Cross-Platform High-Risk (hr) Low Occurrence HPV NAAT Positive Samples for Use in Nucleic Acid Detection Testing as Prospective External Quality Assessment Samples and User Defined Quality Controls

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EUROGIN 2021 THE VIRTUAL EDITION MAY 30 - JUNE 1

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OBJECTIVES

To demonstrate the performance of novel, high-risk, low occurrence (HR-LO) HPV samples for types 31, 33, 39 and low risk, low occurrence HPV 67, designed as liquid formulations, for use on multiple commercially available full genotyping nucleic acid amplification testing (NAAT) platforms.

HPV genotyping NAAT is becoming a cornerstone triage method for stratifying the risk related to cancer development in infected patients. Moreover, monitoring of the general population for the HR-LO HPV types is becoming routine to determine vaccination efficacy and to evaluate oncogenicity of HPV types. However, there is a lack of adequate QC material for full genotype testing, which affects the transition from traditional HPV testing to full genotype HPV testing.

MATERIALS & METHODS

PROCEEDx™ HR-LO HPV Positive Samples contain human epithelial cells with episomal and integrated whole genome HPV types 31, 33 or 39 formulated in Hologic® ThinPrep® PreservCyt® solution. High risk (HR) Negative (HPV 67) contains human epithelial cells with episomal and integrated whole genome HPV type 67 formulated in Hologic® ThinPrep® PreservCyt® solution. The materials are stable at 2-8oC and contain all the diagnostic targets normally found in patient specimen samples.

The products were evaluated by using a three-step approach; first with internal validation of sample performance by the Cepheid® Xpert® HPV test, followed by open label verification by Original Equipment Manufacturer (OEM) laboratories, and last with blinded EQA for HPV 31/HPV 67 organized by Labquality.

The following products were used in the studies:

Product Name	Catalogue	Strain	Volume
PROCEED / PTD HPV 31 Positive Sample	VP-62-31	HPV 31	1 mL
PROCEED HPV 33 Positive Sample	VP-62-33	HPV 33	
PROCEED HPV 39 Positive Sample	VP-62-39	HPV 39	
PROCEED / PTD HR Negative Sample	VP-62-67	HPV 67	

RESULTS

1. Internal validation study and analytical performance

Name	Raw material Batch #	Product Lot#	HR-LO HPV Type ($n_{lot\ testing}$=3) 31	33	39	Variability (CV%) Intra-lot	Inter-lot	Inter-batch
PROCEED HPV 31 Positive Sample	HPV31 Batch#1	Lot#1	+++			1.45%	**1.54**	**1.66%**
		Lot#2	+++			1.08%	**%**	
	HPV31 Batch#2	Lot#3	+++			0.42%	**1.44**	
		Lot#4	+++			1.36%	**%**	
PROCEED HPV 33 Positive Sample	HPV33 Batch#1	Lot#1		+++		3.26%	**2.24**	**2.03%**
		Lot#2		+++		1.33%	**%**	
	HPV33 Batch#2	Lot#3		+++		1.99%	**1.61**	
		Lot#4		+++		1.57%	**%**	
PROCEED HPV 39 Positive Sample	HPV39 Batch#1	Lot#1			+++	0.90%	**1.32**	**1.30%**
		Lot#2			+++	1.32%	**%**	
	HPV39 Batch#2	Lot#3			+++	1.17%	**1.12**	
		Lot#4			+++	1.10%	**%**	

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

PROCEED™

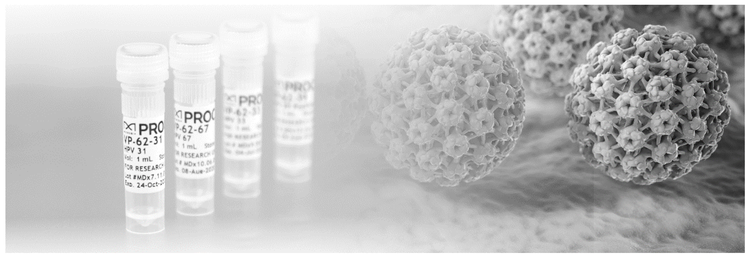
2. OEM feasibility study

2a. Standard genotyping NAAT of PROCEEDx™ HPV positive samples.

OEM	Test	Platform	Target	Gene	HPV Type 67	31	33	39
Roche	**cobas**® 4800 **HPV**	qPCR (Ct)	DNA	L1	-	+++		
	cobas® 6800/8800 **HPV**	qPCR (Ct)	DNA	L1	-	+++		
ELITechGroup	HR – HPV ELITe Panel	qPCR (Ct)	DNA	L1	-	+++		

2b. Extended genotyping NAAT of PROCEEDx™ HPV positive samples

OEM	Test	Platform	Target	Gene	HPV Type 67	31	33	39
BD	BD Onclarity™ HPV	qPCR (Ct)	DNA	E6/E7	-	+++	+++	+++
EUROIMMUN	EUROArray HPV®	qPCR (Ct)	DNA	E6/E7	-	+++	+++	+++
greiner BIO-ONE	GBO PapilloCheck®	Microarray	DNA	E1	-	+++	+++	+++



3. EQA with HPV 31/HPV 67 Samples.

Sample Type	Responded	AVR success rate	Count
HPV 31	HR HPVNAT POSITIVE	93%	27
HPV 67	HR HPVNAT NEGATIVE	89%	27

OEM	Standard HPV genotyping platforms	Method	Gene	Target
Abbott	Abbott High Risk HPV Amplification	qPCR	L1	DNA
Cepheid	Cepheid Xpert HPV	qPCR	E6/E7	DNA
HOLOGIC	Hologic Aptima HPV Assay	TMA	E6/E7	RNA
Roche	Roche cobas HPV 4800	qPCR	L1	DNA
OEM	**Extended HPV genotyping platforms**	**Method**	**Gene**	**Target**
AB ANALITICA	AB ANALITICA AMPLIQUALITY HPV-TYPE EXPRESSv3.0	qPCR	L1	DNA
BD	BD Onclarity HPV Assay	qPCR	E6/E7	DNA
AmpliSens	Amplisens HPV HCR-genotype-FRT PCR kit	FRT	*	DNA
Seegene	Anyplex II HPV HR Detection	DPO qPCR	L1	DNA
GEN-MICA	Genomica CLART HPV2	microarray	L1	DNA
*LDT	LightCycler 480 Probes Master	qPCR	*	DNA
greiner BIO-ONE	Greiner Bio-One (GBO) PapilloCheck®	microarray	E1	DNA
宏瑞生技	Sansure Biotech HPV Genotype	qPCR	*	DNA

**LDT – Laboratory Developed Test*

CONCLUSIONS

The HR-LO HPV Positive Samples formulated in a widely accepted sample transport medium, showed excellent compatibility with several hrHPV genotyping platforms. The labs reporting false negatives for HPV 31 during EQA schemes were identified to be using methodologies utilizing only partial sample volume in the test procedure. Further, HPV 67 has been reported as positive on certain platforms designed to detect this specific HPV type.

Overall, the performance of the HR-LO HPV panel with designated HR Negative sample (HPV67) in multiple NAAT methods targeting sequences in the E6/E7, L1 and E1 regions demonstrates the successful development of cross-platform compatible samples for high-risk, low occurrence (HR-LO) HPV detection.

ACKNOWLEDGEMENTS

We would like to acknowledge that the data used in the poster was provided by:

Roche Molecular Diagnostics, Pleasanton, USA

BD Life Sciences, Sparks, USA

Greiner Bio-One GmbH, Kremsmuenster, Austria

ELITechGroup S.p.A, Torino, Italy

EUROIMMUN AG, Luebeck, Germany

Labquality Oy, Helsinki, Finland

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