

An Evaluation of Newmarket Laboratories TPHA 200

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Background

The aim of this evaluation was to assess and compare the ability of Newmarket Laboratories Syphilis TPHA 200 (product code 60007) to detect anti-treponemal antibodies in human serum and plasma.

Description of the assays

The *T.pallidum* haemagglutination assay (TPHA) is a serological test that detects specific treponemal antibody. Treponemal antigens are attached to red blood cells 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

Evaluation method and specimen panel

The evaluation followed a protocol that was agreed with the manufacturer. All equipment was available at the Evaluations Unit. The assay was performed according to manufacturer's instructions. Batch number 509402 was used in this evaluation.

The sensitivity was assessed by testing each assay against a panel of 114 specimens from individuals with known disease stage and treatment status, along with 121 specimens from individuals whose disease stage and treatment status was unknown. The positive specimens were either serum or plasma. The specificity was assessed by testing each assay against 249 negative blood donor specimens (serum). Details of the specimen panel are shown in Table 1. A quality control was used throughout the evaluation to monitor inter-run variability. All specimens had less than five freeze-thaw cycles. False negative, false positive and discordant specimens were retested in duplicate.

Results were read visually by three laboratory staff who independently recorded their scores. The reactions were read with reference to a scoring card supplied by the manufacturer.

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 Newmarket Laboratories TPHA 200 uses 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 to remove the non-specific reactions, and the specimens can then be retested.

Specimens that gave discordant results were tested by other supplementary assays, including Murex ICE Syphilis EIA (Abbott Laboratories, product code 500E-8E04-01), Newmarket EIA II (Newmarket Laboratories, product code 60093), Serodia TPPA (Fujirebio, product code 9241) Mercia Syphilis IgM EIA (Microgen Bioproducts, product code M404), and INNO-LIA Syphilis (Innogenetics, product code K-1089).

Table 1. Specimen panel

| Sample category | Number |
|---|---------------------------|
| 1. Blood donors' sera | 249 |
| 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. QCRU quality control sample | 1 |
| TOTAL (number of specimens) | 485 |
| Notes: | |
| BCP = Impath-BioClinical Partners Inc., USA | |
| CPHL = Central Public Health Laboratory, HPA, UK | |
| Profile = Profile Diagnostics Inc., USA | |
| SNBTS = Scottish National Blood Transfusion Service | |
| QCRU = Quality Control Reagents Unit, HPA, UK | |

Results

Specificity

The specificity was assessed by testing the assay against 249 blood donors. The assay achieved a final specificity of 100% (Table 2).

Table 2. Specificity

| Assay | Number tested | Number initially reactive | Number repeatedly reactive | % Specificity (95% confidence intervals) |
|----------------|---------------|---------------------------|----------------------------|--|
| Newmarket TPHA | 249 | 0 | 0 | 100 (98.5 - 100) |

Sensitivity

Of the 114 specimens from individuals with known disease stage and treatment status, 113 (including indeterminates) were detected by the assay, giving a final sensitivity of 99.1%. (Table 3). The TPHA repeatedly failed to detect a specimen from an individual who had untreated primary syphilis. Two late-latent specimens from an individual who had been treated gave indeterminate reactions. One specimen was excluded in the initial sensitivity calculation due to agglutination of the control cells.

Table 3. Sensitivity at different disease stages

| Assay | Treatment Status | Number positive (number indeterminate) at different disease stages | | | | % Sensitivity (95% confidence interval) |
|---------|------------------|--|-----------|--------------|-------------|---|
| | | Primary | Secondary | Early latent | Late latent | |
| Initial | Untreated | 32/33 | 37/37* | 15/15 | 4/4 | 99.1 (95.2 - 100) |
| | Treated | 7/7 | 5/5 | 4/4 | 6/8 (2) | |
| Repeat | Untreated | 32/33 | 38/38 | 15/15 | 4/4 | 99.1 (95.2 - 100) |
| | Treated | 7/7 | 5/5 | 4/4 | 6/8 (2) | |

Note: *1 sample excluded due to agglutination with control cells (see 'Evaluation method')

A summary of positive reactions and the overall sensitivity, when the disease stage and treatment status is unknown, is shown Table 4. The Newmarket TPHA gave a sensitivity of 100%. Again, One specimen was excluded in the initial sensitivity calculation due to agglutination of the control cells.

Table 4. Sensitivity when disease stage and treatment status was unknown

| | Number positive (number indeterminate) | % Sensitivity (95% confidence interval) |
|--------------------|--|---|
| Initial (n = 120*) | 120 (0) | 100 (97.0 - 100) |
| Repeat (n = 121) | 121 (0) | 100 (97.0 - 100) |

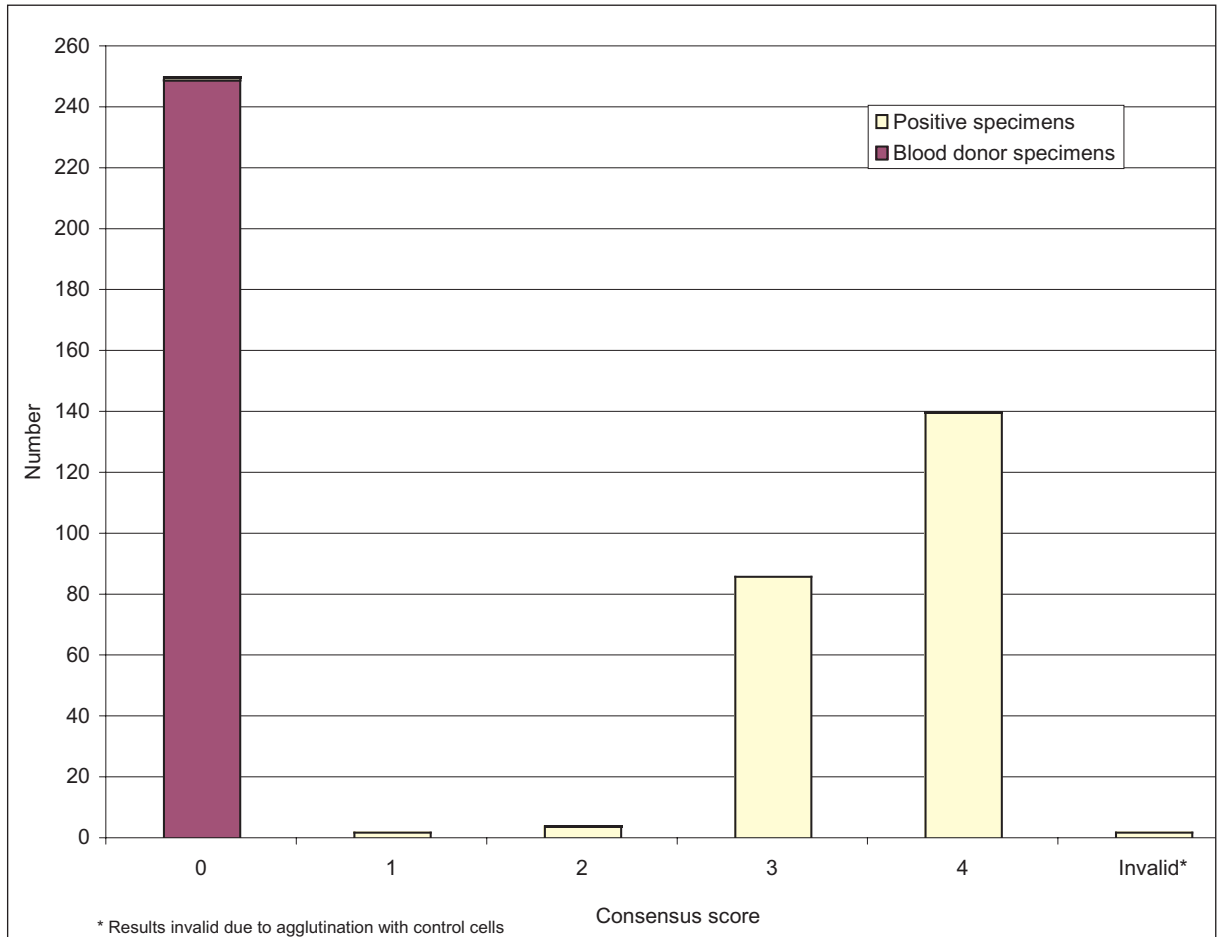
Note: *1 sample was excluded due to agglutination with control cells (see 'Evaluation method')

On the basis of results from other syphilis tests (see Evaluation method), the two late latent treated specimens which gave an indeterminate reaction were deemed to be positive. These results are shown in Table 6. The only specimen not confirmed positive by these methods was positive only by darkfield microscopy only. This specimen was from a patient that had been treated for primary syphilis, and was the same primary specimen not detected by the TPHA.

Distribution of initial reactivities

The distribution of initial reactivities for the 235 syphilis positive specimens and 249 specimens from blood donors are presented in Figure 1. Two positive specimens tested by the assay gave indeterminate reactions and there was one initial false negative specimen. Assays with good discrimination between positives and negatives have few, or no specimens wrongly classified and few reactions in close proximity to the cut off.

Figure 1. Distribution of initial reactivities for the Newmarket TPHA 200 assay



Quality Control Specimens

Manufacturers’ kit controls and an internal quality control specimen (HPA anti-*Treponema pallidum* QC1, bath number 04/B415-01) 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 5. The positive and negative controls provided with the kit and the quality control gave the expected results on all runs.

Table 5. Manufacturers’ kit controls and quality control results

| | Kit controls | | Internal QC |
|-------------------|--------------|----------|-------------|
| | Positive | Negative | |
| Range (mode) | 4 (4) | 0 (0) | 1 - 2 (2) |
| Total no. of runs | 9 | 9 | 9 |

Note: Scores are the consensus of the three readers

Evaluator's comments

The Newmarket Laboratories TPHA assay was very easy to use, and results were available after a 45 minute incubation. The control wells were useful, as they allow non-specific agglutination to be detected. At times, strong positive agglutination patterns were prone to folding at the edges to give a ragged appearance. However, this did not affect the overall interpretation. According to the manufacturer, the folding may be due to a combination of sample age and freeze/thawing. Indeterminate and weak positive reactions were the most difficult to interpret. It should be noted that as the agglutination patterns were read visually, they are subjective.

Conclusion

The final specificity for the Newmarket Laboratories TPHA 200 was 100%.

When the disease stage and treatment status were known the assay gave a final sensitivity of 99.1 %. When the disease stage and treatment status was unknown assay gave a sensitivity of 100%.

The manufacturer's controls and the external quality control gave the expected results on all runs.

Appendix

Table 6. Discordant specimen results and supplementary testing

| Evaluations Unit No. | Disease stage | Treatment Status | Newmarket assay evaluation data | | Supplementary data | | | | | | | | |
|-------------------------|------------------|---------------------|------------------------------------|---------------------|--------------------|---------------|---------------------|-----------------|---------|-------|-------|------|--------|
| | | | Initial consensus | Repeat consensus | EIAs (OD/CO) | | | Serodia TPPA | Innolia | | | | Result |
| | | | | | MUREX ICE | Mercia IgM | Newmarket EIA II | | TpN47 | TpN17 | TpN15 | TmpA | |
| 02S0040* | Primary | Untreated | 0 | 0 | 0.32 | 0.20 | 0.08282 | 0 | - | - | - | - | NEG |
| 02S0015 | Late latent | Treated | 1 | 1 | 6.27 | 0.21 | 26.306 | 3 | +/- | 3 | +/- | 2 | POS |
| 02S0016 | Late latent | Treated | 1 | 1 | 5.15 | 0.16 | 16.806 | 3 | +/- | 3 | - | - | POS |

When OD/CO > 1, the result is positive
Scoring for the TPHA and TPPA; 0 = negative, 1 = indeterminate, 2 = weakly reaction, 3 = medium reaction, 4 = strong reaction
Innolia - scores are the intensities of the antigen lines when compared with the control lines
* Positive by darkfield microscopy