

SOP for Microscopic Examination of Milk Samples

I. Sample Preparation

1. Thaw frozen negative milk samples overnight at 4°C;
2. Preparation of Simulated milk samples: negative milk samples + BCG;
3. Filtration: Frozen milk samples affect the enrichment efficiency. Filtration reduces impurities, which improves enrichment efficiency without affecting the test results;
4. Aliquot the samples and keep them for testing.

II. Sample Pre-treatment

1. Add an equal volume of digestion solution to each tube of sample and digest for 20 min; the digestion time should not be too long or too short;
2. Centrifuge for 5 min;

III. Staining and Microscopic Examination

1. Primary staining: Add carbol-fuchsin staining solution (Solution A) dropwise to cover the entire smear; stain at 70°C for 10–20 s;
2. Decolorization: Rinse off excess stain with running water; add 3% hydrochloric acid-alcohol (Solution B) dropwise to decolorize for 10–30 s, then rinse with running water until the red color is almost completely

removed and the smear appears light pink;

3. Counterstaining: Add methylene blue staining solution (Solution C)

dropwise and counterstain for 30–60 s; rinse with water and drain;

4. Drying: Air-dry naturally or dry with an electric heater;

5. Examine under a microscope.

