QBC F.A.S.T.™ Auramine O Stain Kit

Intended Use
For use as a stain for smears made 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 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...
to “most if not all” the Auramine O binding to the nucleic acid in the mycobacteria. 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. 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.

While standard auramine staining methods are a significant improvement over classical methods, the staining process is still time consuming. The QBC F.A.S.T. Auramine O Stain Kit simplifies the process further by using a proprietary combination of quenching agent and decolorizer to make a rapid four step process that takes just over two minutes to complete.

**Contents**

The contents of this kit include the following items:

- QBC F.A.S.T. Auramine O Stain (120 mL, 250 mL, or 3.8 L)
- QBC F.A.S.T. Decolorizer/Quencher (120 mL, 250 mL, or 3.8 L)
- Package 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 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 kit MSDS for additional information regarding safety and disposal.

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.

**Storage Instructions**

Store at 15-25ºC. Keep stain protected from light. Do not use beyond expiration date.

**Staining Procedure**

The *F.A.S.T.* Auramine O Stain Kit can be used with direct and concentrated sputum and culture specimens.

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.

Quality Control
QBC Diagnostics Quality Control Slides (Catalog Number 427402) should be used routinely to assess the reagents and staining procedure. Quality control should be run in accordance with applicable governing regulations.

If quality control fails, do not report patient results until cause of failure is rectified. A positive control failure can be indicative of stain reagent degradation. If stain is source of no or low-fluorescing positive control, do not use stain for patient specimens.

Expected Results
Mycobacteria and other acid fast bacilli fluoresce green against a dark background 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). All other organisms will not be visible. A fluorescent bacillus is a presumptive identification of *Mycobacterium* spp.

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, so stained specimens should be examined as soon as possible. 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.

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.

**Equipment Required But Not Provided**

The QBC F.A.S.T. Auramine O Stain 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 slides, slide warmer or Bunsen burner.

**References**


Ordering Information

QBC F.A.S.T. Auramine O Stain Kits:

<table>
<thead>
<tr>
<th>Size</th>
<th>Catalog No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial Kit</td>
<td>427422</td>
</tr>
<tr>
<td>120 mL Kit</td>
<td>427404</td>
</tr>
<tr>
<td>250 mL Kit</td>
<td>424424</td>
</tr>
<tr>
<td>3.8 L Kit</td>
<td>424425</td>
</tr>
</tbody>
</table>

QBC Diagnostics, Inc.
200 Shadylane Drive, Philipsburg, PA 16866 U.S.A.

Emergo Europe
Molenstraat 15, 2513 BH The Hague, The Netherlands
Tel: (31) (0) 70-345-8570, Fax: (31) (0) 70-346-7299

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
Caustic
Flammable