

STUDY PROTOCOL TEMPLATE

Evaluation of OMNIgene•SPUTUM

A multicentre study to assess the suitability of OMNIgene•SPUTUM for transportation and processing of samples for TB and MDR-TB diagnosis



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This protocol was prepared by FIND and is made available for adaptation and use by independent investigators and country programmes.

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Because diagnosis matters

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Table of Contents

Protocol Summary	3
1. Background information and rationale	4
2. Objectives	5
2.1 Primary objective	5
2.2 Secondary objectives.....	5
3. Study design.....	5
3.1 Study design	5
3.2 Outcome measures	6
3.3 Study sites	6
3.4 Study population, eligibility criteria and recruitment	6
3.5 Study workflow	7
3.6 Minimization of error and bias	8
4. Study procedures.....	10
4.1 Specimen collection, handling, transport and storage.....	10
4.2 Sample processing procedures.....	10
4.3 Other study procedures	10
5. Analysis plan and statistical methods.....	11
5.1 Definitions of test results	11
5.2 Description of subject characteristics	11
5.3 Exclusion criteria for analyses.....	11
5.4 Analyses of outcome data.....	12
5.5 Sample Size	12

Protocol Summary

Title

A multicentre study to assess the suitability of OMNIgene•SPUTUM for transportation and processing of samples for TB and MDR-TB diagnosis.

Study design

Multicentre, prospective study to assess the performance of OMNIgene•SPUTUM compared to samples processed by NALC-NaOH for solid and liquid culture testing.

Population

Specimens from patients with TB on anti-TB treatment submitted for treatment monitoring by MTB culture (and drug susceptibility testing).

Objectives

Primary objective

- Overall culture-positivity: compare the overall culture-positivity rate between OMNIgene•SPUTUM and standard processing with NALC-NaOH among all cultures (i.e. not excluding contaminated cultures)

Secondary objectives

- Contamination: compare the contamination rate between OMNIgene•SPUTUM and standard processing with NALC-NaOH
- MTB viability: compare the culture-positivity rate and time to culture-positivity between OMNIgene•SPUTUM and standard processing with NALC-NaOH among non-contaminated samples

1. Background information and rationale

Tuberculosis (TB) remains a major global public health problem even today. Despite longstanding availability of treatment, an estimated 9.6 million people developed TB in 2014 and 1.5 million people died from TB^[1]. Early and improved diagnosis of TB, including of multidrug-resistant TB (MDR-TB), has therefore become one of the global priorities for TB prevention and care. In recent years the World Health Organization (WHO) has issued recommendations and guidance for the use of new tests for the diagnosis of MDR-TB, including liquid culture and molecular assays. Despite a significant uptake, access to these new, rapid tests is still a challenge as the testing platforms are mostly located in relatively centralized laboratories, requiring the transport of specimens from remote laboratories and collection sites to these centralized facilities.

Laboratory networks in low- and middle-income countries have generally weak referral systems and lack proper storage facilities; as a result, samples obtained over several days at the collection point are accumulated to limit the number of shipments and stored at room temperature for extended periods, which may lead to the poor recovery of *M. tuberculosis* (MTB) and/or increased contamination rates. Similar issues occur when specimens are transported over long distances and/or when transportation is conducted outside an optimal temperature range. Cetylpyridinium chloride (CPC) has been used as a preservative to improve the recovery of MTB from sputa subjected to long-term storage. However, CPC cannot be used if specimens are destined for inoculating cultures in MGIT tubes. A preservative or transport medium that could increase MTB yield for available diagnostic tests, while reducing transportation costs by removing the need for cold chain, would therefore be of great benefit to many low- and middle-income countries.

OMNIgene•SPUTUM (OMS), a stable, non-toxic reagent manufactured and distributed by DNA Genotek, Ottawa, Ontario, Canada, is a reagent, which could liquefy and decontaminate sputa while preserving the viability of MTB for 8 days when specimens are stored between 4°C to 40°C. The reagent is added in a 1:1 ratio at the point-of-collection and samples can be stored temporarily and shipped at ambient temperature. OMS would thus offer a flexible approach to sputum specimen management and shipping that is most valuable for centralized testing models and provide optimized high quality sputum samples. Specifically, the utility of OMS on specimens used for treatment monitoring of patients with TB requires further evidence through operational research. An added advantage of the OMS reagent is that specimens are decontaminated during transportation, removing a step from the laboratory SOP and thus potentially improving laboratory throughput, reducing the time to test result and minimizing the HR time for undertaking the desired testing.

¹ World Health Organization. Global Tuberculosis Control: WHO report 2015. Geneva, World Health Organization, 2015.

In this study, we will examine the effect of OMS on the recovery of MTB from sputa subjected to various transportation delays and/or subjected to long-term storage ahead of processing in comparison with sputa collected from the same patients and processed by the NALC-NaOH method in the central laboratory. The study will also look into other related laboratory parameters such as transportation time, processing time, contamination rate, and time to culture positivity to explore the feasibility of using the reagent under programmatic settings.

2. Objectives

The general aim of this multi-center study is to compare OMS to NALC-NaOH under real-life conditions to assess suitability for adoption by TB programs.

2.1 Primary objective

- Overall culture-positivity: compare the overall culture-positivity rate between OMNIgene•SPUTUM and standard processing with NALC-NaOH among all cultures (i.e. not excluding contaminated cultures)

2.2 Secondary objectives

- Contamination: compare the contamination rate between OMNIgene•SPUTUM and standard processing with NALC-NaOH
- MTB viability: compare the culture-positivity rate and time to culture-positivity between OMNIgene•SPUTUM and standard processing with NALC-NaOH among non-contaminated samples

3. Study design

3.1 Study design

A total of 500 paired sputum samples collected prospectively and treated with OMS will be compared to NALC-NaOH processed specimens (see section 6.4 for details on sample size calculation). The study will be conducted in accredited culture and drug-susceptibility testing (DST) laboratories with a high specimen workload and among known patients with TB who are currently on anti-TB treatment. Two specimens will be collected from eligible patients. One sample will undergo transport and decontamination with NALC-NaOH in the receiving laboratory as per routine procedures while the other sample will be processed with OMNIgene•SPUTUM at the time of collection and then transported to the receiving laboratory. Both samples will be cultured, tested with microscopy and molecular methods and specified outcomes assessed.

3.2 Outcome measures

Culture

- overall culture positivity rate
- culture contamination rate
- time to culture positivity

Microscopy

- smear positivity rates
- smear grade
- staining quality of the smear

3.3 Study sites

The laboratory testing will be conducted in accredited culture and Drug Susceptibility Testing laboratories. The intent is to select a range of study sites that cover a variety of conditions in terms of climate and duration of sample transport, which influence contamination rates. Sites should be able to perform LJ and MGIT and have access to sites where large numbers of patients with TB are treated and followed clinically and mycobacteriologically to monitor response to anti-TB treatment. Ideally, sites would have strong linkages to the National TB program.

Each laboratory routinely receives specimens from a number of sites where patients with TB are followed. The inclusion of all of these sites is not logistically feasible due to requirements for training, providing informed consent, supplying collection sites with reagents, supervision, etc. Therefore, we will select sites for participation in the study based on the number of samples they send and their representativeness in terms of the patient spectrum, transport time and general performance.

3.4 Study population, eligibility criteria and recruitment

Inclusion criteria

- Patients with diagnosed TB by validated laboratory methods (i.e. culture, Xpert MTB/RIF)
- Specimens from patients on anti-TB treatment
- Providing samples for treatment monitoring (not diagnosis)
- Spontaneously expectorated spot sputa

Exclusion criteria

- age ≤ 18 years
- no informed consent

Recruitment

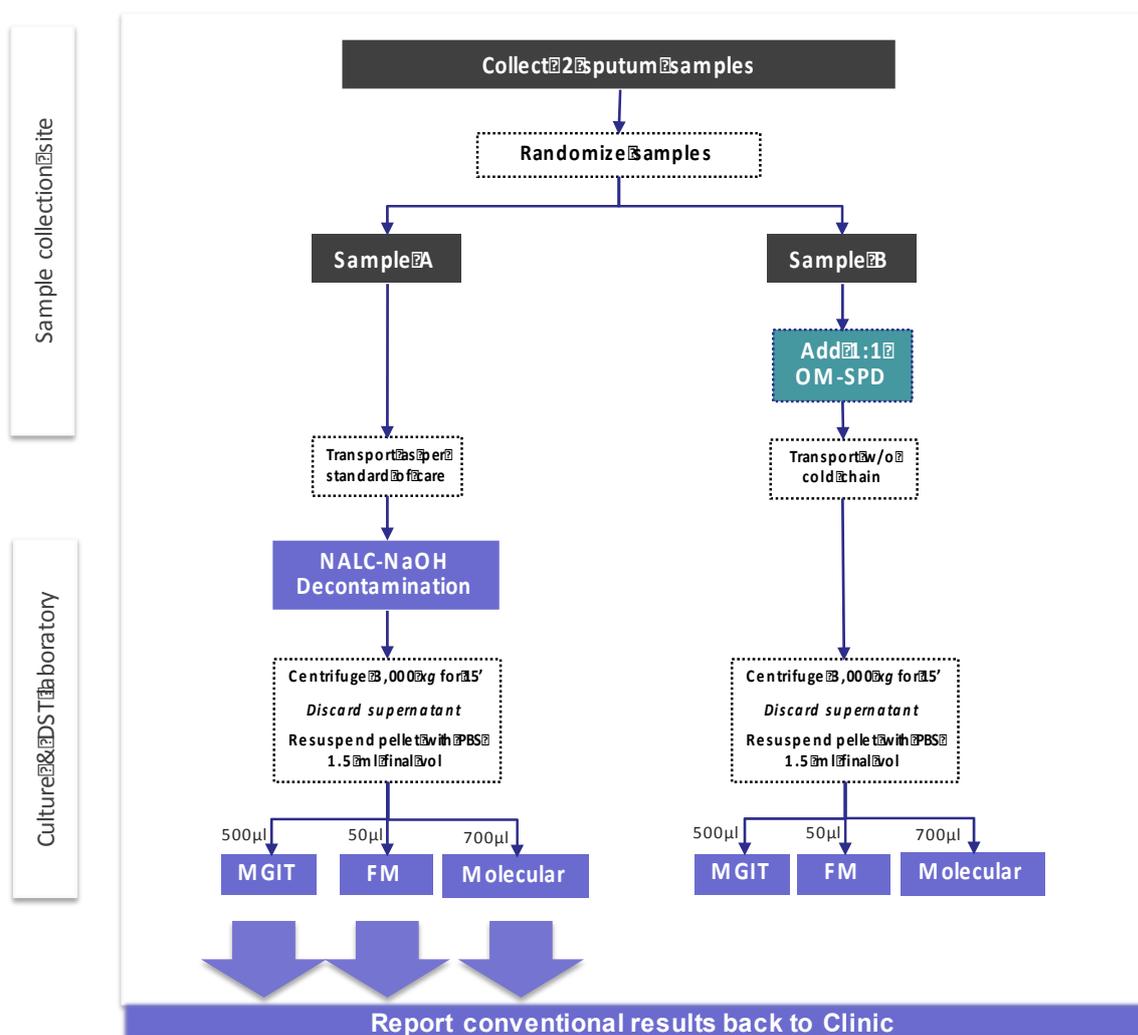
- prospective recruitment
- consecutive eligible patients
- to take place at TB treatment sites

3.5 Study workflow

Patients with TB will be identified at TB treatment or other clinical sites and informed consent obtained. Two samples per patient will be collected (one morning, one spot). Using a blocked randomization scheme, specimens will be randomly assigned to the following procedures: one specimen (Sample A) will be collected and shipped to the project lab as per routine practice for processing and processed by NALC-NaOH method upon arrival. The other specimen (Sample B) will have an equal volume of OMS poured directly in 1:1 ratio and processed as per package insert. This sample will then be shipped to the project lab without cold chain but along with Sample A (i.e. on the same day and with the same shipment method).

At the project lab, Sample A will be treated by established NALC-NaOH decontamination procedure as per current standard protocol (Kent & Kubica). Sample B will be used directly. Both specimens will then be centrifuged, supernatant poured out, and the pellet re-suspended in PBS buffer. Subsequently, samples will be examined by smear microscopy, and inoculated on liquid and solid culture.

Figure: Sample flow



3.6 Minimization of error and bias

Patient selection

We focus on patients with TB and their specimens submitted for treatment monitoring to address the current knowledge gap on OMS utility in this particular population. The focus of this study is the comparative effectiveness of two sputum processing methods while using the *same diagnostic tests*. It is conceivable that the putative advantages of OMS are greater in smear-negative samples, since preserving viability is even more important in paucibacillary samples, as it is often the case for specimens submitted for treatment monitoring. Of note, assessing any negative effect on the time to culture positivity is crucial to ensure that OMS truly does preserve viability, i.e. that OMS does not just reduce contamination rate at the cost of reduced TB viability, in which case there would also be a risk of generating false-negative culture results in paucibacillary specimens.

Sample flow and randomization

As described in section 3.4 (Study workflow) and in the Figure (Sample flow), two specimens per patient will be obtained and allocated to processing with either NALC-NaOH or OMS. It is conceivable that there will be systematic differences between the first and the second sample a patient produces. For example, sample one may have a higher concentration of MTB or a higher risk of being contaminated. To prevent bias, we will use stratified blocked randomization with varying block sizes, with the sequence of the sample being the stratifying variable. This will ensure that each method (NALC-NaOH and OMS) will receive the same number of “first” and “second” samples and also minimise the possibility of any purposeful selection of staff at the TB treatment sites.

Outcome ascertainment

Outcomes and how they will be objectively assessed will be clearly defined a priori to exclude any role of subjective interpretation. Study procedures will ensure that outcomes from the specimen processed with NALC-NaOH are obtained and recorded independently from the outcomes on the (paired) specimen processed with OMS and vice versa. This will ensure independent interpretation of outcomes from the index and reference procedure.

Other sources of error and bias

It is possible that our study could introduce a Hawthorne effect that may in turn lead to some bias towards the Null: being part of a research study may improve adherence to country protocols and overall performance, such that e.g. maintaining a cool chain during transport is improved and contamination under standard of care reduced. Such artificial improvement would make outcomes with NALC-NaOH look better than they normally are and make it more difficult to detect any putative improvement with OMS. We will try to prevent this by emphasizing that it is important that sites don't change their practices during the study period. Since this may not be entirely successful, we will additionally compare study data to historical laboratory records (e.g. past contamination rates from lab registers). This should allow us to assess whether a Hawthorne effect may have been introduced and if so (in a sensitivity analysis) compare OMS during the study to NALC-NaOH before the study.

4. Study procedures

4.1 Specimen collection, handling, transport and storage

Sites will be instructed to follow their routine procedures for sample collection, sample handling, transport and storage and details on these procedures will be recorded. The time between obtaining a sample and arrival and processing in the laboratory will be recorded. The temperature during transport will be monitored (e.g. with Min/Max Thermometers, temperature labels or log tags) and recorded upon arrival in the laboratory.

4.2 Sample processing procedures

The standard sputum processing procedure is NALC-NaOH as described by Kent & Kubica. This is the standard of care in most laboratories in the world and therefore the baseline sample processing procedure to compare against. The NALC-NaOH decontamination procedure will be performed as per National TB Programme guidelines.

The OMNIgene•SPUTUM procedure will be performed according to the manufacturers package insert. Briefly:

- Visually estimate volume of sputum collected.
- Add approximately an equal volume of OMS reagent.
- Recap collection cup tightly. Vigorously invert shake specimen 10 times to mix.
- Specimen can be transported for up to 8 days without cold chain (to a maximum temperature of 40°C). Alternatively, a minimum of 30 minutes must elapse before proceeding to the next step.
- Spin OMS mixed specimen at 3,800 × g for 20 minutes to obtain sediment.
- Gently pour off supernatant into appropriate waste container without disturbing the sediment. Do NOT discard sediment.
- Re-suspend the sediment in sufficient volume of sterile phosphate buffered saline or sterile water as per the standard laboratory test procedures.

4.3 Other study procedures

Smear examination, solid and liquid culture as well as DST will be performed as per National TB Programme guidelines. No attempts will be made by the study to interfere with routine laboratorial procedures. Likewise, routine clinical management will remain the same, according to the clinician's judgment, the NTP guidelines and good clinical practices.

Laboratory results obtained during this phase from NALC-NaOH decontaminated samples will be used for patient management. On the contrary the results from testing done on OMS treated samples will not be used for patient management.

5. Analysis plan and statistical methods

5.1 Definitions of test results

TEST RESULT	DESCRIPTION
Smear-positive	≥ 1 positive smear (inclusive of scanty positive smears) using WHO grading.
Culture-positive	≥ 1 LJ and/or MGIT culture growth confirmed MTB complex.
Culture-negative	At least 1 LJ or MGIT have no culture growth after >56 days and >42 days
Contaminated culture	LJ: Cultures completely overgrown by bacterial or fungal contaminations within 3 weeks (discarded). In case of mixed cultures, isolated MTB colonies transferred to new LJ tube (repeat culture). MGIT: Instrument positivity w/o detection of AFB.

5.2 Description of subject characteristics

Descriptive statistics will be provided on baseline culture positivity rate, contamination rate, number of days that specimens were in transit, ambient temperature during the course of the study and other relevant factors, both by study site and overall.

5.3 Exclusion criteria for analyses

Patients with the any of the following criteria on any of their two (paired) sputum specimens will be excluded from the analyses. As an example, time to culture positivity will be computed in samples with positive cultures in both samples of a patient (i.e. positive culture for both the NALC-NaOH and OMS sample).

Exclusion criterion	Primary outcome	Secondary outcomes		
	Overall % culture-positive	% contaminated	% culture-positive among non-contaminated	time to culture-positive
(a) no valid culture result recorded	X	X	X	X
(b) Cross-contamination likely*	X	X	X	X
(c) culture positive but no MTB speciation available	X	X	X	X
(d) contaminated culture				X
(e) Negative culture			X	X

* Single positive culture with ≤20 colonies (LJ) or >28 days' time to positivity (MGIT)

5.4 Analyses of outcome data

A separate detailed analysis plan will be prepared to detail the analyses and methods planned. The main analyses on the key outcomes will be based on estimating the difference in paired proportions of outcomes (with 95%CI using Tango's score method). Stratified analyses will be performed including analyses:

- by site
- by days in transit
- by baseline contamination/positivity (grouping sputum collection sites across countries)

5.5 Sample Size

To detect a reduction in contamination from 19% with NALC-NaOH to 15% with OMS, a total sample size of 1,901 and 1,433 is required for 90% and 80% power, respectively. The exact precision of the estimated reduction in contamination rate will depend on (and increase with) the baseline contamination rate, comparative effectiveness of OMS and the level of correlation between contamination rates with OMS and to NALC-NaOH. For example, given a sample size of 1,433, 19% contamination rate with NALC-NaOH and 15% contamination with OMS and no correlation, we would estimate the contamination rate to be reduced by 4% (95%CI 1%, 7%).