# Does the Choice of Spot, Morning or Both Sputum Samples Determine Optimal Performance Of a TB Diagnostic Tool?

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## Summary

**INTRODUCTION**

**Spot and morning sputum samples are used in the diagnosis of Pulmonary Tuberculosis (TB). There are no guidelines currently for a choice of two sputum samples to be used in determining optimal performance of a diagnostic tool. Performance has been determined using one of the four approaches of choice of sputum samples in different studies with varying results.**

**The choices include;**

**(a) Final diagnosis based on both spot and morning sample results, whereby the patient is the unit of analysis (point-of-care) [1,3]**
**(b) Spot sample results**
**(c) Morning sample results [1]**
**(d) Pooled results of spot and morning samples [3]**

**OBJECTIVE**

**Whereby the sample is the unit of analysis (laboratory based) respectively, this study was to determine the appropriate choice of sputum sample(s) for optimal performance of TB diagnostic tools in Kenya.**

**METHODOLOGY**

**A cross-sectional study was conducted between February 2013 and October 2016 in nine selected public health, facilities in Kenya. People presumed to have TB aged 18 years and above visiting the facilities and eligible for the study were enrolled after consenting. Spot and early morning samples were collected over two consecutive days. Samples were analysed using Ziehl Neelsen (ZN), Light Emitting Diode-Fluorescent Microscopy (LED-FM).LED-FM and GeneXpert. Lowenstein Jensen LJ Culture was used as the gold standard. TB Positivity, Sensitivity and Specificity were determined using IBM-SPSS statistical software.**

**RESULTS**

**There was significantly high TB identification with LJ culture, using final diagnosis based on both spot and morning sample results compared to separate samples. There was significantly higher incremental detection benefit with GeneXpert than with ZN and FM microscopy. However, there was no significant difference in sensitivities and specificities within the four approaches for choice of sputum sample used.**

**CONCLUSION**

**The findings provide evidence on the need to develop guidelines on choice of sputum samples to be used for accurate TB detection and validation of TB diagnostic tools.**

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# Introduction

A crucial impediment to global tuberculosis control is lack of accurate, rapid diagnostic test for detection of patients with active TB. Spot and morning sputum samples are used in the diagnosis of pulmonary tuberculosis (TB). Currently, there are no guidelines for standard choice of which sputum samples should be used in determining optimal performance of a diagnostic tool.

A new, rapid diagnostic method, (Cepheid) Xpert MTB/RIF Assay (GeneXpert), is an automated sample preparation and real-time PCR instrument, which has shown to have good potential as an alternative to current reference standard sputum microscopy and Culture.

Performance of GeneXpert has been determined using either of the four approaches of choice of sputum samples in different studies with varying results ***(Table 1)***. The four choices are defined within each of the two units of analysis;

Patient as a unit of analysis - point-of-care. The analysis performed by Rachow et al. 2011 used final results based on both spot and morning sample (Approach 1). They reported 88.4% (95% CI = 78.4% to 94.9%) Sensitivity of GeneXpert among patients with a positive culture, and 99.0% (95% CI = 94.7% to 100.0%) Specificity in patients who had no TB.

Hence, using a similar approach, in 2014 reported that GeneXpert achieved;

> 83.2% [77.9% - 89.4%] with specificity of 95.0% [93.2% - 97.2%].

considering sputum sample as the unit of analysis - laboratory based. The study determined the diagnostic performance of GeneXpert using separate spot (Approach 2) and morning (Approach 3) samples [4].

There was reduced performance of GeneXpert with a Sensitivity of 57.0% [49.9%-64.2%] using spot samples, and 66.0% [59.9%-72.1%] using morning samples. There was no report of Specificity for the same. The study of 2014 carried out diagnostic performance using pooled results of spot and morning samples (Approach 4). They determined Sensitivity of GeneXpert to be 83.7% [76.6%-90.8%] and a Specificity of 87.9% [85.1%-90.7%] [3].

***Table 1***: *Previous Studies with Documented Sensitivity and / or Specificity of GeneXpert*

| Publication | Approach of choice of samples | Sensitivity [95% CI] | Specificity [95% CI] |
|---|---|---|---|
| Rachow *et al.* 2011 [1] | Combined spot and morning samples (Approach 1) | 88.4% [78.4%-94.9%] | 99.0%[94.7%-100.0%] |
| Theron *et al.* 2014 [2] | Combined spot and morning samples [Approach 1] | 83.2% [77.9%-89.4%] | 95.0% [93.2%-97.2%] |
| Cavanaugh *et al.* 2016 [3] | Spot samples [Approach 2] | 57.0% [49.9%-64.2%] | Not indicated |
| | Morning samples [Approach 3] | 66.0% [59.9%-72.1%] | Not indicated |
| Githui *et al.* 2014 [4] | pooled spot and morning samples [Approach 4] | 83.7% [76.6%-90.8%] | 87.9% [85.1%-90.7%] |

***ZN*** - *Ziehl-Neelsen Microscopy;* ***LED-FM*** - *Led Emitted Diodides Fluorescent Microscopy;* **GeneXpert** - Xpert MTB/ **RIF;** ***CI*** - 95% *Confidence Interval*

Significant variation in sensitivity and specificity documented in the previous studies, informed the need to investigate the implication of choice of samples. This study aims to determine the appropriate choice of sputum sample(s) for optimal performance of TB diagnostic tools.

# Methodology

A cross-sectional study conducted between February 2013 and October 2016 in nine selected public health, facilities in Kenya. People presumed to have TB aged 18 years attending the facilities and eligible for the study were enrolled after consenting.

Spot and early morning samples were collected over two consecutive days. A total of 3412 sputum samples were collected from 1706 people presumed to have TB. At KEMRI research laboratory, Nairobi, 3366 sputum samples were analysed using Ziehl Neelsen (ZN), 3370 with LED-FM and 1572 with GeneXpert while all the 3412 samples were processed for Lowenstein Jensen (LJ) Culture according to standard procedures.

All LJ positive cultures were subjected to an identification process using Immuno Chromomatogenic Assays (ICA) identification kit (BD MGIT TM TBc identification test) to confirm for MTB. A positive culture for MTB was used as a gold standard for positivity [2].

Data management and analysis was done using MySQL and IBM SPSS respectively. Percentage frequency was used for TB positivity for ZN, LED-FM GeneXpert and a positive culture for MTB.

Diagnostic test values (sensitivity, specificity) values were determined using culture for MTB as a gold standard. Sensitivity and specificity were generated using IBM-SPSS statistical software.

# Results

***Table 2*** presents TB positivity for different diagnostics using different approaches of choice of sputum samples. MTB detection with LJ Culture was significantly higher when both spot and morning sample results were used for final diagnosis (approach choice 1***)*** than when results of either spot or morning samples were separately used (approach choices 2 and 3). Similarly, MTB detection with LJ Culture was significantly higher when both spot and morning sample results were used for final diagnosis (approach choice 1) than when results for pooled spot and morning samples (approach 4) were used.

There was significantly higher incremental detection benefit with GeneXpert than with ZN and FM microscopy in all the four approaches of choice. Similarly, there was significantly higher incremental detection benefit with GeneXpert than LJ culture in all the four approaches of choice.

Despite significant variation between specific diagnostic tool, there was no significant difference in sensitivities and specificities within specific diagnostic tool between the four approaches of choice.

***Table 2:*** *TB Positivity For Different Diagnostics Using Different Approaches Of Choice Of Samples*

| Approach of choice of samples | Diagnostic tool | n | TB positivity (95% CI) |
|---|---|---|---|
| **Approach 1: combined spot and morning samples** | ZN | 1683 | 16.6% [14.9%-18.4%] |
| | LED-FM | 1685 | 16.4% [14.7%-18.2%] |
| | GeneXpert | 786 | 23.4% [20.4%-26.4%] |
| | LJ culture | 1706 | 15.6% [13.9%-17.3%] |
| **Approach 2: spot samples** | ZN | 1683 | 13.5% [11.9%-15.1%] |
| | LED-FM | 1685 | 14.2% [12.5%-15.9%] |
| | GeneXpert | 786 | 20.6% [17.6%-23.5%] |
| | LJ culture | 1706 | 12.5% [11.0%-14.1%] |
| **Approach 3: morning samples** | ZN | 1683 | 13.5% [11.9%-15.2%] |
| | LED-FM | 1685 | 14.1% [ 12.5%-15.8%] |
| | GeneXpert | 786 | 21.0% [18.1%-23.9%] |
| | LJ culture | 1706 | 11.9% [10.4%-13.4%] |
| **Approach 4: pooled spot and morning samples** | ZN | 3366 | 13.5% [12.4%-14.7%] |
| | LED-FM | 3370 | 14.2% [13.0%-15.4%] |
| | GeneXpert | 1572 | 20.8% [18.8%-22.8%] |
| | LJ Culture | 3412 | 12.2% [11.1%-13.3%] |

***Key: ZN*** *- Ziehl-Neelsen Microscopy;* ***LED-FM*** *- Led Emitted Diodides Fluorescent Microscopy;* **GeneXpert** - Xpert MTB/ **RIF;** ***CI*** **- 95%** *Confidence Interval*

***Table 3*** shows the reliability of results generated using homogeneous spot and morning samples for different TB diagnostics. With respect to the Kappa cut-off references, the results from the spot sputum samples and those from the early morning sputum samples agree to a larger extent. The Kappa values for all diagnostic tools are >0.65 which implies there is good agreement in the results generated using spot and early morning sputum samples, the highest being GeneXpert (0.842 [95% CI: 0.795-0.889]) and LED-FM (0.812 [95% CI: 0.7771-0.853]).

***Table 3:*** *Agreement Between Results For Spot and Morning Samples For Different TB Diagnostics*

| Diagnostic tool | Spot | | Total | Discordant [95% CI] | kappa [95% CI] |
|---|---|---|---|---|---|
| | Positive +n | Negative -n | | | |
| **ZN** | | | | | |
| Morning: Positive | 175 | 53 | 228 | 6.2%[5.1%-7.4%] | 0.733[0.684-0.782] |
| Morning: Negative | 52 | 1403 | 1455 | | |
| **Total** | 227 | 1456 | 1683 | | |
| **LED-FM** | | | | | |
| Morning: Positive | 200 | 38 | 238 | 4.6%[3.6%-5.6%] | 0.812[0.771-0.853] |
| Morning: Negative | 39 | 1408 | 1447 | | |
| **Total** | 239 | 1446 | 1685 | | |
| **GeneXpert** | | | | | |
| Morning: Positive | 143 | 22 | 165 | 5.2%[3.7%-6.8%] | 0.842[0.795-0.889] |
| Morning: Negative | 19 | 602 | 621 | | |
| **Total** | 162 | 624 | 786 | | |
| **LJ Culture** | | | | | |
| Morning: Positive | 151 | 52 | 203 | 6.7%[5.5%-7.9%] | 0.686[0.633-0.739] |
| Morning: Negative | 63 | 1440 | 1503 | | |
| **Total** | 214 | 1492 | 1706 | | |

***Key:*** *95%* ***CI*** *- confidence interval;* ***ZN*** *- Ziehl-Neelsen microscopy;* ***LED-FM*** *- Led Emitted Diodide fluorescent microscopy;* ***GeneXpert*** *- Xpert MTB/RIF ; n-Number*

***Table 4:*** presents sensitivity and specificity of TB diagnostic tools using different approaches of choice of samples. Sensitivity of GeneXpert was significantly higher and constant than for both ZN and LED-FM in all the approaches of choice.

However, specificity was significantly lower than that of ZN and LED-FM microscopy, respectively. There was no statistically significant difference in sensitivity and specificity of ZN and LED-FM microscopy in all the approaches of choice, respectively. There was no significant difference in sensitivities and specificities between the four approaches of choice.

The 95% confidence interval for sensitivities and specificities were narrow for the pooled samples compared to those of combined and separate spot and morning samples. Pooling spot and morning samples for same number of patients, improves precision in estimation of performance of a diagnostic tool than using other analytical approaches.

***Table 4***: *Sensitivity and Specificity Of TB Diagnostic Tools Using Different Approaches Of Choice Of Samples*

| Approach of choice of samples | Diagnostic tool | n | Sensitivity (95% CI) | n | Specificity (95% CI) | n |
|---|---|---|---|---|---|---|
| **Approach 1:** cCombined spot and morning samples | ZN | 265 | 69.4%[63.9%-75.0%] | 1418 | 93.2%[91.9%-94.5%] | 1683 |
| | LED-FM | 266 | 71.1%[65.6%-76.5%] | 1419 | 93.8%[92.5%-95.1%] | 1685 |
| | **GeneXpert** | 130 | 83.1%[76.6%-89.5%] | 656 | 88.4%[86.0%-90.9%] | 786 |
| **Approach 2:** Spot samples | ZN | 213 | 67.1%[60.8%-73.4%] | 1470 | 94.3%[93.1%-95.5%] | 1683 |
| | LED-FM | 214 | 73.4%[67.4%-79.3%] | 1471 | 94.4%[93.3%-95.6%] | 1685 |
| | **GeneXpert** | 101 | 88.1%[81.8%-94.4%] | 685 | 89.3%[87.0%-91.7%] | 786 |
| **Approach 3:** Morning samples | ZN | 202 | 63.4%[56.7%-70.0%] | 1481 | 93.2%[92.0%-94.5%] | 1683 |
| | LED-FM | 203 | 69.5%[63.1%-75.8%] | 1482 | 93.5%[92.2%-94.7%] | 1685 |
| | **GeneXpert** | 102 | 84.3%[77.3%-91.4%] | 684 | 88.5%[86.1%-90.8%] | 786 |
| **Approach 4:** Pooled spot and morning samples | ZN | 415 | 65.3%[60.7%-69.9%] | 2951 | 93.8%[92.9%-94.6%] | 3366 |
| | LED-FM | 417 | 71.5%[67.1%-75.8%] | 2953 | 93.9%[93.1%-94.8%] | 3370 |
| | **GeneXpert** | 203 | 86.2%[81.5%-91.0%] | 1369 | 88.9%[87.2%-90.6%] | 1572 |

**ZN** - *Ziehl-Neelsen microscopy;* ***LED-FM*** **-** *Led Emitted Diodide fluorescent microscopy;* ***GeneXpert*** - *Xpert* ***MTB/ RIF****; 95%* ***CI*** *-Confidence interval*

## Discussion

Laboratory diagnosis of pulmonary TB involves analysis of spot and morning sputum samples mainly by microscopy and culture where available. Recent advances in molecular technology have added GeneXpert in TB diagnostic algorithm.

Patient management is optimized where final results of the combined spot and morning samples are used. The TB diagnostic tools have been validated using different approaches of choice of sputum samples.

The validation of TB diagnostic tools in 2014 and 2016 was performed using specific spot and morning sputum samples, whereby the sample was the unit of analysis (***Laboratory based***) [2,4].

The analysis by of 2016 was performed using separate spot and morning sputum samples while that of 2014 was performed using pooled spot and morning sputum samples.

Sensitivity of GeneXpert reported by in 2016 for separate spot and morning samples was significantly lower than the one reported in 2014**.** Using similar approaches used in 2016 and 2014, the results from the studies, indicate no significant difference in the sensitivity of GeneXpert between the two approaches.

The validation of TB diagnostic tools by Rachow et al. 2011 and Theron et al. 2014, were performed using final diagnosis results based on both spot and morning sputum sample, where the patient was the unit of analysis (point-of-care). Using similar approach, the results from the study was not significantly different from the previous one. [2,3]

Generally, validation of TB diagnostics from our study show no significant difference in sensitivities and specificities within the four approaches of choice of sputum samples between specific TB diagnostic tool.

However, when pooled spot and morning samples approach was used, there was increased number of samples, yielding a narrow 95% confidence interval for sensitivities and specificities compared to the number of samples when combined and separate spot and morning sample approaches were used.

When results for pooled spot and morning samples for the same number of patients were used, there was improved precision in estimation of performance of a TB diagnostic tool compared to other analytical approaches.

In addition, the results from this study has confirmed that, sensitivity of GeneXpert was significantly higher and specificity significantly lower than that of ZN and LED-FM, respectively, as observed in previous studies both in Kenya and elsewhere, regardless of the approach of choice [2]

Furthermore, the non-significant difference in sensitivity and specificity of ZN and LED-FM microscopy, respectively in all the approaches of choice, also confirm similar observations in the same previous studies [2].

These results indicate that determination and performance of TB diagnostic tools is not limited by the approach of choice of sputum sample. Variation in performance reported in literature may be influenced by other factors, such as the level of technical skills to perform laboratory procedures, among others.

The Kappa values for all diagnostic tools indicated good expectations in the results generated using spot and early morning sputum samples. The highest being GeneXpert and LED-FM. The findings provide additional evidence that, validation of TB diagnostics is not limited choice of sputum sample.

Clinical significance differences were observed between final diagnosis based on both spot and morning samples for LJ Culture results versus Other separate morning samples. This emphasizes the importance of using results based on both spot and morning samples when making a final diagnosis for patient management.

This approach provides evidence of increased chance of ensuring that people presumed to have TB are promptly and correctly identified for adequate management. Patients identified to be positive using spot sample should immediately be put on treatment while those turning negative should bring the early morning sample the following day for confirmation.

This is the first time to document such findings and to our knowledge there is no policy that provides

guidelines on best approach for choice of sputum sample(s) to achieve optimal performance of a TB diagnostic tool as well as providing adequate diagnosis to people presumed to have TB.

## Conclusion

Clinical significant differences observed between final diagnosis based on both spot and morning samples for LJ culture results versus other separate morning samples, provide additional evidence that informs policy makers on the dare need to emphasize on use of both spot and morning samples for high TB detection.

Patients identified to be positive using spot sample should be put on treatment while those turning negative should bring the early morning sample the following day for confirmation.

Determination and performance of TB diagnostic tools is not limited by the choice of sputum sample. Variation in performance reported in literature may be influenced by other factors, such as the level of technical skills to perform laboratory procedures.

These findings provide evidence on the need to develop guidelines on choice of sputum samples to be used for accurate TB detection and validation of TB diagnostic tools.

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6. The East Africa Public Health Laboratory Networking Project (EAPHLNP) – Operational Research (OR) secretariat.

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