ACID FAST STAINING

1) conc. Carbol fuchsin
2) intermittent heating (5-7 min)
3)wash
4) 20% sulphuric acid (2-3 min)
5)wash
6) methylene blue (3min)
7)wash & keep for drying
8)focus on oil immersion

~ Primary stain: carbol fuchsin
~ Counter stain : methylene blue
~ Mordant : phenol
~ Decoloriser : 20% sulphuric acid

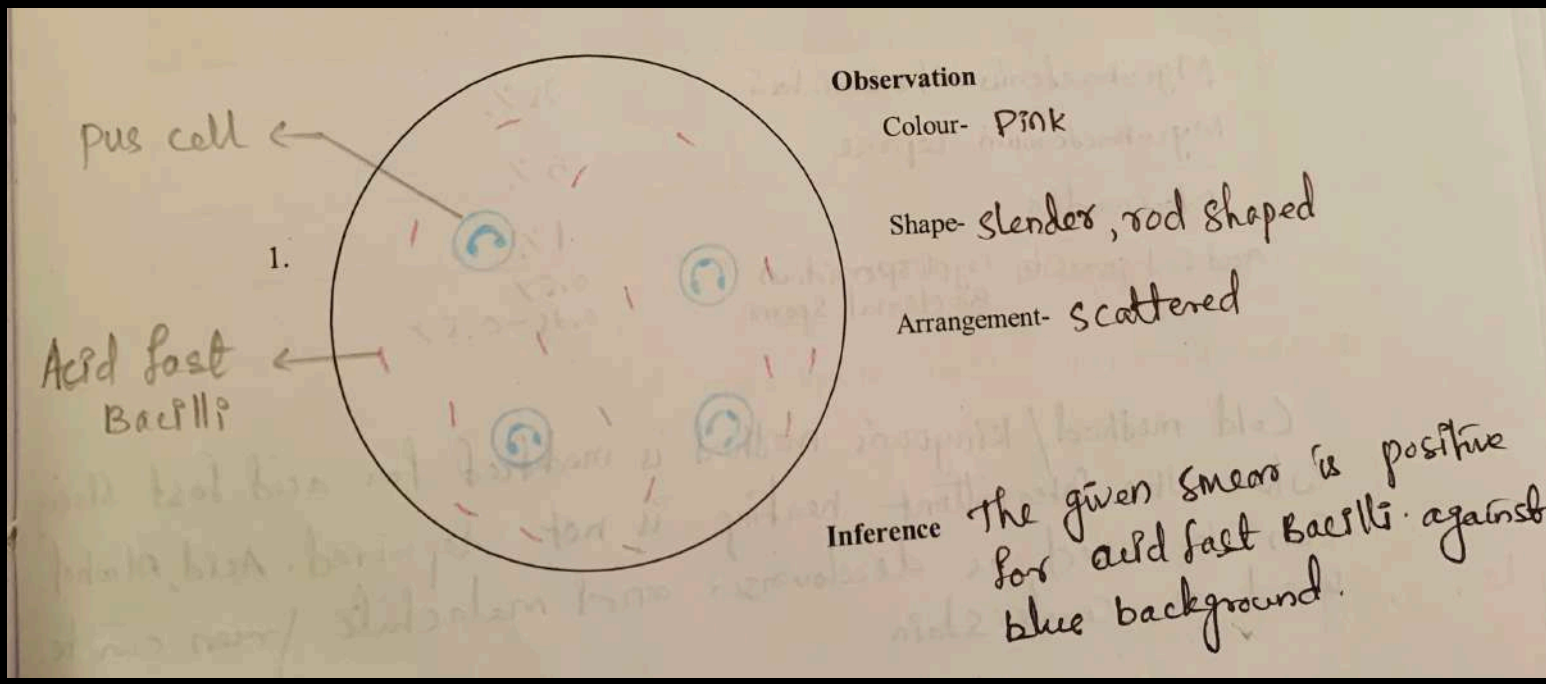


Table 63.3: RNTCP guidelines for grading of ZN stained sputum smears.

No. of AFB seen	OIF to be screened	Grading	Result
No AFB in 100 OIF	100	0	Negative
1–9/ 100 OIF	100	Scanty*	Positive
10–99/100 OIF	100	1+	Positive
1–10/ OIF	50	2+	Positive
>10/ OIF	20	3+	Positive

RNTCP grading is useful for:

- ❑ Monitoring the treatment response of the patients
- ❑ Assessing the severity of disease
- ❑ Assessing the infectiousness of the patient: Higher the grade more is the infectiousness. Smear negative patients (<10,000 bacilli/mL of sputum) are less infectious.

Other organisms giving AF positive:

Table 3.3.2: Acid-fast organisms/structures and percentage of sulfuric acid suitable for staining.

Acid-fast organisms/structures	Sulfuric acid (%) needed for decolorization
Mycobacterium tuberculosis	25%
Mycobacterium leprae	5%
Nocardia	1%
Acid-fast parasites such as *Cryptosporidium, Cyclospora, Cystoisospora,* Microsporidia*	0.5%
Bacterial spore	0.25–0.5%

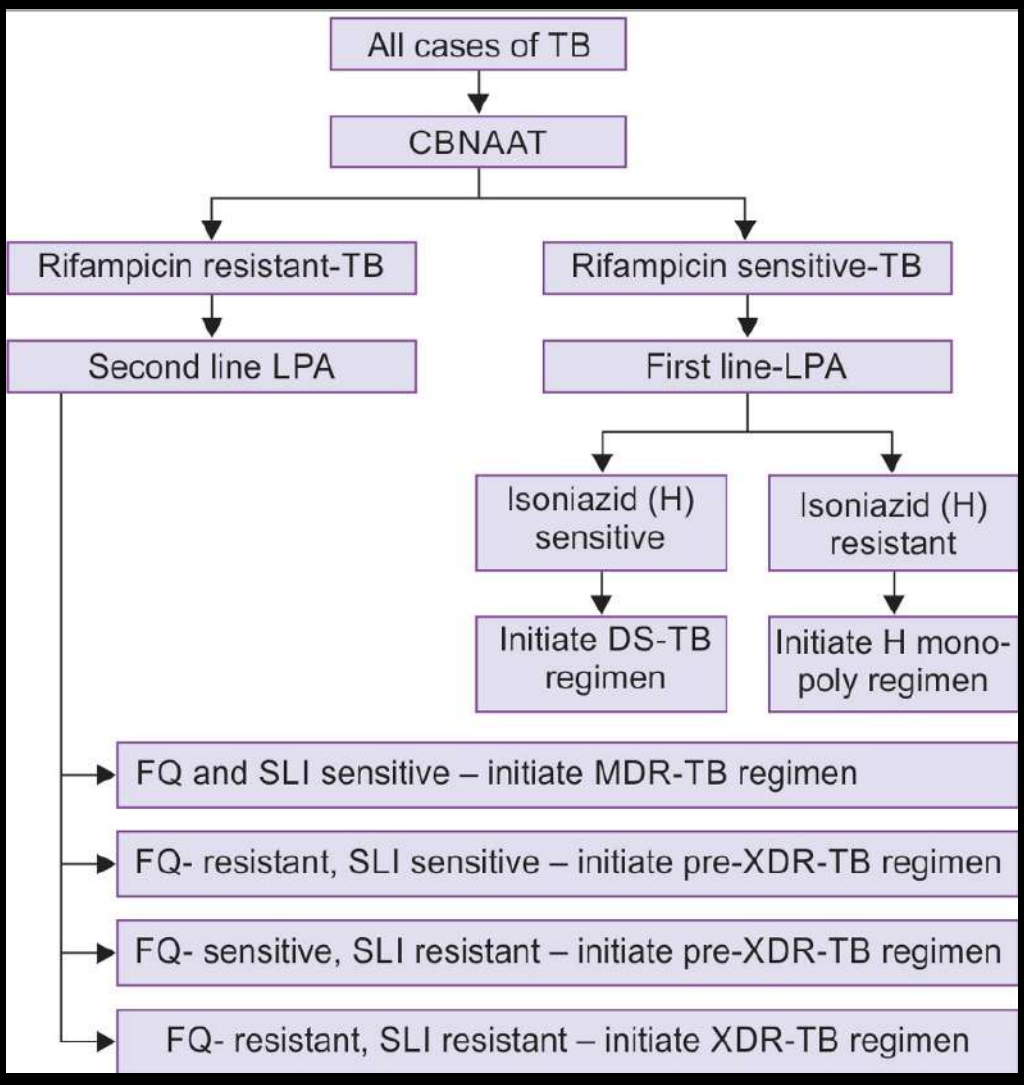
Other staining method:

Fluorescence Staining

It is a fluorescent staining technique, uses auramine-phenol solution (for 7–10 min) as primary stain, 0.5% acid alcohol (for 2 min, twice) as decolorizer and 0.1% potassium permanganate (for 30 sec) as counter stain. Then the slide is examined under fluorescent LED (light-emitting diode) microscope.

- The bacilli appear brilliant yellow against the dark background (Fig. 63.3B)
- Smears are screened by using 20× or 25× objective, hence can be screened faster (2 min for 100 fields)
- It is more sensitive than ZN staining and has been the recommended screening method by RNTCP
- However, artifacts may confound with the interpretation. Hence the reading should be taken by an expert.

Management of a patient with sputum AFB positive:



Modifications of Acid-Fast Staining

Hot method (Ziehl–Neelsen technique) is the most commonly done acid-fast staining technique. Other modifications are as follows:

- **Cold method (Kinyoun's method):** It is modification, where the intermittent heating is not required (described in Chapter 63)
- Acid-alcohol can be used as decolorizer alternatively
- Malachite green can be used as counter stain
- The concentration of sulfuric acid may vary depending on the acid-fastness of the structure to be demonstrated. More the content of mycolic acid in the cell wall, more is the acid-fastness, hence more is the percentage of sulfuric acid required for decolorization (Table 3.3.2).

Kinyoun's Cold Acid-fast Staining

It differs from ZN stain in that—(i) heating is not required, (ii) phenol concentration in carbol fuchsin is increased, and (iii) duration of carbol fuchsin staining is more.

~ why HIV patients are suggested to do CBNAAT directly instead of sputum examination ?

1.High rate of false negatives with sputum examination: as the sputum may not contain enough viable TB bacilli for detection.

2.CBNAAT is more sensitive than sputum examination and can detect TB at an earlier stage.

3. CBNAAT provides results within a few hours, whereas results from sputum examination can take several days.

4.Simplicity: CBNAAT is a simple and easy-to-use test that can be performed at the point of care, whereas sputum examination requires laboratory facilities and specialized training.