Fully Integrated DNA Amplification Devices for Infectious Disease Diagnosis

Angelika Niemz, Keck Graduate Institute, Claremont, CA

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Molecular Diagnostics

- Traditionally restricted to high complexity laboratories
- Physical space separated from the main clinical laboratory
- Typically separate rooms for pre-amplification steps (nucleic acid extraction, reaction setup), and amplification / post-amplification steps
- Move towards moderate complexity integrated systems that can be used in regular clinical laboratory settings

1. Extract Nucleic Acids
2. Set up Reaction (Master-Mix)
3. Amplify and Detect
### Smaller Fully Integrated PCR-Based Systems

<table>
<thead>
<tr>
<th>Vendor</th>
<th>System</th>
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<tbody>
<tr>
<td>Cepheid</td>
<td>GeneXpert</td>
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<tr>
<td>IdahoTech</td>
<td>FilmArray</td>
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<td>Iquum</td>
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<td>Alere</td>
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<td>Luminex</td>
<td>Aries</td>
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<td>Biocartis</td>
<td>Idylla</td>
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<tr>
<td>Enigma</td>
<td>Mini-Laboratory</td>
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“Molecular Diagnostics: Current Research and Applications” (Chapter 10), Horizon Press, 2014
### Systems based on Isothermal Amplification

<table>
<thead>
<tr>
<th>Automated, Integrated Sample Preparation, Amplification &amp; Detection</th>
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<tr>
<td>Great Basin: Portrait Analyzer</td>
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<thead>
<tr>
<th>Manual Sample Prep., Automated Amplification &amp; Detection</th>
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<tr>
<td>Meridian: Illumigene</td>
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<td>TwistDx: Twista</td>
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<td>Optigene: Genie</td>
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<td>Lumora: BART</td>
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<tr>
<th>Manual Sample Prep., Amplif. &amp; Detection</th>
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<td>Biohelix: IsoAmp</td>
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<td>Ustar: EasyNAT</td>
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“Molecular Diagnostics: Current Research and Applications” (Chapter 10), Horizon Press, 2014
Disseminated Nucleic Acid Testing

Driving Factors:

- **Time to result:** Ability to test and treat / contain disease
- **Accessibility:** Is central laboratory network available?
- **Affordability:** Cost of consumables and instrument
- **Clinical performance:** Results equivalent to reference method
- **Operator skills:** Required training, potential for user error
- **Infrastructure:** Power, refrigeration, other equipment

Key Applications:

- **Developed Countries:**
  - Hospital acquired infections
  - Influenza / Respiratory Pathogens

- **Developing Countries:**
  - HIV viral load testing / infant diagnosis
  - TB diagnosis / drug resistance testing
Tuberculosis

- Leading cause of death from a curable infectious disease
- In 2012: 8.6 million incident cases, 1.3 million deaths

**Diagnostic methods for active TB:**
- Sputum smear microscopy
- Chest X-ray
- Culture-based methods
- **Nucleic Acid Testing**
  - Advantages: Faster than culture, high sensitivity and specificity
  - Disadvantages: Complexity, Cost

Source: WHO TB Report 2013
Cepheid GeneXpert MTB/RIF

1. Sputum liquefaction and inactivation with 2:1 sample reagent
2. Transfer of 2 ml material into test cartridge
3. Cartridge inserted into MTB-RIF test platform (end of hands-on work)
4. Sample automatically filtered and washed
5. Ultrasonic lysis of filter-captured organisms to release DNA
6. DNA molecules mixed with dry PCR reagents
7. Seminested real-time amplification and detection in integrated reaction tube
8. Printable test result

Time to result, 1 hour 45 minutes

As of 31 December 2013, a total of 2,021 GeneXpert instruments (comprising 10,561 modules) and 5,219,960 Xpert MTB/RIF cartridges had been procured in the public sector in 98 of the 145 countries eligible for concessional pricing.

http://who.int/tb/laboratory/mtbrifrollout/en/
Pros:
- Enables TB diagnosis and rifampin resistance testing in < 2 hours
- Suitable for low-skilled users, contamination controlled
- Recommended for district and sub-district level laboratories

Cons:
- Expensive: Discount pricing for public sector labs in eligible countries: Instrument ($17,500), cartridge ($9.98), calibration ($ 1,800 per year)
- Requires uninterrupted line power, external computer
- Operating temperatures $\leq$30°C, cartridge storage 2-28°C, cartridges guaranteed shelf life of 6-9 months
- Internet linkage recommended for external QC and result recording

Consequence: patient not diagnosed or lost to follow-up after initial diagnosis
Addressing User Needs: Peripheral Microscopy Centers in High TB Burden Countries

- Implementing TB NAT in peripheral microscopy centers identified as high priority by key stakeholders
- Typically associated with primary health care facilities that can initiate and administer TB treatment
- Possibility to test and treat in the same clinical encounter
- Currently, 42,827 microscopy centers in the public sector of the 22 high TB burden countries perform ~ 77.6 million sputum smear tests annually
- Nucleic acid testing as replacement for sputum smear microscopy for initial TB diagnosis: estimated market potential ~ 30.8 million tests annually
# Addressing User Needs: Peripheral Microscopy Centers in High TB Burden Countries

<table>
<thead>
<tr>
<th>Country</th>
<th>Environment</th>
<th>Infrastructure</th>
<th>Available equipment</th>
<th>Skills</th>
<th>Communication</th>
<th>Current testing</th>
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<tr>
<td></td>
<td>Temperature</td>
<td>Humidity</td>
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<td>Refrigerator</td>
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<td>Pipettes</td>
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<td>Hood</td>
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<td>PCR tests</td>
<td>Computer</td>
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</table>

- **Yes/present**
- **Maybe**
- **No/not present**
- **Unsure/question not answered**
Other Molecular Diagnostics Systems for TB Diagnosis

PCR based systems with real time fluorescence detection

MolBio: TrueLab Uno MTB

Epistem: Genedrive Tuberculosis

Systems based on isothermal DNA amplification

Eiken: LoopAmp TB

Ustar: EasyNAT TB

In Development: AlereQ

Exp. Rev. Mol. Diagn. 2012, 12, 687
## TB NAT systems: Suitable for Low Resource Settings?

<table>
<thead>
<tr>
<th>Method</th>
<th>Sample Prep</th>
<th>Amplification</th>
<th>Detection</th>
<th>Cost</th>
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<tbody>
<tr>
<td>GeneXpert</td>
<td>automated and integrated sample prep + qPCR</td>
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<td>high</td>
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<tr>
<td>Alere Q</td>
<td>automated and integrated sample prep + NEAR</td>
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<td></td>
<td>high</td>
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<tr>
<td>TrueLab</td>
<td>semi-automated</td>
<td>automated / integrated qPCR</td>
<td></td>
<td>medium</td>
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<tr>
<td>GeneDrive</td>
<td>manual</td>
<td>automated / integrated qPCR</td>
<td></td>
<td>medium</td>
</tr>
<tr>
<td>LoopAmp</td>
<td>manual</td>
<td>automated (LAMP)</td>
<td>manual (visual fluorescence)</td>
<td>low</td>
</tr>
<tr>
<td>EasyNAT</td>
<td>manual</td>
<td>heat bath (CPA)</td>
<td>manual (NALF cassette)</td>
<td>low</td>
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</table>
Eiken LoopAmp MTBC Detection test

Diagram showing the process steps:
1. Transfer
2. Heating tube (Heat for 5 min, 90°C)
3. Insert
4. Adsorbent tube
5. Mix
6. Insert
7. Injection cap
8. Squeeze
9. Invert, reconstitute reaction mix
10. LAMP reaction for 40 min, 67°C
11. DNA solution
12. LAMP reaction tube

Int J Tuberc Lung Disease 2011, 15, 1211–1217
USTAR: EasyNAT TB Diagnostic kit

Step 1: Specimen Prep DNA Extraction
- Liquefy sputum & vortex
- Centrifuge, save the supernatant for amplification
- Incubate the mixture at 100°C for 10 min, cool to room temp
- Add DNA Extraction Solution, mix well
- Centrifuge, decant the supernatant
- Wash the pellet, centrifuge, decant the supernatant

Step 2: Isothermal Amplification
- Add re-suspension buffer into the Reaction mix (Glass form) tube, add paraffin oil
- Allow reagents to dissolve completely at room temperature
- Add positive & negative control separately in two separate reaction tubes
- Put the tubes in 63°C bath for 60 minutes.
- Add templates purified in Step 1 to a separate reaction tube, centrifuge all three reaction tubes

Step 3: Quick Detection
- Remove the tubes from the bath
- Place each tube into a separate disposable detection device as instructed (Fig.1)
- Read the results in 15 minutes.
Enable diagnosis of pulmonary tuberculosis in microscopy centers of high burden countries by developing a portable, easy to use, integrated nucleic acid testing device that provides a result in < 1.5 h sample-in-to-answer-out, at a significantly lower cost than currently available fully integrated bench-top nucleic acid testing systems. The device shall be battery operated, with all reagents on board in a thermo-stable form.
Developed POC compatible processes for:

- Sputum liquefaction and disinfection, pathogen lysis / nucleic acid extraction
- Isothermal Loop-Mediated Amplification (LAMP) using thermo-stable mastermix, coupled to lateral flow detection

Device design and integration:

- Developed a prototype integrated cartridge and compact instrument
- Demonstrated process execution in a fully automated manner, following introduction of liquefied and disinfected sputum into the cartridge
The remainder of this presentation has been removed for inclusion in the online course manual, since it contains proprietary or unpublished data. This material will be reinserted for the actual “live” presentation.
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