One-step screening of active hepatitis C virus infection in the community through dried blood spots

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Fighting HCV: Relevance of alternative community outreach strategies

- Promoting HCV diagnosis is a global priority
  Spain: 70% undiagnosed

- Complex two-step testing algorithm for the diagnosis of hepatitis C:
  
  Serology (primary care) → Molecular confirmation by a specialist
  Previous exposure → Viremic infection
  
  Costly, requires venipuncture, cool chain, several return visits
  Problematic in hard-to-reach populations at risk

Screening strategies through community outreach and alternative testing in high-risk groups should complement those performed in healthcare settings. The use of dried blood spots (DBS) facilitate access to HCV testing.
Sexual HCV transmission may not be restricted to HIV-infected men who have sex with men (MSM)

- HCV infection has emerged as a sexually-transmitted infection (STI) among HIV-positive MSM with outbreaks of acute infection in large cities around the world, including Barcelona and Madrid.

Main determinants:
- HIV infection, ulcerative STIs (i.e. syphilis)
- Sexual behaviors (unprotected anal sex, traumatic practices, etc), drug use for sex.

Increasing HCV incidence HIV-negative MSM in large cities in Europe, the US, Asia
- Seldom screened for HCV in Spain.

Scarce data on male sex workers (MSW) and transsexual women sex workers (TWSW)
- Limited access to screening programs and healthcare system (mostly migrants).
- High-risk of STIs, increasing HIV prevalence.

⇒ Community HCV screening among HIV-negative MSM, MSW and TWSW is needed in Spain
Goals of the *HepCdetect* Study

1. To develop an **HCV-RNA detection assay in DBS** as a **one-step screening and confirmatory strategy**.
   - ⇒ Simplification of the HCV diagnostic algorithm, detection of acute & chronic inf.

2. To assess the **feasibility and benefit of this strategy in a community centre attending hard-to-reach populations at risk** (MSW, TWSW, and HIV-negative MSM).
   - ⇒ Improvement of access to HCV diagnosis, identification of silent cases

3. To **provide local HCV epidemiological and bio-behavioural data** in these populations.
   - ⇒ Evidence-based policy making
1. Laboratory set up of an *in house* molecular assay for the detection of the HCV-RNA in dried blood spots (DBS)

- DBS collection, drying & storage at room temp.
- Elution/Lysis & Addition of internal control
- Automatic RNA extraction (easyMAG, BioMérieux)
- Amplification mix* & RNA addition
- Detection of HCV-RNA and Int. control by real time RT-PCR

*Primers and probe detect all HCV genotypes [Daniel. Diagn Microbiol Infect Dis 2008]*


Major modifications in order to simplify the process:
- single-step RT-PCR
- use of a commercial internal RNA control
2. Laboratory validation of the HCV-RNA detection assay in DBS

- **Lower limit of detection:** 541 IU/mL (95% CI, 378-705 IU/mL)

- **Intra-assay and inter-assay variability** ≤ 2.6%

<table>
<thead>
<tr>
<th></th>
<th>Mean Ct</th>
<th>Standard deviation</th>
<th>Maximum Ct</th>
<th>Minimum Ct</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intra-assay variability</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Target HCV-RNA</td>
<td>35.30</td>
<td>0.90</td>
<td>36.46</td>
<td>33.65</td>
<td>0.026</td>
</tr>
<tr>
<td>Internal control RNA</td>
<td>29.00</td>
<td>0.48</td>
<td>29.51</td>
<td>27.96</td>
<td>0.017</td>
</tr>
<tr>
<td><strong>Inter-assay variability</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Target HCV-RNA</td>
<td>35.26</td>
<td>0.92</td>
<td>36.27</td>
<td>33.68</td>
<td>0.026</td>
</tr>
<tr>
<td>Internal control RNA</td>
<td>29.04</td>
<td>0.58</td>
<td>30.51</td>
<td>28.43</td>
<td>0.020</td>
</tr>
</tbody>
</table>

The assay is precise and reproducible and **DBS are stable at room temperature for at least 2 months**

- **Clinical sensitivity and specificity:** 100%

*Viral load range: 3.92-7.48 log_{10} UI/mL (Abbott Molecular)*
Community study design

- **Community-based voluntary counselling and testing centre:**
  Gay district in Barcelona
  Anonymous rapid HIV (Ag/Ab) and syphilis (Ab) testing
  **HCV testing offered for the first time for the HepCdetect study**

- **Study populations:** MSM, MSW, TWSW

- **Study period:** January 2015 to March 2016

- **Exclusion criteria:** < 18 years old
  Previous hepatitis C diagnosis: excluded from testing, questionnaire administered

- **HCV testing was announced:**
  Poster and flyers in gay venues,
  StopSida website, CBVCT centres network,
  Social networks, Apps
Community agents were trained in sample collection, HCV testing and counseling.

- **Written informed consent**
- **Anonymous questionnaire:**
  - Socio-demographic characteristics
  - Sexual practices
  - Drug use
  - Previous diagnosis of HCV/HIV/STIs

- **Bio-behavioural data analysis**

**Two-step HCV screening:**

- **OraQuick HCV Rapid Antibody test in fingerpick blood**
- **Referral** of positives for **confirmation of HCV-RNA in plasma** at a nearby laboratory

**One-step HCV screening:**

- **DBS collection** from fingerpick blood
- **Weekly shipment to the lab at RT for HCV-RNA testing**
Study subjects and HCV tests results

Individuals offered to participate: N=617 (677 instances)

- Accepted to participate: 580 individuals (653 tests; 60 repeated)
  - Acceptability = 95.2%
  - MSM 73.6%
  - MSW 10.2%
  - TWSW 13.2%

- Did not accept: 29 individuals (32 instances)

Previous HCV diagnosis: n=4
  (HIV-neg MSM)

Self-reported HCV prevalence:
  Overall = 0.68%
  HIV-negative MSM = 0.75%

Rapid HCV antibody test: 100% non-reactive

DBS based HCV-RNA test:
- Invalid results: n=26 (4%) technical problems in sample collection or processing
- Negative: n=622
- Positive: n=5 → 1 lost follow-up, 1 negative DBS retesting, 3 negative HCV viral load in plasma
  ≥99.2% specificity when screening such low-prevalence populations

No silent hepatitis C cases were confirmed
Epidemiological and behavioral characteristics of participants

<table>
<thead>
<tr>
<th></th>
<th>MSM</th>
<th>MSW</th>
<th>TWSW</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>419</td>
<td>58</td>
<td>92</td>
<td>569</td>
</tr>
<tr>
<td>Mean age (yr)**</td>
<td>34.2</td>
<td>27.7</td>
<td>30.8</td>
<td>33.0</td>
</tr>
<tr>
<td>Foreign origin**</td>
<td>32.5</td>
<td>79.3</td>
<td>94.6</td>
<td>47.3</td>
</tr>
<tr>
<td>High education level**</td>
<td>67.6</td>
<td>23.9</td>
<td>5.6</td>
<td>55.1</td>
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<tr>
<td>Condomless receptive anal intercourse (non-steady partner)*</td>
<td>30.5</td>
<td>30.6</td>
<td>60.0</td>
<td>31.8</td>
</tr>
<tr>
<td>Condomless receptive anal intercourse (clients)</td>
<td>—</td>
<td>5.6</td>
<td>7.0</td>
<td>6.5</td>
</tr>
<tr>
<td>Group sex (non-steady partner)*</td>
<td>43.6</td>
<td>36.1</td>
<td>5.0</td>
<td>41.3</td>
</tr>
<tr>
<td>Group sex (client)*</td>
<td>—</td>
<td>38.9</td>
<td>15.5</td>
<td>23.4</td>
</tr>
<tr>
<td>Sharing of dildos or sex toys</td>
<td>13.4</td>
<td>17.0</td>
<td>23.6</td>
<td>15.1</td>
</tr>
<tr>
<td>Rectal douching before penetration or fisting**</td>
<td>30.9</td>
<td>23.5</td>
<td>66.2</td>
<td>35.3</td>
</tr>
<tr>
<td>Drug use for sex**</td>
<td>55.2</td>
<td>70.7</td>
<td>93.2</td>
<td>59.9</td>
</tr>
<tr>
<td>HIV (self-reported + positive rapid test) (19 new diagnoses)</td>
<td>4.8</td>
<td>10.3</td>
<td>9.8</td>
<td>7.0</td>
</tr>
<tr>
<td>Syphilis (self-reported + positive Ab rapid test)**</td>
<td>5.0</td>
<td>13.8</td>
<td>13.3</td>
<td>7.2</td>
</tr>
<tr>
<td>Gonorrhoea**</td>
<td>6.6</td>
<td>19.1</td>
<td>1.4</td>
<td>7.0</td>
</tr>
<tr>
<td>Any STI* in the last 12 mo. (other than HIV)**</td>
<td>16.0</td>
<td>36.2</td>
<td>11.3</td>
<td>17.2</td>
</tr>
</tbody>
</table>

**p<0.001, *p<0.05 among groups.

* Any STI including syphilis, gonorrhoea, chlamydia, genital warts, herpes simplex virus, and hepatitis B virus.

Only one TWSW participant reported having injected drugs in her life.
Conclusions

- The HCV-RNA detection assay showed a good performance in DBS.

- This one-step screening and confirmatory strategy was easily implemented and well accepted in the community centre.

- As no silent HCV infections were confirmed, this strategy does not seem currently appropriate in this setting.

- Given the observed high-risk behaviours and prevalence of other STIs, HCV could eventually spread among MSW, TWSW and HIV-negative MSM in Barcelona.

- Sentinel populations should be periodically monitored by risk assessment and rapid antibody testing, while DBS HCV-RNA testing could be useful to facilitate confirmation of antibody positive cases.
The usefulness of this one-step diagnostic strategy is warranted in PWID, and will allow the HRC personnel to focus their efforts on those who really need it, facilitating their linkage to care.
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  - Rafael Muñoz
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