

## Rationale

- Treatment and prevention of further spread of Tuberculosis (TB) in low-income countries is still problematic
- An accurate, cheap and simple to use diagnostic test is lacking
- Especially those patients suspected of TB in whom sputum ZN-smear is negative pose a diagnostic problem
- Exhaled breath analysis showed promising results for diagnosis of active TB (*Phillips, Tuberculosis 2012*)

## Hypothesis

- Breath analysis can accurately distinguish between smear-positive TB and healthy controls, and between smear-negative patients with and without TB

## Aim

- To investigate whether the breath-print of patients with smear-positive TB and healthy controls and of smear-negative patients with and without TB could be discriminated by an integrated electronic nose platform and GC-MS.

## Methods

**Design:** cross-sectional case-control study, Chittagung Bangladesh

### Inclusion criteria:

- patients with suspected TB: chest X-ray abnormalities and/or positive clinical score (*Bangladesh National Guidelines TB, 4th ed.*)
- healthy controls: no suggestive symptoms, negative TB history

### Suspected TB:

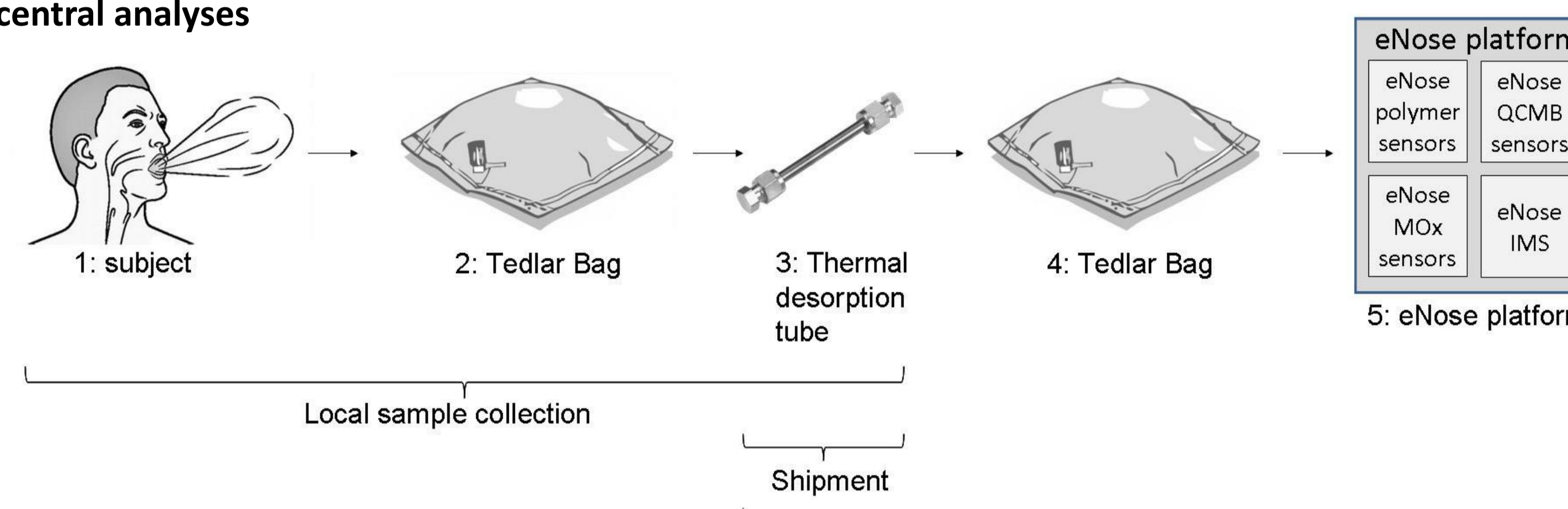
- Positive sputum smear: TB confirmed (case)
- Negative staining:
  - TB confirmed by positive Xpert PCR on sputum or BAL fluid (case)
  - TB excluded by negative Xpert PCR on sputum or BAL fluid (control)

### Breath analysis:

- Exhaled breath was collected by inhalation of VOC filtered air and exhalation of a vital capacity volume into a Nalophan bag
- Breath volatile organic compounds (VOCs) were stored on tenax sorbent tubes and send for transportation
- Exhaled breath was analyzed by:
  - A platform of electronic noses, with a total of 190 sensors (*Brinkman et al. ERS 2012*)
  - Gas Chromatography Mass Spectrometry (GC-MS)

## Methods

**Figure 1: Flowchart of exhaled breath analyses by an eNose platform, through local sample collection and central analyses**



eNose platform: Lonestar, Cyranose, Comon Invent, Tor Vergata

1. adapted from: Kaminsky et al, J Appl Physiol. 2011 Jun; 110(6): 1716-22  
 2 & 4. adapted from: <http://www.skcgulfcoast.com/kbase/images/bags.jpg>  
 3. adapted from: <http://www.perkinelmer.com/Catalog/Product/ID/N9307038>

### Statistics (R 2.11.1):

#### eNose

- principal component (PC) analysis followed by linear discriminant analysis providing a cross-validated bootstrap-based ROC curve
- GC-MS
  - consistent with eNose analysis, preceded by analyte selection through univariate analysis ( $P < 0.1$ ) on log transformed data

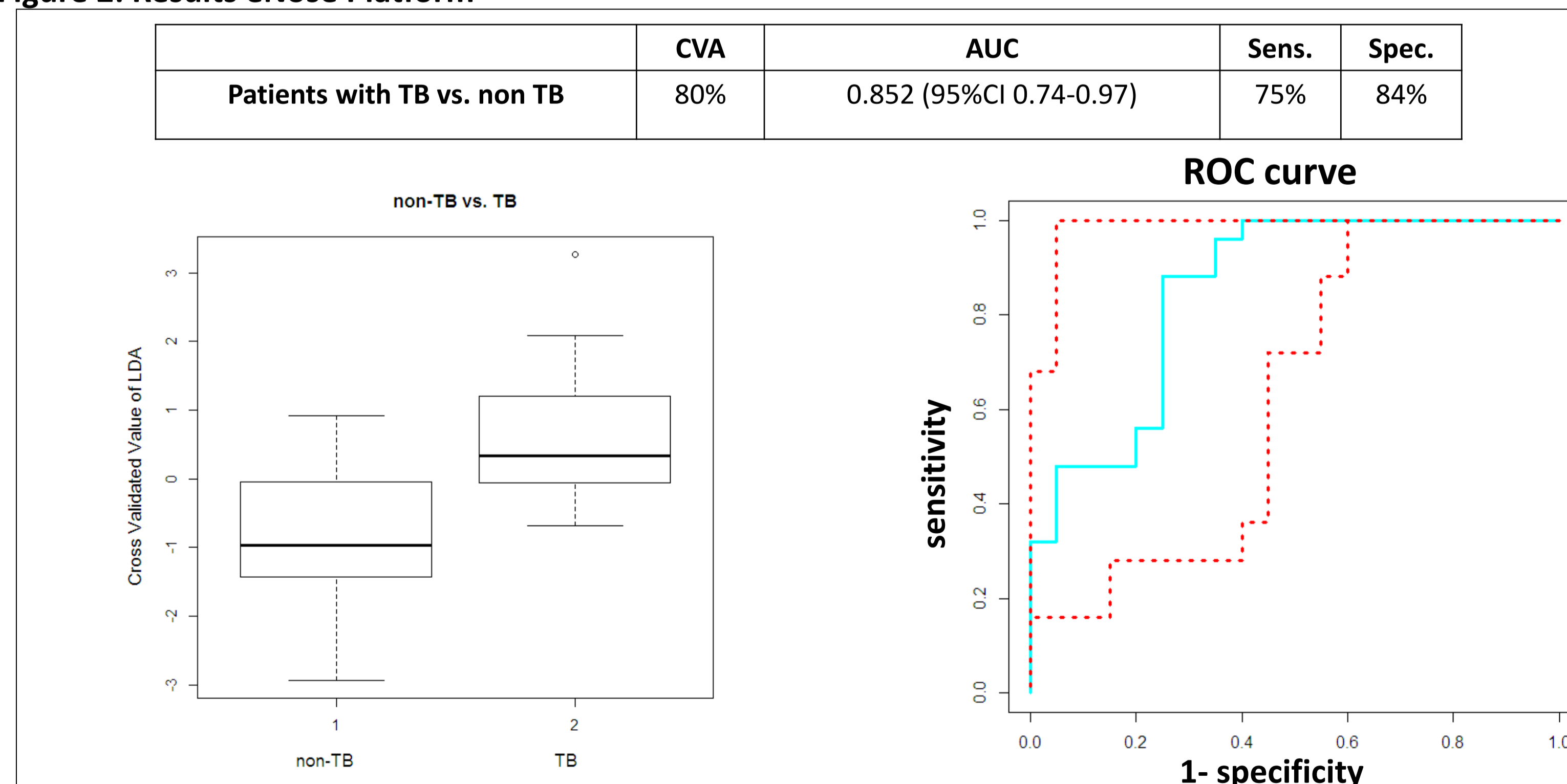
## Results

**Table 1: Subject Characteristics**

	eNose Platform (45)		GC-MS (58)	
	TB (20)	non TB (25)	TB (26)	non TB (32)
Age <sup>a</sup>	X ( )	X ( )	X ( )	X ( )
Gender	X	X	X	X
Smoking	X	X	X	X
Antibiotics (TB treatment) (Other)	X	X	X	X
Suspected TB	33		42	
Positive clinical score <sup>b</sup>	X	X	X	X
Chest X-Ray abnormalities	X	X	X	X
Smear Positive	15	0	19	0
Smear Negative	5 <sup>c</sup>	25	7 <sup>c</sup>	32

<sup>a</sup> Mean (SD)  
<sup>b</sup> Positive clinical score: All major criteria and at least 3 minor criteria are met (based on Bangladesh National Guidelines and Operational Manual for Tuberculosis control, 4th edition)  
<sup>c</sup> TB confirmed by positive Xpert PCR on sputum or BAL fluid

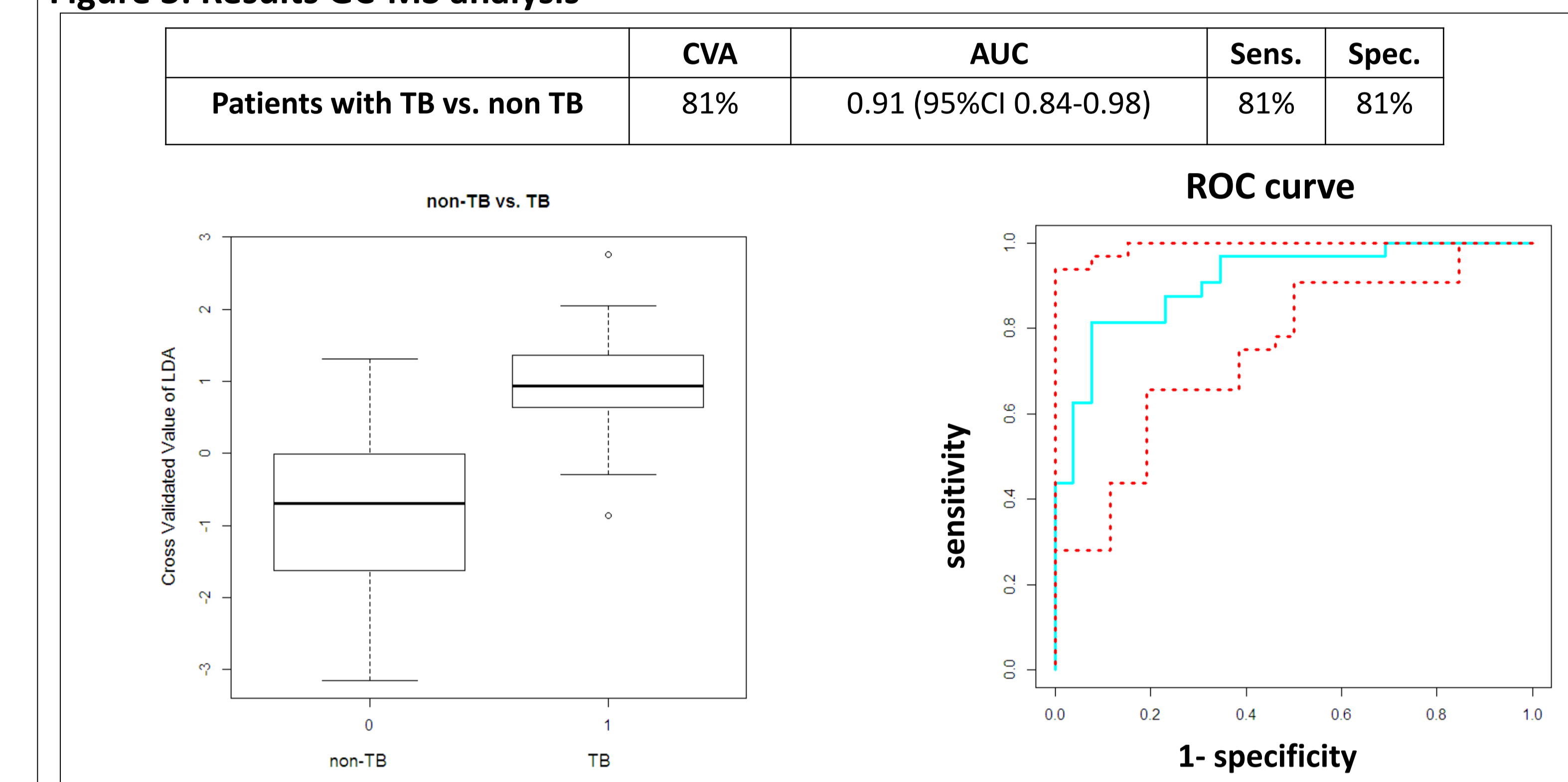
**Figure 2: Results eNose Platform**



- Patients with TB, smear positive and smear negative (positive PCR) could be discriminated from non-TB (negative PCR and healthy controls) using the eNose platform based on 1 PC ( $p < 0.0001$ )

## Results

**Figure 3: Results GC-MS analysis**



- GC-MS analysis could discriminate patients with TB, smear positive and smear negative (positive PCR) from non-TB (negative PCR and healthy controls) with a cross validated accuracy of 81%

**Figure 4: classification overview**

Xpert PCR	eNose	GC/MS	eNose + GC/MS
TB	75%	80%	60%
NON-TB	84%	80%	64%
TOTAL	80%	80%	62%

- Diagnosis of TB by eNose and GC-MS might be derived from different VOCs

## Conclusion

- TB can be accurately diagnosed by exhaled breath measurements using an integrated electronic nose platform or GC-MS
- Diagnosis of TB could also be established in an intention-to-diagnose population of smear-negative patients
- Diagnosis of TB by eNose and GC-MS might be derived from different exhaled biomarkers

## Implications

- Exhaled breath analysis is sensitive and specific for smear positive and smear negative TB, confirmed by Xpert PCR
- Exhaled breath analysis is cheap, easy to perform, non invasive and on site available, which makes it a promising solution for the diagnostic difficulties in patients suffering from TB in low income countries
- The results of GC-MS analysis can be used to develop a tailor-made eNose with sensors specific for differentiating VOCs