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ORIGINAL ARTICLE

# Methodology of collection of spontaneous sputum for microbiological confirmation of the diagnosis of pulmonary tuberculosis, pulmonary disease by non-tuberculous mycobacteria or for folow-up of out- and in-patients under anti-tuberculous therapy

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## INTRODUCTION

The main objective of collection of spontaneous sputum of patients suspected of pulmonary tuberculosis is to support the microbiological diagnosis of this disease (GARAY, 2006). Samples with good quality and volume ranging from 5 to 10 ml (ISENBERG, 1998; MANUAL DE VIGILÂNCIA LABORATORIAL DA TUBERCULOSE E outras MICOBACTÉRIAS, 2008) are recommended because higher the volume and more purulent the sample, more probable will be the isolation of *Mycobacterium tuberculosis* (LAIRD, 1909). Sputum volume of 10 ml or near this value should be collected. The patient should be stimulated as much as possible to expectorate this volume. Volumes ≤ 5 ml should only be considered enough if the patient is not able to expectorate after several unsuccessful attempts.

Among the cavitary pulmonary forms diagnosed at the Clinical Research Center/Núcleo de Doenças Infecciosas/Universidade Federal do Espírito Santo between 2002 and 2006, *Mycobacterium tuberculosis* was isolated of samples of spontaneous sputum collected of these patients in 95% of the cases (data not published). Similar rates were reported in literature (GREENBAUM et al., 1980; LEVY H et al., 1989).

In relation to the patients under anti-tubercu-

lous therapy, the main objective of the collection of spontaneous sputum once a month is to confirm the negativity of direct search of alcohol acid fast bacilli (AFB) and mycobacterial culture. Therefore, all the recommendations should be also respected in order to reach volume of 10 ml and good quality sample.

In order to obtain samples with these features, the health care worker should explain to the patient in a simple and objective manner, the step-by-step for collection of this clinical material (CENTRO DE VIGILÂNCIA EPIDEMIOLÓGICA "PROF. ALEXANDRE VRANJAC", 2002; MANUAL NACIONAL DE VIGILÂNCIA LABORATORIAL DA TUBERCULOSE e outras MICOBACTÉRIAS, 2008). Therefore, it will be possible to collect spontaneous sputum for the diagnostic procedures recommended by the Programa Nacional de Controle da Tuberculose: rapid molecular testing for search of *Mycobacterium tuberculosis,* direct search of alcohol acid fast bacilli (AFB) and mycobacterial culture.

The collection of spontaneous sputum should, preferentially, be performed early in the morning after awaking (ISENBERG, 1998; GARAY, 2006) and prior to the introduction of anti-tuberculous chemotherapy. This sample is usually the most abundant of AFB because it is composed of fluid retained in bronchial tree during the night.

Spontaneous sputum represents bronchopulmonary and oro-pharyngeal fluids because bronchopulmonary fluids mix with the second ones while passing through the upper portion of the respiratory tract. Thus, sputum is a contaminated fluid due to the presence of oro-pharyngeal microbiota. Taking into account the high quantity of mucus, it is considered a viscous fluid (MANUAL NACIONAL DE VIGILÂNCIA LABORATORIAL DA TUBERCULOSE e outras MICOBACTÉRIAS, 2008).

In relation to its macroscopic features, spontaneous sputum sample can be categorized as saliva, mucopurulent, bloody or liquefactive. Good-quality sample is composed of fluid produced in the bronchial tree, obtained after cough and not that obtained of pharynx by aspiration of nasal fluids or saliva. Sputum samples collected for therapy follow-up should always be examined even if the volume is ≤ 5 ml and/or poor-quality ones (MANUAL NACIONAL DE VIGILÂNCIA LABORATORIAL DA TUBERCULOSE e outras MICOBACTÉRIAS, 2008).

The collection procedures should be performed in opened environments. For those settings, where they are performed in closed rooms, these must be well ventilated and its doors must be kept closed. The windows must be kept opened in order to reduce the concentration of infecting particles (Well´s nuclei) in the environmental air. The door must be kept closed during the collection to drive the air flow outside the room environment through the window (MANUAL NACIONAL DE VIGILÂNCIA LABORATORIAL DA TUBERCULOSE e outras MICOBACTÉRIAS, 2008).

The health care worker must use a disposable filtered personal facial mask (N95 mask/PFF2) to wellcome and instruct each patient. Whenever there is indication of respiratory and/or contact isolation, the mask must be the last individual protection equipment to be taken off.

In resource-limited settings, these masks can be stored in paper envelopes or plastic bags previously perforated. The retention of humidity impairs the filter and expose the health care worker to the biological, physical and chemical risks present in the environment.

It is important to avoid the presence of food and/or drug and/or chemical substance residues in the sample of spontaneous sputum because they impair the quality of the sample. Therefore, the following recommendations must be respected:

1. fast after 22:00 h at the previous day,
2. no oral administration of any drug at least 4 h before collection,
3. no oral dministration of anti-tuberculous drugs before collection,
4. do not perform hygiene of oral cavity with dental cream. The anti-septic solutions for gargling like cetylpyridinium chloride (CepacolR) or chlorhexidine hydrochloride (PeriogardR) during 1 min before collection can be used, and
5. tooth brushing with filtered water.

In case of mobile dental prosthesis, it must be taken off before the oral hygiene. If the prosthesis is fixed (like the bridges), advise and observe the dental brushing only with water (without dental cream) and, lately, advise the mouth-wash in order to remove the residues of the dental prosthesis.

The patient must perform the collection of the sample in a sitten position. The collection vial must be positioned in an easy and accessible way during all the procedure. The following features are recommended for the collection vial (MANUAL NACIONAL DE VIGILÂNCIA LABORATORIAL DA TUBERCULOSE e outras MICOBACTÉRIAS, 2008):

1. sterile one
2. plastic and disposable on
3. transparent walls
4. volume ranging from 35 to 50 ml
5. minimal height of 40 mm
6. large mouth and
7. twisting tap with a diameter of 50 mm.

Ask the patient to wash his hands before delivering the vial for collection of spontaneous sputum.

The vial must be delivered to the patient closed and identified with a label adhered to the vial wall with patient name and collection date recorded. The label should be adhered in a place of the vial wall without impairing the observation of the volume scale (MANUAL NACIONAL DE VIGILÂNCIA LABORATORIAL DA TUBERCULOSE e outras MICOBACTÉRIAS, 2008).

Never fix the label over the vial tap. Never deliver the vial without identification because it may incur on the risk of sample change.

Before instructing the patient how to collect the sputum sample, the health care worker must verify if the following elements are present and of easy access to the patient: 1) filtered water for oral ingestion during the collection, 2) closed collection vial with adequate identification and twisting and un-twisting mechanisms properly functioning and 3) disposable towel paper (MANUAL NACIONAL DE VIGILÂNCIA LABORATORIAL DA TUBERCULOSE e outras MICOBACTÉRIAS, 2008).

The following instructions must be given to the patient:

1. deeply inspirate through the noses, stop breathing for some seconds and expirate. After repeating these procedures three times, cough,
2. immediately after the act of a productive cough, the patient should open the collection vial and expectorate the fluid inside the vial without touching any place of the vial with his lips and/or fingers because of the risk of sample contamination and
3. immediately after the end of the expectoration, twist completely the tap to close the vial. Never leave the collection vial opened between two expectorations.

These procedures must be repeated as many times as possible in order to reach the sputum volume of 10 ml.

In addition to these instructions, the health care worker must always refer to the patient by his proper name, welcome him, get closer to him in order to attend his requirements, look to the patient when attending him, concentrate on the patient and always keep a pleasant expression. Verify if the patient has understood all the instructions. If the instructions must be repeated, change the language e give to the patient time enough to ask in case of doubts (CENTRO DE VIGILÂNCIA EPIDEMIOLÓGICA "PROF. ALEXANDRE VRANJAC", 2002).

The quantity of AFB in each sample is variable (WHO, 1998). Therefore all the efforts should be implemented to obtain the sputum volume of 10 ml in order to increase the sensitivity of direct search of AFB and the isolation of M. tuberculosis.

The chronic coughers or with radiographic image suggestive of tuberculosis, should be submitted to collection of 2-3 samples of spontaneous sputum and, if necessary, three other samples for direct search and culture of fungi.

Each patient has his own features for collection of spontaneous sputum, which should be respected. Patient complaints of intercostal pain may occur when the frequency of coughing is too high. At the Clinical Research Center/Núcleo de Doenças Infecciosas/ Universidade Federal do Espírito Santo, most of the patients need 20-30 min to finish the collection procedure. Very few patients need more time (data not yet published).

After the collection of the sample, the collection vial with its tap completely twisted to avoid spill of sputum sample, should be packed in a transparent plastic bag. If the time between the ending of the collection and the beginning of the laboratory procedures is ≤ 2 h, the vial should be sent to the laboratory under environmental temperature protected of the sun light. If this time is more than 2 h, the vial should be stored in a refrigerator (2-8°C) until being sent to laboratory. The sputum sample should not be kept in environmental temperature for more than 2 h because the sample will probably liquefy acquiring a muco-colloidal aspect (MANUAL NACIONAL DE VIGILÂNCIA LABORATORIAL DA TUBERCULOSE e outras MICOBACTÉRIAS, 2008).

Step-by-step guidelines for collection of spontaneous sputum of OUT-PATIENTS ATTENDED AT LOCAL CLINICS AND IN-PATIENTS are mentioned below (LAIRD AT, 1909; ISENBERG, 1998; CENTRO DE VIGILÂNCIA EPIDEMIOLÓGICA "PROF. ALEXANDRE VRANJAC", 2002; GARAY, 2006; MANUAL NACIONAL DE VIGILÂNCIA LABORATORIAL DA TUBERCULOSE e outras MICOBACTÉRIAS, 2008):