Reliability of the chemiluminescence Liaison *Treponema* Screen test for the screening of anti-*Treponema* antibodies

Nicoletta Rusconi[1], Cristiana Passerini Tosi[2], Antonio Goglio[2], Luisa Grassi[3], Giovanni Gesu[3], Maria Parmeggiani[4], Luigi Vecchia[4], Alessandra Avola[5], Giordano Dicuonzo[5], Roberto Vaiani[1]

1 Microbiology Laboratory, Ospedale A. Manzoni, Lecco;
2 Microbiology Laboratory, Ospedali Riuniti, Bergamo;
3 Microbiology Laboratory, Ospedale Niguarda Milano;
4 Microbiology Laboratory, Arcispedale S. Maria Nuova, Reggio Emilia;
5 Laboratory Medicine, Ospedale Università Campus Biomedico, Roma

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SUMMARY

A chemiluminescent test for anti treponemal total antibodies, Liaison *Treponema* Screen test (LTS), was compared with *Treponema Pallidum* Particle Agglutination assay (TPPA) against 1022 regular blood donors and 2627 in-and-outpatients attending the laboratories of five hospitals. All positive and doubtful tests were confirmed by Western Blot (WB) and we have discussed the criteria of WB positivity. The results for blood donors were: nonreactive 1022 TPPA and 1021 LTS and doubtful 1 LTS (WB negative) with a concordance of 99.9%. The mean and median index values of the LTS tests (0.175 and 0.15 respectively) were much lower than the doubtful and positive cut-off index values (0.9 and 1.1 respectively). The results for the 2627 patients were: 2423 (92.2%) LTS and TPPA non reactive; 191 (7.3%) LTS and TPPA reactive; 13 (0.5%) LTS and TPPA discordant. Of the 13 patients with discordant tests (1 TPPA reactive vs. LTS nonreactive and 12 LTS reactive or doubtful vs. TPPA nonreactive) 1 was true positive (LTS reactive and TPPA nonreactive), 10 true negative (LTS reactive/doubtful and TPPA nonreactive) and 2 not verifiable. The sensitivity and specificity of LTS were 99.5% and 99.6% respectively; the sensitivity and specificity of TPPA were 99.0% and 100% respectively. In conclusion, LTS and TPPA are similar in sensitivity and specificity, but an automated analyzer as Liaison is more useful than TPPA in laboratories with high workload in syphilis screening.

INTRODUCTION

Syphilis is a disease in which diagnostic suspicion is more frequently related to anamnestic/epidemiological data than to the clinical picture itself (9, 15); except for the primary infection, the diagnosis is based on the finding of serological positivity but it is often impossible to understand whether the positive serological findings are relate to a past infection, or latent or ongoing disease. The serological diagnostic criteria, which have been redefined on the basis of the more widespread use of new treponemal tests and IgM antibodies, were changed mainly for confirmation criteria of screening tests. Now the screening is recommended with treponemal EIA (that detects both IgG and IgM), or TPPA or VDRL/RPR and TPHA and the positives must be confirmed with a different treponemal test. An immunoblot is recommended when the standard confirmatory test does not confirm the positive result (4-7, 10, 16). The serological tests can be classified in two categories: treponemal tests for specific antibodies against treponema antigens, and aspecific non-treponemal tests for antilipoid antibodies that appear during the initial phase of syphilis but also in other situations, such as autoimmune diseases.

In countries with advanced public healthcare services, syphilis is the sexually transmitted disease that has aroused the least media interest, and this has favoured its recrudescence; currently, screening is restricted to blood and tissue donations, pregnant women, AIDS patients, and patients with known risk behaviours. When screening blood donors, we cannot have any false negative results, therefore the greatest sensitivity is required; on the other hand, it is also important not to unnecessarily block blood bags or suspend donors on the basis of doubtful or false positive results, thus sensitivity needs to be combined with a high degree of specificity. The same consideration is

Corresponding author: **Roberto Vaiani**
Via Carlo Cattaneo 28, 23900 Lecco, Italy.
Tel.: +39-3294128745
E-mail: **arvaiani@libero.it**

valid for pregnant women, in whom non-treponemal tests may give rise to false positive findings.
The optimal evaluation of a new test requires the use of a sufficiently large population of truly negative and positive patients, in whom the phase of the disease is known. In the absence of such a population with a confirmed diagnosis, the alternative is to compare the new test with the currently available reference tests.
The aim of this study is to assess the diagnostic reliability of chemiluminescent Liaison Treponema Screen test (LTS) vs. the Treponema Pallidum Particle Agglutination assay (TPPA), which is considered to be the most reliable of the currently used routine tests. In the case of doubtful or positive findings, confirmation was by western blotting (WB) as a means of identifying the type of significant antibodies present in that sample.

MATERIALS AND METHODS

Donors: Six hundred consecutive regular blood donors attending the blood bank of "Ospedale A. Manzoni", Lecco and 422 consecutive regular blood donors attending the blood bank of "Ospedale Niguarda", Milan, for a total of 1022 donors. This was the true negative population as these subjects had been followed up over time by both clinical and laboratory examinations

Patients: All the samples were of consecutive, different in- and out-patients; 408 plus 31 positive samples previously stored at -20°C at Ospedale A. Manzoni, Lecco; 587 at Ospedale Niguarda, Milan; 761 at Ospedali Riuniti, Bergamo; 685 at Arcispedale S. Maria Nuova, Reggio Emilia; and 155 at Ospedale Campus Biomedico, Rome for a total of 2627 samples.

Tests: Donor serum samples were tested using LTS and TPPA; the patients' serum samples were tested using LTS and TPPA (plus RPR test, if in use at the hospital laboratory and in any case for the samples that were reactive to the treponemal tests). All of the positive and doubtful cases (TPPA >1/40; LTS >0.9, and RPR ≥1/1) were tested for IgG and IgM by a WB.

The characteristics of the tests were:

- Liaison Treponema Screen test (LTS), DiaSorin, Saluggia, Italy is a qualitative chemiluminescent immunoassay (CLIA) that identifies total antibodies against the 17 kD TnpN17 antigen of *T. pallidum,* produced by a recombinant DNA technology. Values are expressed as an index, and the cut-off points are ≤0.9 negative, >0.9 to <1.1 doubtful, and ≥1.1 positive. The test is performed on Liaison that is an automated random-access analyzer.
- Serodia Treponema Pallidum Particle Agglutination assay (TPPA), Fujirebio, Tokyo, Japan is an agglutination test with gel particles sensitized with sonicated *T. pallidum* that identifies total antibodies; the positive cut-off point provided by the manufacturer is ≥ 1/80.
- Rapid Plasma Reagin (RPR), Becton Dickinson is a carbon particle cardiolipin antigen which detects "reagins" from syphilitic persons causing macroscopic flocculation; the positive cut-off point is ≥1/1.
- IgG and IgM *Treponema pallidum* Western Blot (WB), Arnika Diagnostic Line, Milano, Italy, which reveal bands of 47, 44.5, 17 and 15 kD that were considered significant for syphilis immunodiagnosis (2, 11). Bands whose intensity is similar or more than that of the control are considered reactive; those whose intensity is less than that of the control are considered weakly reactive. The WB IgG criteria for a positive diagnosis of syphilis are quite restrictive: a positive sample must have at least two reactive bands; one reactive band plus two or three weakly reactive bands are considered borderline; and one reactive band alone or up to three weakly reactive bands are considered negative. A WB IgM-positive has at least one reactive band (p47, p17 or p15) plus at least one weakly reactive band, whereas borderline cases have only one reactive band (p47, p17 or p15) or one reactive p44.5 band plus at least one other weakly reactive band; all other cases are considered negative. We used different criteria; when LTS and TPPA are reactive (reactivity of screening and confirmation test) the sample is considered a true positive in accordance with new guidelines (5, 6) so every band by WB is positive. When LTS and TPPA are discordant the presence of one reactive (not weakly reactive) IgG significant band may be sufficient to confirm discordant TPPA positivity (every band) or LTS positivity (only the 17 kD band) All of the WB analyses were centralised at the laboratory of Ospedale A. Manzoni, Lecco.

RESULTS

All the 1022 blood donors tested were non reactive by TPPA test; 1021 were non reactive by LTS, while 1 was doubtful by LTS test (index 1.06) and non reactive by WB.

The distribution of the index values of LTS results is summarised in Table 1 and the mean and median analytical index values of the LTS-negative cases were 0.175 and 0.15, much lower than the doubtful and positive cut-off values (0.9 and 1.1 respectively); 90.3% of the negative samples had index values of ≤0.30 and 99.4% of the negative

samples had index values ≤0.6 (respectively 1/3 and 2/3 of the doubtful cut-off index). These data are a guarantee of "clean" values that usually require very little further checking.

Table 1. *Distribution of LTS index values of 1022 donors (< 0.9 non reactive; ≥ 0.9 < 1.1 doubtful; ≥1.1 reactive)*

LTS index values	N° donors by LTS index	%
≤ 0.1	400	39.1
> 0.1 – 0.2	311	30.4
> 0.2 – 0.3	213	20.8
> 0.3 – 0.4	71	6.9
> 0.4 – 0.5	14	1.4
> 0.5 – 0.6	8	0.8
> 0.6 – 0.7	2	0.2
> 0.7 – 0.8	2	0.2
> 0.8 – 0.9	0	0
> 0.9 < 1.1	1	0.1
≥ 1.1	0	0
Total	**1022**	**99.9**

Patient tests: Table 2 shows the LTS and TPPA results obtained in the 2627 samples from different patients tested by the laboratories of the five participating hospitals; 2423 were LTS and TPPA non reactive, 191 were LTS and TPPA reactive, 13 were discordant.

Table 3 reports the distribution of the patients' LTS values vs. TPPA values; the concordance of the LTS and TPPA findings was once again very high: only 13 of the 2627 samples (0.5%) led to discordant results. None of the samples with LTS index values >3.00 showed discordant TPPA and, when associated with TPPA values <1/320, low LTS values #3.00 are likely to reflect the immunological memory of a past infection that is no longer active or, when disagreed with negative TPPA, the result may be non specific.

The RPR tests were 1848 on 2627 samples; however RPR was tested on all the reactive samples of the treponemal tests. There were 84 reactive RPR on 191 reactive LTS and TPPA; 2 reactive RPR on discordant LTS and TPPA; 8 reactive RPR on 1644 nonreactive LTS and TPPA tested. One case was very interesting, since it was RPR positive (1/8) and LTS and TPPA negative; WB showed that the sample was IgG nonreactive and IgM weakly reactive (band 47). Upon a second control,

Table 2. *LTS and TPPA results by labs*

Labs	No. patients*	LTS -TPPA -	LTS+TPPA +	LTS + or ±TPPA -	LTS –TPPA +
A	761	697 (91.6%)	58 (7.6%)	5 (0.7%)	1 (0.1%)
B	408	398 (97.5%)	10 (2.5%)	0	0
	31**	n.a.	31	n.a.	n.a.
C	587	551 (93.9%)	34 (5.8%)	2 (0.3%)	0
D	685	656 (95.8%)	28 (4.1%)	1 (0.1%)	0
E	155	121 (78.1%)	30 (19.3%)	4 (2.6%)	0
Total	**2627**	**2423 (92.2%)**	**191 (7.3%)**	**12 (0.4%)**	**1 (0.04%)**

*Consecutive in- and out-patients

**Previous positive patient samples stored at -20°C.

n.a.= not applicable

Table 3. *Distribution of the LTS index values vs. TPPA in patients*

	LTS index values	No. of samples by LTS index	No. of samples with TPPA values <1/80 by LTS index	No. of samples with TPPA values 1/80-1/160 by LTS index	No. of samples with TPPA values ≥1/320 by LTS index
Non-	≤ 0.10	1381	1380	1	0
reactive	0.11-0.20	753	753	0	0
	0.21-0.30	176	176	0	0
	0.31-0.40	58	58	0	0
	0.41-0.50	18	18	0	0
	0.51-0.60	14	14	0	0
	0.61-0.70	9	9	0	0
	0.71-0.80	8	8	0	0
	0.81-0.89	7	7	0	0
Doubtful	0.90-1.09	1	1	0	0
Reactive	1.10-3.00	24	11	10	3
	3.01-10.00	38	0	21	17
	10.01-20.00	36	0	14	22
	> 20.00	104	0	6	98
	Total	**2627**	**2435**	**52**	**140**

the patient was LTS and TPPA highly reactive (a true positive), therefore the RPR was an early sign of positivity.
The positive samples were confirmed by WB.
Table 4 shows the WB IgG results relating to the simultaneously LTS and TPPA reactive samples (true positives with the new guidelines) (5,6).
In this group the WB had a reactive IgG 17 kD band in 190 of 191 positive samples (one sample had a band p47 and a negative -very weakly- band p17); in 22 WB there was only one IgG band and in 55 WB there were two IgG bands.
All of WB was performed with IgG and IgM tests; all of the WB tests with one or more positive IgM bands had three or more positive IgG bands too (except for one case with two reactive IgG 47 and 17 kD bands and one weak IgG 15.5 kD band).
Table 5 summarises the discordant results, two of which were doubtful. In A/2, the positive findings at first control ten months later (TPPA 1/10240 and RPR 1/32) prevents us from coming to a conclusion concerning the significance of the LTS-positive index value (1.8) and TPPA, RPR and WB negative results. In A/6 we could not control the case (LTS negative, TPPA 1/80, RPR 1/1, WB traces of 47 kD IgM in the absence of IgG bands) to understand whether indicated a case of very early-phase syphilis or whether the findings were simply not specific.
So, we considered 193 patients true positive (191 with concordant LTS and TPPA tests, plus one with discordant results but a 17 kD IgG band at WB which is listed as positive in Table 5, plus the LTS- and TPPA-negative patient with a RPR value of 1/8 who subsequently proved to be a true positive), 2432 true negative (2422 LTS- and TPPA-negative cases – after removing the patient who subsequently proved to be positive – plus the ten patients with discordant LTS and TPPA results without any specific bands at WB), and two not verifiable (Table 5), samples A/2 and A/6).

DISCUSSION

To asses the diagnostic reliability of LTS, we compared its results with those obtained using the TPPA, which is considered the most reliable routine test and that is used by the WHO to select positive and negative samples for the "SDI evaluation of Rapid Syphilis Diagnostics" (www.who.int/std_diagnostics/publications/manuals/ syphilis_evaluation.pdf; 2007). The LTS and TPPA detect IgM antibodies too, thus allowing the early identification of positive cases and making both tests more reliable for screening purposes (5-

Table 4. *WB IgG results in LTS and TPPA positive patients*

Labs	No. of LTS+ and TPPA+ patients	WB IgG with only one band	WB IgG with two bands	WB IgG with ≥ 3 bands	TOTAL WB positive
A	58	4	18	36	58
B	41	6	11	24	41
C	34	5	9	20	34
D	28	3	6	19	28
E	30	4	11	15	30
Total	**191**	**22**	**55**	**114**	**191**

Table 5. *Analytic assessment of the discordances between LTS and TPPA*

Lab / sample	Discordances	RPR	WB IgG	WB IgM	Presence of specific Abs
A / 1	LTS+/TPPA-	Negative	Negative	Negative	NO
A / 2	LTS+/TPPA-	Negative	Negative	Negative	? *
A / 3	LTS±/TPPA-	Negative	Negative	Negative	NO
A / 4	LTS+/TPPA-	Negative	Only traces p17	Negative	NO
A / 5	LTS+/TPPA-	Negative	Negative	Negative	NO
A / 6	LTS-/TPPA+	Pos 1/1	Negative	Traces band 47 kD	?
C / 1	LTS+/TPPA-	Negative	Negative	Negative	NO
C / 2	LTS+/TPPA-	Pos 1/1	Only band p17	Negative	YES
D / 1	LTS+/TPPA-	Negative	Only traces p17	Negative	NO
E / 1	LTS+/TPPA-	Negative	Negative	Negative	NO
E / 2	LTS+/TPPA-	Negative	Negative	Negative	NO
E / 3	LTS+/TPPA-	Negative	Negative	Negative	NO
E / 4	LTS+/TPPA-	Negative	Negative	Negative	NO
Total	**12 LTS+ or ±**	**1**	**3 Pos/traces**	**0**	**1+1? ***
	1 TPPA +	**1**	**0**	**1 traces**	**1 ?**

*LTS index 1.8; ten months later, the patient showed TPPA 1/10240 and RPR 1/32

7, 16). The importance of accuracy of diagnostic tests was highlighted by an analysis of eight external quality control tests carried out by the German Infection Serology Proficiency Testing Program between 2000 and 2003, that put in evidence some problems concerning the ability of laboratory tests to diagnose syphilis (12).

These results underline the importance of careful tests and that any diagnosis cannot rely on laboratory findings alone.

The concordance of the LTS and TPPA findings vas very high (99.9% in the 1022 blood donors with one doubtful test in LTS, and 99.5% in the 2627 patients with 13 discordant results).

None of the samples with LTS index values >3.00 showed discordant TPPA (Table 3) and, when associated with TPPA values of <1/320, low LTS values of ≤3.00 are likely to reflect the immunological memory of a past infection that is no longer active. In the reactive tests we used WB to detect the presence of specific antibody bands which, in the case of discordant test results, can confirm or not that the test reactivity is related to a significant antibody band.

Other authors used WB to confirm positivity (1, 3, 11, 14) and their data indicate that the presence of at least three bands is required to confirm a diagnosis of syphilis (1, 3, 14), but our findings show that sample of positive patients, confirmed by both LTS and TPPA, may have just a single band in WB.

We have 22 of 191 LTS and TPPA-positive cases with a single WB band, and it is worth noting that only 9 of these have their LTS and TPPA values that were respectively less than <3 and <1/320. We think that a rigid band count could reduce the diagnosis of true positive cases (considering true positive also tests with specific antibody bands due to a past non active infection) and that the use of IgG WB in a less strict way, for the rare discordant cases, is useful to understand the presence of possible past infection.

Positive findings with low LTS and/or TPPA values should in any case be reviewed critically and, if the clinical and epidemiological data are doubtful, must be integrated with a search for IgM or RPR and/or checked again after a short time in order to avoid missing early signs of positivity. In our samples there was only one case with an RPR value of 1/8 that was negative by LTS, TPPA and WB IgG with a weakly positive 47 kD IgM band, and which subsequently proved to be a true positive. Of the 204 reactive cases, 13 (6.4%) led to discordant LTS and TPPA results (Table 5); LTS has one more positivity than TPPA but also ten false positive results.

The sensitivity of LTS was 99.5% (192 positive cases out of 193 true positive) and its specificity 99.6% (2422 negative cases out of 2432 true negatives); the sensitivity of TPPA was 99.0% (191 positive cases out of 193 true positives) and its specificity 100% (2432 negative cases out of 2432 true negatives); these excellent data are similar to the results of other two studies on LTS (8, 11).

In conclusion LTS and TPPA are similar in sensitivity and specificity, but LTS moreover may be fully automated and the data can be transferred directly to a laboratory's information system, which is very useful when it is necessary to screen large numbers of samples (such as in a blood bank) and in cases specifically requiring secure and traceable data transfer, such as investigations for organ transplants. Both LTS and TPPA can be used as stand-alone screening tests or as confirmatory tests.

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