Evaluation of five fully-automated immunoassays for diagnosis of syphilis

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INTRODUCTION

Serology is central to the screening and diagnosis of syphilis infection and can be classified into two groups:

1. Non-treponemal tests: Venereal Disease Research Laboratory (VDRL) or Rapid Plasma Reagin (RPR)
2. Treponemal tests which are based on antigens derived from Treponema pallidum: Treponemal Haemagglutination Assay (TPHA), Fluorescent Treponemal Antibody absorption test (FTA-ABS) and native or recombinant antigen Enzyme immunoassay (EIA) tests.

AIM

In this study, the performance of 5 automated tests, using recombinant proteins (Table I), was compared to that of TPHA (Treponema pallidum hemagglutination assay, Biokit, Barcelona, Spain):

- Architect Syphilis TP (Abbott, Delkenheim, Germany):
- BioPlex® 2200 Syphilis IgG, IgM (BioRad, Bio-Rad, Marnes la Coquette, France),
- Elecsys® Syphilis (Roche, Diagnostics GmbH, Mannheim, Germany),
- IMMULITE® 2000 Syphilis (Siemens, Healthcare Diagnostics, Llanberis, United Kingdom),
- LIAISON® Treponema Screen (DiaSorin, Saluggia, Italy).

Table I: Recombinant proteins used in these 5 automated assays.

Assays	Support automate	Antigen Recombinant	Ab detection	Run time (min)
Architect Syphilis TP	Architect	TpN15, TpN17 TpN47	Total Ab	26
BioPlex® Syphilis IgG	BioPlex 2200	TpN15, TpN17 TpN47	IgG	45
BioPlex® Syphilis IgM	BioPlex 2200	TpN17, TpN47	IgM	45
Elecsys® Syphilis	Cobas	TpN15, TpN17 TpN47	Total Ab	18
IMMULITE® Syphilis	IMMULITE 2000	TpN17	Total Ab	35
LIAISON® Treponema Screen	LIAISON	TpN17	Total Ab	39

METHODS

392 unselected sera (except Elecsys®: 385 unselected sera, due to availability of limited volume of samples) and 7 selected samples from patients with primary syphilis were tested by TPHA as well as these 5 assays. Whenever one assay scored reactive, IgG and IgM Line Immunoblot (Genzyme Virotech GmbH, Rüsselsheim, Germany) was used for confirmation.

RESULTS

The following tables summarize the results obtained for the 392 unselected samples tested with each method (equivocal results were excluded).

Concordance between TPHA and five automated assays

Table II: Architect vs TPHA

Architect Syphilis TP	TPHA			
	Positive	Equivocal	Negative	Total
Positive	259	0	6*	265
Equivocal	0	0	1	1
Negative	7**	0	119	126
Total	266	0	126	392

*3/6 of the discordant samples were positive by Immunoblot IgG
**0/7 of the discordant samples were positive by Immunoblot IgG

Percent Agreement

Positive	*97.4%*	*(259/266)*
Negative	*95.2%*	*(119/125)*
Overall	*96.7%*	*(378/391)*

Table III: BioPlex® 2200 Syphilis IgG vs TPHA

BioPlex® 2200 Syphilis IgG	TPHA			
	Positive	Equivocal	Negative	Total
Positive	259	0	7*	266
Equivocal	1	0	1	2
Negative	7**	0	117	124
Total	267	0	125	392

*3/7 of the discordant samples were positive by Immunoblot IgG
**0/7 of the discordant samples were positive by Immunoblot IgG

Percent Agreement

Positive	*97.4%*	*(259/266)*
Negative	*94.3%*	*(117/124)*
Overall	*96.4%*	*(376/390)*

Table IV: Elecsys® vs TPHA

Elecsys® Syphilis	TPHA			
	Positive	Equivocal	Negative	Total
Positive	256	0	4*	260
Equivocal	0	0	0	0
Negative	5**	0	120	125
Total	261	0	124	385

*2/4 of the discordant samples were positive by Immunoblot IgG
**0/5 of the discordant samples were positive by Immunoblot IgG

Percent Agreement

Positive	*98.1%*	*(256/261)*
Negative	*96.8%*	*(120/124)*
Overall	*97.7%*	*(376/385)*

Table V: IMMULITE® vs TPHA

IMMULITE® 2000 Syphilis Screen	TPHA			
	Positive	Equivocal	Negative	Total
Positive	260	0	6*	266
Equivocal	0	0	0	0
Negative	6**	0	120	126
Total	266	0	126	392

*3/6 of the discordant samples were positive by Immunoblot IgG
**0/6 of the discordant samples were positive by Immunoblot IgG

Percent Agreement

Positive	*97.7%*	*(260/266)*
Negative	*95.2%*	*(120/126)*
Overall	*96.9%*	*(380/392)*

Table VI: LIAISON® Treponema Screen vs TPHA

LIAISON® Treponema Screen	TPHA			
	Positive	Equivocal	Negative	Total
Positive	259	0	8*	267
Equivocal	0	0	1	1
Negative	7**	0	117	124
Total	266	0	126	392

*3/8 of the discordant samples were positive by Immunoblot IgG
**0/7 of the discordant samples were positive by Immunoblot IgG

Percent Agreement

Positive	*97.4%*	*(259/266)*
Negative	*93.6%*	*(117/125)*
Overall	*96.2%*	*(376/391)*

Architect, BioPlex® (IgG), Elecsys®, IMMULITE® and LIAISON® show versus TPHA an overall agreement of 96.7%, 96.4%, 97.7%, 96.9% and 96.2% respectively. Their specificity is greater than that of TPHA, based on the Immunoblot results obtained for the discordant samples. Also their sensitivity is grater, since they were able to detect three (LIAISON®, IMMULITE®, Bioplex®, Architect) and two (Elecsys®) more samples as positive compared to TPHA.

Concordance between LIAISON® and four other automated assays

The LIAISON® Treponema test shows an overall agreement of 99.5%, 99.7%, 99.2% and 99.7% versus Architect Syphilis TP, BioPlex® 2200 Syphilis (IgG), IMMULITE® 2000 Syphilis Screen and Elecsys® Syphilis respectively (table VII).

Table VII: Correlation between results of LIAISON® and four other automated assays

LIAISON®	Architect			BioPlex® IgG			Elecsys®			IMMULITE®		
	Pos.	Equiv.	Neg.	Pos.	Equiv.	Neg.	Pos.	Equiv.	Neg.	Pos.	Equiv.	Neg.
Positive	267	0	2	265	2	1	260	0	1	267	0	2
Equivocal	0	0	0	1	0	1	0	0	1	0	0	1
Negative	0	0	122	0	0	122	0	0	123	1	0	121

Percent Agreement

Positive	*100%*	*(267/267)*	*100%*	*(265/265)*	*100%*	*(260/260)*	*99.6%*	*(267/268)*
Negative	*98.4%*	*(122/124)*	*99.2%*	*(122/123)*	*98.4%*	*(123/124)*	*98.4%*	*(121/123)*
Overall	*99.5 %*	*(389/391)*	*99.7%*	*(387/388)*	*99.7 %*	*(383/384)*	*99.2 %*	*(388/391)*

In addition, 7 primary syphilis specimens (i.e., TPHA-negative) scored positive by Architect, BioPlex® IgM, Elecsys®, IMMULITE® and LIAISON® (Table VIII). By contrast, two patients scored negative by BioPlex® IgG.

Table VIII: Primary Syphilis samples

	VDRL	TPHA	Architect	Elecsys®	IMMULITE®	LIAISON®	BioPlex IgM		BioPlex IgG			Immunoblot IgM			
			Abbott	Roche	Siemens	DiaSorin	TpN	TpN	TpN	TpN	TpN	TpN	TmpA	TpN	TpN
							17	47	15	17	47	47		17	15
1	N	N	P	P	P	P	N	P	N	N	N	P	P	P	P
2	N	N	P	P	P	P	N	P	N	N	N	P	N	N	P
3	N	N	P	P	P	P	N	P	N	P	P	P	P	N	P
4	P	N	P	P	P	P	P	P	N	P	N	P	P	P	P
5	N	N	P	P	P	P	P	P	N	P	P	P	P	N	P
6	N	N	P	P	P	P	N	P	N	P	N	P	P	N	P
7	P	N	P	P	P	P	P	P	P	P	P	P	P	P	P

N: negative *P: positive*

DISCUSSION

Architect Syphilis TP, BioPlex® 2200 Syphilis, LIAISON® Treponema, IMMULITE® 2000 Syphilis Screen and Elecsys® Syphilis have excellent sensitivity and specificity performance.
LIAISON® shows an overall agreement › 99% versus Architect, BioPlex® (IgG), IMMULITE® and Elecsys®.
These five fully-automated immunoassays show an overall agreement › 96% versus TPHA and their specificity and sensitivity is greater than that of TPHA, based on the Immunoblot results obtained for the discordant samples.
This evaluation with 7 primary syphilis clearly demonstrates the greater sensitivity of these five fully-automated immunoassays to that of VDRL and TPHA.

CONCLUSION

These five fully-automated immunoassays show an overall agreement › 96% versus TPHA. With their suitability for automation Architect, Elecsys®, IMMULITE® and LIAISON® are an ideal screening test allowing correct identification of all 7 primary infections with one test only. In this study, incorporation of only one recombinant Treponema antigen (TpN17, LIAISON®) is not associated to a lower sensitivity in comparison to the use of three antigens (TpN15, TpN17, TpN47).
The BioPlex® IgM helps to differentiate past infections from recent ones as demonstrated by the negative BioPlex® IgG results for 2 of the 7 primary samples.

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