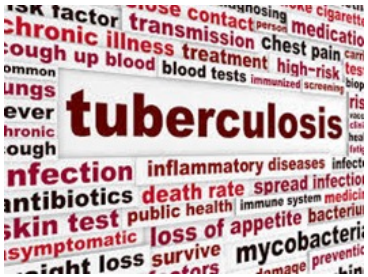
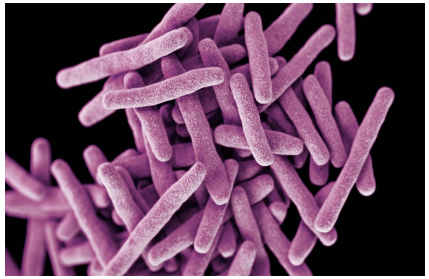
Detección de ADN de *M. tuberculosis* y cultivo negativo. Reto diagnóstico del siglo XXI.





M. Remedio Guna

Servicio de Microbiología

Consorcio Hospital General Universitario de Valencia

CONSORCI HOSPITAL GENERAL UNIVERSITARI VALÈNCIA

GEIM
GRUPO ESTUDIO DE INFECCIONES POR MICOBACTERIAS - SEIMC

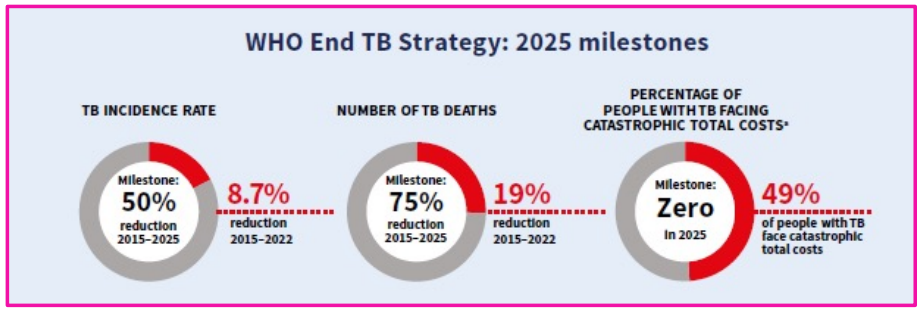
- Grave problema de salud mundial. 1/4 PLANETA
- Afecta a todos los países y grupos de edad
- Probabilidad mayor en VIH, desnutrición, diabetes, tabaquismo, alcoholismo
- Paradójicamente la TUBERCULOSIS es una enfermedad PREVENIBLE y CURABLE

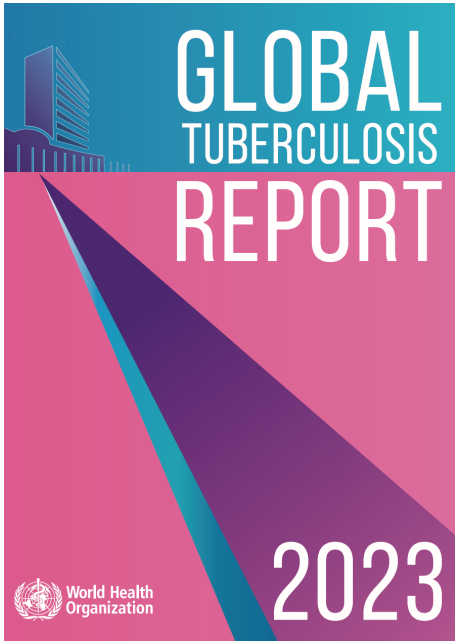
❖1,3 millones de muertes (167 000 VIH).

❖Incidencia de 10,6 millones de nuevos casos 6,3% en VIH (aumento del 4,5% respecto al 2020)

❖ Se da sobre todo en adultos y predomina en hombres (55%)

❖La TB multirresistente gravísimo problema sanitario: Solo 1/3 pacientes con TB MultiR accedió a tratamiento en 2020-21 y las dificultades para recibir tratamiento adecuado se mantuvieron en el 2022 (2/5). Tasas éxito tto MR 63%





Global tuberculosis report 2023

ISBN 978-92-4-008385-1 (electronic version)
ISBN 978-92-4-008386-8 (print version)

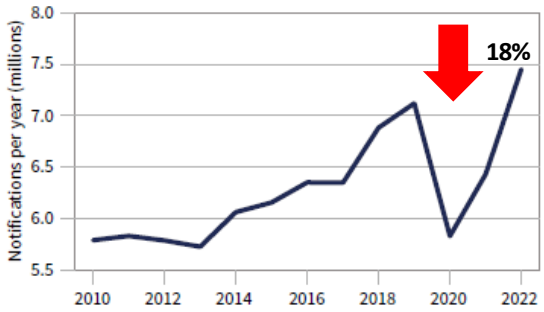
❖Globalmente Disminución de tasas de incidencia 2% al año

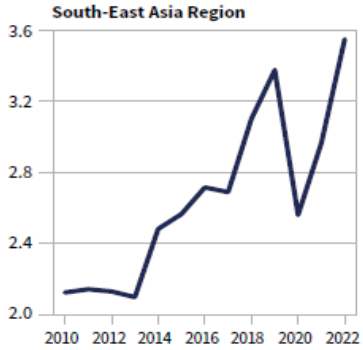
❖ Entre 2015 y 2020 disminución acumulada de la incidencia del 11%

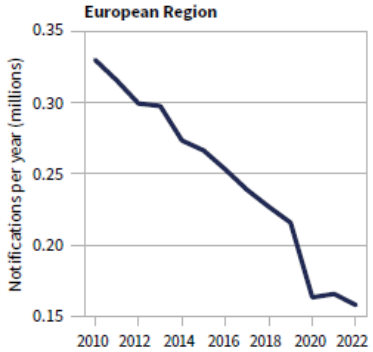
❖A la mitad del camino del objetivo de la OMS en el Programa END TB de reducir la incidencia un 20% entre el 2015 y el 2020.

FIG. 1

Global trend in case notifications of people newly diagnosed with TB, 2010–2022



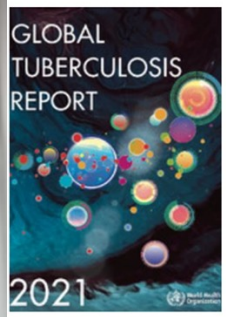




Big drops in TB case notifications

The most obvious impact on TB of disruptions caused by the COVID-19 pandemic is a large global drop in the number of people newly diagnosed with TB and reported in 2020, compared with 2019 (Fig. 1). Following large increases between 2017 and 2019, there was a fall of 18% between 2019 and 2020, from 7.1 million to 5.8 million.

Dramatic setback to TB Elimination

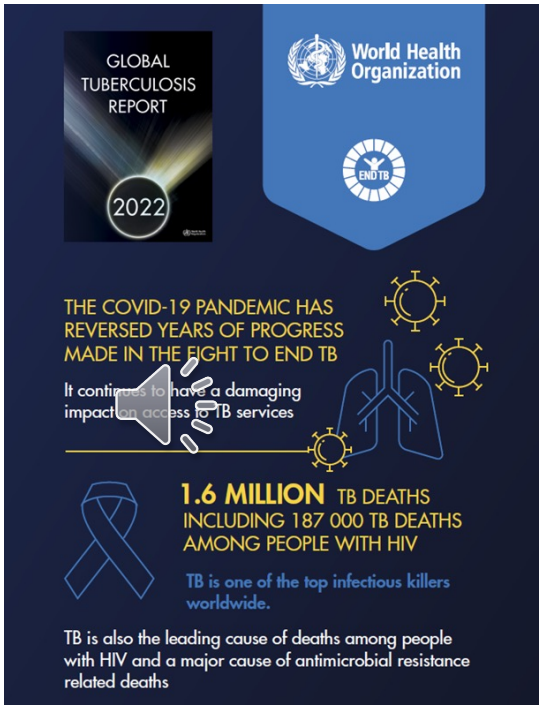


TB deaths increased for the first time in over a decade

Only **5.8 Million** people reported to have access to TB care in 2020. Down from **7.1 Million** in **2019**

In most countries, **resources** (human and financial) were **reallocated** from **tackling TB to the COVID-19 response**

A minimum of 5 years of progress lost and 6 million additional people infected



Perfil de tuberculosis: España

Población 2022: 48 millones

Estimaciones de la carga de TB*, 2022

	Número	(Tasa por 100 000 habitantes)
Incidencia total de TB	3 300 (2 800-3 800)	6.9 (5.9-8)
Incidencia de TB en VIH-positivos	220 (85-430)	0.47 (0.18-0.9)
Incidencia de TB-MDR/RR**	170 (71-260)	0.35 (0.15-0.55)
Mortalidad por TB en VIH-negativos	170 (170-170)	0.35 (0.35-0.36)
Mortalidad por TB en VIH-positivos	59 (20-120)	0.12 (0.04-0.25)

Proporción estimada de casos de TB con TB-MDR/RR*, 2022

Casos nuevos	3.7% (1.9-6.4)
Casos previamente tratados	19% (3.8-46)

Cobertura universal de salud y protección social*

Cobertura del tratamiento de TB (casos notificados/incidencia estimada), 2022	79% (68-92)
Pacientes con TB que enfrentan costos totales catastróficos	
Tasa de letalidad de TB (mortalidad estimada/incidencia estimada), 2022	10% (8-12)

Notificaciones de casos de TB, 2022

Total casos nuevos y recaídas	2 580
- % con prueba rápida al momento del diagnóstico	44%
- % con estado serológico de VIH conocido	52%
- % pulmonar	72%
- % confirmados bacteriológicamente ^	84%
- % niños de 0 a 14 años	4%
- % mujeres (≥15 años)	35%
- % hombres (≥15 años)	61%
Total casos notificados	3 698

2019: 4.300 casos=9,3 casos/100.00 hab
2020: 3.400 casos= 7,3 casos /100.000 hab
2021: 3,900 casos=8,2 casos/100.000hab

Atención de la TB drogo-resistente**, 2022

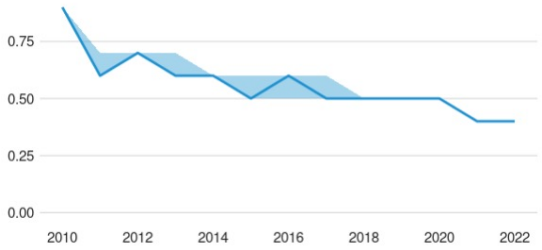
% de casos de TB confirmados bacteriológicamente con prueba de resistencia a la rifampicina - Casos nuevos ^	77%
% de casos de TB confirmados bacteriológicamente con prueba de resistencia a la rifampicina - Casos previamente tratados ^	82%

2020: 32%
2021: 39%

2020: 74%
2021:88%

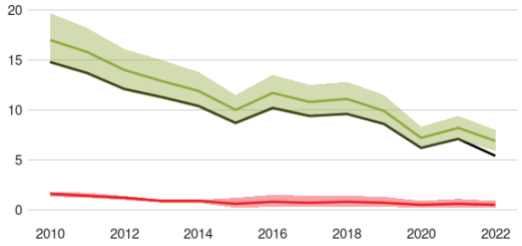
Mortalidad por TB en VIH-negativos

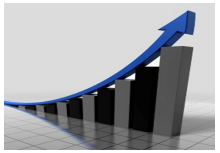
(Tasa por 100 000 habitantes por año)



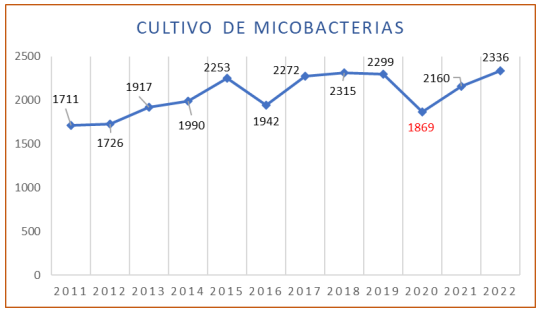
Incidencia, Casos nuevos y recaídas de TB notificados, Incidencia de TB en VIH-positivos

(Tasa por 100 000 habitantes por año)





En relación con Protocolo COVID



AÑOS	Nº PCR	Nº PCR Positivas
2019	1144	36 (3,1%)
2020	1032	35 (3,4%)
2021	1132	40 (3,5%)
2022	1197	33 (2,9%)
2023	1443	54 (3,74%)

2021 : Seguimos adelante !!!

2022-2023 : Aumento del número de muestras!!!

Control TBC

- ✓ Diagnóstico y Tratamiento precoz de la TBC, detección de posibles resistencias
- ✓ Estudio de la ITL

→ Disminución Carga de Enfermedad

Diagnóstico microbiológico directo de enfermedad tuberculosa

- ✓ Visualización de BAAR en la muestra
- ✓ Cultivo
- ✓ Métodos Moleculares
- ✓ Secuenciación

ANNEX 1

Basic facts about TB

Tuberculosis (TB) is an old disease. Studies of human skeletons show that it has affected humans for thousands of years *(1)*. Its cause remained unknown until 24 March 1882, when Dr Robert Koch announced his discovery of the bacillus responsible, subsequently named *Mycobacterium tuberculosis (2)*. The disease is spread when people who are sick with TB expel bacteria into the air (e.g. by coughing). TB typically affects the lungs (pulmonary TB) but can also affect other sites (extrapulmonary TB). Most people who develop the disease (about 90%) are adults and there are more cases among men than women.

Diagnostic tests for TB disease have improved substantially in recent years. There are now several rapid molecular tests recommended by WHO as the initial diagnostic test for TB, some of which can detect drug resistance simultaneously *(3)*. These tests can be used at the lower levels of the health system. A point-of-care lateral-flow test performed on urine is also recommended by WHO; its main use is to assist with diagnosis of TB in people with advanced HIV disease, in combination with rapid molecular tests. There are additional rapid molecular tests specifically for the detection of resistance to a variety of first- and second-line anti-TB drugs, while sequencing technologies can be used to provide a comprehensive individual profile of drug resistance. The older method of sputum smear microscopy (developed >100 years ago) is still widely used for TB diagnosis in low and middle-income countries but is increasingly being replaced with rapid tests.

Culture testing remains the reference standard for TB diagnosis. In addition, culture is required for the detection of resistance to newer anti-TB drugs and may also be used as a confirmatory test in settings and situations in which people have a low pre-test probability of having TB disease. Following diagnosis, culture or smear (as opposed to rapid molecular tests) are necessary to monitor an individual's response to treatment.

Without treatment, the death rate from TB is high. Studies of the natural history of TB disease in the absence of treatment with anti-TB drugs (conducted before drug treatments became available) found that about 70% of individuals with sputum smear-positive pulmonary TB died within 10 years of being diagnosed, as did about 20% of people with culture-positive (but smear-negative) pulmonary TB *(4)*.

Effective drug treatments were first developed in the 1940s. The latest WHO guidelines *(5)* include a strong recommendation for a 6-month regimen of isoniazid (H), rifampicin (R), ethambutol (E) and pyrazinamide (Z) for people with drug-susceptible TB (both pulmonary and extrapulmonary): all four drugs for the first two months, followed by H and R for the remaining 4 months. They also include newer recommendations that people aged 12 years and older with drug-susceptible pulmonary TB may be treated with a 4-month regimen of rifapentine (P), H, Z and moxifloxacin (M), and that children and adolescents between 3 months and 16 years of age with non-severe TB (and without suspicion or evidence of resistance to R and H) may be treated with a 4-month regimen (2 months of H, R, Z and sometimes also E, followed by 2 months of H and R). Treatment success rates of at least 85% for people enrolled on the 6-month regimen are regularly reported to WHO by its 194 Member States.

Treatment for people diagnosed with R-resistant TB (RR-TB) and multidrug-resistant TB (MDR-TB, defined as resistance to H and R) requires other regimens. The latest WHO guidelines *(6)* prioritize a new 6-month regimen consisting of bedaquiline (B), pretomanid (Pa), linezolid (L) and moxifloxacin (M), referred to as BPaLM; for people who have pre-extensively drug-resistant TB (pre-XDR-TB, defined as TB that is resistant to R and any fluoroquinolone), the regimen can be used without moxifloxacin (BPaL). Based on currently available safety data, this regimen is recommended only for people aged 14 years and above. For people not eligible for the 6-month regimen, other 9-month or longer regimens can be used *(6)*. Nationally, treatment success rates for RR-TB reported to date have typically been in the range of 50–75%; the global average has been improving in recent years, reaching 63% in the most recent patient cohort for which data are available. This may further improve with expanded use of BPaLM, for which clinical trial data showed a treatment success rate of 89% *(7)*. Treatment for XDR-TB (resistance to R, any fluoroquinolone and at least one of bedaquiline or linezolid) remains much more difficult and treatment success rates are typically low.

A global modelling study published in 2016 estimated that about a quarter of the world's population had been infected with *M. tuberculosis (8)*. Recent analyses and commentary suggest that the number of those currently infected is lower, given that some people will clear

Diagnostic tests for TB disease have improved substantially in recent years. There are now several rapid molecular tests recommended by WHO as the initial diagnostic test for TB, some of which can detect drug resistance simultaneously *(3)*. These tests can be used at the lower levels of the health system. A point-of-care lateral-flow test performed on urine is also recommended by WHO; its main use is to assist with diagnosis of TB in people with advanced HIV disease, in combination with rapid molecular tests. There are additional rapid molecular tests specifically for the detection of resistance to a variety of first- and second-line anti-TB drugs, while sequencing technologies can be used to provide a comprehensive individual profile of drug resistance. The older method of sputum smear microscopy (developed >100 years ago) is still widely used for TB diagnosis in low and middle-income countries but is increasingly being replaced with rapid tests.

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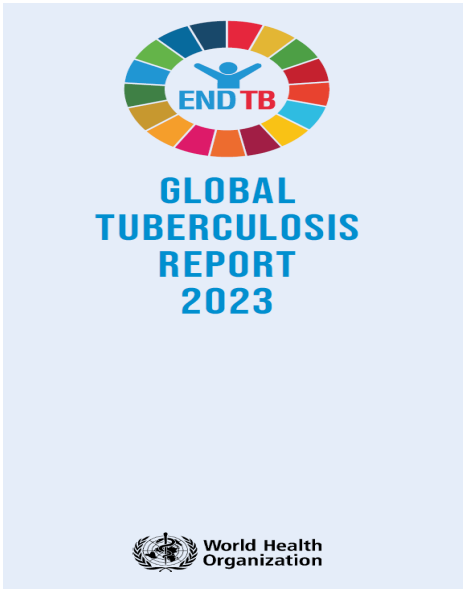


- Test Moleculares rápidos
- Secuenciación
- Baciloscopia
- Cultivo (permanece como Gold standard)

Aplicación de estas técnicas en pacientes con sospecha de TBC supone un ahorro potencial significativo.

Herraez *et al* (Enferm Infecc Microbiol Clin. 2017;35.7) estudio de coste-efectividad en DX de TBC por GeneXpert MTB/RIF

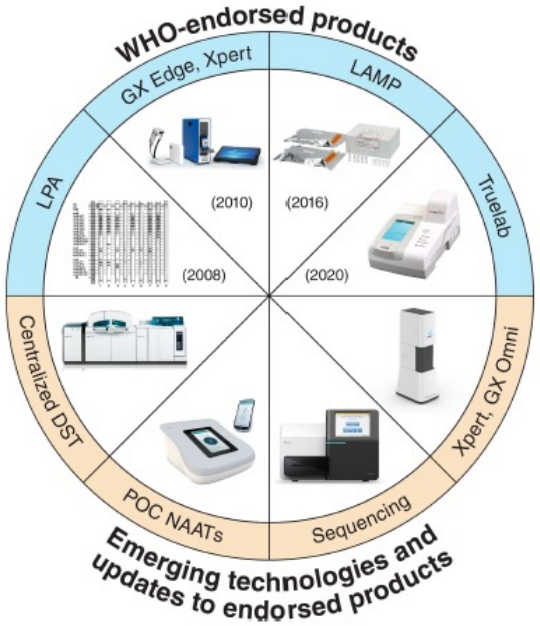
Advances in Molecular Diagnosis of Tuberculosis

Emily MacLean,[a,b] Mikashmi Kohli,[a,b] Stefan F. Weber,[c] Anita Suresh,[d] Samuel G. Schumacher,[d] Claudia M. Denkinger,[b,c] Madhukar Pai[a,b,e]

TABLE 1 WHO-endorsed molecular tests for pulmonary TB detection and drug susceptibility testing[a]

Technology	Year endorsed	Method principle	Intended use	Sensitivity (%)[b]	Specificity (%)[b]	Target setting of use	Turnaround time (h)	Amenable to rapid test-and-treat?	Reference for policy guidance
Xpert MTB/RIF	2010	qPCR	MTB diagnosis and RIF resistance detection	85 (pooled), 96 (RIF resistance)	99 (MTB detection) 98 (RIF resistance)	District or subdistrict laboratory	<2	Yes, especially on Omni platform	WHO 2020 (21), WHO 2016 (84)
Xpert MTB/RIF ultra	2017	qPCR/melting temperature analysis (RIF resistance)	MTB diagnosis and RIF resistance detection	90 (pooled), 94 (RIF resistance)	96 (MTB detection), 98 (RIF resistance)	District or subdistrict laboratory	<2	Yes, especially on Omni platform	WHO 2020 (21)
First-line probe assays (e.g., GenoType MTBDRplus and NIPRO)	2008	PCR, hybridization	Diagnosis of RIF and INH resistance	98 (RIF resistance), 84 (INH resistance)	99 (RIF resistance), >99 (INH resistance)	Reference laboratory	5	No	WHO 2008 (14)
Second-line probe assays (e.g., GenoType MTBDRsl)	2016	PCR, hybridization	Diagnosis of FLQ and SLID resistance	86 (FLQ resistance), 87 (SLID resistance)	99 (FLQ resistance), 99 (SLID resistance)	Reference laboratory	5	No	WHO 2016 (15)
Loopamp MTBC assay	2016	Loop-mediated isothermal amplification	MTB diagnosis	78 (pooled)	98 (MTB detection)	Peripheral laboratory	<2	Yes	WHO 2016 (16)
Truenat MTB plus	2020	Micro RT-PCR	MTB diagnosis	80 (pooled)	96 (MTB detection)	Peripheral laboratory	<2	Yes, on Truelab platform	WHO 2020 (21)
Truenat MTB-RIF Dx	2020	Micro RT-PCR	Diagnosis of RIF resistance	84 (RIF resistance)	97 (RIF resistance)	Peripheral laboratory	<2	Yes, on Truelab platform	WHO 2020 (21)

[a]FLQ, fluoroquinolone; INH, isoniazid; LAMP, loop-mediated isothermal amplification; NAAT, nucleic acid amplification tests; RIF, rifampin; RT-PCR, reverse transcriptase PCR; SLID, second-line injectable drugs; SSM+/C−, sputum smear microscopy positive/culture positive; SSM−/C+, sputum smear microscopy negative/culture positive; WHO, World Health Organization.
[b]Performance estimates have been retrieved from different studies and are not the result of head-to-head comparisons. Therefore, comparing performances between tests must be made with caution. All reported values are from the policy guidance document cited.



Detección de MTBC sobre muestra directa: PCR-RT

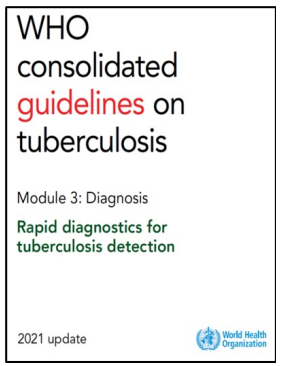


Table 1.1. Classes of technologies and associated products included in current guidelines

Technology class	Products included in the evaluation
	Xpert® MTB/RIF and Xpert® MTB/RIF Ultra (Cepheid)*
	Truenat™ (Molbio) *;
Moderate complexity automated NAATs for detection of TB and resistance to rifampicin and isoniazid	Abbott RealTime MTB and Abbott RealTime MTB RIF/INH (Abbott) BD MAX™ MDR-TB (Becton Dickinson) cobas® MTB and cobas MTB-RIF/INH (Roche) FluoroType® MTBDR and FluoroType® MTB (Hain Lifescience/Bruker)
	TB-LAMP (Eiken) *
Antigen detection in a lateral flow format (biomarker-based detection)	Alere Determine™ TB LAM Ag (Alere)
Low complexity automated NAATs for the detection of resistance to isoniazid and second-line anti-TB agents	Xpert® MTB/XDR (Cepheid)
Line probe assays (LPAs)	GenoType® MTBDR*plus* v1 and v2; GenoType® MTBDRs*l*, (Hain Lifescience/Bruker), Genoscholar™ NTM+MDRTB II; Genoscholar™ PZA-TB II (Nipro)

*These recommendations are currently product specific but will be changed to class-based to align with the other recommendations.

WHO diagnostic guidelines included in these consolidated guidelines

- Xpert MTB/RIF and Xpert MTB/RIF (Ultra). Issued in 2020 for the first time as a part of the WHO consolidated guidelines on tuberculosis. Module 3: diagnosis – rapid diagnostics for tuberculosis detection
- Truenat MTB, Truenat MTB Plus and Truenat MTB-RIF Dx. Issued in 2020 for the first time as a part of the WHO consolidated guidelines on tuberculosis. Module 3: diagnosis – rapid diagnostics for tuberculosis detection
- Moderate complexity automated NAATs for detection of TB and resistance to rifampicin and isoniazid. Issued in 2021 for the first time as a part of the present document.
- The use of loop-mediated isothermal amplification (TB-LAMP) for the diagnosis of pulmonary tuberculosis: policy guidance (WHO/HTM/TB/2016.11). Geneva: World Health Organization; 2016.
- Lateral flow urine lipoarabinomannan assay (LF-LAM) for the diagnosis of active tuberculosis in people living with HIV. Policy update 2019 (WHO/CDS/TB/2019.16). Geneva: World Health Organization; 2019.
- Low complexity automated NAATs for detection of resistance to isoniazid and second-line anti-TB agents. Issued in 2021 for the first time as a part of the present document.
- The use of molecular line probe assays for the detection of resistance to isoniazid and rifampicin (WHO/HTM/TB/2016.12). Geneva: World Health Organization; 2016.
- The use of molecular line probe assays for the detection of resistance to second-line anti-tuberculosis drugs: policy guidance (WHO/HTM/TB/2016.07). Geneva: World Health Organization; 2016.
- High complexity hybridization based NAATs for detection of resistance to pyrazinamide. Issued in 2021 for the first time as a part of the present document.

Sistemas comerciales de amplificación genética para la detección en muestra directa del MTBC (y R a RIF e INH)

Detección de MTBC sobre muestra directa: PCR-RT (GeneXpert)

Next-generation Xpert® MTB/RIF Ultra assay recommended by WHO. 24.03.2017

- Xpert MTB/Rif Ultra: MTBC (y R a Rif) (< 80 min)
- Sensibilidad analitica: 11,8 ufc/mL
- Sobre todo en Baciloscopias negativas, VIH. Muestras pediátricas, Muestras extrapulmonares
- Mayor precisión detección R a Rifampicina
- Mayor probabilidad de detectar bacilos no viables

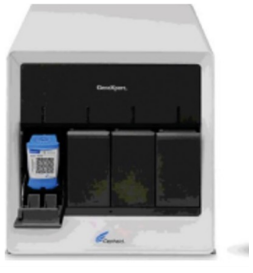


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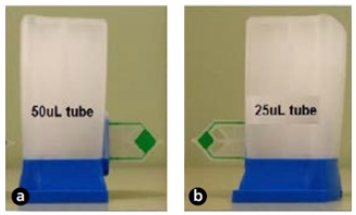
Fig. 2.1.2. (a) The Xpert MTB/RIF Ultra cartridge with its 50 µL reaction tube (green) and (b) the Xpert MTB/RIF cartridge with its 25 µL reaction tube (green)



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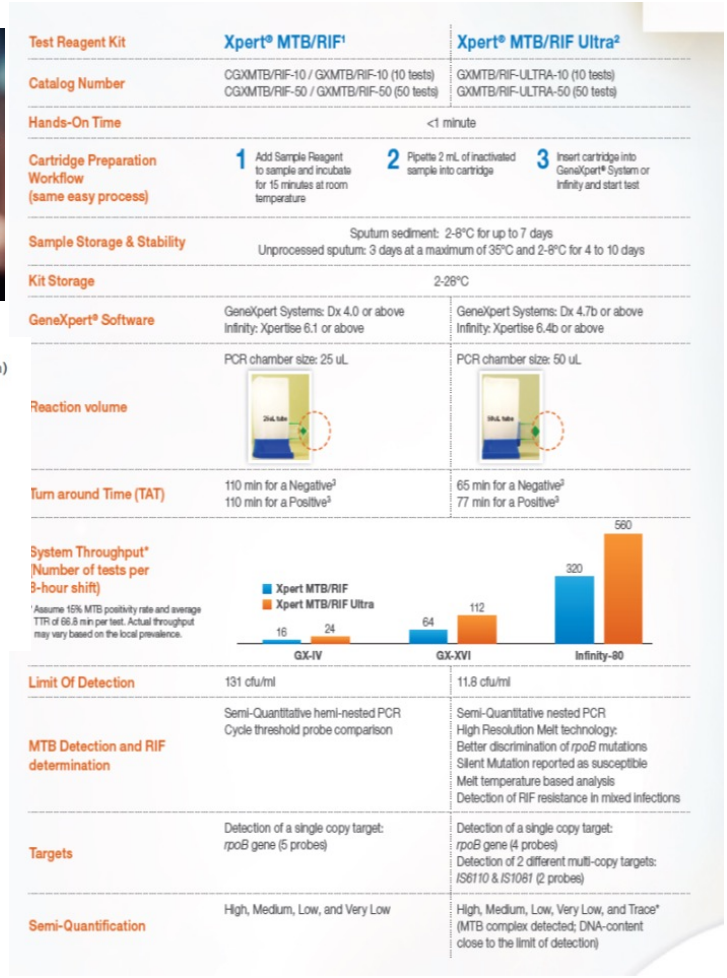

Xpert MTB/RIF Ultra for detection of *Mycobacterium tuberculosis* and rifampicin resistance: a prospective multicentre diagnostic accuracy study

Interpretation For tuberculosis case detection, sensitivity of Xpert Ultra was superior to that of Xpert in patients with paucibacillary disease and in patients with HIV. However, this increase in sensitivity came at the expense of a decrease in specificity.

www.thelancet.com/infection Vol 18 January 2018

WHO meeting report of a technical expert consultation: non-inferiority analysis of Xpert MTB/RIF ultra compared to Xpert MTB/RIF

Findings

In the main study, 1,520 persons with signs and symptoms of TB were enrolled. Overall, sensitivity of the Ultra was 5% higher than that of Xpert MTB/RIF (95%CI +2.7, +7.8) but specificity was 3.2% lower (95%CI -2.1, -4.7).

Additional retrospective studies demonstrated that in low TB burden settings where there is very limited TB transmission the specificity of Ultra is very high (99.3%, 95%CI 96-99). For EPTB and paediatric TB, studies highlighted the benefit of the increased sensitivity (primarily due to the 'trace call') with a sensitivity of 95% for Ultra versus 45% for Xpert MTB/RIF for detection of TB meningitis using cerebrospinal fluid and 71% for Ultra on respiratory samples from children versus 47% for Xpert MTB/RIF.

WHO meeting report of a technical expert consultation: non-inferiority analysis of Xpert MTB/RIF ultra compared to Xpert MTB/RIF

Interpretación del resultado de PCR positiva con “ trazas de genoma de MTBC ”

- En pacientes VIH, niños y muestras extrapulmonares : debe ser considerado como verdadero positivo para su uso en decisiones clínicas y seguimiento del paciente.
- En pacientes sin riesgo de contraer VIH, se debe repetir la prueba en una muestra fresca y tomar decisiones clínicas y seguimiento del paciente en función de este resultado.
- Aunque la clínica e información radiológica debe ser siempre considerado en el diagnóstico de tuberculosis, una segunda prueba de PCR positiva con trazas de genoma de MTBC es suficiente para hacer un diagnóstico de TB pulmonar a menos que haya una historia reciente de tuberculosis.
- Xpert/Ultra tiene alta sensibilidad y especificidad para detección de mutaciones que determinan la resistencia a la rifampicina.
- El hecho de que Ultra emplee un análisis basado en la temperatura de fusión (melting) permite mejor diferenciación de mutaciones silentes
- En personas con bajo riesgo de resistencia a la rifampicina, si se detecta resistencia positiva a la rifampicina, debe repetirse la determinación en una nueva muestra para descartar cualquier posible error preanalítico y/o postanalítico.

Diagnostic Performance of Xpert MTB/RIF Ultra Compared with Predecessor Test, Xpert MTB/RIF, in a Low TB Incidence Setting: a Retrospective Service Evaluation

Microbiology Spectrum

TABLE 1 Comparative Performance of the Xpert and Ultra Assays

	Sensitivity % (95%CI)[a]			Specificity % (95%CI)	
Sample Type	Xpert	Ultra	*P* value	Xpert	Ultra
All samples ($n = 1404$)	82.19 (75.01–88.02)	93.22 (87.08–97.03)	0.0078	95.76 (93.71–97.30)	94.81 (92.71–96.44)
Smear positive ($n = 197$)	97.27 (92.24–99.43)	100 (95.85–100)	0.1206		
Smear negative ($n = 67$)	36.11 (20.82–53.78)	74.19 (55.39–88.14)	0.0018		
All pulmonary samples ($n = 830$)	90.32 (82.42–95.48)	95.35 (88.52–98.72)	0.1955	97.44 (95.01–98.89)	96.46 (93.90–98.16)
Smear positive pulmonary ($n = 150$)	98.73 (93.15–99.97)	100 (94.94–100)	0.3415		
Smear negative pulmonary ($n = 29$)	42.85 (17.66–71.14)	73.33 (44.9–92.21)	0.0959		
All extrapulmonary samples ($n = 574$)	67.92 (53.68–80.08)	87.5 (71.01–96.49)	0.0426	93.51 (89.52–96.32)	92.64 (88.74–95.51)
Smear positive extrapulmonary ($n = 47$)	93.55 (78.58–99.21)	100 (79.41–100)	0.2991		
Smear negative extrapulmonary ($n = 38$)	31.8 (13.86–54.87)	75 (47.62–92.73)	0.0086		

[a]95% CI: 95% confidence interval; Xpert: Xpert MTB/RIF; Ultra: Xpert MTB/RIF Ultra; PPV: positive predictive value; NPV: negative predictive

Conclusiones del estudio : genXpert ULTRA

Mayor sensibilidad en muestras con baciloscopia negativa y extrapulmonares

Resultados semicuantitativos podrían correlacionarse con carga bacilar: transmisibilidad, aislamiento de pacientes

Assessment of the Xpert MTB/RIF Ultra assay on rapid diagnosis of extrapulmonary tuberculosis

Xiaocui Wu[a], Guangkun Tan[b], Rongliang Gao[a], Lan Yao[c], Dexi Bi[d], Yinjuan Guo[a], Fangyou Yu[a,1,*], Lin Fan[c,1,*]

International Journal of Infectious Diseases 81 (2019) 91–96

Objective: To evaluate the diagnostic performance of Xpert MTB/RIF Ultra for EPTB (Extrapulmonary Tuberculosis) patients on different types of extrapulmonary specimens from different anatomic sites.
Methods: Patients with suspected EPTB were prospectively included, extrapulmonary specimens were collected and subjected to culture, Xpert and Xpert Ultra assays in accordance with relevant guidelines.

Conclusions: Xpert Ultra assay had a higher sensitivity than those of Xpert and culture on extrapulmonary specimens, which could be a promising approach for rapid EPTB diagnosis.

Conclusion

Xpert Ultra represents the most recent advancement in the rapid molecular diagnosis of TB. Many studies have shown a potential for the use of Xpert Ultra in extra-pulmonary specimens. Xpert Ultra sensitivity differs by specimen type, with higher sensitivity among specimens obtained from lymph nodes (ranging from 50 to 100%) and CSF (ranging from 71.4 to 96.4%), and lower sensitivity when using pleural fluids (ranging from 47.6 to 84.2%). Moreover, the performance of Xpert Ultra proved to be particularly high in special populations, such as pediatric TB and HIV co-infected TB. In addition, the use of Xpert Ultra on extra-pulmonary samples, i.e., gastric aspirate and stool, has been demonstrated to be helpful for detecting pulmonary TB.

Although negative Xpert Ultra results are not sufficient to rule out TB, positive Xpert Ultra results may be useful in rapidly identifying EPTB cases, thus suggesting that Xpert Ultra is a useful rule-in rapid diagnostic test that can improve the definitive diagnosis of EPTB.

Rapid Molecular Diagnosis of Extra-Pulmonary Tuberculosis by Xpert/RIF Ultra

*Laura Rindi**

Frontiers in Microbiology

MINI REVIEW
published: 11 May 2022
doi: 10.3389/fmicb.2022.817661

TABLE 1 | Diagnostic performance of Xpert MTB/RIF Ultra (Xpert Ultra) on extra-pulmonary TB (EPTB) specimens.

Specimen type	Sensitivity		Number of specimens	
	vs Culture	vs CRS	Culture-Positive	Total
Pleural fluid	84.2%	61.1%	57	108
	83.6%	44.2%	55	208
		43.7%		103
	47.6%		21	24
		37.5%		48
	50%		6	118
	66.7%		9	77
	50%	48.1%	6	27
Pleural tissue	100%		2	2
	100%		2	41
	100%	81.48%	9	27
Lymph node tissue	94.1%		17	25
	75%		8	51
	95.8%		24	196
	90%	66.7%	10	24
		91.3%		46
	50%		10	10
	100%	40%	1	5
		33.3%		3
Lymph node aspirate	77.8%	70%	9	30
	100%	26.7%	4	15
CSF		44.2%		43
		33.3%		6
	90%	95.4%	10	22
	80%	63.6%	5	11
		92.9%		42
	90.9%	59.5%	22	42
	96.4%	72%	56	204
		46.7%		60
	71.4%	45%	14	76
Joint fluid	87.5%		8	9
Osteoarticular pus	62.5%		8	8
	96.1%	90.9%	52	132
Osteoarticular biopsy	100%		2	21
	100%		3	27
Urine	100%		12	24
	100%		2	6
		18%[a]		84[a]
		17.2%[b]		203[b]
		33.9%[c]		56[c]
Gastric aspirate	75%		4	5
	50%		2	2
		60%		5
	87.5%	60.5%	48	129
	85.4%	52.5%	48	141
Stool	80%		5	8
		60.3%		141
	83.3%		72	111
		45.5%		126

[a]*TB lymphadenitis;* [b]*pulmonary TB;* [c]*TB meningitis.*

Diagnosis of paediatric TB using Xpert® MTB/RIF Ultra on fresh respiratory samples

INT J TUBERC LUNG DIS 26(9):862–868

http://dx.doi.org/10.5588/ijtld.22.0007

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OBJECTIVE: To evaluate the diagnostic accuracy of Xpert® MTB/RIF Ultra (Ultra) on fresh respiratory samples for the diagnosis of pulmonary TB (PTB) in children.

METHODS: Between July 2017 and December 2019, children with presumed TB were prospectively enrolled at clinical sites in three African countries. Children were assessed using history, physical examination and chest X-ray. Sputum or gastric aspirate samples were analysed using Ultra and culture. The diagnostic accuracy of Ultra was calculated against culture as the reference standard.

RESULTS: In total, 547children were included. The median age was 4.7 years, 77 (14.1%) were HIV infected and 77 (14.1%) had bacteriologically confirmed TB. Ultra detected an additional 20 cases in the group of children with negative culture results. The sensitivity of Ultra was 66.3% (95% CI 47–82), and the specificity was 95.4% (95% CI 89–99) when assessed against culture as the reference standard.

CONCLUSION: Despite the improved performance of Ultra as compared to Xpert as was previously reported, its sensitivity remains sub-optimal for the detection of TB in children. Ultra detected additional 20 cases which otherwise could not have been detected by culture alone, suggesting that the latter is an imperfect reference standard.

Diagnostic accuracy of Xpert MTB/RIF Ultra for tuberculous meningitis in HIV-infected adults: a prospective cohort study

Nathan C Bahr, Edwin Nuwagira, Emily E Evans, Fiona V Cresswell, Philip V Bystrom, Adolf Byamukama, Sarah C Bridge, Ananta S Bangdiwala, David B Meya, Claudia M Denkinger, Conrad Muzoora, David R Boulware, on behalf of the ASTRO-CM Trial Team

Summary

Background WHO recommends Xpert MTB/RIF as initial diagnostic testing for tuberculous meningitis. However, diagnosis remains difficult, with Xpert sensitivity of about 50–70% and culture sensitivity of about 60%. We evaluated the diagnostic performance of the new Xpert MTB/RIF Ultra (Xpert Ultra) for tuberculous meningitis.

Methods We prospectively obtained diagnostic cerebrospinal fluid (CSF) specimens during screening for a trial on the treatment of HIV-associated cryptococcal meningitis in Mbarara, Uganda. HIV-infected adults with suspected meningitis (eg, headache, nuchal rigidity, altered mental status) were screened consecutively at Mbarara Regional Referral Hospital. We centrifuged CSF, resuspended the pellet in 2 mL of CSF, and tested 0·5 mL with mycobacteria growth indicator tube culture, 1 mL with Xpert, and cryopreserved 0·5 mL, later tested with Xpert Ultra. We assessed diagnostic performance against uniform clinical case definition or a composite reference standard of any positive CSF tuberculous test.

	Sensitivity vs composite endpoint (95% CI; n/N)	Sensitivity vs case definition (95% CI; n/N)	Assay error rate
Xpert Ultra	95% (77–99; 21/22)	70% (47–87; 16/23)	2·3% (3/129)
Xpert	45% (24–68; 10/22)	43% (23–66; 10/23)	4·7% (6/129)
MGIT culture	45% (24–68; 10/22)	43% (23–66; 10/23)	1·6% (2/129)

All three tests were done in all 129 participants. Composite endpoint included any positive CSF Xpert Ultra, Xpert, or Bactec 960 MGIT culture. Sensitivity vs uniform clinical case definition for definite (n=14) or probable (n=9) tuberculous meningitis excluded Xpert Ultra results in defining case status.[a] Error in culture reflects contamination with non-tuberculous mycobacterium growth. Xpert=Xpert MTB/RIF. MGIT=mycobacteria growth indicator tube. CSF=cerebrospinal fluid.

Table 3: CSF diagnostic performance for tuberculous meningitis of Xpert, culture, and Xpert Ultra

Findings From Feb 27, 2015, to Nov 7, 2016, we prospectively evaluated 129 HIV-infected adults with suspected meningitis for tuberculosis. 23 participants were classified as probable or definite tuberculous meningitis by uniform case definition, excluding Xpert Ultra results. Xpert Ultra sensitivity was 70% (95% CI 47–87; 16 of 23 cases) for probable or definite tuberculous meningitis compared with 43% (23–66; 10/23) for Xpert and 43% (23–66; 10/23) for culture. With composite standard, we detected tuberculous meningitis in 22 (17%) of 129 participants. Xpert Ultra had 95% sensitivity (95% CI 77–99; 21 of 22 cases) for tuberculous meningitis, which was higher than either Xpert (45% [24–68]; 10/22; p=0·0010) or culture (45% [24–68]; 10/22; p=0·0034). Of 21 participants positive by Xpert Ultra, 13 were positive by culture, Xpert, or both, and eight were only positive by Xpert Ultra. Of those eight, three were categorised as probable tuberculous meningitis, three as possible tuberculous meningitis, and two as not tuberculous meningitis. Testing 6 mL or more of CSF was associated with more frequent detection of tuberculosis than with less than 6 mL (26% *vs* 7%; p=0·014).

Interpretation Xpert Ultra detected significantly more tuberculous meningitis than did either Xpert or culture. WHO now recommends the use of Xpert Ultra as the initial diagnostic test for suspected tuberculous meningitis.

Lancet Infect Dis 2018; 18: 68–75

Case Report

Detection of *Mycobacterium tuberculosis* in urine by Xpert MTB/RIF Ultra: A useful adjunctive diagnostic tool in HIV-associated tuberculosis

International Journal of Infectious Diseases 75 (2018) 92–94

In January 2017, the World Health Organisation recommended the Xpert® MTB/RIF Ultra assay (Ultra) for tuberculosis (TB) diagnosis. Ultra offers improved analytical sensitivity when compared with the initial Xpert® MTB/RIF (Xpert) assay for the detection of *Mycobacterium tuberculosis*. Ultra is therefore likely to be of particular benefit for detecting paucibacillary TB.

We present a case from Uganda demonstrating Ultra positivity in urine from an HIV-infected patient presenting with altered mental status and urinary incontinence, and no other signs of active pulmonary or extrapulmonary TB. This represents the first published instance of a diagnosis of extrapulmonary TB made on the basis of a positive urine Ultra assay.

The use of Ultra on urine may be a useful addition to the diagnostic armamentarium for disseminated TB in persons with HIV co-infection. The diagnostic accuracy of urine Ultra should be characterised further via prospective studies.

Xpert® MTB/XDR*

Prueba de sensibilidad a fármacos para la tuberculosis de nueva generación

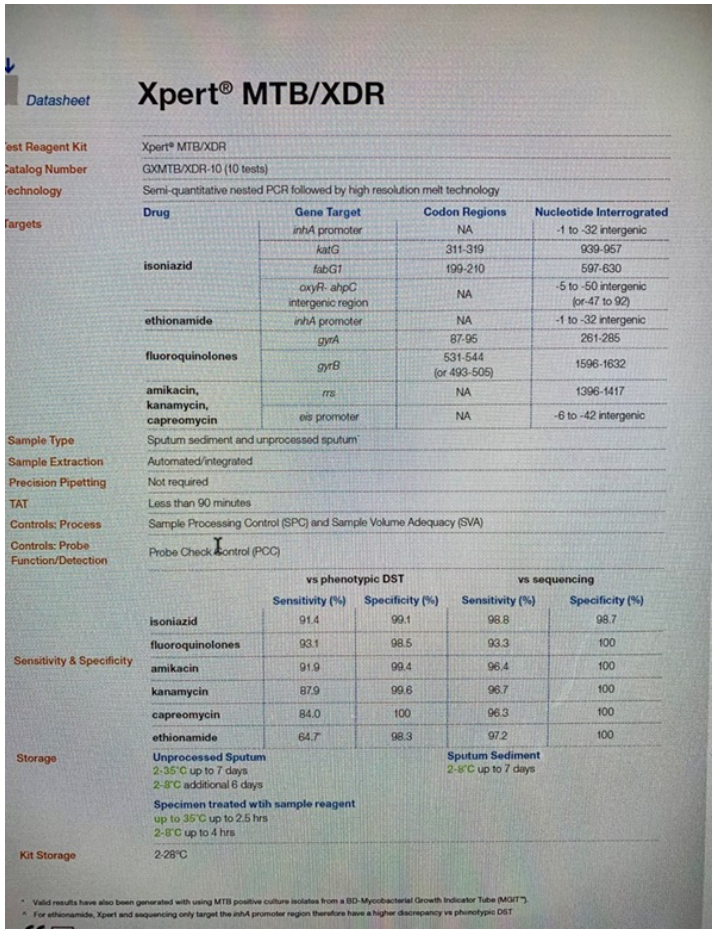
MUTACIONES ASOCIADAS	RESISTENCIA A FÁRMACOS DETECTADA
katG *fabG1* región intergénica *ahpC-oxyR* promotor *inhA*	**isoniazid**
promotor *inhA*	**etionamida**
gyrA *gyrB*	**fluoroquinolonas**
rrs *eis* promotor	**amikacina, kanamicina, capreomicina**

La solución

Xpert MTB/XDR

El ensayo Xpert MTB/XDR puede detectar la resistencia a isoniacida, etionamida, fluoroquinolonas e inyectables de segunda línea (amikacina, kanamicina y capreomicina) en menos de 2 horas.

Puede utilizarse en las plataformas GeneXpert® existentes^ en entornos centralizados o descentralizados y con el mismo procesamiento de muestras y el mismo flujo de trabajo sencillos que los ensayos Xpert® MTB/RIF# y Xpert® MTB/RIF Ultra†.



Datasheet

Xpert® MTB/XDR

Test Reagent Kit	Xpert® MTB/XDR			
Catalog Number	GXMTB/XDR-10 (10 tests)			
Technology	Semi-quantitative nested PCR followed by high resolution melt technology			
Targets	**Drug**	**Gene Target**	**Codon Regions**	**Nucleotide Interrogated**
	isoniazid	*inhA* promoter	NA	-1 to -32 intergenic
		katG	311-319	939-957
		fabG1	199-210	597-630
		oxyR- ahpC intergenic region	NA	-5 to -50 intergenic (or-47 to 92)
	ethionamide	*inhA* promoter	NA	-1 to -32 intergenic
	fluoroquinolones	*gyrA*	87-95	261-285
		gyrB	531-544 (or 493-505)	1596-1632
	amikacin, kanamycin, capreomycin	*rrs*	NA	1396-1417
		eis promoter	NA	-6 to -42 intergenic
Sample Type	Sputum sediment and unprocessed sputum			
Sample Extraction	Automated/integrated			
Precision Pipetting	Not required			
TAT	Less than 90 minutes			
Controls: Process	Sample Processing Control (SPC) and Sample Volume Adequacy (SVA)			
Controls: Probe Function/Detection	Probe Check Control (PCC)			

Sensitivity & Specificity	vs phenotypic DST		vs sequencing	
	Sensitivity (%)	**Specificity (%)**	**Sensitivity (%)**	**Specificity (%)**
isoniazid	91.4	99.1	98.8	98.7
fluoroquinolones	93.1	98.5	93.3	100
amikacin	91.9	99.4	96.4	100
kanamycin	87.9	99.6	96.7	100
capreomycin	84.0	100	96.3	100
ethionamide	64.7^	98.3	97.2	100

Storage

Unprocessed Sputum
2-35°C up to 7 days
2-8°C additional 6 days

Sputum Sediment
2-8°C up to 7 days

Specimen treated wtih sample reagent
up to 35°C up to 2.5 hrs
2-8°C up to 4 hrs

Kit Storage: 2-28°C

* Valid results have also been generated with using MTB positive culture isolates from a BD-Mycobacterial Growth Indicator Tube (MGIT™).
^ For ethionamide, Xpert and sequencing only target the *inhA* promoter region therefore have a higher discrepancy vs phenotypic DST

XXV Jornadas Internacionales sobre Tuberculosis

uitb
Unidad de Investigación en Tuberculosis de Barcelona

15 de noviembre de 2021

Evaluación del Xpert MTB/XDR para la detección de resistencias a isoniacida, fluoroquinolonas e inyectables de segunda línea en un área de baja incidencia

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Microbióloga. Unidad de Salud Internacional-TB Drassanes. Hospital Vall d'Hebron. Barcelona.

Detección de MTBC sobre muestra directa descontaminada : PCR-RT

- **Tecnología LiquidArray** basada en la amplificación de ADN por PCR asimétrica y detección del amplificado por sondas de hibridación Lights-ON y Lights-OFF.
- Lectura mediante curvas de melting, en el sistema **FluoroCycler® XT**
- **Interpretación** de resultados **automatizada** gracias al **FluoroSoftware XT-IVD**
- **Detección** de **todos los miembros** del **Complejo *Mycbacterium tuberculosis***.
- Identificación mutaciones silenciosas que se informan como sensibles
- Validado para realizar a partir de Muestra clínica descontaminada y de cultivo

Detección de **todos los miembros** del **Complejo *Mycbacterium tuberculosis***.

Identificación de hasta **48 mutaciones** en el **gen *rpoB***, **5 mutaciones** en el **gen *katG*** y **7 mutaciones** en el **gen *inhA*:**

FluoroType® MTBDR VER 2.0

rpoB

Substitutions			Delections	Silent mutations
T508A	D516F	H526N	Del at 514-516	F514F
Q510H	D516G	H526R	Del at 515	G523G
Q510P	D516N/D516Y	H526Y	Del at 515-516	A532A
L511P	N518I	H526Q	Del at 517	
S512T	S522L	R529K	Del at 517-518	
S512K	S522Q	S531F	Del at 517-519	
Q513K	H526D/H526S	S531L	Del at 526-527	
Q513L/D513V	H526F	S531Q	Del at 531-533	
Q513P	H526G	S531W		
Q513R/M513V	H526L	L533P		Combined mutations
D516A	H526P	L533E	Insertions	
			Ins at 514-515	Q513H + L533P M515I + D516Y D516G + L533P

inhA	*katG*
T -8A	S315T1
T -8C	S315T2
C -15T	S315N
A -16G	S315I
G -9A	S315R
G -17T	

- Requiere mínima manipulación (extracción con reactivos ya preparados)
- Control interno de amplificación
- Límite de detección 14 UFC/ml en muestra descontaminada
- Resultados fiables, reproducibles y rápidos en menos de 3 horas.
- Generación automática de informe de resultados.

FluoroType® MTBDR VER 2.0

MDR-TB is resistant to Isoniazid and Rifampicin – two most important first-line drugs in TB treatment.

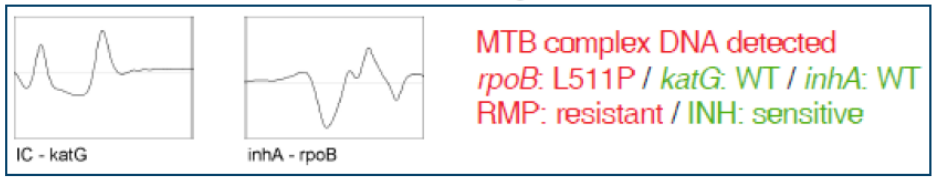
WHO endorsement!

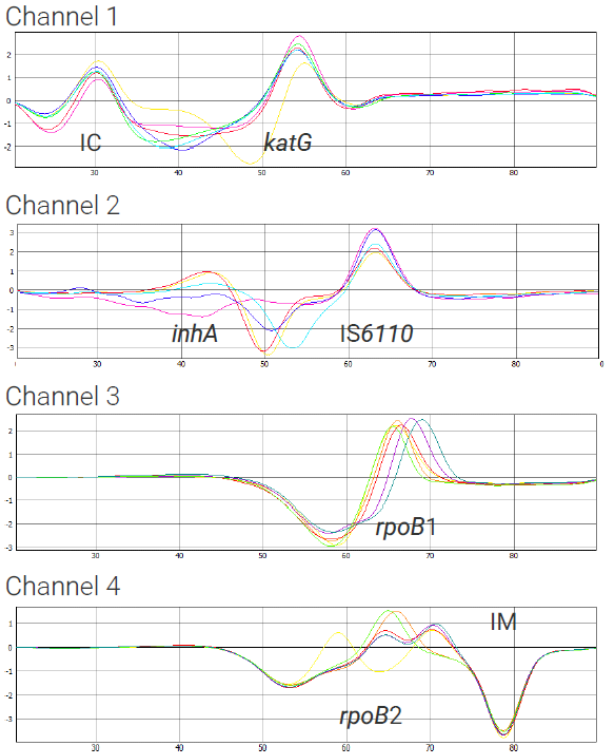
First assay leveraging LiquidArray® technology

- Detection of the MTBC
  - *rpoB* + *IS6110* * → Improved sensitivity
- Resistance-mediating mutations – detect MDR-TB and monoresistances
  - 45 mutations
  - *rpoB* (Rifampicin)
  - *katG*, *inhA* (Isoniazid)
  - Adjusted to WHO catalogue * → State of the art, even more mutations
- Native sputum as an additional sample material *
  - Liquefaction Reagent * → Faster sample-to-result

* Coming Q4 2023

Results at a glance





Evaluación de la técnica FluoroType MTBDR ver 2.0 para la detección de genoma de *Mycobacterium tuberculosis* complex a partir de biopsias parafinadas

Lucas Aranda Núñez[1], Laura Diab Casares[1], Mariana Lamas Santangelo[1], Francisco Javier Hernández Felices[1], Marta Moreno Córdoba[1], Marta Toval Sánchez[1], Rafael Medina González[1], Lara Navarro[1], M Dolores Ocete Mochón[1], Concepción Gimeno Cardona[1,2], M Remedio Guna Serrano[1,2]

[1] Consorcio Hospital General Universitario de Valencia [2] Facultad de Medicina y Cirugía. Universidad de Valencia

OBJETIVO

Evaluar la técnica FluoroType MTBDR ver 2.0 para detección de genoma de *Mycobacterium tuberculosis* complex (MTBC) y resistencia a rifampicina e isoniacida en biopsias parafinadas, comparando los resultados obtenidos con la técnica empleada habitualmente en nuestro laboratorio en este tipo de muestras, así como las ventajas de su implementación.

MATERIAL Y MÉTODOS

Se seleccionaron 15 muestras clínicas (biopsias parafinadas) correspondientes a 12 pacientes, remitidas al Servicio de Microbiología del Consorcio Hospital General Universitario de Valencia para detección de genoma de MTBC. Las muestras habían sido remitidas inicialmente al Servicio de Anatomía Patológica donde se realizó la desparafinación. Se llevó a cabo extracción automática del ADN de las muestras con el equipo QIAsymphony DSP DNA mini kit. La PCR se realizó mediante el método comercial de rutina utilizado en nuestro laboratorio que permitía la detección de genoma de MTBC. Los resultados se compararon con los obtenidos mediante FluoroType MTBDR ver 2.0 (que permite detección de genoma de MTBC y resistencia a rifampicina e isoniacida), modificando el protocolo, puesto que no se empleó el kit FluoroLyse para extracción manual de DNA: se utilizó el eluido de ADN obtenido mediante el mismo método de extracción automática descrito anteriormente y se añadió el control interno diluido en 1/5 para iniciar el proceso de amplificación; a partir de este punto, se siguieron las instrucciones del fabricante para la realización de la PCR en el termociclador Fluorocycler® XT.

Muestra de biopsia	PCR rutinaria	PCR Fluorotype
1	MTBC-negativo	MTBC-negativo
2	MTBC-negativo	MTBC-negativo
3	MTBC-negativo	MTBC-negativo
4	MTBC-negativo	MTBC-negativo
5	MTBC-negativo	MTBC-negativo
6	MTBC-negativo	MTBC-negativo
7	MTBC-positivo	MTBC, no R-rifampicina/ isoniazida indeterminado
8	MTBC-negativo	MTBC-negativo
9	MTBC-negativo	MTBC-negativo
10	MTBC-negativo	MTBC, R a rifampicina e isoniazida indeterminado
11	MTBC-negativo	MTBC-negativo
12	MTBC-negativo	MTBC-negativo
13	MTBC-negativo	MTBC-negativo
14	MTBC-negativo	MTBC-negativo
15	MTBC-negativo	MTBC-negativo

Tabla 1. Resumen Resultados

CONCLUSIONES

1. Fluorotype MTBDR ver 2.0 presenta alta sensibilidad en la detección de MTBC en muestras de biopsias parafinadas en las que partimos de ADN extraído por una técnica diferente a la recomendada por la casa comercial, a pesar de ello sería conveniente ampliar el número de muestras evaluadas para concretar la sensibilidad de la técnica con esta modificación del protocolo para este tipo de muestras.
2. Es un sistema de fácil implementación en la rutina de laboratorio que permite realizar un elevado número de PCRs y aporta información sobre la detección de genoma de MTBC

LiquidArray® MTB-XDR VER 1.0

LiquidArray® MTB-XDR VER 1.0 for detecting second-line drug resistance in TB

Tuberculosis (TB) affects over 10 million people worldwide, leading to an estimated 1.4 million deaths per year. TB requires multidrug therapy, which can fail due to emerging drug resistance, with more extensively drug resistant strains progressively more difficult to treat. Rapid and reliable detection of resistance-mediating mutations is essential for fast intervention and effective disease management at individual and public health levels.

- Detect *M. tuberculosis* complex with high specificity and sensitivity
- Identify resistances to five WHO-recommended drugs
- Differentiate between high/low-level resistance to moxifloxacin
- Sequencing-like results in under 5.5 hours (up to 94 samples)

Sample	Extraction	Targets	28 mutations in genes mediating resistances to
Decon-tami-nated sputum Culture	**Manual (1-94 samples):** FluoroLyse **Automated (12-94 samples):** GenoXtract® fleXT GXT96 X2 extraction kit	*IS6110* *gyrA* *gyrB* *rplC* *rrl* *rrs* *embB*	√ Moxifloxacin (high level) √ Moxifloxacin (low level) √ Levofloxacin √ Linezolid √ Amikacin √ Ethambutol

Same DNA extract can be re-used in all validated LiquidArray® mycobacteria assays

Powered by LiquidArray®

LiquidArray® MTB-XDR VER 1.0 is a highly sensitive multiplex assay for detection of up to 28 mutations across six genes mediating resistances to five anti-TB drugs in one PCR well. Manual or automated DNA extraction and PCR setup using decontaminated sputum or culture specimens is followed by amplification and detection in the FluoroCycler® XT. Users benefit from receiving results at a glance with individual mutations and resistance interpretation displayed in a clear, colour-coded report.

The LiquidArray® MTB-XDR VER 1.0 assay complements FluoroType® MTBDR VER 2.0, which can use the same DNA extract and reports resistances to first-line drugs, rifampicin and isoniazid. Together with FluoroType® Mycobacteria VER 1.0 assay, which detects up to 32 clinically relevant NTM species, the three assays form a core comprehensive molecular diagnostic workflow, essential for every mycobacteria laboratory.

- Detection of the ***M. tuberculosis*** **complex** from sputum specimens and cultivated samples (*IS6110* and others)
- Resistance to **fluoroquinolone**: the most significant associated mutations of the *gyrA* gene and the *gyrB* gene
- Resistance to **linezolid**: *rplC* gene and the *rrl* gene
- Resistance to **amikacin**: *rrs* gene
- Resistance to **ethambutol**: *embB* gene
- **Most comprehensive XDR-TB molecular test in one well**
- Can be run in the same plate as FluoroType® MTBDR 2.0 and FluoroType® Mycobacteria (if same sample material is used)

Ficha técnica

SD BIOSENSOR

STANDARD *M10*

MDR-TB

Kit de reactivo de prueba	STANDARD M10 MDR-TB
Número de catálogo	11MTB10A
Descripción/Tecnología	Ensayo molecular que realiza la detección y diferenciación simultánea del ADN de los genes IS1081 y IS6110 de cepas de complejo M. tuberculosis y los genes rpoB, katG e inhA como parámetro de resistencia a múltiples fármacos, aplicando la tecnología de PCR en Tiempo Real. Cartuchos precargados con todos los reactivos necesarios para realizar la extracción y amplificación de los ácidos nucleicos de manera totalmente automatizada.

Dianas

MTB	RIF	INH
Gen IS1081 / Gen IS6110	Gen rpoB	Gen katG / Gen inhA

Tipo de muestra	Muestra pretratada de: Esputo sin procesar y Esputo sedimentado.
Volumen de muestra	Mínimo 500 µL de muestra de partida, o 1.4 ml de muestra pretratada.
Tiempo procesamiento	80 minutos[A]
Control Interno	Gen exógeno: verifica procesos de extracción y amplificación.

Límite de detección

Especies de micobacterias (cepa)	Diana del gen		Tipo de muestra	LoD (CFU/mL)
M. tuberculosis (H37Rv)	MTB	IS1081	Esputo	9.3
		IS6110		3.4
		IS1081	Sedimento de esputo	4.2
		IS6110		1.9
M. bovis	MTB	IS1081	Esputo	84.5
		IS6110		209.7
		IS1081	Sedimento de esputo	93.3
		IS6110		125.0
M. tuberculosis with RIF and INH resistance (4B252)	INH		Esputo	46.6
			Sedimento de esputo	37.0
	RIF		Esputo	96.7
			Sedimento de esputo	34.5

Sensibilidad y especificidad

	Sensibilidad	Especificidad
MTB	100.00 %	100.00 %

	Sensibilidad	Especificidad
RIF	100.00 %	100.00 %

Conservación del kit	2–28 °C
Conservación de las muestras	Refrigeración (2-8 °C), protegida de la luz. / **Número de pruebas/kit** 10 pruebas.
Medidas del kit	8.4 x 32 x 13.2 cm
Peso del kit	1.2 kg

[A]Con detección precoz en caso de resultados positivos.

Nota: Para más información consultar el manual de uso.

CE IVD Producto sanitario para diagnóstico *in vitro*.

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Nuevas plataformas para diagnóstico molecular sobre muestra directa

Evaluation of the SD Biosensor Standard™ M10 MDR-TB kit

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Flow Cytometry Centre
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4. Conclusion

The results of this assessment found the standard M10 MDR-TB kit as a viable alternative to the GeneXpert MTB/RIF kit and is suitable for use at Flow Cytometry Centre.

TITLE: Evaluation of STANDARD™ M10 MDR-TB for Detection of Mycobacterium tuberculosis, Rifampicin (RIF) and Isoniazid (INH) Resistance

Laboratory Name: National Microbiology Reference Laboratory-Tuberculosis (NMRL-TB)

sensitivity and specificity of the assay for the diagnosis of TB were 99% and 97.9% respectively. The assay is able to detect MDR-TB with sensitivity and specificity of 97.8% and 100% respectively. The assay is also able to detect MTB in samples with low bacterial load that give MTB detected TRACE with a high sensitivity of 91.6%. However, because of the small sample size for this type of samples, were recommend further studies with larger sample size to ascertain true sensitivity and specificity.

The diagnostic accuracy of STANDARD ™ M10 MDR-TB assay was found to be high for MTB detection and MTB-RIF and INH resistance detection. This assay can be used as point of care test (POCT) for detection of MTB and MDR-TB. With the results from this evaluation study we therefore deem this assay suitable for diagnosis of MDR-TB.

Evaluación del equipo SD Biosensor Standard™ M10 MDR-TB como método de detección genotípica de *Mycobacterium tuberculosis* complex y resistencia a rifampicina e isoniazida.

Nº de muestras	Xpert Rif/Ultra: detección MTBC y resistencia a Rifampicina	Biosensor Standard M10 MDR-TB: detección MTBC y resistencia a Rifampicina e Isoniaida
30	Negativa	Negativa
4	Positiva No resistencia	Positiva No resistencia
4	Trazas Resistencia indeterminada	Carga baja Resistencia indeterminada

Al comparar con resultados del cultivo: en 7 de las 8 muestras con PCR positiva (Biosensor Standard™) se aisló MTBC con estudio de sensibilidad concordante. Solo una PCR con carga baja tuvo un cultivo negativo a los 38 días de incubación. La sensibilidad de la técnica fue del 100%, especificidad del 96,7%, VPP del 87,5%, VPN del 100%.

Conclusión

Biosensor Standard™ M10 MDR-TB es una técnica rápida, sencilla y con alta sensibilidad, especificidad y valores predictivos que permite su implementación para uso rutinario en el laboratorio de Microbiología para la detección rápida de genoma de MTBC en diferentes tipos de muestras con detección simultanea de genes de resistencia a rifampicina e isoniacida. Dado el bajo tamaño muestral, es recomendable realizar más estudios para afianzar los valores de sensibilidad y especificidad.

Evaluation of Xpert MTB/RIF Ultra assay for detection of *Mycobacterium tuberculosis* and rifampicin resistance

Pathology (August 2023) 55(5), pp. 688–697

Wenjie Huang[1], Melody Kee Tai Lee[1], Amanda Teo Kai Sin[1], Reyan Shah Nazari[2], Syn Yu Chua[1], Li-Hwei Sng[1]

MICROBIOLOGY

Table 3 Samples which tested positive by molecular methods for *Mycobacterium tuberculosis* complex DNA but were culture-negative

Case	Lab number	Xpert	Ultra	Culture isolation of MTBC	Clinical correlation
1	LN21	Not detected	Trace	No	Previously treated for TB 9 months ago
2	LN58	Low	Low	No	Sample came from same patient as Case 1
3	LN84	Very low	Very low	No	Previously treated for TB 1 year ago
4	LN138	Low	Medium	No	Previously treated for TB 2 years ago
5	LN46	Low	Low	No	On TB treatment for 4 months
6	LN139	Low	Low	No[a]	Clinically TB and pre-treated with Tazocin
7	LN140	Very low	Very low	No	Clinically TB and pre-treated with levofloxacin
8	LN79	Very low	Very low	No	Clinically TB and pre-treated with Augmentin
9	LN72	Low	Very low	No	Clinically TB and pre-treated with ceftriaxone
10	LN61	Low	Low	No	Clinically TB
11	LN98	Low	Low	No	Possible TB (differential of malignancy); not pre-treated with antibiotics

MTBC, *Mycobacterium tuberculosis* complex; TB, tuberculosis.
[a] Contaminated with fungus.

In total, 149 samples collected between January 2019 and November 2020 were analysed. *Mycobacterium tuberculosis* complex (MTBC) was isolated from 55 cultures. Using culture as the reference standard, Ultra demonstrated higher sensitivity (96.4% vs 85.5%) and marginally lower specificity (88.3% vs 89.4%) compared to Xpert in the full cohort. When considering only paucibacillary specimens such as extrapulmonary and smear-negative samples, similar results were obtained. Reclassifying Ultra trace

Trace results obtained using Ultra may require careful interpretation to prevent overtreatment of false positive cases, especially in countries with high tuberculosis prevalence.[8,17] WHO has recommended that a trace result should be regarded as a true positive in people living with HIV, children and extrapulmonary specimens, whereas it may be worthwhile repeating the test in adults not at risk for HIV infection prior to starting treatment.[9] The FIND foundation noted that

Performance of microbiological tests for tuberculosis diagnostic according to the type of respiratory specimen: A 10-year retrospective study

TYPE Original Research
PUBLISHED 02 March 2023
DOI 10.3389/fcimb.2023.1131241

frontiers | Frontiers in Cellular and Infection Microbiology

Background: The microbial diagnosis of tuberculosis (TB) remains challenging and relies on multiple microbiological tests performed on different clinical specimens. Polymerase chain reactions (PCRs), introduced in the last decades has had a significant impact on the diagnosis of TB. However, questions remain about the use of PCRs in combination with conventional tests for TB, namely microscopy and culture. We aimed to determine the performance of microscopy, culture and PCR for the diagnosis of pulmonary tuberculosis according to the type of clinical specimen in order to improve the diagnostic yield and to avoid unnecessary, time and labor-intensive tests.

Methods: We conducted a retrospective study (2008-2018) on analysis (34'429 specimens, 14'358 patients) performed in our diagnostic laboratory located in the Lausanne University Hospital to compare the performance of microbiological tests on sputum, induced sputum, bronchial aspirate and bronchoalveolar lavage (BAL). We analysed the performance using a classical "per specimen" approach and a "per patient" approach for paired specimens collected from the same patient.

Results: The overall sensitivities of microscopy, PCR and culture were 0.523 (0.489, 0.557), 0.798 (0.755, 0.836) and 0.988 (0.978, 0.994) and the specificity were 0.994 (0.993, 0.995), 1 (0.999, 1) and 1 (1, 1). Microscopy displayed no significant differences in sensitivity according to the type of sample. The sensitivities of PCR for sputum, induced sputum, bronchial aspirate and BAL were, 0.821 (0.762, 0.871), 0.643 (0.480, 0.784), 0.837 (0.748, 0.904) and 0.759 (0.624, 0.865) respectively and the sensitivity of culture were, 0.993 (0.981, 0.998), 0.980 (0.931, 0.998), 0.965 (0.919, 0.988), and 1 (0.961, 1) respectively. Pairwise comparison of specimens collected from the same patient reported a significantly higher sensitivity of PCR on bronchial aspirate over BAL ($p < 0.001$) and sputum ($p < 0.05$) and a significantly higher sensitivity of culture on bronchial aspirate over BAL ($p < 0.0001$).

Conclusions: PCR displayed a higher sensitivity and specificity than microscopy for all respiratory specimens, a rational for a smear-independent PCR-based approach to initiate tuberculosis microbial diagnostic. The diagnosis yield of bronchial aspirate was higher than BAL. Therefore, PCR should be systematically performed also on bronchial aspirates when available.

Article

Xpert MTB/RIF Ultra Trace Results: Decision Support for the Treatment of Extrapulmonary Tuberculosis in Low TB Burden Countries

J. Clin. Med. **2023**, *12*, 3148. https://doi.org/10.3390/jcm12093148

Abstract: Objectives. Extrapulmonary tuberculosis (EPTB) can be difficult to diagnose, especially in severe forms. The Xpert MTB/RIF Ultra test introduced an additional category called trace to reference very small amounts of *Mycobacterium tuberculosis* complex (MTBC) DNA. The objective of our multicenter study was to evaluate whether the trace result on an extrapulmonary (EP) sample is a sufficient argument to consider diagnosing tuberculosis and starting treatment, even in severe cases. Methods. A retrospective, multicenter cohort study was conducted from 2018 to 2022. Patients strongly suspected of EPTB with a trace result on an EP specimen were included. Hospital records were reviewed for clinical, treatment, and paraclinical data. Results. A total of 52 patients were included, with a severe form in 22/52 (42.3%) cases. Culture was positive for MTBC in 33/46 (71.7%) cases. Histological analysis showed granulomas in 36/45 (80.0%) cases. An Ultra trace result with a presumptive diagnosis of TB led to the decision to treat 41/52 (78.8%) patients. All patients were started on first-line anti-TB therapy (median duration of 6.1 months), with a favorable outcome in 31/35 (88.6%) patients. The presence of a small amount of MTBC genome in EPTB is a sufficient argument to treat patients across a large region of France.

Among the 13 culture-negative patients, 6 had a favorable outcome without any other associated diagnosis, 3 very old patients died (pleural forms n = 2, disseminated form n = 1), and 4 were lost to follow-up (epididymis, lymph node, pleural, and neuromeningeal forms).

Among the six patients for whom culture could not be performed, three had a favorable evolution without any other associated diagnosis and three were lost to follow-up (lymph node, pleural and disseminated forms with granulomatous lesions and suppurative necrosis).

RESEARCH ARTICLE

Evaluation of trace calls by Xpert MTB/RIF ultra for clinical management in low TB burden settings

Citation: Amedeo A, Beci G, Giglia M, Lombardi G, Bisognin F, Chiarucci F, et al. (2022) Evaluation of trace calls by Xpert MTB/RIF ultra for clinical management in low TB burden settings. PLoS ONE 17(8): e0272997. https://doi.org/10.1371/journal.pone.0272997

Abstract

Background

Clinical interpretation of trace results by Xpert MTB/RIF Ultra assay (Ultra) used as an initial diagnostic test for tuberculosis (TB) may be challenging. The aim of the study was to evaluate the frequency and epidemiology of trace readouts in routine clinical practice in a low TB prevalence setting and to propose guidance on how to manage patients with trace calls considering the data available (clinical, radiological, bacteriological etc.).

Materials and methods

A retrospective, observational, monocentric study was conducted at IRCCS Azienda Ospedaliero-Universitaria of Bologna, Italy between November 2017—December 2020. Presumptive TB patients with at least one Ultra trace result during diagnostic workup before treatment were included in the study. Patients with ongoing anti-TB treatment at the time of the trace call result or with no clinical data available were excluded from the study.

Results

Fifty-nine presumptive TB patients with Ultra trace readouts were included in the study (mean age 37.0 years, 61% males). Four patients had a history of TB in the last 2 years. Twenty-five (42.4%) of the 59 samples with trace results were respiratory material. 57/59 (96.6%) patients started anti-TB treatment soon after obtaining trace results, based on clinical, radiological or other information available, while for two patients with a recent history of TB the trace result did not lead to anti-TB treatment. Culture was positive for *M. tuberculosis* for 31/59 (52.5%) samples with trace calls: 13/25 (52.0%) were respiratory samples and 18/33 (54.5%) non-respiratory samples. The clinical and/or radiological findings of 47/57 (82.4%) patients given anti-TB therapy improved during treatment.

Conclusion

In low TB incidence settings, Ultra trace calls in presumptive TB patients should be considered as true-positive and treatment should be started promptly, except in cases of recent history of TB, where careful evaluation of other diagnostic criteria is necessary before starting anti-TB treatment. A decisional algorithm for clinical management is proposed.

Based on our experience, we propose using the following diagnostic algorithm (summarized in Fig 1). In patients with presumptive TB and a trace call on biological samples, diagnosis of TB can be formulated (and anti-TB treatment started) in the following scenarios:

- Trace result on non-respiratory sample (extra-pulmonary disease localization);
- Paediatric population;
- PLHIV;
- Positive Ultra on a different sample in patients with no recent history of TB;
- Suggestive histopathology and exclusion of other causes;
- MTB positive culture (on the same sample with trace call or a different sample), regardless of TB treatment history;
- Clinical deterioration and/or radiological progression in patients with recent successful treatment history and no other elements available (and other causes excluded).

Positive IGRA cannot be considered confirmatory evidence of TB, but in the paediatric population and when other criteria are lacking, a positive IGRA test can support the diagnosis

La OMS recomienda el uso de las técnicas moleculares rápidas como prueba inicial para diagnosticar la Tuberculosis ya que tienen una alta precisión diagnóstica y conducirán a importantes mejoras en la detección temprana de la Tuberculosis y la Tuberculosis resistente a los medicamentos.

Los esfuerzos acelerados para diagnosticar la Tuberculosis y la resistencia a los fármacos son esenciales para poner fin a la epidemia mundial de TB y lograr los objetivos de la OMS en su Programa END TB así como los Objetivos de Desarrollo Sostenible de la ONU.

¡Muchas gracias por vuestra atención!