NOVEL CROSS-PLATFORM HIGH-RISK (HR) HPV NAAT POSITIVE CONTROLS FOR USE IN L1 AND E6/E7 TARGETED NUCLEIC ACID (DNA AND RNA) DETECTION METHODS

P.Zhelev, A. Alagic, M. Luscher, K. Hughes, J.Li

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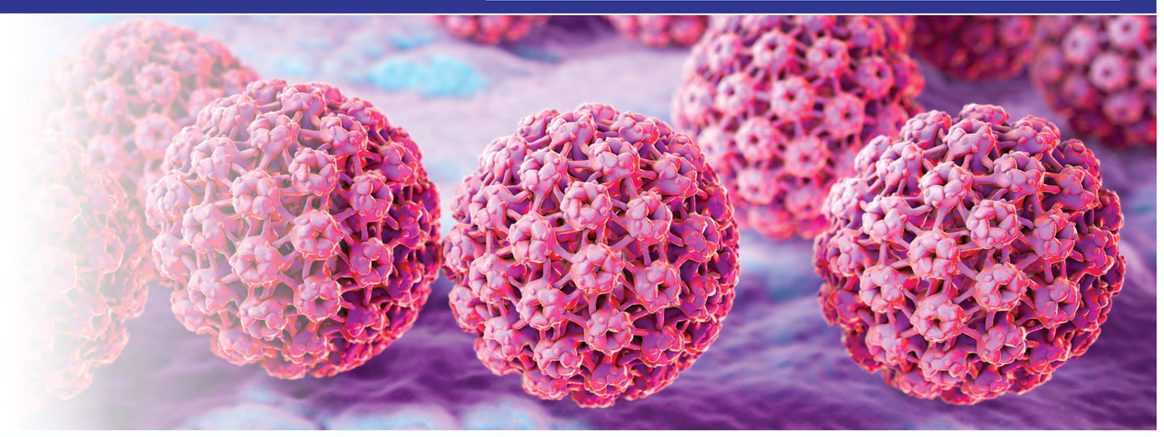

Objectives

To demonstrate the performance of a high-risk (hr)HPV Panel containing hrHPV types 16, 18 and 45 in multiple L1 and E6/E7 nucleic acid targeted hrHPV detection methodologies.

Materials and Methods

REDx™ HPV Quality Controls (QC, CE-IVD) and EQA HPV positive samples (hrHPV Panel) were manufactured by Microbix Biosystems inc. to contain all the diagnostic targets normally found in infected patient specimen samples - namely integrated and episomal viral DNA, viral RNA and proteins, as well as the host epithelial cells.

The hrHPV panel performance was evaluated using a two-step approach, with feasibility studies followed by validation studies in Clinical IVD laboratories, Original Equipment Manufacturer (OEM) laboratories and by internal (Microbix) validation runs.

The following products were used in the study:

Product Name	Catalogue	Viral strain	Volume and format
RED™ HPV 16 Positive Control	RED-62-16	HPV 16	1 mL liquid sample in PreservCyt® Solution
RED™ HPV 18 Positive Control	RED-62-18	HPV 18	
RED™ HPV 45 Positive Control	RED-62-45	HPV 45	

Table 1. RED™ HPV Positive Controls used in the study.

Product Name	Catalogue	Viral strain	Volume and format
HPV 16 Positive Sample	PT-62-16	HPV 16	1 mL liquid sample in PreservCyt® Solution
HPV 18 Positive Sample	PT-62-18	HPV 18	
HPV 45 Positive Sample	PT-62-45	HPV 45	

Table 2. EQA HPV Samples used in the study.

Results

Results from the feasibility and validation studies are presented in the tables below.

OEM	Test	Platform	Target	Gene	HPV Type		
					16	18	45
Roche	cobas® 4800 HPV	qPCR (Ct)	DNA	L1	+++		+++
HOLOGIC	Aptima HPV 16 18/45 GT	TMA (RLU)	RNA	E6/E7	+++		+++
Cepheid	Xpert® HPV	qPCR (Ct)	DNA	E6/E7	+++		+++
Seegene	Anyplex™ II HPV HR	DPO qPCR (TOCE - Ct)	DNA	L1	++	++	++

Table 3. Data from the hrHPV Panel feasibility study.

OEM	HPV Type				Sample detectability	Sample specific genotype detection
	16	18	45/Other	Total		
HOLOGIC	71	64+43		178	100.00%	98.86%
Roche	51	50	34	135	100.00%	100.00%

Table 4. EQA HPV Samples used in the API's HPV Genotyping testing panel (2019).

OEM	hrHPV	Sample-specific hrHPV detection
HOLOGIC	242	100.00%
Roche	139	100.00%

Table 5. EQA HPV Samples used in the API's hrHPV testing panel (2019).

Legend/ Symbol	Test detection range
+	Low positive
++	Medium positive
+++	High positive

Roche	Test	Platform	Target	HPV Type		
				16	18	45/Other
cobas® 4800	cobas® HPV Test	qPCR	DNA-polymorphic L1	+++	+++	+++
cobas® 6800/8800		qPCR		+++	+++	+++

Table 6. Results from RED™ HPV Positive Controls used in the Roche supported validation study.

BD	Test	Platform	Target	HPV Type		
				16	18	45
BD Viper™ LT	BD Onclarity™ HPV	qPCR	DNA – E6/E7	+++	+++	+++

Table 7. Results from RED™ HPV Positive Controls used in the BD supported validation study.

ELITechGroup EMPOWERING IVD	Test	Platform	Target	HPV Type		
				16	18	45
ELITe InGenius®	HPV	qPCR	DNA – L1	+++	+++	+++

Table 8. Results from RED™ HPV Positive Controls used in the EliTech Group supported validation study.

Product Name	Viral type	Sequencing
RED™ HPV 16 Positive Control	HPV 16	Confirmed
RED™ HPV 18 Positive Control	HPV 18	Confirmed
RED™ HPV 45 Positive Control	HPV 45	Confirmed

Table 9. Results from RED™ HPV products sequence analysis (LOXO GmbH)

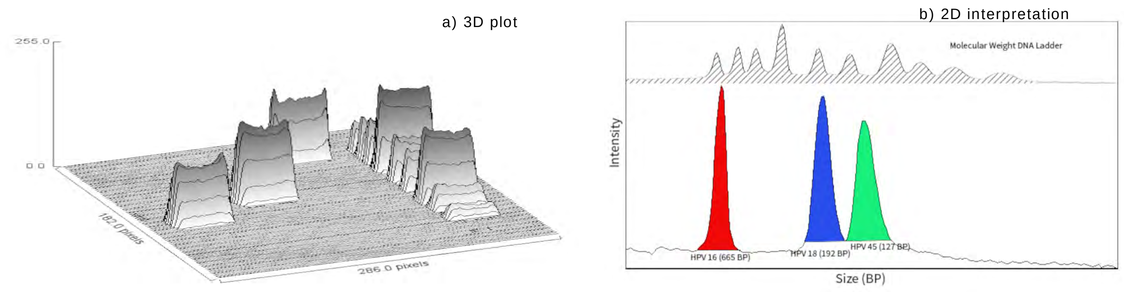

Fig1 (a,b). Lab Developed Test (LDT) for detection of hrHPV (LOXO GmbH).

Cepheid / Name	Raw material Batch #	Product Lot#	HPV Type ($n_{lot\ testing}$=3)		Variability (CV%)		
			16	18/45	Intra-lot	Inter-lot	Inter-batch
RED™ HPV 16 Positive Control	HPV16 Batch#1	Lot#1	+++	-	1.2%	1.4%	1.8%
		Lot#2	+++	-	1.4%		
	HPV16 Batch#2	Lot#3	+++	-	0.5%	1.5%	
		Lot#4	+++	-	1.4%		
RED™ HPV 18 Positive Control	HPV18 Batch#1	Lot#1	-	+++	0.9%	2.3%	1.7%
		Lot#2	-	+++	3.1%		
	HPV18 Batch#2	Lot#3	-	+++	1.3%	0.9%	
		Lot#4	-	+++	0.7%		
RED™ HPV 45 Positive Control	HPV45 Batch#1	Lot#1	-	+++	1.8%	2.0%	1.8%
		Lot#2	-	+++	1.0%		
	HPV45 Batch#2	Lot#3	-	+++	1.7%	1.4%	
		Lot#4	-	+++	1.4%		

Table 10. Results from RED™ HPV products internal validation run on twelve lots.

Conclusions

Based on both feasibility and validation studies, REDx™ HPV Positive Controls and EQA Positive samples for hrHPV types 16, 18 and 45 (hr HPV Panel) are confirmed to contain all the diagnostic targets normally found in infected patient specimens (integrated and episomal viral DNA, viral RNA and host epithelial cells). In all cases, material formulated in a widely accepted sample transport medium showed excellent compatibility with multiple hrHPV genotyping platforms. Overall, performance of the hrHPV panel in TMA, qPCR and TOCE-DPO methods, with HPV target sequences in E6/E7 or L1 genes, demonstrates excellent cross-platform compatibility for hrHPV detection.

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Contact information

Microbix Biosystems Inc.
265 Watline Ave, Mississauga, Ontario,Canada L4Z 1P3
+1-800-794-6694
+1-905-361-8910
www.microbix.com

Pavel Zhelev, Director Quality Assessment Products
email: pavel.zhelev@microbix.com