



PERFORMANCE OF FULL AND EXTENDED GENOTYPING ASSAYS USING A NEW HIGH-RISK HPV MULTIPLEX PANEL

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INTRODUCTION

The development of extended (beyond HPV 16/18) and full genotyping Human Papillomavirus (HPV) assays has resulted in a demand for external control panels that can confirm assay performance across multiple high-risk genotypes. To address this need, we have developed high-risk HPV (hr-HPV) whole-genome raw materials that can be formulated into multiplex samples to provide end-users with a complete hr-HPV panel. These samples are designed to facilitate in-house assay verification, provide known positive material for personnel training, and assist with routine Quality Control Assurance. The objectives of this study were to confirm: (1) hr-HPV raw material suitability by internal and external testing and (2) targeted performance of a prototype HPV multiplex panel with the BD Onclarity™ HPV Assay.

MATERIALS & METHODS

Microbix Biosystems Inc. designed and manufactured inactivated hr-HPV raw materials that were used to develop liquid multiplex samples formulated in PreservCyt® (Table 1). The HPV multiplex samples were developed by using full-length viral genomes grown in cell culture, thereby including all possible diagnostic targets (DNA, RNA, protein). Samples also demonstrate 100% clinical sample equivalence and include human housekeeping genes. Raw materials were preformulated as single-plex samples and tested internally (Cepheid, E6/E7 target) and externally (EliTech, L1 target) in order to confirm sample cross-platform compatibility. All multiplex samples were verified internally with the Anyplex II HPV28 Detection Assay (L1 target) and externally with the BD Onclarity™ HPV Assay (E6/E7 target), in order to prove sample compatibility with full and extended genotyping assays, respectively.

PROCEED	Product Description
VP-62-M1	Multiplex HPV 16, HPV 18, HPV 45, Human Fibroblast Cells
VP-62-M3	Multiplex HPV 39, HPV 51, HPV 52, Human Fibroblast Cells
VP-62-M4	Multiplex HPV 31, HPV 33 Human Fibroblast Cells

Table 1: HPV Multiplex Samples



RESULTS

1. Verifying raw material hr-HPV performance with Partial HPV Genotyping Assays

A. Xpert® HPV (E6/E7 target) – Internal Testing

Assay Target	Raw Material HPV Type							
	16	18	45	31	33	39	51	52
HPV 16	+	-	-	-	-	-	-	-
HPV 18/45	-	+	+	-	-	-	-	-
Other hr-HPV types (31, 33, 35, 39, 51, 52, 56, 58, 59, 66, 68)	-	-	-	+	+	+	+	+
Outcome	HPV 16, Positive	HPV 18/45, Positive	Other hr-HPV Positive					

Table 2 HPV single-plex pre-formulation performance using the Xpert® HPV kit

B. HR-HPV ELITe Panel (L1 target) – External Testing

Assay Target	Raw Material HPV Type					
	16	18	31	33	39	45
HPV 16	+	-	-	-	-	-
HPV 18	-	+	-	-	-	-
Other hr-HPV types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68)	-	-	+	+	+	+
Outcome	HPV 16, Positive	HPV 18, Positive	Other hr-HPV Positive			

Table 3: HPV single-plex pre-formulation performance using the HR-HPV ELITe Panel

RESULTS CONTINUED

3. Verifying Sample Performance with Full and Extended HPV Genotyping Assays

A. Anyplex™ II HPV28 Detection Assay – Internal Testing

Assay Target	PROCEED VP-62-M1	PROCEED VP-62-M3	PROCEED VP-62-M4
HPV 16	+	-	-
HPV 18	+	-	-
HPV 31	-	-	+
HPV 33	-	-	+
HPV 39	-	+	-
HPV 45	+	-	-
HPV 51	-	+	-
HPV 52	-	+	-
Outcome	HPV 16, 18, 45 Positive	HPV 39, 51, 52 Positive	HPV 31, 33 Positive

Table 4: HPV Multiplex Sample Performance using the Anyplex™ II HPV28 Detection Assay

B. BD Onclarity™ HPV Assay – External Testing

Assay Target	PROCEED VP-62-M1	PROCEED VP-62-M3	PROCEED VP-62-M4
HPV 16	+	-	-
HPV 18	+	-	-
HPV 31	-	-	+
HPV 45	+	-	-
HPV 51	-	+	-
HPV 52	-	+	-
HPV 33, 58	-	-	+
HPV 35, 39, 68	-	+	-
HPV 59, 56, 66	-	-	-
Outcome	HPV 16, 18, 45 Positive	HPV 51, 52, (35,39,68) Positive	HPV 31, (33,58), Positive

Table 5: HPV Multiplex Sample Performance using the BD Onclarity™ HPV Assay

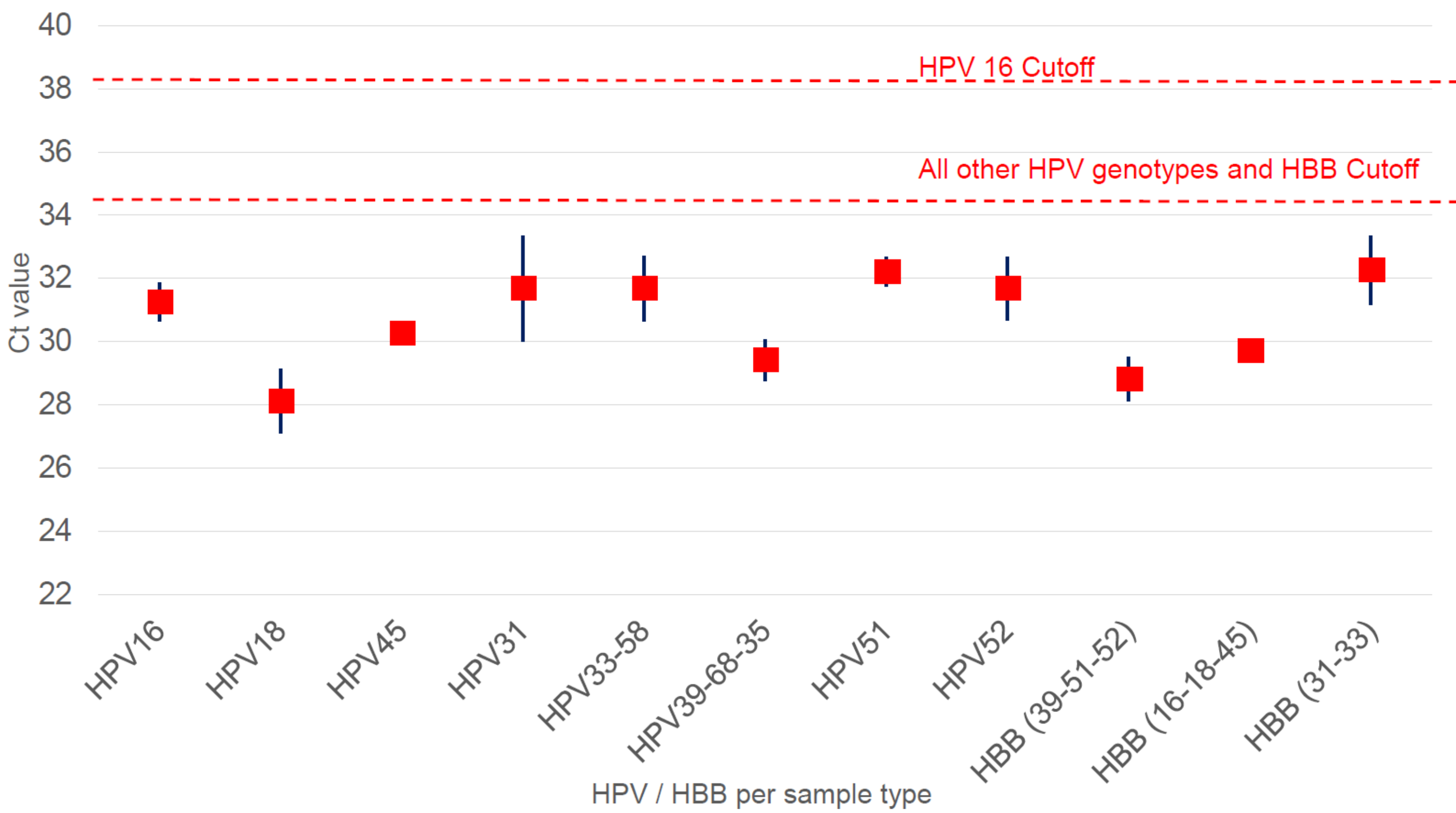


Figure 1: HPV Multiplex Sample Performance Per Target using the BD Onclarity™ Assay*

CONCLUSION

Microbix Biosystems Inc. has successfully designed hr-HPV multiplex samples to support verification/validation, personnel training, and quality assurance for extended and full HPV genotyping assays. The samples exhibited acceptable performance using assays that detect E6/E7 and L1 HPV targets, demonstrating their potential use as cross-platform compatible quality controls, verification panels and External Quality Assessment samples. Overall, Microbix's unique sample formulations with full-length viral genomes and human fibroblast cells, provide end-users with material that control whole workflows and are compatible with any molecular assay target region.

* We are currently formulating a 3-tube high-risk panel that will enable all 14 high-risk genotypes to be identified using the BD Onclarity™ HPV assay

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