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**Evaluation of the culture-enhanced Xpert MTB/RIF assay for the diagnosis of smear-  
negative tuberculosis**

**Évaluation du test Xpert MTB/RIF sur une pré-culture pour le diagnostic de la tuberculose  
à frottis négatif**

Latifa Noussair<sup>1\*§</sup>, Frédéric Bert<sup>1§</sup>, Véronique Leflon-Guibout<sup>1</sup>, Roselyne Métivier<sup>1</sup>, Chantal  
Chauvet<sup>1</sup>, Catherine Napol<sup>1</sup>, Marie-Hélène Nicolas-Chanoine<sup>1, 2, 3</sup>

<sup>1</sup> AP-HP, Hôpital Beaujon, Service de Microbiologie, Clichy, France

<sup>2</sup> Faculté de Médecine D. Diderot, Université Paris VII, Paris, France

<sup>3</sup> INSERM U 773, Centre de Recherche Biomédicale Bichat-Beaujon, CRB3, Paris, France

(\*) **Current address:** 2IC, UMR1173, Inserm, université Versailles St Quentin, Versailles et  
APHP, Hôpital Raymond Poincaré, Service de microbiologie, Garches, France

(§) **Corresponding authors:** Dr L. Noussair ([latifa.noussair@aphp.fr](mailto:latifa.noussair@aphp.fr)) and Dr F. Bert  
([frederic.bert@aphp.fr](mailto:frederic.bert@aphp.fr))

**Keywords:** tuberculosis, GeneXpert, molecular diagnosis, rifampicin resistance

**Mots clés :** tuberculose, GeneXpert, diagnostic moléculaire, résistance à la rifampicine

**Contribution of authors**

MHNC and LN designed the study protocol.  
RM, CC, CN, and LN performed the experiments.  
LN, FB, VLG, and MHNC wrote the article.

**Abstract**

**Objectives.** To evaluate a new tool for the early diagnosis of tuberculosis.

**Methods.** A total of 374 smear-negative clinical specimens from patients presenting with suspected tuberculosis were evaluated using a new procedure consisting of a preliminary step of culture in broth bottles followed by the detection of *Mycobacterium tuberculosis* complex (*Mtb*) and rifampicin resistance by the Xpert MTB/RIF assay (XMTB-RIF).

**Results.** A total of 30 *Mtb* strains were isolated, all susceptible to rifampicin. When broth cultures were subjected to XMTB-RIF analysis after 15 days of incubation, sensitivity, specificity, PPV, and NPV were each 100% when compared with liquid culture.

**Conclusion.** The XMTB-RIF assay used in 15-day broth cultures may provide a final culture result for smear-negative specimens. This process, combined with clinical signs, may contribute to rapidly diagnosing tuberculosis and also to the early re-evaluation of empirical antituberculosis treatment.

## Résumé

**Objectifs.** Évaluer un nouvel outil pour le diagnostic précoce de la tuberculose.

**Méthodes.** 374 échantillons cliniques à frottis négatif issus de patients suspects de tuberculose ont été analysés avec une nouvelle procédure : culture en bouillon suivie de la détection du complexe tuberculosis (Mtb) et de la résistance à la rifampicine par test Xpert MTB/RIF (XMTB-RIF).

**Résultats.** 30 souches de Mtb sensibles à la rifampicine ont été isolées. Lorsque les cultures en bouillon ont été soumises à une analyse XMTB-RIF après 15 jours d'incubation, la sensibilité, spécificité, VPP et VPN étaient toutes de 100 % comparativement à la culture liquide.

**Conclusion.** Le test XMTB-RIF appliqué à des cultures en bouillon incubé 15 jours peut fournir un résultat définitif de la culture pour les échantillons à frottis négatif. Cette procédure peut aider au diagnostic précoce de la tuberculose et contribuer à la réévaluation précoce de la thérapie antituberculeuse empirique.

## Introduction

Tuberculosis (TB) remains one of the major public health problems in developing and industrialized countries [1]. The worldwide control of the disease has been hampered by diagnostic difficulties relating to poor sensitivity of smear microscopy and slow growth of mycobacteria. Recently, the ability of nucleic acid amplification tests to rapidly and accurately detect *Mycobacterium tuberculosis* complex (*Mtb*) and its resistance to the first-line drug rifampicin in clinical specimens has offered significant advantages for the management and control of TB [2].

However, the major drawback of these molecular assays is still their limited ability to provide an accurate result when testing smear-negative specimens. Whilst the Xpert MTB/RIF (XMTB-RIF) provides 96%-100% sensitivity on smear-positive respiratory and non-respiratory specimens [3-12], this is reduced to only 57%-83% for smear-negative respiratory specimens and to 37%-75% for non-respiratory specimens [3-14]. Its negative predictive value (NPV) to rule out the diagnosis of tuberculosis on smear-negative specimens is therefore far from optimal.

We previously developed a culture-enhanced PCR test consisting in a preliminary step of broth culture followed by the molecular detection of *Mtb* [15]. This procedure was shown to be highly sensitive and specific for the detection of *Mtb* in paucibacillary specimens. On this occasion we set out to evaluate the performance of the XMTB-RIF directly on liquid cultures of 374 respiratory and non-respiratory specimens from patients presenting with a suspicion of TB, after 5, 10, or 15 days of incubation. We were thus able to confirm the true negativity of smear-negative specimens after 15 days of incubation as opposed to eight weeks of conventional microbiological incubation. The antituberculosis treatment could therefore be re-evaluated sooner alongside clinical sign and symptom evaluation.

## Materials and methods

Three hundred and seventy-four smear-negative specimens (respiratory and non-respiratory) (Table 1) were obtained from 320 patients presenting with a suspicion of TB. Consecutive specimens were considered as a single specimen with the sediments obtained from the three bottles pooled to perform a single XMTB-RIF test. Respiratory and non-respiratory specimens were analyzed as previously described [15]. Liquid (BactT/Alert, BioMérieux) and solid (Lowenstein-Jensen, BioMérieux) cultures were monitored for up to eight weeks at 37°C. A 2.5 ml aliquot, after centrifugation, was subjected to XMTB-RIF assay according to the manufacturer's recommendations. A total of 177 smear-negative specimens were tested after a 5-day broth culture (December 2010 - June 2011), 89 smear-negative specimens were tested after a 10-day broth culture (July 2011 - October 2011), and 108 smear-negative specimens were tested after a 15-day broth culture (November 2011 - February 2012). Isolated *Mtb* strains were tested for rifampicin (RMP) resistance by conventional drug susceptibility testing. A positive culture of *Mtb* in liquid medium was considered the gold standard for calculating the clinical values of the XMTB-RIF assay.

## Results

Three hundred and seventy-four smear-negative specimens obtained from 320 patients, including 165 respiratory specimens and 209 non-respiratory specimens, were tested using the culture-enhanced XMTB-RIF procedure. Respiratory specimens included sputum and gastric aspirates (n=86), bronchial washings (n=44), and bronchoalveolar lavage (n=35). Non-respiratory specimens included lymph nodes (n=46), vertebral biopsies (n=31), peritoneal fluid (n=31), pleural fluid (n=29), gastrointestinal and liver biopsies (n=23), cerebrospinal

fluid (n=11), cerebral biopsies (n=8), cutaneous biopsies (n=7), and other specimens (n=23). Overall, 30 out of 374 specimens were liquid culture-positive for *Mtb*. Twenty-seven out of 374 specimens were solid culture-positive for *Mtb*. The positivity rates according to the specimen type are detailed in Table 1. The mean time to positive detection by the BacT/Alert instrument was 25 days (range 12-43).

From December 2010 to June 2011, 177 smear-negative specimens obtained from 163 patients were tested. Fourteen (7.9%) specimens were culture-positive. The XMTB-RIF assay detected *Mtb* on Day 5 in 9 of 14 culture-positive specimens (64.3%) (Table 1). The remaining 163 culture-negative specimens were also XMTB-RIF-negative. Sensitivity, specificity, positive predictive value (PPV), and NPV were 64%, 100%, 100%, and 97% respectively, as compared with liquid culture (Table 2).

From July 2011 to October 2011, 89 smear-negative specimens obtained from 65 patients were tested. Seven (7.9%) specimens were positive in culture (Table 1). The XMTB-RIF assay was positive in five of seven (71.4%) culture-positive specimens. All remaining specimens, negative in liquid culture, were also negative with the XMTB-RIF assay. Sensitivity, specificity, PPV, and NPV were 71.4%, 100%, 100%, and 97.6% respectively, as compared with liquid culture (Table 2).

From November 2011 to February 2012, 108 smear-negative specimens obtained from 92 patients were tested. Broth culture was positive in 9 of 108 specimens (8.3%). The XMTB-RIF assay was positive on all culture-positive specimens on Day 15 (Table 1). All culture-negative specimens (n=99) were XMTB-RIF-negative. Sensitivity, specificity, PPV, and NPV were each 100% when compared with liquid culture (Table 2).

The 30 *Mtb* isolates were found susceptible to RMP by conventional biochemical drug susceptibility assays. The same result was observed with the XMTB-RIF assay.

## Discussion

The sensitivity and specificity of nucleic acid amplification tests are usually evaluated against conventional bacteriological culture as the gold standard diagnosis. These tests, including the recently developed XMTB-RIF assay, have shown high specificity and high PPV but highly variable sensitivity and NPV, especially for smear-negative specimens [3, 7, 9-12].

The XMTB-RIF assay failed to detect *Mtb* in 5 of the 14 culture-positive specimens on Day 5 and in 2 of the 7 culture-positive specimens on Day 10. A 15-day incubation period was required to reach perfect concordance between culture results and XMTB-RIF results. Thus, the efficacy of the culture-enhanced XMTB-RIF procedure to predict final broth culture result was similar to that previously reported with the GTMD assay [15].

The early diagnosis of smear-negative tuberculosis cases, particularly extra-pulmonary tuberculosis, remains challenging because patients often present with atypical clinical signs and symptoms. The length of time required to obtain microbiological confirmation by culture may result in delays in appropriate therapy initiation or in unnecessarily prolonged empirical treatment. In our study, negative XMTB-RIF assay on Day 15 led to ruling out with confidence the presence of *Mtb* in tested specimens several weeks before final broth culture result availability. Alongside a high level of clinical judgement, this procedure may be used for the early reevaluation of antituberculosis treatment.



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**Table 1.** Culture-positive specimens: type and Xpert assay result**Tableau 1.** Échantillons à culture positive : type et résultat du test Xpert

<b>Day of culture-enhanced PCR</b>	<b>Clinical specimen</b>	<b>Time to detection in liquid culture (days)</b>	<b>Culture enhanced-Xpert assay</b>
<b>Day 5</b>			
	Sputum	33	Negative
	Sputum	14	Positive
	Lymph node	21	Positive
	Lymph node	23	Positive
	Lymph node	28	Positive
	Lymph node	32	Negative
	Vertebral biopsy	13	Positive
	Vertebral biopsy	34	Positive
	Vertebral biopsy	35	Positive
	Psoas abscess	12	Positive
	Psoas abscess	14	Positive
	Peritoneal fluid	30	Negative
	Pleural fluid	41	Negative
	Cerebral biopsy	20	Negative
<b>Day 10</b>			
	Sputum	28	Negative
	Sputum	43	Negative
	Sputum	40	Positive
	Sputum	21	Positive
	Bronchoalveolar lavage	32	Positive
	Vertebral biopsy	21	Positive
	Vertebral biopsy	21	Positive
<b>Day 15</b>			
	Bronchial aspirate	30	Positive
	Sputum	19	Positive
	Sputum	25	Positive
	Lymph node	25	Positive
	Lymph node	23	Positive
	Lymph node	18	Positive
	Lymph node	19	Positive
	Pleural fluid	19	Positive
	Pleural fluid	25	Positive

**Table 2.** Comparison of culture-enhanced PCR (Enhanced Xpert) results with culture results in respiratory and non-respiratory specimens on days 5, 10, and 15 of incubation in liquid culture

**Tableau 2.** Comparaison des résultats de la pré-culture/PCR avec les résultats de culture dans des échantillons respiratoires et non-respiratoires aux jours 5, 10 et 15 de l'incubation en culture liquide

<b>Day 5</b>		<b>Culture</b>		<b>Total</b>
		<b>P*</b>	<b>N<sup>§</sup></b>	
<b>Respiratory specimens</b>				
	P	1	0	1
Enhanced-Xpert	N	1	75	76
<b>Non-respiratory specimens</b>				
	P	8	0	8
Enhanced-Xpert	N	4	88	92
<b>Total specimens</b>		14	163	177
<b>Day 10</b>		<b>Culture</b>		<b>Total</b>
		<b>P</b>	<b>N</b>	
<b>Respiratory specimens</b>				
	P	3	0	3
Enhanced-Xpert	N	2	41	43
<b>Non-respiratory specimens</b>				
	P	2	0	2
Enhanced-Xpert	N	0	41	41
<b>Total specimens</b>		7	82	89
<b>Day 15</b>		<b>Culture</b>		<b>Total</b>
		<b>P</b>	<b>N</b>	
<b>Respiratory specimens</b>				
	P	4	0	4
Enhanced-Xpert	N	0	38	38
<b>Non-respiratory specimens</b>				
	P	5	0	5
Enhanced-Xpert	N	0	61	61
<b>Total</b>		9	99	108