IFU

Instructions for Use

Q-SLIDE™ AFB

Cat. no. Z301	Q-Slide™ AFB	5 slides/box

INTENDED USE

Hardy Diagnostics Q-Slide™ AFB is used to qualify reagents and methods used in carbol fuchsin (Ziehl-Neelsen and Kinyoun methods) and flourescent acid-fast staining procedures.

SUMMARY

An acid-fast stain is performed on any type of clinical specimen or recovered organism that is suspect of containing mycobacteria. Results obtained in an acid-fast smear can be critical to the early diagnosis of mycobacterial infection due to their slow rate of growth. Mycobacteria can be difficult to stain due to the lipid content of their cell wall. It is this cell wall structure that dictates the acid-fast properties of the cell. Hardy Diagnostics Q-Slide™ AFB is used to ensure the quality of reagents and efficacy of acid-fast staining procedures. The format of the control slides allows for simultaneous staining of positive and negative control organisms, and up to four patient specimens. Fixed smears of *Mycobacterium gordonae* and *Corynebacterium* spp. serve as the positive and negative controls, respectively.

Acid-fast stain procedures typically consist of three staining steps; primary stain, decolorization, and counterstain. The primary stain (Ziehl-Neelsen, Kinyoun, Auramine O, or Auramine-Rhodamine) dictates how the acid-fast positive organisms appear. Ziehl-Neelsen and Kinyoun stained cells appear red in color. Auramine O and Auramine-Rhodamine stained cells fluoresce yellow to orange. Decolorization with acid alcohol removes the primary stain from acid-fast negative cell walls. The lipid content in the walls of acid-fast bacteria resists decolorization by the acid alcohol. Cells that are decolorized will pick up the color of the counterstain (methylene blue, brilliant green, potassium permanganate, or acridine orange).

STORAGE AND SHELF LIFE

Upon receipt store at 15-30ºC. in the original container. Do not use if there are any signs of deterioration. Protect from moisture and dust.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

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

Caution: Always use appropriate biohazard techniques and work in a biological safety cabinet when materials are suspect to contain mycobacteria.

SMEAR PREPARATION[1]

Concentrated or Unconcentrated Specimens (not CSF)

1. Vortex or thoroughly mix specimen.
2. Transfer a representative portion of the specimen to the slide using a loop, applicator stick or pipette.
3. Smear the specimen within the test area of the slide (space number 3, 4, 5, or 6).
4. Allow the smear to air dry.
5. Fix according to one of the procedures below.

CSF Specimens

1. Vortex CSF sediment thoroughly.
2. Place one drop within the test area of the slide (space number 3, 4, 5, or 6).
3. Allow the drop to air dry.
4. Repeat steps 2 and 3 two more times, placing the new drop directly over the previous drop.
5. Fix the smear by one of the methods below.

Heat Fixing*

1. After the smear has air dried, pass the slide through a flame or hold next to an incinerator for 5 to 10 seconds. Take care not to overheat the slides. Allow slide to cool prior to staining.
2. Alternatively, leave the slide on an electric slide warmer (65-75ºC.) for at least 2 hours. The slide may be left on the warmer overnight.

Methanol Fixing

1. Air dry the smear, and place a few drops of methanol over the smear for 1 minute. Drain off excess without rinsing. Allow to air dry.

STAIN PROCEDURE[1]

Follow the manufacture's instructions for performing the stain. The following is provided as a reference.

Ziehl-Neelsen (Hot Carbol Fuchsin):

Paper Strip Method

1. Place a filter paper strip over the smear on the slide.

2. Flood paper strip with Ziehl-Neelsen carbol fuchsin stain.
3. Using a bunsen burner, or electric staining rack, heat slide slowly until it is steaming.
4. Let the slide stand for 5 minutes. Add more stain if the smear begins to dry.
5. Remove the paper from the slide and discard the strip.
6. Rinse the slide thoroughly with water.
7. Flood the slide with acid alcohol decolorizer, and let stand for 2 minutes.
8. Rinse the slide with water, and drain excess.
9. Flood slide with counterstain (methylene blue or brilliant green) and let stand for 20 to 30 seconds.
10. Rinse slide thoroughly, drain excess and allow to air dry. Do not blot.

Direct Method

1. Flood slide with Ziehl-Neelsen carbol fuchsin stain.
2. Using a bunsen burner, or electric staining rack, heat slide slowly until it is steaming.
3. Maintain steaming for 3 to 5 minutes using low or intermittent heat.
4. Cool the slide and rinse briefly with water.**
5. Decolorize with acid alcohol until no more color drains from the slide.
6. Rinse the slide with water, and drain excess.
7. Flood slide with counterstain (methylene blue or brilliant green) and let stand for 20 to 30 seconds.
8. Rinse slide thoroughly, drain excess and allow to air dry.

Kinyoun's Carbol Fuchsin (Cold Carbol Fuchsin):

Paper Strip Method

1. Place a filter paper strip over the smear on the slide.
2. Flood paper strip with Kinyoun's carbol fuchsin stain.
3. Let the slide stand for 5 minutes. Do not heat.
4. Remove the paper from the slide and discard the strip.
5. Rinse the slide thoroughly with water.
6. Flood the slide with acid alcohol decolorizer, and let stand for 2 minutes.
7. Rinse the slide with water, and drain excess.
8. Flood slide with counterstain (methylene blue or brilliant green) and let stand for 1 to 2 minutes.
9. Rinse slide thoroughly, drain excess and allow to air dry.

Direct Method

1. Flood slide with Kinyoun's carbol fuchsin stain.
2. Let the slide stand for 2 minutes.
3. Rinse briefly with water.
4. Decolorize with acid alcohol until no more color drains from the slide.
5. Rinse the slide with water, and drain excess.
6. Flood slide with counterstain (methylene blue or brilliant green) and let stand for 20 to 30 seconds.
7. Rinse slide thoroughly, drain excess and allow to air dry.

FLUOROCHROME ACID-FAST STAINS[1]

1. Flood the slide with fluorochrome stain (Auramine O or Auramine-Rhodamine).
2. Allow the smear to stain for 15 minutes, making sure the smear is covered in stain at all times. Do not heat this stain or use paper strips to cover the smear.
3. Rinse the slide with water, and drain excess.
4. Flood the slide with acid alcohol decolorizer, and let stand for 2 minutes.
5. Rinse the slide with water, and drain excess.
6. Flood slide with counterstain (potassium permanganate, or acridine orange) and let stand for 2 minutes.***
7. Rinse the slide with water, and drain excess.
8. Allow to air dry. Do not blot.

MICROSCOPIC EVALUATION

1. Using a low power objective, locate and focus on the cells.
2. Use the oil immersion lens (100x) to determine acid fast reaction, cell morphology and arrangement.

* Heat fixing may not kill all mycobacteria. Slides should be treated as biohazardous material, and discarded as such.

** Rinsing a hot slide with cold water may crack the slide, take care to allow the slide to cool prior to rinsing with water.

*** Counterstain timing is critical with potassium permanganate counterstain. Extended time may quench the fluorescence of acid-fast organisms.

INTERPRETATION OF RESULTS

Expected results with carbol fuchsin based staining protocols:

Stain	**Acid-Fast Positive Organism Appearance**	**Acid-Fast Negative Organism Appearance**
Ziehl-Neelsen Carbol Fuchsin w/Methylene Blue	Cells appear red	Cells appear blue
Ziehl-Neelsen Carbol Fuchsin w/Brilliant Green	Cells appear red	Cells appear green
Kinyoun's Carbol Fuchsin w/Methylene Blue	Cells appear red	Cells appear blue
Kinyoun's Carbol Fuchsin w/Brilliant Green	Cells appear red	Cells appear green

Cells within the positive control circle should appear as irregular rods that stain red. The cells inside the negative control circle should appear as irregular rods that stain blue or green depending on which counterstain is used.

Expected results with fluorescent based staining protocols:

Stain	**Acid-Fast Positive Organism Appearance**	**Acid-Fast Negative Organism Appearance**
Flurochrome (Auramine O or Auramine-Rhodamine)	Cells fluoresce yellow to orange	No fluorescence

Cells within the positive control circle should appear as irregular, yellow to orange fluorescent rods. The cells inside the negative control circle should appear as irregular rods that do not fluoresce.

LIMITATIONS OF THE PROCEDURE

Do not use the acid-fast smear in place of mycobacterial culture methods.[1]

Organisms other than mycobacteria may exhibit some acid-fastness.[1]

Rapidly growing mycobacteria may give false-negative acid-fast results.[1]

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological equipment such as microscopes, inoculating loops, incinerators, acid-fast stain reagents, methanol, etc., are not provided.

QUALITY CONTROL

Appearance of the positive and negative wells when stained according to acid-fast stain procedures using Carbol Fuchsin with a Methylene Blue counterstain:

Test Organism	Appearance	Results
Positive well:		
Mycobacterium gordonae	Irregular rods, stained red	Acid-fast positive
Negative well:		
Corynebacterium spp.	Irregular rods, stained blue	Acid-fast negative

PHYSICAL APPEARANCE

Q-Slide™ AFB should have a methanol fixed smear in each the positive and negative control wells.

REFERENCES

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

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

IFU-10698[A]

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

HDQA 2207F [D]