Comparison between different auramine staining kits: Mauguen, S. Vacher, S. Degrange
Laboratoire de Bactériologie, hôpital Haut Lévêque, 33604 PESSAC

Microscopic detection of mycobacteria is the first step in the diagnosis of a Mycobacteria infection. This rapid and cheap test can directly be carried out on the sample or after homogenization-decontamination. Its sensitivity ranges from 50 to 80% depending on the report writer. Note that the factors influencing the sensitivity rate not only include the sample nature but also the staining technique, the biologist’s experience and the last but not the least, the tuberculosis prevalence.

All staining techniques are based on the Acid-Fast-Bacilli principle. In 1957 in the Pasteur institute’s annals, Degommier described a new staining technique for the mycobacteria detection using fluorescence. Its principle is based on the property of fluorescent stains: they emit a visible radiation when excited by an ultraviolet radiation produced by a mercury gas lamp or a well filtered intense blue light. Auramine O and Rhodamine B are described to bind with the mycolic acids of the bacterium wall. The “non-bound” stain is then eliminated by an acid-alcohol discolouring solution and the counter-stain or potassium permanganate removes the non-specific interfering fluorescence. The fluorescence microscopy is actually from far the most used method, essentially thanks to its reading rapidity. Several kits are marketed in the world.

According to the manufacturers, several pack variations exist, depending on the stain composition and preparation, the counter-stain used, the product reaction length ...

It seemed then interesting to list and compare the different existing techniques.

- Material and methods

Comparison of 5 different products:
- Tb-fluor and Tb-fluor, phenol-free marketed by Merck
- TB Fluorescent Stain kit M (BD kit M) and TB Fluorescent Stain kit T (BD kit T) sold by Becton Dickinson
- Kit Fluo-RAL commercialised by Réactifs RAL
- TB Fluorescent Stain kit M (BD kit M) and TB Fluorescent Stain kit T (BD kit T) sold by Becton Dickinson
- Tb-fluor and Tb-fluor, phenol-free marketed by Merck

Smears have been made from clinical samples, urines and pulmonary secretions showing a different richness of Mycobacterium tuberculosis.

- Negative smears (checked with a negative culture)
- Positive smears detected with a Ziehl staining (objective 50):
  - 1 BAAR / 10 fields with Ziehl staining

Each kit has been tested on smears, all coming from the same samples, after decontamination-homogenization.

They all have been read by two persons used to look at auramine stained smears daily.

Mycobacterium tuberculosis

Smears have been made from clinical samples, urines and pulmonary secretions showing a different richness of Mycobacterium tuberculosis.

Comparison between different auramine staining kits

<table>
<thead>
<tr>
<th>Test lengths</th>
<th>BD kit T</th>
<th>BD kit M</th>
<th>Kit Fluo-RAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rinse</td>
<td>30 s</td>
<td>30 s</td>
<td>30 s</td>
</tr>
<tr>
<td>Staining</td>
<td>2 min</td>
<td>2 min</td>
<td>2 min</td>
</tr>
<tr>
<td>Counter-stain</td>
<td>3 min</td>
<td>3 min</td>
<td>3 min</td>
</tr>
<tr>
<td>TOTAL</td>
<td>41 min</td>
<td>41 min</td>
<td>41 min</td>
</tr>
</tbody>
</table>

Reading: whatever the kit used, the Acid-Fast Bacilli (A.F.B.), M. tuberculosis like M. intracellulare, have all been detected, but with more or less easiness.

For Pulmonary samples, the orangey red fluorescence (auramine-rhodamine) against the black background (BD kit T, Tb-fluor and Tb-fluor, phenol-free) enables to observe a greater number of bacilli/microscopy field than the yellow green fluorescence (auramine).

But, only Kit Fluo-RAL thanks to its counter-stain quality enables a quick pinpointing of the elements and smears.

For samples like urines and some fluids, the background often reveals orange and as a matter of fact, the orangey red fluorescence lacks contrast while the green one seems to be brighter and leads to a better AFB detection.

Finally, artefacts are more numerous with BD kit T and BD kit M than with others.

As a conclusion, the reading and the cost being two major points in evaluating the test quality, kit Fluo-RAL seems to give one of the best results, followed by BD kit T and Tb-fluor, phenol-free.

Besides slides stained with those techniques can easily be re-stained with a Ziehl Neelsen staining, essential step to confirm the presence of M. tuberculosis in the sample.

Urines Samples

<table>
<thead>
<tr>
<th>Kit Fluoro-RAL</th>
<th>BD kit T</th>
<th>BD kit M</th>
<th>Tb-fluor</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD kit T</td>
<td>BD kit M</td>
<td>Tb-fluor</td>
<td></td>
</tr>
</tbody>
</table>

Bibliography