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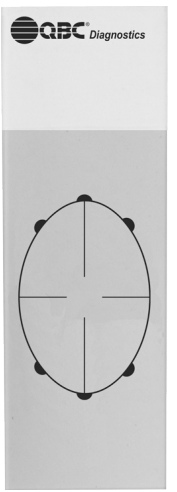


SureFocus™ Microscope Slide

Instruction Manual

CE

4277-400-128 Rev. G
2017-08-14

English

SureFocus™ Microscope Slides

Patent-Pending

Intended Use

To aid in fluorescence microscopy based detection of acid fast bacilli (AFB)

Summary and Principles

The worldwide incidence of tuberculosis (TB) 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 TB is critical for effective treatment and control. The most common method for detection of acid fast bacteria (AFB), such as the prinicpal causitive agent of tuberculosis (*Mycobacterium tuberculosis*), is through the use of sputum smear microscopy[1], which can provide both an initial presumptive diagnosis as well as a quantification of mycobacterial load.

Fluorescence microscopy based detection of tuberculosis in sputum smears has been shown to be more sensitive than bright field methods[2]. However, despite its recognized benefits, fluorescence microscopy introduces problems that affect its utility and indication for widespread use. These problems center around the fact the fluorescence microscopy is a dark field technique, and, as such, requires the sample to provide any light signal. In the case of fluorescent stained TB smears, background fluorescence is quenched, which is important for providing significant contrast

for AFB detection. As a result of this quenching, the examination field provides little to no signal on which to focus in samples where bacilli burden is low to non-existent. Therefore, it is difficult to ensure the quality of assays where a negative result is found. Furthermore, the signal from fluorescently stained AFB is relatively low (compared to bright field methods) and can only be seen within a small distance from the focal plane. Here, inexperienced users can easily overlook positive samples by missing the small range where signal is distinguishable.

The *F.A.S.T.* SureFocus slides are designed to solve the problem of uncertainty of focus in smear examination by providing fluorescent landmarks on which microscopists can find initial focus and maintain focus throughout examination. The landmarks, which take the form of an ellipse with circles and lines, are also strategically formatted to provide helpful tools for smear preparation, reading, training, and quality control. The ellipse demarcates a 3 cm x 2 cm area, which is standard for TB sputum smears, and is punctuated with 6 circles on and around the apex of the widest dimension. Circles provide a greater surface area that facilitates easy centering of the objective for initial focusing. Circles also provide coordinates that can be used for standardization of laboratory examination procedures and can be used in conjunction with the fluorescent lines within the smear area to gauge distances required for complete examination. In addition to the smear prep and examination aids provided by the slides, the fluorescent landmarks can be useful for providing a fluorescence positive control when reagents, staining procedure, instrument function, or operator performance are in question.

SureFocus slides are designed with similar fluorescence characteristics as Auramine O (i.e., excited by blue light and

fluoresce green light), so the slides can be used with fluorescence microscopes formatted for viewing smears stained by Auramine O.

Contents

Contains:

- 432 SureFocus microscope slide
- 1 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* species should be performed using Biosafety Level 3 procedures and practices.

This product is designed to aid in the detection of acid fast 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.

Caution: Product made with glass. Handle with care.

Storage Conditions

Avoid excess heat. Store away from direct light.

Procedure

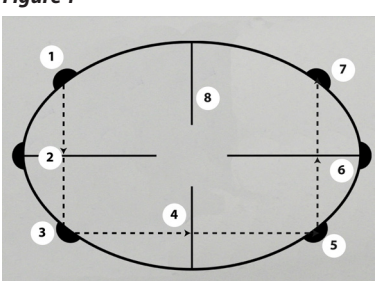
SureFocus slides can be used with direct patient specimens, digested specimens, or cultured specimens.

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. 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:

Figure 1



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 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

The fluorescent lines on the SureFocus slide should fluoresce brightly in a fluorescence microscope. Use the lines to ensure that your microscope light source and optics are functioning correctly. In the event lines are not seen or are dim in the fluorescence microscope, the instrument, the slides, and the user should be considered for determining the cause of the malfunction. Do not perform microscopy for patient diagnosis until the problem is

rectified.

Expected Results

SureFocus slides should fluoresce green when excited by blue light (in the range of 435 – 480 nm) and observed through fluorescence filters that permit green light (in the range of 510-600 nm) to pass to the observer.

Limitations

Fluorescence wanes over time and materials that fluoresce can degrade with excessive heat or light. If fluorescence signal is not seen from the lines on the SureFocus slides, do not use the slides for fluorescence microscopy.

If the SureFocus slides do not fluoresce, the fluorescence microscopy system should also be checked to ensure that it is functioning properly.

Equipment Needed But Not Included

- Applicator for applying and smearing patient specimen
- Burner or slide warmer
- Staining reagents and supplies
- Fluorescence microscope with the following characteristics:
    - Capable of exciting specimens from 435 to 480 nm
    - Capable of transmitting emitted light in the range of 510 to 600 nm
- Immersion oil (if needed)

References

The QBC *F.A.S.T.* SureFocus Slides are an integral part of overall fluorescence microscopy quality assurance. For addition guidance on quality assurance for the mycobacteriology lab, the following resources are available:

1. World Health Organization. (2009) Global tuberculosis control: epidemiology, strategy, financing: WHO Report 2009. WHO Press, Geneva, Switzerland.
2. Steingart, K.R., *et al*. (2007) Fluorescence Versus Conventional for Sputum Smear Microscopy for Tuberculosis: a Systematic Review. *Lancet Infect Dis* 6:570-81.
3. Essential Procedures for Clinical Microbiology. (1998) American Society of Microbiology. Washington, D.C.
4. Laboratory Diagnosis of Tuberculosis by Sputum Microscopy. (2005) Institute of Medical and Veterinary Science. Adelaide, Australia.
5. Manual of Clinical Microbiology. (2007) Volumes 1 and 2. 9th Edition. American Society of Microbiology. Washington, D.C.

Ordering Information

QBC *F.A.S.T.* SureFocus Slides: Catalog No. 427411

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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