# Aim

To describe the procedure to presumptively identify *Mycobacterium tuberculosis* (TB) infection by detection of acid fast bacilli (AFB) in gastric aspirate or sputum specimens.

# Principle

Tuberculosis is caused by the organism *Mycobacterium tuberculosis.* The lung is the most frequent site of infection. Infection can be presumptively identified by detection of acid fast bacilli in sputum or gastric aspirate specimens. Early morning gastric aspirates consist of sputum swallowed overnight and are useful in young children who cannot easily cough up sputum.

Ziehl-Neelsen (ZN) stain renders mycobacteria pink as a result of the mycolic acid in their cell wall. Most other bacteria have a lower fat content and hence the carbol fuchsin is washed away by the acid-alcohol, resulting in their being stained blue by the counterstain (methylene blue). Hence, mycobacteria are referred to as being “acid fast”.

# Method

## Specimen collection

Specimens should be collected into wide-mouthed leak-proof sterile plastic pots.

Three sequential day gastric aspirates (young children) or sputum specimens (older children) should be obtained for investigation of suspected TB infection.

## Specimen transport and storage

Specimens should ideally be stored and transported in sealed plastic bags. Laboratory processing should occur as soon as possible after specimen collection. Specimens should be refrigerated if delays in processing over two hours are unavoidable.

## Specimen processing

### Reception

Log the specimen in the appropriate specimen book and assign a specimen number.

The specimen should also be logged in for molecular detection of *Mycobacterium tuberculosis* (GeneXpert, SOP MOL-002).

### Microscopic examination

Preparation of the smear must be done in the Class II biosafety cabinet.

Label a clean glass slide with the patient code specimen number.

Using a bamboo/wooden stick, select a blood-stained or purulent portion of the specimen.

Smear the specimen in the centre of the slide (2cm x 1cm).

Discard the wooden stick into the 1% Virkon discard container.

Leave the smears in the cabinet to dry for 15-30 minutes.

Follow the ZN stain procedure (SOP MID-001)

Examine systematically using the 100x oil objective lens.

# Interpretation

Acid fast bacilli (AFB) are slender rods which vary from 0.5-10 µm in length and stain red. Some may appear beaded. TB bacilli may occur singly, as v-shaped forms, or as clumps of bacilli.

All other organisms and background material stain blue (if methylene blue counterstain is used).

## Reporting

Report the absence or quantity of AFB seen as outlined in the table.

|  |  |
| --- | --- |
| **Number of AFB****(x100)** | **Report** |
| No AFB in 100 fields  | AFB negative |
| 1 - 9 AFB in 100 fields | +/- AFB(report number seen) |
| 10 - 99 AFB in 100 field  | 1+ AFB |
| 1 - 10 AFB per field(check 50 fields) | 2+ AFB |
| >10 AFB per field(check 20 fields) | 3+ AFB |

# Quality assurance

ZN stain reagents should be quality controlled according to SOP MID-001.

# Limitations

Spores and artefacts may stain with Ziehl-Neelsen’s stain and appear as positive to untrained eyes.

Poor quality specimens may yield false negative results.

# References

1. Health Protection Agency, UK SOP B40: Investigation of Specimens for Mycobacterium species (Issue 6.0; August 2012).
2. Cheesbrough, M. District Laboratory Practice in Tropical Countries, Part 2. 2nd Edition Update (2006). Cambridge University Press.
3. Standard Operating Procedures from LOMWRU, SMRU and AHC.

# Synopsis / Bench aid

Not applicable

# Risk assessment

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| **COSHH risk assessment - University of Oxford COSHH Assessment Form** |
| **Description of procedure**Microscopy of sputum or gastric aspirates to identify acid fast bacilli | **Substances used**ZN stain reagents (carbol fuchsin, 3% hydrochloric acid in isopropyl alcohol, methylene blue) |
| **Quantities of chemicals used**Small | **Frequency of SOP use**Daily |
| **Hazards identified**1. Potentially infectious material in sample 2. Stain reagents can cause burns, are harmful by inhalation, skin contact and ingestion | **Could a less hazardous substance be used instead?** No |
| **What measures have you taken to control risk?** 1. Training in good laboratory practices (GLP)2. Appropriate PPE (lab coat, gloves, eye protection)3. Use of biosafety cabinet for preparation of slides3. Small amounts of stain are in use only (stocks kept in a closed chemical cupboard)4. Laboratory is well ventilated |
| **Checks on control measures**Observation and supervision by senior staff |
| **Is health surveillance required?**No | **Training requirements:**GLP |
| **Emergency procedures**:1. Report all incidents to Safety Adviser2. Use eyewash for splashes3. Clean up spills using 1% Virkon or chemical spill kit | **Waste disposal procedures**:1. Sharps discarded into appropriate rigid containers for incineration2. Infectious waste discarded into autoclave bags or 1% Virkon solution prior to autoclaving and subsequent incineration3. All stains are washed down the sink with copious amounts of water |