



Blood and Transplant



Evaluation of Abbott Architect Syphilis TP Assay Product code 8D06

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Introduction

The Microbiological Diagnostics Assessment Service (HPA-MiDAS) in conjunction with the National Transfusion Microbiology Reference Laboratory (NTMRL), North London Blood Centre carried out evaluations of six assays for the Abbott Architect i2000SR analyser. The analyser was installed at the NTMRL where all testing took place.

The object of the evaluations was to assess the ability of the Architect HBsAg, HIV Ag/Ab combo, Anti-HCV, Rubella IgG, anti-CMV and Syphilis TP assays to detect serological evidence of each respective marker in human serum and plasma specimens.

The results of the evaluation of the Syphilis TP assay are presented in this report. The kits were tested against a panel of serum/plasma samples found to be either reactive or unreactive by relevant screening assays used in Europe. In addition, national quality control samples were incorporated in this evaluation.

Abbott Diagnostics provided all equipment, reagents and consumables required for this evaluation. They were responsible for the training of the operators in the use of the analyser and for the installation and ongoing maintenance and repair of any faulty equipment.

HPA-MiDAS was responsible for the testing of the evaluation panel which consisted of anti-*Treponema pallidum* (TP) positive and negative specimens and quality control samples. NTMRL was responsible for the specificity testing of a panel of anti-TP negative ante-natal patients' specimens and sensitivity testing of a panel of specimens previously confirmed as anti-TP positive and also a panel of samples used for the NBS Lot Release Testing program.

Description of the assay

The Abbott Architect Syphilis TP assay is a two-step sandwich chemiluminescent microparticle immunoassay (CMIA) for the qualitative detection of antibody to *Treponema pallidum* (TP) in human serum or plasma. Antibody present in the sample binds to TP antigen (*E. coli*, recombinant) coated paramagnetic particles. After a wash step, murine anti-IgG/anti-IgM acridinium-labelled conjugate is added. Following a further wash step, pre-trigger solution (hydrogen peroxide) and trigger solution (sodium hydroxide) are added. The resulting chemiluminescent reaction is measured in relative light units (RLUs) which are directly proportional to the amount of anti-TP present in the sample.

Table 1: Assay information

General	
Assay name	Architect Syphilis TP
manufacturer/UK agent	Abbott Diagnostics
Product number	8D06
Number of tests per pack	100 / 500
Total sample volume (including 'dead volume')	150µL
Presentation	
Assay type	Two-step chemiluminescent sandwich immunoassay
Solid phase	Paramagnetic microparticles coated with <i>T pallidum</i> antigen (<i>E coli</i> recombinant)
Conjugate	Acridinium-labelled anti-IgG/anti-IgM (murine)
Substrate	Pre-trigger - hydrogen peroxide solution Trigger - sodium hydroxide solution
Negative control	1
Positive control	1
Reading wavelength	n/a - chemiluminescent
Cut-off computation	calibrator 1 mean RLU x 0.20
Equivocal zone	None
Stages	
Preparation/sample well loading	5 minutes
Specimen volume (excluding 'dead volume')	30µL
Incubation status	37°C
Sampling time - 1 sample	1 minute
Total time to completion (from initial loading of first sample)*	
- 1 sample	30 minutes
- 10 samples	34 minutes
- 100 samples	60 minutes
Additional equipment requirement	
Centrifuge	
Latex/nitrile gloves & personal protective equipment	
Note: *These data were observed timings by the evaluator. Information provided by Abbott Diagnostics: Throughput approximately 100 tests in the first hour and 200 tests per hour after the first result is generated.	

Evaluation panel and method

A total of 1466 samples were included in the evaluation panel, Table 2.

The main specificity study was carried out by the NTMRL, by testing 1002 ante-natal patients' specimens previously screened by the Biokit Syphagen TpHA Auto assay (Product code: 3000-5705) and found to be anti-TP negative. NTMRL also tested 206 specimens that had been previously confirmed as positive for anti-TP by the BiomerieuxTrepanostika TP Recomb, Newmarket TPHA 1000, VDRL Murex VD24, Mercia Syphilis M and Innogenetics Syphilis line assays. In addition, a panel of 10 samples which comprised the NBS Lot Release Testing panel were tested.

The evaluation panel used by the HPA-MiDAS totalled 248 specimens. One hundred and seven were anti-TP positive specimens for which disease stage and treatment status were known, 121 anti-TP positive specimens for which disease stage and treatment status were unknown. Specimens whose result was discordant with the expected result were retested in duplicate.

The characterisation of the samples was determined by testing in 15 different anti-TP assays, five of which were agglutination assays and 10 were EIAs^{1,2}. The status of each specimen that gave a discordant result in the assays was confirmed by supplementary assays; the Mercia Syphilis IgM EIA (Microgen Bioproducts, Camberley, England), to confirm or exclude active syphilis, and the INNO-LIA Syphilis (Innogenetics, Ghent, Belgium). The INNO-LIA is a line immunoassay which uses recombinant and synthetic polypeptide *T. pallidum* antigens. Its use as a confirmatory test for syphilis has previously been evaluated³.

Seven anti-TP IgM positive obtained from a commercial source were included in the panel, however confirmation of the IgM status has not been carried out by HPA-MiDAS. Ten anti-TP negative specimens were also included. In addition, three quality control samples, from the HPA and from a commercial source, were included in the panel.

Table 2: Evaluation panel

Sample category	Number
NTMRL	
1. Anti-treponemal negative (antenatal patients' sera)	1002
2. Anti-treponemal positive (antenatal and donors' sera)	206
3. Lot release testing panel	10
HPA-MiDAS	
1. Anti-Treponema negative	10
2. Positive samples	
Known disease stage (Impath-BCP & CFI) (n = 107)	
Primary syphilis	5 treated 29 untreated
Secondary syphilis	5 treated 37 untreated
Early latent syphilis	4 treated 15 untreated
Late latent syphilis	8 treated 4 untreated
Unknown disease stage (n = 128)	
Profile specimens / SNBTS plasma / CFI specimens	121
Marcel Merieux IgM Positive specimens	7
3. QC specimens	
HPA: Syphilis QC1 3x	1
HPA: Syphilis QC2 3x	1
Virotrol Syphilis Total 3x	1
Total (number of specimens)	1466
Notes:	
NLBC = North London Blood Centre, Colindale	
BCP = Impath-BioClinical Partners Inc., USA (now known as ZeptoMetrix Corporation). All clinical information provided by Impath-BCP.	
Specimens from individuals that have been treated range from > 1 month to many years post treatment. If bled within 1 month of treatment, then specimens placed in 'untreated' category (2 E-L and 1 SEC).	
SNBTS = Scottish National Blood Transfusion Service	
HPA = Health Protection Agency	

The method described in the kit insert was strictly followed. Abbott Architect i2000SR is a fully automated analyser; all processing steps are performed on the instrument. The Architect assay parameters are factory set and defined in the system software.

A daily maintenance program is followed each day, the steps for which are prompted on the display screen. Principally, Probe Conditioning Solution and sodium hypochlorite solution are loaded onto the analyser by the operator and the analyser completes the program automatically. This process takes approximately 20 minutes. In addition, a weekly maintenance program is also required in which the sample, reagent and wash probes are cleaned with cotton-wool swabs soaked in distilled water and the air filters are cleaned.

Prior to running the analyser, test reagents, pre-trigger solution, trigger solution, wash buffer and reaction vessels are loaded onto the analyser and automatically primed and loaded as appropriate. The latter two may also be added whilst the analyser is in 'Running' mode.

Prior to running a new batch of an assay, a calibration must first be performed. The calibrators are provided in dropper bottles and an appropriate volume is placed into sample cups and loaded onto the analyser. The calibration is valid for all subsequent tests using that particular lot number; it is not time limited. For the Syphilis TP assay, one calibrator is supplied which is run in triplicate. The mean of the triplicate RLU values x 0.20 is calculated to provide the cut-off for the reagent lot.

Two Syphilis TP kit controls are provided by Abbott. It is recommended that these are run at least once within every 24 hours that the test is in use. The Syphilis TP controls consist of a Negative Control (S/CO ≤ 0.4) and a Positive Control (S/CO range 1.25-3.75).

Specimens may be loaded in their primary tubes, if suitable for the analyser, or aliquots made into Architect sample cups. Sample processing is initiated by the loading of the samples onto the analyser. The reactions occur in the following processing sequence: -

- A reaction mixture is formed combining sample, sample diluent and microparticles in the reaction vessel.
- After the first incubation is complete, the reaction mixture undergoes a wash step. A magnetic field is applied to retain the paramagnetic microparticles within the reaction vessel during the wash procedure.
- The anti-IgG/anti-IgM/acridinium conjugate is then added and a further incubation takes place.
- Following a second wash step, pre-trigger (hydrogen peroxide) and trigger (sodium hydroxide) solutions are added to the reaction vessel
- The resultant chemiluminescent signal is measured and expressed as Relative Light Units.

The time taken from loading a sample to obtaining a result was 30 minutes for the Syphilis TP assay. Subsequent results are obtained every 18 seconds, assuming continuous loading of samples. (18 seconds is a set cycle time and does not vary.)

The results are expressed as sample/cut-off (S/CO); S/CO value < 1.0 is considered nonreactive and S/CO ≥ 1.0 is considered reactive.

Specificity

One thousand and two antenatal specimens were tested in the Architect Syphilis TP assay by NTMRL.

All 1002 samples were nonreactive by the Architect Syphilis TP assay to give a specificity of 100% (95% confidence interval 99.6-100%). The S/CO range was 0.02-0.83 with mean and median values of 0.07 and 0.06, respectively, Table 3.

Table 3: Specificity of the Architect syphilis assay when tested against 1002 anti-TP negative specimens

Number tested	Number initially reactive	Mean	Median	Range
		S/CO		
1002	0	0.07	0.06	0.02-0.83

Ten anti-TP negative specimens were interspersed among the anti-TP positive specimens when tested by the HPA-MiDAS. All 10 samples were nonreactive by the Architect Syphilis TP assay, Table 4.

Table 4: Architect syphilis assay results for 10 anti-TP negative specimens interspersed among anti-TP positive specimens

Number tested	Number initially reactive	Mean	Median	Range
		S/CO		
10	0	0.09	0.08	0.04-0.25

Sensitivity

Two hundred and twenty-eight anti-TP positive samples were tested in the Architect Syphilis TP assay, lot number 50033LP22, by the HPA-MiDAS. One hundred and seven anti-TP positive specimens were from individuals whose disease stage and treatment status was known and 121 specimens from individuals whose disease stage and treatment status was unknown. On initial testing, 227 of the 228 samples were reactive by the assay, to give an overall sensitivity of 99.56% (95% confidence interval 97.6-100%), and one was nonreactive (see below).

Two hundred and thirteen of the 228 anti-TP positive samples had been tested in 12 other assays previously evaluated. The comparative initial sensitivities for the thirteen assays are presented in Table 5.

The reactivities in the Architect Syphilis TP for the 228 anti-TP positive samples according to disease stage and treatment status are shown in Table 6.

One of the anti-TP positive samples was initially nonreactive in the Architect Syphilis TP assay and again nonreactive on repeat testing. This sample was from the primary syphilis untreated category and had been nonreactive when tested previously in enzyme immunoassays but was positive by darkfield microscopy, Table 7.

Table 5: Comparative initial sensitivity of the Architect Syphilis TP assay when tested against 213 anti-TP positive specimens (HPA-MiDAS panel)

Assay	Product code	Number tested	Number positive	Sensitivity (95% confidence interval)	Mean	Median	Range
Architect Syphilis TP	8D06	213	213	100% (97.6-100%)	19.98	22.2	1.14-29.62
Adaltis EIAGEN Syphilis New Generation	81043	213	213	100% (98.3-100%)	16.91	16.71	1.95-24.36
Bioelisa Syphilis 3.0	3000-1149	213	213	100% (98.3-100%)	8.26	8.36	2.72-10.38
Abbott Murex ICE Syphilis	500E-8E04-01	213	213	100% (98.3-100%)	12.32	13.39	1.44-13.91
BioKit Bioelisa Syphilis 3.0	3000-1148	213	213	100% (98.3-100%)	9.26	9.73	1.10-11.90
bioMérieux Trepanostika TP Recombinant	285034	213	213	100% (98.3-100%)	7.92	8.77	1.32-9.20
Bio-Rad Syphilis Total	72514	213	211	99.1% (96.6-99.9%)	23.64	28.01	0.42-32.67
Dade Behring Enzygnost Syphilis	OWV021	213	211	99.1% (96.6-99.9%)	11.35	8.09	0.78-103.40
Diesse Enzywell Syphilis	91100	213	212	99.5% (97.4-100%)	6.48	7.04	0.89-9.82
Microgen Bioproducts Mercia Syphilis	M4033	213	204	95.8% (92.1-98.1%)	3.81	3.48	0.69-9.93
Newmarket Laboratories Syphilis EIA II	60093	213	213	100% (98.3-100%)	19.18	19.85	2.91-33.05
Omega Pathozyne Syphilis Competition	OD117	213	204	95.8% (92.1-98.1%)	4.24	3.8	0.73-11.55
Trinity Biotech Captia Syphilis	850-065	213	209	99.1% (96.6-99.9%)	23.69	28.87	0.42-32.67

Table 6: Results for 228 anti-TP positive specimens according to disease stage and treatment status

Treatment status	Number reactive/number tested				
	Primary	Secondary	Early-latent	Late latent	Unknown
Treated	5/5	5/5	4/4	8/8	N/A
Untreated	28/29	37/37	15/15	4/4	N/A
Unknown	N/A	N/A	N/A	N/A	121/121

Note: N/A = not applicable

Table 7: Results for nonreactive sample

Sample number	Architect Syphilis TP			Report MHRA 04109									
	Initial result	Repeat results		Assay 1	Assay 2	Assay 3	Assay 4	Assay 5	Assay 6	Assay 7	Assay 8	Assay 9	Assay 10
02S0040*	0.065	0.056	0.057	0.120	0.263	0.254	0.245	0.058	0.276	0.127	0.492	0.457	0.435

Notes. Assays 1-10: results from MHRA evaluation carried out in 2004.
* This specimen was TP positive by dark ground immunofluorescence.

NTMRL tested a panel of specimens that had been previously confirmed anti-TP positive according to the NTMRL confirmatory algorithm. 204 of the 206 specimens were initially reactive in the Architect Syphilis TP assay to give an initial sensitivity of 99.03% (95% confidence interval 96.5-99.9%). The S/CO values of the two specimens that tested negative were 0.57 and 0.85. After retesting in duplicate, the two initial negative samples were reactive (S/CO values 1.60/1.64 and 1.12/1.14, respectively) to give a repeat sensitivity of 100% (95% confidence interval 98.52-100%), Table 8.

Table 8: Sensitivity of the Architect syphilis assay when tested against 206 anti-TP positive specimens confirmed by the NTMRL syphilis algorithm

	Number tested	Number positive	Sensitivity	95 % Confidence interval	Range	Mean	Median
Initial sensitivity	206	204	99.03%	96.5-99.9%	0.57-32.21	17.70	18.41
Repeat sensitivity	206	206	100%	98.2-100%	1.13-32.21	17.71	18.41

A panel of ten samples, eight anti-TP positive and 2 anti-TP negative, used for lot release testing by the NBS were tested in the Architect Syphilis TP assay by NTMRL. Six of the eight anti-TP positive samples were reactive and two were nonreactive in the Architect Syphilis TP assay. Both anti-TP negative samples were nonreactive in the Architect Syphilis TP assay, Table 9.

Table 9: NTMRL Syphilis lot release testing panel tested by Architect Syphilis assay

Panel/Ctrl Member	Expected Ratios	Expected Result	Architect S/CO	Architect Result
1	>0.6	Negative	0.63	Nonreactive
2	>1	Positive	1.31	Reactive
3	>4.5	Positive	2.38	Reactive
4	>5.5	Positive	4.83	Reactive
5	<1	Negative	0.12	Nonreactive
6	>2.5	Positive	1.46	Reactive
7	>1.2	Positive	1.43	Reactive
8	>2	Positive	1.90	Reactive
9	>1	Positive	0.46/0.42	Nonreactive
10	>1	Positive	0.68/0.62	Nonreactive

HPA-MiDAS tested a panel of seven anti-TP IgM positive samples that had been sourced from Marcel Mérieux, France (now Biomnis). Five of the seven samples were reactive in the Architect Syphilis TP assay. Of the two samples nonreactive in the Architect Syphilis TP assay, one was nonreactive in both comparative assays and one was positive in one comparative assay and negative in the other, Table 10.

Table 10: Results of testing 7 anti-TP IgM positive specimens

Assay	Product number	Sample number						
		OD/CO						
		1	2	3	4	5	6	7
Architect Syphilis TP	8D06	25.43	26.06	0.11	28.03	0.62	19.06	21.05
Adaltis EIAGEN Syphilis New Generation*	81043	11.54	15.81	0.18	15.02	1.17	7.38	22.83
Bioelisa Syphilis 3.0*	3000-1149	10.09	10.37	-0.02	10.64	0.05	7.58	10.22
Newmarket Syphilis IgM**		24.236	25.462	3.344	0.936	0.357	4.413	1.914
VDRL**		Pos	Neg	Pos	Pos	Pos	Neg	Pos

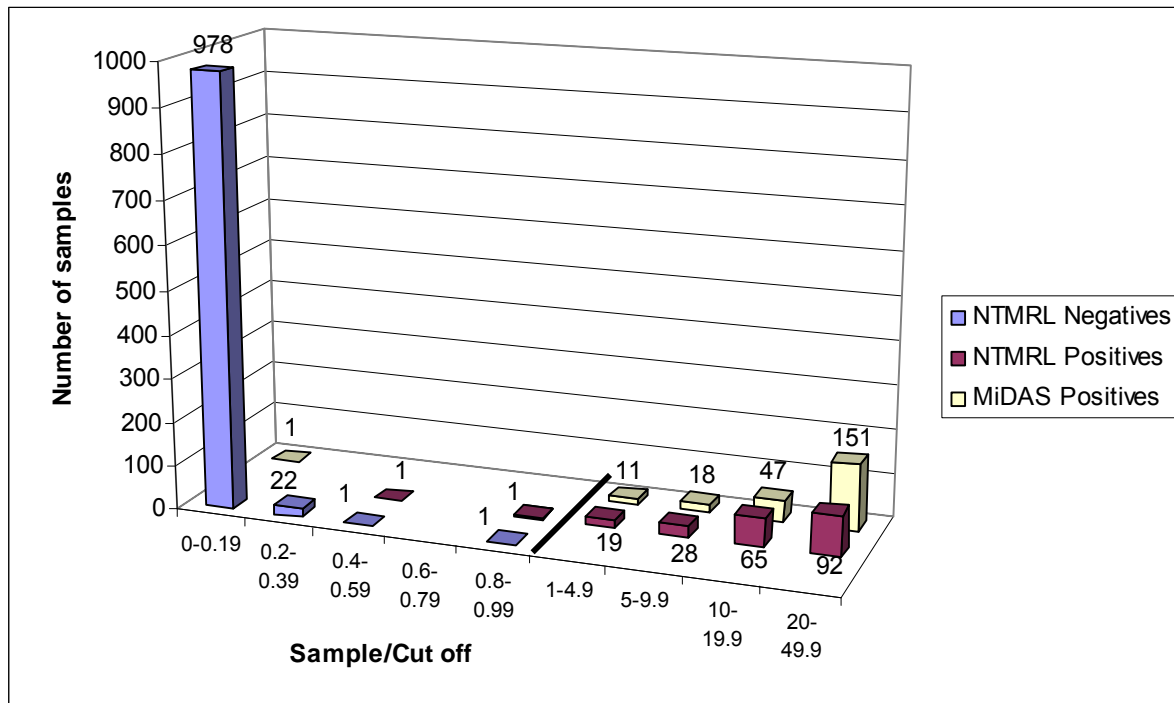
Notes: *Results from MiDAS evaluation PER07001. **Results generated by NTMRL.

Distribution of reactivities

The distribution of initial reactivities for the 1002 anti-TP negative and 206 anti-TP positive samples tested by NTMRL and the 228 anti-TP positive samples tested by HPA-MiDAS are shown in Figure 1. Assays with good discrimination have few or no samples wrongly classified and few reactions close to the cut-off.

All 1002 anti-TP negative specimens were nonreactive by the Architect Syphilis TP assay. Two of 206 specimens from the NTMRL anti-TP positives panel were initially falsely negative which were reactive after retesting. One of 228 specimens from the HPA-MiDAS anti-TP positive panel was initially non-reactive and remained unreactive after retests.

Figure 1: Distribution of initial reactivities



Note: The scale used for the S/CO values is not continuous.

Lot comparison

A subset of the main evaluation panel was tested in a second lot of the Architect Syphilis TP assay (Lot number 50031LP22). Forty anti-TP positive specimens, 10 negative specimens and three quality control samples were compared, Tables 11a, 11b and 11c. The S/CO results obtained from both lots were similar for all specimens tested.

Table 11a: Two kit lots tested against 40 anti-TP positive specimens

Anti-TP positive	Lot 1 50033LP22	Lot 2 50031LP22
Number tested	40	40
Number reactive	40	40
Mean	17.89	18.71
Median	21.59	22.66
Range	2.35-29.62	2.32-29.97

Table 11b: Two kit lots tested against 10 anti-TP negative specimens

Anti-TP negative	Lot 1 50033LP22	Lot 2 50031LP22
Number tested	10	10
Number reactive	0	0

Table 11c: Two kit lots tested against 3 quality control samples

Quality control sample	Lot 1 50033LP22				Lot 2 50031LP22			
	Test 1	Test 2	Test 3	Mean	Test 1	Test 2	Test 3	Mean
HPA QC1	1.77	1.61	1.65	1.68	1.86	1.97	2.02	1.95
HPA QC2	16.36	15.93	16.55	16.28	16.81	16.90	16.73	16.81
Virotrol Total	13.93	13.72	13.27	13.64	13.37	14.33	14.27	13.99

A quality control sample/statistical assay control should be chosen to have a reactivity within the linear dynamic range of the assay. Our findings suggest that all three controls tested would be suitable for use in the Architect Syphilis TP assay.

Conclusion

All 1012 anti-TP negative specimens were nonreactive by the Architect Syphilis TP assay to give a reactive rate of 0.0%.

When tested with the HPA-MiDAS anti-TP positive panel, the Architect Syphilis TP assay detected 227 of the 228 specimens to give an initial sensitivity of 99.6%. After retesting, the initially nonreactive specimen was again nonreactive (this specimen was also nonreactive in all other EIAs in which it was tested but had been positive by dark-ground microscopy).

When tested with the NTMRL anti-TP positive panel, the Architect Syphilis TP assay detected 204 of the 206 specimens to give an initial sensitivity of 99.03%. After retesting, the two initially nonreactive specimens were weakly reactive (repeat sensitivity 100%).

A small lot comparison was undertaken in which both lots of the Architect Syphilis TP assay gave similar results for all samples tested.

References

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