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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

Drucker Diagnostics
Innovative Solutions for a Healthier World

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

Instruction Manual

CE

4277-400-085 Rev. H
Revised 2019-03-13

English

QBC *F.A.S.T.* AFB Smear Kit

Intended Use

For use in preparing and staining smears from patient specimens or cultures in the detection or characterization of acid fast bacilli such as *Mycobacterium tuberculosis*.

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 data[1]. 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 microscopy[1], which can provide both an initial presumptive diagnosis as well as a quantification of the mycobacterial load.

Acid fast bacilli, 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 bacilli 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 mycobacteria[2] and the stain binding

2. Baron, E.J. and S.M. Finegold. (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 *Coccidiae*. *Transactions of the Royal Society 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 Infectious Disease* 6:570-81.

For more information on ordering *F.A.S.T.* AFB Kits and accessories, consult our website at www.druckerdiagnostics.com. Drucker Diagnostics sales staff is also available at 814-692-7661 (U.S.A.) and via email at qbcsales@druckerdiagnostics.com.

Product Numbers

QBC *F.A.S.T.* AFB Smear Kit	427409

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.

F.A.S.T. Stain reagents can degrade when exposed to excessive heat. Always perform quality control to determine integrity of the stain before reporting patient results. Do not use stain if quality control fails.

Materials Needed But Not Included

The QBC *F.A.S.T.* AFB Smear Kit 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 includes slide warmer or flame burner (e.g., spirit lamp or Bunsen burner).

References

1. World Health Organization. (2009) Global tuberculosis control: epidemiology, strategy, financing: WHO Report 2009. WHO Press, Geneva, Switzerland.

to "most if not all" the Auramine O binding to the nucleic acid in the mycobacteria[3]. It has been demonstrated, though, that Auramine O based staining methods are more sensitive than light microscopy methods for the detection of acid fast bacilli (AFB)[4]. This increase in sensitivity is due, in large part, to the significant contrast the fluorescent stains impart to the acid fast bacilli, 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 QBC *F.A.S.T.* AFB Smear Kit includes the reagents and disposables necessary for performing fluorescence microscopy of AFB smears. 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 smear samples and contains the following items:

400 Sputum Collection Cups
400 Applicator Sticks
432 SureFocus Microscope Slides

120 mL QBC *F.A.S.T.* Auramine O Stain
120 mL QBC *F.A.S.T.* Decolorizer/Quencher
5 *F.A.S.T.* QC Control Slides
1 Product Insert

Storage Conditions

Store at 2-25 ºC. Avoid excessive heat. Do not use beyond expiration date.

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. Use in a well ventilated area with proper personal protective equipment. Keep product away from open flames. Consult MSDS for additional information regarding safety and disposal.

This product is designed to aid in the detection of acid fact bacilli. Sputum smear microscopy and the procedures involved with sample preparation and processing for AFB detection should be performed only by those trained in the techniques involved as well as general laboratory practices and procedures.

Failure of the QC positive control well to fluoresce could indicate stain reagent degradation. Do not use stain for patient specimens until problem is rectified.

Expected Results

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 bacilli fluoresce green against a dark background. All other bacilli, such as those in the negative control, should exhibit the background staining characteristics. A fluorescent bacillus is a presumptive identification of *Mycobacterium* species.

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

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.

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 examination. 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.

Caution: Product contains glass slides. Handle with care.

Instructions for Use

For use with direct or concentrated sputum or cultured specimens.

Sputum Collection Cups; Applicator Sticks, and SureFocus™ Slides are for single use only.

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. For direct smears, the lines of the SureFocus slide should still be visible through the specimen. Heat fix the slide using a burner or slide warmer.

Staining Procedure:

1. Heat fix slide containing sputum 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
6. Dry slide

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 if fields (distance traveled if movement was continuous) dictated by your standard operating procedures. Report results.

Smear Examination Example:

Figure 1

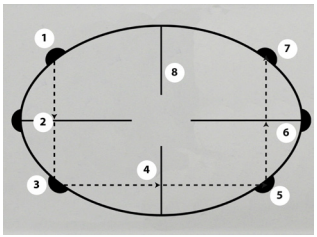

Figure 1 above depicts a SureFocus slide with a suggested examination path. For this path, obtain initial focus using starter circle 1. Examine slide vertically and systematically, moving toward starter circle 3. When moving from field-of-view to field-of-view, scan with a continuous motion being careful 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