Diagnostica Microbiologica della Malattia di Chagas

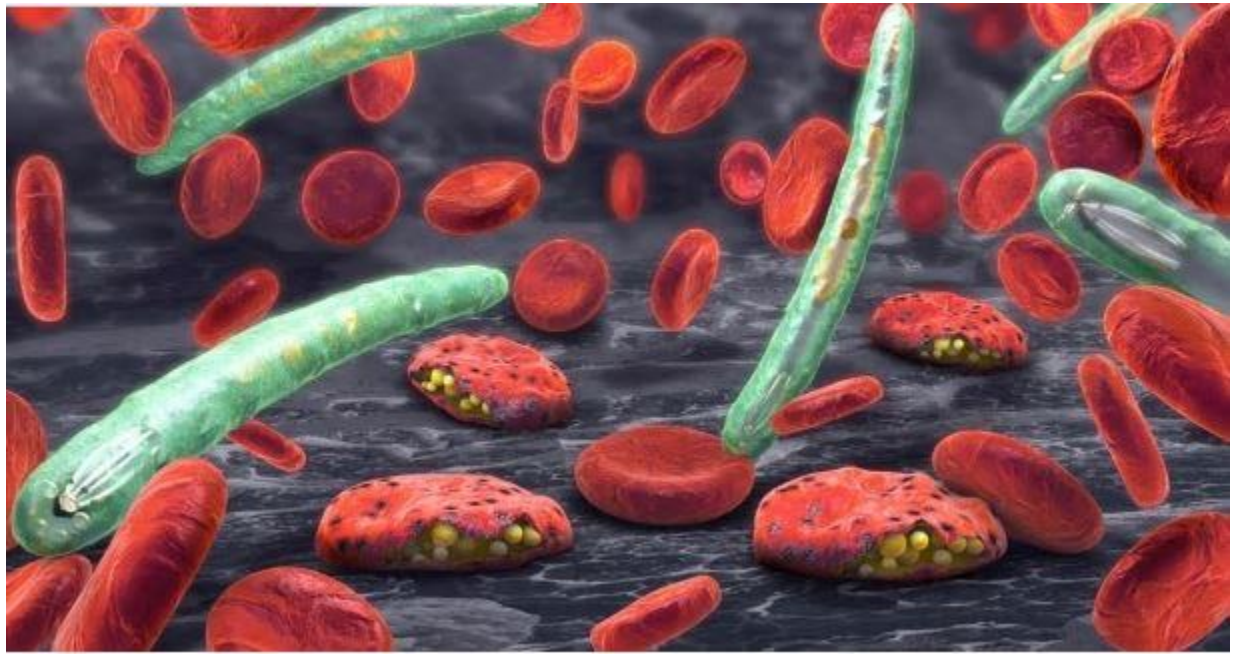
Romualdo Grande, MD

Ospedale Luigi Sacco Polo Universitario
ASST FBF Sacco Milano

CLIMVIB
LABORATORY OF CLINICAL MICROBIOLOGY, VIROLOGY AND BIOEMERGENCIES

Il problema diagnostico delle emoparassitosi

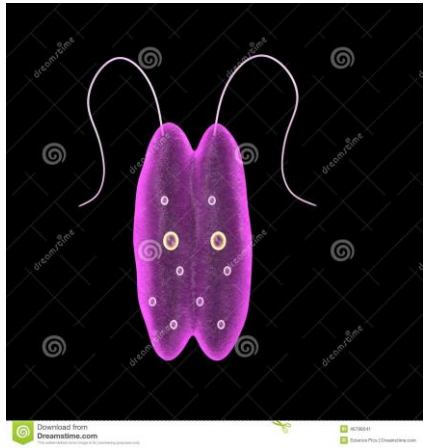
Plasmodium

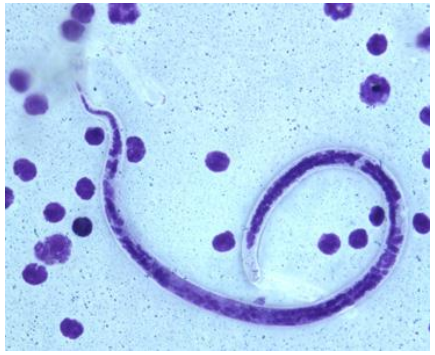


Babesia

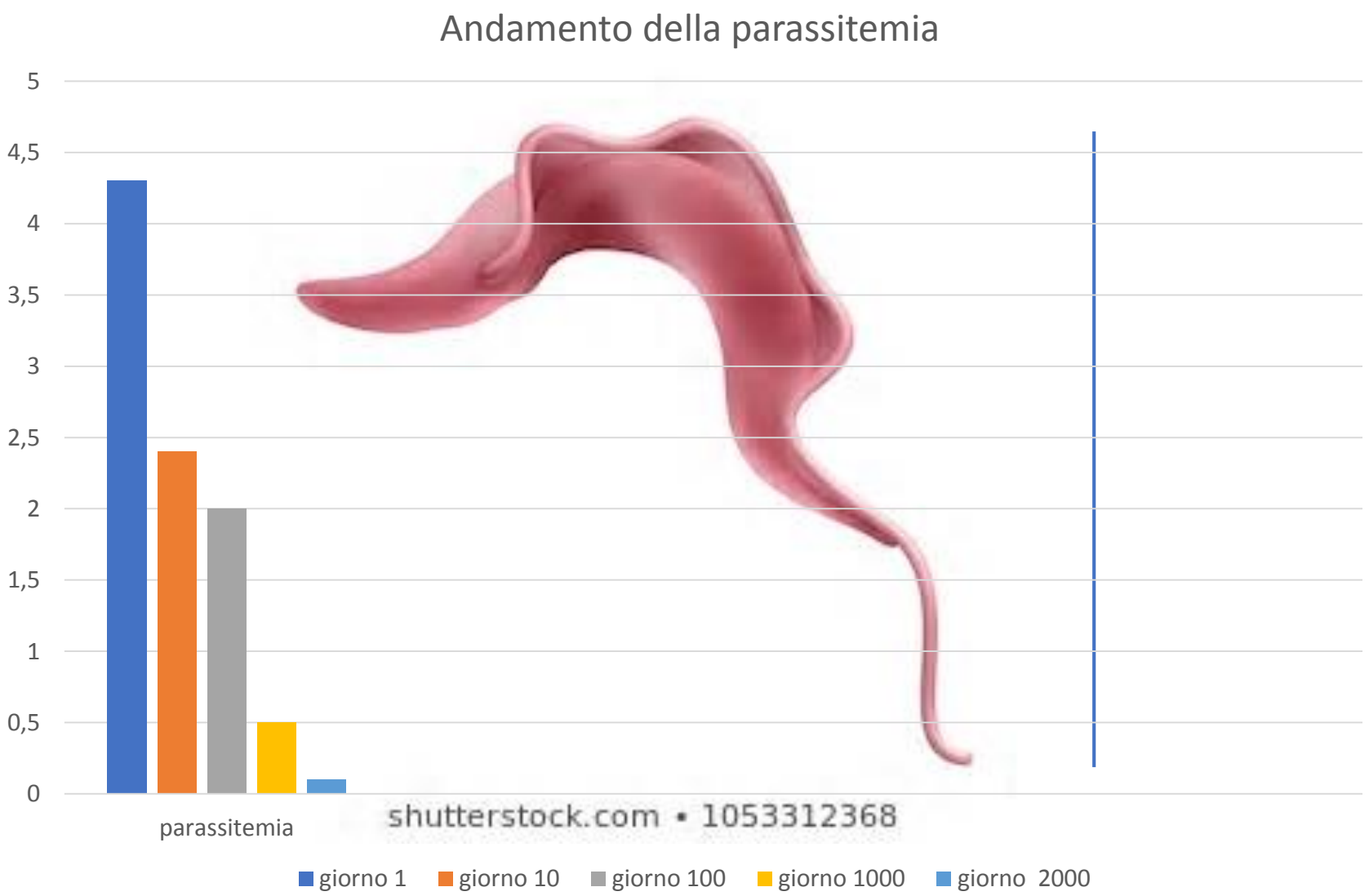
Il problema diagnostico delle emoparassitosi

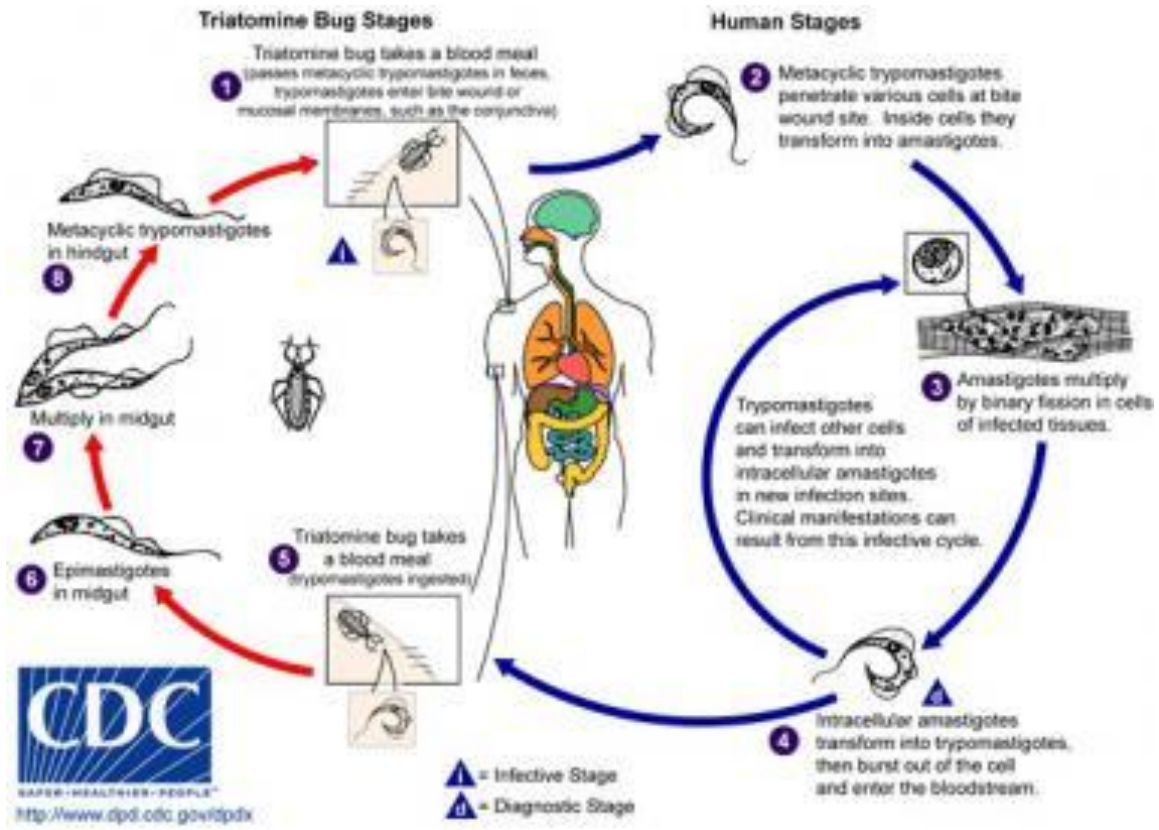
Quelli del reticolo endoteliale



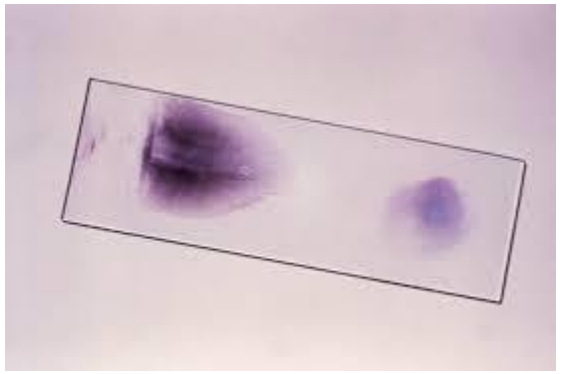


Trypanosoma cruzi




Triatomine Bug Stages
Human Stages
1 Triatomine bug takes a blood meal
(passes metacyclic trypomastigotes in feces, trypomastigotes enter bite wound or mucosal membranes, such as the conjunctiva)
2 Metacyclic trypomastigotes penetrate various cells at bite wound site. Inside cells they transform into amastigotes.
3 Amastigotes multiply by binary fission in cells of infected tissues.
Trypomastigotes can infect other cells and transform into intracellular amastigotes in new infection sites. Clinical manifestations can result from this infective cycle.
4 Intracellular amastigotes transform into trypomastigotes, then burst out of the cell and enter the bloodstream.
5 Triatomine bug takes a blood meal
(trypomastigotes ingested)
6 Epimastigotes in midgut
7 Multiply in midgut
8 Metacyclic trypomastigotes in hindgut
= Infective Stage
= Diagnostic Stage
CDC
SAFER·HEALTHIER·PEOPLE
http://www.dpd.cdc.gov/dpdx

La diagnosi diretta è un problema



L'ATTIMO FUGGENTE!!

TABLE 3 Diagnostic methods to detect Chagas' infections

Method[a]	Infection stage		
	Acute	Indeterminate	Chronic
Direct			
Direct microscopy	+	−	−
Thick and thin blood films	+	−	−
PCR	+	+	+
Blood culture	+	−	−
Xenodiagnosis	+	+	+

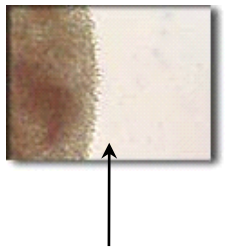
Lennet 10 ed ASM ed

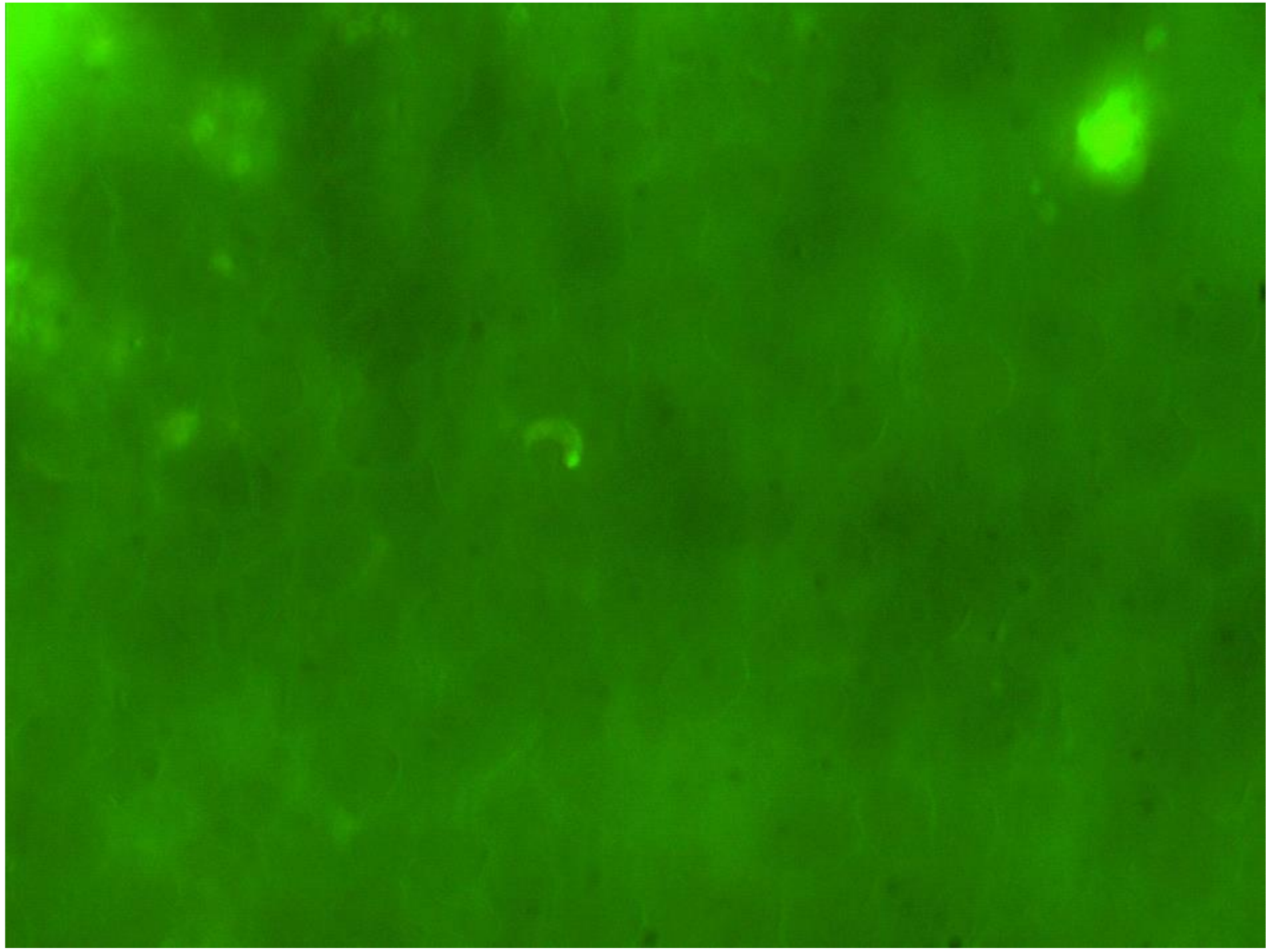
Ricerca Trypanosoma in provetta da microematocrito (MHT)

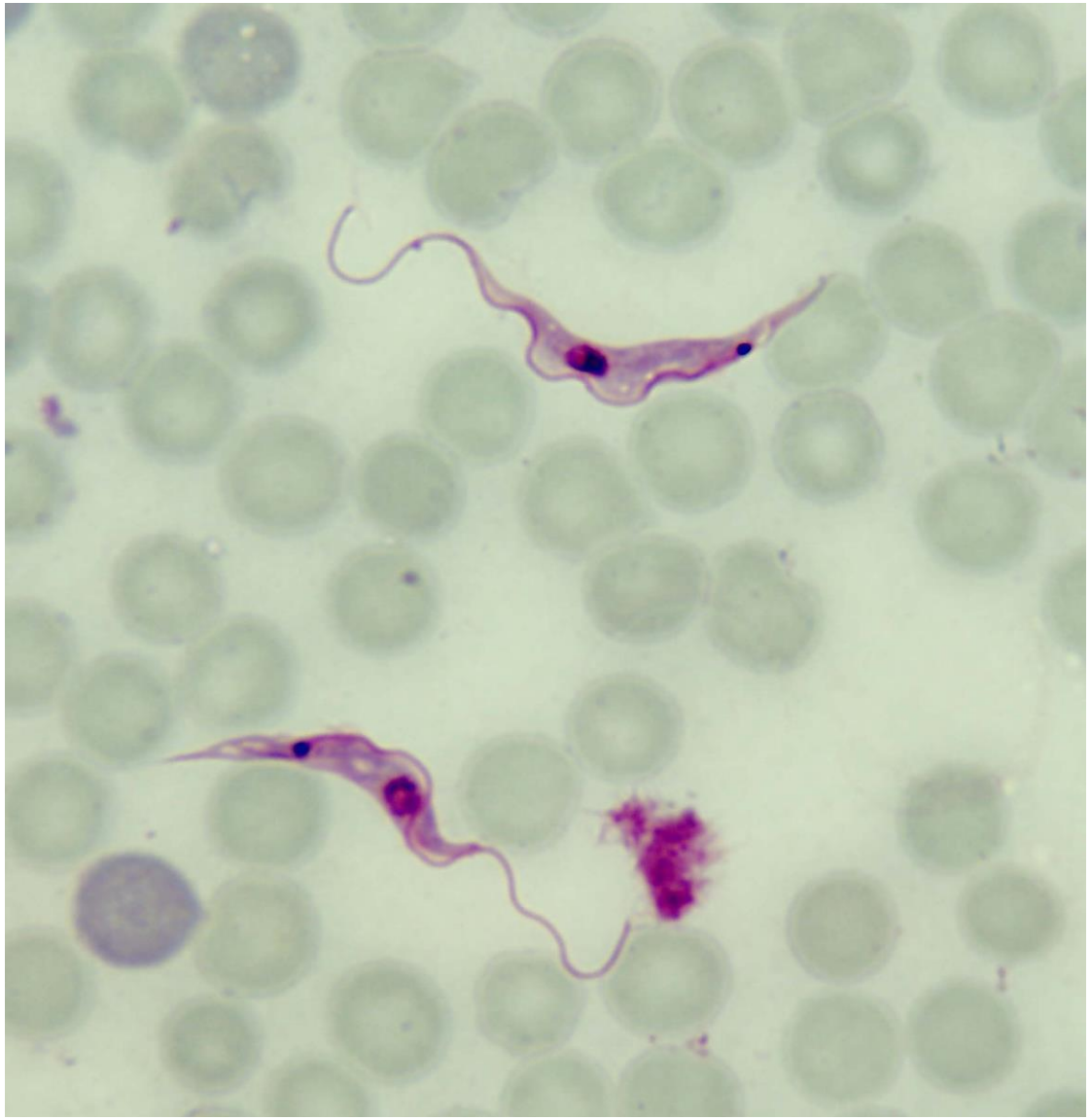
L'esame tramite capillare da ematocrito si basa sul principio della forza centrifuga la quale fa in modo che, un corpo che si ponga in movimento circolare è sottoposto ad una forza che lo allontana dal centro del circolo.

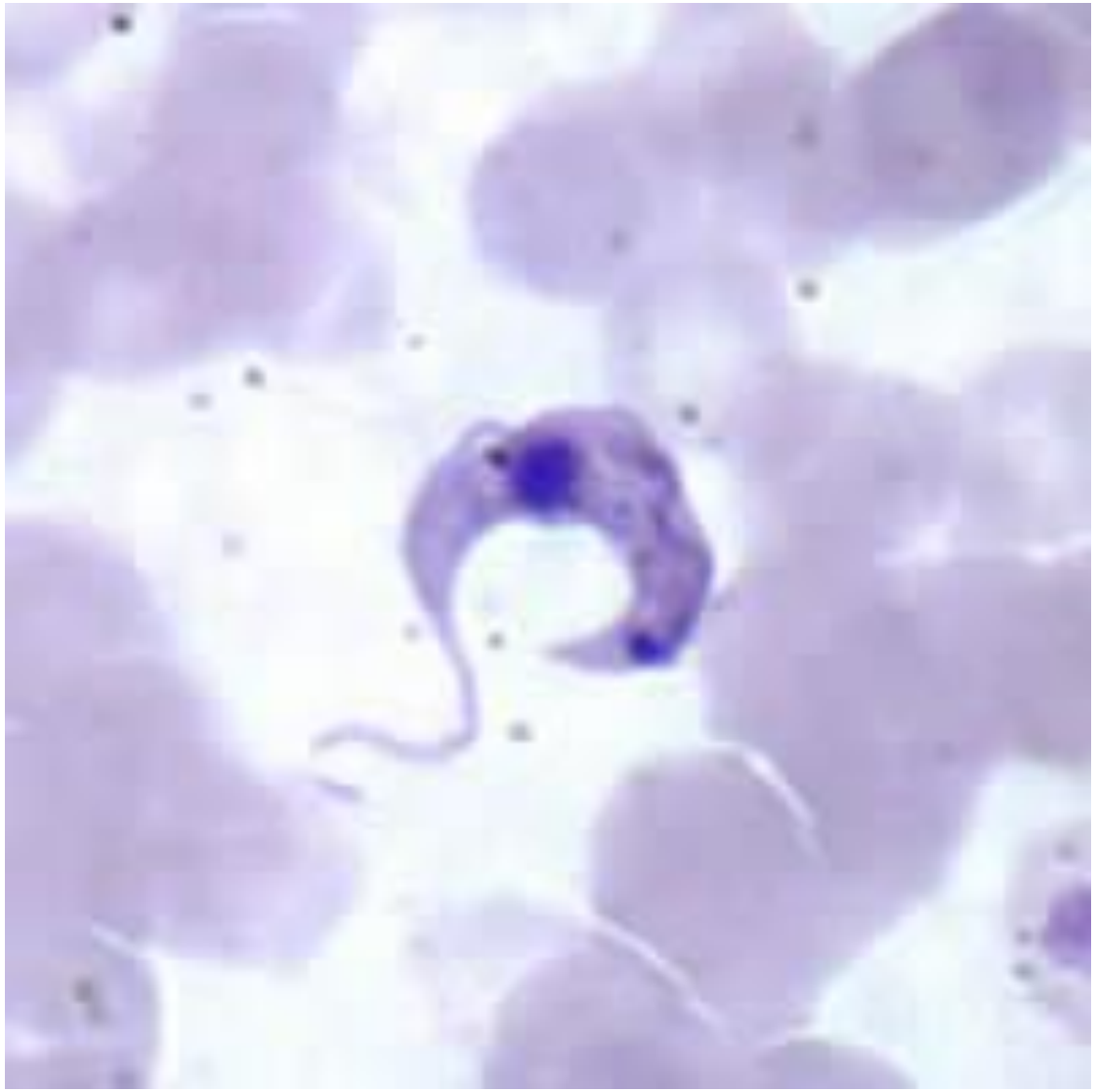
Le stratificazioni degli elementi dovute alla forza centrifuga sono in funzione della densità e del volume degli stessi e della viscosità del liquido di sospensione.

In un sangue centrifugato in capillare la concentrazione degli eventuali tripanosomi è tra il buffy–coat e il plasma.









Altri metodi di concentrazione ematica

- Concentrazione secondo Strout (su sangue coagulato)(1962)
- Concentrazione secondo Hoff (sangue EDTA)

Concentrazione secondo Strout
5 ml di sangue coagulato incubato a 37° C x 1 ora
Trasferire la parte non corpuscolata in provetta a fondo conico e centrifugare a 400g x 5 minuti
Osservare il sedimento a 40x

Strout RG A method to concentrate haemoflagellates
J. Parasitol.1962; 48:100

Concentrazione secondo Hoff
2,5 ml di sangue EDTA (agitare gentilmente)
Aggiungere in provetta conica assieme a 7 ml di soluzione lisante di cloruro di ammonio
Lasciare agire la soluzione lisante per 3 minuti
Centrifugare a 400x per 15 minuti
Osservare il sedimento a 40x

Metodi colturali

- Xenodiagnosi
- NNN
- Flacone di emocoltura addizionato con antibiotici
- (Cheesebrough M. District Lab Practise in Tropical Countries 2° ed Cambridge University Press)

Parametri ematologici più sensibili nella fase acuta

- VES ⬆
- Linfocitosi (emoscopia molto simile ad una mononucleosi in fase acuta)

Biologia Molecolare

- Utile in fase acuta
- Monitoraggio dell’evento accidentale
- Monitoraggio del ricevente di trapianto e organo solido
- Sorveglianza della riattivazione del paziente immunodefedato (immunodepressione indotta o secondaria)

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.

TABLE 3 Diagnostic methods to detect Chagas' infections

Method[a]	Infection stage		
	Acute	Indeterminate	Chronic
Direct			
Direct microscopy	+	−	−
Thick and thin blood films	+	−	−
PCR	+	+	+
Blood culture	+		−
Xenodiagnosis	+	+	+


depositphotos

Diagnosi sierologica

IHA; IFAT; ELISA basati su ag crudi e ricombinanti, WB, test immunocromatografici)

Le linee guida più importanti chiedono l’esecuzione di due test

Lineages T.cruzi: nuovo consenso 2009

Mem Inst Oswaldo Cruz, Rio de Janeiro, Vol. *104*(7): 1051-1054, November 2009 **1051**

A new consensus for *Trypanosoma cruzi* intraspecific nomenclature: second revision meeting recommends TcI to TcVI

B Zingales[1/+], SG Andrade[2], MRS Briones[3], DA Campbell[4], E Chiari[5], O Fernandes[6], F Guhl[7], E Lages-Silva[8], AM Macedo[9], CR Machado[9], MA Miles[10], AJ Romanha[11], NR Sturm[4], M Tibayrenc[12], AG Schijman[13]

I cloni vanno classificati in **TC I → TC VI**

(su base delle **Discrete Typing Units** → a set of stocks that are genetically more similar to each other than to any other and are identifiable by common genetic, molecular, or immunological markers

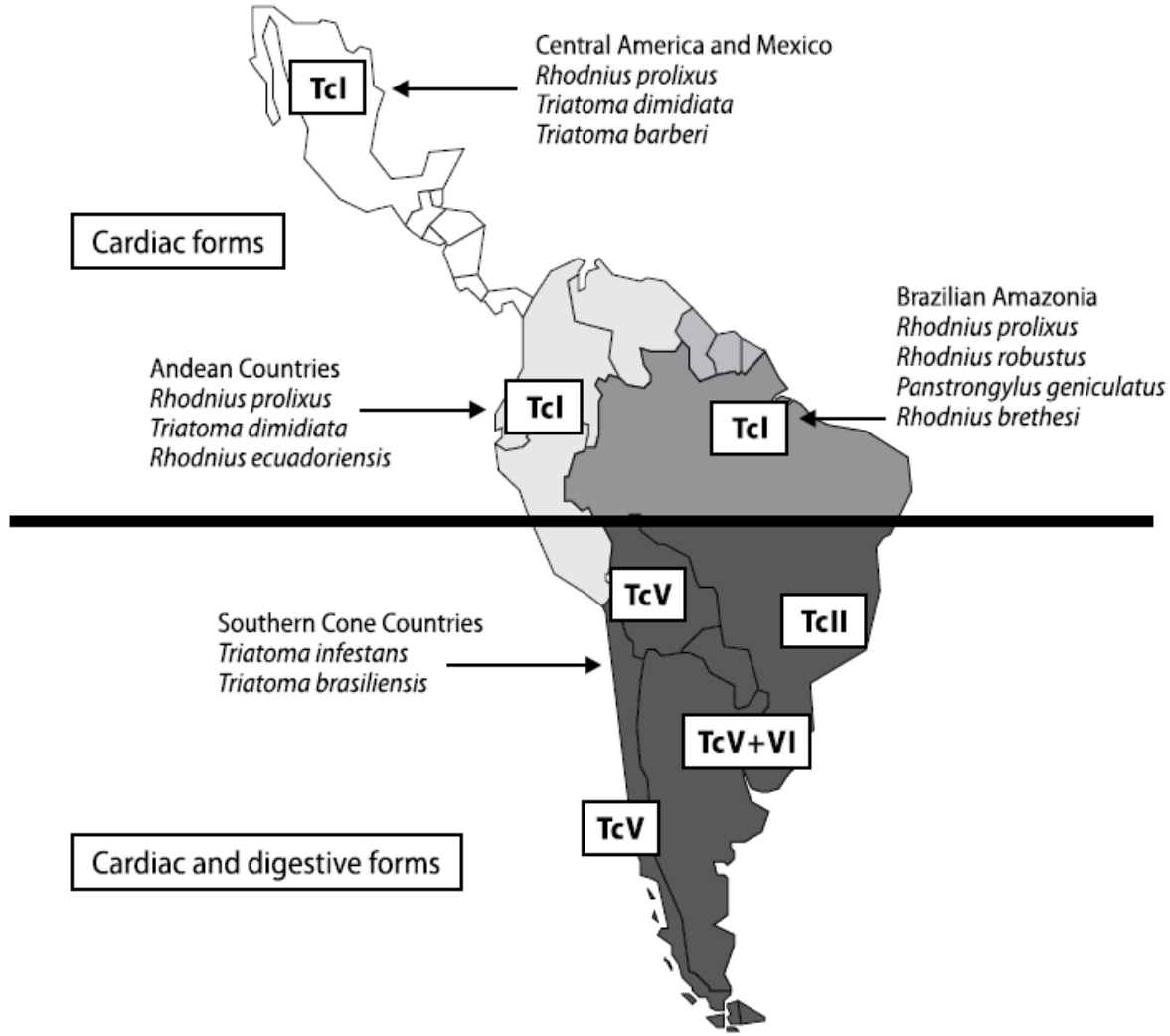
2009 nomenclature for *Trypanosoma cruzi* divisions

DTU designation	Abbreviation	Equivalence to former *T. cruzi* grouping schemes
T. cruzi I	TcI	*T. cruzi* I[a, b] and DTU I[c]
T. cruzi II	TcII	*T. cruzi* II[a] and DTU IIb[c]
T. cruzi III	TcIII	Z3/Z1 ASAT[d], Z3-A[e], DTU IIc[c] and *T. cruzi* III[f]
T. cruzi IV	TcIV	Z3[d], Z3-B[e] and DTU IIa[c]
T. cruzi V	TcV	Bolivian Z2[d], rDNA 1/2[g], clonet 39[h] and DTU IId[c]
T. cruzi VI	TcVI	Paraguayan Z2[i], Zymodeme B[j] and DTU IIe[c]

a: Anonymous 1999; *b*: Falla et al. 2009; *c*: Brisse et al. 2000; *d*: Miles et al. 1981; DTU: discrete typing units; *e*: Mendonça et al. 2002; *f*:Freitas et al. 2006; *g*: Souto et al. 1996; *h*: Tibayrenc and Ayala 1991; *i*: Chapman et al. 1984 ; *j*: Carneiro et al. 1990.

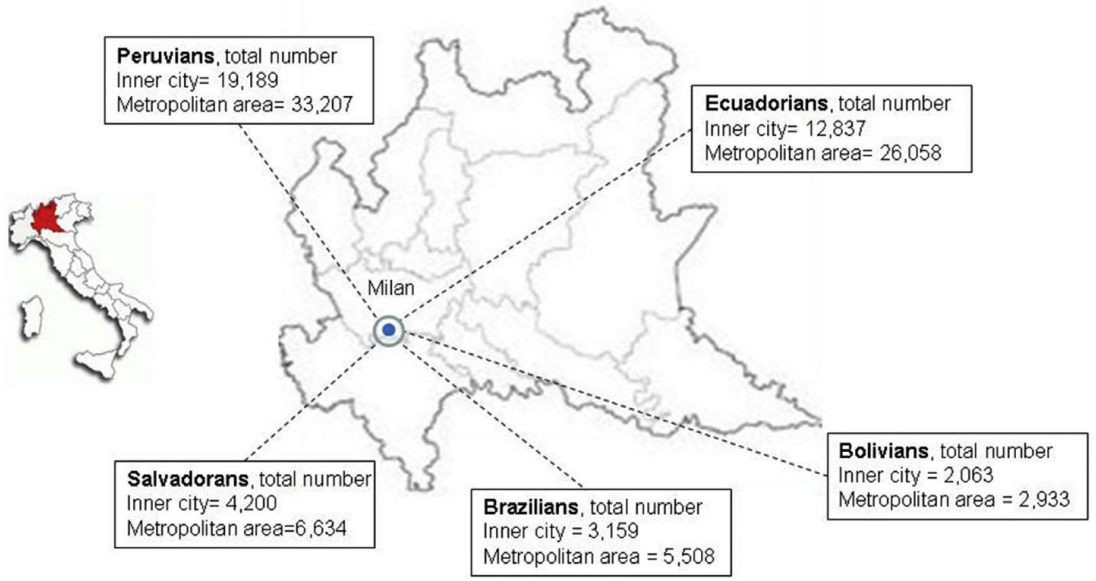
Figure 2

Geographical distribution of *T. cruzi* discrete typing units in human patients



Geographical distribution of *T. cruzi* discrete typing units (DTUs) in human patients. At present, *T. cruzi* is partitioned into six discrete typing units (DTUs), *T. cruzi* I-VI (TcI–TcVI) (ref 56). Data were compiled from a review article (ref 57). Triatomine vector species of major epidemiological importance in Chagas disease are indicated on the map (ref 133). Andean countries: Colombia, Ecuador, Peru, and Venezuela; Southern Cone Countries: Argentina, Bolivia, Brazil, Chile, Paraguay and Uruguay. The line separates the prevalence of Chagas disease clinical manifestations (see ref 57). DTUs, discrete typing units (I to IV); TcI –TcVI, *T. cruzi* type I-IV.

Il problema dei discordanti



Original article

Chagas disease knocks on our door: a cross-sectional study among Latin American immigrants in Milan, Italy

S. Antinori [1, 2, *], L. Galimberti [2], R. Grande [3], R. Bianco [4], L. Oreni [2], L. Traversi [2], D. Ricaboni [2], G. Bestetti [2], A. Lai [1], D. Mileto [3], M.R. Gismondo [3], M. Petullà [4], S. Garelli [6], G. De Maio [6], C. Cogliati [5], D. Torzillo [5], A.M. Villa [7], A.M. Egidi [6], E.C. Repetto [6], A.L. Ridolfo [2], M. Corbellino [2], M. Galli [1, 2]

Clin Microbiol Infect 2018;24:1340.e1-1340.e6

Table 1
Performance of four serological tests for the diagnosis of *T.cruzi* infection[a]

Measure	ARCHITECT Chagas[b] ($n = 501$)		BioELISA Chagas III[c] ($n = 501$)		Chagas Liaison XL[d] ($n = 263$)		Chagas Rapid ImmunoSpark[e] ($n = 95$)	
	Number/total	95% CI	Number/total	95% CI	Number/total	95% CI	Number/total	95% CI
Sensitivity (%)	100 (49/49)	91.01–100	95.92 (47/49)	85.34–99.56	96.77 (30/31)	82.15–100	89.29 (25/28)	71.79–96.96
Specificity (%)	99.78 (451/452)	98.60–100	99.78 (451/452)	98.60–100	99.14 (230/232)	96.66–99.95	92.54 (62/67)	83.21–97.08
Validity index (%)	99.80 (500/501)	99.41–100	99.40 (498/501)	98.73–100	98.86 (260/263)	97.58–100	91.58 (87/95)	85.99–97.16
PPV (%)	98.00 (49/50)	94.12–100	97.92 (47/48)	93.88–100	93.75 (30/32)	85.36–100	83.33 (25/30)	70.00–96.67
NPV (%)	100 (451/451)	100–100	99.56 (451/453)	98.95–100	99.57 (230/231)	98.72–100	95.38 (62/65)	90.28–100
LR+	452.00	63.81–3201.83	433.55	61.15–3073.76	112.26	28.20–446.85	11.96	5.10–28.07
LR-	0.00	—	0.04	0.01-0.16	0.03	0.00-0.22	0.12	0.04-0.34
Youden's index	1.00	1.00–1.00	0.96	0.86–1.00	0.96	0.83-1.00	0.82	0.65–0.92

CI, confidence interval; LR+, positive likelihood ratio; LR-, negative likelihood ratio; NPV, negative predictive value; PPV, positive predictive value.

[a] In case of discordant results serology was repeated using a new sample after 2–4 months.

[b] The chemiluminescent reaction is measured in relative light units (RLUs). The results are expressed as sample RLUs/cutoff value (S/CO) with ratio < 0.8 considered negative, inconclusive from ≥0.8 to < 1 and ≥ 1 positive.

[c] For the ELISA test results were expressed as the index between the absorbance of the test serum and the cutoff value. Tests were considered negative if the index was <0.9, equivocal if ≥ 0.9 and < 1, and positive if ≥1.

[d] The chemiluminescent reaction is measured in relative light units (RLUs). The results are expressed as sample RLUs/cutoff value (S/CO) with ratio < 0.9 considered negative, inconclusive from ≥0.9 < 1 and ≥1 positive.

[e] 5 μL of serum were added in the sample port following the transfer of two full drops of conjugate into the reagent port. The results were read within 5 min after the sample application. The test was considered positive if a pink/purple line was seen in the test and control areas. The test was considered negative if a pink/purple line was seen only in the control area. The test was invalid (and was repeated) if the control line failed to appear.

Discordanti: 4

International Conference on

MIGRATION HEALTH

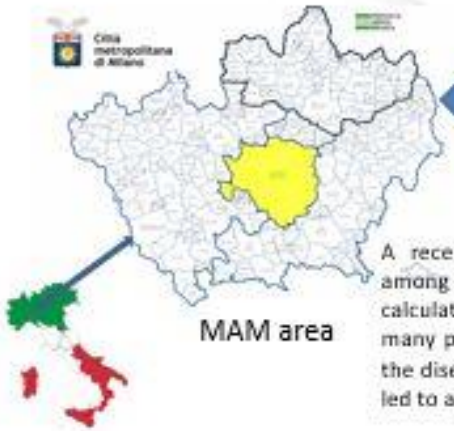
International Society of Travel Medicine
Established 1991

Rome, Italy
1 - 3 October 2018

Prevalence of Positive Serology for *T. cruzi* in a sample population of El Salvador and Honduras Migrants Residents in Metropolitan Area of Milan (MAM)

Grande R[1], Pilisfax A[1], Morlando R[2], Gismondo M.R[1], Antinori S[3],Galimberti L[3], Fadelli S[4], Adamoli M[4], Oliveri E.A.[4] Villa A.M[4]
[1] Clinical Microbiology, Virology and Bioemergency Diagnosis Lab(CLIMVIB), L.Sacco Teaching Hospital, University of Milan, Italy
[2] Biology Department, Bicocca University, Milan, Italy
[3]Tropical and Infectious Diseases University Ward, L. Sacco Teaching Hospital, University of Milan, Italy
[4] Outpatient Clinic For Not Guaranteed People Opera S.Francesco Milan, Italy.
romualdo. grande@unimi.it

BACKGROUND



MIGRATION

The El Salvador community in MAM is the largest of Italy

A recent meta-analysis of studies of Chagas Disease(CD) among Latin Americans living in the European Union (EU) has calculated a pooled prevalence of 4.2%. Like Spain, Italy hosts many people who have migrated to the EU but, despite this, the disease is largely unknown to Italian physicians, which has led to a call to "break this epidemiological silence"

AIM

To improve data collected in previous surveys about the seroprevalence for *T. cruzi* antibody in Central American migrants living in MAM, especially El Salvador and Honduras community.

328 soggetti COINVOLTI

7 discordanti
5 considerati negativi (III test negativo)
2 sono ancora sotto valutazione

TUTTI HANNO VALORI BORDERLINE

Il problema dei discordanti

- Test di conferma o terzo test → quale?
- WB? LD Bio
- - TESA (immuno blot con ag escretorio-secetorio)? Biomerieux Brasil, non CE
- - RIPA (radioimmunoprecipitation assay)? CDC Atlanta

Adv Parasitol. 2017 ; 97: 1–45. doi:10.1016/bs.apar.2016.10.001.

Chagas disease diagnostic applications: present knowledge and future steps

Virginia Balouz, **Fernán Agüero**, and **Carlos A. Buscaglia**[#]

Instituto de Investigaciones Biotecnológicas - Instituto Tecnológico de Chascomús (IIB-INTECH), Universidad Nacional de San Martín (UNSAM) - Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), San Martín, B 1650 HMP, Buenos Aires, Argentina

			Diagnostic Performance[1]							
	Technique/Tool		Chronic cases	Acute cases	Congenital cases	Drug efficacy	Disease prognosis	Parasite typing	PoC setting[2]	Remarks
Parasitological Methods	Directbloodexaminationtests						N.A.	N.A.		Microscopy-based methods, gold standard for diagnosis of acute and congenital cases
	Hemoculture					N.A.	N.A.	N.A.		Improved sensitivity due to parasite amplification, time-consuming, expensive
	Xenodiagnosis				N.A.	N.A.	N.A.	N.A.		Improved sensitivity due to parasite amplification, time-consuming, expensive, not well tolerated
Serological Methods	Whole Parasite-based	IHA[3]					N.A.	N.A.		Moderate specificity, trained personnel required
		IFA[3]					N.A.	N.A.		Moderate specificity, trained personnel and infrastructure required
		ELISA[3]					N.A.	N.A.		Method of choice for diagnosis of most clinical situations, cost-effective, easy to perform
		CoML	N.A.	N.A.	N.A.		N.A.	N.A.		Measures antibodies that are cleared in parasitological cured hosts early after drug treatment
	Parasite fraction	TESA[4]					N.A.	N.A.		Secreted/Excreted Antigens from trypomastigotes
		F2/3		?			?	N.A.		Highly *O*- glycosylated trypomastigote mucins, expensive, low purification yields
	Recombinant Antigens	Parental Repertoire[3,5]					N.A.	N.A.		High specificity but impaired sensitivity as compared to whole parasite-based methods.
		SAPA					?	?		Repetitive, surface antigen associated to enzymatically active *trans*-sialidases
		Ag13/TcD				?	?	?		Repetitive, surface antigen associated to enzymatically inactive *trans*-sialidases
		F29/FCaBP/Tc-24/Tc-28/1F8		?	?		?	?		Flagellar Calcium Binding Protein
		Ag1/JL7/FRA/H49						N.A.		Repetitive, internal antigen
		JL5		?	?	?		N.A.		Ribosomal antigen that elicits 'auto-antibodies' able to cross-recognize endogenous receptors
		TSSA		?						Mucin-like, surface antigen showing polymorphisms among strains
Molecular Methods	PCR, qPCR						N.A.			High specificity, trained personnel required
	Genotypification Markers[6]		N.A.	N.A.	N.A.	N.A.	N.A.			High specificity, trained personnel required, manual technique, expensive
	Biochemical Markers[7]		N.A.	?	?			?		Low specificity and sensitivity, in most cases only 'indicative/predictive' of cardiomyopathy

[1] Performance is arbitrarily indicated as appropriate (green boxes), non-appropriate (vermillion boxes) or intermediate/needs to be improved (yellow boxes) according to available data. N.A. and question marks (?) stand for non-applicable or not enough experimental data available to assess the performance, respectively.

[2] Adaptability to be deployed in PoC settings, which depends on specific features of the tool/technique and which is arbitrarily indicated as above.

[3] Commercially available from different vendors.

[4] TESA assays include diverse techniques that measure anti-TESA antibodies in serum samples (i.e. TESA-ELISA, TESA-dot blot, TESA-blot) and techniques that capture TESA antigens directly in urine samples (i.e. Chunap assays)

[5] The term 'parental' repertoire refers to *T. cruzi* antigens identified in large-scale initiatives carried out in the 80's, and includes: Ag1/JL7/FRA/H49; Ag2/B13/TCR39/PEP-2; Ag10; Ag13/TcD; Ag19; Ag26; Ag30/CRA/JL8/TCR27; Ag36/JL9/MAP; Ag54; JL1; JL5; JL9; SAPA; B12; TcE; A13; F29/FCaBP/Tc-24/Tc-28/1F8; Tc-40; HSP70; HSP78 and FL-160, according to current nomenclature. These antigens were assayed for conventional diagnosis in different combinations using a variety of technological platforms (ELISA, LFIA, Western blot, dot blot), some of which are commercially available from different vendors. The individual performance of some of these antigens showing particular features is indicated below.

[6] Include RFLP, PCR-RFLP, RAPD, Southern blot and other DNA hybridization methods, karyotyping methods and sequence-based methods either using a single locus or MLST.

[7] Include TNF-α, ACE2, BNP, ANP, ET-1 and other biochemical markers indicated in text.

All of abbreviations are defined in the text. Briefly: PoC, Point-of-Care; HIA, Hemagglutination Inhibition Assay; IFA, Immunofluorescence Assay; ELISA, Enzyme Linked Immunosorbent Assay; F2/3: Purified highly *O*-glycosylated and antigenic trypomastigote mucins; CoML, Complement-Mediated Lysis; TESA, Trypomastigote Excreted-Secreted Antigens; LFIA, Lateral-Flow Immunochromatographic Assays; SAPA, Shed Acute-Phase Antigen; TSSA, Trypomastigote Small Surface Antigen; PCR, Polymerase Chain Reaction; PCR-RFLP, PCR-based Restriction Fragment Length Polymorphism assay; MLST, Multi-Locus Sequence Typing assay; RAPD, Random Amplification of Polymorphic DNA; TNF-α, Tumor Necrosis Factor α; ACE2, Angiotensin-Converting Enzyme 2; BNP, Brain Natriuretic Peptide; ANP, Atrial Natriuretic Peptide; ET-1, Endothelin-1.

Legenda della tabella

Conclusioni

- Necessità di un protocollo diagnostico multistep
- Necessità di adeguare il tariffario nazionale a indagini sierologiche multistep
- Necessità di expertise nella valutazione dei risultati

GRAZIE A

ANNA MARIA VILLA
SARA FADELLI
MARTINA ADAMOLI
ERICA OLIVIERI

POLIAMBULATORIO DEI NON GARANTITI DI VIA A. DA MESSINA

Sistema Socio Sanitario
Regione Lombardia
ASST Fatebenefratelli Sacco

SPINELLO ANTINORI
LAURA GALIMBERTI
MARIA RITA GISMONDO
DAVIDE MILETO
ALKIS PILIAFAS
ALBERTO RIZZO