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Review Article

Decontamination of Sputum in the Context of Implementation of Mycobacterial Culture in the Republic of Congo

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Introduction

Tuberculosis is a serious disease. TB is caused by bacteria that spread through the air when a person with contagious TB in their lungs coughs, sneezes, sings, or talks. Pulmonary tuberculosis is still a major health problem in the Republic of Congo and other developing countries despite the major advances in diagnosis and treatment over the past years [1,2]. Sputum smear microscopy is the first-line diagnostic procedure for pulmonary TB in Congo because it is simple, relatively low cost and monitors patient's response to anti-TB treatment. Sputum culture is more sensitive than sputum smear microscopy and though being the gold standard diagnosis for TB [3], remains in limited use due to its cost of implementation, time required to obtain the result and culture contamination [4]. Decontamination is an operation which aims to eliminate, kill or inhibit unwanted microorganisms depending on the objectives set. Only the microorganisms present at the time of the operation are destroyed. Decontamination is partially bacteriostatic, that is to say that under well-defined conditions, the proliferation of microorganisms is momentarily inhibited during the decontamination process.

Culture contamination is a major limitation as it reduces the proportion of interpretable results and diminishes the diagnostic value of culture systems [5]. Factors such as sputum collection method, storage temperature, transport conditions, duration between sample collection and processing, and lab methodology affect contamination rates. The aim of this brief communication is to highlight the main factors linked to contamination through each step from the collection of the sputum sample to its inoculation in culture and which impedes mycobacterial culture establishment in the Congo Table.

Comments

Sputum is mucous that an individual coughs up from deep inside the lungs. It is usually thick, cloudy and sticky. Sputum is not saliva (spit). Saliva comes from your mouth and is thin, clear and watery. The sputum collection must be done very early in the morning, fasting and in the absence of any cigarette intake. These specifications contribute to reducing the effect of contamination of the sample by the bacterial flora of the oral cavity [6].

The multiplication of the common flora bacteria could mask the pathogenic agent (s) responsible for the pulmonary infection. Sputum is thick mucus that is secreted by the lower respiratory tract (bronchi and lungs), which is different from saliva. Teeth should be brushed, the mouth rinsed with a glass of water, two or three deep breaths taken, and finally cough to bring forth the expectoration from the lungs.

Sample collection should be done carefully to ensure that it comes from the lower respiratory tract and not the upper respiratory tract. If the sample contains mainly saliva, the microorganisms identified in culture will not necessarily be responsible for the infection. In addition, the presence of saliva and salivary bacteria in a sputum sample will make it more difficult to identify pathogenic bacteria in the lungs [13]. All samples taken must be sent to the laboratory for mycobacteriological analysis in order to avoid the development of commensal bacteria at the expense of fragile pathogenic bacteria.

The quality of the sputum sample must be checked by the laboratory technician before performing the analysis [14]. In order for the sputum culture to be interpretable, the collection of the sample must be of irreproachable quality [5,15]. A sample of inadequate quality must be rejected and another sample collected [16]. Transport the specimen to the lab the same day it is collected. Within two hours of collection is the preferred time frame for optimal specimen processing.

Indeed, the sample is often contaminated by salivary bacteria (*staphylococci, streptococci, coryneforms, Neisseria*)b [17,18] or commensal bacteria of the respiratory system (that cause no infections under normal conditions, such as *Haemophilus influenzae, Streptococcus pneumoniae, Staphylococcus aureus*, strict anaerobic bacteria) [19].

Conclusion

The rising rate of contamination of mycobacterial culture shows an urgent need for the improvement of Health center systems in Republic of Congo, patient education, and quality trained personnel at all levels to ensure better specimen collection, handling and processing in order to maintain the viability of the tuberculosis bacilli

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Table: Different levels of Sources of Mycobacterial Sputum Contamination.

Test	Conditions	Preservation	Source of contamination
Expectoration	-Well brushed teeth;	- Send the sterile jar containing the sputum within 2 hours following the sample collection;	- Oral flora is a source of contamination for spit [7];
	-Inside of mouth well rinsed with an antiseptic solution;		- Saliva production is a source of contamination [4];
	-Done early in the morning;	- Protected from light, kept refrigerated.	- Heat favors contamination [8]
	-Well washed hands;		- Unsupervised collection of sputum by patient [9];
	-Producing mucus from the lungs by coughing heavily.		- Poor Storage and transportation may lead to contamination [10].
			- Environment and poorly disinfected laboratory equipment.
Microscopy	-Thoroughly sterilize platinum loop with Bunsen burner flame before use;	-The preservation of the sputum container is put immediately in the refrigerator after staining the slides.	- Poorly sterilized platinum loop [11];
	-Close the sterile jar immediately and store in the refrigerator.		- Open jar exposed to the open air ;
			- Sputum container left open at room temperature for hours is a source of multiplication of pathogenic microorganisms contained inside the sputum [12].
Decontamination of expectorates	-Sputum should be decontaminated within 3 days before culture;	-Sputum should be stored at refrigerator temperature (+2°C and +8°C) during the 3 day interval.	-A concentration below the standard does not eliminate all contaminants;
	- Final concentration of decontaminating reagent		- the reduced decontamination time does not eliminate all contaminants;
	- sample treatment time is critical		- A non-sterile decontaminant solution will be useless.
	- Use of sterile decontaminating solution		
Culture	-Acceptable contamination for solid media (3-5%)	**-** Decontaminated expectorates should be cultured immediately;	-The concentration of the decontamination solution must be that recommended for decontamination;
	-Acceptable contamination for liquid media (3-10%)		- Respect of the duration during the process of decontamination must be respected; For example, for NALC-NAOH, a duration of less than 15 minutes would not destroy all the microorganisms contained in the sputum);
	-Good quality reagents (Expiry date)		- Prolonged storage in the refrigerator favours contamination;
			- Poor storage of decontaminated expectorates in the freezer will favor loss of sample integrity;
	-Sterile culture medium		-Insufficient PANTA volume or expired reagents may favour contamination to >10% (for liquid culture)
	-Sufficient PANTA volume		

with increasing sensitivity while reducing contamination.

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