SUPPLEMENT No 2 1978

Bulletin of the INTERNATIONAL UNION AGAINST TUBERCULOSIS AND LUNG DISEASES

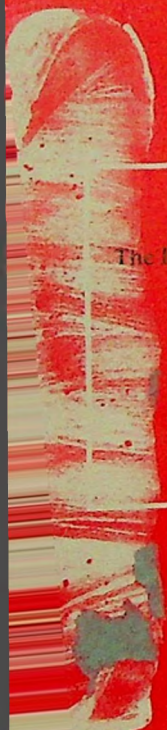
tuberculosis / respiratory disease / community health

TECHNICAL GUIDE FOR SPUTUM EXAMINATION FOR TUBERCULOSIS BY DIRECT MICROSCOPY

WHO EXPERT COMMITTEE ON TUBERCULOSIS

..."The object of tuberculosis control is to break the chain of transmission of infection. This can be achieved by detecting the sources of infection as early as possible and rendering them non-infectious by chemotherapy. Transmission is maintained in the community particularly by subjects whose sputum is so heavily positive that tubercle bacilli can be detected by smear microscopy"...

Abstract from page 14 of the Ninth report of the WHO Expert Committee on Tuberculosis (Technical Report Series, 1964, No. 272)



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1978 - 3rd edition (revised)
Reprinted 1986

BULLETIN OF THE INTERNATIONAL UNION
AGAINST TUBERCULOSIS AND LUNG DISEASES

... to disseminate knowledge and promote the continuing education of physicians and health personnel ...

SUPPLEMENT No 2 DECEMBER 1978

TECHNICAL GUIDE

FOR SPUTUM EXAMINATION FOR TUBERCULOSIS BY DIRECT MICROSCOPY

1978 - 3rd edition (revised)
Reprinted 1986

PREFACE

This Guide is based upon one initiated as early as 1969 by Dr. J. Holm (then Executive Director of the International Union Against Tuberculosis). It was felt that the auxiliary personnel, specially in developing countries, needed a simple guide for collection, storage and transport of sputum specimens and for examination for tuberculosis by direct microscopy.

This document, the third edition, has been carefully examined and revised by the members of the two IUAT Scientific Committees on Bacteriology/ Immunology and Diagnostic Methods ; account was also taken of suggestions made by other experienced authorities, as well as those of workers who have been using the Guide in the field.

The Guide is intended for field laboratories which may often have very limited facilities and personnel. It presents the basic general principles for collection, transportation, and examination by smear of sputum possibly containing tubercle bacilli.

While the Guide provides basic procedures for the detection of infectious tuberculous patients, it is recognized that local modifications of methods may be both desirable and appropriate.

To clarify all points of procedure or detail all possible modifications of methods would be prohibitive. It is anticipated that some users of this Guide, in consultation with colleagues, supervisors, and central laboratory personnel, may modify certain procedures to accommodate local facilities and equipment.

December 1978

Prof. V. Farga
Dr. Annik Rouillon

CONTENTS

I. COLLECTION OF SPUTUM SPECIMENS

Collection of sputum specimens will be made in peripheral health centres and in various types of outpatient clinics. The specimens will be sent to the laboratory for examination.

Special sputum containers are used. They must be rigid to avoid crushing in transit and possess a wide-mouthed screw top hermetically sealable to prevent dessication and to minimize contamination by leakage.

There are two acceptable types of container. One, available from UNICEF, is plastic with a black bottom, a translucent lid, and is readily destroyed by burning ; the patient's identification must be made on the container (not on the lid). The other is a heavy glass, screw-capped jar that may be re-used after disinfection by boiling (10 min.) and thorough cleaning.

A. Number of sputum specimens requested from each patient suspect of tuberculosis

Three specimens should be requested from each suspect patient ; for instance, one spot specimen when the patient presents himself at the clinic ; an early morning specimen consisting of all sputum raised within one or two hours after rising and a second spot specimen collected at the time the early morning sample is brought to the clinic.

B. Recording of sputum examinations

It is very important to keep a full and accurate register of all sputum examinations performed by the laboratory.

The information necessary for the precise identification of each sputum specimen studied must be accurately recorded. The system of registering may be, for instance, the following : on one line of the sputum register corresponding to the specimen, write the health centre's code letter and accession number, the patient's surname, given name, age, sex and address and the date the sputum specimen was collected (Annex I).

The health centre's code letter and the accession number are those listed on sputum container. It should be noted that the three specimens from the same patient will have different identification numbers.

C. Place for collecting the specimen at the health centre

The risk of infection is very great when the patient coughs, therefore specimens should be collected in the open air and as far away as possible from other people.

If conditions do not permit procuring the sputum out of doors, it is best to use a separate, well-ventilated room.

D. Technique for collection

A trained person must :

1) Give the patient confidence by explaining to him the reason for the examination, and also explain to him how to cough so that the expectoration will come from as deep down in the chest as possible.

2) Open the sputum container, keep the lid and give only the bottom part to the patient.

3) Stand behind the patient, and ask him to hold the sputum container close to his lips and spit into it.

4) Check the quality and the quantity of the sputum ; a specimen of sufficient volume (3 to 5 ml) containing solid or purulent particles and not just saliva, should be obtained. If the expectoration is insufficient, the technician should encourage the patient to cough again until a satisfactory result is obtained. It must be realized that many patients cannot produce sputum from deep in the respiratory tract in a few minutes, consequently sufficient time should be given the patient to produce expectoration which he himself feels is produced by a deep cough.

If there is no expectoration, the sputum container must be considered as used, and must be properly disposed of.

5) Close the sputum container securely and if it must be sent to the laboratory, put it into a special box for transport.

6) Wash hands with soap and water.

7) Give the patient a new sputum container and
– make quite sure that the patient has understood that he must spit into this container as soon as he coughs up sputum in the morning ;
– demonstrate how the container should be securely closed ;
– instruct him to bring it back to the health centre.

II. STORAGE AND TRANSPORT OF SPUTUM SPECIMENS

If the health centre does not make its own examinations for acid-fast bacilli, the collected sputum specimens must be brought to the laboratory where they will be examined. This transport should normally take place once or twice a week. Consequently, the specimens collected over a

period of a few days must be kept at the health centre and transported all together in one batch to the laboratory.

For this storage and transport, special transport boxes are used, each holding 10-20 sputum containers.

A. Storage (if transport is needed)

1) The special box with the sputum containers should be kept in as cool a place as possible until it is dispatched.

2) Sputum can also be processed in the health centre and the fixed smears be sent to the laboratory (see section V).
Storage and transport of fixed smears is easier than storage of sputum samples. When it is intended to make cultures from the same sample, the sputum specimen must be kept under refrigeration.

B. Dispatch

With each transport box, an accompanying list must be prepared which identifies the sputum specimens it contains and the data for the patients from whom the specimens were collected. When fixed smears are sent they should be accompanied by the same list. This list is prepared by copying from the health centre's sputum register (as in Annex II).

Before the dispatch from the health centre, the trained person must verify for each transport box :

1) that the total number of sputum containers in the box corresponds to that on the accompanying list ;

2) that the identification number on each sputum container corresponds to the identification number on the accompanying list ;

3) that the accompanying list contains the necessary data for each patient.

When this check has been made, the trained person :
– marks the date of dispatch on the accompanying list,
– puts the list in an envelope which he attaches to the outside of the transport box,
– closes the transport box carefully.

Results of examination will be reported from the laboratory to the health centre on the same accompanying list which the health centre sent with the transport box (cf. Annex III). Details for recording results of examination are presented in Section VIII.

III. THE LABORATORY

A. Safety

Each laboratory worker is responsible for his own safety and that of his co-workers. Transmission of tuberculosis results essentially from micro-aerosols – droplet nuclei of 5 microns diameter – containing tubercle bacilli, the inhalation of which produces a focus of infection in the alveoli of the lung. Efforts should be made to avoid, minimize or control those laboratory operations which create potentially infectious aerosols.

Some common sources of aerosol production in the laboratory are :
– opening of specimen containers ; this is especially dangerous if sputum has dried between the cover and the side of the sputum container, or if the container has been shaken just prior to opening,
– preparing smears on microscope slides,
– flaming of transfer loops.

Equipment : For maximum protection of laboratory workers, it is highly desirable to have a laboratory biological safety cabinet for use in preparation and fixing of smears or for processing specimens for culture (see Fig. 21). In the absence of a safety cabinet, extreme care must be taken to protect workers from infection.

B. Laboratory arrangement

The detailed arrangement for the laboratory will vary greatly as to whether other work is also done and what size and shape of room is available and also whether electricity or day light is used for microscopic examination. For example, the laboratory can be arranged so as to include three separate sections (Fig. 1) :
– one well-lighted area for (A) preparing and (B) staining smears,
– one area for microscopy,
– one area for the register and storage space.

It should contain at least three tables :
– one table (1), divided into two parts : one for smear-preparation within the safety cabinet, if available (A), the other for staining (B),
– one table (2) for microscopic examination. If there is no electricity, this table should be placed directly before a window (3),
– one table (6) for register books and slide storage.

The laboratory should also contain :
– a sink or basin (4), if possible with running water,
– a chair, or preferably a stool, the height of which can be adjusted,
– a closed wardrobe (5) or locker.

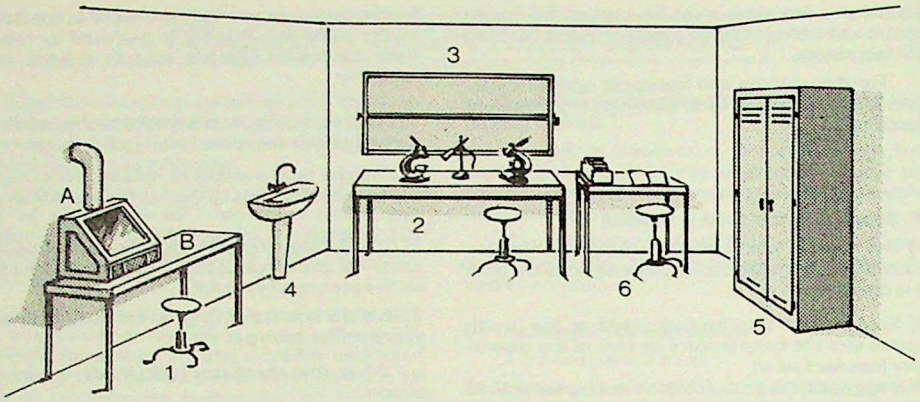

Figure 1

Each time he enters, the technician must put on his laboratory coat and wash his hands with soap and water (Basin 4, Fig. 1). Similarly, each time he goes out, he must wash his hands and leave his coat hanging in the wardrobe.

It is forbidden to smoke or eat in the laboratory, or to sit on the tables.

Before the preparation of smears is started, the material on the table should have been arranged in accordance with that shown in Figure 2.

All the manipulations for preparing a smear should be completely standardized ; the arrangements of the material on the table should always be the same, to ensure maximum safety, and to achieve a satisfactory standardization for the manipulations.

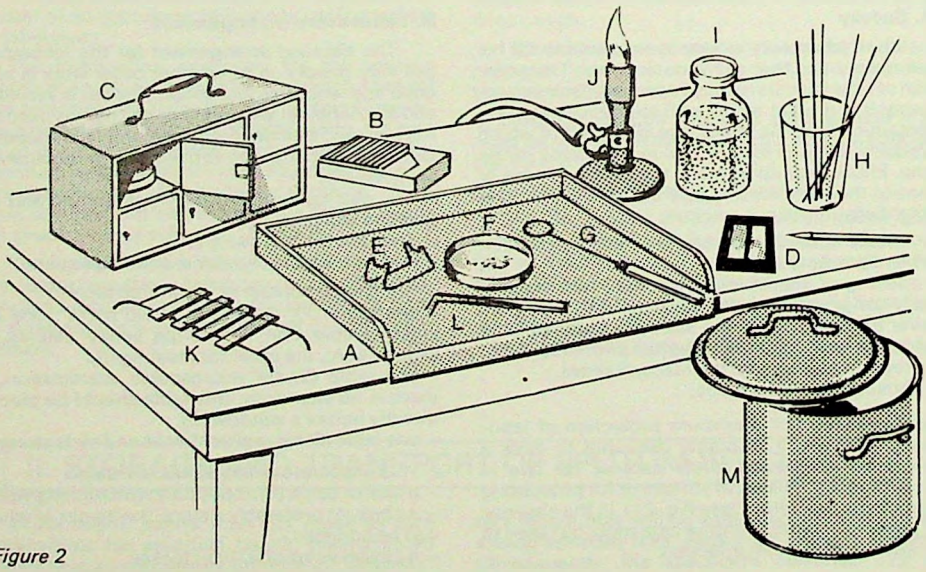

Figure 2

Details of material shown in Fig. 2

A. A non-porous surface plate, e.g. formica, galvanized metal or aluminium : this plate must be about 80 cm wide, and its borders must be 5 cm high. The front edge must be bent down at an angle of 90° to meet the edge of the table, thus facilitating manipulations. These must be made strictly over the surface plate, which must be sterilized every day after use, either by flaming or by soaking with a TB-germicide (e.g. 5% phenol, 3% cresol, or other phenolic germicide). See Fig. 13 for details of surface plate.

B. Box of engraved slides for the smears. (Slides must have no scratches on the area to be smeared. If the slides are greasy, they must be cleaned with methylated spirit and then carefully wiped with a fine cloth).

C. Special box for transporting with specimens to be examined.

D. Diamond-pointed stylus.

E. Slide-holder for the preparation of smears (see also Fig. 4, 5 and 10).

F. Sputum container placed as near as possible to the slide-holder on the right.

G. A wire loop (which may be flame-sterilized between specimens).

H. Wooden applicators (if used) (see later).

I. Alcohol sand flask.

J. Bunsen-burner or spirit lamp.

K. Dryer on which to place the finished smears. (This dryer must be *as far as possible* from the place where the smears are prepared so as to avoid any contamination from other specimens when they are being handled.)

L. Forceps.

M. Metal waste receptacle and lid to receive waste septic material.

NOTE : If the technician is left-handed, it may be more convenient to arrange all (or most) items in Fig. 2 in exactly the opposite position on the table (i.e. in a mirror-image location).

IV. RECEPTION AND REGISTRATION OF SPUTUM SPECIMENS

The sputum specimens or fixed smears together with identification data (see dispatch list, Annex II) are delivered to the laboratory.

It is necessary to make sure that the request for examination on the list and the indications on the sputum container (or fixed smears) are the same. These written indications as well as any other coded information must be carefully recorded on the examination register. This must also include the date of collection of the sputum, the date of arrival at the laboratory and the identification data of the patient and of his health centre.

V. PREPARATION OF SMEARS

A. Engraving slides for smears

This is done at the microscope table.

1) The technician washes his hands.

2) He takes a new box of slides.

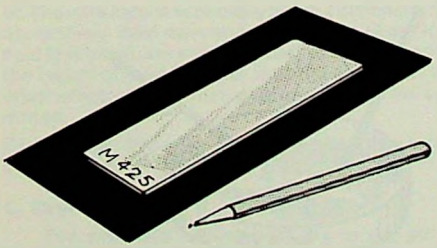

Figure 3

3) He takes the diamond-pointed stylus and engraves the sputum specimen identification number on the end of a slide. Using the list, he numbers a slide for each specimen.
He must avoid making fingerprints on the rest of the slide.
To make engraving easier, he may place the slide on a piece of black paper (Fig. 3).

4) After engraving, he checks that the identification on the slide is the same as that on the list, and puts it in the slide box reserved for engraved slides for this batch of specimens (See B, Fig. 2).

B. Smear preparation

The maximum chance of finding bacilli is in the solid particles of the sputum. **The result of the examination depends to a great extent on the choice of these particles.** Do not attempt processing at one time more than 10 or 12 specimens.

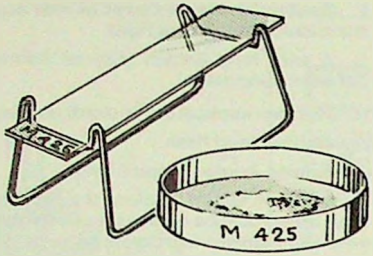

Figure 4

This stage of the procedure **(the opening of the sputum containers and the preparation of the smears)** is most dangerous ; therefore, it must be done carefully to prevent the formation of infectious aerosols.

Here is an example of a suitable smear preparation :

1) Take a slide from the slide box, holding it by the part on which the number is engraved, place it across the slide-holder (E) with the engraved side uppermost and turned towards the operator (see Fig. 4).

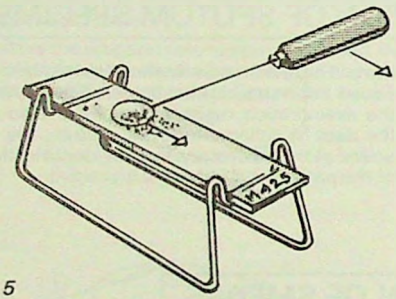

Figure 5

2) Take the sputum container corresponding to the number on the slide. Check that the number engraved on the slide corresponds to that on the sputum container. Open the container, deposit the lid in the waste receptacle (M, Fig. 2) and place the sputum container to the right of the slide-holder (Fig. 4).

3) When using the loop :
– a) flame it and allow to cool ;
– b) pick a small portion of sputum **selecting purulent particles** if present. A second loop may facilitate selection of particles from very viscous specimens ;
– c) spread the sputum sample as thinly as possible over two thirds of the slide (Fig. 5) ;
– d) sterilize the loop between successive specimens by holding in the flame of a Bunsen-burner or spirit lamp until the wire is red hot. Before sterilizing, larger particles of adherent sputum may be removed from the wire by moving it up and down through a flask containing sand and alcohol or lysol (Fig. 6). (If the sand and alcohol or lysol are placed in a screw top 300-500 ml Erlenmeyer flask, this may be used for long periods merely by refreshing the alcohol or lysol to maintain the level at least 3 cm above the sand.)

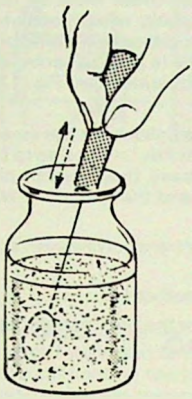

Figure 6

4) When using wooden applicators :
– a) take a wooden applicator between the thumb and index finger of each hand about three centimetres from its centre and break it (Fig. 7) ;

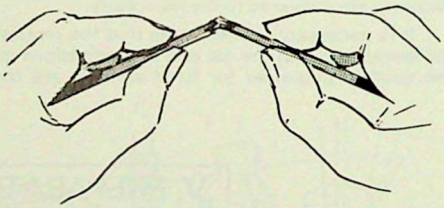

Figure 7

– b) choose yellowish, opaque, purulent particles which will be placed on the slide. In order to do this, without changing position of the hands,

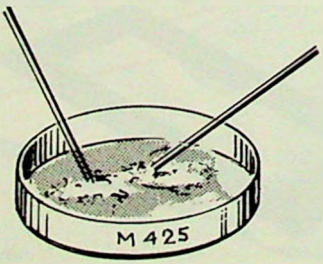

Figure 8

use the broken ends of the two pieces of the applicator to break up the larger particles (Fig. 8) ;

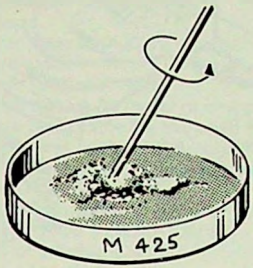

Figure 9

– c) if the particles are difficult to detach from the bottom of the sputum container, turn it with one of the sticks with a rapid circular movement (Fig. 9);

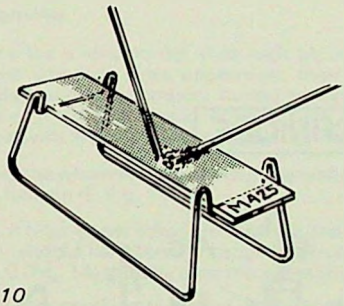

Figure 10

– d) finally, using the two sticks in a pincer movement, raise the particle and place it on the slide (Fig. 10) (very often the particle is not homogenous and one must, with the end of one stick, roll the dependent part around the other stick) ;
– e) with one of the sticks, mix the particles placed on the slide ; hold the stick firmly, and while applying downward pressure to the purulent particles, spread them evenly to cover 2/3 of the slide (as indicated in Fig. 5).

5) The wire loop is especially helpful in transferring excessively fluid specimens onto the slide. If no acid fast bacilli are seen in "fluid" sputum samples, the report should indicate that the specimen was unsatisfactory and that another should be submitted.

6) After the smear is prepared, place the smear on the dryer.

C. Drying

The prepared slides must dry in the air for about 15-30 minutes. Do not use flame for drying.

Neither fixed nor unfixed slides should be left exposed overnight. Not only is there a chance of accidental breakage and infection, but insects and rodents may enter the laboratory and eat the smears off the slides. The technician must arrange his working day so that slide preparation, staining and reading are completed ; if reading cannot be completed, slides should be placed in a covered box for overnight storage.

D. Fixation

The dryer with slides and the Bunsen-burner or spirit lamp are placed in the work area.

– Using forceps, take a slide from the dryer at the engraved end, with the smear uppermost.

– Pass it three times through the flame of the Bunsen-burner or the spirit lamp (Fig. 11) ; this should take 3-5 seconds.

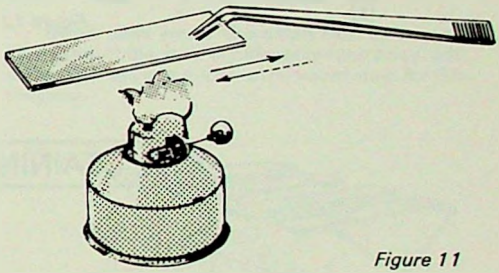

Figure 11

– Place it on the clean dryer.

When all the slides have been treated in this way, flame the empty dryer.

The dryer with the smeared and fixed slides is brought to the staining part of the working table (area B, Fig. 1).

E. Disinfection and sterilization of contaminated material

When the manipulations are finished, infected materials and sputum containers must be thrown into the waste receptacle.

1) The waste receptacle (M, Fig. 2) containing used materials and plastic sputum containers is half-filled with water containing disinfectant (see E. 3) covered with its lid and brought to the boil and boiled for 10 minutes (Fig. 12). The contents are then collected for later burning.

2) In the event both burnable materials and glass sputum jars are used, the latter should be discarded into a separate container so that they may be boiled and washed for re-use.

3) Other items such as the slide holder, the dryer and the work surface should be flamed (Fig. 13) or literally soaked in a TB germicidal solution. (It should be noted that a spirit lamp has limited usefulness for this particular application). TB germicides are only those that have been shown to kill tubercle bacilli by approved tests ; 5% phenol or one of the phenol-derivative soap mixtures may be used.

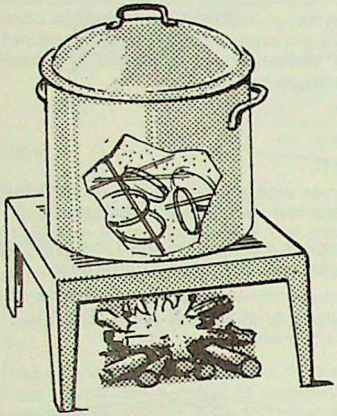

Figure 12

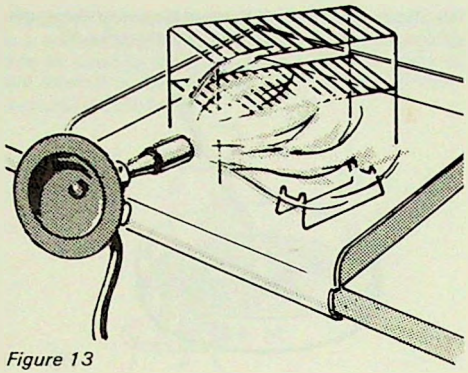

Figure 13

VI. STAINING TECHNIQUE

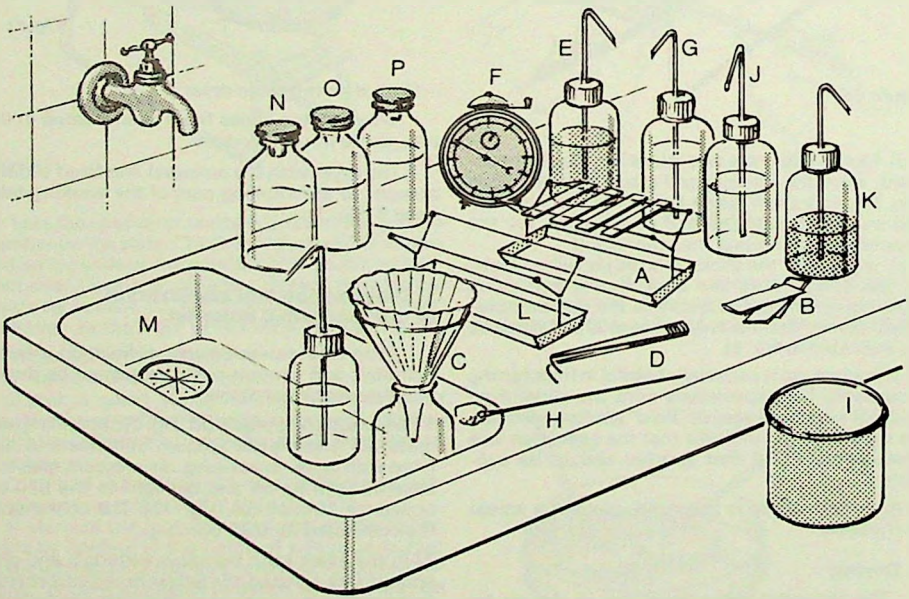

Figure 14

Arranging the table is shown in Figure 14.

A. Slide-rack for staining (can be used for 12 or more slides)
B. Filter-paper (cut up beforehand) or
C. Funnel with filter paper
D. Forceps
E. Plastic flask with Ziehl's carbol fuchsin
F. Alarm clock
G. Plastic flask with spirit
H. Cotton holder (in metal or wood)
I. Waste receptacle for the used filter papers
J. Plastic flask with 25% sulphuric acid
K. Plastic flask with 0,3% methylene blue
L. Extra slide rack
M. Basin (with running water if possible ; otherwise have an additional plastic flask containing water)
N. Flask of Ziehl's carbol fuchsin
O. Flask of sulphuric acid } Stock solutions to refill bottles E, J, K
P. Flask of methylene blue

Formulations for the above reagents are to be found in Section XII.

The smeared and fixed slides are stained in batches of up to about 12.

A. Staining

1) Place the slides on the slide-rack (A, Fig. 14) with the smeared sides uppermost, their edges separated and the numbers turned towards the operator. The smeared part of each slide can be covered with a piece of filter-paper (B, Fig. 14).

2) Cover the whole surface of the slides with Ziehl's carbol fuchsin (E, Fig. 14).

NOTE : If filter-paper strips are not used, the carbol fuchsin should be filtered through filter-paper in funnel C (Fig. 14) directly onto the slides (Fig. 15).

3) Heat very gently until vapour rises. For this, use the flame of a Bunsen-burner or of a wad of cotton-wool in methylated spirit (G, Fig. 14) fixed on the end of a metal rod or a fairly strong stick of wood (Fig. 16).

In no case must the stain boil or dry on the slide. If the stain accidentally runs away, add more and heat again. Leave the warm stain for five minutes.

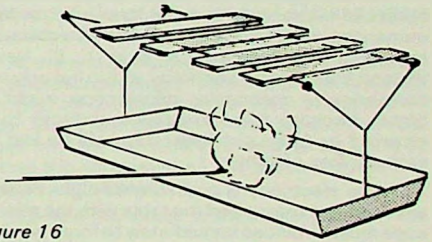

Figure 16

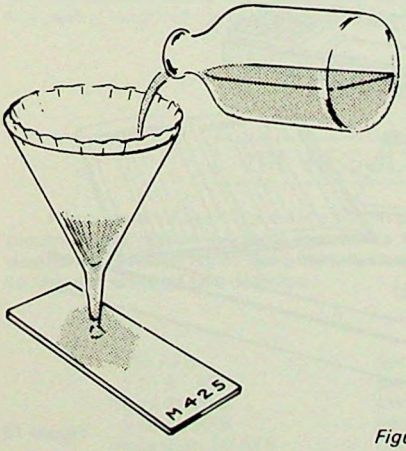

Figure 15

B. Decolorization

1) With forceps, remove the filter-papers and deposit them in the waste receptacle (I, Fig. 14).

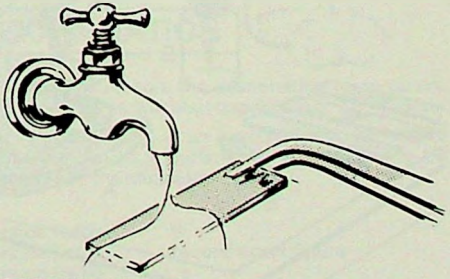

Figure 17

2) Rinse each slide individually in a **gentle** stream of running water (tap water, or bottled water (M, Fig. 14)) until all free stain is washed away (Fig. 17).

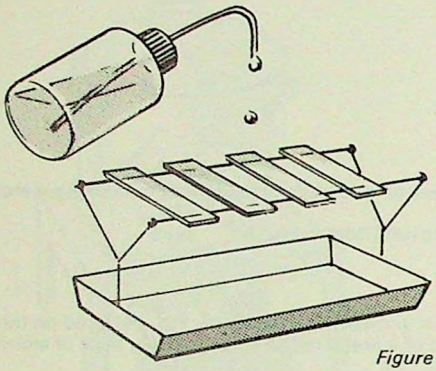

Figure 18

NOTE : Bulk staining, rinsing, acid decolorizing and counter staining must be avoided because of the real possibility of cross contamination from one slide to another.

3) Replace all slides on the slide-rack (A, Fig. 14) and cover each one individually with 25% sulphuric acid (J, Fig. 14) for 3 minutes (Fig. 18).

4) Rinse as in B.2) above.

5) Decolorize again for 1-3 minutes (as in B.3) above) until all color has practically disappeared.

6) Rinse as in B.2) above.

C. Counter-staining

1) Replace decolorized, rinsed slides on slide-rack (A, Fig. 14) and flood smear with 0,3% methylene blue counterstain (K, Fig. 14) for 60 seconds.

2) Rinse as in B. 2) above and allow to dry in open air.

VII. EXAMINATION BY MICROSCOPY

For the examination of stained specimens, a binocular microscope is most convenient, with an immersion objective (× 100) and eye pieces of moderate magnification (× 6 or × 8). Nevertheless, if there is no electricity, and in hot or humid conditions, a monocular microscope might be better, because there are fewer surfaces to be attacked by fungi and fewer days will be lost because of lack of light.

If no electricity is available, daylight must be used as light source and the table with the microscope must be placed immediately before a window.

A. Arranging the working table (Fig. 19)

In addition to the microscope (A), the microscopist must have on the table :
- a bottle with immersion oil (B)
- toluene and clean cotton (C)
- a note book and pencil (D)
- the stained slides to be examined (E)
- the dispatch list for these sputum specimens (F)
- a slide box for examined slides (G).

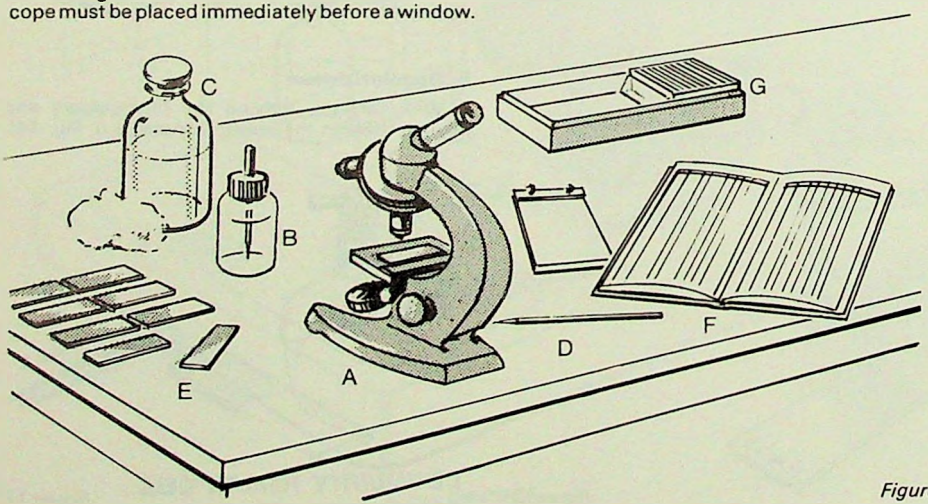

Figure 19

B. Use of the microscope

Before starting the actual examination of smears, the technician must make sure that all elements of his microscope are correctly set. He should, in particular, check that the source of light is well regulated and focused, that the condenser is in the upper position, with the diaphram open and that the immersion objective and the ocular lenses are clean.

Put a drop of immersion oil on the left edge of the stained smear (near the engraved number) and place the slide on the microscope stage. To avoid possible contamination of the immersion oil, **do not touch the slide with the oil applicator,** but permit the drop of oil to fall freely onto the slide. With the macrometric screw, lower the immersion lens, keeping continuous watch until it touches the drop of oil. Looking through the eye-piece(s) bring the immersion lens slowly upwards, by means of the macrometric screw, until the image of the smear appears. Complete the focusing by means of the micrometric screw. All during the reading, the correct focusing is ensured by using the micrometric screw.

C. Technique of reading

Examine at least 100 microscopic fields. For a skilled microscopist, this will take 5 minutes.

The reading must be systematic and standardized. For instance, begin the reading of the slide in the centre of the left end of the smear, by slight adjustements of the micrometric screw, systematically examine the field, beginning at the periphery and ending at the centre.

After examining a microscopic field, move the slide longitudinally so that the neighbouring field to the right can be examined. In this manner, all the microscopic fields from beginning to end of this central length of the slide should be examined.

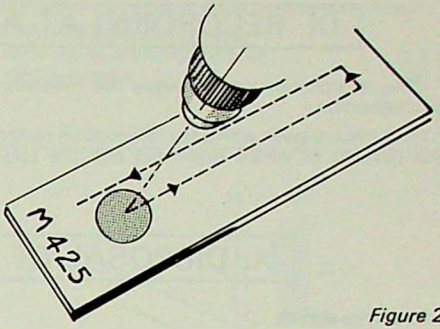

Figure 20

The number of microscopic fields in one length of the slide corresponds to at least 100

When no Acid-Fast Bacilli (AFB) are found in 100 fields, a more thorough search should be made in 100 new fields.

As shown in Fig. 20, move the slide a few millimeters towards its back and read a second length (from right to left).

Tubercle bacilli look like fine red rods, slightly curved, more or less granular, isolated, in pairs, or in groups, standing out clearly against the blue background. Count the number of AFB and report this number on the note book.

At the end of examination, take the slide from the microscope stage, check the identification engraved on it, and enter the result of the examination in the last column of the dispatch list (see Annex III). Dip the slide into toluene (or xylol) to remove the immersion oil and place it in the box for examined slides.

Examine the slides in the order given on the dispatch list.

Before examining the next slide, wipe the immersion lens with a piece of clean cotton.

VIII. RESULTS OF EXAMINATION

The number of bacilli found is a very important piece of information because **it relates to the degree of infectivity of the patient, as well as to the severity of the disease.**

For this reason, the examination must be not only qualitative, but also quantitative.

The following is an example of a method of reporting which is sufficiently quantitative to be valuable to the clinician :

– No AFB –	per 100 immersion fields	0
1 to 9 AFB	per 100 immersion fields	record exact figure
10 to 99 AFB	per 100 immersion fields	+
1 to 10 AFB	per field	+ +
more than 10 AFB	per field	+ + +

IX. RECORDING AT A MICROSCOPY CENTRE

The microscopy centre keeps the following documentation :

1) Work-record book with daily information on the total number of slides examined and the total number of positive slides, by health centre (Annex IV).

2) Positive sputum register with detailed information on all positive patients identified at the microscopy centre (Annex V).

X. DISPOSAL OF EXAMINED SLIDES

A. Positive slides

A slide in which acid-fast bacilli have been demonstrated is a document on which the diagnosis of pulmonary tuberculosis of a person depends. It must be recorded and kept in the laboratory, and the reading should be confirmed by a second reader. The record of all positive slides is made in a special record book of the laboratory (cf. Annex V), in the order in which the slides have been examined.

The positive slides are removed to a special box and kept for about one year. Before discarding, they must be broken and buried to prevent their re-use.

B. Negative slides

All the negative slides must be kept in the laboratory for at least one week, in order to allow for a control of the reading. After this time, they may be discarded (e.g. buried).

XI. DISPATCH OF RESULTS OF EXAMINATION

Upon completion of a series of microscopic examinations, the date of examination is recorded on the dispatch list which is returned to the health centre as soon as possible. The transport box is cleaned by swabbing with a cloth wet with a TB-germicide (e.g. 3% cresol or other phenol derivative) and also returned to the health centre.

XII. FORMULATION OF REAGENTS

A. Ziehl's carbol fuchsin

FORMULA 1

To prepare 100 ml of stain, use the following formula. Larger volumes may be made for stock solutions, if desired :

1) Saturated alcoholic solution of fuchsin
- basic fuchsin ..... 3 g
- 95% ethyl alcohol ..... 100 ml

2) Working solution
- phenol crystals ..... 5 g

Heat gently in a flask to liquefy, and bring to 90 ml with water.

- add : saturated fuchsin solution ..... 10 ml

FORMULA 2 (needs no scales)

- saturated solution of fuchsin, filtered . 100 ml
- aqueous phenol 5% ..... 900 ml

Saturated solution of fuchsin : the contents of a 25 g flask of basic fuchsin is introduced into a 250 ml bottle, which is then filled with methylated spirits. The bottle is shaken vigorously and is shaken three times more on the same day It is allowed to settle. The solution is ready for use the following day.

Methylated spirits may be added until the deposit is used up.

The 5% aqueous phenol is prepared by adding 5 ml of crystalised phenol melted at 45° C (over 100 ml of water).

B. Decolourization reagents

SULPHURIC ACID 25%

Empty 300 ml of water into a 1-litre flask. Slowly add 100 ml of sulphuric acid, allowing it to flow along the side of the flask. Mix. The contents will heat up. **Never empty the water into the sulphuric acid.**

Sulphuric acid 25% may be replaced by acid alcohol, prepared as follows :

ACID ALCOHOL
- methylated spirits ..... 970 ml
- hydrochloric acid ..... 30 ml

C. Counter-staining with methylene blue

- methylene blue chloride or hydrosoluble methylene blue..... 0.3 g
- distilled water..... 100 g

Diagram of a transfer cabinet (Fig. 21)

A transfer cabinet can be made of wood or other material and placed on the laboratory bench or ordinary table. The dimensions, which may be varied, are as follows :

length : 1 m, height : 85 cm, width of base : 60 cm, width of top : 33 cm.

X. outlet for contaminated air to be disposed of safety. The contaminated air must be drawn by exhaust fan through filter. If no ventilation is possible, a transfer cabinet can be more dangerous than no cabinet at all.

W. large window (thick glass) as from automobile. May be framed and hinged for opening.

P. slit to allow the introduction of the technician's hands and forehands (20 cm high).

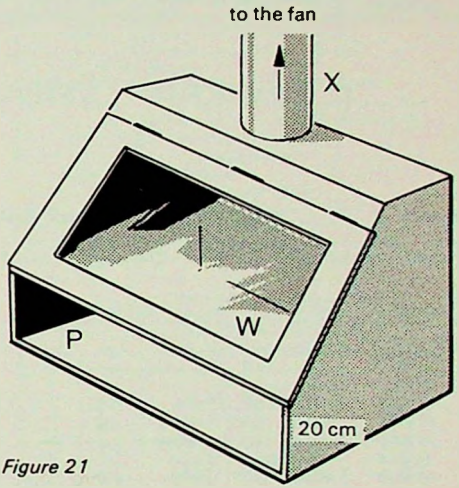

Figure 21

ANNEXES

ANNEX I **HEALTH CENTRE'S SPUTUM REGISTER**

No. sputum container	Name and surname	Age	Sex	Address	Date of collection	Date of results	AFB results
M 421	Abdelazyz ben Ahamed	38	M	Kisar	10/11/69		
M 422	Habara bent Jounes	44	F	Kisar	"		
M 423	Fatma bent Amor	32	F	Kisar	12/11/69		
M 424	Othmen ben Ali	28	M	Kisar	"		
M 425	Zina bent Jounes	22	F	Slita	13/11/69		
M 426	Heddia bent Mohamed	46	F	Slita	"		
M 427	Alkazzis ben Ahamed	38	M	Kisar	"		
M 428	Zina bent Jounes	22	F	Slita	14/11/69		
M 429	Aziza bent Salah	27	F	Djerda	"		
M 430	Heddia bent Mohamed	46	F	Slita	"		
M 431	Aziza bent Salah	27	F	Djerda	15/11/69		
M 432	M'Barka ben Amor	26	M	Djerda	"		
M 433	Ammar ben Husnian	25	M	Kisar	"		
M 434	M'Barka ben Amor	26	M	Djerda	16/11/69		
M 435	Abdelazzis ben Ahamed	38	M	Kisar	17/11/69		

ANNEX II

DISPATCH LIST
(accompanying the transport box containing sputum containers sent by the health centre to the laboratory)

Health centre : Bahra | Sent on : 18/11/69 | Laboratory : | Received on :
Examined on :
Technician : Salah | Results on : | Microscopist : | Sent back on :

No. sputum container	Name and surname	Age	Sex	Address	Date of collection	AFB results

(COPY FROM SPUTUM REGISTER)

ANNEX III

DISPATCH LIST SENT BACK TO THE HEALTH CENTRE

Health centre : Bahra | Sent on : 18/11/69 | Laboratory : Tunis | Received on : 18/11/69
Examined on : 19/11/69
Technician : Salah | Results on : | Microscopist : Ali | Sent back on : 20/11/69

No. sputum container	Name and surname	Age	Sex	Address	Date of collection	AFB results
M 425	Zina bent Touna	22	F	Slita	13/11/69	0/2L
M 426	Hedda bent Mohamed	46	F	Slita	"	36/1L
M 427	Abdelazzis ben Ahmed	38	M	KSar	"	8/2L
M 428	Zina bent Touna	22	F	Slita	14/11/69	0/2L
M 429	Aziza bent Salah	27	F	Djerda	"	0/2L
M 430	Hedda bent Moh.	46	F	Slita	"	>50/L
M 431	Aziza bent Salah	27	F	Djerda	15/11/69	0/2L
M 432	M'Barka ben Amor	26	M	Djerda	"	0/2L
M 433	Ammar ben Hassen	25	M	Kitour	"	0/2L
M 434	M'Barka ben Amor	26	M	Djerda	16/11/69	50/2L
M 435	Abdelazzis ben Ahmed	38	M	KSar	17/11/69	0/2L

ANNEX IV

WORK-RECORD

Date received	Health centre	First and last number	Total number examined	Total number positive	Date results sent off
16/11/69	C	825 - 836	12	2	17/11/69
16/11/69	R	415 - 424	10	1	17/11/69
17/11/69	K	250 - 261	12	0	17/11/69
18/11/69	M	425 - 435	11	4	18/11/69

ANNEX V

POSITIVE SPUTUM REGISTER

Number container	Name and given name	Age	Sex	Address	Date of collection	Date of examination	AFB results	Confirmation *
M 426	Hedda bent Mohamed	46	F	Slita	13/11/69	19/11/69	36/1L	
M 427	Abdelazzis ben Ahmed	38	M	Ksar	13/11/69	19/11/69	8/2L	x
M 430	Hedda bent Mohamed	46	F	Slita	14/11/69	19/11/69	>50/L	
M 434	M'Barka ben Amor	26	M	Djerda	16/11/69	19/11/69	50/2L	x

* By the second reader.

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