



F.A.S.T. AFB Smear Kit with Digestion Solution (400 count)

Instruction Manual

QBC F.A.S.T. AFB Smear Kit with Digestion Solution

Intended Use

For use in digesting/decontaminating and preparing patient respiratory specimens or cultures for subsequent detection or characterization of acid fast organisms.

Summary and Principles

The worldwide incidence of tuberculosis has been on an increasing trend since at least 1990, when the World Health Organization began tracking incidence data¹. Early and accurate detection of tuberculosis (TB) is critical for both effective control and treatment of the disease. The most common method for detection of *Mycobacterium tuberculosis* is the use of sputum smear microscopy¹, which can provide both an initial presumptive diagnosis as well as a quantification of the mycobacterial load.

Acid fast organisms, such as *Mycobacterium tuberculosis*, can be stained by aniline dyes and are resistant to decolorization by acid and alcohol. When followed by a counterstain, this treatment results in the acid fast organisms staining with contrast to other organisms and debris that have retained only the counterstain. However, the staining methods classically used for acid fast microscopy result in a smear that can be difficult and time consuming to read. Auramine O and auramine-rhodamine

stains have been successfully used for fluorescence based microscopy of mycobacteria. Reports of mechanism of staining are conflicting; these include Auramine O binding to the cell wall of the mycobacteria² and the stain binding to “most if not all” the Auramine O binding to the nucleic acid in the mycobacteria³. It has been demonstrated, though, that Auramine O based staining methods are more sensitive than light microscopy methods for the detection of acid fast bacteria (AFB)⁴. This increase in sensitivity is due, in large part, to the significant contrast the fluorescent stains impart to the acid fast organisms, which appear green against a dark background. This increase in distinction permits the use of objectives with larger fields-of-view, thereby decreasing the total examination time.

The reference method for TB diagnosis is culture and is often performed in conjunction with smear microscopy or following detection of a smear positive specimen. Preparation of sputum specimen for culture requires both reduction of sample viscosity through digestion as well as decontamination by reducing the levels of viable common flora. For digestion and decontamination, the NALC-NaOH method is often employed⁴. Here the NALC (N-acetyl-L-cysteine) acts as a mucolytic agent by breaking the associations between individual mucin molecules. NaOH (sodium hydroxide) will inactivate common flora and acid fast organisms, but with controlled exposure (time and concentration) it will kill common flora and will leave acid fast organisms viable. Following digestion, decontamination and subsequent concentration, the resultant sample can be used for culture and smear microscopy.

The QBC *F.A.S.T.* AFB Smear Kit with Digestion Solution includes the

reagents and disposables necessary for performing fluorescence microscopy of TB smears, including specimen digestion and decontamination. The components are specially designed to work as a complete system and include the innovative, labor saving *F.A.S.T.* Auramine O Stain Kit and SureFocus Microscope Slides (patent pending). The *F.A.S.T.* Auramine O Stain Kit uses a rapid four step process that takes just over two minutes to complete, compared to conventional auramine methods that take approximately 15 to 20 minutes to complete. The SureFocus Microscope Slides simplify fluorescence microscopy by providing fluorescent landmarks throughout the smear area for finding and maintaining focus. This helps to reduce operator stress and fatigue and to ensure quality of results.

Kit Components

The contents of this kit are sufficient for processing approximately 400 specimens and contains the following items:

432	SureFocus Microscope Slides
120 mL	QBC <i>F.A.S.T.</i> Auramine O Stain
120 mL	QBC <i>F.A.S.T.</i> Decolorizer/Quencher
5	<i>F.A.S.T.</i> QC Control Slides
9	Sputum Digestion Solution (75 mL)
4	Digestion Buffer Packets
1	Kit Product Insert

Warnings and Precautions

For *in vitro* diagnostic use

Human clinical specimens can harbor infectious diseases such as the causative agents of tuberculosis, hepatitis, human immunodeficiency virus (HIV) and others. Universal Precautions and local guidelines and regulations should be followed when handling clinical specimens. All activities that could generate aerosols from clinical specimens should be performed in a biosafety cabinet. Activities that involve culturing of *Mycobacterium tuberculosis* should be performed using Biosafety Level 3 procedures and practices

The chemicals in this kit are hazardous and can be harmful or fatal. Reagents contain strong alkali and can cause burns. Avoid eye and skin contact. In case of eye contact, flush immediately with copious amounts of water. If Sputum Digestion Solution is ingested, give milk, egg white or large amounts of water. In case of eye contact or ingestion call a physician immediately. Consult kit MSDS for additional information regarding safety and disposal.

Sputum smear microscopy and the procedures involved with sample preparation and processing should be performed only by those trained in the techniques involved as well as general laboratory practices and procedures.

Instructions for Use

For use with patient respiratory specimens.

Sputum Prep Tubes; Sterile Transfer Pipettes, and SureFocus Slides are for single use only.

Phosphate Buffer Preparation

1. Pour contents of Phosphate Buffer Powder packet into

- a 500 mL volumetric flask or autoclavable bottle with a 500 mL gradation.
2. Add water to 500 mL and mix well.
 3. If a volumetric flask was used, pour contents into an autoclavable bottle.
 4. Autoclave buffer.

Digestion procedure

1. Prior to use, loosen but do not remove the screw-cap on the Sputum Digestion Solution plastic bottle.
2. Locate ampoule in bottle and squeeze bottle in the upright position until the ampoule breaks.
3. Close the lid and shake the bottle gently to dissolve the NALC; avoid foaming the solution.
4. In an aerosol-free sterile 50 mL centrifuge tube, add equal amounts of clinical specimen and NALC containing solution.
5. Cap the centrifuge tube and mix specimen until it is liquefied. Allow digestion reaction to take place for 15 minutes at room temperature. Avoid longer digestion times as mycobacteria will become inactivated with prolonged exposure to the digestion solution.
6. Add sterile phosphate buffer for a final volume of 50 mL and recap the centrifuge tube.
7. Centrifuge sample for 15 minutes at 2200 to 3000 x g.
8. Decant supernatant into appropriate biohazard container.
9. Resuspend sediment in 1 to 2 mL of phosphate buffer. Specimen should now be pH 6.8.
10. Specimen is now ready for diagnostic testing and culturing.

Smear preparation and staining:

Add specimen to the center of the SureFocus slide and smear to create a uniform smear that extends to fill the entire area of the ellipse. The smear should be thick enough to ensure adequate specimen has been added. However, the lines of the SureFocus slide should still be visible through the specimen. Heat fix the slide using a burner or slide warmer.

1. Heat fix slide containing specimen smear
2. Cover smear with *F.A.S.T.* Auramine O Stain and let stand for 1 minute
3. Rinse smear gently with deionized or tap water and drain
4. Cover smear with *F.A.S.T.* Decolorizer/Quencher and let stand for 1 minute
5. Rinse smear gently with deionized or tap water and drain Dry slide
6. Examine slide using the QBC ParaLens or equivalent fluorescence microscopy apparatus

Examination Procedure:

Examine slide using the QBC ParaLens or equivalent fluorescence microscopy apparatus Stain heat fixed slide using an Auramine O staining procedure such as *F.A.S.T.* Auramine O. Note: it is advisable to include a positive and negative control sample with each batch of stained slides to ensure reagent and instrument integrity as well as technicians' performance.

Smear Examination:

Place stained slide on the microscope stage and center objective over a starter circle. Using bright field mode, focus on starter

circle using a lower power objective and progress to the desired smear examination objective. Change to fluorescence mode. Alternatively, the microscope can be focused in fluorescence mode using the following procedure: center objective over starter circle and adjust stage height to just above the working distance of the objective; with the fluorescence light source on, look through the eyepiece and focus downward with the fine focus until the field comes into focus. (Tip: as the fluorescent line is coming into focus, the field-of-view should become brighter green. If the field remains dark, the correct focal plane has been passed.) Move to the edge of the fluorescent line and readjust focus.

Begin examining the smear from the starter circle and traveling to the next landmark. The landmarks can be used as milestones for number of fields-of-view examined if fields-of-view are examined sequentially without jumping through the smear (i.e., a stage movement is continuous). When the next landmark is reached, ensure the scope is in focus. Continue examining moving from landmark to landmark until the appropriate number of fields (distance traveled if movement was continuous) dictated by your standard operating procedures. Report results.

Smear Examination Example:

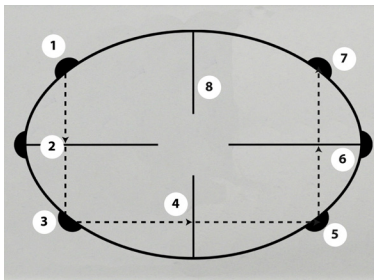


Figure 1 above depicts a SureFocus slide with a suggested examination path. For this path, obtain initial focus using starter circle 1. Examine slide vertically and systematically, moving toward starter circle 3. When moving from field-of-view to field-of-view, scan with a continuous motion being careful not to jump between fields. Once line 2 is reached, ensure that the microscope is in focus. Proceed vertically to starter circle 3 and ensure the microscope is in focus. Take a horizontal course toward line 4. When line 4 is reached, ensure that the scope is in focus. At this point, the following number of fields has been scanned if fields-of-view were read in a continuous motion:

Magnification	Number of Fields Examined
200x	26
400x	52
600x	78
1000x	130

The following table provides the approximate distances and fields-of-view at standard magnifications between landmarks:

Examination Pathway	Distance (mm)	Fields of View		
		200x	400x	600x
1 to 2; 2 to 3; 5 to 6; 6 to 7	6.5	7	14	21
1 to 8; 8 to 7; 3 to 4; 4 to 5	11	12	24	36

Quality Control Procedure:

Slides should be stained with reagents to be used for patient specimen diagnosis. The technician performing patient specimen staining should perform the control slide staining using specimen staining procedures. Both positive and negative smears should be examined by laboratory technicians performing patient specimen diagnosis. QC should be performed routinely and in accordance with governing regulations. Results should be documented.

1. Stain the QBC *F.A.S.T.* Quality Control Slide along with the test slide, using the *F.A.S.T.* Auramine O Stain Kit per the above procedure
2. Keep slides well separated during the staining procedure to avoid cross-contaminating carry over of stain reagents from slide to slide.
3. Read the stained slide with a microscope appropriate

for the type of stain and record the results.

Expected Results

The QBC *F.A.S.T.* Sputum Digestion Solution is used for the digestion and decontamination of clinical respiratory specimens (sputum or bronchial lavage) suspected to contain mycobacteria.

If procedures are followed correctly, viscous specimens will be liquefied and contamination by normal flora will be reduced or eliminated.

When viewed with a fluorescence microscope having a blue excitation and green emission filter configuration (e.g., Excitation Filter: 435 - 480 nm; Emission Filter 510 - 600 nm), the markings on the SureFocus slide should fluoresce green and provide a useful means for focus. When in focus, Mycobacteria, such as the ones in the positive control, and other acid fast organisms fluoresce green against a dark background. All other organisms, such as those in the negative control, should exhibit the background staining characteristics. A fluorescent bacillus is a presumptive identification of *Mycobacterium* spp.

The mycobacteria in the positive well of the quality control slide, should fluoresce bright green. The negative control should exhibit the background staining characteristics.

If expected results are not obtained, investigate cause of failure, which may include failure of reagents, instrument, and operators. If control failure is suspected, use another means to test system such as patient specimen (known positive or

negative). Do not report patient results until system failure is corrected.

Limitations

Sputum Prep Tubes and Sterile Transfer Pipettes are provided sterile. If primary packaging is damaged or opened, do not use product.

No one method of digestion-decontamination is suitable for all clinical specimens in all situations. When selecting a procedure, choose the mildest procedure that will reduce contamination.

Some rapid growing mycobacteria may not fluoresce with this stain. Ziehl-Neelsen, Kinyoun, or other methods should be used on these specimens. Fluorescence of smears will wane over time and can degrade with excessive heat and light, so stained specimens should be examined as soon as possible.

If fluorescence signal is not seen from the lines on the SureFocus slides, do not use the slides for fluorescence microscopy.

While a positive result provides evidence of mycobacteria, a negative result does not rule out an infection. Other diagnostic methods such as culture or PCR should be used for positive identification.

If the SureFocus slides do not fluoresce or the quality control slides exhibit little or no fluorescence, the fluorescence microscopy system should also be checked to ensure that it is functioning properly. Investigate cause of failure, which may

include failure of reagents, instrument, and operators. If control failure is suspected, use another means to test system such as patient specimen (known positive or negative). Do not report patient results until system failure is corrected.

Materials Needed But Not Included

The QBC *F.A.S.T.* AFB Smear Kit with Digestion Solution is designed to work with a fluorescent microscope system capable of exciting specimens from 425-480 nm and transmitting fluorescence of at least 510-600 nm. Additional equipment needed includes the following items:

- Slide warmer or flame
- Centrifuge capable of spinning Sputum Prep Tubes at 2200-3000 x g
- Biosafety cabinet for specimen handling
- Personal protective equipment
- Sterile Sputum Prep Tubes
- Sterile Transfer Pipettes

References

1. World Health Organization. (2009) Global tuberculosis control: epidemiology, strategy, financing: WHO Report 2009. WHO Press, Geneva, Switzerland.
2. Baron, E.J. and S.M. Tenover. (1990) Baily & Scott's Diagnostic Microbiology, 8th Edition. The CV Mosby Company, Baltimore, Maryland.
3. Hanscheid, T. (2008) The future looks bright: low-cost fluorescent microscopes for detection of *Mycobacterium tuberculosis* and *Coccidia*. *Transactions of the Royal Soci-*

ety of Tropical Medicine and Hygiene.

4. Steingart K. R., *et al.* (2007) Fluorescence versus conventional for sputum smear microscopy for tuberculosis: a systematic review. *Lancet Infect Dis* 6:570-81.
5. Essential Procedures for Clinical Microbiology. (1998) American Society of Microbiology. Washington, D.C.

Ordering Information

- QBC *F.A.S.T.* TB Smear Kit with Digestion Solution 427408
- QBC *F.A.S.T.*™ Centrifuge (115V, 60Hz), catalog number 42741
- QBC *F.A.S.T.*™ Centrifuge (220V, 50Hz), catalog number



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Manufacturer



Authorized Representative in the European Community



Use By



Catalog Number



In Vitro Diagnostic Medical Device



Temperature Limitation



Batch Code (Lot)



Consult Instructions for Use



Single Use Only



Caustic



Flammable