# ECCMID

**COPENHAGEN** 25-28 April 2015 P1194

## **Comparison of four commercial real-time PCR assays for detection** of Mycobacterium tuberculosis complex, associated or not to detection of anti-tuberculosis drug-resistance genes

Sibel Berger, Catherine Laurain, Christine Larcher, Alain Lozniewski, Corentine Alauzet Bacteriology Laboratory, University Medical Center of Nancy, Nancy, FRANCE

#### BACKGROUND

- Tuberculosis (TB) is one of the world's most important infectious causes of morbidity and mortality.
- Emergence and large spread of drugresistant TB cause major therapeutic problems worldwide.
- ✤ Early detection, with rapid and reliable diagnosis methods, is essential to reduce the death rate and interrupt transmission of TB, particularly drug-resistant TB.
- Various PCR-based assays are currently available and they enable the direct and fast detection of the Mycobacterium tuberculosis complex (MTBC) in clinical samples.
- Some of these kits also permit the detection of resistance to the most important antituberculosis drugs.
- However, their sensitivity varies according to the technique or the type of clinical sample, particularly in the case of smearnegative specimens.

### **OBJECTIVES**

- Comparison of diagnostic performances of four commercial PCR-based kits for direct detection of MTBC in the respiratory and non-respiratory samples.
- Evaluation of two of them for their ability to detect resistance genes to rifampicin (RMP) and/or isoniazid (INH).

<b>Clinical samples</b>	N = 90	MATERIAL AND METHODS						
Respiratory	59				Manufacturer	Torrat	Detection of	
bronchial aspirate	29	Microscopic examination		PCR assay	(provider in France)	Target	resistance	
sputum	13	(EM) of Ziehl-Neelsen		Anyplex™ II MTB/MDR/XDR *	Seegene (Eurobio)	MPB64	YES (RMP + INH)***	
bronchoalveolar lavage	11	stained sinears						
gastric aspirate	6		$\longrightarrow$	FluoroType®MTB **	Hain (Biocentric)	IS6110	NO	
Extra-pulmonary	31	Phenotyp	ic drug-		Coorena (Europia)	IS6110 /	NO	
liquid puncture (pleural,	15	Culture susceptibil	ity testing	Seeplex®MIBACE *	Seegene (Europio)	MPB64	NU	
urine	6	Lowenstein-Jensen	cation	Xpert™ MTB/RIF	Cepheid	rpoB	YES (RMP)	
cerebrospinal fluid	5	Iiquid medium     SD Bioline TB	• SD Bioline TBAg MPT64®	<ul> <li>* DNA extraction with QiaAMP DNA minikit (Qiagen)</li> <li>** DNA extraction with Fluorolyse extraction kit (Hain)</li> <li>*** also detects some mutations associated with resistance to fluoroguinolones and injectable aminosides</li> </ul>				
biopsy specimen	5	MGIT BACTEC	IRC					

#### Patients with very low clinical suspicion of TB :

73 specimens were negative by smear, culture and PCR techn

### Patients with high clinical suspicion of TB :

- 2 culture-negative specimens (patients under anti-TB treatm 1 ME<sup>+</sup>/PCR<sup>+</sup> (for all kits tested)
  - 1 ME<sup>-</sup>/PCR<sup>+</sup> (for 3 kits) : Anyplex = 1 false negative result
- 15 culture-positive specimens (13 respiratory + 2 others)
  - 11 ME<sup>+</sup>/PCR<sup>+</sup> (for all kits tested)
  - $\circ$  4 ME<sup>-</sup>/PCR<sup>+</sup>: Xpert <sup>4/4</sup> > FluoroType/Seeplex <sup>3/4</sup> > Anyplex

PCR sensitivity : 82,4 to 100% PCR specificity : 100%

#### Results for anti-TB drug-resistance detection :

- resistance to RMP (n=3): Xpert  $^{3/3}$  > Anyplex  $^{2/3}$
- resistance to INH (n=2): Anyplex  $^{2/2}$
- This study compare for the first time the performances of these four commercial molecular assays.
- resistance to RMP.

We thank Florent Girardier for its technical assistance. We are grateful to Biocentric, Cepheid and Eurobio for providing reagents. Inscription and travel fees for the participation of C. Alauzet to this congress were supported by Cepheid.



#### c.alauzet@chu-nancy.fr

				1							
niques	PCR assay	PCR result	EM⁺, culture⁺		EM⁺, culture⁻		EM⁻, culture⁺		EM⁻, culture⁻		
iiques	Xnert	+	11		1			4		1	
	хрен	—	0		0			0	73		
	FluoroType	+	11		1		3		1		
ent)		—	0		0		1		73		
	Seenlex	+	11		1		3		1		
t		_		0	0			1		73	
	Anyplex	+	11		1		2		0		
		-		0	0			2	•	74	
< <sup>2/4</sup>	Method	т	'N	ТР	FN		FP	SEN (%	) S	PE (%)	
	ME	7	'3	12	5		0	70,6		100	
	culture	7	'3	15	2		0 88,2			100	
	XPert	7	'3	17	0		0	100		100	
	FluoroType	7	'3	16	1		0	94,1		100	
	Seeplex	7	'3	16	1		0	94,1		100	
	Anyplex	7	'3	14	3		0	82,4		100	

TN: true negative; TP: true positive; FN: false negative; FP: false positive; SEN

### CONCLUSION

\* All molecular methods tested exhibited excellent specificity and higher sensitivity than ME for the detection of MTBC. ☆ The Xpert<sup>™</sup> MTB/RIF test provided the best performances for MTBC detection associated to simultaneous diagnosis of