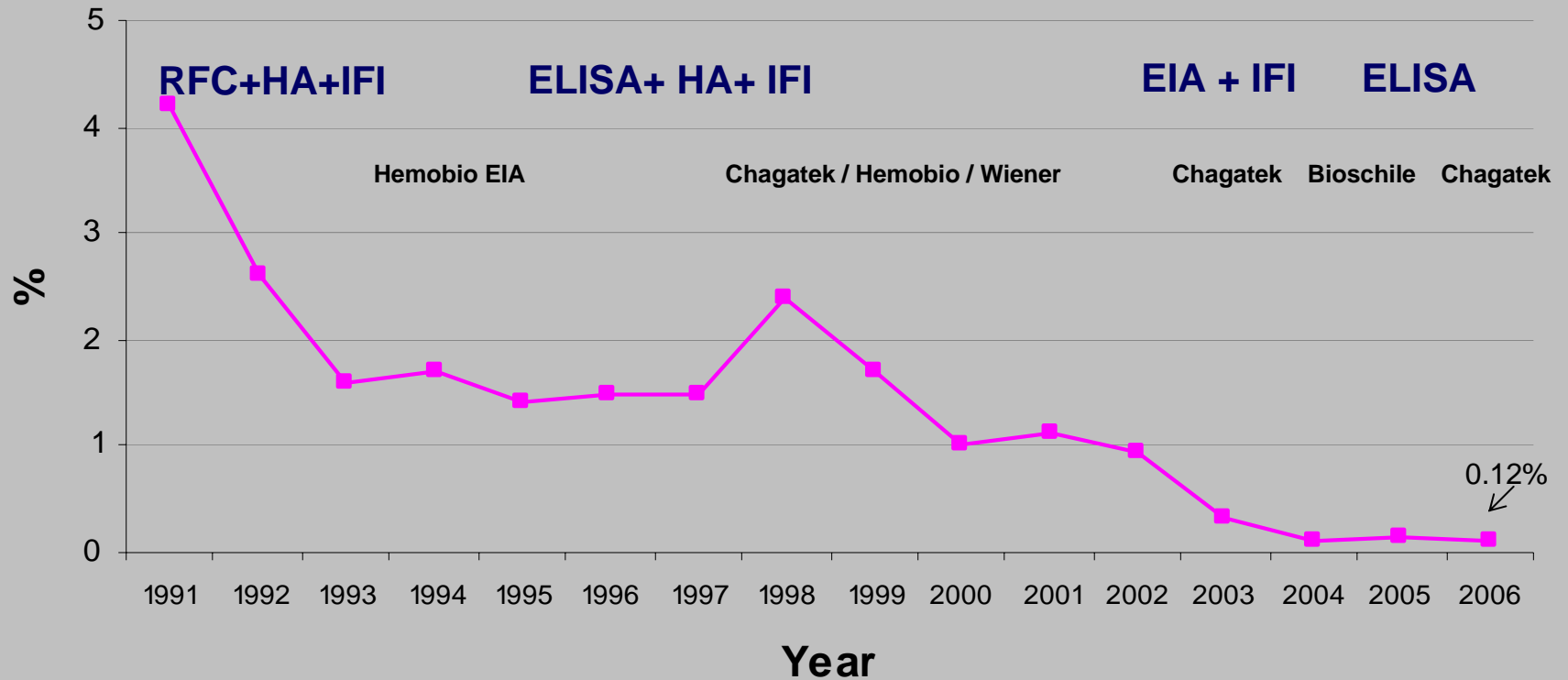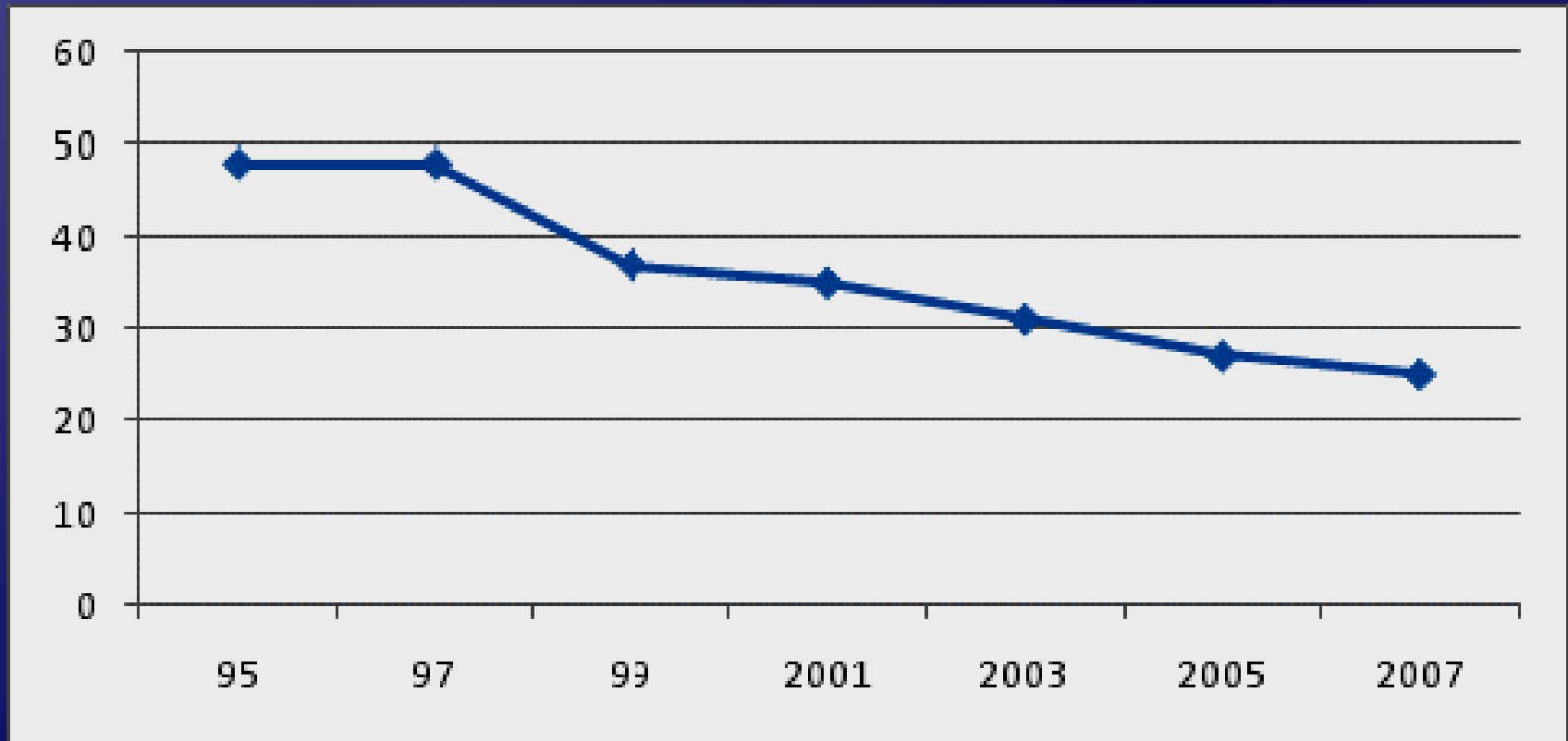# Chagas disease and blood screening

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Hemocentro de São Paulo Brasil

Trends in % of Chagas discarded units

Year Prevalence (Positive cases/10.000 donors) of Chagas disease among first-time blood donors

Sabino EC Transfusion. 2003;43(7):853-6

Difficulties with Chagas tests

- Initial tests developed for Chagas RFC, IHA and IFI were not easy to be standardized.
- Lack of a gold standard
- Parasitic tests not sensitive
- Cross reaction with other parasitic infections
- Serological response between individuals

  - Use of 2 tests for screening

Results of parallel testing

Test or combination of tests	Number	Percentage
IIF	2,809	40.5
IHA	902	13.0
ELISA	541	7.8
IIF+IHA	215	3.1
IIF+EIA	277	4.0
IHA+EIA	97	1.4
IHA+IIF+ELISA	2,095	30.2
Total	6,936	100

6,936 units (1.7%) among 411,617 units screened in 1993-94.

Test results on follow-up samples

Results at screening	Results at return				
	Reactive			Negative	total
	3 tests+	2 tests+	1 test+		
	n%	n%	n%	n%	n%
3 tests +	370 (95)	16 (4)	5(1)	0	391 (100)
2 tests+	21 (18)	39(33)	31(27)	26(22)	117(100)
1 test +	1 (0.1)	29 (4)	192(25)	537 (71)	795(100)
Total	392(31)	84(7)	228(18)	393(44)	1267(100)

What is the epidemiological evidence of Chagas infection among inconclusive samples?

Serological results at follow up	Yes to one of the 2 questions	Total
3 tests +	141(74%)	190
2 tests+	16(57%)	28
1 test+	30(29%)*	103
Control neg 1	6(13%)*	45
Control neg 2	18(15%)*	119

Does someone in your family have Chagas disease?

Have you ever lived in a house where the Chagas bug was present?

How could we evaluate a confirmatory test?

- What samples should be used?

- Only those reactive to all tests? Inconclusive samples?

Experience with InnoLia assay

- Samples collected from 1995 -1996

- Reactive at screening and returned for confirmation

Saez-Alquézar A, et al. J Clin Microbiol. 2000, 38(2):851-4

TABLE 1. INNO-LIA Chagas confirmatory assay results compared to CSS

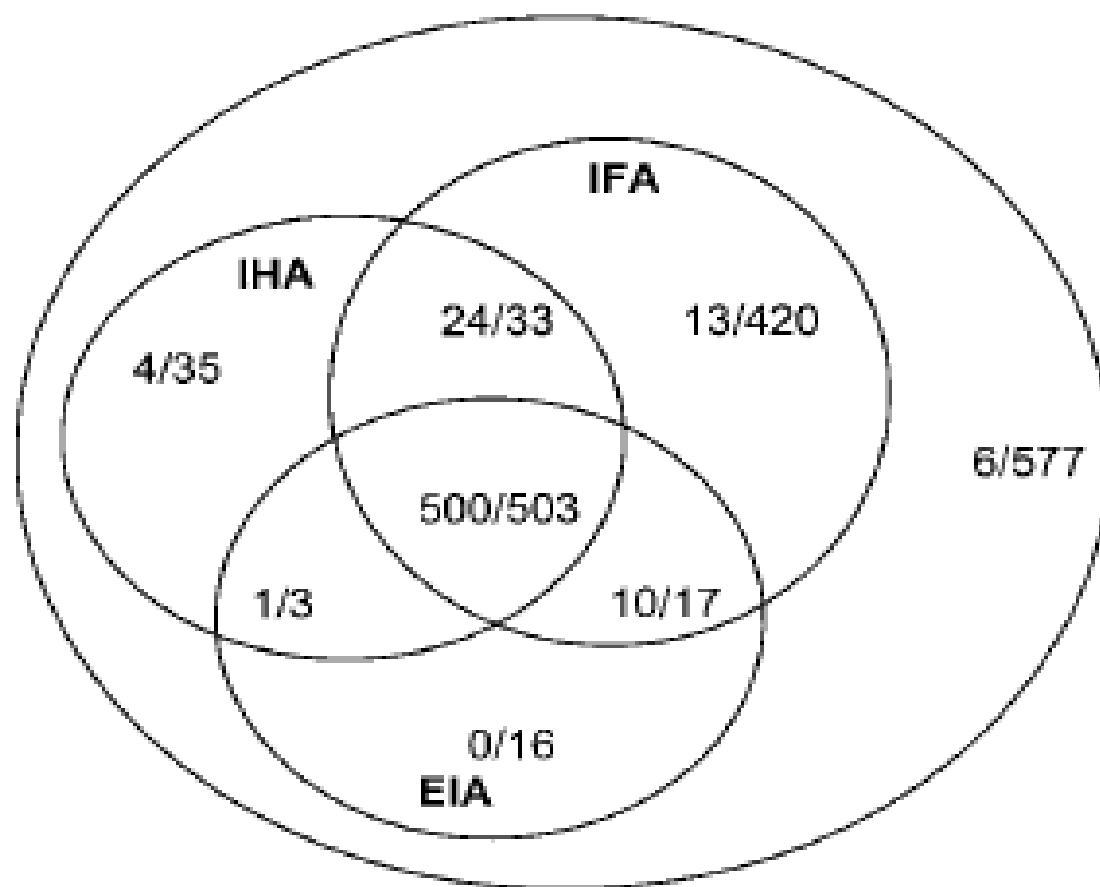| CSS	No. (%) of samples with the following result by INNO-LIA Chagas assay:			
	Negative	Indeterminate	Positive	Total
0	566 (98.1)	5 (0.9)	6 (1.0)	577
1	438 (93.0)	16 (3.4)	17 (3.6)	471
2	17 (32.1)	1 (1.9)	35 (66.0)	53
3	3 (0.6)	0 (0.0)	500 (99.4)	503
Total	1,024 (63.8)	22 (1.4)	558 (34.8)	1,604

FIG. 2. Comparison of screening and confirmation assay results. The outer circle includes 1,604 screened serum samples. Each inner circle represents one of the screening techniques (IFA, IHA, and EIA). Ratios indicate the number of INNO-LIA Chagas assay-positive samples/the number of samples reactive by the technique being considered.

WHO panel Methods Summary

- 437 samples from: Argentina, Bolivia, Brazil, Colombia, Ecuador, El Salvador, Honduras, Mexico, Nicaragua, Paraguay

- Different assays used at screening

- Number of screening kits: 18 (11EIA, 5IHA, 2PA)

- Supplemental test performed: IFI, IB, WB, RIPA

Otani et al Transfusion 2009

Institutions

- Argentina - Hospital de Pediatria Prof. Dr. Juan P. Garrahan
- Bolivia - Hospital Clinico "Viedma"
- Brazil - Fundação Pró - Sangue Hemocentro de SP
- Colombia - Instituto Nacional de Salud
- Ecuador - Programa de Sangre Cruz Roja Ecuatoriana
- El Salvador - Programa de Sangre Cruz Roja Salvadoreña
- Honduras - Programa de Sangre Cruz Roja Hondureña
- Mexico - Centro Nacional de Transfusión
- Nicaragua - Programa de Sangre Cruz Roja Nicaragüense
- Paraguay - Instituto de Investigación en Ciências de la Salud

Results according to number of reative tests
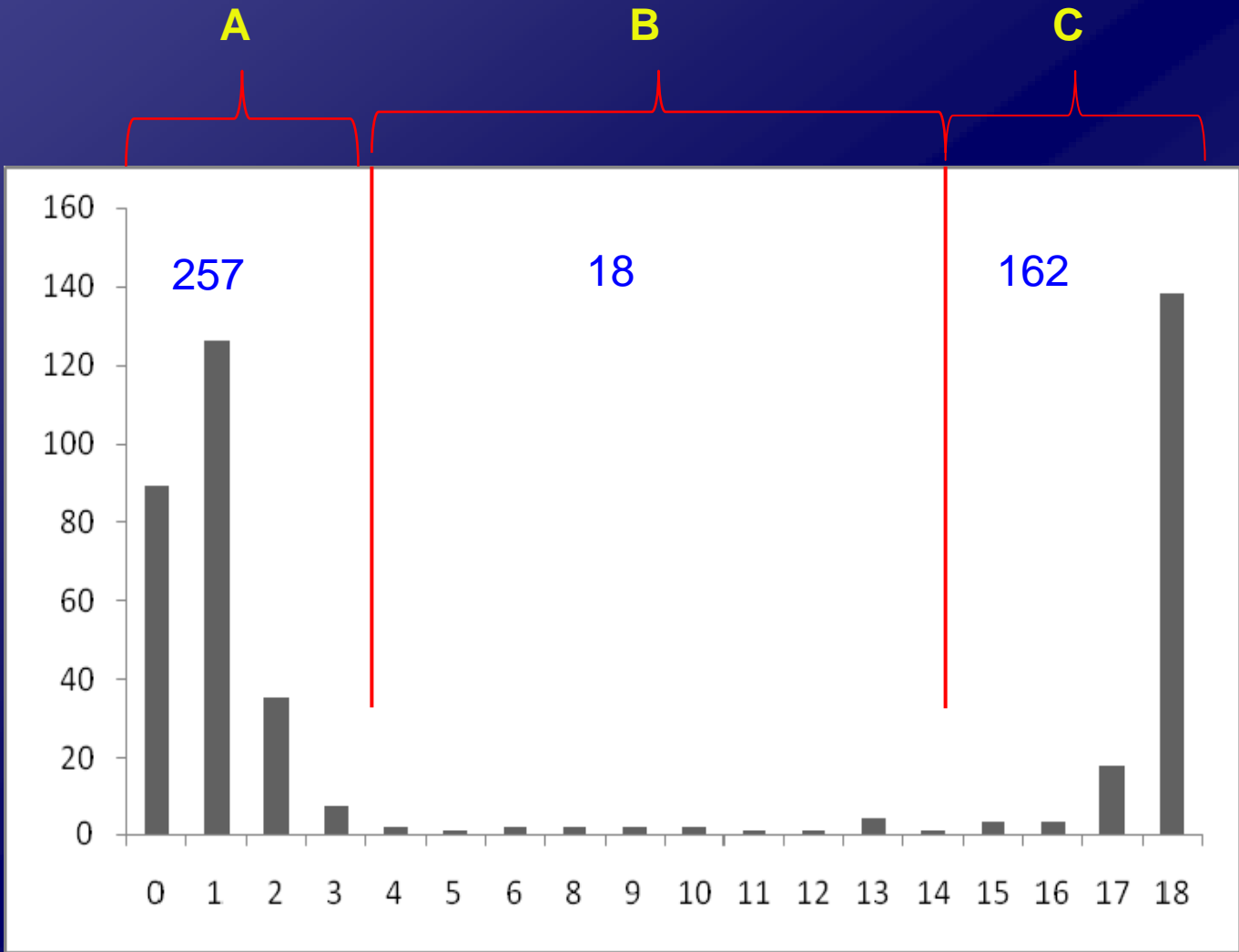
Algorithm

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┌─────────────────────────────────────┐
│ Imunofluorescencia (Biolab)         │
│ Western Blot (TESA)                 │
│ Imuno Blot (Innolia)                │
│ RIPA (Univ. IOWA)                   │
└─────────────────────────────────────┘
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+ + + + / + + + -	Ind	- - - - / - - - +
Positive	Ind	Negative
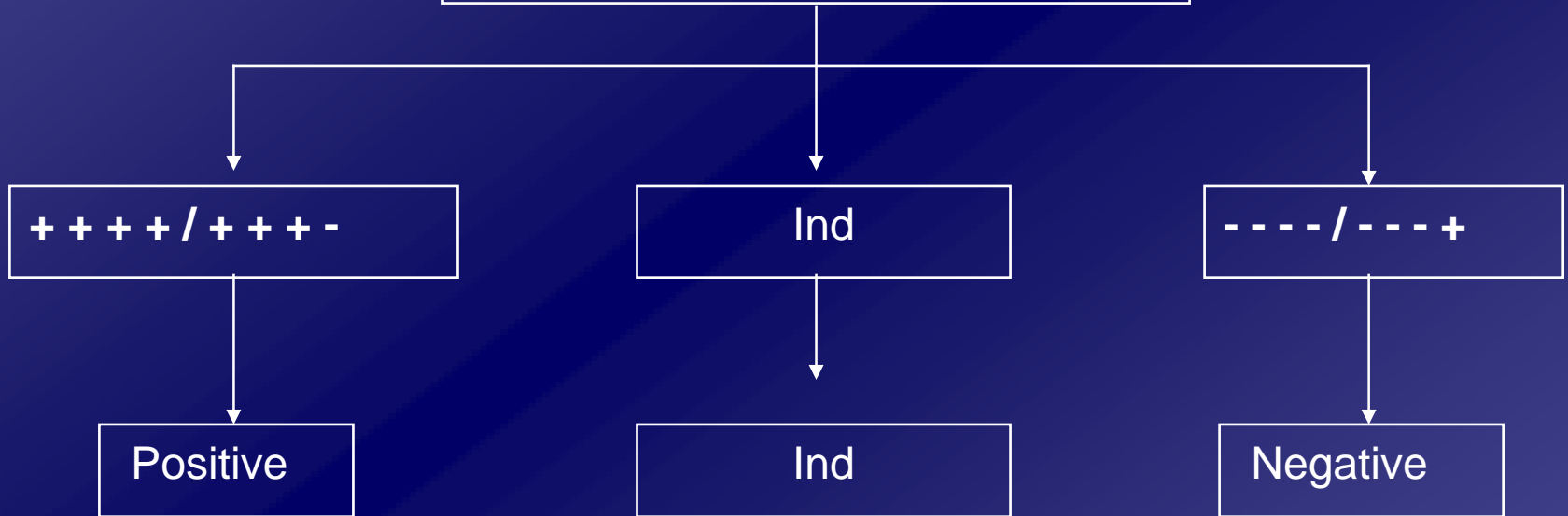
Final status of the samples

Group	IFI Pos	IFI Inc	IFI Neg	IB Pos	IB Inc	IB Neg	WB Pos	WB Neg	RIPA Pos	RIPA Neg	Final status Pos	Final status Inc	Final status Neg	Total
A	0	2	255	0	1	259	5	252	0	257	0	0	257	257
B	5	6	7	7	2	9	15	3	7	11	6	7	5	18
C	161	1	0	160	1	1	162	0	162	0	162	0	0	162

Inconclusive samples

Sample ID	Country	N of reactive screening test	IFI(titer)	IB	WB	RIPA	Final criteria
41	Bolívia	6	1/40	neg	pos	Neg	inc
33	Argentina	6	inc	neg	pos	Neg	inc
40	Bolívia	9	inc	neg	pos	Neg	inc
31	Argentina	10	neg	pos	pos	Neg	inc
27	Argentina	12	neg	pos	pos	Neg	inc
36	Paraguay	11	inc	inc	pos	Pos	inc
34	Argentina	13	inc	inc	pos	Neg	inc

EIA assays	Sensitivity (95% CI)	Specificity (95% CI)
HBK 401 HEMOBIO CHAGAS	100 (97.8 – 100)	99.62 (97.9 – 100)
CHAGAS – ELISA	97.62 (94.0 – 99.3)	97.71 (95.1 – 99.2)
CHAGATEK ELISA	99.40 (96.7 – 100)	99.24 (97.3 – 99.9)
Premier™ Chagas'IgG ELISA Test	94.04 (89.3 – 97.1)	100 (98.6 – 100)
TEST ELISA PARA CHAGAS	99.40 (91.2 – 98.1)	99.62 (97.9 – 100)
BIOELISACRUZI®	98.21 (94.9 – 99.6)	99.24 (97.3 – 99.9)
ABBOTT CHAGAS ANTICORPOS EIA	99.40 (96.2 – 100)	98.09 (95.6 –99.4)

| EIA assays	Sensitivity (95% CI)	Specificity (95% CI)
CHAGAS – TEST *IICS*, método ELISA	97,02 (93.2-99.0)	99,24 (97.3-99.9)
Chagatest Elisa	98.81 (95.8 – 99.9)	99.62 (97.9 – 100)
Bioelisa CHAGAS	100 (97.8 – 100)	99.24 (97.3 – 99.9)
HEMAGEN® ELISA	100 (97.8 – 100)	96.56 (93.6-98.4)

Hemaglutination assay	Sensitivity (95% CI)	Specificity (95% CI)
CHAGAS HAI IMUNOSERUM	97.62 (94.0 – 99.3)	78.62 (77.2 – 83.4)
CHAGAS – HAI	88.09 (82.2 – 92.6)	59.92 (53.7 – 65.9)
Imuno-HAI	100 (97.2 – 100)	95.80 (92.6 – 97.9)
HEMAGEN® HA	92.26 (87.1 – 95.8)	89.31 (84.9 – 92.8)
HEMACRUZI®	99.40 (96.7 – 100)	97.33 (94.6 – 98.9)
Aglutination assays		
SERODIA – Chagas	100 (97.2 – 100)	97.70 (95.1 – 99.2)
"ID-Chagas Antibody Test"	97.02 (93.2 – 99.0)	99.62 (97.9 – 100)
Rapid test		
Chagas Stat-pak	94.08 (89.1-97.3)	95.75 (92.1-98.0)

Argentinian Chaco region
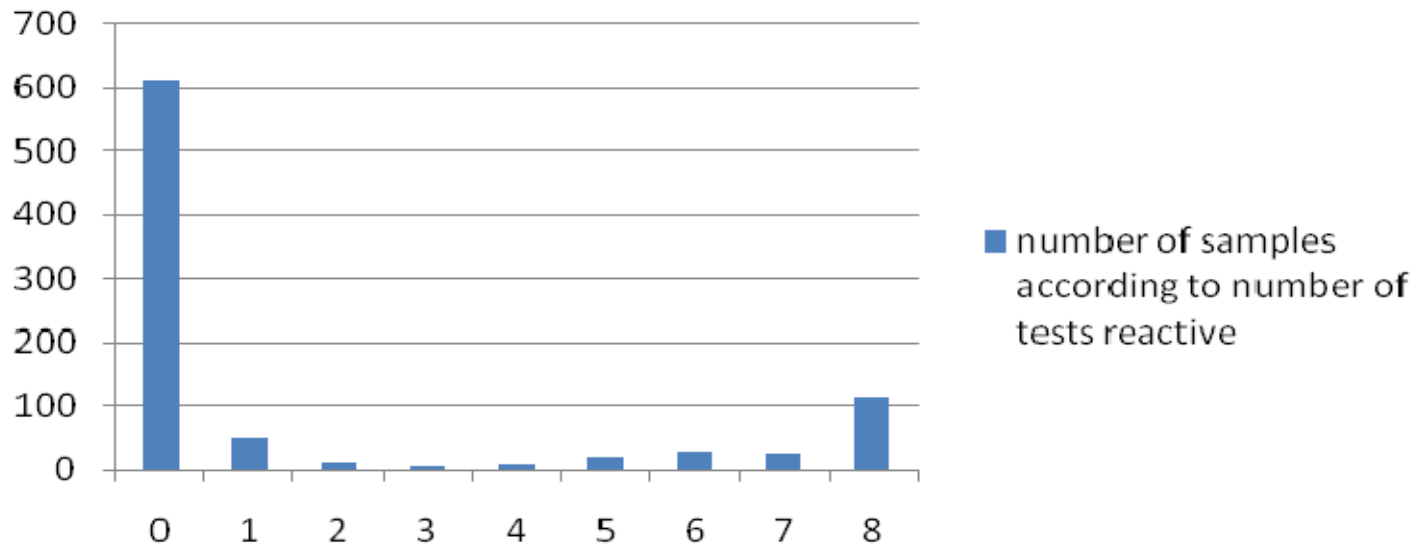
Percentage of reactivity of each test in the blood donor population
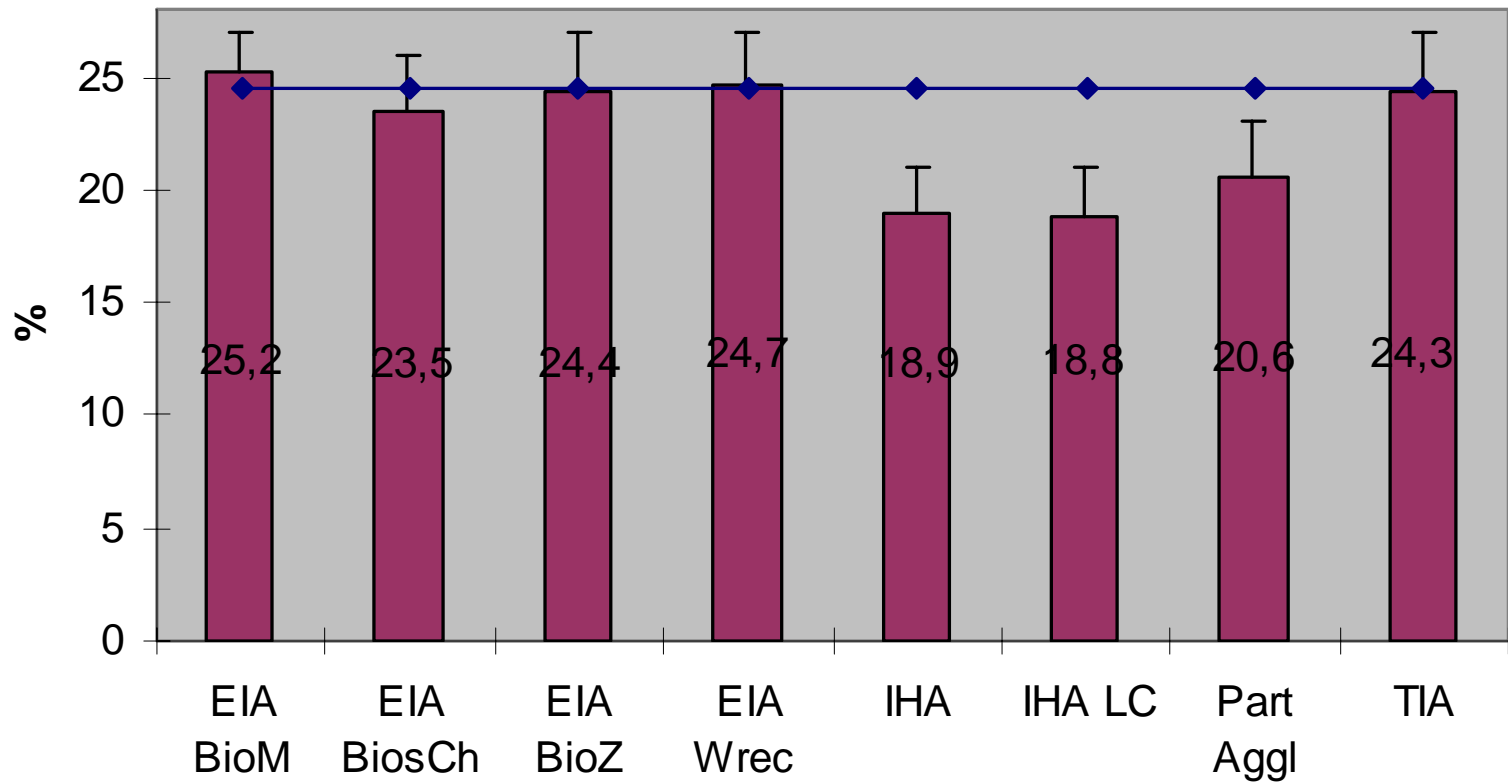
Sensitivity and Specificity by latent class analysis

	Sensitivity	Specificity
EIA BioM: Chagatek	99.7	99.0
EIA BiosCh: Chagas BiosChile	95.6	99.8
EIA BioZ: BioZima Chagas	97.5	99.3
EIA Wrec: Chagatest rec. 3.0	95.9	98.3
TIA: trans-Sialidase Inhibition Assay	90.5	97.1
Chagas Serodia	84.1	99.9
Chagas HAI	73.9	98.9
HAI LC	65.5	96.3

REDS-II International /NHLBI

Blood Systems Research Institute

Fund-Pro-Sangue – HEMOMINAS - HEMOPE

Fonte: IBGE, CENSO 2000

REDS Chagas Procedures

- All donations are screened with one EIA assay

- Repeat reactive samples are sent to São Paulo lab that performs a different EIA assay and IFA

Serological Patterns of the 877 discarded units (615,433)from 2007-8

Pattern	EIA Screening	EIA Confirmatory	IFA	Total	Final Status
1	P	P	P	222	P
2	P	P	N	30	I
3	I	P	N	28	I
4	I	I	N	8	I
5	P	P	I	3	I
6	I	P	P or I	5	I
7	P	N	P or I	3	I
8	I	N	P or I	6	I
9	P or I	N	N	506	N
10	P or I	S	S	66	S
TOTAL				877	

Serological Patterns and distribution

Pattern	EIA Screening	EIA Confirmatory	IFA	Total	Final Status
1	P	P	P	222	P
2	P	P	N	30	I
3	I	P	N	28	I
4	I	I	N	8	I
5	P	P	I	3	I
6	I	P	P or I	5	I
7	P	N	P or I	3	I
8	I	N	P or I	6	I
9	P or I	N	N	506(60%)	N
10	P or I	S	S	66	S
TOTAL				877	

Serological Patterns and distribution

Pattern	EIA Screening	EIA Confirmatory	IFA	Total	Final Status
1	P	P	P	222(27%)	P
2	P	P	N	30	I
3	I	P	N	28	I
4	I	I	N	8	I
5	P	P	I	3	I
6	I	P	P or I	5	I
7	P	N	P or I	3	I
8	I	N	P or I	6	I
9	P or I	N	N	506	N
10	P or I	S	S	66	S
TOTAL				877	

Serological Patterns and distribution

Pattern	EIA Screening	EIA Confirmatory	IFA	Total	Final Status
1	P	P	P	222	P
2	P	P	N	30	I
3	I	P	N	28	I
4	I	I	N	8	I
5	P	P	I	3	I
6	I	P	P or I	5	I
7	P	N	P or I	3	I
8	I	N	P or I	6	I
9	P or I	N	N	506	N
10	P or I	S	S	66	S
TOTAL				877	

9.1%

Serological Patterns and distribution

Pattern	EIA Screening	EIA Confirmatory	IFA	Total	Final Status
1	P	P	P	222	P
2	P	P	N	30	I
3	I	P	N	28	I
4	I	I	N	8	I
5	P	P	I	3	I
6	I	P	P or I	5	I
7	P	N	P or I	3	I
8	I	N	P or I	6	I
9	P or I	N	N	506	N
10	P or I	S	S	66	S
TOTAL				877	

1.1%

Trying to understand inconclusive results for Chagas

Screening assay	2nd EIA	IFA	Recombinant EIA	Conclusion
+	+	+	+	Chagas (CP)
+/I	+/I/neg	+/I/neg	+	Chagas low(PP)
+/I	+/I/neg	+/I/neg	-	Other infection?(OPI)
+/I	-	-	-	FP at screening

Geographical distribution of Chagas serology pattern

Blood Center Region: # donations tested: Classification	RE 204,124 #(n/100,000)	BH 139,429 #(/100,000)	SP 271,880 #(n/100,000)	Total 615,433 #(n/100,000)
CP	31(15)	46(33)	141(52)	218
PP	10(5)	13(9)	37(14)	60
OPI	19(9)	12(8)	13(5)	44
FP	74(36)	250(179)	165(61)	489
N/A for testing	22(11)	44(31)	0	66
Total	134(65)	321(230)	356(131)	811
Ratio PP/CP	0.32	0.28	0.26	0.28
Ratio OPI/CP	0.61	0.26	0.09	0.20

Risk factors in inconclusive reactive blood donors for *Trypanosoma cruzi* in Argentina

Remesar et al

EIA tests

	Type of antigen	Manufacturer
Chagatek ELISA	Parasite lysate	Distributed by BioMerieux Argentina. Made by Lemos Lab., Buenos Aires, Arg.
Chagas recombinante ELISA	Recombinant	Wiener Lab., Rosario, Argentina
BioChagas	Parasite lysate	BiosChile, Chile
BioZima Chagas	Parasite lysate	Lemos Lab., Buenos Aires, Argentina
Ortho Chagas	Parasite lysate	Ortho Lab. U.S.A.

Groups classified by reactivity

Blood donor samples were classified in the following groups

Reference Group (0): Negative by the five EIA

Group 1: Reactive by 1 or 2 EIA

Group 2: Reactive by 3 or 4 EIA

Group 3: Reactive by the 5 EIA tested
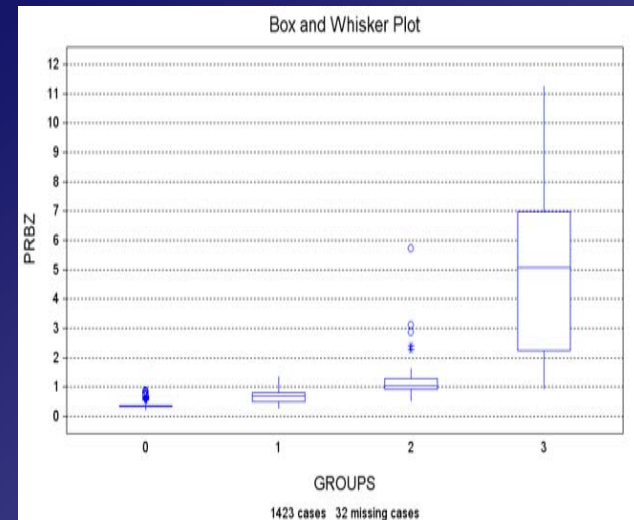
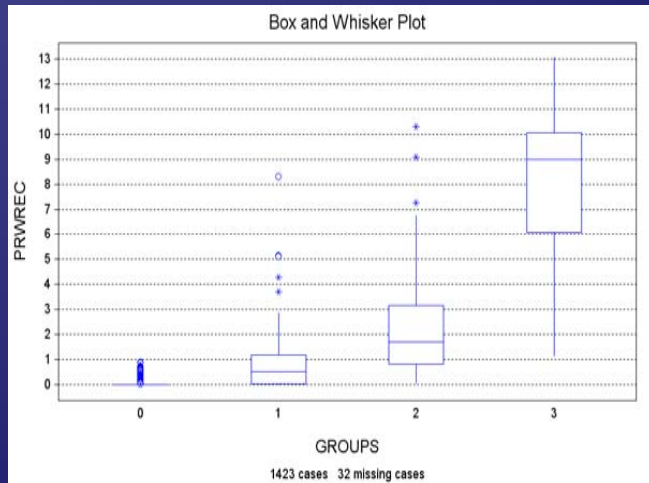# Sample OD/CO by Group of Reactivity:

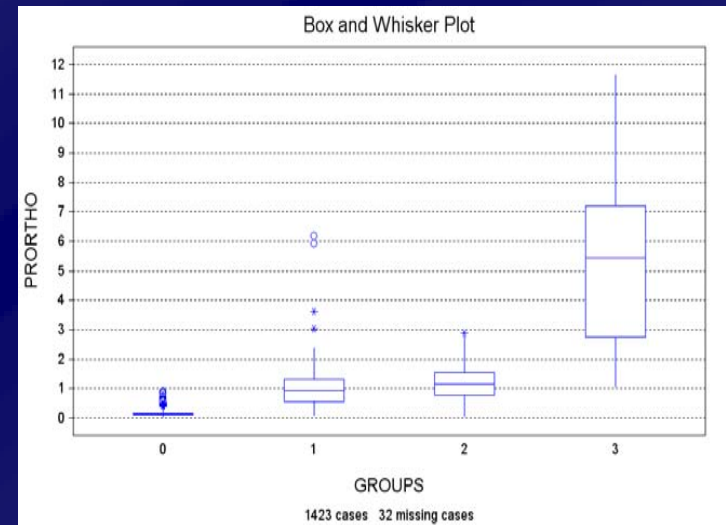Chagatek, BioMerieux
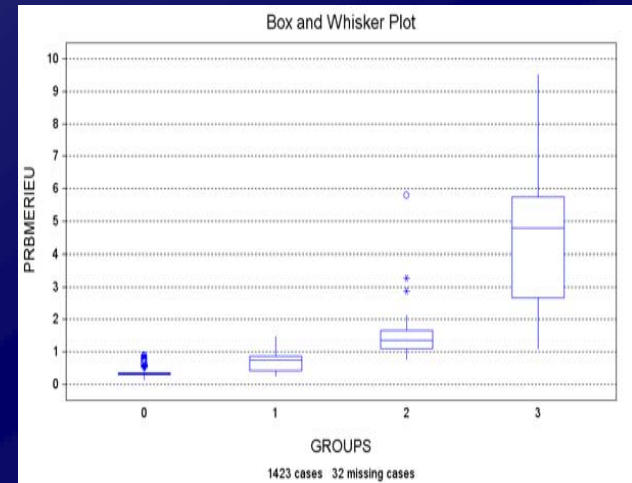
BioChagas, BiosChile
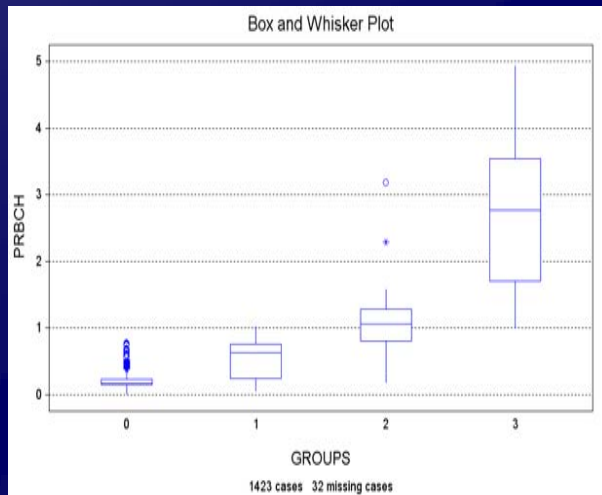
BioZima Chagas

Chagas rec. ELISA, Wiener Lab

Ortho Chagas

Classification according to reactivity to recombinant EIA

1) Reference (NR): Non-reactive by all tests (n= 1026);

2) Probable cross-reactive to Leismania **(L c-r)**: samples reactive by 1 or more lys-EIAs, but non-reactive by r-EIA;

3) T. cruzi low-level reactive **(TC l-r)**: samples reactive by 1, 2 or 3 lys-EIAs and reactive by r-EIA or reactive only by r-EIA;

4) T cruzi high-reactive **(TC h-r)**: samples reactive by 4 lys-EIAs and r-EIA.

Pattern according to risk factor

	L c-r (n= 48) (OR +/- 95%CI)	TC l-r (n=45) (OR +/- 95%CI)	TC h-r (n=304) (OR +/- 95%CI)
Lived in Rural Area	7.5 (3.3- 16.9)	2.8 (1.5-5.4)	6.3 (4.5-8.7)
Bugs (vectors) in your house	1.7 (0.8-3.7)	5.0 (2.6-9.5)	5.8 (4.2-7.8)
Relatives with Chagas Disease	1.4 (0.5-3.6)	2.5 (1.1-5.6)	3.2 (2.2-4.6)

Conclusions

- EIA tests should be used for blood testing (parallel testing with IHA and IFA will not improve screening)

- Confirmation of Chagas results is still difficult

- Low titer samples are common:
  - Cross reactivity explain part of the results
  - Low Chagas antibody level - represent 30% of all infections
    - self cured Infection? Low response? Infectious?

Acknowledgment

- Fundaçao Pró-Sangue Hemocentro de São Paulo:

  - Marcia Mitiko Otani
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  - Gaby Vercauteren

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  - Elisabeth Vinelli

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  - Ana Del Pozo
  - Mirta Remesar

- Blood Systems Research Institute
  - Michael Busch
  - Edward Murphy
  - Brian Custer