

Comparative Evaluation of Ziehl Neelsen Staining and Knowledge, Attitudes and Practices of Laboratory Personnel in Relation to Ziehl Nielsen

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ABSTRACT

Objective: To describe laboratory personnel's attitude and practices toward phenol exposure during Ziehl Neelsen (ZN) acid fast staining method and to evaluate the feasibility of an alternate modified Kinyoun cold (MKC) stain.

Method: A total of 187 sputum samples were collected from suspected tuberculosis cases and stained by the MKC method and ZN stain and were read by an experienced microscopist and a researcher. A cross-sectional questionnaire survey of 35 laboratory personnel was also conducted.

Results: Modified Kinyoun cold stain gave sensitivity, specificity, positive and negative predictive values of 100%, 99.4%, 94.1% and 100%, respectively. Both stains corresponded with an agreement rate of 99.5%. Almost 94.7% of respondents reported that they worked in a closed area when staining and 57.1% did the staining method without ventilation. Material safety data sheet (MSDS) of phenol was not known to 77.1% of laboratory personnel. All of the participants (100%) in this study welcomed a similar, non heating method for acid-fast bacillus (AFB). There was significant association between those not comfortable with phenol exposure (77.1%) and complaints of irritation (48.6%) and headache (2.9%) [$\chi^2 = 10.98$, $r = 0.55$, $p = 0.001$].

Conclusions: The MKC is suitable for use as a substitute for the ZN method for the demonstration of AFB in the primary diagnosis and treatment assessment of pulmonary tuberculosis. Focus should be given on educating laboratory staff on the hazards, risks and precautions associated with the phenol/ZN method.

Keywords: Acid fast staining, cold staining method, knowledge attitudes practices, laboratory personnel, tuberculosis

Evaluación Comparativa de la Tinción de Ziehl Neelsen y los Conocimientos, Actitudes y Prácticas del Personal de Laboratorio en Relación con Ziehl Nielsen

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RESUMEN

Objetivo: Describir las actitudes y prácticas del personal del laboratorio hacia la exposición al fenol durante la aplicación del método de tinción ácido-rápida de Ziehl Neelsen (ZN), y la viabilidad de la alternativa de una tinción de Kinyoun en frío modificada (MKC).

Método: Un total de 187 muestras de esputo fueron recogidas de casos con sospecha de tuberculosis, y teñidas por el método MKC y la tinción de ZN, tras lo cual fueron leídas por un microscopista y un investigador con experiencia. También se realizó una encuesta transversal a manera de cuestionario, entre las 35 personas que conformaban el personal del laboratorio.

Resultados: La tinción de Kinyoun en frío modificada arrojó sensibilidad, especificidad, y valores predictivos positivos y negativos de 100%, 99.4%, 94.1% y 100%, respectivamente. Ambas tinciones se correspondieron con una tasa de acuerdo de 99.5%. Casi el 94.7% de los encuestados informó que trabajaban en un área cerrada en el momento de la tinción, y 57.1% tuvo el método de la tinción sin ventilación. La ficha de datos de seguridad (FDS) de fenol era desconocida para el 77.1% del personal de laboratorio. Todos los participantes (100%) en este estudio dieron la bienvenida a un método similar, sin

calentamiento, para los bacilos acidoresistentes (BAR). Hubo una asociación significativa entre aquellos para quienes la exposición al fenol les era incómoda (77.1%) y las quejas de irritación (48.6%) y dolor de cabeza (2.9%) [$\chi^2 = 10.98$, $r = 0.55$, $p = 0.001$].

Conclusiones: El MKC es adecuado para uso como sustituto del método de ZN para la demostración de BAR en el diagnóstico primario y la evaluación del tratamiento de la tuberculosis pulmonar. Debe centrarse la atención en educar al personal de laboratorio en los peligros, los riesgos, y la precaución asociados con el método de fenol/ZN.

Palabras claves: Tinción ácido-rápida, método de tinción en frío, conocimientos-actitudes-prácticas, personal de laboratorio, tuberculosis

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INTRODUCTION

Although once thought to be eradicated, today tuberculosis (TB) remains a global health problem with its resurgence primarily associated with the advent of the HIV/AIDS epidemic. The World Health Organization (WHO) estimates that there are over two million TB-related deaths each year with the emergence of multi-drug resistant strains of the TB bacilli associated with HIV infection. Thus, TB remains a global public concern with approximately one-third of the world's population being infected at an infection rate of one per second (1). Early diagnosis and treatment of pulmonary TB remains the most effective and reliable means of controlling the spread of the disease. Diagnosis and assessment of treatment depends on microscopic expression of acid-fast bacilli (AFB) in sputum for initial diagnosis and drug sensitivity by means of culture (2).

The Ziehl Neelsen (ZN) method remains most popular throughout the majority of laboratories across Guyana. It is doubtlessly the paramount and most frequently used method for staining for *Mycobacterium tuberculosis*. The heating of carbol fuchsin employed by the ZN method causes aerosolization of phenol which is hazardous when it exceeds the permissible exposure limit (PEL) of 5 ppm (parts per million) or 19 milligrams per cubic metre or in an eight-hour time weighted average (TWA) concentration. The short-term exposure limit (STEL) is 15.6 ppm (60 mg/m³) for periods not to exceed 15 minutes (3).

To prevent the exposure of laboratory personnel to this undetermined concentration of the mutagen phenol, this research seeks to introduce another method – modified Kinyoun cold stain (MKC), which is equally effective in demonstrating the tubercle bacilli. The modified Kinyoun cold stain requires no heating, thus it eliminates phenol vapour contamination risks. This study was therefore to assess the efficiency of the MKC method as a replacement for the ZN and to assess the knowledge, attitudes and practices (KAP) of laboratory personnel in relation to the ZN method in Guyana.

SUBJECTS AND METHODS

A sample size of 187 sputum smears of TB suspects was randomly collected from the different laboratories in Guyana.

Four laboratories in Guyana which use the ZN technique for staining of sputum samples for AFB were selected for the study. A simple survey was also conducted by randomly distributing questionnaires to thirty-five laboratory personnel in laboratories performing the ZN method for the analysis of KAP. The study design adopted both cross-sectional and experimental studies.

The mucopurulent part of the sputum was evenly spread over an area of 1 x 2 cm. The smears were air dried and heat fixed, by passing the slide through the flame. Each original smear was stained using the conventional ZN and MKC stain method and recorded by the researcher and by an experienced microscopist of the department.

Staining procedure

Ziehl-Neelsen stain: The fixed smears were flooded with a solution prepared by dissolving 2.5 g of basic fuchsin in 100% alcohol and diluted with 250 ml of water and 12.5 ml melted phenol crystals. The smear was gently heated until steaming with a Bunsen burner for five minutes. The smear was then rinsed with water and decolourized with 1% acid-alcohol (10 ml hydrochloric acid and 70% alcohol – 990 ml) and allowed to stand for two to five minutes. After standing, the smear was rinsed with water and counterstained with methylene blue for one or two minutes. The slide was finally rinsed with water and air dried before examination. The stained smears were scanned with x100 oil immersion lens for the presence of red thin rods or coccobacilli.

Modified Kinyoun cold stain: Fixed smears were flooded with a solution prepared by dissolving 4 gm of basic fuchsin in 8 ml of phenol and diluted with 100 ml distilled water and 20 ml of 95% alcohol. One to two drops of Tergitol no. 7 agent was added to every 30–40 ml of MKC to accelerate the acid fast organism (4). The smears were allowed to stand for 10 minutes and then rinsed with water. Following rinsing, the slide was counterstained with methylene blue for two minutes (dissolving 5 ml of methylene blue stock in 45 ml distilled water). The slide was finally rinsed with water and dried before examination. The stained smears were scanned with x100 oil immersion lens for the presence of red thin rods or coccobacilli.

Microscopy reports

Smear examination reports included quantification by expressing the actual number of bacilli seen per field or by giving a 1⁺ to 3⁺ rating according to the convention of the American Thoracic Society (5).

Data analysis

The data were double entered in Microsoft Excel data sheets, cross-checked and analysed using SPSS 11.0. Descriptive statistics were carried out to measure relative frequencies, prevalence percentages, Chi-squared test (χ^2) and odds ratio.

Ethical clearance was obtained from the Ethical Review Committee (ERC) under the Ministry of Health, Guyana. After explaining the objectives of the study, written consent was obtained from the participants.

RESULTS

Table 1 shows comparative results obtained with MKC against ZN staining methods. Of the 187 sputum samples, the ZN method detected a total of 16 (8.6%) positive slides and MKC detected 17 (9.1%) slides positive for AFB. Modified Kinyoun cold stain gave sensitivity, specificity, positive and negative predictive values of 100%, 99.4%, 94.1% and 100%, respectively. Both stains corresponded with an agreement rate of 99.5%. All slides negative by the ZN method were also negative by the MKC method.

Comparing the ZN method with the MKC method, the grading results were the same for 1⁺, 2⁺, 3⁺ as 6, 5 and 5, respectively except that MKC had one positive for 3 AFB (Table 2).

A total of 35 laboratory personnel working in the acid fast staining section were interviewed. The working area and characteristics of AFB staining are presented in Table 3. About 45.7% of the respondents reported that they prepared more than 20 slides per day whereas 22.9%, 5.7% and 2.9% said they prepared > 100, > 200 and > 300 slides per day, respectively. The majority of the respondent (60%) reported exposure to phenol for ≥ 1 year and 28.6% for almost six months to > 1 year; 94.3% reported working in a closed working area and 57.1% reported working without any facility for ventilation; 31.4% of respondents claimed that phenol could be smelled

Table 2: Cross comparison of the Kinyoun cold staining method with the Ziehl Neelsen (ZN) method by grading of acid-fast bacilli smear positivity

Kinyoun Staining	ZN Staining				Total
	Negative	1 ⁺	2 ⁺	3 ⁺	
Negative	170	–	–	–	170
1 ⁺	–	6	–	–	6
2 ⁺	–	–	5	–	5
3 ⁺	–	–	–	5	5
3 acid-fast bacilli	1	–	–	–	1
Total	171	6	5	5	187

$\chi^2 = 561$, $df = 12$, p -value < 0.05

Table 3: Characteristics of work nature for Ziehl Neelsen (ZN) staining method

Variable	Number	%
No. of slides		
0	1	2.9
0–5	7	20.0
> 20	16	45.7
> 100	8	22.9
> 200	2	5.7
> 300	1	2.9
Duration of exposure		
Never	1	2.9
< 6 Months	3	8.6
6 Month – < 1 Year	10	28.6
1 Year – < 2 Year	1	2.9
2 Year – < 5 Year	9	25.7
5 Year – < 10 Year	8	22.9
> 10 Year	3	8.6
Working in closed area		
Yes	33	94.3
No	2	5.7
Working in ventilation		
Yes	15	42.9
No	20	57.1
Can persons smell phenol outside staining section		
Yes	11	31.4
No	19	54.3
Sometimes	5	14.3

Table 1: Smear results by Ziehl Neelsen (ZN) method and modified Kinyoun cold staining methods

Kinyoun staining	ZN staining			Accuracy	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	Positive predictive value (%)	Negative predictive value (%)
	Positive	Negative	Total					
Positive	16	1	17	99.5	100 (82.1–100)	99.4 (97.7–99.4)	94.1 (77.3–94.1)	100 (98.3–100)
Negative	0	170	170					
Total	16	171	187					

$\chi^2 = 174.97$, $df = 1$, p -value < 0.05, kappa = 9.7, Pearson correlation = 0.97

outside the staining section. Univariate analysis of the associations between working in a closed area and ventilation predicted the risk of exposure of laboratory personnel to phenol (OR = 0.74, 95% CI 0.04, 12.8).

Knowledge and preventive measures regarding AFB staining

Table 4 shows that 68.6% used gloves and laboratory coat, 28.6% used gloves, laboratory coat and face shield and 2.9%

Table 4: Measures used and knowledge about acid-fast bacilli (AFB) staining method among lab personnel

Variable	Number	%
PPE used		
Gloves and lab coat	24	68.6
Gloves, lab coat and face shield	10	28.6
Gloves, lab coat and goggles	1	2.9
Are you aware of MSDS?		
Yes	16	45.7
No	19	54.3
Are you aware of MSDS of phenol?		
Yes	8	22.9
No	27	77.1
Are you comfortable to phenol exposure?		
Yes	8	22.9
No	27	77.1
Symptoms		
Irritation	17	48.6
Headache	1	2.9
None	17	48.6

PPE = personal protective equipment

MSDS = material data safety sheet

used gloves, laboratory coat and goggles as personal protective equipment (PPE). Only 45.7% of respondents were aware of the general material data safety sheet (MSDS) with only 22.9% being aware of the MSDS of phenol. There was a significant association between awareness of general MSDS and awareness of phenol with a correlation of 0.59 and $\chi^2 = 12.31$. There was significant association between those not comfortable with phenol exposure (77.1%) and complaints of irritation (48.6%) and headache (2.9%) [$\chi^2 = 10.98$, $r = 0.55$, $p = 0.001$].

Table 5 represents the attitude and practices of laboratory personnel toward AFB staining. Although 71.4% of lab personnel were aware of the fact that phenol inhalation could cause risk to the body, the majority of the respondents were not aware of any other staining method to replace ZN. All the 11.4% of respondents who knew about other staining method were satisfied with the alternate method.

The majority of laboratory personnel (77.1%) did not have any appraisal regarding the hazards related to ZN or phenol exposure, whereas 65.7% said that the appropriate safety equipment were not provided. All the lab personnel in this

Table 5: Attitude and practices of lab personnel toward acid-fast bacilli (AFB) staining method

Variable	Number	%
Can phenol inhalation cause risk?		
Yes	25	71.4
No	9	25.7
Is there any other method to replace Ziehl Neelsen?		
Yes	4	11.4
No	30	85.7
Don't know	1	2.9
Satisfied with other staining method?		
Yes	4	100
No	0	0
Properly appraised of hazards associated with Ziehl Neelsen?		
Yes	8	22.9
No	27	77.1
Appropriate safety equipment provided?		
Yes	12	34.3
No	23	65.7
Would you welcome similar effective, faster, and more economical non heating method than Ziehl Neelsen?		
Yes	35	100
No	0	0

study welcomed a similar effective, faster and more economical non heating method than ZN.

DISCUSSION

Tuberculosis is a public health problem in the developing world today and case detection constitutes an important directly observed treatment, short-course (DOTS) strategy according to WHO (6). For successful reduction in tuberculosis morbidity and mortality, identification of TB cases in the community and chemotherapy is foremost important. Culture of TB bacilli remains the gold standard diagnostic method but is lengthy because of slow growth; the results require weeks to identify positive culture. On the other hand, several automated systems and polymerase chain reaction (PCR) are rapid, very specific and sensitive but are not very cost-effective especially in the developing world. The sputum smears examination, although low sensitive, is relatively cheap.

In Guyana, sputum smear examination using ZN method remains one of the most common methods of detecting TB. Phenol is one of the important components used in ZN method, which is a toxin, mutagen and poison.

To improve the efficiency of the diagnostic process and to reduce the toxic effect of phenol to laboratory personnel, an alternative method, MKC, was introduced. The MKC incor-

porates the use of the chemical tergitol. This compound replaces the heat utilized in the ZN method. It works by reducing the surface tension of the highly resistant and fatty cell wall of *Mycobacterium*, thus making it permeable to the carbol fuschin, facilitating successful staining of the bacilli (4).

The most important advantage of the MKC method is the elimination of the risk of exposure to phenol aerosols. However, in one slide positive for AFB by the MKC method and negative for AFB by the ZN method, the number of AFB seen was only three for the entire slide. This could have been as a result of: the presence of scanty AFB in the specimen, disproportionate allotment on the slides and thoroughness and experience of the microscopist, poor specimen, patient on treatment or new infection.

From the method outlined earlier, the MKC is both easier to perform than the ZN method, more comfortable and more economical since it eliminates the expenditure of fuel for heat. Because of its simplicity, it does not require highly trained or experienced technical laboratory personnel to carry out the staining procedure as compared with the ZN method (7, 8).

Phenol is a highly flammable, poisonous, corrosive chemical that is well absorbed by all routes of exposure. It is readily absorbed through inhalation, eye and skin contact/absorption, and ingestion. Exposure through any of the aforementioned routes can result in systemic poisoning. Severe irritation is caused by all forms of phenol, but the greatest effect occurs through skin contact which produces chemical burns at the site of contact. Phenol is not a carcinogen but it is a promoter of tumours (MSDS of phenol, Appendix 1). "It is also a possible teratogenic agent and the fumes are irritating to the eyes and affect the pupil's response to light (miosis)" (9).

The current Occupational Safety and Health Administration (OSHA) permissible exposure limit for phenol is 5 ppm (19 milligrams per cubic metre (mg/m³) as an eight-hour time-weighted average (TWA) concentration). The National Institute for Occupational Safety and Health (NIOSH) has established a recommended exposure limit (REL) for phenol of 5 ppm (19 mg/m³) as a TWA for up to a 10-hour work-day and a 40-hour work-week and a short-term exposure limit (STEL) of 15.6 ppm (60 mg/m³) for periods not exceeding 15 minutes. The NIOSH also assigns a 'skin' notation to phenol (3, 10, 11).

The American Conference of Governmental Industrial Hygienists (ACGIH) has assigned phenol a threshold limit value (TLV) of 5 ppm (19 mg/m³) as a TWA for a normal eight-hour workday and a 40-hour workweek. The ACGIH also assigns a 'skin' notation to phenol (12).

Phenol is of known toxicity and its lethal limit has been established by science, thus its use in poorly vented spaces is hazardous. There is a high potential for chronic systemic poisoning due to prolonged exposure to low concentrations of this toxin (13).

This study shows very low awareness among laboratory personnel regarding the risks of phenol exposure. The infor-

mation about the particular properties of chemicals, listing everything from toxicity and health effects to necessary safety equipment, handling and storage procedures should be provided to laboratory personnel. The employer is also responsible for educating staff on the hazardous nature of exposure in the workplace. The study also illustrates the emission of ZN fumes to areas other than the staining room.

Although laboratory personnel feel uncomfortable being subjected to the fumes associated with this method, many are unaware of the availability of other methods which are safer and obtain the same objective. All of the participants said they would welcome another staining method which is just as effective as the ZN, faster and more economical, without the use of heat.

CONCLUSION

The modified Kinyoun cold method has proven to be a suitable replacement for the ZN method for the demonstration of AFB in the primary diagnosis of pulmonary TB in sputum. Despite all the transparent information available about the dangerous nature of phenol, the ZN technique remains the standard for sputum staining for the demonstration of AFB without the utilization of appropriate safety requirements in almost all laboratories throughout Guyana. Looking at the toxic/harmful effect of phenol on laboratory personnel, the ZN method should be replaced by the KMC method and laboratory personnel should be adequately equipped and informed about the hazards of ZN method and the availability of other methods.

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