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Regulatory Agency

NUMBER

Evaluation Report **MHRA 04007**

Five Syphilis Agglutination Assays

MHRA Evaluation Report
MHRA Report number 04007



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Five Syphilis Agglutination Assays

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Background

Four *T.pallidum* haemagglutination assays (Biokit S.A., Axis-Shield Diagnostics Ltd., Newmarket Laboratories Ltd., Randox Laboratories Ltd.) and one *T.pallidum* particle agglutination assay (Fujirebio Inc.) were evaluated to determine their ability to detect anti-treponemal antibodies.

Evaluation panel

The evaluation panel consisted of 488 specimens. Of these, 250 were anti-treponemal negative specimens from blood donors, 235 were anti-treponemal positive specimens and three were quality control samples. The positive category comprised 114 specimens from individuals whose disease stage and treatment status was known and 121 specimens from individuals whose disease stage and treatment status was unknown.

Specificity findings

Of the 250 anti-treponemal negative specimens, one was initially reactive by the Newmarket and the Biokit TPHAs, two by the Randox TPHA and four by the Axis-Shield TPHA. The Serodia TPPA gave no false positive reactions. Following retests the Biokit TPHA gave one repeatedly reactive specimen, to give a final specificity of 99.6%. The other four kits gave no repeatedly reactive specimens resulting in final specificities of 100%.

Sensitivity

Known disease/treatment category

Of the 114 specimens from individuals with known disease stage and treatment status the Serodia TPPA and Randox TPHA detected 113, both giving initial sensitivities of 99.1%. The initial sensitivities of the Biokit, Axis-Shield and Newmarket TPHAs were 98.2%, 97.4%, and 96.5% respectively. Following retests, the Serodia TPPA was still the most sensitive kit with a sensitivity of 99.1%. Biokit was the most sensitive TPHA with a repeat sensitivity of 97.3%, although one specimen was not included in the sensitivity calculation. This specimen agglutinated the control cells but in the absence of an absorption procedure no interpretation could be made. The Randox, Axis-Shield and Newmarket TPHAs had repeat sensitivities of 95.6%, 93.9% and 93.9% respectively.

Unknown disease/treatment category

Of the 121 specimens from individuals with unknown disease stage and treatment status, Serodia TPPA detected 121 (100%). For this group the initial sensitivities were 100% for Biokit (nine specimens not included in the sensitivity calculation), 98.3% for Randox TPHA, 97.5% for Axis-Shield TPHA and 96.7% for Newmarket TPHA. After repeat testing, the Serodia TPPA had a sensitivity of 100%, Biokit TPHA 98.3% (two specimens not included in the sensitivity calculation), Randox TPHA 95.9%, Axis-Shield TPHA 95.9% and Newmarket TPHA 91.7%.

The sensitivity calculations included positive and indeterminate reactions. All the repeat sensitivities, other than the Serodia TPPA, decreased after retesting. This was because many of the initial indeterminate reactions were negative after retesting.

Technical appraisal

All the agglutination assays were similar and very easy to use. The main difficulty experienced was the subjective reading of results by eye. Of the five kits, the Serodia TPPA was the easiest to interpret and the agglutination patterns were the most stable.

Conclusion

Overall the Serodia TPPA was the most suitable assay for screening of all disease stages. Compared with Serodia, the TPHAs had lower sensitivities, particularly related to specimens from primary, late latent, and the unknown disease stage categories. TPHA/TPPAs generally detect infection during the fourth week⁶, so access to follow up specimens, the patient's clinical data and other confirmatory tests are all important in making a diagnosis.

Syphilis is a sexually transmitted infection (STI) caused by the spirochaete *Treponema pallidum* subspecies *pallidum*. There were 1193 diagnoses of syphilis in genitourinary medicine (GUM) clinics in England, Wales and Northern Ireland in 2002, which was the highest number identified since 1984¹. The increase in syphilis diagnoses started in Bristol in 1997 where it was associated with heterosexually acquired infection, commercial sex work, and drug use². Since then Manchester, Brighton and London have all experienced syphilis outbreaks, mainly among men who have sex with men (MSM), several of whom were HIV positive³⁻⁵.

Syphilis can be divided into three infectious disease stages; primary, secondary, early latent and two non-infectious disease stages; late latent and tertiary.

During infection, a broad immune response is produced that consists of anti-treponemal and non-specific (cardiolipin) antibodies. During primary syphilis, anti-treponemal IgM may be detected within approximately two weeks postinfection, and IgG after about four weeks postinfection⁶. Antibodies can usually be detected by the time clinical symptoms appear⁷. Non-specific antibodies and anti-treponemal IgM decline after successful treatment of early syphilis, however anti-treponemal IgG antibody can persist for longer⁶. Concurrent infection with HIV may alter the serological response to *T.pallidum* infection by delaying or enhancing the response, although there is generally no effect. This has been reviewed elsewhere⁸.

T.pallidum is a human obligate parasite, which is difficult to culture *in vitro*, so the laboratory diagnosis of syphilis is usually by serology. The exception is for very early syphilis, where treponemes may be directly detected in material from lesions by darkfield or fluorescent microscopy. This enables a diagnosis of primary syphilis to be made in the absence of reactive serology. Serological tests for syphilis can be classified into two groups:

1. Non-treponemal tests *e.g.* Venereal Disease Research Laboratories (VDRL) or Rapid Plasma Reagin (RPR). These detect non-specific antibodies and are important for monitoring treatment.
2. Treponemal tests which are based on antigens derived from *T.pallidum*, allow detection of specific anti-treponemal antibodies. These include the *Treponema pallidum* haemagglutination assay (TPHA), *Treponema pallidum* particle agglutination (TPPA), the fluorescent treponemal antibody-absorbed test (FTA-abs) and native antigen and recombinant enzyme immunoassay (EIA) tests.

These serological tests and their application were previously reviewed in the PHLS (Public Health Laboratory Service) guidelines where a testing algorithm was recommended⁹.

In the UK, GUM attenders who are at risk of acquiring an STI, blood donors at each attendance and all pregnant women, with the aim of preventing congenital syphilis, are screened for antibodies to *T.pallidum*.

This report describes results obtained from the evaluation of four TPHA kits and one TPPA kit. These have been tested against a moderately sized panel designed to give an indication of kit specificity and sensitivity, including ability to detect early infection. The performance of TPHA and the TPPA is of interest to the National Blood Service where they are used to screen blood donations, and to clinical laboratories where they are used for syphilis screening and diagnosis.

The advantages and disadvantages of using TPHA and TPPA assays can be found in the [Appendix \(Table 14\)](#).

Description of the assays

The *T. pallidum* haemagglutination assay (TPHA) and the *T. pallidum* particle agglutination assay (TPPA) are serological tests that detect specific treponemal antibody. Treponemal antigens are attached to red blood cells (TPHA) or to gelatin particles (TPPA) which agglutinate in the presence of specific antibodies in serum or plasma. The agglutination patterns may be interpreted by eye or by using a microplate reader.

All the kits have a similar procedure. Briefly, in U-well microplates, a specimen dilution of 1/20 is created in diluent for all the TPHAs, and 1/40 for the TPPA. Sensitised (treponemal antigens attached) red blood cells or gelatin particles are then added to create a final dilution of 1/80. Unsensitised control cells are used with the Biokit TPHA (1/80 final dilution) and the Serodia TPPA (1/40 final dilution). The contents of the wells are mixed for 30 seconds on a microplate shaker. The microplates are covered and incubated on a static surface for 60 minutes (TPHAs) or two hours (TPPA). The results are then read and interpreted. A summary of the characteristics of the evaluated kits is shown in [Table 1](#).

Additional equipment required for the kits, as well as manufacturers' claims and limitations for each assay are noted in the [Appendix](#). Examples of agglutination patterns can be viewed in [Figures 1 and 2](#).

Figure 1. Example of a TPPA agglutination pattern



Figure 2. Example of a TPHA agglutination pattern



Table 1. Assay information

General					
Kit name (Manufacturer)	Microsyph TP1000+ (Axis-Shield Diagnostics Ltd.)	Syphagen TPHA (Biokit S.A.)	TPHA Screening (New market Laboratories Ltd.)	Syphilis TPHA (Randox Laboratories Ltd.)	Serodia TPPA (Fujirebio Inc.)
UK agent	Axis-Shield Diagnostics Ltd.	Launch Diagnostics Ltd	New market Laboratories Ltd.	Randox Laboratories Ltd.	Mast Group Ltd.
Product number	FTPH1000	300615700	60005A	SY2215	9241
Lot number used in evaluation	212PROT01	H-0802	231802	0313J	YN20903
Number of tests in one pack	1000	200	1000	1000	600
Sample volume	10µL	25µL	10µL	10µL	25µL
FDA registered	No	No	No	No	Yes
CE marked	Yes*	Yes	Yes*	Yes*	Yes*
ISO9000 series certificate	Yes	Yes	Yes	Yes	Yes
UK launch date	December-03	December-95	January-95	April-96	October-96
Presentation					
Assay type	Indirect haemagglutination	Indirect haemagglutination	Indirect haemagglutination	Indirect haemagglutination	Passive particle gelatin agglutination
Sample addition colour change	Yes	No	No	No	No
Control cells	No	Yes	Yes	No	Yes
Absorption procedure to remove non-specific reactions	No	No	Yes	No	Yes
Maximum number of tests / plate	48	24	48	48	24
Negative control	1	1	1	1	1 (serum diluent)
Positive control	1	1	1	1	1
Reading	Visual	Visual	Visual	Visual	Visual
Equivocal zone	Yes	Yes	Yes	Yes	Yes
Stages					
Preparation time	30 minutes	30 minutes	30 minutes	30 minutes	30 minutes
Incubation status	Static / level surface	Static / level surface	Static / level surface	Static / level surface	Static / level surface
Sample incubation	1 hour / room temperature	1 hour / room temperature	1 hour / room temperature	1 hour / room temperature	2 hours / room temperature
Reading	After 45-60 mins incubation	After 45-60 mins incubation	After 45-60 mins incubation	After 45-60 mins incubation	After 2 hour incubation**
Notes:					
*Kits used in the evaluation were not CE marked. CE status received December 2003.					
**May be left overnight					

The evaluation was performed in accordance with the protocol that was agreed by all participating companies (see [Appendix](#)). All equipment was available at MiDAS. The manufacturers were offered the opportunity to train the evaluator, but all decided that this was not necessary.

Each kit was tested against a panel of 235 anti-treponemal positive specimens (plasma and serum), 250 negative blood donor specimens (serum), and three quality control sera ([Table 2](#)). Of the 235 positive specimens, 114 were from individuals whose disease stage and treatment status was known and 121 from individuals whose disease stage and treatment status was unknown. All specimens tested had been subjected to fewer than five freeze-thaw cycles. All specimens with false negative, false positive, indeterminate and discordant results were retested in duplicate.

All assays were performed according to the manufacturers' instructions. Manufacturers' claims and limitations for their assays are shown in the [Appendix \(Tables 15 - 19\)](#). Results were read visually by three laboratory staff who independently recorded their scores on separate sheets. The reactions were read with reference to scoring cards supplied by the manufacturers, either in the kit inserts or requested separately.

The following scoring system was adhered to:

0 = negative reaction, 1= indeterminate, 2 = very weak reaction, 3 = medium reaction, 4 = strong reaction.

The consensus of the three readers was taken. A consensus of 2 or greater equalled a positive reaction, a consensus of 1 equalled an indeterminate reaction and a consensus of 0 was deemed to be a negative reaction.

The sensitivity and specificity were calculated as follows:

Specimen Status	Results of assays - consensus			Total
	Positive	Indeterminate	Negative	
Positive	a	b	c	a+b+c
Negative	d	e	f	d+e+f

Sensitivity: $(a+b) / (a+b+c) \times 100$

Specificity: $(f) / (d+e+f) \times 100$

The Biokit TPHA and Serodia TPPA kits both use a control cell procedure. Control cells that agglutinate in the presence of the specimen may be caused by non-specific agglutinins. An absorption procedure is described for the Serodia TPPA to remove the non-specific reactions, and the specimens can then be retested. However, no absorption procedure is described for the Biokit TPHA, so any specimens that cause agglutination with the control cells cannot be interpreted.

Specimens that gave discordant results were tested by a range of other syphilis kits including Murex ICE Syphilis EIA (Abbott Laboratories), Newmarket Syphilis EIA (Newmarket Laboratories), Mercia Syphilis IgM EIA (Microgen Bioproducts), and INNO-LIA Syphilis (Innogenetics). The IgM EIA was used to detect early infection, the INNO-LIA was used to assess the response to individual antigens, and the two other EIAs were used as a different method of detecting anti-treponemal antibodies from the TPHAs and TPPA.

All participating manufacturers were sent a draft report of the results obtained with their products. Any comments received back from the manufacturers prior to the publication of the final report are [appended](#).

Table 2. Specimen panel

Sample category	Number
1. Blood donors' sera	250
2. Positive samples from Impath-BCP (stages known)	
Primary syphilis	7 treated 16 untreated
Secondary syphilis	5 treated 4 untreated
Early latent syphilis	4 treated 3 untreated
Late latent syphilis	8 treated 4 untreated
3. Positive samples from CPHL (stages known)	
Primary syphilis	17 untreated
Secondary syphilis	34 untreated
Early latent syphilis	12 untreated
Total where disease stage and treatment status known = 114	
4. Positive samples (syphilis stage not known)	
Profile specimens	78
SNBTS plasma	33
CPHL specimens	10
Total where disease stage and treatment status unknown = 121	
5. Quality control samples	
QAL, CPHL (QC-1)	1
QAL, CPHL (QC-2)	1
Fortress (QC-3)	1
TOTAL (number of specimens)	488
Notes:	
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).	
CPHL = Central Public Health Laboratory, Health Protection Agency, UK	
Profile = Profile Diagnostics Inc., USA	
SNBTS = Scottish National Blood Transfusion Service	
QAL = Quality Assurance Laboratory	
Fortress = Fortress Diagnostics Ltd., UK	

Key to the presentation of results

The presentation of results is intended to allow readers to draw their own conclusions from the data. Unless otherwise stated, the results are shown as a consensus, as described in the *Evaluation Method* section.

Specificity ([Table 3](#))

Sensitivity ([Tables 4 - 9](#), [Figure 3](#))

Distribution of initial reactivities ([Figure 4](#))

Inter-reader variability ([Figures 5 - 10](#))

Manufacturers' kit controls and quality control results ([Tables 10 - 11](#))

Specificity

Anti-treponemal negative specimens from 250 blood donors were tested against all five kits to determine specificity. All 250 were unreactive by Serodia TPPA, giving an initial specificity of 100% (Table 3). The other kits gave between 1 and 4 initially reactive specimens. Of these, only one specimen was repeatedly reactive with the Biokit TPHA. This specimen was confirmed negative by the supplementary tests described in the [Evaluation method](#).

A specificity claim of 99.6 to 100% is given in the kit insert for the Biokit TPHA, and a claim of 100% is given in the kit insert for the Newmarket TPHA. Both of these specificity claims were achieved in this study. Serodia, Randox and Axis-Shield make no specificity claims in their kit inserts.

Table 3. Specificity

Assay	Number tested	Number initially reactive	Number repeatedly reactive	% Specificity (95% confidence intervals)
Serodia TPPA	250	0		100 (98.5 - 100)
Axis-Shield TPHA	250	4	0	100 (98.5 - 100)
Newmarket TPHA	250	1*	0	100 (98.5 - 100)
Randox TPHA	250	2*	0	100 (98.5 - 100)
Biokit TPHA	250	1*	1	99.6 (97.8 - 100)
Note: * one specimen gave an indeterminate reaction				

Sensitivity

The sensitivity of the five syphilis agglutination assays was assessed by testing each kit against a panel of 114 syphilis positive specimens from individuals whose disease stage and treatment status was known, and 121 specimens from individuals whose disease stage and treatment status was unknown. The results from these two specimen categories are considered separately below.

Known disease stage and treatment status

Of the 114 positive specimens with known disease/treatment category, 113 (including indeterminates) were initially detected by Serodia TPPA and Randox TPHA giving both kits a sensitivity of 99.1% (Table 4). Biokit detected 111 specimens giving a sensitivity of 98.2%, although one less specimen was included in the calculation for Biokit TPHA; this specimen agglutinated the control cells but in the absence of an absorption procedure no interpretation could be made. Axis-Shield and Newmarket TPHAs gave initial sensitivities of 97.4 and 96.5% respectively.

Following retesting of false negative, indeterminate and discordant results, Serodia TPPA detected 113 of the 114 specimens giving a sensitivity of 99.1% (Table 5). However, a decrease in sensitivity was noted for all the TPHA kits. Specimens that initially gave indeterminate reactions gave negative results following retests. The number of positive reactions remained the same as initial tests, except for Serodia TPPA which gave one more positive than previously. All discordant specimens were confirmed positive by a combination of other syphilis assays ([Appendix, Table 20](#)). The only specimen not confirmed positive by these methods was positive by darkfield microscopy only. This specimen was from a patient who had untreated primary syphilis, and was the only specimen not to be detected by the Serodia TPPA.

Table 4: Initial sensitivities for TPHA/TPPA kits tested against specimens from individuals with known disease stage and treatment status

Assay	Number of reactive specimens (total = 114, Biokit = 113*)			Initial sensitivity % (95% confidence interval)
	Positive	Indeterminate	Negative	
Serodia TPPA	112	1	1	99.1 (95.2 - 100)
Randox TPHA	109	4	1	99.1 (95.2 - 100)
Biokit TPHA*	109	2	2	98.2 (93.8 - 99.8)
Axis-Shield TPHA	107	4	3	97.4 (92.5 - 99.5)
Newmarket TPHA	107	3	4	96.5 (91.3 - 99.0)
Note: * One sample was excluded due to agglutination with control cells (see <i>Evaluation method</i>)				

Table 5: Repeat sensitivities for TPHA/TPPA kits tested against specimens from individuals with known disease stage and treatment status

Assay	Number of reactive specimens (total = 114, Biokit = 113*)			Repeat sensitivity % (95% confidence interval)
	Positive	Indeterminate	Negative	
Serodia TPPA	113	0	1	99.1 (95.2 - 100)
Biokit TPHA*	109	1	3	97.3 (92.4 - 99.4)
Randox TPHA	109	0	5	95.6 (90.1 - 98.6)
Axis-Shield TPHA	107	0	7	93.9 (87.8 - 97.5)
Newmarket TPHA	107	0	7	93.9 (87.8 - 97.5)
Note: * One sample was excluded due to agglutination with control cells (see <i>Evaluation method</i>)				

A breakdown of results according to disease/treatment category is shown in Tables 6 and 7. Most variation between kits was observed in the untreated primary and treated late latent groups. Initially there was also some variation in the secondary untreated specimens due to the occurrence of indeterminate results. Specimens from patients with primary syphilis are likely to have low antibody levels, and patients with treated late latent syphilis will have decreasing antibody levels. The Serodia TPPA was the best assay for detecting antibodies within these disease stages.

Table 6: Initial reactivity of TPHA/TPPA kits at different disease stages

Assay	Treatment Status	Number of reactive specimens (number indeterminate) at different disease stages			
		Primary	Secondary	Early latent	Late latent
Serodia TPPA	Untreated	31/33 (1)	38/38	15/15	4/4
	Treated	7/7	5/5	4/4	8/8
Randox TPHA	Untreated	31/33 (1)	37/38 (1)	15/15	4/4
	Treated	7/7	5/5	4/4	6/8 (2)
Biokit TPHA	Untreated	31/33 (1)	37/37	15/15	4/4
	Treated	7/7	5/5	4/4	6/8 (1)
Axis-Shield TPHA	Untreated	29/33 (2)	37/38 (1)	15/15	4/4
	Treated	7/7	5/5	4/4	6/8 (1)
Newmarket TPHA	Untreated	30/33 (1)	37/38 (1)	15/15	4/4
	Treated	7/7	5/5	4/4	5/8 (1)

Table 7: Repeat reactivity of TPHA/TPPA kits at different disease stages

Assay	Treatment Status	Number of reactive specimens (number indeterminate) at different disease stages			
		Primary	Secondary	Early latent	Late latent
Serodia TPPA	Untreated	32/33	38/38	15/15	4/4
	Treated	7/7	5/5	4/4	8/8
Biokit TPHA	Untreated	31/33	37/37	15/15	4/4
	Treated	7/7	5/5	4/4	6/8 (1)
Randox TPHA	Untreated	31/33	37/38	15/15	4/4
	Treated	7/7	5/5	4/4	6/8
Axis-Shield TPHA	Untreated	29/33	37/38	15/15	4/4
	Treated	7/7	5/5	4/4	6/8
Newmarket TPHA	Untreated	30/33	37/38	15/15	4/4
	Treated	7/7	5/5	4/4	5/8

Unknown disease stage and treatment status

Of the 121 positive specimens with unknown disease and treatment categories, 121 were positive by Serodia TPPA giving a sensitivity of 100% (Table 8). Biokit TPHA also gave 100% sensitivity, although initially nine specimens were excluded in the calculation for Biokit; these specimens agglutinated the control cells and in the absence of an absorption procedure no interpretation could be made.

Following retests (Table 9) there was a decrease in sensitivity of all TPHAs. Specimens that initially gave indeterminate reactions gave negative results following retests. The number of positive reactions either increased or remained the same as initial tests, except Newmarket TPHA for which one previously reactive sample was found to be unreactive upon retesting. Of the TPHAs, Biokit was the most sensitive (98.3%); although two specimens were excluded due to agglutination of control cells. All discordant specimens were confirmed positive ([Appendix, Table 20](#)). It initially appears that the sensitivities of the TPHA kits were lower when the disease stage and treatment status was unknown, however the confidence intervals do overlap. [Figure 3](#) shows an overall representation of the repeat sensitivities and specificities of all the kits.

Table 8. Initial sensitivity of TPHA/TPPA kits for specimens from individuals whose disease stage and treatment status was unknown

Assay	Number of reactive specimens (number indeterminate) Total = 121, Biokit = 112*	Initial sensitivity % (95% confidence interval)
Serodia TPPA	121	100 (97 - 100)
Biokit TPHA*	110 (2)	100 (96.8 - 100)
Randox TPHA	115 (4)	98.3 (94.2 - 99.8)
Axis-Shield TPHA	113 (5)	97.5 (92.9 - 99.5)
Newmarket TPHA	110 (7)	96.7 (91.8 - 99.1)

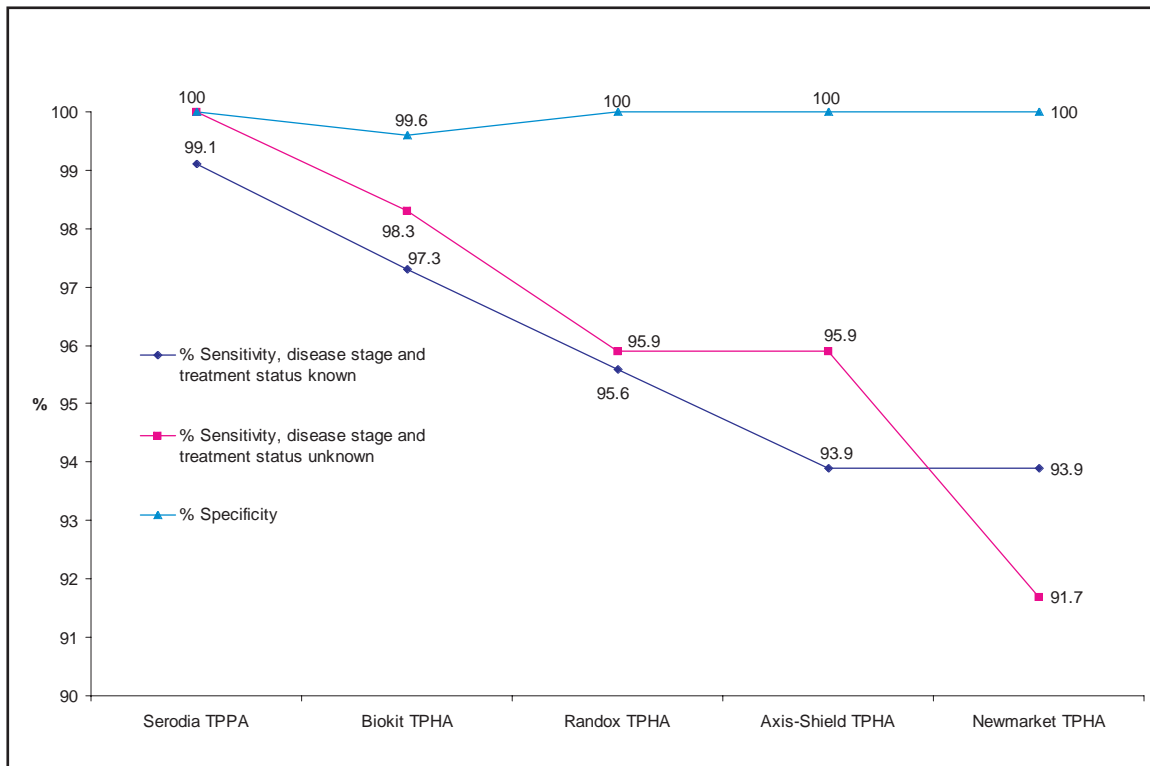
Note: * Nine samples were excluded due to agglutination with control cells (see *Evaluation method*)

Table 9. Repeat sensitivity of TPHA/TPPA kits for specimens from individuals whose disease stage and treatment status was unknown

Assay	Number of reactive specimens (number indeterminate) Total = 121, Biokit = 119*	Repeat sensitivity % (95% confidence interval)
Serodia TPPA	121	100 (97 - 100)
Biokit TPHA*	117	98.3 (94.1 - 99.8)
Randox TPHA	115 (1)	95.9 (90.6 - 98.6)
Axis-Shield TPHA	114 (2)	95.9 (90.6 - 98.6)
Newmarket TPHA	109 (2)	91.7 (85.3 - 96)

Note: * Two samples were excluded due to agglutination with control cells (see *Evaluation method*)

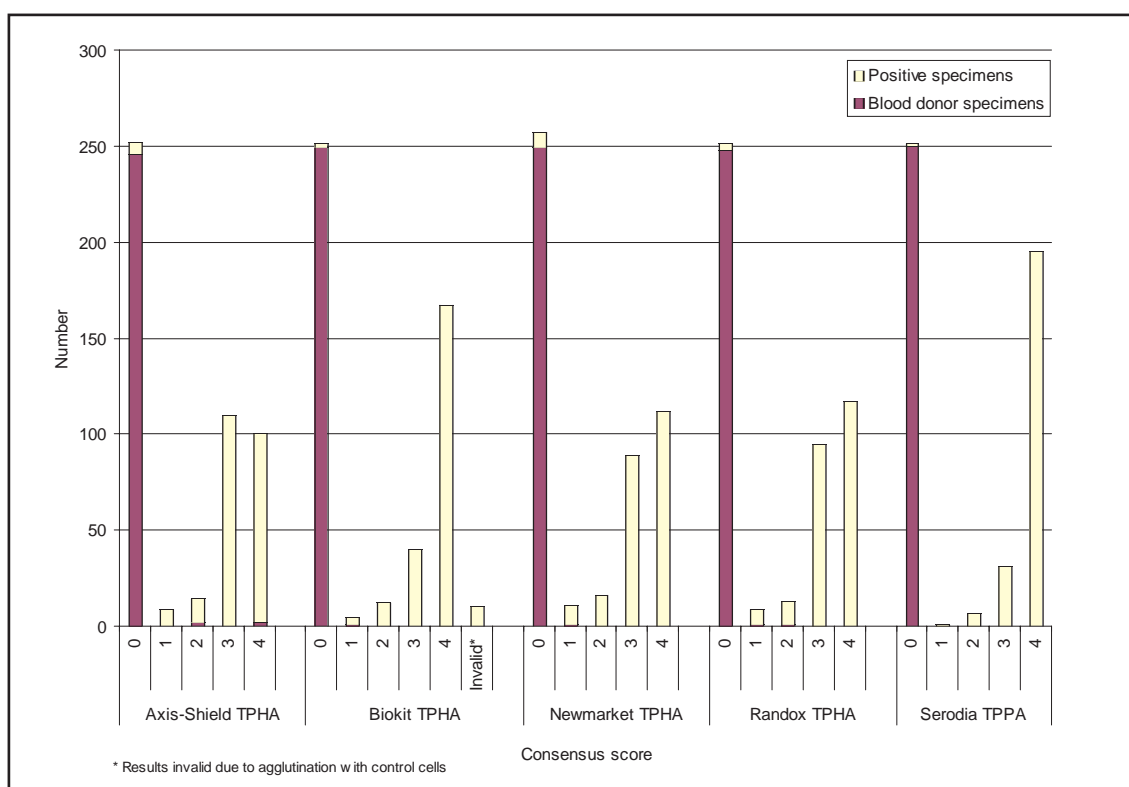
Figure 3. Graph showing the repeat % sensitivity and specificity of the TPHA and TPPA kits



Distribution of initial reactivities

The distribution of initial reactivities for the 235 syphilis positive specimens and 250 negative specimens from blood donors are presented in Figure 4. Assays with good discrimination between positives and negatives have few, or no, specimens wrongly classified and few indeterminate or weak reactions. The Serodia TPPA gave the best discrimination, and of the 235 positive specimens, had the highest number of strong positive reactions (score of 4), and the lowest number of other reaction scores. All the assays initially gave some false negative results, ranging from eleven for the Axis-Shield TPHA and three for the Serodia TPPA.

Figure 4. Distribution of initial reactivities



Scoring system; 0 = negative reaction, 1= indeterminate, 2 = very weak reaction, 3 = medium reaction, 4 = strong reaction.

Inter-reader variability

The variation in scoring between the three readers is shown in Figures 5 to 9. Other than the Axis-Shield TPHA, there appears to be wider variation between readers in scoring the higher scores, although this doesn't affect the overall interpretation. Reader 2 tended to score lower than the other two readers giving fewer scores of 4 and more scores of 3.

Figure 5. Frequency of initial test scores given by each reader for the Axis-Shield TPHA

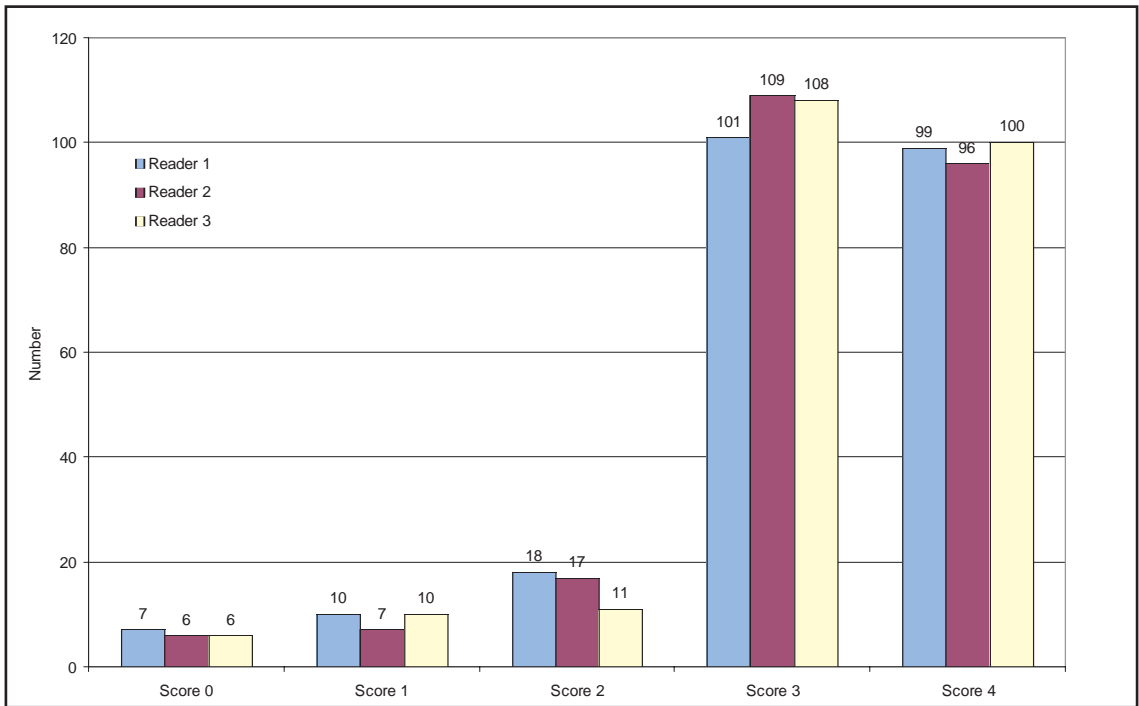


Figure 6. Frequency of initial test scores given by each reader for the Biokit TPHA

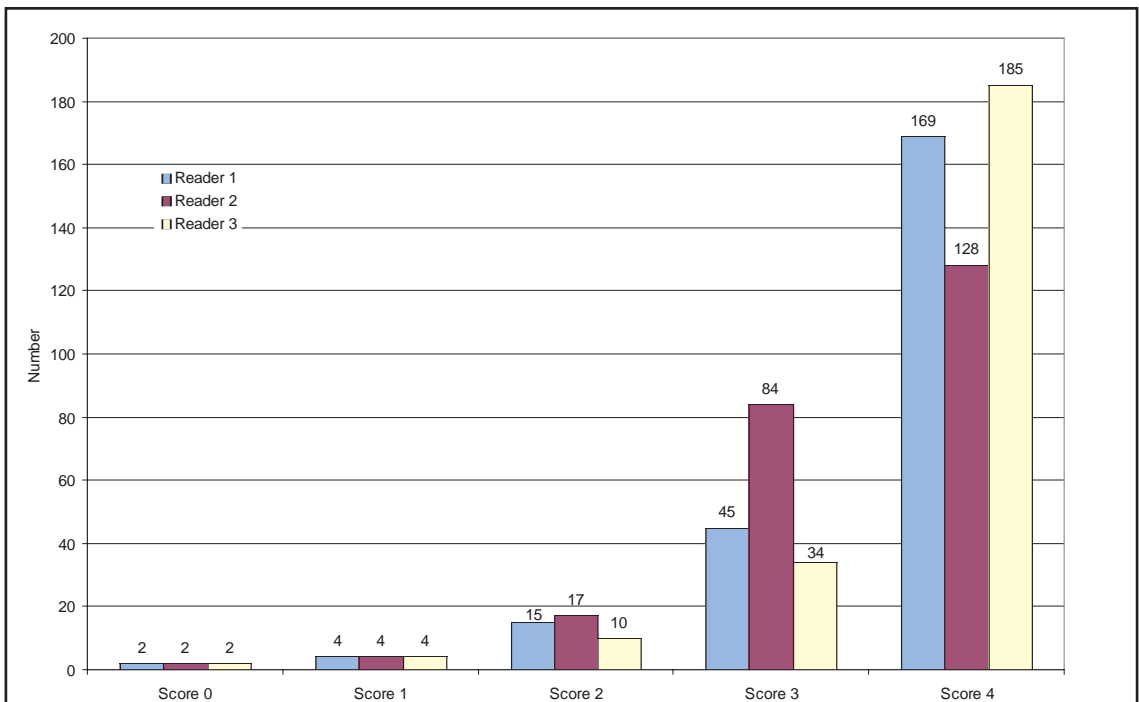


Figure 7. Frequency of initial test scores given by each reader for the Randox TPHA

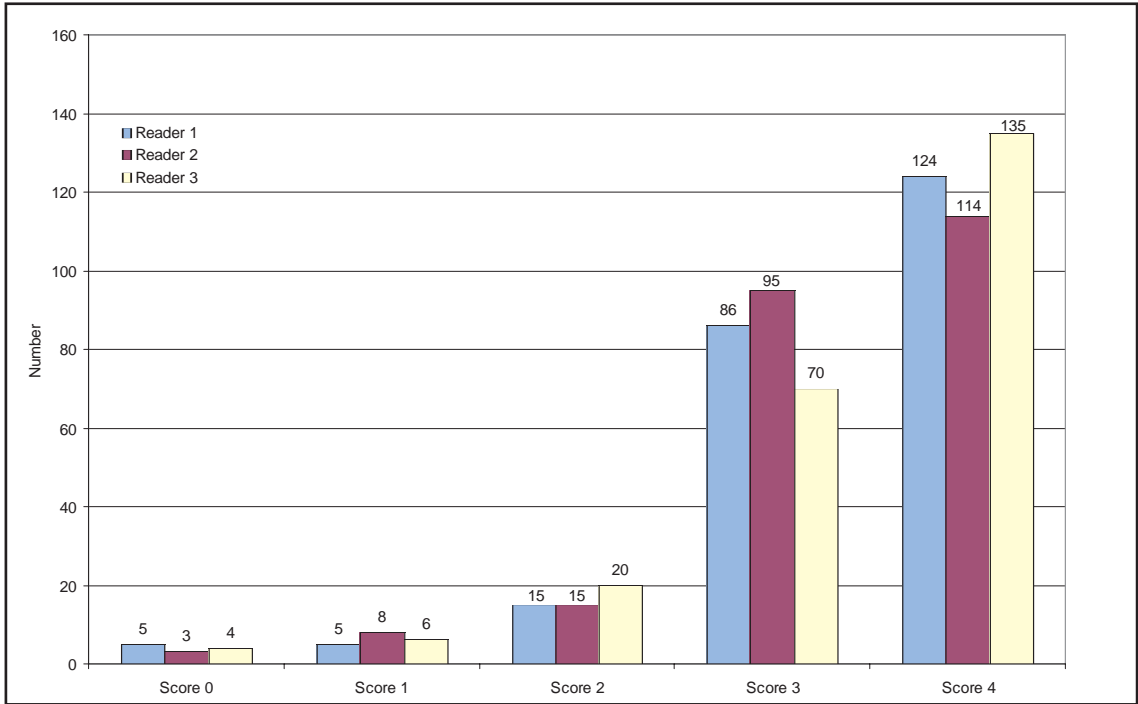


Figure 8. Frequency of initial test scores given by each reader for the Newmarket

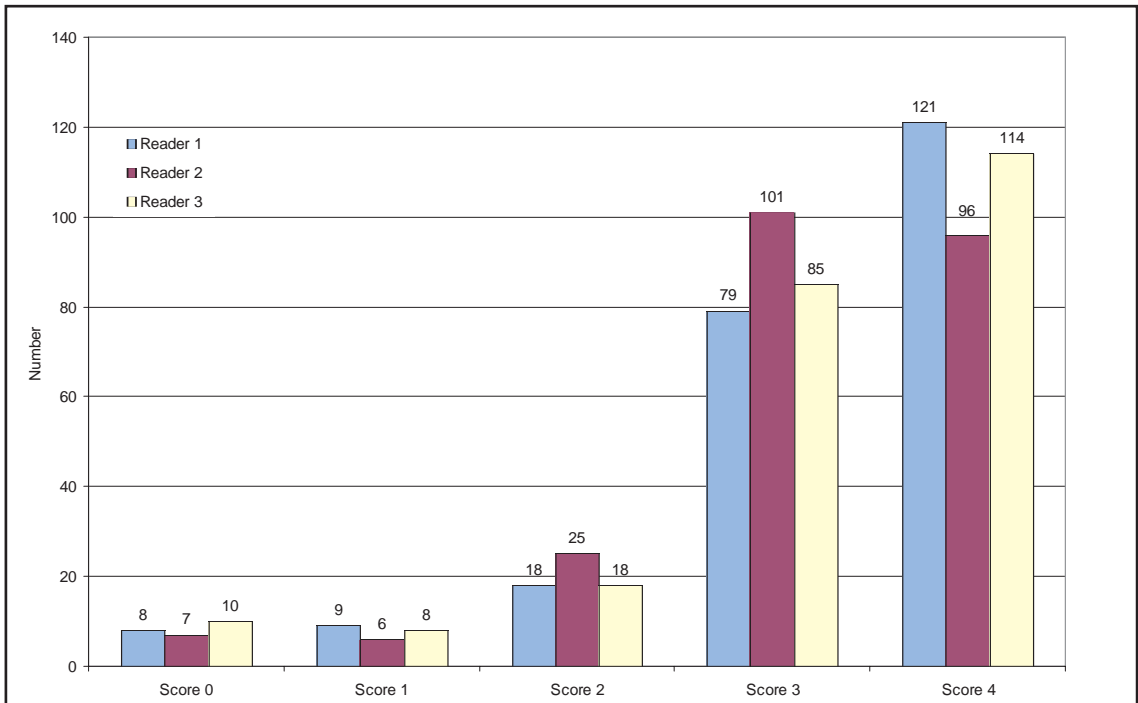
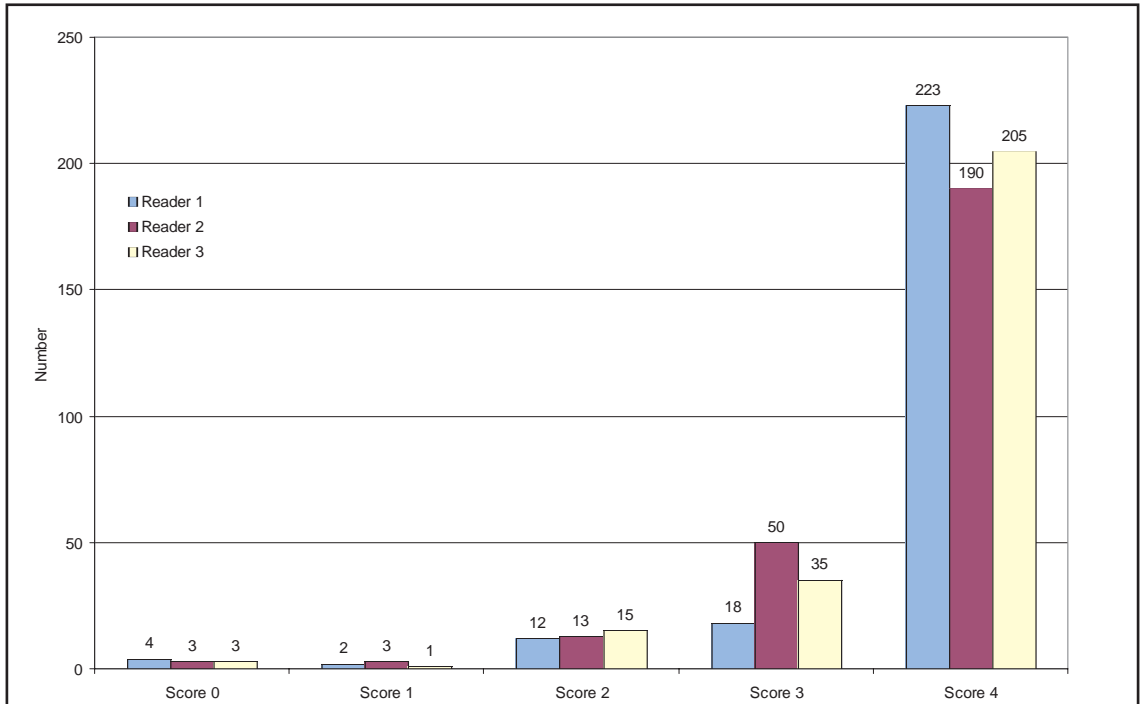
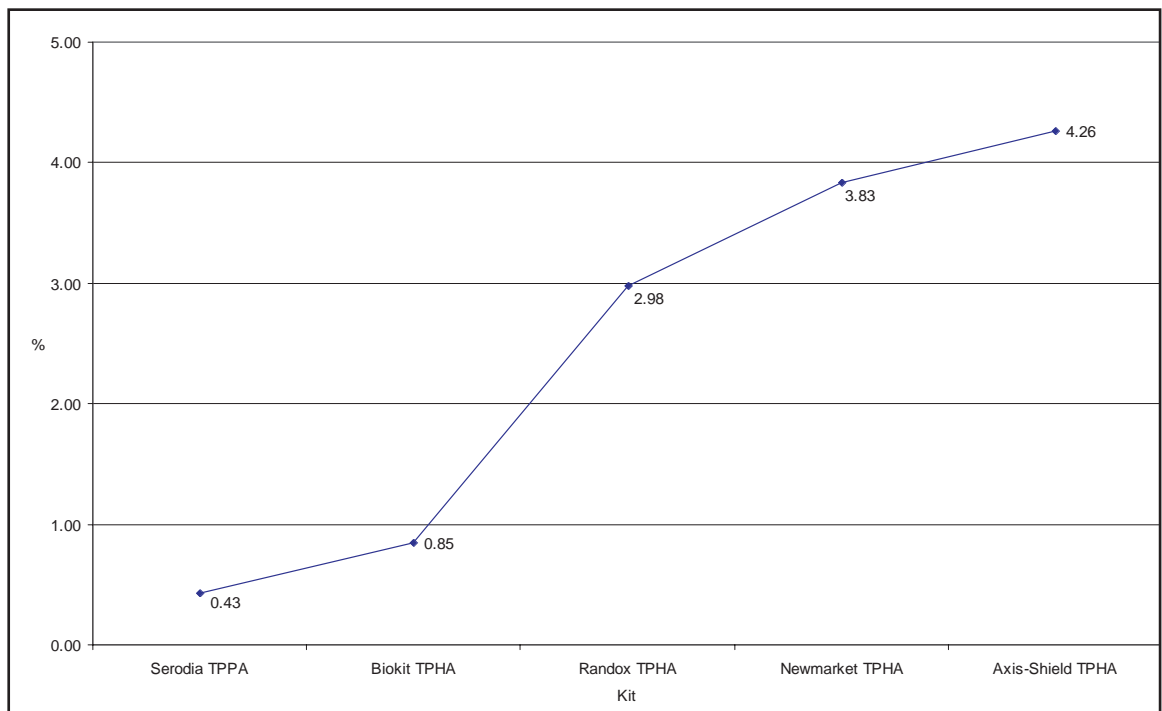


Figure 9. Frequency of initial test scores given by each reader for the Serodia TPPA



The inter-reader variability was calculated as the percentage of specimens for which initial test results were interpreted differently as positive, indeterminate or negative, by the three independent readers. Figure 10 shows that the Serodia TPPA gave the least variation between readers, and the Axis-Shield TPHA gave the largest variation. The kits with the lower variation have the highest sensitivities and greatest number of strong positive reactions.

Figure 10. % inter-reader variability



Manufacturers' kit controls and quality control results

Manufacturers' kit controls and quality control specimens from other sources (Table 2) were tested on every assay run. Controls were used on one plate per assay run to monitor the assay reagents, and to monitor reproducibility between runs. The results are shown in Table 10. The positive and negative controls provided with the kit and the quality controls gave the expected results on all runs.

Table 10. Manufacturers' kit controls and quality control results

Assay		Kit Controls		External Controls		
		Positive	Negative	QC-1	QC-2	QC-3
Axis-Shield TPHA	Range (mode)	4 (4)	0 (0)	3 (3)	3 (3)	4 (4)
	Total no. or runs	7	7	7	7	7
Biokit TPHA	Range (mode)	4 (4)	0 (0)	4 (4)	4 (4)	4 (4)
	Total no. or runs	9	9	9	9	9
Newmarket TPHA	Range (mode)	4 (4)	0 (0)	3 - 4 (3)	3 (3)	4 (4)
	Total no. or runs	10	10	10	10	5
Randox TPHA	Range (mode)	4 (4)	0 (0)	3 (3)	3 (3)	4 (4)
	Total no. or runs	10	10	10	10	10
Serodia TPPA*	Range (mode)	4 (4)	0 (0)	4 (4)	4 (4)	4 (4)
	Total no. or runs	8	8	8	8	8
Notes:						
Scores are the consensus of the three readers						
* No negative control supplied. Reagent control used.						

The end point titres (the highest titre to give a reading of 2 or greater) were determined for each control (Table 11). The Serodia TPPA gave the highest titres for the quality control specimens. Of the TPHA kits, Axis-Shield gave the highest titre for the external quality control specimens, however the other TPHA kits all gave results that were within one doubling dilution.

Table 11. End point titres for controls

Assay	End point titres			
	Positive kit control	QC-1	QC-2	QC-3
Axis-Shield TPHA	1:2560	1:640	1:640	1:1280
Biokit TPHA	1:1280	1:640	1:640	1:640
Newmarket TPHA	1:640	1:320	1:320	1:640
Randox TPHA	1:1280	1:320	1:320	1:640
Serodia TPPA	1:640	1:2560	1:2560	1:5120

All the kits were very similar and easy to use. An overall appraisal of the kits used in the evaluation can be found in Table 12 and further comments follow on each kit.

Table 12. Technical appraisal of the kits used in the evaluation

	Axis-Shield TPHA					Biokit TPHA					Newmarket TPHA					Randox TPHA					Serodia TPPA									
	Good		Poor			Good		Poor			Good		Poor			Good		Poor			Good		Poor							
Kit instructions	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5					
Clarity	x						x						x			x										x				
Presentation	x						x				x					x										x				
Content	x						x						x			x										x				
Safety instructions	x						x						x			x										x				
Kit and reagent packaging / labelling																														
Clear identification	x						x				x					x										x				
Labelling	x						x				x					x										x				
Reagent packaging	x						x				x							x										x		
Safety (i.e. contains sodium azide)	x							x			x							x										x		
Ease of use																														
Performing test	x						x				x					x										x				
Sample preparation	x						x				x					x										x				
Reagent preparation	x						x				x					x												x		

Axis-Shield TPHA

The Axis-Shield TPHA was the only kit to have a sample addition monitor, and this was extremely useful.

Biokit TPHA

Biokit provided a small reference sheet showing a summary of the Biokit TPHA assay procedure. This was useful when undertaking the test, but the reference sheet mentioned a three well procedure, as well as a four well procedure, whereas the full kit insert only described the four well procedure. It was clear to see when a sample had been added due to the obvious increase in well volume. Control cells were supplied with the kit to identify non-specific agglutination. However, no absorption procedure was described. Therefore, any samples that agglutinated with the control cells could not be interpreted. The Biokit TPHA was the only kit to supply a visual diagram of the agglutination patterns in the kit insert. All other score cards were supplied or requested independently from the manufacturers.

Randox TPHA

The test cells with the Randox TPHA were contained in a dark brown bottle. This made it difficult to see when the test cells had been reconstituted.

Newmarket TPHA

In the Newmarket TPHA kit insert, it mentioned control cells in the 'interpretation of results' section, but there was no mention of the use of control cells in the assay protocol. An absorption procedure also describes the use of control cells, but this procedure is dependent upon the control cells being used in the assay initially.

It was sometimes difficult to visualise whether a sample had been added to the sample diluent with the Randox and Newmarket TPHAs. However this problem can be overcome by the use of a spectrophotometer as described in the Newmarket TPHA kit insert.

Serodia TPPA

The Serodia TPPA kit contains lyophilised particle bottles with thin metal caps. To remove the caps, they had to be torn off, and this had the potential to create a sharps accident. When removing the rubber stopper from the lyophilised particle bottles it was difficult to contain all the

powder as some adhered to the stopper. Users need to be careful not to cross contaminate unsensitised particles and any open microtitre plates, with sensitised particles. Once the lyophilised particles were reconstituted, they were only stable for 7 days at 2 - 10°C. Since the particles are sufficient to perform 300 tests, these will be wasted if 300 tests are not performed within one week with this particular kit size (2 x 300).

Control cells

Three of the five kits provided control cells (see [Description of the assay, Table 1](#)), and two of the three described an absorption procedure. Control cells are important to detect non-specific agglutination. The routine use of control cells means that fewer tests can be done per plate. However, control cells could be used only when repeat testing of initially reactive samples. An absorption procedure to remove non-specific antibodies is useful, so sera that react with control cells can be retested.

Ease of interpretation

The three laboratory staff who visually read the TPHA/TPPA results were asked to score, on a scale of 1 to 10, how easy it was to interpret the end-point reactions. Their assessments for each kit are given in Table 13.

Of the five kits, the Serodia TPPA was the easiest to interpret and the agglutination patterns were the most stable. The patterns were usually either clearly positive or negative. The Axis-Shield and Newmarket TPHA reactions were slightly more difficult to interpret. These two kits also gave the highest inter-reader variability ([Figure 10](#)).

All the TPHAs sometimes gave strongly positive agglutination patterns that were prone to folding at the edges to give a ragged appearance. The Serodia TPPA did not suffer from this problem and the agglutination patterns were stable and could be left overnight. For the TPHAs, it was noted that sometimes the weakly positive reactions tended to collapse soon after the end of the incubation period. Interpretation was most difficult for specimens that gave weak positive, indeterminate or negative reactions.

Table 13. Ease of interpretation

Assay	Reader	Ease of Interpretation Score									
		1	2	3	4	5	6	7	8	9	10
Serodia TPHA	1	x									
	2	x									
	3	x									
Randox TPHA	1		x								
	2		x								
	3		x								
Biokit TPHA	1		x								
	2		x								
	3			x							
Axis-Shield TPHA	1			x							
	2		x								
	3			x							
Newmarket TPHA	1			x							
	2		x								
	3				x						

Notes: 1 = very easy, 10 = very difficult (i.e. much deliberation over final decision)

Readers are encouraged to carefully study the results presented and to draw their own conclusions. We offer the following comments:

The final specificities for the five syphilis agglutination assays were from 99.6% to 100%. The final sensitivities for the five kits ranged from 93.9% to 99.1% when tested against the 114 positive specimens of known disease stage, and between 91.7% and 100% when tested against the 121 specimens of unknown disease stage.

Biokit and Newmarket claim sensitivities of 100%, and 99.5% to 100%, respectively for their TPHAs. In this study, both kits fell outside this range, although performance with respect to disease status is not specified in the kit inserts. Serodia, Randox and Axis-Shield make no sensitivity claims in their kit inserts. In this evaluation, the number of indeterminate reactions after retesting decreased and resulted in lower final sensitivities for the TPHAs compared with initial results. The reason for this is not known. However, every effort was made to minimise variation of evaluation methods during the study. To this end, the same three laboratory staff read the results independently, and with reference to score cards. Nevertheless the readings are subjective and interpretation of indeterminate results is difficult. No specimens had been subjected to more than five freeze-thaw cycles in total. Quality control samples were used throughout the evaluation and we would suggest that these should be used to routinely monitor TPHA/TPPA assay runs, especially as there may be difficulties in standardising batches for these assay types.

Overall the Serodia TPPA was the most suitable assay for screening of all disease stages. Compared with Serodia, the TPHAs had lower sensitivities, particularly related to specimens from primary, late latent, and unknown disease stage categories. A number of TPHAs gave negative results in the unknown disease category that were reactive by Serodia TPPA, microplate EIAs, and Inno-LIA. In most of these cases the IgM assay was negative and therefore did not indicate early infection. In using TPHAs, users should be aware of their lower sensitivities. TPHA/TPPAs generally detect infection during the fourth week⁶, so access to follow up specimens, the patient's clinical data and other confirmatory tests are all important in making a diagnosis.

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TPHA/TPPAs advantages and disadvantages

Table 14. Advantages and disadvantages of using TPAs and the TPPA

Advantages
<p>Quick, simple and easy to use</p> <p>Cheap</p> <p>No requirement for specialist and expensive equipment</p> <p>Can detect both IgM and IgG anti-treponemal antibody</p> <p>Can be used to test a small number of samples as well as screening large numbers</p> <p>Short reaction time</p>
Disadvantages
<p>Interpretation of results can be subjective</p> <p>Weak and indeterminate reactions can be difficult to interpret</p> <p>End point may only be stable for a short time</p> <p>A prozone effect may be exhibited at lower dilutions</p> <p>May detect antibodies from other treponemal infections</p> <p>May give false positive reactions from patients with infectious mononucleosis, leprosy and autoimmune diseases</p> <p>If control cells are not used, a positive result may be due to a non-specific reaction with the test cells (although a reaction with control cells does not rule out a specific reaction)</p> <p>Cannot distinguish antibodies from past or present infection</p>

Additional equipment required

Below is a list of additional equipment required for all kits evaluated.

- Adjustable micropipettes: 5 - 40µL
- Adjustable multichannel pipette: 5 - 50µL
- Adjustable multichannel pipette: 50 - 300µL
- Disposable pipette tips
- Reagent reservoir
- U-well microplates (uncoated)
- Tray mixer
- Tray viewer

Claims for the assays and their limitations

Table 15. Axis-Shield TPHA, claims and limitations (quoted from kit insert)

Claims
<p>MICROSYPH™ TP1000+ is a rapid assay for the detection of specific antibodies to <i>Treponema pallidum</i> in human serum or plasma by indirect haemagglutination.</p> <p>MICROSYPH™ TP1000+ has been shown to be a convenient and specific test for the diagnosis of treponemal infection, having a similar specificity similar to that of the TPI test and a sensitivity comparable to that of the FTA-ABS test.</p> <p>The MICROSYPH™ TP1000+ test detects antibodies to <i>T.pallidum</i> by means of an indirect haemagglutination (IHA) method. Preserved avian erythrocytes are coated with antigenic components of pathogenic <i>T.pallidum</i> (Nichol's strain). These test cells agglutinate in the presence of specific antibodies to <i>T. pallidum</i>, and show characteristic patterns in microwell plates.</p> <p>Antibodies to non-pathogenic treponemes are absorbed by an extract of Reiter's treponemes included in the cell suspension.</p>
Sample usage/limitations
<p>Serum or plasma samples may be used. Store at 2-8°C if a preservative such as 0.1% azide is added prior to storage. For long term storage samples should be stored at -20°C. Any visible particulate matter should be removed by centrifugation.</p> <p>This test is to be used only with individual (unpooled) serum or plasma samples.</p> <p>Use of haemolysed samples, incompletely clotted sera, plasma samples containing fibrin or samples with microbial contamination may give rise to erroneous results.</p> <p>Erroneous results may be obtained with samples from cadavers.</p>
Limitations
<p>The kit is intended to be used as initial screening test for routine donor samples and must not be used for clinical specimens, as no Control Cells are included.</p> <p>The MICROSYPH™ TP1000+ kit does not contain control cells. A positive result may therefore be due to a non-specific reaction of the sample with the cells. In order to exclude this possibility any sample reactive in the test should be retested using the MICROSYPH™ TP200 kit (FTH200).</p> <p>For confirmation the FTA-ABS test should be used, since it allows a differentiation between IgG and early IgM antibodies. The FTA-ABS test is also useful in very early syphilis where the haemagglutination test may be negative.</p> <p>For therapeutic control it is advisable to use a quantitative test such as an RPR test. This reagent is available from Axis-Shield Diagnostics Ltd.</p> <p>Although the MICROSYPH™ TP1000+ test is highly specific, false positive results have been known to occur in patients suffering from leprosy, infectious mononucleosis and connective tissue disorders.</p> <p>Serological test, including MICROSYPH™ TP1000+, cannot distinguish between syphilis and other forms of pathogenic treponemal infections e.g. yaws. Clinical evidence should be used to determine which condition is present.</p> <p>Syphilis antibodies detected in the MICROSYPH™ TP1000+ test persist after successful treatment. Therefore, a positive test may indicate past or present infection.</p> <p>Following infection with <i>T. pallidum</i>, antibodies (both anti-lipoidal and anti-treponemal) may not appear until 1 to 4 weeks after the characteristic syphilis lesion (chancre) has formed. Thus, in early primary syphilis, tests such as MICROSYPH™ TP1000+ may give a negative result for some samples. In these cases, alternative testing procedures e.g. microscopic identification of <i>T. pallidum</i> should be used.</p> <p>Results obtained using plate reading systems must be checked manually. Depending on the reading parameters some indeterminate or collapsed patterns may be misread as borderline or negative.</p>
Claimed specificity and sensitivity
None

Table 16. Biokit Syphagen TPHA, claims and limitations (quoted from kit insert)

Claims
<p>Stabilized chicken erythrocytes are sensitized with an antigenic extract of <i>Treponema pallidum</i> (Nichols strain). These cells will agglutinate with specific antibodies present in either the serum or plasma of syphilitic patients.</p> <p>Non-specific reactions are detected by the unsensitized control reagent.</p> <p>Non-pathogenic treponemal antibodies are absorbed by an extract of Reiter's treponemes included in the diluent solution.</p> <p>A positive result indicates the presence of antibodies to <i>T. pallidum</i>, resulting from past or present infection.</p> <p>A negative result indicates the absence of antibodies to <i>T. pallidum</i> (see limitations of the procedure).</p> <p>A borderline result in the qualitative test may correspond to a low level of antibodies in early stages of syphilis or to residual antibodies in treated syphilis. In the case a further sample should be tested to demonstrate a rise in the antibody titer.</p>
Sample usage/limitations
<p>Use fresh serum. Serum may be stored at 2-8°C for 5 days. For longer periods serum samples should be frozen (-20°C).</p> <p>Although serum is the specimen of choice for all tests for syphilis, for screening purposes in blood banks, EDTA plasma samples may be used. Other anticoagulants should be evaluated before use. Plasma can be stored at 2-8°C and tested within 72 hours of blood collection.</p>
Limitations
<p>Any agglutination seen in the control well indicates the presence of non-specific agglutinins. Although non-specific reactions are very rare using chicken erythrocytes, a sample giving this result cannot be interpreted with this kit.</p> <p>Although serum is the specimen of choice for all tests for syphilis, for screening purposes in blood banks, EDTA plasma samples may be used. However, some incidence of false positive reactions has been reported. Therefore, serum must be used for all repeat testing of initial positive or borderline results obtained from plasma samples.</p> <p>Specific antibodies may persist for a long period of time, even after successful treatment of the disease. In order to assess the response to treatment, the use of a reagin test (RPR reditest) is recommended.</p> <p>The TPHA technique can give cross reactions with other forms of treponemal infections and false positive reactions with samples from patients with infectious mononucleosis, leprosy, autoimmune diseases and drug addiction.</p> <p>Occasionally, in early primary syphilis, the specific antibodies could not be detected by the TPHA technique.</p>
Claimed specificity and sensitivity
<p>Several evaluations have been performed with the syphagen TPHA kit. In one evaluation performed in Germany, the specificity found was 99.6% in front of 695 negative samples (serum and plasma) including samples from patients of different ages, of pregnant women, and also samples that can cause false positive reactions such as lipemic and icteric samples, samples with auto-antibodies, and from patients with different infections. In the same evaluation the sensitivity was 100% in front of a panel of 191 known positive samples</p> <p>In a second evaluation performed in France, 100% of sensitivity and 100% specificity was found in front of 55 positive and 201 negative serum samples, also including samples with heterophile antibodies, rheumatoid factor, EBV and Borrelia.</p>

Table 17. Newmarket TPHA, claims and limitations (quoted from kit insert)

Claims
Will detect antibodies to <i>T. pallidum</i> in human sera and plasma using micro haemagglutination. TPHA Screening 1000 uses preserved avian erythrocytes coated with antigens of <i>T. pallidum</i> (Nichol's strain) to bind with specific antibody present in patient sera. The cells are suspended in diluent containing components to eliminate non-specific reactions. Positive reactions are characterised by haemagglutination.
Sample usage/limitations
Use fresh sera.
Limitations
None
Claimed specificity and sensitivity
Specificity - Two independent studies on 2900 donor sera have shown 100% consensus with existing test methods. Initial reactive rate was 0.1%. Repeat reactive rate was 0%. An independent study on 200 antenatal sera has shown 100% specificity. Sensitivity - In house studies on 110 characterised positive samples gave 100% positive results. This included 2 samples tested negative by other commercial TPHA tests but confirmed FTA positive and IgM EIA positive. An independent study on characterised sera including positive samples from various stages of syphilis disease conditions other than syphilis have shown excellent performance characteristics. Clinical samples - 467 samples were run showing : Sensitivity 99.5%. Specificity 100%. (All data on file).

Table 18. Randox TPHA, claims and limitations (quoted from kit insert)

Claims
<p>The Syphilis TPHA test is an indirect haemagglutination test for the detection of specific antibodies against <i>Treponema pallidum</i>.</p> <p>Avian erythrocytes are sensitised with antigens of the Nichol's strain of <i>Treponema pallidum</i>. In the presence of syphilitic antibodies, these test cells aggregate to form characteristic patterns on the surface of the microplate wells.</p> <p>Antibodies directed against other non-pathogenic treponemes are absorbed by an extract of Reiter's treponemes present in the cell suspension thus greatly reducing false positives.</p>
Sample usage
<p>The samples (serum or EDTA plasma) should be free from contamination and non-haemolysed. Fresh serum or EDTA plasma samples may be stored for 24 hours at +2 to +8°C or 4 weeks at -20°C.</p>
Limitations
<p>Despite TPHA's high specificity, false positive results have been known to occur with patients suffering from leprosy, infectious mononucleosis and some autoimmune diseases.</p> <p>Syphilis antibodies persist after a successful course of treatment. Therefore, a positive result with the TPHA test may indicate a past or current infection.</p> <p>This TPHA kit does not contain control cells. A positive result may therefore be due to a non-specific reaction of the sample with the cells. In order to exclude this possibility, any sample reactive in this test should be retested using either Randox's TPHA kits SY 1480 or SY 1481.</p> <p>For confirmation the FTA-Abs test should be used, since it allows a differentiation between IgG and the early IgM antibodies.</p> <p>For therapeutic monitoring it is advisable to use a quantitative test such as VDRL or RPR which are both available from Randox.</p>
Claimed specificity and sensitivity
None

Table 19. Serodia TPPA, claims and limitations (quoted from kit insert)

Claims
<p>SERODIA-TP-PA kit is manufactured using gelatin particle carriers sensitized with purified pathogenic <i>Treponema Pallidum</i> (Nichols Strain). The test is based on the principle that sensitized particles are agglutinated by the presence of antibodies to <i>Treponema Pallidum</i> in human serum/plasma.</p> <p>The kit uses artificial particles, specifically developed by Fujirebio Inc. as carriers. These particles can minimize the nonspecific agglutination usually observed with use of other carriers.</p>
Sample usage
<p>Erythrocytes of other visible components present in the serum or the plasma samples should be removed by centrifugation prior to testing in order to prevent interference with test results. Serum inactivation has no effect on test results. Do not inactivate Plasma.</p>
Limitations
<p>For specimens showing positive or indeterminate results with SERODIA-TP-PA test, the results should be confirmed by testing with other methods and retesting on another day using a specimen freshly collected. A comprehensive assessment of the patient's condition should comprise the careful analysis of the patient's clinical symptoms and interpretation of results of available tests for the disease.</p> <p>If a specimen causes agglutination with both Unsensitized and Sensitized Particles or shows indeterminate, it should be retested after following the absorption procedure.</p> <p>When a specimen shows reactive or indeterminate in the Qualitative Assay, the specimen should be retested in the Quantitative Assay. A repeated reactive or indeterminate specimen should be confirmed by other methods (FTA-ABS).</p> <p>This kit is designed for the sole purpose of detecting <i>Treponema Pallidum</i> antibodies in serum/plasma specimens. It does not, however, detect TP directly. The test results should not be used in isolation but used in conjunction with the patient's clinical symptoms, clinical history, and any other available data to produce an overall clinical diagnosis.</p> <p>At the early stage of infection, in case of extremely low concentration of the antibodies, it is recognized that presently available methods (including this kit) for detection of antibodies to TP are not sensitive enough to detect existing antibodies. Therefore, in case infection is suspected, even if test results are negative, specimens should be retested and interpreted in conjunction with the results of other test methods and also with patient's clinical symptoms, clinical history, and any other available data to produce an overall clinical diagnosis.</p> <p>Note that some specimens with very high antibody titer may exhibit the prozoning phenomenon at lower dilutions.</p> <p>When patients specimen injected blood derivatives/preparations including immunoglobulin is interpreted, positive reaction might be observed.</p>
Claimed specificity and sensitivity
None

Evaluation protocol

Procurement of product for assessment, duration of assessment and training of evaluators

The evaluator will require for assessment a package that contains sufficient syphilis kits to test a panel of specimens, together with ancillary reagents and consumables that are recommended by the manufacturer. The kits will be used in conjunction with equipment, that is either provided by the manufacturer or is available at MiDAS and has been agreed to meet the requirements of the manufacturer's representative.

Before assessment starts, the manufacturer will be invited to train the evaluator in the use of the kits and equipment and to satisfy themselves that the evaluator is properly trained.

Conduct of the assessment

The product will be used in exactly the manner laid down in the manufacturer's instructions. Any modifications to the instructions provided with the kit that are described during the training period, or any subsequent changes must be confirmed in writing. Agglutination assays will be scored by three observers each working independently.

All data will be stored on the syphilis kit database for the duration of the assessment. The data may subsequently be downloaded to long-term backup system. In addition the evaluators will keep clear records of the practical work. All observer scores, original printouts from the plate reader or automated systems will be retained, together with any reader printouts from confirmatory assays.

Content of the assessment / specimen panel

The object of this assessment is to assess the ability of syphilis kits to detect, with a high degree of sensitivity and specificity, syphilis antibodies in human serum and plasma.

To do this, the kits will be tested against a panel of specimens obtained from primary, secondary and latent syphilis (early and late) cases, as well as negative blood donors. Available quality control samples will also be included.

Specimens previously tested because of false positive screening reactions will not be included in this study because of the potential bias that could be introduced against particular assays.

Storage of samples

Aliquots of each serum specimen will be distributed into plastic tubes with screw-cap lids with sealers. The aliquots will be stored at -20°C or below until required and at 4°C for the duration of the assessment. Thawing will be carried out at room temperature.

Other aspects of the assessment

The following features of the kits will be noted and may be remarked on in the report:

- packaging and labelling of the materials
- clarity of the operating instructions
- ease of use and reliability of the products, including equipment supplied for the assessment
- health and safety considerations

Discordant results

A discordant result will arise when the kit under assessment gives a result that disagrees with the observed consensus by other syphilis assays. If this occurs tests will be repeated in duplicate on the same aliquot of the serum.

Analysis of results and evaluation report

Raw data will be transferred from the laboratory computer onto a database specifically prepared for these evaluations. The data entry will be checked by a second person.

A detailed report in the usual style will be prepared for publication by the MHRA. The manufacturers will be given the opportunity to comment on the results of the evaluation of their product before the MHRA evaluation report is published. Results obtained in other manufacturers' tests undergoing evaluation will not be disclosed at this time. Manufacturers' written comments, where relevant, will be appended to the report.

Discordant specimens results and confirmatory testing

Table 20. Discordant and confirmatory results

MiDAS no	Disease stage	Treatment Status	Evaluation data										Supplementary data							
			Axis-shield		Biokit		New market		Randox		Serodia		EIAs (OD/CO)			INNO-LIA				
			Initial	Repeat	Initial	Repeat	Initial	Repeat	Initial	Repeat	Initial	Repeat	Mercia IgM	Murex ICE	New market	TpN47	TpN17	TpN15	TmpA	Result
02S0008	Late latent	Treated	2	2	2		1	0	2		4		0.25	8.71	28.83	1	3	+/-	+/-	POS
02S0015	Late latent	Treated	1	0	1	1	0	0	1	0	2	3	0.21	6.27	25.80	+/-	3	+/-	2	POS
02S0016	Late latent	Treated	0	0	0	0	0	0	1	0	2	3	0.16	5.15	13.77	+/-	3	-	-	POS
02S0040*	Primary	Untreated	0		0		0		0		0		0.20	0.32	0.15	-	-	-	-	NEG
02S0057	Unknown	Unknown	2		3		1	1	2		4		0.16	2.80	8.14	3	3	3	-	POS
02S0058	Unknown	Unknown	1	0	2		0	0	1	0	4		0.25	2.18	21.04	-	3	1	1	POS
02S0071	Unknown	Unknown	1	1	2		1	0	2		3		0.46	5.98	19.40	1	3	3	1	POS
02S0074	Unknown	Unknown	2		VOID	2	1	1	4		3		0.15	12.54	27.24	1	3	+/-	-	POS
02S0077	Unknown	Unknown	0	0	1	0	0	0	0	0	2	2	0.15	6.08	15.18	1	3	+/-	-	POS
02S0078	Unknown	Unknown	2		1	0	1	0	1	0	2		0.18	3.06	6.80	1	1	1	-	POS
02S0079	Unknown	Unknown	2	2	3		1	0	2		3		0.20	8.07	5.26	1	1	3	+/-	POS
02S0082	Unknown	Unknown	1	1	2		1	0	1	1	3		0.19	5.44	8.29	1	2	1	-	POS
02S0087	Unknown	Unknown	0	0	2		0	0	0	0	3		0.88	3.94	6.93	1	3	1	-	POS
02S0088	Unknown	Unknown	0	0	2		0	0	1	0	3		0.22	5.58	8.65	3	3	3	1	POS
02S0110	Primary	Untreated	1	0	3		1	0	2		2		1.01	6.38	5.38	1	1	1	1	POS
02S0121	Secondary	Untreated	1	0	2		1	0	1	0	3		1.65	2.25	2.35	1	2	2	2	POS
02S0188	Unknown	Unknown	1	2	2		2	0	2		2		2.38	3.46	5.71	1	1	1	1	POS
03S0281	Primary	Untreated	1	0	2		2		2		1	2	1.19	1.29	1.10	1	+/-	-	-	POS
03S0287	Primary	Untreated	0	0	1	0	0	0	1	0	2	2	1.49	0.72	0.58	+/-	+/-	+/-	-	POS
03S0316	Unknown	Unknown	1	0	2		1	0	2		3		0.19	10.46	22.34	1	3	1	+/-	POS

Notes:

Scoring for the TPHA and TPPA; 0 = negative, 1 = indeterminate, 2 = weak reaction, 3 = medium reaction, 4 = strong reaction

Innolia - scores are the intensities of the antigen lines when compared with the control lines (- = negative, +/- = weakest, 3 = strongest)

Repeat results - performed in duplicate

OD/CO >1 = positive

* Positive by darkfield microscopy

VOID - agglutination with control cells

Contact details for participating manufacturers

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Manufacturers' comments

Axis-Shield Diagnostics Ltd.



Axis-Shield Diagnostics Ltd

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12 March 2004

Dear Michelle

Re: Report on the evaluation of 'Five Syphilis Agglutination Assays'

Thank you for the opportunity to comment on your report. In summary, we believe the report to be well structured, objective and excellently presented covering all the major issues associated with syphilis testing. There is nothing in this report that we would contest and consider it to be a true reflection of the performance of the Axis-Shield Microsyph TP1000+ TPHA kit.

Subsequent to submitting this kit for evaluation, our in-house studies revealed that the kit was not as sensitive as we had hoped. This is endorsed by the findings of the report. We therefore decided not to launch this particular version of the assay and sought to improve the test sensitivity. Recently, a new version of the assay (Product code FTPHA1000) which has now been CE marked was released onto the market with a clinical sensitivity of 98% using our own cohort of syphilitic samples (n = 137). It would have been interesting to test our new kit with the positive syphilitic samples used in your study, but I understand that these are not available to Axis-Shield.

Yours sincerely,

Richard B Seaton
CUSTOMER SERVICES MANAGER
Laboratory Division



Registered in Scotland No: 77359

Biokit S.A.

biokitEvaluations Unit
Health Protection Agency

Lliçà d'Amunt, 18 February 2004

Dear Sirs,

Thank you for allowing us to see the data and the opportunity to comment. We appreciate the time and effort put in by all of the team.

We are pleased to see the sensitivity and specificity figures of our test with the different samples studied, and we are also pleased with your comments.

The figures of sensitivity mentioned in the insert correspond to the specific evaluations performed at two different sites with known positive and negative samples. As these figures do not exactly match with your results, we are going to perform additional studies to update our insert.

Regarding the assay procedure, we recommend the one mentioned in the instructions for use, which needs a total of four wells (two for sample dilution, one for antigen reagent and one for control reagent).

We do not indicate an absorption procedure for non-specific samples, because the incidence of such samples is very rare. We just indicate that these results can not be interpreted with our kit.

Yours sincerely



J. Guixer
QA&RA Director
BIOKIT, S.A.

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Mast Laboratories Ltd.



Michelle Cole
Health Protection Agency
Evaluation and Standards Laboratory
Evaluations Unit
61 Colindale Avenue
London
NW9 5HT

10/2/04

Dear Miss Cole

Re: Five Syphilis Agglutination Assays/ FUJIREBIO SERODIA TPPA TEST

Thank you for sending us the draft copy of the above report.

We are very pleased with the results reported about the FUJIREBIO SERODIA TPPA TEST.

In response to some comments in the report we wish to make the following observations

1) Kit size and particle stability

To overcome any operational difficulties associated with the seven day stability of the re-constituted particles Fujirebio supply three different pack sizes, 2 x 300 tests as evaluated in the report, 4 x 55 tests, and 5 x 20 tests which is available to special order.

2) Metal caps

The metal capped freeze dried vials have been shown to give the best long term stability of freeze dried material. We recommend that these caps are removed with forceps

3) Freeze dried particles in the stopper.

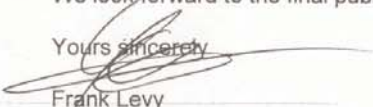
We have not had any reports that this causes reduction in sensitivity.

4) Possible cross contamination of sensitised and un-sensitised particles/microtitre plates

The incorporation of un-sensitised control particles in these kits has been shown to help increase the specificity of test results by detecting non-specific agglutination.
We would expect users to be competent in avoiding cross contamination of all test materials.

We look forward to the final publication of the report

Yours sincerely


Frank Levy
Technical Support Specialist

Newmarket Laboratories Ltd.



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Michelle Cole
MiDAS
Health Protection Agency
Evaluations and Standards Laboratory
Evaluations Unit
61 Colindale Avenue
London NW9 5HT

2004-02-12

Dear Michelle,

Re: MHRA Evaluation 'Five Syphilis Agglutination Assays'

Thank you for the opportunity to comment on your draft report.

Our comments concern two areas of the report.

1. Sensitivity

Results from samples of both known and unknown status were unexpected and disappointing. On being made aware of this you kindly agreed to retest the discrepant samples. As there was a time lag of 6 months since we first supplied trial material it was not possible to re supply the original batch.

The second batch you received would have produced results of:

Sensitivity for samples of known status: 98.2 %

Sensitivity for samples of unknown status: 99.3 %

We would very much welcome the opportunity to investigate the cause of this discrepancy at the end of your trials should discrepant sample material be available.



A Limited Company Number 2957012 Registered Office, Lanwades Business Park

2. Product claims in the Instructions for Use

Since trial material was supplied the Instructions for Use have been revised.

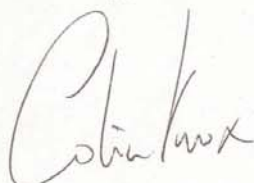
The main changes are the statements on Sample Usage and Limitations which now reads:

Specimens

Serum or plasma specimens should be free of blood cells and of obvious microbial contamination. They may be stored at 2-8 °C for up to 7 days before testing.

Specimens needing longer storage should be frozen at -20 °C or lower. Frozen specimens should be thawed and well mixed before testing.

Yours sincerely,

A handwritten signature in black ink, appearing to read 'Colin Knox'. The signature is written in a cursive style with a large initial 'C'.

Colin Knox.

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