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EFFECTIVENESS OF NALC (N-ACETYL-L-CYSTEINE) TO IMPROVE MICROSCOPIC EXAMINATION OF ACID FAST BACILLI

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Effectiveness of Nalc (N-Acetyl-L-Cysteine) To Improve Microscopic Examination of Acid Fast Bacilli

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Abstract – We compared the NaOH-N-acetyl cysteine (NaOH-NALC) and the sulfuric acid decontamination procedure in the detection of mycobacteria using the Mycobacteria Growth Indicator Tube (MGIT). In total 219 sputum specimens were collected from 142 Zambian patients and subjected to mycobacterial culture. One half of the specimen was decontaminated with NaOH-NALC and the other half was decontaminated with sulfuric acid. From the 438 samples a total of 261 (60%) cultures yielded growth of mycobacteria, consisting of 22 different species. The sulfuric acid method was more successful than the NaOH-NALC method in recovering mycobacteria in MGITs (146 versus 115 respectively, p=0.001). Of the 146 positive mycobacterial cultures recovered after sulfuric acid decontamination 28 were Mycobacterium tuberculosis, 84 nontuberculous mycobacteria (NTM) and 34 acid fast bacterial isolates which could not be identified to the species level. The 115 mycobacteria recovered by the NaOH-NALC method consisted of 34 M. tuberculosis strains, 55 NTM and 26 acid fast bacteria that could not be identified.

Keywords: NALC, Decontaminated, Mycobacteria, Culture, Growth, Versus, Recovered

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INTRODUCTION

Tuberculosis continues to be a tremendous public health problem in both developing and industrial countries. Mycobacterium tuberculosis complex strains are still responsible for the majority of Mycobacterium infections worldwide (5). However, there has been an increase in infections caused by nontuberculous mycobacteria (NTM) mainly due to the AIDS pandemic (12). Culture is considered the gold standard for the detection of M. tuberculosis, but most clinical sputum samples contain a variety of microorganisms that may overgrow M. tuberculosis. Decontamination of these samples is therefore crucial in preventing contamination of the mycobacterial culture. However, also the recovery of mycobacteria is negatively influenced by decontamination (13,15). The decontamination method applying sulfuric acid was used in our laboratory in the former days and NaOH-N-acetyl cysteine (NaOH-NALC) is currently most widely used. It is unclear which method provides maximum decontamination while maintaining the viability of M. tuberculosis complex and which species of mycobacteria are more sensitive to a specific decontamination method (9,10). The isolation of M. tuberculosis complex and/or NTM has epidemiological implications and is also relevant for the management of patients with regard to appropriate treatment, isolation and contact tracing. Antibiotic treatment may vary according to the species encountered, and certain species may require no antibiotic therapy at all.

REVIEW OF LITERATURE:

From March to June 2001, 219 sputum specimens were collected from 142 chronically ill patients and cultured for mycobacteria in Zambia. After informed consent each patient admitted on the medical ward in the hospital and who was coughing for more than two weeks was included in the study. During three consecutive days morning sputum was collected from these patients and two separate specimens were cultured for mycobacteria. The third morning sputum specimen was stored at −20°C. About 5% of the patients were not able to produce sputum and about 35% of the patients could not submit a second sputum sample because they did not have a productive cough anymore, discharged themselves or died. Before decontamination, the 219 specimens were divided in two samples. The specimen was macroscopically divided and the (muco)purulent parts were separated equally. The specimen was assessed microscopically according to the criteria from the ASM-manual for clinical microbiological procedures, i.e. basically on the basis of the number of squamous epithelial cells and leukocytes (11). One half of the specimen was decontaminated with NaOH-NALC and

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the other half was decontaminated with sulfuric acid. All specimens were collected, decontaminated by NaOH-NALC and sulfuric acid, inoculated and incubated in Zambia. After transport of all tubes the work-up of the positive cultures was done in The Netherlands. Each specimen of the patient was collected in a container that was never used before and was imported from The Netherlands. The stock of decontamination fluid was sterilised twice a week and each day a fresh tube or bottle was opened.

1.Decontamination with NaOH-N-acetyl cysteine :

For the NaOH-NALC procedure 2.5 ml of the sputum specimen was put in a 15 ml glass centrifuge tube with a screw cap(9). An equal volume of NaOH-NALC decontamination solution (6.8% NaOH, 2.9% sodium citrate, 0.5% NALC, final concentration of NaOH: 1.7%) was added. After vortexing the specimens was left at room temperature for 15 min. Subsequently 5 ml of sterile 0.067 M phosphate buffer (pH 6.8) was added and the mixture was centrifuged at 3660×g for 15 min. The supernatant was discarded and the sediment was inoculated in a Mycobacteria Growth Indicator Tube (MGIT; Becton Dickinson Microbiology Systems, Cockeysville, MD).

Decontamination with sulfuric acid (15):

For the sulfuric acid (6.0%) decontamination, 2.5 ml of the specimen was put in a 15 ml glass centrifuge tube with a screw cap and an equal volume of sulfuric acid (final concentration of sulfuric acid: 3.0%) was added. After vortexing the specimen was left at room temperature for 15 min for decontamination. Then, 5 ml sterile normal saline was added and the mixture was vortex-mixed again. After centrifugation for 15 min at 3660×g, the supernatant was discarded and the sediment was suspended in 10 ml of normal saline. After another centrifugation for 15 min at 3660×g, the supernatant was discarded and the sediment was inoculated in an MGIT.

3.Media, incubation, reading, identification:

Half a ml of the decontaminated specimens was inoculated into an MGIT with PANTA (polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin) according to the protocol of the manufacturer. The inoculated tubes were incubated for at least 8 weeks at 37°C in Zambia or in The Netherlands(14). The tubes were incubated between 2 and 12 weeks in Zambia and the time of incubation was extended for at least 8 weeks in The Netherlands. They were read macroscopically every week and smears stained by Ziehl- Neelsen were made from all tubes in The Netherlands. Mycobacteria were identified by the Accuprobe (bioMérieux) or 16S rDNA sequencing (8). From 60 samples, identifica tion of the acid fast bacteria (AFB) was not possible due to contamination or due to loss of viability of the bacteria during the process of culture, transport, and/or identification. From 142 patients 219 sputum specimens were collected. These specimens were divided in two parts (in total 438 samples) that were decontaminated with NaOH-NALC or sulfuric acid. At least 70% of the sputum specimens had a quality representative for the lower respiratory tract (Quality score≥1). Of the 142 patients 37 had a sputum culture positive for M. tuberculosis and from these patients 18 had also a positive smear for AFB made from these samples. The sulfuric acid method was more successful than the NaOH-NALC method in recovering mycobacteria via MGITs (146 and 115 respectively, p=0.001). Of the 146 mycobacteria (recovered by sulfuric acid method) 28 belonged to M. tuberculosis, 84 to NTM and 34 AFB with no identification. The 115 mycobacteria recovered by NaOH-NALC method consisted of 34 strains of M. tuberculosis, 55 NTM and 26 AFB with no identification. Comparing the samples decontaminated with NaOH-NALC and those decontaminated with sulfuric acid no significant difference was found in recovering M. Tuberculosis (p=0.39). However, the sulfuric acid method appeared a significantly better decontamination method than the NaOH-NALC method for recovering NTM (p=0.001). The 146 positive mycobacterial cultures detected by the sulfuric acid method consisted mostly of M. tuberculosis (5), M. lentiflavum (2,6) and M. intracellulare (13). The 115 mycobacteria isolated after decontamination with NaOH-NALC were mainly identified as M. tuberculosis (3,4), M. intracellulare (11), and M. lentiflavum (7). Of the 62 M. Tuberculosis isolates 14 were detected by the NaOH-NALC method as well as by the sulfuric acid method. 14 M. tuberculosis isolates where detected exclusively by the sulfuric acid method and 20 only by the NaOH-NALC method.

CONCLUSION:

In the study of Burdz et al. no clearly superior method between NaOH-NALC, modified Petroffs' method or the Yamane procedure was found in affecting the viability of M. Tuberculosis H37Ra (2). A limited number of earlier studies showed differences between the recovery of mycobacteria by different decontamination methods. Salfinger and Kafader compared NaOH-NALC with sodium dodecyl sulfate (SDS)-sodium hydroxide for the detection of mycobacteria by BACTEC Middlebrook 7H12 medium and Löwenstein- Jensen slants (17). They concluded that SDS-NaOH was superior to NaOH-NALC as a pre-treatment method. On the other hand, in the study of Pfyffer et al. clinical specimens were processed with SDS and NaOH-NALC using the MGIT (16). Specimens pre-treated with SDS resulted in both poor recovery and delayed mean time to detection of AFB. In both studies the number of NTM strains isolated were much less than in our study.

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