IFU

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Instructions for Use

ACID-FAST STAIN KIT

Cat. no. AF900	Acid Fast Stain Kit with Methylene Blue Counterstain
Cat. no. 483KB	Acid Fast Stain Kit with Brilliant Green Counterstain
Cat. no. CF008	Carbol Fuchsin, 250ml
Cat. no. AA008	Acid Alcohol, 250ml
Cat. no. MB008	Methylene Blue, 250ml
Cat. no. BG008	Brilliant Green, 250ml

INTENDED USE

The Acid-Fast Stain Kit is used as a differential method to detect the difference between those organisms which decolorize with treatment of acid alcohol, and those that do not, such as the mycobacteria.

SUMMARY

Based on the Kinyoun modified staining technique, the acid-fast stain is a very useful tool for differentiating acid-fast bacteria (AFB), such as *Mycobacterium* spp., from those that do not resist the acidified decolorization step. The mechanism for acid-fastness is not clearly understood, however it is suspected that it is determined by selective permeability of the cytoplasmic membrane. The brilliance of red coloration is due to retention of the carbol fuchsin dye within the cell membrane. Should the cell be mechanically disrupted, its acid-fast property is lost.

After staining, decolorizing, and counterstaining, any acid-fast bacteria present are brilliant pink to red in color, and other bacteria present are seen as blue (methylene blue counterstain) or green (brilliant green counterstain). No heating or time dependent decolorization steps are required.

REAGENT FORMULA

	CAS No.	PRECAUTIONS
Carbol Fuchsin Stain, Cat. no. CF008		**Caustic, Poison**
Basic Fuchsin	58969-01-0	
Phenol, Liquid	108-95-2	
Ethyl Alcohol	64-17-5	
Isopropanol	67-63-0	
Methyl alcohol	67-56-1	
Dimethyl sulfoxide	67-68-5	

Deionized Water		
Acid Alcohol Decolorizer, Cat. no. AA008		**Flammable, Poison**
Hydrochloric Acid	7647-01-0	
Ethanol	64-17-5	
Isopropanol	67-63-0	
Methyl alcohol	67-56-1	
Methylene Blue Counterstain, Cat. no. MB008		
Methylene Blue	61-73-4	
Deionized Water		
Brilliant Green (optional counterstain), Cat. no. BG008		
Brilliant Green	633-03-4	
Deionized Water		

STORAGE

Storage: Upon receipt store at 2-30ºC. Products should not be used if there are any signs of deterioration or if the expiration date has passed.

After prolonged storage, phenol may be seen to separate from the carbol fuchsin stain. Shake the tightly capped bottle well to remix before use.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual Universal Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Specimen Collection: Consult listed references for information on specimen collection.[2-4]

Specimen Preparation, Sputum: Using a portion of blood, caseous, or purulent material, spread a loop full of the specimen over a small area of clean, dry glass slide in a thin smear. When dry, heat fix and proceed with staining.

Urine and Gastric Samples: To concentrate organism, centrifuge 10-50ml of the specimen. Follow smear preparation

instructions as stated above using the resulting sediment.

Histology Sections: Prepare the sample following published techniques.

Staining Procedure:

Note: All times are approximate. The user may wish to adjust staining times as appropriate to achieve desired results.

1. Flood the heat fixed smear with the carbol fuchsin stain for 3-6 minutes. No heat is required.

2. Rinse slide with water.

3. Using the acid alcohol decolorizer, direct a gentle stream on the smear. When no more color is seen coming off, stop decolorizing.

4. Rinse with water again.

5. Flood the smear with the methylene blue (or brilliant green, if preferred) counterstain for 2 minutes.

6. Rinse with water, and allow slide to dry or blot gently with absorbent paper.

Histology Sections:

1. In the normal manner, section the paraffin tissue blocks.

2. Process the sections through deparaffinization.

3. Rehydrate to water.

4. Stain slides individually as described within the staining procedure above, with the exception of adding occasional gentle agitation to the staining steps.

5. Finish processing the slides for permanent mounting as follows: Acetone for 10 seconds, acetone:xylene (1:1) for 1 minute, xylene for 2 minutes, and mount the specimen.

INTERPRETATION OF RESULTS

The appearance of bright red stained bodies that are slender, slightly curved, long or short rods, and sometimes granular or beaded are typically acid-fast bacteria (AFB). Some atypical forms appear thick or diptheriod, sometimes coccoid, or are very long. Other non-acid-fast organisms will stain blue (or green when using brilliant green counterstain), as will background material.

LIMITATIONS

If the cell walls of the bacteria in the stained sample are disrupted, the Acid-Fast Stain will yield false-negatives. Therefore, care must be taken to avoid this type of damage. In stained preparations, observation of acid-fast bacilli is only presumptive evidence for the presence of *Mycobacterium* organisms. Cultures for *Mycobacterium* should also be carried out, in conjunction with other biochemical tests to identify the organism. Tap water used for rinsing slides may contain mycobacteria, which could produce false-positives.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, swabs, applicator sticks, other culture media, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

USER QUALITY CONTROL

It is recommended that each new lot or shipment of reagent be tested with known positive and negative controls and retested each day of use hereafter.[1]

PHYSICAL APPEARANCE

- Carbol Fuchsin Stain should appear deep red in color.
- Acid Alcohol should appear colorless.
- Methylene Blue should appear deep royal blue in color.
- Brilliant Green should appear bright green in color.
- All reagents should have no precipitate present.

Acid-Fast Stain Kit with Methylene Blue Counterstain (Cat. no. AF900)

PACKAGING

Acid-Fast Stain Kit

Cat. no. AF900 contains 8oz. of each (with dropper caps):

- Carbol Fuchsin
- Acid Alcohol
- Methylene Blue

Cat. no. 483KB contains 8oz. of each (with dropper caps):

- Carbol Fuchsin
- Acid Alcohol
- Brilliant Green

REFERENCES

1. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

2. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.

3. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.

4. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.

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