BIOLOGICAL HEALTH RISKS
QUALITY OF LABORATORIES

CLINICAL BIOLOGY
COMMITEE OF EXPERTS

EXTERNAL QUALITY ASSESSMENT
IN CLINICAL BIOLOGY

# DEFINITIVE GLOBAL REPORT

## Molecular Microbiology

## HBV-HCV

## SURVEY 2023/S5

**Sciensano/Molecular Microbiology (HBV-HCV)-2-E**

Biological health risks
Quality of laboratories
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# 1 INTRODUCTION

## 1.1 The samples

The samples were prepared from a negative serum (HBV and HCV negative) spiked or not with HBV or HCV positive sera. These positive patient sera were provided by the NRC (UCL Saint-Luc, Brussels).

### 1.1.1 PRODUCTION

100 µl of sample of patient 1 (sample 19292) (HBV) was diluted in 110 mL of negative serum (13155) and then divided in 2 mL aliquots to constitute sample HBV23-1.
100 µl of sample of patient 2 (sample 19289) was diluted in 110 mL of negative serum (13155) and then divided in 2 mL aliquots to constitute sample HBV23-2.
The negative serum (13155) was divided in 2 mL aliquots to constitute sample HBV23-3.
200 µl of serum of patient 3 (sample 19294) (HCV) was diluted in 110 mL of negative serum (13155) and then divided in 2 mL aliquots to constitute sample HCV23-1.
100 µl of serum of patient 4 (sample 19296) (HCV) was diluted in 110 mL of negative serum (13155) and then divided in 2 mL aliquots to constitute sample HCV23-2.
The negative serum (13155) was divided in 2 mL aliquots to constitute sample HCV23-3.
Therefore the panel HBV consists of 3 samples (HBV23-1, HBV23-2, HBV23-3) and the panel HCV consists of 3 samples (HCV23-1, HCV23-2, HCV23-3).

### 1.1.2 HOMOGENEITY

Randomly selected samples were sent to the NRC to check the homogeneity.
The sample HBV23-1 was detected as positive (6.20 Log IU/mL)
The sample HBV23-2 was detected as positive (5.31 Log IU/mL)
The sample HBV23-3 was detected as negative.
The sample HCV23-1 was detected as positive (4.06 Log IU/mL)
The sample HCV23-2 was detected as positive (3.69 Log IU/mL)
The sample HC23-3 was detected as negative.
The samples were considered as homogeneous.

### 1.1.3 STABILITY

The results before and during the surveys were compared.

Table I.1. Samples stability.

| Sample | Result pre-PT | Result PT |
|---|---|---|
| HBV23-1 | Positive (6.2 log IU/mL) | Positive (6.55 Log IU/mL) |
| HBV23-2 | Positive (5.31 IU/mL) | Positive (5.52 IU/mL) |
| HBV23-3 | Negative | Negative |
| HCV23-1 | Positive (4.06 Log IU/mL) | Positive (4.32 Log IU/mL) |
| HCV23-2 | Positive (3.69 Log IU/mL) | Positive (3.97 Log IU/mL) |
| HCV23-3 | Negative | Negative |

The qualitative results are the same and the quantitative results are similar (<10% difference), therefore the samples were considered as stable.

## 1.2 Scoring system

The scoring system is based on the scoring system of QCMD.

### 1.2.1 QUALITATIVE RESULTS

Table I.2. Qualitative scoring system.

| Sample type | Result | Score |
|---|---|---|
| Negative | Negative | 0 |
| Negative | Positive | 3 |
| Negative | Not determined | 3 |
| Frequently detected | Positive | 0 |
| Frequently detected | Negative/Not determined | 3 |
| Detected | Positive | 0 |
| Detected | Negative/not determined | 2 |
| Infrequently detected | Positive | 0 |
| Infrequently detected | Negative/not determined | 1 |

Frequently detected if detected by more than 95% of the participants.
Detected if detected by more than 65% of the participants.
Infrequently detected if detected by less or equal to 65% of the participants.

### 1.2.2 QUANTITATIVE RESULTS

A Z-score is calculated
Z= R-TV/SD
R= result
TV=target value= the median of all the results
SD=standard deviation= (Percentile 75-percentile 25)/1,349

If $|Z|<1$, the score is 0
If $1\leq|Z|<2$, the score is 1
If $2\leq|Z|<3$the score is 2
If $|Z|\geq3$, the score is 3 and the laboratory is cited.

## 1.3 Survey dates

Sending of the samples:26/09/2023
Closure date: 10/10/2023
Preliminary report: 17/10/2023

# 2 RESULTS

## 2.1 HBV

### 2.1.1 THE PARTICIPANTS

25 laboratories sent results, 24 sent quantitative results and 1 sent qualitative results only.

### 2.1.2 RESULTS PER SAMPLE

#### 2.1.2.1 Qualitative results

Table II.1. Qualitative results.

| Sample ID | Expected qualitative result | Observed qualitative results |
|---|---|---|
| HBV23-1 | Positive | 25 positive results |
| HBV23-2 | Positive | 25 positive results |
| HBV23-3 | Negative | 25 negative results |

All the 25 participants obtained the expect results for the qualitative detection of HBV in the serum.

#### 2.1.2.2 Quantitative results

24 laboratories encoded quantitative results for the 2 positive samples. The median of all the results per sample was calculated and used as target value to calculate Z scores: Z=R-T/SD where R=result, T: target, SD: standard deviation. A Z score below 3 is considered as acceptable and a Z score upper or egal to 3 is unacceptable and means that the result was incorrect.

Table II.2. quantitative results

| Sample ID | Median±SD (Log10 IU/mL) | Z<1 | 1≤Z<2 | 2≤Z<3 | Z≥3 | Comment |
|---|---|---|---|---|---|---|
| HBV23-1 | 6.52±0.11 | 16 | 6 | 0 | 2 | 2 incorrect results |
| HBV23-2 | 5.445±0.078 | 16 | 2 | 0 | 6 | 6 incorrect results |

Out of the 48 results (24 per sample), 40 (83.3%) were acceptable (Z<3) and 8 (16.7%) were incorrect (Z≥3).

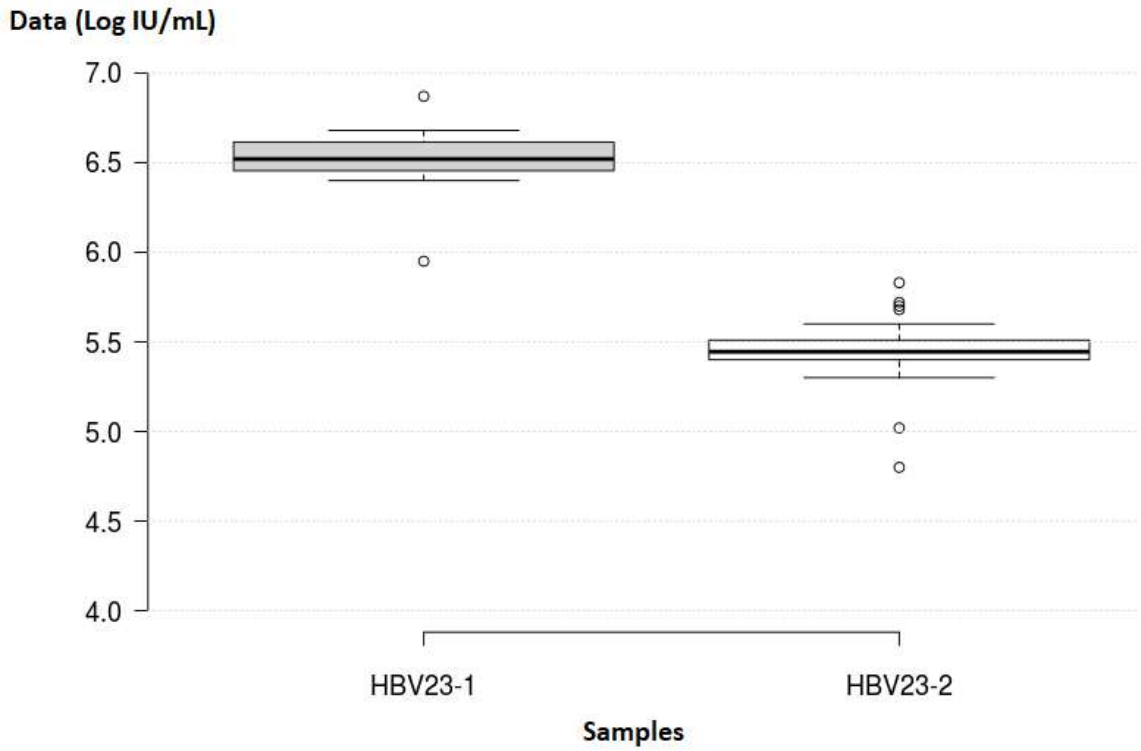


Figure 1. Distribution of results (boxplots) per sample.

### 2.1.3 RESULTS PER METHOD

Table II.3. Quantitative results per method

| Method | N | NR | NCR | % | Z<1 | 1<Z<2 | 2<Z<3 | Z≥3 | ranking |
|---|---|---|---|---|---|---|---|---|---|
| Cobas 5800 HBV test | 2 | 4 | 4 | 100 | 4 | 0 | 0 | 0 | 1 |
| Cobas 6800 HBV | 2 | 4 | 4 | 100 | 4 | 0 | 0 | 0 | 1 |
| NeuMoDx HBV quant Assay | 1 | 2 | 2 | 100 | 2 | 0 | 0 | 0 | 1 |
| Cepheid Xpert HBV viral load | 9 | 18 | 17 | 94.4 | 16 | 1 | 0 | 1 | 2 |
| Abbott ALINITY M HBV AMP KIT | 7 | 14 | 10 | 71.4 | 4 | 6 | 0 | 4 | 3 |
| Aptima HBV Quant assay | 1 | 2 | 1 | 50 | 1 | 0 | 0 | 1 | 4 |
| In house | 2 | 4 | 2 | 50 | 1 | 1 | 0 | 2 | 4 |
| Total | 24 | 48 | 40 | 83.3 | 32 | 8 | 0 | 8 | |

## 2.2 HCV

### 2.2.1 THE PARTICIPANTS

32 laboratories sent results for HCV; 30 sent quantitative results and 2 sent qualitative results only.

### 2.2.2 QUALITATIVE RESULTS

All the participants find the samples HCV23-1 and HCV23-2 as positive and the sample HCV23-3 as negative.

### 2.2.3 QUANTITATIVE RESULTS

#### 2.2.3.1 Results per sample

Table II.4. Quantitative results per sample.

| Sample ID | Median±SD (Log10 IU/mL) | Z<1 | 1≤Z<2 | 2≤Z<3 | Z≥3 | Comment |
|---|---|---|---|---|---|---|
| HCV23-1 | 4.045±0.22 | 20 | 8 | 2 | 0 | ok |
| HCV23-2 | 3.71±0.13 | 22 | 7 | 1 | 0 | ok |

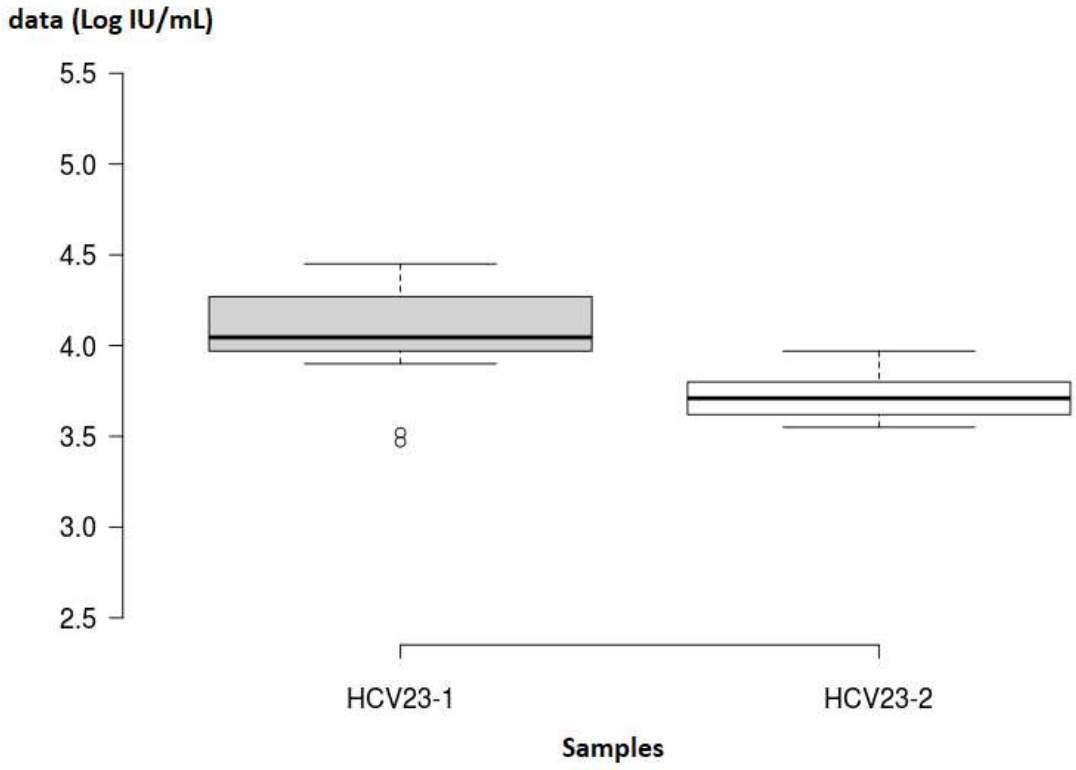


Figure 2. Distribution of the results (boxplots) per sample.

#### 2.2.3.2 Results per method

Table II.5. Quantitative results per method.

| Method | N | NR | NCR | % | Z<1 | 1<Z<2 | 2<Z<3 | Z>3 |
|---|---|---|---|---|---|---|---|---|
| Cobas 4800 HBV test | 1 | 2 | 2 | 100 | 1 | 1 | 0 | 0 |
| Cobas 5800 HCV test | 2 | 4 | 4 | 100 | 2 | 2 | 0 | 0 |
| Cobas 6800 HCV test | 4 | 8 | 8 | 100 | 5 | 3 | 0 | 0 |
| Cepheid Xpert HCV viral load | 14 | 28 | 28 | 100 | 24 | 4 | 0 | 0 |
| Abbott ALINITY M HCV AMP KIT | 7 | 14 | 14 | 100 | 9 | 4 | 1 | 0 |
| Aptima HCV Quant assay | 2 | 4 | 4 | 100 | 1 | 1 | 2 | 0 |
| Total | 30 | 60 | 60 | 100 | 42 | 15 | 3 | 0 |

### 2.2.4 GENOTYPING

Only 10 laboratories sent results for the genotyping of HCV.

Table II.6. Genotyping results.

| Sample | Expected genotype* | Obtained results |
|---|---|---|
| HCV23-1 | 3a | 7 answers 3a<br>3 answers 3 |
| HCV23-1 | 4f | 5 answers 4f<br>1 answer 4<br>1 answer 4 and 5<br>1 answer non-1<br>1 answer “not determined”<br>1 answer “Invalid” |

- As determined by the NRC

Used methods:

4 laboratories used the HCV genotype LIPA 2.0
2 laboratories used a Sanger sequencing method
2 used a real-time qPCR method
2 used a NGS method (Nanopore or Ion torrent)
The individual results are presented in the annexes.

# 3 ANNEXES

1. Individual results for HBV detection and/or quantification.

Table III.1. Individuals results for the HBV detection and quantification.

| Sample → | HBV2301 | | HBV2302 | | HBV2303 |
|---|---|---|---|---|---|
| Status → | Positive | | Positive | | Negative |
| Target value Median (Log IU/mL) -→ | 6.52 | | 5.445 | | NA |
| Detection/quantification kit | | Z1-score | | Z2-score | |
| Abbott ALINITY M HBV AMP KIT | 6.63 | 1.00 | 5.72 | 3.53 | NEG |
| Abbott ALINITY M HBV AMP KIT | 6.53 | 0.09 | 5.42 | -0.32 | NEG |
| Abbott ALINITY M HBV AMP KIT | 6.55 | 0.27 | 5.52 | 0.96 | NEG |
| Abbott ALINITY M HBV AMP KIT | 6.68 | 1.45 | 5.7 | 3.27 | NEG |
| Abbott ALINITY M HBV AMP KIT | 6.67 | 1.36 | 5.83 | 4.94 | NEG |
| Abbott ALINITY M HBV AMP KIT | 6.65 | 1.18 | 5.68 | 3.01 | NEG |
| Abbott ALINITY M HBV AMP KIT | 6.4 | -1.09 | 5.3 | -1.86 | NEG |
| Aptima HBV Quant Assay | 6.49 | -0.27 | 5.02 | -5.45 | NEG |
| Cepheid Xpert HBV viral load | 6.56 | 0.36 | 5.43 | -0.19 | NEG |
| Cepheid Xpert HBV viral load | 6.59 | 0.64 | 5.5 | 0.71 | NEG |
| Cepheid Xpert HBV viral load | 6.42 | -0.91 | 5.43 | -0.19 | NEG |
| Cepheid Xpert HBV viral load | 6.5 | -0.18 | 5.45 | 0.06 | NEG |
| Cepheid Xpert HBV viral load | 6.51 | -0.09 | 5.48 | 0.45 | NEG |
| Cepheid Xpert HBV viral load | 6.49 | -0.27 | 5.5 | 0.71 | NEG |
| Cepheid Xpert HBV viral load | 6.53 | 0.09 | 5.43 | -0.19 | NEG |
| Cepheid Xpert HBV viral load | 6.46 | -0.55 | 5.38 | -0.83 | NEG |
| Cepheid Xpert HBV viral load | 6.87 | 3.18 | 5.6 | 1.99 | NEG |
| Cobas 5800 HBV test | 6.42 | -0.91 | 5.37 | -0.96 | NEG |
| Cobas 5800 HBV test | 6.44 | -0.73 | 5.44 | -0.06 | NEG |
| Cobas 6800 HBV | 6.45 | -0.64 | 5.4 | -0.58 | NEG |
| Cobas 6800 HBV | 6.5 | -0.18 | 5.46 | 0.19 | NEG |
| In house | 6.64 | 1.09 | 5.5 | 0.71 | NEG |
| In house | 5.95 | -5.18 | 4.8 | -6.27 | NEG |
| NeuMoDx HBV quant Assay | 6.6 | 0.73 | 5.4 | -0.58 | NEG |
| MULTIPLEX HIV HCV HBV ROCHE 6800* | POS | | POS | | NEG |

*: qualitative detection only
POS: positive
NEG: Negative
NA : not applicable
Color code : green : $|Z|<1$ ; yellow : $1\leq|Z|<2$ ; red : $|Z|\geq3$

2. Individual results for HCV detection and/or quantification.

Table III.2. Individual results for HCV detection and/or quantification.

| Samples → | HCV2301 | | HCV2302 | | HCV2303 |
|---|---|---|---|---|---|
| Status → | Positive | | Positive | | Negative |
| Target value (Log IU/mL) → | 4.045 | | 3.71 | | NA |
| Detection/quantification Method | | Z1 | | Z2 | |
| Abbott Allinity m HCV amp kit | 4.26 | 0.98 | 3.71 | 0.00 | NEG |
| Abbott Alinity m HCV amp kit | 4.36 | 1.43 | 3.82 | 0.85 | NEG |
| Abbott Alinity m HCV amp kit | 4.04 | -0.02 | 3.69 | -0.15 | NEG |
| Abbott Alinity m HCV amp kit | 4.24 | 0.89 | 3.9 | 1.46 | NEG |
| Abbott Alinity m HCV amp kit | 4.32 | 1.25 | 3.97 | 2.00 | NEG |
| Abbott Alinity m HCV amp kit | 4.17 | 0.57 | 3.69 | -0.15 | NEG |
| Abbott Alinity m HCV amp kit | 4 | -0.20 | 3.57 | -1.08 | NEG |
| APTIMA HCV quant dx assay | 3.47 | -2.61 | 3.55 | -1.23 | NEG |
| Cepheid Xpert HCV viral load | 3.97 | -0.34 | 3.77 | 0.46 | NEG |
| Cepheid Xpert HCV viral load | 3.96 | -0.39 | 3.55 | -1.23 | NEG |
| Cepheid Xpert HCV viral load | 4 | -0.20 | 3.6 | -0.85 | NEG |
| Cepheid Xpert HCV viral load | 3.97 | -0.34 | 3.69 | -0.15 | NEG |
| Cepheid Xpert HCV viral load | 4 | -0.20 | 3.82 | 0.85 | NEG |
| Cepheid Xpert HCV viral load | 3.97 | -0.34 | 3.8 | 0.69 | NEG |
| Cepheid Xpert HCV viral load | 4.08 | 0.16 | 3.7 | -0.08 | NEG |
| Cepheid Xpert HCV viral load | 3.9 | -0.66 | 3.56 | -1.15 | NEG |
| Cepheid Xpert HCV viral load | 3.91 | -0.61 | 3.71 | 0.00 | NEG |
| Cepheid Xpert HCV viral load | 4.05 | 0.02 | 3.82 | 0.85 | NEG |
| Cepheid Xpert HCV viral load | 3.95 | -0.43 | 3.59 | -0.92 | NEG |
| Cepheid Xpert HCV viral load | 4.14 | 0.43 | 3.88 | 1.31 | NEG |
| Cepheid Xpert HCV viral load | 3.92 | -0.57 | 3.88 | 1.31 | NEG |
| Cepheid Xpert HCV viral load | 4.01 | -0.16 | 3.71 | 0.00 | NEG |
| Aptima HCV quant Dx | 3.52 | -2.39 | 3.72 | 0.08 | NEG |
| Roche Cobas 4800 HCV | 4.45 | 1.84 | 3.61 | -0.77 | NEG |
| Roche Cobas 5800 HCV | 4.33 | 1.30 | 3.62 | -0.69 | NEG |
| Roche Cobas 5800 HCV | 4.32 | 1.25 | 3.74 | 0.23 | NEG |
| Roche Cobas 6800 HCV | 4.35 | 1.39 | 3.72 | 0.08 | NEG |
| Roche Cobas 6800 HCV | 4.16 | 0.52 | 3.62 | -0.69 | NEG |
| Roche Cobas 6800 HCV | 4.35 | 1.39 | 3.72 | 0.08 | NEG |
| Roche Cobas 6800 HCV | 4.27 | 1.02 | 3.64 | -0.54 | NEG |
| Procleix Ultrio Elite Assay HCV* | POS | | POS | | NEG |
| Roche Cobas 6800 multiplex * | POS | | POS | | NEG |

*: qualitative detection only
POS: positive
NEG: Negative
NA : not applicable
Color code : green : $|Z|<1$ ; yellow : $1\leq|Z|<2$ ; orange: $2\leq|Z|<3$

3. HCV genotyping.

Table III.3. Individual genotyping results.

| Samples → | HCV2301 | HCV2302 |
|---|---|---|
| Genotyping method | genotype | genotype |
| Abbott m2000RT HCV genotype II | 3 | 4 et 5 |
| HCV genotype LIPA | 3a | Non 1 |
| in house sequencing (ABI3500) | 3a | ND |
| NGS : nanopore sequencing | 3a | 4f |
| NGS: Sentosa SQ301 (ion torrent) | 3a | 4f |
| RT-qPCR cobas 4800 | 3 | 4 |
| Sanger sequencing | 3 | invalid |
| Versant HCV genotype LIPA | 3a | 4F |
| Versant HCV genotype LIPA | 3a | 4f |
| Versant HCV genotype LIPA | 3a | 4f |

**END**