# Performance of High-Risk HPV Single Analyte and Multiplex Panel External Quality Controls for Verification of Extended Genotyping on the BD Onclarity™ HPV Assay

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## INTRODUCTION

There is abundant evidence that primary cervical cancer screening based on molecular detection of high-risk human Papillomavirus (HPV) has significant advantages over traditional Papanicolaou (Pap) testing. The adoption of molecular HPV methods in a diagnostic lab setting requires a rigorous program of training, method validation, and quality assessment, relying in part on the availability of well-characterized external assay controls. Microbix® has designed and developed high-risk HPV (HR-HPV) whole-genome raw materials as liquid single analytes and multiplex panels. The Queen Elizabeth Hospital Laboratory, an agency of Health PEI, employed Microbix controls to perform verification of the BD Onclarity™ HPV assay prior to their launch of a provincial HPV-based cervical cancer screening program.

## MATERIALS & METHODS

HR-HPV positive samples were developed, formulated in PreservCyt®, and delivered as IVD controls, comprising single analytes, HPV subtypes 16, 18, 31, 33, 39, 45, and 67, and multiplex panels: Panel 1, HPV 16/18/45; Panel 2, HPV 39/51/52; Panel 3, HPV 31/33/66. All samples were provided as **PROCEED**™ RUO products, and are also available as **RED**® IVD unassayed controls. All include human cells as a source of human DNA to satisfy sample adequacy assay criteria, and all incorporate full length viral genomes including all possible diagnostic targets.

The BD Onclarity assay is approved for testing cervical specimens collected in SurePath™ and PreservCyt ThinPrep® liquid cytology medium. The performance of Microbix HPV controls in either medium was verified by testing “contrived specimens”. A working suspension of HPV (single analyte or multiplex panel) was prepared in both ThinPrep and SurePath by combining 1 ml of the HPV product with 1 ml of the respective cytology medium. Each suspension was vortexed to ensure homogeneity prior to removal of aliquots for testing. Contrived specimens were prepared by adding different amounts of each suspension (0.5, 0.3 or 0.2ml) to the BD Onclarity HPV liquid cytology diluent tube and tested on the BD Viper™ LT system. Each sample was assayed separately, representing single infection (with HPV 16, 18, 31, 45, 51, 52) or different coinfection combinations (multiplex HR HPV panels; group 1: 33/58; group 2: 56/59/66; group3: 35/39/68) to simulate various scenarios and examine detection by the BD Onclarity HPV Assay.

***Table 1: Microbix HPV Controls***

| Catalogue | PROCEED™ Product Description |
|---|---|
| VP-62-M1 | Multiplex HPV 16, HPV 18, HPV 45, Human cells |
| VP-62-M2 | Multiplex HPV 31, HPV 33, HPV 66, Human cells |
| VP-62-M3 | Multiplex HPV 39, HPV 51, HPV 52, Human cells |
| VP-62-X | HPV subtype ’X’, Human cells (X = 16, 18, 31, 33, 39, 45, 51, 52, 66) |
| VP-99-M1 | Negative, with Human cells |

***Table 2: BD Onclarity assay detection targets and channels***

| Assay Target & Channel | Detects | Well |
|---|---|---|
| IC / Sample adequacy (ROX) | Human β-globin | 1, 2, 3 |
| HPV16 (FAM) | HPV 16 | 1 |
| HPV18 (HEX) | HPV 18 | 1 |
| HPV31 (HEX) | HPV 31 | 2 |
| HPV33-58 (FAM) | HPV 33 or 58 | 2 |
| HPV35-39-68 (Cy5) | HPV 35, 39, or 68 | 3 |
| HPV45 (Cy5) | HPV 45 | 1 |
| HPV51 (FAM) | HPV 51 | 3 |
| HPV52 (HEX) | HPV 52 | 3 |
| HPV56-59-66 (Cy5) | HPV 56, 59, or 66 | 2 |

## VERIFICATION TESTING PLAN

- Verify staff competency to prepare contrived specimens and perform testing on the BD Onclarity HPV assay, for individual and extended genotype panel detection.
- Confirm compatibility of Microbix HPV controls (formulated in ThinPrep medium) for testing of contrived specimens prepared in SurePath medium.
- Test a dilution series to compare BD Onclarity detection in ThinPrep and SurePath across the expected reportable range.
- Clinical verification for accuracy, reproducibility, specificity, comparing pre-qualified BD SurePath cytology specimens previously assayed on Roche cobas®, obtained from Newfoundland (n=123)
- Verify detection of individual HPV types and genotype groups by the BD Onclarity assay with a large panel of contrived specimens prepared in SurePath medium.
- Completion of Verification Report to meet ISO 15189 Accreditation Standards

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Conflict of interest statement: A Rebbapragada, P Zhelev, T Gerbaba, J Li, A Alagic, and presenter Mark A Luscher are employees of Microbix, which manufactures and provides for sale the HPV single and mixed control preparations described in this work.

## RESULTS

### *1. Microbix HPV contrived positive samples tested with BD Onclarity*

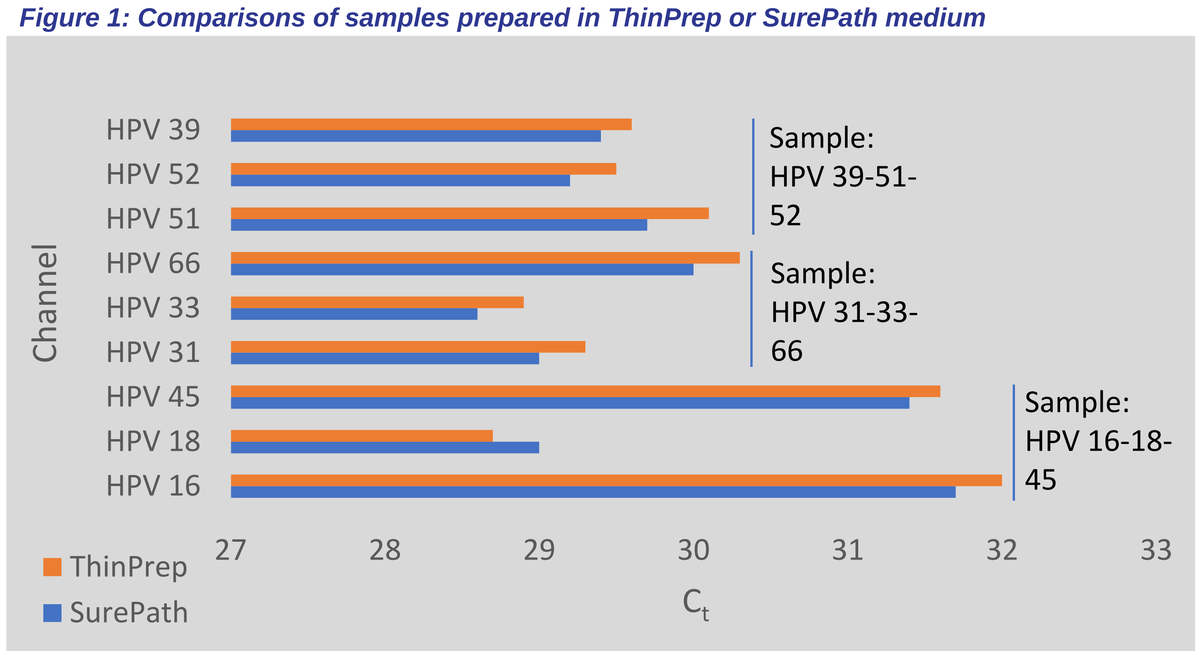
***Table 3: HPV detection in samples prepared with Microbix controls in BD SurePath medium***

| Channel / Sample | IC | HPV16 | HPV18 | HPV31 | HPV33-58 | HPV35-39-68 | HPV45 | HPV51 | HPV52 | HPV56-59-66 |
|---|---|---|---|---|---|---|---|---|---|---|
| HPV 16, 18, 45 | ✓ | ✓ | ✓ | ✗ | ✗ | ✗ | ✓ | ✗ | ✗ | ✗ |
| HPV 39, 51, 52 | ✓ | ✗ | ✗ | ✗ | ✗ | ✓ | ✗ | ✓ | ✓ | ✗ |
| HPV 31, 33, 66 | ✓ | ✗ | ✗ | ✓ | ✓ | ✗ | ✗ | ✗ | ✗ | ✓ |
| HPV 16 | ✓ | ✓ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ |
| HPV 18 | ✓ | ✗ | ✓ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ |
| HPV 45 | ✓ | ✗ | ✗ | ✗ | ✗ | ✗ | ✓ | ✗ | ✗ | ✗ |
| Negative | ✓ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ | ✗ |

The results demonstrate specificity in the recognition of these single analyte and multiplex whole-genome controls, and the compatibility of contrived samples prepared in SurePath on the Onclarity assay at multiple dilutions, indicating that PreservCyt is non-interfering.

### *2. Comparisons of samples prepared in ThinPrep or SurePath medium*

***Figure 1: Comparisons of samples prepared in ThinPrep or SurePath medium***




The results showed no significant differences between the $C_t$ values for samples prepared in the respective medium, indicating compatible performance of Microbix controls and the BD Onclarity assay with testing in SurePath and ThinPrep.

The results demonstrate accuracy for detection of all individual HPV types and extended genotypes by the BD Onclarity HPV assay.


## CONCLUSION

The Microbiology Laboratory of the Queen Elizabeth Hospital, Health PEI, successfully verified the BD Onclarity HPV assay on the Viper LT system, in preparation for deployment of an HPV-based cervical cancer screening program for the province. The verification utilized residual clinical BD SurePath specimens (previously tested on the Roche cobas HPV assay at the Eastern Health Public Health Laboratory) and contrived specimens prepared with whole genome HPV controls (quality assessment products) from Microbix to verify the accuracy, reproducibility, specificity, and reportable range for the assay. Microbix’s HPV whole-genome controls were fully compatible for testing on the Onclarity HPV assay when resuspended in BD SurePath medium, demonstrating accurate detection of human beta-globin on the sample adequacy channel, and the expected HPV genotypes in the individual and extended genotype channels.

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