Evaluation of Performance of the RIBA Processor System for Automated Analysis of the Strip Immunoblot Assay for Detection of Antibodies to Hepatitis C Virus

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The performance of a new automated analyzer for the processing and interpretation of the RIBA Strip Immunoblot Assay (SIA), used in the diagnosis of hepatitis C virus (HCV) infection, was evaluated. Laboratory performance of the RIBA SIA was compared with that of two manually processed supplementary anti-HCV tests (RIBA HCV 3.0 SIA and INNO-LIA HCV Antibody III). Specificity of the automated processing of SIA was 100% for 90 selected anti-HCV-negative samples. On the other hand, 119 of 120 (99.2%) previously confirmed anti-HCV-positive samples were also positive when assayed on the automated processor. Results for all specimens except one (51 of 52) were concordant for manual and automated RIBA, while 15 of 68 sera tested with automated RIBA and the INNO-LIA assay showed different patterns of reactivity. Three HCV sensitivity panels and one seroconversion panel were also compared. The results show a high sensitivity for SIA NS3- and NS5-encoded antigens. Moreover, data obtained for the anti-HCV seroconversion panels and for samples with borderline or discordant anti-HCV enzyme-linked immunosorbent assay results suggest that bands with a relative intensity of >0.5 on the automated analyzer (theoretically negative) should be evaluated with care. Coefficients of variability ranged from 9 to 14.8% in an interassay reproducibility study. Overall, the performance of the automated analysis of SIA is comparable to that of the manual RIBA assay. The new automated processor for SIA bands proved to be sensitive and specific. Its use makes the optical scoring of bands unnecessary by indicating relative intensity values, which could be particularly useful in the follow-up care of anti-HCV-positive patients receiving antiviral therapy.

The discovery of a specific reactivity to the hepatitis C virus (HCV) in 1989 (5) was a landmark in the diagnosis of the so-called parenterally transmitted non-A, non-B hepatitis. Since then, continuous progress in research has led to the availability of three different generations of screening and supplementary assays for the detection of antibodies to HCV. Indirect diagnostic methods are particularly important, since no immunological technique for the direct detection of HCV antigens is presently available. Nucleic acid amplification procedures are the only means to show the occurrence of viral replication, but they are difficult to apply for routine diagnostic purposes due to economic and/or organizational constraints and standardization problems.

The addition of antigens encoded by the genomic regions of “core” and NS3 in second-generation screening and supplementary assays led to a clear increase in sensitivity and specificity (8). The use of NS3-derived antigens, c100 and c22 peptides, and NS5-encoded antigens in the third-generation assay further improves its performance (4, 9).

A rising demand for virological tests precipitated the development of automated analyzers comparable to those largely used in biochemistry laboratories. A new automated analyzer for the processing and interpretation of the RIBA Strip Immunoblot Assay (SIA) has recently been developed (the RIBA Processor System). Its use in the diagnosis of HCV infection was evaluated both with regard to its performance relative to that of two manually processed supplementary anti-HCV tests and to the reproducibility of its results.

MATERIALS AND METHODS

The RIBA Processor System is an analyzer which, after manually dispensing sera, allows the automated addition of reagents, shaking, incubation, disposal of waste material, strip drying, reading, and interpretation of results.

The key features of the RIBA Processor System include high-precision syringe pumps; separate systems for dispensing specimen diluent, conjugate, and substrate and for the removal of waste; temperature control of the reagent holders and the reaction chamber; a solid-state, high-resolution camera for strip imaging; and a microprocessor-based personal computer with validated software to control and document assay processing, interpret strip results, and print various reports. Each strip is stuck to a single holder, which makes the use of an “ad hoc” kit necessary, unlike manual processing of SIA.

Elaboration of the results consists of capturing strip images, locating bands, measuring the density of reflectance (DR) for each band, calculating relative intensity (RI), interpreting the results, and determining the number on each strip. Measurement of band intensity is based on mean band luminescence, which is calculated by averaging the pixels at the center of the specified band coordinates and the background luminescence on each side of the band. The DR of the band is obtained by calculating the logarithm of the ratio between mean band luminescence and mean background luminescence. DRs for each band are calculated by averaging the pixels at the center of the specified band coordinates and the background luminescence on each side of the band.

For scoring band intensity, two internal control bands (immunoglobulin G [IgG] I and IgG II), corresponding to a low-positive and a high-positive control, are included in each strip. The R.I. of each band is quantitated by comparing the DR of each band with the DRs of the two IgG internal controls on each strip as follows: (i) Bands with DRs less than or equal to the low IgG I control are scaled such that the low IgG I R.I. of 1. (ii) Bands with DRs greater than the low IgG I control band are scaled to a straight line, with the low IgG I control band having an R.I. of 1 and the high IgG control band having an R.I. of 3.

Scaled values for each band are printed under the heading R.I. in each sample report. The scoring of each band is determined by the ranges of R.I. Scores are expressed semi-quantitatively as follows: negative, R.I. of 0.00 to 0.10; +/−, R.I. of 0.11 to 0.95; +, R.I. of 0.96 to 1.05; 2+, R.I. of 1.06 to 2.85; 3+, R.I. of 2.86 to 3.15; and 4+, R.I. of >3.15.

The overall result is interpreted as follows: positive, at least two bands with a
score of $\geq 1+; \text{indeterminate, only one band with a score of } \geq 1+; \text{negative, no bands with a score of } \geq 1+; \text{invalid, manual} \text{reading, which means that reading needs to be performed optically.}$

Two SIAs were used as references for this study: the RIBA HCV 3.0 SIA (manual) (Chiron Corporation, Emeryville, Calif.) and the INNO-LIA HCV Antibody (Ab) III (Innogenetics N.V., Zwijndrecht, Belgium).

The RIBA HCV 3.0 SIA is designed to detect specific antibodies to HCV in human serum or plasma. It is based on two recombinant antigens (c33c, produced in Saccharomyces cerevisiae, and NS5, obtained from Escherichia coli) and three synthetic peptides (c100p, 5-1-1p, and c22p). Human superoxide dismutase (hSOD) is added as a control band, since recombinant antigens are produced as fusion proteins with such an enzyme.

In the first stage of the test, the diluted sample is incubated with the strip. Anti-HCV, if present, binds to the corresponding band on the strip. After a washing step, peroxidase-labeled goat anti-human IgG conjugate is added. This will bind to antigen-antibody complexes. Another washing will remove unbound materials. The third stage consists of the addition of a detection system composed of hydrogen peroxide and 4-chloro-1-naphthol. If bound conjugate is present, an insoluble blue-black-colored product will be produced at each specific HCV antigen, peptide, and control band as a result of the enzymatic reaction.

After color develops, a final washing step stops the reaction and removes the staining materials. The fourth stage consists of the addition of a color-stopping solution. The detection system is composed of hydrogen peroxide and 4-chloro-1-naphthol. If bound conjugate is present, an insoluble blue-black-colored product will be produced at each specific HCV antigen, peptide, and control band as a result of the enzymatic reaction.

The INNO-LIA HCV Ab III is a line immunoassay for the detection of antibodies to HCV in human serum or plasma. Synthetic peptides corresponding to four genomic regions of HCV—core (four epitopes), E2, NS1, and NS4 (three epitopes), and NS5 (three epitopes)—together with a recombinant NS3 protein produced in E. coli are bound to a nylon strip with plastic backing.

A total of six HCV lines are present on each strip. Lines 1 and 2 consist of core epitopes, line 3 contains NS1 epitopes, line 4 contains NS3 epitopes, line 5 contains NS4 epitopes, and line 6 contains NS5 epitopes. In addition, four internal control bands on each strip: one antistreptavidin control line, one strongly positive control line (anti-human IgG), one moderately positive control line (all human IgG), and one weakly positive control line (all human IgG).

In brief, an LIA test strip is placed with the membrane side facing up onto a test trough with tweezers. Troughs are placed into a tray. Ten microliters of each specimen and one milliliter of sample diluent are dispensed into troughs. One milliliter each of prediluted positive and negative controls is also assayed in each test run. Samples are incubated overnight (16 to 24 h) at room temperature (15 to 30°C) by placing the tray on an orbital mixer (at 160 rpm).

Conjugate solution is prepared 10 min prior to the end of the incubation by diluting 1:100 the concentrate conjugate (goat anti-human IgG, heavy plus light chains, labeled with alkaline phosphatase in Tris buffer containing protein stabilizers and 0.1% sodium azide as a preservative).

After each test strip is washed three times with a solution containing phosphate buffer, sodium chloride, and 0.1% sodium azide as a preservative, 1 ml of prepared conjugate solution is added to each test trough and incubated for 30 min at room temperature (15 to 30°C) on the orbital mixer.

After each test strip is washed twice with the wash solution and once with a substrate buffer (Tris buffer containing sodium chloride and 0.1% sodium azide as a preservative), 1 ml of diluted (1:100) BCIP substrate (bromochloro-indolylphosphate in dimethyl formamide) is added to each test trough and incubated for 30 min at room temperature (15 to 30°C) on the orbital mixer.

To read the test, the strips are removed from the test trough and placed on absorbent paper with tweezers. Results are interpreted as such:

(i) The intensity of the color of each antigen line is compared with that of the strongly positive control line (anti-human IgG), heavy plus light chains, labeled with alkaline phosphatase in Tris buffer containing protein stabilizers and 0.1% sodium azide as a preservative.

(ii) The intensity of the antigen line reaction as negative (lower than level 1), moderately positive (level 1+), moderately positive (level 2+), strongly positive (level 3+).

The intensity of the color of each antigen line is compared with that of the weakly positive control line (anti-human IgG), heavy plus light chains, labeled with alkaline phosphatase in Tris buffer containing protein stabilizers and 0.1% sodium azide as a preservative.

(iii) A sample is considered positive when at least two HCV bands show 1+ or greater reactivity or when the hSOD band is present alone and the bands have 1+ intensity evaluation: absent, negative; $+, lower than the level 1$ IgG control band; 1+, equal to level 1 IgG control band; 2+, between levels 1 and 2 IgG control bands; 3+, equal to level 2 IgG control band; 4+, greater than level 2 IgG control band.

Results are interpreted as follows. (i) A sample is considered negative when no bands have 1+ or greater reactivity or when the hSOD band is present alone and the bands have 1+ or greater reactivity. (ii) A sample is considered positive when at least two HCV bands show 1+ or greater reactivity or when the hSOD band is present with one or more HCV bands having 1+ or greater reactivity.

The INNO-LIA HCV Ab III is a line immunoassay for the detection of antibodies to HCV in human serum or plasma. Synthetic peptides corresponding to four genomic regions of HCV—core (four epitopes), E2, NS1 and NS4 (three epitopes), and NS5 (three epitopes)—together with a recombinant NS3 protein produced in E. coli are bound to a nylon strip with plastic backing.

FIG. 1. R.I. values of automated RIBA SIA antigens in 90 anti-HCV-negative samples and 120 anti-HCV-positive samples.
TABLE 1. Interpretation of the results for the automated RIBA SIA and the INNO-LIA for 65 samples of the anti-HCV mixed-titer panels PHV 201 and PHV 202 and the anti-HCV low-titer panel PHV 102 (BBI)

<table>
<thead>
<tr>
<th>Type of result for the automated RIBA SIA</th>
<th>Type of result for the INNO-LIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. positive</td>
<td>No. positive</td>
</tr>
<tr>
<td></td>
<td>No. indeterminate</td>
</tr>
<tr>
<td></td>
<td>No. negative</td>
</tr>
<tr>
<td>45</td>
<td>3</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
</tr>
</tbody>
</table>

than level 1 (but lower than level 3), 3 (equivalent to level 3), or 4 (higher than level 3).

Results are interpreted as follows. (i) A sample is considered nonreactive for HCV antibodies if all antigen lines have a negative reactivity rating. (ii) A sample is considered reactive to HCV if one HCV antigen line has a reactivity rating of ≥2+ or if at least two HCV antigen lines have a reactivity rating of ≥1+.(iii) A sample is considered indeterminate if a reactivity rating of 1+ or +/+ is seen on one antigen line with or without other antigen lines presenting a reactivity rating of ≥1.

Several groups of sera were selected for the study of specificity and sensitivity.

Ninety samples negative for anti-HCV by two commercially available screening tests (Ortho HCV 3.0 enzyme-linked immunosorbent assay [ELISA]; Ortho Diagnostic Systems, Raritan, N.J., and Innogenetics N.V.) were collected from 30 healthy subjects from the general population, 30 blood donors, and 30 individuals with potentially interfering factors (hemodialysis patients, subjects with autoimmune diseases or acute viral infections, etc.) for the study of clinical specificity.

One hundred twenty serum samples positive for anti-HCV with different antibody patterns in supplementary assays (Chiron RIBA HCV 3.0 SIA; Chiron Corporation [assay A] and Innogenetics N.V. [assay B]) were selected for the study of clinical sensitivity. Of the 52 samples positive for assay A (manually processed), 22 were reactive for all four bands; 20 were reactive for c100, c33, and c22; and 10 were reactive for c33 and c22. Of the 68 sera positive for assay B, 20 were reactive for all bands; 20 were reactive for the core, NS3, and NS4; 18 were reactive for the core and for at least two bands other than NS4; and 10 were reactive for the core and for NS3.

Four commercially available panels were also used in order to evaluate the sensitivity of the automated test: two anti-HCV mixed-titer performance panels (PHV 201 and PHV 202; Boston Biomedica Inc.), one anti-HCV low-titer performance panel (PHV 102, Boston Biomedica Inc.), and one seroconversion panel (Hepatitis C Virus Seroconverter 30994B; Serologicals Inc., Clarkston, Ga.).

A series of six samples from a case of seroconversion to HCV in a hemodialysis patient was also used in order to evaluate the comparative sensitivities of assays B and A performed with the RIBA Processor System.

Sixty-five samples with discordant results for two anti-HCV screening assays were tested in parallel by assays B and A performed with the automated system.

With regard to the reproducibility of results, one serum sample reactive for all four bands by RIBA HCV 3.0 SIA, one sample reactive for all bands but NS5, and one sample reactive only for the c33 and c22 bands were selected for the study of intra-assay (eight replicates per sample) and interassay precision (15 runs). Mean R.I. values of each band and relative coefficients of variability (CV) were calculated.

RESULTS

Figure 1 reports the R.I.s of the four RIBA antigens for 90 selected anti-HCV-negative and 120 selected anti-HCV-positive sera.

The specificity of the RIBA HCV 3.0 SIA performed on the RIBA Processor System was 100%, since the analyzer was able to interpret properly all 90 selected anti-HCV-negative samples. R.I.s observed in most cases (35% of 360, or 98.3%) ranged between 0 and 0.2, while three samples had an R.I. of 0.3. Two of the remaining three specimens had an R.I. of 0.4, and one had an R.I. (corresponding to the c33 band) of 0.7.

With regard to sensitivity, all but 1 of the 120 previously confirmed anti-HCV-positive samples (52 by assay A and 68 by assay B) were also positive for assay A performed with the automated system. The only discordant specimen was characterized by the result c22:1+ and c33:1+ for the manual RIBA assay, which for the automated system showed the two bands with R.I.s of 0.5 and 0.8, respectively. The remaining 51 samples tested by both RIBA SIA procedures (manual and automated) gave concordant results.

Of the 68 sera tested with assays B and A performed on the automated processor, 53 had comparable antibody patterns; the remaining 15 specimens, though positive by both methods, showed different patterns of reactivity. Specifically, 11 samples were NS5 positive by the automated RIBA assay but negative by the INNO-LIA assay, and 4 sera without the NS3 band by the INNO-LIA assay were c33 positive by the automated RIBA assay. Thus, for the c22 and the c33 bands, 88.3 and 93.3% of the R.I.s were ≥3, respectively. R.I.s of ≥3 were observed for 79.3% of the c100 bands and 80.3% of the NS5 bands.

With regard to the three HCV sensitivity panels, results were 100% concordant for the manual and the automated RIBA SIA, while complete concordance of results for the INNO-LIA and the automated RIBA SIA was obtained only for panel PHV 202. On the other hand, these two supplementary assays gave different results for two and three serum samples for panels PHV 201 and PHV 102, respectively. Tables 1 and 2 show the interpretation of the results for the INNO-LIA and the automated RIBA SIA for the three panels and the band scores of the discordant specimens.

The results of the two supplementary tests on the anti-HCV

TABLE 2. Results of screening tests and band scores for the automated RIBA SIA and the INNO-LIA for five discordant samples for panels PHV 201 and PHV 102 (BBI)

<table>
<thead>
<tr>
<th>Panel and no. of samples</th>
<th>ELISA optical density</th>
<th>Automated RIBA SIA score (R.I.) for band:</th>
<th>INNO-LIA result* (band)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ortho</td>
<td>Innogenetics</td>
<td>c100</td>
</tr>
<tr>
<td>PHV 201</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>0.3</td>
<td>0.8</td>
<td>2+</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0.1</td>
<td>+/+</td>
</tr>
<tr>
<td>PHV 102</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>2.3</td>
<td>2.0</td>
<td>—</td>
</tr>
<tr>
<td>13</td>
<td>5.9</td>
<td>3.5</td>
<td>+/+</td>
</tr>
<tr>
<td>15</td>
<td>5.1</td>
<td>0.5</td>
<td>+/+</td>
</tr>
</tbody>
</table>

* For INNO-LIA results, the band is indicated in parentheses.
seroconversion panel 30994B are reported in Fig. 2. The first anti-HCV-positive sample (screening test) showed a reactivity for the c33 band in the automated RIBA SIA, with an R.I. of 3.1. The INNO-LIA assay was negative.

The next sample, collected 3 days later, had an automated RIBA assay pattern of 3.1 for c33 and 0.9 for c22. The INNO-LIA assay C1 band was reactive (1+). All the subsequent sera were positive in the RIBA assay run on the RIBA Processor System and the two reference tests, with at least two positive bands.

Figure 3 shows the profile of anti-HCV reactivity in a hemodialysis patient who seroconverted 1 year after entering follow-up care. The two ELISA-reactive samples were c33 and c22 positive in the automated RIBA assay and core positive in the INNO-LIA assay.

Table 3 reports the patterns of reactivity in the automated RIBA SIA, the manual RIBA SIA, and the INNO-LIA for 65 samples with borderline or discordant results for two ELISA screening tests.

Intra-assay reproducibility was tested with a sample reactive to all four RIBA bands (eight replicates). The mean R.I.s (M) and CV were as follows: for c100, M = 4.19 and CV = 12.9%; for c33, M = 5.95 and CV = 6.7%; for c22, M = 3.74 and CV = 12.2%; and for NS5, M = 1.22 and CV = 29.2%. Two samples were used to study interassay reproducibility (15 runs). One of them was reactive for the three bands c100, c33, and c22, and the other was positive only for c22 and c33. Mean R.I.s and CV were as follows: for c100, M = 1.25 and CV = 14.8%; for c33, M = 4.18 and CV = 10.3%; and for c22, M = 6.25 and CV = 9.0% for the first sample and for c33, M = 1.54 and CV = 9.7%; and for c22, M = 1.1 and CV = 11.7% for the second sample.

**DISCUSSION**

The new automated RIBA HCV SIA performed on the RIBA Processor System for the confirmation of anti-HCV-reactive results showed very good specificity and sensitivity, as demonstrated by the results of anti-HCV-negative samples and anti-HCV-positive sera with different RIBA SIA and INNO-LIA patterns. The R.I. of antigens in anti-HCV-negative samples was <0.2 in less than 2% of serum samples, with only 1 of 360 with an R.I. between 0.5 and <0.95.

Furthermore, only 6% of all reactive bands in 120 anti-HCV-positive specimens showed R.I.s between 1 and 2. This means that, in general, R.I.s for anti-HCV-negative and -positive sera differ widely and that the misclassification of bands should be uncommon.

However, it must be noted that, according to the manufacturer’s instructions, R.I.s ranging between 0.11 and 0.95 (+/-) should be interpreted as negative. Nevertheless, the above-mentioned results for the distribution of R.I.s and data obtained for the anti-HCV seroconversion panel and for samples with borderline or discordant ELISA results suggest that bands with R.I.s of >0.5 should be interpreted with care.

As a matter of fact, the finding of an R.I. of 0.9 for c22 in sample no. 6 for the seroconversion panel (the R.I. for c22 was 3.2 for the following sample) and of several R.I.s in the range of 0.6 to 0.9 for ELISA borderline and discordant sera indicates that further testing is needed (follow-up tests and/or
HCV-RNA detection) in order to elucidate the meaning of such low-level reactivities.

Moreover, caution in the interpretation of R.I.s of >0.5 is also suggested by the only discordant result between the manual and the automated RIBA for anti-HCV-positive samples, for which recommended classification criteria would lead to a false-negative result with the RIBA Processor System.

It must not be forgotten, however, that the interpretation of the results between the automated and the manual RIBA SIA and between the automated RIBA SIA and the INNO-LIA was 99.1% concordant for our series of anti-HCV-positive specimens.

With regard to reproducibility of results, CV were generally influenced by the mean R.I.s of the bands. Both intra-assay and interassay precision tests gave acceptable results, taking into account that the automation of strip reading gives objective results and that inaccurate interpretations of band intensity due to individual variations in optical reading can hence be avoided.

However, it must be noted that the intra-assay CV for the NS5 band was more than double the interassay CV for the c100 and c22 bands with similar R.I.s. Since this variability was not due to a single outlier among the NS5 bands, it seems reasonable to conclude that a misclassification of low-reactive NS5 bands might have occurred in some cases.

In spite of this relatively scarce reliability of NS5 band low reactivities, several studies have shown that third-generation anti-HCV supplementary assays are more sensitive and specific than second-generation ones (2, 3, 11–13). As a matter of fact, earlier anti-HCV detection during seroconversion and resolution of second-generation indeterminate results has generally been attributed more to a better orientation of the conformation of NS3-derived epitopes than to the introduction of NS5-encoded antigens in third-generation tests (4, 10).

![Figure 3](http://jcm.asm.org/) Profile of anti-HCV reactivity in a seroconverter hemodialysis patient.

| TABLE 3. Pattern of reactivity (single or prevalent band) in the automated RIBA SIA, the manual RIBA SIA, and the INNO-LIA for 65 samples with borderline or discordant results for two ELISA screening tests |
|---|---|---|---|---|---|---|---|---|---|---|
| Band | Automated | Manual | INNO-LIA (1+) |
| | R.I. >1.0 | R.I. 0.6-0.7 | >1+ | +/- |
| c100/NS4 | 4 | 3 | 6 | 3 | 1 |
| c33/NS3 | 5 | 1 | 8 | 3 | 7 |
| c22/core | 19 | 1 | 19 | 6 | 15 |
| NS5 | 6 | 3 | 12 | 5 | 2 |

* Negative results were found as follows: n = 24 for automated RIBA SIA, n = 20 for manual RIBA SIA, and n = 23 for INNO-LIA.
* Three samples gave the following results: 1.0 (c100), n = 1; 0.9 (c100), n = 1; and 0.9 (c22), n = 1.
* Three samples gave the following results: +/- (NS4), n = 2; +/- (NS4), n = 1; and +/- (NS5), n = 1.
* Four samples gave the following results: 0.9 (c33), n = 1; 0.8 (c33), n = 1; 0.6 (c33), n = 1; and 0.8 (c100), n = 1.
* Seven samples gave the following results: +/- (NS4), n = 1; 2+ (NS4), n = 1; +/- (NS3 and NS4), n = 2; +/- (NS3 and NS5), n = 1; +/- (NS3, NS4, and NS5), n = 1; and +/- (NS3, NS4, and NS5), n = 1.
* One sample gave the following result: 1+ (c22), n = 1.
* One sample gave the following result: +/- (NS1), n = 1.
It has also been demonstrated that isolated NS5 reactivities with Chiron RIBA HCV 3.0 SIA were not confirmed by the use of synthetic peptides and HCV-RNA detection, while reactivities with the same recombinant NS5 in proven HCV carriers were confirmed by synthetic peptides 85% of the time (7).

Therefore, specificity issues for the NS5 band are suggested by several studies, including ours, although we did not test sera with isolated NS5 reactivity.

In a previous paper, we showed that NS3-encoded antigens used in Ortho and Chiron kits are more sensitive than those used in other immunoassays (1). These data are confirmed by our experience with the automated RIBA SIA. The 15 anti-HCV confirmed-positive samples with different patterns of reactivity in the automated RIBA SIA and the INNO-LIA test had an additional c33 band with the former test in 4 cases and an additional clearly reactive recombinant NS5 band in 11 cases. Furthermore, NS3-encoded antigens were detectable earlier with the automated RIBA SIA than with the INNO-LIA test, both in the seroconversion panel and in the seroconverter hemodialysis patient. Also, four of five discordant samples of anti-HCV mixed-titer panels PHV 102 and PHV 201 were clearly reactive for c33 in the automated RIBA SIA and NS3 negative in the INNO-LIA.

In conclusion, our study shows that the newly available RIBA Processor System for the automated processing and reading of RIBA HCV SIA strips is sensitive and specific and able to supply results which are comparable to those obtained by the manual RIBA SIA procedure. The sensitivity of NS3- and NS5-encoded antigens seems to be higher for the automated RIBA SIA than for the INNO-LIA test. Moreover, the system makes the semiquantitative scoring of bands unnecessary by indicating R.I.s, which could be useful in the follow-up care of patients, particularly those receiving antiviral therapy (6).

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