Use of a Novel Chagas Urine Nanoparticle Test (Chunap) for Diagnosis of Congenital Chagas Disease

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Abstract

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Introduction

Trypanosoma cruzi is transmitted to humans via vector, organ transplantation, blood transfusion and from mother to fetus [1]. Initiatives to control Chagas disease have achieved remarkable success, as demonstrated by the decrease in estimated prevalence of infected individuals from 20 million in 1990 to 7.8 million in 2005 [2], [3]. Detection of congenital T. cruzi transmission is considered one of the pillars of control programs. Congenital transmission accounts for 25% of new infections with an estimate of 15,000 infected infants per year in Latin America [2]–[5]. The transmission is considered one of the pillars of control programs of Chagas disease. Congenital transmission accounts for 25% of new infections with an estimated 15,000 infected infants per year. Current programs to detect congenital Chagas disease in Latin America utilize microscopy early in life and serology after 6 months. These programs suffer from low sensitivity by microscopy and high loss to follow-up later in infancy. We developed a Chagas urine nanoparticle test (Chunap) to concentrate, preserve and detect T. cruzi antigens in urine for early, non-invasive diagnosis of congenital Chagas disease.

Methodology/Principal Findings: This is a proof-of-concept study of Chunap for the early diagnosis of congenital Chagas disease. Poly N-isopropylacrylamide nano-particles functionalized with trypan blue were synthesized by precipitation polymerization and characterized with photon correlation spectroscopy. We evaluated the ability of the nanoparticles to capture, concentrate and preserve T. cruzi antigens. Urine samples from congenitally infected and uninfected infants were then concentrated using these nanoparticles. The antigens were eluted and detected by Western Blot using a monoclonal antibody against T. cruzi lipophosphoglycan. The nanoparticles concentrate T. cruzi antigens by 100 fold (western blot detection limit decreased from 50 ng/ml to 0.5 ng/ml). The sensitivity of Chunap in a single specimen at one month of age was 91.3% (21/23, 95% CI: 71.92%–98.68%), comparable to PCR in two specimens at 0 and 1 month (91.3%) and significantly higher than microscopy in two specimens (34.8%, 95% CI: 16.42%–57.26%). Chunap specificity was 96.5% (71/74 endemic, 12/12 non-endemic specimens). Particle-sequestered T. cruzi antigens were protected from trypsin digestion.

Conclusion/Significance: Chunap has the potential to be developed into a simple and sensitive test for the early diagnosis of congenital Chagas disease.

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**Methods**

**Ethics statement**

The protocols were approved by the institutional review boards of Hospital Universitario Japones, Asociacion Benefica PRISMA (Lima, Peru), Universidad Peruana Cayetano Heredia (Lima, Peru), Universidad Catolica Boliviana (Santa Cruz, Bolivia) and Johns Hopkins Blooomberg School of Public Health (Baltimore, MD).

**Study design and human samples**

The present study is a proof-of-concept study to evaluate the performance of Chunap in the early diagnosis of congenital Chagas disease. Specimens were collected during studies of congenital Chagas disease conducted in Hospital Universitario Japones and Centro de Salud 18 de Marzo in Santa Cruz, Bolivia from 2009 to 2012. The samples included in this analysis represent a select subset (74 specimens from infants without congenital infection and 23 specimens from infants with congenital infection whose urine collection occurred prior to initiation of antitrypanosomal treatment). Urine samples from 12 seronegative infants from Lima, Peru were collected as non-endemic negative controls. Trained study nurses explained the protocol to women presenting for delivery and obtained written informed consent. A specimen was collected to screen for maternal *T. cruzi* infection. The specimen used for screening was a maternal venous blood specimen if labor was not far advanced, or cord blood if the mother was admitted straight to the delivery room in late stages of labor. The screening specimen was tested by 2 rapid diagnostic tests (RDTs): the indirect hemagglutination test (IHA) (PolyChaco, sensitivity and specificity according to manufacturer’s instructions: 98% and 99%, respectively) and Trypanosoma Detect, an immunochromatographic strip assay (InBios International) (Sensitivity: 90.7%, Specificity: 100% [29]). A study nurse attended each delivery. For women diagnosed as infected in cord blood, a maternal blood specimen was collected after the mother recovered from the delivery and before discharge from the hospital. Mothers with positive results by one or more rapid tests and their infants were asked to return for follow-up at 1, 6 and 9 months. At the 1-month visit, 5-cc urine samples were collected. Blood samples from infants obtained at 0 and 1 months were evaluated by the micromethod and PCR. The micromethod is the technique used routinely in Bolivian hospitals to screen for congenital Chagas disease. In this technique, cord or neonatal blood is collected in 4–6 heparinized microhematocrit tubes, centrifuged and the buffy coat layer examined microscopically for parasites [30]. Maternal sera and the 6- and 9-month infant sera were tested by at least two of the following IgG serology assays (sensitivity and specificity according to manufacturer’s instructions): Chagatest Recombinant ELISA (Wiener Laboratories, Argentina. Sensitivity: 99.3%, and Specificity: 100%), Chagatest ELISA with *T. cruzi* cytoplasmic and membrane antigens (Wiener Laboratories, Argentina. Sensitivity: 100%, and Specificity: 99.6%), and the IHA (PolyChaco). Infants were considered to have congenital infection if they had positive results by microscopy or PCR in 0 or 1 month specimens, or positive results by two or more serologic tests at 6 or 9 months. The nurses were blinded to the Chagas status of each infant. Infants with confirmed infection were referred for treatment by the physicians designated by the Bolivian National Chagas Disease Control Program [31].

**Nanoporous particles synthesis**

Hydrogel nanoporous particles were synthesized as previously described [23]–[28]. Briefly, N-isopropylacrylamide (NIPAm, copy, but the technical requirements and cost preclude routine use in resource-limited settings. A sensitive, specific and field-friendly screening test is needed to enable effective Chagas disease screening [7]–[9].

Urine antigen detection is an attractive alternative to improve the diagnosis of congenital *T. cruzi* infection. The non-invasive nature promotes high acceptability by parents. Reported sensitivity varies from 32.6% to 100% [18]–[22], depending on the phase of the infection and the methodology used. *T. cruzi* antigens were detected with a sensitivity of 80–90% in urine samples from a small number of congenitally infected infants by a sandwich ELISA test using a panel of monoclonal antibodies [20], [21]; however, this observation was never replicated. In our experience, we achieved a sensitivity of 67.5% in urine ultrafiltrate from acutely infected guinea pigs using a polyclonal antibody to trypomastigote excretory-secretory antigen [19].

Antigens exist in urine in very low concentrations and are susceptible to degradation within minutes after collection. A novel nanotechnology, using capturing nanoporous hydrogel particles produced with poly (N-isopropylacrylamide) (poly(NIPAm)) and N,N'-methylenebisacrylamide (BAAm) and coupled to chemical antigens from enzymatic degradation. Chunap allows for the early diagnosis of congenital Chagas disease, and with appropriate adaptation, may allow early point-of-care intervention.
lyophilized particles. The concentration of particles was determined by weighing the supernatant was adjusted to pH 5–6 with 1M HCl. To avoid aggregation particles were incubated with the activated particles at room temperature and remazol brilliant blue R) were coupled by condensation to the poly(NIPAm-co-AAc) particle suspension was centrifuged, the supernatant was discarded and the particle pellet was re-suspended in 0.2 M Na 2HPO4 buffer pH 7.0. The Chunap was carried out by a laboratory biologist who was also blinded to the Chagas status of the patient.

Protection of antigens from degradation

In order to evaluate the ability of poly(NIPAm) particles to protect T. cruzi antigens from enzymatic degradation, H49 and 1F1 T. cruzi antigens (1 µg) were incubated with 3 ng of trypsin (Promega, WI, USA) at 37°C for one hour in 50 mM Tris-HCl pH 7.2, in the presence or absence of particles. After the incubation, antigens were eluted as described above and analyzed with SDS-PAGE analysis.

Gel electrophoresis

SDS-PAGE analysis was performed using 4–20% Tris Glycerine polyacrylamide gel (Invitrogen Corporation, CA-USA) using a Novex X-Cell IITM Mini-Cell (Invitrogen Corporation, CA-USA), at 200 V for 50 minutes. Visualization of bands was performed by silver staining or by Western blot.

Detection of T. cruzi antigens by western blot

Resuspended antigens (20 µl) were mixed with 4 µl of sample buffer (50 mM TrisHCl pH 6.8 and treated with 2% SDS, 144 mM 2-mercaptoethanol, 10% glycerol and 0.01% bromphenol blue) and heated to 100°C for 7 min. The antigens were separated on 4–20% Tris Glycerine polyacrylamide gels and then transferred to polyvinylidene difluoride membranes (PVDF) (Millipore, MA-USA). The membranes were blocked with casein-based buffer: PBS supplemented with 0.1% Tween 20 (PBST) and 0.2% 1-Block (Applied Biosciences, CA-USA) for one hour. The membranes were incubated overnight with mouse monoclonal antibody anti-LPG diluted 1/250 in the casein-based buffer. After six washing steps with PBST, membranes were incubated with peroxidase conjugated goat anti-mouse IgG and IgM (Invitrogen Corporation, CA-USA) diluted 1/5 000 in casein-based buffer for 60 minutes. Visualization of antigenic bands was done using an enhanced chemiluminescence system (Supersignal West Dura, Thermo Fisher Scientific, MA-USA). In each PVDF membrane we included 5 ml of normal urine sample containing 1 ng of TESA antigen. The Western blot analysis was performed by silver staining or by Western blot.
75 kDa and 82 kDa) was considered as a positive result for the Chunap. The criteria for defining a positive band depended on the judgment of a trained analyst.

**DNA extraction and real time PCR**

Real time PCR was performed to evaluate levels of parasitemia in 500 μl of cord blood at birth or 200 μl of blood obtained at 1, 6 and 9 months-old. DNA extraction and quantitative real time PCR (qPCR) were performed based on published methods [33,34] with the modifications detailed in a previous publication [7]. The primer set Cruzi 1 (5’-ASTCG-G-C-G-A-T-C-G-T-T-T-T-CGA-3’) and Cruzi 2 (5’-AAT-T-T-C-G-C-A-G-A-G-A-G-G-A-G-G-A-ATA-3’) was used to amplify a 166-base pair DNA fragment. The probe Cruzi 3 (5’-CAG-A-C-A-G-G-G-A-G-A-C-A-A-G-G-A-CAA-3’) was labeled with 5’FAM (6-carboxylfluorescein) and 3’MGB (minor groove binder).

**Statistical analysis**

STATA 10.0 software was used to calculate the sensitivity and specificity of each diagnostic test with 95% confidence interval.

**Results**

**Selection of optimal protein affinity bait for concentration of T. cruzi antigens**

Poly (N-isopropyl acrylamide) (NIPAm) particles functionalized with all molecular baits tested in this study (acid blue 22, acid black 48, bismarck brown Y, pararosaniline base, trypan blue and remazol brilliant blue R) captured H49 and TLA antigens to some degree; but poly(NIPAm/trypan blue) [poly(NIPAm/TB)] particles were the most effective because they completely sequestered the target protein from the solution (Figure 1A). In order to further characterize the yield of

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**Figure 1. Sequestration and concentration of T. cruzi antigens by bait functionalized poly(NIPAm) particles.** Silver stain analysis: A. H49 antigen (50 ng) was spiked in 250 μl of normal urine samples, and incubated with different NIPAm particles for 15 min at room temperature. Particles were separated by centrifugation, and a SDS-PAGE analysis was performed of the particles (containing bound proteins) and the supernatant (20 μl, containing unbound proteins). NIPAm based particles functionalized with different affinity baits successfully captured and concentrated T. cruzi antigens (H49 recombinant protein). Poly(NIPAm/trypan blue) (TB) particles completely captured H49 antigen and deplete the supernatant. IS: Initial Solution. S: Supernatant (unbound proteins). P: Particles (containing bound proteins). B. 250 μl of H49 antigen (20 ng) was incubated with poly (NIPAm/trypan blue) particles. After incubation and centrifugation the supernatant (S, unbound proteins) was saved and particles were washed with 250 μl of miliQ water. After centrifugation, the wash solution (W) was saved and the elution of antigens from particles was performed using acetonitrile-based elution buffer (E). A complete elution of H49 antigen from poly(NIPAm/TB) particles was obtained, and T. cruzi antigens were not lost during the washing step. E: Elute, W: washing solution (20 μl), P: Particle content after elution indicating not presence of H49 antigen, S: supernatant (20 μl, unbound proteins), IS: Initial Solution (20 μl, corresponds to 1.6 ng). C. Poly(NIPAm/TB) particles completely capture different types of T. cruzi antigens. TESA: Trypomastigote excretory-secretory antigen and 1F8: recombinant antigen 1F8. E: Elute from particles. S: Supernatant (unbound proteins). Western Blot analysis using a mouse monoclonal antibody to LPG of T. cruzi: D. Concentration of trypomastigote lysate antigen (TLA) in urine using poly(NIPAm/TB) particles. Poly(NIPAm/TB) particles capture and concentrate TLA antigen in the presence of excess competing proteins in urine. The limit of detection of TLA antigen by Western Blot substantially improves when urine samples were treated with particles, from 50 ng/ml without particle treatment to 0.5 ng/ml with particle treatment.

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poly(NIPAm/TB) particles pre-processing step, we demonstrated by SDS PAGE analysis that H49 antigen was not lost during the washing step and was eluted from the particles with a yield higher than 95% (Figure 1B) (similar results were obtained for TLA). In order to investigate whether poly(NIPAm/TB) particles have high affinity also for other T. cruzi antigens, particles were incubated with TESA and recombinant 1F8 antigen. SDS PAGE analysis demonstrated that poly(NIPAm/TB) particles completely sequestered all the T. cruzi antigens tested (Figure 1C).

Our Chagas urine nanoparticle test (Chunap) uses poly(NIPAm/TB) particles for concentration of T. cruzi antigens in urine. A detection limit (DL) of 0.5 ng/ml was achieved with an initial urine volume of 5 milliliters after concentration of antigens by poly(NIPAm/TB) particles. The DL of western blot without particle concentration preprocessing step was 50 ng/ml, yielding a concentration factor of 100 fold (Figure 1D).

**Poly(NIPAm/trypan blue) particles characterization**

Hydrogel particles hydrodynamic dimensions typically exhibit a temperature responsive behavior. The diameter of the poly (NIPAm/TB) particles decreased with increasing temperature, as expected (from 780 nm at 20°C to 320 nm at 45°C). The diameter of particles at 25°C and pH 4.5 was 758.6 nm±15.03.

**Protection from degradation of T. cruzi antigens by poly(NIPAm/trypan blue) particles**

Bait functionalized capturing particles protect captured analytes from enzymatic degradation even if the degradative enzyme is small enough to penetrate inside the particles [24]. In this study, trypsin was captured and concentrated by poly[NIPAm/TB] particles (Figure 2A). Even if trypsin was fully captured by poly[NIPAm/TB] particles, H49 and 1F8 T. cruzi antigens were completely protected from enzymatic digestion in the presence of poly(NIPAm/TB) particles (Figure 2B). As a positive control, complete degradation of H49 and 1F8 T. cruzi antigens was observed in presence of trypsin at 37°C after 1 hour (Figure 2B).

**Detection of T. cruzi antigens in urine of infants**

The table 1 shows the results of diagnostic testing for each infected infant. Combining the results from the birth and 1-month specimens, the cumulative sensitivity of micromethod and PCR was 34.8% (8/23) and 91.3% (21/23), respectively. Bands of 22 kDa, 42 kDa, 58 kDa, 75 kDa and 82 kDa were detected in urine samples of infected babies (Figure S2). The presence of any of these five bands was considered as a positive result for the Chunap. Chunap showed 91.3% sensitivity (95% CI: 71.92%–90.68%) in a single specimen at one month of age, comparable to the 88.2% sensitivity of PCR at this time point (95% CI: 63.5%–90.2%) (Table 1). Parasitemia levels determined by qPCR peaked at one month of age with subsequent decrease over time (Figure 3). Chunap specificity was 96.5% (95% CI: 90.1% to 99.2%), 71 negative results/74 specimens from uninfected babies in the endemic site, 12 negative results/12 specimens from babies in the non-endemic site).

**Discussion**

In this study, we demonstrate for the first time that our Chagas urine nanoparticle assay (Chunap) detects congenital Chagas disease in a single urine specimen at one month of life with more than 90% sensitivity and more than 95% specificity. The study also shows that poly(NIPAm) particles coupled with trypan blue dye efficiently capture and concentrate T. cruzi antigens in urine, and under experimental conditions these particles protect T. cruzi antigens in urine from enzymatic degradation. Evaluation at one month of age provides high sensitivity because this time point is characterized by the highest levels of parasitemia and therefore also excretion of high levels of antigen. Nanotechnology-based tests can be adapted to point-of-care and cost-effective detection of microbial agents, as shown for other infectious diseases [35]. The non-invasive nature of the test will also greatly enhance parental acceptability.

We evaluated the performance of poly(NIPAm) particles coupled with five different high affinity dyes. Optimal results were obtained with poly(NIPAm) particles with trypan blue (TB), which achieved a 100-fold increase in antigen concentration in urine. Our data demonstrate that these nanoparticles can capture and
Table 1. Diagnostic test results in infants with congenital *T. cruzi* infection.

| Infant code | Micromethod | PCR | Serology | Chunap |
|-------------|-------------|-----|----------|--------|
|             | Birth | 1 month | Birth | 1 month | 6 to 12 months | Chunap 1 month | Chunap Bands |
| 1           | NEG   | NEG      | POS   | POS      | POS                | POS            | 22 kDa, 75 kDa |
| 2           | NEG   | NEG      | NEG   | POS      | POS                | POS            | 22 kDa, 75 kDa |
| 3           | NEG   | POS      | POS   | POS      | PT                 | POS            | 22 kDa |
| 4           | NEG   | NEG      | NEG   | NEG      | POS                | POS            | 82 kDa |
| 5           | NEG   | NEG      | POS   | POS      | POS                | POS            | 42 kDa |
| 6           | NEG   | NEG      | NEG   | POS      | PT                 | POS            | 42 kDa |
| 7           | POS   | PT       | POS   | POS      | PT                 | PT             | 42 kDa |
| 8           | POS   | PT       | POS   | POS      | PT                 | PT             | 42 kDa |
| 9           | NEG   | NEG      | NEG   | POS      | PT                 | PT             | 42 kDa |
| 10          | NEG   | POS      | NEG   | POS      | POS                | POS            | 82 kDa |
| 11          | NEG   | NEG      | POS   | POS      | POS                | POS            | 82 kDa |
| 12          | NEG   | POS      | NEG   | POS      | PT                 | PT             | 82 kDa |
| 13          | NEG   | POS      | PT    | PT       | POS                | POS            | 82 kDa |
| 14          | NEG   | NEG      | POS   | POS      | POS                | POS            | 82 kDa |
| 15          | NEG   | NEG      | NEG   | no data  | POS                | POS            | 82 kDa |
| 16          | NEG   | NEG      | POS   | no data  | POS                | POS            | 82 kDa |
| 17          | NEG   | NEG      | POS   | no data  | POS                | POS            | 82 kDa |
| 18          | NEG   | POS      | POS   | no data  | PT                 | POS            | 22 kDa, 58 kDa |
| 19          | NEG   | NEG      | POS   | no data  | POS                | POS            | 22 kDa, 42 kDa, 58 kDa |
| 20          | NEG   | NEG      | no data | NEG | POS                | POS            | 22 kDa, 42 kDa, 58 kDa |
| 21          | NEG   | NEG      | NEG   | POS      | POS                | POS            | 22 kDa, 42 kDa, 58 kDa |
| 22          | NEG   | NEG      | NEG   | POS      | POS                | NEG            | POS |
| 23          | NEG   | NEG      | POS   | no data  | POS                | NEG            | POS |
| **Total**   | 4/23  | 4/19     | 13/22 | 15/17    | 15/15              | 21/23          | |
| **Sensitivity (%)** | 17     | 21       | 59    | 88       | 100                | 91             | |
| (95% CI)    | (5.1–38.8) | (6.2–45.6) | (36.4–79.3) | (63.5–98.2) | (78.0–100.0) | (71.9–98.7) | |

The results of each test are reported only if the samples were obtained before the treatment was initiated.

1 Specimen taken at 3 months.

95% CI: 95% Confidential Interval.

PT: Post-treatment.

POS: Positive. NEG: Negative.

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concentrate \emph{T. cruzi} analytes of different chemical structures, including proteins (H49 and 1F8), glycoproteins (TESA) and lipophosphoglycan. The broad range of antigens captured provides an advantage over other methodologies that target a specific chemical structure [20], enabling the use of these nanoparticles as a single pre-processing step for sensitive multiplex analysis of several urinary analytes simultaneously.

Bait-loaded hydrogel nanoparticles also preserve captured proteins from enzymatic degradation, even when the proteolytic enzyme (e.g. trypsin, as in this study) is small enough to penetrate inside the particles. We hypothesize that the mechanism of protection stems from the immobilization of trypsin by the nanoporous particle, which prevents the enzyme from binding substrate proteins. Another possibility is the steric hindrance associated with trapping of the substrate by the affinity-bait groups in the particle, which may prevent enzymes from productively binding target proteins [24]. Antigens in urine can be potentially degraded in the urinary tract or bladder; however, studies indicate that this degradation is minimal [36]. Degradation of antigens after urine collection is enhanced due to bacterial contamination, so that the advantage of hydrogel particles is their protective effect immediately after urine collection. Further studies using urine samples collected at different times (first morning urine vs random spot urine collection) may help evaluate the extent of degradation in the urinary tract or bladder. However, random spot urine collection makes the test simpler [37].

Two previous studies have utilized urine antigen detection to diagnose congenital Chagas, reporting sensitivities of 80%–100% [20], [21]. However, these studies had small sample sizes (n = 10 and 14) and all but two of the congenital cases had parasitemia detected by microscopy. By contrast, nearly two-thirds of our infected infants were missed by micromethod, implying lower levels of parasitemia.

In this study, PCR showed good sensitivity early in life (91.3% when results from birth and 1-month specimens were combined), as previously reported by other studies [7], [9]. Two infants had positive results by Chunap but negative results by PCR; the large volume concentrated by the hydrogel nanoparticles may enable detection in some cases when antigen loads in the urine are low. Similar to qPCR, antigenuria also has the advantage of permitting early treatment which is associated with higher cure rates and fewer side effects compared to treatment later in life. Early diagnosis also translates to a much lower rate of missed infections compared to an algorithm requiring 6–12 months of follow-up [7], [15].

To our knowledge, this study is the first to successfully and consistently detect \emph{T. cruzi} LPG in urine of Chagas infected infants and to use this analyte for diagnostic purposes. Similarly, LPG of \emph{Leishmania} has been detected in urine of patients [38], LPG has been shown to play a key role in host-cell recognition/invasion and in parasite survival. LPG is highly expressed in \emph{T. cruzi} with a cellular copy number of 4×10⁵ molecules of LPG glycoconjugates/cell making it a good candidate for diagnosis [39] and a monoclonal antibody is commercially available. The monoclonal antibody that we used was directed against the LPG of \emph{T. cruzi} CL Brener strain, corresponding to the hybrid genotype VI [40]. This antibody also recognizes LPG fractions (band of 82 kDa) in TLA and TESA of \emph{T. cruzi} Y strain (genotype II) and of a genotype I strain isolated from a patient from Bolivia (Figure S3), suggesting that this antibody can identify LPG preparations of most strains of \emph{T. cruzi}. However, further studies must be performed in order to determine the ability of this antibody to recognize the LPG of other genotypes.

With appropriate adaptation to a field-friendly format, Chunap has the potential to enable early point-of-care diagnosis of congenital Chagas disease in peripheral health facilities. Further steps will be necessary to apply this nanotechnology in developing countries. We are currently optimizing a novel separation method based on magnetic labeling of capturing nanoparticles to enable particle separation from urine without the need for a high speed centrifuge. Finally, although used in this study for Chagas disease, this method could be adapted for detection of other parasitic infections in urine and other body fluids.

 Supporting Information

**Checklist S1** “Use of a Novel Chagas Urine Nanoparticle Test (Chunap) for Diagnosis of Congenital Chagas Disease.”

**Figure S1** Flow diagram of the study. “n” represents the number of individuals in each group.

**Figure S2** Detection of \emph{T. cruzi} antigens in nanoparticles-concentrated urine samples of infants. Bands of 22 kDa, 42 kDa, 58 kDa, 75 kDa and 82 kDa were detected by Western Blot using a mouse monoclonal antibody against lipophosphoglycan of \emph{T. cruzi}. Lanes 1–21: Patient codes of infants with congenital \emph{T. cruzi} infection (See Table 1 for more details). Ns: Infants without congenital \emph{T. cruzi} infection.

**Figure S3** Detection of lipophosphoglycan in trypomastigote excretory-secretory antigen (TESA) of \emph{T. cruzi} Bolivia and Y strains. A. Two bands of 42 kDa and 82 kDa were detected by Western Blot using a monoclonal antibody to lipophosphoglycan of \emph{T. cruzi} CL Brener strain (genotype VI). 1. \emph{T. cruzi} Bolivia strain (genotype I); 2. \emph{T. cruzi} Y strain (genotype II). B. Periodic acid–Schiff stain demonstrating the polysaccharide content of the 82 kDa band of \emph{T. cruzi} Bolivia strain (lane 1) and \emph{Y} strain (lane 2).

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References

1. Rassi A Jr, Rassi A, Marin-Neto JA (2010) Chagas disease. Lancet 375(9725):1380–82.
2. Organización Panamericana de la Salud (2006) Estimación cuantitativa de la enfermedad de Chagas en las Américas. Montevideo, Uruguay: Organización Panamericana de la Salud.
3. World Health Assembly (2010) Chagas disease: control and elimination. In: Sixty-third World Health Assembly. Resolutions, Geneva. Recommendations and decisions, annexes (WHA63/2010/REC/1), resolution WHA63/20:39–42.
4. Voelcker R (2012) Congenital Chagas disease reported in United States. JAMA 308(5):447.
5. Schenone H, Gaggero M, Sapunar J, Contreras MC, Rojas A, et al. (2001) Congenital Chagas disease of second generation in Santiago, Chile. Report of two cases. Rev Inst Med Trop Sao Paulo 43(4):231–2.
6. Blanco SB, Segura EL, Corti EN, Chirri F, Tabías R, et al. (2000) Congenital transmission of Trypanosoma cruzi: an operational outline for detecting and treating infected infants in north-western Argentina. Trop Med Int Health 5(4):293–301.
7. Bern C, Verastegui M, Gilman RH, Lafuente C, Galdos-Cardenas G, et al. (2009) Congenital Trypanosoma cruzi transmission in Santa Cruz, Bolivia. Clin Infect Dis 49(11):1667–74.
8. Mora MC, Sanchez Negrette O, Marco D, Barrio A, Ciaccio M, et al. (2005) Early diagnosis of congenital Trypanosoma cruzi infection using PCR, hemoculture, and capillary concentration, as compared with delayed serology. J Parasitol 91:464–73.
9. Bern C, Martin D, Gilman RH (2011) Acute and congenital Chagas disease. Advances in Parasitology 75:19–47.
10. Segura EL, Quintero ML, Salomón O, Gómez AO, Sosa Estani S, et al. (1994) Community participation in the National Program for Transmission Control of Chagas Disease. Medicina (B Aires) 54(5 Pt 2):610–14.
11. Blanco SB, Segura EL, Gurtler RE (1999) Control of congenital transmission of Trypanosoma cruzi in Argentina. Medicina (B Aires) 59 Suppl 2:130–42.
12. Oliveira I, Torrico F, Muñoz J, Gascon J (2010) Congenital transmission of Chagas disease: a clinical approach. Expert Rev Anti Infect Ther 8(3):945–56.
13. Sánchez Negrette O, Mora MC, Basombrio MA (2005) High prevalence of congenital Trypanosoma cruzi infection and family clustering in Salta, Argentina. Pediatrics 115(6):e668–72.
14. Romero M, Postigo J, Schneider D, Chipaux P, Santalla JA, et al. (2011) Door-to-door screening as a strategy for the detection of congenital Chagas disease in rural Bolivia. Trop Med Int Health 16(3):562–9.
15. Cardoso EJ, Valdés GC, Campos AC, de la Luz Sanchez R, Mendoza CR, et al. (2012) Maternal fetal transmission of Trypanosoma cruzi: a problem of public health little studied in Mexico. Exp Parasitol 131(4):425–32.
16. Carlier Y, Torrico F, Sosa-Estani S, Rusomando G, Luqueti A, et al. (2011) Congenital Chagas disease: recommendations for diagnosis, treatment and control of newborns, siblings and pregnant women. PLoS Negl Trop Dis 5(10):e1250.
17. Howard EJ, Xiong X, Carlier Y, Sosa-Estani S, Barquero P (2013) Frequency of the congenital transmission of Trypanosoma cruzi: a systematic review and meta-analysis. BJOG 121(1):22–33.
18. Umezawa E, S, Shikata-Sawada MA, Daisuke J, C, Courbon P, Paranhos G, et al. (1993) Trypanosoma cruzi: Detection of a Circulating Antigen in Urine of Chagas Diseased Patients Sharing Common Epitopes with an Immunodominant Repetitive Antigen. Experimental Parasitology 75:352–357.
19. Carlier Y, Torrico F, Sosa-Estani S, Rusomando G, Luqueti A, et al. (2011) Congenital Chagas disease: recommendations for diagnosis, treatment and control of newborns, siblings and pregnant women. PLoS Negl Trop Dis 5(10):e1250.
20. Carpio S, Carlier Y, Sosa-Estani S, Barquero P (2013) Frequency of the congenital transmission of Trypanosoma cruzi: a systematic review and meta-analysis. BJOG 121(1):22–33.
21. Freilij HL, Corral RS, Katzin AM, Grinstein S (1987) Antigenuria in infants and acute and congenital Chagas’ disease. J Clin Microbiol 25(1):133–7.
22. Katzin A, Manso M, Abúñi G, Colli W (1989) Antigenuria in chronic chagasic patients detected by a monoclonal antibody raised against Trypanosoma cruzi. Trans R Soc Trop Med Hyg 83(4):341–343.
23. Luchini A, Longo C, Espina V, Petricoin EF 3rd, Liotta LA (2010) Nanoparticle technology: addressing the fundamental roadblocks to protein biomarker discovery. Curr Mol Med 10(2):133–41.
24. Luchini A, Gehe DH, Bishop B, Tran D, Xia C, et al. (2008) Smart hydrogel particles: biomarker harvesting one-step affinity purification, size exclusion, and protection against degradation. Nano letters 8(1):50–61.
25. Douglas TA, Tamburro D, Fredolini C, Espina VH, Lepeque BS, et al. (2010) The use of hydrogel microparticles to sequester and concentrate bacterial antigens in a urine test for Lyme disease. Biomaterials 31(4):1157–1166.
26. Fredolini C, Meami F, Reeder KA, Rucker S, Paparastou G, et al. (2008) Concentration and Preservation of Very Low Abundance Biomarkers in Urine, such as Human Growth Hormone [hGH], by Cibacron Blue F3G-A Loaded Hydrogel Particles. Nano Res 1(6):502–518.
27. Longo C, Patanarut A, George T, Bishop B, Zhou W, et al. (2009) Core-shell hydrogel particles harvest, concentrate and preserve labile low abundance biomarkers. PLoS One 4(3):e4763.
28. Tamburro D, Fredolini C, Espina VH, Douglas TA, Ranganathan A, et al. (2011) Multifunctional core-shell nanoparticles: discovery of previously invisible biomarkers. J Am Chem Soc 133(47):19178–88.
29. Verani JR, Seitz A, Gilman RH, Lafuente C, Galdos-Cardenas G, et al. (2009) Geographic variation in the sensitivity of recombinant antigen-based rapid tests for chronic Trypanosoma cruzi infection. Am J Trop Med Hyg 80(3):410–5.
30. Frelij H, Muller L, González Cappa S, et al. (1983) Direct micromethod for diagnosis of acute and congenital Chagas’ disease. J Clin Micro 10:327–328.
31. Programa Nacional de Control de Chagas (2007) Chagas Congenito: Estrategias de Diagnostico y Control. In: 2nd ed: Digital Dreams, Cochabamba, Bolivia: 1–89.
32. Umezawa ES, Nascimento MS, Kesper N Jr, Coura JR, Borges-Pereira J, et al. (1994) Immunoblot assay using secreted-antigens of Trypanosoma cruzi in serodiagnosis of congenital, acute, and chronic Chagas’ disease. J Clin Microbiol 34(9):2143–7.
33. Fitzwater S, Calderon M, Lafuente C, Galdos-Cardenas G, Ferrufino L, et al. (2008) Polymerase chain reaction for chronic Trypanosoma cruzi infection yields higher sensitivity in blood clot thanuffy coat or whole blood specimens. Am J Trop Med Hyg 78(5):767–70.
34. Piron M, Fisa R, Casamitjana N, López-Chejade P, Puig L, et al. (2007) Development of a real-time PCR assay for Trypanosoma cruzi detection in blood samples. Acta Trop 103(3):195–200.
35. Syed MA (2014) Advances in nanodiagnostic techniques for microbial agents. Biosens Bioelectron 51:391–400.
36. Zhou H, Yuen PS, Rastan A, Gonzales BA, Yasuda H, et al. (2006) Collection, storage, preservation, and normalization of human urinary exosomes for biomarker discovery. Kidney Int 69(8):1471–6.
37. Thomas CE, Sexton W, Benson K, Surphen R, Koomen J (2010) Urine collection and processing for protein biomarker discovery and quantification. Cancer Epidemiol Biomarkers Prev 19(4):953–9.
38. Sarker B, Chample CA, Hommel M (2002) Antigenicity in visceral leishmaniasis: detection and partial characterisation of a carbohydrate antigen. Acta Trop 82(3):339–48.
39. Singh BN, Lucas JJ, Beach DH, Costello CE (1994) Expression of a novel cell surface lipopolysaccharide-like glycoconjugate in Trypanosoma cruzi epimastigotes. J Biol Chem 269(32):21972–82.
40. Zingales B, Andrade SG, Brocq MRS, Campbell DA, Chiar I, et al. (2009) A new consensus for Trypanosoma cruzi intraspecific nomenclature: second revision meeting recommends TcI to TcVI. Mem Inst Oswaldo Cruz 104(7):1051–1054.

Author Contributions

Conceived and designed the experiments: YECS RHG CB LL AL. Performed the experiments: YECS LF GS EVC GG. Analyzed the data: YECS RHG CB AL. Obtained permission for use of cell line: RHG CB.