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BIOLOGICAL HEALTH RISKS
QUALITY OF LABORATORIES

CLINICAL BIOLOGY COMMISSION
COMMITEE OF EXPERTS

EXTERNAL QUALITY ASSESSMENT
IN CLINICAL BIOLOGY

DEFINITIVE GLOBAL REPORT

Molecular biology: microbiology

HBV-HCV: pilot study

SURVEY 2022/6

.be

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INTRODUCTION

For the first time, Sciensano (Quality of laboratories= QL) organized an external quality assessment for the detection and the quantification of HCV and HBV viruses in collaboration with the National reference centre (NRC for Hepatitis) which is a consortium between Sciensano (Virology) and the clinical laboratory of UCL St Luc. In practice, the NRC possesses sera from patients with the titres and the genotype. Among this collection, we selected several positive samples to spike negative sera and we aliquoted them to constitute a panel of 5 samples. These panels were tested by the NRC before the shipment to the participants.

1.1 The samples

HCV

Sample ID	Matrix	Content	Qualitative result	Quantitative result (Log IU/mL) *
HCV22-1	plasma	HCV	Positive	3.2
HCV22-2	plasma	HCV	Positive	3.83
HCV22-3	plasma	HCV	Positive	3.39
HCV22-4	plasma	HCV	Positive	3.7
HCV22-5	plasma	No HCV	Negative	Not applicable

* : it is the value obtained by the NRC during the validation of the panel

HBV

Sample ID	Matrix	Content	Qualitative result	Quantitative result (Log IU/mL) *
HBV22-1	plasma	HBV	Positive	5.44
HBV22-2	plasma	HBV	Positive	5.26
HBV22-3	plasma	HBV	Positive	4.62
HBV22-4	plasma	HBV	Positive	4.07
HBV22-5	plasma	No HBV	Negative	Not applicable

* : it is the value obtained by the NRC during the validation of the panel

1.1.1   HOMOGENEITY

The samples were tested by the reference laboratory before sending to the participants to determine the qualitative and quantitative target values.

1.1.2   STABILITY

Stability was verified by comparing the results obtained by the reference laboratory before and during the investigation. Qualitatively the results are identical and quantitatively the results do not differ by more than 10%. We considered the samples to be stable.

1.2 Evaluation

For Qualitative detection, participants' results will be compared to the pre-survey results of the reference laboratory. The QCMD scoring system will be applied (Table 2).

Sample status	Score in case of correct answer	Score in case of wrong answer
Negative	0	+3
Frequently detected*	0	+3
Detected*	0	+2
Infrequently detected*	0	+1

* : Frequently detected : sample detected by more than 95% of the participants, detected : sample detected by more than 65% of the participants infrequently detected : Sample detected by less than 65% of the participants.

For the quantitative results, a Z-score is calculated

Z= (Res-target value)/SD

Target value= median of the participants

SD= (P75-P25)/1.349

if lZl score <1, score 0

if lZl score between 1 and 2, score =1

If lZl score between 2 and 3= score 2

Si lZl score $\geq$ 3, Score=3 (cited result)

A not determined result is considered as a wrong answer but not Z score can be calculated.

1.3 Survey Timeline

Samples shipment :06/09/2022

Encoding deadline : 26/09/2022*

Publication of the preliminary report : 06/10/2022

* : the closing date has been extended by one week due to many requests for second shipments.

2 RESULTS

2.1 HBV

2.1.1 PARTICIPANTS

28 laboratories had registered. 24 (85.7%) encoded results.

2.1.2 QUALITATIVE RESULTS

2.1.2.1 Results per sample

The table R1 presents the results per sample. All the encoded results were correct.

Table R1. Results per sample

Sample ID	Expected	Positive	Negative
HBV22-1	Positive	24	0
HBV22-2	Positive	24	0
HBV22-3	Positive	24	0
HBV22-4	Positive	24	0
HBV22-5	Negative	0	24

2.1.2.2 .Used methods

The methods used to detect HBV are resumed in table R2. Cepheid Genexpert method is the most used one (9/24=37.5%)

Table R2. The methods

Method	N (%)
Abbott Allinity	4 (16.7%)
Abbott real time PCR	3 (12.5%)
APTIMA HBV Quant Assay	1 (4.2%)
Cepheid Genexpert	9 (37.5%)
In house real time PCR	2 (8.3%)
NeuMoDx HBV Quant Assay	1 (4.2%)
Roche Cobas 4800 HBV	3 (12.5%)
Roche Cobas 6800 HBV	1 (4.2%)
Total	24

2.1.2.3 Score per laboratory

For the qualitative results, all the laboratories obtained the ideal score of 0.

2.1.3 QUANTITATIVE RESULTS

24 laboratories encoded quantitative results.

2.1.3.1 Results per sample

Table R3 summarizes the results per sample. If the Z score is less than 3, the result was considered as correct. Out of the 96 results, 84 (87.5%) were correct and 12 (12.5%) were not correct (Z score $\geq$3).

Table R3. Quantitative results par sample.

Sample	Z score <1	1$\leq$Z score<2	2$\leq$Z score<3	Z score $\geq$3	% citation
HBV22-1	14	5	0	5	20.8
HBV22-2	16	5	0	3	12.5
HBV22-3	12	5	6	1	4.2
HBV22-4	14	6	1	3	12.5
Total	56	21	7	12	12.5

2.1.3.2 Results per method

Table R4 summarizes the quantitative results per method. The percentage of correct results ranged from 25% (Aptima) to 100 % (Abbott Allinity, NeuMoDx, Roche Cobas).

Table R4. Quantitative results per method

Method	N	NR	NCR	%	Z score <1	1$\leq$Z score<2	2$\leq$Z score<3	Z score$\geq$3
Abbott Allinity	4	16	16	100	11	5	0	0
NeuMoDx HBV Quant	1	4	4	100	3	1	0	0
Roche Cobas 6800 HBV	1	4	4	100	0	4	0	0
Cepheid Genexpert	9	36	34	94.4	26	6	2	2
Roche Cobas 4800 HBV	3	12	11	91.7	10	1	0	1
Abbott real time PCR	3	12	9	75	4	2	3	3
In house real time PCR	2	8	5	62.5	2	2	1	3
APTIMA HBV Quant Assay	1	4	1	25	0	0	1	3
Total	24	96	84	87.5	56	21	7	12

N : number of laboratories, NR : number of results, NCR : number of correct results, % : % of correct results.

2.1.3.3 Score per laboratory

The score can range from 0 for 100% of correct results to 12 for 100% of wrong results.

4 laboratories obtained the ideal score of 0 ; 5 laboratories obtained the score of 1 ; 3 laboratories obtained the score of 2 ; 6 laboratories obtained the score of 3 ; 2 laboratories obtained the score of 4, and 3 laboratories obtained the score of 11. The individual results per laboratory are presented in annex 1.

2.2 HCV

2.2.1 QUALITATIVE RESULTS

29 out of 33 registered laboratories (87.9%) encoded qualitative results.

2.2.1.1 Results per sample

The table R5 summarizes the results per sample.

Table R5. Qualitative results per sample

Sample	Expected results	Positive	Negative	ND	status
HCV22-1	Positive	27	1*	1	detected
HCV22-2	Positive	28	0	1	Frequently detected
HCV22-3	Positive	29	0	0	Frequently detected
HCV22-4	Positive	28	0	1	Frequently detected
HCV22-5	Negative	1*	28	0	Negative

* : The laboratory that responded positive for the negative sample also responded negative for a positive sample, likely switch.

Out of 145 results collected, 140 or 96.6% were correct. Following the reversal, 1 result was a false negative and one result a false positive while 3 results were indeterminate results.

2.2.1.2 Results per method

The table R6 summarizes the results per method. The percentage of correct result per method ranged from 86.7% (Roche Cobas 6800) to 100% (Abbott Allinity, Aptima, Hologic, Procelix, Roche Cobas 4800)

Table R6. Results par method

Method	N	NR	NCR	%	FP	FN	ND
Abbott Allinity HCV	4	20	20	100	0	0	0
Abbott RT PCR	2	10	9	90	0	0	1
APTIMA HCV Quant Assay	1	5	5	100	0	0	0
Cepheid Genexpert	14	70	68	97.1	0	0	2
Hologic HCV qyuant Dx assay	1	5	5	100	0	0	0
Procleix Ultrio Elite Assay	1	5	5	100	0	0	0
Roche Cobas 4800 HCV	3	15	15	100	0	0	0
Roche Cobas 6800 HCV	3	15	13	86.7	1	1	0
Total	29	145	140	96.6	1	1	3

N : number of participants, NR : number of results, NCR : number of correct results ; % : percentage of correct results, FP : false positive ; FN : false negative ; ND : not determined.

2.2.1.3 Score per laboratory

25 laboratories obtained the ideal score of 0. One lab scored 2, two labs scored 3, and one lab scored 5. The maximum possible score was 14.

2.2.2 QUANTITATIVE RESULTS

26 laboratories encoded quantitative results.

2.2.2.1 Results per sample

The table R7 summarizes the results per sample. Out of the 104 results, 95 (91.3%) were correct and 9 (8.7%) were not correct (3 Z scores ≥3 and 6 Not determined).

Table R7. Results per sample

Samples	Z score <1	1≤Z score<2	2≤Z score<3	Z score ≥3	ND	% citation
HCV22-1	14	8	0	1	3	14.4
HCV22-2	19	4	1	1	1	7.7
HCV22-3	17	3	4	1	1	7.7
HCV22-4	18	5	2	0	1	3.8
Total	68	20	7	3	6	8.7

2.2.2.2 Quantitative results per method

The table R8 summarizes the results per method. The percentage of correct results ranged from 75% (Abbott real time PCR and Hologic) to 100% (Abbott Allinity, Aptima).

Table R8. Results per method

Method	N	NR	NCR	%	Z score <1	1≤Z score<2	2≤Z score<3	Z score≥3	ND
Abbott Allinity HCV	4	16	16	100	11	4	1	0	0
Abbott RT PCR	2	8	6	75	3	1	2	1	1
APTIMA HCV Quant Assay	1	4	4	100	3	0	1	0	0
Cepheid Genexpert	13	52	48	92.3	42	6	0	0	4
Hologic HCV quant Dx assay	1	4	3	75	2	1	0	1	0
Roche Cobas 4800 HBV	3	12	11	91.7	3	7	1	1	0
Roche cobas 6800 HCV	2	8	7	87.5	4	1	2	0	1
	26	104	95	91.3	68	20	7	3	6

N: number of participants, NR: number of encoded results, NCR: number of correct results, %: percentage of correct resutls. ND: not determined result.

2.2.2.3 Score per laboratory

The score can range from 0 for 100% of correct results to 12 for 100% of wrong results.

7 laboratories obtained the ideal score of 0 ; 4 laboratories obtained the score of 1 ; 4 laboratories obtained the score of 2 ; 4 laboratories obtained the score of 3 ; 2 laboratories obtained the score of 4 ; 3 laboratories obtained the score of 5 ; 2 laboratories obtained the score of 6.

2.2.3 HCV GENOTYPING

7 laboratories encoded results for HCV genotyping but 15 laboratories indicated that they performed this analysis. Most laboratories did not perform genotyping due to the sample volume too small to do and detection and genotyping.

The results of the genotyping were summarized in table R9. Out of the 28 answers, 20 (71.4%) were correct, 8 were not correct among them 4 were not determined results.

Table R9. Genotyping results

SAMPLE	Expected genotype*	Encoded results (number)	comment
HCV22-1	1a	Genotype 1a (4) Genotype 1 (1) ND (2)	3 not correct results
HCV22-2	1b	Genotye 1b (4) Genotype 1 (2) ND (1)	3 not correct results
HCV22-3	3a	Genotype 3a (4) Genotype 3 (1) Genotype 6 (1) ND (1)	2 not correct results
HCV22-4	1a	Genotype 1a (7)	ok

* : as determined by the NRC

Among the 7 laboratories, 4 used the Versant HCV genotype 2 method, 1 the Sentosa® SQ HCV Genotyping Assay, one the Abbott real time HCV genotype II and one a homemade method.

3 CONCLUSIONS.

Globally, the pilot study was successful. The major problem came from the volume of the sample for HCV analysis. It seems that for Cepheid method, 1 mL is not enough. Therefore, for the next survey, it is important to foresee samples of 2 mL. It is also important if we ask the genotyping on the same sample as the detection and the quantification.

4.1 HBV : Results per laboratory

Table A1: results par laboratory for HBV

method	Qual. HBV 22-1	Quant HBV 22-1	Z sc. Z1	Qual. HBV 22-2	Quant HBV 22-2	Z sc. Z2	Qual. HBV 22-3	Quant HBV 22-3	Z sc. Z3	Qual. HBV 22-4	Quant HBV 22-4	Z sc. Z4	Qual HBV 22-5	Quant HBV 22-5	score
Abbott Allinity	1	5,51	0,43	1	5,26	0,61	1	4,81	0,13	1	4,05	0,65	0	NA	0
Abbott Allinity	1	5,61	1,86	1	5,36	1,62	1	4,81	0,13	1	4,14	1,43	0	NA	3
Abbott Allinity	1	5,57	1,29	1	5,13	-0,71	1	4,8	0,00	1	4,02	0,39	0	NA	1
Abbott Allinity	1	5,54	0,86	1	5,31	1,11	1	4,79	-0,13	1	3,96	-0,13	0	NA	1
ABbott RT PCR	1	5,44	-0,57	1	5,04	-1,62	1	4,6	-2,53	1	3,9	-0,65	0	NA	3
ABbott RT PCR	1	5,8	4,57	1	5,74	5,45	1	4,99	2,41	1	4,46	4,22	0	NA	11
ABbott RT PCR	1	5,49	0,14	1	5,2	0,00	1	4,61	-2,41	1	3,84	-1,17	0	NA	3
APTIMA HBV Quant Assay	1	5,19	-4,14	1	4,87	-3,33	1	4,57	-2,91	1	3,59	-3,35	0	NA	11
Cepheid Genexpert	1	5,25	-3,29	1	5,26	0,61	1	4,91	1,39	1	3,88	-0,83	0	NA	4
Cepheid Genexpert	1	5,73	3,57	1	5,11	-0,91	1	4,86	0,76	1	4	0,22	0	NA	3
Cepheid Genexpert	1	5,48	0,00	1	5,25	0,51	1	4,88	1,01	1	3,97	-0,04	0	NA	1
Cepheid Genexpert	1	5,48	0,00	1	5,32	1,21	1	4,81	0,13	1	3,98	-0,04	0	NA	1
Cepheid Genexpert	1	5,45	-0,43	1	5,12	-0,81	1	4,98	2,28	1	4,15	1,52	0	NA	3
Cepheid Genexpert	1	5,44	-0,57	1	5,16	-0,40	1	4,84	0,51	1	4,03	0,48	0	NA	0
Cepheid Genexpert	1	5,45	-0,43	1	5,18	-0,20	1	4,63	-2,15	1	3,98	-0,04	0	NA	2
Cepheid Genexpert	1	5,49	0,18	1	5,19	-0,10	1	4,77	-0,34	1	3,98	-0,06	0	NA	0
Cepheid Genexpert	1	5,48	0,00	1	5,25	0,51	1	4,9	1,27	1	3,86	-1,00	0	na	2
In house real time PCR	1	5,61	1,86	1	5,11	-0,91	1	4,68	-1,52	1	4,02	0,39	0	NA	2
In house real time PCR	1	4,98	-7,14	1	4,67	-5,35	1	4,24	-7,09	1	3,68	-2,57	0	NA	11
Neu%pDx HBV Quant Assay	1	5,6	1,71	1	5,2	0,00	1	4,8	0,00	1	3,9	-0,65	0	NA	1
Roche Cobas 4800 HBV	1	5,41	-0,93	1	5,27	0,73	1	4,80	0,06	1	3,87	-0,92	0	NA	0
Roche Cobas 4800 HBV	1	5,494	0,20	1	5,265	0,66	1	4,788	-0,15	1	3,827	-1,29	0	NA	1
Roche Cobas 4800 HBV	1	5,43	-0,72	1	5,21	0,15	1	4,76	-0,47	1	4,67	6,09	0	NA	3
Roche Cobas 6800 HBV	1	5,342	-1,97	1	5,09	-1,11	1	4,665	-1,71	1	3,79	-1,61	0	NA	4

For qualitative results: 1=positive; 0=negative. For Z scores: green=Z<1; yellow= $1 \le Z$ score<2; orange: $2 \le Z$ score<3; red: Z score ≥ 3.

4.2 HCV results per laboratory

Table A2. Results per laboratory for HCV

method	Qual. HCV22-1	Quant. HCV22-1	Z score Z1	Genotyp HCV22-1	Qual. HCV22-2	Quant. HCV22-2	Z score Z2	Genotyp HCV22-2	Qual. HCV22-3	Quant. HCV22-3	Z score Z3	Genotyp HCV22-3	Qual. HCV22-4	Quant. HCV22-4	Z score Z4	Genotyp HCV22-4	Qual.; HCV22-5	socre
Abbott Allinity HCV	1	3,17	1,42	ND	1	3,85	0,79	ND	1	3,46	2,88	ND	1	3,78	1,28	ND	0	2
Abbott Allinity HCV	1	2,97	-0,25	ND	1	3,51	-1,21	1b	1	3,2	-0,33	3a	1	3,48	-0,39	1a	0	1
Abbott Allinity HCV	1	2,9	-0,83	1a	1	3,59	-0,74	1b	1	3,37	1,75	3a	1	3,71	0,89	1a	0	1
Abbott Allinity HCV	1	2,99	-0,08	ND	1	3,6	-0,68	ND	1	3,42	2,38	ND	1	3,41	-0,78	ND	0	2
ABbott RT PCR	ND	INH	ND	ND	1	3,83	0,68	ND	1	3,46	2,88	ND	1	3,79	1,33	ND	0	6
ABbott RT PCR	1	3,6	5,00	1	1	3,59	-0,74	1b	1	3,44	2,63	3a	1	3,39	-0,89	1a	0	5
APTIMA HCV Quant Assay	1	3,02	0,17	ND	1	3,6	-0,68	ND	1	3,03	-2,50	ND	1	3,48	-0,39	ND	0	0
Cepheid Genexpert	1	2,94	-0,50	1a	1	3,67	-0,26	1	1	3,29	0,75	3	1	3,49	-0,33	1a	0	0
Cepheid Genexpert	1	3,08	0,67	ND	1	3,86	0,85	ND	1	3,27	0,50	Nd	1	3,66	0,61	ND	0	0
Cepheid Genexpert	1	3,03	0,25	ND	1	3,7	-0,09	ND	1	3,28	0,62	ND	1	3,46	-0,50	ND	0	0
Cepheid Genexpert	3	3,03	0,25	ND	ND	ND	ND	ND	1	3,29	0,67	ND	1	3,6	0,28	ND	0	3
Cepheid Genexpert	1	2,98	-0,17	ND	1	3,56	-0,91	ND	1	3,23	0,00	ND	1	3,43	-0,67	1a	0	0
Cepheid Genexpert	1	2,96	-0,33	ND	1	3,48	-1,38	ND	1	3,12	-1,38	ND	1	3,48	-0,39	ND	0	2
Cepheid Genexpert	1	3	0,00	ND	1	3,67	-0,26	ND	1	3,23	0,00	ND	1	3,59	0,22	ND	0	0
Cepheid Genexpert	1	3,12	1,00	ND	1	3,73	0,09	ND	1	3,32	1,00	ND	1	3,54	-0,06	ND	0	2
Cepheid Genexpert	1	2,97	-0,25	ND	1	3,78	0,38	ND	1	3,17	-0,75	ND	ND	error	ND	ND	0	3
Cepheid Genexpert	1	error	ND	ND	1	3,76	0,26	ND	1	error	ND	ND	1	3,41	-0,78	ND	0	6
Cepheid Genexpert	1	2,829947	-1,42	1a	1,00	3,67	-0,27	1b	1	3,2014	-0,36	3a	1,00	3,54	-0,07	1a	0	1
Cepheid Genexpert	1	2,87	-1,08	ND	1	3,81	0,56	ND	1	3,2	-0,33	ND	1	3,56	0,06	ND	0	1
Cepheid Genexpert	1	2,98	-0,17	ND	1	3,83	0,68	ND	1	3,27	0,44	ND	1	3,56	0,06	ND	0	0
Hologic HCV quuant Dx assay	1	2,93	-0,58	1a	1	3,51	-1,21	1	1	2,99	-3,00	6	1	3,44	-0,61	1a	0	4
Roche Cobas 4800 HBV	1	3,1959	1,63		1,00	3,07	-3,78		1	3,269513	0,49		1,00	3,77	1,21		0	5
Roche Cobas 4800 HCV	1	3,220108	1,83	ND	1,00	4,06	2,01	ND	1	3,1959	-0,43	ND	1,00	3,76	1,19	ND	0	4
Roche Cobas 4800 HCV	1	3,152	1,27	ND	1	3,908	1,14	ND	1	3,212	-0,22	ND	1	3,823	1,52	ND	0	3
Roche Cobas 6800 HCV	0	na	ND	ND	1	3,787	0,42	ND	1	3,029	-2,51	ND	1	3,944	2,19	ND	1	5
Roche cobas 6800 HCV	1	3,22	1,83	ND	1	3,84	0,74	ND	1	3,17	-0,67	ND	1	4	2,50	ND	0	3

For qualitative results: 1=positive; 0=negative. For Z scores: green=Z<1; yellow= 1≤Z score<2; orange: 2≤Z score<3; red: Z score≥3. ND: not determined.

END