

# Evaluation of the OMNIgene® SPUTUM reagent for long term transportation of samples for Xpert testing in a high TB-HIV setting



Patrick Oriquiriza<sup>1,2</sup>; Ssuuna Charles<sup>3</sup>; Dan Nyehangane<sup>1</sup>; Erick Gryzbowski<sup>1</sup>; Martina Casenghi<sup>4</sup>; Maryline Bonnet<sup>2,3,5</sup>; Céline Langendorf<sup>6</sup>; Elisa Ardizzoni<sup>7</sup>

<sup>1</sup>Epicentre Mbarara Research Centre, Uganda; <sup>2</sup>University of Montpellier, France; <sup>3</sup>Mbarara University of science and Technology, Uganda; <sup>4</sup>MSF-Access Campaign, Switzerland; <sup>5</sup>IRD UMI233/ INSERM U1175, France; <sup>6</sup>Epicentre Paris, France; <sup>7</sup>Institute of Tropical Medicine, Belgium

contact: [patrick.oriquiriza@epicentre.msf.org](mailto:patrick.oriquiriza@epicentre.msf.org)

## Background and objective

- Xpert MTB/RIF has greatly improved tuberculosis diagnosis.
- The use of Xpert is limited by the need of electricity, supplies and maintenance costs.
- As a result, in many settings samples still require long transportation to reference laboratories to be tested.
- Currently, Xpert testing is recommended within 7 days from sample collection.
- OMNIgene® (DNA Genotek, Canada) is a new reagent for sample preservation.
- Ethanol is a known preservative for DNA but with limited evidence for Xpert MTB/RIF testing
- This laboratory proof of concept study assessed the Xpert detection yield for samples stored in ethanol, Omnigene and preservative-free up to 15 days at room temperature

## Methods

**Study site:** Mbarara, South West Uganda.

### Study population

- Newly diagnosed smear positive pulmonary adults
- Informed consent to participate

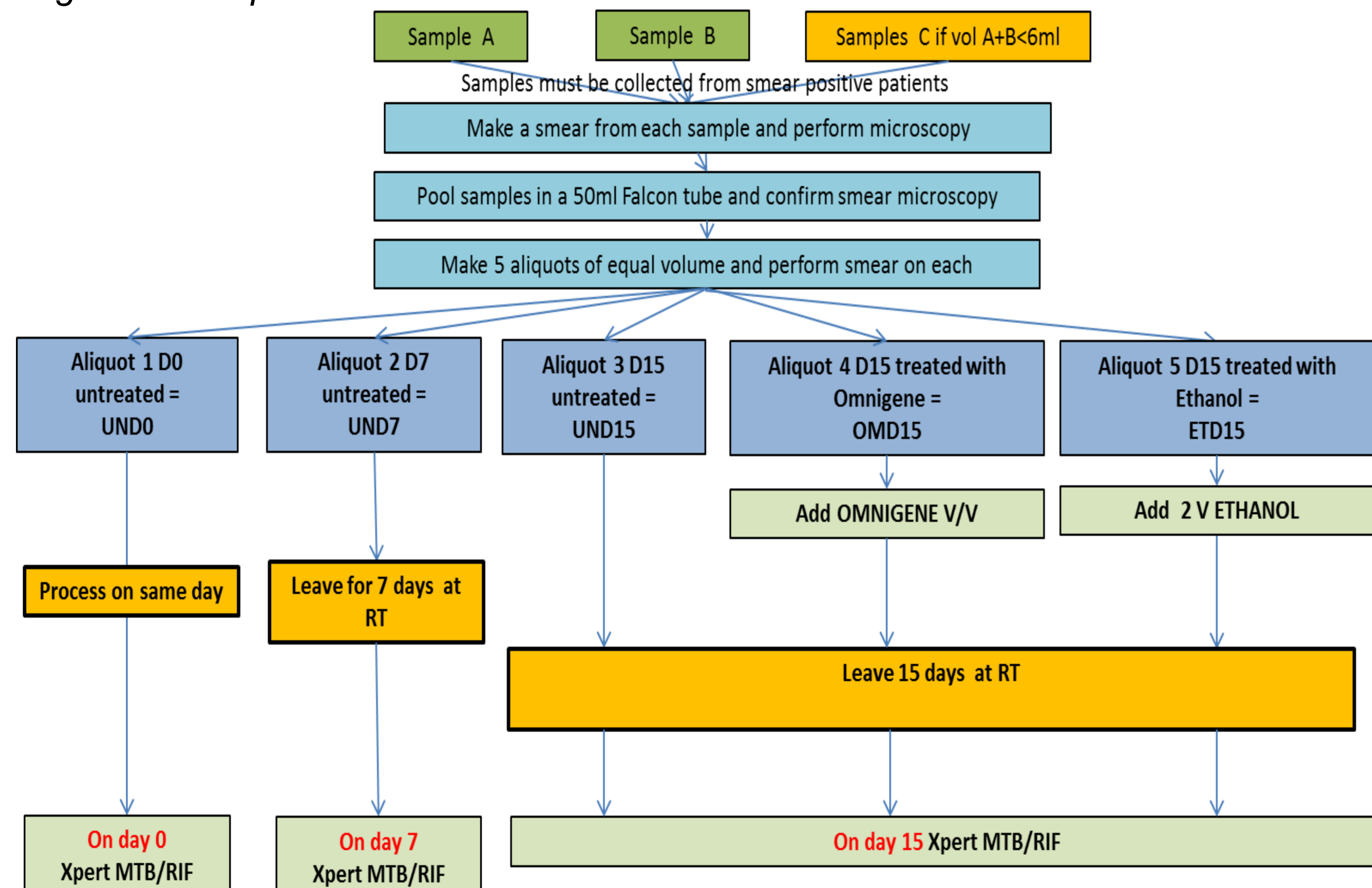
### Sample size

50 consecutive patients

### Laboratory procedures

- Two to three Smear positive samples (min volume of 5ml) were pooled together
- Samples were gently vortexed and split into five aliquots (Fig 1), checked for microscopy homogeneity
- Untreated sputum aliquots were tested with Xpert MTB/RIF at day 0, 7 and 15, with OMNIgene® and ethanol at day 15 (Figure 1)
- Xpert MTB/RIF testing followed manufacturer's guidelines

Figure 1: Sample flow chart



References: Cepheid. Operator Manual test Xpert MTB / RIF. 2009.

## Results

- 55 participants screened and 50 enrolled  
5 excluded because of smear negativity
- Majority of participants 70% (35/50) were males
- 250 aliquots tested with smear-microscopy
  - Smear negative: 18 (7.2%)
  - Smear positive scanty: 53 (21.2%)
  - Smear positive 1+: 67 (26.8%)
  - Smear positive 2+: 52 (20.8%)
  - Smear positive 3+: 60 (24%)
- Xpert MTB/RIF results of the 250 aliquots globally (Table 1)  
4 (1.6%) invalid results (not re-tested)

Table 1: Xpert results by smear grade

Xpert results	Negative, scanty, 1+ (n=138 aliquots)					≥ 2+ (n= 112 aliquots)				
	UND0 N=28	UND7 N=30	UND15 N=26	OMD15 N=26	ETD15 N=28	UND0 N=22	UND7 N=20	UND15 N=24	OMD15 N=24	ETD15 N=22
<b>N, Pos</b>	<b>28</b>	<b>28</b>	<b>26</b>	<b>26</b>	<b>26</b>	<b>22</b>	<b>20</b>	<b>24</b>	<b>24</b>	<b>22</b>
High	6 (21.4)	1 (3.6)	3 (11.5)	2 (7.7)	2 (7.7)	11 (50)	16 (80)	15 (62.5)	16 (66.7)	9 (40.9)
Medium	11 (39.3)	18 (64.3)	15 (57.7)	14 (53.8)	15 (57.7)	11 (50)	4 (20)	8 (33.3)	8 (33.3)	11 (50)
Low	10 (35.7)	8 (28.6)	6 (23.1)	7 (26.9)	7 (26.9)	0	0	1 (4.2)	0	1 (4.5)
Very Low	1 (3.6)	1 (3.6)	2 (7.7)	3 (11.3)	2 (7.7)	0	0	0	0	1 (4.5)
<b>N, Neg</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>
<b>N, Invalid</b>	<b>0</b>	<b>2</b>	<b>0</b>	<b>0</b>	<b>2</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>

- At day 15, 40/50 (80%) of aliquots treated with OMNIgene® and 37/50 (74%) with Ethanol had Xpert TB detected with high or medium grade vs 41/50 (82%) for untreated aliquots, p=0.92.
- Low smear grades (<2+), at day 15; Xpert detection was high or medium grade in 16/26 (61.5%) and 17/26 (65.4%) for OMNIgene® and ethanol treated aliquots vs 18/26 (69.2%) for untreated, p=0.91 and p=0.68.

## Conclusion

- OMNIgene® did not improve TB detection in aliquots preserved up to 15 days.
- No difference with the delay in time for samples without preservatives.
- OMNIgene® did not add value to untreated sample; no significant difference in detection.
- Ethanol did not add value to untreated sample as well.
- Additional evaluation could be explored with a large number of smear-negative samples and also for aliquots stored beyond 15 days (mainly for surveys).

## Acknowledgements:

We express our gratitude to the study participants, the staff of Epicentre and all our collaborating institutions for the invaluable contribution at various stages. We appreciate MSF for funding