Multicenter Evaluation of Xpert MTB/RIF Ultra Tests Reporting Detection of "Trace" of *Mycobacterium tuberculosis* DNA

Dear Editor,

The Xpert RIF/TB (Xpert) (Cepheid, Sunnyvale, CA, USA) test for the detection of *Mycobacterium tuberculosis* DNA in clinical specimens has been recently replaced by the new Xpert MTB/RIF Ultra (Ultra).[1] A major difference with previous kit is the introduction of the new semi-quantitative category "trace" to report paucibacillary samples, with still detectable multicopy targets (IS*6110* and/or IS*1081*) but without measurable amount of the single copy *rpoB* gene. This innovation, aiming to improve the sensitivity of the test, made the report of "trace" very frequent. uncertainty about its significance led to different interpretations of "trace" calls, including the suggestion to repeat the test.[2-4]

Trying to make clarity on this point, we planned, in a low-prevalence high-resource setting, a multicenter study involving 21 laboratories performing routine Ultra test. The centers were requested, for each test resulting "trace," to entry a number of microbiological and clinical information in an *ad hoc* questionnaire. The entries included the incidence of tuberculosis (TB) in the country of origin of the patient, the type of clinical sample, the results of microbiological tests (microscopy, culture in solid and liquid medium, identification), the presence of signs and symptoms of TB (cough, fever, night sweat, thoracic pain, and hemoptysis), and information about previous TB, contacts with TB patients, chest X-ray, anti-TB treatment, and clinical diagnosis.

The decision of repeating the tests scoring "trace" was left to single laboratories; they were requested instead to extend up to 56 days the incubation of MGIT (mycobacteria growth indicator tube) cultures still negative at 42 days. Liquid cultures become positive after 42 days were, however, less than 5%. The identification of mycobacteria grown in culture was performed by molecular tests. This study was retrospective and the patients' information was anonymized before analysis.

In a period of approximately 24 months, 32,835 tests Ultra were performed; of them, 29,882 were negative, 2,659 positive (any category including "trace"), and 305 invalid or error. Of positive tests, 317 (11.9%) scored "trace."

Most of patients originated from high-incidence TB countries (29.5%) or from high–middle-incidence countries (27.0%), 4.9% from low–middle-incidence countries, and 38.6% from low-incidence countries. The proportion of patients with diagnosis of TB was significantly lower in patients from low-incidence countries (61.1%) in comparison with patients from other countries (89.2%–100%), $P = 0.00001$ (Chi-square test).

Out of the 122 tests that were repeated, a positive result, "trace" or higher, was reported in 86 (70.5%) cases such that confirming the common experience of the low repeatability of amplification results in deeply paucibacillary specimens.

Microbiological features of the samples scoring "trace" are reported in Table 1.

To interpret the significance of tests flagged "trace" they were assigned to different categories. (a) Were regarded as true-positive (148, 46.7%) samples with growth of *M. tuberculosis* in culture (solid, liquid, or both). (b) Equally true positive were considered the samples without growth in culture but collected from patients (91, 28.7%) with definite or probable diagnosis, some of which were under TB treatment. (c) Were in contrast considered false-positive the samples from 25 patients (7.9%) with

Table 1: Microbiological characteristics of tests scoring "trace"

Sample type	Smear negative	Smear positive	Solid medium positive	Solid medium negative	MGIT positive 42 days	MGIT positive >42 days	MGIT negative
Sputum	123/125	2/125	45/125	80/125	46/125	2/125	77/125
BA/BAL	69/70	1/70	25/70	45/70	23/70	1/70	46/70
Biopsy	55/55	0/55	25/53[b]	28/53[b]	29/53[b]	0/53[b]	24/53[b]
Gastric aspirate	21/21	0/21	10/21	11/21	10/21	1/21	10/21
Pleural fluid	8/8	0/8	2/8	6/8	3/8	1/8	4/8
Cerebrospinal fluid	9/9[a]	0/9[a]	3/10	7/10	3/10	0/10	7/10
Other	28/28	0/28	13/28	15/28	15/28	0/28	13/28
Total	313/316	3/316	123/315	192/315	129/315	5/315	181/315

[a]Microscopy not performed on 1 sample, [b]Culture not performed on 2 samples. MGIT: Mycobacteria growth indicator tube, BA: Bronchial aspirates, BAL: Bronchoalveolar lavage

history of previous TB and from 53 patients (16.7%) with excluded or improbable diagnosis of TB. Our data confirm the well-known poor sensitivity of molecular diagnosis of TB in smear-negative specimens,[5,6] with 75.4% of our tests scoring "trace" being true positive and 24.6% false positive. The introduction of the semi-quantitative category "trace" allowed achieving a substantial increase of sensitivity versus a reasonable loss of specificity.

With the aim of excluding technical false-positive results, most of the cartridges detecting "trace" were frozen at − 80°C at the end of the test. Of them, the ones corresponding to samples negative in culture were retrospectively tested with a home-made PCR to detect, in the amplification cell of the cartridge, IS*6110* and IS*1081*. At least one of the two insertion elements above was detected in all of them.

Although Ultra and Xpert as well are recommended for use with pulmonary specimens only, both revealed effective with extrapulmonary samples too.[7,8] With biopsies and gastric aspirates in particular, the sensitivity of Ultra was 89% and 100%, respectively.

Several studies investigated sensitivity and specificity of Ultra, but none of them evaluated the significance of "trace." Different approaches have been proposed to interpret the test flagged as "trace":[2] (a) to consider all of them negative, (b) to consider negative the tests from patients with history of TB (therefore attributing the positivity to previous disease), (c) to repeat the test on the same or a new sample, and (d) to validate the result obtained at repetition. Using rule (a), 148 tests from samples culture positive and 91 from patients with diagnosis and/or treatment of TB would be reclassified as negative. According to criterion (b), a decrease of specificity would be produced due to the grading as positive of 78 samples from patients not diagnosed to have TB. According to criterion (c), a decrease in sensitivity would be produced by the reclassification as negative of 66 tests from patients with diagnosis of TB. In any case, the cost is higher than the benefit.

In our opinion, the introduction of the semi-quantitative category "trace" represents an important improvement in comparison with the previous Xpert. The proportion of true-positive results clearly exceeds the false positives with the latter being easily avoidable by not performing the Ultra test for samples from patients with diagnosis of TB clinically excluded.

The detection of "trace" is anyway an objective finding confirmed by the presence in clinical specimen of amplicons specific for *M. tuberculosis* complex. This is understandable in patients with history of previous TB; in others, subclinical forms of TB[9] are assumable. Should this be the case, an added value of the test would emerge.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Ester Mazzola[1], Paola Dal Monte[2], Claudio Piersimoni[3], Annalisa Del Giudice[4], Anna Camaggi[5], Cristina Pedrotti[6], Francesca Gurrieri[7], Cristina Russo[8], Claudio Farina[9], Alessandra Lombardi[10], Pietro Viggiani[11], Elio Cenci[12], Salvatore Nisticò[13], Vanina Rognoni[14], Eugenio Sala[15], Paola Cichero[16], Eliana Frizzera[17], Vincenzina Monzillo[18], Fulvia Morini[19], Claudio Scarparo[20], Emanuele Borroni[16], Daniela M. Cirillo[16], Enrico Tortoli[16]

[1]ASST Grande Ospedale Metropolitano Niguarda, [10]ASST Sacco Hospital, [16]IRCCS S. Raffaele, Hospital, Milan, [2]S. Orsola-Malpighi Polyclinic, Bologna, [3]Ospedali Riuniti, Ancona, [4]AO dei Colli, Naples, [5]AOU Maggiore della Carità Hospital, Novara, [6]APSS S. Chiara Hospital, Trento, [7]ASST Spedali Civili, Brescia, [8]Bambino Gesù Pediatric Hospital, Roma, [9]Papa Giovanni XXIII Hospital, Bergamo, [11]ASST della Valtellina e alto Lario, Sondalo, [12]S. Maria della Misericordia Hospital, Perugia, [13]Pugliese Ciaccio Hospital, Catanzaro, [14]ASST Lodi, Lodi, [15]S. Anna Hospital, Como, [17]Centrale Hospital, Bolzano, [18]IRCCS S. Matteo, Pavia, [19]S. Gerardo Hospital, Monza, [20]AULSS Serenissima, Mestre, Italy

Address for correspondence: Dr. Enrico Tortoli, Emerging Bacterial Pathogens Unit, San Raffaele Scientific Institute, Via Olgettina 58, 20132 Milan, Italy.
E-mail: tortoli.enrico@hsr.it

ORCID:
https://orcid.org/0000-0001-5512-704X

Submitted: 29-Oct-2020
Accepted: 30-Nov-2020 **Published:** 28-Feb-2021

References

1. World Health Organization. WHO Meeting Report for a Technical Expert Consultation: No-Inferiority Analysis of Xpert MTB/RIF Ultra Compared to Xpert MTB/RIF. Geneva: World Health Organization; 2017 (WHO/HTM/TB/2017.04).
2. Dorman SE, Schumacher SG, Alland D, Nabeta P, Armstrong DT, King B, *et al.* Xpert MTB/RIF ultra for detection of *Mycobacterium tuberculosis* and rifampicin resistance: A prospective multicentre diagnostic accuracy study. Lancet Infect Dis 2018;18:76-84.
3. Kendall EA, Schumacher SG, Denkinger CM, Dowdy DW. Estimated clinical impact of the xpert MTB/RIF ultra cartridge for diagnosis of pulmonary tuberculosis: A modeling study. PLoS Med 2017;14:e1002472.
4. Chakravorty S, Simmons AM, Rowneki M, Parmar H, Cao Y, Ryan J, *et al*. The new xpert MTB/RIF ultra: Improving detection of *Mycobacterium tuberculosis* and resistance to rifampin in an assay suitable for point-of-care testing. mBio 2017;8:mBio-17.
5. Sohn H, Aero AD, Menzies D, Behr M, Schwartzman K, Alvarez GG, *et al.* Xpert MTB/RIF testing in a low tuberculosis incidence, high-resource setting: Limitations in accuracy and clinical impact. Clin Infect Dis 2014;58:970-6.

6. Akkerman OW, van der Werf TS, de Boer M, de Beer JL, Rahim Z, Rossen JW, *et al.* Comparison of 14 molecular assays for detection of *Mycobacterium tuberculosis* complex in bronchoalveolar lavage fluid. J Clin Microbiol 2013;51:3505-11.
7. Tortoli E, Russo C, Piersimoni C, Mazzola E, Dal Monte P, Pascarella M, *et al.* Clinical validation of xpert MTB/RIF for the diagnosis of extrapulmonary tuberculosis. Eur Respir J 2012;40:442-7.
8. Bisognin F, Lombardi G, Lombardo D, Re MC, Dal Monte P. Improvement of *Mycobacterium tuberculosis* detection by xpert MTB/RIF ultra: A head-to-head comparison on xpert-negative samples. PLoS One 2018;13:e0201934.
9. Pai M, Behr MA, Dowdy D, Dheda K, Divangahi M, Boehme CC, *et al.* Tuberculosis. Nat Rev Dis Primers 2016;2:16076.

Access this article online

Quick Response Code:

Website: www.ijmyco.org

DOI: 10.4103/ijmy.ijmy_200_20

How to cite this article: Mazzola E, Monte PD, Piersimoni C, Giudice AD, Camaggi A, Pedrotti C, *et al.* Multicenter evaluation of Xpert MTB/RIF ultra tests reporting detection of "Trace" of mycobacterium tuberculosis DNA. Int J Mycobacteriol 2021;10:101-3.