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BIOLOGICAL HEALTH RISKS
QUALITY OF LABORATORIES

EXTERNAL QUALITY ASSESSMENT*

DEFINITIVE GLOBAL REPORT

Molecular Microbiology

High Risk HPV

SURVEY 2024/S4

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Quality of laboratories
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A draft version of this report was submitted to the experts on 11/11/2024.
The expert(s) was/were invited to send his/their comments via e-mail.
This report was discussed at the meeting of the Committee of experts on 14/11/2024.

Responsibilities:
The Committee of experts was consulted for advice about the content of the global report, the interpretation of the results, the evaluation criteria and the organization of the next evaluations. The responsibility for the choice of the samples used and the final design of the EQA survey is carried out by the department Quality of laboratories of Sciensano.

Authorization of the report: by Bernard China, coordinator

Date of publication: 26/11/2024

All the reports are also available on our webpage:

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1 INTRODUCTION

THE SAMPLES

The samples originated from the samples library of the National reference center (NRC).
10 samples were considered as core samples because the results were reproducible for all tested methods.
4 samples were considered as educational because the results were not reproducible (at least one method gave a different result).
Table I1. The samples

Samples	Matrix	Content	Expected result	Status
HPV24-01	PreservCyt (Thinprep)	HPV16; HPV58; HPV59	Positive	Core
HPV24-02	PreservCyt (Thinprep)	HPV52	Positive	Core
HPV24-03	PreservCyt (Thinprep)	HPV16	Positive	Educational
HPV24-04	PreservCyt (Thinprep)	HPV39; HPV51; HPV56; HPV58	Positive	Core
HPV24-05	PreservCyt (Thinprep)	HPV18	Positive	Educational
HPV24-06	PreservCyt (Thinprep)	HPV33	Positive	Core
HPV24-07	PreservCyt (Thinprep)	HPV18	Positive	Core
HPV24-08	PreservCyt (Thinprep)	HPV16	Positive	Core
HPV24-09	PreservCyt (Thinprep)	HPV31	Positive	Educational
HPV24-10	PreservCyt (Thinprep)	HPV66	Positive	Educational
HPV24-11	PreservCyt (Thinprep)	HPV35	Positive	Core
HPV24-12	PreservCyt (Thinprep)	No HPV , Human DNA	Negative	Core
HPV24-13	PreservCyt (Thinprep)	No HPV , No Human DNA	Invalid	Core
HPV24-14	PreservCyt (Thinprep)	HPV16 ; HPV18 ; HPV31; HPV45	Positive	Core

HOMOGENEITY

4 specimen of each sample were tested and the results were reproducible. The samples were considered as homogeneous.

STABILITY

The samples were send to different expert laboratories and tested with 7 different methods.
Abbott m2000 , Abbott Alinity , Roche Cobas 6800 ,Fujirebio INNO-LiPA , Hologic Panther , Seegene Allplex HR HPV , Next Generation Sequencing .
The samples with consistent results were considered as stable and included in the core panel. The samples with only one discrepancy were included in the panel as educational samples.

PARTICIPANTS

56 laboratories were registered. 52 laboratories encoded results (92.9%).

THE STAGES OF THE SURVEY

The samples were sent on: 25/06/2024
The deadline for results encoding was: 16/07/2024
The preliminary report was diffused on: 23/07/2024

2 RESULTS

2.1 Results per sample

52 laboratories encoded results. 47 laboratories encoded one dataset, 3 laboratories encoded 2 datasets and 2 laboratories encoded 3 datasets. Finally, 59 datasets were encoded.

Table R1. Results per sample

Samples	Content	Expected result	Encoded results	Status
HPV24-01	HPV16; HPV58; HPV59	Positive	59 positive results	Core
HPV24-02	HPV52	Positive	59 positive results	Core
HPV24-03	HPV16	Positive	56 positive results 3 negative results	Educational
HPV24-04	HPV39; HPV51; HPV56; HPV58	Positive	59 positive results	Core
HPV24-05	HPV18	Positive	53 positive results 6 negative results	Educational
HPV24-06	HPV33	Positive	59 positive results	Core
HPV24-07	HPV18	Positive	59 positive results	Core
HPV24-08	HPV16	Positive	59 positive results	Core
HPV24-09	HPV31	Positive	53 positive results 6 negative results	Educational
HPV24-10	HPV66	Positive	56 positive results 3 negative results	Educational
HPV24-11	HPV35	Positive	59 positive results	Core
HPV24-12	No HPV , Human DNA	Negative	58 negative results 1 invalid result	Core
HPV24-13	No HPV , No Human DNA	Invalid	51 invalid results 1 not determined result 7 negative results*	Core
HPV24-14	HPV16 ; HPV18 ; HPV31; HPV45	Positive	59 positive results	Core

*: among the 7 negative results, 5 can be considered as correct (no human DNA internal control) and 2 can be considered as incorrect.

59 datasets and 14 samples per dataset giving 826 results. Out of the 826 results, 805 (97.5%) were considered as correct. Among the 21 incorrect results, 18 were false negative results, one was an invalid result and 2 were negative results for an invalid sample.
If we considered the core samples only, among the 590 results, 587 (99.5%) were correct. Among the 3 incorrect results, one was an invalid result and 2 were negative results for an invalid sample.

2.2 Results per method.

Table R2. Results per method

Method	N	NR	NCR	%	IR
Cepheid genexpert	3	42	42	100,00	0
in house qPCR	2	28	28	100,00	0
Roche Cobas 5800 HPV kit	2	28	28	100,00	0
Roche Cobas 6800 HPV kit	6	84	84	100,00	0
Sacace HPV genotype 14	1	14	14	100,00	0
Seegene allplex 28	3	42	42	100,00	0
seegene Anyplex II HPV HR	4	56	56	100,00	0
Roche Cobas 4800 HPV Kit	10	140	139	99,29	1 invalid result
seegene allplex HPV HR	12	168	166	98,81	1 false negative 1 negative for 1 invalid
Abbott Alinity m HR HPV assay	5	70	69	98,57	1 negative for 1 invalid
Abbott realtime High risk HPV	5	70	68	97,14	2 false negative
BD biosciences Onclarity HPV assay	1	14	12	85,71	2 false negative
Hologic Aptima HPV Assay	5	70	57	81,43	13 false negative
	59	826	805	97,46	21

N. number of datasets; NR: number of results, NCR: number of correct results. IR: incorrect results.

13 different methods were used. The percentage of correct results ranged from 81.43% (Aptima) to 100%. Seven methods gave 100% of correct results.

Conclusion. A large majority of encoded results were correct independently of the used methods. Nevertheless, method based on RNA (Transcription Mediated Amplification) gives less correct results. It is maybe due to the fact that the EQA samples were not appropriate for this method due RNA instability.

3 ANNEX: GENOTYPING

The precise genotyping was facultative since the nomenclature only asks for the presence or absence of High risk HPV.
The answer for the genotype depends of the used method. It is important for each participant to compare their result with the genotype present in the sample and to see if their results are consistent regarding to the used method.
58 datasets for the genotyping were encoded.

Table A1. Encoded genotype per method

			Sample ID/expected genotype					
			HPV24-01	HPV24-02	HPV24-03	HPV24-04	HPV24-05	HPV24-06
Method	Detected genotype	N	HPV16; 58; 59	HPV52	HPV16	HPV39; 51; 56; 58	HPV18	HPV33
Abbott Alinity m HR HPV assay	16, 18, 45 + A(31/33/52/58)+ B(35/39/51/56/59/66/68)	8	16, other (3) ; 16, A, B (5)	Other A (5); Other (3)	HPV16 (8)	HPV A+ HPV B (6) Other HR HPV (2)	HPV18 (8)	other HPV A (8)
Abbott realtime High risk HPV	16, 18, + (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68)	2	HPV16; 58; 59 (2)	Other (2)	HPV16 (2)	Other HR HPV (2)	HPV18 (2)	Other HR HPV (2)
BD biosciences Onclarity HPV assay	16, 18, and 45 to include types 31, 51, 52, 33/58, 35/39/68, and 56/59/66	1	16, 33/58, 56/59/66	HPV52 (1)	HPV16 (1)	33/58, 51, 35/39/68	NEG (1)	33/58
Cepheid genexpert	16, 18/45 P3= 31, 33,35, 52,58 P4= 51, 59 P5= 39, 56, 66, 68	3	16, P3, P4 (3)	P3 (3)	HPV16 (3)	P3, P4, P5 (3)	HPV18/45 (3)	P3 (3)
Hologic Aptima HPV 16 18/45	16, 18/45	4	16 (3) ; Other (1)	Other (4)	HPV16 (2) NEG (2)	Other HR HPV (3) NEG (1)	NEG (4)	Others HR-HPV(3) NEG (1)
in house qPCR	HPV6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 56, 58, 59, 66, 67, 68	2	HPV16; 58; 59 (2)	HPV52 (2)	HPV16 (2)	39, 51, 56, 58 (2)	HPV18 (2)	HPV33 (2)
Roche Cobas 4800 HPV Kit	16, 18 + (31,33,35,39,45,51,52,56,58,59,66,68)	10	16, other (9) ; 16 (1)	Other (9); ND (1)	HPV16 (10)	Other HR HPV (9) ND (1)	HPV18 (10)	Others HR-HPV (1) ND (1)
Roche Cobas 5800 HPV kit	16, 18 + (31,33,35,39,45,51,52,56,58,59,66,68)	2	16, other (2)	Other (2)	HPV16 (2)	Other HR HPV (2)	HPV18 (2)	Others HR-HPV (2)
Roche Cobas 6800 HPV kit	16, 18 + (31,33,35,39,45,51,52,56,58,59,66,68)	6	16, other (6)	Other (6)	HPV16 (6)	Other HR HPV (6)	HPV18 (6)	Others HR-HPV (6)
Sacace HPV genotyp 14	16,18,45 and (31, 33, 35, 39, 51, 52, 56, 58, 59, 66, 68) + 6/11 (low risk HPV)	1	16, other (1)	HPV52 (1)	HPV16 (1)	39, 51, 58 (1)	HPV18 (1)	HPV33 (1)
Seegene allplex 28	19 HR (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 69, 73, 82) + 9 low-risk HPV types (6, 11, 40, 42, 43, 44, 54, 61, 70)	3	16/58/59/73 (3)	HPV52 (3)	HPV16 (3)	HPV 58/HPV 51/ HPV 39/ (HPV 42) / (HPV 44) (1) 39, 42, 51, 58 (1) 58;51;39;56;42;44 (1)	HPV18 (3)	HPV33 (3)
seegene allplex HPV HR	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68	12	16,58, 59 (12)	HPV52 (12)	HPV16 (12)	39, 51, 58 (3) 39, 51, 56, 58 (9)	HPV18 (12)	HPV33(12)
seegene Anyplex II HPV HR	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68	4	16,58, 59 (4)	HPV52 (4)	HPV16 (4)	39, 51, 58 (3) 39, 51, 56, 58 (1)	HPV18 (4)	HPV33 (4)

Method	Detected genotype	N	HPV24-07 HPV18	HPV24-08 HPV16	HPV24-09 HPV31	HPV24-10 HPV66	HPV24-11 HPV35	HPV24-14 HPV16, 18, 31, 45
Abbott Alinity m HR HPV assay	16, 18, 45 + A (31/33/52/58)+ B(35/39/51/56/59/66/68)	8	HPV18 (8)	HPV16 (8)	Other A (6) NEG (2)	Other B (7) Other HR HPV (1)	Other B (7) Other HR HPV (1)	16/18/45/A (7) HPV16, 18, 45 (1)
Abbott realtime High risk HPV	16, 18, + (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68)	2	HPV18 (2)	HPV16 (2)	Other HPV (2)	Other HR HPV (2)	Other HR HPV (2)	HPV16, 18, Other (2)
BD biosciences Onclarity HPV assay	16, 18, and 45 to include types 31, 51, 52, 33/58, 35/39/68, and 56/59/66	1	HPV18 (1)	HPV16 (1)	NEG (1)	56/59/66 (1)	35/39/68 (1)	HPV16, 18, 31, 45 (1)
Cepheid genexpert	16, 18/45 P3= 31, 33,35, 52,58 P4= 51, 59 P5= 39, 56, 66, 68	3	HPV18/45 (3)	HPV16 (3)	P3 (3)	P5 (3)	P3 (3)	HPV16, 18/45 (3)
Hologic Aptima HPV 16 18/45	16, 18/45	4	HPV18/45 (4)	HPV16 (4)	Other HPV (2) NEG (2)	Other HPV (2) NEG (2)	Other HR HPV(4)	16, 18/45 (2) 18/45 (1) Other HR HPV (1)
in house qPCR	HPV6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 56, 58, 59, 66, 67, and 68	2	HPV18 (2)	HPV16 (2)	HPV31 (2)	HPV66 (2)	HPV35 (2)	HPV16, 18, 31, 45 (2)
Roche Cobas 4800 HPV Kit	16, 18 + (31,33,35,39,45,51,52,56,58,59,66,68)	10	HPV18 (10)	HPV16 (10)	Other HR HPV (9) ND (1)	Other HR HPV (9) ND (1)	Other HR HPV (9) ND (1)	HPV 16, HPV 18, other (10)
Roche Cobas 5800 HPV kit	16, 18 + (31,33,35,39,45,51,52,56,58,59,66,68)	2	HPV18 (1) Other (1)	HPV16 (2)	Other HR HPV (2)	Other HR HPV (2)	Other HR HPV (2)	HPV 16, HPV 18, other (2)
Roche Cobas 6800 HPV kit	16, 18 + (31,33,35,39,45,51,52,56,58,59,66,68)	6	HPV18 (6)	HPV16 (6)	Other HR HPV (6)	Other HR HPV (6)	Other HR HPV (6)	HPV 16, HPV 18, other (6)
Sacace HPV genotyp 14	16,18,45 and (31, 33, 35, 39, 51, 52, 56, 58, 59, 66, 68) + 6/11 (low risk HPV)	1	HPV18 (1)	HPV16 (1)	HPV31 (1)	HPV66 (1)	Other HR HPV (1)	HPV16, 18, 31, 45 (1)
Seegene allplex 28	19 HR (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 69, 73, 82) + 9 low-risk HPV types (6, 11, 40, 42, 43, 44, 54, 61, 70)	3	HPV18 (3)	HPV16 (3)	HPV31 (3)	HPV66 (3)	HPV35 (3)	HPV 45, 16, 18, 31,(44) 16.31,44,45 (1) 45;16;18;31;44 (1)
seegene Allplex HPV HR	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68	12	HPV18 (12)	HPV16 (12)	HPV31 (11) ND (1)	HPV66 (11) HPV66, 39 (1)	HPV35 (12)	HPV16, 18, 31, 45 (9) HPV 16, HPV 18, other (1) HPV16, 18, 45 (2)
seegene Anyplex II HPV HR	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68	4	HPV18 (4)	HPV16 (4)	HPV31 (3) HPV31, 39 (1)	HPV66 (4)	HPV35 (4)	HPV16, 18, 31, 45 (3) HPV16, 18, 31 (1)

N: number of encoded datasets, ND: not determined

END