

## Fully Integrated DNA Amplification Devices for Infectious Disease Diagnosis

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AACC Emerging Technologies for Clinical & Laboratory Diagnostics  
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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



Extract Nucleic Acids



Set up Reaction (Master-Mix)



Amplify and Detect

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## Smaller Fully Integrated PCR-Based Systems

Cepheid: GeneXpert



IdahoTech: FilmArray



Iquum: LIAT



Alere: Alere Q



Luminex: Aries



Biocartis: Idylla



Enigma: Mini-Laboratory



3

"Molecular Diagnostics: Current Research and Applications" (Chapter 10), Horizon Press, 2014

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### Systems based on Isothermal Amplification

Automated, Integrated Sample Preparation, Amplification & Detection	<div style="display: flex; justify-content: space-between;"> <div style="width: 48%;"> <p><b>Great Basin: Portrait Analyzer</b></p>  </div> <div style="width: 48%;"> <p><b>Alere: Alere i</b></p>  </div> </div>
Manual Sample Prep, Automated Amplification & Detection	<div style="display: flex; justify-content: space-between;"> <div style="width: 22%;"> <p><b>Meridian: Illumigene</b></p>  </div> <div style="width: 22%;"> <p><b>TwistDx: Twista</b></p>  </div> <div style="width: 22%;"> <p><b>Optigene: Genie</b></p>  </div> <div style="width: 22%;"> <p><b>Lumora: BART</b></p>  </div> </div>
Manual Sample Prep, Amplif. & Detection	<div style="display: flex; justify-content: space-between;"> <div style="width: 48%;"> <p><b>Biohelix: IsoAmp</b></p>  </div> <div style="width: 48%;"> <p><b>Ustar: EasyNAT</b></p> <p style="font-size: 8px;">"Molecular Diagnostics: Current Research and Applications" (Chapter 10), Horizon Press, 2014 4</p> </div> </div>

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### Disseminated Nucleic Acid Testing

**Driving Factors:**

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

**Key Applications:**

<b>Developed Countries:</b> Hospital acquired infections Influenza / Respiratory Pathogens	<b>Developing Countries:</b> HIV viral load testing / infant diagnosis TB diagnosis / drug resistance testing
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### 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

Estimated TB Incidence Rate for 2012




Source: WHO TB Report 2013

AFB Microscopy



Sputum Sample

BD MGIT TB Culture



Mycobacterium tuberculosis

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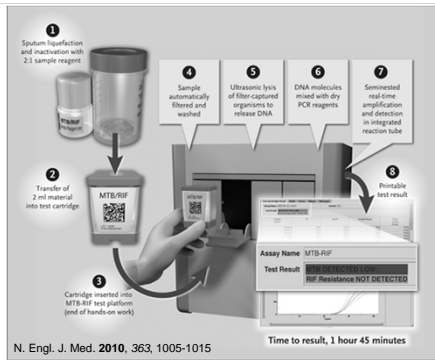
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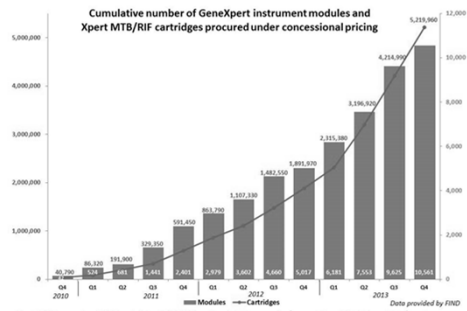
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## Cepheid GeneXpert MTB/RIF



7

## GeneXpert MTB/RIF: Global Roll-Out



8

## 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  $\leq 30^{\circ}\text{C}$ , cartridge storage 2-28 $^{\circ}\text{C}$ , 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

- 9 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



Expert Rev. Mol. Diagn. 2013, 13, 763-767  
Eur Respir J. 2014 Feb 13. [Epub ahead of print]

10

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### Addressing User Needs: Peripheral Microscopy Centers in High TB Burden Countries

Country	Environment				Infrastructure				Available equipment				Skills				Communication				Current testing			
	Temp. stable	Humidity	Power	Water	GIS	Printer	Range test	Incubator	Centrifuge	Wash tank	hood	Plumbing	PCR test	Computer	Landline	Mobile	Internet	QA assurance	Zn	ITB				
Congo																								
Zimbabwe																								
Mozambique																								
Ethiopia																								
Algeria																								
Myanmar																								
Uganda																								
Tanzania																								
Kenya																								
Bangladesh																								
Cambodia																								
Nigeria																								
Pakistan																								
Vietnam																								
Philippines																								
Indonesia																								
Thailand																								
India																								
China																								
South Africa																								
Brazil																								
Russia																								

Legend:  Yes/present  Maybe  No/not present  Unsure/question not answered

Expert Rev. Mol. Diagn. 2013, 13, 763-767

11

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### 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

12

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### TB NAT systems: Suitable for Low Resource Settings?

	Sample Prep	Amplification	Detection	Cost
GeneXpert	automated and integrated sample prep + qPCR			high
Alere Q	automated and integrated sample prep + NEAR			high
TrueLab	semi-automated	automated / integrated qPCR		medium
GeneDrive	manual	automated / integrated qPCR		medium
LoopAmp	manual	automated (LAMP)	manual (visual fluorescence)	low
EasyNAT	manual	heat bath (CPA)	manual (NALF cassette)	low

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Exp. Rev. Mol. Diagn. 2012, 12, 687

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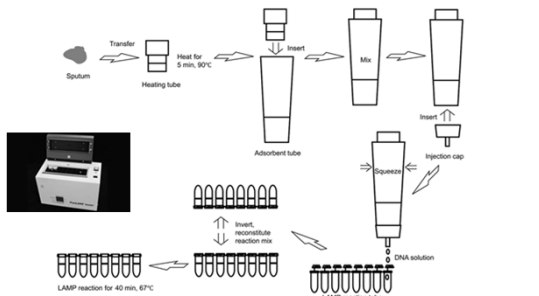
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### Eiken LoopAmp MTBC Detection test



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

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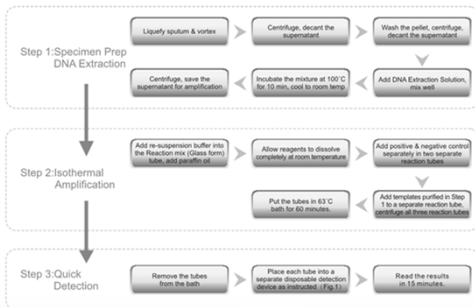
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### USTAR: EasyNAT TB Diagnostic kit



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USTAR EasyNAT Package Insert

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## 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.

16

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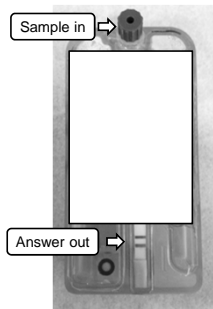
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## Current Status



### 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

17

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## 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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Innovation  
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Megan Savlov



Delia Ontengco

19

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