

Performance evaluation of the MultiStainer AT-3001 for auramine staining and hot Ziehl-Neelsen staining.

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Objective

The search for acid-fast bacilli (AFB) in samples by microscopic examination is an essential step in the diagnosis of mycobacterial infections. Rapid auramine staining is often performed as a first step. The Ziehl-Neelsen hot stain (ZNC) is still the standard method. The MultiStainer AT-3001 (MT, Biocentric) is the first system that can perform both stains in an automated way. The objective was to compare the performance of the stainer (MT) to the AutoStainer AT-2000F (AT, Biocentric) for auramine staining and to compare the performance of the ZNC staining (MT) to the cold Ziehl-Neelsen staining (ZNF).

Material and Methods

- ❖ 844 clinical respiratory specimens (sputum n=498, traps n=211, gastric tubes n=70, BAL n=65) were prospectively collected and decontaminated using the MycoPrep® technique. Using the decontamination pellet obtained for each sample, 2 smears were made and stained with auramine (Biocentric) by MT stain and AT stain. Auramine-positive smears were then stained with ZNF (Biomérieux, Tan-Thiam-Hok method) and ZNC (Biocentric, MT). Where there were discrepancies between ZNC and ZNF staining, 6 additional smears were taken from each decontamination pellet and stained in the same manner.
- ❖ Using liquid (MGIT, BD) and solid (Coletsos, Biomérieux) cultures, whether or not they were positive for mycobacteria belonging to the *tuberculosis* complex (MCT or MNCT), 2 smears were made and stained with ZNC (MT) and ZNF.
- ❖ The stained smears were performed and read in a blinded fashion by different experienced technicians. The results were interpreted according to WHO recommendations for the smears, and according to a quality score based on the intensity of the BAARs and the contrast for the cultures.

Results

❖ Results obtained for samples stained with auramine by MT and AT stainers

844 respiratory specimens analyzed (Table 1):

- ✓ 840 samples (777 negative and 63 positive) were concordant between the 2 stainers, i.e. 99.5% concordance.
- ✓ 4 samples were not concordant, including 3 with ≤ 1 -9 BAAR /100 ch.

❖ Results for ZNC and ZNF stained samples

Of the 67 auramine positive (or discordant) DEs, 64 could be stained with ZNC and ZNF stainers (Table 2):

- ✓ 30 results were concordant between ZNC and ZNF
- ✓ 34 results were discordant

To resolve these discrepancies, 171 additional smears were analyzed (Table 3):

- ✓ 68 (40%) became concordant,
- ✓ 103 (60%) remained discordant, of which 93 results were more sensitive with the ZNC and 10 more sensitive with the ZNF.

❖ Results obtained for ZNC and ZNF staining on cultures

Smears could be stained with ZNF/ZNC for 105 cultures (Table 4):

- ✓ 51 MGIT positive (MCT n=25, MAC n=11, other MNCT n=15)
- ✓ 54 positive on Coletsos medium (MCT n=29, MAC n=8, other MNCT n=17)

A score of 2 (strong intensity and contrast) was obtained in 86% (91/105) with ZNC and 14% (15/105) with ZNF.

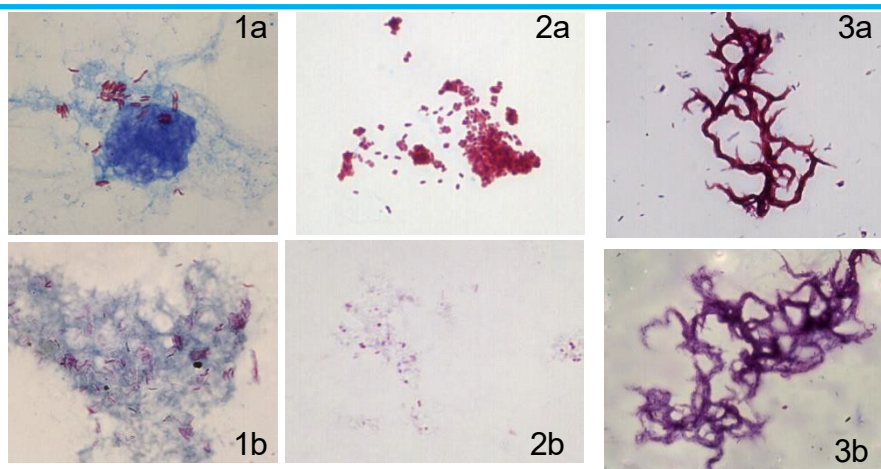


Figure 1: ZNC staining (sputum 1a, culture of *M. avium* 2a and *M. tuberculosis* 3a) and ZNF staining (sputum 1b, culture of *M. avium* 2b and *M. tuberculosis* 3b).

Auramine		MultiStainer AT 3001 (MT)						Total
		None	+/-	Rare	1+	Some 2++	Several 3+++	
AutoStainer AT 2000 F (AT)	None	777	1					778
	+/-		12					12
	Rare 1+		2	7				9
	Some 2++					17	1	18
	Several 3+++						27	27
	Total	777	15	7		17	28	844
Concordant results		Discordant results						

Table 1: Results obtained for auramine staining with the AT and MT from the samples.

		ZNC (MT)					Total
		None	+/-	Rare 1+	Some 2++	Several 3+++	
ZNF	None	5	7	4	1		17
	+/-		10	4	2	3	19
	Rare 1+	1		5	1	4	10
	Some 2++				2	3	5
	Several 3+++					12	12
	Total	6	17	13	6	22	64

Table 2: Results obtained for ZNC (MT) and ZNF staining from Auramine-positive specimens.

		ZNC (MT)					Total
		None	+/-	Rare 1+	Some 2++	Several 3+++	
ZNF	None	12	12	11	1	1	37
	+/-	2	16	23	4	7	52
	Rare 1+		5	15	5	12	37
	Some 2++			3	5	17	25
	Several 3+++					20	20
	Total	14	33	52	15	57	171

Table 3: Results obtained for the additional ZNC-stained smears (MT) and for ZNF, with discordance between 2 ZN stains.

Cultures (n=105)		ZNC (MT)		
		Score 0	Score 1	Score 2
ZNF	Score 0		7	1
	Score 1	1	5	76
	Score 2		1	14

score 0: no BAAR; score 1: low intensity and contrast; score 2: high intensity and contrast

Table 4: Results obtained for ZNC and ZNF staining performed on the cultures.

Conclusion

The MultiStainer AT-3001 stainer is the first automated system to perform auramine staining and hot ZN staining. The AT and MT machines have comparable performance for auramine staining. On the other hand, ZN staining is considerably improved by heat, both in terms of sensitivity from samples, and in terms of intensity and contrast from cultures, and is becoming routinely accessible thanks to its automation.