



An assessment of Adaltis EIAgen™ Syphilis New Generation assay

Christine Burgess, Keith Perry

Microbiological Diagnostics Assessment Service
Evaluations and Standards Laboratory
Health Protection Agency - Centre for Infections
61 Colindale Avenue
London
NW9 5EQ

**Report PER07001_1
October 2007**

Study Aim

The ability of the Adaltis EIAgen™ Syphilis New Generation to correctly identify anti-treponemal positive and negative serum/plasma specimens was assessed. The status of the specimens had previously been determined by application of other screening and supplementary assays for detection of *Treponema pallidum*. The specimens were also tested concurrently by the Bioelisa Syphilis v3.0 assay to ensure that no deterioration of the samples had taken place during storage and after freeze-thawing. The Bioelisa Syphilis v3.0 and nine other EIA assays and five agglutination assays for the detection of antibody to *T. pallidum* have previously been evaluated by us^{1,2,3}.

Description of the assay

The Adaltis EIAgen™ Syphilis New Generation (product number 081043/081044) is a semi-quantitative enzyme immunoassay for the detection of *Treponema pallidum* antibodies in human serum or plasma. Antigens representing immunodominant epitopes of *T. pallidum* are coated onto polystyrene microtitre wells. Serum or plasma is added to the wells and, after a short incubation, antigen conjugated to biotin is added. If antibodies specific to the antigens are present in the sample, they form stable complexes with the *T. pallidum* antigens on the microtitre plate and with the biotinylated antigens. A second conjugate consisting of streptavidin-horseradish peroxidase is added and further incubated. This step is followed by a wash step and the addition of tetramethylbenzidine substrate. The wells are incubated in the dark and the development of a blue colour indicates the presence of antibody to *T. pallidum* in the test sample. If the specimen does not contain specific antibodies there will be no colour development in the assay wells. See Table 1 for further information on the assay.

Evaluation method

The assays were performed according to the manufacturer's instructions. The assessment panel comprised 462 specimens of which 221 were anti-treponemal positive specimens (213 from a previous comparative evaluation and eight additional anti-treponemal IgM positive specimens), 240 were anti-treponemal negative, unselected blood donors' serum/plasma specimens and one was a quality control sample, *Appendix Table 8*. Of the 213 positive specimens, 28 were primary infection specimens, 38 secondary, 18 early latent, 11 late latent, and the remaining 118 specimens were positives of unknown disease stage.

The specific anti-treponemal status of specimens was confirmed using a range of other syphilis kits including the Serodia TPPA (Fujirebio Inc), Biokit TPHA (Biokit Ltd), Murex ICE Syphilis EIA (Abbott Laboratories) and the Newmarket Syphilis EIA II (NewMarket Laboratories). Any specimens that gave discordant results were additionally tested using the Mercia Syphilis IgM EIA (Microgen Bioproducts) and the INNO-LIA Syphilis (Innogenetics). The IgM EIA was used to detect early infection and the INNO-LIA was used to assess the response to individual antigens.

Table 1. Assay information

General	
Assay name	EIAgen™ Syphilis New Generation
Manufacturer / UK agent	Adaltis Italia
Product number	081043 / 081044
Number of tests in one pack	192 / 480
Specimen volume	50µL
Presentation	
Assay type	One-step sandwich ELISA
Solid phase	12 x 8 microtitre plate wells
Coating	Antigens representing immunodominant epitopes of <i>T pallidum</i>
Conjugate 1	Biotinylated <i>T pallidum</i> antigens
Conjugate 2	Horseradish peroxidase-streptavidin
Substrate	Tetramethylbenzidine
Controls per plate	6
Negative control	1 (3 replicates)
Antibody positive control	1 (3 replicates)
Reading wavelength	450 / 600-650
Cut-off computation	(Mean neg control + mean pos control) / 8
Equivocal zone	=>0.85<1.0 OD/CO
Stages	
Preparation / sample well loading*	30 minutes
Prewash of reaction plate	n/a
Incubation status	Static
Sample + conjugate 1 incubation*	15 minutes 18-25°C
Conjugate 2 incubation	30 minutes 37°C
Number of washes	5
Substrate incubation (time/temp)	15 minutes 18-25°C
Stop solution incubation	5 minutes
Reading	5 minutes
Total incubation times	70 minutes
Approximate time to completion	110 minutes
Number of optional procedures	none
Additional equipment required	
Incubator, type not specified (**Dry incubator)	
Microplate spectrophotometer (**EL 808)	
Micropipettes: 40 - 200µL, 200 - 1000µL & 2 - 10mL	
Multichannel pipettes: 50 - 300µL	
Disposable tips	
Reagent troughs and bottles	
Measuring cylinder	
Distilled water	
Notes:	
* Due to the short R° first incubation, the samples were first aliquotted into an uncoated plate and transferred into the test plate using a multichannel pipette. ** Equipment used in this evaluation.	

Specificity

The panel used to assess specificity comprised 240 anti-treponemal negative, unselected blood donors' specimens. On initial testing 235 of the 240 were unreactive by the Adaltis EIAgen™ New Generation assay to give an initial reactive rate of 2.1% (95% confidence interval 0.7-4.8%). In comparison, all 240 samples were unreactive by the Biolisa Syphilis 3.0 assay.

Table 2a shows the comparative initial reactive rate and specificity against 10 kits evaluated in 2004, using the same anti-TP negative specimen panel.

After retesting, all five samples initially reactive by Adaltis EIAGEN™ New Generation were unreactive to give a repeat specificity of 100% (95% confidence interval 98.5-100%), Table 2b. The OD/CO values for the initial and repeat testing of the five specimens are shown in Table 3.

Table 2a: Comparative initial reactivity

Assay	Product code	Number tested	Number reactive	Initial reactive rate	Initial specificity (95% confidence interval)	Mean OD/CO	Median OD/CO	Range OD/CO
Adaltis EIAGEN Syphilis New Generation	81043	240	5	2.10%	97.9% (95.2 - 99.3%)	0.34	0.23	0.09-11.36
Bioelisa Syphilis 3.0	3000-1149	240	0	0%	100% (98.5 - 100%)	0.03	0.03	-0.04 - 0.43
Abbott Murex ICE Syphilis	500E-8E04-01	240	2	0.83%	99.17% (97.0 - 99.9%)	0.32	0.30	0.19 - 1.56
Biokit Bioelisa Syphilis 3.0	3000-1148	240	1	0.42%	99.58% (97.7 - 100%)	0.04	0.02	0.01 - 2.39
bioMérieux Trepanostika TP Recombinant	285034	240	0	0%	100% (98.5 - 100%)	0.14	0.13	0.09 - 0.29
Bio-Rad Syphilis Total	72514	240	2	0.83%	99.17% (97.0 - 99.9%)	0.22	0.20	0.09 - 1.52
Dade Behring Enzygnost Syphilis	OWV021	240	6	2.50%	97.5% (94.6 - 99.1%)	0.58	0.52	0.41 - 3.77
Diesse Enzywell Syphilis	91100	240	1	0.42%	99.58% (97.7 - 100%)	0.23	0.22	0.17 - 2.17
Microgen Bioproducts Mercia Syphilis	M4033	240	0	0%	100% (98.5 - 100%)	0.49	0.49	0.37 - .70
Newmarket Laboratories Syphilis EIA II	60093	240	0	0%	100% (98.5 - 100%)	0.10	0.09	-0.01 - 0.38
Omega Pathozyme Syphilis Competition	OD117	240	0	0%	100% (98.5 - 100%)	0.53	0.52	0.38 - 0.83
Trinity Biotech Captia Syphilis	850-065	240	0	0%	100% (98.5 - 100%)	0.14	0.13	0.07 - 0.32

Table 2b: Comparative repeat reactivity

Assay	Product code	Number tested	Number reactive	Repeat reactive rate	Repeat specificity (95% confidence interval)	Mean OD/CO	Median OD/CO	Range OD/CO
Adaltis EIAGEN Syphilis New Generation	81043	240	0	0%	100% (98.5 - 100%)	0.25	0.23	0.09-0.66
Bioelisa Syphilis 3.0	3000-1149	240	0	0%	100% (98.5 - 100%)	0.03	0.03	-0.04 - 0.43
Abbott Murex ICE Syphilis	500E-8E04-01	240	2	0.83%	99.17% (97.0 - 99.9%)	0.32	0.30	0.19 - 1.34
Biokit Bioelisa Syphilis 3.0	3000-1148	240	0	0%	100% (98.5 - 100%)	0.03	0.02	0.01 - 0.52
bioMérieux Trepanostika TP Recombinant	285034	240	0	0%	100% (98.5 - 100%)	0.14	0.13	0.09 - 0.29
Bio-Rad Syphilis Total	72514	240	0	0%	100% (98.5 - 100%)	0.21	0.20	0.09 - 0.43
Dade Behring Enzygnost Syphilis	OWV021	240	0	0%	100% (98.5 - 100%)	0.53	0.52	0.41 - 0.75
Diesse Enzywell Syphilis	91100	240	0	0%	100% (98.5 - 100%)	0.22	0.22	0.17 - 0.38
Microgen Bioproducts Mercia Syphilis	M4033	240	0	0%	100% (98.5 - 100%)	0.49	0.49	0.37 - .70
Newmarket Laboratories Syphilis EIA II	60093	240	0	0%	100% (98.5 - 100%)	0.10	0.09	-0.01 - 0.38
Omega Pathozyme Syphilis Competition	OD117	240	0	0%	100% (98.5 - 100%)	0.53	0.52	0.38 - 0.83
Trinity Biotech Captia Syphilis	850-065	240	0	0%	100% (98.5 - 100%)	0.14	0.13	0.07 - 0.32

Table 3. OD/CO values for initially reactive specimens

Sample number	Initial result	Repeat results	
	OD/CO	Rep 1 OD/CO	Rep 2 OD/CO
03S0033c	1.711	0.265	NT
03S0049c	11.361	0.237	NT
03S0105c	1.582	0.170	0.193
03S0142c	2.414	0.137	0.137
03S0242c	4.314	0.147	0.162

Sensitivity

Two hundred and thirteen anti-treponemal positive specimens were included in the specimen panel. The Adaltis EIAGEN™ Syphilis New Generation assay detected all 213 specimens to give a sensitivity of 100% (95% confidence interval 98.3-100%). Table 4 shows results compared with the Bioelisa Syphilis 3.0 assay, with which the samples were tested contemporaneously, and with the results for ten assays that were evaluated in 2004.

Table 4: Comparative initial sensitivity for the Adaltis EIAGEN™ Syphilis New Generation assay

Assay	Product code	Number tested	Number positive	Sensitivity (95% confidence interval)	Mean	Median	Range
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 Pathozyme 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

The panel of 213 positive specimens consisted of 118 specimens of unknown disease stage and 95 specimens for which the disease stage and treatment status was known. A breakdown of the results for these samples is shown in Table 5.

Table 5: Results 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	23/23	33/33	14/14	3/3	N/A
Unknown	N/A	N/A	N/A	N/A	118/118

Note: N/A = not applicable

Eight IgM anti-treponemal positive specimens were tested that were not part of the 2004 comparative evaluation panel. The samples had been obtained from Marcel Merieux, France, and supplied pre-characterised as anti-TP IgM positive. No further confirmatory testing has been performed by HPA-MiDAS.

Of the eight specimens, seven were reactive and one was unreactive in the Adaltis EIAgen™ New Generation assay. The Bioelisa Syphilis 3.0 assay detected six of the samples and two were unreactive, Table 6.

Table 6: Results for eight IgM anti-treponemal specimens

Assay	Product number	Sample number								OD/CO
		1	2	3	4	5	6	7	8	
Adaltis EIAgen Syphilis New Generation	81043	11.54	15.81	0.18	15.02	12	1.17	7.38	22.83	
Bioelisa Syphilis 3.0	3000-1149	10.09	10.37	-0.02	10.64	9.47	0.05	7.58	10.22	

Results for Quality Control sample

The HPA Anti-Syphilis QC1, product code QCRSYPHQC1, was included in the evaluation to fulfill internal quality control procedures. The QC sample was run up to three times on each test plate for the Adaltis EIAgen™ New Generation assay evaluation.

For lot 617012 of the Adaltis EIAgen™ New Generation assay, the S/CO range for the 19 replicates of HPA QC1 was 13.7-20.9, with mean and median values of 17.6 and

17.8 respectively. An overview of the 19 results generated for lot 6170012 and the three results for lot 617022 of the Adaltis EIAgen™ New Generation is shown in Table 7.

Comparison of two kit lots

Two lot numbers of the Adaltis EIAgen™ New Generation assay were compared using a subset of the main evaluation panel. A total of 49 samples were compared; 24 anti-treponemal negative, 24 anti-treponemal positive and one quality control sample. All specimens gave similar results in both lots of the Adaltis EIAgen™ New Generation assay, Table 7.

Table 7: Results for two lots of the Adaltis EIAgen™ Syphilis New Generation assay

Sample category	Number tested	Number reactive	Mean	Median	Range
Positive					
Lot 1: 6170012	24	24	18.94	19.34	12.78-20.48
Lot 2: 6170022	24	24	25.28	27.28	11.53-31.70
Negative					
Lot 1: 6170012	24	0	0.26	0.23	0.12-0.45
Lot 2: 6170022	24	0	0.34	0.32	0.25-0.64
HPA QC1					
Lot 1: 6170012	19 replicates	19	17.60	17.77	13.74-20.97
Lot 2: 6170022	3 replicates	3	18.67	18.99	17.60-19.43
Total number of samples	49				

Technical Appraisal

The kit instructions were clear and well presented with sufficient explanation and safety instructions. The packaging was clearly labelled and easy to identify. All reagents were supplied in clear plastic bottles except for the substrate solution which was in a brown opaque bottle. Conjugate 1 was coloured red and conjugate 2 was coloured blue, all other reagents were clear. The controls, conjugates 1 and 2, substrate and stop solution were supplied as 'ready-to-use'. The wash buffer was supplied as a concentrated solution requiring dilution at 1:25 in distilled water.

Conclusions

When tested against a panel of 240 negative serum/plasma specimens the Adaltis EIAgen™ New Generation assay gave an initial reactive rate of 2%. The final specificity, following retests, was 100%.

The sensitivity was 100%, based on testing anti-treponemal positive specimens from various disease stages and known treatment status, and those for which disease stage and treatment was unknown. An additional eight anti-treponemal IgM positive specimens were tested of which seven were detected by Adaltis EIAgen™.

In a small study of lot comparability, both lots of the assay gave very similar results for both anti-treponemal positive and negative samples.

The high sensitivity for all disease stages and treatment status of the samples included in this evaluation of the Adaltis EIAgen™ New Generation assay would make this a suitable assay for screening purposes.

References

1. **Cole M, Dean L, Perry KR, Parry JV** (2004): Five syphilis agglutination assays; *Medicines and Healthcare products Regulatory Agency Report 04007*, p1-39; HMSO - ISBN 1 84182 816 5. Available from www.hpa-midas.org.uk/reports
2. **Cole M, Perry K** (2004): Ten Syphilis EIAs; *Medicines and Healthcare products Regulatory Agency Report 04109*, p1-47; HMSO - ISBN 1 84182 889 0. Available from www.hpa-midas.org.uk/reports
3. **Cole MJ, Perry KR, Parry JV** (2007) Comparative evaluation of 15 serological assays for the detection of syphilis infection; *European Journal of Clinical Microbiology & Infectious Diseases* 26 (10) 705-13 <http://dx.doi.org/10.1007/s10096-007-0346-9>

Appendix

Table 8: Evaluation panel

Sample category	Number
1. Blood donors' sera from NLBC	240
2. Positive samples with known disease stage (Impath-BCP & Cfl) (n = 95)	
Primary syphilis	
Treated	5
Untreated	23
Secondary syphilis	
Treated	5
Untreated	33
Early latent syphilis	
Treated	4
Untreated	14
Late latent syphilis	
Treated	8
Untreated	3
3. Positive specimens with unknown disease stage	
Profile specimens / SNBTS / Cfl specimens	118
Marcel Merieux IgM positive specimens	8
4. QC specimen	
HPA QCRU Syphilis QC1	1
Total number of specimens	462
Notes	
NLBC = North London Blood Centre, Colindale BCP = Impath-BioClinical Partners Inc., USA. 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). Cfl = Centre for Infections, Health Protection Profile = Profile Diagnostics Inc., US SNBTS = Scottish National Blood Transfusion Service QCRU = Quality Control Reagents Unit, HPA, UK	



October 3rd 2007

Dr. Keith Perry
Microbiological Diagnostics Assessment Service
61, Colindale Avenue
London NW9 5HT

Re: Report, An Assessment of Adaltis EIAgen™ Syphilis assay.

Dear Dr. Perry,

First and foremost, we would like to thank you for giving us the opportunity to comment on this report. We would also like to mark our appreciation to Dr. Christine Burgess for the quality of her work and her availability throughout this evaluation.

We are very pleased with the results that you obtained, as sensitivity and specificity of 100% were observed with the various panels tested; these results make EIAgen Syphilis New Generation one of the best Syphilis assays on the market.

The excellent accuracy you observed and the results obtained with the Quality Control Reagents confirm our internal results and our other external evaluations. Finally, your conclusion about the user-friendliness and the suitability of our kit for screening is also well appreciated.

Yours sincerely,

A handwritten signature in black ink, appearing to read "Michel Houde".

Michel Houde Ph.D.

Head of Research
Adaltis Development inc.
Laval, Quebec, Canada

Adaltis inc.
10900 Hamon street
Montreal (Qc)
Canada H3M 3A2
www.adaltis.com