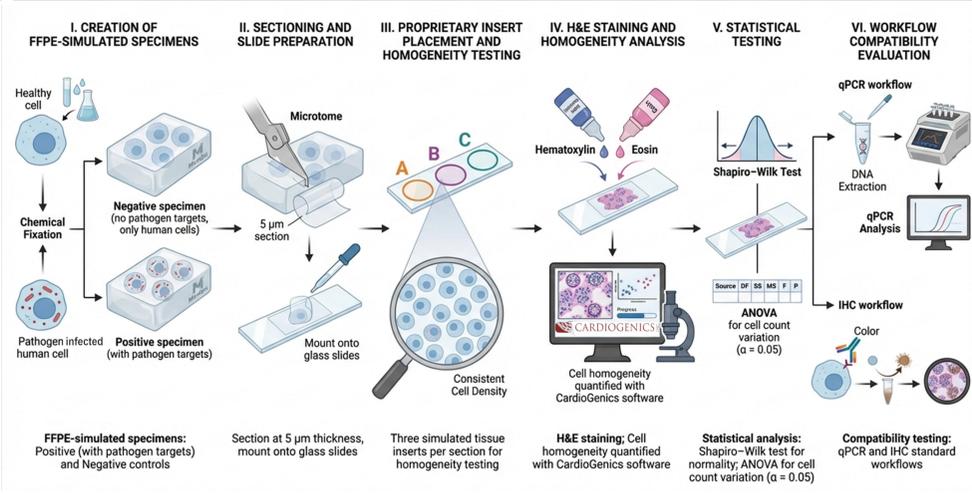


INTRODUCTION & AIM

Formalin-Fixed Paraffin-Embedded (FFPE) tissue is indispensable in histopathology and reflex molecular diagnostics. However, the absence of standardized, reproducible FFPE-based quality control (QC) materials limits NAAT/IHC assay comparability and diagnostic reliability across laboratories. To address this need, Microbix developed simulated FFPE QC materials for key infectious agents relevant to pathology and molecular workflows—Human Papillomavirus (HPV), Herpes Simplex Virus (HSV-1/2), and *Chlamydia trachomatis*.

Our objective was to develop standardized FFPE-based reference materials for HPV, HSV, and *Chlamydia trachomatis* to address variability in diagnostic workflows. These controls will improve reproducibility and validation of PCR and IHC assays for OPSCC, HSV-related disease, and *Chlamydia* rectal biopsies, ensuring accurate diagnosis across laboratories.

MATERIALS & METHODS



RESULTS

1. Homogeneity Assessment- Cell Counting – Descriptive Statistics

Slide ID (A,B,C sections)	Total Cell Count Average	Total SD	Total CV(%)
1 st - 40 th Slide HPV-Positive (A, B, C)	1092	148	13.55
1 st - 40 th Slide HPV-Negative (A, B, C)	5819	657	11.29

Slide ID (A,B,C sections)	Total Cell Count Average	Total SD	Total CV(%)
1 st - 50 th Slide HSV-1 (A, B, C)	1642	111	6.77
1 st - 50 th Slide HSV-2 (A, B, C)	1545	95	6.12

Slide ID (A,B,C sections)	Total Cell Count Average	Total SD	Total CV(%)
1 st - 25 th Slide CT (A, B, C)	4222	168	3.98

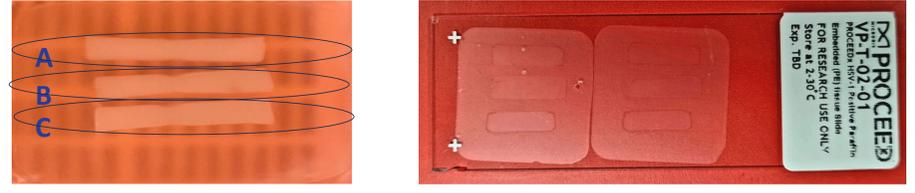


Figure 1: A, B, C inserts in paraffin block and PORCEEDx™ tissue sections (thickness of 5 µm)

2. H&E Staining, Cell Counting and IHC – Inferential Statistics

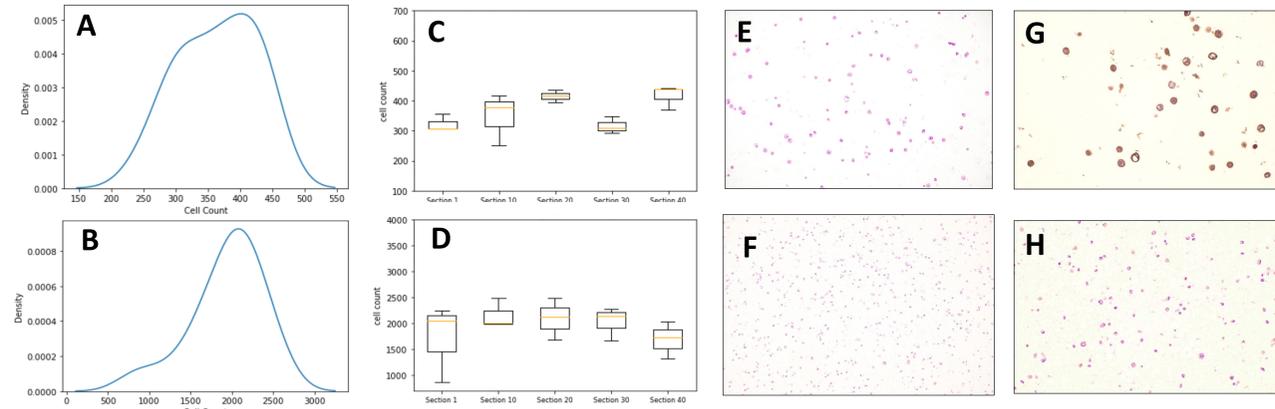


Figure 2: (A) Kernel Density Estimation Plot of HPV-positive samples ($s=0.933$, $p=0.301$), (B) Kernel Density Estimation Plot of HPV-negative samples ($s=0.914$, $p=0.157$), (C) HPV-positive slide cell count ($f=3.33$, $p=0.0561$), (D) HPV-negative slide cell count ($f=0.70$, $p=0.6118$), (E) HPV16 cell line H&E 10x, (F) HPV-Negative cell line H&E 10x (G) p16 expression with HPV16 cell line, (H) HPV-Negative control

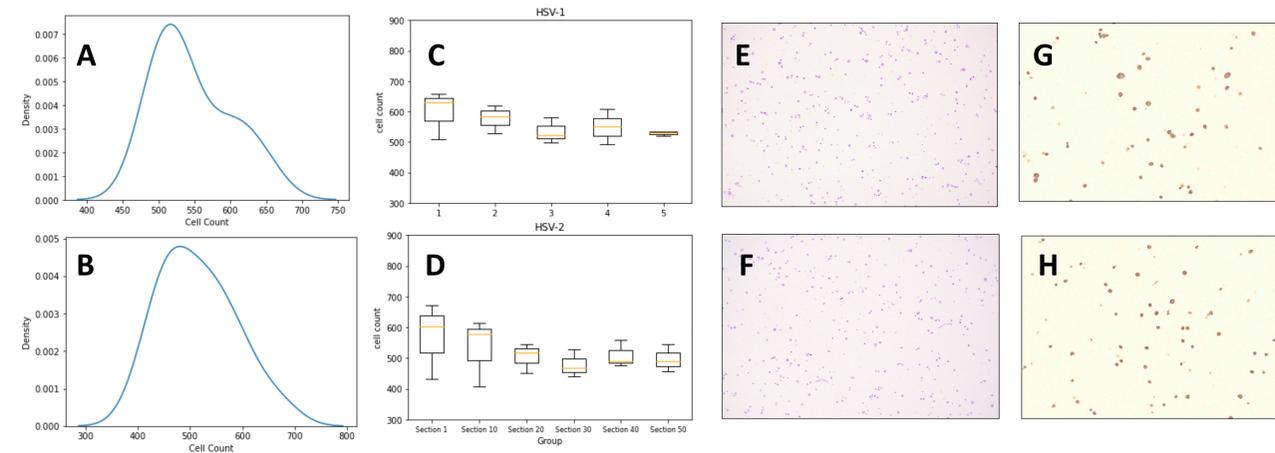


Figure 3: (A) Kernel Density Estimation Plot of HSV-1 positive samples ($S=0.914$, $p=0.101$), (B) Kernel Density Estimation Plot of HSV-2 positive samples ($S=0.967$, $p=0.742$), (C) HSV-1 positive slide cell count ($f=1.78$, $p=0.1916$), (D) HSV-2 positive slide cell count ($f=0.50$, $p=0.7715$), (E) HSV-1 cell line H&E 10x, (F) HSV-2 cell line H&E 10x, HSV antigen detection with HSV-1 (G) and HSV-2 (H) cell line.

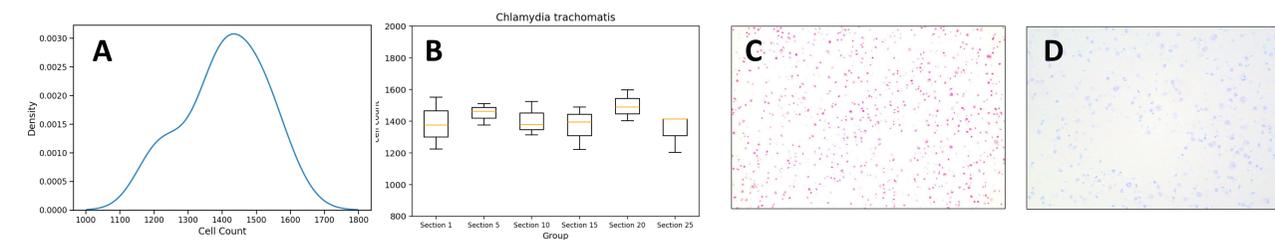


Figure 4: (A) Kernel Density Estimation Plot of CT positive samples ($S=0.946$, $p=0.395$), (B) CT positive slide cell count ($f=0.65$, $p=0.6646$), (C) CT cell line H&E 10x, (D) CT Giemsa

3. Reflex Testing using Nucleic Acid Amplification Tests (NAAT)

Table 1: Sections tested on validated QuanDx MeltPro High-risk HPV Genotyping LDT

Sample (n=8)	Outcome	Average DNA Concentration
HPV-Positive Slide	HPV16 (+)	0.71ng/ul
HPV-Negative Slide	hr-HPV (-)	4.4 ng/ul

Table 2: Sections tested on Cepheid Xpert® HPV Assay off-label workflow

Sample (n=3)	Outcome
HPV-Positive Slide	HPV16 (+)
HPV-Negative Slide	hr-HPV (-)(SAC+)

Table 3: Sections tested on Alinity m HR HPV Assay

Sample (n=3)	Outcome
HPV-Positive Slide	HPV16 (+)
HPV-Negative Slide	hr-HPV (-)(SAC+)

Table 4: Sections tested on Savanna HSV 1+2/VZV Assay

Sample (n=3)	Outcome
HSV-1 Positive Slide	HVS-1(+), HSV-2 (-)
HSV-2 Positive Slide	HSV-1 (-), HSV-2 (+)

Table 5: Sections tested on Allplex™ Seegene Meningitis V1

Sample (n=3/dilutions)	Outcome
HSV-1 Positive Slide	HVS-1(+)*, HSV-2(-)
HSV-2 Positive Slide	HSV-1 (-)*, HSV-2(+)

*Sections tested positive for CMV and EBV with very high Ct value

Table 6: Sections tested on Allplex™ Seegene CT/NG/MG/TV Assay

Sample (n=3)	Outcome
CT Positive Slide	CT(+), NG(-), TV(-), MG(-)

CONCLUSIONS

Microbix developed a reproducible platform for FFPE-simulated QC materials targeting HPV, HSV, and *Chlamydia trachomatis*. These standardized slides—available as positive and negative FFPE sections and scrolls—are homogeneous, stable, and compatible with histological and molecular techniques. The platform enables laboratories to:

- Standardize methodologies across sites and assays
- Monitor diagnostic accuracy and workflow consistency
- Support External Quality Assessment (EQA) programs

By bridging the gap between clinical tissue variability and assay validation needs, this FFPE QC platform enhances inter-laboratory reproducibility and reliability in infectious disease diagnostics. Future development will expand to additional pathogens and refine tissue scaffold design to strengthen diagnostic quality assurance.

ACKNOWLEDGMENTS

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