

Performance study of the automated immunoassay test anti-hepatitis C virus VIDAS® for the qualitative detection of antibodies anti-hepatitis C virus

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Summary

Background and aims: Hepatitis C virus (HCV) represents a major worldwide public health problem requiring global action for the diagnosis, treatment and prevention of this infection. HCV is the leading cause of chronic liver disease, including chronic hepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma and it is responsible for about 350,000 deaths yearly. Anti-HCV assays remain the first choice for screening HCV infection in most clinical laboratories. The anti-HCV VIDAS® (bioMérieux, Marcy L'Etoile, France) test has been recently launched and we aimed to evaluate its performance compared with other widely used methods.

Materials and methods: Routine anti-HCV screening of clinical sam-

ples was carried out on the ARCHITECT® i2000_{SR} platform (Abbot Laboratories, Abbott Park, IL, USA); out of 8876 samples tested from October 2012 to May 2013, 70 sera with low positive anti-HCV results ($1 \leq S/CO < 8$) were collected. These samples were tested for the presence of anti-HCV antibodies using anti-HCV VIDAS® and INNO-LIA™ HCV score assays (Innogenetics NV, Ghent, Belgium).

Results and conclusions: Positive anti-HCV results were obtained in 61.4% and 41.4% of sera tested with VIDAS® and INNO-LIA™, respectively. Concordance between methods was 63.2% for ARCHITECT® and VIDAS®, 42.6% for ARCHITECT® and INNO-LIA™, and 79.4% for VIDAS® and INNO-LIA™. Anti-HCV VIDAS® demonstrated superior specificity compared to the anti-HCV test ARCHITECT®; therefore, this assay has been introduced in our routine analysis as a second level screening test to select samples to be subjected to confirmatory anti-HCV immunoblot.

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Key words: Hepatitis C virus; anti-hepatitis C virus antibodies; hepatitis C virus infection.

Acknowledgements: the authors would like to thank Dr. Claudio Galli for critically reading the manuscript, helpful discussions and suggestions.

Contributions: SS, data collection, analysis and interpretation of data, study concept and design, drafting of the manuscript; FL, analysis and interpretation of data, critical revision of the manuscript; IM, study concept and design, critical revision of the manuscript, final approval of the revised version.

Conflict of interest: the authors declare no potential conflict of interest.

Received for publication: 29 October 2014.

Revision received: 26 June 2015.

Accepted for publication: 25 November 2015.

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Microbiologia Medica 2016; 31:4808
doi:10.4081/mm.2016.4808

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Introduction

The hepatitis C virus (HCV) is a RNA virus belonging to the flaviviridae family. It has been first recognised as a cause of transfusion-associated acute and chronic hepatitis (formerly known as non-A, non-B hepatitis) in 1989 (5). At present, it is estimated that about 170 million people, roughly 3% of the world population, are chronically infected with HCV leading to about 350.000 deaths yearly, related to complications such as cirrhosis and liver cancer (14,16). Italy is the European country with the largest number of people chronically infected by HCV (10), which is the leading cause of hepatic disease in this country. For these reasons, HCV represents a major worldwide public health problem requiring global action for the diagnosis, treatment and prevention of this infection (13).

HCV infection is often asymptomatic, thus diagnosis relies heavily on clinical laboratory assays, including the serological immunoassays for antibodies to HCV (anti-HCV) or core antigen, and nucleic acid testing (NAT) for HCV RNA (12,15). Anti-HCV assays were widely used in the screening and diagnosis of HCV infection, leading to a marked decline in the incidence of transfusion-associated hepatitis in the early 1990s (9). As *indirect* tests, these assays detect infection up to 50 days after obtaining positive results from HCV RNA or core antigen tests (seroconversion window) and might report false-negative results in severely immunosuppressed populations (2,7). However, anti-HCV assays offer the great advantages of technical simplicity, short turnaround time, high-speed throughput, and full automation. Therefore these tests remain the preferred method for screening HCV infection in most clinical laboratories.

Over the last few years, several anti-HCV assays have been developed;

only recently, a new bioMérieux diagnostic test, anti-HCV VIDAS® (bioMérieux, Marcy L'Etoile, France), has been marketed for the detection of antibodies against HCV in human serum or plasma. The assay is based on the enzyme linked fluorescent assay technique and uses synthetic core, NS3 and NS4 polypeptides as the detection antigens.

In this study, we comprehensively evaluated the performance of the anti-HCV VIDAS® test for the qualitative detection of anti-HCV antibodies. To this aim, samples with low positive signal to cut-off ratios were collected from daily routine anti-HCV screening carried out on the automated platform ARCHITECT® i2000_{SR} (Abbot Diagnostic, Abbott Park, IL, USA); sera were analysed with the anti-HCV VIDAS®, and immunoblotting method was used as a gold standard to assess the presence of anti-HCV antibodies (3,4,11,15). Additionally, we propose a renewed algorithm for the confirmation of HCV infection status.

Materials and Methods

Anti-HCV screening of serum samples was carried out using the anti-HCV test on the automated platform ARCHITECT® i2000_{SR}, according to the manufacturer's instructions. The anti-HCV test (Abbott) is a third-generation chemiluminescence microparticle immunoassay (CMIA) for the qualitative detection of immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies to hepatitis C virus (anti-HCV) in human serum or plasma. The results were expressed as signal to cutoff (S/CO) ratios; specimens with S/CO values <1.00 (*not reactive*) are considered negative, while specimens with S/CO values ≥1.00 (*reactive*) are considered positive.

Selected samples were assayed with the anti-HCV test VIDAS®, a third-generation test that employs three recombinant HCV antigens (core, NS3 and NS4) for the qualitative detection of anti-HCV antibodies. The assay combines a two-step enzyme immunoassay sandwich method with a final fluorescent detection (ELFA). All of the assay steps are performed automatically by the instrument and the results expressed as negative if the index value is <1.00 while positive if ≥1.00.

Anti-HCV results were confirmed by immunoblot analysis using the INNO-LIA™ HCV Score test (Innogenetics NV, Ghent, Belgium). This is a third generation line immunoassay (LIA) which incorporates HCV antigens derived from the core region, the E2 hypervariable region (HVR), the NS3 helicase region, the NS4A, NS4B, and NSB5A regions. The antigens were coated as 6 discrete lines on a nylon strip that also contains four control lines (streptavidin, 3+ positive control anti-human IgG, 1+ positive control human IgG, and the ± cut-off line human IgG). A sample is negative for HCV antibodies if all HCV antigen lines have a negative reactivity rating or if only one HCV antigen has a reactivity of ±, except when the reactivity is observed for NS3. A sample is positive for HCV antibodies if at least two HCV antigen lines have a reactivity of ± minimum or higher. A sample is considered as indeterminate if one HCV antigen line has a reactivity rating of 1+ or higher or if the NS3 line reacts with a reactivity of ± or higher and all other antigen line are negative.

Specimen preparation and testing were carried out according to instructions given in the package inserts of the respective suppliers.

Results and Discussion

From October 2012 to May 2013, a total of 8876 consecutive sera were submitted to the Microbiology Laboratory of San Giuseppe-AUSL11 Hospital (Empoli, FI, Italy), for routine anti-HCV screening. Of the samples tested on the ARCHITECT® platform, 8603 yielded negative anti-HCV results. Between positive sera, 70 were selected using as inclusion criteria a S/CO value ranging from 1 to 8. These samples with first time detected *reactive* anti-HCV were analysed with the anti-HCV test VIDAS®; positive results were obtained in 61.4% of sera (Table 1).

Immunoblot analysis of the samples revealed that 29 sera were positive (41.4%) while 31 negative for anti-HCV antibodies. Notably, 10 samples (14.3%) tested with the INNO-LIA™ HCV Score assay gave an indeterminate result (Table 1). Immunoblot test has very high specificity and can determine whether a positive ELISA test represents true HCV infection. However, several authors highlight the inefficiency of this assay to resolve samples with borderline S/CO ratios (1,6,19). Indeterminate anti-HCV supplemental test results have been observed in recently infected persons who are in the process of seroconversion and occasionally in persons chronically infected with HCV (17). Especially in a low-risk HCV-infection population, indeterminate immunoblot might also indicate a false-positive screening test result (8,18).

Overall the analysis indicates that out of the 70 sera tested, 29 showed the same positive result with the three methods used in this study, while 39 showed agreement only between two methods (Table 2). In particular, 8 and 6 samples yielded positive results with the anti-HCV test VIDAS® showed respectively indeterminate and negative results with the INNO-LIA™ HCV Score assay (Table 3). 25 samples were revealed as negative with both anti-HCV VIDAS® and INNO-LIA™ HCV Score assays (Table 2); notably, the majority of samples *reactive* with the anti-HCV ARCHITECT®, which were not confirmed as positive by immunoblot analysis, had an S/CO ratio ranging from 1 to 2 (Table 2). Only two sera showed unclear status being anti-HCV positive when tested with ARCHITECT® (S/CO ratio 1.42 and 3.33),

Table 1. Number of samples with positive, negative, or indeterminate result using the three different anti-hepatitis C virus assays.

	ARCHITECT®	VIDAS®	INNO-LIA®
Positive	70	43	29
Negative	0	27	31
Indeterminate	NA	NA	10

NA, not applicable.

Table 2. Distribution of analysed samples considering results obtained with the three tests and the S/CO ratios.

ARCHITECT®	VIDAS®	INNO-LIA®	S/CO ratios (ARCHITECT®)			
			1≤S/CO<2	2≤S/CO<4	4≤S/CO<6	6≤S/CO≤8
29 positive	29 positive	29 positive	5	8	8	8
41 positive	8 positive	8 indeterminate	2	5	1	0
	6 positive	6 negative	3	2	1	0
	25 negative	25 negative	16	7	2	0
	2 negative	2 indeterminate	1	1	0	0

negative with VIDAS® (index value 0.77 and 0.27), and indeterminate with INNO-LIA™ HCV Score assay. These samples were excluded from further analysis.

ARCHITECT® and anti-HCV VIDAS®, 42.6% between anti-HCV ARCHITECT® and INNO-LIA™ HCV Score assay, and 79.4% between anti-HCV VIDAS® and INNO-LIA™ HCV Score assay. Interestingly, excluding the eight samples unresolved by immunoblot analysis, the concordance between anti-HCV VIDAS® and INNO-LIA™ HCV Score assay increased up to 90%.

In agreement with a previous experience (11), the anti-HCV VIDAS® demonstrated an efficient antibody detection and a specificity higher than ARCHITECT®, and this allowed us to propose a renewed algorithm for the confirmation of anti-HCV serological status: in cases of first time diagnosed *reactive* anti-HCV, sera are additionally analysed with the anti-HCV test VIDAS®; only samples with positive result with ELFA technology are subjected to confirmatory anti-HCV immunoblot (Figure 1). This strategy enhances the identification of false positive samples and limits the number of specimens that are subjected to further confirmatory testing by the line blot assay, that adds direct and indirect costs and delays substantially the diagnostic response. Though it seems unlikely that the same interfering factors may affect two assays using antigens from different sources for each genome region, the usefulness of the supplemental assay on samples reactive by both screening is justified by the occurrence of six samples that were reactive by both ARCHITECT® and VIDAS® and yielded a negative result by INNO-LIA™. In our opinion, such samples should be considered as false positives and flagged with a request for a further specimen from the same patients if the clinical suspicion of an HCV infection is high.

Conclusions

In the case of a *reactive* anti-HCV result, further testing are needed to confirm HCV infection status or to discover unspecific reactivity of the anti-HCV test. However, as many assays utilize similar HCV recom-

Table 3. Comparison between the results obtained in selected samples showing discrepancy.

Sample	ARCHITECT® (S/CO)	VIDAS® (index value)	INNO-LIA® (result)
1	1.17	1.19	Indeterminate
2	1.60	1.8	Indeterminate
3	2.14	4.17	Indeterminate
4	2.16	2.23	Indeterminate
5	2.43	2.74	Indeterminate
6	2.67	2.09	Indeterminate
7	3.42	5.04	Indeterminate
8	4.18	2.28	Indeterminate
9	1.14	3.14	Negative
10	1.27	2.59	Negative
11	1.55	1.15	Negative
12	2.64	4.81	Negative
13	3.55	7.55	Negative
14	4.89	5.71	Negative

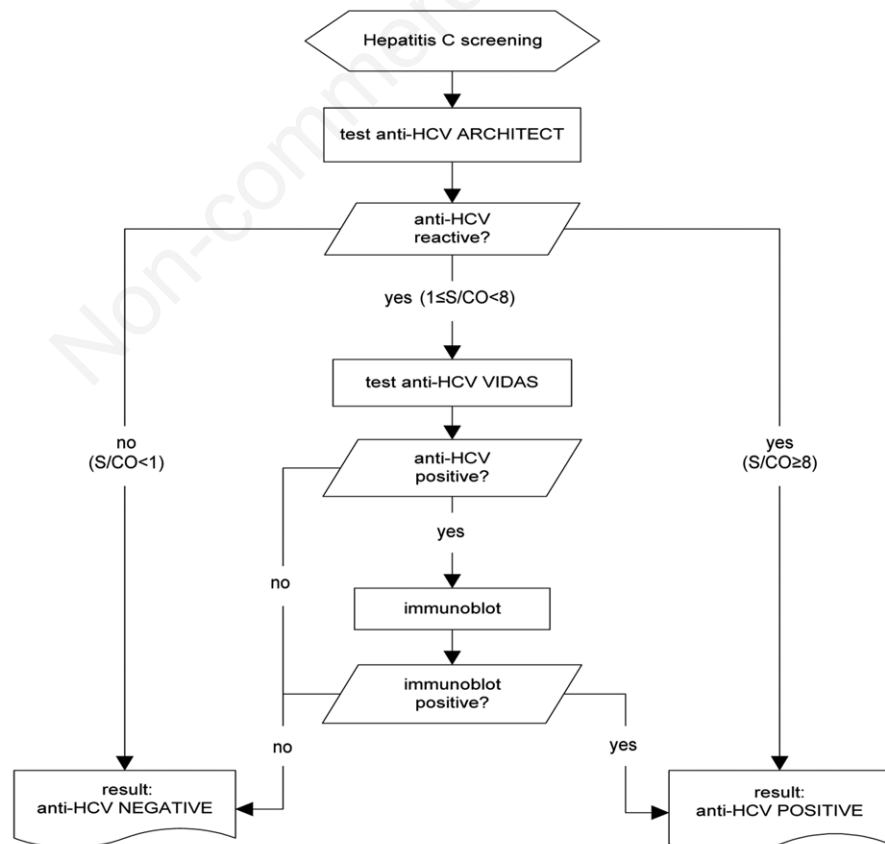


Figure 1. Proposed algorithm for screening and confirmation of hepatitis C.

binant antigens as a solid phase, shared *non-specific* reactions frequently occur. This study demonstrates that anti-HCV test VIDAS® possess a good sensitivity and a specificity higher than ARCHITECT® in detection of anti-HCV antibodies. Since the anti-HCV VIDAS® format is not suitable for large scale screening, the test has been introduced in our routine as a second level screening for specimens showing a S/CO value on ARCHITECT® ranging from 1 to 8. This choice allows to save costs and to reduce the execution of confirmatory anti-HCV immunoblot tests, which are still useful to detect false reactive anti-HCV results by screening tests.

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