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Journal of INFECTION CONTROL

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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-

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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 VIGI-LÂNCIA 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 MICOBACTÉRIAS, 2008).

In relation to its macroscopic features, spontaneous sputum sample can be categorized as saliva, mucopurulent, bloody or liquefactive. Goodquality 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 followup 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,
- no oral administration of any drug at least 4 h before collection,
- no oral dministration of anti-tuberculous drugs before collection,
- 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):

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

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).

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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:

- 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 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):

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STEP-BY-STEP GUIDELINES FOR COLLECTION OF THE FIRST SAMPLE OF SPONTANEOUS SPUTUM AT THE LOCAL CLINICS FOR CONFIRMATION OF THE DIAGNOSIS OF PULMONARY TUBERCULOSIS

VIAL PREPARATION

The health care worker should:

1. WASH THE HANDS PRIOR TO VIAL PREPARATION;



2. CHOOSE A STERILE AND SCALED VIAL WITH TRANSPARENT WALLS, MINIMAL AND MAXIMAL VOLUME CAPACITIES OF 35 ml and 50 ml, respectively, MAXIMAL HEIGHT of 40 mm and TWISTING TAP with a DIAMETER of 50 mm, according to the illustration below:

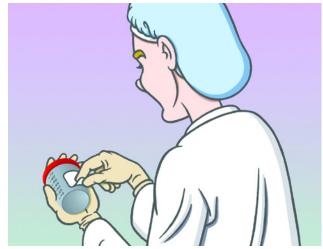


Boca larga= large mouth; tampa rosqueável=twisting tap; plástico transparente= transparent plastic, capacidade de 35 ml a 50 ml= minimal and maximal volumes of 35 ml and 50 ml, respectively; pote descartável= disposable vial; altura mínima= minimal height; etiqueta do frasco – Nome completo= full name, data=date: xx/yy/zz

3. OPEN THE PLASTIC BAG OF THE COLLECTION VIAL, TAKE THE VIAL OFF WITHOUT UNTWISTING THE TAP and DISCARD THAT IN THE COMMON TRASH;



4. RECORD THE PATIENT NAME AND THE COLLECTION DATE IN A LABEL;

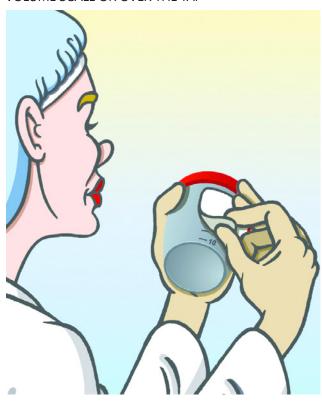


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5. FIX THE LABEL ON THE EXTERNAL WALL OF THE COLLECTION VIAL. DO NOT FIX THE LABEL OVER THE VOLUME SCALE OR OVER THE TAP



6. 10 ml line must be pointed out with black reto-projector pen to make easier the observation of the level to be reached with the sputum.

ATTENTION: FOAM WILL NOT BE REGARDED.



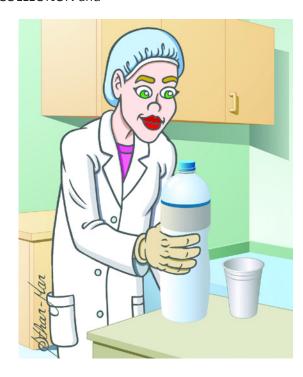
THE HEALTH CARE WORKER, PROPERLY WORN WITH N95 MASK, SHOULD:

- 1. WELLCOME THE PATIENT;
- 2. DELIVER A WHITE SURGICAL MASK TO THE PATIENT AND ADVISE THE PATIENT HOW TO FIX THE MASK OVER THE FACE;
- 3. EXPLAIN TO THE PATIENT THAT THIS MASK SHOULD BE FIXED OVER HIS FACE UNTIL THE PLACE OF THE COLLECTION PROCEDURE or IN CASE THE PATIENT MOVES TO OTHER PLACES (e.g.: GO TO THE BATHROOM or TO THE RADIOLOGY SERVICE);



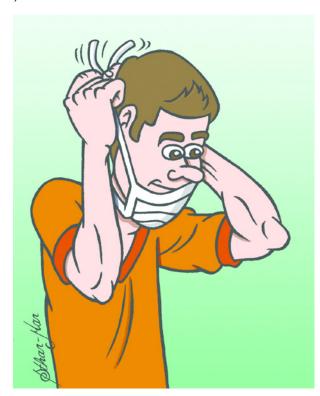


4. RECOMMEND TO THE PATIENT TO SWALLOW WATER AS MUCH AS POSSIBLE DURING THE COLLECTION and



5. ASK THE PATIENT TO GO TO THE BATHROOM FOR THE FOLLOWING PROCEDURES:

a) TAKE THE WHITE SURGICAL MASK OFF THE FACE



b) WASH THE HANDS WITH WATER AND SOAP AND LATER ON, DRY THEM WITH PAPER TOWEL;





c) REMOVE ANY TOOTH PROTHESIS;







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d) BRUSH THE TEETH WITH TOOTH BRUSH DAMPENED WITH FILTERED WATER TO REMOVE ANY FOOD RESIDUE OF THE TEETH, GINGIVAS and TONGUE;



e) RINSE THE MOUTH AND GARGLE WITH FILTERED WATER;



- f) FIX AGAIN THE WHITE SURGICAL MASK OVER THE FACE.
- 6. ADVISE THE PATIENT NOT TO MAKE ORAL HYGIENE WITH TOOTH CREAM and
- 7. INFORM THAT TOOTH BRUSHING, MOUTH RINSING and GARGLE WITH WATER ARE ENOUGH AND INDISPENSABLE.

ENVIRONMENTAL PREPARATION FOR SPUTUM COLLECTION

THE HEALTH CARE WORKER, PROPERLY WORN WITH N95 MASK, SHOULD:

1. CHOOSE AN OPENING PLACE AT THE LOCAL CLINICS FOR SPUTUM COLLECTION;



- 2. IN CASE OF NO OPENING PLACE, CHOOSE A VENTILATED ENVIRONMENT IN WHICH THE PATIENT CAN REMAIN ALONE;
- 3. ASK THE PATIENT TO SIT TO BEGIN THE COLLECTION. THE PATIENT MUST REMAIN SITTEN THOROUGHOUT THE COLLECTION PROCEDURE;
- 4. KEEP A TABLE NEAR THE PATIENT TO SUPPORT THE COLLECTION VIAL;

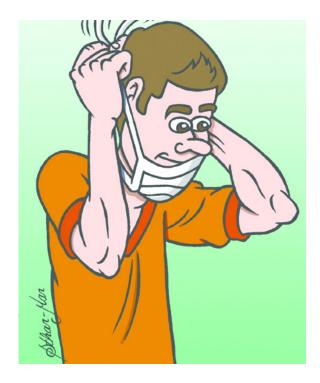


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- 5. PUT THE COLLECTION VIAL IN AN EASY AND ACCESSIBLE PLACE TO THE PATIENT;
- 6. PUT A FILTERED WATER BOTTLE AND A CUP OVER THE TABLE IN ORDER THE PATIENT CAN DRINK DURING THE COLLECTION PROCEDURES and



7. ASK THE PATIENT TO TAKE THE WHITE SURGICAL MASK OFF THE FACE;



SIMULATION OF THE PROCEDURES FOR SPUTUM COLLECTION

THE HEALTH CARE WORKER, PROPERLY WORN WITH N95 MASK, SHOULD EXPLAIN THE PROCEDURES TO COLLECT THE SAMPLE OF SPONTANEOUS SPUTUM TO THE PATIENT IN FEW WORDS USING A SIMPLE AND OBJECTIVE LANGUAGE. SIMULATE THE PROCEDURES TO MAKE EASIER THE UNDERSTANDING BY THE PATIENT:

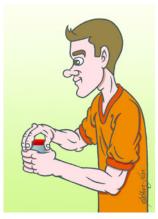
- 1. EXPLAIN THAT THE SPUTUM IS A FLUID WHICH COMES FROM THE LUNGS, AFTER COUGHING. THEREFORE, PATIENT'S COLLABORATION TO COUGH IS INDISPENSABLE;
- 2. ADVISE THE PATIENT TO:
- a) BREATH DEEPLY, KEEP THE AIR INSIDE THE LUNGS FOR SOME SECONDS and, LATELY, EXPIRATE;



b) HESE PROCEDURES SHOULD BE REPEATED THREE TIMES and

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- c) IMMEDIATELY AFTER, STIMULATE THE PATIENT TO COUGH;
- d) WHILE COUGHING, THE PATIENT SHOULD UN-TWIST THE COLLECTION VIAL TAP, OPEN THE VIAL, CURVE THE HEAD OVER THE VIAL, WITHOUT TOUCHING THE LIPS, CHIN or CHEEKS IN ANY AREA OF THE VIAL OR OF ITS TAP AND EXPECTORATE INSIDE THE VIAL:







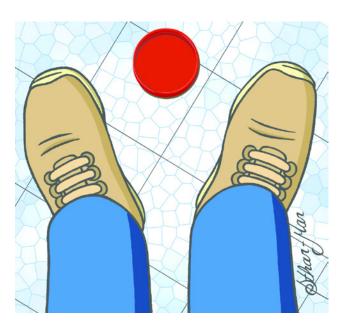
e) DO NOT TOUCH THE FINGERS IN THE INTERNAL PART OF THE VIAL:



f) AT THE END OF EACH EXPECTORATION, ADVISE THE PATIENT TO CLOSE THE VIAL COMPLETELY TWISTING THE TAP;



3. ALERT THE PATIENT TO AVOID THE VIAL TAP FALLING ON THE FLOOR. IN CASE IT HAPPENS, THE PATIENT SHOULD ASK THE SUBSTITUTION OF THE VIAL TAP TO THE HEALTH CARE WORKER;



- 4. THE IMPORTANCE OF COLLECTING 10 ml MUST BE EXPLAINED TO THE PATIENT; POINT OUT THAT THE FOAM WILL NOT BE REGARDED TO REACH THIS VOLUME;
- 5. THERE IS NO TIME LIMITS FOR SPUTUM COLLECTION;
- 6. THE COLLECTION PROCEDURE WILL BE ENDED WHEN THE VOLUME of 10 ml IS REACHED;
- 7. ADVISE THE PATIENT TO REPEAT THESE PROCEDURES AS MANY TIMES AS NECESSARY UNTIL REACHING THE 10 ml LINE IN THE VIAL WALL and
- 8. IN CASE THE PATIENT IS NOT ABLE TO EXPECTORATE 10 ml, THE HEALTH CARE WORKER SHOULD STIMULATE THE PATIENT TO EXPECTORATE AS MUCH AS POSSIBLE; AT LEAST 5 ml.

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SPUTUM COLLECTION

THE HEALTH CARE WORKER, PROPERLY WORN WITH N95 MASK, SHOULD:

- 1. DELIVER A PROPERLY IDENTIFIED COLLECTION VIAL WITH THE 10 ml LINE POINTED OUT TO MAKE THE OBSERVATION OF THE VOLUME RECOMMENDED EASIER BY THE PATIENT;
- 2. DELIVER A DISPOSABLE PAPER TOWEL TO REMOVE EXPECTORATION IN CASE IT REMAINS OVER THE LIPS. IT IS FORBIDDEN TO REMOVE THE EXPECTORATION WITH THE VIAL DUE TO THE RISK OF SAMPLE CONTAMINATION;



- **3.** OBSERVE THE FIRST EXPECTORATIONS AND THE PROCEDURES THE PATIENT ADOPTS TO EXPECTORATE INSIDE THE VIAL. CORRECT THE PATIENT WHENEVER NECESSARY;
- 4. SUPERVISE THE COLLECTION AS MUCH AS POSSIBLE TO EVALUATE IF THE INSTRUCTIONS ARE BEING RESPECTED. DURING THE SUPERVISIONS, STIMULATE THE PATIENT TO CONTINUE THE COLLECTION UNTIL REACHING THE VOLUME OF 10 ml;
- 5. IN CASE OF DIFFICULTIES, ASSIST THE PATIENT DURING THE COLLECTION PROCEDURES UNTIL HE IS ABLE TO PERFORM ALL THE PROCEDURES BY HIMSELF:
- 6. IN CASE THE EXPECTORATION FALLS OVER THE EXTERNAL WALL OF THE VIAL, THE HEALTH CARE WORKER SHOULD IMMEDIATELY REMOVE THAT USING PAPER TOWEL AND DESCONTAMINATE WITH PHENOL SOLUTION 5%;
- 7. THE COLLECTION WILL BE FINISHED WHEN THE VOLUME OF 10 ml IS REACHED;

- 8. WAIT THE TIME THAT YOU CONSIDER NECESSARY TO THE PATIENT COLLECT 10 ml OF SPUTUM OR THE MAXIMAL VOLUME NEAR THIS VALUE and
- 9. IN CASE THE PATIENT IS NOT ABLE TO EXPECTORATE 10 ml OF SPUTUM, THE HEALTH CARE WORKER SHOULD STIMULATE THE PATIENT TO EXPECTORATE THE MAXIMAL VOLUME; AT LEAST 5 ml.



STORE OF THE SPUTUM VIAL IN THE REFRIGERATOR UNTIL ITS TRANSPORT TO THE LABORATORY

AT THE END OF THE COLLECTION, THE HEALTH CARE WORKER, PROPERLY WORN WITH N95 MASK, SHOULD:

- 1. PUT THE SPUTUM VIAL INSIDE A TRANSPARENT PLASTIC BAG AND CLOSE THE BAG WITH A KNOT and
- 2. STORE THE VIAL INSIDE THE REFRIGERATOR RESERVED FOR CONTAMINATED MATERIAL UNDER 2-8oC TEMPERATURE UNTIL ITS TRANSPORT TO THE LABORATORY;

ATTENTION: IF THE HEALTH CARE WORKER CONSIDERS THAT THE PATIENT IS ABLE TO RESPECT ALL THE RECOMMENDATIONS ABOUT THE COLLECTION IN THE ABSENCE OF ANY SUPERVISION, THE NEXT COLLECTION SHOULD BE PERFORMED AT THE PATIENT'S HOUSE. IF NO, THE COLLECTION SHOULD BE PERFORMED AT THE LOCAL CLINICS OR AT ANYOTHER REFERENCE HEALTH SERVICE.





STEP-BY-STEP GUIDELINES FOR COLLECTION OF THE SPONTANEOUS SPUTUM SAMPLE AT THE PATIENT'S HOUSE FOR CONFIRMATION OF THE DIAGNOSIS OF PULMONARY TUBERCULOSIS, PULMONARY DISEASE BY NON-TUBERCULOUS MYCOBACTERIA OR FOR TREATMENT FOLLOW-UP OF ANY OF THESE DISEASES

RECOMMENDATIONS OF FAST AND WATER INGESTION PRIOR TO COLLECTION

AT THE DATE PRIOR TO SPUTUM COLLECTION, THE HEALTH CARE WORKER, PROPERLY WORN WITH N95 MASK, SHOULD:

- 1. RECOMMEND TO THE PATIENT REMAIN ON FAST OF AT LEAST 6 h and
- 2. RECOMMEND TO THE PATIENT TO INGEST WATER AS MUCH AS POSSIBLE DURING THE NIGHT PRIOR TO THE COLLECTION DATE. WATER INGESTION IS ALLOWED DURING THE FAST PERIOD.

VIAL PREPARATION

THE HEALTH CARE WORKER SHOULD:

1. WASH THE HANDS PRIOR TO THE VIAL PREPARATION;



2. CHOOSE A STERILE AND SCALED VIAL WITH TRANSPARENT WALLS, MINIMAL AND MAXIMAL

VOLUMES OF 35 ml and 50 ml, respectively, MAXIMAL HEIGHT of 40 mm and TWISTING TAP with a DIAMETER of 50 mm, according to the illustration below;



Boca larga= large mouth; tampa rosqueável= twisting tap; plástico transparente= transparent plastic, capacidade de 35 ml a 50 ml= minimal and maximal volumes of 35 ml and 50 ml, respectively; pote descartável= disposable vial; altura mínima= minimal height; etiqueta do frasco – Nome completo= full name, data=date: xx/yy/zz

3. OPEN THE PLASTIC BAG OF THE COLLECTION VIAL, TAKE THE VIAL OFF WITHOUT UNTWISTING THE TAP AND DISCARD THAT IN THE COMMON TRASH;



4. RECORD THE PATIENT NAME AND THE COLLECTION DATE IN A LABEL;

5. FIX THE LABEL ON THE EXTERNAL WALL OF THE **COLLECTION VIAL;**



6. DO NOT FIX THE LABEL OVER THE VOLUME SCALE OR OVER THE TAP;



7. 10 ml LINE MUST BE POINTED OUT WITH BLACK RETO-PROJECTOR PEN TO MAKE EASIER THE OBSERVATION OF THE LEVEL TO BE REACHED WITH THE SPUTUM. ATTENTION: FOAM WILL NOT BE REGARDED.

- 8. DELIVER THE COLLECTION VIAL TO THE PATIENT TAKE THAT TO HIS RESIDENCE, PROPERLY IDENTIFIED AND WITH THE 10 ml LINE POINTED OUT and
- 9. IN ADDITION, DELIVER A TRANSPARENT PLASTIC BAG IN WHICH, THE SPUTUM VIAL WILL BE PUT AT THE END OF THE COLLECTION.

HAND WASHING, HYGIENE OF THE TEEH AND ORAL CAVITY

THE HEALTH CARE WORKER, PROPERLY WORN WITH N95 MASK, SHOULD:

- 1. IN THE MORNING OF THE COLLECTION DATE. ADVISE THE PATIENT TO GO TO THE BATHROOM FOR THE FOLLOWING PROCEDURES;
- a) WASH THE HANDS WITH WATER AND SOAP, LATER ON, DRY THEM with PAPER TOWEL;





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b) REMOVE ANY TOOTH PROTHESIS;



d) RINSE THE MOUTH AND GARGLE WITH FILTERED WATER;



- e) IN THE MORNING OF THE COLLECTION DATE, ADVISE THE PATIENT TO NOT MAKE ORAL HYGIENE WITH TOOTH CREAM or SOLUTIONS FOR RINSING/ **GARGLING** and
- f) ATTENTION: INFORM THAT TOOTH BRUSHING, RINSING AND GARGLING WITH WATER ARE ENOU-GH AND INDISPENSABLE.

ENVIRONMENTAL PREPARATION FOR SPUTUM COLLECTION

AT HIS HOUSE, THE PATIENT SHOULD:

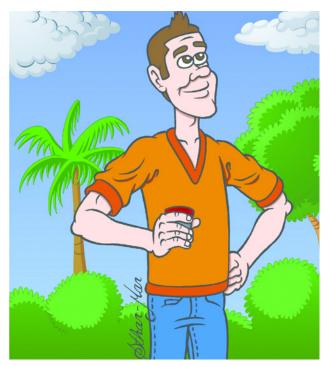
1. CHOOSE AN OPENING PLACE AT HIS RESIDENCE FOR SPUTUM COLLECTION;





c) BRUSH THE TEETH WITH TOOTH BRUSH DAMPENED WITH FILTERED WATER TO REMOVE ANY FOOD RESIDUE OF THE TEETH, GINGIVAS and TONGUE;





2. IN CASE OF NO OPENING PLACE, CHOOSE A VENTILATED ENVIRONMENT IN WHICH THE PATIENT CAN REMAIN ALONE;

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- 3. KEEP A TABLE NEAR THE PATIENT TO SUPPORT THE VIAL;
- 4. PUT THE VIAL IN AN EASY AND ACCESSIBLE PLACE OVER THE TABLE;
- 5. PUT A FILTERED WATER BOTTLE AND A CUP OVER THE TABLE IN ORDER THE PATIENT CAN DRINK DURING THE COLLECTION PROCEDURES;



6. ADVISE THE PATIENT TO SIT IN ORDER TO BEGIN THE COLLECTION PROCEDURES. THE PATIENT MUST REMAIN SITTEN DURING ALL THE COLLECTION PROCEDURE.

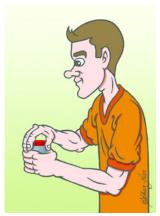
SIMULATION OF THE PROCEDURES FOR SPUTUM COLLECTION

THE HEALTH CARE WORKER, **PROPERLY** WORN WITH N95 MASK, SHOULD EXPLAIN TO THE PATIENT IN FEW WORDS USING A SIMPLE AND OBJECTIVE LANGUAGE THE PROCEDURES TO COLLECT THE SAMPLE OF SPONTANEOUS SPUTUM. SIMULATE THE PROCEDURES TO MAKE EASIER THE UNDERSTANDING BY THE PATIENT:

- 1. EXPLAIN THAT THE SPUTUM IS A FLUID WHICH COMES FROM THE LUNGS, AFTER COUGHING. THEREFORE, PATIENT'S COLLABORATION TO COUGH IS INDISPENSABLE;
- 2. ADVISE THE PATIENT TO:
- a) BREATH DEEPLY, KEEP THE AIR INSIDE THE LUNGS FOR SOME SECONDS and, LATELY, EXPIRATE;



- b) THESE PROCEDURES SHOULD BE REPEATED THREE TIMES;
- c) IMMEDIATELY AFTER, STIMULATE THE PATIENT TO COUGH:
- d) WHILE COUGHING, THE PATIENT SHOULD UN-TWIST THE COLLECTION VIAL TAP, OPEN THE VIAL and CURVE THE HEAD OVER THE VIAL, WITHOUT TOUCHING THE LIPS, CHIN or CHEEKS IN ANY AREA OF THE VIAL OR IN ITS TAP;







g) IN CASE THE EXPECTORATION FALLS OVER THE EXTERNAL WALL OF THE TAP, THE PATIENT SHOULD IMMEDIATELY REMOVE THAT USING HYGIENIC PAPER;



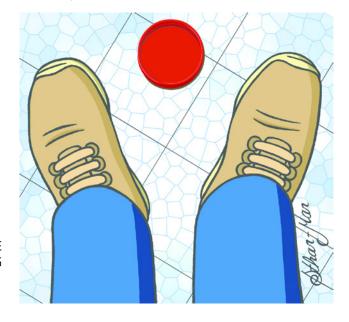
e) DO NOT TOUCH THE FINGERS IN THE INTERNAL PART OF THE VIAL;



3. ALERT THE PATIENT TO AVOID THE VIAL TAP FALLING ON THE FLOOR. IN CASE IT HAPPENS, THE PATIENT SHOULD COMMUNICATE TO THE HEALTH CARE WORKER THE EVENT AT THE MOMENT OF DELIVERING THE VIAL;



f) AT THE END OF EACH EXPECTORATION, ADVISE THE PATIENT TO CLOSE THE VIAL COMPLETELY TWISTING THE TAP;



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- 4. THE IMPORTANCE OF COLLECTING 10 ml MUST BE EXPLAINED TO THE PATIENT; POINT OUT THAT THE FOAM WILL NOT BE REGARDED TO REACH THIS VOLUME:
- 5. THERE IS NO TIME LIMITS FOR SPUTUM COLLECTION;
- 6. ADVISE THE PATIENT TO REPEAT THESE PROCEDURES AS MANY TIMES AS NECESSARY UNTIL REACHING THE 10 ml LINE IN THE VIAL WALL:
- 7. IN CASE THE PATIENT IS NOT ABLE TO EXPECTORATE 10 ml, THE HEALTH CARE WORKER SHOULD STIMULATE THE PATIENT TO EXPECTORATE THE MAXIMAL VOLUME; AT LEAST 5 ml and
- 8. THE COLLECTION PROCEDURE WILL BE ENDED WHEN THE VOLUME of 10 ml IS REACHED or THE MAXIMAL VOLUME NEAR THIS VALUE.

TRANSPORTATION OF THE VIAL UNTIL THE HEALTH SERVICE or LABORATORY

AT THE PATIENT'S HOUSE, AFTER THE END OF THE COLLECTION PROCEDURES, THE PATIENT SHOULD:

1. PUT THE SPUTUM VIAL INSIDE A TRANSPARENT PLASTIC BAG AND CLOSE THE BAG WITH A KNOT and



2. TRANSPORT THIS VIAL UNTIL THE LOCAL CLINICS or LABORATORY PROTECTED OF THE SUNLIGHT. THIS TRANSPORTATION SHOULD BE MADE IN LESS THAN 2 h;



DELIVERY OF THE VIAL AT THE LOCAL CLINICS

PRIOR TO THE PATIENT GETS INTO THE LOCAL CLINICS, THE HEALTH CARE WORKER, PROPERLY WORN WITH N95 MASK, SHOULD:

- 1. WELLCOME THE PATIENT;
- 2. DELIVER THE WHITE SURGICAL MASK TO THE PATIENT AND ASK HIM TO FIX THAT ON HIS FACE;
- 3. CERTIFY THAT THE PATIENT RESPECTED THE FAST OF AT LEAST 6 h PRIOR TO THE SPUTUM COLLECTION;
- 4. CERTIFY THAT THE PATIENT PERFORMED ORAL HYGIENE AND TOOTH BRUSHING IMMEDIATELY BEFORE THE BEGINNING OF THE COLLECTION AT HIS HOUSE; CERTIFY THAT TOOTH CREAM or ANTI-SEPTIC SOLUTIONS WERE NOT USED and
- 5. STORE THE VIAL INSIDE THE REFRIGERATOR RESERVED FOR CONTAMINATED MATERIAL UNDER 2-8°C TEMPERATURE UNTIL ITS TRANSPORT TO THE LABORATORY.



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STEP-BY-STEP GUIDELINES FOR COLLECTION OF THE SPONTANEOUS SPUTUM SAMPLE AT THE LOCAL CLINICS OR AT THE HOSPITAL OF OUT-PATIENTS WHOSE COLLECTIONS WERE NOT SATISFACTORY AT THE PATIENT'S HOUSE. THE OBJECTIVE OF THIS COLLECTION IS TO CONFIRM THE DIAGNOSIS OF PULMONARY TUBERCULOSIS, PULMONARY DISEASE BY NON-TUBERCULOUS MYCOBACTERIA OR FOR TREATMENT FOLLOW-UP OF ANY OF THESE DISEASES

RECOMMENDATIONS OF FAST AND WATER INGESTION PRIOR TO COLLECTION

AT THE DATE PRIOR TO SPUTUM COLLECTION, THE HEALTH CARE WORKER, PROPERLY WORN WITH N95 MASK, SHOULD:

- 1. ADVISE TO THE PATIENT TO ARRIVE AT THE PLACE AT TIME AND DATE SCHEDULED;
- 2. RECOMMEND TO THE PATIENT REMAIN ON FAST OF AT LEAST 6 h;
- 3. RECOMMEND TO THE PATIENT TO INGEST WATER AS MUCH AS POSSIBLE DURING THE NIGHT PRIOR TO THE COLLECTION DATE. WATER INGESTION IS ALLOWED DURING THE FAST PERIOD;
- 4. CERTIFY THAT THE PATIENT KNOWS THE ADDRESS AND THE WAY TO ARRIVE AT THE PLACE;
- 5. IN THE MORNING OF THE COLLECTION DATE, ADVISE THE PATIENT NOT TO MAKE ORAL HYGIENE WITH TOOTH CREAM;
- **6.** INFORM THAT TOOTH BRUSHING, RINSING THE MOUTH and GARGLING WITH WATER ARE ENOUGH AND INDISPENSABLE. THEY WILL BE PERFORMED AT THE PLACE OF THE COLLECTON AND NOT AT HIS HOUSE.

VIAL PREPARATION

THE HEALTH CARE WORKER SHOULD:

1. WASH THE HANDS PRIOR TO THE VIAL PREPARATION;



2. CHOOSE A STERILE AND SCALED VIAL WITH TRANSPARENT WALLS, MINIMAL AND MAXIMAL VOLUMES OF 35 ml and 50 ml, respectively, MAXIMAL HEIGHT of 40 mm and TWISTING TAP with a DIAMETER of 50 mm, according to the illustration below;



Boca larga= large mouth; tampa rosqueável= twisting tap; plástico transparente= transparent plastic, capacidade de 35 ml a 50 ml= minimal and maximal volumes of 35 ml and 50 ml, respectively; pote descartável= disposable vial; altura mínima= minimal height; etiqueta do frasco – Nome completo= full name, data=date: xx/yy/zz

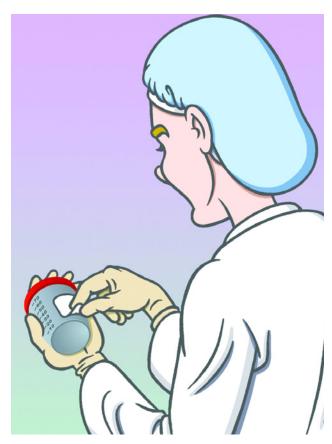
- 3. OPEN THE PLASTIC BAG OF THE COLLECTION VIAL TAKE THE VIAL OFF WITHOUT UNTWISTING THE TAP and DISCARD THAT IN THE COMMON TRASH;
- 4. RECORD THE PATIENT NAME AND THE COLLECTION DATE IN A LABEL;



5. FIX THE LABEL ON THE EXTERNAL WALL OF THE COLLECTION VIAL;



7. 10 ml LINE MUST BE POINTED OUT WITH BLACK RETO-PROJECTOR PEN TO MAKE EASIER THE OBSERVATION OF THE LEVEL TO BE REACHED WITH THE SPUTUM.



6. DO NOT FIX THE LABEL OVER THE VOLUME SCALE OR OVER THE TAP;

HYGIENE OF THE TEEH AND ORAL CAVITY

BEFORE THE PATIENT GETS INTO THE PLACE, THE HEALTH CARE WORKER, PROPERLY WORN WITH N95 MASK, SHOULD:

- 8. WELLCOME THE PATIENT;
- 9. DELIVER THE WHITE SURGICAL MASK TO THE PATIENT AND ADVISE HIM WHO TO FIX THAT ON HIS FACE;



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- 10. EXPLAIN TO THE PATIENT THAT THIS MASK SHOULD BE FIXED OVER HIS FACE UNTIL THE PLACE OF THE COLLECTION PROCEDURE or IN CASE THE PATIENT MOVES TO OTHER PLACES (e.g.: GO TO THE BATHROOM or TO THE RADIOLOGY SERVICE);
- 11. CERTIFY THAT THE PATIENT RESPECTED FAST OF AT LEAST 6 h PRIOR TO THE SPUTUM COLLECTION;
- 12. CERTIFY THAT THE PATIENT DID NOT PERFORM ORAL HYGIENE AND TOOTH BRUSHING AT HIS HOUSE;
- 13. ASK THE PATIENT TO GO TO THE BATHROOM FOR THE FOLLOWING PROCEDURES:
- a) TAKE THE WHITE SURGICAL MASK OFF THE FACE;



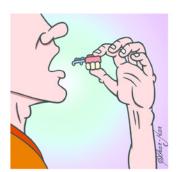
b) WASH THE HANDS WITH WATER AND SOAP and, LATER ON, DRY THEM WITH PAPER TOWEL;





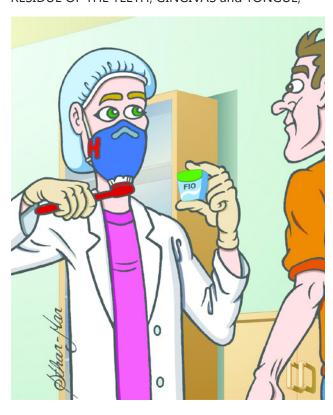
c) REMOVE ANY TOOTH PROTHESIS;



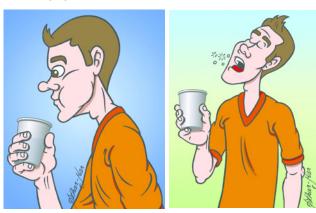


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d) BRUSH THE TEETH WITH TOOTH BRUSH DAMPENED WITH FILTERED WATER TO REMOVE ANY FOOD RESIDUE OF THE TEETH, GINGIVAS and TONGUE;



e) RINSE THE MOUTH and GARGLE WITH FILTERED WATER and



f) FIX AGAIN THE WHITE SURGICAL MASK OVER THE FACE.



ENVIRONMENTAL PREPARATION FOR SPUTUM COLLECTION

THE HEALTH CARE WORKER, PROPERLY WORN WITH N95 MASK, SHOULD:

1. ASK THE PATIENT TO SIT TO BEGIN THE COLLECTION;



2. KEEP A TABLE NEAR THE PATIENT TO SUPPORT THE COLLECTION VIAL;



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- 3. PUT THE COLLECTION VIAL IN AN EASY AND ACCESSIBLE PLACE TO THE PATIENT;
- 4. DELIVER DISPOSABLE PAPER TOWEL TO REMOVE EXPECTORATION IN CASE IT REMAINS OVER THE LIPS. IT IS FORBIDDEN TO REMOVE THE EXPECTORATION WITH THE VIAL DUE TO THE RISK OF SAMPLE CONTAMINATION;



- **5.** PUT A FILTERED WATER BOTTLE AND A CUP OVER THE TABLE IN ORDER THE PATIENT CAN DRINK DURING THE COLLECTION PROCEDURES and
- **6.** ASK THE PATIENT TO TAKE THE WHITE SURGICAL MASK OFF THE FACE AND PUT THAT IN ANY EASY AND ACCESSIBLE PLACE.

SIMULATION OF THE PROCEDURES FOR SPUTUM COLLECTION

THE HEALTH CARE WORKER, PROPERLY WORN WITH N95 MASK, SHOULD EXPLAIN TO THE PATIENT IN FEW WORDS USING A SIMPLE AND OBJECTIVE LANGUAGE THE PROCEDURES TO COLLECT THE SAMPLE OF SPONTANEOUS SPUTUM. SIMULATE THE PROCEDURES TO MAKE EASIER THE UNDERSTANDING BY THE PATIENT:

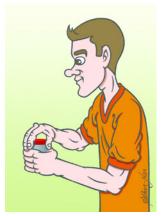
- 1. INFORM TO THE PATIENT THE IMPORTANCE OF THE COLLECTION PROCEDURE FOR TUBERCULOSIS DIAGNOSIS;
- 2. EXPLAIN THAT THE SPUTUM IS A FLUID WHICH COMES FROM THE LUNGS, AFTER COUGHING.

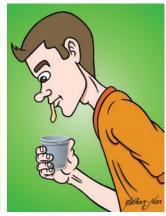
THEREFORE, PATIENT'S COLLABORATION TO COUGH IS INDISPENSABLE;

- 3. ADVISE THE PATIENT TO:
- a) BREATH DEEPLY, KEEP THE AIR INSIDE THE LUNGS FOR SOME SECONDS and, LATELY, EXPIRATE;



- b) THESE PROCEDURES SHOULD BE REPEATED THREE TIMES and
- c) IMMEDIATELY AFTER, STIMULATE THE PATIENT TO COUGH;
- d) WHILE COUGHING, THE PATIENT SHOULD UN-TWIST THE COLLECTION VIAL TAP, OPEN THE VIAL, CURVE THE HEAD OVER THE VIAL, WITHOUT TOUCHING THE LIPS, CHIN or CHEEKS IN ANY AREA OF THE VIAL OR OF ITS TAP and EXPECTORATE INSIDE THE VIAL;





e) DO NOT TOUCH THE FINGERS IN THE INTERNAL PART OF THE VIAL;

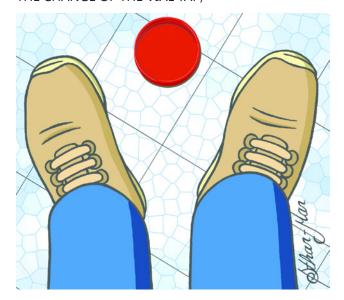
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f) AT THE END OF EACH EXPECTORATION, ADVISE THE PATIENT TO CLOSE THE VIAL COMPLETELY TWISTING THE TAP;



4. ALERT THE PATIENT TO AVOID THE VIAL TAP FALLING ON THE FLOOR. IN CASE IT HAPPENS, THE PATIENT SHOULD ASK TO THE HEALTH CARE WORKER THE CHANGE OF THE VIAL TAP;



- 5. OBSERVE THE FIRST EXPECTORATIONS AND THE PROCEDURES THE PATIENT ADOPTS TO EXPECTOR ATE INSIDE THE VIAL. CORRECT THE PATIENT WHENEVER NECESSARY;
- 6. SUPERVISE THE COLLECTION AS MANY TIMES AS POSSIBLE TO EVALUATE IF THE INSTRUCTIONS ARE BEING RESPECTED. DURING THE SUPERVISIONS, STIMULATE THE PATIENT TO CONTINUE THE COLLECTION UNTIL THE VOLUME OF 10 ml;
- 7. IN CASE OF DIFFICULTIES, ASSIST THE PATIENT DURING THE COLLECTION PROCEDURES UNTIL HE IS ABLE TO PERFORM ALL THE PROCEDURES BY HIMSELF;
- 8. IN CASE THE EXPECTORATION FALLS OVER THE EXTERNAL WALL OF THE VIAL, THE HEALTH CARE WORKER SHOULD IMMEDIATELY REMOVE THAT USING PAPER TOWEL AND DECONTAMINATE WITH PHENOL SOLUTION 5%:



- 9. THE IMPORTANCE OF COLLECTING 10 ml MUST BE EXPLAINED TO THE PATIENT; POINT OUT THAT THE FOAM WILL NOT BE REGARDED TO REACH THIS VOLUME;
- 10. THERE IS NO TIME LIMITS FOR SPUTUM COLLECTION; WAIT THE TIME THAT YOU CONSIDER NECESSARY TO COLLECT 10 ml OF SPUTUM OR ANY **VOLUME NEAR THIS VALUE;**
- 11. ADVISE THE PATIENT TO REPEAT THESE PROCEDURES AS MANY TIMES AS NECESSARY UNTIL REACHING THE MARK IN THE VIAL WALL;
- 12. IN CASE THE PATIENT IS NOT ABLE TO EXPECTORATE 10 ml, THE HEALTH CARE WORKER SHOULD STIMULATE THE PATIENT TO EXPECTORATE MAXIMAL VOLUME NEAR THIS VALUE; AT LEAST 5 ml and
- 13. THE COLLECTION PROCEDURE WILL BE ENDED WHEN THE VOLUME of 10 ml IS REACHED or THE POSSIBLE MAXIMAL VOLUME.

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TRANSPORTATION OF THE VIAL TO THE FREEZER

THE HEALTH CARE WORKER, PROPERLY WORN WITH N95 MASK, SHOULD:

- 1. ONCE THE COLLECTION PROCEDURE IS CONSIDERED ENDED BY THE HEALTH CARE WORKER, HE MUST PUT THE VIAL CLOSED INSIDE A TRANSPARENT PLASTIC BAG AND CLOSE THIS BAG WITH A KNOT and
- 2. STORE THE VIAL INSIDE THE REFRIGERATOR RESERVED FOR CONTAMINATED MATERIAL UNDER 2-8°C TEMPERATURE UNTIL ITS TRANSPORT TO THE LABORATORY.



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STEP-BY-STEP GUIDELINES FOR COLLECTION OF THE SPONTANEOUS SPUTUM SAMPLE OF IN-PATIENTS. THE OBJECTIVE OF THIS COLLECTION IS TO CONFIRM THE DIAGNOSIS OF PULMONARY TUBERCULOSIS, PULMONARY DISEASE BY NON-TUBERCULOUS MYCOBACTERIA OR FOR TREATMENT FOLLOW-UP OF ANY OF THESE DISEASES

RECOMMENDATIONS OF FAST AND WATER INGESTION PRIOR TO COLLECTION

AT THE NIGHT PRIOR TO THE DATE OF SPUTUM COLLECTION, THE HEALTH CARE WORKER, PROPERLY WORN WITH N95 MASK, SHOULD:

- 1. COMMUNICATE TO THE PATIENT THAT A SPUTUM SAMPLE WILL BE COLLECTED IN THE MORNING OF THE NEXT DAY;
- 2. RECOMMEND TO THE PATIENT REMAIN ON FAST OF AT LEAST 6 h;
- 3. RECOMMEND TO THE PATIENT TO INGEST WATER AS MUCH AS POSSIBLE DURING THE NIGHT PRIOR TO THE COLLECTION DATE. WATER INGESTION IS ALLOWED DURING THE FAST PERIOD;
- 4. ADVISE THE PATIENT TO NOT MAKE ORAL HYGIENE WITH TOOTH CREAM or SOLUTIONS for RINSING THE MOUTH/GARGLING IN THE MORNING OF THE COLLECTION DATE and
- 5. INFORM THAT TOOTH BRUSHING, RINSING THE MOUTH and GARGLING WITH WATER ARE ENOUGH AND INDISPENSABLE.

VIAL PREPARATION

THE HEALTH CARE WORKER SHOULD:

- 1. WASH THE HANDS PRIOR TO VIAL PREPARATION;
- 2. CHOOSE A STERILE AND SCALED VIAL WITH TRANSPARENT WALLS, MINIMAL AND MAXIMAL VOLUMES OF 35 ml and 50 ml, respectively, MAXIMAL HEIGHT of 40 mm and TWISTING TAP with a DIAMETER of 50 mm, according to the illustration below;





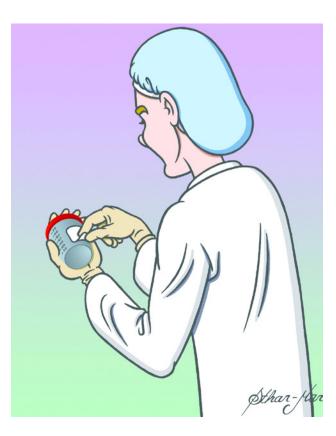
Boca larga= large mouth; tampa rosqueável= twisting tap; plástico transparente= transparent plastic, capacidade de 35 ml a 50 ml= minimal and maximal volumes of 35 ml and 50 ml, respectively; pote descartável= disposable vial; altura mínima= minimal height; etiqueta do frasco – Nome completo= full name, data=date: xx/yy/zz

3. OPEN THE PLASTIC BAG OF THE COLLECTION VIAL, TAKE THE VIAL OFF WITHOUT UNTWISTING THE TAP and DISCARD THAT IN THE COMMON TRASH;



- 4. RECORD THE PATIENT NAME AND THE COLLECTION DATE IN A LABEL;
- 5. FIX THE LABEL ON THE EXTERNAL WALL OF THE COLLECTION VIAL;

7. 10 ml LINE MUST BE POINTED OUT WITH BLACK RETO-PROJECTOR PEN TO MAKE EASIER THE OBSER-VATION OF THE LEVEL TO BE REACHED WITH THE SPUTUM.



6. DO NOT FIX THE LABEL OVER THE VOLUME SCA-LE OR OVER THE TAP;



HAND WASHING, HYGIENE OF THE TEEH AND ORAL CAVITY

IN THE MORNING OF THE COLLECTION DATE. THE HEALTH CARE WORKER, PROPERLY WORN WITH N95 MASK, SHOULD:

- 1. ASK THE PATIENT TO GO TO THE BATHROOM FOR THE FOLLOWING PROCEDURES:
- a) WASH THE HANDS WITH WATER AND SOAP and, LATER ON, DRY THEM WITH PAPER TOWEL;





b) REMOVE ANY TOOTH PROTHESIS;







c) BRUSH THE TEETH WITH TOOTH BRUSH DAMPENED WITH FILTERED WATER TO REMOVE ANY FOOD RESIDUE OF THE TEETH, GINGIVAS and TONGUE and

d) RINSE THE MOUTH and GARGLE WITH FILTERED WATER:





ENVIRONMENTAL PREPARATION FOR SPUTUM COLLECTION

IN THE MORNING OF THE COLLECTION DAY, THE HEALTH CARE WORKER, PROPERLY WORN WITH N95 MASK, SHOULD:

1. ASK THE PATIENT TO SIT OR RAISE THE BED HEAD 90° TO BEGIN THE COLLECTION. HE/SHE MUST REMAIN SITTEN THOROUGHOUT THE COLLECTION PROCEDURE;



2. KEEP A TABLE NEAR THE PATIENT TO SUPPORT THE COLLECTION VIAL;



- 3. PUT THE COLLECTION VIAL IN AN EASY AND ACCESSIBLE PLACE TO THE PATIENT;
- **4.** PUT A FILTERED WATER BOTTLE AND A CUP OVER THE TABLE IN ORDER THE PATIENT CAN DRINK DURING THE COLLECTION PROCEDURES;

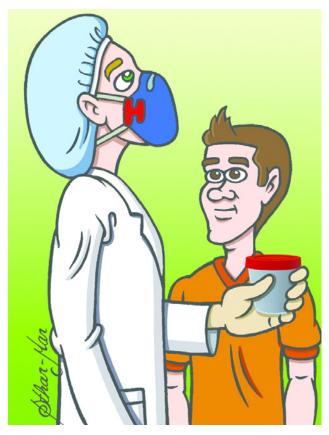


5. BEFORE DELIVERING THE COLLECTION VIAL, ASK THE PATIENT IF HE HAS ALREADY PERFORMED THE TOOTH BRUSHING AND THE RINSING WITH WATER. IF NOT, ASK HIM TO MAKE BOTH PROCEDURES. IF THE PATIENT IS RESTRICTED TO THE BEDROOM; DELIVER A TOOTH BRUSH WITH A WATER CUP FOR BOTH PROCEDURES.

SIMULATION OF THE PROCEDURES FOR SPUTUM COLLECTION

THE HEALTH CARE WORKER, PROPERLY WORN WITH N95 MASK, SHOULD EXPLAIN TO THE PATIENT IN FEW WORDS USING A SIMPLE AND OBJECTIVE LANGUAGE THE PROCEDURES TO COLLECT THE SAMPLE OF SPONTANEOUS SPUTUM. SIMULATE THE PROCEDURES TO MAKE EASIER THE UNDERSTANDING BY THE PATIENT:

- 1. EXPLAIN THAT THE SPUTUM IS A FLUID WHICH COMES FROM THE LUNGS, AFTER COUGHING. THE-REFORE, PATIENT'S COLLABORATION TO COUGH IS INDISPENSABLE;
- 2. ADVISE THE PATIENT TO:
- a) BREATH DEEPLY, KEEP THE AIR INSIDE THE LUNGS 4. DO NOT TOUCH THE FINGERS IN THE INTERNAL FOR SOME SECONDS and, LATELY, EXPIRATE;



- b) THESE PROCEDURES SHOULD BE REPEATED THREE TIMES and
- c) IMMEDIATELY AFTER, STIMULATE THE PATIENT TO COUGH.
- 3. WHILE COUGHING, THE PATIENT SHOULD UN-TWIST THE COLLECTION VIAL TAP, OPEN THE VIAL and CURVE THE HEAD OVER THE VIAL, WITHOUT TOUCHING THE LIPS, CHIN or CHEEKS IN ANY AREA OF THE VIAL OR OF ITS TAP;





PART OF THE VIAL;

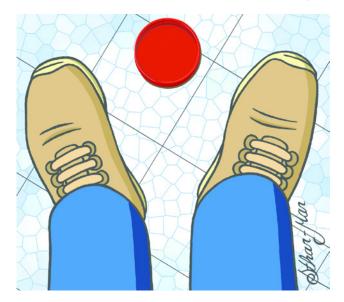
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- 5. EXPECTORATE INSIDE THE VIAL;
- **6.** AT THE END OF EACH EXPECTORATION, ADVISE THE PATIENT TO CLOSE THE VIAL COMPLETELY TWISTING THE TAP;



7. ALERT THE PATIENT TO AVOID THE VIAL TAP FALLING ON THE FLOOR. IN CASE IT HAPPENS, THE PATIENT SHOULD COMMUNICATE TO THE HEALTH CARE WORKER THAT THE VIAL TAP BE CHANGED;



- **8.** THE IMPORTANCE OF COLLECTING 10 ml MUST BE EXPLAINED TO THE PATIENT; POINT OUT THAT THE FOAM WILL NOT BE REGARDED TO REACH THIS VOLUME;
- 9. THERE IS NO TIME LIMITS FOR SPUTUM COLLECTION and
- 10. ADVISE THE PATIENT TO REPEAT THESE PROCEDURES AS MANY TIMES AS NECESSARY UNTIL REACHING THE 10 ml LINE IN THE VIAL WALL.

SUPERVISION OF THE SPUTUM COLLECTION (whenever possible)

IN THE MORNING OF THE COLLECTION DAY, THE HEALTH CARE WORKER, PROPERLY WORN WITH N95 MASK, SHOULD:

1. DELIVER A DISPOSABLE PAPER TOWEL TO REMOVE EXPECTORATION IN CASE IT REMAINS OVER THE LIPS. IT IS FORBIDDEN TO REMOVE THE EXPECTORATION WITH THE VIAL DUE TO THE RISK OF SAMPLE CONTAMINATION;



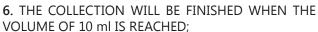
- 2. OBSERVE THE FIRST EXPECTORATIONS AND THE PROCEDURES THE PATIENT ADOPTS TO EXPECTORATE INSIDE THE VIAL. CORRECT THE PATIENT WHENEVER NECESSARY;
- 3. SUPERVISE THE COLLECTION AS MUCH AS POSSIBLE TO EVALUATE IF THE INSTRUCTIONS ARE BEING RESPECTED. DURING THE SUPERVISIONS, STIMULATE THE PATIENT TO CONTINUE THE COLLECTION UNTIL REACHING THE VOLUME OF 10 ml;

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- 4. IN CASE OF DIFFICULTIES, ASSIST THE PATIENT DURING THE COLLECTION PROCEDURES UNTIL HE IS ABLE TO PERFORM ALL THE PROCEDURES BY HIMSELF;
- 5. IN CASE THE EXPECTORATION FALLS OVER THE EXTERNAL WALL OF THE VIAL, THE HEALTH CARE WORKER SHOULD IMMEDIATELY REMOVE THAT USING PAPER TOWEL AND DECONTAMINATE WITH PHENOL SOLUTION 5%:





7. IN CASE THE PATIENT IS NOT ABLE TO EXPECTORATE 10 ml OF SPUTUM, THE HEALTH CARE WORKER SHOULD STIMULATE THE PATIENT TO EXPECTORATE THE MAXIMAL VOLUME, AT LEAST 5 ml and

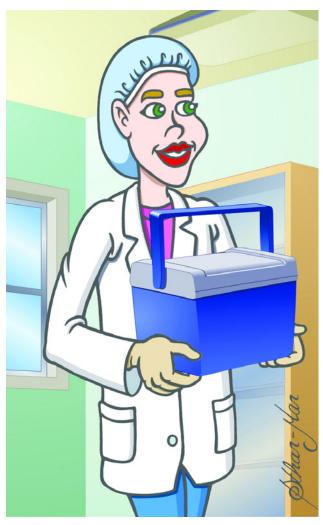
8. WAIT THE TIME THAT YOU CONSIDER NECESSARY TO THE PATIENT COLLECT 10 ml OF SPUTUM OR ANY VOLUME NEAR THIS VALUE.

TRANSPORTATION OF THE VIAL TO THE FREEZER

THE HEALTH CARE WORKER, PROPERLY WORN WITH N95 MASK, SHOULD:

- 1. PUT THE VIAL INTO A TRANSPARENT PLASTIC BAG AND CLOSE THIS BAG WITH A KNOT and
- 2. STORE THE VIAL INSIDE THE REFRIGERATOR RESERVED FOR CONTAMINATED MATERIAL UNDER 2-8°C TEMPERATURE UNTIL ITS TRANSPORT TO THE LABORATORY.





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IMPORTANT OBSERVATIONS

- 1. THE IMPORTANCE OF COLLECTING 10 ml MUST BE EXPLAINED TO THE PATIENT; POINT OUT THAT THE FOAM WILL NOT BE REGARDED TO REACH THIS VOLUME;
- 2. THERE IS NO TIME LIMIT FOR SPUTUM COLLECTION. THE NURSE SHOULD EVALUATE IF THE PATIENT IS ABLE TO EXPECTORATE; NOTE: THERE ARE FEW PATIENTS WHO REQUIRE MORE THAN 30 min TO EXPECTORATE 10 ml OF SPUTUM.
- 3. THE COLLECTION WILL BE CONSIDERED ENDED AFTER CERTIFICATION BY THE NURSE THAT VOLUME OF 10 mL OF SPUTUM WAS REACHED;
- 4. IF THE PATIENT IS NOT ABLE TO REACH 10 mL OF SPUTUM, THE NURSE SHOULD STIMULATE THE PATIENT TO COLLECT AT LEAST 5 ml or THE POSSIBLE MAXIMAL VOLUME;
- 5. DO NOT DISCARD VIALS WITH VOLUMES OF SPUTUM INFERIOR TO 5 ml;
- **6.** IN CASE IT IS IMPOSSIBLE TO COLLECT THE SPUTUM SAMPLE, THE VIAL MUST BE DISCARDED AND THE PATIENT ADVISED TO TRY THE COLLECTION IN A FUTURE DATE;
- 7. IN CASE OF BLOODY SPUTUM OR HEMOPTYSIS, IMMEDIATELY CALL THE PHYSICIAN TO EVALUATE THE INTERRUPTION OF THE COLLECTION PROCEDURE;
- **8.** IN CASE OF VOMITING DURING THE COLLECTION, STOP THE PROCEDURE. THE VIAL MUST BE DISCARDED;
- **9.** RECORD THE BEGINNING AND ENDING TIMES OF THE SPUTUM COLLECTION IN THE MEDICAL RECORD FOR BACILOSCOPY AND MYCOBACTERIAL CULTURE;
- 10. RECORD ANY PROTOCOL DEVIATION DURING THE COLLECTION PROCEDURE IN THE MEDICAL REQUIREMENT FOR BACILOSCOPY AND MYCOBACTERIAL CULTURE;
- 11. AT THE END OF THE COLLECTION, THE PATIENT SHOULD WASH HIS HANDS, FIX AGAIN THE MASK OVER HIS FACE IN ORDER TO LEAVE THE PLACE IN CASE HE IS NOT HOSPITALIZED;
- 12. THE HEALTH CARE WORKER, RESPONSIBLE FOR THE COLLECTION, SHOULD PUT THE COLLECTION VIAL INTO A TRANSPARENT PLASTIC BAG AND CLOSE THAT;
- 13. THE NURSE MUST CERTIFY THAT THE INFORMATIONS RECORDED IN THE VIAL WALL ARE CONCORDANT WITH THOSE RECORDED IN THE MEDICAL REQUIREMENT OF BACILOSCOPY AND MYCOBACTERIAL CULTURE;
- 14. STORE THE VIAL WITH THE SPUTUM SAMPLE INSIDE THE REFRIGERATOR RESERVED FOR CONTAMINATED MATERIAL UNDER 2-8°C TEMPERATURE UNTIL ITS TRANSPORTATION TO THE LABORATORY, IN CASE THE INTERVAL BETWEEN THE ENDING OF THE COLLECTION AND THE BEGINNING OF THE LABORATORY PROCEDURES IS SUPERIOR TO 2 h;
- **15.** PUT EACH SPUTUM VIAL INTO A WELL (TO AVOID AGITATION AS MUCH AS POSSIBLE) INSIDE A REFRIGERATED BOX (2-8°C);

- **16.** TRANSPORT THIS REFRIGERATED BOX CLOSED TO THE LABORATORY PROTECTED OF THE SUN LIGHT;
- 17. THE MEDICAL REQUIREMENTS MUST BE KEPT FAR AWAY OF THE PATIENT DURING ALL THE PROCEDURES TO AVOID ANY DIRTINESS CAUSED BY THE CONTACT WITH THE SPUTUM;
- 18. THE SPUTUM VIALS MUST BE TRANSPORTED TO THE LABORATORY PROTECTED OF THE SUNLIGHT AS QUICKLY AS POSSIBLE and
- 19. THE INTERVAL BETWEEN THE ENDING OF THE SPUTUM COLLECTION AND THE BEGINNING OF THE LABORATORY PROCEDURES WITH EACH SPUTUM SAMPLE SHOULD BE LESS THAN 2 h.

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