

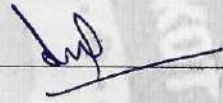


	All India Institute of Medical Sciences, Rajkot Department of Clinical Microbiology	
	SOP for Collection and transport of Sputum sample for AFB stain	

Proprietary statement: This document contains information. It is provided to institution staff under AIIMS Rajkot only. Do not share verbally or otherwise without written consent of the Prof & Head, Department of Clinical Microbiology, AIIMS, Rajkot.

DOC. NO.		TITLE		ISSUE NO.
AIIMS/Rajkot/Micro/SOP/1		SOP for Collection and transport of Sputum sample		1.0
Effective Date: 20/06/2022				
Name	Designation	Function	Signature	
Dr Mayuri Bhise	Assistant Professor	Preparation	 20/6/22	
Dr Ashwini Agrawal	Prof & Head	Review and Approval		
Distribution List:				
Collection Centre				
REVISION SUMMARY				
Revision No.	Issue No.	Effective Date	Revision History	
0	1.0	20/06/2022	NA	

	All India Institute of Medical Sciences, Rajkot Department of Clinical Microbiology SOP for Collection and transport of Sputum sample for AFB stain	
--	--	--

1.0 PURPOSE AND SCOPE

Purpose of this SOP is to describe the process involved in collection of sputum sample
This SOP is applied to the collection of Sputum sample for AFB stain

2.0 DEFINATIONS AND ABBREVIATIONS

2.1 SOP: Standard Operating Procedure

3.0 RESPONSIBILITY

It is a responsibility of Laboratory Technician /Resident to instruct the patient regarding the collection of sputum sample

4.0 PROCEDURE

4.1 Container required:



- Clean, sterile leak proof screw capped wide mouth container
- Spot and next day early morning sample and or two spot samples collected one hour apart.



4.2 Collection Instructions

- Introduce yourself and identify the patient
- Check the specific test requisition form prefilled from Resident/Clinician.
- Verify the identity of the patient and check for patient details before labelling.
- Label the container previously before giving to patient
- For two different samples give two separate sterile prelabelled container as A and B (A -SPOT and B -Early Morning)
- Patient should be asked to produce the sputum specimen in open air, (Sputum collection area / Cough Corner)
- Patient should be instructed not to eat, drink, smoke, brush teeth, or use mouthwash
- If Patient is staying far away from hospital and difficult for him to come on next day for giving second sample so instruct the patient to give two spot samples one hour apart
- Early-morning sputum samples should be obtained because they contain pooled overnight secretions in which pathogenic bacteria are more likely to be concentrated
- Having the patient brush his or her teeth and gargle with water immediately before obtaining the sputum specimen reduces the number of contaminating oropharyngeal bacteria.
- To prevent contamination of the outside of the container, the patient should be instructed to press the rim of the container under the lower lip to catch the entire expectorated cough sample.
- Sputum must be thick, yellowish, mucopurulent, expectorant material directly from

Document Type: Standard Operating Procedure	Issue No.	Issue Date	20/06/2022
Document No.: AIIMS/Rajkot/Micro/ SOP/1	Page No: 2 of 19		

	All India Institute of Medical Sciences, Rajkot Department of Clinical Microbiology	
	SOP for Collection and transport of Sputum sample for AFB stain	

deep of the lungs. The optimal volume of sputum required is 2-5 ml is collected in in a sterile screw-cap container

- Tightly close the cap of the container.



4.3 Storage/Transport

The sample has to be immediately transported to the laboratory in transportation box with container preferably within 2 hours

5.0 REFERENCES:

- 5.1 Handbook for Specimen collection and handling in Microbiology 2 nd Edition CDC Atlanta
- 5.2 Standard operating procedures Bacteriology -ICMR New Delhi India 2 nd Edition 2019
- 5.3 Manual for Laboratory technicians, Central TB Division, Directorate General of health services (NTEP)

6.0 APPENDICES AND FORMS:

6.1 Test Requisition Form (NTEP)

Document Type: Standard Operating Procedure	Issue No.	Issue Date	20/06/2022
Document No.: AIIMS/Rajkot/Micro/ SOP/1		Page No: 3 of 19	



**All India Institute of Medical Sciences, Rajkot
Department of Clinical Microbiology**

**SOP for Collection and transport of Sputum sample for
AFB stain**



7.0 VALIDITY STATEMENT

This SOP is valid for one year from the date of issue.

Annexure 15A

RNTCP Request Form for examination of biological specimen for TB
(Required for Diagnosis of TB, Drug susceptibility Testing and follow up)

Patient Information			
Patient name	Age (in yrs):	Gender: <input type="checkbox"/> M <input type="checkbox"/> F <input type="checkbox"/> TO	
Patient mobile no. or other contact no.	Specimen collection date (DD/MM/YY)	<input type="checkbox"/> Sputum <input type="checkbox"/> Other (specify) _____	
Aadhaar no. (if available)	HIV Status: <input type="checkbox"/> Reactive <input type="checkbox"/> Non-Reactive <input type="checkbox"/> Unknown		
Patient address with landmark	Key populations: <input type="checkbox"/> Contact of known TB Patient <input type="checkbox"/> Diabetes <input type="checkbox"/> Tobacco <input type="checkbox"/> Prison <input type="checkbox"/> Miner <input type="checkbox"/> Migrant <input type="checkbox"/> Refugee <input type="checkbox"/> Urban slum <input type="checkbox"/> Health-care worker <input type="checkbox"/> Other (specify) _____		
Name and Type of referring facility (PHC/DMC/TU/OTC/CT/ART/Medical College/DR-TB Centre/Private Others, specify)		Type of patient: <input type="checkbox"/> Public sector <input type="checkbox"/> Private sector	
Health Establishment ID (NIKSHAY):		Episode ID: _____	
State: _____ District: _____		Tuberculosis Unit (TU): _____	
Reason for Testing			
Diagnosis and follow up of TB			
Diagnosis of TB		Follow up (Smear and culture)	
H/O and TB Rx for >1 month: <input type="checkbox"/> Yes <input type="checkbox"/> No		Reason: <input type="checkbox"/> End IP <input type="checkbox"/> End CP	
<input type="checkbox"/> Presumptive TB	Predominant symptom _____	Post treatment: <input type="checkbox"/> 6m <input type="checkbox"/> 12m <input type="checkbox"/> 18m <input type="checkbox"/> 24m	
<input type="checkbox"/> Repeat Exam	Duration _____ days		
<input type="checkbox"/> Presumptive NTM			
<input type="checkbox"/> Contact of DR TB			
Diagnosis and follow up Drug-resistant TB			
Diagnosis of DR TB (DRT/ DST)		Follow up (Smear & culture)	
<input type="checkbox"/> New <input type="checkbox"/> Previously treated		Treatment follow up month: _____	
Presumptive MDR TB	<input type="checkbox"/> At TB diagnosis	Type of case: <input type="checkbox"/> H mono/poly TB <input type="checkbox"/> MDR/RR TB <input type="checkbox"/> XDR TB	
	<input type="checkbox"/> Follow up Sm +ve	Regimen Type:	
	<input type="checkbox"/> Presumptive H mono/poly	<input type="checkbox"/> All oral H mono/poly TB	
Presumptive XDR TB	<input type="checkbox"/> MDR/RR TB at Diagnosis	<input type="checkbox"/> Shorter MDR TB regimen	
	<input type="checkbox"/> Failure of MDR/RR TB regimen	<input type="checkbox"/> All oral longer regimen	
	<input type="checkbox"/> Recurrent of MDR/RR TB regimen	<input type="checkbox"/> Any other regimen _____	
		Regimen composition:	
		<input type="checkbox"/> 1R <input type="checkbox"/> Mfx <input type="checkbox"/> D3sq <input type="checkbox"/> Lzd <input type="checkbox"/> Cfx <input type="checkbox"/> Cs <input type="checkbox"/> Z <input type="checkbox"/> E <input type="checkbox"/> Etb <input type="checkbox"/> Dm <input type="checkbox"/> Am <input type="checkbox"/> Km <input type="checkbox"/> Dcns <input type="checkbox"/> _____	
Test requested:			
<input type="checkbox"/> Microscopy <input type="checkbox"/> TST <input type="checkbox"/> IGRA <input type="checkbox"/> Chest X-ray <input type="checkbox"/> Cytopathology <input type="checkbox"/> Histopathology <input type="checkbox"/> CBNAAT <input type="checkbox"/> TruNAAT			
<input type="checkbox"/> Culture <input type="checkbox"/> DST <input type="checkbox"/> FL -LPA <input type="checkbox"/> SL -LPA <input type="checkbox"/> Gene Sequencing <input type="checkbox"/> Other (Please Specify) _____			
Requested by (Contact No. & Designation and Signature): _____			
Contact Number: _____		Email ID: _____	
Results:			
Microscopy (<input type="checkbox"/> ZN <input type="checkbox"/> Florescent) Test ID: _____			
	Lab Sr. No	Visual appearance	Result
		Negative Scanty	1+ 2+ 3+
Sample A		S M B	
Sample B		S M B	
Date tested: _____		Date Reported: _____	
Laboratory Name: _____		Reported by: _____ (Name and Signature)	



**All India Institute of Medical Sciences, Rajkot
Department of Clinical Microbiology**

**SOP for Collection and transport of Sputum sample for
AFB stain**



Nucleic Acid Amplification Test (NAAT) Lab serial _____ Test ID: _____		
Type of test	<input type="checkbox"/> CBNAAT <input type="checkbox"/> TrueNat	
Sample	<input type="checkbox"/> A <input type="checkbox"/> B	
M. Tuberculosis	<input type="checkbox"/> Detected <input type="checkbox"/> Not Detected <input type="checkbox"/> N/A	
Rif Resistance	<input type="checkbox"/> Detected <input type="checkbox"/> Not Detected <input type="checkbox"/> Indeterminate <input type="checkbox"/> N/A	
Test	<input type="checkbox"/> No Result <input type="checkbox"/> Invalid <input type="checkbox"/> Error – Error Code _____ (Please arrange for fresh sample)	
Date tested:	Date Reported: _____	
Laboratory Name:	Reported by: _____ (Name and Signature)	
Culture (<input type="checkbox"/> LJ <input type="checkbox"/> LC) Test ID: _____		
Lab Sr. No	Negative Positive NTM (write species) Contamination	
Date Result:	Date Reported: _____	
Laboratory Name:	Reported by: _____ (Name and Signature)	
First line LPALab serial Test ID: _____		
<input type="checkbox"/> Direct <input type="checkbox"/> Indirect <input type="checkbox"/> Valid <input type="checkbox"/> Invalid <input type="checkbox"/> MTB detected <input type="checkbox"/> MTB not detected		
Drug	Resistant detected Final interpretation Remark	
Rifampicin (R)	<input type="checkbox"/> Yes <input type="checkbox"/> Inferred <input type="checkbox"/> No if yes or inferred, R should not be given	
Isoniazid (Kat G)	<input type="checkbox"/> Yes <input type="checkbox"/> Inferred <input type="checkbox"/> No if yes or inferred, H(h) should not be given	
Isoniazid (Inh A)	<input type="checkbox"/> Yes <input type="checkbox"/> Inferred <input type="checkbox"/> No if yes or inferred, H(h) can be considered & Iso should not be given	
Date Result:	Date Reported: _____	
Laboratory Name:	Reported by: _____ (Name and Signature)	
Second line LPALab serial Test ID: _____		
<input type="checkbox"/> Direct <input type="checkbox"/> Indirect <input type="checkbox"/> Valid <input type="checkbox"/> Invalid <input type="checkbox"/> MTB detected <input type="checkbox"/> MTB not detected		
Drug	Resistant detected Final interpretation Remark	
Levofloxacin	<input type="checkbox"/> Yes <input type="checkbox"/> Inferred <input type="checkbox"/> No if yes or inferred, Lfx should not be given. Mfx (h) can be considered.	
Moxifloxacin (h)	<input type="checkbox"/> Yes <input type="checkbox"/> No if yes, Lfx & Mfx (h) should not be given	
Amikacin	<input type="checkbox"/> Yes <input type="checkbox"/> Inferred <input type="checkbox"/> No if yes or inferred, Am should not be given	
Kanamycin	<input type="checkbox"/> Yes <input type="checkbox"/> Inferred <input type="checkbox"/> No if yes or inferred, Km should not be given	
Capreomycin	<input type="checkbox"/> Yes <input type="checkbox"/> Inferred <input type="checkbox"/> No if yes or inferred, Cm should not be given	
Date Result:	Date Reported: _____	
Laboratory Name:	Reported by: _____ (Name and Signature)	
Drug Susceptibility Test (DST) results Test ID: _____		
Lab Sr.No	1 st line drugs SLI FO Other	
ac	H(0) H(4) No W Mh S An Is Mh (1) Mh (2) PAS Lfx Of Or Ar Mfx Dm Cs	
Date Result:	Date Reported: _____	
Laboratory Name:	Reported by: _____ (Name and Signature)	
R: Resistant; S: Susceptible; C: Contaminated – Not done		
Other tests for TB diagnosis Test ID: _____		
Test (Please Specify): _____		
Result: _____		
Date reported: _____		
Reported by: _____ (Name and Signature)		





All India Institute of Medical Sciences, Rajkot
Department of Clinical Microbiology
SOP on Sample acceptance and rejection



Proprietary statement: This document contains information. It is provided to institution staff under AIIMS Rajkot only. Do not share verbally or otherwise without written consent of the Prof & Head, Department of Clinical Microbiology, AIIMS, Rajkot.

DOC. NO.	TITLE	ISSUE NO.
AIIMS/Rajkot/Micro/SOP/2	SOP on Sample acceptance and rejection	1.0
Effective Date:20/06/2022		

Name	Designation	Function	Signature
Dr Mayuri Bhise	Assistant Professor	Preparation	
Dr Ashwini Agrawal	Prof & Head	Review and Approval	

Distribution List:
Laboratory

REVISION SUMMARY			
Revision No.	Issue No.	Effective Date	Revision History
0	1.0	20/06/2022	NA



1.0 INTRODUCTION

The Samples that are collected in the Collection centre OPD campus need to be screened for acceptance/rejection and received in the Laboratory in a proper way.

2.0. PURPOSE

This SOP details the procedure to be followed for receiving, accepting and rejecting the samples at Designated Microscopy Centre.

3.0. SCOPE

This SOP is applicable to DMC laboratory for collection of sputum for acid fast bacilli for ZN microscopy.

4.0 RESPONSIBILITY

It is the responsibility of the Laboratory Technicians to read the SOP for sample acceptance and rejection for sputum sample.

5.0 PROCEDURE

5.1 Sample Acceptance:

- Check and verify the details mentioned on the sample container & on the Test Requisition form are same.
- Check that the samples are brought to the laboratory as early as possible without delay
- Check the sample containers are intact and there is no leakage

5.2 Sample Rejection Criteria:

The sample is rejected if any of the following is observed.

- Sample is salivary
- Sample container is leaked
- Sample volume insufficient
- Unlabelled/mislabelled samples
- Sample without requisition form

Document Type: Standard operation procedure	Issue No.	Issue Date	20/06/2022
Document No.: AIIMS/Rajkot/Micro/ SOP/2			Page No: 7 of 19



6 REFERENCES:

- 6.1 Technical and operational guidelines for Tuberculosis control October 2005
- 6.2 Manual for Laboratory Technicians Central TB Division May 1999

7 APPENDICES AND FORMS:

NA

8 VALIDITY STATEMENT

This SOP is valid for one year from the date of issue.



Document Type: Standard operation procedure	Issue No.	Issue Date	20/06/2022
Document No.: AIIMS/Rajkot/Micro/ SOP/2		Page No: 8 of 19	



**All India Institute of Medical Sciences, Rajkot
Department of Clinical Microbiology**

SOP for sputum smear preparation and ZN staining



Proprietary statement: This document contains information. It is provided to institution staff under AIIMS Rajkot only. Do not share verbally or otherwise without written consent of the Prof & Head, Department of Clinical Microbiology, AIIMS, Rajkot.

DOC. NO.	TITLE	ISSUE NO.
AIIMS/Rajkot/Micro/SOP/3	SOP for sputum smear preparation and ZN staining	1.0
Effective Date:20/06/2022		

Name	Designation	Function	Signature
Dr Mayuri Bhise	Assistant Professor	Preparation	
Dr Ashwini Agrawal	Prof & Head	Review and Approval	

Distribution List:
DMC Laboratory

REVISION SUMMARY			
Revision No.	Issue No.	Effective Date	Revision History
0	1.0	20/06/2022	NA



SOP for sputum smear preparation and ZN staining

1.0 PURPOSE AND SCOPE

Purpose of this SOP is to describe the process involved in smear preparation and ZN staining of sputum sample

This SOP is applied to the smear preparation and staining of Sputum sample for AFB stain

2.0 DEFINATIONS AND ABBREVIATIONS

2.1 SOP: Standard Operating Procedure

3.0 RESPONSIBILITY

It is a responsibility of Laboratory Technician /Resident to instruct the patient regarding the smear preparation and staining of sputum sample

4.0 PROCEDURE

4.1 Container required:

- Clean, sterile leak proof screw capped wide mouth container early morning sample and spot or two spot samples collected one hour apart.
- Assess and record visual appearance of the sample
- Indicate the visual appearance by writing M, B or S (mucous, blood stained and salivary)



A good sputum sample is:

- thick (semi-solid), coughed out deeply from the lungs;
- purulent (yellowish mucus);
- sufficient in amount (at least 2 ml).

A poor-quality sputum sample:

- contains only saliva (watery fluid) or nasal mucus;
- is small in quantity (less than 2 ml).

4.2 Smear preparation

- Select new, clean, grease-free, unscratched slides, and be careful not to leave fingerprints on the slide. Write the Laboratory Serial No. with a diamond marker on one end of the slide.
- Break a broomstick (wooden/ bamboo) in two halves with uneven ends.
- Using the jagged ends of the broken stick, select and pick up the larger, yellow, purulent portion and transfer them onto the slide. Use a separate stick for each sample.
- With one of the sticks, spread the sputum evenly to cover 2/3 of the central portion of the slide, using a continuous, rotational movement.
- Place the applicators (broken wooden sticks) into a bucket containing disinfectant.

Document Type: Standard operative procedure	Issue No.	Issue Date	20/06/2022
Document No.: AIIMS/Rajkot/Micro/ SOP/3		Page No:	10 of 19



SOP for sputum smear preparation and ZN staining

- Place the smeared slide on the drying rack and replace the lid of the sputum container.
- The size of the smear should be approximately 3x2 cm. The smear should neither be too thick nor too thin.
- A good smear is
 - Made from mucopurulent sputum
 - Spread evenly
 - 3 cm × 2 cm in size
 - Not too thick
 - Thin enough to read newsprint through
 - Air-dried before being fixed
- A bad smear is
 - Made from saliva
 - Too small
 - Not in the centre of slide
 - Too big
 - Too thick
 - Uneven
 - Too thin
- Let the slides air-dry for 15–30 minutes. Do not use flame for drying
- Fix the dry slide by heating it briefly
- After the slide is dry, hold the slide using forceps with the smeared side facing upwards.
- Pass the slide over the flame 3– 5 times, for 3–4 seconds each time.
- Do not heat the slide for too long or keep it stationary over the flame.
- Place the slide in the clean slide tray.

4.3 Smear staining

- Place the slides on the staining rack with the smeared sides facing upwards
- Pour 1% carbol fuchsin to cover the entire surface of the slide
- Do not allow the carbol fuchsin to drain off the slide.
- Do not leave the carbol fuchsin on the slide for a long time or it will dry.
- Add more carbon fuchsin if required
- Heat the slides from underneath until vapors start rising approx. 5 mins
- Gently rinse the slides with tap water to remove excess carbol fuchsin stain
- Decolorize the stained slides by pouring 25% sulphuric acid onto the slides and let it stand for 2–4 minutes.
- The red color should have almost completely disappeared from the smears.
- Lightly wash away sulphuric acid and excess stain with tap water

Document Type: Standard operative procedure	Issue No.	Issue Date	20/06/2022
Document No.: AIIMS/Rajkot/Micro/ SOP/3		Page No:	11 of 19



making sure that the smear itself is not washed away.

- Tilt the slide to drain off the water.
- Counterstain with 0.1% methylene blue for 30 seconds.
- Gently rinse the slide with tap water
- Tilt the slide to drain off the water and allow to air dry'

5.0 REFERENCES:

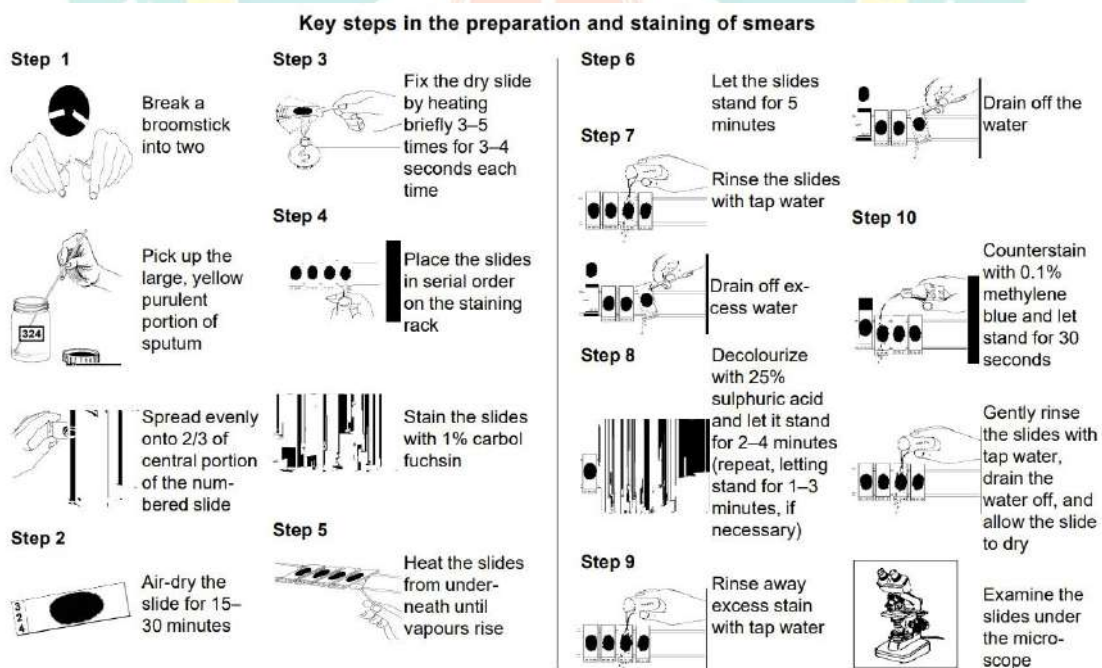
- 5.1 Handbook for Specimen collection and handling in Microbiology 2 nd Edition CDC Atlanta
- 5.2 Standard operating procedures Bacteriology -ICMR New Delhi India 2 nd Edition 2019
- 5.3 Manual for Laboratory technicians, Central TB Division, Directorate General of health services (NTEP)



6.0 APPENDICES AND FORMS:

NA

7.0 VALIDITY STATEMENT

This SOP is valid for one year from the date of issue.



	All India Institute of Medical Sciences, Rajkot Department of Clinical Microbiology	
	SOP for sputum smear microscopy	

Proprietary statement: This document contains information. It is provided to institution staff under AIIMS Rajkot only. Do not share verbally or otherwise without written consent of the Prof & Head, Department of Clinical Microbiology, AIIMS, Rajkot.

DOC. NO.	TITLE	ISSUE NO.
AIIMS/Rajkot/Micro/SOP/4	SOP for sputum smear microscopy	1.0
Effective Date:20/06/2022		

Name	Designation	Function	Signature
Dr Mayuri Bhise	Assistant Professor	Preparation	
Dr Ashwini Agrawal	Prof & Head	Review and Approval	

Distribution List:
DMC Laboratory

REVISION SUMMARY			
Revision No.	Issue No.	Effective Date	Revision History
0	1.0	20/06/2022	NA

Document Type: Standard operative procedure	Issue No.	Issue Date	20/06/2022
Document No.: AIIMS/Rajkot/Micro/ SOP/4	Page No: 13 of 19		



SOP for sputum smear microscopy

1.0 PURPOSE AND SCOPE

Purpose of this SOP is to describe the process involved in microscopy of sputum sample

This SOP is applied to the microscopy of Sputum sample for AFB stain

2.0 DEFINATIONS AND ABBREVIATIONS

2.1 SOP: Standard Operating Procedure

3.0 RESPONSIBILITY

It is a responsibility of Laboratory Technician to perform the microscopy of sputum sample

4.0 MICROSCOPY

4.1 Examine the slide under the microscope

- Put one drop of immersion oil on the left edge of the stained smear.
- Bring the slide into focus with the x40, then the x100 lens.
- Systematically examine at least 100 fields
- The examination must be systematic and standardized
- Count the number of AFB and record the results as: 3+, 2+, 1+, scanty, or negative, as given in Table.
- If 1–9 bacilli are found in 100 oil immersion fields, examine another 100 oil immersion fields

Examination	Result	Grading
More than 10 AFB per oil immersion field	Positive	3+
1-10 AFB per oil immersion field	Positive	2+
10-99 AFB per 100 oil immersion field	Positive	1+
1-9 AFB per 100 oil immersion field	Scanty	Record exact number seen
No AFB in 100 oil immersion field	Negative	0

4.2 Precautions to prevent false-positive sputum results

- Always use new, unscratched slides
- Always use filtered carbol fuchsin
- Do not allow the carbol fuchsin to dry during staining
- Decolorize adequately with sulphuric acid
- Make sure there are no food particles or fibers in the sputum sample.



SOP for sputum smear microscopy

- Never allow the oil immersion applicator to touch a slide
- Never allow the oil immersion lens to touch a slide
- Label sputum containers, slides, and Laboratory Forms accurately
- Cross-check the number on the Laboratory Form and sputum container before recording
- Record and report results accurately

4.3 Precautions to prevent false-negative sputum results

- Make sure the sample contains sputum, not just saliva
- Make sure there is enough sputum (at least 2 ml)
- Select thick, purulent particles to make the smear
- Prepare smears correctly—not too thick, too thin or too little material
- Fix the slide for the correct length of time, not too short or too long
- Stain with carbol fuchsin for the full 5 minutes
- Do not decolorize with sulphuric acid too intensively
- Examine every smear for at least five minutes before recording it as negative
- Label the sputum containers, slides and Laboratory Forms carefully
- Cross check the number on the Laboratory Form and sputum container before recording
- Record and report result accurately

4.4 Clean and store the microscope and slides

- Clean the slides with xylene and preserve them in the appropriate slide box for the supervisor to review.
- Xylene will not damage the slide or stain, facilitating neat and clean slide storage.
- Pour a small amount (2–3 ml) of xylene onto the stained side of the slide, and then allow it to air dry.
- Do not clean too vigorously or the stain itself may come off.
- All positive slides should be preserved in one box, and negative slides in a different box.
- Clean the x100 microscope lens with lens paper
- Keep the microscope and lenses clean.
- Clean the microscope with lens paper before and after use.
- Do not leave immersion oil on the surface of the immersion lens.
- Never use spirit or alcohol to clean the lenses, as these can damage them.
- Never let the oil immersion lens touch the smear.
- Use the fine focusing knob only while using the oil immersion lens.
- All the lenses should be cleaned with dry lens paper.

Document Type: Standard operative procedure	Issue No.	Issue Date	20/06/2022
Document No.: AIIMS/Rajkot/Micro/ SOP/4		Page No:	15 of 19



SOP for sputum smear microscopy

- Lens paper can be moistened with xylene if necessary. Do not clean lenses with an ordinary cloth.

5.0 REFERENCES:

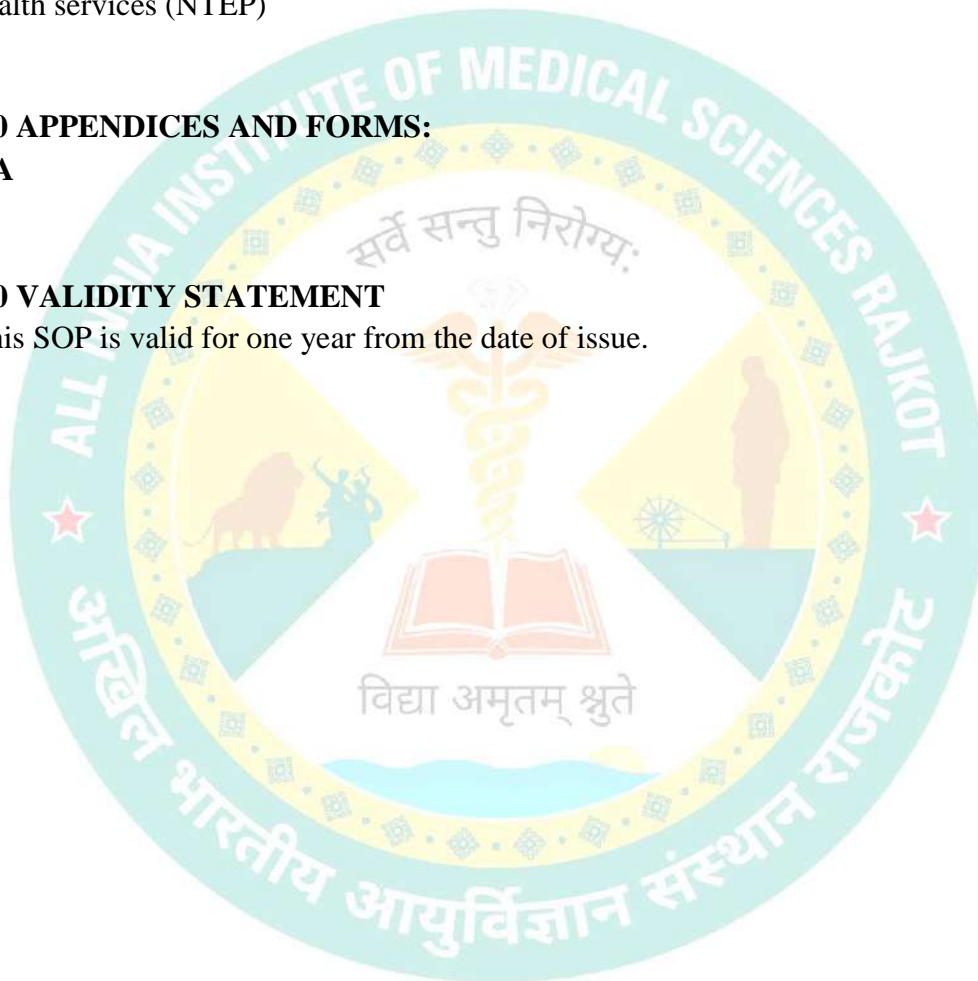
- 5.1 Handbook for Specimen collection and handling in Microbiology 2 nd Edition CDC Atlanta
- 5.2 Standard operating procedures Bacteriology -ICMR New Delhi India 2 nd Edition 2019
- 5.3 Manual for Laboratory technicians, Central TB Division, Directorate General of health services (NTEP)

6.0 APPENDICES AND FORMS:

NA

7.0 VALIDITY STATEMENT

This SOP is valid for one year from the date of issue.



Document Type: Standard operative procedure	Issue No.	Issue Date	20/06/2022
Document No.: AIIMS/Rajkot/Micro/ SOP/4		Page No:	16 of 19



**All India Institute of Medical Sciences, Rajkot
Department of Clinical Microbiology**

SOP on Sample storage and transport



Proprietary statement: This document contains information. It is provided to institution staff under AIIMS Rajkot only. Do not share verbally or otherwise without written consent of the Prof & Head, Department of Clinical Microbiology, AIIMS, Rajkot.

DOC. NO.	TITLE	ISSUE NO.
AIIMS/Rajkot/Micro/ SOP/5	SOP on Sample storage and transport	1.0
Effective Date:20/06/2022		

Name	Designation	Function	Signature
Dr Mayuri Bhise	Assistant Professor	Preparation	
Dr Ashwini Agrawal	Prof & Head	Review and Approval	

Distribution List:
DMC Laboratory

REVISION SUMMARY			
Revision No.	Issue No.	Effective Date	Revision History
0	1.0	20/06/2022	NA

Document Type: Standard operative procedure	Issue No.	Issue Date	20/06/2022
Document No.: AIIMS/Rajkot/Micro/ SOP/5	Page No: 17 of 19		



1 INTRODUCTION

This standard operating procedure defines the procedures to be followed to store and transport the sputum samples

2. PURPOSE

To define methods for storage and transport of sputum samples after testing is performed.

3. SCOPE

The DMC Laboratory staff will follow this SOP.

4. RESPONSIBILITY

- It is the responsibility of the Laboratory staff (DMC) to follow this SOP for storage of the samples after sputum smear microscopy is over.

5. STORAGE AND TRANSPORT:

5.1. Sputum sample

- Store the sputum samples in leak proof container with proper labels in refrigerator
- Keep the tested positive sputum smear samples in a separate box with appropriate label.

5.2 Positive Samples

- Transfer the positive samples in falcon tube in transport chamber by cold chain in Thermocol box to City TB centre

5.3 Negative Samples

- Transfer the clinically suspected sputum smear negative samples in transport chamber by cold chain in Thermocol box to City TB centre

5.4 Transport of samples in cold chain

- Transfer the sputum sample from wide mouth container to falcon tube or if already collected in falcon tube, tightly seal and wrap the falcon tube with parafilm tape.
- Label the falcon tube with name of patient, date of sample collection, specimen type (A/B) and name of DMC

Document Type: Standard operative procedure	Issue No.	Issue Date	20/06/2022
Document No.: AIIMS/Rajkot/Micro/ SOP/5		Page No:	18 of 19



- Keep one prefrozen ice gel pack in Thermocol box.
- Keep falcon tubes in zip log bag_in box.
- Keep another prefrozen ice gel pack on falcon tubes
- Keep test requisition form in polythene bag and keep in box
- Wrap the box with brown tape/ adhesive tape with Biohazard logo and address

6 REFERENCES:

6.1 Technical and operational guidelines for Tuberculosis control October 2005

6.2 Manual for Laboratory Technicians Central TB Division May 1999

7 APPENDICES AND FORMS:

NA

8 VALIDITY STATEMENT

This SOP is valid for one year from the date of issue.

Document Type: Standard operative procedure	Issue No.	Issue Date	20/06/2022
Document No.: AIIMS/Rajkot/Micro/ SOP/5		Page No: 19 of 19	