Point-of-care testing and other new diagnostics for HCV

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- Consulting: Abbvie, Gilead, Janssen, Merck
- Research: Abbvie, Gilead, Janssen, Merck
- Speaking: None
Outline

• The need for new tests
• Different approaches
  – New tests
    • Rapid Diagnostic Tests (RDTs) vs Point-of-Care tests (POC)
      – serology & RNA
    • Core antigen
  – New testing strategies
    • Dried Blood Spot (DBS)
    • Matching the test to the setting
The current paradigm

- **Delay = LTFU**
  - HCV antibody
    - Nonreactive
      - No HCV antibody detected
        - STOP*
      - LTFU = unaware
    - Reactive
      - HCV RNA
        - Not Detected
          - No current HCV infection
            - Additional testing as appropriate†
        - Detected
          - Current HCV infection
            - Link to care
          - LTFU = Not linked to care
          - LTFU = ‘may assume infected’
          - Delay = LTFU

LTFU = unaware
Many assumed that with DAAs…suddenly the cascade of care would no longer cascade.
Does this get better with DAAs?

24,966 boomers tested in Urban HC settings → 11.6% HCV Ab+!

**Problems:** Poor f/u for **RNA testing** and poor attendance at 1st appt

Patel Public Health Rep 2016
It doesn’t look so different

Reminder in EPR → 92,012 visits → 16,772 (18%) tested → 715 Ab+ (4.2%)

Even with effective treatment, major gaps in cascade of care!

Mera MMWR 2016
Consistent themes

Problem

• RNA confirmation

• Attendance in care

Potential Solution

• Reflex RNA for all Ab +
• RDT for Ab → immediate draw for RNA if Ab +
• Skip Ab test → direct to test of viremia
• Combine linkage with testing → RDT for RNA (+/- Ab) → immediate linkage to care & → discuss treatment
RDTs vs POCT

- **RDT** – rapid but requires special equipment +/- trained personnel
  - Antibody (blood, serum or saliva)
  - RNA (blood, serum)

- **POCT** – rapid and no special equipment or electricity required – easier to perform, no cold chain required
  - Antibody (blood, saliva)
  - RNA (blood)

Not all tests are created equal!
POCT - Simple to perform

**Fingerstick**

**Step 1** - Collect sample.

**Step 1b** - Mix sample in buffer.

**Step 2** - Insert the device into the buffer.

**Step 3** - Read between 20 and 40 minutes.

- Non-Reactive
  - Line in the C Zone
- Reactive
  - Line in the C and T Zones
Principle behind POCTs

Abs bind to protein A Gold
Colorimetric reagent

HCV
Antigens

Control line
e.g. anti-IgG Ab

Test sample

Capillary Flow

Sample Pad
Conjugate Pad
Membrane
Wicking Pad

Test Line
(positive)

Control Line
(valid test)
Rapid antibody tests

• Meta-analysis
• >13,000 individuals included in 18 studies (11 in LMIC) between 1992 and 2012

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Specificity</th>
<th>Sensitivity</th>
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</thead>
<tbody>
<tr>
<td>Whole blood POCTs</td>
<td>99.5%</td>
<td>98.9%</td>
</tr>
<tr>
<td>Serum &amp; Plasma POCTs</td>
<td>99.7%</td>
<td>98.9%</td>
</tr>
<tr>
<td>Serum &amp; Plasma RDTs</td>
<td>98.6%</td>
<td>98.4%</td>
</tr>
<tr>
<td>Saliva POCTs</td>
<td>98.2%</td>
<td>97.1%</td>
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</tbody>
</table>

Other issues: Co-infection, accuracy across genotypes
Co-infection can be an issue

- CDC study of 3 HCV RDTS
- All 3 – high specificity (99.5-99.8%)
- But sensitivity varied
  - Orasure (HCV) – 99.3%
  - Chembio (HCV) – 97.8%
  - Medmira (HIV & HCV) – 88.3% (also lower Kappa scores)
- Co-infection important
  - HIV co-infection 11-fold increase in false negative result with Chembio & Medmira (not with Orasure)
  - Other studies report higher false negative rates
  - *Orasure does not have this issue* – approved in US & Europe

Smith JID 2011, Honge HIV Med 2014
Availability

WHO Prequalified Tests

**HCV Ab**

- **Rapid/POCT**
  - Orasure – blood/serum/oral
    - Blood/serum also FDA
    - 20-40 minutes
  - SD Bioline – blood/serum
    - 5-20 minutes
- **Standard EIA**
  - INNO-LIA HCV Score
  - Bioelisa HCV 4.0
  - Murex anti-HCV 4.0

**HCV RNA**

- **Rapid**
  - Xpert HCV Viral Load (Cepheid)
  - Plasma/serum, lab equipment and reader required, individual cartridges
  - rt-PCR, 10-100E6 IU/mL
  - ~100 minutes
Testing sites with very different capacity

1 - Primary care
   • Health-care workers
   • Health centres, health posts and outreach

2 - District hospital
   • Technicians and assistants

3 - Regional/provincial laboratories
   • Specialists/senior technicians

4 - National reference laboratory
   • Senior health specialists
The right test for the right setting

• **Type of Technology**
  – Output (Ab vs viremia)
  – Lab or POC-based
  – Instrument vs kit
  – Electricity

• **Sample**
  – Type (blood, serum, saliva)
  – Stability (cold chain)

• **Cost**
  – Lab instrument
  – Reagents
  – Quality control reagents
  – Additional supplies (pipette, vortex, scanner, printer etc.)

• **Lab considerations**
  – Throughput & turnaround time
  – Complexity – training
  – Reagent stability
  – Environment – heat/cold/altitude
Oral Fluid (saliva)

OraQuick test crevicular fluid – 513 patients – **Specificity 100%, Sensitivity 97.6%**

3rd Generation EIA – Ortho in Crevicular Fluid

- 8 False negatives
  - 6 CHC, 2 resolved

- Despite excellent performance – **not FDA approved** but approved in UK & Europe
- Significant advantages for screening in certain settings

Chevaliez Clin Micro Infect 2016
Rapid RNA test

- Serum/plasma sample (1mL)
- Cartridge to reader

HCV RNA by Xpert (Log IU/mL)
HCV RNA by m2000 (Log IU/mL)

~100 mins

- Relatively easy-to-use rapid HCV RNA test – platform present in many LMIC
- Broad diagnostic range – 10-100E6 IU/mL, all genotypes
- High correlation with standard PCR assays – analytical performance $r=0.99$
- Real-world performance very good
  - Venipuncture - Sens 100%, Spec 99%
  - Finger-prick – Sens 95%, Spec 98%
- Other similar assays in development

McHugh J Clin Micro 2017, Grebely Lancet Gastro Hep 2017
Future true POC RNA tests

- Isothermal PCR
- Cross-priming amplification
- Detection of RNA quickly – 20’
- Longer duration increases sensitivity…but sensitivity less and less important
- All truly point-of-care – self-contained kit for RNA extraction from whole blood and amplification
- Promising…but not yet available
- Other technologies also in the works – less sensitive but faster

Xu Science Reports 2012, Ustart Biotech
An alternative to HCV RNA

- **HCV core antigen (Ag)**
  - Nucleocapsids are released into the serum and can be detected earlier than HCV Abs
  - Correlates well with HCV RNA but less sensitive
  - Lower sensitivity → LLOD ~3,000 IU/mL of HCV RNA
  - More stable than HCV RNA
  - Can be done on the same sample as used for Ab test
  - Cheaper – 15-25% cost of HCV RNA
  - Fully automated (but requires central lab)
  - Will be difficult to make ‘POC’ – lysis of sample, dissociation from Abs and signal amplification for sensitivity all required…challenging!
HCV Core Ag performance

- Similar performance for Abbott Architect & Ortho ELISA-Ag but more Architect data
- **Specificity 98.9% & Sensitivity >93%** with good correlation if HCV RNA>3000 IU/mL
- Could not assess HIV & HBV co-infection (small n) or genotype
Performance of core Ag: Screening & Treatment Monitoring

• Screening
  – 10,000 samples of residual sera tested for HCV Ab
  – All Ab+ and ~10% of Ab –ve samples → RNA & core Ag

• Treatment Monitoring
  – HCV RNA and core Ag at:
    • Pre-treatment
    • Week 4
    • End-of-treatment
    • SVR12
Performance of core Ag: screening and treatment monitoring

10,006 Residual sera

155 HCV Ab +

HCV RNA + 80/145

75/80 core Ag + - 5 RNA<3 log

9851 HCV Ab-

993 tested

17 RNA + (all<2 log)

0 core Ag +

- If RNA reserved for Ab +ve/core Ag –ve  Avoid 52% of HCV RNA tests
- Alternatively if core Ag cheap enough  screen with core Ag alone (~3% miss rate)
Conclusions:

1. Core Ag effective for confirming viremia for screening/baseline
2. On-treatment and EOT monitoring had poor predictive value – Core Ag & RNA similar but neither necessary
3. SVR12 – RNA preferred – 3 missed SVRs, 1 missed relapse

Collectively: If RNA used only for SVR & eliminate w4 & EOT tests → avoid 75% of all HCV RNA tests

Bottom line on core Ag

Pros

• Sensitivity
  – Detects almost all CHC
  – Confirmation of SVR…does it matter?

• Cost
  – Cheaper than RNA
  – Price arbitrary!

• Stable
  – Protein vs RNA

Cons

• Sensitivity
  – Still miss some CHC ~3% - is this acceptable?

• Cost
  – But still need central lab!

• Not rapid
  – Technically challenging to make it an RDT

Useful – now recommended by EASL guidelines, should be used more particularly for screening
Beyond core antigen

- Similar assay but added NS3, NS4b and NS5A to core

- Additional HCV antigens improves sensitivity → down to 250 IU/mL
- Important to use non-denaturing conditions to avoid false positive for resolved/SVR
Not only the test, but the sample collection method
Dried Blood Spot (DBS) Testing

Pros:
- No blood draw (screening drives, PWID)
- Peer testing
- Easy storage → mail to lab
- No need for 2nd visit for confirmatory RNA test

Cons:
- Smaller volume – may need multiple pricks – better with capillary
- Lower HCV RNA titre
- No immediate result
HCV RNA off DBS

CAP/CTM (Roche)

$m2000$ (Abbott)

Predictably lower HCV RNA titre - $\sim 1.5$-$2$ log IU/mL

Soulier JID 2016
**Does DBS handling matter?**

Assessed Ab, core Ag and HCV RNA off DBS samples with improper handling: **Unfrozen (4C/RT), heated (37C), hot/cold**

<table>
<thead>
<tr>
<th></th>
<th>-80° C (100%)</th>
<th>+4° C (100%)</th>
<th>RT (100%)</th>
<th>+37° C (100%)</th>
<th>Hot/Cold (100%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ab</strong></td>
<td>93/93 (100%)</td>
<td>91/91 (100%)</td>
<td>89/89 (100%)</td>
<td>91/91 (100%)</td>
<td>89/89 (100%)</td>
</tr>
<tr>
<td><strong>Core Ag</strong></td>
<td>85/94 (90%)</td>
<td>84/90 (93%)</td>
<td>79/89 (89%)</td>
<td>79/91 (87%)</td>
<td>81/89 (91%)</td>
</tr>
<tr>
<td><strong>HCV RNA</strong></td>
<td>66/66 (100%)</td>
<td>72/74 (97%)</td>
<td>75/75 (100%)</td>
<td>74/75 (99%)</td>
<td>67/67 (100%)</td>
</tr>
</tbody>
</table>

- Core Ag somewhat less sensitive (low titre samples)
- Handling conditions had no effect on HCV Ab or RNA (with 2 spots)

Van Tilborg *In Preparation*
DBS in remote settings

- High burden of HCV in Canadian Aboriginal populations
- Very remote communities → no road access
- Very limited resources

HCV Screening
- Community leaders (Chief & council) support
  - Peer screeners → DBS
  - Peer & RN counseling

Linkage to care
- Local MD/RN – treatment with ECHO model
- OST clinics
RDT/POCTs not suitable for all settings

Rapid testing is not always ‘rapid’ – pre and post-test counseling + linkage
Matching the test to the setting

• **Current model** (Ab then RNA or Ab reflex RNA)
  – Boomer/hospital screening, OST clinics → Reliable F/U

• **RDT/POCT blood** (Ab)
  – Above + Screening drives, prison

• **POCT saliva** (Ab)
  – Screening drives, opportunistic screening (ER, prison)

• **RDT (RNA or viremia – core Ag)**
  – Very high prevalence population – active PWID, ?OST

• **DBS**
  – Rural remote (no lab), hard-to-reach, time issues (ER)
Conclusions

• Current testing approach is cumbersome and not ideal for high volume screening
  – Leads to drop-offs in cascade of care
• New tests increasingly available
  – Rapid – Ab and RNA, blood and saliva – WHO prequalified
  – More tests in the pipeline…cheaper, similar/better performance
  – Core Ag testing could be used more (not rapid yet)
  – DBS – not rapid but attractive in many settings – would be nice to add APRI for fibrosis assessment to DBS…
• Future tests may use new platforms – multiplex HIV/HBV and HCV
• Different tests preferred in different settings
  – Niklas…show us how they’re being used around the world!