BIOLOGICAL HEALTH RISKS
QUALITY OF LABORATORIES

CLINICAL BIOLOGY& PATHOLOGICAL ANATOMY COMMISSIONS
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EXTERNAL QUALITY ASSESSMENT
IN CLINICAL BIOLOGY & PATHOLOGICAL ANATOMY

DEFINITIVE GLOBAL REPORT

Molecular Microbiology

high-risk HPV (pilot scheme III)

SURVEY 2023/S4

Sciensano/Molecular Microbiology (HPV)/1-E

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The results were discussed with the NRC on: 26/01/2024
The results were discussed in a expert meting on: 29/02/2024.

Authorization of the report: by Bernard China, scheme coordinator

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1 INTRODUCTION

1.1 Preparation of the samples

The samples were prepared by the Belgian NRC (AML, Antwerp). The samples consisted of 3 mL of Thinprep medium with or without HPV (Table 1). In total 30 different samples were analyzed using following assays prior to survey in order to determine the HPV genotype present:

- RIATOL qPCR HPV genotyping assay
- Hologic Aptima HPV Assay (Panther System)
- Abbott Alinity m HR HPV Assay
- Abbott RealTime High Risk HPV Assay
- Seegene Allplex HPV HR Detection
- Sequencing

Finally, 10 samples were selected for their reproducibility results.

Table 1. The samples.

Sample ID	Content	Sample status
HPV23-1	HPV16, HPV18	Core
HPV23-2	No DNA	Core
HPV23-3	HPV18	Core
HPV23-4	HPV6*	Educational
HPV23-5	HPV33	Core
HPV23-6	HPV53*	Educational
HPV23-7	HPV16, HPV45	Core
HPV23-8	HPV51, 52, 56, 58, 59	Core
HPV23-9	HPV39	Core
HPV23-10	Control human DNA, no HPV	Core

*: not considered as a high-risk genotype

1.2 Sample homogeneity

The NRC tested 5 aliquots of each sample with the RIATOL qPCR HPV genotyping assay starting from primary sample. A dedicated ThinPrep liquid handling robot was used for preparation of aliquots. Additionally, the extracted DNA from two aliquots was tested in duplo with the Riatol assay.
The results were qualitatively as well as quantitively concordant in every validation round, and sample homogeneity was confirmed.

1.3 Sample stability

The NRC tested the panel before, during and after the survey. In addition, robustness of samples was also confirmed prior to survey by subjecting samples to multiple real-life shipping conditions. During validation experiments samples were stored according to manufactures instructions. The results obtained at different time points were again consistent both qualitatively and quantitively. The samples were considered stable.

1.4 Participation

19 laboratories of pathologic anatomy and 25 laboratories of clinical biology participated in this EQA scheme.

2 COURSE OF THE SURVEY

Sending samples: 27/06/2023
Closure of results encoding: 20/07/2023
Preliminary report: 31/07/2023

3 RESULTS

3.1 Detection of high-risk HPV

In the INAMI/RIZIV nomenclature, the following nomenclature is applicable:
588932 588943 Test for high-risk HPV using a molecular diagnostic method, according to service 589853-589864 or 588873-588884, on the same cervico-vaginal sample(s).
This EQA scheme is dedicated to assess the capability of the laboratories to detect high-risk HPV using molecular methods.

3.1.1 RESULTS PER SAMPLE

47 datasets were submitted. 41 laboratories submitted one dataset and 3 laboratories submitted 2 datasets.

Table 2. Results per sample.

Sample ID	Content	Expected result	HR-HPV Positive	HR-HPV Negative	Other
HPV23-1	HPV16, HPV18	Positive	44	3	0
HPV23-2	No DNA	ND/INH/NEG	0	15	18 INH 4 Not determined 9 Invalid 1 No DNA
HPV23-3	HPV18	Positive	47	0	0
HPV23-4	HPV6	Negative	5*	42	0
HPV23-5	HPV33	Positive	47	0	0
HPV23-6	HPV53	Negative	5**	42	0
HPV23-7	HPV16, HPV45	Positive	47	0	0
HPV23-8	HPV51, 52, 56, 58, 59	Positive	47	0	0
HPV23-9	HPV39	Positive	47	0	0
HPV23-10	No HPV	Negative	0	47	0

*: the participants used a detection kit able to detect HPV6.
**: the participants used a detection kit able to detect HPV53.

47 datasets and 10 samples equals 470 results. Of the 470 results, 467 results (99.4%) were correct. Only 3 false negative results were recorded for sample HPV23-1. This sample contained very low concentrations of HPV16 and HPV18. Technically, the detection of non-HR-HPV genotypes was evaluated on the basis of the method's ability to detect these genotypes. However the final report must clarify that a high-risk genotype was not detected.

3.1.2 RESULT PER METHOD

Table 3. Results per method

Method	N	NR	NCR	%
Abbott Alinity m HR HPV Assay	2	20	20	100
Abbott RealTime High Risk HPV Assay	5	50	50	100
Cepheid GeneXpert HPV Assay	5	50	50	100
Roche cobas HPV Test	13	130	130	100
Seegene Allplex HPV HR Detection	10	100	100	100
In-house qPCR	1	10	10	100
ELITechGroup HR-HPV ELITe Panel InGenius	1	10	10	100
INNO-LiPA HPV Genotyping Extra II	1	10	10	100
BD Onclarity HPV Assay	1	10	10	100
Hologic Aptima HPV Assay (Panther System)	7	70	67	95.7
Total	47	470	467	99.4

N: number of datasets, NR: number of results; NCR: number of correct results; %: concordance percentage. Hologic Aptima HPV Assay is the only method that targets mRNA, while other assays all target DNA. The lower stability of mRNA as compared to DNA could explain the lower concordance percentage of the Hologic Aptima HPV Assay.

3.2 Genotyping

The genotyping results were analyzed for educational purposes since genotyping is not yet mandatory in the official nomenclature.
45 datasets were recorded for the genotyping.

3.2.1 GENOTYPE PER SAMPLE

Table 4. Genotypes recorded in each sample.

Sample ID	Expected result	Encoded results	Number of results
HPV 23-1	HPV16, 18	HPV 16, 18	**35**
		HPV 16/18, 45	**4**
		HPV16	3
		Other	2
HPV23-3	HPV18	HPV18	**37**
		HPV18/45	**8**
HPV23-4	HPV6	HPV6	**5**
HPV23-5	HPV33	HPV33	**15**
		Group A	**4**
		P3	**4**
		HR	**2**
		Other	**19**
		35, 38	1
HPV23-6	HPV53	HPV53	**5**
HPV23-7	HPV16, 45	HPV16, 45	**22**
		HPV16, Other	**14**
		16/18, 45	**6**
		16	2
		Other	1
HPV23-8	HPV51, 52, 56, 58, 59	51, 52, 56, 58, 59	**11**
		51, 52, 53/56, 58/59/66	**1**
		51, 52, 56	**2**
		Other	**17**
		HR	**3**
		P3, P4, P5	**3**
		Group A and B	**2**
		Group A	2
		16	1
		P3	1
HPV23-9	HPV39	HPV39	**15**
		Group B	**4**
		Other	**17**
		P5	**4**
		HR	**4**
		35/39/68	**1**

Group A: 31, 33, 52, 58
Group B: 35, 39, 51, 56, 59, 66, 68
P3: 31, 33, 35, 52, 58
P4: 51, 59
P5: 39, 56, 66, 68
Other: Positive for another genotype then HPV16, 18 or 45
HR: 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 or 68

Results that are considered correct are indicated in bold. Out of the 360 recorded results, 347 (96.4%) were correct. 13 results were incorrect.

3.2.2 GENOTYPING METHODS

Table 5. Genotyping tests.

Method	N	Detected genotypes
Seegene Anyplex II HPV HR (14)	6	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68
Seegene Anyplex HPV28	5	HR-HPV 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 69, 73, 82, LR-HPV 11, 40, 42, 43, 44, 54, 6, 61, 70.
Roche cobas HPV Test	11	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68
Sacace HPV genotypes 14 Real-TM Quant	1	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59
In-house qPCR	1	Not explained
ELITechGroup HR-HPV ELITe Panel InGenius	1	The test specifically identifies HPV16 and HPV18 while concurrently detecting other high risk types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68).
Cepheid GeneXpert HPV Assay	4	Optimized detection of 14 HR-HPV reported as: HPV16, HPV18/45 or other HR-HPV (31, 33, 35, 52, 58; 51, 59; 39, 56, 66, 68)
Hologic Aptima HPV Assay (Panther System)	7	16, 18/45
INNO-LiPA HPV Genotyping Extra II	1	HR-HPV 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 69, 73, 82, LR-HPV 11, 40, 42, 43, 44, 54, 6, 61, 70.
Abbott Alinity m HR HPV Assay	2	The assay specifically identifies HPV genotypes 16, 18, and 45 while reporting the concurrent detection of the other high-risk genotypes (31/ 33/ 52/ 58) and (35/ 39/ 51/ 56/ 59/ 66/ 68)
Abbott RealTime High Risk HPV Assay	5	The Abbott RealTime HR HPV assay is intended to detect 14 high risk HPV genotypes: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 and to partially genotype 16, 18 from other 12 high risk genotypes.
BD Onclarity HPV Assay	1	16, 18, 45, 31, 51, 52+ (33, 58)+ (35, 39, 68)+ (56, 59, 66)

HR: high risk
LR: low risk

The genotyping results must be analyzed in regard to the used method.

END