

## Supplementary Material. Annex A

**Table S1.** Literature search strategy in MEDLINE used for this meta-analysis.

Search	Query	Items found
<a href="#">#16</a>	Search (#6 AND #14) Filters: <b>Publication date from 2013/07/01</b>	<a href="#">194</a>
<a href="#">#15</a>	Search (#6 AND #14)	<a href="#">1122</a>
<a href="#">#14</a>	Search (#7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13)	<a href="#">1273</a>
<a href="#">#13</a>	Search <b>Lampit</b> [tiab]	<a href="#">25</a>
<a href="#">#12</a>	Search <b>nifurtimox</b> [tiab]	<a href="#">556</a>
<a href="#">#11</a>	Search <b>Nifurtimox</b> [Mesh]	<a href="#">378</a>
<a href="#">#10</a>	Search <b>Radanil</b> [tiab]	<a href="#">3</a>
<a href="#">#9</a>	Search <b>benzonidazol*</b> [tiab]	<a href="#">39</a>
<a href="#">#8</a>	Search <b>benznidazol*</b> [tiab]	<a href="#">785</a>
<a href="#">#7</a>	Search <b>Benzonidazole</b> [Supplementary Concept]	<a href="#">561</a>
<a href="#">#6</a>	Search (#1 OR #2 OR #3 OR #4 OR #5)	<a href="#">35077</a>
<a href="#">#5</a>	Search <b>T.Cruzi</b> [tiab]	<a href="#">6737</a>
<a href="#">#4</a>	Search <b>Cruzi</b> [tiab]	<a href="#">12376</a>
<a href="#">#3</a>	Search <b>Trypanosom*</b> [tiab]	<a href="#">29520</a>
<a href="#">#2</a>	Search <b>Chagas</b> [tiab]	<a href="#">9768</a>
<a href="#">#1</a>	Search <b>Chagas Disease</b> [Mesh]	<a href="#">10561</a>

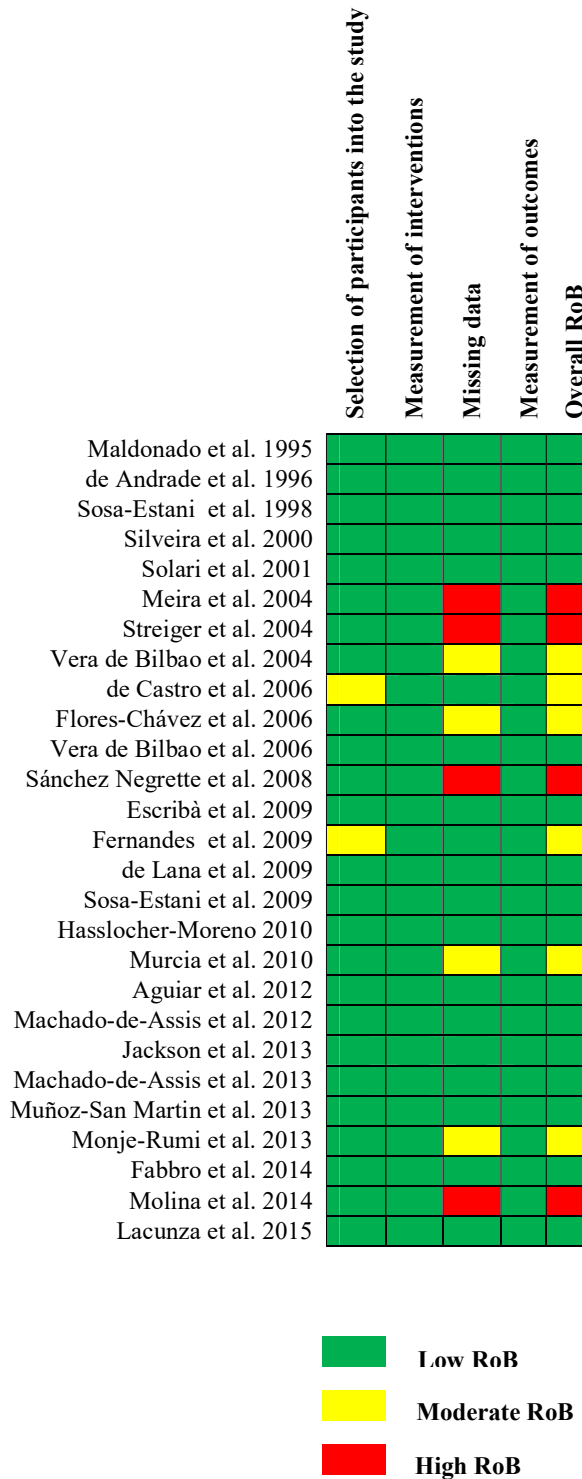
**Table S2.** Studies for which individual participant data could be available but were not obtained (12 studies, 1899 treated subjects with chronic *Trypanosoma cruzi* infection).

Source	Study ID <sup>a</sup>	Duration of follow-up (months)	Study population	Anti-trypanosomal drug	Number of treated subjects according to test										Total number of subjects <sup>b</sup>
					Parasitological/molecular tests		Conventional serological tests			Others					
					XD	PCR	ELISA	IIF	IHA	CoML	CF	DA-2MD	Non-conventional ELISA		
Argentina	Viotti et al. 2011	36 (median)	Adults	Benznidazole	-	-	53	53	53	-	-	-	32	53	
	Viotti et al. 2009	124.8 (mean)	Adults	Benznidazole	-	-	760	760	760	-	-	-	-	760	
	Viotti et al. 2006	117.6 (median)	Adults	Benznidazole	-	-	158	158	158	-	-	-	-	158	
	Gallerano and Sosa 2000	64 (mean)	Adults & children	Benznidazole & Nifurtimox	226	-	-	226	226	-	-	-	-	226	
	Fabbro et al. 2000	180 (mean)	Adults	Benznidazole & Nifurtimox	38	-	38	38	38	-	-	-	-	55	
	Fabbro et al. 2007	247 (mean)			41	-	-	54	54	-	-	54	-		
	Fabbro et al. 2010	276 (mean)			-	-	-	55	55	-	-	55	29		
Fabbro et al. 2013	-				-	-	55	55	-	-	55	29			
Viotti et al. 1994	96 (mean)	Adults	Benznidazole	24	-	-	110	110	-	110	-	-	110		
<b>Total number of subjects-Argentina</b>													<b>1362</b>		
Brazil	Andrade et al. 2013	48	Adults	Benznidazole	-	-	13	-	-	-	-	-	-	13	
	Diniz Marques et al. 2003	24	Children	Benznidazole	-	-	46	46	46	-	-	-	-	46	
	Coura et al. 1997	12	Adults	Benznidazole & Nifurtimox	42	-	-	42	-	-	42	-	-	42	
<b>Total number of subjects-Brazil</b>													<b>101</b>		
Colombia	Bianchi et al. 2015	30	Children	Nifurtimox	-	43	43	-	43	-	-	-	-	43	
	Gulh et al. 2004	5	Children	Benznidazole	-	-	36	-	36	-	-	-	-	36	
<b>Total number of subjects-Colombia</b>													<b>79</b>		
Spain	Pérez-Ayala et al. 2011	12 (median)	Adults	Benznidazole	-	357	357	-	357	-	-	-	-	357	
<b>Total number of subjects-Spain</b>													<b>357</b>		
<b>Grand total</b>													<b>1899</b>		

<sup>a</sup> References of all studies not providing individual participant data are available in Annex B of the Supplementary Material. <sup>b</sup> The estimation of total number of subjects was based on published data.

CF, complement fixation; CoML, complement-mediated lysis test; DA-2MD, direct agglutination with 2-mercaptoethanol; ELISA, enzyme-linked immunosorbent assay; IIF, indirect immunofluorescence assay; IHA, indirect hemagglutination assay; PCR, polymerase chain reaction; XD, xenodiagnosis.

**Figure S1.** Risk of bias (RoB) assessment of each included study.



**Table S3.** Anti-trypanosomal drugs used in treated subjects with *Trypanosoma cruzi* chronic infection and duration of treatment.

<b>Duration of treatment (days)<sup>a</sup></b>	<b>Benznidazole</b>	<b>Nifurtimox</b>	<b>Benznidazole &amp; Nifurtimox</b>	<b>Total number of subjects</b>
<b>≤ 30</b>	63	13	-	76
<b>31-60</b>	848	110	1	959
<b>&gt; 60</b>	242	14	-	256
<b>Total number of subjects</b>	1153	137	1	<b>1291</b>

<sup>a</sup> Treatment duration not known in five subjects.

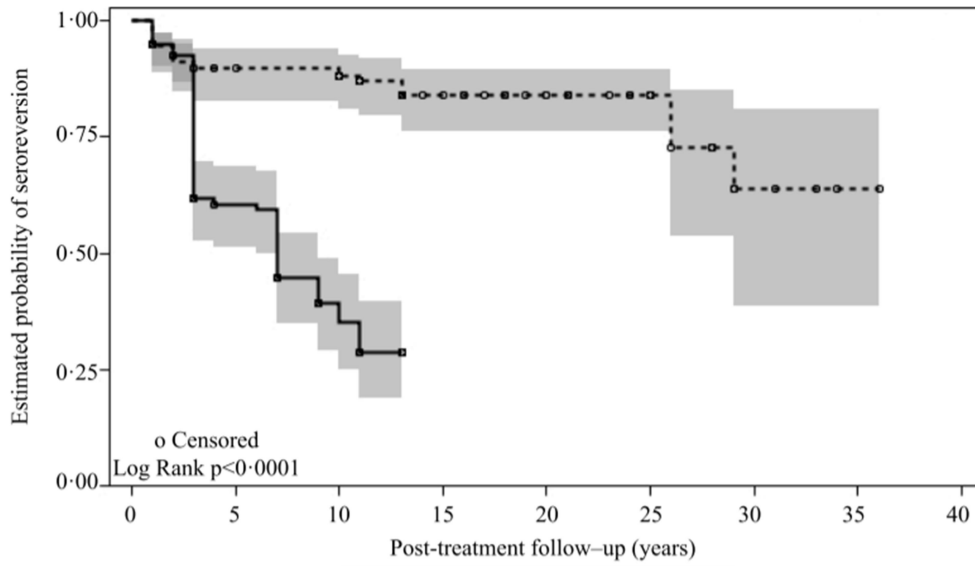
**Table S4.** Datasets reporting on conventional and non-conventional serological tests in treated subjects with chronic *Trypanosoma cruzi* infection.

Study ID	Number of subjects		Total number of subjects
	Non-conventional tests	Conventional ELISA	
de Andrade et al. 1996	58 AT-ELISA	58	116
Flores-Chávez et al. 2006	33 SAPA	33	66
Sánchez Negrette et al. 2008	13 IRA ELISA	18	31
Machado-de-Assis et al. 2012	94 TESA ELISA	94	188
de Lana et al. 2009			48
Machado-de Assis et al. 2013	22 FC-ALTA	26	
Sosa-Estani et al. 1998			106
Sosa-Estani et al. 2009	53 ELISA F29	53	
<b>Total number of subjects</b>	<b>273</b>	<b>282</b>	<b>555</b>

AT-ELISA, antigen trypomastigote ELISA; CF-ALTA, flow cytometric analysis of anti-live trypomastigote antibodies; ELISA, enzyme-linked immunosorbent assay; ELISA F29, enzyme-linked immunosorbent assay using a *Trypanosoma cruzi* flagellar calcium-binding protein; IRA-ELISA, ELISA with individual recombinant antigens; SAPA, shed acute-phase antigen; TESA ELISA, recombinant ELISA and *Trypanosoma cruzi* excreted-secreted antigen blotting.

**Figure S2.** Kaplan-Meier plots of the progression of conventional and non-conventional serological tests in treated subjects with chronic *Trypanosoma cruzi* infection stratified by the age at treatment, with 95% IC.

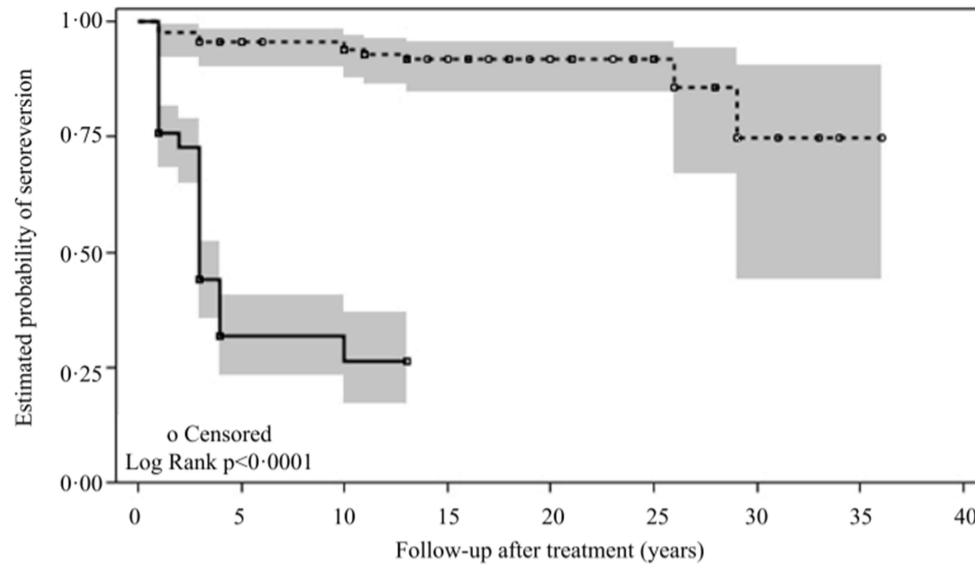
1)



Number at risk

1-19 years	157	50	19	0					
>19 years	125	109	108	38	29	20	4	1	0

2)



Number at risk

1-19 years	156	12	12	0					
>19 years	117	118	104	38	29	20	4	1	0

Plots show the proportion of treated subjects progressing towards seroreversion according to (1) conventional serology and (2) non-conventional serology results during the follow-up, stratified by age at treatment (1-19 years vs. > 19 years). IC, interval confidence; ELISA, enzyme-linked immunosorbent assay.

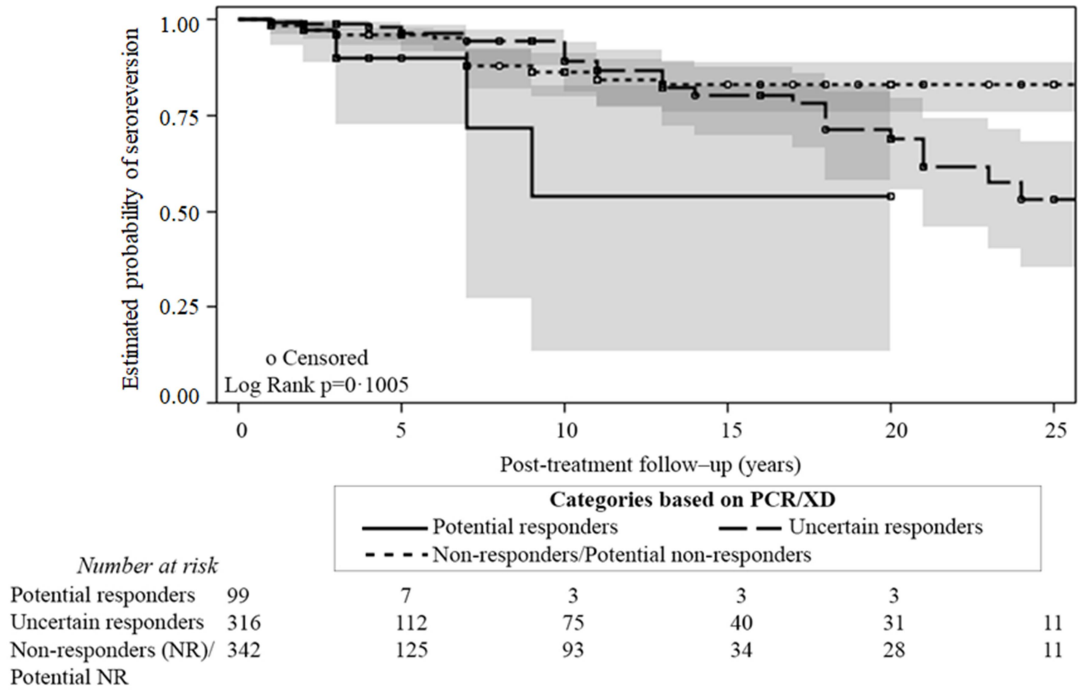
**Table S5.** Summary of censored & uncensored values for conventional serological tests in treated subjects with chronic *Trypanosoma cruzi* infection according to categories based on PCR or xenodiagnosis results.

Category	Type of test	Number of subjects	Number of events	Number of censures	% censored
<b>Potential responders</b>	ELISA	99	6	93	93.94
	IIF	64	7	57	89.06
	IHA	35	4	31	88.57
<b>Uncertain responders</b>	ELISA	316	30	286	90.51
	IIF	224	46	178	79.46
	IHA	156	38	118	75.64
<b>Non-responders / potential non-responders</b>	ELISA	342	27	315	92.11
	IIF	239	44	195	81.59
	IHA	200	21	179	89.50

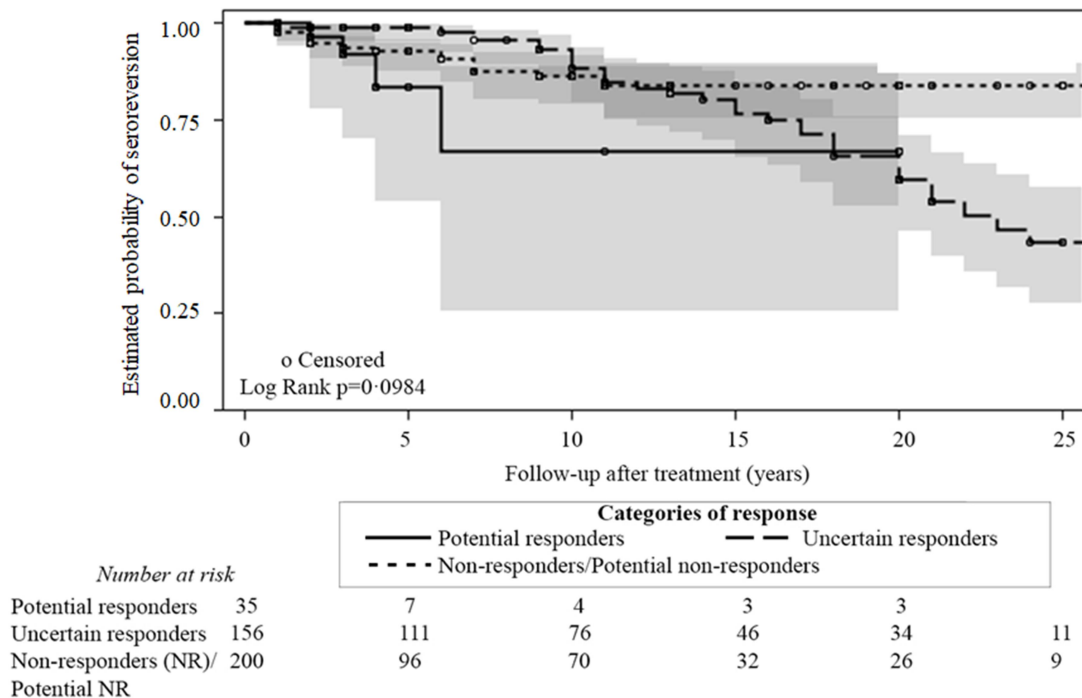
‘Potential responders’, i.e. subjects with three or more negative PCR or xenodiagnosis results; ‘uncertain responders’, i.e. subjects with at least two negative PCR or xenodiagnosis results; and ‘non-responders/potential non-responders’, i.e. subjects with just one PCR or xenodiagnosis (positive or negative). ELISA, enzyme-linked immunosorbent assay; IIF, indirect immunofluorescence assay; IHA, indirect hemagglutination assay; PCR, polymerase chain reaction.

**Figure S3.** Kaplan-Meier plots of the progression of conventional serology in treated subjects with chronic *Trypanosoma cruzi* infection stratified by categories based on PCR or xenodiagnosis, with 95% IC.

1)

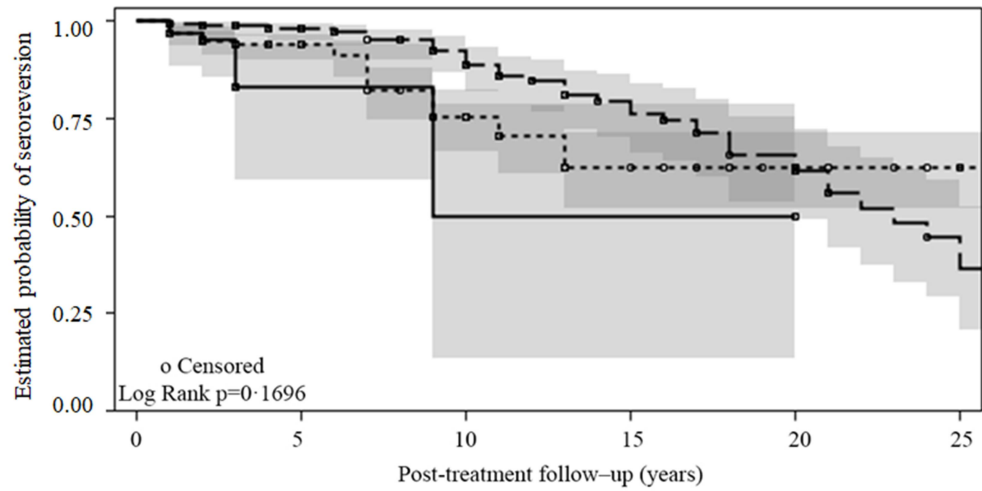


2)





3)



Number at risk	Categories based on PCR/XD					
	Potential responders	Uncertain responders	Non-responders/Potential non-responders	Potential responders	Uncertain responders	Non-responders/Potential non-responders
Potential responders	64	5	3	3	3	
Uncertain responders	224	150	100	50	34	11
Non-responders (NR)/ Potential NR	239	105	72	34	28	11

Plots show the proportion of treated subjects progressing towards seroreversion according to (1) ELISA, (2) IIF, and (3) IHA tests during the follow-up, stratified by category based on PCR or xenodiagnosis. 'Potential responders', i.e. subjects with three or more negative PCR or xenodiagnosis results; 'uncertain responders', i.e. subjects with at least two negative PCR or xenodiagnosis results; and 'non-responders/potential non-responders', i.e. subjects with just one PCR or xenodiagnosis (positive or negative). ELISA, enzyme-linked immunosorbent assay; IIF, indirect immunofluorescence assay; IHA, indirect hemagglutination assay; PCR, polymerase chain reaction.

**Table S6.** Hazard ratios (95% confidence interval) corresponding to the adjusted Cox interaction model for conventional serology in treated children or adolescents (1–19 years) versus adults (> 19 years) with chronic *Trypanosoma cruzi* infection based on studies with low risk of bias.

Serological test	HR (95% IC)		<i>p</i> -value <sup>a</sup>
	Brazil	Argentina, Bolivia, Chile and Paraguay	
<b>ELISA</b>	8.39 (2.45–28.78)	1.73 (0.72–4.13)	0.036
<b>IIF</b>	8.86 (3.20–24.53)	1.46 (0.59–3.56)	0.007
<b>IHA</b>	5.42 (1.48–19.90)	1.23 (0.52–2.90)	0.048

IC, interval confidence; ELISA, enzyme-linked immunosorbent assay; IIF, indirect immunofluorescence assay; IHA, indirect hemagglutination assay; HR, hazard ratio.

<sup>a</sup> The *p*-value corresponds to the effect of the interaction obtained from adjusted Cox proportional hazards model.