GeneXpert Technology

Indira Soundiram

2012
A Better Way to Platform Design
Defining Molecular Diagnostics

Any Test
Any Time
Any Sample
Any Place

The GeneXpert Family of Systems

ICORE® Module

SmartCycler® System

SmartCycler® Tube

GeneXpert® Cartridge

GeneXpert Module
Delivering A Better Way

Delivering a Better Way to Realize the Benefits of Molecular Diagnostics

ACCURATE Results

FAST Answers

EASY To Use
GeneXpert Dx System Components

GeneXpert System includes:

- **Modules**
  Thermal and optical system

- **Cartridge**
  Self-Contained
  Disposable

- **Computer System**
  Software
  Barcode scanner

- **Optional Accessories**
  Printer
  UPS
GeneXpert Technology

Integrated micro-fluidic based system comprised of

- Instrument platform
- Cartridges
- Protocols and Controls
GeneXpert® Technology

- Employ random access fluidic systems that bridge microfluidics with macrofluidics
- Uses multiplex, rapid real-time PCR technique (6-colors)
- Precision of microfluidics-based reconstitution, and simplification of automated filling
- Exploits proven SmartCycler® technology (Incorporate same “solid-state” thermal cycler/fluorometer module)
- Utilize families of cartridges to address wide range of applications and specimen types
GeneXpert® Design Approach

- No wet interface between instrument and cartridge to eliminate carry-over
- Integrated ultrasonic horn for rapid lysis of spores
- Total internal control of reagent system –
  - No separate external positive or negative controls required
- Smart fluidics
  - Encoded software driven motors for valve movement and integral hydraulic drives
  - Advanced micro fluidics technologies to enable complex sample prep processing protocols (many milliliters down to 10 micro liters)
- Automated data reduction and results interpretation
Cepheid Technology

GeneXpert

Patient Sample

Washing

DNA/RNA extraction

DNA or RNA purification

MasterMix

SmartCycler

Positive

Analysis

Detection

Amplification

Négative

DNA or RNA purification

DNA/RNA extraction

Washing

Patient Sample
Automated Xpert MTB/RIF Protocol

1. Put the eluted sample in the cartridge, and then put the cartridge in the instrument.

2. The sample combines with the SPC.

3. A filter captures the sample and the SPC.

4. Ultrasonically lysed cells release nucleic acid.

5. Eluted DNA mixes with dried-down bead reagents.

6. Simultaneous PCR amplification and detection occurs.

7. Results are ready to view and print in less than 2h.
Platforms

- I-CORE module
- GX module
- SmartCycler® Instrument
- GeneXpert® Instrument
- BDS
The I-Core Module

Building Block of the Smart Cycler and GeneXpert
### GeneXpert I-Core module Excitation & Detection

<table>
<thead>
<tr>
<th>Optical channel</th>
<th>Excitation (nm)</th>
<th>Emission (nm)</th>
<th>Calibrated reporter dyes</th>
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<tbody>
<tr>
<td>1</td>
<td>375–405</td>
<td>420–480</td>
<td>CF 1</td>
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<tr>
<td>2</td>
<td>450–495</td>
<td>510–535</td>
<td>FAM</td>
</tr>
<tr>
<td>3</td>
<td>500–550</td>
<td>565–590</td>
<td>Alexa Fluor® 532</td>
</tr>
<tr>
<td>4</td>
<td>555–590</td>
<td>606–650</td>
<td>Texas Red®</td>
</tr>
<tr>
<td>5</td>
<td>630–650</td>
<td>665–685</td>
<td>Alexa Fluor 647®</td>
</tr>
<tr>
<td>6</td>
<td>630–650</td>
<td>&gt;700</td>
<td>CF 6</td>
</tr>
</tbody>
</table>
A micro lab: GeneXpert module + the cartridge

- Syringe Motor
- Motherboard
- I-CORE
- Module Door
- Valve Drive Motor
- Processing Chambers
- Reaction tube
- Valve Body
Cepheid Technology

GeneXpert

SmartCycler
Syringe drive

I-CORE

Valve drive

Ultrasonic horn
Inside Chamber

- Syringe drive shaft
- ICORE tube insertion
- Valve drive (wedge)
- Ultrasonic horn

¾ inches
GeneXpert Cartridge

- GX cartridge has 11 chambers
- Lower numbered chambers are used for sample processing
- Higher numbered chambers are used for reagents
- There are basically two types of cartridges
  - Cartridge A
  - Cartridge C
- Both cartridge A and C has identical body construction. Only difference is the bottom part
Cartridge Side View

Retaining balls

Sample preparation bead:
- Full process control; excipients

Enzyme reagent bead:
- Hot start Taq polymerase; dNTPs; Hepes buffer; BSA; Mg 2+; salts; excipients

Target-specific reagent bead:
- Primers; Probes; Internal control; Hepes buffer; Mg2+; excipients
Cartridge Exploded View

- Cartridge Body (11 Fluid Chambers and overmolded gasket)
- Valves Body Ports
- PCR Tube
- Ports for PCR tube
- Syringe Barrel
- Rotary Valve/Filter and Ultrasonic Lysis or SPB Region
- Cap/Ultrasonic Interface
- Cartridge Foot
Cartridge Valve Body Types

Cartridge A

Cartridge C
Cepheid Assay Control Strategy

• Each GeneXpert cartridge is a self-contained unit test device
  – The use of external controls will have limited use in assuring proper assay performance
  – Cepheid designed specific molecular methods to include internal controls that enable the system to detect specific failure modes

• Three elements of the molecular design were created to address all the key failure modes that could result in a false negative result
  – Probe Check
  – Specimen Processing Controls
Probe Check

What is it?

• Just prior to starting thermal cycling, multiple fluorescence readings at different temperatures are made.
• The results are automatically compared to pre-established factory settings in the software.

What does it do?

Probe check verifies

• Bead re-hydration
• PCR tube filling
• Probe integrity
• Dye or quencher instability
Sample Processing Control (SPC)

What is it?

- Intact organisms, DNA or RNA
- **Detection probe**: A fluorescently-labeled hybridization probe with different color reporter dye than that of the target probe(s) or the internal control (IC); present in primer-probe bead
- **Primers**: Can be the same as those for the target or IC or can be unique; present in primer-probe bead

What does it do?

- **Co-processed** with target organism through entire sample prep process
- **Co-amplified** with targets
  - The SPC assesses the effectiveness of the sample processing steps, including and up to reaction tube filling
  - Detects degradation of enzyme(s) or other components of system
  - Detects sample inhibition
Regular Real-Time PCR Assay

- DNA Positive Control (PC) - Assay Run control
  - Verifies reagent integrity

- DNA Negative Control (NC) - Assay Run control
  - Detects environmental or reagent contamination and verifies reagent integrity.

- Internal control (IC) – Tube control
  - Detects PCR inhibition and verifies reagent integrity.
  - Does not compete with target DNA.
GeneXpert Real-Time PCR Assay

• DNA Positive Control (PC) - Assay Run control
  • ProbeCheck

• DNA Negative Control (NC) - Assay Run control
  • No need! No wet interface between instrument and cartridge and 1 cartridge for 1 sample!

• Internal control (IC) – Tube control
  • Better: SPC, not only amplification but extraction too
  • Does not compete with target DNA.
SPC does not compete with target DNA
SPC does not compete with target DNA

- SPC must be Positive when the Target is Negative

- SPC can be Positive or Negative when the Target is Positive
Interpretation of Results

Probe Check

Pass

Target

Pos

Positive

Fail

Error

No Value

Test

Stopped

No Value

No Value

SPC – IC

Pos

Negative

Pos

No Value

Invalid