Novel high-risk (hr) HPV NAAT Positive Controls for cross-platform quality control

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Objectives

To assess the performance of novel high-risk (hr) Human papillomavirus (HPV) Nucleic Acid Amplification Test (NAAT) Positive controls for use in qPCR and TMA testing methods to monitor the entire workflow, including nucleic acid extraction, amplification, and detection.

Materials and Methods

The REDx™ Quality Control (QC) and EQA HPV positive samples (hrHPV Panel) used in this study were manufactured by proprietary methods. HPV DNA is presented in the samples in epithelial cells. The positive samples for hrHPV types 16, 18 and 45 contain all the diagnostic targets normally found in the infected patient specimen sample such as integrated and episomal viral DNA, viral RNA and proteins. The cells comprising the preparation were further processed to produce high-quality control materials by quantification, stabilization, inactivation, and dilution in widely-compatible preservation medium functionally equivalent to clinical universal transport media (UTM).

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

OEM lab validation runs were supported by Roche and BD Life Sciences. Data from participating labs in the American Proficiency Institute's US EQA scheme, part of the hrHPV and the HPV Genotyping testing panel, were used for the Clinical laboratory validation study.

Additional sequencing for the presence of the HPV 16, 18 and 45 genotype was performed by LOXO GmbH.

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 UTM
RED™ HPV 18 Positive Control	RED-62-18	HPV 18	
RED™ HPV 45 Positive Control	RED-62-45	HPV 45	

Table 1. RED™ HPV 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 UTM
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.

All the participants in the feasibility and validation runs were asked to use the hrHPV Panel samples as per normal patient sample handling methods described in the OEMs' 'Instructions for Use' for NAAT methods. The hrHPV Panel samples were used to monitor the whole process of extraction, separation and detection for the NAAT methods.

The reported values for all the tests were categorized relative to the normal range of reported values for each test in order to compare the sample performance between diagnostic platforms.

Results

Results from the feasibility and validation studies are presented in the tables below.The following symbols were used:

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

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

Table 3. Data from the hrHPV Panel feasibility study.

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

Table 4. Results from RED™ HPV Positive Controls used in the Roche supported validation study.

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

Table 5. Results from RED™ HPV Positive Controls used in the BD supported validation study.

OEM	HPV Type				Sample detectability	Sample specific genotype detection
	16	18	45/Other	Total		
HOLOGIC	39	44+27		110	100.00%	99.33%
Roche	33	36	22	91	100.00%	100.00%

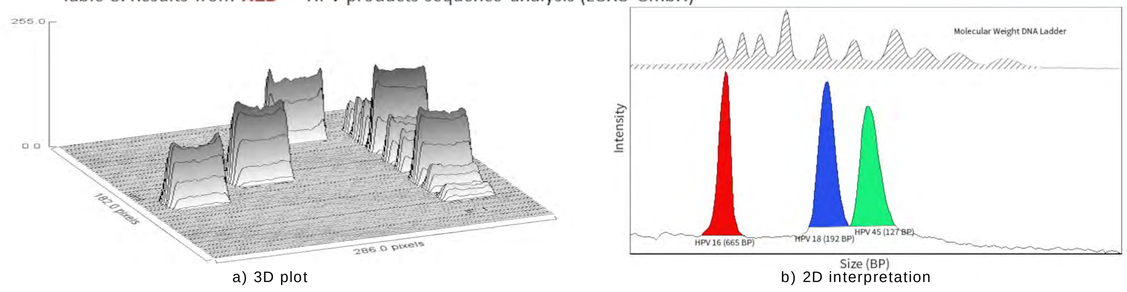
Table 6. EQA HPV Samples used in the API's HPV Genotyping testing panel.

OEM	hrHPV	Sample detection	Sample-specific hrHPV detection
HOLOGIC	153	100.00%	100.00%
Roche	91	100.00%	100.00%

Table 7. EQA HPV Samples used in the API's hrHPV testing panel.

Product Name	Viral type	Confirmed by 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 8. Results from RED™ HPV products sequence analysis (LOXO GmbH)



a) 3D plot b) 2D interpretation

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

Conclusions

Based on our feasibility and validation studies, REDx™ HPV Positive Controls and EQA Positive samples for hrHPV types 16, 18 and 45 (hr HPV Panel) contain all the diagnostic targets normally found in the infected patient specimen, such as integrated and episomal viral DNA, viral RNA as well as the host epithelial cells. Throughout the study, the material formulated in a widely acceptable sample transport medium showed excellent compatibility with several hrHPV genotyping platforms. Overall, the excellent performance of the hrHPV panel in TMA, qPCR and TOCE-DPO methods with HPV target sequences in E6/E7 or L1 regions demonstrates the achievement of constructing cross-platform compatible samples for hrHPV detection.

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