BenchMark Special Stains AFB Staining Kit

REF 860-043

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IVD Σ 75

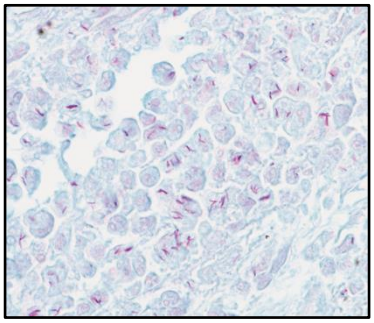

Figure 1. BenchMark Special Stains AFB Staining Kit on Diseased Lung Tissue.

INTENDED USE

The BenchMark Special Stains AFB Staining Kit is intended for laboratory use as a qualitative histologic stain to demonstrate acid-fast bacteria (AFB) by light microscopy in sections of formalin-fixed, paraffin-embedded (FFPE) tissue stained on a BenchMark Special Stains instrument.

This product should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information, and proper controls.

This product is intended for in vitro diagnostic (IVD) use.

SUMMARY AND EXPLANATION

BenchMark Special Stains AFB Staining Kit is a modification of the Ziehl-Neelsen stain.[1] A fuchsin solution is used to stain acid fast organisms and components red. Aniline Blue counterstain is applied to provide contrasting blue background.

The property of acid-fastness is related to the cell wall structure of AFB.[2] This group of bacteria contain an additional hydrophobic layer composed of complex lipids, specifically mycolic acid, that surrounds the peptidoglycan cell wall.[2] This waxy lipid layer acts as a selective barrier, allowing certain phenolic stains like fuchsin to permeate through, while preventing acid-alcohol decolorizing agent from entering, rendering these organisms acid-resistant or "acid-fast".[2] Therefore, acid-fast bacteria can retain the fuchsin stain and appear red upon visualization. Non-AFB organisms lack the extra lipid layer and allow the acid-alcohol to enter and decolorize the fuchsin stain. Upon staining with Aniline Blue, non-AFB organisms stain blue, making them easily distinguishable from AFB.

The BenchMark Special Stains AFB Staining Kit is used to aid the pathologist in the diagnosis of an infection with acid-fast bacteria (AFB).

PRINCIPLE OF THE PROCEDURE

The staining reaction is based on the application of new fuchsin with a surfactant and base in alcohol, which enhances staining and dissolves the dye, to stain all components in the section. Decolorizer, an acid alcohol reagent, is applied to remove the color from all tissue elements other than the acid-fast components. Organisms that remain stained are believed to have selective permeability to the fuchsin.[2] The Aniline Blue counterstain provides contrast in the tissue section to enhance organism visibility.

This kit is optimized for use on BenchMark Special Stains instruments. The reagents are applied to tissue on microscope slides and mixed over the entire specimen.

MATERIAL PROVIDED

The reagent vials are supplied in barcode labeled carriers to insert into the reagent tray of the instrument. Each kit contains sufficient reagent for 75 tests:

One 27 mL vial of AFB Core Stain contains 1.75% new fuchsin and 2.25% non-ionic alcohol ethoxylate surfactant in reagent alcohol.

One 27 mL vial of AFB Core Decolorizer contains 64% methanol and 18% sulfuric acid.

One 27 mL vial of Aniline Blue for AFB contains 0.015% aniline blue and 0.25% acetic acid.

Three vial inserts with sipping straws.

Reconstitution, Mixing, Dilution, Titration

No reconstitution, mixing, dilution, or titration of kit reagents is required. Further dilution of any of the reagents may result in unsatisfactory staining.

The reagents in this kit have been optimally diluted for use on BenchMark Special Stains instruments.

MATERIALS REQUIRED BUT NOT PROVIDED

Not all products listed in the method sheet may be available in all geographies. Consult your local support representative.

The following reagents and materials may be required for staining but are not provided:

1. Recommended control tissue
2. Microscope slides, positively charged
3. BenchMark Special Stains instrument
4. BenchMark Special Stains Deparaffinization Solution (10X) (Cat. No. 860-036 / 06523102001)
5. BenchMark Special Stains Liquid Coverslip (Cat. No. 860-034 / 06523072001)
6. BenchMark Special Stains Wash II (Cat. No. 860-041 / 08309817001)
7. General purpose laboratory equipment

STORAGE AND STABILITY

BenchMark Special Stains AFB Staining Kit should be stored at 15-30°C.

When properly stored, unopened and opened reagents are stable to the date indicated on the label.

Do not use reagent beyond the expiration date indicated on the kit.

There are no obvious signs to indicate instability of these reagents; therefore, controls should be run simultaneously with unknown specimens. Contact your local support representative if positive control material shows a decrease in staining as it could indicate reagent instability.

SPECIMEN PREPARATION

Routinely processed FFPE tissues are required for use with this assay and BenchMark Special Stains instrument. The recommended tissue fixative is 10% neutral buffered formalin.[3]

Perform specimen collection and storage according to Histotechnology: A Self Instructional Text.[4] Cut sections to the appropriate thickness, approximately 4 µm, and place the sections on positively charged glass slides.

1. Dry the slides.[3]
2. Print appropriate barcode label(s).
3. Apply barcode labels to the frosted end of the slides prior to loading the slides onto the instrument (see the Instrument User Guide for correct application of labels).

Refer to the Instructions for Use section for the recommended protocol for the BenchMark Special Stains instrument.

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic (IVD) use.
2. For professional use only.
3. **CAUTION:** In the United States, Federal law restricts this device to sale by or on the order of a physician. (Rx Only)
4. Do not use beyond the specified number of tests.
5. Positively charged slides may be susceptible to environmental stresses resulting in inappropriate staining. Ask your Roche representative for more information on how to use these types of slides.
6. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions. In the event of exposure, the health directives of the responsible authorities should be followed.[5,6]
7. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
8. Avoid microbial contamination of reagents as it may cause incorrect results.
9. For further information on the use of this device, refer to the BenchMark Special Stains instrument User Guide, and instructions for use of all necessary components located at navifyportal.roche.com.
10. Consult local and/or state authorities with regard to recommended method of disposal.
11. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.

12. To report suspected serious incidents related to this device, contact the local Roche representative and the competent authority of the Member State or Country in which the user is established.

This product contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Table 1. Hazard information.

Hazard	Code	Statement
Danger	H225	Highly flammable liquid and vapour.
	H301 + H311 + H331	Toxic if swallowed, in contact with skin or if inhaled.
	H314	Causes severe skin burns and eye damage.
	H351	Suspected of causing cancer.
	H370	Causes damage to organs.
	H412	Harmful to aquatic life with long lasting effects.
	P210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
	P260	Do not breathe mist or vapours.
	P280	Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection.
	P301 + P310 + P330	IF SWALLOWED: Immediately call a POISON CENTER/ doctor. Rinse mouth.
	P303 + P361 + P353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water.
	P304 + P340 + P310	IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/ doctor.
	P305 + P351 + P338 + P310	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor.
	P308 + P311	IF exposed or concerned: Call a POISON CENTER/ doctor.
	P370 + P378	In case of fire: Use dry sand, dry chemical or alcohol-resistant foam to extinguish.
	P403 + P233	Store in a well-ventilated place. Keep container tightly closed.

INSTRUCTIONS FOR USE

Prepare Reagent Vial

Before first use, a vial insert and sipping straw must be placed in the reagent vial.

Remove the shipping cap from the vial and place the insert and straw into the vial. The insert and sipping straw should be left in the vial, once the vial has been opened.

Staining Procedure

1. Load reagents and slides onto the instrument.
2. Place the soft cap into the slot on the reagent holder when the reagent is in use.
3. Perform the staining run according to the recommended protocol (see Table 2), and the instructions in the User Guide.
4. When the run is complete, remove the slides from the instrument.
5. Use the soft cap to cover the reagent vial when reagent is not in use.
6. After use, store the reagents according to the recommended storage conditions.

Recommended Protocol

The parameters for the automated procedures can be displayed, printed, and edited according to the procedure in the instrument User Guide.

The following procedures allow flexibility to accommodate user preference. This product has been optimized for the BenchMark Special Stain instrument and users must validate results obtained with this product.

Table 2. Recommended staining protocol for BenchMark Special Stains AFB Staining Kit on a BenchMark Special Stains instrument.

Staining Procedure	S AFB
Protocol Step	**Method**
Deparaffinization	Select to automate paraffin removal.
Baking (optional)	The default is not selected. 75°C for 4 minutes is recommended.
Optimize Stain Intensity	The default time is 16 minutes. Select to enable the adjustment of staining intensity.* Select an incubation time from 12 to 20 minutes: 12 minutes, lighter AFB staining with less pink blush 20 minutes, darker AFB staining with more pink blush
Optimize Aniline Blue Intensity	The default time is 4 minutes. Select to enable the adjustment of Aniline Blue intensity.* Select an incubation time from 4 to 12 minutes: 4 minutes, lighter counterstain 12 minutes, darker counterstain

* To adjust staining preferences, increment the incubation time one parameter at a time.

Recommended Post-Instrument Processing

1. Rinse slides in two changes of 95% ethanol to remove the leftover solution, followed by three changes of 100% ethanol.
2. Clear slides in three changes of 100% xylene.
3. Coverslip with permanent mounting media.
4. Compatible with the VENTANA HE 600 system coverslipping protocol. For further instructions, refer to the VENTANA HE 600 system User Guide.

QUALITY CONTROL PROCEDURES

An example of a positive control material would be FFPE human tissue positive for acid-fast bacteria.[3] Control tissue should be fresh autopsy, biopsy, or surgical specimen prepared or fixed as soon as possible in a manner identical to test sections. Such tissues should monitor all steps of the analysis, from tissue preparation through staining.

Use of a tissue section fixed or processed differently from the test specimen provides control for all reagents and method steps except fixation and tissue processing. The cellular components of other tissue elements may serve as the negative control.

Optimal laboratory practice is to include a positive control section on the same slide as the test tissue. This helps identify any failures applying reagents to the slide. Control tissue may contain both positive and negative staining elements and serve as both the positive and negative control.

The control tissue must be tested with each run.

Known positive tissue controls should only be utilized for monitoring the correct performance of processed tissues and test reagents, not as an aid in formulating a specific diagnosis of patient samples.

If the positive tissue components fail to demonstrate positive staining, results with the test specimens should be considered invalid. If the negative components demonstrate positive staining, results with patient specimens should also be considered invalid.

Unexplained discrepancies in control results should be referred to the local support representative immediately. If quality control results do not meet specifications, patient

results are invalid. The cause must be identified and corrected, and the patient samples repeated.

STAINING INTERPRETATION / EXPECTED RESULTS

The BenchMark Special Stains AFB Staining Kit is tested to demonstrate acid-fast bacteria.

- Acid-fast Bacteria (AFB): Red
- Background: Blue

The bacteria appear as red patches on lower magnification. At 100x, under oil, they appear as overlapping rod shaped structures. Some types of acid-fast bacteria may appear pleomorphic depending on the type of organism and the plane of the section.

Non-specific pink to red cytoplasmic granular staining has been observed in some inflammatory cells.

Red blood cells, particularly within blood vessels within fatty areas, may stain pink.[7]

SPECIFIC LIMITATIONS

Only positively charged microscope slides have been used and validated for this assay.

Only AFB positive organisms were tested. Specific organisms were not identified.

This method is not recommended for the detection of mycobacterium leprae.[2,3]

PERFORMANCE CHARACTERISTICS

ANALYTICAL PERFORMANCE

Staining tests for sensitivity, specificity, and precision were conducted and the results are listed below.

Sensitivity and Specificity

Analytical sensitivity and specificity for acid fast bacteria was evaluated in multiple studies comparing to two different methods of detection. Acid-fast bacteria staining is expected in cases of acid-fast bacteria infected lung whereas absence of acid-fast bacteria staining is expected in cases of normal appendix and lung. A total of 98% (62/63) of evaluated tissue cases passed for acceptable staining as shown in Table 3.

Table 3. Sensitivity/Specificity of BenchMark Special Stains AFB Staining Kit was determined by testing the following FFPE normal and infected tissues.

Tissue	# Cases Passed / # Tested
Appendix (normal)	22 / 23*
Lung (normal)	15 / 15
Lung (AFB infection)	25 / 25

* Artifact on a single slide prevented interpretation

Precision

Precision studies were compared across multiple slides using 9 AFB infected tissue cases and 1 normal tissue case. Stained test and control slides were evaluated in two ways: 1) ≥95% of test sample slides showed the acid-fast bacteria stained bright red, and background stained light blue, and 2) blinded pairwise comparison of test slides to control slides shows ≥90% of test slides were scored as non-inferior to the control slides. All acceptance criteria were fully met. Precision studies were performed for the BenchMark Special Stains AFB Staining Kit as follows:

1. Instrument-to-Instrument: 180 slides were tested across 3 different BenchMark Special Stains instruments (60 slides per instrument and 18 slides per tissue case) with the BenchMark Special Stains AFB Staining Kit. The slides were evaluated for staining with pass or fail criteria. The study met acceptance criteria.
2. Run-to-Run: 180 slides were tested across 3 BenchMark Special Stains instruments (20 slides per run/3 runs per day per instrument and 18 slides per tissue cases) on 2 consecutive days with the BenchMark Special Stains AFB Staining Kit. The slides were evaluated for staining with pass or fail criteria. The study met acceptance criteria.
3. Lot-to-Lot: 180 slides (3 lots with 60 slides per lot) were tested across 3 BenchMark Special Stains instruments with the BenchMark Special Stains AFB Staining Kit. The slides were evaluated for staining with pass or fail criteria. The study met acceptance criteria.

CLINICAL PERFORMANCE

Clinical performance data relevant to the intended purpose of BenchMark Special Stains AFB Staining Kit were assessed by systematic review of the literature. The data gathered support the use of the device in accordance with its intended purpose.

TROUBLESHOOTING

1. Section thickness may affect quality and intensity of staining. If staining is inappropriate, contact your local support representative for assistance.
2. Necrotic or autolyzed tissue may exhibit nonspecific staining.
3. If the positive control is negative, tissue may have been improperly collected, fixed, or deparaffinized. Follow the proper procedure for collection, storage, and fixation.
4. If the positive control is negative, check that the slide has the proper barcode label. If the slide is labeled properly, check the other positive controls from the same run to determine if the controls were properly stained.
5. If excessive background staining occurs: incomplete paraffin removal could cause staining artifacts or no staining. If all paraffin is not removed from the slide, repeat the staining run using the extended deparaffinization option, if available.
6. If tissue sections wash off the slide, confirm the slides are positively charged.
7. The intensity and hue of the aniline blue counterstain is affected by tissue section thickness. Thinner sections will result in lighter counterstain.
8. Extended stay of the slides on-board the instrument after run completion may affect quality and intensity of the staining. If the staining is inappropriate, remove slides promptly at the end of the run and proceed to post-instrument processing.
9. For corrective action, refer to the Instructions for Use section, the instrument User Guide or contact your local support representative.

REFERENCES

1. Sheehan DC, Hrapchak BB. Theory and Practice of Histotechnology. 2nd edition. St. Louis, MO: C.V. Mosby Company; 1980.
2. Vilchèze C, Kremer L. Acid-Fast Positive and Acid-Fast Negative Mycobacterium Tuberculosis: The Koch Paradox. Microbiology spectrum. 2017;5(2).
3. Bancroft JD, Gamble M. Theory and Practice of Histological Techniques. 2nd edition. Edinburgh: Churchill-Livingston; 1982.
4. Carson F, Hladik C. Histotechnology: A Self Instructional Text, 3rd edition. Hong Kong: American Society for Clinical Pathology Press; 2009.
5. Occupational Safety and Health Standards: Occupational exposure to hazardous chemicals in laboratories. (29 CFR Part 1910.1450). Fed. Register.
6. Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.
7. Horobin RW, Bancroft JD. Troubleshooting Histology Stains. New York: Churchill Livingstone; 1998.

NOTE: A point (period/stop) is always used in this document as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

The summary of safety and performance can be found here: https://ec.europa.eu/tools/eudamed

Symbols

Ventana uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see elabdoc.roche.com/symbols for definition of symbols used):

GTIN	Global Trade Item Number
UDI	Unique Device Identification
	Indicates the entity importing the medical device into the European Union

REVISION HISTORY

Rev	Updates
D	Updates to Precision Section

INTELLECTUAL PROPERTY

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