PACIFIC JOURNAL OF MEDICAL SCIENCES

{Formerly: Medical Sciences Bulletin}

ISSN: 2072 – 1625

Pac. J. Med. Sci. (PJMS)

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SODIUM CHLORIDE 0.9% INTRAVENOUS SOLUTION IS ACCEPTABLE AS A PRE-ANALYTICAL PREPARATION SOLUTION FOR FINE NEEDLE ASPIRATES PRIOR TO GENEXPERT TESTING FOR THE DETECTION OF MYCOBACTERIUM TUBERCULOSIS COMPLEX IN PORT MORESBY GENERAL HOSPITAL, PAPUA NEW GUINEA.

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Running title: 0.9% NaCl is acceptable as pre-analytical sample preparation solution for Xpert testing of fine needle aspirates.

Submitted: June 2020; Accepted: July 2020
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Keywords: mycobacterium tuberculosis complex, fine needle aspiration, tuberculous lymphadenitis, Genexpert, sodium chloride.
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Dear Editor,

Conventional laboratory confirmation of tuberculous lymphadenitis (TBLN) is by fine needle aspirate biopsy (FNA) and identifying features of granulomatous inflammation (caseous necrosis, epithelioid cells and granuloma) with or without the presence of acid fast bacilli (AFB) using a microscope. This method requires interpretation of the smear by a trained cytologists or pathologist. The turn-around-time is long causing delay in treatment. Genexpert test (Xpert) has been shown to detect mycobacterium tuberculosis complex (MTB) in FNA specimens [1] and is now routinely used for TBLN diagnosis in some settings [2]. The assay is fully automated and results are available in two hours.

The Xpert diagnostic system (Cepheid, Sunnyvale, CA, USA) was originally developed for rapid detection of anthrax for use by the United States postal service following mail contamination in sorting offices [3]. As described by Lawn et al [3], it is a cartridge-based system

that incorporates microfluid technology and automated nucleic acid analysis to purify, concentrate, detect, and identify targeted nucleic acid sequences from unprocessed clinical specimens. The assay uses molecular beacon technology to detect deoxyribonucleic acid (DNA) sequences amplified in a heminested real-time polymerase chain reaction (PCR) assay. The assay uses single use cartridges with several chambers that are preloaded with liquid buffers and lyophilised reagent beads necessary for sample processing, DNA extraction and PCR [3]. Sample reagent included in the assay is used for decontamination of specimen prior to processing.

Pre-analytical sample preparation of FNA aspirates for Xpert testing is an important procedure because poor sample preparation can affect the accuracy of the results. Various types of solutions have been used for mixing FNA aspirates to generate a suitable aspirate suspension which can be used for further analysis. For example N-acetyl-L-cysteine/sodium hydroxide (NALC/NaOH) [4] or phosphate buffered saline (PBS) [5] have been used.

In a study evaluating the performance of Xpert in detecting MTB in FNA aspirates [6] we used 0.9% sodium chloride (NaCl) intravenous solution as the pre-analytical sample preparation liquid for suspending FNA aspirates for Xpert testing. Patients clinically diagnosed with probable TBLN and referred to the Port Moresby General Hospital (PMGH) for FNA were recruited.

Those that gave consent were clinically examined and FNA samples obtained from enlarged lymph nodes that were more than two centimeters in diameter. Where multiple sites and glands were involved, the largest palpable node and or the most superficially enlarged glands were chosen for sampling. The procedure for FNA sampling including the results of that study has been published [6,7].

The FNA aspirate suspensions were generated by rinsing the needle in 2.0ml of 0.9% NaCl in a standard urine specimen container. Although any suitable container can be used, we chose urine container because it was readily available in our laboratory. Rinsing was done by drawing 2.0ml of 0.9% NaCl completely into the syringe and expressing the aspirate-NaCl mixture back into the container. This process was repeated several times to maximize aspirate material in the needle hub to be mixed with NaCl. Care was taken not to create visible aerosols. Biohazard and safety protection measures were implemented throughout the procedure. The aspirate-NaCl suspension was transported in room temperature to the Central Public Health Laboratory (CPHL) housed within PMGH and stored at two to eight degree Celsius for Xpert assay. On the day of Xpert testing the aspirate-NaCl mixture underwent further pre-analytical sample

preparation following an in-house protocol as outlined:

- One milliliter of the aspirate-NaCl mixture was pipetted into 1.0ml 0.9% NaCl to make up to 2.0ml.
- Then manufacturer supplied Xpert sample buffer was added at 2:1 ratio.
- The mixture was vortexed and incubated at room temperature for 10 minutes.
- Samples with high mucoid matrix were vortexed again and incubated at room temperature for further 5 minutes. The additional 5 minutes of vortexing was required to lyse pus cells, increase yield and reduce viscosity to prevent test failure. The time interval and length were divided in this manner to allow the laboratory scientist to determine whether an extra 5 minutes was needed to lyse the sample further.
- Finally 2.0 ml of the whole solution from the final mixture was analyzed using Xpert.
- Results were reported as positive if MTB detected, negative if MTB not detected and invalid if there was test failure. Invalid tests were repeated using fresh sample. Instrument settings were established following manufacturer's guidelines [8].The results of this study were used to establish standard operating procedures at CPHL for TBLN Xpert testing [9].

A total of 107 FNA samples were processed. Mycobacterium tuberculosis complex was detected in 66 samples (61.7%; 66/107). Of the 66 positive cases, rifampicin resistance was detected in 19 samples (28.8%; 19/66). There were no invalid results. The remainder of the aspirate-NaCl mixture was used for culture. Samples for culture were sent to Australia due to unavailability of MTB culture facility within CPHL at the time of this study. Culture was completed on 24 samples (22.4%, 24/107) with a culture yield of 58.3% (14/24).

Xpert detected MTB in all culture positive samples (100%, 24/24). Drug susceptibility testing pattern of the MTB isolates have been published [7].

Previous studies evaluating diagnostic performance of Xpert in detecting MTB in FNA aspirates used NALC/NaOH [4], PBS [5] and other bactericidal reagents [9] for mixing FNA aspirates to suspend the aspirate material in the liquid. The rate of MTB detection in FNA aspirates using Xpert is variable and the type of fluid used for suspending aspirate material may affect test accuracy. For example, MTB detection rate with NALC/NaOH is 60% [4] and 64.6% with PBS [5]. Biadglegne et al [10] used a bactericidal reagent and the MTB detection rate in their study

was 39.0%. Fantahum et al [11] directly inoculated aspirate material into the manufacturer provided buffer solutions for Xpert testing and obtained a detection rate of 49.3%. The Xpert MTB detection rate in the present study was 61.7%. However, other factors could also influence Xpert MTB detection rates. Some of these factors may include quantity of material aspirated, immune status of the patient, whether the patient is on anti-tuberculous drugs or not, storage temperature and length of time sample is stored before Xpert testing. Further studies would need to be conducted to determine if these factors have any influence on the Xpert test outcome.

Although the culture yield of 58.3% is high compared to Wright et al [12] who used a MTB specific transport media, the present study is small and a larger study will need to be conducted at PMGH to confirm these findings. Culture yield of patients infected with Human Immunodeficiency Virus (HIV) is also higher compared to HIV negative patients [13]. The design of the present study did not permit investigating the HIV status of the patients.

In conclusion, the results obtained in this study demonstrated that 0.9% intravenous NaCl is suitable for pre-analytical sample preparation for Xpert testing for detecting MTB in FNA aspirates at PMGH. The laboratory protocol used for pre-analytical FNA aspirate preparation for Xpert testing of FNA samples is suitable for use at PMGH and can be adopted by laboratories for use in PNG. It is hoped that widespread use of Xpert testing of FNA samples in PNG will reduce the result turn-around time ensuring prompt commencement of anti-tuberculous chemotherapy.

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