Point-of-Care (PoC) Testing: Hepatitis B and C

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Hepatitis B – Global Summary

• 248 million people HBsAg-positive (Ott 2012; Schweitzer 2015)

• 786,000 attributable deaths from hepatitis B annually (Lozano 2012)

• Without appropriate management, 15-25% of people with CHB will develop advanced liver disease &/or HCC (Lavanchy 2004)

• Vast majority unaware of their HBsAg status
Hepatitis C – Global Summary

• HCV - globally > 184 million infected \( (Mohd\ Hanafiah\ 2013) \)

• In limited resource countries < 1% aware of having infection

• 499,000 attributable deaths from hepatitis C annually
  - 1.3 million from hepatitis B & C collectively \( (GBD\ 2010;\ Lozano\ 2012) \)

• Viral hepatitis the 9th ranked cause of human death; similar numbers of deaths to HIV, malaria and TB \( (GBD\ 2010;\ Lozano\ 2012;\ Cowie\ 2013) \)

• Liver cancer is the 3rd most common cause of cancer death globally \( (Lozano\ 2012) - \) GBD 2013 - 2nd most common)
Hepatitis C Cure

• No refractory or archived genetic material

• Effective new Direct Acting Agents (DAAs)
  – Still evolving
  – Pan-genotypic
  – 8-12 week treatment regimens (>90% cure rates)
  – Low cost treatments to limited resource countries
  – < $1,000 per cure

• Cure bottleneck - need **DIAGNOSIS** before treatment
Alternative HCV Diagnostic Tools

• Dried Blood Spot (DBS) testing

• Point-of-Care (PoC) testing

Ideally suited to fingerprick blood
Dried Blood Spot (DBS) Testing

- Around > 50 years (Guthrie cards)
- In viral studies, DBS mostly used for antibody testing
- More recent applications for HIV, HBV & HCV in:
  - Viral load testing
  - Genotyping (*HIV Aitken 2015; HBV Vinikoor 2015; HCV Greenman 2015*)
- Protocols available for elution of DNA/RNA
  - QIAGEN – manual method with kit
  - Abbott *m2000* – semi-automated
  - Hologic Panther - automated
DBS – Advantages & Disadvantages

• Advantages
  – Minimal invasiveness (fingerprick)
  – Low volume required
  – No specialized equipment
  – Ease of storage and transport (room temperature)

• Disadvantages
  – Reduced sensitivity
  – Long term storage requires -20°C for DNA/RNA
  – No standardized approach for sampling, storing, and processing DBS samples

Greenman 2015 HCV JVH
What is Point-of-Care Testing?

- Test near or on-site of the patient
- Quick result turnaround
  - Patient available for counselling
  - Reduce loss to follow-up
  - Allows immediate decisions about patient care
- Helpful in remote and regional areas that lack access to traditional lab-based testing
- In well resourced countries, PoC testing can also be useful in disenfranchised high-risk populations
Point-of-Care Tests
WHO Guidelines - ASSURED

• **A** Affordable
• **S** Sensitive
• **S** Specific
• **U** User-friendly
• **R** Rapid
• **E** Equipment-free
• **D** Delivery

* WHO Mantra - Screening linked to care
Point-of-Care Tests

1. Lateral flow immunoassay
   - Most common PoC test – usually simple capture format
   - In ID, used for antibody/antigen detection (HIV, TB, malaria)
   - Detection usually visual – qualitative
   - Built in Quality Control checks
   - Can be adapted to quantification using hand-held devices which incorporate multichannel light detectors
   - Signal amplification – fluorescence, luminescence

Drawbacks
   - Large Coefficient of Variation (CV)
   - Reduced sensitivity
   - Inability to multiplex (additional strips, cartridges required)
Point-of-Care Tests for Anti-HCV

- Meta-analysis conducted on 30 anti-HCV PoC tests
- Several CE-marked, currently only one FDA approved
  - OraQuick Rapid Antibody Test
- Nine of 30 had poor sensitivity and specificity
- OraQuick had highest sensitivity and specificity

(Khuroo³ 2015 PLoS One)
OraQuick Test Results

- Two lines:
  - “C” - Control line
  - “T” - Test line
- Negative result
- Reactive result
- Invalid result
Point-of-Care Test – HCV cAg

- HCV cAg testing is available as a conventional lab-based chemiluminescent EIA (Abbott)

- Alternative to molecular-based HCV RNA assays

- Lower cost, faster turnaround but reduced sensitivity

- Potential to be developed as a PoC test

Tillmann 2014 WJ Gastro
HCV Load determined by the Bayer Versant HCV RNA 3.0 bDNA test
From the table, the average viral load is approximately 500,000 IU/mL
Daktari Enters Collaboration Agreement with Merck to Develop Test for Hepatitis C Virus

Cambridge, MA (Daktari Diagnostics)
August 31, 2015

Daktari Diagnostics today announced a collaboration with Merck, known as MSD outside the United States and Canada, to develop Daktari’s rapid hepatitis C virus (HCV) screening test. The deal, worth up to $50 million over the next 3.5 years, will support an accelerated development timeline for the clinical validation and regulatory approval of Daktari’s HCV test.

The Daktari technology forms the basis for a point-of-care instrument that can detect low levels of virus directly in a single drop of blood in approximately 30 minutes, making on-the-spot HCV treatment decisions possible. The Daktari test is based on high-sensitivity measurement of the HCV core antigen, which is used in Europe and Japan for the diagnosis of chronic hepatitis C infection, but has never been available as a point-of-care diagnostic.

The Daktari™ System includes an embedded connectivity platform, Daktari InSight, which provides real-time data management through mobile networks, connectivity and a web-based dashboard, allowing rapid monitoring of test results. The Daktari™ System has the potential to transform public health screening programs and be made available at retail clinics, pharmacies, and doctor’s offices.

“For many patients chronic hepatitis C has become a curable disease,” said Bill Rodriguez, M.D., Founder and CEO of Daktari. “Merck’s collaboration provides support for an accelerated development and regulatory timeline for our HCV diagnostic.”

Globally, HCV is “severely underdiagnosed.” Some 130 to 150 million people are infected with chronic HCV worldwide, including 2.7 million people in the United States, but fewer than 2 percent are aware of their infection. Simpler screening tests are expected to greatly expand diagnosis of individuals infected with HCV who need treatment. The World Health Organization recommends that HCV testing be offered in settings of high HCV prevalence or to people at risk for HCV: in the United States, the Centers for Disease Control and the U.S. Preventive Services Task Force (USPSTF) both recommended HCV testing for all adults born between 1945 and 1965, regardless of risk, and persons of all ages who are at risk for HCV infection.

About Daktari

Daktari Diagnostics, Inc. (www.daktaridx.com) is a venture-backed company based in Cambridge, USA. Daktari’s mission is to address the world’s biggest health problems through a new generation of simple, affordable molecular diagnostic tests that can be deployed anywhere in the world. The Daktari connectivity platform, Daktari InSight, provides real-time data management through mobile network connectivity and a web-based dashboard, allowing instantaneous monitoring of test results and disease outbreaks from any health facility in the world. In addition to hepatitis C, HIV, and other infectious diseases, Daktari is currently developing tests for tuberculosis and influenza.

Telephone: +1 617 336 3299  •  Web: www.daktaridx.com  •  Email: media@daktaridx.com

ChipCare Device - HIV
(www.chipcare.ca)
Point-of-Care Tests - Molecular

2. Microfluidics – Lab-on-a-Chip & Molecular PoC tests

The Device, Gene Xpert (Cepheid)

- Single use cartridges
- Extraction and amplification: in the cartridge
- Fully Automated

Available for some infectious diseases – not yet for HBV
Do not yet meet ASSURED criteria
**BASIS**

All molecules vibrate at particular frequencies depending on the strength of the bond and masses of the atoms. The absorption of infrared light at these frequencies gives rise to peaks in the infrared spectrum. The infrared spectrum is therefore a snapshot of the entire chemistry of the sample. Because viruses have specific nucleic acids, proteins, glycoproteins, etc. we can diagnose them directly in a blood sample.

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<td>Phospholipids, DNA</td>
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<tr>
<td>3000-3020</td>
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**EXPERIMENTAL**

1) Analysis performed on whole blood and serum from blood collected in heparinised tubes

2) 1-5 μL of sample is deposited onto the ATR crystal and dried or alternatively deposited on a glass or paper based substrate and then dried. The spectra is measured in 1 minute

3) Spectra are compared with a database in order to investigate the presence/phenotype of the infectious agent
RESULTS

Principle Component Analysis (PCA) scores plots showing each spectrum (sample) represented as a point in 2D space. Although the sample number is small a clear separation is observed between HBV, HCV and HIV infected samples demonstrating the potential of the technology as a point-of-care diagnostic.

Slides courtesy of Bayden Wood, School of Chemistry, Monash University
Hepatitis B Cure

- cccDNA difficult to eliminate
- Integrated HBV DNA

But

HBsAg loss/seroconversion = functional cure
Point-of-Care Tests HBV

- Lateral flow technologies for:
  - HBsAg, anti-HBs, anti-HBc, HBeAg, anti-HBe

Ideally, molecular testing for HBV DNA
REVEAL data – association of viral load with cirrhosis & HCC

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<td>258</td>
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HBsAg titers are indicated under each strip in IU/ml. The positive control line is on the right with the sample line on the left.
Frequency Distribution of HBsAg Titres in Serum Samples

median = 2.8
n = 7380
LOD = 0.7
Point-of-Care - HBsAg

- Opportunity for counselling
- Allows management of contacts
- Reduce maternal transmission
- Indicative of the risk of liver disease progression
- Used for serosurveys in limited resource countries
Quantification of HBsAg

- High levels in HBeAg-positive indicative of immune tolerance

- Low levels in HBeAg-positive pregnant women indicative of low viral load and reduced risk of mother-to-baby transmission with infant vaccination

- Low levels in HBeAg-negative indicative of low risk of HCC

- On-treatment response marker for interferon-based therapy

_Honer zu Siederdissen & Cornberg 2014 Ann Gastro_
HBV Treatment Landscape in 2015

1990: Interferon alfa-2b (IFN)
1998: Peginterferon alfa-2a (PEG-IFN)
1998: Lamivudine (LMV)
2002: Entecavir (ETV)
2002: Adefovir (ADV)
2005: Tenofovir (TDF)
2006: Telbivudine (LdT)
2008: Tenofovir (TDF)
<table>
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<tr>
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<td>cccDNA inhibitors</td>
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<td>TetraLogic</td>
<td>Phase 1</td>
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</tbody>
</table>
Together for Cure: International Coalition to Eradicate HBV - ICE-HBV

ICE-HBV will promote and advocate for the four pillars of HBV cure research worldwide.

Revill, Testoni, Locarnini, Zoulim. 2015 Nature Reviews - Gastroenterology and Hepatology Perspectives In Press