

## 短 報

EVALUATION OF SENSITIVITY AND SPECIFICITY OF COLD STAIN  
VIS-A-VIS ZIEHL NEELSEN STAIN ON CULTURE RESULTS

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Various cold stain techniques have been tried with varying success since early part of the century<sup>1)</sup>. Dr. S. Kudoh of Japan and Dr. A. J. Pathan of Pakistan discussed to evolve a simple process for cold method (see the foot note)<sup>2)</sup> which is a simple procedure for staining tubercle bacilli in sputum specimen. And the cold method was found as sensitive, as specific and almost as reliable as Ziehl Neelsen on direct comparison of two techniques<sup>3)</sup>. In this paper, the cold method was further evaluated on culture results with Ziehl Neelsen stain during 1987-90 in our institute.

## MATERIAL AND METHODS

Duplicate smears were prepared first from each of 1659 sputum specimens for staining independently by cold and Ziehl Neelsen methods, then 0.1ml of each sputum specimen was inoculated for growth on acid Lowenstein Jensen medium<sup>4)</sup> after treatment with NaOH. Both Slides stained with Ziehl Neelsen and cold methods were scanned under microscope for AFB following IUAT technical guide (1978). The

seeded slant was incubated for a minimum of six weeks and observed for buff coloured colonies weekly.

## RESULTS

Observations were made at two stages : (a) comparison of Ziehl Neelsen and cold stained sputum smears under direct microscope as shown in table 1. (b) Comparison of results of Ziehl Neelsen and cold stains with the results of seeding of the respective sputa on acid Lowen-

Cold stain method		Procedure
Reagents		1. Cover air dried smear with A) for 25 minutes
A) Basic fuchsin	1.0 g	2. Rinse off stain with water
95 % Ethanol	10 ml	3. Cover smear with B) for 30 seconds (this step is repeated again till the smear become pale blue)
Phenol (liquefied)	3.0 g	4. Rinse off with water
Distilled water	100 ml	5. Airdry the slide
B) 10 % NaCl water solution	10 ml	
Methanol	90 ml	
Methylene blue	0.1 g	

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**Table 1** Correlation between Conventional Ziehl Neelsen and Cold Methods

		ZIEHL NEELSEN METHOD		TOTAL
		0/±	Positive	
C O L D  M E T H O D	0/±	483	42	525
	+ve	0	1134	1134
TOTAL		483	1176	1659

**Table 2** Comparison of Ziehl Neelsen and Cold Smear Microscopy with Culture

		ZIEHL NEELSEN MICROSCOPY		TOTAL			COLD MICROSCOPY		TOTAL
		+	-				+	-	
C U L T U R E	+	1147	189	1336	C U L T U R E	+	1105	231	1336
	-	29	294	323		-	29	294	323
TOTAL		1176	483	1659	TOTAL		1134	525	1659

stein Jensen media as shown in table 2.

By comparing the results of Ziehl Neelsen and cold techniques total positive yield by cold method was slightly lower than Ziehl Neelsen by 1134 or 68.3% against 1176 or 70.8%. Disregarding the scores, 1617 pairs of smears gave identical results i.e., an agreement of 97.4% or a disagreement of 2.5%. The negative result by cold method had 8% chances of being positive by Ziehl Neelsen method. Comparison of cold and Ziehl Neelsen techniques on culture results showed: the positive yield by cold on culture positive specimens (1105 or 82.7%) was slightly lower than that of Ziehl Neelsen (1147 or 85.8%). The agreement between the results of cold smear microscopy and culture was on 1399 or 84.3% against the agreement with Ziehl Neelsen 1441 or 86.8%. Thus the agreement between cold and Ziehl Neelsen methods on culture including all negative and positive cases is 97.08% or a

disagreement of 2.9%. Sensitivity for cold method was 82.7% against 85.8% of Ziehl Neelsen method. The specificity of each method was 91%.

#### DISCUSSION

The comparison of cold and Ziehl Neelsen methods on culture has almost coincided with the comparison of their respective results, thus supporting the said accuracy and reliability of cold method against Ziehl Neelsen method. The sensitivity of cold is 3% less than Ziehl Neelsen method and this disparity may be because of detachment of smears as they are not heat fixed like Ziehl Neelsen but it is obvious that by not fixing with heat, we don't compromise the results much. This can be improved by prolong drying. On the other hand, specificity of cold method is as high as Ziehl Neelsen technique suggesting that where regular and adequate supply of material can not be maintained, the

cold stain is other way a practicable and rewarding technique.

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