

EVALUATION OF A NEW GENERATION OF AN ACID-FAST BACILLI (AFB) AEROSPRAY AUTOMATED SLIDE STAINER FOR DIRECT SPUTUM SMEARS

Kevin MULLEN¹, Elizabeth SHIPIKI², Chris HELME³

¹ELITechGroup Inc. (Logan, UT USA), ²Namibia Institute of Pathology (Windhoek, Namibia), ³Nyala Technologies (Johannesburg, South Africa)

Objectives: Staining sputum samples with Auramine O stain is an essential part of screening for Mycobacterium tuberculosis. ELITechGroup Inc. (Logan, UT) has developed a new Aerospray[®] TB stainer (Model 7722). The stainer automates the Auramine O staining process. It helps labs standardize the staining process and stains slides uniformly. It also uses less stain and is less time consuming than manual staining methods without any risk of cross-contamination. This study was carried out to assess the performance of this new TB stainer.

Methods: Sputum specimens were collected from human subjects. These samples were applied directly on to microscope slides as **direct sputum smears**. Slides were made in duplicate. Slides were prepared by smearing the sputum sample directly onto a microscope slide as thinly and evenly as possible. The slides were heat fixed by placing them on a hot plate (65-70°C) for at least 15 minutes. One slide was stained with Auramine O using manual methods. The other slide was stained with Auramine O in the Aerospray TB stainer. The slides were stained 30 at a time in the stainer using the available 30-slide staining carousel. The stainer utilized Aerospray TB reagents supplied by ELITechGroup Inc. The Decolorizer Concentrate (REF: SS-161AF, was diluted as instructed), Counterstain: Potassium Permanganate (REF: SS-061BP), and Primary Stain: Auramine O (REF: SS-061CA) were used. Stains supplied by National Health Service Reagent Division (South Africa) were used for the manually stained slides.

The slides were stained using the default program for fluorescence stains. The stainer does offer programming flexibility where the primary stain, counterstain, and decolorizer stain applications can be adjusted. These adjustments were not varied as part of this study. The lab found the default setting to be satisfactory for staining by running 20 random samples and confirming the staining was satisfactory on those sample prior to starting the study.

The slides were then examined microscopically and rated. The slide examination was a blind study. Slides were reviewed by a microscopist randomly without them knowing the result of the corresponding slide by the alternative method at any time during the study. The microscopist determined the rating of each slide and reported it. The rating results for each slide were then compared to its respective duplicate slide and the ratings were compiled so that the slides stained manually were compared to the slides stained with the Aerospray TB stainer.

An additional study has been conducted on **digested sputum samples** after the original call for ECCMID 2014 Abstracts. This study was conducted at National Institute of Communicable Disease (NICD) in Johannesburg, South Africa. Sputum samples were digested with NALC (N-acetyl-L-cysteine) and Sodium Hydroxide (NaOH). Slides were stained in duplicate and fixed by putting the slides in an incubator set at 75°C overnight. One slide from the sample was stained manually by conventional means. The other side was stained on the Aerospray TB Stainer (Model 7722) using the default Fluorescence staining program. Slides were stained using the Auramine O primary stain and Potassium Permanganate counterstain. The slides were viewed by two microscopists in a blind study.

Slide Rating	Number of Slides Stained with Manual Methods	Number of Slides and Corresponding Rating for Slides Stained with Aerospray TB Stainer				
		Negative	Rare	1+	2+	3+
Negative	151	151	0	0	0	0
Rare	0	0	0	0	0	0
1+	3	0	1	1	1	0
2+	7	0	0	5	2	0
3+	6	0	0	0	2	4
Total	167	151	1	6	5	4

Results: A total of 139 samples were stained and rated. Eighty-three(59.7%) of the samples were rated as AFB negative with both staining methods. Fifty-five(39.6%) of the samples were rated as AFB positive with both staining methods. One (0.7%) of the samples rated as positive when stained with the Aerospray TB stainer, but negative when stained manually. One (0.7%) of the samples rated as positive when stained manually, but negative when stained with the Aerospray TB stainer. Thirty-seven (67.3%) of the positive samples rated exactly the same with both staining methods. Six (10.9%) of the positive samples had a higher positivity rating when stained with the Aerospray TB stainer versus the comparative slide that was stained manually. Twelve (21.8%) of the positive samples had a lower positivity rating when stained with the Aerospray TB stainer versus the comparative slide that was stained manually.

Slide Rating	Number of Slides Stained with Manual Methods	Number of Slides and Corresponding Rating for Slides Stained with Aerospray TB Stainer				
		Negative	Rare	1+	2+	3+
Negative	84	83	0	0	0	1
Rare	16	0	12	4	0	0
1+	10	0	4	5	1	0
2+	12	1	1	1	8	1
3+	17	0	0	1	4	12
Total	139	84	17	11	13	14

Conclusions: 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. These data prove that the new Aerospray TB stainer can be used for routine samples in the Namibia Institute of Pathology in Windhoek, Namibia.

A total of 167 samples were stained at NICD and were part of this study. Out of these 167 samples 151 (90.4%) were negative for both staining methods. Out of the 16 positive samples, 16 (100%) of the Aerospray slides were within one rating of the rating assigned to the manually stained slide. It was observed that there was a trend that some samples were rated as positive but with a lower rating. Out of the 16 positive samples, eight (50.0% of positive samples) were rated with a lower rating than the comparative manually stained sample. Seven (43.8% of positive samples) were rated the same when stained with both methods. One (6.3% of the positive samples) was rated with a higher rating than the comparative manually stained sample.

The NICD lab concluded that these results were acceptable. They have implemented the use of the stainers in their lab. They will be conducting another study with some changes to the stain program used. They plan on increasing the primary stain setting on the stainer to determine if the numerical ratings of positive slides will converge with these changes.

Acknowledgements: Zacharia Mebena for his efforts performing this additional study at NICD.