance was compared with that of a standard rtPCR (the comparator test),[18] operated in a well-equipped laboratory (Fundacion CEADES, Cochabamba, Bolivia). The outcome of both molecular tests was compared with that of the reference algorithm to detect *T cruzi* in newborns. Due to the low estimated incidence

of vertical transmission per 100 livebirths,[4] a statistical approach that involved the analysis of all samples collected over a determined period (7 months) was applied.[19] We considered a sample size of 240 positive pregnant women to obtain a minimum of 12 positive children with vertical *T cruzi* transmission (5% prevalence).[20]

The study complied with the principles of the Declaration of Helsinki. The Ethics Committee from the Hospital Clinic of Barcelona (Spain) and from Fundacion CEADES (Bolivia) reviewed and approved the study protocol (reference numbers HCB/2018/0083 and CE-LAMP1-23022-018). All study participants signed the corresponding informed consent form. If they were younger than 18 years, this was signed by the mother, father, or assigned legal tutor. Participants who tested positive for *T cruzi* (by the current algorithm) were referred for treatment with benznidazole.[17]

Procedures

Mothers' blood samples (approximately 3·0 mL) were collected by arm venous puncture, centrifuged, and the isolated serum samples used in the ELISA tests or stored frozen at –20 °C until needed. The Wiener Recombinant version 3.0 ELISA (Wiener Lab, Rosario, Argentina), and the Lemos Chagatek (Laboratorios Lemos, Buenos Aires, Argentina) were used as primary tests. In case of discordancy, the Werfen BioELISA (Lliçà d'Amunt, Spain) was used. All tests were conducted as per manufacturers' instructions. Data are shown as signal-to-cutoff (S/CO) distance—ie, difference between the optical density readout by ELISA and the kit-established cutoff—as a measure of the level of positivity.

Newborns' whole-blood samples (approximately 0·6 mL) were collected at birth and at 2 months of age from the veins on the back of the hand, and by arm venous puncture at 8 months of age (approximately 1·0 mL). Part of the blood sampled at birth and at 2 months of age (approximately 0·2 mL) was loaded in heparinised capillary tubes and observed under the microscope in search of parasites by the micromethod.[7] The rest of the blood collected at those timepoints was anticoagulated in EDTA-K2 tubes and stored frozen at –20 °C in the hospital laboratory until use with the PrintrLab-LAMP or PCR. Blood collected at 8 months of age was left to coagulate and the isolated serum stored frozen for use in the serological assays. Some of these serum samples were additionally analysed by haemagglutination inhibition assay (HAI) with the Lemos Polychaco kit,[21] and by PCR amplification of *T cruzi* DNA.[22]

The protocol for the PrinterLab-LAMP was described by Wehrendt and colleagues (see procedure D in their paper).[16] Results were read by the naked eye. rtPCR amplification of *T cruzi* satellite DNA was performed as described by Duffy and colleagues.[18] It was first run qualitatively as single determinations, and samples registered as positive were analysed in duplicate through quantitative PCR (qPCR), using a standard quantification curve expressed in parasite equivalents per mL (parEq/mL) of blood samples.[18] Results were reported as mean with SD, unless the amplification was only achieved with one of the two duplicate determinations. PrintrLab-LAMP and PCR were performed in different laboratories by different operators, and results were respectively unknown to each other.

Statistical analysis

Data are described as frequencies and mean (with SD) for discrete and continuous variables, respectively. The agreement between classification tools was estimated using the kappa statistic (κ).[23,24] The classification performance of the LAMP and PCR was compared with the gold standard (microscopy at birth and 2 months and serological study several months later).[17] We calculated their sensitivity and specificity as follows:

$$\text{Sensitivity} = \frac{\text{True positives}}{\text{True positives} + \text{false negatives}} \times 100$$

$$\text{Specificity} = \frac{\text{True negatives}}{\text{True negatives} + \text{false positives}} \times 100$$

Sensitivity and specificity were also calculated for the use of the micromethod. To calculate the 95% CIs of the sensitivity and specificity, we used the formulas described in appendix E of Berkman and colleagues' study.[25] The analysis was carried out using Stata (release 16).[26]

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

A total of 986 mothers were screened between April 23 and Nov 17, 2018. We found 276 confirmed *T cruzi*-positive cases—ie, a prevalence of 28·0% (95% CI 25·6–31·2)—based on concordant ELISA tests. The study flowchart and general description of the cohort are in figure 1 and table 1, respectively. Of the 276 mothers with confirmed *T cruzi*, the newborns of 259 (93·8%) were involved in the study. 262 newborns completed follow-up at birth, 253 (96·6%) completed follow-up at 2 months, and 224 (85·5%) had serological data at 8 months.

Regarding the place of collection of the samples, at month 2, 64·0% (95% CI 57·8–69·9; 162 of 253) were obtained at the hospital and 36·0% (30·1–42·2; 91 of 253) at domiciliary visits. At the following visit at month 8, 45·5% (38·9–52·3; 102 of 224) of the samples were obtained during hospital visits and 54·5% (47·7–61·1; 122 of 224) upon domiciliary visits.

For the early diagnosis of vertical *T cruzi* infections, the micromethod at birth and at 2 months of age detected nine cases, which meant a transmission rate of 4·0% (95% CI 1·9–7·5; nine of 224); six were found at birth and the other three at month 2 (table 2). One of the children was positive by the micromethod at both timepoints (table 2,

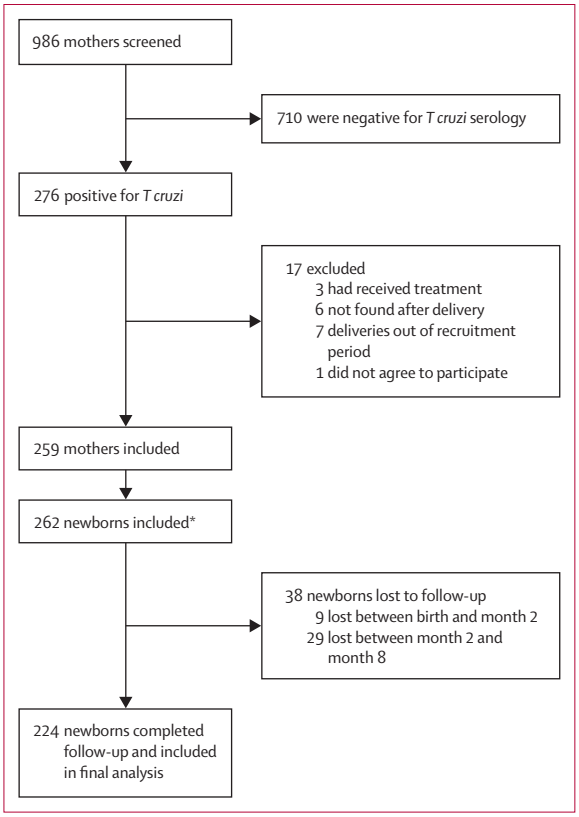

Figure 1: **Flow of participants**
*Three mothers had twins.

figure 2). All these children were referred for treatment, which was completed. When the children positive for *T cruzi* at birth were serologically studied at month 8, they all had negative results, indicating successful treatment (figure 2). In contrast, the three children who were positive for *T cruzi* at 2 months of age remained serologically positive, despite having received treatment (figure 2).

The PrintrLab-LAMP and PCR assays detected all the micromethod-positive cases (table 2, figure 2). Moreover, the PrintrLab-LAMP assay yielded three additional detections of the parasite DNA on samples collected at birth, and another two on samples collected at month 2 (figure 2). All were confirmed serologically, except one sample which was positive at birth only through the PrintrLab-LAMP assay, negative for all three tests at month 2 (micromethod, PrintrLab-LAMP, and PCR) and then negative for serology at month 8 (figure 2).

See Online for appendix 2

	Villa Montes	Yacuiba	Overall
Mothers			
Participating mothers	81 (36·7%)	140 (63·3%)	221
Age of mother, years	28·3 (24·0–32·0)	30·5 (25·0–36·0)	29·9 (25·0–34·8)
Gave birth to twins	1 (0·5%)	2 (0·9%)	3 (1·4%)
Newborns			
Total	82 (36·6%)	142 (63·4%)	224
Female	43 (52·4%)	58 (40·8%)	101 (45·1%)
Male	39 (47·6%)	84 (59·2%)	123 (54·9%)

Data are N, n (%), or median (IQR).

Table 1: **Characteristics of study participants who completed all follow-up visits**

	At birth	At 2 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% increase)	8 *vs* 4 (100% increase)
Specificity (95% CI)	99% (86–100)	98% (86–100)
rtPCR accuracy§		
Increased detection of positivity *vs* microscopy	9 *vs* 6 (50% increase)	10 *vs* 4 (150% increase)
Specificity (95% CI)	99% (86–100)	97% (85–100)
Agreement between PrintrLab-LAMP and rtPCR¶	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

Data are n (%) or N, unless otherwise stated. Cases are ordered with respect to their first positive detection by micromethod either at birth or month 2, and then by PrintrLab-LAMP or PCR positivity. For a full summary of microscopy, PrintrLab-LAMP, or PCR positive cases see appendix 2 p 1. Ct=cycle threshold. LAMP= loop-mediated isothermal amplification. qPCR=quantitative PCR. rtPCR=real-time PCR. *Three of the four positive microscopy detections at month 2 were new cases, and one corresponded to a child already positive by microscopy at birth. †Five of the eight positive PrintrLab-LAMP detections at month 2 were new cases, and the other three were already positive at birth. ‡Five of the ten positive rtPCR detections at month 2 were new cases, and the other five were already positive at birth. §Accuracy was calculated against the results of the microscopy-based assays. ¶The agreement between molecular tools was estimated using the κ statistic (see Methods).

Table 2: **Summary of positive cases by microscopy or either of the two molecular-based techniques (LAMP or PCR)**

The PCR confirmed one of the PrintrLab-LAMP-positive samples at birth and detected two extra cases at that timepoint compared with the micromethod and the PrintrLab-LAMP (figure 2). Notably, these two cases had very low parasite loads (0·011 parEq/mL and 1·48 parEq/mL; appendix 2 p 1). The PrintrLab-LAMP-positive determinations at month 2 that were micromethod-negative were also positive by PCR, which further detected an extra case at this timepoint, albeit with a very low parasite load (YAC141; figure 2 and appendix 2 p 1). Altogether, the agreement between the PrintrLab-LAMP and the PCR on samples collected at birth was 98·2% (255 of 259) with a κ of 0·77 (95% CI 0·64–0·90), corresponding to substantial agreement. With samples from 2 months of age, the level of concordance between both molecular tests was 99·2% (251 of 253) with a κ of 0·88 (0·75–1·01), indicative of nearly perfect agreement. The sensitivity of the LAMP was slightly lower than that of the PCR as it detected two fewer cases (table 2).

For the serological study, children's samples were collected at a mean age of 9·3 months (SD 0·6). ELISA tests detected 27 positive children: ten were positive with the

	Birth			2 months			8 months
	Microscopy	PrintrLab-LAMP	PCR	Microscopy	PrintrLab-LAMP	PCR	Serology
Newborns who were treated							
YAC34	Positive	Positive	Positive	Negative	Positive	Positive	Negative
YAC97	Positive	Positive	Positive	Negative	Negative	Negative	Negative
YAC106	Positive	Positive	Positive	Negative	Negative	Negative	Negative
YAC142	Positive	Positive	Positive	Negative	Negative	Negative	Negative
VM5	Positive	Positive	Positive	Negative	Negative	Negative	Negative
VM70	Positive	Positive	Positive	Positive	Positive	Positive	Negative
YAC47	Negative	Negative	Negative	Positive	Positive	Positive	Positive
VM49	Negative	Negative	Negative	Positive	Positive	Positive	Positive
VM61	Negative	Negative	Positive	Positive	Positive	Positive	Positive
Newborns who were not treated (within the study period)							
YAC158*	Negative	Positive	Negative	Negative	Negative	Negative	Negative
VM22	Negative	Positive	Negative	Negative	Negative	Negative	Positive
VM45	Negative	Positive	Positive	Negative	Positive	Positive	Positive
VM28	Negative	Negative	Positive	Negative	Negative	Positive	Positive
YAC23	Negative	Negative	Negative	Negative	Positive	Positive	Positive
VM36	Negative	Negative	Negative	Negative	Positive	Positive	Positive
YAC141	Negative	Negative	Negative	Negative	Negative	Positive	Positive

Figure 2: **Evolution of the positive cases detected by microscopy or either of the two molecular-based techniques (LAMP or PCR) including their serological status**
In the study number identifiers, YAC stands for Yacuiba and VM for Villa Montes. LAMP=loop-mediated isothermal amplification. *Study subject YAC158 did not receive treatment as the serological study at month 8 determined it was a negative case.

two main ELISAs (Wiener and Lemos), but 17 were discordant and required the third test (Werfen) to confirm a positive determination obtained only by the lysate-based Lemos. Among the ten positive determinations with the main ELISAs, three had been positive by the micromethod, PrintrLab-LAMP, and PCR at month 2 (YAC47, VM49, and VM61; figure 2 and appendix 2 p 1). Another three and four serologically positive children had been detected positive by PrintrLab-LAMP and PCR at month 2, respectively (YAC23, VM36, and VM45 for both, and VM28 specifically by PCR; figure 2 and appendix 2 p 1). Notably, S/CO distance for all these samples in the two main ELISAs was generally more than 0·5 arbitrary units (appendix 2 p 1).

Only three of the ten serologically positive cases with the two main ELISAs escaped detection by molecular methods (YAC1, YAC35, and YAC155; appendix 2 p 2). The presence of triatomine vectors was not reported in the domicile of any of them. Nonetheless, the reported S/CO distance was mostly below 0·4 arbitrary units with both ELISAs, closer to the assays' threshold of detection (appendix 2 p 2).

Among the 17 positive cases that were discordant by the main ELISAs and needed the Werfen BioELISA for confirmation, only one was positive by PrintrLab-LAMP at birth (VM22; appendix 2 pp 1–2) and another was positive by PCR at month 2 (YAC141; see appendix 2 pp 1–2). Notably, those 17 samples were weakly positive, with the S/CO distance often below 0·1 arbitrary units (appendix 2 p 2). With respect to vector presence in the household, only one participant lived in a house with a positive report (YAC37; appendix 2 p 2).

To understand the serological outcome of those samples, we performed HAI and PCR in a subgroup of serum samples that included: the 17 samples positive by Lemos and Werfen ELISAs (group 1 in appendix 2 p 2); 16 samples that were negative by all techniques (group 2 in appendix 2 p 2); six samples that were positive by PrintrLab-LAMP, PCR, and the main ELISAs (group 3 in appendix 2 p 2); and another six samples that were positive by the micromethod, PrintrLab-LAMP, and PCR, but negative by ELISA (group 4 in appendix 2 p 2).

The PCR did not amplify parasite DNA from any of the serum samples, while the internal amplification control (human ribonuclease P) confirmed the presence and reactivity of DNA in those samples (appendix 2 p 2). Regarding HAI results, only four of the 17 Lemos and Werfen-positive samples (group 1) were reported positive, all of them at the limit of detection of the assay (serum dilution 1/16; appendix 2 p 2). In 12 of the 13 samples registered as HAI-negative in group 1, the S/CO distance at the Lemos ELISA was below 0·1 arbitrary units. In contrast, HAI confirmed the positivity of those samples registered as positive by the two main ELISAs and both molecular techniques (group 3; appendix 2 p 2). Similarly, HAI determined as negative 15 of the 16 fully negative samples included, and the false positive HAI result was at the limit of detection of the assay (group 2; appendix 2 p 2). In summary, 14 cases of vertically acquired *T cruzi* infection were diagnosed serologically, accounting for a transmission rate of 6·3% (95% CI 3·6–10·3) determined by this methodology. All samples from children who received timely treatment upon

testing positive by the micromethod were HAI-negative (group 4; appendix 2 p 2), further confirming treatment success.

In total, the congenital transmission rate was 10·3% (95% CI 6·6–15·0; 23 of 224) when the micromethod (nine positive newborns) and serology (14 positive children) results were combined. Among the 14 serologically positive children, ten were positive by the main ELISAs (appendix 2 pp 1–2), and four more positive detections by Lemos and Werfen ELISAs were confirmed by HAI (appendix 2 p 2). In comparison, the PrintrLab-LAMP managed to detect shortly after birth 13 of 23 cases (sensitivity 56·5%; 95% CI 34·5–76·8) with a specificity of 99·5% (97·3–99·9) due to a single false positive (YAC158; figure 2). The PCR detected 14 of 23 cases (sensitivity 60·9%; 36·6–77·9) with a specificity of 100% (98·2–100·0). The micromethod rendered a sensitivity of 39·1% (18·8–59·4) and a specificity of 100% (98·2–100·0). When the LAMP or PCR were compared with the outcome of the micromethod, both outperformed it by around 20%.

The qPCR analysis of the newborns' positive samples indicated that the levels of *T cruzi* parasites ranged from 0·011 parEq/mL to 6040·01 parEq/mL (appendix 2 p 1). Hence, the micromethod managed to detect parasitaemia above 80 parEq/mL (with the exception of YAC97), missing those cases with levels of circulating parasites below that threshold (appendix 2 p 1).

No adverse events were observed from performing the blood extractions, the DNA purification, or running the index and comparator tests.

Discussion

Inability to make an early diagnosis of congenital Chagas disease is a shame given the efficacy of currently available drugs is near 100% when administered in the first year of life, at a time when both drugs (benznidazole and nifurtimox) are well tolerated.[6] In this study, all positive newborns detected by the micromethod were treated, and all of those detected at birth were serologically negative when evaluated at 8 months of age, confirming treatment success.

Remarkably, the PrintrLab-LAMP and the PCR not only mirrored the micromethod detection capacity (nine cases), but outperformed it, respectively finding four and five more vertical infections (PCR additionally detected a case at birth that was not microscopy-positive until month 2). Direct comparison of the LAMP and PCR showed that the performance of the former was only slightly lower despite being a point-of-care test used for the first time in a poorly equipped laboratory.

Both molecular tests yielded a similar sensitivity to that reported elsewhere.[27] Regarding the specificity, the PCR registered a 100% capacity to determine true negatives, while the PrintrLab-LAMP yielded one false positive determination that made its specificity stay at 99·5%. That false positive could be due to the use of EDTA anticoagulated blood, since EDTA can interfere with the calcein-based detection of the LAMP reaction (Eiken Chemical, personal communication). We did not have an opportunity to collect blood treated with an alternative anticoagulant (eg, heparin), and relying on fluorescence detectors instead of the naked eye could guarantee better visualisation of LAMP results.[28] This would help to avoid any doubtful interpretation.

Despite being involved in a study with specifically dedicated personnel, only 45·5% (102 of 224) of the participants attended the hospital for the last visit. This emphasises the importance of information, education, and communication. It also points to how advantageous it would be to have highly sensitive and specific diagnostics for timely detection of congenital Chagas disease. In this respect, the promising results obtained in this work encourage further investigation of the use of the PrintrLab-LAMP to find out whether the satellite DNA target of the prototype yields a similar performance in other regions. Moreover, a study involving several hospitals covering a wide geographical area will enlighten logistical issues concerning the distribution of the PrintrLab-LAMP reagents and that of the ELISAs or rapid diagnostic tests (RDTs). Pregnant women attending the health centres or hospitals for delivery might be unaware that they are positive for *T cruzi*, and the availability of RDTs for fast screening could make a difference considering that the return of ELISA results can take several weeks. Bearing in mind that births also take place in health centres without laboratories nor the possibility to keep and transport frozen samples, the use of alternative sampling supports for LAMP, such as filter paper, should be considered.[28]

A limitation of this study is the small number of cases identified, which is a common hurdle in clinical studies of the vertical transmission of Chagas disease. In the 2–3 years of a project, a large number of pregnant women must be screened to identify those positive for *T cruzi*, the opportunity for their newborns to participate offered, and the 5–10% of vertically transmitted cases eventually identified. Considering that the estimated incidence of *T cruzi* vertical transmission in Bolivia is 0·235 per 100 livebirths,[4] we would have had to enrol more than 80 000 children to assess the agreement between classification tools, an impossible target with the resources and time (17 months) allotted. Another limitation was the time available in which to follow up the infants and perform the serological study. We did not have the opportunity to collect extra serum samples beyond the last visit. Serological analysis of such extra samples would have been very informative,[29] especially to understand the results of the 17 children who had discordant serological results and required the third ELISA.

In its current format, the PrintrLab-LAMP might not fully comply with the ASSURED criteria, as it is not an equipment-free test.[30] However, point-of-care also refers to tests performed near to the patient, improving access to treatment and the disease outcome. In contrast to serological RDTs, which are suitable even for remote areas, the *T cruzi*-LAMP will require a minimum level of

infrastructure. For congenital Chagas disease, taking into account that a large proportion of births in endemic regions occur in primary health centres or secondary level hospitals equipped with laboratories, deployment of the technology would be feasible in many settings.

Already commercialised for malaria and tuberculosis, the attributes of LAMP make it a very promising tool for the diagnosis of acute *T cruzi* infection, including vertical transmission, relapse in immunocompromised patients, or oral outbreaks,[12] and treatment efficacy follow-up.[14] To adopt the technology, besides wider operational evaluation studies, cost–benefit and health economics analyses will be key.

Contributors

Conceptualisation, investigation, methodology, project administration, resources, validation: DFL, LO, M-JP, AP, MA, JG, SW, AGS, FT, JA-P. Data collection, data analysis, data interpretation, formal analysis, investigation, methodology, validation, visualisation: LRP, SRN, DPW, LP, C-WK. Data curation, data verification, formal analysis, software, validation, visualisation: AC, SM, SS, JA-P. Funding acquisition, project administration, resources, supervision: MA, JG, FT, JA-P. Writing—original draft: JA-P. Writing—review and editing: all authors. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

C-WK and SW are employees at AI Biosciences, the biotechnology company that developed the PrintrLab. All other authors declare no competing interests.

Data sharing

We will make available the study protocol and all individual participant data that underlie the results reported in this Article, after de-identification (text, tables, figures, and appendices) after publication. Data will be shared with researchers who provide a methodologically sound proposal to achieve aims in the approved protocol. Proposals should be directed to julio.a.padilla@isglobal.org; to gain access, data requestors will need to sign a data access agreement.

Acknowledgments

This study was funded by the Inter-American Development Bank (Promoting Chagas Disease Control in the Gran Chaco Region; Technical Cooperation ATN/CF-14808-RG), implemented by Fundación Mundo Sano. We are grateful to our colleagues Mirko Rojas and Carlos Ramos for accompanying the project in the Chaco, and to Sebastián Cañon for his administrative expertise. We also want to thank the directors and laboratory personnel of the hospitals Rubén Zelaya in Yacuiba and Municipal of Villa Montes for their great collaboration. Finally, we would like to acknowledge the authorities at SEDES Tarija (Departmental Health Services, Tarija, Bolivia) for their support.

References

1 Lee BY, Bacon KM, Bottazzi ME, Hotez PJ. Global economic burden of Chagas disease: a computational simulation model. *Lancet Infect Dis* 2013; **13:** 342–48.

2 Requena-Méndez A, Aldasoro E, de Lazzari E, et al. Prevalence of Chagas disease in Latin-American migrants living in Europe: a systematic review and meta-analysis. *PLoS Negl Trop Dis* 2015; **9:** e0003540.

3 Irish A, Whitman JD, Clark EH, Marcus R, Bern C. Updated estimates and mapping for prevalence of Chagas disease among adults, United States. *Emerg Infect Dis* 2022; **28:** 1313–20.

4 WHO. Weekly Epidemiological Record (WER), 6 February 2015, vol. 90, no. 6 (pp. 33–44) [EN/FR]. https://reliefweb.int/report/world/weekly-epidemiological-record-wer-6-february-2015-vol-90-no-6-pp-33-44-enfr (accessed May 11, 2023).

5 Pan American Health Organization. EMTCT Plus. Framework for elimination of mother-to-child transmission of HIV, Syphilis, Hepatitis B, and Chagas. https://iris.paho.org/handle/10665.2/34306 (accessed May 11, 2023).

6 Carlier Y, Altcheh J, Angheben A, et al. Congenital Chagas disease: updated recommendations for prevention, diagnosis, treatment, and follow-up of newborns and siblings, girls, women of childbearing age, and pregnant women. *PLoS Negl Trop Dis* 2019; **13:** e0007694.

7 Picado A, Cruz I, Redard-Jacot M, et al. The burden of congenital Chagas disease and implementation of molecular diagnostic tools in Latin America. *BMJ Glob Health* 2018; **3:** e001069.

8 Gallego M, Schijman AG, Alonso-Padilla J. Diagnosis of *Trypanosoma cruzi* infection: challenges on laboratory tests development and applications. In: Pinazo Delgado MJ, Gascón J, eds. Chagas disease. Cham: Springer, 2020: 75–94.

9 Benatar AF, Danesi E, Besuschio SA, et al. Prospective multicenter evaluation of real time PCR Kit prototype for early diagnosis of congenital Chagas disease. *EBioMedicine* 2021; **69:** 103450.

10 Notomi T, Okayama H, Masubuchi H, et al. Loop-mediated isothermal amplification of DNA. *Nucleic Acids Res* 2000; **28:** E63.

11 Besuschio SA, Llano Murcia M, Benatar AF, et al. Analytical sensitivity and specificity of a loop-mediated isothermal amplification (LAMP) kit prototype for detection of *Trypanosoma cruzi* DNA in human blood samples. *PLoS Negl Trop Dis* 2017; **11:** e0005779.

12 Besuschio SA, Picado A, Muñoz-Calderón A, et al. *Trypanosoma cruzi* loop-mediated isothermal amplification (*Trypanosoma cruzi* Loopamp) kit for detection of congenital, acute and Chagas disease reactivation. *PLoS Negl Trop Dis* 2020; **14:** e0008402.

13 Flores-Chavez MD, Abras A, Ballart C, et al. 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. *J Clin Microbiol* 2021; **59:** e01860–20.

14 Muñoz-Calderón AA, Besuschio SA, Wong S, et al. Loop-mediated isothermal amplification of *Trypanosoma cruzi* DNA for point-of-care follow-up of anti-parasitic treatment of Chagas disease. *Microorganisms* 2022; **10:** 909.

15 Chan K, Coen M, Hardick J, et al. Low-cost 3D printers enable high-quality and automated sample preparation and molecular detection. *PLoS One* 2016; **11:** e0158502.

16 Wehrendt DP, Alonso-Padilla J, Liu B, et al. Development and evaluation of a 3D Printer-based DNA extraction method coupled to loop mediated isothermal amplification (LAMP) for point-of-care diagnosis of congenital Chagas disease in endemic regions. *J Mol Diagn* 2021; **23:** 389–98.

17 Ministerio de Salud y Deportes. Programa Nacional de Control de Chagas. Chagas congénito. Estrategias de diagnóstico y control. 2ª Edición. Cochabamba: Ministerio de Salud y Deportes, 2007 (in Spanish).

18 Duffy T, Cura CI, Ramirez JC, et al. Analytical performance of a multiplex Real-Time PCR assay using TaqMan probes for quantification of *Trypanosoma cruzi* satellite DNA in blood samples. *PLoS Negl Trop Dis* 2013; **7:** e2000.

19 Liao JJZ. Sample size calculation for an agreement study. *Pharm Stat* 2010; **9:** 125–32.

20 Bujang MA, Adnan TH. Requirements for minimum sample size foe sensitivity and specificity analysis. *J Clin Diagn Res* 2016; **10:** YE01–06.

21 Egüez KE, Alonso-Padilla J, Terán C, et al. Rapid diagnostic tests duo as alternative to conventional serological assays for conclusive Chagas disease diagnosis. *PLoS Negl Trop Dis* 2017; **11:** e0005501.

22 Melo MF, Moreira OC, Tenório P, et al. Usefulness of real time PCR to quantify parasite load in serum samples from chronic Chagas disease patients. *Parasit Vectors* 2015; **8:** 154.

23 Cohen J. A coefficient of agreement for nominal scales. *Educ Psychol Meas* 1960; **20:** 37–46.

24 Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics* 1977; **33:** 159–74.

25 Berkman ND, Wallace IF, Watson L, et al. Screening for speech and language delay and disorders in children age 5 years or younger: a systematic evidence review for the U.S. Preventive Services Task Force. Rockville, MD: Agency for Healthcare Research and Quality, 2015. https://www.ncbi.nlm.nih.gov/books/NBK305674/ (accessed Dec 6, 2023).

26 StataCorp. Stata Statistical Software: Release 16. College Station, TX: StataCorp LLC, 2019.

27 Messenger LA, Gilman RH, Verastegui M, et al. Towards improving early diagnosis of congenital Chagas disease in an endemic setting. *Clin Infect Dis* 2017; **65:** 268–75.

28 Longhi SA, García Casares LJ, Muñoz-Calderón AA, Alonso-Padilla J, Schijman AG. Combination of ultra-rapid DNA purification (PURE) and loop-mediated isothermal amplification (LAMP) for rapid detection of *Trypanosoma cruzi* DNA in dried blood spots. *PLoS Negl Trop Dis* 2023; **17:** e0011290.

29 Abras A, Muñoz C, Ballart C, et al. Towards a new strategy for diagnosis of congenital *Trypanosoma cruzi* infection. *J Clin Microbiol* 2017; **55:** 1396–407.

30 Land KJ, Boeras DI, Chen XS, Ramsay AR, Peeling RW. REASSURED diagnostics to inform disease control strategies, strengthen health systems and improve patient outcomes. *Nat Microbiol* 2019; **4:** 46–54.