Short Report: Polymerase Chain Reaction for Chronic Trypanosoma cruzi Infection Yields Higher Sensitivity in Blood Clot Than Buffy Coat or Whole Blood Specimens

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Abstract. Trypanosoma cruzi polymerase chain reaction (PCR) is widely used, but sensitivity varies widely. We compared PCR using 121/122 primers targeting kinetoplast minicircle DNA in whole blood, buffy coat, and clot from Bolivian women. Sensitivity was significantly higher in clot (60.1%) than buffy coat (46.5%) or whole blood (40%). The use of clot could simplify specimen collection while improving sensitivity.

Chagas disease, caused by the protozoan Trypanosoma cruzi, infects an estimated 8–10 million people in the Americas.1,2 After an initial acute phase, lasting 60–90 days and marked by readily detectable parasitemia, infected individuals enter the chronic phase, which, in the absence of successful treatment, is life-long. In 20–30% of those infected, cardiomyopathy or gastrointestinal disease develops years to decades later.3 The efficacy of drug treatment has been shown in randomized, double-blinded, placebo-controlled trials in children 6–12 years of age,4,5 and recent data suggest that trypanocidal treatment in adults with early cardiac lesions may decrease disease progression.6 Parasitemia in the chronic phase is undetectable by microscopy, and diagnosis relies on serologic tests. Because no single serologic test is sufficiently sensitive and specific, the accepted criteria for diagnosis of chronic T. cruzi infection consist of having positive results on two or more assays using different techniques or antigens (e.g., enzyme-linked immunosorbent assay [ELISA] and indirect fluorescent antibody [IFA]).7

Polymerase chain reaction (PCR) in the acute phase is highly sensitive, but published sensitivity in the chronic phase ranges from 40% to 100% and seems to depend on age and other characteristics of those tested, as well as PCR primers and methods.8,9,11

Our objective was to develop and evaluate a practical sensitive method for PCR diagnosis of chronic T. cruzi infection. T. cruzi PCR is most commonly performed on whole blood specimens.8,10,11 Although some authors recommend mixing the fresh specimen with guanidine,12 this reagent can no longer be shipped by air under new International Air Transport Association regulations, and its use complicates the logistics of specimen collection. A preliminary analysis showed no difference in sensitivity between whole blood in EDTA and whole blood mixed with guanidine (M. Calderon, unpublished data). Based on the literature, we hypothesized that circulating trypanomastigotes would sediment in the buffy coat,13 but might also be trapped with the cellular portion in clot. We therefore compared detection PCR in three types of specimens: whole blood in EDTA, buffy coat, and clot, from women in Santa Cruz, Bolivia.

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Women admitted for delivery from November 13, 2006 to June 12, 2007 were asked to participate in serologic screening, as the first step in a study of congenital T. cruzi transmission. After informed consent, blood was collected into two 5-mL tubes with EDTA and one 5-mL tube without additives. One EDTA tube was centrifuged at 1,000g for 12 minutes, and the buffy coat (volume ~500 µL) was extracted; the other specimen was frozen as whole blood. The tube without additive was centrifuged at 1,000g for 5 minutes, and serum was drawn off to leave the clot (volume ~2 mL). All specimens were maintained at ~20°C and shipped to the Universidad Peruana Cayetano Heredia, Lima, Peru. The study protocol was approved by ethical review boards of Johns Hopkins University Bloomberg School of Public Health, Asociacion Benefica PRISMA, Universidad Peruana Cayetano Heredia, Hospital Universitario Japones, and US Centers for Disease Control and Prevention.

Serum specimens were screened for antibodies to T. cruzi using a commercial ELISA (Chagatek, bioMérieux-Argentina, Buenos Aires, Argentina); the threshold for positive was calculated at 0.100 units above the mean absorbance of two negative control specimens on each plate, following the manufacturer’s instructions. All specimens were also tested by IFA, using a titer of 1:32 as the positive cut-off.14 Specimens positive by ELISA and IFA were considered confirmed positive for T. cruzi infection.7 Specimens with discordant results were excluded.

Specimen aliquots (500 µL of whole blood or clot and 300 µL of buffy coat) were combined with buffer (10 mmol/L Tris HCl, pH 7.6, 5 mmol/L MgCl2, 10 mmol/L NaCl), homogenized, and centrifuged. The supernatant was removed, and the pellet was resuspended in buffer and centrifuged. This procedure was repeated three times for buffy coat and whole blood and five times for clot. SDS and Proteinase K (Invitrogen, Carlsbad, CA) were added to reach concentrations of 0.5% and 0.5 mg/mL, respectively and the specimens were incubated for 2 hours at 56°C. DNA was extracted following a standard phenol-chloroform extraction protocol15 and resuspended in 100 µL Tris HCl, 10 mmol/L, and EDTA 1 mmol/L. For the PCR reaction, the total volume was 25 µL (23 µL reaction mix and 2 µL DNA). This is equivalent to between 30 and 50 ng DNA (human DNA, and, in positive specimens, parasite DNA).

PCR amplifications were performed using the 121/122 primer set targeting the kinetoplast minicircle (5'-AAATAA-
T. CRUZI PCR IN BLOOD CLOT

TABLE 1
Performance of PCR in clot, buffy coat, and whole blood specimens from women in Santa Cruz, Bolivia, relative to T. cruzi infection status as indicated by results of two serologic assays

<table>
<thead>
<tr>
<th>Specimen type</th>
<th>Specimens processed by PCR (N)</th>
<th>Seropositive specimens* [N (%)]</th>
<th>PCR sensitivity† (95% CI)</th>
<th>PCR specificity‡ (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clot</td>
<td>516</td>
<td>148 (28.7%)</td>
<td>60.1% (53.7–68.6)</td>
<td>100% (99.0–100)</td>
</tr>
<tr>
<td>Buffy coat</td>
<td>208</td>
<td>71 (34.1%)</td>
<td>46.5% (36.2–59.7)</td>
<td>100% (97.3–100)</td>
</tr>
<tr>
<td>Whole blood</td>
<td>520</td>
<td>150 (28.8%)</td>
<td>40.0% (32.9–48.7)</td>
<td>100% (99.0–100)</td>
</tr>
</tbody>
</table>

* Positive by both ELISA and IFA (see text for explanation).
† P values for pairwise comparisons of PCR sensitivity in clot versus buffy coat, 0.0237; clot versus whole blood, < 0.0001; buffy coat versus whole blood, 0.233.
‡ Performance relative to confirmed positive or negative serologic results.

Received May 20, 2008. Accepted for publication July 17, 2008.

Acknowledgments: The Chagas Disease Working Group in Peru and Bolivia includes Susan Espetia Anco, Elizabeth Chavez, Viviana Pinedo Cancino, Elizabeth de La Fuente, Michael Levy, Lilia Cabrera, Hugo Aparicio, Mark Brady, Faustino Torrico, Daniel Lozano, and James H. Maguire. The authors thank John Williamson for statistical support.

Financial support: This work was supported by NIH Global Research Training Grant 3 D43 TW006581, NIH Training Grant in Infectious and Tropical Diseases 5 T35 AI065385, and NIH 1R21 AI072093-01.

Disclaimer: The authors declare no potential conflicts of interest. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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