Evaluating novel diagnostics in an outbreak setting: lessons learned from Ebola

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Development and evaluation of novel diagnostic tools

- Opportunities and challenges, throughout the development cycle
  - Proof of principle demonstration
    - Novel platform technologies
    - Novel assays
    - Both
  - Method comparison
    - POC: how “good” does the POC test need to be, given potential advantages of speed, cost, and/or ease of use?
    - All new tests: how “good” are the gold standards?
- Clinical validation
  - “Controlled settings”
  - “The field”
Laboratory diagnosis of Ebola Virus Disease (EVD), 2014-16 epidemic: challenges

- Testing, if available: standard high-complexity RT-PCR performed in biocontainment laboratories

- **Specimen collection challenges**
  - Resource limitations: unsteady supply chain for venipuncture and packaging materials
  - Inadequate training
    - Compromised safety of those handling blood
    - Compromised sample integrity (hemolysis, cracked tubes)
  - Inadequate sample volume

- **Logistic challenges**
  - Incomplete specimen submission forms
  - Lack of unique identifiers
  - Sample transport delays
  - Results reporting delays

- Results could take days to return to clinical sites
- Potential for diagnostic errors
EVD Outbreak: 2014

- International mobile lab deployment robust—but access remained inadequate in many areas

- Urgent clinical need for rapid sample-to-answer/POC tests that could be run by less-experienced operators
  - Explosion in test development
  - Acute need for test evaluation

- Winter, 2014: was asked to help Partners In Health urgently develop field studies in Sierra Leone to evaluate most promising novel diagnostics
  - Funding available from the Abundance Foundation
Novel diagnostic evaluation: what I knew before the Ebola outbreak

- **Consider all variables**
  - Sample handling
  - Case definition
  - Choice of reference test
  - Cutoffs
  - Disease status
  - Co-morbidities
  - Site of test implementation
  - Operator

- **Expect the unexpected**
  - Lot-to-lot variability
  - Difference between results in fingerstick (FS) vs venipuncture samples
  - Analytical interferents
  - Variation between reference methods
    - Which reference method gives the “right” answer?
  - Challenges of testing “in the field” versus a “controlled setting”
Novel diagnostic evaluation: what I learned from the Ebola outbreak

- There are systematic challenges to evaluating novel diagnostics in an emergency setting.
- Without collective consideration of these challenges and their solutions, development and evaluation of diagnostics in future outbreak scenarios will be similarly handicapped.
- We need to address these issues now so that in the future we can respond more rapidly-- and save more lives.
- A Global Emergency Diagnostic Framework is needed.

Pollock and Wonderly, JCM 2017
Ebola Dx evaluation landscape: snapshot, winter 2014

- **Multiple novel assays under development--?which to prioritize?**
  - Novel assay AND novel platform—too risky?
  - Assays/platforms never tested in this version of “the field”
  - Production capability, commercial viability variables
    - Only want to evaluate tests with potential for use!
  - Assay development and planning for evaluation(s) in parallel
    - Impact on sample type, SOPs for POC/lab testing
    - Impact on protocol design/IRB approval process
Ebola Dx evaluation landscape: snapshot, winter 2014

- **Multiple RT-PCR assays in use**—optimal reference method?
  - At start of outbreak, no EVD Dx with either FDA or WHO approval
  - Multiple LDTs: CDC, DoD, PHE, “home brews”
  - Nov 2014: first WHO EUAL for commercial assay (altona RealStar Filovirus Screen RT-PCR Kit 1.0)
  - Nov 2014: first FDA EUA for commercial assay (altona RealStar Ebolavirus RT-PCR Kit 1.0)

- **Insufficient data sharing** re: design and performance to guide choice of reference method
Ebola Dx evaluation landscape: snapshot, winter 2014

- **Multiple regulatory and governmental bodies (who’s in charge?)**
  - WHO, FDA, CDC, DoD, PHE, DfID
  - Sierra Leone (SL): SL Ethics and Scientific Review Committee, SL Pharmacy Board, SL National Laboratory Technical Working Group
  - Formal vs informal processes for study approval
  - Processes for regulatory approval—how to anticipate

- **Unique biosafety considerations for sample collection and testing**

- **Outbreak scenario: speed essential**
Step 1: learn (quickly) about diagnosis of Ebola
Traditional testing methods

- Ebola virus (EBOV): Single-stranded RNA genome encoding seven viral proteins
  - Five known Ebola virus species: key is Ebola-\textit{Zaire}
- Traditional gold-standard: cell culture (BSL-4, slow)
- Serologic tests: minimal utility in acute disease
  - Variable onset of Ab responses, persistent IgG in survivors
- Protein antigen detection by ELISA
  - Viral proteins accumulate to detectable levels in blood within a few days of disease onset
- RT-PCR
  - more sensitive than serology/ELISA
  - Lower Ct values (higher viral RNA copy numbers) associated with higher mortality
  - Can detect viral RNA in other sample types (saliva, seminal fluid)

<table>
<thead>
<tr>
<th>Analyte (method)</th>
<th>Outcome</th>
<th>Incubation period</th>
<th>Acute illness</th>
<th>Convalescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG (ELISA)</td>
<td>Non-fatal</td>
<td>2-21 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fatal</td>
<td></td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>IgM (ELISA)</td>
<td>Non-fatal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fatal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antigen (ELISA)</td>
<td>Non-fatal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fatal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RNA (RT-PCR)</td>
<td>Non-fatal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fatal</td>
<td></td>
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</tr>
</tbody>
</table>

- **IgG antibodies** are detectable for years following infection; loss of IgG antibodies has not been characterized.
- **IgM antibodies** are undetectable by day 80 of illness in nearly all patients; however, IgM antibody detection has been reported at day 168 (17).
- **Viral antigen and RNA** levels increase until the time of death in fatal cases, with death typically between days 6-14 of illness (2,4,10,11,17,18,35,36).
Reducing time to diagnosis of EVD is key

- Clinical management and infection control
  - Clinical presentation is non-specific
  - Separation of patients while awaiting diagnostic results is challenging
- Discharge and community re-integration of non-EVD patients and survivors
  - Negative test results often required for acceptance and receipt of health care at non-Ebola facilities
- Testing of dead bodies
  - Allowing families to proceed with desired burial practices
  - Assist with surveillance and contact tracing
- Contact tracing

Step 2: design and execute (quickly) studies of the most promising Ebola Dx—in the hopes that they can be used to save lives
Fall, 2014: what was needed?

- EVD diagnostics that were safe, rapid, and cost-effective (from user perspective)\(^1\)
- Useable at or near the POC
- Performed by local laboratory technicians and/or healthcare workers

\(^1\) World Health Organization. Target Product Profile for Zaire ebolavirus rapid, simple test to be used in the control of the Ebola outbreak in West Africa. 03 October, 2014.
Field Evaluation of the Corgenix ReEBOV Antigen Rapid Test Kit
Sierra Leone

- RDT selected in consultation with the WHO/FIND Ebola Diagnostics Access Collaboration
- Detects the EBOV VP40 matrix protein
  - Zaire, Sudan, Bundibugyo
- Dipstick immunoassay, designed for testing of whole blood (FS or venipuncture) or plasma
- No external instrumentation
- Requires cold chain

Broadhurst et al (Pollock), Lancet 2015; kits provided by Corgenix
Study Design

- **Data collection, RDT**
  - POC testing of FS blood from *EVD suspects* (PIH sites)
    - Test performed at bedside, by MoHS technicians, in “red zone” (full PPE)
  - Reference lab (RL) testing of consecutive venous WB (PHE Port Loko)
    - Test performed in flexible film isolator

- All RDT results compared (blinded) to clinical test results on contemporaneous plasma (RT-PCR, altona assay, performed in PHE laboratory)

- All RDTs read by 2 operators; disagreements resolved by a 3rd reader

Broadhurst et al (Pollock), Lancet 2015
POC testing: PIH clinical site, February 2015
Key Study Conclusions

- Corgenix ReEBOV RDT had 100% sensitivity and 92% specificity against the benchmark of the altona RT-PCR
  - Two readers required to achieve this sensitivity
- Feasible to carry out the test in the red zone
  - FS performed successfully despite dehydration, PPE
  - Technicians able to perform and read test with minimal training
- Inter-operator agreement at POC was high (95%)
- Test reliably detected all patients with Ct values $\leq 26.3$ (all expected to be highly infectious)

HOWEVER:

Broadhurst et al (Pollock), Lancet 2015
Study conclusions, continued

- altona assay used in this study was an imperfect reference standard
  - Determined by comparison to PHE LDT (Trombley)

- altona sensitivity suboptimal in specimens with Trombley Ct >30
  - ? due to extraction and amplification (SmartCycler II) methodology (selected for speed, cost, biosafety, efficiency..)\(^1,2\)

- **Use of this reference standard caused us to overestimate the true sensitivity and underestimate the true specificity of the ReEBOV RDT...but by how much?**
  - Unable to retest all samples by Trombley

- How would the RDT perform in a larger set of samples with Ct>30?
- What is the clinical significance of viral loads in this range?\(^3\)

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Integration of the RDT into testing algorithms……?

• Disagreement between WHO/FIND and many “on the ground” regarding use, given imperfect sensitivity and specificity

• Concern about misunderstanding/misuse of our data

• Difference between our data and lab data produced by WHO
  • Sensitivity 91.8%, specificity 84.6% in frozen plasma/fresh WB
  • No methodological details provided to allow comparison

• Approval of in-country regulatory bodies required (and process uncertain), regardless of EUA status
  • E.g. Sierra Leone Ministry of Health and Sanitation, Laboratory Technical Working Group, Pharmacy Board

• →Stalemate (and some frustration)……
OraQuick Ebola RDT *used in DRC*


**FDA package insert, Oct 2019:**

- Frozen venous WB from SL: PPA 84%, NPA 98%
- Specificity in non-endemic venous WB/FS controls: 100%
- Positive FS/venous samples (non-human primate): 20/0% detection D3; 100/60% detection D5; 100% D7-8
- Cadaveric samples (WHO) (frozen, retested by EUA PCR in parallel): PPA 97.1%, NPA 100%
- Cadaveric samples (CDC) (field?lab testing, PCR negatives only): NPA 100%
- Pos/neg results must be confirmed by additional testing

- CDC surveillance of “low-risk” febrile patients (n = 1000), Guinea²; all RDT neg (no RT-PCR performed)

**No data for accuracy, operational feasibility, or inter-reader reliability when performed at POC in Ebola care centers... wasn’t ready in time**

(1) OraQuick Ebola IFU, Oct 2019; (2) Huang et al, MMWR 2016
GeneXpert Ebola assay  
*(used in DRC)*

- GeneXpert Ebola (Cepheid)
  - FDA EUA: March, 2015
  - WHO EUAL: May, 2015
  - CE-IVD: August, 2015

- Automated “sample-to-answer” system
  - Outside cartridge: sample inactivation
  - Inside cartridge: sample processing, NA extraction, amplification, detection

- Targets: EBOV NP and GP genes

- Samples placed directly into sample reagent (SR) vial for >20'; aliquot loaded into cartridge, run on Xpert instrument
PHE/PIH study (Sierra Leone): Sensitivity and specificity of Xpert Ebola versus Trombley assay performed on clinical WB and BS samples

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Adjusted Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N = 211)</td>
<td>22/22</td>
<td>181/189</td>
<td>181/182</td>
</tr>
<tr>
<td></td>
<td>(100; 84.6-100)</td>
<td>(95.8; 91.8-98.2)</td>
<td>(99.5; 97.0-100)</td>
</tr>
<tr>
<td>Buccal swab</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N = 64)</td>
<td>20/20</td>
<td>44/44</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>(100; 83.2-100)</td>
<td>(100; 92.0-100)</td>
<td></td>
</tr>
</tbody>
</table>

*revised based on discrepant analysis; 7/8 Xpert+/Trombley-WB samples were from previously Trombley-positive EVD patients receiving follow-up testing to monitor for clearance of viremia (Xpert Ct 37.7-43.4)

Semper et al (Pollock), PLoS Medicine, 2016; kits provided by Cepheid
MSF Xpert study, Guinea

- Xpert performed in MSF Ebola treatment center (TC)
  - Benchmark was LDT performed at nearby national ref lab
  - Xpert had **100% sensitivity, 96.0% specificity** in fresh venous WB specimens (n=218; 26 pos)
  - All discordant specimens (LDT neg/Xpert pos) were from convalescing EVD patients
  - Demonstrated feasibility of operating Xpert platform at site of patient care; however, biosafety/logistical concerns noted (?? blood on inner threads of SR vial)
Laboratory diagnosis of EVD in the 2014-16 outbreak: collective accomplishments

- ~40 international laboratories ultimately deployed to west Africa
  - All performing real-time RT-PCR
  - All maintaining rigorous biocontainment

- At start of outbreak no EVD diagnostics had regulatory approval for clinical use; by May, 2016, multiple tests had WHO/FDA EUA (next slide)

- A small number of field studies were successfully performed for novel test evaluation
<table>
<thead>
<tr>
<th>Assay name (manufacturer)</th>
<th>FDA/WHO EUA</th>
<th>Assay format</th>
<th>Detection target (Ebola species)</th>
<th>Approved specimen types under EUA</th>
<th>Pre-analytic processing (approved kits/reagents)</th>
<th>Approved instrument platform(s) under EUA</th>
<th>Facility restrictions for FDA EUA</th>
<th>Infrastructure requirements¹</th>
<th>Time to results²</th>
</tr>
</thead>
<tbody>
<tr>
<td>RealStar Filovirus Screen RT-PCR Kit 1.0 (altana Diagnostics GmbH)</td>
<td>WHO</td>
<td>Real-time RT-PCR</td>
<td>L gene RNA (EBOV, BDBV, RESTV, SUDV, TAFV)</td>
<td>Plasma</td>
<td>Sample lysis, RNA extraction (QIAamp Viral RNA Mini Kit, Qiagen)</td>
<td>ABI Prism 7500 SDS and 7500 Fast SDS (Applied Biosystems), LightCycler 480 II (Roche), CFX96 system/Dx (Bio-Rad), Mx 3005P QPCR (Stratagene), Rotor-Gene 3000/6000 (Corbett Research), Rotor-Gene Q 5/6 plex (Qiagen), Versant kPCR Molecular System AD (Siemens)</td>
<td>N/A</td>
<td>-20°C storage, UPS</td>
<td>4-6 hours</td>
</tr>
<tr>
<td>RealStar Ebolavirus RT-PCR Kit 1.0 (altana Diagnostics GmbH)</td>
<td>FDA</td>
<td>Real-time RT-PCR</td>
<td>L gene RNA (EBOV, BDBV, RESTV, SUDV, TAFV)</td>
<td>Plasma</td>
<td>Sample lysis, RNA extraction (QIAamp Viral RNA Mini Kit, Qiagen)</td>
<td>ABI Prism 7500 SDS and 7500 Fast SDS (Applied Biosystems), LightCycler 480 II (Roche), CFX96 system/Dx (Bio-Rad)</td>
<td>CLIA high complexity or similar qualification</td>
<td>-20°C storage, UPS</td>
<td>4-6 hours</td>
</tr>
<tr>
<td>EZ1 Real-time RT-PCR Assay (US Dept of Defense, LDT)</td>
<td>FDA</td>
<td>Real-time RT-PCR</td>
<td>GP gene RNA (EBOV)</td>
<td>Venous WB, plasma</td>
<td>Sample lysis, RNA extraction (QIAamp Viral RNA Mini Kit, Qiagen)</td>
<td>ABI Prism 7500 Fast SDS (Applied Biosystems), LightCycler 480 II (Roche), JBAIDS instrument (BioFire Defense)</td>
<td>Laboratories designated by the US DoD</td>
<td>-20°C storage*, UPS</td>
<td>4-6 hours</td>
</tr>
<tr>
<td>CDC Ebola Virus NP Real-time RT-PCR Assay (US CDC, LDT)</td>
<td>FDA</td>
<td>Real-time RT-PCR</td>
<td>NP gene RNA (EBOV)</td>
<td>Venous WB, plasma, serum, urine (if paired with blood)</td>
<td>Sample lysis, RNA extraction (MagMax Pathogen RNA-DNA kit, Applied Biosystems; Dynal Bead Retriever, Invitrogen)</td>
<td>ABI Prism 7500 SDS and 7500 Fast SDS (Applied Biosystems), CFX96 system/Dx (Bio-Rad)</td>
<td>Laboratories designated by the US CDC</td>
<td>-20°C storage*, UPS</td>
<td>4-6 hours</td>
</tr>
<tr>
<td>CDC Ebola Virus VP40 Real-time RT-PCR Assay (US CDC, LDT)</td>
<td>FDA</td>
<td>Real-time RT-PCR</td>
<td>VP40 gene RNA (EBOV)</td>
<td>Venous WB, plasma, serum, urine (if paired with blood)</td>
<td>Sample lysis, RNA extraction (MagMax Pathogen RNA-DNA kit, Applied Biosystems; Dynal Bead Retriever, Invitrogen)</td>
<td>ABI Prism 7500 SDS and 7500 Fast SDS (Applied Biosystems), CFX96 system/Dx (Bio-Rad)</td>
<td>Laboratories designated by the US CDC</td>
<td>-20°C storage*, UPS</td>
<td>4-6 hours</td>
</tr>
<tr>
<td>LightMix Ebola Zaire rRT-PCR Test (TIB MOLBIOL Syntheselabor GmbH)</td>
<td>FDA</td>
<td>Real-time RT-PCR</td>
<td>L gene RNA (EBOV)</td>
<td>WB</td>
<td>Sample lysis (TriPure, Roche), RNA extraction (MagNA Pure 96 DNA and Viral Nucleic Acid Kit, Roche; High Pure Viral Nucleic Acid Kit, Roche)</td>
<td>LightCycler 480 (Roche), cobas z 480 Analyzer (Roche)</td>
<td>CLIA high complexity or similar qualification</td>
<td>2-24°C storage, UPS</td>
<td>4-6 hours</td>
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<tr>
<td>Liferiver – Ebola Virus (EBOV) Real Time RT-PCR Kit (Shanghai ZJ BioTech Co, Ltd)</td>
<td>WHO</td>
<td>Real-time RT-PCR</td>
<td>NP gene RNA (EBOV, SUDV, TAFV, BDBV)</td>
<td>WB, plasma, serum</td>
<td>Sample lysis, RNA extraction (QIAamp Viral RNA Mini Kit, Qiagen; QIAamp DSP virus Spin kit, Qiagen; Liferiver RNA isolation kit, Liferiver)</td>
<td>ABI Prism 7500 SDS and 7500 Fast SDS (Applied Biosystems), LightCycler 480 II (Roche), CFX96 system/Dx (Bio-Rad), SLAN-96 (Hongsh)</td>
<td>N/A</td>
<td>-20°C storage*, UPS</td>
<td>4-6 hours</td>
</tr>
</tbody>
</table>

¹ Infrastructure requirements may vary based on the specific testing environment.

² Time to results may vary based on the specific testing environment.
<table>
<thead>
<tr>
<th>Test Name</th>
<th>Approval Body</th>
<th>Automation</th>
<th>Target RNA</th>
<th>Sample Type</th>
<th>Target Inactivation</th>
<th>Instrument</th>
<th>Lab Qualification</th>
<th>Storage</th>
<th>Handling</th>
</tr>
</thead>
<tbody>
<tr>
<td>FilmArray NGDS BT-E Assay (BioFire Defense, LLC)</td>
<td>FDA</td>
<td>Automated</td>
<td>L gene RNA (EBOV)</td>
<td>WB, plasma, serum</td>
<td>Inoculation of sample injection vial containing viral inactivation buffer</td>
<td>FilmArray instrument (Biofire)</td>
<td>Laboratories designated by the US DoD</td>
<td>15-25°C storage*, UPS</td>
<td>75 minutes</td>
</tr>
<tr>
<td>FilmArray Biothreat-E Test (BioFire Defense, LLC)</td>
<td>WHO, FDA</td>
<td>Automated</td>
<td>L gene RNA (EBOV)</td>
<td>WB: urine (if paired with blood)</td>
<td>Inoculation of sample injection vial containing viral inactivation buffer</td>
<td>FilmArray instrument (Biofire)</td>
<td>CLIA moderate or high complexity or similar qualification</td>
<td>15-25°C storage UPS</td>
<td>75 minutes</td>
</tr>
<tr>
<td>Xpert Ebola Assay (Cepheid)</td>
<td>WHO, FDA</td>
<td>Automated</td>
<td>NP, GP gene RNA (EBOV)</td>
<td>Sample inactivation</td>
<td>GeneXpert platform (Cepheid)</td>
<td>CLIA moderate or high complexity or similar qualification</td>
<td>2-28°C storage, UPS</td>
<td>100 minutes</td>
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<tr>
<td>ReEBOV Antigen Rapid Test (Corgenix, Inc)</td>
<td>WHO, FDA</td>
<td>Chromatographic</td>
<td>VP40 protein antigen (EBOV, SVUD, BDBV)</td>
<td>None</td>
<td>N/A</td>
<td>Adequately equipped facilities, including treatment centers and public health clinics</td>
<td>2-8°C storage</td>
<td>15-25 minutes</td>
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<tr>
<td>OraSure Ebola Rapid Antigen Test (OraSure Technologies, Inc)</td>
<td>WHO, FDA</td>
<td>Chromatographic</td>
<td>VP40 protein antigen (EBOV, SVUD, BDBV)</td>
<td>None</td>
<td>N/A</td>
<td>Adequately equipped facilities, including treatment centers and public health clinics</td>
<td>2-30°C storage</td>
<td>30 minutes</td>
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<tr>
<td>SD Q Line Ebola Zaire Ag (SD Biosensor, Inc)</td>
<td>WHO</td>
<td>Chromatographic</td>
<td>VP40 protein antigens (EBOV)</td>
<td>None</td>
<td>N/A</td>
<td>N/A</td>
<td>1-40°C storage</td>
<td>20-30 minutes</td>
<td></td>
</tr>
</tbody>
</table>

*Storage temperature requirements are taken from the following package inserts, except where otherwise indicated: Real Star Filovirus Screen RTPCR Kit v1.0 IVD package insert, August 2014; Real Star Filovirus RT/PCR Kit v1.0 EUA package insert, November 2014; LightMix Ebola & Zaire RT/PCR Test v1.0 EUA package insert, MDx407 0666796; FilmArray Biothreat-E EUA package insert, PRF1/FRT030201, October 2014; Xpert Ebola Assay IVD package insert, 3014826, Revision A, June 2015; ReEBOV Antigen Rapid Test EUA package insert, 14005701, March 2015; OraQuick Ebola Rapid Antigen Test EUA package insert, March 2016; SD Q Line Ebola Zaire Antigen Test EUA package insert, R120150901, Lidd, September, 2015. For assays without accessible package inserts (marked with '*'), storage requirements were taken from the WHO guidance document on Ebola in vitro diagnostic assays (S1). |

1 Including preanalytic processing, if applicable! |
2 Venipuncture whole blood, swab biofingertick blood, and swab biofloral fluid are all listed in the CE IVD approved package insert (June 2015), while only venipuncture whole blood is included in the FDA and WHO EUAs! |
Pre-qualification (WHO)/IVD (FDA) status as of January 2020

- WHO: in absence of Ebola Public Health Emergency of International Concern (PHEIC), encourages manufacturers of products listed through EUAL to apply for full prequalification
  - No products yet on WHO prequalified list
  - WHO EUAL products can still be purchased/used

- FDA: only OraQuick has full regulatory approval\(^1\)
  - Ebola remains an active Emergency Situation so EUA authorizations remain active and products can be purchased\(^2\)
  - EUA\(\rightarrow\)full regulatory approval: TBD!

\(^{1}\) [https://www.fda.gov/media/131706/download](https://www.fda.gov/media/131706/download); \(^{2}\) [https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations](https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations).
Even if approved, Ebola diagnostics may not be accessible.

Of the 7 PCR-based tests investigated, only 4 were **readily available** (<2 weeks lead time).

OraQuick **available in DRC** but only through CDC or WHO.

The larger problem....

- Throughout the outbreak, lack of clarity regarding local and global processes and priorities, in addition to unique biosafety considerations for sample collection and testing, led to substantial delays in the overall diagnostic evaluation process.

- Tests could not be developed, evaluated, approved, and implemented fast enough to save lives.

- We can and should do better.
Specific challenges and questions encountered during evaluation of novel diagnostics during the Ebola outbreak
Sample ownership

- Who “owns” clinical samples, once tested?
  - The gov’t of country where patients were tested (e.g. SL, for testing performed there)?
  - The gov’t of country whose patients were tested (e.g. Guinea, for Guinean patients tested in SL)?
  - The gov’t of country directing lab in which samples were tested (e.g. UK, operating in SL)?
  - The organization responsible for testing and storing patient samples (e.g. PHE, operating in SL)?
  - The organization funding the lab testing/sample storage? (e.g. DFID)
  - The WHO or other global governing body?
  - The patient?

Pollock and Wonderly, JCM 2017
Sample ownership, continued

- Can samples be shipped out of the country of origin for test development (or research use) elsewhere?
- Should test developers have to pay for clinical samples?
- Who decides if—and with whom—samples should be shared?
- Who determines research priorities?
- What efforts receive priority?
  - Academic researchers?
  - Companies developing tests?
Data ownership—all the same questions

- Who owns data generated as a result of clinical testing? (e.g. clinical results, operational data)

- Can clinical data be transmitted out of the country of origin for test development, optimization of disease management, or research use elsewhere during an outbreak?

- Should data consumers (WHO, test developers, etc.) have to pay for data access?

- Who decides if--and with whom--data should be shared?

- Who pays for data transmission, where applicable, and what system(s) should be used for transmission of clinical data?

- What efforts receive priority?
  - Disease management?
  - Academic research?
  - Commercial development of tests, therapies, or vaccines?

Pollock and Wonderly, JCM 2017
Human subjects

- Do patients need to provide consent for research testing done on their own excess clinical samples?
  - Legislation being considered in US
  - Logistically, how would this be carried out?
    - Clinical condition/competency for consent
    - Lack of literacy/education/understanding
    - Language/cultural barriers

- Which bodies in-country need to sign off on a human subjects research protocol?
  - Formal, vs informal (courtesy) notifications
  - E.g. SL: MOHS, LTWG, Pharmacy Board?
Regulatory authority

- WHO EUAL vs FDA EUA: will countries accept either or neither as sufficient for in-country use of a product?

- Will countries require additional in-country data to be generated prior to in-country approval for use?

- Which group, within a given country, is legally responsible for clearing a test for clinical use?
  - E.g. SL: LTWG, Pharmacy Board, both? Role of MOHS?
Regulatory authority

- How do manufacturers validate their system when access to samples is limited and biosafety concerns complicate testing?

- Who should be responsible for organizing/facilitating test validation?
  - Who decides which tests to prioritize for evaluation?

- Which approval determines which sample types can be used for diagnostic testing in-country?
  - E.g. Xpert Ebola, May 2016:
    - WHO EUA: Venous WB
    - FDA EUA: Venous WB
    - CE-Mark: Venous WB, swab of FS blood, swab of oral fluid
  - E.g. Corgenix ReEBOV RDT, May 2016:
    - WHO EUA: “WB,” plasma, serum
    - FDA EUA: Fingerstick WB, venous WB, plasma

- Can/should a clinical lab validate an “unapproved” sample type and then report clinical results?

Pollock and Wonderly, JCM 2017
Identifying a “gold standard” reference technology

- How do we approach LDTs that are not available commercially?
  - Should an LDT be considered as a candidate reference method?
    - If so, what comparative evaluations (vs commercial assays or other LDTs) should be required?
  - Should efforts be made to make an LDT widely available during an outbreak?
  - Who should pay for distribution of an LDT?

- How might determination of cutoff thresholds (e.g. Ct values) be standardized and/or publicized to better allow inter-lab comparison of assays and results?
Effective communication

- How do we effectively communicate to clinicians and programs working in the field, where infrastructure may be lacking?
  - Research findings
  - Product approvals
  - Testing algorithm updates
  - Guideline updates
  - Biosafety concerns
One vision to address these challenges: “Global Emergency Diagnostic Framework”

- Framework/standards specifically for diagnostic evaluation (or, optimally, development and evaluation) in an outbreak setting

- Standards could be developed by an expert committee led by the WHO/FDA, with international input

Pollock and Wonderly, JCM 2017
**Global Emergency Dx Framework: goals**

- Develop systematic framework for diagnostic evaluations
  - Provide templates (for customization) to field labs, test developers, and clinical sites
  - Flexibly accommodate test development/evaluation work already in progress at time of emergency declaration

- Develop guidelines summarizing required IRB approvals and pathways to regulatory approval
  - Share publicly and provide to manufacturers and groups preparing for test evaluations
  - In-country IRBs should provide protocol templates for guidance
  - Clarify formal vs informal approvals needed (and order of approval)
  - Anticipate/accommodate different types of evaluations (discarded samples, POC testing)

Pollock and Wonderly, JCM 2017
Global Emergency Dx Framework: goals

- Optimize collaboration between global leaders (WHO and CDC/FDA)
  - Eliminate redundant efforts; synergize
  - Avoid controversies/bias regarding which tests to evaluate
  - Collaborative biobank development
  - Facilitate efficiency and synergy in test evaluations
    - Multiple tests run in parallel on a given sample
    - Provision of standardized samples for external QA
  - Develop effective communication plan for relaying new developments to clinicians/programs in the field
    - Acknowledge infrastructure limitations (wifi/cellular coverage)

- Increase transparency
  - Develop standards for data sharing, including in advance of publication
  - If not publishing, increase level of detail in EUA documentation to allow critical analysis and test comparison

Pollock and Wonderly, JCM 2017
Additional points from the test development perspective

- Development of diagnostics for outbreak pathogens: **companies will need outside funding to do it**
  - If test will only be used intermittently/briefly during an outbreak, it is difficult for a company to expend R+D resources on development
    - Particularly important if test will be used in resource-poor settings and test cost must be kept as low as possible
  - Similar barriers apply re: purchasing samples for test development, payments for in-country clinical studies/clearance applications

- Once tests are developed, companies can’t easily maintain large stocks on the shelf

- Greater investment in surveillance testing could help ensure that ongoing market exists (Cnops et al, Comment in Nature, Jan 2019)

Personal communication; Ellen Jo Baron, Cepheid
Additional points from the development perspective

- **Availability of a standardized and well-annotated biobank is critical for test development/validation**
  - Should biobanks be reserved for tests meeting pre-defined performance criteria?

- **Requirements for “EUA” should have more international consistency**
  - Rules for allowing use of an “emergency” diagnostic need to be clarified/outlined in advance
  - Country-specific/unique rules create confusion and barriers

- **Common procurement systems (i.e. for groups of countries) would facilitate access to novel Dx**
  - Without such a system, each country has to negotiate a price, **AFTER** regulatory approval

Personal communication; Mark Miller, bioMerieux
Status of biobanks


- ~162,000 stored samples identified
  - ~28,000 in Liberia, ~58,000 in Guinea, ~33,000 in Sierra Leone, ~42,000 stored elsewhere
  - ~15,000 positive samples

- Short-term sample preservation tenuous

- Recommends increase in laboratory capacity including coordination across labs, strengthening lab human resources

https://www.who.int/blueprint/priority-diseases/key-action/biobanking_ebola_samples/en/
MOHS-PHE Ebola Biobank

- Residual clinical samples and accompanying data from PHE-led lab testing in SL
  - Funded by Wellcome Trust Bioresource Grant
  - Acknowledges that sample quality/data completeness consistent with collection under outbreak conditions (e.g. potential for incorrect classification, multiple freeze/thaw)

- MOHS in SL retains ownership of data and materials, but most samples stored in UK
  - ~9955 samples (WB, plasma, swabs), including 1108 positive
  - Data includes age, gender, original vs follow-up sample, RT-PCR result, data of symptom onset/hospitalization, clinical chemistry results, malaria test results

- Ebola Biobank Governance Group (EBGG) established to review proposals for use of biobank samples
  - Includes SL, WHO, Wellcome, UK DFID, PHE members
  - Accessible to academia/government/commercial applicants
  - Must also obtain ethical approval for use (in SL and locally) and obtain MTA
  - Option to obtain inactivated samples (RNA/DNA pre-extracted, future X-ray inactivation) or non-inactivated (to BSL4 facility only)

Hannigan et al, Wellcome Open Research 2019, 4:115
Progress

“An R&D Blueprint for Action to Prevent Epidemics; Plan of Action”
Original May 2016—updated 2017 and 2018
Stated goal: road map to accelerate development and evaluation of therapeutics, vaccines, and diagnostics for pathogens with outbreak potential
• Includes list of priority diseases
• As of 2020, still primarily focused on development of therapeutics and vaccines
• 2020 website does mention biobanking efforts²
  • “To ensure that biological samples can be available for R&D and that new interventions can be evaluated, partners (MSF and Oxford University, notably) are developing open platforms for samples’ biobanking and sharing.”
• Elements focused on funding, data sharing, biobanking²,³, and regulatory approval will be equally relevant to Dx development/evaluation

R+D blueprint:

- Acknowledges that the entire global community will benefit if development efforts during outbreaks are "facilitated through adoption of fair and transparent principles which will have been negotiated by all stakeholders ahead of an emergency"

- We must work together now to develop such principles and a framework for development and evaluation of Dx in an outbreak setting
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