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► **To cite this version:**

Juliette Besombes, Charlotte Pronier, Charles Lefevre, Gisèle Lagathu, Anne Maillard, et al.. Performances of NeuMoDx<sup>TM</sup>, a random-access system for hepatitis B virus DNA and hepatitis C virus RNA quantification. *Clinical Microbiology and Infection*, 2021, 27 (11), pp.1693.e9-1693.e15. 10.1016/j.cmi.2021.02.023 . hal-03216472

**HAL Id: hal-03216472**

**<https://hal.science/hal-03216472>**

Submitted on 25 Oct 2021

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Contents lists available at ScienceDirect

## Clinical Microbiology and Infection

journal homepage: [www.clinicalmicrobiologyandinfection.com](http://www.clinicalmicrobiologyandinfection.com)

## Original article

## Performances of NeuMoDx™, a random-access system for hepatitis B virus DNA and hepatitis C virus RNA quantification

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## ARTICLE INFO

## Article history:

Received 6 November 2020  
 Received in revised form  
 22 February 2021  
 Accepted 24 February 2021  
 Available online xxx

Editor: F. Allerberger

## Keywords:

Correlation analysis  
 Hepatitis  
 Molecular biology  
 Viral load

## ABSTRACT

**Objective:** Monitoring of viral loads (VL) for hepatitis B and C viruses (HBV; HCV) is essential to evaluate disease progression and treatment response. Automated, random-access rapid systems are becoming standard to provide clinicians with reliable VL. The aim of this study was to evaluate the analytical performances of the recently launched NeuMoDx™ for HBV-DNA and HCV-RNA quantification.

**Methods:** Clinical samples routinely quantified on the Beckman–Veris system were either retrospectively (frozen samples; HBV  $n = 178$ , HCV  $n = 249$ ), or in parallel (fresh primary tubes; HBV  $n = 103$ , HCV  $n = 117$ ) tested using NeuMoDx™. Linearity range was assessed on serial dilutions of high-titre plasmas containing different genotypes for HBV (A–E,  $n = 9$ ) and HCV (1a,1b,2–5,  $n = 12$ ).

**Results:** Overall test failure, mostly internal control amplification failure, was 2.3% and was not influenced by matrix types (fresh or frozen). For HBV VL,  $\kappa$  agreement was 74%, with 27 (12.6%) discrepancies. Correlation between HBV assays on 72 quantified samples by both methods was excellent ( $r = 0.963$ ) with a mean bias (NeuMoDx™–Veris) of 0.21 log IU/mL. For HCV VL,  $\kappa$  agreement reached 94%, with 9 (2.8%) discrepancies. The  $r$  correlation factor between assays on 104 samples was 0.960 with a mean bias of  $-0.14$  log IU/mL (NeuMoDx™–Veris). Serial dilutions confirmed the claimed linear ranges for all analysed HBV and HCV genotypes. The mean turnaround time was 72 minutes (range 55–101 minutes) for HBV and 96 minutes (range 78–133 minutes) for HCV.

**Conclusion:** Results obtained on the NeuMoDx™ confirmed the overall good functionality of the system with a short turn-around-time, full traceability and easy handling. These results on HBV and HCV VL look promising and should be challenged with further comparisons. **Juliette Besombes, Clin Microbiol Infect 2021;•:1**

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## Introduction

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are the most common chronic viral infections and remain an important global health problem with more than 250 million chronic HBV carriers and 70 million people living with chronic HCV infection [1]. These chronic infections may lead to chronic liver diseases and to primary liver cancer. The incidence and prevalence of these infections have notably decreased as a result of HBV vaccination programmes [2,3] and to HCV direct-acting antiviral treatment [4], but HBV and HCV are still a major cause of deaths worldwide, especially in low-

middle-income countries, and are responsible for 96% of the mortality from viral hepatitis [1].

Anti-HCV antibody detection alone does not differentiate an active from a past infection, the former being necessarily confirmed by viral RNA detection [5]. Determination of HBV viral load (VL) in serum or plasma is essential to diagnose a chronic infection, to establish the prognosis, to decide the course of treatment and to monitor the virological response to antiviral therapy [6]. The ultimate objective is to achieve an undetectable VL in both cases. The risk of disease progression is reduced when a sustained reduction of HBV-DNA to undetectable levels is achieved, whatever the course of the infection [6]. In a similar way, remission from an HCV infection following treatment with direct-acting antivirals is defined by the achievement of a sustained virological response, which is an undetectable HCV-RNA level 12 weeks or 24 weeks

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<https://doi.org/10.1016/j.cmi.2021.02.023>

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after treatment completion [7]. International clinical practice guidelines recommend the use of sensitive nucleic acid amplification technologies for HBV-DNA or HCV-RNA detection [6–9]. Assays with a lower limit of detection  $\leq 10$  International Units (IU)/mL for HBV-DNA and  $\leq 15$  IU/mL for HCV-RNA are strongly recommended [6,7].

Fully automated, random-access, closed systems that integrate the entire molecular diagnostic process from sample to result are routinely used for this purpose [10,11]. By opposition to systems working on batches, random access systems allow samples to be loaded on a one-by-one basis at any time even if other analyses are in progress. They include reagent storage, specimen preparation, nucleic acid extraction, real-time PCR set-up, amplification and detection, as well as result analysis and reporting, on a single platform. Among many advantages, they usually offer high throughput, full traceability, minimal cross-contamination risk and high safety standard for end users.

The recently launched NeuMoDx™ 96 Molecular System (QIAGEN Sciences (Waltham, MA, USA)) fits this definition with a microfluid-based technology. The aim of this study was to evaluate the analytical performances of the NeuMoDx™ for HBV-DNA and HCV-RNA quantification. We used a panel of specimens, including different HBV and HCV genotypes, with a wide range of VL values as determined on a routine basis with the VERIS MDx System (Beckman Coulter France S.A.S., Villepinte, FRANCE) to compare the concentrations obtained with the new system. A comparison of the turnaround times of both tests was also performed.

## Materials and methods

### HBV and HCV viral load assays

NeuMoDx™ Molecular System is a fully automated sample-to-result molecular diagnostic system: it integrates the extraction, purification, quantification and result interpretation of infectious disease nucleic acid targets. It integrates magnetic particle affinity capture and real-time PCR chemistry in a multi-sample microfluidic cartridge. The plasma input volume is 1.0 mL. An exogenous Sample Processing Control is incorporated in the Extraction Plate. The NeuMoDx™ HBV and HCV Quant Assays target highly conserved sequences in the regions encoding X and preCore proteins in the HBV genome and 5' untranslated region sequences in the HCV genome. Across genotypes, the dynamic ranges of quantification for the HBV and HCV assays are 7.6 to  $1.05 \times 10^9$  IU/mL (0.88–9.02 log IU/mL) and 7.7 to  $1.6 \times 10^8$  IU/mL (0.9–8.2 log IU/mL), respectively. Both assays are CE-IVD cleared.

VERIS MDx System (Beckman Coulter) is also a random access integrated automated nucleic acid extraction and real-time PCR system that uses a plasma input volume of 1.0 mL. Quantification is based on the amplification of the S-gene for HBV and the 5'-untranslated region for HCV. The dynamic range of quantification of the HBV assay is 10 IU/mL to  $1 \times 10^9$  IU/mL (1–9 log IU/mL) and 12 to  $1 \times 10^8$  IU/mL (1.08–8 log IU/mL) for the HCV assay. An internal control is included in each test to monitor process variations. The acquisition time is 70 minutes for one patient after loading a sample tested for HBV and 105 minutes for HCV.

These tests establish traceability to the fourth WHO International Standard for HBV assay and to the fifth WHO International Standard for HCV assay. External quality controls were passed daily before the start of testing for both assays on both systems.

### Sample collection

The study included 281 samples for HBV assay and 366 samples for HCV assay from patients regularly followed up at the University

Hospital Pontchaillou of Rennes, France. Human whole blood was collected in sterile blood collection tubes containing EDTA as anticoagulant for plasma preparation, and stored at  $-80^\circ\text{C}$ . Plasma VL was initially quantified on the Veris MDx system then either retrospectively (frozen samples;  $n = 178$  HBV,  $n = 249$  HCV) or in parallel (fresh primary tubes;  $n = 103$  HBV,  $n = 117$  HCV) tested using NeuMoDx™-specific reagents.

### Research ethics

As required by French law, patients were informed that the samples could be used for research purposes and were free to refuse. The samples were used anonymously to ensure medical confidentiality.

### Linearity

The linearity was assessed on serial dilutions of 9 HBV (genotype A to E) and 12 HCV (genotype 1a, 1b, 2 to 5) high-titre frozen plasmas (around 7 log IU/mL) to cover the assay full quantification ranges. Tenfold dilutions were performed in HBV- and HCV-negative human plasmas supplied by the Blood Transfusion Centre. The measured values were compared with the expected values calculated using the initial Veris-determined VL.

### Reproducibility

To assess inter-assay reproducibility, a set of two HBV and HCV daily controls, including a low-positive and a high-positive control, were tested 11 times (on different days).

### Statistical analysis

Passing-Bablok regression was performed to evaluate the correlation between the NeuMoDx™ and the Veris, and while Bland–Altman analysis was used to analyse the concordance and mean difference between assays. Overall agreement was measured using weighted Cohen's  $\kappa$  coefficient. Means of all differences and standard deviations (SD) were calculated. The 95% limits of agreement between assays were determined as the mean  $\pm 1.96$  SD. Statistical significance was set at  $p < 0.05$ . All analyses were performed using ANALYSE-IT v5.30 software (<https://analyse-it.com/>).

## Results

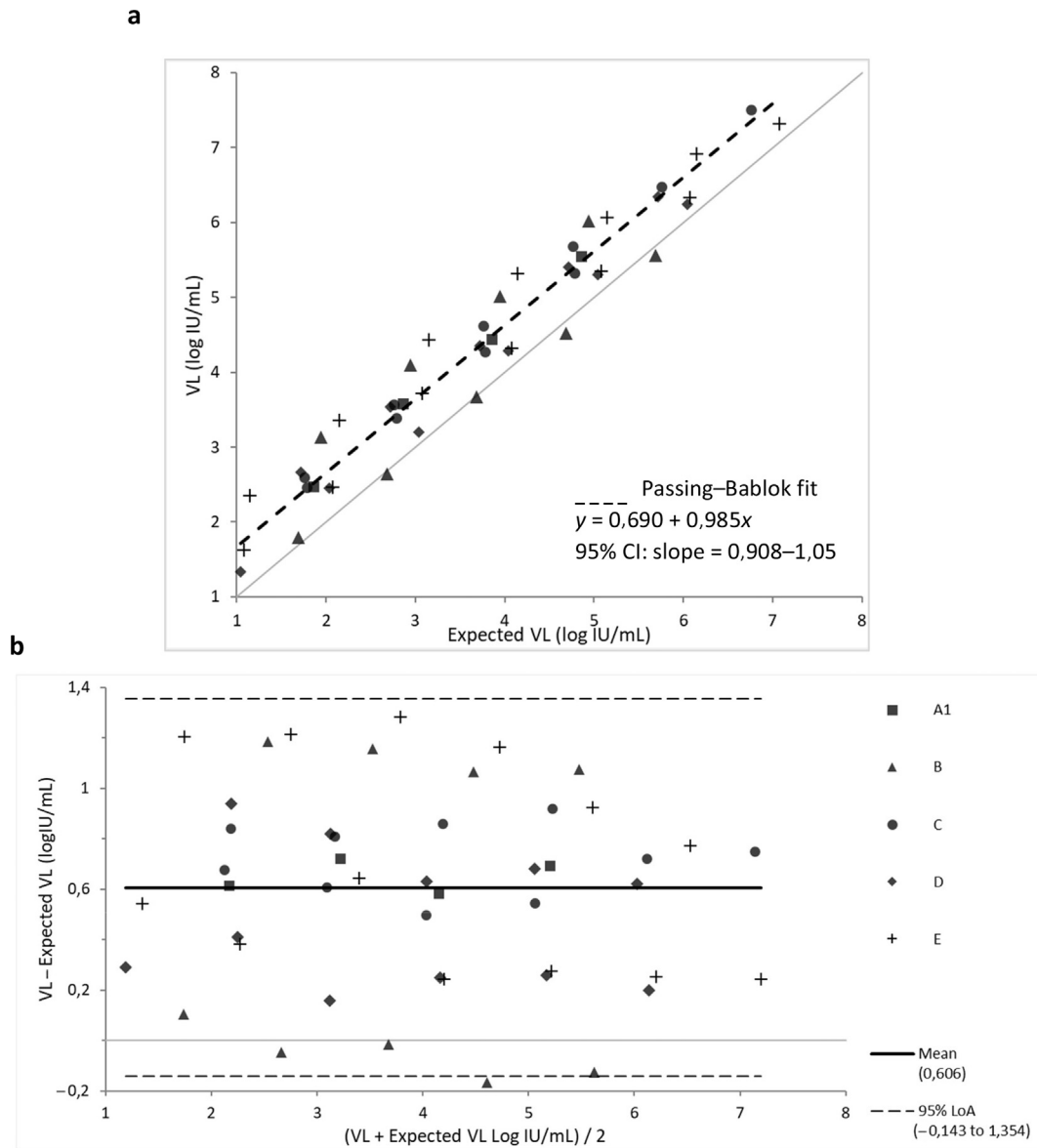
### Linearity

#### HBV assay

Statistical analysis was performed on the 47 dilutions quantified by both methods out of 67, with VL ranging from 1.33 log IU/mL to 7.51 log IU/mL covering the linearity range of the NeuMoDx™ HBV assay. Correlation between HBV-DNA levels measured by the NeuMoDx™ HBV assay and the expected values was excellent ( $r = 0.99$ ) (Fig. 1a). A mean bias of 0.61 log IU/mL was observed (Fig. 1b). These differences seemed to be more important in lower values, close to the quantification threshold of the technique. When comparing with the expected values, a clinically significant difference in quantification ( $>0.5$  log) was observed throughout the linearity range of the assay. No influence of genotype on VL quantification was identified.

#### HCV assay

Statistical analysis was performed on the 52 dilutions quantified by both methods out of 84, with a VL ranging from 1.1 log IU/mL to 6.19 log IU/mL covering the linearity range of the



**Fig. 1.** Passing–Bablok regression and Bland–Altman plot analysis of hepatitis B virus (HBV) DNA levels as determined by the NeuMoDx HBV assay on 47 samples obtained after dilution of nine clinical specimens from patients infected with HBV genotypes A ( $n = 1$ ), B ( $n = 2$ ), C ( $n = 2$ ), D ( $n = 2$ ) and E ( $n = 2$ ). (a) Passing Bablok regression of NeuMoDx HBV assay versus expected values. Dashed line represents the regression line. (b) Bland–Altman plots of NeuMoDx HBV versus expected values. On the Bland–Altman graph, the bold and dashed lines represent the mean and the 95% limits of agreement, respectively.

NeuMoDx™ HCV assay. Correlation between HCV-RNA levels measured by the NeuModx™ HCV assay and the expected HCV-RNA levels with the Veris was very good ( $r = 0.89$ ) (Fig. 2a). A mean bias of  $-0.095$  log IU/mL was observed (Fig. 2b). This dispersion was more important in lower values, close to the quantification threshold of the technique. No influence of genotype on VL quantification was identified.

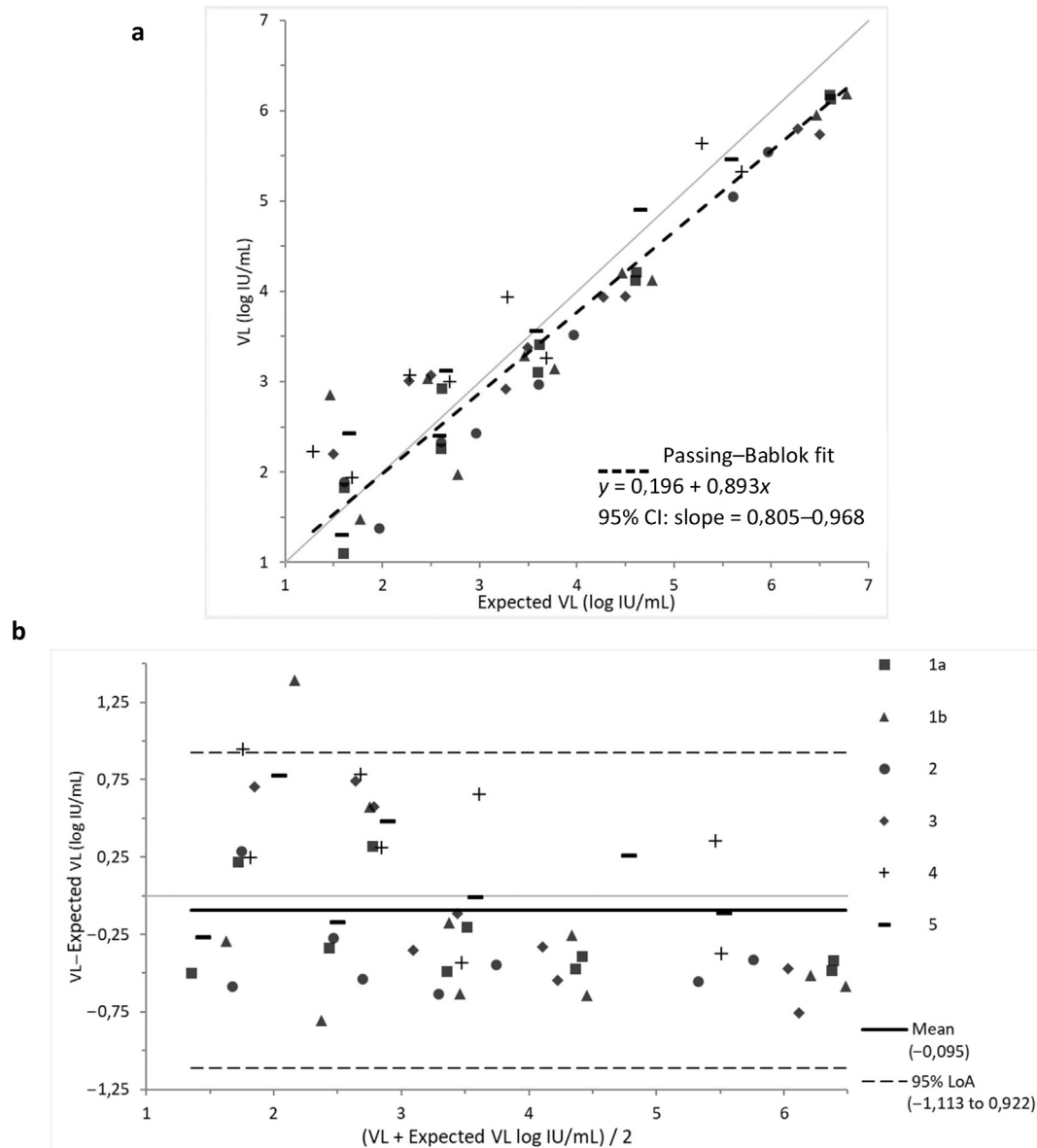
#### Reproducibility

For each assay, low and high control specimens were measured 11 times by different users and over several days. The inter-assay coefficients of variation (CV) for high and low HBV-DNA levels were 1.48% and 4.86%, respectively. For high and low HCV-RNA levels, they were 1.77% and 6.81%, respectively.

#### Performances

##### HBV assay

HBV-DNA levels were measured in parallel with both NeuMoDx™ and Veris systems in 281 samples from patients infected with HBV genotypes A to E (Table 1). Seventy-two samples were quantified with the NeuMoDx™ and the Veris assays with a quantification range of 0.74–8.82 log IU/mL and of 1.08–8.75 log IU/mL respectively. The overall  $\kappa$  qualitative agreement was 74%, with 30 discrepancies (Veris positive while NeuMoDx™ negative); discrepant sample VL ranged from 1.1 to 2.6 log IU/mL. Correlation between both HBV assays on 72 quantified samples was excellent ( $r = 0.967$ ) (Fig. 3a) with a mean bias (NeuMoDx™–Veris) of 0.21 log IU/mL (Fig. 3b). Overall test failure, representing all unsuccessful processes for tested samples and



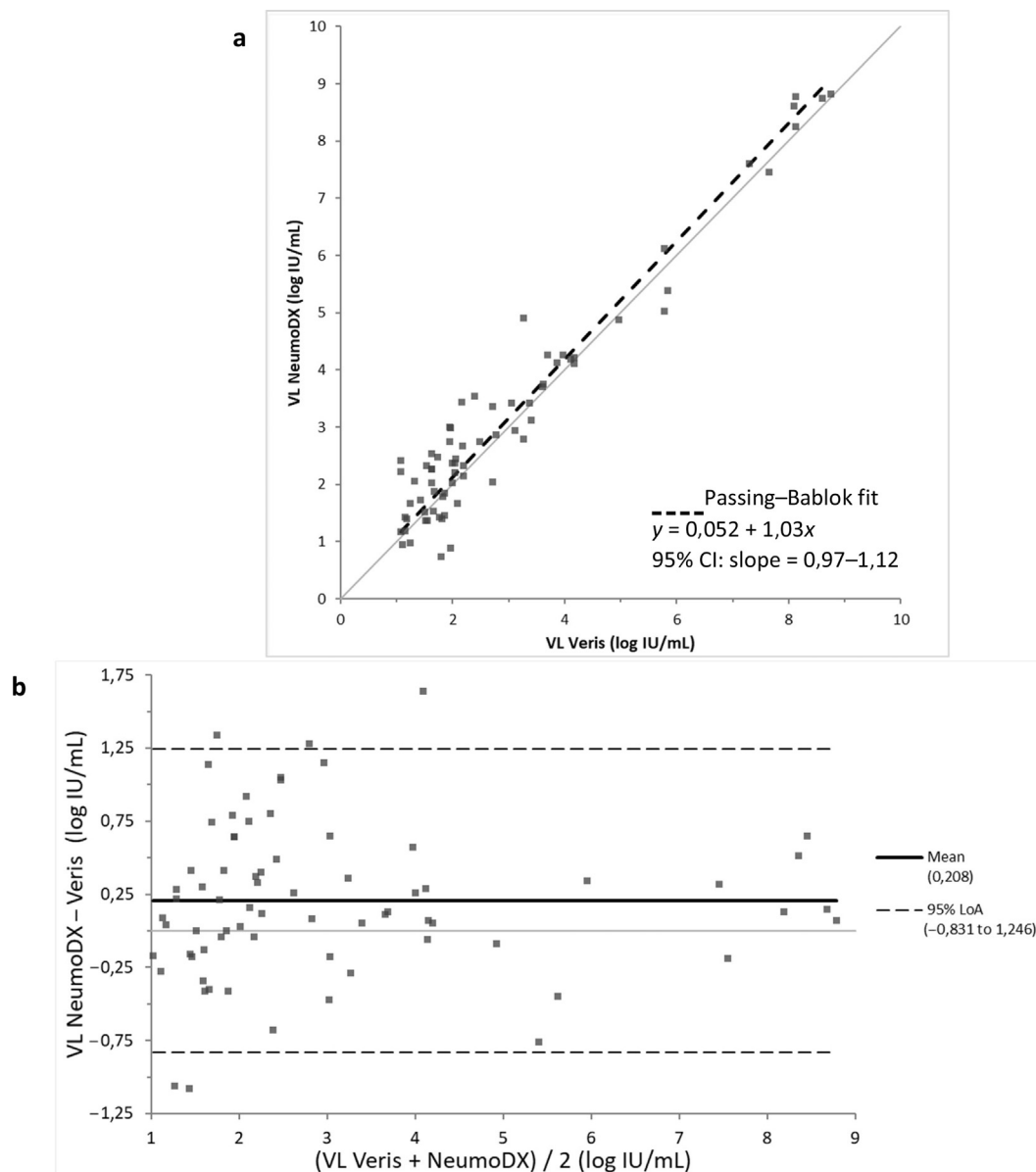
**Fig. 2.** Passing Bablok regression and Bland–Altman plot analysis of hepatitis C virus (HCV) RNA levels as determined by the NeuMoDx HCV assay on 52 samples obtained after dilution of 12 clinical specimens from patients infected with HCV genotypes 1a ( $n = 2$ ), 1b ( $n = 2$ ), 2 ( $n = 2$ ), 3 ( $n = 2$ ), 4 ( $n = 2$ ) and 5 ( $n = 2$ ). (a) Passing Bablok regression of NeuMoDx HCV assay versus expected values. Dashed line represents the regression line. (b) Bland–Altman plots of NeuMoDx HCV versus expected values. On the Bland–Altman graph, the bold and dashed lines represent the mean and the 95% limits of agreement, respectively.

**Table 1**

Comparison between NeuMoDx™ and Veris (reference) HBV and HCV assays among the selected samples (281 for HBV and 366 for HCV)

Status	HBV assay, $n$ (%)					HCV assay, $n$ (%)					
	Veris	NeuMoDx <sup>a</sup>				Veris	NeuMoDx <sup>a</sup>				
		Positive	Negative	Detected	Unresolved		Indeterminate	Positive	Negative	Unresolved	Indeterminate
Positive	102 (36.3)	72 (70.6)	18 (17.6)	4 (3.9)	1 (1.0)	7 (6.9)	110 (29.5)	104 (94.5)	0	2 (1.8)	4 (3.6)
Negative	141 (50.2)	0	117 (83.3)		4 (2.8)	20 (14.2)	223 (59.8)	8 (3.6)	195 (87.4)	4 (1.8)	16 (7.2)
Detected	17 (6.0)	4 (23.5)	12 (70.6)	1 (5.9)			7 (1.9)	6 (85.7)	1 (14.3)		
Indeterminate	1 (0.4)				1 (100)		5 (1.3)	2 (40.0)		3 (60.0)	
Not tested	20 (7.1)	3 (15.0)	13 (65.0)			4 (20.0)	21 (5.6)	3 (14.3)	10 (47.6)	1 (4.8)	7 (33.3)

<sup>a</sup> Possible statuses are: positive, negative, detected, unresolved (usually defines an amplification failure) or indeterminate (usually indicates a sample processing failure like low volume).



**Fig. 3.** Passing Bablok regression and Bland–Altman plot analysis of hepatitis B virus (HBV) DNA levels measured by the NeuMoDx and Veris HBV assays in 72 samples from patients infected with HBV. (a) Passing Bablok regression of NeuMoDx HBV assay versus expected values. Dashed line represents the regression line. (b) Bland–Altman plots of NeuMoDx HBV versus expected values. On the Bland–Altman graph, the bold and dashed lines represent the mean and the 95% limits of agreement, respectively.

corresponding mostly to internal control amplification failure, was 2.1% (6/281) and was not influenced by the matrix types (fresh: 1/103 or frozen: 5/178;  $p$  0.5).

#### HCV assay

HCV-RNA levels were measured in parallel with both NeuMoDx™ and Veris systems in 366 samples from patients infected with HCV genotypes 1a, 1b, 2 to 5 (Table 1). One hundred and four samples were quantified with the NeuMoDx™ and the Veris assays with a quantification range of 1.32–7.44 log IU/mL and of 1.11–7.61 log IU/mL, respectively. The overall  $\kappa$  qualitative agreement reached 94%, with eight discrepancies (Veris negative while NeuMoDx™ positive). Among those, eight HCV VL were detected positive (HCV VL from 17 to 71 IU/mL) on NeuMoDx™ but negative on Veris. The  $r$  correlation factor between both HCV assays on 106 samples was also excellent ( $r = 0.960$ ) (Fig. 4a) with a mean bias (NeuMoDx™–Veris) of  $-0.14$  log IU/mL (Fig. 4b). Overall test failure,

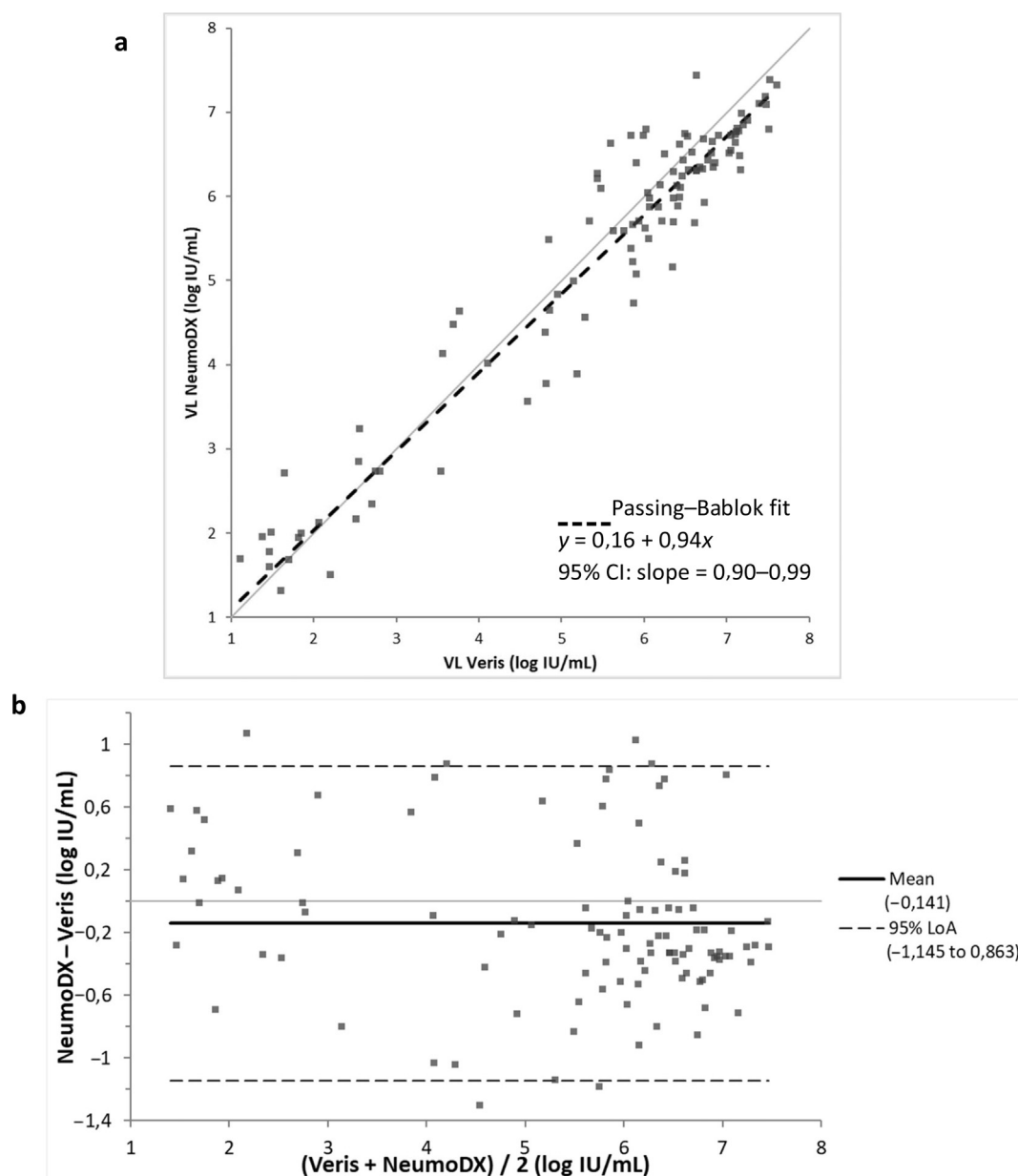
corresponding mostly to internal control amplification failure, was 2.5% (9/366) and was not influenced by the matrix type (fresh: 2/117 or frozen: 7/249;  $p$  0.8).

#### Turnaround time

On NeuMoDx™ the mean turnaround time was 72 minutes (55–101 minutes) for HBV and 96 minutes (78–133 minutes) for HCV, and depended on the number of concomitantly loaded samples on the system.

#### Discussion

As recommended by international guidelines, molecular methods have become the reference standard to provide reliable quantification of HBV and HCV VL to clinicians in reasonable time. This measurement is necessary to diagnose chronic HBV or HCV



**Fig. 4.** Passing Bablok regression and Bland-Altman plot analysis of hepatitis C virus (HCV) RNA levels measured by the NeuMoDx and Veris HCV assays in 104 samples from patients infected with HCV. (a) Passing Bablok regression of NeuMoDx HCV assay versus expected values. The dashed line represents the regression line. (b) Bland-Altman plots of NeuMoDx HCV versus expected values. On the Bland-Altman graph, the bold and dashed lines represent the mean and the 95% limits of agreement, respectively.

infection, to make decisions for therapeutic intervention and to monitor antiviral treatment responses and resistance [6,7]. Fully automated and continuous random-access analysers are routinely used, they offer a fast turn-around time and are usually simple to use and accessible to individuals with no background in molecular biology or virology.

The NeuMoDx™ possesses several of these characteristics. The specificities of this system are its microfluid-based process and the use of dried reagents that are stable at room temperature. Its ability to accept different sample types and tube formats is also an interesting feature. In this study, we evaluated the performance of the system as a routine system.

The Veris MDx System is suitable for high-throughput HBV-DNA and HCV-RNA monitoring in large hospital laboratories like ours [10]. Both HBV and HCV NeuMoDx™ assays were compared with those performed on the Veris. We found that both NeuMoDx™

assays have a good linearity over the claimed quantification ranges. Assay reproducibility was good, particularly for high VL controls; the CV of both HBV and HCV assays for controls at 5 log IU/mL was below 2%. At lower viral loads, the HBV assay CV appeared better than the one observed on the Veris or GeneXpert assays but worse than that reported on the Cobas AmpliPrep/Cobas TaqMan HBV test [10–12]. We noticed a more pronounced dispersion in the low VL values for the HCV test, which may require further investigation even though it remained acceptable. The CV for low HCV-RNA levels is similar to those observed with the GeneXpert or Veris assays but better than the one with the VERSANT 1.0 assay [13–15]. Higher CV at the lower end of quantification is commonly seen with other systems and we cannot affirm from our study whether this observation was more important on NeuMoDx™.

The concordance with the Veris was better for the HCV ( $\kappa = 0.94$ ) than for the NeuMoDx™ HBV assay ( $\kappa = 0.74$ ). Yet, an

overall very good quantification correlation was observed between NeuMoDx™ and Veris for both HBV ( $r = 0.929$ ) and HCV ( $r = 0.960$ ) assays. Even though the NeuMoDx™ assays possess performances comparable to those provided by the Veris, discrepant quantifications were mostly observed on low VL samples. Indeed, eight HCV VL were detected positive on NeuMoDx™ but negative on Veris and conversely 27 HBV VL were detected positive on Veris but negative on NeuMoDx™. The mean bias estimated for both HBV ( $m = 0.21$  log IU/mL) and HCV ( $m = -0.14$  log IU/mL) assays should not translate into any major clinical impact. Indeed, for patient follow up, a variation below 0.5 log IU/mL is usually not considered as clinically relevant [16]. However, these subtle differences confirmed the importance of following an infected patient with the same technique over-time, so as not to miss a relapse or a potential recontamination with HCV.

Not only previously frozen samples, but also freshly drawn samples were run, and no interference from freezing and thawing was noticed. We did not observe any difference of quantification according to genotype in our set of samples and linearity was similar whatever the genotype. Subtle observed differences could possibly be explained by differences in the extraction methods or in primer and probe sequences. Indeed, the NeuMoDx™ HBV assay targets regions encoding HBx and preC proteins whereas the Veris HBV assays targets the S gene. Mutation of the target region may influence the results of two techniques [17].

Finally, the NeuMoDx™ can deliver results in roughly 1.2 hours for the HBV test and 1.6 hours for the HCV test, a fast turn-around time that is useful for emergencies, such as in a transplantation context. The volume required for testing is comparable to that required in other molecular assays such as the Veris or GeneXpert [10,14].

The system works at room temperature because of the use of dry reagents (NeuDry™ technology). This enables waste reduction compared with traditional reagents and easier storage. Moreover, the absence of cooling systems reduces noise emission and energy consumption. These specificities could make this automated system suitable for use in countries with limited resources, particularly in Africa where the prevalence of chronic HBV infection is higher than in Europe (6.1% versus 1.6%) [1].

Some study limitations may be raised, particularly the difficulty to further document discrepant results. Indeed, as VL reaches values close to the limit of detection, one sample can be detected positive on a first run and then not confirmed as positive on a second run. Unless high volume of sample is available, confirmation is always difficult and should then rely on clinical investigation or medical history. Despite our best efforts, we were not able to solve all discrepant situations and sample volumes were too low to control the results. Yet, these first results obtained on the NeuMoDx™ confirm the overall good functionality of the system with a short turnaround time, simple training, full traceability and easy handling. Preliminary results on HBV and HCV VL look promising and should be challenged with further comparisons.

#### Author contributions

The study was conceptualized by VT. Methodology was by JB, CL and VT. Validation and formal analysis were by JB, CP, CL and VT. Investigation was by CP, PB, CH and VT, resources were the responsibility of JB, CP, CL, GL, AM, CG and VT, and data curation was by JB, CL, AM and VT. The original draft was written by JB, CP, VT, and review and editing were by JB, CP, CL and VT. Visualization was

by JB, CP, CL, GL, AM, CG and VT, and supervision was by JB, CP, AM and VT.

#### Transparency declaration

The authors have not received any financial support to conduct the study but all reagents and the NeuMoDx system were provided free of charge from NeuMoDx. JB, CP, CG, CL, GL and AM have not received any support from the industry in the past 3 years. VT has received travel support from Cepheid, Eurobio and Beckman Coulter and speaker honorarium from Abbott.

#### Funding

The NeuMoDx™ 96 system and the reagents used in the present study were provided by QIAGEN Sciences (Waltham, MA, USA).

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