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Decontamination Method for Tuberculosis: A Review

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Keywords: Tuberculosis (TB); Mycobacterium tuberculosis; Pulmonary infectious disease; Drug- resistance; Decontamination method; Decontamination method for TB.

Keywords: TB: Tuberculosis; MDR: Multi-Drug Resistant; WHO: World Health Organization; DOTS: Directly Observed Treatment Short Course; ZN: Ziehl-Neelsen; LJ: Lowenstein-Jensen Medium; NALC: N-Acetyl-L-Cysteine; Naoh: Sodium Chloride; CPC: Cetylpyridium Chloride; Nacl: Sodium Chloride; AFB: Acid Fast Bacilli; HCL: Hydrochloric Acid; Naocl: Sodium Hypochlorite; HIV: Human Immune Deficiency Virus; BC-TSP: Benzalkonium Chloride- Tri-Sodium Phosphate; USP: Universal Sample Processing; EPTB: Extrapulmonary Tuberculosis; PTB: Pulmonary Tuberculosis; PCR: Polymerase Chain Reaction.

Abstract

Tuberculosis is a communicable disease spread from one person to another; it is caused by Mycobacterium tuberculosis. It's a very dangerous bacterial infection liable for severe increase in death cases. It's a chronic granulomatous infectious disease. Infection happens via aerosol and inhalation of some droplets containing mycobacteria bacilli. The disease also affects animals like cattle; this is often referred to as bovine tuberculosis, which can sometimes be communicated to man. The pulmonary tuberculosis is the most important type of tuberculosis which effects man.

India is the highest Tuberculosis burden country within the world in terms of absolute number of incident cases that occur every year. It accounts for fourth part of the estimated global incident Tuberculosis cases in 2007.

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Introduction

Tuberculosis is one of the oldest diseases affecting mankind and has been found in skeletal remains from the ancient mummies of Egypt and Peru [7]. Tuberculosis is a major threat killing about 2 million people each year. World Health Organization estimates, one billion people will be newly infected in the period 2000-2020. Resulting in thirty five million more deaths, nearly one billion more people will be freshly infected, two hundred million will get & seventy million can die from TB if control not strengthened & active TB left [8].

Infection happens via aerosol and inhalation of some droplets containing M. Tuberculosis bacilli. It's remain one of the major public health issue worldwide, with ninety five percent of cases and ninety eight percent of death occurring in developing countries [1]. Geographically, the incidence is higher in southeast Asia (India and China along account for nearly forty percent of the worldwide TB cases) (WHO, 2012) [3]. In humans, the Tuberculosis is the primary causative bacterium although other mycobacteria like *M. Bovis, M. Microti, M. Africanum and M. Canetti* are also infective [1]. But, the prevalence and transmission of TB and other mycobacterial infections may be significantly reduced by detecting in the early stage of the disease.

Epidemology of tuberculosis

In the year 1993, World Health Organization (WHO) declared TB a worldwide public health emergency. About one-third of the world's population (>2 billion), are infected with TB bacilli. 10% of the people infected with TB bacilli will become side with active TB in their lifetime. According to world health organization report, world population with burden of disease caused by TB from 1990-2011 was 6948 million and total range of Multi-Drug Resistant (MDR) cases from 2005-2011 were 61690 [10].

In 2011, there are calculable 8.7 million incident cases of TB (range, 8.3million-9.0million) globally. Highest ranges of incidence were reported in Asia (59%) and Africa (26%) estimates of the burden of TB disease among youngsters have also been carried out. In 2011, largest ranges of cases were reported from India, China, Indonesia and Pakistan. Republic of India and China alone, accounted for 26% and 12% of world wide cases, the 8.7 million TB incident cases reports in 2011 [10].

In 2011, 1.3 million new TB cases were reported in India. About 0.5 million people died of TB and 4237 MDR cases. Male & Female population at the age of 25-34 is that the most affected. Directly observed treatment short course (DOTS), introduced by the world health organization consists of mainly standard short course regimen with first line drugs (isonized, pyrazinamide, rifampicin and streptomycin or ethambutol or both antibiotic).the medical therapy has long course of duration (6-12 months & 1-2 years for MDR-TB), with severe drug side effects.[10].

Source of Infection

The source of infection of Mycobacterium tuberculosis may be

1- Human e.g. cases of pulmonary tuberculosis.

2- Bovine source e.g. consumption of unpasteurized infected milk.

Mode of transmission

1- **Inhalation mode:** Mycobacterium tuberculosis is mainly transmitted by inhalation of droplet nuclei, produced while infected person coughing, sneezing and speaking. There may be as many as 2000-3000 infectious nuclei per cough of the infected patient.

2- **Inoculation:** The transmission of infection through direct skin contact with an infected person.

3- **Ingestion:** Swallowing of sputum or consumption of unpasteurized milk.

Clinical manifestations

1- Pulmonary Tuberculosis (PTB)

Pulmonary tuberculosis accounts for 80% of all cases of tuberculosis. It can be further categorized into primary or secondary types of tuberculosis.

2- Extrapulmonary Tuberculosis

Extrapulmonary tuberculosis involves the body organs different from the lungs (e.g., pleura, lymph nodes, abdomen, genitourinary tract, joints, skin and bones or meninges). A patient with both pulmonary and extra pulmonary tuberculosis infection is assessed as a case of pulmonary tuberculosis. Though EPTB constitutes about 15-20% of all cases of TB, in HIV –positive patients, the frequency is much higher accounting for 20-50% of all cases of tuberculosis infection.

Symptoms

Latent Tuberculosis: In this condition, you've got a tuberculosis infection, but the bacteria remain in your body in an inactive state and cause no symptoms. Latent tuberculosis is also known as inactive tuberculosis or tuberculosis infection, isn't contagious. It can become active tuberculosis, so treatment is vital for the person with latent tuberculosis and to assist control the spread of tuberculosis. An estimated 2 billion peoples have latent tuberculosis infection.

Active Tuberculosis: This condition causes you to sick and in most cases can spread infection to healthy persons. It can occur within the first few weeks after infection with the tuberculosis bacteria, or it'd occur years later.

Signs and symptoms of active TB include

- Coughing that lasts 3 or more weeks.
- Unintentional weight loss.
- Chest pain or pain with breathing or coughing.
- Fatigue.
- Chills.
- Coughing up blood.
- Night sweets.
- Loss of appetite.

When tuberculosis occurs outside your lungs, signs and symptoms very consistent with the organs involved.

Methodology of review

This is a chemical analysis of methods of sputum decontamination administered through review of published articles, literature from print and electronic (i.e. internet sources and books) materials relevant to research in TB diagnosis and con-

trol of tuberculosis. Reports created in such the simplest way to reflect the results, opinions, data and knowledge of authors and relevance of the methods used in enhancing sputum decontamination prior to Ziehl Neelsen (ZN) staining and culture of tuberculosis on Lowenstein Jensen (LJ) media.

Specimens containing normal flora

STOOL – For disseminated tuberculosis in HIV infected patients and infants.

SWABS: Considered suboptimal specimen.

The only recommended swabs are:

- Laryngeal swabs: These swabs are collected early morning in empty stomach.
- Swabs from discharging sinus.

Urine: Early morning three specimens of urine collected (500ml/specimen, centrifuged) on different days as tuberculosis bacilli in urine are shed intermittently.

Other respiratory samples: Bronchial secretions (2-5 ml), Bronchoalvelar lavage (20-30 mL), Transbronchial and other biopsies.

Sterile site samples collected aseptically

Optimum samples – Spinal, CSF, synovial, pericardial, ascetic, blood and pleural biopsy, bone marrow, tissues (collected in sterile saline).

Suboptimal samples (organism load is less) – Pleural fluid (20-50mL is collected and centrifuged). Blood (indicated only for disseminated TB and co-infected with HIV).

Decontamination methods

NALC-NaOH

NALC-2% sodium hyper oxide –sodium citrate solution was prepared as described by kent & kubica. Following 15 minutes of incubation at room temperature, the quantity was delivered to 50ml with 0.067M phosphate buffer (pH 6.8) and therefore the content was mixed by inversion. Bacteria were pelleted by centrifugation at 3000xg for 15minutes the supernatant was discarded, & the pelleted material was resuspended in 1ml.

From the pellet 0.5ml of sample is inoculated on LJ media slopes and to smears is prepared. The culture slant was incubated at 37˚C in a incubator.

NALC-NaOH method

The use of N-acetyl- L-cysteine (NALC) as a digestant in the isolation and culture of mycobacteria from sputum was first suggested by Webb while Sheffner *et al.* observed that acetylcysteine had a mucolytic effect.

The use of NaOH citrate along with side the mucolytic agent N-acetyl- L-cysteine (NALC) to decontaminate sputum samples is probably the most commonly used method of decontamination in most laboratories in different developed countries because it is rapid and relatively effective in reducing the numbers of contaminants.

If properly performed, it provides more positive culture than other methods, leading t to the killing of only approximately 30% of tubercle bacilli. Again, the indicated specimen exposure time must be strictly adhered to and reagents such as bovine albumin and the required filter are expensive therefore, it is generally an expensive method for local laboratories because it requires centrifugation which brings in additional cost. Finally, the reagents aren't readily available to be use in local laboratories; therefore, making its use mostly limited to well funded laboratories.

Cetylpyridinium chloride-NaCl method: Cetylpyridinium chloride is a cationic quaternary ammonium compound that is utilized in some kind of toothpastes, lonzenges, mouthwashes, throat sprays and nasal sprays. It is an antiseptic that kills bacteria and other types of microorganisms. The use of cetylpyridinium chloride–sodium chloride (CPC-NaCl) was proposed to decontaminate sputum specimens that are transported to the laboratory for quite 24hrs [11]. Essentially, if delay of more than 48 hours between collection and processing is anticipated, the sputum should be collected with 1%CPC and 2%NaCl. However, it requires an extended treatment period than is required with NALC-NaOH. Therefore, those specimens who are treated with CPC should be inoculated in the egg- based media [12,11]. Cetylpyridinium chloride eliminates the associated flora in sputum specimens and treated specimens shouldn't be submitted to further decontamination before activation [13]. Also, the detection of AFB with Ziehl- Neelsen staining is often significantly reduced in specimens preserved by this method [14, 15]. Hence, this method won't be ideal in local laboratories that perform only ZN.

Petroff's concentration or NaOH method: This method was first described in 1968, and is widely used, because it increases the sensitivity of sputum smears also as those of cultures, because the mycobaceria concentrated within the deposit are viable [16,17]. It involves the utilization of NaOH at concentrations ranging between 2% and 4% to digest, and at an equivalent time decontaminate the specimen [13]. It is widely utilized in developing countries due to its relative simplicity and therefore the incontrovertible fact that the reagents are affordable. The advantages of the method include the very fact that it's simple and cheap. It provides fairly effective control of contaminants and therefore the time needed to process a one sample is approximately 1hr. However, the disadvantages include: Strict adherence to specimen exposure time, failure of this might result to kill of the bacilli and therefore the incontrovertible fact that the NaOH procedure is extremely robust and should kill up to 60% of tubercle bacilli in clinical specimens [13]. However, specimens that form clots could even be digested with this method after the sample has been homogenized [18]. This method is effective under local setting.

Modified petroff's method (4% NaOH): Kent and Kubica, to optimize the NaOH method [12] described a standard Petroff's procedure which involves mixing of sputum during a tube with equal amount of 4% NaOH solution with phenol red and incubated for 15 minutes at room temperature. The tubes are going to be centrifuged for 20 min at 2500 g and therefore the sediment are going to be neutralized with 1 n HCl. The neutralized sediment will then be inoculated into two tubes containing Lowenstein–Jensen medium. This method is effective because it leads to minimal destruction of the bacilli in comparison to the NaOH method. It is valuable under nearby laboratory setting since the reagents to be used are often sourced locally; however, the main drawback is that the use of centrifuge, which cannot be feasible in some settings.

Bleach method: This is often a rapid concentration method with the utilization of sodium hypochlorite (NaOCL). It was

described in 1942 and had shown a crucial increase on smear sensitivity [20] Furthermore, Githiu and his colleagues [22] also suggested that exposure of sputum to 5% NaOCL for at minimum of 15hrs could also be a safety and security procedure in smear microscopy for the diagnosis of TB. It is because of NaOCL, being a potent disinfectant, which reduces the dangerous risk of laboratory acquired infections [13]. It requires a minimum of 15 minutes. It has also been demonstrated that the simple liquefaction and overnight sedimentation of sputum with NaOCL would significantly augment smear microscopy up to 70% [23]. Moreover, a big improvement within the proportion of positive AFB smear results has been reported, it's about from 70% to 83% [24].The major advantage of this method is that it is simple and cheap, because the only reagent required is sodium hypochlorite which is definitely available. However, the disadvantage is the tactic isn't suitable for culturing [24]. Bonnet and co-workers suggest the further evaluation of Bleach method under operational conditions [25].

Benzalkonium chloride-tri-sodium phosphate method: The primary description of the effect of benzalkonium chloride (BC) on tubercle bacilli was made in 1954 [26], Patterson and associates [27] who tried it for daily diagnostic culture of mycobacterium bacilli, reported the results superior to those which are obtained by the NaOH pre-treatment of sputum sample. When the treatment of lungs sample with BC-trisodium phosphate for the recovery of tubercle bacillus from sputum has been described [28]. When compared with four other decontamination methods, it had been shown to be effective within the recovery of *M. avium*; with BC-TSP and cetylpyridinium chloride (CPC)-sodium chloride (NaCl) showing the smallest amount of decontamination rates [29]. BC may be a quaternary ammonium compound that acts as a decontaminating agent. The advantage of this method is the procedure needn't be as critically timed as NaOH digestion procedure. However, it's laborious and leads to killing of roughly 30% tubercle bacilli [29]. The time required for one specimen is almost 2hrs while 20 specimens would require 4hrs [18]. Another limitation of this method to be utilized in local laboratories could even be the unavailability of the materials needed since BC isn't easily available.

The oxalic acid method: Corper and Uyei in 1930 described the oxalic acid method. It involves the utilization of oxalic acid as a decontaminant for the recovery of *Mycobacterium tuberculosis* from respiratory samples [29]. The use of oxalic acid for decontamination for the mycobacterial samples was tested and located to be highly effective in reducing contaminating bacterial populations. The reason for the very high reduction of mycobacteria may be because of non-neutralization of the sediment. The oxalic acid treatment was recommended to be utilized in recovery of AFB from heavily contaminated sputum sample such as those consistently overgrown with *Pseudomonas* species [18,12]. This method involves a half hour incubation of the specimen with 5% oxalic acid, centrifugation, and neutralization of the sediment [29]. It can also be used for *M. tuberculosis* recovery from laryngeal swab samples which involves covering the swab with 5% oxalic acid for 15 minutes, transfer into sterile saline for few minutes and later removed and allowed to empty and wont to make smear [18]. This can be resolved by neutralization with 2-3 ml of 5% sterile sodium citrate kept for 5 minutes before inoculation in the media [31].

Simplified concentration method: This method involves the utilization of ammonium sulphate for sputum decontamination and was described by Garay within the year 2000 [17]. The technique is reasonable, easy and appropriate for rural hospitals in Africa, especially those with no constant electricity supply. This methodology involves the preparation of the solutions of 3% ammonium sulphate and 1% of NaOH which can be added together. 2mls of this could be mixed with sputum sample of 1ml; it is shaken by hand and left at room temperature for between 12-15 hours after this process the smears are made. The limitations are there would be 1-day delay in results because of the overnight sedimentation of the sample [17]. With this system, fast smear TB diagnosis was enhanced by quite 30%.

Chitin method: This method was described in 2002 by Farnia and co- workers [32] in their attempt to improve the efficiency of direct smear microscopy through liquefaction and sedimentation of sputum by chitin. It has a high molecular weight and considered the second most abundant natural polymer after cellulose [32]. It was reported that the sensitivity of chitin treated sputum method was significantly increased (80%) in comparison to that of direct sputum microscopy (46%). The advantage does not require any specific instrument and can be used under existing conditions of Tuberculosis laboratories in developing countries [32].

Sulphuric acid method: It involves the usage of 4% H2SO4 [12]. Sulphuric acid method is usually helpful for urine and other thin water body fluids that consistently yield contaminated cultures when processed with one among the alkaline digestant [18]. This method also can be used for other specimens like gastric lavage which doesn't require decontamination. Other samples which will be treated with this method include: lymph nodes biopsies and other surgical tissues if suspected to be contaminated [18].

The kudoh and kudoh method: This method is very easy and practical decontamination method that obviates the utilization of sample centrifugation before culturing. In this method, sputum is gathered onto a cotton swab; the swab is decontaminated for 2 minutes during in solution of 4% NaOH, and then directly smeared onto a suitable culture medium. It doesn't require laboratory facilities and may be performed within the field. The applicator is removed and inoculated on Ogawa medium and with same applicator; a smear for AFB microscopic examination are often made [13]. The advantages of this method are: Centrifugation and concentration are not necessary, it's low cost, and can be used on the field. The method needs no laboratory instrument and, due to the minimal manipulation of the sample, it's a low bio-security risk method. In a study administered by Jaspe *et al.* [34] under both research laboratory and field setting in Venezuella, the Kudoh swab method was as sensitive because the Petroff decontamination procedure within the diagnosis of pulmonary TB. Thus, it indicates a valuable alternative method for culturing mycobacteria that's especially appropriate for rural laboratories without the right infrastructure and instrumentation for traditional culturing [33].

Tri-sodium phosphate (TSP) method: This is often an easy, one step decontamination and concentration method. Tri-sodium phosphate may be an easy to buy and cheap "soft" decontaminant- cum- liquefying agent. Sputum samples are collected directly into Tri-Sodium Phosphate containing screw cap Mc Cartney bottles. These bottles are vortexed and left overnight at the room temperature. On the next morning, the supernatant are discarded and smear made up of the deposit. This will be helpful especially for collection of sputum samples from distant places and their transport to the nearest hospital and laboratory, as well as sputum samples arriving late in a working day's

schedule [34,35]. TSP alone is claimed to possess considerable advantages, namely: being a single step culture procedure, having less effect on mycobacterium and with low contamination rate in the sample. The procedure is not requires sophisticated equipment or instrument nor technically skilled personnel. Another advantage of using the Tri-Sodium Phosphate method is that it is operationally more convenient as the use of costly equipment like shaker and bio-safe centrifuge are avoided.

Universal sample processing (USP) method: The USP method is suitable for both Pulmonary Tuberculosis (PTB) and Extra-Pulmonary Tuberculosis (EPTB). It draw on the chaotropic (water soluble) properties of guanidium hydrochloride [HNC (NH2)2] for sample processing and involves incubating the specimen with Universal Sample Processing solution, concentrating tuberculosis bacilli by centrifugation method, and using the processed specimen for microscopy, culture, and PCR test. It requires about 45 minutes. The detection limit for Acid Fast Bacilli (AFB) in spiked sputum by smear microscopy is approximately three hundreds (300) bacilli per ml of specimen.

Conclusion

Decontamination methods are mostly used for the recovery of Mycobacterium tuberculosis from the sputum sample of pulmonary tuberculosis and extrapulmonary tuberculosis. Some decontamination methods are used to kill the normal flora and other type of microorganisms other then mycobacterium. Therefore, in support of challenges faced by each laboratory, some adaptations/modifications should be made to cause adequate specimen decontamination for effective TB diagnosis to cause optimal patient care.

References

1. Dipiro TJ, Taibert LR, Yee CG, Matzke RG, Wells GB, et al. Tuberculosis, Pharmacotherapy a physiologic approach Mc Graw Hill 7th ed. 2008; 1839-1844.
2. World Health Organization. guidelines for the management of drug-resistant tuberculosis. WHO/T.B/96.210 (rev.1). 1997.
3. Shinu P, Singh V, Nair A, et al. Application of Cetylpyridinium Chloride and Sodium Chloride Decontamination Method for recovery of Mycobacterium tuberculosis from clinically suspected cases of pulmonary tuberculosis. J.R Diagnostic Microbiology and infectious Disease. 2013; 77: 150-157.
4. Kumar NS, Sekar MG, Ilampuranan KJ, et al. Increased detection by restaining of acid fast bacilli in sputum samples transported in cetylpyridinium chloride solution. Tuberculosis Research centre (ICMR), Chennai, India. INTT Tuberc Lung Dis.2005: 9: 195-199.
5. Ramarokoto H, Randriamiharisoa H, Rakotoarisaunia A, et al. Bacteriological follow-up of tuberculosis treatment: a comparative study of smear microscopy and culture results at the second month of treatment. Int. J Tuberc Lung Dis. 2002; 6: 909-912.
6. Manuela Pardini, Francis Varaine, Elisabettalona, Erchanik Arzumanian, Francesco checchi, Marco Rinaldo Oggiuni, Graziella Oreficia and Lanfranco fattorini. 2004. Cetylpyridinium Chloride is useful for isolation of mycobacterium tuberculosis from sputa subjected to long-term storage. JR of clinical microbiology. 2005; 442-444.
7. Donaghue HD, Soigelum M, Green blatt CL, et.al. Tuberculosis: From prehistory to Robert Koch, as revealed by ancient DNA. Lancet Infect dis. 2004; 4: 584-592.
8. Satoskar RS, Bhandarkar SD, Ainapure SS, et al. Pharmacology and Pharmacotherapeutics. Popular prakashan, Mumbai. 19th ed. 2005; 727-747.
9. There were an estimated 1.1 million deaths due to HIV, including 0.4 million deaths from TB among HIV-positive people. 2015.
10. Design, synthesis and evaluation of Antimycobacterial activity of triazoles. Manipal college of Pharmaceutical Sciences. 2019; 1-2.
11. Smithwick RW, Stratigos CB, David HL. Use of cetylpyridinium chloride and sodium chloride for the decontamination of sputum specimens that are transported to the laboratory for the isolation of Mycobacterium tuberculosis. J Clin Microbiol 1975; 1: 411-413.
12. Kent PT, Kubica GP. Public health mycobacteriology: A guide for the level III laboratory. Atlanta. Center for disease control. 1985.
13. Della LP. Mycobacteriology and mycobacterial susceptibility tests. In: Clinical Microbiology Procedures Handbook. 2004; 2.
14. Selvakumar N, Sudhamathi S, Duraipandian M, et al. Reduced detection by Ziehl-Neelsen method of acid-fast bacilli in sputum samples preserved in cetylpyridinium chloride solution. Int J Tuberc Lung Dis. 2004; 8: 248-252.
15. Selvakumar N, Gomathi SM, Kumar V, et al. Sensitivity of Ziehl-Neelsen method for centrifuged deposit smears of sputum samples transported in cetylpyridinium chloride. Indian J Med Res. 2006; 124: 439-442.
16. Allen BW, Baker FJ. Mycobacteria isolation, identification and sensitivity testing. London: Butterworths. 1968; 10.
17. Garay JE. Analysis of a simplified concentration sputum smear technique for pulmonary tuberculosis diagnosis in rural hospitals. Tropical Doctor. 2000; 30: 70-72.
18. Karin W, Roxanna R., Valerie M., Paul VH. Part 111: culture homogenization and decontamination. Tuberculosis. 2006.
19. Weyer K, Rustomjee R, Mizrahi V, van Helden P. Tuberculosis; Part III: Culture homogenization and decontamination. 2010.
20. Oliver J, Reusser TR. Rapid method for the concentration of tubercle bacilli. Am Rev Tuberc. 1942; 45: 450-452.
21. Loretta AC, Lucy BC, Lee AB, et al. Efficacy of chemical dosing methods for isolating nontuberculous mycobacteria from water supplies of dialysis centers. Appl Environ Microbiol. 1998; 54: 1756-1760.
22. Githui WA, Matu SW, Tunge N, et al. Biocidal effect of bleach on Mycobacterium tuberculosis: a safety measure. Int. J Tuberc Lung Dis. 2007; 11: 798-802.
23. Miomer H, Gebre N, Karlsson U. Diagnosis of pulmonary tuberculosis. Lancet 1994; 344: 127.
24. Angeby KA, Hoffner SE, Diwan VK. Should the 'bleach microscopy method' be recommended for improved case detection of tuberculosis? Literature review and key person analysis. In J Tuberc Lung Dis. 2004; 8: 806-815.
25. Bonnet M, Ramsay A, Githui W, et al. Bleach sedimentation: an opportunity to optimize smear microscopy for tuberculosis diagnosis in settings of prevalence of HIV. Clin Infect Dis. 2008; (46): 1710-1716.
26. Hirsch JG. The resistance of tubercle bacilli to the bactericidal action of benzalkonium chloride (Zephiran). Amer Rev Tuber. 1954; 70: 312-319.

27. Patterson RA, Thompson TL, Larsen DH. The use of Zephiran in the isolation of tubercle bacilli. Amer Rev Tuber 1956; 74: 284.

28. Wayne LG, Krasnow I, Kidd G. Finding the "Hidden Positive" in tuberculosis eradication programs. The role of the sensitive trisodium phosphate-benzalkonium (Zephiran) culture technique. Am Rev Respir Dis. 1962; 86: 537-541.

29. Yajko DM, Nassos PS, Sanders CA. Comparison of four decontamination methods for the recovery of Mycobacterium avium complex from stools. J Clin Microbiol. 1993; 31: 302-306.

30. Dorothy YM, Thomas B, Ernestina MQ, et al. Evaluation of decontamination methods and growth media for primary isolation of Mycobacterium ulcerans from surgical specimens. J Clin Microbiol. 2004; 42: 5875 – 5876.

31. Nassau E. Bacteriological examination in tuberculosis. Tubercle (London). 1954; 35.

32. Farnia M, Mohammadi F, Zarifi Z, et al. Improving sensitivity of direct microscopy for detection of acid- fast bacilli in sputum: use of chitin in mucus digestion. J. Clin. Microbiol. 2002; 40: 508-511.

33. Jaspe RC, Rojas YM, Flores LA, et al. Evaluation of the Kudoh swab method for the culturing of Mycobacterium tuberculosis in rural areas. Trop Med Int Hlth. 2009; 14: 468-471.

34. Jena N, Panda BN. Rapid slide culture using a simple concentration method for rapid and better primary isolation of Mycobacterium tuberculosis. Ind J Tuberc. 1998; 45: 176.

35. Jena J, Panda BN. Evaluation of trisodium phosphate as a transport medium and its utility in a single-step decontamination technique for the culture of M. tuberculosis. Ind J Tuberc. 2004; 51: 137-141.