Comparative performance measurement of agglutination assays for antibody detection against *Treponema pallidum*

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Introduction

The agglutination assay is an essential part of the stepwise diagnostic of syphilis according to the current guideline. The assay confirms the presence of antibodies against Treponema pallidum. Agglutination assays differ in the use of gelatin particles (TPPA), bird erythrocytes (TPHA) or latex particles (TPLA) as antigen carriers. The TPPA from Fujirebio is regarded as the gold standard in syphilis diagnostics. However, Fujirebio decided to stop the production in 2022. The Paul-Ehrlich-Institute published a recommendation against the use of the TPHA after a series of false-negative results for blood donations in 2020. Hence, it is essential to identify an alternative assay for the TPPA using a comparative performance measurement of agglutination assays to accordingly define new guidelines for future syphilis diagnostics.

Material and Methods

Serum specimens were pre-analyzed with the screening test Alinity i Syphilis TP CMIA (Abbott). Based on in-house validation results of 0.3–0.9 were defined as borderline. The following agglutination assays were included for comparison: Sekure TPLA Assay (SEKISUI Diagnostics, TPLA), TPPA (Serodia Fujirebio Inc.), TPHA (Bio-Rad Laboratories Inc.) and TPHA (Newmarket Biomedical Ltd.).

Results

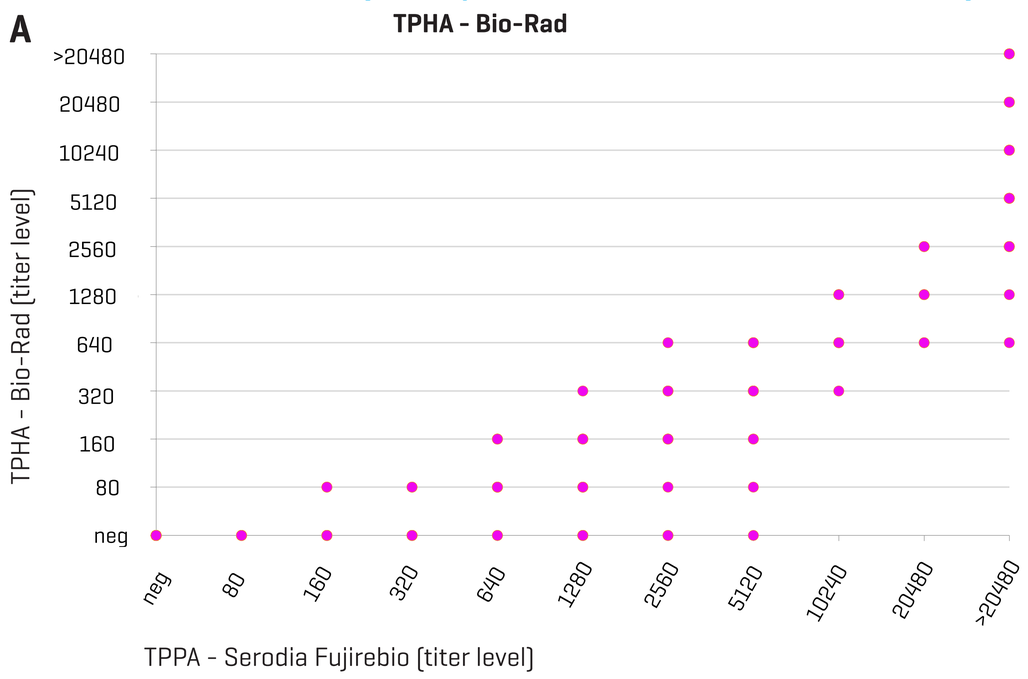
TPLA deviates significantly from TPPA results

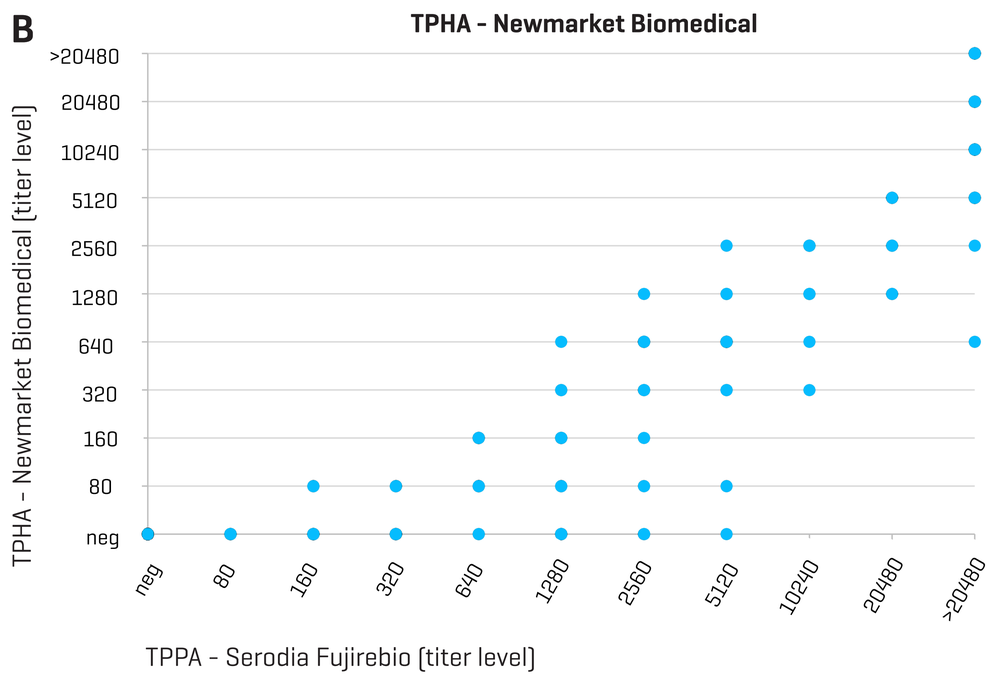
Table 1: Comparison of TPLA and TPPA

	TPLA positive	TPLA negative	Total
TPPA positive	107	22	129
TPPA negative	12	535	547
Total	119	557	676
Sensitivity	82.9 %		
Specificity	97.8 %		

Table 1: Comparison of TPLA and TPPA:
In total, 676 samples were analyzed, of which 535 samples were scored negative in both tests and 107 samples were scored positive in both tests. There were 22 samples scored false negative and 12 samples scored false positive in the TPLA. The correlation of TPPA titer levels and titer units of TPLA was calculated for all positive study samples, showing a correlation coefficient of R=0.73. Thus TPLA deviates significantly from the TPPA results. The sensitivity for the measured group was calculated with 82.9 % and the specificity with 97.8 %.

Lower titer levels were frequently measured with the TPHA compared to the TPPA





TPLA positiv > 10 TU

Figure A and B: Comparison of TPHA and TPPA titer levels.
A total of 684 samples were analyzed on both TPHA test systems and the TPPA. Of these, 555 samples showed a negative result in the TPHA test systems and in the TPPA and 94 samples showed a positive result in both tests. Based on these results, a correlation of R=0.87 was calculated for both TPHA test systems compared to the TPPA, for the group used in this study. It can be seen that significantly lower titer levels were frequently measured with the TPHA compared to the TPPA.

The TPHA and TPLA detect highly infectious samples less reliably compared to the TPPA

Table 2 a-c: Results of patient samples with well-defined medical history.

a: Samples with primary affection

Sample	TPPA (Fujirebio)	CMIA (Abbott)	TPLA (SEKISUI)	TPHA (Bio-Rad)	TPHA (New Bio)
1	1:640	0.52	1	negative	negative
2	1:2560	0.76	5	negative	negative
3	1:1280	1.93	21	negative	negative

b: Samples with seroconversion

Sample	TPPA (Fujirebio)	CMIA (Abbott)	TPLA (SEKISUI)	TPHA (Bio-Rad)	TPHA (New Bio)
1	1:160	5.18	12	negative	negative
2	1:320	0.73	1	negative	negative
3	1:2560	0.78	8	negative	negative

c: Samples with Syphilis *satis curata*

Sample	TPPA (Fujirebio)	CMIA (Abbott)	TPLA (SEKISUI)	TPHA (Bio-Rad)	TPHA (New Bio)
1	1:160	0.61	7	negative	negative
2	1:320	4.76	42	negative	negative
3	1:80	1.49	33	negative	negative

Table 3: Detection rate of specimen with known primary affection.

n=22	TPPA (Fujirebio)	CMIA (Abbott)	TPLA (SEKISUI)	TPHA (Bio-Rad)	TPHA (New Bio)
Positive	100.0 %	100.0 %* (63.6 %)**	40.9 %	13.6 %	13.6 %
False Negative	0.0 %	0.0 %* (36.4 %)**	59.1 %	86.4 %	86.4 %

Table 4: Detection rate of specimen with residual findings after treated infection.

n=27	TPPA (Fujirebio)	CMIA (Abbott)	TPLA (SEKISUI)	TPHA (Bio-Rad)	TPHA (New Bio)
Positive	100.0 %	100.0 %* (81.5 %)**	92.6 %	74.1 %	77.8 %
False Negative	0.0 %	0.0 %	8.1 %	21.6 %	18.9 %
TPPA ≥640 (n=18)	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %
TPPA ≤320 (n=9)	100.0 %	100.0 %* (44.4 %)**	77.8 %	22.2 %	33.3 %

**Borderline values are included. **According to the manufacturer interpretation criteria.*

TPPA/TPHA positiv ≥1:80

Table 2 a-c, 3 and 4: Detection rate of specimens with known medical history.
One aim of our study was to test the reliability of TPHA and TPLA regarding the detection of highly infectious specimens, e.g., from patients with primary affection. The results of patients with well-defined medical histories show significant under-detection for the compared TPHA/TPLA tests in the early phase of infection (table 2a&b). Analysis of a group with known primary affection (n=22) revealed a detection rate of 13.6 % for both TPHA assays (table 3). Detection rate of TPLA was 40.9 % and 100 % in CMIA, if our internal borderline values were included. Another important study group consists of specimen with residual antibody findings after infection and treatment. Here, differences can be seen according to the titer level in TPPA. Specimens (n=27) with residual antibodies and titer level ≤320 were detected with a rate of 22.2 and 33.3 % in TPHA tests (table 2c and 4).

Conclusion

The aim of our study was to compare particle agglutination assays with the gold standard TPPA to identify alternative assays. Analysis of TPLA showed insufficient correlation and instable titer level detection. TPHA assays show almost no differences between manufacturers. Both assays fail in the detection of highly infectious specimen from patients with primary affection. Additionally, specimen with low titer levels of residual antibodies were almost not detected. Taken together, it appears that none of the tested agglutination assays could replace the diagnostic reliability of the no longer produced TPPA. Therefore, the current guideline has to be revised and ongoing investigation is needed to find an alternative procedure for the stepwise diagnostic of syphilis.