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Characterization of antigenic preparations for anti-HCV screening tests: Issue of test performance

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Abstract

Screening for anti-HCV antibodies is often subject to contradiction between different laboratories due to the use of tests from different manufacturers. In this study, we have proceeded to the identification of the various screening tests for anti-HCV antibodies with the main concerns of the characterization of each brand according to its principle, its antigenic preparation, and its evaluation in relation to the PCR RNA HC Positive and Negative samples.

Our results identified thirty-two anti-HCV tests, of which 90.7% of the anti-HCV antibody detection tests had the following principles: simple liquid phase immunochromatography and solid support, the other tests, their principles were based either on the immunobolt (3.125%) or on sandwich immunochromatography (6.25%). 22 tests (68.75%) had no specification of the antigen preparation 9 tests mentioned (28.125%) recombinant proteins and only 1 test (3.125%) Recombinant proteins + synthetic peptides The correspondence was the antigen preparation, and its composition was such that 12 tests of recombinant protein preparations consisted of 8 tests contained the core, NS3, NS4 and NS5 proteins; 1 tests nucleocapsid and non-structural proteins. The evaluation of the anti-HCV tests presents on the Lubumbashi market, it turns out that the antigenic preparation tests: Core, NS3, NS4, NS5, meet the description of third-generation anti-HCV tests, because they consist of core antigens, NS3 and NS4 antigens combined and incorporated an NS5 epitope, showed significantly improved performance in terms of sensitivity and specificity.

Keywords: Test; HCV; Screening; identification; Characterization

1. Introduction

Hepatitis remains a significant public health threat in Africa. Progress in prevention, diagnosis and treatment was hampered between 2019 and 2021, due to insufficient implementation of hepatitis interventions in countries.).(1) In 2021, WHO established the 2021-2030 Framework for an integrated multisectoral response to tuberculosis, HIV infection, sexually transmitted infections, and hepatitis in the African Region.(2)

WHO has provided technical support to countries in their national hepatitis response and 28 African countries now have a national hepatitis program, either as a stand-alone program or integrated into HIV services . Hepatitis strategic plans have been developed in 21 countries while 17 countries have treatment and screening guidelines aligned with WHO guidelines.(3)

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Diagnosis and treatment rates are also particularly low, as the dashboard shows. In 2021, only 2% of people infected with the hepatitis B virus have been diagnosed and barely 0.1% of them have been treated. For hepatitis C, an estimated 5% of those infected have been diagnosed and almost 0% have been treated.(4)

Viral hepatitis is a long-neglected silent killer in sub-Saharan Africa. Most countries in the region do not have national programs for the prevention and treatment of hepatitis.(5). Only the program to fight against HIV is organized in the country and the dispensing of antiretrovirals, two molecules of which are active on the hepatitis B virus (Tenofovir and lamivudine of the DTL complex) benefits patients with hepatitis B from a free treatment, which is not the case for patients who are probably positive for anti-HCV antibody screening tests.

Screening for anti-HCV antibodies is increasingly contradictory from one laboratory to another, the basis of the contradiction being due to the fact of the various screening tests available on the local market, these tests which do not benefit from any control of reliability when entering the country. It is within this framework that this work falls, which has set itself the following objectives:

Identify the different screening tests for hepatitis C in the city of Lubumbashi and Likasi

Check their principles, their antigenic preparation, and their performance in terms of sensitivity and specificity according to the information provided by the manufacturers.

Evaluate their performance based on the test that will meet WHO requirements for rapid tests.

2. Methodology

This is a cross-sectional descriptive study conducted in the Democratic Republic of Congo precisely in the province of Haut-Katanga, the case of the city of Lubumbashi and that of Likasi. It took place:

Through an interview which aimed to identify the varied brands of rapid screening tests for hepatitis C virus infection and conducted based on a structured questionnaire consisting of open and closed questions preformed in the laboratories and medical structures of the city of Lubumbashi and Likasi. After having conducted the quality control on the consistency of the data collected, we will proceed to the codification of certain (open) questions. Data encoding and analysis using Epi Info 7.3 software.

By an evaluation of the tests according to the composition of their antigenic preparation compared to twenty-one samples examined PCR RNA HCV detected and 65 samples examined PCR RNA HCV Not detected.

Calculation of Sensitivity and Specificity by group of tests according to the composition of their antigenic preparation

3. Results and discussion

We have identified thirty-two different screening tests for hepatitis C infection, all having immunochromatography as a principle and whose distribution is as follows:

Table 1 Distribution of anti-HCV antibody detection tests according to principle and antigen preparation

Principles	**Recombinant Proteins**	**Recombinant Proteins + Synthetic Peptides**	**Not specified**	**Total**
Immunochromatography	6	1	22	29
Immunoblot	1	0	0	1
Sandwich immunochromatography	2	0	0	2
Total	9	1	22	32

This table tells us that 90.7% of the anti-HCV antibody detection tests had the following principles: simple liquid phase immunochromatography and solid support, the other tests, their principles were based either on immunobolt (3.125%) or on sandwich immunochromatography (6.25%). 22 tests (68.75%) had no specification of the antigen preparation 9 tests mentioned (28.125%) recombinant proteins and only 1 test (3.125%) Recombinant proteins + synthetic peptides.

Table 2 Distribution of anti-HCV antibody detection tests according to the principle and composition of the antigen preparation

Proteins / **Principle**	**Core, NS3, NS4, NS5**	**Nucleocapsid and Non-Structural**	**Not Specified**	**p value**
Immunochromatography	**5**	**1**	**22**	**29**
Immunoblot	**1**	**0**	**0**	**1**
Sandwich immunochromatography	**2**	**0**	**0**	**2**
Total	**9**	**1**	**22**	**32**

The distribution of the principles of the anti-HCV antibody detection tests according to the composition of the antigenic preparation is such that twenty-two tests were of unspecified composition, 1 test composed of Nucleocapsid proteins and non-structural proteins and 9 composed of core proteins and of NS3, NS4 and NS5. It is known for the hepatitis C virus an antigenic structure consisting of structural (C, E1 and E2) and non-structural (p7, NS2, NS3-4A, NS4B, NS5A and NS5B) viral proteins.(7) However, in the clinical diagnosis of HCV, the presence of viral RNA, anti-HCV antibodies and/or Core viral antigen confirms the infectious status of a patient (8). Nucleic acid testing (NAT) is the gold standard for validating active HCV infection within days of infection. It should be noted that standard serological assays for HCV measure anti-Core antibodies, NS proteins NS3, NS4 and NS5, but rarely anti-Env(9) antibodies. surface viral HCV E1E2 (or Env) are targets of traps and are important antigens for vaccine development(10, 11).

Table 3 Breakdown of HCV tests according to the antigenic preparation and its composition

Principles	**Recombinant proteins**	**Recombinant Proteins + Synthetic Peptides**	**Not specified**	**p value**
Core, NS3, NS4, NS5	8	1	0	9
Nucleocapsid and Non-Structural	1	0	0	1
Not Specified	3	0	19	22
Total	12	1	19	32

The correspondence to be the antigenic preparation and its composition was such that 12 tests of recombinant protein preparations consisted of 8 tests contained the proteins core, NS3, NS4 and NS5: 1 tests nucleocapsid and non-structural proteins. Most had an unspecified composition. In this regard, Xinyi Jiang claims that all commercial hepatitis C virus (anti-HCV) antibody tests use a combination of recombinant antigens to detect the antibody response(12) and in his study based on four immunoassays: hepatitis C virus antibodies against different antigens may have unequal contributions to detection found that anti-core and anti-NS3/4 antibodies were simultaneously detected in 99.2% of samples positive for HCV RNA and showed high consistency with total anti-HCV signals . Also, responses to the core region were more robust than those to the NS3/4 region in the coherent anti-HCV group and that the anti-NS5 antibody gave responses only in association with responses to the core antigens. ;While NS3/4 and failed to affect final anti-HCV positive signals(12,13). Thus, antibody responses to core and NS3/4 antigens were strongest, while responses to NS5 antigen were weakest, indicating that individual antigenic regions played distinct roles in total anti-HCV signals(12)

Table 4 Distribution of means of reading time, sensitivity, and specificity of tests Anti-HCV antibody detection test.

Settings	X± SD	Minimum Value	Fashion	Maximum Value
Sensitivity	100±1.81	92	100	100
Specificity	99±11.93	60	100	100
Reading time	17± 8.82	1	15	40

In general, the average sensitivity of the tests in the city of Lubumbashi was 100% for a specificity of 99 ± 11.93 and a reading time of 17 ± 8.82 minutes. It is recommended at this level that any rapid test for antibodies against HCV (anti-HCV). must be validated by the FDA. These tests should be extremely sensitive and specific. And must provide results in less than sixty minutes for clinical use (8)

and use as follows: an immunocompetent person without risk of HCV infection who assesses negative for HCV is not infected with HCV and no further HCV testing is required. Additional testing may be needed for people with current or recent risks of HCV exposure (egg, injection drug use) and severely immunocompromised people (egg, some HIV/AIDS patients or those on hemodialysis).

Table 5a Evaluation of anti-HCV antibody detection tests according to the composition of the Antigenic preparation compared to PCR: RNA HCV: Core, NS3, NS4, NS5

PCR RNA HCV Detected	Positive #21	Negative #21	PCR RNA HCV Not Detected	Positive #65	Negative #65
1	20	1	1	0	65
2	21	0	2	0	65
3	20	1	3	1	64
4	21	0	4	0	65
5	21	0	5	0	65
6	21	0	6	0	65
7	21	0	7	0	65
8	21	0	8	0	65
9	21	0	9	0	65
Total	187	2	Total	1	584

The calculated Specificity value: 98.94% and calculated Sensitivity: 99.74

Table 5b Nucleocapsid + Non-structural proteins

PCR RNA HCV Detected	Positive #21	Negative #21	PCR RNA HCV Not Detected	Positive #65	Negative #65
1	20	1	1	0	65
Total	20	1	Total	0	65

From this table the calculated Specificity: 100% and calculated Sensitivity: 95.24%

Table 5c Composition Not specified.

PCR RNA HCV Detected	Positive #21	Negative #21	PCR RNA HCV Not Detected	Positive #65	Negative #65
1	21	0	1	2	63
2	21	0	2	0	65
3	21	0	3	3	62
4	18	3	4	0	65
5	21	0	5	0	65
6	19	2	6	0	65
7	21	0	7	0	65
8	18	3	8	0	65
9	20	1	9	0	65
10	21	0	10	4	61
11	20	1	11	5	60
12	19	2	12	1	64
13	21	0	13	0	65
14	18	3	14	3	62
15	17	4	15	6	59
16	21	0	16	2	63
17	19	0	17	4	61
18	20	1	18	1	64
19	21	0	19	0	65
20	20	1	20	3	62
21	19	2	21	1	64
22	21	0	22	0	65
Total	437	23	Total	35	1395

Calculated Specificity: 95.00% and Calculated Sensitivity: 97.55%

By these two evaluation tables of anti-HCV tests present on the Lubumbashi market, it turns out that the antigenic preparation tests: Core, NS3, NS4, NS5, meet the description of third-generation anti-HCV tests ,as consisting of the core antigens, NS3 and NS4 antigens combined and incorporated an NS5 epitope, show significantly improved performance in terms of sensitivity and specificity with a seroconversion period of approximately 8 weeks (14,15) and Commercial tests currently available are mainly based on third-generation anti-HCV tests. However, the composition of each antigenic component and the size of the antigen in these tests vary between manufacturers (16) . Core antigens have been shown to elicit an early antibody response more frequently in vivo (17). This is Anti-core generated shortly after seroconversion and reacting to coated NS3 antigens, covering the entire NS3 helicase region and most of the NS4 region, the combination Core +NS3 and NS4 enhanced sensitivity of third-generation HCV screening methods and detected a strong anti-NS3/4 signal early in serological conversion and persists during HCV infection resolved (18), Expression of these responses were higher in individuals with viral persistence than in those with viral clearance(19). This supports that anti-core and anti-NS3/4 are two significant indicators of HCV infection. Viremia is proportionally linked to both the presence of anti-core and anti-NS3. The NS5 antigen was weakly immunogenic and responses to NS5 were weakest and likely to be missing (18-20) . In this regard, the WHO recommends that the analytical sensitivity of most rapid tests

vary from 2 to 10 IU/ml.(21) These indications, unfortunately, are not defined in the prospectus of the tests used in Lubumbashi and it is thus difficult to prove compliance with this recommendation by the manufacturer of the tests.

Through their simplicity, rapid tests have proven to provide equitable, near-site access to diagnostic services for patients, regardless of their geographic location and socio-economic status. This has increased demand in the in-house diagnostics market. in vitro (IVD). And to ensure the reliability of these tests, guidelines and recommendations for quality control and performance evaluations of IVD tests should be updated and updated regularly(22). And unlike high-income countries, resource-limited and hard-to-reach settings face challenges such as inaccessible and expensive conventional HCV laboratory diagnostics that inconvenience end users. This promotes health inequalities due to poor access to HCV diagnostic services (23-25)

It now turns out that the introduction of confirmation tests using an algorithm based on the evaluation of the signal/threshold ratio could limit the occurrence of false positives. Indeed, a study in Uganda shows that eighty-four percent of hepatitis C virus (HCV) blood donations reactive to screening in Ugandan blood transfusion services were identified as presumptive false positives. Active HCV infection is present in only 8.1% of reactive HCV samples (26).

Table 6 Distribution of tests according to the type of samples recommended.

Samples	**N=32**	**%**
Whole Blood	24	75
Plasma	26	81.25
Serum	32	100
Mouth Fluid	1	3.125

From this table, it follows that all anti HCV tests use serum as samples, 81.25% use plasma, 75% serum and only one test uses oral fluid. The use of whole blood is subject to the optimal and mandatory use of wash buffers.

However, these oral tests have lower sensitivities than blood tests. This may be due to a lower concentration of antibodies in oral fluid than in blood, or sample dilution by collection buffer. Additionally, oral fluid positivity for HCV may be affected by oral pathology or the collection of oral fluid after subjects use mouthwashes or acidic beverages (27).

4. Conclusion

The development of serological tests for the detection of anti-HCV antibodies has been conducted considerable progress. It should be noted here that the manufacturers will have to consider not only the antigenic preparation and its nature, but also specify its concentration in the test on the prospectus. This is even more useful for the choice of import tests and the purchase. As far as high-performance tests are concerned, our recommendation is such that only diagnostic tests with an antigenic preparation grouping together the antigens: Core, NS3, NS4, NS5) which have revealed excellent sensitivity and specificity.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no conflict of interest in relation to this article.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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