Fully Integrated DNA Amplification Devices for Infectious Disease Diagnosis

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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

Smaller Fully Integrated PCR-Based Systems

Cepheid: GeneXpert
Illumina: FilmArray
Iquum: LIAT
Enigma: Mini-Laboratory

[Images of various molecular diagnostic systems]

From "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
GeneXpert MTB/RIF: Global Roll-Out

As of 31 December 2013, a total of 1,193 GeneXpert instruments (comprising 10,545 modules) and 5,255,900 GeneXpert MTB/RIF cartridges had been procured in the public sector in 98 of the 144 countries eligible for concessional pricing.

http://who.int/tb/laboratory/mtbrifrollout/en/

GeneXpert MTB/RIF: Pros and Cons

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 <30°C, cartridge storage 2-28°C, cartridges guaranteed shelf life of 6-9 months
  - Internet linkage recommended for external QC and result recording
  - Unsuitable for low resource peripheral settings that serve the majority of affected patients

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

Other Molecular Diagnostics Systems for TB Diagnosis

- PCR based systems with real-time fluorescence detection
  - Molecular: TrueLab Uno MTB
  - Genestein: Eiken Tuberculosis
- Systems based on isothermal DNA amplification
  - Epistem: EasyNAT TB
  - In Development: AlexQ
TB NAT systems: Suitable for Low Resource Settings?

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<th>Sample Prep</th>
<th>Amplification</th>
<th>Detection</th>
<th>Cost</th>
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<td>manual (visual fluorescence)</td>
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<td>EasyNAT</td>
<td>manual</td>
<td>heat bath (CPA)</td>
<td>manual (NALF cassette)</td>
<td>low</td>
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Exp. Rev. Mol. Diagn. 2012, 12, 687

Eiken LoopAmp MTBC Detection test

Int J Tuberc Lung Disease 2011, 15, 1211–1217

USTAR: EasyNAT TB Diagnostic kit

USTAR EasyNAT Package Insert
Our Goal

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.

Current Status

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

Disclaimer

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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