

Alcohol-based conservation of sputum : quantitative and qualitative impact among referred samples from distant centres for Genexpert MTB/Rif analysis

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INTRODUCTION

Introduction

The Democratic Republic of Congo (DRC) lies among the countries with the highest burden of Tuberculosis (TB), but under-diagnosis of TB remains an important challenge, particularly in the South-Kivu Province, where only about 30%-40% of the estimated smear positive cases are effectively detected and treated. An ongoing TB Reach Wave 2 grant permitted to implement 8 Genexpert (Cepheid) in this particular province, and over 7.000 tests have been performed among smear negative TB suspects, with the aim to increase bacteriologically confirmed TB cases.

An increasing number of tests are being performed on samples referred from remote peripheral health facilities. In order to overcome deterioration of samples during transport, a low-cost and easy preservation method has been proposed and validated on-site.

Polymerase Chain Reaction (PCR) is a very sensitive and specific technique, but it can be inhibited by a number of inhibitors. In addition, enzymes present in the sputum sample may deteriorate DNA structure in parallel with the deterioration due to storage. For these reasons, a bibliographic review of available and validated sample- preservation techniques has been performed-, and the choice has been made towards the most simple and low-cost technique, considering that the cold chain is not an existing option in the South-Kivu rural context.

Ethanol 95% 1/1 dilution of sputum may be considered as a low-cost alternative for the preservation of sputum samples referred for Xpert MTB/Rif testing. The use of a preservation technique increases the availability of this new technique among rural population in low-income countries. The impact of this technique on the diminution of biohazard risk has still to be validated in a culture lab, but Ethanol is known to have antiseptic activity on *Mycobacterium tuberculosis*.

Technical validation

1. Absence of interference linked to alcohol preservation.

5 different sputum samples have been diluted 1/1, 1/2, 1/5 and 1/10 in 95% ethanol, and Xpert MTB/Rif tests have been performed on these samples. No interference or error has been reported, and the impact of 1/1 and 1/2 dilution did not interfere significantly with the Ct, when considering the intra-run variation of this technique.

2. Comparison with other locally available conservation methods (table 1)

One same sample has been analyzed 3 times on day 1, and then 3 times over 3 weeks after 3 different locally available transportation conditions (4°C, room temperature, and Ethanol 95% preservation). Analysis of Ct values show that, although not significative, alcohol preservation of sample does not interfere significantly with the Ct of the test.

3. Absence of increased errors/invalid results linked to alcohol preservation of samples (table 2)

A 1/1 dilution of samples in 95% ethanol has been performed among 214 referred samples, and general indicators (Error rate, Invalid results rate, Yield) have been compared with samples that have been processed without any pre-treatment method. Although not significative, these results show a decreased level of errors and an increased yield among the samples that received a pre-treatment.

4. (ongoing validation) Decreased biohazard risk

Alcohol is known to kill *Mycobacterium tuberculosis*, but an ongoing technical validation, in a culture lab, will confirm the exact alcohol dilution of the sample needed to avoid (or diminish to an acceptable level) the biohazard risk linked to the transport of TB samples. This technique will eventually permit to outsource the transport of samples to individuals or local transport companies and avoid extra costs linked to specialized transport systems.

Table 1 : Impact of temperature and Ethanol pre-treatment of sputum samples

Pre-treatment of sample	Day 0	Day 7	Day 14	Day 21
Room temperature	Ct=13,8	Ct=15,1	Ct=16,7	Ct=15,3
4°C		Ct=12,9	Ct=12,5	Ct=11,8
95% ethanol 1/1 dilution		Ct=12,9	Ct=12,3	Ct=13,5

Table 2 : Impact of Ethanol preservation on tests performed among referred sputum samples

Pre-treatment of sample	Error, n (%)	Invalid, n (%)	Positive, n (%)
No pre-treatment, n=1090	64 (5,9%)	11 (1,0%)	62 (6,1%)
95% ethanol 1/1 dilution, n=214	40 (4,7%)	9 (1,0%)	64 (7,9%)

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