Aerospray® TB series 2

Multi-Site Performance Summary of

# AEROSPRAY® TB SLIDE STAINER/CYTOCENTRIFUGE (Model 7722)

ELITechGroup
BIOMEDICAL SYSTEMS

## Abstract

The new Aerospray TB Slide Stainer/Cytocentrifuge - Model 7722 (ELITechGroup Inc., www.elitechgroup.com) has been evaluated in a multi-national study by three independent laboratories. Each lab conducted evaluation studies, and then the study plans, protocols, and results were reviewed by ELITechGroup Biomedical Systems (Logan, UT, USA). The stainer was evaluated using samples suspected of containing acid-fast bacilli (AFB). Samples were prepared in duplicate under the microscope. One slide was stained by traditional manual methods, and its duplicate was stained on the Aerospray TB stainer using Aerospray TB stains. Slides were read and rated first as AFB positive or negative: If positive, the positive slides were rated as rare (or scanty), 1+, 2+, or 3+. The slides from the same specimen demonstrated a high correlation between manual staining methods and Aerospray staining methods with 99.6% agreement across 519 samples.

## Introduction

Tuberculosis (TB) is caused by *Mycobacterium tuberculosis* that most often affects the lungs. TB occurs in every part of the world. According to the World Health Organization (WHO) in 2012 8.6 million people fell ill with TB and 1.3 million of those died.

Microscopic examination of acid-fast stained smears is one of the first, easiest, most inexpensive, and rapid methods for demonstrating the presence of mycobacteria in clinical specimens and cultures[2].

Mycobacteria retains the primary stain even after exposure to decolorizing acid-alcohol, hence the term “acid-fast.” There are several methods of determining the acid-fast nature of mycobacteria. In carbol fuchsin procedures, acid-fast organisms appear red against a blue or green background, while in the fluorochrome procedure (auramine-O, auramine-rhodamine), the acid-fast organisms appear as fluorescent rods, yellow to orange against a darker background. The advantage of fluorescence microscopy is that a low-magnification objective is used to scan smears, allowing a much larger area of the smear to be seen and resulting in more rapid examination[3].

The Aerospray TB (Model 7722) automatic slide stainer automates the staining routine using either carbol fuchsin or fluorescence stains and without any risk of cross-contamination. The stainer can also be used as a cytocentrifuge by utilizing the option Cytopro® Cytocentrifuge Rotor (REF: AC-160).

The stainer offers programming flexibility where the primary stain, counterstain, and decolorizer stain applications can be adjusted to achieve the needs of AFB staining in the laboratory and make it comparable to manual staining results.

The fluorescence stain mode was recently validated by three laboratories in an international study. The laboratories that participated in this study were as follows:

- Department of Microbiology and Infection Control, Universitair Ziekenhuis Brussel, Vrije Universiteit Brussel (VUB), Belgium

- National Institute of Pathology (NIP) - Windhoek, Namibia
- National Institute of Communicable Diseases (NICD) – Johannesburg, South Africa

Each site worked independently and conducted their own internal study. ELITechGroup Biomedical Systems (EBS) reviewed the plans, protocols, and results for each study. The results of these studies were used to validate the use of the Aerospray TB (Model 7722) at each laboratory.

## Methods

The three laboratories prepared routine samples that were suspected of TB. Microscope slides were prepared in duplicate from these samples. One slide was stained using manual staining methods and its duplicate was stained using the Aerospray TB. A microscopist reviewed each slide using 400x magnification and diagnosed the slide based on the following table:

| Rating | Organism Count |
|---|---|
| Negative | 0-4 AFB organisms observed per 40 fields |
| Scanty or Rare | 5-19 organisms observed per 40 fields |
| 1+ | 20-199 organisms observed per 40 fields |
| 2+ | 5-50 organisms observed per 1 field |
| 3+ | 50+ organisms observed per 1 field |

**Table 1:** Method for Reporting Average Number of AFB Observed in Clinical Specimens

Each laboratory had some unique methods:

- UZ Brussel (Brussels Belgium) used Auramine O (REF: SS-061CA) as the primary stain and Thiazine Red (REF: SS-061BRT-EU and SS-061BCS-EU) as the counter stain. They used the 12-slide carousel for the staining. Samples were prepared from various sample types. Samples were **digested** with Benzalkonium chloride or Chlorhexidine. Slides were made in duplicates. The slides were fixed by putting the slides on a hot plate set at 65-75 °C for at least two hours. After heat fixation, the slides were sprayed with methanol and then were air dried for approximately 5 minutes. After drying Mycohold® (REF: SS-061M) was spread over the sample on the slides. The slides were heated again for 15 minutes in order to solidify the Mycohold. (Mycohold is an albumin-based fixative reagent used to improve fixation of samples on microscope slides.)

- NIP (Windhoek, Namibia) used Auramine O (REF: SS-061CA) as the primary stain and potassium permanganate (SS-061BP) as the counter stain. They used the 30-slide carousel for the staining. Samples were prepared from **direct sputum**, meaning that sputum was not digested. It was mixed with diluted Mycohold (REF: SS-161M) and smeared onto a microscope slide. Slides were made in duplicate. The slides were fixed for 15 minutes on a hot plate set to 65-70 °C.

- NICD (Johannesburg, South Africa) used Auramine O (REF: SS-061CA) as the primary stain and potassium permanganate (REF: SS-061BP) as the counter stain. They used the 30-slide

carousel for the staining. Samples were prepared with **sputum digested** with NALC (N-acetyl-L-cysteine) and Sodium Hydroxide (NaOH). Slides were made in duplicate. The slides were fixed by putting the slides in an incubator set at 75 °C overnight.

## Results

There were no noticeable trends between the different staining methods used. The data from the three tests sites were combined in the following table. Out of a total of 519 samples tested, 83 of those were positive and 436 were negative samples. There was one false positive and one false negative reported, which means 99.6% (517/519) of the samples tested were diagnosed as negative or positive correctly.

| | | Number of Slides and Corresponding Rating for Slides Stained with 7722 Stainer | | | | |
|---|---|---|---|---|---|---|
| **Slide Rating** | **Number of Slides Stained with Manual Methods** | **Negative** | **Rare** | **1+** | **2+** | **3+** |
| **Negative** | 436 | 435 | 0 | 0 | 0 | 1 |
| **Rare** | 23 | 0 | 17 | 5 | 1 | 0 |
| **1+** | 16 | 0 | 4 | 8 | 4 | 0 |
| **2+** | 20 | 1 | 1 | 7 | 10 | 1 |
| **3+** | 24 | 0 | 0 | 1 | 6 | 17 |
| **Total** | 519 | 436 | 22 | 21 | 21 | 19 |

**Table 2:** Results of sample slides stained at the three laboratories. The samples were clinical samples used to prepare microscope slides. Slides were prepared in duplicate from each sample. One slide was stained by manual methods and the duplicate was stained using the Aerospray TB (Model 7722). The slide were rated independently. The ratings are displayed in this table.

## Discussion

The results indicated two discrepant results, one false positive and one false negative. Both of these discrepant results occurred in the Namibian lab which stained direct sputum samples. The laboratory did not elaborate on these discrepant results and considered them to normal. It is known that direct sputum samples can be thick and inconsistent. It is possible that direct sputum samples can be spread too thickly on the slide which can lead to poor fixation and the specimen washing off of the slide. This can occur with both manual and automated staining. The laboratory did not indicate how these samples were treated after staining, it is uncertain if the sample was truly positive or negative. It is recommended to digest sputum samples when possible to make the sample more uniform and able to be spread more thinly and evenly onto the slide.

Finally, outside of the scope of this evaluation, it has been demonstrated that cytocentrifugation of sputum samples increases the sensitivity of smear microscopy for the diagnosis of Tuberculosis[4]. In that way, the optional cytocentrifuge function of the TB stainer can also contribute to the increased overall sensitivity of TB screening, especially in patients with extrapulmonary TB or with HIV co-infection. Conclusion

Although there are some minor discrepancies in slide ratings between the Aerospray TB stainer and manual staining methods the overall results of the two methods correspond well. These data are consistent with expected results when comparing slides from the same sample. The three test sites have implemented the use of the Aerospray TB (Model 7722) in their labs for routine samples suspect of TB. The three test sites used slightly different sample preparation and/or stain protocols, but the results of their staining proved that the Aerospray stainer stains the slides the same or close to the same as manual staining techniques.

## References


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