Significant Improvement in Diagnosis of Hepatitis C Virus Infection by a One-Step Strategy in a Central Laboratory: an Optimal Tool for Hepatitis C Elimination

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HEPATITIS C

Catalonia (7.5 M): HCV 1.1%; 20% not still diagnosed
Screening for chronic hepatitis C virus infection

WHO GUIDELINES FOR THE CARE AND TREATMENT OF PERSONS DIAGNOSED WITH CHRONIC HEPATITIS C VIRUS INFECTION. JULY 2018

- Screening for chronic hepatitis C virus infection is based on the detection of anti-HCV antibodies. Recommending the use of both laboratory immunoassays and the use of rapid diagnostic tests.

- If antibodies against the hepatitis C virus are detected, we have to determine the presence of RNA (or the presence of core antigen) to identify those patients with active infection by virus C.

Source: WHO. GUIDELINES FOR THE CARE AND TREATMENT OF PERSONS DIAGNOSED WITH CHRONIC HEPATITIS C VIRUS INFECTION. JULY 2018
The diagnosis of active HCV infection is based on the detection of HCV RNA in serum or plasma by a sensitive, molecular method. An assay with a lower limit of detection ≤15 international units (IU)/ml is recommended. However, the vast majority of patients with an indication for anti-HCV therapy have an HCV RNA level above 50,000 IU/ml.

A study in patients with chronic HCV due to genome 1 found only 4 patients out of 2,472 (0.16%) with an HCV RNA level below 1,000 IU/mL.

Therefore when sensitive HCV RNA assays are not available, HCV RNA assay with a lower limit of detection ≤1,000 IU/mL can be used.
HCV-Antibody Detection Tests

Enzyme Immunoassays (EIA)
- The gold standard is the detection of antibodies in serum or plasma by EIA. Currently with chemiluminescent detection (GUIDELINES ON HEPATITIS B AND C TESTING, WHO, 2017)

Rapid Detection Tests (RDTs)
- In order to facilitate the screening of HCV infection, a multitude of RDTs have been developed for the detection of antibodies against HCV.
- ADVANTAGE: RDTs are easy to perform, they do not require specific instrumentation and the reagents are stored at room temperature.
- RDTs use various matrices, including serum and plasma, but also fingerstick capillary whole blood or oral (crevicular) fluid, facilitating detection without the need for venipuncture.
- Rapid diagnostic tests using serum, plasma, fingerstick whole blood or crevicular fluid (saliva) as matrices can be used instead of classical EIAs as point-of-care tests to facilitate anti-HCV antibody screening and improve access to care (A1).

HCV-Nucleic Acid Detection Tests

Centralized NAT

High Throughput

Near Patient NAT

e.g. Cobas 6800 (Roche)

Volume: 0.5-1 mL
More than 90,000 HCV serologies per year→ 70% non-hospital (PC / CAS).

**Situation in 2015, at the beginning of VHCL:**
- In patients anti HCV positive-
  - (PC) Primary Care in 60% of patients Viral Load (HCV-RNA) was requested.
  - (CAS) Centres for detoxification of drug dependency less than 20% of patients Viral Load (HCV-RNA) was requested.

**Measures from the laboratory since 2015**
- Recommendation to doctors in the laboratory report: "Viral load needs to be done as soon as a patient gets positive by a serology assay".
- In spite of this measure, 30% did not get tested for Viral Load. → Something else had to be done…
Diagnostic algorithms: Conventional vs “One Step”

A - Conventional diagnosis

Best scenario 4 weeks, but frequently months
Loss of follow-up (>40% PC, >80% CAS)

B – “One Step” Diagnosis (also known as “HCV RNA reflex”) 2 days

If negative discharge

Reflex testing for HCV RNA or HCV core antigen in patients found to be anti-HCV antibody-positive should be applied to shorten pathways to care (A1).


https://www.hcvguidelines.org/evaluate/testing-and-linkage

Recommended for Initial HCV Testing and Follow-Up

- reflex laboratory-based virological NAT of positive serology samples;
- HCV-antibody testing with reflex HCV RNA polymerase chain reaction (PCR) testing is recommended for initial HCV testing.

World Health Organization

https://www.aasld.org/
Our experience in “One Step”: published peer-reviewed article

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Rosa López-Martínez, Andrea Arias-García, Francisco Rodríguez-Algarra, Laura Castellote-Bellés, Ariadna Rando-Segura, Guillermo Tarraso, Elena Vargas-Accarino, Isabel Montserrat-Lloan, Albert Blanco-Grau, Andrea Caballero-Garralda, Roser Ferrer-Costa, Tomas Pumarola-Sunye, Maria Buti-Ferret, Rafael Esteban-Mur, Josep Quer, Ernesto Casis-Saez, Francisco Rodríguez-Frias.
“The one-step diagnosis consists of the investigation of viremia in all patients with a new serological diagnosis….. …. the determination of viremia must be carried out (without mediating a new clinical request) under the same security conditions as carried out up to this point, that is, ensuring the absence of contamination and degradation of the biological sample, ensuring the stability of the sample and guaranteeing the final result”.

Although the samples for hematimetry are accessible in centralized laboratories, they may not be accessible in other laboratories, in these cases the specimens, such as those previously processed for serology, can be used after being thoroughly tested to guarantee this safety.
% RNA Analysis (VL) Performed

<table>
<thead>
<tr>
<th>Setting</th>
<th>6-step protocol</th>
<th>Reflex testing protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>HCVRNA positive</td>
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<tr>
<td>Hospital care</td>
<td>20,446</td>
<td>2,583 (12.6)</td>
</tr>
<tr>
<td>Non-hospital care</td>
<td>62,340</td>
<td>2,612 (4.2)</td>
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<tr>
<td>CAS</td>
<td>1,335</td>
<td>211 (15.8)</td>
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<td>Incurrent</td>
<td>2,644</td>
<td>383 (14.5)</td>
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<tr>
<td>Primary care</td>
<td>57,830</td>
<td>2,010 (3.5)</td>
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<tr>
<td>Other</td>
<td>531</td>
<td>81 (1.5)</td>
</tr>
<tr>
<td>Total</td>
<td>82,786</td>
<td>5,195 (6.3)</td>
</tr>
</tbody>
</table>

**CAS:** Centres for detoxification of drug dependency (e.g. Methadone replacement therapy) 10 times more HCV seropositives than general population (61 in all Catalonia, 9 in Barcelona city under our responsibility for in vitro testing)

Our experience in “One Step”: % HCV RNA (VL) Performed:

A decrease in % viremic!!: probably "patients cured by treatment": Spain more than 200,000 treated (Catalonia 26,000) with 95% Sustained Virological Response.

"one step" activity in the centers for detoxification of drug dependencies (CAS) until a month ago

<table>
<thead>
<tr>
<th>Year</th>
<th>Months</th>
<th>N</th>
<th>aVHC+</th>
<th>HCV RNA (VL) detectable</th>
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<tr>
<td>2018</td>
<td>10</td>
<td>1595</td>
<td>206 (13%)</td>
<td>100 (49%)</td>
</tr>
<tr>
<td>2019</td>
<td>12</td>
<td>1793</td>
<td>196 (17%)</td>
<td>124 (42%)</td>
</tr>
<tr>
<td>2020</td>
<td>12</td>
<td>1343</td>
<td>209 (16%)</td>
<td>68 (33%)</td>
</tr>
<tr>
<td>2021</td>
<td>6</td>
<td>902</td>
<td>103 (11%)</td>
<td>27 (26%)</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Setting</th>
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<th>aHCV positive</th>
<th>HCV RNA analysis omitted</th>
<th>HCV analysis omitted</th>
<th>HCV RNA</th>
<th>n</th>
<th>aHCV positive</th>
<th>HCV RNA analysis omitted</th>
<th>HCV RNA</th>
<th>n</th>
<th>aHCV positive</th>
<th>HCV RNA analysis omitted</th>
<th>HCV RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospital care</td>
<td>20,446</td>
<td>2,583 (12.6%</td>
<td>2,998</td>
<td>1,697 (8.3%)</td>
<td>979 (32.7%)</td>
<td>10,740</td>
<td>616 (5.7%)</td>
<td>831</td>
<td>70 (11.4%)</td>
<td>1,093</td>
<td>82 (7.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-hospital care</td>
<td>62,340</td>
<td>2,612 (4.2%)</td>
<td>1,885</td>
<td>776 (29.7%)</td>
<td>752 (39.9%)</td>
<td>35,195</td>
<td>1,126 (3.2%)</td>
<td>1,093</td>
<td>82 (7.3%)</td>
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</tr>
<tr>
<td>CAS</td>
<td>1,335</td>
<td>211 (15.8%)</td>
<td>39</td>
<td>172 (81.5%)</td>
<td>21 (53.8%)</td>
<td>1,052</td>
<td>235 (22.3%)</td>
<td>245</td>
<td>12 (5.1%)</td>
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</tr>
<tr>
<td>Itinerant</td>
<td>2,644</td>
<td>383 (14.5%)</td>
<td>360</td>
<td>35 (9.1%)</td>
<td>199 (55.5%)</td>
<td>401</td>
<td>53 (13.2%)</td>
<td>57</td>
<td>3 (3.8%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary care</td>
<td>57,830</td>
<td>2,010 (3.5%)</td>
<td>1,480</td>
<td>570 (28.4%)</td>
<td>530 (35.8%)</td>
<td>33,399</td>
<td>831 (2.5%)</td>
<td>343</td>
<td>7 (2.0%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>531</td>
<td>8 (1.5%)</td>
<td>6</td>
<td>2 (25.0%)</td>
<td>2 (33.3%)</td>
<td>343</td>
<td>7 (2.0%)</td>
<td>343</td>
<td>7 (2.0%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>82,786</td>
<td>5,195 (6.3%)</td>
<td>4,883</td>
<td>2,476 (47.7%)</td>
<td>1,731 (35.4%)</td>
<td>45,935</td>
<td>1,742 (3.8%)</td>
<td>343</td>
<td>7 (2.0%)</td>
<td></td>
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</tr>
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</table>

6.4% HCV RNA omitted

Spanish experience in “One Step”

Hepatitis C reflex testing in Spain in 2019: A story of success

Abstract

Background: Reflex testing is necessary to achieve the objectives of hepatitis C elimination. However, in 2017 only 31% of Spanish hospitals performed reflex test. As a consequence of that finding, reflex testing was recommended by scientific societies involved in the diagnosis and treatment of hepatitis C.

Objective: To evaluate the degree of implementation of reflex testing in 2019 and to know the implementation of rapid diagnostic and/or dried blood spot testing (RDT and/or DBS) in Spanish hospitals.

Methods: Cross-sectional study through a survey conducted in October 2019 to Spanish general hospitals with at least 200 beds, public or private with teaching accreditation.

Results: 129 (80%) hospitals responded. Reflex testing is performed by 89% of the centers vs. 31% in 2017 (P<.001). From 2017 to 2019, centers using alerts to improve continuity of care increased from 69% to 86% (P=.002). In 2019, 11% of centers can determine anti-HCV in dried spot, 15% viremia in dried spot, 0.85% anti-HCV in saliva, and 37% of antibodies and/or viremia with point of care test. 43% of hospitals have at least one diagnostic method with RDT and/or DBS.

Conclusion: The implementation of reflex testing has increased significantly, reaching 89% of hospitals in 2019. The recommendations of scientific societies could have contributed to the implementation of reflex testing. On the other hand, access to RDT and/or DBS is insufficient and initiatives are needed to improve their implementation.

“The one-step diagnosis consists of the investigation of viremia in all patients with a new serological diagnosis.....

..., the determination of viremia must be carried out (without mediating a new clinical request) under the same security conditions as carried out up to this point, that is, ensuring the absence of contamination and degradation of the biological sample, ensuring the stability of the sample and guaranteeing the final result”.


“The implementation of “reflex testing” has increased significantly, reaching 89% of hospitals in 2019. The recommendations of scientific societies could have contributed to the implementation of reflex testing.”
Limited access to health care systems is a major challenge to eliminate HCV

Two main Groups:

**Immigrant population**

**Drug addicts**

**Foreign population in Barcelona:**
360,000 from 1,7M (21%), 137 different countries.
32% (120,000) people from regions with high hepatitis prevalence. E.G. from Pakistan 5% HCV +

**PWID antiHCV prevalence 80% (59% viremic) and 40% unaware. DBS “one step” testing.**
10% reinfections after cure.

These patients do not usually follow the normal procedures of health care and nursing services are not always available to do conventional blood draws in the centers where they attend for processes of cessation (CAS) or rooms for active drug consumption or due to situations of illegal immigration, they remain marginalized).

Other alternatives are needed!
HCV diagnosis: Alternative algorithms

A - One-step diagnosis with dried blood

- Whole blood sampled on DBSs can be used as an alternative to serum or plasma obtained by venipuncture for anti-HCV antibody testing, after shipment to a central laboratory where the EIA will be performed (A1).

Source: Velasquez-Orozco F; Diagnostics 2021;11:473

A - One-step diagnosis with dried blood

Including a prospective study in Hospital Nossa Senhora da Paz. Cubal (Angola) N= 93
11% HBsAg positive in PSC-DBS . HBV-DNA was detected 60 %. Non anti HCV or anti-HDV positive cases.

Reflex viral load testing in dried blood spots generated by plasma separation card allows the screening and diagnosis of chronic viral hepatitis

Joan Martínez-Campreciós a,b,c, Adriana Rando-Segura b,c, María Buti a,b,d,e, Fernando Rodrigo-Velásquez b,c, Mar Riveiro-Barciela a,b,d, Ana Barreira-Díaz a,b, Patricia Álvarez-López a,b, Paula Salmerón b,c,d, Adriana Palom a,b, David Taberner a,b, Nieves Palomo a,b, Arlette Nindia f, Gisela Barbosa f, Eva López f, Vicentina Ferreira f, Nelsa Saiago f, Alison Kuchta h, Roser Ferrer-Costa h, Rafael Esteban a,b,d, Israel Molina j, Francisco Rodríguez-Frias d,i,k

HBV, HDV and HCV
HCV diagnosis: Alternative algorithms, including molecular characterization

A - One-step diagnosis with dried blood

Visit #1

Visit #2

Diagnostics 2021, 11, 473. https://doi.org/10.3390/diagnostics11030473

HCV Viral Load & Genotyping (qPCR and NGS)

Article

Utility of the Cobas® Plasma Separation Card as a Sample Collection Device for Serological and Virological Diagnosis of Hepatitis C Virus Infection

Fernando Velásquez-Orozco 1,2, Ariadna Rando-Segura 1,2,3,4, Joan Martínez-Camprecios 4, Paula Salmeron 1,2, Adrián Najarro-Centeno 3,5,6, Àngels Esteban 3,5,6, Josep Quer 5, Maria Buti 3,5,7, Tomás Pumarola-Suñe 1,2 and Francisco Rodriguez-Frias 1,2,3,4,7,8
Decentralized alternative diagnostic algorithms: starting with “Rapid Detection Tests “ (RDTs”)

A-Diagnosis based on rapid antibody detection tests (RDTs) followed by HCV-RNA conventional study:

In the same place where patient is located, based in “one step strategy”.

- Conventional venopunction if available (1)

- Capillary finger blood if venopunction not available (2)

- Rapid diagnostic tests using serum, plasma, fingerstick whole blood or crevicular fluid (saliva) as matrices can be used instead of classical EIAs as point-of-care tests to facilitate anti-HCV antibody screening and improve access to care (A1).
Decentralized alternative diagnostic algorithms: starting with “Rapid Detection Tests “ (RDTs)

A- Diagnosis based on rapid antibody detection tests (RDTs) followed by HCV-RNA conventional study:

In the same place where patient is located, based in “one step strategy”.

- Conventional venopunction if available (1)
- Capillary finger blood if venopunction not available (2)

B - Diagnosis based on RDTs, both, antibody and nucleic acid detection tests

Visit #1
Visit #2
(1) Rapid anti-HCV antibody test (Health care worker)
Phlebotomy (Phlebotomist)

receive diagnosis (Physician)

Treatment if indicated

Visit #1
Visit #2
(2) Rapid anti-HCV antibody test (Health care worker)
Dried blood spot sample

receive diagnosis (Physician)

Treatment if indicated

T & T ?

Especially indicated for active drug addicts, some times even homeless. Extremely high HCV prevalence (60-90% of antiHCV positive vs 1% in general population)
TAKE HOME MESSAGES

1: ONE-STEP diagnosis of hepatitis C simplifies the process and prevents many patients from not completing the diagnostic process. The quality of the sample used for HCV RNA testing must be guaranteed.

2: Its application in dry blood samples fixed to a solid support (ex: PSC) allows to improve monitoring patients worst linked to health systems like Immigrants and PWID.

3: In populations with more difficulties to be linked to health systems, the use of “rapid diagnostic tests” (RDT) for serology and decentralized viral load tests helps to link these patients.

4: The “TEST AND TREAT" strategy may be an optimal solution for these patients such as PWID.
Barcelona 2,000 years of history