Evaluation of Mediace Treponema Pallidum Latex Agglutination (TPLA) and Rapid Plasma Reagin (RPR) Assay on the Roche Cobas c702 Platform

Muna Osman1,2, Stephen Phoenix2, Megan Northam3, James Ezard2, James Barnes2, Alan Lord2
1University of Staffordshire, College Rd, Stoke-on-Trent ST4 2DE. 2Virology laboratory Manchester Medical Microbiology Partnership, Clinical Sciences Building, Manchester University NHS Foundation Trust, Oxford Road, Manchester M13 9WL, 3 Biochemistry laboratory, Clinical Sciences Building, Manchester University NHS Foundation Trust, Oxford Road, Manchester M13 9WL

Introduction

Syphilis, caused by Treponema pallidum bacterium, remains a major global concern as untreated tertiary-stage syphilis is fatal in 60% of cases (Kojima and Lausner, J. 2018). The current syphilis testing algorithm in Virology at Manchester Foundation Trust, employ a reverse algorithm where treponemal screens are followed by treponemal and non-treponemal manual confirmatory tests. The evaluation of the automated assay performance will facilitate the replacement of the labour-intensive manual process.

Aims and Objectives

•Verify the automated Mediace TPLA and RPR assay on Roche Cobas c702 platform by assessing specificity, sensitivity, reproductivity and repeatability.

•Investigate the efficiency and accuracy with the goal of minimising subjective interpretation, human error and increase throughput capacity using automated system.

•Support UKHSA's mission to address the rising prevalence of syphilis in the UK.

Methods

•158 frozen reactive samples, previously tested with manual TPPA, RPR, TPHA, Line Blot, and EQA samples, retested with Mediace TPLA and RPR assays, on Roche Cobas 8000 c702 photometric analyser.

•Samples selected from a 12-month pool (Jan 2023 - Jan 2024) using the Slicer Dicer tool via LIMS Beaker.

•Sensitivity, specificity, PPV, NPV, repeatability, and reproducibility (CV) calculated from true/false positive/negative results to investigate reactivity levels.

Results

The performance characteristics of the TPLA assay against TPPA samples demonstrated in Figure 1. The study results for TPLA assay against TPHA samples are shown in figure 2. Furthermore, the study results for automated RPR assayare shown in figure 3.

Performance Characteristic	IFU Results for TPLA Assay	Study Results using TPPA Samples
Sensitivity	100%	100%
Specificity	99.60%	69%
PPV	100%	92%
NPV	99.60%	100%

Figure 1: Performance Characteristics of the TPLA Assay Using TPPA samples

Performance Characteristic	IFU Results for TPLA Assay	Study Results using TPHA Samples
Sensitivity	100%	95%
Specificity	99.60%	59%
PPV	100%	75%
NPV	99.6	91%

Figure 2: Performance Characteristics of the TPLA Assay Using TPHA samples

Performance Characteristic	IFU Results for RPR Assay	Study Results
Sensitivity	99.50%	91%
Specificity	99.50%	88%
PPV	99.50%	93%
PPV	99.60%	85%

Figure 3: Performance Characteristics of RPR Assay

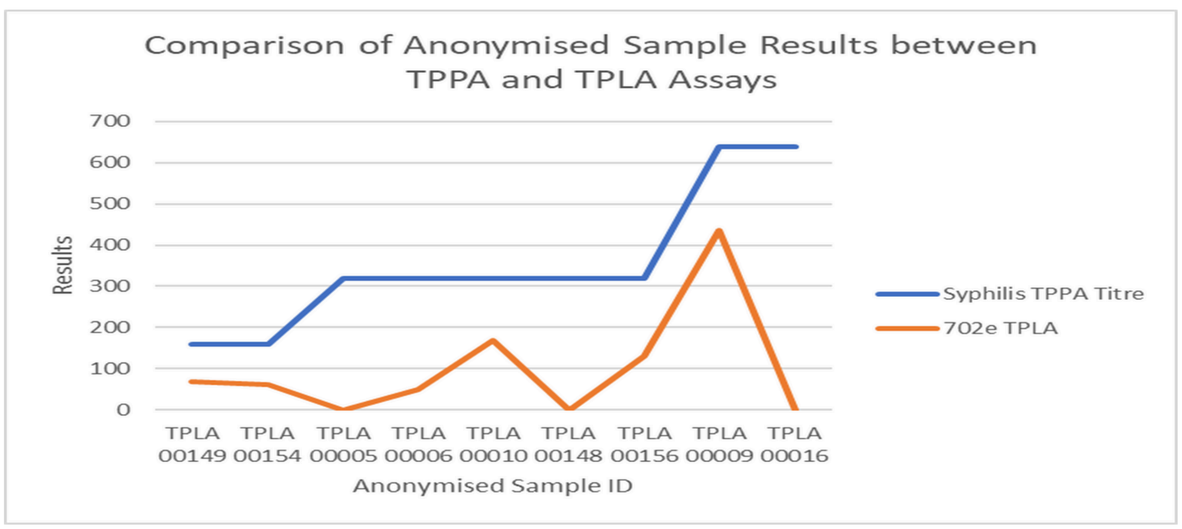

Figure 4: Comparison of Anonymised Sample results between TPPA and TPLA Assays

Discussion

The TPLA sensitivity assay on TPPA samples was consistent with IFU claims, confirming the study hypothesis thus could potentially replace the current TPHA manual tests (Murai, R. et al. 2019). The low specificity attained (figure1) is likely due to archived frozen samples subjected to two freeze–thaw cycles (Park, 2016). Sample integrity could be compromised due to temperature fluctuations leading to cell protein degradation causing nonspecific binding (Chen et al., 2017)

The results suggest that TPLA assay correlates better with TPPA then TPHA. Both TPLA and TPPA promote better antibody -antigen complex formation compared to erythrocytes utilised by TPHA (Sidana et al, 2011). The weaker agreement with TPHA is likely due to assay design. erythrocytes are less stable more prone to nonspecific binding compared to the denser sonicated gelatin or latex particles employed in TPPA and TPLA (Park et al, 2016).
The lack of TPHA previously tested samples in this study restricted statistical power. This was due to the recent introduction of TPHA in the Virology laboratory replacing the discontinued TPPA, thus the low specificity of TPLA assay of 59% expressed in the study does not give a true reflection of the automated assay capability (Leeflang, et al 2013).

The reduced sensitivity of the RPR assay, due to 8 false-negatives samples, can be explained by the prozone effect. At very high antibody concentrations, antigen–antibody lattice formation is inhibited masking reactivity and producing falsely negative results (Sidana et al, 2011).
Reduced specificity caused by false positives, can be attributed to the structural fragility of the reagin antibodies detected by RPR. These antibodies target lipid antigens. The lipid–antibody interactions rely on precise hydrophobic and charge-based binding. Freeze–thaw cycles can disrupt antibody conformation, leading to denaturation and reduced cross-linking capacity, thereby promoting nonspecific agglutination (Chen, Werner and Brenner, 2017).

Conclusion

Automated Mediace TPLA and RPR assays on the Cobas c702 offers superior advantages to manual syphilis testing, including higher throughput, greater consistency, faster turnaround and lower operator variability. Although not all the performance characteristics align with the IFU claims. The assay is suitable for the intended purpose. Further evaluation with a larger sample size and fresh clinical samples is necessary.

Follow Up

Between January to July 2025, a follow up validation report was performed to evaluate the performance characteristics of the RPR and TPLA on fresh samples. Frozen samples previously tested using TPPA/TPHA and recent serum samples following routine testing by TPHA were retested and yielded 95% sensitivity, 85.71% specificity 97% PPV and 75% NPV. Findings concluded TPHA processed by multiple operators without a clearly defined positive/negative interpretation caused significant variation in result. The TPLA assay represents a promising alternative to the manual TPHA. Fresh and refrigerated samples previously tested within the last 7 days using manual RPR assay were re-tested and generated 84.29% sensitivity, 71.43% specificity, 78.67% PPV and 78.43% NPV. Findings concluded automated RPR RU values does not correlate linearly with the manual RPR titres, which limits its use for monitoring disease progression and assessing treatment response. Automated RPR could be used as an initial screening test to filter negative specimen, then use manual RPR for specimens with a R.U./ml greater than or equal to 1.0, so that a precise titre can be determined.

References

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