คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล

Diagnosis for infectious diseases

" Molecular based techniques "

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Real-life situations

- Hemoculture grows bacterial colonies on blood agar
- Automated blood culture flagged positive
- CSF sample from patients with meningoencephalitis
  - Culture:no growth or previous antibiotic Rx
- Acid fast positive from clinical specimens or from mycobacterial culture

How molecular techniques assist our practice?

สมาคมโรคติดเชื้อแห่งประเทศไทย
การประชุมใหญ่วิชาการ ประจำปี ครั้งที่ 43

Outline

- Culture-based molecular identification
- Direct molecular detection

สมาคมโรคติดเชื้อแห่งประเทศไทย
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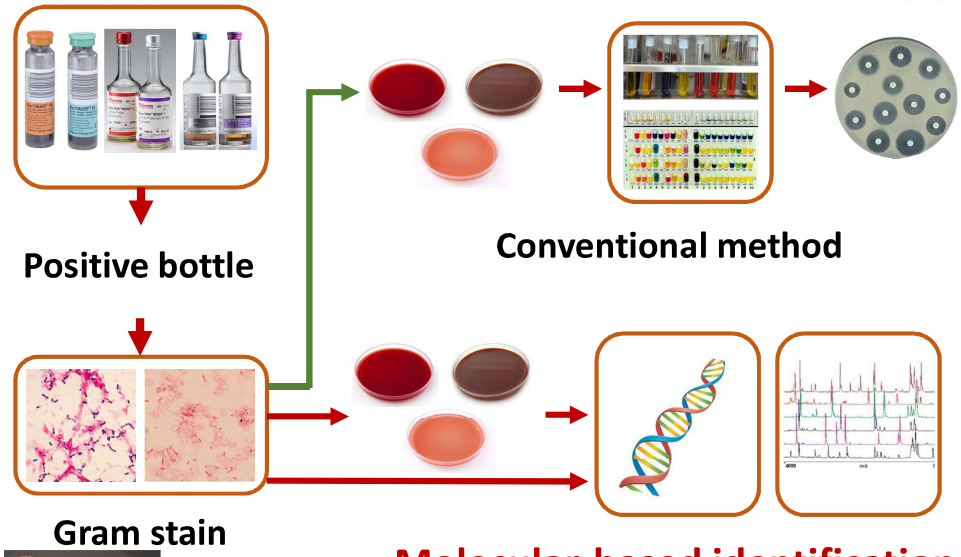
Culture-based molecular identification



สมาคมโรคติดเชื้อแห่งประเทศไทย
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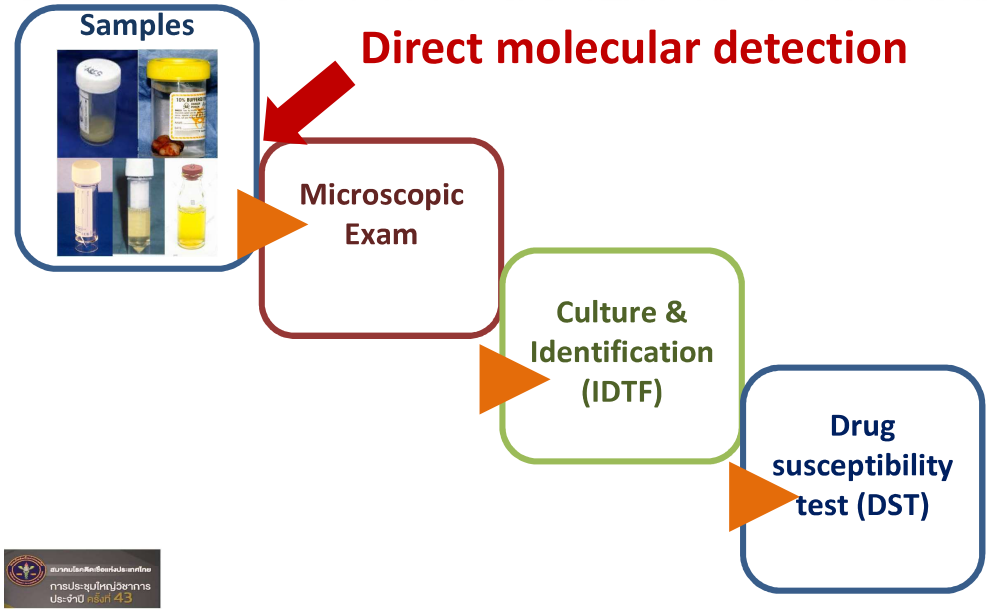
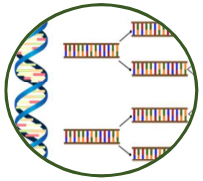
Direct molecular detection

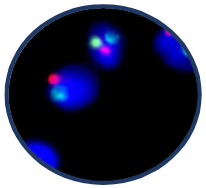


Basic molecular techniques for organism identification



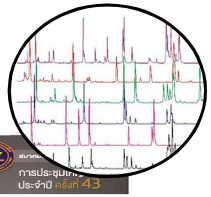
Amplified nucleic acid-based

e.g. polymerase chain reaction (PCR)



Nucleic acid hybridization

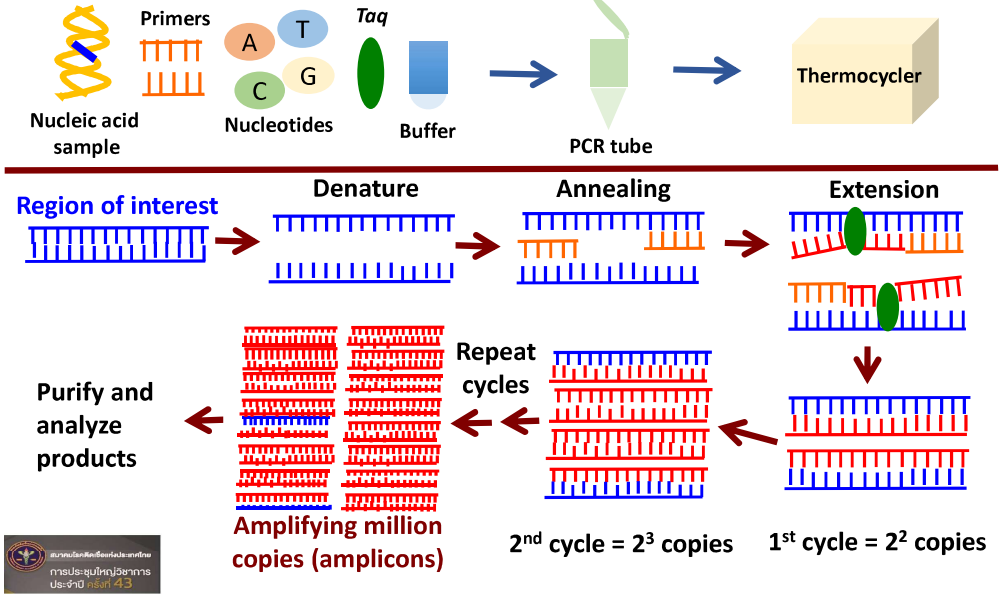
e.g. fluorescent in situ hybridization (FISH), line probe assay, microarray



Non-nucleic acid-based

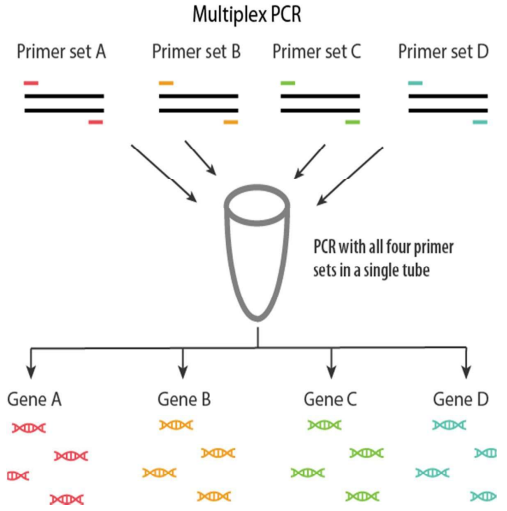
e.g. Mass spectometry

PCR-based method: Basic concept



Multiplex PCR

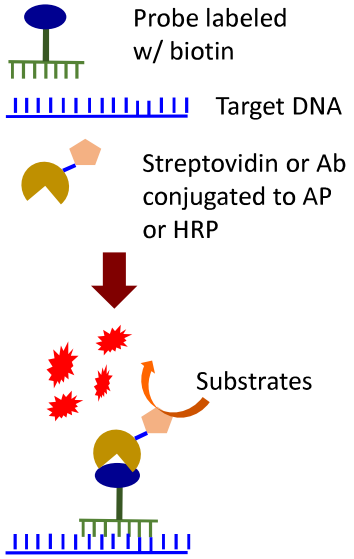
- Use > 1 primer sets at once
- Target multiple organisms or genes
- Fluorescence discriminates each amplicon product
- Useful application in clinical microbiology
  - Respiratory sample, CSF, stool, genital swab



https://www.abmgood.com/marketing/knowledge_base/img/PCR/Multiplex_PCR.png

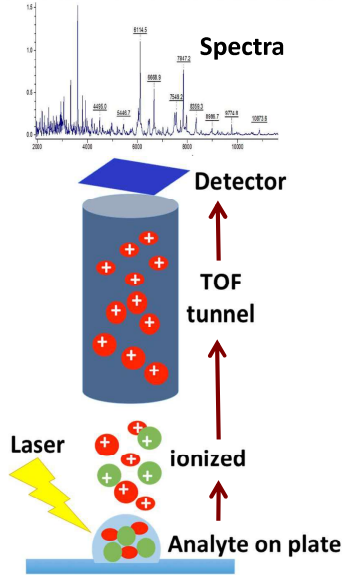
Nucleic-acid hybridization

- Use probes to identify target DNA or RNA and are labelled with biotin or digoxygenin
- Probes hybridize to the target
- Detect the hydridization by conjugated enzyme/substrate reactions
  - Color change (chromogenic)
  - Chemiluminescent or light signals under optical camera



Non-nucleic acid-based: Matrix-assisted laser desorption ionization time-of-flight mass spectometry (MALDI-TOF MS)

- Analyte preparation (sample + matrix)
- Laser desorb the analyte on plate into ionized molecules, and run through an electrostatic tunnel
- Small molecules go fast, followed by larger molecules (time-of flight)
- The molecules detected by an ion detector
- Mass spectra are unique to specific genera and species



Clin Microbiol Infect 2010;16:1604-13., Clin Infect Dis 2013;57:564-72.

MALDI-TOF MS

- 2 commercial MALDI-TOF systems

Biotyper (Bruker, USA)

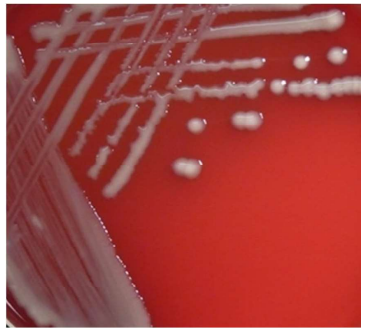
Vitek MS (Biomerieux, France)

- Overall performance is comparable
- Differences are associated with software databases

Future Microbiol 2014;9:543-59.

Case 1

- A patient has a prolonged admission in an ICU
- Previously receiving broad spectrum antibiotics for weeks
- Previous colonization with XDR *A. baumannii* (sputum) and CRE *K. pneumoniae* (urine)
- The patient has a new onset of sepsis
- Blood culture grew the isolate as shown

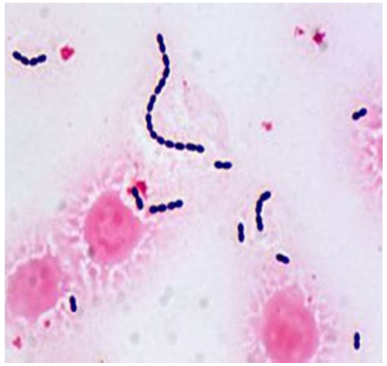


Blood agar plate

Any methods for early organism identification?

Case 2

- A patient, Dx AML post CMT, has prolonged neutropenia, fever and mucositis
- On meropenem, colistin, vancomycin and amphotericin B
- New onset of sepsis with shock
- Automated blood Cx is alarmed at 4 hrs; Gram stain as shown

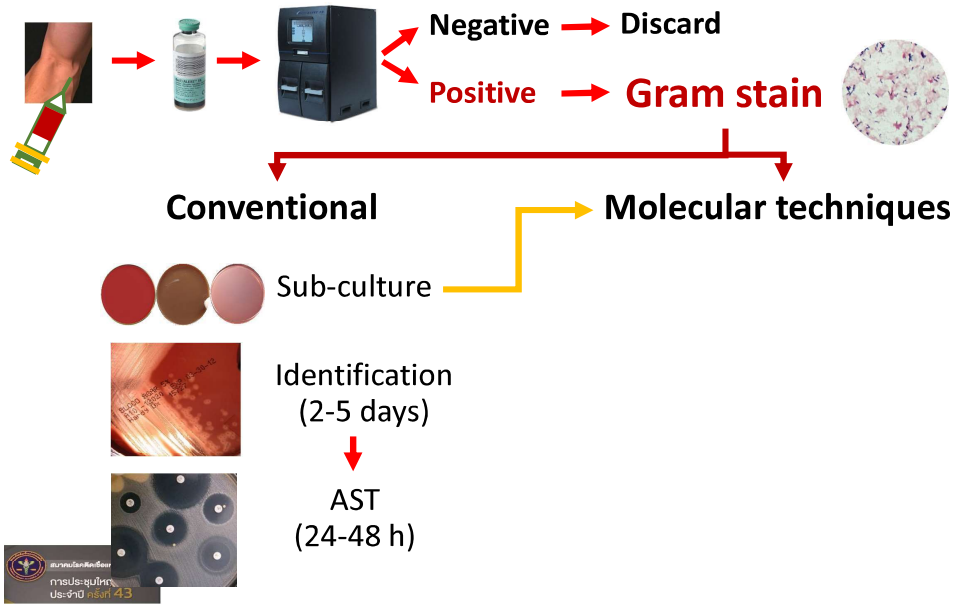


Gram stain from blood culture bottle

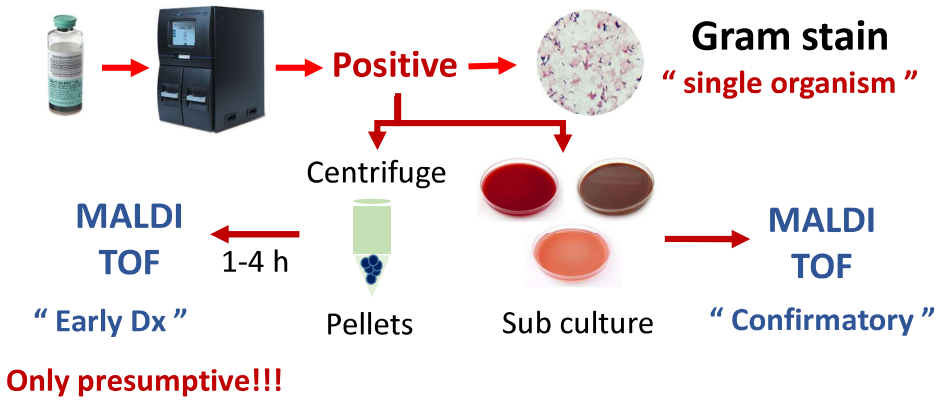
Any methods for early organism identification?

Exploitation of molecular techniques in blood culture positive

Identifying organisms from positive blood culture



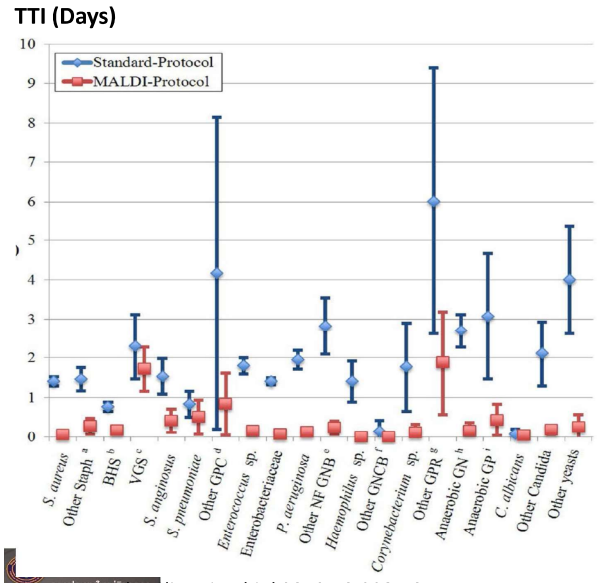
MALDI-TOF MS for positive blood culture



Only presumptive!!!

Clin Microbiol Rev 2013;26:547-603.
Future Microbiol 2014;9:543-59., Clin Microbiol Infect 2015;21:313-22.

Time to identification (TTI) and cost effectiveness studies



The MALDI provided the identifications

- 1.45 days earlier (P <0.001)

Cost effectiveness

Cost/12 months	Standard protocol ($)	MALDI ($)
Reagents	158,645	29,613
Labor	31,323	26,669
Fixed annual		31,272
Total	189,969	87,555

Save cost 53.9%

Tan KE et al. J Clin Microbiol 2012;50:3301-8.

MALDI-TOF MS for positive blood culture

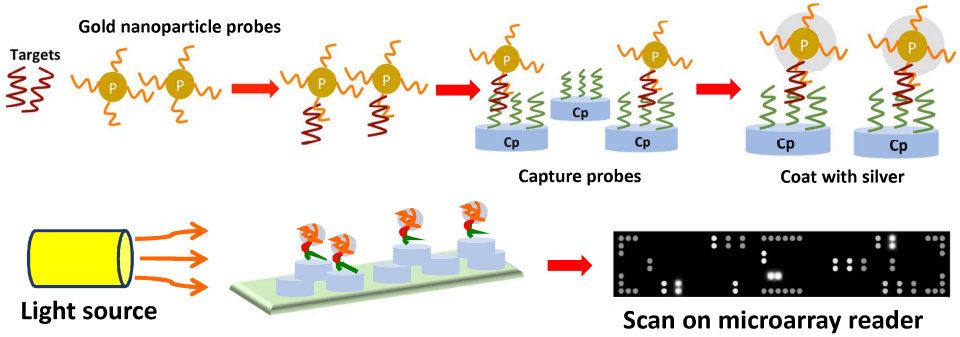
- **Limitations**
  - Polymicrobial infection
  - Anaerobic bacteria
  - GNR non fermenters
  - Closely related species such as
    - *Streptococcus pneumoniae* vs. *S. mitis* group
    - *Bacillus* spp.
    - *E. coli* vs. *Shigella*
    - *Candida* spp.

"Only preliminary report, require back-up from conventional identification"

Clin Microbiol Rev 2013;26:547-603.
Future Microbiol 2014;9:543-59., Clin Microbiol Infect 2015;21:313-22.

Microarray-based for positive blood culture

- Probes and targets hybridization on microarray glass slide
- Detect the targets by an optical camera in the microarray reader



Clin Microbiol Rev 2014;27:783-822., Clin Chem Lab Med 2015;53:1013-24.

Commercial microarray for positive blood culture

- Reduce the cost per target tested
- Simultaneous testing for multiple pathogens with similar symptoms

Test	Targets	Sensitivity (%)	Specificity (%)	Time to result (h)
Verigene BC-GP	12 Gram positive, 3 resistance genes (*mec*A, *van*A, *van*B)	92-100	98-100	2.5
Verigene BC-GN	8 Gram negative, 6 resistance genes (KPC, NDM, CTX-M, VIM, IMP, OXA)	81-100	98-100	2
Prove-it Sepsis	60 bacteria, 13 fungi, *mec*A	95%	99%	3.5

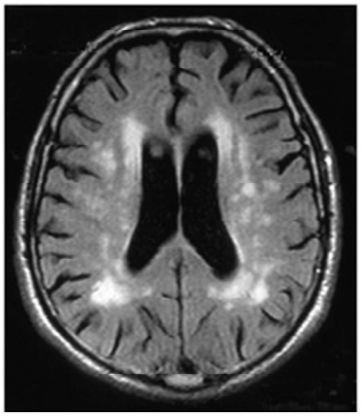
Limitations

- *S. pneumoniae*, polymicrobial infection

Clin Microbiol Rev 2014;27:783-822., Clin Microbiol Infect 2015;21:313-22.

Case 3

- A patient with SLE with active LN receiving prednisolone and cyclophosphamide Rx
- She developed altered mental status and seizure
- CSF culture: No growth
- MRI brain suspected infectious meningoencephalitis vs. vasculitis

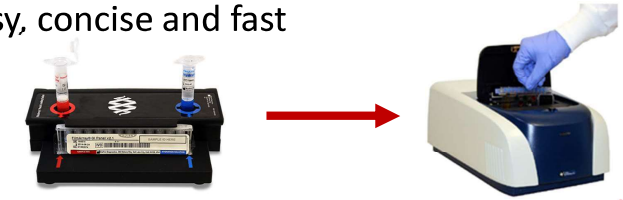


Any molecular methods for identifying infectious etiology?

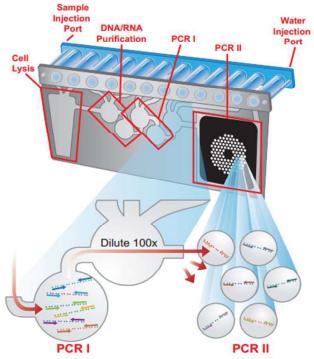
Exploitation of molecular techniques in CNS infection

Automated multiplex real time PCR (BioFire FilmArray®)

- Easy, concise and fast



- A qualitative method using 2 stages PCR
  - 1st PCR to enrich the target
  - 2nd PCR using specific primers, detect the target by fluorescent dye



http://www.biofiredx.com/wp-content/uploads/2016

BioFire FilmArray® Meningitis/Encephalitis panel

- Uncentrifuged CSF specimens at least 200 µL
- Identify the following organisms

S. pneumoniae	CMV	*C. neoformans/gattii*
S. agalactiae	Enterovirus	
N. meningitides	HSV-1	
L. monocytogenes	HSV-2	
E. coli K1	HHV-6	
H. influenzae	VZV	
	Human parechovirus	

- **Limitations**
  - Latent vs. active CMV and HHV-6 infections
  - Non-K1 *E. coli*, non encapsulated *N. meningitides*
  - CSF samples from shunt devices
  - Data in immunocompromised populations

http://www.biofiredx.com/wp-content/uploads/2016

Multicenter study of BioFire FilmArray ME Panel

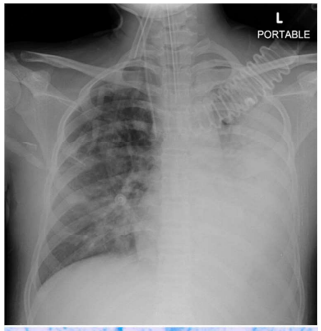
- **1,560 CSF specimens**
  - Positive 136 (8.7%), negative 1,424 (91.3%),
  - 84.4% positive and >99.9% negative agreement with standard
  - **False POSITIVE ME panel (22)**
    - *S. pneumoniae* (7), HHV-6 (3), CMV (2), EBV (2), HSV-1 (2), *C. neoformans/gattii* (2)
    - *E. coli* (1), *S. agalactiae* (1), HSV-2 (1), VZV (1)

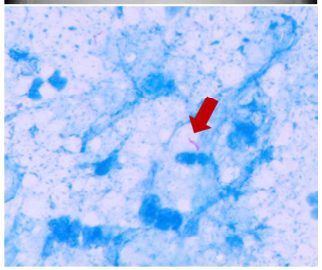
" CSF culture or conventional methods are still needed, regardless of the FilmArray ME Panel "

J Clin Microbiol 2016;54:2251-61.

Case 4

- A 16-year-old woman has pneumonia and ARDS
- Her symptoms was not responsive after IV cefepime and azithromycin Rx
- Sputum culture: no growth
- Sputum AF stain as shown

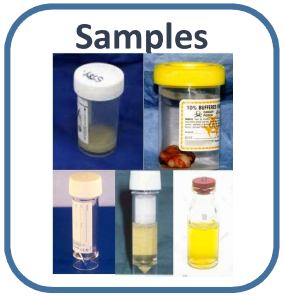




Is it likely MTB?

Any molecular methods for Dx MTB?

Direct molecular detection



Culture and identification (2-6 weeks)

New molecular detection

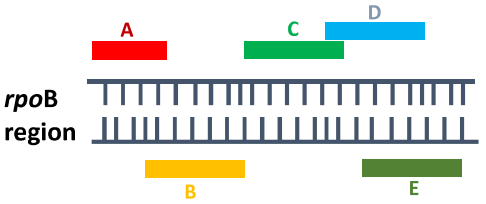
- Detection of mycobacterial DNAs
- Amplify the targets by PCR assay
- Presumptive diagnosis

Xpert MTB/RIF®

- Approved by WHO since 2010
- Semi-automated technique

WHO. Policy update, 2013

Xpert MTB/RIF



- Target: MTB *rpo*B gene
- PCR and detect the target by 5 specific probes (A-E)
- Semi-quantitative assay
- Detect *rpo*B gene mutation

Semi-automated technique

Sputum → Processing → Transfer to cartridge → Run → Result

Turn-around time ≈ 2 hrs

WHO. Policy update, 2013.

Xpert MTB/RIF

For Dx pulmonary TB in adults

	Pooled sensitivity	Pooled specificity
Initial test regardless of smear	88%	99%
Add-on test following smear (-)	68%	99%
Detect RIF resistance	95%	98%

For Dx extra-pulmonary TB

	Pooled sensitivity	Pooled specificity
Pleural fluid	17-43%	98-99%
CSF	55-79%	98%
LN Bx and aspirate	83-85%	92-99%
Tissue BX	81%	98%

WHO. Policy update, 2013.

Limitations

- Presumptive diagnosis
- Pediatric and other samples has not been validated
- A positive test does not indicate viable organisms
- False negativity (low MTB load, post treatment, or processing errors)
- False positivity (*M. scrofulaceum*, $\geq 10^8$ CFU/mL)

Xpert® MTB/RIF. Package insert, Cepheid 2015.

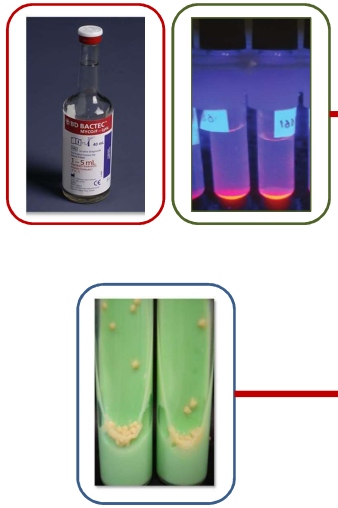
Case 5

- A bottle from an automated mycobacterial liquid media culture alarmed positive
- Gram stain and AF stain compatible with mycobacteria

Is it MTB or NTM?

Any molecular methods for mycobacteria species identification?

Hybridization technique for mycobacterial identification



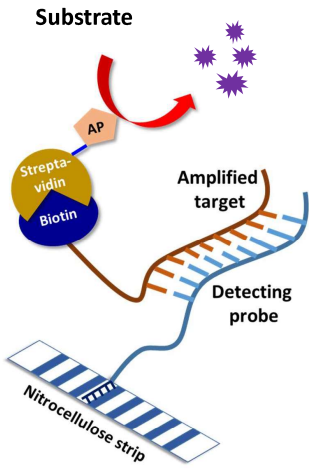
Molecular identification assays

- Gold standard
- Provide early diagnosis

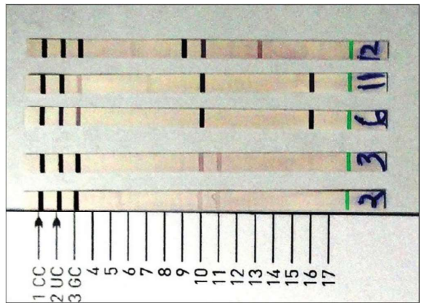
Line probe assays

- Amplify targets by PCR
- Pretreat the amplicons with biotin
- Probe hybridize with target DNA or RNA
- Detect color change or fluorescence
- Commercial kits
  - INNO-LiPA®, GenoType®

Line probe assay



- Species specific probes immobilized on nitrocellulose strip
- Biotinylated amplicons hybridize with the probes
- Streptavidin-AP reacts with substrate
  → purple brown band

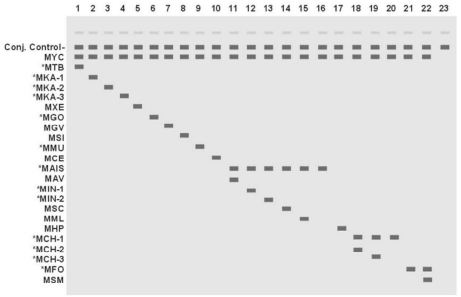


"Reverse hybridization"

INNO-LiPA MYCOBACTERIA v2, Innogenetics, Journal of Laboratory Physicians, 2013;5:83-9

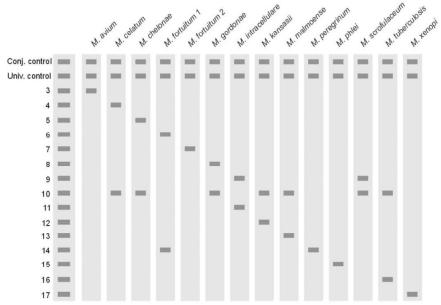
INNO-LiPA® vs. GenoType®

INNO-LiPA®



Sensitivity 100%, specificity 94.4%

GenoType® CM



Sensitivity 97.9%, specificity 92.4%

Uncommon mycobacteria can be misidentified as *M. fortuitum* or *M. avium-intracellularare*

None of the systems misidentified MTB complex

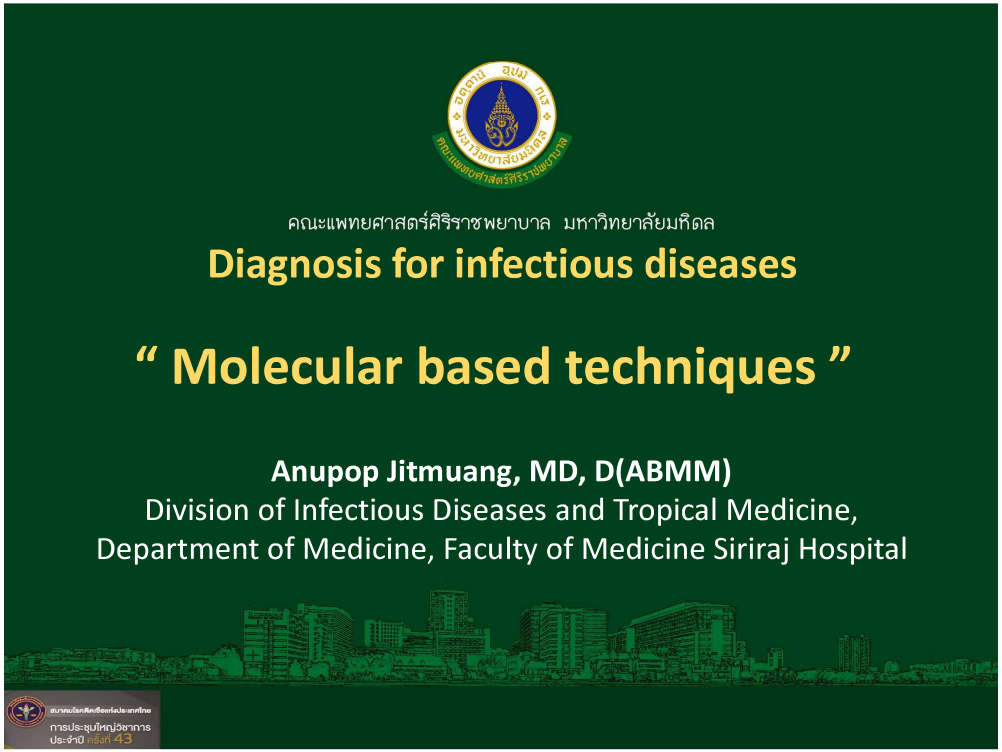
Tortoli E, et al. J Clin Microbiol 2003;41:4418-20., Russo C, et al. J Clin Microbiol 2006;44:334-9., Tortoli E, et al. J Clin Microbiol 2010;48:307-10.

Pros and Cons

Molecular diagnosis: Pros and Cons

Pros	Cons
• Rapid identification	• Lack of a gold standard
• No influence of antibiotics	• No susceptibility data
• High sensitivity	• Detect nucleic acids instead of viable organisms
• Semi-automated or automated system	• Restricted availability
• Less labor-intensive	• Presence of contamination
• Appropriate for highly fastidious microorganisms	• Cost-effectiveness?

Lancet Infect Dis 2004;4:751-60.
Clin Microbiol Infect 2015;21:313-22.


คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล
Diagnosis for infectious diseases
“ Molecular based techniques ”
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การประชุมใหญ่วิชาการ
ประจำปี ครั้งที่ 43