Technologies for Multiple Pathogen Detection

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Technologies for Multiple Pathogen Detection

- Molecular diagnostic technologies
- Challenges of multiplex pathogen detection
- Future of molecular microbiology
Traditional Multiplex Pathogen Detection
Limitations of Traditional Methods

- Speed
- Sensitivity
- Taxonomy-based
- Spectrum of targets
- Specialized training
Ideal Microbiology Test

- Rapid
- Sensitive and specific
- Syndrome-based multiplexing
- Shared technology platform
- Common laboratory training
Viruses
Bacteria
Fungi
Parasites
Detection of Host Response

Serology
(Host Immune Response)
Detection of Pathogen

- Microscopy
- Antigens
- Culture
- Nucleic Acids
Nucleic Acid Detection

• Non-amplified Nucleic Acid Probe
  – labeled DNA or RNA probe (enzyme, fluorescence, etc.)

• Signal Amplification
  – increase concentration of labeled molecules attached to target

• Target Amplification
  – enzyme-mediated synthesis of copies of the target nucleic acid

• Probe Amplification
  – amplification products generated only from probes

• Metagenomics (sequencing)
Non-Amplified Nucleic Acid Probe

- Liquid-phase hybridization protection assay (Gen-Probe)
- Single-stranded DNA probe labeled with acridinium ester is added to sample
- If the probe binds to its complementary target sequence, the acridinium ester is protected from alkaline hydrolysis
  - otherwise, acridinium ester will be hydrolyzed
- Acridinium ester emits light upon addition of peroxides
Quantum Dots

- Semiconductor nanocrystals
- Quantum confinement of electrons
- Shape and size determine fluorescence spectrum
- 100 times brighter than regular fluorescent report dyes
GeneFluidics

- Electrochemical detection
- Capture probe is bound to sensor surface
- HRP-linked detection probe binds to second site on target DNA
- HRP reaction generates current proportional to target concentration
Signal Amplification

- Branched DNA (Siemens)
  - sandwich hybridization assay with multiple sets of probes
  - bDNA has 15 identical branches, each can bind 3 labeled probes
Nanosphere

- Microarray-based platform
- Gold nanoparticle detection
- Signal amplification through deposition of elemental silver onto gold nanoparticles
- Measure light scatter from nanoparticles
Target Amplification

• Polymerase Chain Reaction (PCR)
• Transcription-mediated amplification (TMA) / Nucleic acid sequence-based amplification (NASBA)
• Strand displacement amplification (SDA)
• plus many other strategies...
Multiplexing
Multiplex PCR

- Electrophoresis
- Fluorescent probes (FRET)
- Hybridization
  - Microarrays
  - Bead-based arrays
- Mass spectroscopy detection
- Sequencing
- Spatial multiplexing
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Line Probe Assays
Multiplex PCR

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Multiplex PCR

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Luminex
Multiplex PCR

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Abbott PLEX-ID
Multiplex PCR

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Life Technologies
Ion Torrent

Illumina MiSeq

Roche GS Junior
Multiplex PCR

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LAMP

• Loop mediated isothermal amplification
• Precipitation of pyrophosphates released during DNA amplification
• Visible turbidity
  – Can modify to produce color or fluorescence
• Inexpensive
  – No thermal cycler
  – Visible to naked eye
Nucleic Acid Lateral Flow

• Simple, fast, cheap
  – potential for developing world POC diagnostics
• May incorporate target amplification or signal amplification to increase sensitivity
  – detection using enzymes, quantum dots, colloidal gold, etc.
• Future devices may incorporate integrated solid phase isothermal nucleic acid amplification
Which is the best one for my lab?
challenges
Technical Challenges of Multiplexing

- Nucleic acid extraction
- Loss of sensitivity
- Mispriming
- Cross-hybridization
- Competition
Expensive
Centralized Testing
Batch Testing
New Pathogens
Perpetuating the *Old* Microbiology
Where are we headed?
The Future...

- Integration of new point-of-care diagnostics onto wards
  - Improved rapid serologic tests (antigen or antibody)
  - Simple, rapid chip-based or lab-in-a-box molecular tests
    - Real-time PCR, microarrays, single-molecular detection
  - Target the most common pathogens
    - Syndromic-based testing

- Expansion of clinical team
  - Physicians, nurses, pharmacists
  - *plus* laboratory technologists
    - Operation and troubleshooting of advanced diagnostic equipment
The Future...

- **Hospital laboratories**
  - Focus on rapid tests targeting common pathogens (multiplexing based on syndrome)
  - More molecular tests, fewer culture-based tests

- **Reference laboratories**
  - Traditional culture and phenotypic tests
    - Maintenance of sequence databases to ensure molecular assays reflect the circulating strains of various organisms
  - High volume tests
    - Tests which do not require rapid turn-around times
  - Advanced molecular tests
    - Microarrays
    - Metagenomics
  - Genotyping and characterization of organisms
    - Whole genome sequencing
Point-of-Care Molecular Tests

- Integrated sample preparation
- Sealed lab-in-a-box systems
- Fast turn-around time
- High sensitivity

Cepheid GeneXpert
Point-of-Care Molecular Tests

Idaho Technologies
FilmArray

IQuum Liat Analyzer
Advancing the Molecular Revolution

- Revolution requires re-thinking of the entire system of delivering laboratory services
- Many new technologies emerging
  - What is hype and what is the real thing?
- New technology inherently costs more
  - How do we pay for it all?
- POC molecular tests are here
  - How will we manage POC testing?
Questions

Goodbye, ABI 9700?