ISVHLD 2012
Shanghai
22 – 25 June
SA00329

# Sensitivity and specificity evaluation of a new automated immunoassay test, VIDAS® Anti-HCV, for the qualitative detection of antibodies anti-HCV in human samples

B. Seignères[1], N. Ripoll[1], L. Mercier[1], F. Forge[1], C. Prétis[1], B. Riou[1], JM Dugua[1], AM Avellon[2], JM Echevarria[2], M. Hausmann[1].
[1]bioMérieux, Marcy l'Etoile, France. [2]National Centre of Microbiology, Majadahonda, Spain

## INTRODUCTION AND PURPOSE

**In diagnosis of hepatitis C infection**, updated assays for anti-HCV detection perform very high on positive samples from chronic carriers infected with any viral genotype. Differences in performance can be recorded on samples from patients undergoing the early moments of the anti-HCV response after an acute primary infection or having very low anti-HCV levels (i.e. patients with anti-HCV in serum after a past resolved HCV infection). In both cases, differences between tests originate mostly from detection ability for antibodies to NS3 and at a second level to core and NS4.

**The VIDAS® automated system (bioMérieux) is suitable for routine, emergency or complementary testing, notably in infectious diseases diagnosis.**

**To complete its existing HIV-HAV-HBV panel, VIDAS® Anti-HCV has been developped. Following the assessment of its specificity performance, we performed a sensitivity evaluation of this prototype by comparison with CE-marked tests, notably on well-characterized positive clinical samples.**

## METHODS

VIDAS® Anti-HCV is a third-generation test using antigens corresponding to HCV core, NS3 and NS4 for the qualitative detection of anti-HCV IgG antibodies. The CE-marked tests used in this study are Architect (Abbott) and Vitros (Ortho-Clinical diagnostics). Two immunoblots, RIBA (Ortho-Clinical diagnostics) and LIA (Innogenetics), were used to characterize antibody nature of samples.

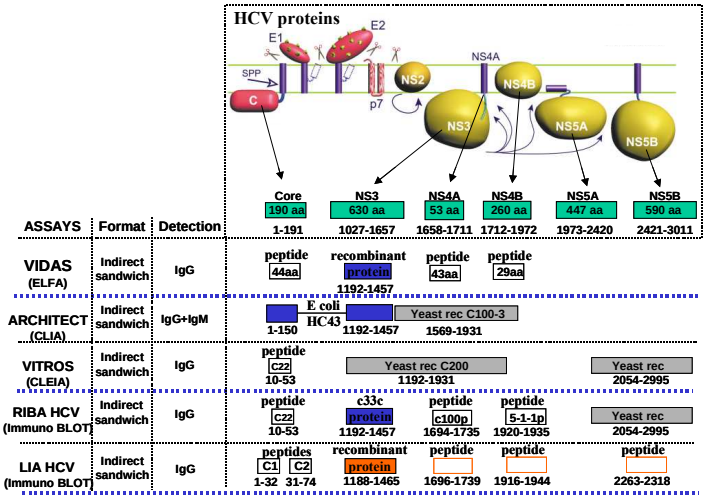



*FIGURE 1: Formats and representative HCV proteins used in the different Anti-HCV assays*

**The specificity study was performed on:**

➢ 4766 French blood donor samples (drawn from different plants in France), HCV negatives after being tested with molecular biology assay. The false positive sera were characterized, notably those linked to the NS3 recombinant protein used in VIDAS® Anti-HCV by a 2D-PAGE analysis: on Figure 2, see the 2 different profiles of interferences: detection of molecules fixed directly on the NS3 sequence (A) or on co-purified proteins with NS3 (B).

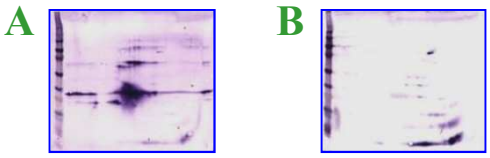



*FIGURE 2: 2D-PAGE analysis*

**The sensitivity study was performed on a collection of samples:**

➢ 402 positive samples collected from patients at different stages of HCV disease and covering the 6 genotypes (1 to 6) (Biomnis Laboratory).

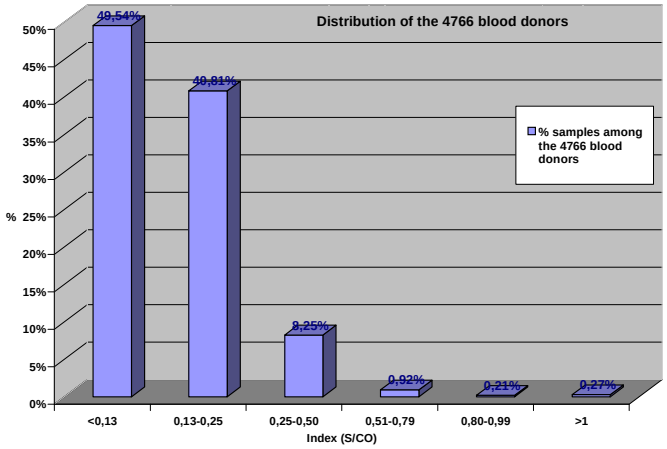
➢97 anti-HCV well-characterized sera, notably on their antibody nature (anti-core, anti-NS3) from chronic or resolved past infection patients.

All of them have been tested in comparison with **Architect® or VITROS**.

## RESULTS

### 1. SPECIFICITY:

Performed on 4766 negative samples, this analysis demonstrated a high specificity performance of the **VIDAS® Anti-HCV** assay (≥99.7%) with an excellent discrimination: >98 % of negatives have index under 0.5




**Analysis of false positive** shows that most of interferences are linked to NS3 raw material (>84.5%) which can be due to interferences recognizing directly either NS3 seq or contaminant co-purified proteins.

| Number of false positives on 4766 donors | PTBs | NAME | VALUE | Test Values PTB3 | PTB4 | PTB5 | PTB6 | interference identified | |
|---|---|---|---|---|---|---|---|---|---|
| 13 (100% with S/CO<5) | PTB3 | EFS D676 | 1,08 | 1,08 | 0,65 | 0,82 | 0,37 | NS3 (ND) | 11/13 are linked to NS3 |
| | PTB3 | EFS B0105 | 1,09 | 1,09 | 0,57 | 0,58 | empty | NS3 (ND) | |
| | PTB3 | EFS B0267 | 1,15 | 1,15 | 0,66 | empty | | NS3 (ND) | |
| | PTB3 | S16-M005 | 1,18 | 1,18 | 0,73 | 0,56 | 0,32 | NS3 E coli | |
| | PTB3 | S11-M018 | 1,43 | 1,43 | 0,59 | 1,36 | 0,87 | NS3 seq | |
| | PTB3 | EFS B0114 | 1,88 | 1,88 | 1,04 | 1,11 | 0,71 | NS3 (ND) | |
| | PTB3 | EFS B0274 | 2,40 | 2,4 | 2,36 | 1,59 | 2,47 | NS3 seq | |
| | PTB3 | S11-F018 | 2,74 | 2,74 | 1,65 | 0,98 | 0,39 | NS3 E coli | |
| | PTB5 | ECH1017 | 1,01 | 1,37 | 1,14 | 1,01 | 1,18 | NS3 (ND) | |
| | PTB5 | EFS0311 | 1,17 | 1,34 | 1,44 | 1,17 | 1,21 | NS3 seq | |
| | PTB5 | EFS0654 | 1,33 | 1,97 | 1,95 | 1,33 | 0,95 | Format | |
| | PTB5 | EFS0291 | 2,48 | 4,62 | 4,66 | 2,48 | 4,76 | NS3 seq | |
| | PTB5 | EFS0308 | 3,13 | 3,13 | 2,45 | 3,13 | 4,67 | N27T | |

### 2. SENSITIVITY:

VIDAS® Anti-HCV sensitivity demonstrated an efficient antibody detection for the six HCV genotypes whatever the antibody titer and response,

| | STUDY | POSITIVE SERA TOTAL | LOW* | HIGH |
|---|---|---|---|---|
| VIDAS Anti-HCV | Positive samples | 402 (100%) | 52 (12,94%) | 350 (87,06%) |
| | % sensitivity | 100% | 100% | 100% |
| | % samples with s/CO>5 | 92% | 35% | 100% |

* Low positive as CDC guidelines, i.e.<5 for Architect

**with notably a performing anti-NS3 detection evidenced on the panel of the 97 anti-HCV well-characterized sera**

| SAMPLES | PCR | Immunoassays VIDAS | VITROS | BLOTS RIBA | LIA |
|---|---|---|---|---|---|
| anti-NS3 alone | - | 25/30 | 17/29 | 27/30 | 24/30 |
| anti-CORE alone | - | 23/29 | 24/29 | 23/28 | 23/28 |
| Weak positive (only two antibodies) | - | 14*/16 | 16/16 | 5/16 | 16/16 |
| Weak positive (only two antibodies) | + | 3/3 | 3/3 | 2/3 | 2/2 |
| Chronic infections | + | 19/19 | 18/18 | ND | ND |
| Total | 75 RNA - | 84/97 | 78/95 | 57/77 | 65/76 |
| | 22 RNA + | 86,6% | 82,1% | 74,0% | 85,5% |

*** 2 samples have index within the grey zone (0,8-0,99)**

## CONCLUSION

**Evaluation of VIDAS® Anti-HCV sensitivity and specificity showed that this new prototype VIDAS assay is as performant as other CE-marked tests and suitable for the qualitative detection of antibodies anti-HCV in human samples.**