Novel HPV Reference Materials Representing Normalized Viral Load for Use in Assay Validation

Zhelev P[1], Rivers S[1], Alagic A[1], Li J[1], Lutz K[2], Vaughan L[2], Randall C[1]

[1]Microbix Biosystems, Mississauga, ON, Canada

[2]BD Life Sciences, Sparks, United states

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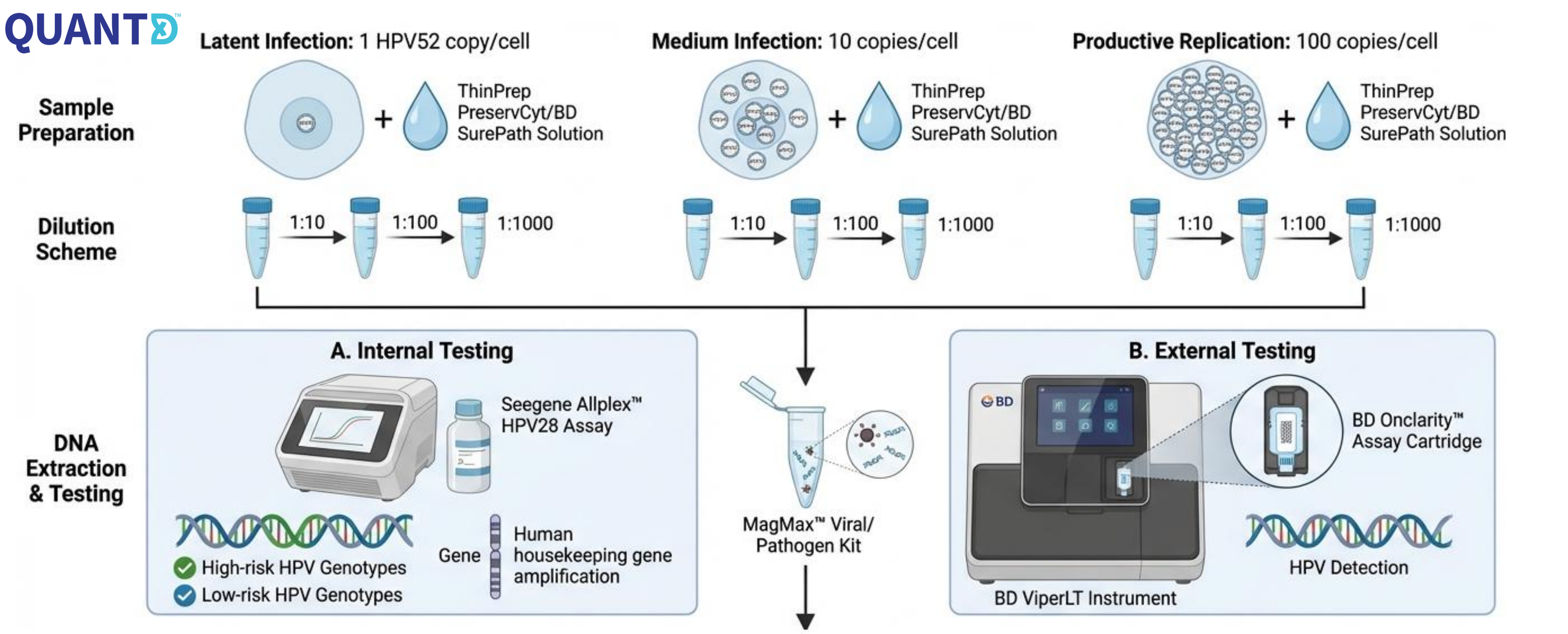
INTRODUCTION

Quantifying human papillomavirus (HPV) DNA copy number per cell is a key metric for understanding viral infection dynamics, disease progression, and oncogenic risk—especially with the emergence of second-generation assays aimed at improving quantification and qualification. Microbix has initiated the development of prototype normalized copy number controls that reflect distinct stages of HPV infection: latent (1–10 copies/cell), medium (10–100), and productive replication (>100). These levels correlate with lesion severity and viral activity.

Elevated copy numbers are frequently associated with high-risk HPV types and may indicate viral genome integration—a critical step in carcinogenesis. Copy number also fluctuates during the cell cycle, amplifying during S-phase and reducing during mitosis, which underscores the complex interaction between viral and host mechanisms. Clinically, normalized HPV copy number serves as a valuable biomarker for screening, prognosis, and treatment monitoring. However, reliable controls for assay validation remain limited. Recent studies report copy numbers ranging from 2.3 to over 100 copies per cell in cervical smears, reinforcing its utility in stratifying infection risk and guiding therapeutic strategies [1,2,3] .

MATERIALS & METHODS



RESULTS

Both assays demonstrated high sensitivity for detecting different HPV 52 infection stages. Medium and Productive stages were resolved down to extremely low cellularity. Latent infection was consistently detected at low cellularity (1:100) but dropped at extremely low levels (1:1000), confirming the expected viral-to-cell ratio in the sample. Notably, both assays exhibited no cross-interference between targets or infection stages, confirming consistent performance and independent titration of HPV52 and HBB markers.

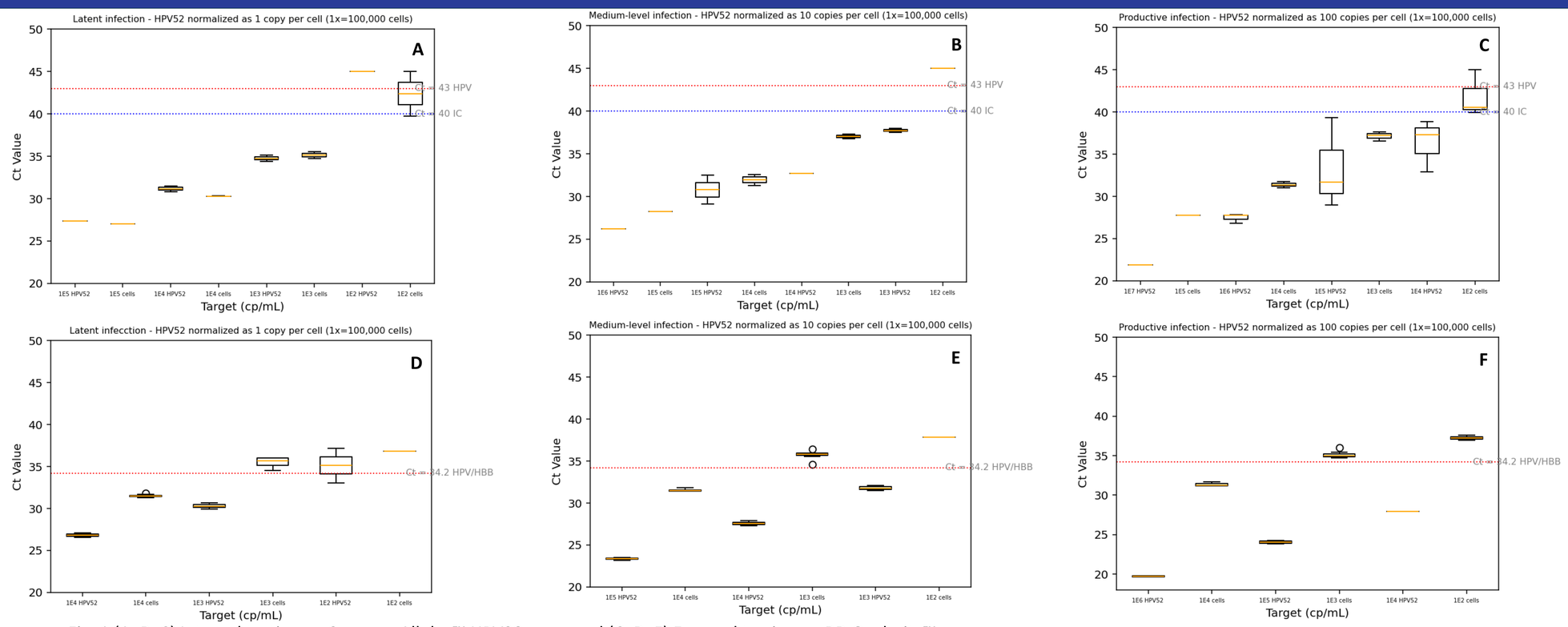


Fig. 1 (A, B, C) Internal testing on Seegene Allplex™ HPV28 assay and (C, D, F) External testing on BD Onclarity™ assay

CONCLUSIONS

These findings confirm that QUANTDx-normalized HPV samples provide a robust framework for evaluating assay performance across varying disease states and sample qualities, as reflected in the dilution series. The ability of both assays to consistently detect HPV52—even under poor cellularity conditions—supports the refinement of minimum target cut-offs for defining “true” negatives and strengthens the reliability of co-detection algorithms. This, in turn, can significantly improve triage accuracy in clinical practice.

Moreover, the consistent detection of HBB alongside HPV52 highlights the importance of normalized internal controls. By ensuring equivalent representation of target and housekeeping genes, these controls establish the minimum sample quality and quantity required for reproducible results. Given that detection sensitivity and PCR efficiency may differ across HPV genotypes, the integration of such controls is essential to enhance diagnostic confidence and optimize patient management.

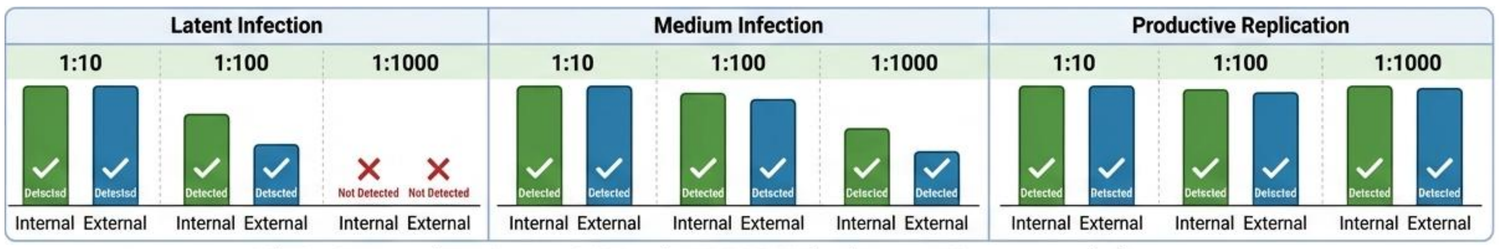


Fig. 2 Dilution impacts detection sensitivity at low HPV DNA levels; comparison across platforms.

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3. **Terzi, N.K., & Yulek, O. (2024).** Assessment of Cervicovaginal Smear and HPV DNA Co-Test for Cervical Cancer Screening: Implications for Diagnosis and Follow-Up Strategies. **Diagnostics 14(6):611.**

CONTACT US

Pavel Zhelev, Director of Product Management (QAPs)
Email: pavel.zhelev@microbix.com

Microbix Biosystems Inc.
+1-800-794-6694
+1-905-361-8910
www.microbix.com