Malattia di Chagas: le tecniche di diagnosi disponibili

Corso Precongressuale A

SOSTENIBILITÀ, FATTIBILITÀ

E STRATEGIE DA ADOTTARE NELLA DIAGNOSTICA PARASSITOLOGICA …

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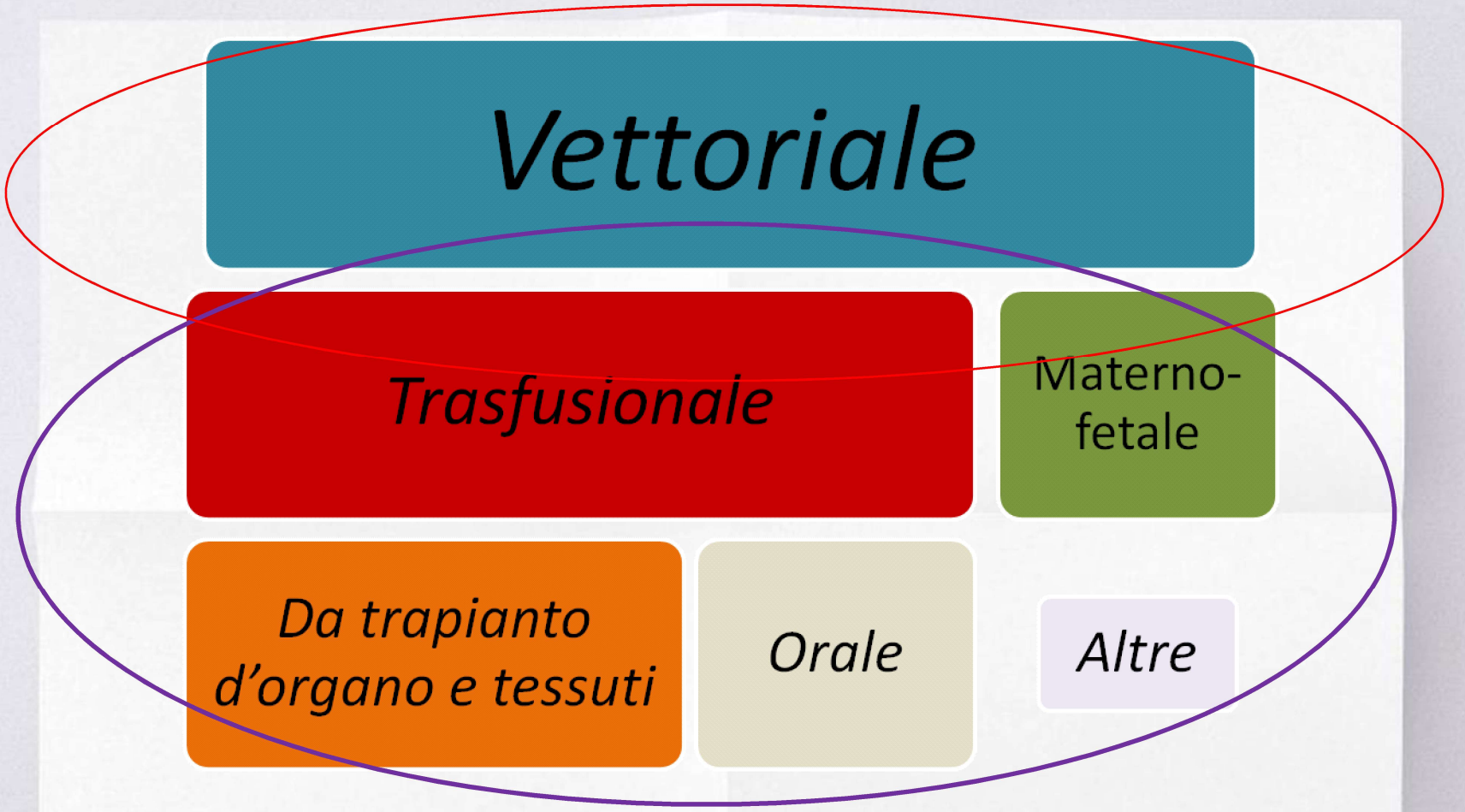
CHAGAS DISEASE

- Chagas disease, also known as American trypanosomiasis, is caused by infection with the protozoan parasite Trypanosoma cruzi. The organism T cruzi and infection in humans were first described in 1909 by the Brazilian physician **Carlos R. J. Chagas**

Modalità di trasmissione



Rischio di trasmissione da:

- **contatto con Triatomina infetta: 0,1%**
- **singola trasfusione di sangue (500 ml): 12-20%**
- **trapianto renale da donatore in fase indeterminata: 35%**
- **riattivazione in corso di immunodepressione: 30%**
- **materno-fetale: 0,1-12%**

MALATTIA DI CHAGAS
SERBATOI D'INFEZIONE

Circa 150 specie di mammiferi , selvatici e domestici, sono "naturalmente infettati " da *T. cruzi*

- **OSPITI SELVATICI:**
  Marsupiali (opossum), roditori, primati, chirotteri, carnivori, armadilli
- **OSPITI DOMESTICI:**
  Cani, gatti, ovini
  Più raramente bovini, suini, equini
- *Uccelli e rettili non suscettibili all'infezione da* T. cruzi

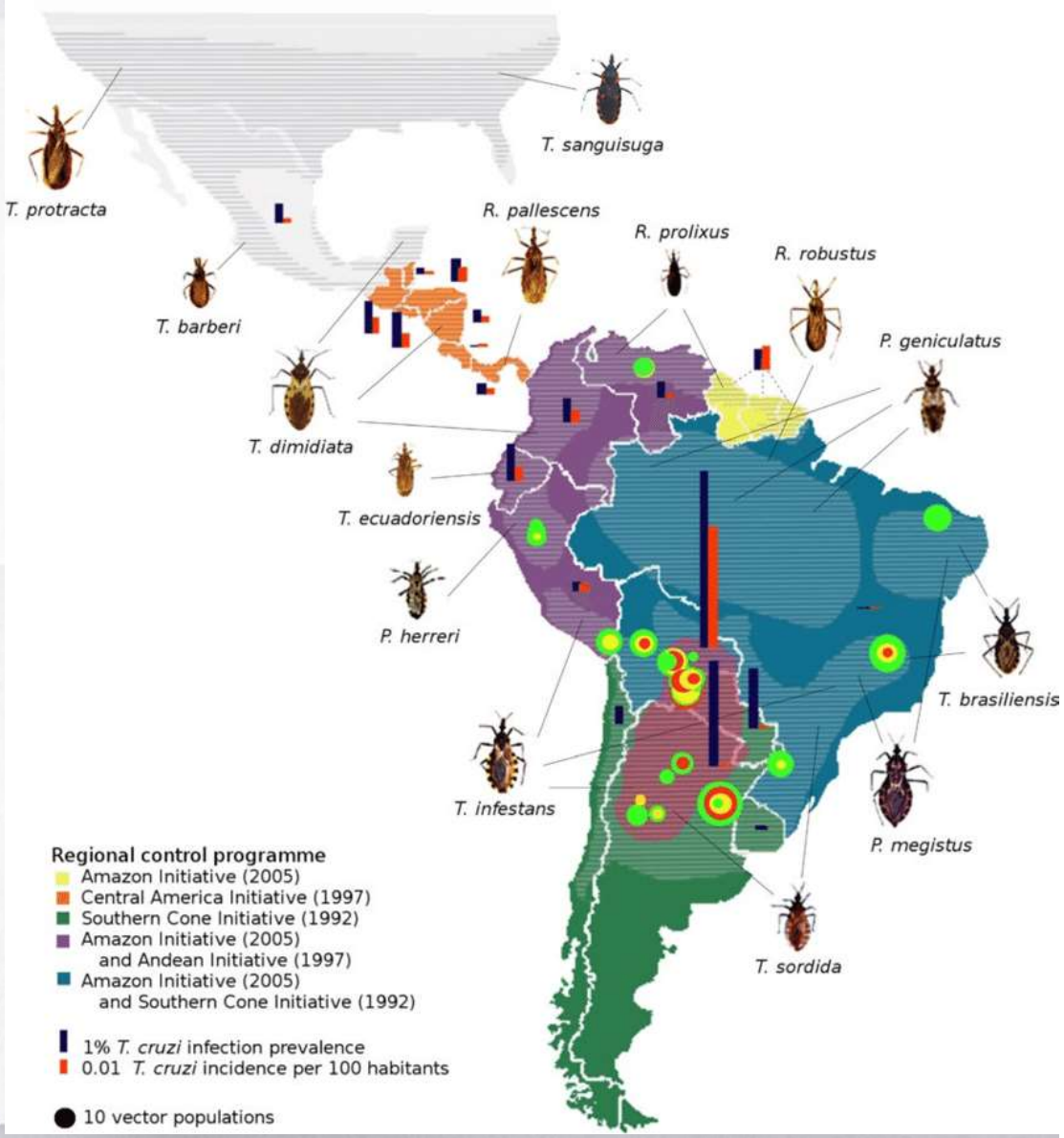
MALATTIA DI CHAGAS
I VETTORI

- **TRIATOMINE**: emitteri ematofagi, ~ 130 specie, almeno 40 possono ospitare *T. cruzi*
- Maggioranza delle specie vive in habitat naturale e si nutre a spese di animali (uccelli, mammiferi, rettili)

TRIATOMINE SELVATICHE	Diffusione: USA → Patagonia Ciclo selvatico di *T. cruzi*, mantenimento serbatoio animale

- ~ 10 specie appartenenti a 3 generi (*Triatoma*, *Rhodnius*, *Panstrongylus*) adattate all'ambiente antropico (abitazioni di aree rurali endemiche), responsabili di infezione umana da *T. cruzi*

TRIATOMINE DOMICILIARI	Diffusione: Messico → Argentina/Cile (esclusa Amazzonia) Ciclo domestico di *T. cruzi*



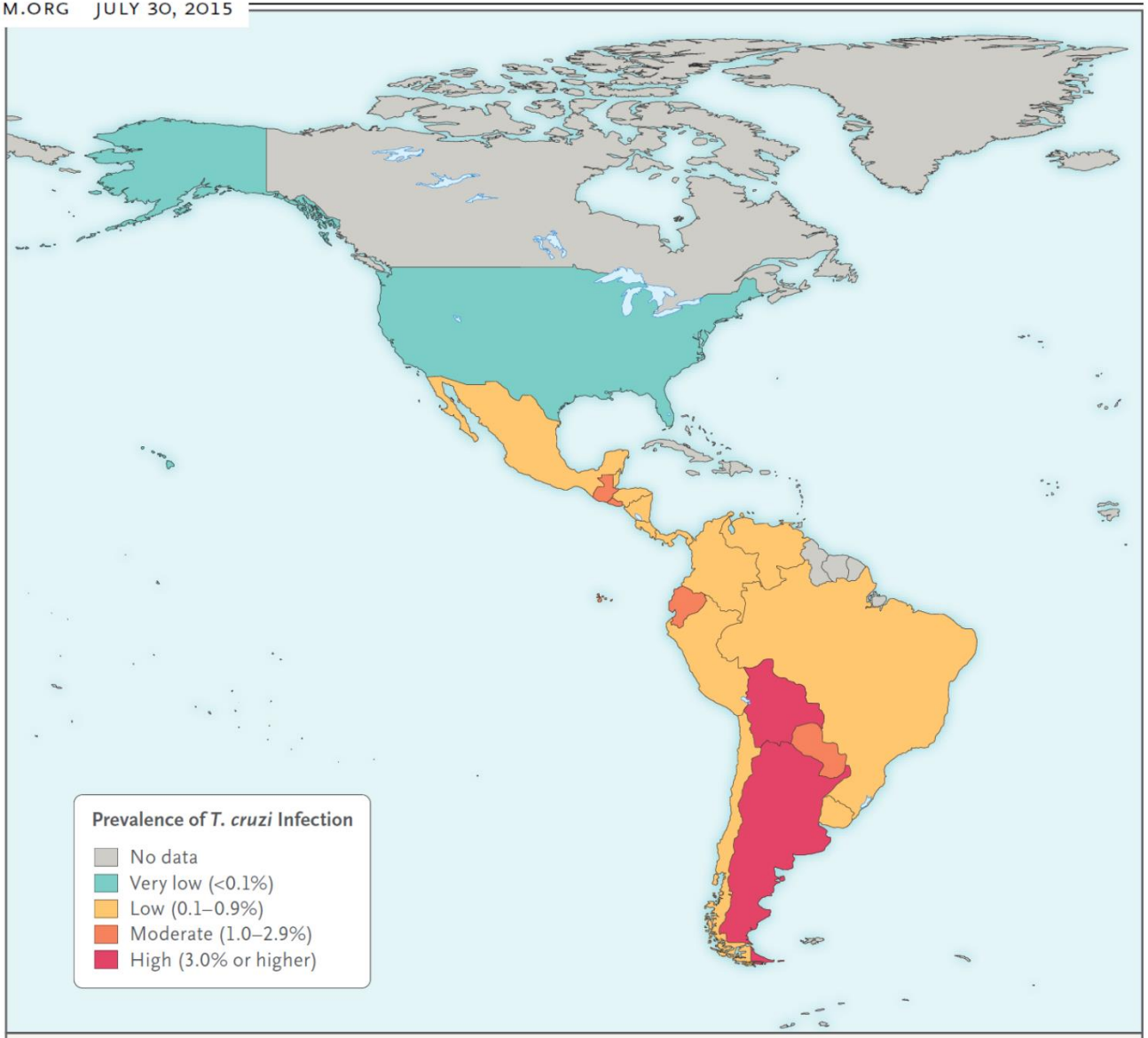


Figure 2. Estimated Prevalences of *Trypanosoma cruzi* Infection.

Shown are the prevalences at the national level, calculated on the basis of published data.[5] Transmission is concentrated in areas with domestic vector infestation and therefore shows strong spatial clustering. However, because infection is lifelong in the absence of treatment, the prevalence reflects past transmission over decades and human migration patterns. Existing data are insufficient to allow mapping at the subnational level.

Figure 1. Estimated number of Chagas disease cases in North America.

Hotez PJ, Dumonteil E, Betancourt Cravioto M, Bottazzi ME, Tapia-Conyer R, et al. (2013) An Unfolding Tragedy of Chagas Disease in North America. PLoS Negl Trop Dis 7(10): e2300. doi:10.1371/journal.pntd.0002300
http://127.0.0.1:8081/plosntds/article?id=info:doi/10.1371/journal.pntd.0002300

PLOS | NEGLECTED TROPICAL DISEASES

La migrazione di individui affetti da malattia di Chagas pone un PROBLEMA DI SALUTE PUBBLICA NEI PAESI NON ENDEMICI…

Clin Res Cardiol
DOI 10.1007/s00392-013-0613-y

REVIEW

Epidemiology of Chagas disease in Europe: many calculations, little knowledge

Jörn Strasen · Tatjana Williams · Georg Ertl · Thomas Zoller · August Stich · Oliver Ritter

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Fig. 1 Incidence of Chagas disease from *light* to *dark* color, showing the biggest incidences in Spain, followed by Italy and Portugal and, surprisingly, Sweden, Switzerland, and the Netherlands. The maximum absolute number of cases is shown by *blue spots*. By far again Spain is most affected, again followed by Italy and again the Netherlands and then Germany and France. In the *red shares*, the portion of indentified patients is illustrated, showing visible shares only for Switzerland, Spain and Belgium [11] (Tables 2, 3, and 4)

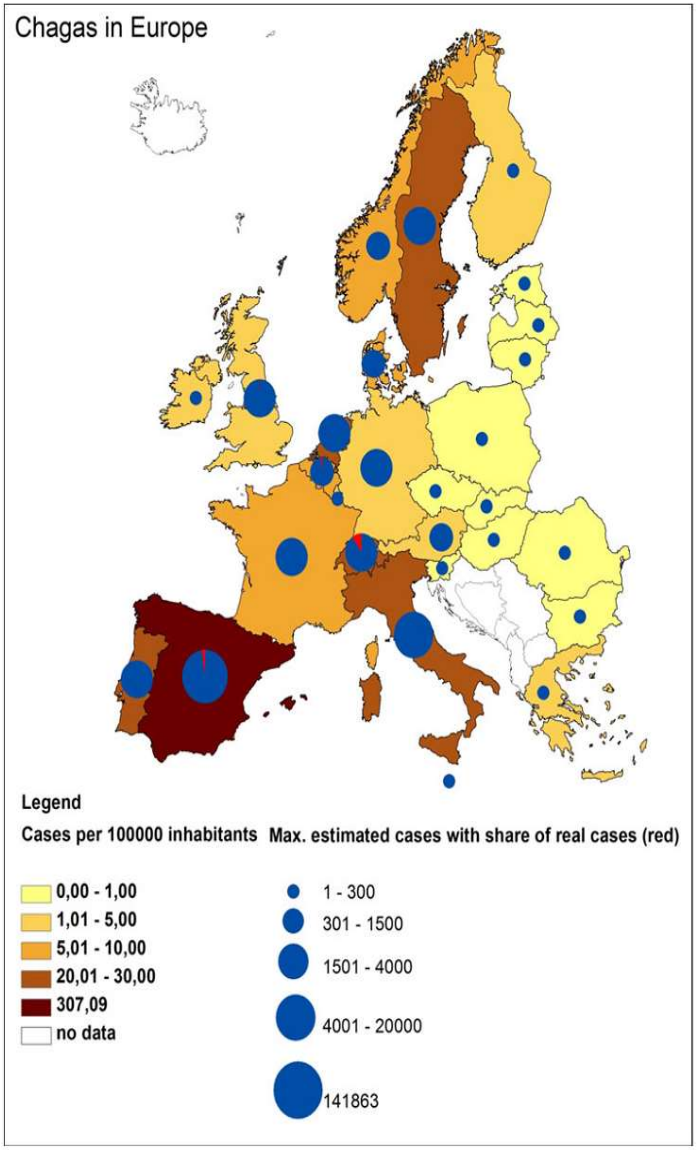


Table 4. Pooled *T. cruzi* prevalence by country of origin in Latin American migrants from European countries.

Country	Number screened	Number of seropositives	Country-specific prevalence* (%)	95% CI	Prevalence in country of origin (National level) PAHO (%)[39]	Prevalence ratio
Argentina	875	16	2.2	0.80–4·13	4.13	0.53
Bolivia	2264	541	18	13.9–22.66	6.75	2.67
Brazil	954	4	0.6	0.16–1.12	1.02	0.59
Chile	290	1	1	0.17–2.36	0.99	1.01
Colombia	1627	6	0.5	0.15–0.92	0.96	0.52
Ecuador	2131	7	0.4	0.18–0.72	1.74	0.23
El Salvador	67	2	3.7	1.62–11.7	3.37	1.10
Honduras	136	3	4.2	1.27–7.36	3.05	1.38
Mexico	166	0	1.5^	0.24–3.76	1.03	1.46
Nicaragua	50	1	4.6	0.76–11.3	1.14	4.04
Paraguay	385	19	5.5	3.46–7.91	2.54	2.17
Peru	1029	4	0.6	0.23–1.18	0.69	0.87
Uruguay	248	0	0.8^	0.08–2.24	0.66	1.21
Venezuela	311	0	0.9^	0.16–2.22	1.16	0.78

CI: Confidence Interval; PAHO: Pan American Health Organization;

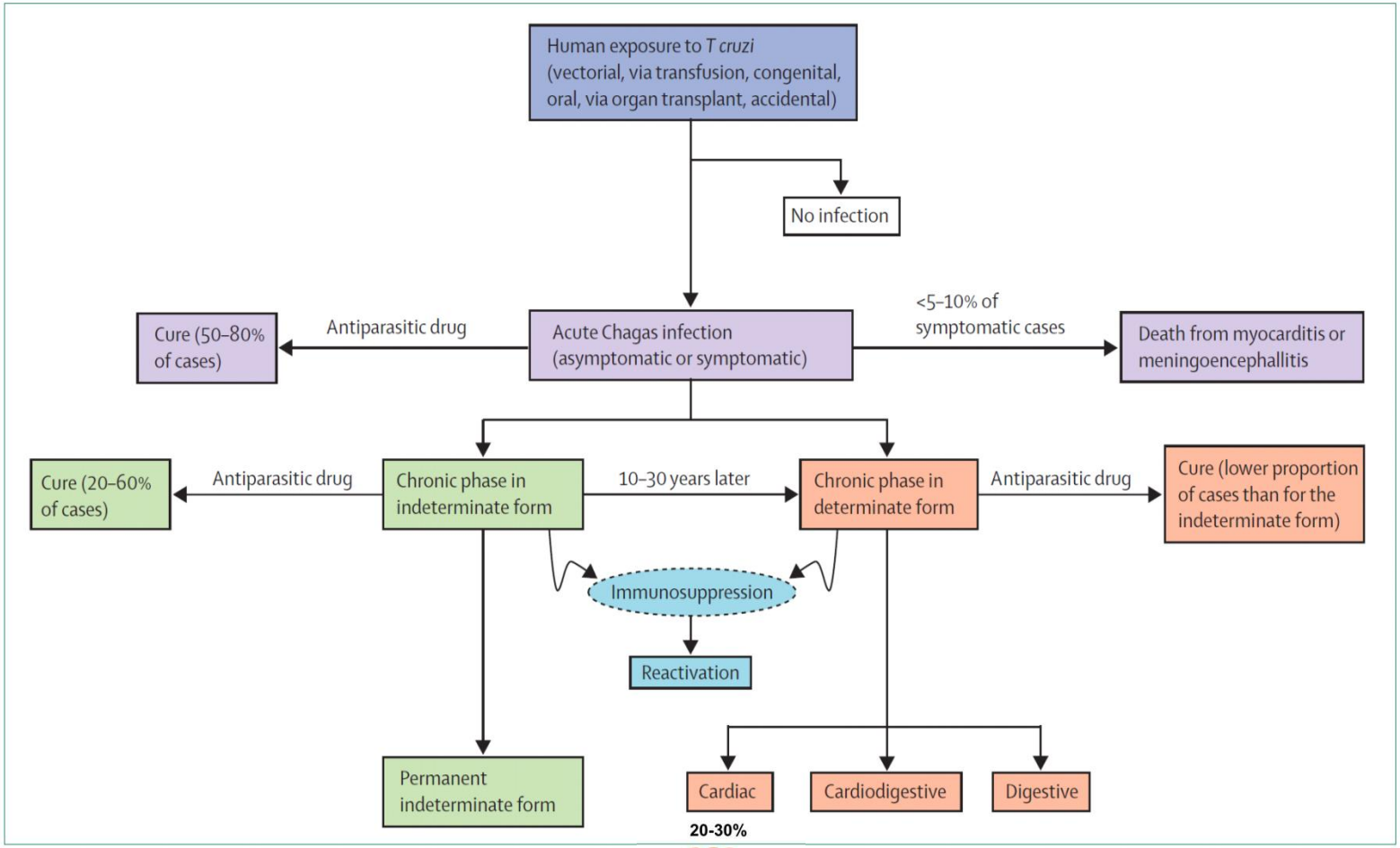
*Weighted prevalence with Random effect model;

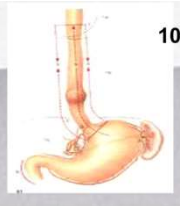
^ although there was not any reported case of Chagas disease in migrants coming from this country, the weighted prevalence is not "0" due to the Random Effect model

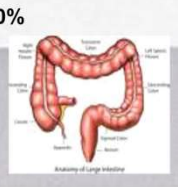
doi:10.1371/journal.pntd.0003540.t004

CLINICA

Storia naturale della malattia di Chagas



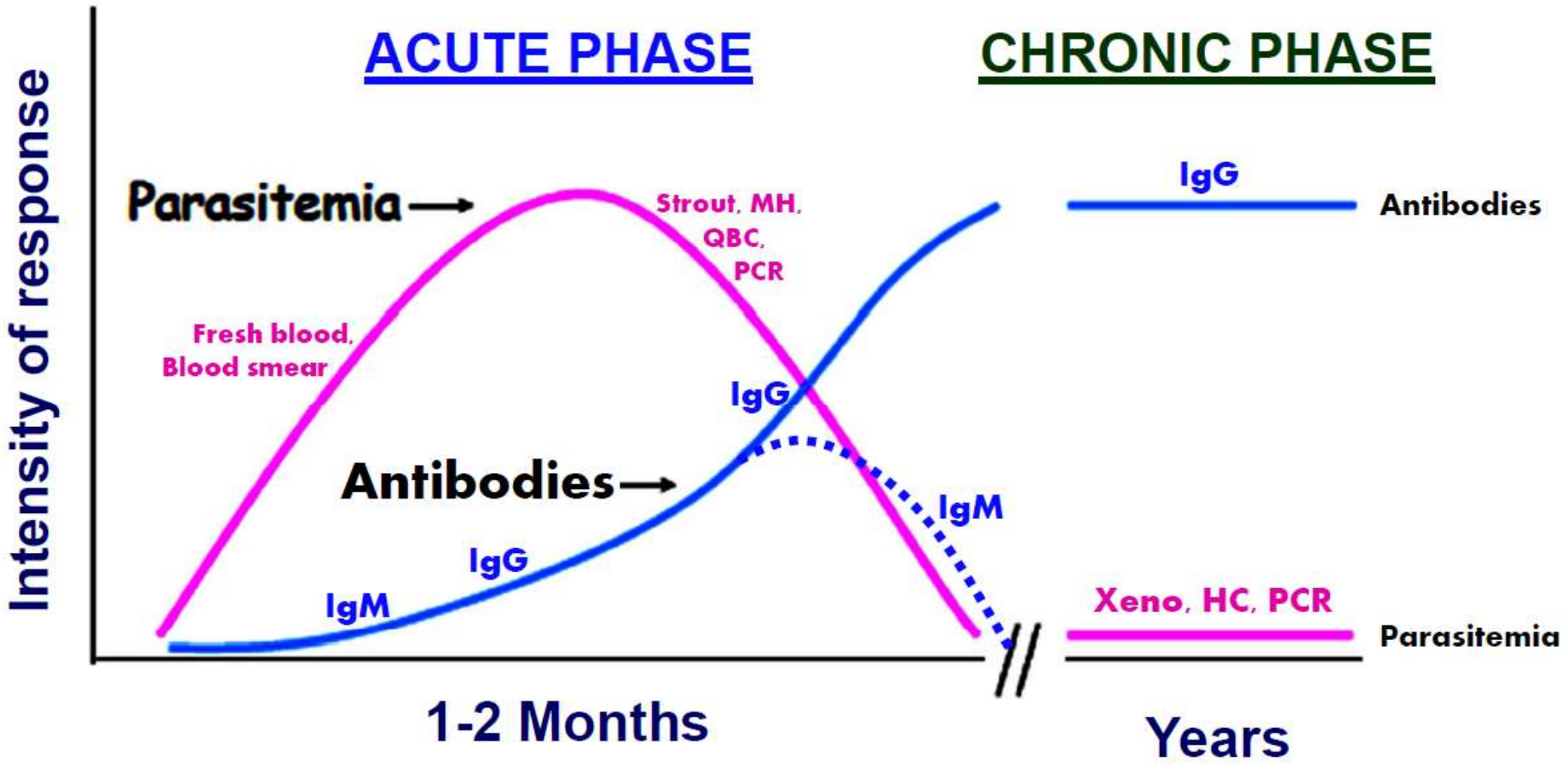




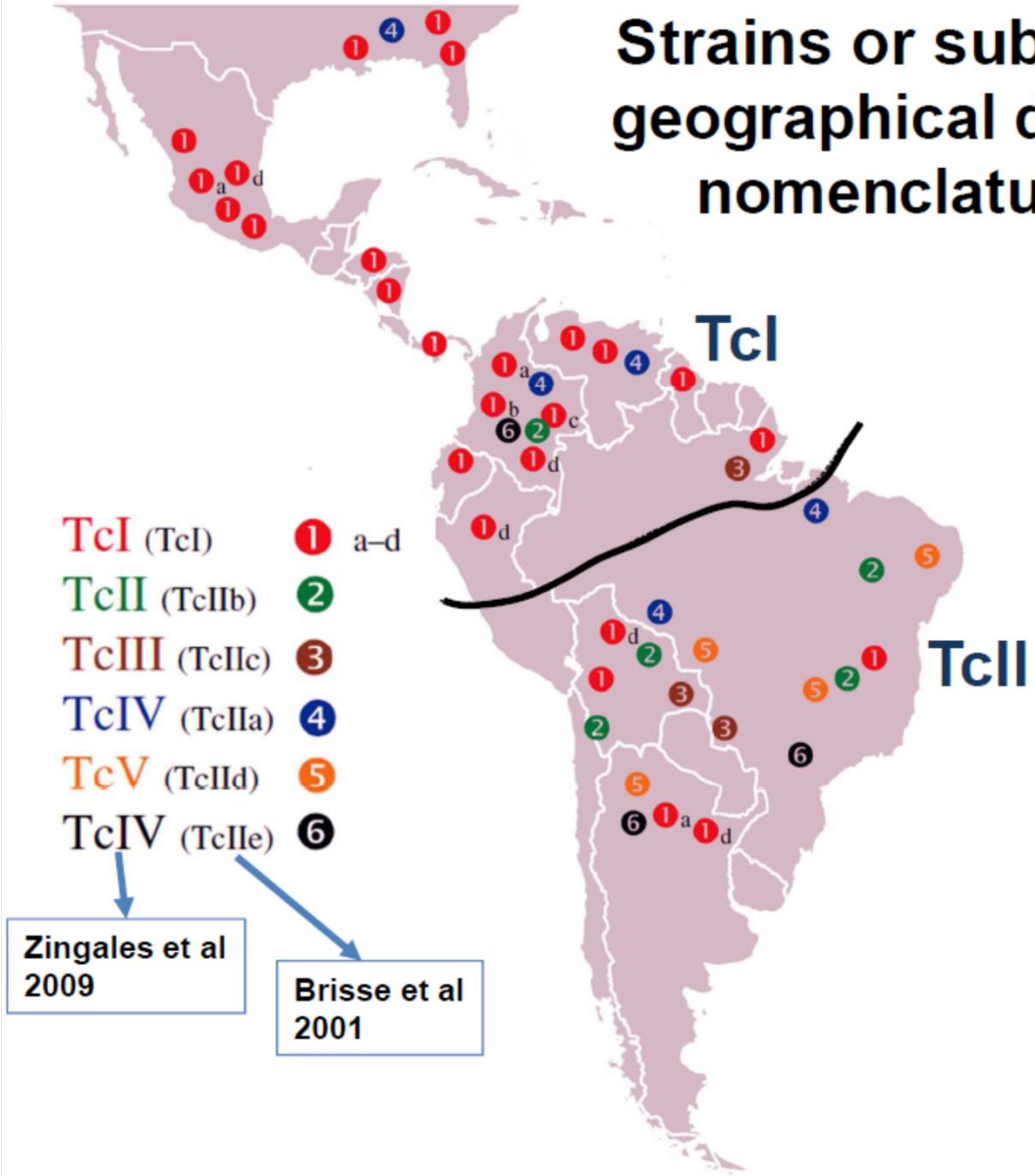
Rassi, The Lancet 2010

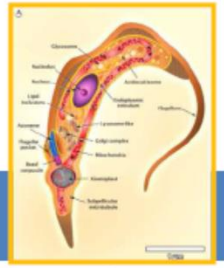
DIAGNOSI

Serological and Parasitological Evolution in Chagas Disease (untreated patients)



Strains or subtypes of *T. cruzi* and their geographical distribution using the latest nomenclature (Zingales et al., 2009)



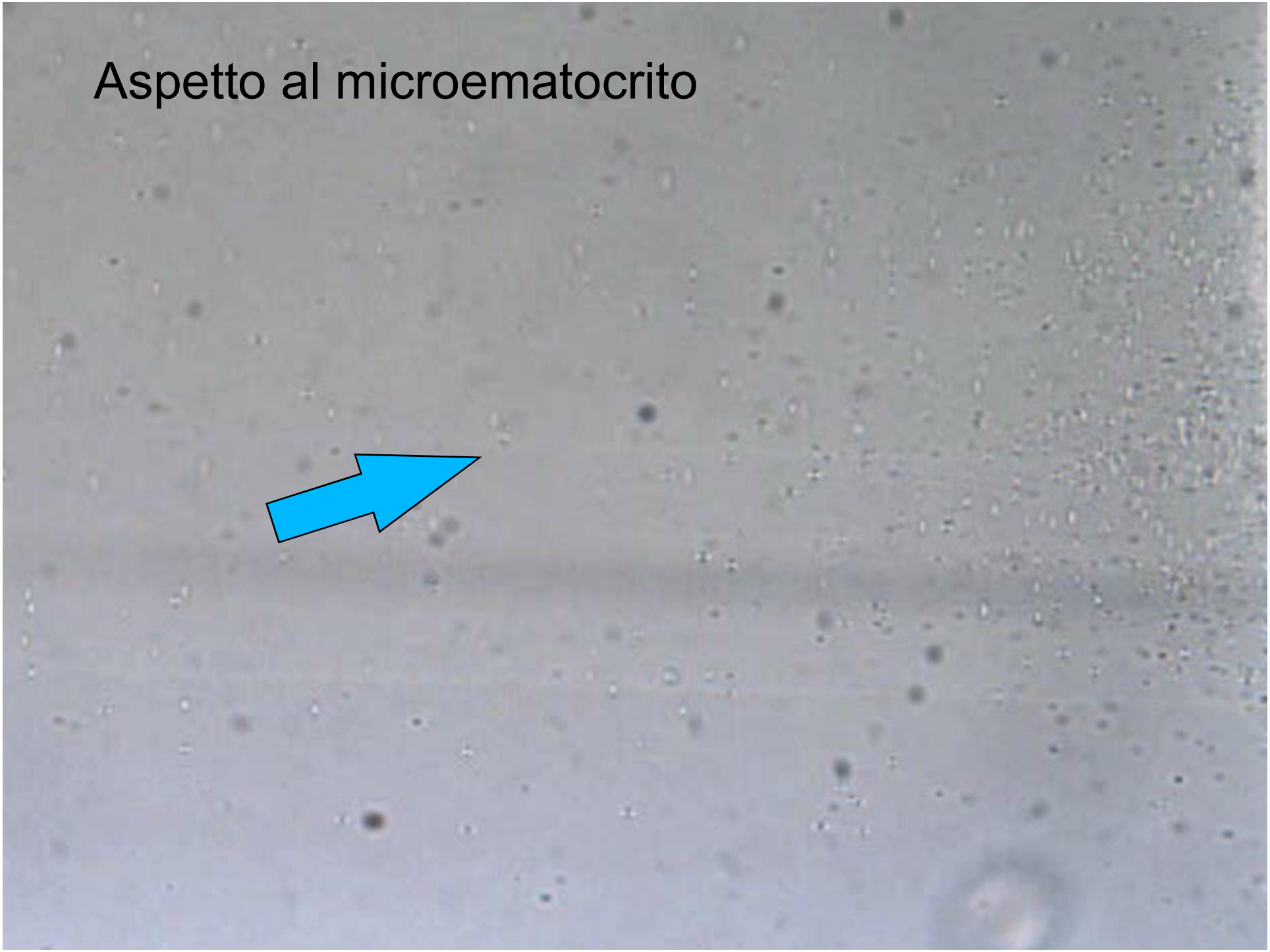


TcI	TcII
North of the Amazon	Southern Cone region
Less pathogenic	More pathogenic
Associated with cardiac lesions	Associated with cardiac and digestive lesions
Less resistant to antiparasite drug	More resistant to antiparasite drug
Faster negativization of CS tests	Slower negativization of CS tests

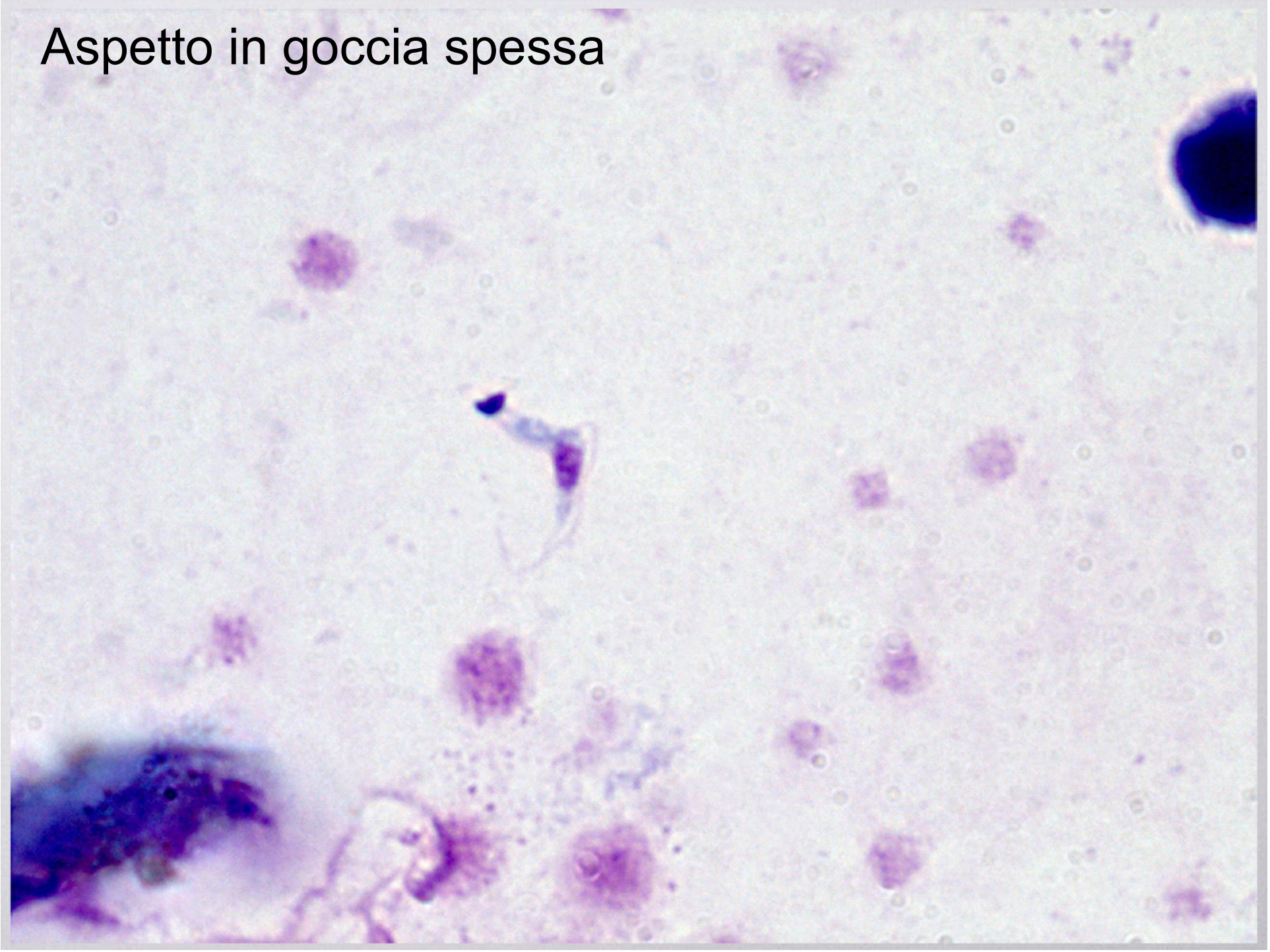
Tecniche diagnostiche

- Parassitologica diretta (MHT, QBC, goccia spessa e striscio sottile, xenodiagnosi)
- Sierologica (due tecniche che sfruttino diversi antigeni; 3° test di conferma)
- PCR

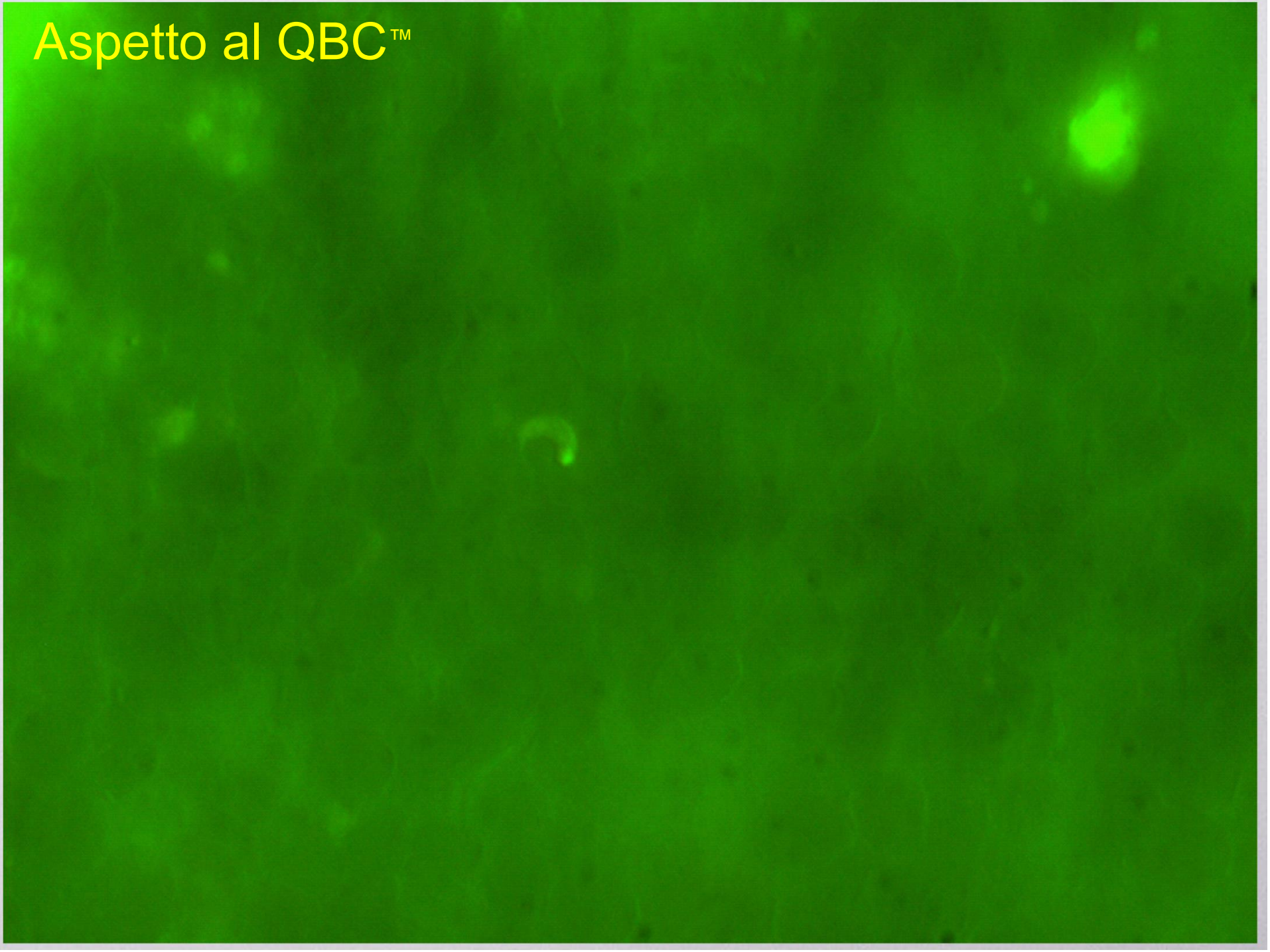
Aspetto al microematocrito



Aspetto in goccia spessa



Aspetto al QBC™



Diagnosi sierologica

- Test convenzionali (IHA; IFAT; ELISA basati su ag crudi)
- Test non convenzionali (ELISA basati su ag ricombinanti, PCR)

Table 2
Laboratory diagnosis of Chagas disease

Objectives	Serological and molecular methods				
	Conventional			Non-conventional	
	ELISA	IIF	IHA	Recombinant antigens	PCR
Serological evidence (two tests recommended)	X	X	X	X	
Blood-bank screening (one test recommended)	X				
Transplacental and perinatal transmission (two tests recommended)	X	X		X	X
Epidemiological surveys (one test recommended)	X	X			
Treatment follow-up (two tests recommended)	X	X	X		X

Different procedures can be adopted for the diagnosis of *T. cruzi* infection depending on the situation (see also Table 2), as follows:

- To confirm a clinical suspicion, two conventional tests should be used. If the results are not in agreement, a third test should be performed, either conventional or non-conventional.
- For blood-bank screening, ELISA is recommended.
- With congenital transmission, one conventional test on the mother should be performed, and a positive result confirmed by another conventional test. In children of seropositive mothers, a conventional IgG test should be done 8 months after birth. Parasitological tests are desirable wherever these can be carried out.
- In epidemiological surveys, a single conventional test should be used. Sera, plasma, or blood collected on filter paper can all be used for this purpose.
- In treatment follow-up, two serological tests are recommended. In specialized centres, PCR assays may be performed to verify parasitaemia.

World Health Organization
Geneva 2002

Sierologie a confronto

Tabla 1
Pruebas para la determinación de anticuerpos anti-*Trypanosoma cruzi*

Grupo de prueba	Prueba	Nombre abreviado de la prueba
Convencional	Inmunofluorescencia indirecta *in house* (CNM)	IFI-CNM
	Inmunoensayo *in house* (CNM)	ELISA-CNM
	CERTEST Chagas ELISA test (BiosChile, Abbott Laboratories, Zaragoza, España)	Certest
	ORTHO® *Trypanosoma cruzi* ELISA Test System (Johnson and Johnson, High Wycombe, Reino Unido)	Ortho
	T. cruzi (Chagas' disease) IgG ELISA (BLK Diagnostics, Barcelona, España)	BLK
	ELISA cruzi (Enfermedad de Chagas), (bioMérieux®, Mercy-l'Etoile, Francia)	BioMérieux
No Convencional	Bioelisa Chagas (Biokit, Lliçà d' Amunt, España)	Biokit
	ID-PaGIA antibody test (DiaMed, Cressier sur Morat, Suiza)	
	Versión con 2 antígenos recombinantes	*ID-PaGIA2*
	Versión con 3 antígenos recombinantes	*ID-PaGIA3*
	Simple Stick Chagas (Operon S. A., Zaragoza, España)	ICT Operon
	OnSite Chagas Ab Combo-Cassette (CTK, Biotech, Inc., San Diego, California, EE. UU.)	ICT CTK

CNM: Centro Nacional de Microbiología; ELISA: enzimoinmunoanálisis; ICT: prueba inmunocromatográfica (*immunochromatographic test*); ID-PaGIA2: prueba de aglutinación en gel, versión con 2 péptidos; ID-PaGIA3: prueba de aglutinación en gel, versión con 3 péptidos; IFI: inmunofluorescencia indirecta.

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www.elsevier.es/eimc

Original

Comparación de técnicas serológicas convencionales y no convencionales para el diagnóstico de la enfermedad de Chagas importada en España

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[a] Servicio de Parasitología, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Madrid, España
[b] Centro de Transfusión Sanguínea de Sevilla-Huelva y Banco de Tejidos, Sevilla, España

Prueba	Antígeno	Conjugado
IFI-CNM	Epimastigotes (T. Mc, Dm28)	Anti-IgG humana (FITC)
ELISA-CNM	Extracto soluble (T. Mc, Dm28)	Anti-IgG humana (Biot, Strep-HRP)
Certest	Extracto total (Tulahuen, Mn)	Anti-IgG humana (HRP)
Ortho	Extracto total	Anti-IgG humana (HRP)
BLK	Extracto total	Anti-IgG humana (HRP)
bioMérieux	Extracto total	Anti-IgG humana (HRP)
Biokit	TcD, TcB, Pep2, TcLo1.2	Anti-IgG humana, anti-IgM humana (HRP)
ID-PaGIA2	Ag2, TcB	Anti-Ig humana
ID-PaGIA3	Ag2, TcB, TcD	
ICT Operon	TcD, TcB, Pep2, SAPA	
ICT CTK	Antígenos recombinantes	Anti-IgG humana (oro coloidal)

Sierologie a confronto

Tre pannelli di sieri

- un pannello da impresa esterna: 38 siero+ per Chagas endemici, 2 controlli non reattivi
- un II pannello di 128 sieri: 28 pts con Chagas cronico diagnosticato in Spagna, 73 LA sani e 22 spagnoli sani
- un III pannello di 60 soggetti spagnoli, non viaggiatori in LA, 30 siero+ per leishmania e 30 per malaria

Sierologie a confronto: risultati

Tabla 5
Sensibilidad y especificidad de las pruebas de detección de anticuerpos anti-*Trypanosoma cruzi*

	Chagásicos N.° positivos (total sueros)	Sensibilidad (IC del 95%)	No chagásicos N.° positivos (total sueros)	Especificidad 1[a] (IC del 95%)	Otras enfermedades N.° de positivos (total sueros)	Especificidad 2[b] (IC del 95%)
IFI-CNM	65 (66)	98,5 (94,8–100)	0 (97)	100 (99,5–100)	23 (60)	85,4 (79,5–91,2)
ELISA-CNM	66 (66)	100 (99,2–100)	0 (97)	100 (99,5–100)	18 (60)	88,5 (83,2–93,8)
Certest	66 (66)	100 (99,2–100)	0 (97)	100 (99,5–100)	25 (60)	84,1 (78,0–90,1)
Ortho	66 (66)	100 (99,2–100)	0 (97)	100 (99,5–100)	18 (60)	88,5 (83,2–93,8)
BLK	41 (42)	97,6 (91,8–100)	0 (74)	100 (99,3–100)	13 (60)	90,3 (84,9–95,7)
bioMérieux	66 (66)	98,5 (94,8–100)	0 (97)	100 (99,5–100)	9 (60)	94,3 (90,3–98,2)
Biokit	66 (66)	100 (99,2–100)	1 (97)	99 (96,4–100)	7 (60)	94,9 (91,2–98,7)
ID-PaGIA2	61 (66)	92,4 (85,3–99,6)	1 (97)	99 (96,4–100)	1 (60)	98,7 (96,7–100)
ID-PaGIA3	65 (66)	98,5 (94,8–100)	2 (97)	97,9 (94,6–100)	2 (60)	98,1 (95,6–100)
ICT Operon	61 (66)	92,4 (85,2–99,6)	2 (97)	97,9 (94,6–100)	10 (60)	92,4 (85,3–99,6)
ICT CTK	63 (66)	95,5 (89,7–100)	3 (97)	96,9 (93–100)	9 (60)	93 (88,7–97,3)

CNM: Centro Nacional de Microbiología; ELISA: enzimoinmunoanálisis; ICT: prueba inmunocromatográfica (*immunochromatographic test*); ID-PaGIA2: prueba de aglutinación en gel, versión con 2 péptidos; ID-PaGIA3: prueba de aglutinación en gel, versión con 3 péptidos; IC: intervalo de confianza; IFI: inmunofluorescencia indirecta.

[a] Especificidad calculada a partir de los datos de individuos no chagásicos (controles sanos).

[b] Especificidad calculada teniendo en cuenta los resultados de todos los individuos no chagásicos de todos los paneles (controles sanos, leishmaniasis y malaria).

World Health Organization

Anti-*Trypanosoma cruzi* ASSAYS: Operational Characteristics

Report 1

2010

Table 1. Description of plasma units and their original test results as submitted by each institution

Country of collection	Collecting institution	Assays used for screening of the blood units	Total
Argentina	Hospital de Pediatría	Chagatek ELISA + Serodia Chagas	45
Bolivia	Hospital Clínico "Viedma"	Chagatek ELISA + Chagas HAI Imunoserum	40
Brazil	FPS/HSP	Hemacruzi + Imunocruzi + Hemobio Chagas	146
Colombia	Instituto Nacional de Salud	IFI Freddy Chaparro	24
Ecuador	Cruz Roja Ecuatoriana	Chagatek ELISA+ CRE ELISA	26
El Salvador	Cruz Roja Salvadoreña	Chagas Anticorpos EIA + Hemobio Chagas	31
Honduras	Cruz Roja Hondureña	Chagas Anticorpos EIA + Hemobio Chagas	40
Mexico	Centro Nacional de Transfusión	Chagas Anticorpos EIA + Chagatest ELISA	10
Nicaragua	Cruz Roja Nicaragüense	Chagas Anticorpos EIA + Hemobio Chagas	31
Paraguay	Instituto de Investigación en Ciencias de la Salud	Chagas-Test IICS + IFI caseiro	44
Total			437

ANNEX 1

ALGORITHM FOR CHARACTERIZATION OF THE WHO ANTI-*TRYPANOSOMA CRUZI* SPECIMEN REFERENCE PANEL

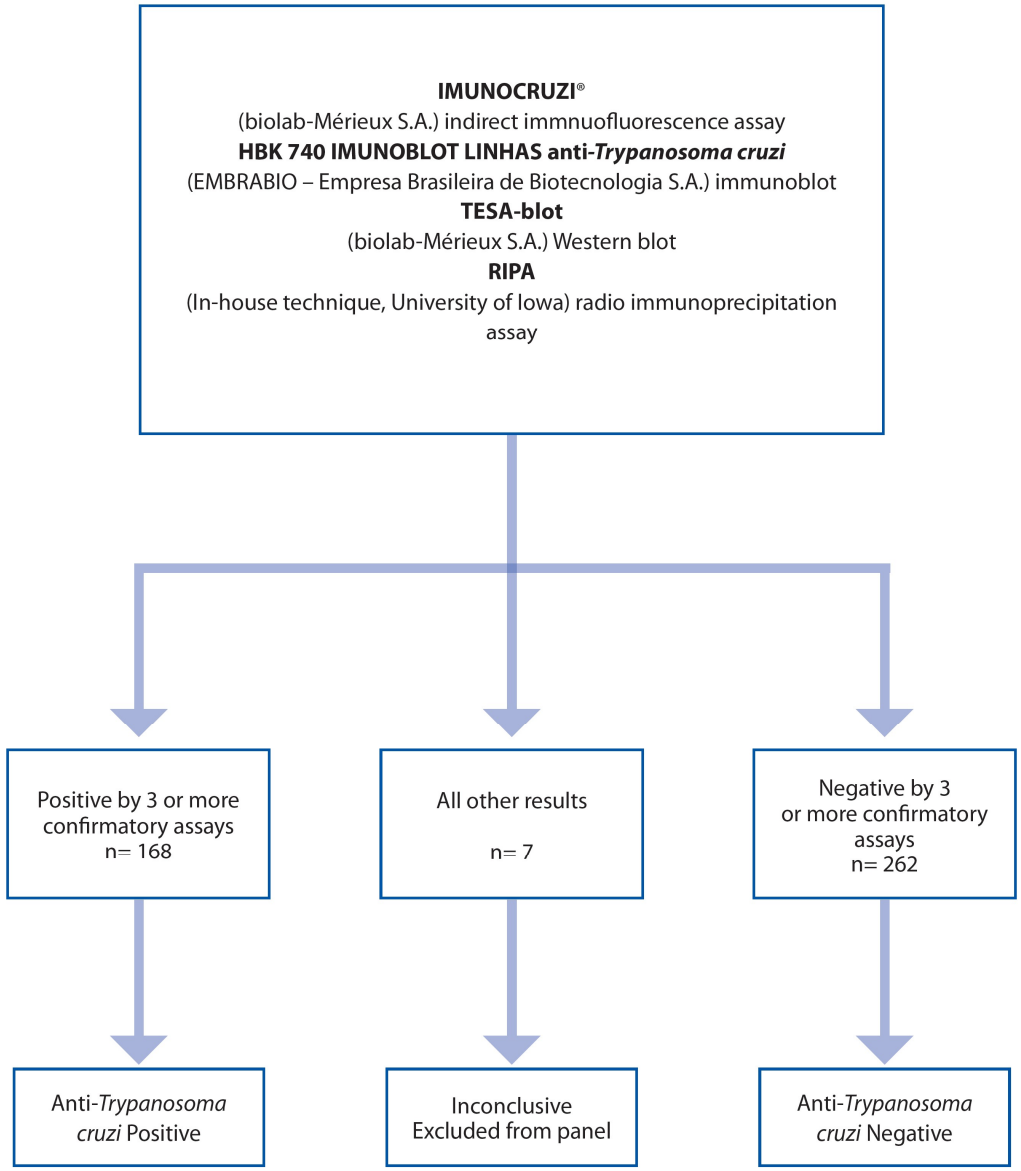


Table 8. Comparison of the assays with reference results: Group 2, Agglutination and rapid assays

NAME		CHAGAS HAI IMUNOSERUM	CHAGAS – HAI	Imuno-HAI	CHAGAS HEMAGEN®	HEMACRUZI®	SERODIA – Chagas	ID-Chagas Antibody Test	Chagas STAT-PAK®
Final Sensitivity % (95 CI) n = 168		97.60 (94.0–99.3)	88.09 (82.2–92.6)	100 (97.2–100)	92.26 (87.1–95.8)	99.40 (96.7–100)	100 (97.2–100)	97.02 (93.2–99.0)	93.0* (87.9–96.5)
Specificity % (95 CI) n = 262		78.62 (77.2–83.4)	59.92 (53.7–65.9)	95.80 (92.6–97.9)	89.31 (84.9–92.8)	97.33 (94.6–98.9)	97.70 (95.1–99.2)	99.62 (97.9–100)	95.3 (91.5–97.7)
Indeterminate results %		0.46– 0.69	1.14	0.23	3.20	1.14	0.46	2.97	1.86
Initial inter-reader variability %		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/D
PPV	0.1%	0	0	2	1	4	4	20	2
	5%	19	11	55	31	66	69	93	51
	10%	34	20	72	49	81	82	97	68
NPV	0.1%	99.99	99.98	100	99.99	99.99	100	99.99	99.99
	5%	99.84	98.96	100	99.96	99.96	100	99.84	99.61
	10%	99.66	97.84	100	99.93	99.93	100	99.66	99.18

CI: Confidence intervals; N/A: not applicable; ND: not determined; NPV: negative predictive value; PPV: positive predictive value.

* Note: The evaluation of the Chagas STAT-PAK (Chembio Diagnostics Systems, INC) was performed later and it was not possible to test the entire WHO Chagas Specimen Reference Panel using the assay. An abbreviated panel with a total of 212 anti-*Trypanosoma cruzi* positive specimens and 158 anti-Trypanosoma cruzi negative specimens was used to determine these performance characteristics.

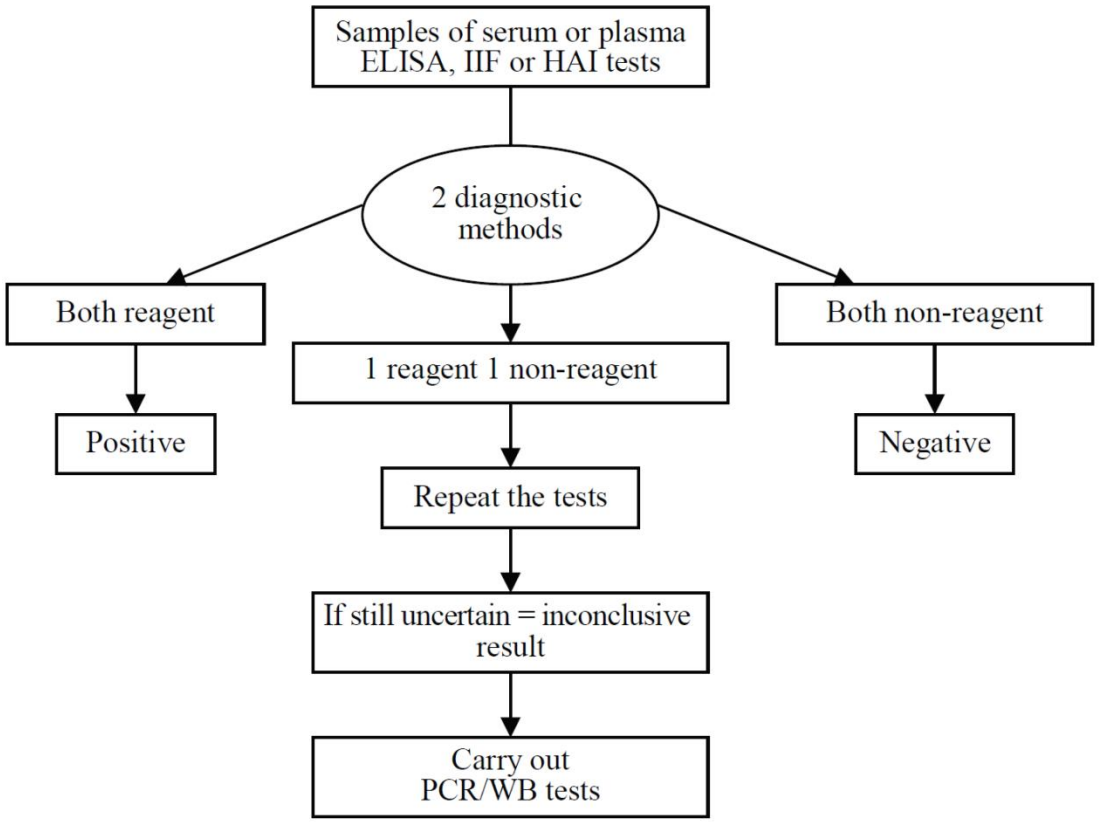


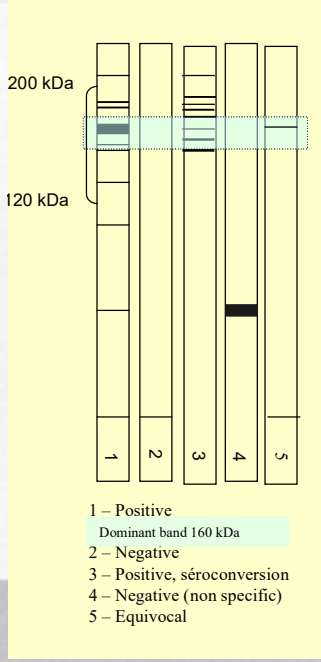
Fig. 2: flow chart of serological diagnosis for the detection *Trypanosoma cruzi* infection (MS 2005). ELISA: enzyme-linked immunosorbent assay; IIF: indirect immunofluorescence; HAI: indirect haemaglutination; PCR: polymerase chain reaction; WB: western blot.

Mem Inst Oswaldo Cruz, Rio de Janeiro, Vol. *104*(Suppl. I), 2009

Il problema dei discordanti

- Si possono avere meno del 2% di risultati discordanti appaiando 3 test...
- Test di conferma o terzo test → quale?

- WB? → LDBIO Diagnostics + marchio CE
- TESA (immuno blot con ag escretorio-secetorio) Biomerieux Brasil, non CE
- RIPA (radioimmunoprecipitation assay)? CDC Atlanta

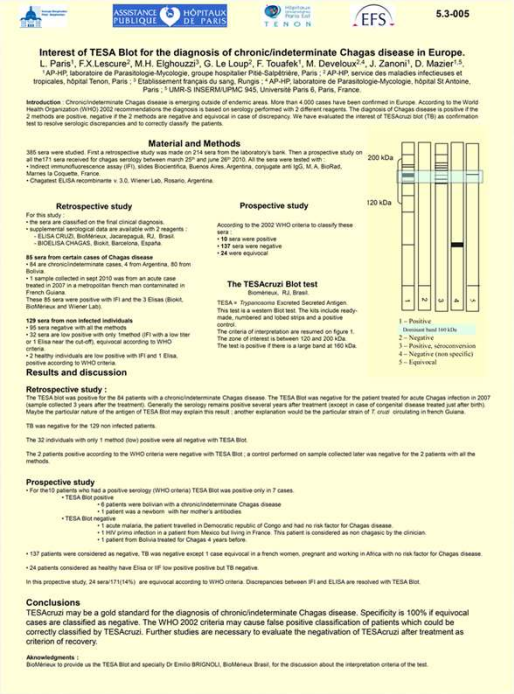


TESAcruzi - Parameters

Serum panel bioMérieux + serum from blood banks

Sensitivity	100%
Specificity	99,5%
PPV	98,7%
PNV	100%
Efficiency	99%
Kappa index	0,99
N positives	795
N negatives	1946

The TESA-blot (Y strain of *T.cruzi*) showed that a 150-160 kDa band was recognised in the serum of 100% of chronic Chagas disease patients and did not cross-react in case of leishmaniasis (Umezawa et al. 1996).



5.3-005

Interest of TESA Blot for the diagnosis of chronic/indeterminate Chagas disease in Europe.

L. Paris[1], F.X.Lescure[2], M.H. Elghouzzi[3], G. Le Loup[2], F. Touafek[1], M. Develoux[2,4], J. Zanoni[1], D. Mazier[1,5].
[1] AP-HP, laboratoire de Parasitologie-Mycologie, groupe hospitalier Pitié-Salpêtrière, Paris ; [2] AP-HP, service des maladies infectieuses et tropicales, hôpital Tenon, Paris ; [3] Etablissement français du sang, Rungis ; [4] AP-HP, laboratoire de Parasitologie-Mycologie, hôpital St Antoine, Paris ; [5] UMR-S INSERM/UPMC 945, Université Paris 6, Paris, France.

Introduction : Chronic/indeterminate Chagas disease is emerging outside of endemic areas. More than 4.000 cases have been confirmed in Europe. According to the World Health Organization (WHO) 2002 recommendations the diagnosis is based on serology performed with 2 different reagents. The diagnosis of Chagas disease is positive if the 2 methods are positive, negative if the 2 methods are negative and equivocal in case of discrepancy. We have evaluated the interest of TESAcruzi blot (TB) as confirmation test to resolve serologic discrepancies and to correctly classify the patients.

Material and Methods

385 sera were studied. First a retrospective study was made on 214 sera from the laboratory's bank. Then a prospective study on all the171 sera received for chagas serology between march 25th and june 28th 2010. All the sera were tested with :
• Indirect immunofluorescence assay (IFI), slides Biocientifica, Buenos Aires, Argentina, conjugate anti IgG, M. A. BioRad, Marnes la Coquette, France.
• Chagatest ELISA recombinante v. 3.0, Wiener Lab, Rosario, Argentina.

Retrospective study

For this study :
• the sera are classified on the final clinical diagnosis.
• supplemental serological data are available with 2 reagents :
- ELISA CRUZI, BioMérieux, Jacarepaguá, RJ, Brasil.
- BIOELISA CHAGAS, Biokit, Barcelona, España

85 sera from certain cases of Chagas disease
• 84 are chronic/indeterminate cases, 4 from Argentina, 80 from Bolivia.
• 1 sample collected in sept 2010 was from an acute case treated in 2007 in a metropolitan french man contaminated in French Guiana.
These 85 sera were positive with IFI and the 3 Elisas (Biokit, BioMérieux and Wiener Lab).

129 sera from non infected individuals
• 95 sera negative with all the methods
• 32 sera are low positive with only 1method (IFI with a low titer or 1 Elisa near the cut-off), equivocal according to WHO criteria.
• 2 healthy individuals are low positive with IFI and 1 Elisa, positive according to WHO criteria.

Prospective study

According to the 2002 WHO criteria to classify these sera :
• **10** sera were positive
• **137** sera were negative
• **24** were equivocal

The TESAcruzi Blot test

BioMérieux, RJ, Brasil.

TESA = *Trypanosoma* Excreted Secreted Antigen.
This test is a western Blot test. The kits include ready-made, numbered and lobed strips and a positive control.
The criteria of interpretation are resumed on figure 1.
The zone of interest is between 120 and 200 kDa.
The test is positive if there is a large band at 160 kDa.

200 kDa
120 kDa
Ut
Pos
Neg
Ab
Ut

1 – Positive
Dominant band 160 kDa
2 – Negative
3 – Positive, seroconversion
4 – Negative (non specific)
5 – Equivocal

Results and discussion

Retrospective study :
The TESA blot was positive for the 84 patients with a chronic/indeterminate Chagas disease. The TESA Blot was negative for the patient treated for acute Chagas infection in 2007 (sample collected 3 years after the treatment). Generally the serology remains positive several years after treatment (except in case of congenital disease treated just after birth). Maybe the particular nature of the antigen of TESA Blot may explain this result ; another explanation would be the particular strain of *T. cruzi* circulating in french Guiana.

TB was negative for the 129 non infected patients.

The 32 individuals with only 1 method (low) positive were all negative with TESA Blot.

The 2 patients positive according to the WHO criteria were negative with TESA Blot ; a control performed on sample collected later was negative for the 2 patients with all the methods.

Prospective study
• For the10 patients who had a positive serology (WHO criteria) TESA Blot was positive only in 7 cases.
- TESA Blot positive
  - 6 patients were bolivian with a chronic/indeterminate Chagas disease
  - 1 patient was a newborn with her mother's antibodies
- TESA Blot negative
  - 1 acute malaria, the patient travelled in Democratic republic of Congo and had no risk factor for Chagas disease.
  - 1 HIV primo infection in a patient from Mexico but living in France. This patient is considered as non chagasic by the clinician.
  - 1 patient from Bolivia treated for Chagas 4 years before.

• 137 patients were considered as negative, TB was negative except 1 case equivocal in a french women, pregnant and working in Africa with no risk factor for Chagas disease.

• 24 patients considered as healthy have Elisa or IIF low positive positive but TB negative.

In this propective study, 24 sera/171(14%) are equivocal according to WHO criteria. Discrepancies between IFI and ELISA are resolved with TESA Blot.

Conclusions
TESAcruzi may be a gold standard for the diagnosis of chronic/indeterminate Chagas disease. Specificity is 100% if equivocal cases are classified as negative. The WHO 2002 criteria may cause false positive classification of patients which could be correctly classified by TESAcruzi. Further studies are necessary to evaluate the negativation of TESAcruzi after treatment as criterion of recovery.

Aknowledgments :
BioMérieux to provide us the TESA Blot and specialty Dr Emilio BRIGNOLI, BioMérieux Brasil, for the discussion about the interpretation criteria of the test.

Poster presented at ECTMIH, Barcelone, 2012

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Original article

Serodiscordance in chronic Chagas disease diagnosis: a real problem in non-endemic countries

Z. Moure [1,*], A. Angheben [2,3], I. Molina [4], F. Gobbi [2,3], M. Espasa [1], M. Anselmi [5], F. Salvador [4], S. Tais [6], A. Sánchez-Montalvá [4], T. Pumarola [1], P. Albajar-Viñas [7], E. Sulleiro [1]

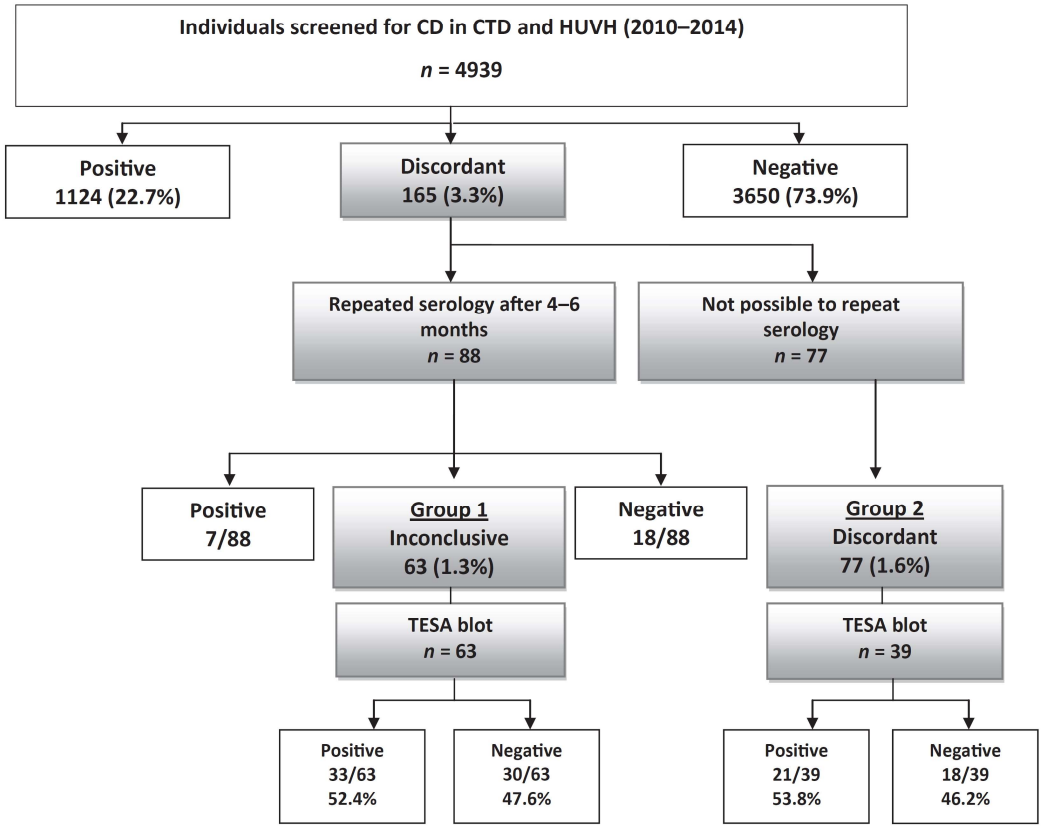

Fig. 1. WHO algorithm applied to our cohort of patients.

Usefulness of a Western blot method for serodiagnosis of Chagas' disease in cases of inconclusive serological results

Cristina Riera[1], Elena Sulleiro[2], Eva Dopico[3], Laura Iniesta[1], Silvia Tebar[1], Roser Fisa[1] and Carmen Guillen[1]

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Introduction. Although many serological tests are available, there is no gold-standard method for diagnosis of Chagas' disease in patients with low antibody titers or inconclusive results. A qualitative method, such as Western blot (WB), could be an alternative way to confirm the diagnosis of Chagas' disease when other tests are discordant.

Materials and Methods. We examined serum samples from forty-three patients from endemic areas: Bolivia (27), Colombia (3), Ecuador (2), Paraguay (1), El Salvador (1), Peru (1), unknown origin (5) and Spanish travelers to America (3) with discordant serological results by a recombinant enzyme immunoassay BioELISA Chagas; Biokit® S.A. (r-ELISA) and a native enzyme immunoassay Ortho® T. cruzi Elisa Test System (n-ELISA). All sera were tested by a Western blot (WB) method using a lysate from *Trypanosoma cruzi* (Maracay strain) epimastigotes. Sera were considered positive when they recognized at least five antigenic bands of the standard pattern (28, 32, 38, 39, 40 and 48 kDa), indeterminate when they recognized one to four bands, and negative when no band was present.

Results. Of the 43 sera tested by WB, 16 (45.7%) were positive, 19 (44.2%) were indeterminate and 8 were (18.6%) negative. Of the 27 sera positive by r-ELISA, 5 (18.5%) were positive by WB, 15 (55.5%) were indeterminate and 7 (25.9%) were negative. Of 16 sera positive by n-ELISA 11 (68.8%) were positive by WB, 4 (25%) were indeterminate and 1 (6.3%) was negative. The WB confirmed 10 false negative sera by r-ELISA and 6 by n-ELISA. The correlation between number of specific bands was better in n-ELISA (r=0.511) than in r-ELISA. (r = 0,271).

Conclusions. The Western blot confirmed 46% of the sera with discordant results by r-ELISA and n-ELISA. Therefore, it could be considered a good alternative method to confirm serodiagnosis of Chagas' disease.

CHAGAS CE

Western blot IgG

AUTORISE

Technique d'immunoblot pour usage diagnostique *in vitro*

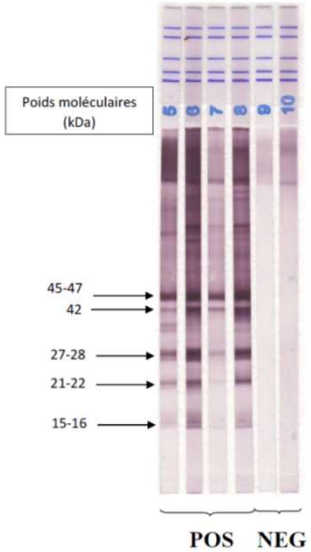

Fig. 1 : Exemples de quatre résultats positifs et deux négatifs

- Interprétation :

La présence d'un minimum de **2 bandes bien définies** parmi les 5 précédemment décrites, **P15-16, P21-22, P27-28, P42 et P45-47**, est indicative de la présence d'anticorps anti-*Tryponosoma cruzi* dans l'échantillon.

		EIA Ag Ric	EIA Ag Crudo	ICT	PCR /		45-47	42	27-28	21-22	15-16	P/N
1	**C+**						X	X	X	X	X	P
2	000-PNCQ	2,294	2,075				X	X	X	X		P
3	000-PNCQ	2,334	3,136				X	X	X	X		P
4	000-PNCQ	2,41	2,91				X	X	X	X		P
5	000-PNCQ	6,351	5,726				X	X	X	X	X	P
6	000-PNCQ	5,593	5,166				X	X	X	X	X	P
7	000-PNCQ	6,756	4,37				X	X	X	X	X	P
8	000-PNCQ	8,476	9,087				X	X	X	X	X	P
9	000-PNCQ	9,636	8,105				X	X	X	X	X	P
10	000-PNCQ	9,14	7,853				X	X	X	X	X	P
11	000-PNCQ	0,071	0,184									N
12	000-PNCQ	0,07	0,102									N
13	**C+**						X	X	X	X	X	P
14	PZ01	7,3	1,43	P			X	X	X	X	X	P
15	PZ02	9,1	5,08	P			X	X	X	X	X	P
16	PZ03	9,1		P			X	X	X	X	X	P
17	PZ04	9,1		P			X	X	X	X	X	P
18	PZ05	9,1		P	P		X	X	X	X	X	P
19	PZ06	1,1		P			X	X	X	X	X	P
20	PZ07	9,1	5,31	P			X	X	X	X	X	P
21	PZ_ALTRO_01	leish										N
22	PZ_ALTRO_02	leish					D	D	X			P
23	PZ_ALTRO_03	schisto										N
24	PZ_ALTRO_04	strongy										N

E i test rapidi?

Journal of Clinical Microbiology

Comparative Evaluation of 11 Commercialized Rapid Diagnostic Tests for Detecting Trypanosoma cruzi Antibodies in Serum Banks in Areas of Endemicity and Nonendemicity

Claudia L. Sánchez-Camargo, Pedro Albajar-Viñas, Patricia P. Wilkins, Javier Nieto, David A. Leiby, Luc Paris, Karenina Scollo, Carolina Flórez, Carmen Guzmán-Bracho, Alejandro O. Luquetti, Nidia Calvo, Kenji Tadokoro, Amadeo Saez-Alquezar, Pedro Pablo Palma, Miguel Martin and Laurence Flevaud

J. Clin. Microbiol. 2014, 52(7):2506. DOI: 10.1128/JCM.00144-14.
Published Ahead of Print 7 May 2014.

- The first multicenter study evaluation of all commercially available RDTs for Chagas disease;
- It is a case-control study on 11 (9) commercially available RDTs fro T.cruzi Ab
- 11 National Reference Labs (endemic and non-endemic countries)
- A total of 474 samples (424 serum and 50 plasma samples) previously tested for *T. cruzi* infection following two conventional tests (ELISA/IHA/IFA),
- 6 RTDs can be recommended for screening and surveillance in areas of endemicity and nonendemicity, but the results should be confirmed in a reference laboratory.

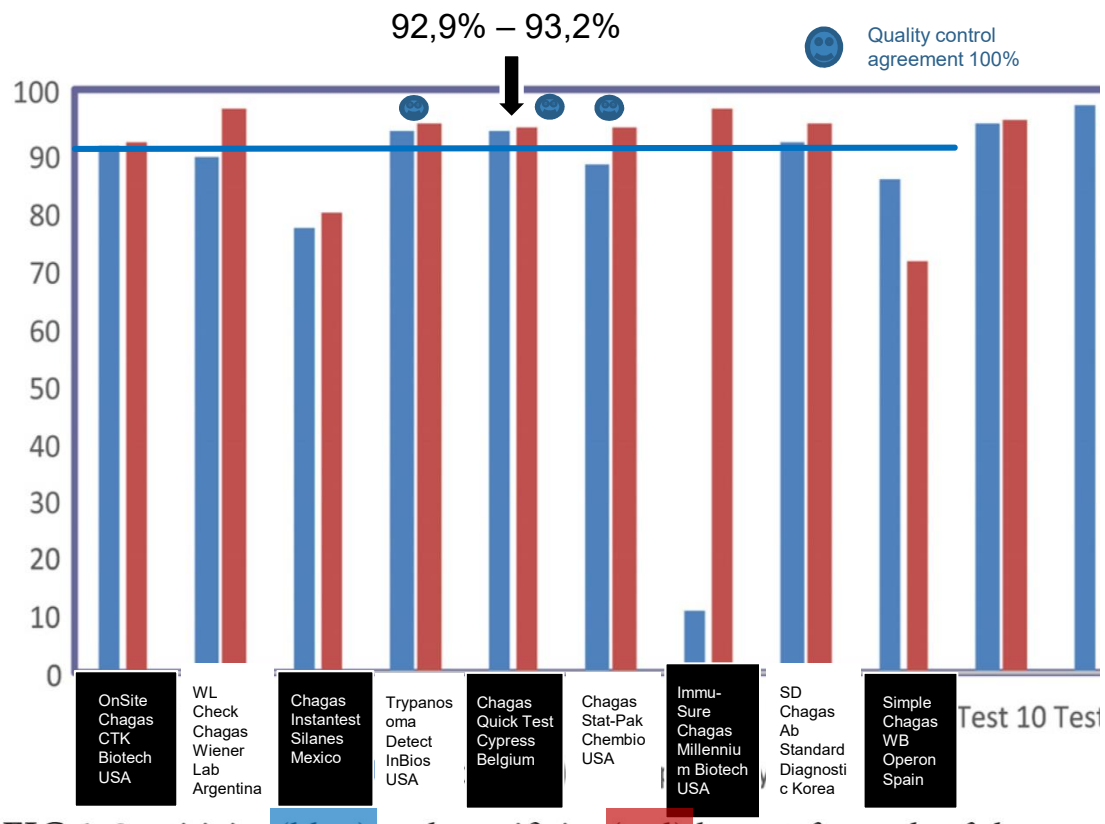

FIG 1 Sensitivity (blue) and specificity (red) by test for each of the 11 commercialized *T. cruzi* detection tests.

Am. J. Trop. Med. Hyg., 80(3), 2009, pp. 410–415
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Geographic Variation in the Sensitivity of Recombinant Antigen-based Rapid Tests for Chronic *Trypanosoma cruzi* Infection

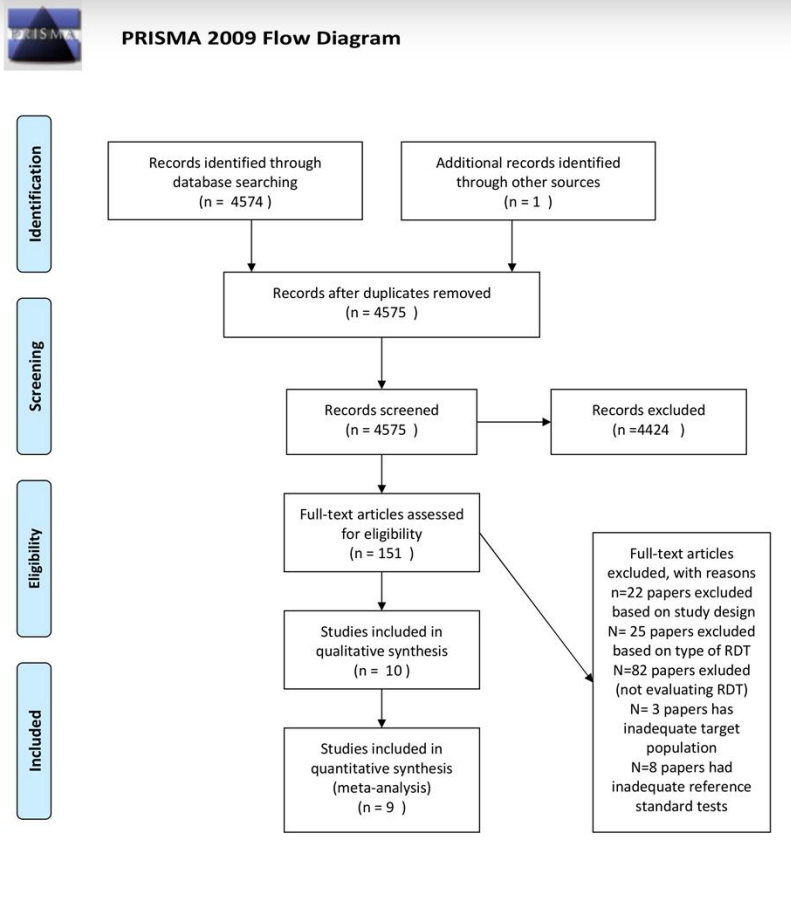
Jennifer R. Verani,* Amy Seitz, Robert H. Gilman, Carlos LaFuente, Gerson Galdos-Cardenas, Vivian Kawai, Elizabeth de LaFuente, Lisbeth Ferrufino, Natalie M. Bowman, Viviana Pinedo-Cancino, Michael Z. Levy, Francis Steurer, Charles W. Todd, Louis V. Kirchhoff, Lilia Cabrera, Manuela Verastegui, and Caryn Bern

Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia; Emory University, Atlanta, Georgia; Johns Hopkins University Bloomberg School of Public Health, Baltimore, Maryland; Asociación Benéfica PRISMA, Lima, Peru; Universidad Peruana Cayetano Heredia, Lima, Peru; Hospital Unversitario Japonés, Santa Cruz, Bolivia; Fogarty International Center, National Institutes of Health, Bethesda, Maryland; Carver College of Medicine, University of Iowa, Iowa City, Iowa

Abstract. Chagas disease affects 8–11 million people throughout the Americas. Early detection is crucial for timely treatment and to prevent non-vectorial transmission. Recombinant antigen-based rapid tests had high sensitivity and specificity in laboratory evaluations, but no Peruvian specimens were included in previous studies. We evaluated Stat-Pak and Trypanosoma Detect rapid tests in specimens from Bolivia and Peru. Specimens positive by three conventional assays were confirmed positives; specimens negative by two or more assays were confirmed negatives. In Bolivian specimens, Stat-Pak and Trypanosoma Detect tests were 87.5% and 90.7% sensitive, respectively; both showed 100% specificity. Sensitivity in Peruvian specimens was much lower: 26.6–33.0% (Stat-Pak) and 54.3–55.2% (Trypanosoma Detect); both had specificities > 98%. Even in Bolivian specimens, these sensitivities are inadequate for stand-alone screening. The low sensitivity in Peru may be related to parasite strain differences. Chagas disease rapid tests should be field tested in each geographic site before widespread implementation for screening.

Rapid immunochromatographic tests for the diagnosis of chronic Chagas disease in at-risk populations. A systematic review and meta-analysis

Andrea Angheben[1,*,§], Dora Buonfrate[1,§], Mario Cruciani[2], Yves Jackson[3,4], Julio Alonso-Padilla[5], Joaquim Gascon[5], Federico Gobbi[1], Giovanni Giorli[1], Mariella Anselmi[6], Zeno Bisoffi[1,7]



Angheben *et al* submitted to Plos NTD

Interpretative criteria: Endemicity / Stat-Pak	Effect (95% CI)	# of studies	Mean Prevalence[a] (95% CI)	What do these results mean?
All areas	Sensitivity: 96.60% (91.3-98.7) Specificity: 99.27% (98.4-99.7)	12	30.33% (18.6 to 42.0)	Of the 30 out of 100 patients with CD, 1 will be missed by a single RDT (3.40% of 30). Of the other 67, not even 1 will have a false positive result of the RDT.
Endemic areas	Sensitivity: 98.07% (91.6-99.6) Specificity: 99.30% (98.3-99.7)	8	35.93% (20.7 to 51.1)	Of the 36 out of 100 patients with CD, not even 1 will be missed by a single RDT (1.93% of 33). Of the other 64, not even 1 will have a false positive result of the RDT.
Non-Endemic areas	Sensitivity: 89.77% (79.7-95.1) Specificity: 98.52% (95.0-99.5)	4	19.14% (4.3 to 42.6)	Of the 19 out of 100 patients with CD, 2 will be missed by a single RDT (10.23% of 19). Of the other 81, 1 will have a false positive result of the RDT.
Stat-Pak tests only	Sensitivity: 97.02% (87.6-99.3) Specificity: 99.44% (98.6-99.8)	4	26.37% (2.9 to 49.8)	Of the 26 out of 100 patients with CD, not even 1 will be missed by a single Stat-Pak test (2.98% of 26). Of the other 74, not even 1 will have a false positive result of the Stat-Pak test.

Table 1: Summary of findings from the review of RDTs for the diagnosis of chronic CD in at risk populations

[a] Estimates of true prevalences for each study were calculated as described by Rogan and Gladen (1978) [ref.] .
CI: confidence interval
RDT: Rapid Diagnostic Test
CD: Chagas disease.

[ref.] Rogan WJ, Gladen B. Estimating prevalence from the results of a screening test. Am J Epidemiol. 1978; 107:71-6

Angheben *et al* submitted to Plos NTD

Rapid immunochromatographic tests for the diagnosis of chronic Chagas disease in at-risk populations. A systematic review and meta-analysis

Andrea Angheben[1,*,§], Dora Buonfrate[1,§], Mario Cruciani[2], Yves Jackson[3,4], Julio Alonso-Padilla[5], Joaquim Gascon[5], Federico Gobbi[1], Giovanni Giorli[1], Mariella Anselmi[6], Zeno Bisoffi[1,7]

Conclusions

The accuracy of all RDTs under study can be considered sufficiently good to recommend their use in endemic settings, particularly in the Southern Cone of LA, in order to increase access to diagnosis.

The Stat-Pak test can be recommended for use in screening surveys when the expected prevalence is moderately high or high, in the setting of Southern Cone or for migrants from that area in case of non endemic countries.

On the other hand, the pooled sensitivity of all RDTs studied resulted too low to recommend them as stand-alone tests for detection of CD affected individuals in a non-endemic context, as a negative result cannot rule out a *T. cruzi* infection with reasonable certainty.

The WHO's principle which states the need of diagnosis confirmation through another serologic test remains a prudent approach that should be followed at least for the confirmation of positive results.

Still, further studies conducting head-to-head comparisons of different available RDTs are needed, and it would be particularly important to extend these studies to the Andean countries, the Amazon basin, Central America and Mexico. Robust evidence from studies of high quality is also needed to advocate adequate control policies and quality assurance in endemic countries, mainly in those with lower prevalence of the disease.

Angheben *et al* submitted to Plos NTD

Brasil *et al. BMC Infectious Diseases* 2010, **10**:337
http://www.biomedcentral.com/1471-2334/10/337

BMC
Infectious Diseases

RESEARCH ARTICLE Open Access

ELISA versus PCR for diagnosis of chronic Chagas disease: systematic review and meta-analysis

Pedro EAA Brasil[1,2*], Liane De Castro[1], Alejandro M Hasslocher-Moreno[1], Luiz HC Sangenis[1], José U Braga[2,3]

- La PCR ha una sensibilità del 50-90% (PCR kDNA Studio BENEFIT 1896 pz, 61,7% PCR+)
- Una specificità praticamente del 100%
- Allo stato attuale la sierologia ELISA ha una performance migliore

Brasil *et al. BMC Infectious Diseases* 2010, **10**:337
http://www.biomedcentral.com/1471-2334/10/337

BMC
Infectious Diseases

RESEARCH ARTICLE Open Access

ELISA versus PCR for diagnosis of chronic Chagas disease: systematic review and meta-analysis

Pedro EAA Brasil[1,2*], Liane De Castro[1], Alejandro M Hasslocher-Moreno[1], Luiz HC Sangenis[1], José U Braga[2,3]

ized. Visual comparison of the area under the summary ROC curves for ELISA and PCR indicates that ELISA has better performance than PCR. At this point, PCR test cannot be considered a tool for diagnosis of chronic Chagas disease in clinical practice.

Evaluation of two commercial real-time PCR kit for in blood *T. cruzi* detection.

Author: Formenti F., Angheben A., Bisoffi Z. e Perandin F.
Centro per le Malattie Tropicali, Ospedale Sacrocuore, Negrar, Verona

Introduzione: La malattia di Chagas […]

Metodi: Nello studio sono stati presi in considerazione 14 campioni di sangue positivi per *T. cruzi*. 13 sono stati conservati in Guanidina 6M ed uno in EDTA a -80°C. Dei 13 campioni, 3 sono stati forniti da CAEDES, Cochabamba, Bolivia, i rimanenti 10 sono stati forniti dal Centro Nazionale di Microbiologia Carlo III, Madrid. Il campione conservato in EDTA proviene da un paziente ricoverato presso il Centro Malattie Tropicali di Negrar.

Tutti i campioni sono stati estratti con il sistema automatico MaGnaPure LC.2 (Roche) utilizzando il KIT DNA-I. In ciascun campione è stato messo un controllo esogeno di estrazione/presenza di inibitori (PhHV). L'eluato (100 ul) è stato utilizzato per le reazioni di amplificazioni in Real-Time PCR (TaqMan), seguendo le istruzioni riportate nel kit. Ciascun kit prevede l'amplificazione di un controllo interno e di un controllo positivo di amplificazione.

I due sistemi commerciali saggiati sono: Quantification of Trypanosoma cruzi (Genesig PrimerDesign, England) e CHAG-U (Progenie Molecular, Valencia).

Risultati: 9 su 14 campioni amplificati con kit Molecular Progenie sono risultati positivi, mentre solo 1 è risultato positivo quando amplificato con kit Genesig-PrimerDesign

*: campione conservato in EDTA a -80°C

Conclusioni: […] Attualmente il panorama commerciale per diagnosi molecolare di Chagas è molto scadente; i pochi kit disponibili peccano di scarsa sensibilità; mentre nel mondo della ricerca esistono miriadi di differenti approcci (differenti target amplificati) per cui non si riesce a standardizzare un protocollo. Questo scenario e i risultati ottenuti in questo studio preliminare evidenziano quanto sia necessario unire le forze per trovare un sistema di amplificazione specifico, sensibile e standardizzato.

Campione	Genesig PrimerDesign (Ct)	Progenie Molecular (Ct)
1*	neg	neg
2	neg	pos (29)
3	pos (33.51)	pos (26)
4	neg	pos (20.99)
5	neg	pos (31)
6	neg	pos (34)
7	neg	pos (34.43)
8	neg	pos (34.13)
9	neg	neg
10	neg	neg
11	neg	neg
12	neg	neg
13	neg	pos (38.90)
14	neg	pos (33.54)

Poster presentato al Convegno «Dalle malattie tropicali alla salute globale, Verona, 16-18/09/2014

28 pazienti con Chagas certo

Campione	in home SAT/K PCR (ct)	Real Time Progenie (ct)
1	neg/neg	NEG
2	neg/neg	NEG
3	neg/neg	NEG
4	neg/neg	NEG
5	neg/neg	NEG
6	**neg/pos (35,63)**	NEG
7	neg/neg	NEG
8	neg/neg	NEG
9	neg/neg	NEG
10	neg/neg	NEG
11	neg/neg	NEG
12	**neg/pos (37,25)**	NEG
13	neg/neg	NEG
14	**pos (29,26)/pos (29,74)**	**pos (29,48)**
15	neg/neg	NEG
16	**pos (32,61)/pos (32,11)**	**pos (30,11)**
17	neg/neg	NEG
18	neg/neg	NEG
19	**pos (28,89)/pos (34.32)**	**pos (30,44)**
20	neg/neg	NEG
21	neg/neg	NEG
22	**pos (27,82)/pos (33,14)**	**pos (27)**
23	neg/neg	NEG
24	neg/neg	NEG
25	**neg/pos (31,08)**	NEG
26	**neg/pos (31,06)**	NEG
27	neg/neg	NEG
28	neg/neg	NEG

PCR: lavoro da tenere sul comodino

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]

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Acta Tropica 102 (2007) 182–189

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Species-specific loop-mediated isothermal amplification (LAMP) for diagnosis of trypanosomosis

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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

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The analytical specificity was 100%.
The analytical **sensitivity** was **between 0,1 and 0,01 parasite equivalent/mL** depending on the strain DNA.
All DTUs.

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

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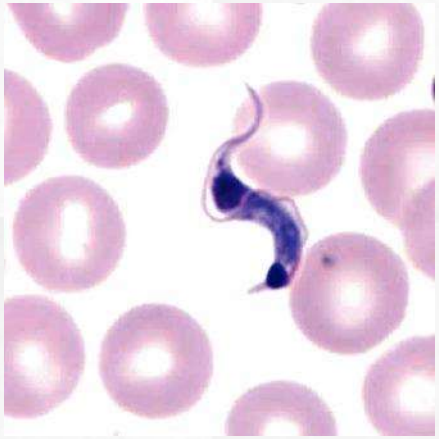
The analytical sensitivity of the assay was evaluated using artificial control samples (non-infected human blood spiked with *T. cruzi*). The limit of detection of the LAMP assay was **50 parasites/mL**.
The analytical sensitivity was approximately equal to thatof the conventional parasitological method (**40 parasites/mL**) (Torrico et al., 2005).

All DTUs.



Grazie

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