



105年全國結核病防治檢討會議

結核病檢驗新知介紹

慢性傳染病組

分枝桿菌實驗室

黃偉倫

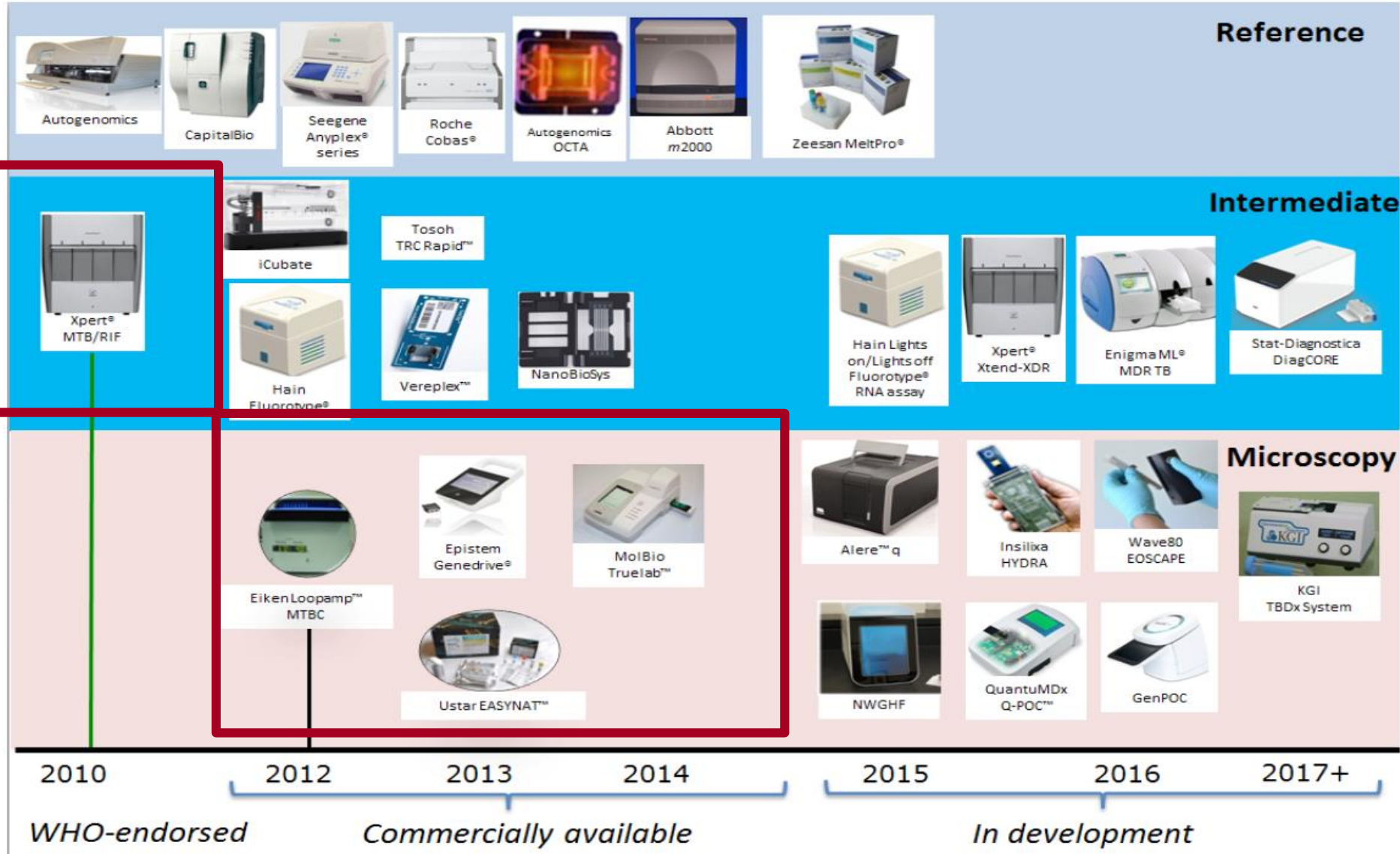


大綱

- 分子檢測應用在結核病防治的趨勢
- 世衛組織推薦結核病檢驗流程
- 具潛力的新一代分子檢測技術/工具



結核病分子檢測發展



不同區域/功能實驗室 採用不同分生檢測法

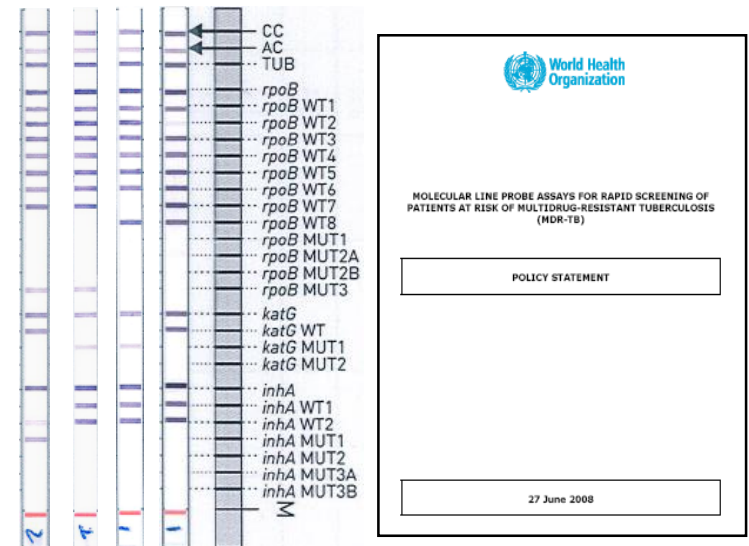
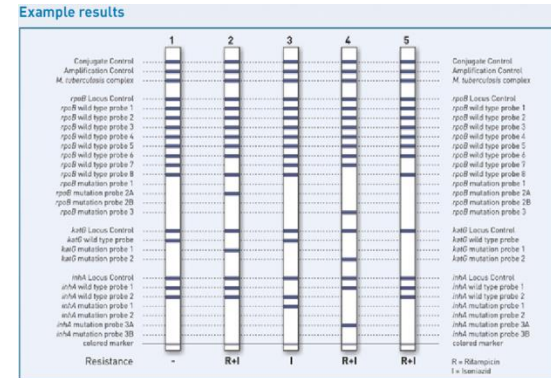
Laboratory level	Function	Tests
Peripheral (subdistrict and community)	Screening , case-finding, referral, treatment	AFB smear exams using either Ziehl–Neelsen stain with light microscopy, or fluorochrome stain with fluorescence microscopy (preferably with LED illumination); Xpert MTB/RIF assay
Intermediate (regional and district)	Case-finding, treatment follow up	All tests performed at the peripheral level and possibly culture on solid media and LPA directly from AFB smear-positive sputum
Central (reference)	Case-finding, treatment follow up, surveillance, development and provision of reference methods and standards, supervision of laboratories in the network	All tests performed at the peripheral and intermediate levels plus liquid culture, DST for first-line and second line anti-TB agents (including fluoroquinolones and injectable agents) on solid or in liquid media; LPA on positive cultures and AFB-positive sputum; and rapid speciation tests

AFB, acid-fast bacilli; LED, light-emitting diode; LPA, line-probe assay; DST, drug-susceptibility testing.

The GenoType MTBDRplus test (GenoType MDR)

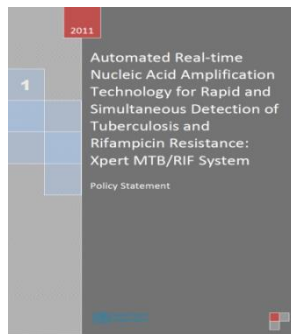
- 2008年WHO 推薦使用於
檢測isoniazid (INH)及
rifampin (RIF)抗藥性

- WHO 強烈推薦使用於檢測
TB及MDR-TB高危險族群

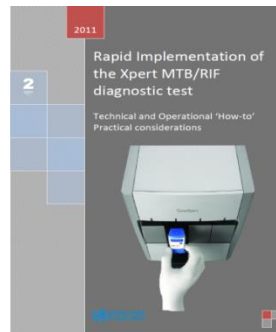


Xpert MTB/RIF (Xpert)

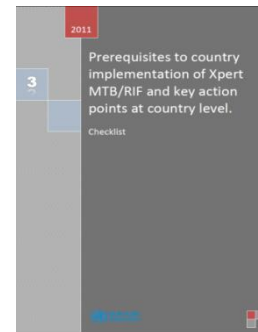
- 2010年9月，Xpert MTB/RIF 檢驗方式通過WHO STAG-TB (Strategic & Technical Advisory Group) 審查
- 2010年12月, WHO 推薦Xpert MTB/RIF進行快速檢驗
- 2011年出版使用介紹及指引



http://whqlibdoc.who.int/publications/2011/9789241501545_eng.pdf



http://whqlibdoc.who.int/publications/2011/9789241501569_eng.pdf

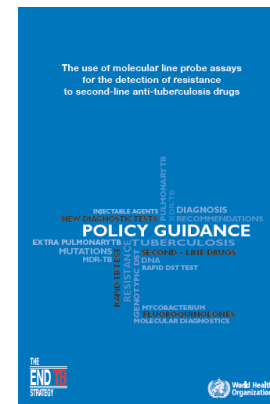
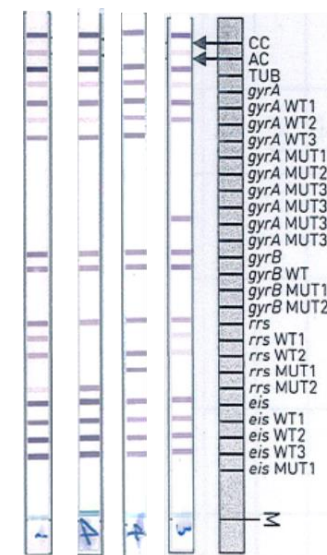
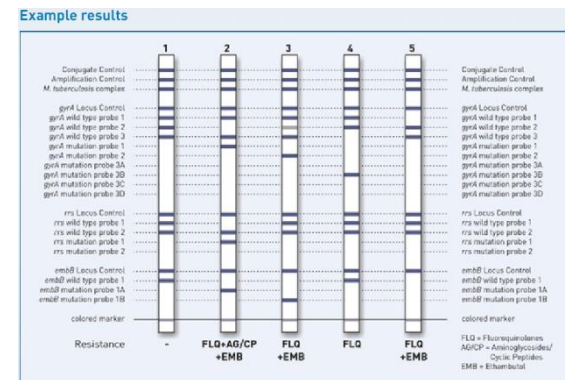


http://whqlibdoc.who.int/hq/2011/WHO_HTM_TB_2011.12_eng.pdf

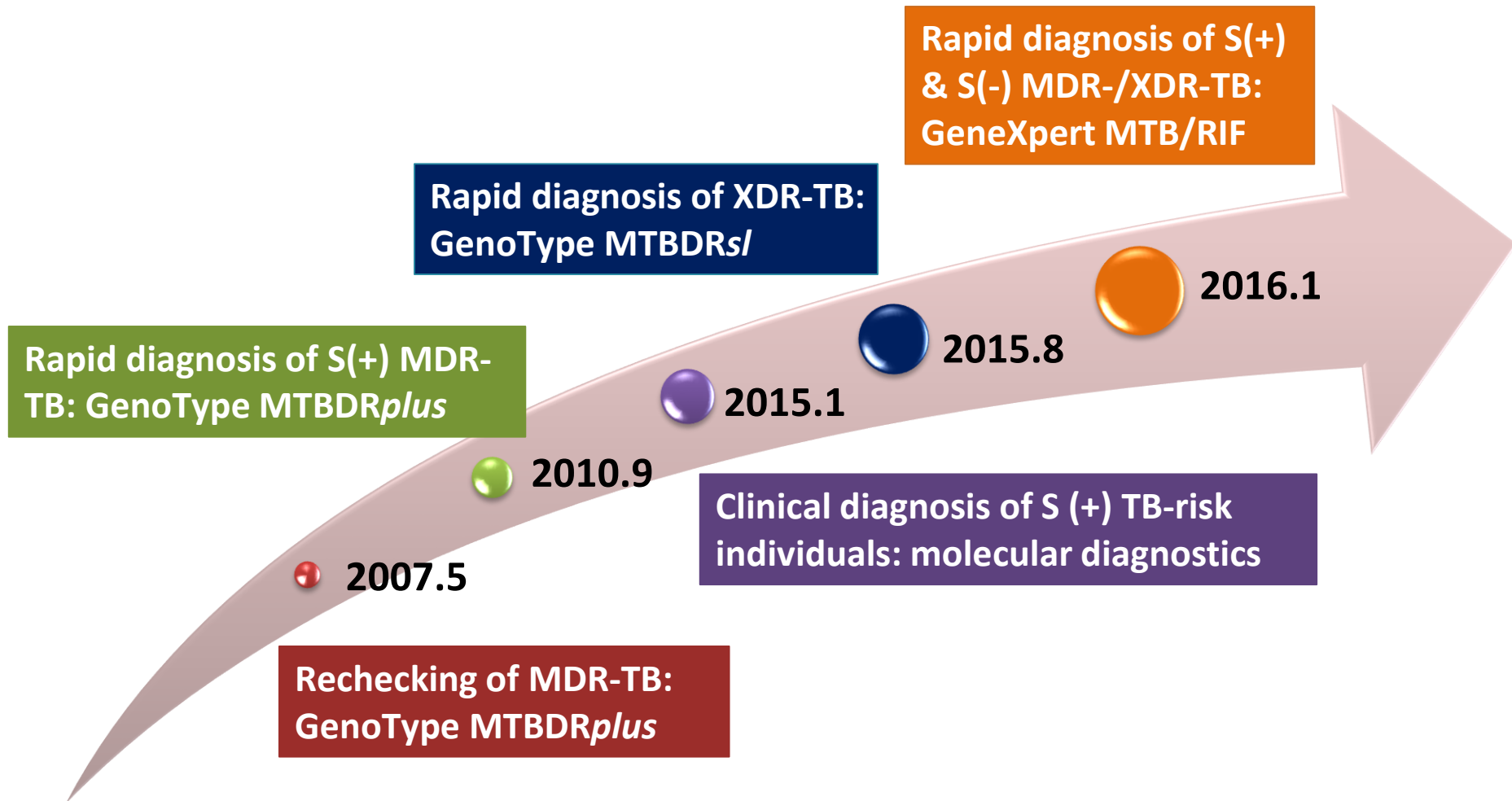
- WHO強烈建議使用於檢測 TB個案和MDR-TB高危險族群

The GenoType MTBDRs/ v2.0 test (GenoType二線)

- 2016年5月，WHO 建議使用線性探針檢測法 (line probe assay, LPA) 進行二線藥物抗藥性檢測 (SL-LPA)
- 對RIF抗藥或MDR-TB確診個案，使用SL-LPA初步檢 fluoroquinolone、二線針劑藥物抗藥性，以取代傳統藥物感受性試驗



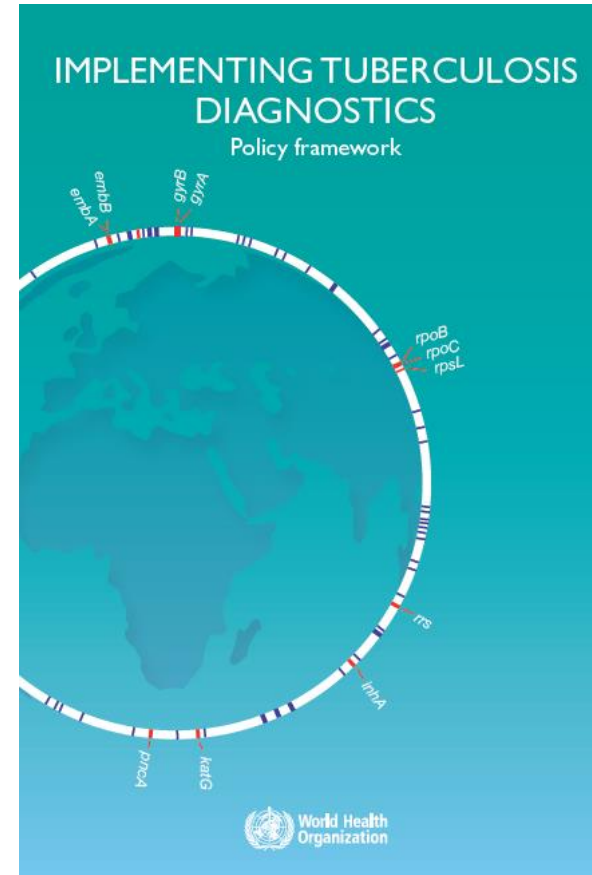
台灣應用結核菌新檢測技術的時程



大綱

- 分子檢測應用在結核病防治的趨勢
- **世衛組織推薦結核病檢驗流程**
- 具潛力的新一代分子檢測技術/工具

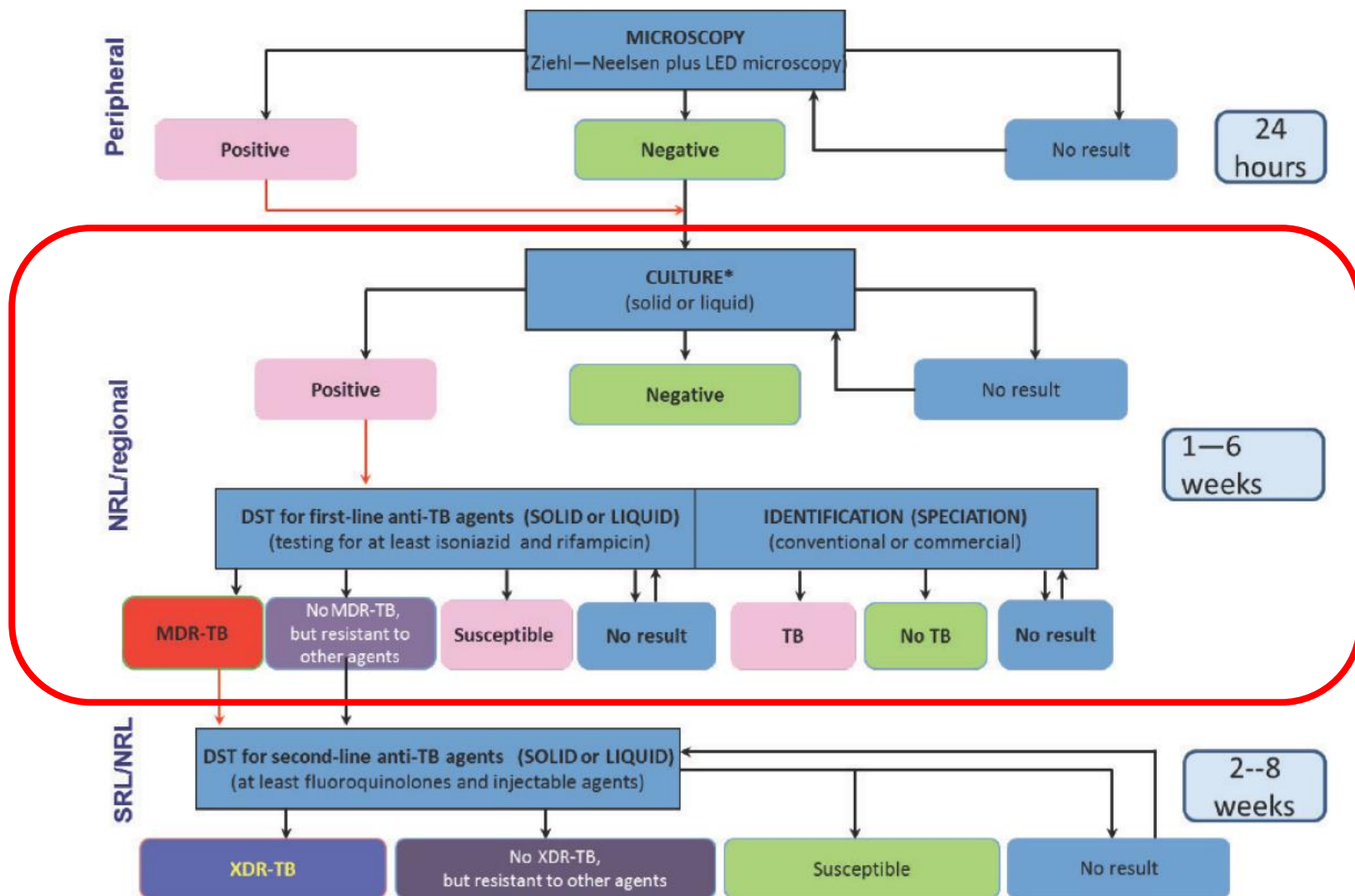
- **2015年WHO 推薦各國參考使用的結核病檢驗流程**
- 傳統檢驗(抹片、培養、藥物感受性試驗)
- 分生檢驗
(GenoType、Xpert)



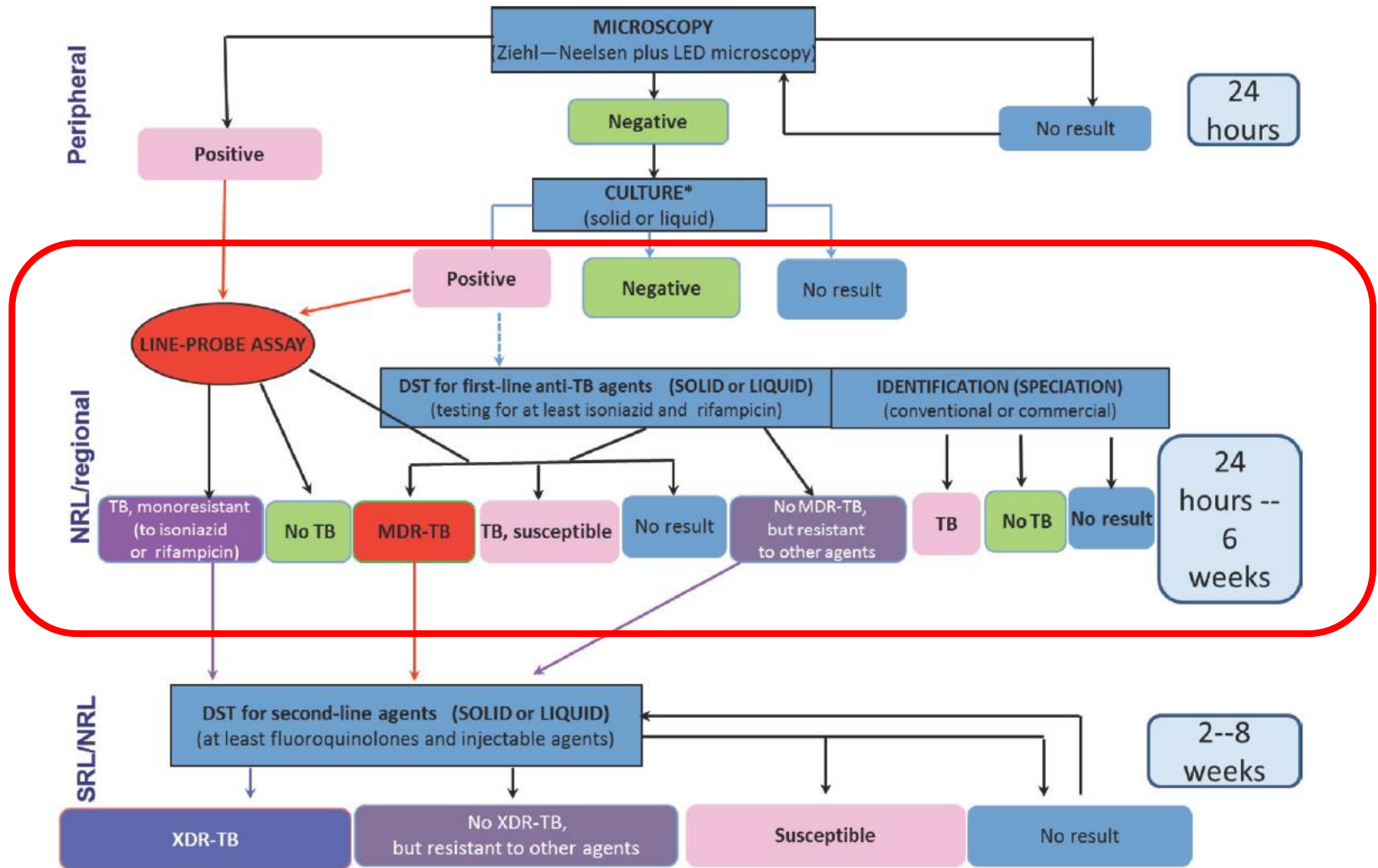
http://www.who.int/tb/publications/implementing_TB_diagnostics/en/



沒有任何分子檢測工具時 傳統流程 (抹片、培養、鑑定、藥物感受性試驗)

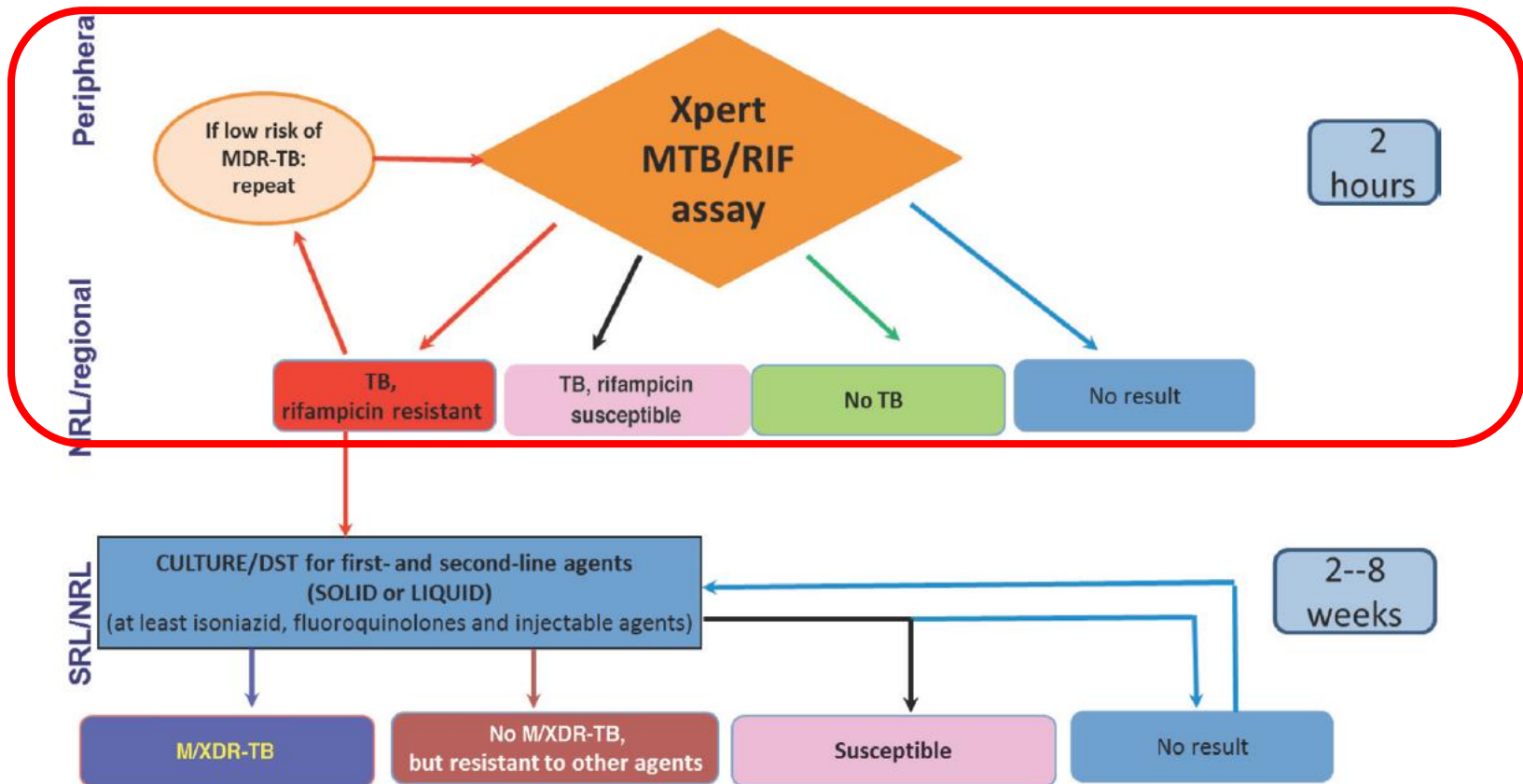


傳統流程 (抹片、培養、鑑定、藥物感受性試驗) 加GenoType

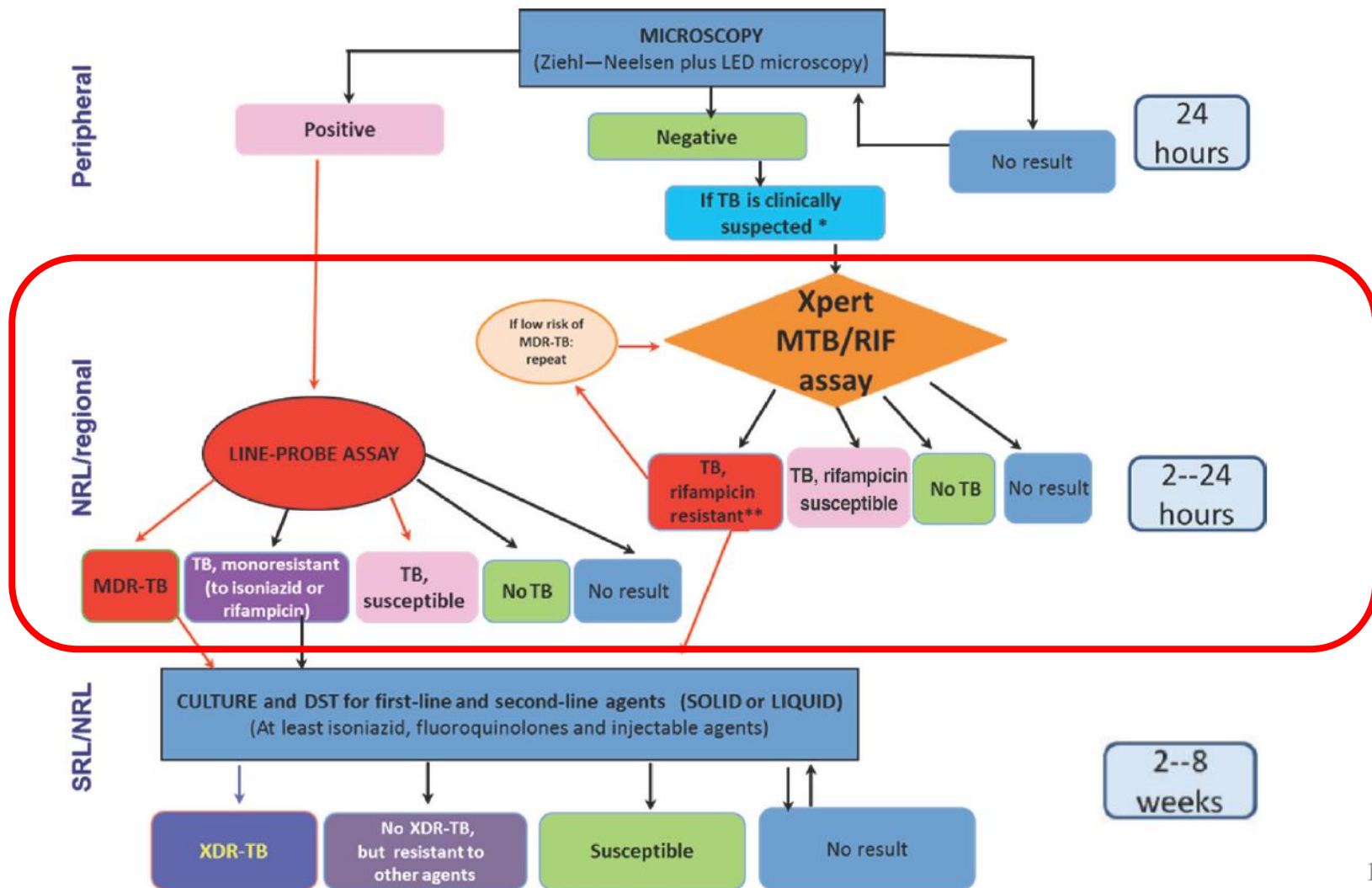


Xpert 加部分傳統流程

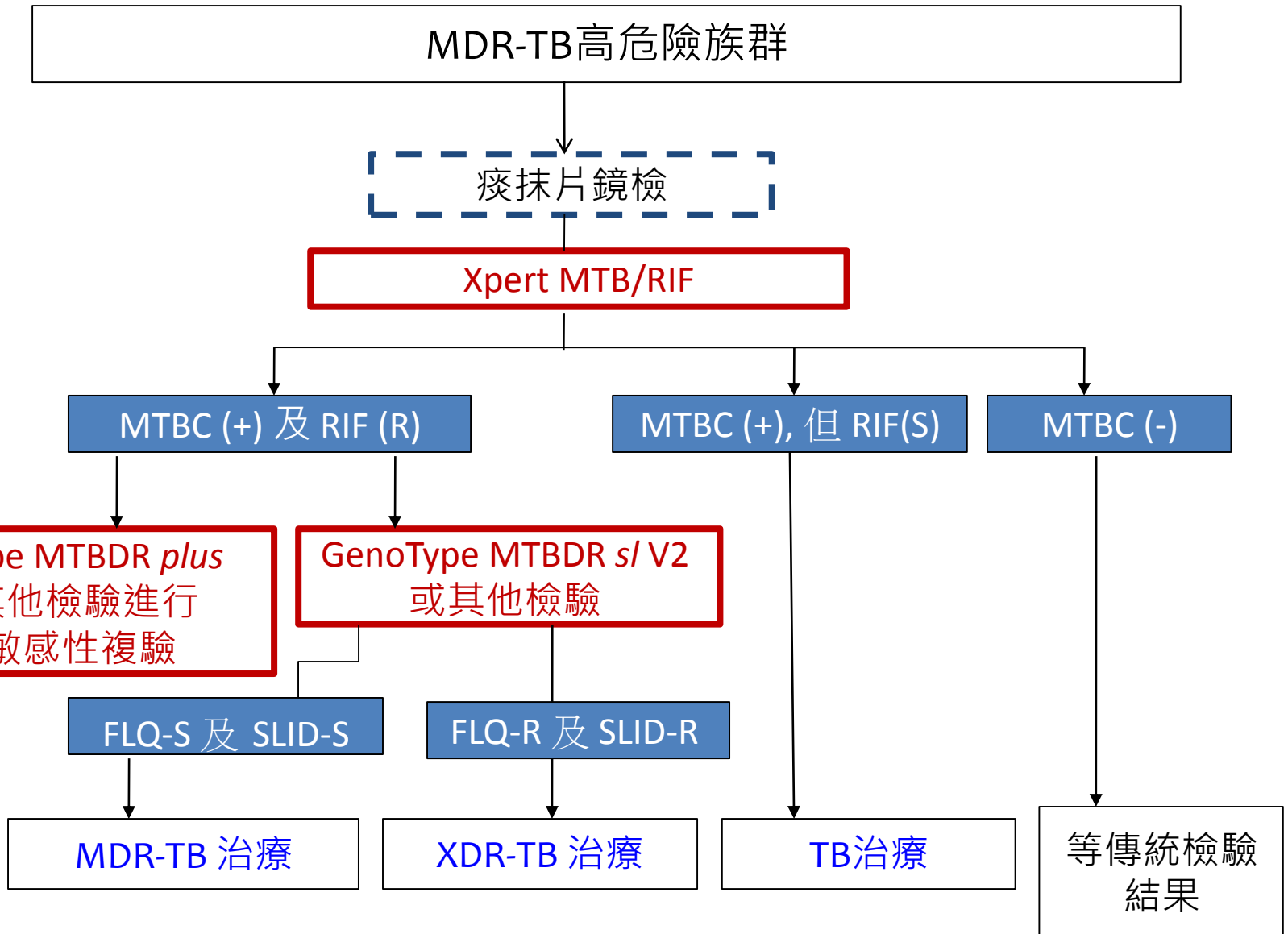
(抹片、培養、鑑定、藥物感受性試驗)



合併GenoType、Xpert 分子檢測工具 加傳統流程 (抹片、培養、鑑定、藥物感受性試驗)



現階段我國高危險群個案分子快速檢測流程 (2016)



大綱

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- 世衛組織推薦結核病檢驗流程
- **具潛力的新一代分子檢測技術/工具**



分子快速檢測

對床邊檢測(point-of-care)的重要性

- 時效性
傳統檢驗方法
- 即時性
醫療處方開立
- 簡易性
操作步驟簡化

核酸恆溫複製技術

Technology	Abbreviation	Target	Initial denaturation	Incubation (°C)	Reaction time (min)	Multiplex	Primers required	Commercial source (Manufacturer)	FDA approved Tests
Helicase-dependent amplification	HDA	DNA	N	65	30-120	Y	2	Y (Biohelix)	Y
Recombinase polymerase amplification	RPA	DNA	N	37-42	20-40	Y	2	Y (TwistDx)	N
Nucleic acid sequence-based amplification	NASBA	RNA (DNA)	Y	41	60-180	Y	2	Y (Organon Teknika Corporation/bioMérieux)	Y
Transcription-mediated amplification	TMA	RNA (DNA)	Y	42	60-180	Y	2	Y (Hologic/Gen-Probe)	Y
Loop-mediated amplification	LAMP	DNA	Y	60-65	60	Y	4-6	Y (Meridian Bioscience)	Y
Rolling circle amplification	RCA	DNA	Y	30-65	60-240	N/A	1	N/A	N
Strand displacement amplification	SDA	DNA	Y	30-55	60-120	Y	4	Y (BD)	Y
Nicking enzyme amplification reaction	NEAR	DNA	N	55-60	60-120	Y	2	Y (Alere)	Y
Ribonuclease-mediated amplification	RMA	DNA	N	65	60-90	Y	2	Y (Great Basin)	N
Cross priming amplification	CPA	DNA	N	63-65	60	N/A	5-8	N/A	N

TB-LAMP test

- 2016年7月，WHO 建議使用環型恆溫核酸增幅法(TB-LAMP) 取代抹片對具肺結核症狀的成人進行檢測
- TB-LAMP能搭配抹片進一步對抹片陰性檢體的成人進行檢測

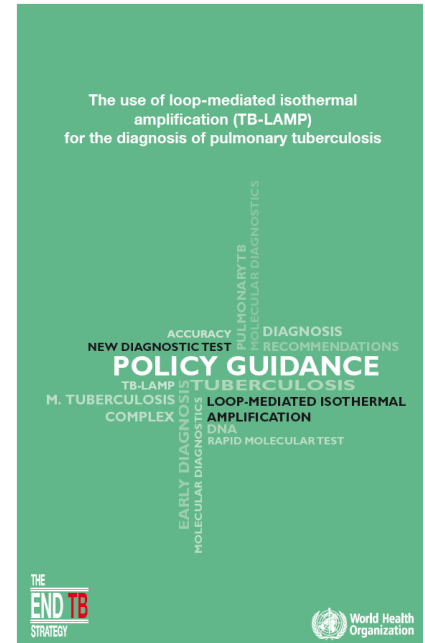
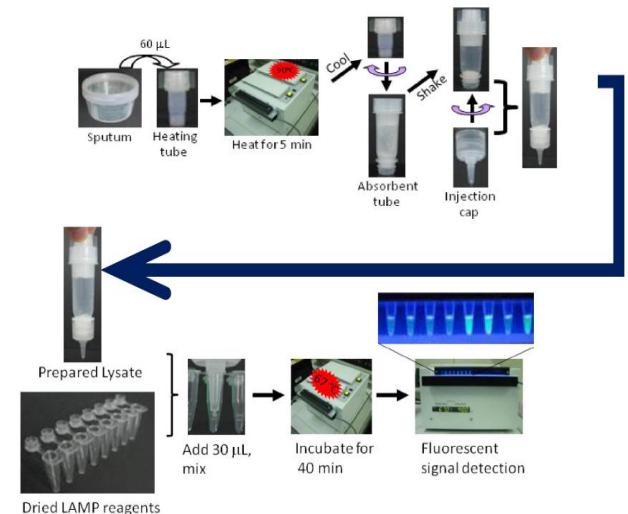
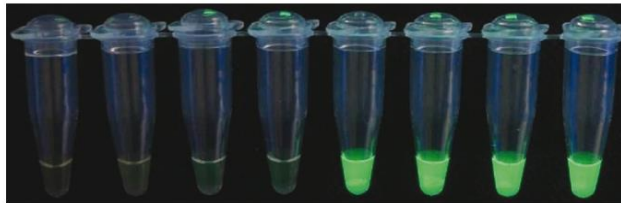


Figure 2. Visual readout of TB-LAMP results applying ultraviolet (UV) light



RPA test

- 37-42°C 20-40 min

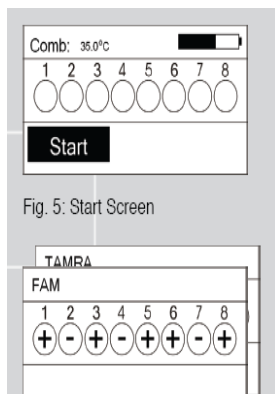
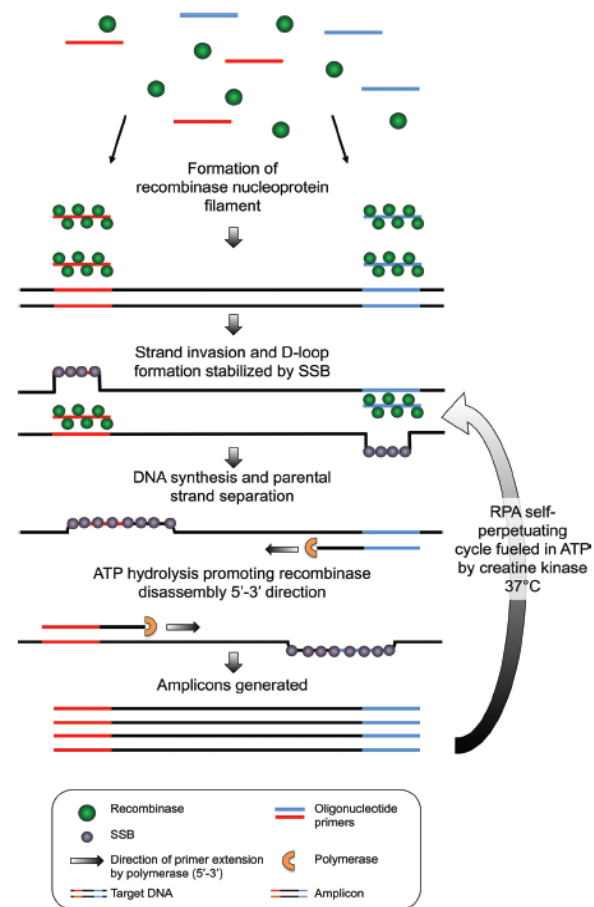


Fig. 5: Start Screen

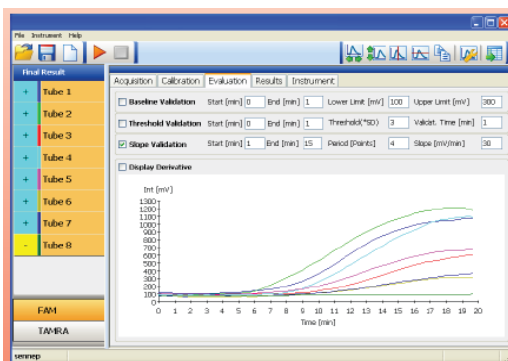
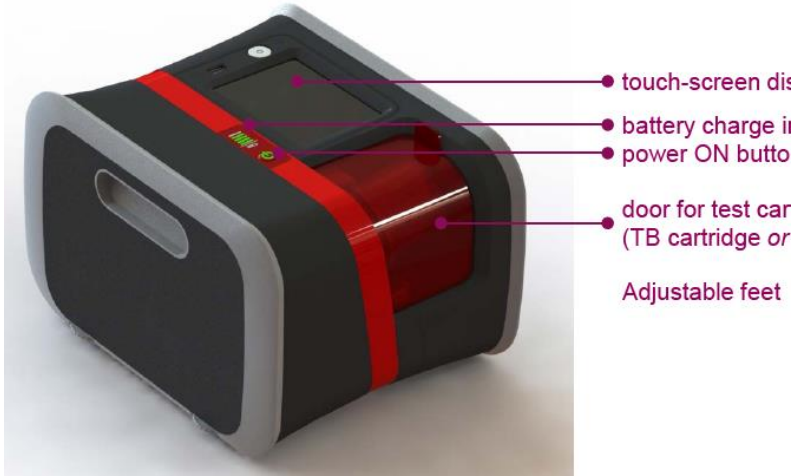


Fig. 1. RPA cycle.

(Daher *et al.*, 2016)

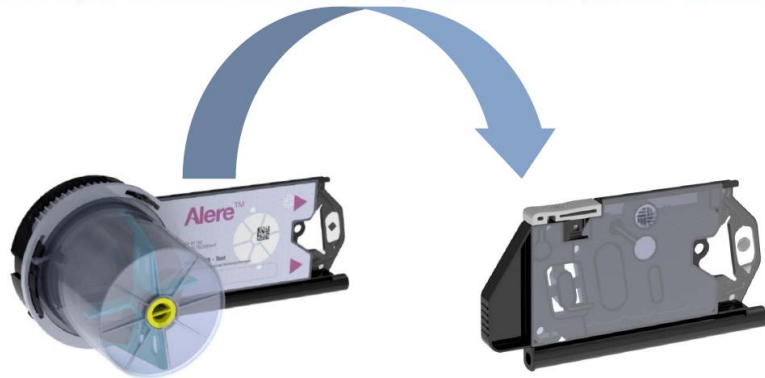
Alere q TB/drug resistance platform



- touch-screen display
- battery charge indicator
- power ON button
- door for test cartridge (TB cartridge or drug resistance profile cartridge)
- Adjustable feet

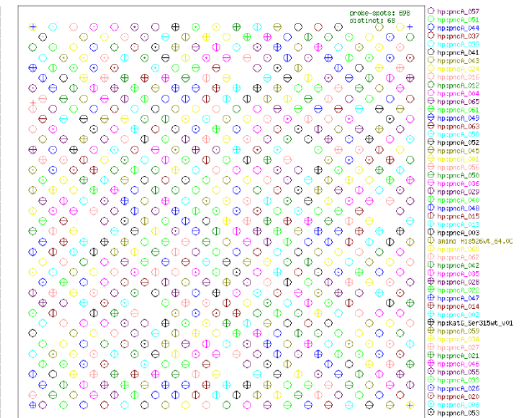
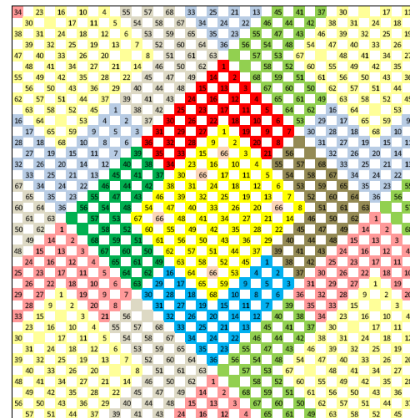
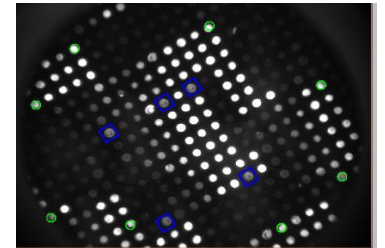
- active TB with DST profile
- sensitivity < 100 CFU/mL
- ID < 20 min; DST < 40 min
- RIF, INH, FLQ, PZA

Transfer of purified DNA (pre-amplification) with simple transfer tool to drug resistance profile cartridge



D 2.0 LVSM cartridge with cup for TB-Test

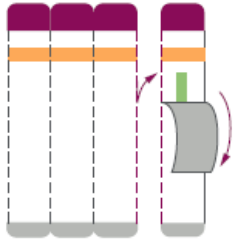
R1.0 TB resistance profile cartridge



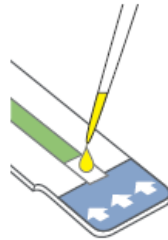
Alere Determine TB LAM Ag

Procedure

- 1** Prepare Test
Tear one strip from the right and remove cover.



- 2** Add Sample
Apply 60 µL of urine to sample pad.



- 3** Read Results
Wait 25 minutes and read the results.



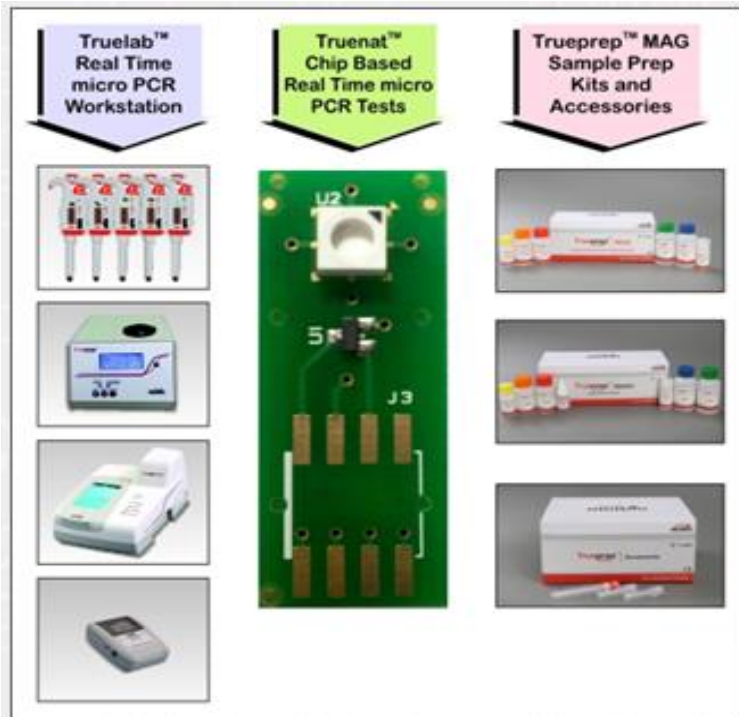
- 4** Check Results
Against the Reference Scale Card.



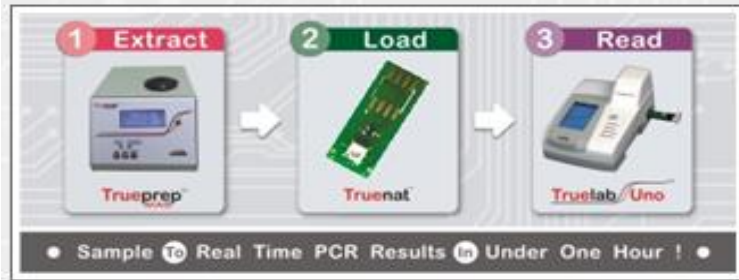
- rapid rule in TB-HIV coinfection
- detect smear negative patient
- urine sample
- 25 min

Truenat MTB

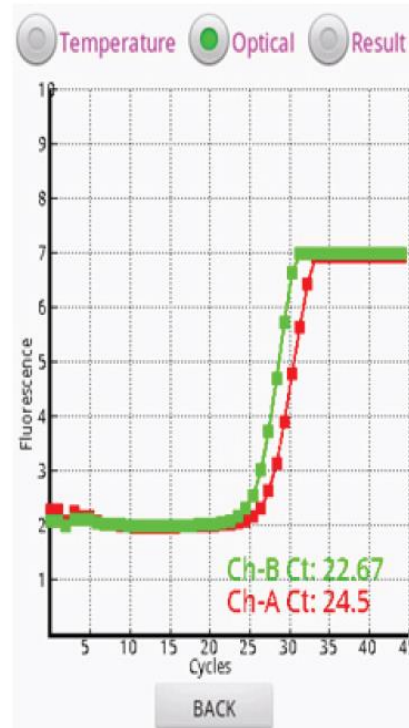
Positive result (MTB detected)



The Truelab™ Real Time micro PCR system works on the following simple principle:



Optical plot (displayed during the run)



Result summary screen

Truenat™ MTB	
Center	molbio
Date	Friday 24 August 2012 08:40:16
Operator	Satheesh
Profile	MTB
Lot Number	12345
Expiry Date	0912
Sample	Sputum
Patient Details	
Name	
ID	PN5063
Age	Sex
Referred By	
Result	
Control C _t	24.5
Test C _t	22.67
Run Status	Valid
MTB	DETECTED 5.8x10 ⁰⁶ CFU/ml
Print	SMS
Share	Back

- 35 minutes
- an Android-based
- global positioning system (GPS)

Genedrive



01

Collect patient sample & process on sample processing cassette.



02

Load MTB/RIF cartridge.

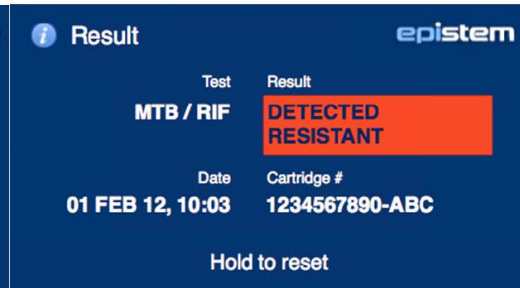
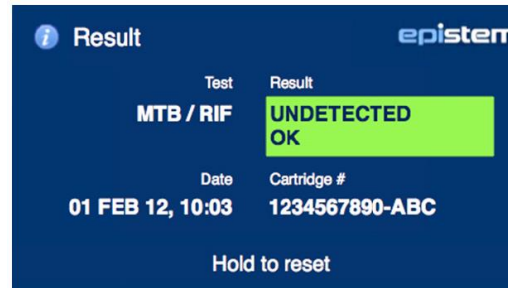


03

Insert cartridge and start.



- 75 minutes
- sample volume
- biosafety





新一代核酸複製平台希望解決


New NAAT platforms

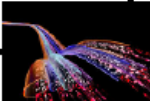



Needs addressed

Decentralization 

Improving time to diagnosis 

Improving MTB detection 

Higher throughput, multiplexing 

Extended, timely DST 
Antibiotic Resistance

Catharina Boehme, 2015

謝謝聆聽，敬請指教！