Development of a Novel Multiplex PCR for Rapid Diagnosis of Mpox, Syphilis, and Herpes Infections

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PLAIN LANGUAGE SUMMARY

This poster shows the development of an investigational laboratory test that can look for mpox, syphilis, and herpes at the same time using a single sample. In this early study, the test showed promising agreement with existing laboratory methods and suggests that combined testing could help public health labs evaluate genital lesion cases more efficiently, though further validation is needed before any clinical use.

BACKGROUND

Mpox is a significant emerging public health concern, with an increasing number of community transmission cases reported in the US. The virus spreads through close contact with infected individuals such as, but not limited to exposure during intercourse, often leading to the development of genital lesions. This pattern of transmission overlaps with a rise in sexually transmitted infections, further complicating diagnosis, specially in cases of coinfection. At the Dallas County Health and Human Services (DCHHS), the syphilis positivity rate has been as high as 25% in 2023, with current positivity rate at 17.8% in 2026[2]. Meanwhile, herpes remains a common STI in the US[1]. Given the overlap in lesion presentation among Mpox, syphilis, and herpes, a multiplex PCR (MPCR) assay could detect these infections simultaneously following syndromic approach to testing. This MPCR assay may provide streamlined testing protocols to enable more efficient response to public health needs, while ensuring accurate, timely results. The developed MPCR addresses current and emergent diagnostic needs of the community where commercial testing options are currently limited.

METHODS

The protocol was evaluated using the TaqPath™ BactoPure™ Microbial Detection Master Mix for its high specificity and sensitivity for microbial DNA detection, the CDC primers/probe[1] sets for the Mpox and extraction control detection, and primers/probe sets for HSV1/2, and *Treponema pallidum* detection previously reported by the DCHHS[2,3]. DNA was extracted from dry swabs from genital lesion collected for surveillance using the QiAamp DSP DNA Mini Kit. Genital lesion and archived Mpox eluates were tested, then results were compared to current comparable laboratory tests.

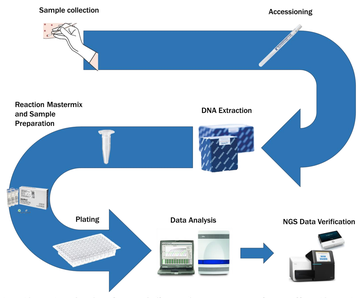


Figure 1: MPCR testing technical workflow from sample collection to data analysis.

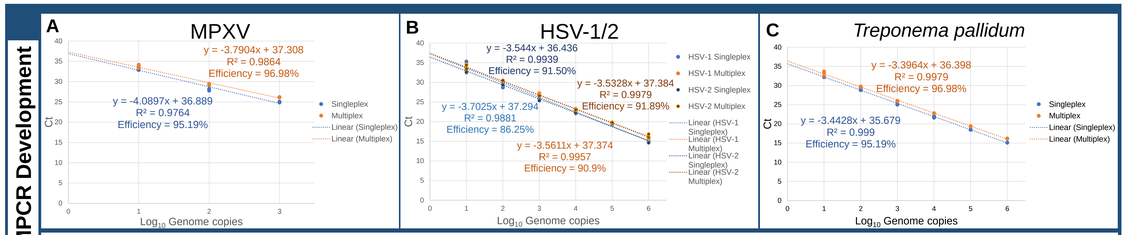


Figure 2: (A-C) Standard curve of the primers and probe set for the detection of Mpox virus, HSV-1/2, and *Treponema pallidum*. Graphs contain R^2 and replication efficiency analysis of the primers and probe sets in singleplex and multiplex.

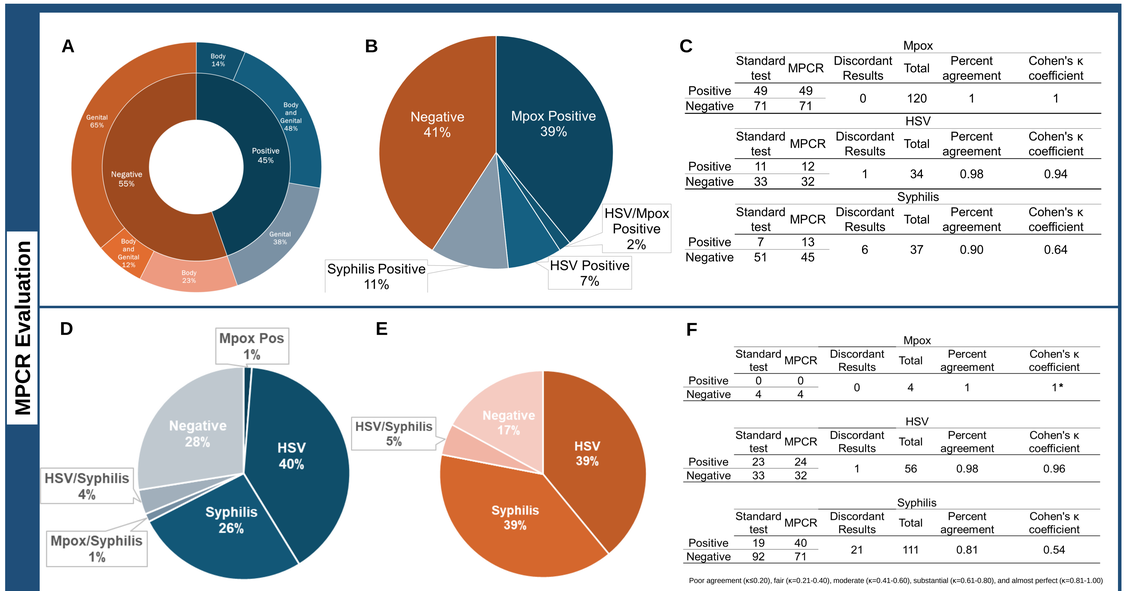


Figure 3: (A-C) Analysis of blinded archived Mpox extraction samples. **(A)** Lesion site distribution stratified by Mpox test results, showing the anatomical locations of lesions in Mpox positive and negative cases (N=47). **(B)** MPCR results of the archived samples (N=120) and **(C)** concordance analysis to comparable laboratory test. **(D-F)** Analysis of blinded clinical samples from **(D)** darkfield remnants (N=80) and **(E)** direct genital lesions (N=41) collected in dry swabs matrix. **(F)** Concordance analysis to comparable laboratory test of the clinical samples.

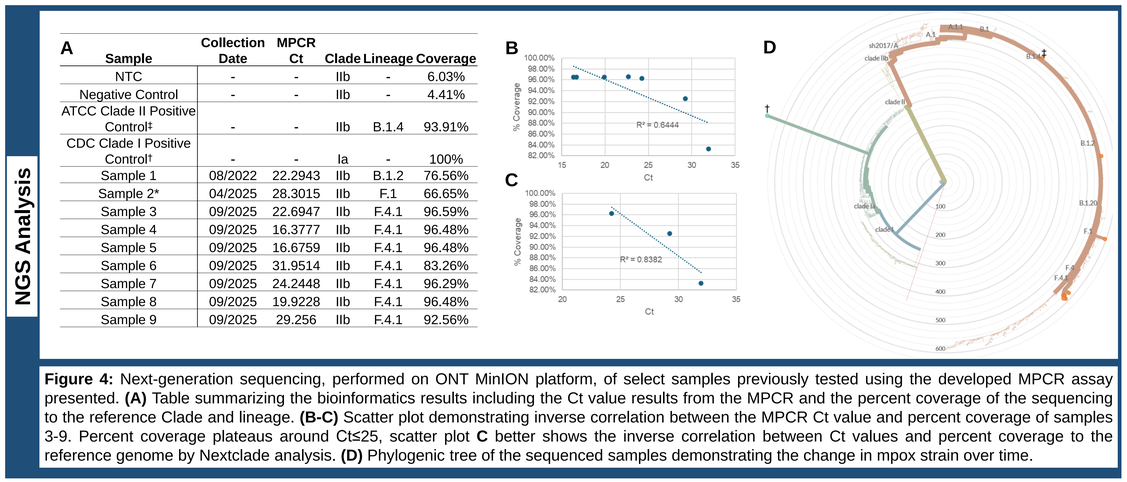


Figure 4: Next-generation sequencing, performed on ONT MinION platform, of select samples previously tested using the developed MPCR assay presented. **(A)** Table summarizing the bioinformatics results including the Ct value results from the MPCR and the percent coverage of the sequencing to the reference Clade and lineage. **(B-C)** Scatter plot demonstrating inverse correlation between the MPCR Ct value and percent coverage of samples 3-9. Percent coverage plateaus around Ct≤25, scatter plot **C** better shows the inverse correlation between Ct values and percent coverage to the reference genome by Nextclade analysis. **(D)** Phylogenic tree of the sequenced samples demonstrating the change in mpox strain over time.

RESULTS

The developed MPCR assay showed >90% efficiency with an LoD of 10 genomes copies (Figure 2). Lesion collection site of archived Mpox samples showed that samples were mostly associated with genital lesions (Figure 3A). The MPCR assay identified 13 *T. pallidum* and 9 HSV previously tested negative for Mpox and 2 samples coinfected with Mpox and HSV (Figure 3B). Overall, the MPCR assay demonstrated high concordance with prior results (κ=1) (Figure 3C). HSV samples also showed strong agreement (κ=0.94), while syphilis had lower concordance, potentially reflecting known limitations of darkfield microscopy, such as spirochete motility required for positive results (Figure 3C). When surveillance clinical samples were evaluated, the MPCR assay demonstrated similar performance as the archived Mpox samples (Figure 3D-F). Samples sequenced using the Oxford Nanopore Technology (ONT) further corroborated the MPCR results showing how different molecular techniques can be used to enhance diagnostic testing and public health surveillance efforts simultaneously (Figure 4).

CONCLUSIONS

The MPCR assay was designed to enable simultaneous detection of Mpox virus, HSV-1/2 and *T. pallidum* with a goal to improve efficiency and aid in diagnosis of atypical genital lesion cases. It can reduce testing time and resources for public health labs and introduces a novel MPCR-based method for direct detection of *T. pallidum* at lesion sites. The MPCR was able to detect two Mpox from darkfield slide remnants* and detected *T. pallidum* even in an RPR-negative case. These findings shows the potential for molecular detection of pathogens that may not be identified in some cases using current diagnostic approaches, warranting further evaluation. Reflex tests are being developed for Mpox virus clade determination and syphilis confirmation. The assay is planned for CLIA and FDA validation for potential integration into the DCHHS SHC testing algorithm.

ADDITIONAL KEY INFORMATION

Acknowledgements/ Sources

APHL-CDC Fellowship Program

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Healthy People Healthy Communities Health and Social Equity