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Sensitivity of hepatitis C virus core antigen and antibody combination assays in a global panel of window period samples

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Abstract

BACKGROUND—Hepatitis C virus (HCV) antigen and antibody combination assays have been launched as a cost-effective alternative to nucleic acid testing (NAT) for reducing the antibody-negative window period (WP). Later, a HCV antigen chemiluminescence immunoassay (CLIA) became available.

STUDY DESIGN AND METHODS—A panel composed of 337 HCV NAT–yield samples that were characterized for viral load (VL) and genotype was used to compare the sensitivity of two combination enzyme-linked immunosorbent assays (Monolisa, Bio-Rad; and Murex, formerly Abbott) and a HCV antigen CLIA (Abbott). Analytic sensitivity was compared with HCV RNA detection using Ultrio (Grifols) by testing serial dilutions of 10 genotype (gt)1 to gt4 samples.

RESULTS—HCV antigen CLIA detected 92.4% of samples, whereas Monolisa and Murex detected 38.3 and 47.5%, respectively. In the HCV RNA VL range of 10^5 to 10^7 IU/mL, Monolisa and Murex detected 38% to 56% of gt1, 85% to 78% of gt2, and 21% to 37% of gt3. The overall geometric mean 50% limit of detection (range) of Ultrio on gt1 to gt4 dilution series was 3.5 (1.2–7.7) copies/mL, compared to 3.3×10^6 (4.4×10^5 – 2.7×10^7), 3.4×10^6 (2.2×10^5 – 4.2×10^7), and 2728 (415–7243) copies/mL for Monolisa, Murex, and HCV antigen CLIA, respectively.

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CONFLICT OF INTEREST

NL works as a consultant for Grifols Diagnostic Solutions. The other authors have disclosed no conflicts of interest.

CONCLUSION—Analytical sensitivity of NAT was on average 1 million- and 780-fold higher than combination assays and HCV antigen CLIA, respectively. Relative sensitivities of combination assays differed for genotypes with Murex being more sensitive for gt1 and gt3 and Monolisa more sensitive for gt2. Although being less sensitive than NAT, combination assays could be considered in resource-limited settings since they detect 38% to 47% of seronegative WP donations.

Blood donation screening using nucleic acid testing (NAT) has been reported to efficiently detect serologically negative donors who are infected with human immunodeficiency virus (HIV), hepatitis B virus (HBV), or hepatitis C virus (HCV), leading many countries to mandate NAT for these viruses over the past two decades.¹ Despite the proven efficacy of NAT in preventing HCV transmission by blood transfusion, financial or organizational limitations prevent some countries, especially in developing and resource-limited regions, from implementing this technology for screening the blood supply. HCV antigen detection, even though less sensitive than NAT, has been proposed as an alternative to improve the safety of blood transfusions in these circumstances,^{2–5} either by using antigen and antibody combination assays or by HCV core antigen detection.^{6,7} At the time of the study, two HCV combination assays were available: the Monolisa HCV antigen and antibody Ultra from Bio-Rad and the Murex antigen and antibody HCV combination assay from then Abbott, now DiaSorin. In studies on seroconversion panels the Monolisa assay detected HCV infection 28 days before antibody assays and 5 days after minipool (MP)-NAT.^{3,4} The Murex assay has been reported as more sensitive than the Monolisa assay in the detection of a panel of HCV window period (WP) samples, particularly in recognizing genotype (gt)3a infections.⁵ More recently a more sensitive HCV core antigen chemiluminescence immunoassay (CLIA) became available (Architect HCV antigen assay, Abbott Diagnostics). This assay has been proposed as a reliable alternative to HCV RNA detection for confirming or excluding active infection⁸ in subjects with acute hepatitis or belonging to high risk groups^{9,10} and for monitoring antiviral response in patient with gt1 infection.¹¹ However, this assay has not been considered yet for screening of blood donations (although it has been used for this purpose in a few locations).

The course of viremia in early HCV infection has been studied in plasma donor seroconversion panels from the United States. Plateau viremia levels in these panels varied between 4×10^4 and 7×10^7 copies/mL.¹² In these seroconversion panels approximately 80% of WP samples with viral loads (VLs) of higher than 100,000 IU/mL were detectable by HCV core antigen enzyme-linked immunosorbent assay (ELISA),¹³ although some donors with low viremia levels were found to remain HCV antigen negative during the plateau phase.¹⁴ Most studies on the relative sensitivity of HCV combination ELISAs have been performed with samples obtained from Western Europe and the United States, where HCV gt1a is predominant. However, in other regions of the world other genotypes are more prevalent. Therefore, we collected a large number of anti-HCV-negative WP samples identified by either individual-donation (ID) or MP-NAT screening in different regions of the world. This allowed us to establish the differences in sensitivity between the above-mentioned HCV antigen and antibody combination and HCV antigen assays, in detecting viremia before antibody conversion. Moreover, by testing serial dilutions of a number of the sourced HCV NAT-yield samples of different (sub)genotypes, the analytical sensitivity of

these assays was compared with that of one NAT blood screening assay (Ultrio, Grifols Diagnostic Solutions). Finally, we examined the distribution of VLs in WP samples with and without reactivity in the HCV antigen and antigen and antibody combination assays.

MATERIALS AND METHODS

Samples

HCV RNA–positive and antibody-negative samples from donors who donated blood from 1997 to 2008 in seven countries using HCV NAT routinely were included in the study (Table 1). Samples were stored at -70°C by most of the centers. A total of 337 NAT-yield samples were shipped on dry ice to the Institut National de la Transfusion Sanguine (INTS) and stored at -20°C until handled. All samples were tested for HCV antibody with a third-generation assay and for HCV NAT with the methods that were routinely used in the participating centers. These assays included the Procleix Duplex or Ultrio assays (Chiron-Novartis/Gen-Probe, currently Grifols/Hologic, Basel, Switzerland); the Ampliscreen, AmpliNAT, or TaqScreen assays (Roche Molecular Systems, Indianapolis, IN); and noncommercial polymerase chain reaction (PCR) assays performed in national donor screening NAT laboratories.¹ The assays were performed in either ID or MP format with pool sizes varying between six and 500 donations (Table 1). Investigators provided the locally measured VLs and, if VL permitted, also the genotype. Samples comprised six genotypes: 157 samples were of gt1, 55 gt2, 85 gt3, 14 gt4, two gt5, and one gt6, and in 23 samples the genotype could not be determined due to too low VL. The overall mean VL was 5.63 ± 1.24 log IU/mL with no difference according to genotypes. There was an equal distribution of samples in different VL ranges for gt1 to gt4 (not shown); however, the proportion of samples with a VL of higher than 6 log IU/mL was significantly ($p = 0.01$) higher for gt2 (63.6%) compared with the other genotypes (overall, 42.1%; range, 29.4% for gt4 to 6 to 44.6% for gt1).

Assays

Clinical sensitivity study—The Monolisa HCV antigen and antibody Ultra (Bio-Rad, Marne-la-Coquette, France) and Murex antigen and antibody HCV combination (Abbott Diagnostics Division, Wiesbaden, Germany) were used according to the manufacturers' instructions. Results were considered reactive if the sample-to-cutoff (S/CO) ratio was equal or greater than 1.0. A 10% gray zone was also used for this analysis. Each sample was tested individually with both assays performed simultaneously. Samples were retested in duplicate in case of discrepant results between the two combination assays or when the S/CO ratio in either assay was found between 0.9 and 2. In these cases, the mean of the ratios was taken as the final result. The clinical sensitivity of the assays was expressed as the percentage of HCV antigen–reactive samples of the total number of NAT-yield samples and this was also analyzed by country and by genotype.

The HCV antigen assay was performed on Architect platform on a subset of 331 (of 337) samples (155 gt1, 55 gt2, 84 gt3, 13 gt4, two gt 5, one gt6, 21 not genotyped) in the Paul Ehrlich Institute when sufficient volume was left over. This assay quantitates HCV antigen

in fmol/L, and a result was considered reactive when the concentration was higher than 3 fmol/L.

Analytical sensitivity study—To evaluate the analytical sensitivity for the detection of HCV genotypes by the HCV antigen and the two combination assays, 10 samples of different genotypes, that is, gt1a (n = 1), gt1b (n = 2), gt2a (n = 2), gt2b (n = 2), gt3a (n = 2), and gt4 (n = 1), were selected on the basis of their high VL for the preparation of serial dilution panels prepared by Biologicals Quality Control (Rijswijk, the Netherlands). These panels were prepared in suitable volumes and concentration ranges for evaluation of the different assays and tested in duplicate by the two combination assays and HCV antigen CLIA. Samples were also tested in duplicate in quantitative NAT assays for cross-calibration in the third-generation bDNA assay (Siemens Laboratories, Berkeley, CA) and the TaqMan HCV assay (Roche, Meylan, France) against the secondary Sanquin HCV gt1 standard quantified in copies/mL and IU/mL (1 IU equals 2.73 copies¹⁵). Finally suitable dilutions of genotype samples and the Sanquin HCV gt1 standard were tested in 12 replicates with the Procleix Ultrio assay on the TIGRIS system (Hologic | Gen-Probe, San Diego, CA).

Statistical analysis

Differences in the proportion of reactive results overall and in different VL ranges for each genotype were compared by chi-square test. A difference was considered significant when p values were not more than 0.05.

The log HCV concentrations in the undiluted NAT-yield samples were plotted against either a positive (value 1) or a negative result (value 0) in the HCV antigen and the two combination assays, respectively. Probit analysis was then used to determine the 50% limit of detection (LOD) and 95% confidence interval (CI) in IU/mL and in copies/mL by using a conversion factor of 2.73 copies/IU.¹⁵ The correlation between log values of either HCV antigen results in fmol/L or combination assay S/CO ratios versus log VL was analyzed by linear regression analysis and t test. The cutoff crossing point of the regression line was calculated and expressed in IU/mL or copies/mL at S/CO ratio of 1 for combination assays and at fmol/L of 3.0 for HCV antigen assay.

Similarly, for comparing the analytical sensitivity in detecting different genotype dilution series the log values of results were plotted against the log values of the HCV concentrations. The HCV concentrations were determined by comparing the geometric mean of the VL results calibrated against the Sanquin HCV gt1 standard. Probit analysis was used on the proportions of reactive results to determine the 50% and 95% LOD and 95% CI by Ultrio for each genotype. The cutoff crossing points of the core antigen CLIA and the two combination ELISAs were determined by regression analysis after log transformation of the quantitative values (i.e., fmol/L or S/CO ratios) and the HCV concentrations.

RESULTS

Clinical sensitivity of combination assays

As shown in Table 1, the overall percentage of HCV NAT–yield samples reactive (or gray zone) with combination assays was 38.3% for Monolisa and 47.5% for Murex ($p = 0.015$). The highest proportion of combination ELISA-reactive samples was found in countries where samples were identified by MP-NAT with large pool sizes (50, 96, and 500) and lowest rates were found in countries where ID-NAT and MP-NAT with small pool sizes (six, 16, 24) were used. Some samples had discrepant results: seven (5.4%) of the 129 Monolisa-reactive samples were Murex nonreactive (four samples were gt2, three gt3 with an overall mean log VL of 6.09 ± 0.61 IU/mL) and 37 (23.1%) of 160 Murex-reactive samples were nonreactive with Monolisa (21 samples were gt1, two gt2, and 12 gt3 with an overall mean log VL of 6.18 ± 0.49 IU/mL).

The HCV RNA levels were not correlated with the S/CO values obtained with the two combination assays as shown in Fig. 1. However, nonreactive samples (i.e., those with S/CO values on combination assays of < 1.0) had significantly lower VLs than reactive samples with both assays (5.07 ± 1.18 log IU/mL vs. 6.52 ± 0.68 log IU/mL, $p < 10^{-4}$ with Monolisa; and 4.87 ± 1.14 log IU/mL vs. 6.47 ± 0.66 log IU/mL, $p < 10^{-4}$ with Murex).

Table 2 compares the proportions of reactive NAT–yield samples in the two combination assays according to genotype. The highest rate of reactive results was observed in gt2 (69.1 and 67.3% for Monolisa and Murex, respectively) while the lowest rate was found in gt3 for both combination assays (23.5 and 34.1%). However, as mentioned there was a selection bias by the screening protocols that had an impact on the VL distribution by genotype. gt2 samples came exclusively from the United States, Germany, and Japan, where MP sizes of 16 to 500 have been used for screening, whereas gt3 samples were mainly obtained in Poland, Lithuania, and the United States, where a large proportion was identified by ID-NAT and the rest by MP6-48 NAT. To exclude a bias by different VL distributions per genotype, the proportion of combination ELISA-reactive samples in different HCV concentration ranges for each genotype was compared (Table 3). In the critical VL ranges of 10^5 to 10^6 and 10^6 to 10^7 IU/mL, the proportion of Murex-reactive gt2 samples was comparable to that of gt1 (38% vs. 24% and 88% vs. 88%, respectively). However, Monolisa detected a significantly higher proportion of gt2 than gt1 samples in these ranges (50% vs. 12% and 94% vs. 63%, respectively). Both assays detected gt3 with significantly lower sensitivity than gt1 in the VL range of 10^6 to 10^7 IU/mL, although Murex detected a higher proportion (61%) than Monolisa (29%).

Clinical sensitivity of HCV antigen assay

Of the 331 tested samples with a mean VL of 5.57 ± 1.34 log IU/mL, 92% were HCV core antigen positive with the Abbott HCV antigen CLIA. There was no difference in sensitivity by genotypes: 95% in 155 gt1, 98% in 55 gt2, 94% in 84 gt3, 93% in 13 gt4, and 58% in the remaining 24 samples. The overall mean HCV core antigen concentration was $0.92 \times 10^4 \pm 1.51^4$ fmol/L and varied from $0.21 \times 10^4 \pm 0.32 \times 10^4$ for gt4 to $1.1 \times 10^4 \pm 1.93 \times 10^4$ for

gt1. The HCV core antigen levels were highly correlated with VLs for all samples combined (Fig. 2) and when analyzed by genotypes (data not shown).

Table 3 shows the clinical sensitivity of the HCV antigen CLIA with increasing HCV RNA VL ranges. In the range below 10^4 IU/mL, 26% of samples were detected by HCV antigen CLIA and none by the combination assays. In the range of 10^4 to 10^5 IU/mL, 100% were HCV antigen CLIA reactive, while the combination assays detected 3% to 5%. Higher than 10^5 IU/mL, 100% were HCV antigen CLIA reactive except for three samples. In these latter VL ranges, the combination assays were significantly less sensitive and detected 16% to 23% and 64% to 81%, for Monolisa and Murex, respectively.

VL distribution and core antigen reactivity in NAT-yield samples

When probit analysis was used on the VLs that were detected and not detected by Monolisa and Murex, we calculated 50% LODs of 1.6×10^6 and 0.8×10^6 IU/mL (4.5×10^6 and 2.3×10^6 copies/mL), respectively (Table 4). The more sensitive HCV antigen CLIA reached a 50% LOD of 942 IU/mL (2572 copies/mL). When the 50% LODs were calculated for the most prevalent gt1 to gt4 in a parallel line probit model, Murex was found to be 2.6- to 2.7-fold more sensitive than Monolisa in detecting gt1, gt3, and gt4 samples but 1.6-fold less sensitive for gt2 ($p > 0.05$).

Analytical sensitivity in dilution series of NAT-yield samples

Table 5 compares the detection limits of the assays based on testing of half-log dilution series of 10 NAT-yield samples of different (sub)genotypes followed by probit analysis for the Ultrio assay results and regression analysis for the HCV antigen and combination assay results. Although similar, the 95% and 50% LODs in the Ultrio assay tended to be somewhat higher on the gt2 samples than on gt1 and gt3 samples. If the quantification of HCV RNA was accurate the gt2 samples may be detected with five- to sixfold lower sensitivity than the gt1a sample by the Ultrio assay. Much larger variability was observed in the genotype detection limits of the combination assays, varying up to 100-fold. When comparing the relative analytical sensitivity of Monolisa and Murex by calculating the ratio of the detection limits we found differences varying between a factor of 0.28 and 4.0, but the overall geometric mean factor was similar (factor 0.99). By contrast, the detection limits in the HCV antigen CLIA were much more consistent over the genotypes and varied up to a sixfold lower sensitivity for one gt2a sample than for gt1a.

When comparing the overall geometric mean 50% LOD of all genotypes in Ultrio with the overall geometric mean cutoff crossing points of the combination assays, it can be concluded that HCV RNA detection by the ID-NAT system was a factor of 9.5×10^5 times more sensitive than the combination assays (ranging between 8.5×10^4 for a gt2b to 9.5×10^6 for a gt3a sample for Monolisa and between 9.4×10^4 for a gt2b to 3.36×10^7 for a gt3a sample for Murex). The difference in analytical sensitivity between the HCV antigen CLIA and the Ultrio assay was on average 780-fold and sensitivity difference factors were more consistent across genotypes (varying between 65-fold for a gt2b and 2490-fold for a gt3a sample).

The overall detection limits in the HCV antigen and combination assays found by regression analysis on the dilution panels were comparable with those estimated by probit analysis on the VLs of undiluted samples (Table 4). The HCV antigen CLIA conversion point in undiluted samples was estimated at on average 942 IU/mL (2572 copies/mL) of HCV RNA, whereas on the dilution series this was 999 IU/mL (2728 copies/mL). The mean conversion point in undiluted samples for Monolisa was estimated at 4.5×10^6 copies/mL whereas it was 3.3×10^6 copies/mL in the dilution series; the conversion points for Murex were 2.3×10^6 and 3.4×10^6 copies/mL with the undiluted samples and dilution series, respectively.

DISCUSSION

To our knowledge this is the first study that compared the sensitivity of antigen and antibody combination ELISAs and HCV antigen CLIA in HCV RNA-positive and antibody-negative WP samples ($n = 337$) identified by ID- and MP-NAT screening of blood donors. These samples were sourced from 1997 to 2008 from blood donors in several parts of the world with the objective to create a representative global panel of HCV NAT-yield samples of different genotypes. Previous evaluation studies of core antigen-based assays were mainly targeting specific groups of patients, such as hemodialysis patients,^{4,10,16–19} HIV-coinfected individuals,²⁰ and antibody-positive chronically infected patients.^{9,11,21–28} Other studies focused on WP antibody-negative subjects included in commercial seroconversion panels,^{3,5,9,29,30} or in a few studies samples were evaluated from blood donors from a restricted source.^{4,7,13}

Our findings in the global panel of NAT-yield samples demonstrate that assays detecting HCV antigen are significantly less sensitive during the antibody-negative WP than ID- and MP-NAT methods used by the participating countries. In this international sample collection, HCV antigen was detected at a rate of 38 and 48% by the Monolisa and Murex combination ELISAs, respectively, but in a significantly higher proportion (92%) by the HCV antigen CLIA. These results are in line with those obtained previously showing that combination assays detected lower rates of HCV RNA-positive and antibody-negative samples than core antigen assays. Sensitivity ranged from 29%⁵ to 70%³ depending on the combination assay and panels and for core antigen assays from 71%¹³ with one of the first developed assays to 99% with a CLIA.³⁰ In addition, we observed that, contrary to the HCV antigen CLIA, reactivity of both combination assays did not correlate well with HCV RNA concentrations in NAT-yield samples.

The influence of HCV genotype on the capacity of combination assays and antigen assays to detect WP samples has not been clearly established. Tuke and colleagues⁵ reported failures in the detection of gt3a with Monolisa, but this was not confirmed elsewhere.³ According to some previous reports, the intrinsic properties of antigen assays seem not to be affected by genotype,^{8,9,28,29} but Ross and coworkers⁹ showed that the analytical sensitivity of HCV antigen CLIA was the lowest for gt2 (13.5 fmol/L against < 10 fmol/L for the other genotypes). In our study, the clinical sensitivity of both combination assays was lower in detecting gt3 than gt1 (Table 2). Murex had a significantly higher capacity in detecting gt1 (50.3%) than Monolisa (36.9%), whereas the latter assay was slightly more sensitive in detecting gt2 (69.1% vs 67.3%). However, a selection bias due to differing sensitivities of

NAT screening methods had an impact on the VL distribution for the different genotypes, which prompted us to compare the diagnostic sensitivity of the assays in different VL ranges. The results confirmed the significantly higher sensitivity of Monolisa for gt2 than for gt1, and a significantly lower sensitivity for gt3, whereas Murex seemed to detect gt1 and gt2 with similar sensitivity and gt3 with lower sensitivity.

A limitation of our analysis was that VLs were not detected by one standardized method but by different techniques used by the participating centers. To exclude the impact of variability due to VL assays multiple bDNA assays were performed to calibrate HCV RNA concentration in the dilution series for comparing the analytical sensitivity of the assays. The bDNA assay was used as the reference method since it has been validated for equal sensitivity of gt1 to gt5.³¹ Considerable variation was found in detection limits of the combination assays in the dilution series (Table 5), which were on average 1 million-fold less sensitive than Ultrio (with factors varying up to 100 fold for the individual genotypes). By contrast, the HCV antigen CLIA was on average 780-fold less sensitive than Ultrio and factors were far more consistent across genotypes, except for the two gt2b samples, which were detected with only 65- to 185-fold lower sensitivity, because the analytical sensitivity of Ultrio was significantly lower for gt2b than for the other genotypes. This may be due to mismatches in oligonucleotides utilized in this assay. A lower sensitivity of Ultrio was also noticed on a low VL HCV gt2b donation that has caused transmission to a recipient.³²

The detection limit of the HCV antigen CLIA assay has been established in chronically infected patients at approximately 500 to 3000 IU of HCV RNA/mL (except for HCV gt2) with bDNA assay as reference for HCV RNA quantification⁹ and between 250 and 32,000 IU/mL irrespective of genotype with Cobas Ampliprep/TaqMan assay (Roche).⁸ According to our analytical sensitivity analysis in the genotype dilution series, the detection limits of the core HCV antigen CLIA assay varied between 150 and 2600 IU/mL, similar to the range found by Ross and colleagues.⁹ This range of HCV antigen assay detection limits is equivalent to 415 to 7243 (mean, 2728) copies/mL; that is, three log higher than the range of 50% LODs in the Ultrio assay varying between 1.2 and 7.7 (mean, 3.5) copies/mL. In our analytical sensitivity study the HCV antigen CLIA detection limits were the highest for gt2a and gt4 and the lowest for gt1a and gt2b. This variation can be explained by the influence of genetic diversity on quantification of HCV RNA and core antigen, which has a particularly high degree for gt2 strains³³ or by the stage of infection during which the samples were collected. One-third of gt2 samples investigated here were retrieved from blood donors from Japan where gt2b is predominant, whereas Ross and coworkers⁹ studied only a few gt2a specimens from antibody-positive chronically infected patients.

In summary, our results demonstrate that the HCV antigen CLIA showed a clinical sensitivity approaching that of HCV RNA detection by NAT, whereas the current generation of combination assays was significantly less sensitive. The HCV antigen CLIA only missed few samples in the early ramp up phase, whereas the combination assays also failed to detect a proportion of samples in the late ramp-up or plateau phases of viremia. It is not known what the difference in design of the assays is that causes the higher sensitivity of HCV antigen CLIA. Possibly, the chemistry in standalone core antigen assays (e.g., to expose capsid protein or dissociate immune complexes) cannot be applied in combination assays.

The HCV antigen CLIA is currently not used for blood screening as it would be required as an additional test to mandatory HCV antibody screening. It has been suggested that the replacement of anti-HCV assays by a combination assay would be an efficient measure to ensure blood safety at a cost-effectiveness greater than that of NAT.^{34–36} As a result, the World Health Organization³⁷ recommends the use of these assays, particularly in resource-limited countries. The results of our study demonstrate that the combination ELISA reduce the WP by 37 and 45% for the Monolisa and Murex, respectively, according to modeling data.³⁸ Although significantly less sensitive than NAT, these assays could be an important contribution to blood safety when compared to anti-HCV testing in countries where NAT is not affordable or technically not feasible, especially since they can be easily used in poor resource setting as they demand much less advanced equipment and no unique platform. In addition, high clinical and analytical sensitivity of the HCV antigen CLIA observed in this study suggest that it may be possible to develop next-generation combination assays that approach the performance of NAT.

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ABBREVIATIONS

CLIA	chemiluminescence immunoassay
gt	genotype (when followed by a number)
ID	individual donation
LOD	limit of detection
MP	minipool
S/CO	sample to cutoff
VL(s)	viral load(s)
WP	window period

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Fig. 1. Distribution of log S/CO obtained with Monolisa HCV Ag/Ab Ultra (top) and Murex HCV combination (bottom) plotted against log HCV RNA concentration in IU/mL.

Fig. 2. Scatterplot of log HCV core antigen concentration (fmol/L) versus log HCV RNA concentration (IU/mL) determined in samples tested by the Architect HCV antigen assay. Samples with concentration of more than 100,000 fmol/L were tested in dilutions (up to 1:20).

TABLE 1

Clinical sensitivity of combination assays per country

Country	Number of samples	Period of collection	Genotypes	NAT method	Monolisa reactive		Murex reactive	
					Number (%) reactive *	Number Murex nonreactive	Number (%) reactive *	Number Monolisa nonreactive
Egypt	15	2007	1-3-4	Ultrio IDT	5 (33.3)	0	6 (40)	1
France	10	2001–2008	1-3-4	Ultrio MP8 or Ampliscreen MP24	4 (40.0)	0	5 (50)	1
Germany	25	1997–2008	1-2-3-5	In-house PCR MP96	13 (52)	0	17 (68)	4
Japan	33	2001	1-2	AmpliNAT MP500, MP50	21 (63.6)	1	21 (63.6)	1
Lithuania	19	2006–2007	1-3	Ultrio IDT	4 (21.1)	0	4 (21.1)	0
Poland	69	1999–2006	1-3-4	Duplex, Ultrio IDT, Ampliscreen MP24, or MP48, TagScreen MP6	18 (26.1)	2	23 (33.3)	7
US	166	1999–2008	1-2-3-6	Duplex or Ultrio MP16	64 (38.5)	4	84 (50.6)	23
TOTAL	337				129 (38.3) [†]	7 (5.4) [‡]	160 (47.5) [‡]	37 (23.1) [§]

* Including gray zone (S/CO, 0.9–1). The percentage of samples with $0.89 < S/CO < 1$ was 6.2% with Monolisa and 5% with Murex.

[†] $p = 0.06$.

[‡] Four samples were gt2, and three gt3; the overall mean log VL was 6.09 ± 0.614 IU/mL.

[§] Twenty-one samples were gt1, two gt2, and 12 gt3; the overall mean VL was 6.18 ± 0.490 IU/mL.

IDT = individual-donation testing; MP = minipool testing.

TABLE 2
Clinical sensitivity of combination assays in WP-NAT–yield samples according to genotype

Genotype	Number	Mean VLs Log IU/mL*	Reactive [†]		p value	Nonreactive [‡]
			Monalisa	Murex		
1	157	5.67 ± 1.29	58 (36.9)	79 (50.3)	0.01	78 (50)
2	55	5.89 ± 1.10	38 (69.1)	37 (67.3)	NS	15 (27.3)
3	85	5.59 ± 1.09	20 (23.5)	29 (34.1)	NS	53 (62.4)
4	14	5.13 ± 0.89	4 (28.6)	4 (35.7)	NS	9 (64.2)
5	2	NA	2 (100)	2 (100)	NA	0
6	1	NA	1 (100)	1 (100)	NA	0
ND	23	4.45 ± 1.74	6 (26.1)	7 (30.4)	NS	16 (66.7)
TOTAL	337	5.63 ± 1.24	129 (38.3) [‡]	160 (47.5) [§]	0.01	171 (50.7)

* No difference in VLs (measured locally and given by each center) according to the genotype.

[†] Data are reported as number (%). Including gray zone (S/CO, 0.9–1).

[‡] Percentage of positive samples significantly different ($p < 10^{-4}$) according to genotypes (gt1 to gt4).

[§] Percentage of positive samples significantly different ($p = 10^{-3}$) according to genotypes (gt1 to gt4).

NA = not applicable.

Clinical sensitivity of HCV antigen and combination assays in WP samples of different VL ranges per genotype

TABLE 3

HCV RNA range (IU/mL)	Genotype	Number	Geomean HCV-RNA (IU/mL) *	Geomean HCV antigen (fmol/L)	HCV antigen [†]	Monolisa [‡]	Murex [‡]
<10 ⁴	1	12	1,693	2.4	6 (50)	0	0
	2	1	10	0.1	0	0	0
	3	2	80	0.3	0	0	0
	4	2	653	3.7	1 (50)	0	0
	IND	10	54	0.2	0	0	0
	All	27	137	0.6	7 (26)	0	0
10 ⁴ -9.9 × 10 ⁴	1	29	26,850	70	29 (100)	2 (7)	2 (7)
	2	11	30,610	81	11 (100)	0	1 (9)
	3	18	30,324	85	18 (100)	0	0
	4	2	62,907	55	2 (100)	0	0
	IND	3	53,314	135	3 (100)	0	0
	All	63	38,409	82	63 (100)	2 (3)	3 (5)
10 ⁵ -9.9 × 10 ⁵	1	42	404,222	1,073	42 (100)	5 (12)	10 (24)
	2	8	354,032	1,098	8 (100)	4 (50)	3 (38)
	3	26	293,332	250	24 (92)	3 (12)	2 (8)
	4	8	262,122	1,078	8 (100)	2 (25)	3 (38)
	IND	5	368,295	1,999	5 (100)	0	2 (40)
	All	89	332,310	913	87 (98)	14 (16)	20 (23)
10 ⁶ -9.9 × 10 ⁶	1	43	3,059,299	20,123	43 (100)	27 (63) [‡]	38 (88) [‡]
	2	33	3,250,044	13,435	33 (100)	31 (94) [‡]	29 (88)
	3	31	3,199,623	4,980	30 (97)	9 (29) [‡]	19 (61) [‡]
	4	2	1,281,249	14,974	2 (100)	1 (50)	2 (100)
	5	2	4,183,300	79,741	2 (100)	2 (100)	2 (100)
	6	1	8,857,143	12,652	1 (100)	1 (100)	1 (100)
>10 ⁶	IND	1	3,700,000	443,798	1 (100)	1 (100)	1 (100)
	All	113	3,430,225	26,437	112 (99)	72 (64) [‡]	92 (81) [‡]
	1	26	17,574,231	215,629	26 (100)	24 (92)	24 (92)
	2	2	11,880,356	15,201	2 (100)	2 (100)	2 (100)

HCV RNA range (IU/mL)	Genotype	Number	Geomean HCV-RNA (IU/mL) *	Geomean HCV antigen (fmol/L)	HCV antigen [†]	Monolisa [‡]	Murex [‡]
	3	4	16,255,608	38,043	4 (100)	3 (75)	4 (100)
	All	32	15,028,063	49,959	32 (100)	29 (91)	30 (94)

* Based on VLs provided by centers. Three samples reported as containing 3.7×10^7 , 2.8×10^5 , and 2.2×10^5 IU/mL tested negative in HCV antigen.

[†] Data are reported as number (%).

[‡] $p < 10^{-3}$.

TABLE 4

Comparison of the 50% LOD (95%CI) in the combination assays and relative sensitivity factors (95% CI) estimated by using probit analysis on the distribution of VL in WP samples with and without HCV core antigen reactivity

Genotype	Number	50% LOD (95% CI) in IU/mL*			HCV antigen	Sensitivity of Murex relative to Monolisa (95%CI)
		Monolisa	Murex			
1	157	2.1 × 10 ⁶ (7.3 × 10 ⁵ –6.7 × 10 ⁶)	7.4 × 10 ⁵ (3.0 × 10 ⁵ –1.8 × 10 ⁶)	ND	2.73 (0.65–14.8)	
2	55	2.2 × 10 ⁵ (2.4 × 10 ⁴ –1.3 × 10 ⁶)	4.0 × 10 ⁵ (8.0 × 10 ⁴ –1.6 × 10 ⁶)	ND	0.65 (0.05–7.9)	
3	85	5.8 × 10 ⁶ (1.4 × 10 ⁶ –4.1 × 10 ⁷)	1.8 × 10 ⁶ (6.0 × 10 ⁵ –6.5 × 10 ⁶)	ND	2.62 (0.38–22.5)	
4	14	1.2 × 10 ⁶ (4.5 × 10 ⁴ –4.7 × 10 ⁷)	3.9 × 10 ⁵ (2.9 × 10 ⁴ –5.5 × 10 ⁶)	ND	2.73 (0.03–291)	
All [†]	334	1.6 × 10 ⁶ (9.8 × 10 ⁵ –2.8 × 10 ⁶)	8.3 × 10 ⁵ (4.9 × 10 ⁵ –1.4 × 10 ⁶)	942 [‡] (0.004–8,540)	1.97 (0.95–4.43)	
		50% LOD (95% CI) in copies/mL				
All [†]	334	4.5 × 10 ⁶ (2.7 × 10 ⁶ –7.7 × 10 ⁶)	2.3 × 10 ⁶ (1.3 × 10 ⁶ –3.8 × 10 ⁶)	2572 [‡] (1–23,314)	1.97 (0.95–4.43)	

* Based on VLs provided by centers.

† Including two gt5, one gt6, and 20 samples with undetected genotype.

‡ Detected in 324 WP samples.

ND = not done since samples cannot be detected with VLs near 50% HCV antigen LOD.

TABLE 5

Analytical sensitivity of HCV antigen and combination assays on genotype dilution series quantified in copies/mL (1 IU is equal to 2.73 copies) compared to HCV RNA detection by ID-NAT

Genotype	Ultrio HCV-RNA LODs (copies/mL)*		HCV antigen cutoff crossing point (copies/mL)†			Factors difference in analytical sensitivity compared to 50% LOD in Ultrio for		
	50% (CI)	95% (CI)	Monolisa	Murex	HCV antigen	Monolisa	Murex	HCV antigen
1a	1.2 (0.6–2.3)	9.9 (4.8–22.5)	438,000	429,000	1160	207,000	357,000	967
1b	3.9 (1.9–7.9)	33.5 (16.0–78.1)	10,000,000	2,480,000	3096	2,560,000	636,000	794
1b	2.6 (1.3–5.2)	22.4 (10.9–51.2)	2,270,000	1,300,000	2269	873,000	500,000	873
2a	7.7 (3.9–15.3)	65.8 (32.0–150)	3,640,000	42,000,000	7243	473,000	5,460,000	941
2a	2.9 (1.5–5.8)	25.2 (12.3–57.1)	5,870,000	19,200,000	5425	2,020,000	6,630,000	1871
2b	6.3 (3.2–12.5)	54.2 (26.5–123)	1,600,000	591,000	1168	2,540,000	93,900	185
2b	6.4 (3.2–12.7)	54.7 (26.6–124)	547,000	216,000	415	85,400	33,800	65
3a	1.9 (0.9–3.8)	16.3 (7.9–37.3)	4,650,000	2,460,000	4726	2,450,000	1,300,000	2487
3a	2.8 (1.4–5.5)	23.7 (11.5–54.4)	26,700,000	9,420,000	4985	9,530,000	33,600,000	1780
4	5.1 (2.5–10.4)	44.0 (21.2–102)	13,100,000	5,680,000	6244	2,570,000	1,110,000	1224
All‡	3.5 (ND)		3,300,000	3,400,000	2728	946,000	955,000	779

* Estimated by probit analysis.

† Estimated by regression analysis.

‡ Geometric mean values.

ND = not determined.