HIGHLY PERFORMANT MULTIPLEX PCR FOR TB AND NTM INFECTIONS IN PERSON WITH HIV

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BACKGROUND

The prevalence of non-tuberculous mycobacteria (NTM) has been increasing worldwide in both developed and developing countries. NTM infection is clinically indistinguishable from tuberculosis and therefore poses significant challenges in patient management, especially in patients chronically treated for pulmonary TB. In this study, we evaluated a new highly sensitive Multiplex MTB/NTM assay that can differentiate M. tuberculosis complex (MTBC) from all NTM, including the treatable and most common NTM, M. avium complex (MAC) in HIV and non-HIV infected patient with tuberculosis.

METHODS

We developed and optimized a new open- Multiplex MTB/NTM assay with two gene-targets for MTBC (IS6110/senX3-regX3) and two targets for MAC (IS1311/DT1) with samples spiked with stored isolates and testing 20 replicates. Patients with presumptive TB and NTM were enrolled at the Respiratory Disease Department of The University Teaching Hospital of Point G, in Mali for the clinical evaluation.

Table 1. Multiplex and Xpert MTB/RIF diagnostic performances relative to sputum culture

		Multiplex RT-PCR	Xpert MTB-RIF
All participants (N=190)	Sensitivity (%) [CI]	80·4 [66·8-89·3]	93·4 [82·85-97·7]
	Specificity (%) [CI]	85·7 [77·7-91·1]	61·9 [52·3-70·6]
	PPV (%) [CI]	71·1 [57·7-81·6]	51·8 [41·2-62·2]
	NPV (%) [CI]	90·9 [83·6-95·1]	95·5 [87·8-98·4]
TB Patient Naïve to treatment (N=30)	Sensitivity (%) [CI]	82·4 [58·9–93·8]	94·1 [73·0–98·9]
	Specificity (%) [CI]	90·9 [62·2–98·3]	81·8 [52·3–94·8]
	PPV (%) [CI]	93·3 [70·1–98·8]	88·9 [67·2–96·9]
	NPV (%) [CI]	76·9 [49·7–91·8]	90.0 [59·5–98·2]
HIV Patient (N=36)	Sensitivity (%) [CI]	71·4 [52·9–84·7]	96·4 [82·2–99·3]
	Specificity (%) [CI]	96·2 [81·1–99·3]	76.9 [57·9–88·9]
	PPV (%) [CI]	95·2 [77·3–99·1]	81·8 [65·6–91·3]
	NPV (%) [CI]	75·8 [58·9 – 87·1]	95·2 [77·3–99·1]
Treatment failure, retreatment and NTM suspected patients (N=77)	Sensitivity (%) [CI]	75·0 [50·5-89·8]	87·5 [63·9-96·5]
	Specificity (%) [CI]	71·1 [56·6-82·2]	37·7 [25·1-52·3]
	PPV (%) [CI]	48·0 [30·0-66·5]	33·3 [21·0-48·4]
	NPV (%) [CI]	88·8 [74·6-95·5]	89·4 [68·6-97·0]
All (without treatment failure participants) (N=113)	Sensitivity (%) [CI]	83·3 [66·4-92·6]	96·7 [83·3-99·4]
	Specificity (%) [CI]	96·6 [88·6-99·0]	80·0 [68·2-88·1]
	PPV (%) [CI]	92·5 [82·3-96·5]	70·7 [55·5-82·3]
	NPV (%) [CI]	92·2 [82·7-96·5]	97·9 [89·3-99·6]

An RT-PCR assay have been successfully set-up and able to diagnose tuberculosis and non tuberculosis mycobacteria in the sputum and have the same or more performance diagnosis like the Xpert-MTB/RIF® with NTM diagnosis as added value.

RESULTS

In the development stage, the new assay showed a high analytic performance with 100% detections of MTBC and MAC at only 5 colony forming units (CFUs). Overall, without the treatment failure cases, the Multiplex assay and the Xpert showed a sensitivity, specificity, PPV and NPV of 83.3% [66.4-92.6], 96.6% [88.6-99.0], 92.5% [82.3-96.5] and 92.2% [82.7-96.5] and the Xpert had values of 96·7% [83.3-99.4], 80.0% [68.2-88.1], 70·7 [55.5-82.3] and 97.9% [89.3-99.6], respectively. For the HIV infected group, the Xpert assay showed a better sensitivity (96.4% vs. 71.4%, respectively), but the Multiplex won on the specificity (76.9 vs. 96.2%) (See Table). The Multiplex assay successfully detected all (5/5) the MAC cases.

Table 2. Interpretation Criteria of the Multiplex MTB/NTN Assay

	Criteria					
Identification	IS*6110/ senX3-regX3*	IS*1311* + *DT1*	Pan-*Mycobacterium*	Extraction control: *cotJC* gene	Positive controls MTBC/MAC	Negative control
M. tuberculosis complex	+	-	+	+	+	-
M. avium complex	-	+	+	+	+	-
Other NTM	-	-	+	+	+	-
Negative results	-	-	-	+	+	-

Table 3. Clinical Performance of the Multiplex MTB/MTN in the Detection of *Mycobacterium avium* complex using Wilson confidence interval

Sputum Culture + Accuprobe MAC	Multiplex MTB/NTM		
	Positive	Negative	Total
Positive (n)	5	0	5
Negative (n)	0	185	185
Sensitivity % (CI)	100 [56·6-100]		
Specificity % (CI)	100 [97·9-100]		
Positive Predictive Value % (CI)	100 [56·6-100]		
Positive Predictive Value % (CI)	1000 [98·0-100]		

CONCLUSIONS

Our new Multiplex assay demonstrates better specificity than Xpert for all group studied, in addition to detecting potential NTM cases. The assay could therefore complement the widely used Xpert assay and enhance discrimination of TB and NTM infections

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