

Improving Diagnostic Tools



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Priority Issues:

- Diagnostics are inadequate - particularly with respect to the detection of cure.
- Inability to determine treatment efficacy undermines the use of current treatments and the development of new treatments.
- Researchers, Developers, Funders and Practitioners need to be more critical of the data and its interpretation and more careful with how the data are used to set policy.

Serologic Diagnostic Tests for *T. cruzi* infection

Test types: ELISA, IFA, hemagglutination, complement fixation

Target antigens: whole epimastigotes, crude epimastigote lysates, processed epimastigote components, recombinant antigens

Standard procedures: positive reaction on 2 of 3 tests is considered infected; confirmatory parasitological tests (e.g. PCR, hemaculture, xenodiagnosis) rarely done

Some of the issues:

Inappropriate target antigens for detection

Inadequate/insensitive confirmatory tests

Poor quality control/test evaluation

Time, expense, poor field applicability

no "gold standard"

TABLE 2. Frequencies of the different observed test patterns (n = 396)*

Response pattern†	Observed frequency	Percentage
0 0 0 0 0 0	189	47.7
0 0 0 0 0 1	4	1.0
0 0 0 0 1 0 0	5	1.3
0 0 0 1 0 0 0	3	0.8
0 0 1 0 0 0 0	3	0.8
0 1 0 0 0 0 0	9	2.3
1 0 0 0 0 0 0	23	5.8
0 0 0 1 0 0 1	1	0.3
0 0 1 0 0 0 1	1	0.3
1 0 0 0 0 1 0	1	0.3
0 0 1 1 0 0 0	1	0.3
1 0 0 1 0 0 0	1	0.3
0 1 1 0 0 0 0	1	0.3
1 1 0 0 0 0 0	2	0.5
1 0 0 0 0 1 1	1	0.3
1 0 0 0 1 0 1	2	0.5
0 1 1 0 0 0 1	1	0.3
1 0 1 1 0 0 0	1	0.3
0 1 0 0 1 1 1	1	0.3
1 1 1 0 0 0 1	1	0.3
1 0 1 1 1 0 1	1	0.3
1 1 1 1 1 1 0	1	0.3
1 1 1 1 1 0 1	1	0.3
1 1 1 1 0 1 1	2	0.5
1 0 1 1 1 1 1	3	0.8
0 1 1 1 1 1 1	5	1.3
1 1 1 1 1 1 1	132	33.3
Total	396	100.0

* Four samples were excluded for incompleteness of data.
† Negative results reported as 0, and positive results as 1. Order of results: IHA in RBB, IHA in CENETROP, IFA, crude ELISA 1, crude ELISA 2, recombinant ELISA 1, and recombinant ELISA 2.

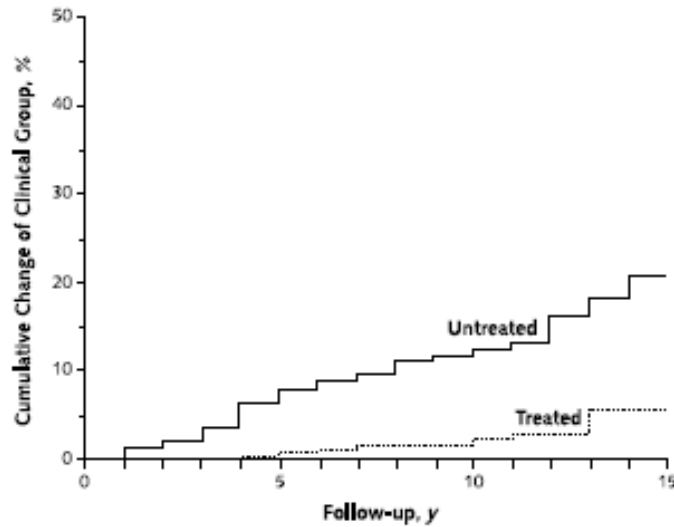
Current serodiagnostics are inadequate -

"Positive serology" depends on the test.

Results of random blood bank screening in Santa Cruz, Bolivia

Efficacy of Benznidazole Treatment in Chronic Chagas Disease

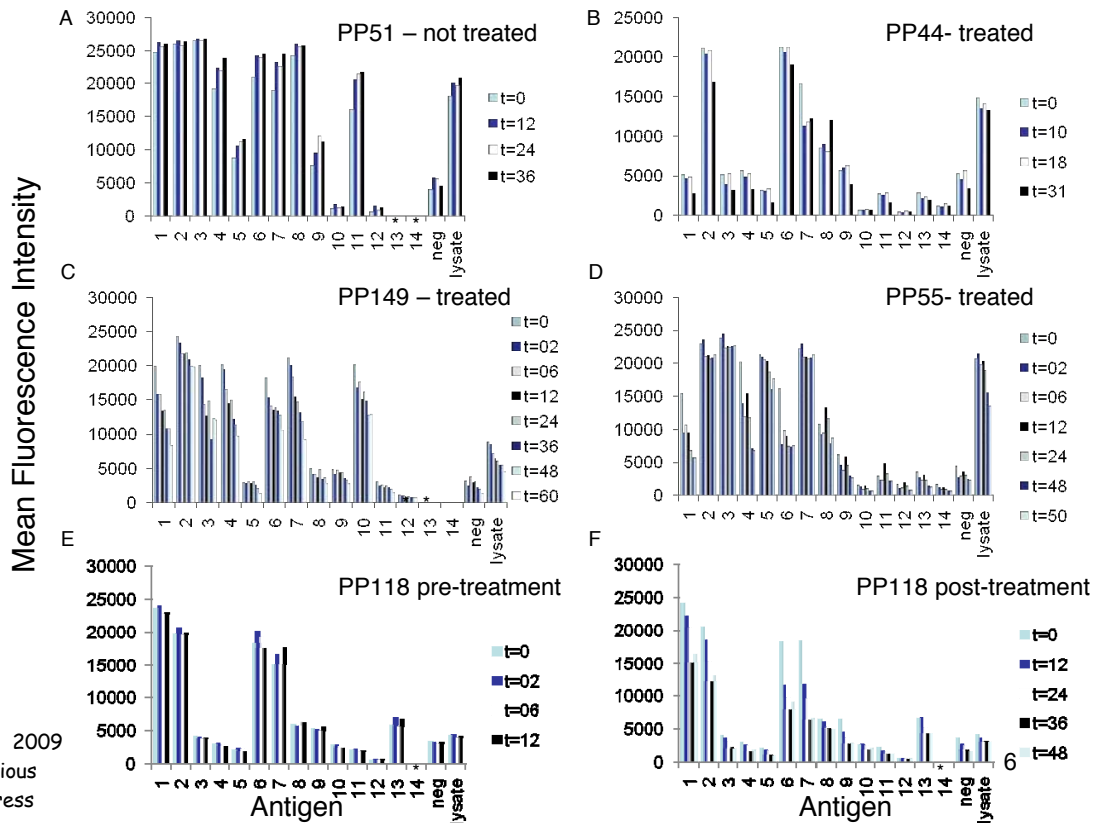
Figure 2. Kaplan–Meier curves of cumulative percentage of patients who changed clinical group.



Viotti, et al. 2006 Ann. Intern. Med. 144:724-734

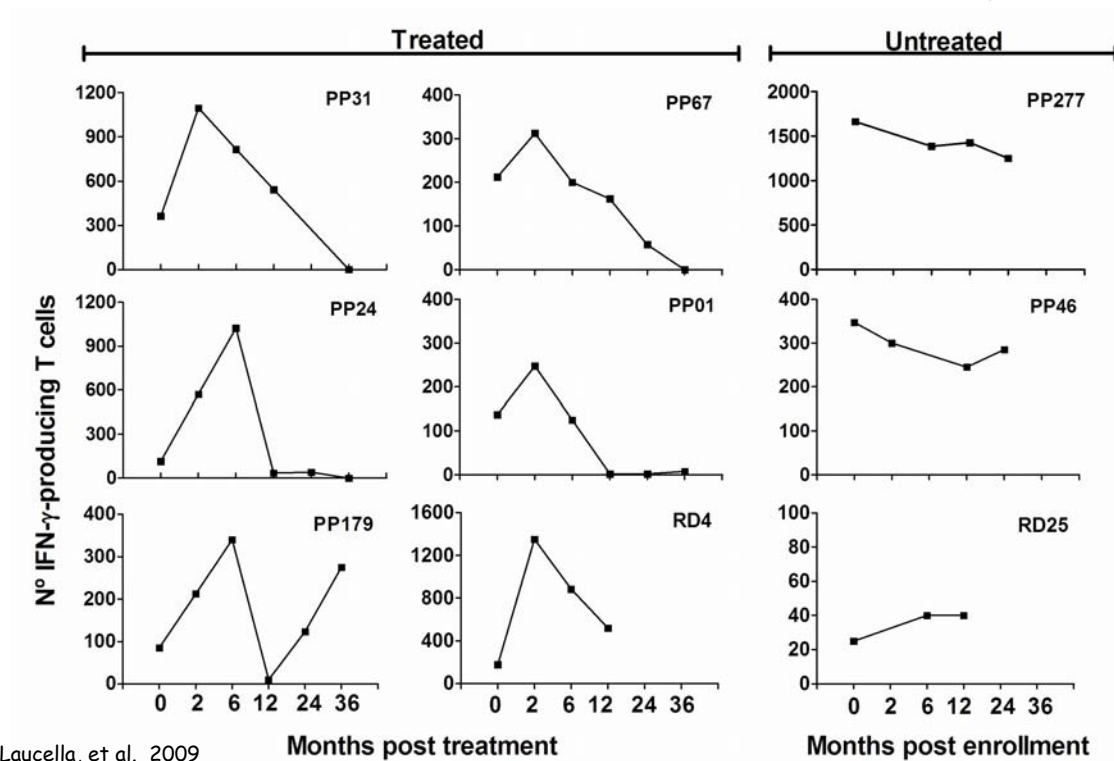
5

Multiplex diagnostic detects treatment effects in humans



Laucella, et al. 2009 Clinical Infectious Diseases, in press

Increased IFN- γ ELISPOT responses precede significant decreases in many benznidazole-treated but not in untreated subjects



Laucella, et al. 2009
Clinical Infectious Diseases in press

7

BZ treatment.

Correlation of decreases in T cell responses to changes in serological responses following BZ treatment

	BZ-treated	Non-treated	Stats
Decrease in both ELISPOT and serology	21	0	< 0.0001
Decrease in ELISPOT responses only	6	1	ns
Decrease in serology only	6	1	ns
No decrease in ELISPOT or serology	7	30	< 0.0001
Insufficient data/complex pattern	3	0	ns
Number with altered immune profile/total	33/43 (74%)	2/32 (6%)	< 0.0001

Laucella, et al. 2009
Clinical Infectious Diseases in press

8

Why does drug treatment not always work?

TABLE I
Indirect immunofluorescence titers for the follow-up of 80 chagasic patients from the state of Rio Grande do Sul following treatment with benznidazol

Serology titer	Before treatment n	After treatment		
		1st year n	2nd year n	3rd year n
Negative	0	0	1	4
1/40	6	6	10	39
1/80	6	15	38	35
1/160	30	33	24	2
1/320	14	24	7	0
1/640	24	2	0	0
Total	80	80	80	80

TABLE III
Comparison between serology and PCR results from the 80 patients treated with benznidazol after a three-years follow-up

Methodology	Positive (%)	Negative (%)
Serology	76 (95.0)	4 (5.0)
PCR	71 (88.3)	9 (11.3)

NOTE: all 80 subjects became hemaculture negative following treatment.

Fernandes, et al. 2009 Mem Inst Oswaldo Cruz

Table 2. Clinical characteristics, adverse side effects and interruption of treatment with benznidazole according to the clinical group (Kuschnir) in 1047 patients treated in our center.

Variables	Clinical group at admission				Total n = 1047
	0 (n = 724)	I (n = 241)	II (n = 73)	III (n = 9)	
Mean age (SD), years	33.9 (13.2)	42.6 (10.6)	46.2 (9.2)	52 (9.7)	36.9 (13.4)
Male, n (%)	322 (44.5)	115 (47.7)	36 (49.3)	7 (77.8)	480 (45.8)
Years of residence in endemic areas, median (25–75th percentiles)	14 (2–19)	16 (10–20)	15 (5.5–21)	10 (3.5–19)	14 (2–19)
Years of follow-up, median (25–75th percentiles)	4.0 (0.2–10.2)	4.9 (0.4–13.4)	6.0 (2.1–13.4)	2.2 (0.2–3.6)	4.2 (0.2–11.1)
Symptoms, n (%)	258 (35.6)	150 (62.2)	55 (75.3)	9 (100)	472 (45.1)
Adverse side effects, n (%)	221 (30.5)	83 (34.4)	22 (30.1)	2 (22.2)	328 (31.3)
Allergic dermatitis, n (%)	181 (25.0)	73 (30.3)	11 (15.1)	0 (0.0)	265 (25.3)
Gastrointestinal intolerance, n (%)	30 (4.1)	11 (4.6)	9 (12.3)	1 (11.1)	51 (4.9)
Headache, n (%)	13 (1.8)	4 (1.7)	1 (1.4)	0 (0.0)	18 (1.7)
Pruritus, n (%)	12 (1.7)	2 (0.8)	2 (2.7)	0 (0.0)	16 (1.5)
Edema, n (%)	6 (0.8)	2 (0.8)	2 (2.7)	0 (0.0)	10 (1.0)
Fever, n (%)	5 (0.7)	3 (1.2)	1 (1.4)	0 (0.0)	9 (0.9)
Incomplete treatment with benznidazole, n (%)	125 (17.3)	46 (19.1)	8 (11.0)	1 (11.1)	180 (17.2)

Group 0: Normal ECG without cardiomegaly; Group I: Abnormal ECG without cardiomegaly; Group II: Abnormal ECG and cardiomegaly, without heart failure; Group III: Abnormal ECG, cardiomegaly and heart failure.
SD: Standard deviation.

Summary:

- It is possible to design serological and T cell assays that both detect infection and assess treatment efficacy

Accurate serology may require a multiples platform and monitoring treatment efficacy requires serial assessment

- Treatment efficacy can be documented in both experimental animals and in humans

Therefore, current treatments should be more widely used, especially given that adverse effects can be managed, and new therapies should be more aggressively pursued

Researchers, Developers, Funders and Practitioners need to be more critical of the data and its interpretation and more careful with how the data are used to set policy