Contamination of specimen container surfaces during sputum collection

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SUMMARY Sputum specimens from culture-positive tuberculosis patients were examined for the presence of *Mycobacterium tuberculosis* on container surfaces. Although specimens were in transit for several days, *M. tuberculosis* was isolated from 18 (6.5%) of 279 containers examined. Sputum specimens from local patients were examined for evidence of upper respiratory bacterial flora on the outside of containers as an indicator of contamination with sputum. Of 300 containers examined, 41 (14%) were contaminated. A technique for disinfecting specimen containers from tuberculosis patients has been evaluated and recommendations are made for handling sputum containers.

Sputum is generally regarded as the clinical specimen most likely to contain large numbers of tubercle bacilli and therefore to present a potential hazard to laboratory staff. The difficulties encountered in the collection of sputum without contaminating the outside of the container, have led to recommendations for disinfecting containers prior to transit to and handling in the laboratory. The United States Public Health Service¹ recommends wiping the surface of sputum containers with gauze soaked in 5% phenol. Mitchison² has recommended immersing sputum containers in a suitable disinfectant for 30 min to ensure that the surface is not infective. The laboratory examination of sputum necessitates the transportation of specimens from hospital clinics or wards to laboratories. Many diagnostic laboratories are attached to hospitals. However, the laboratory diagnosis of tuberculosis is often performed by reference centres, which in developing countries may be located a considerable distance from the clinic. As sputum containers may be handled by numerous personnel during transit it is important to demonstrate that containers may become contaminated with *Mycobacterium tuberculosis* and ensure that decontamination is reliable. In this investigation sputum specimens collected from tuberculous and non-tuberculous patients were examined for evidence of specimen contamination on the outside of the container, and the value and risk of sample contamination from immersing in disinfectant has been evaluated.

Material and methods

SPUTUM FROM TUBERCULOSIS PATIENTS

Pretreatment sputum from patients admitted to studies of short-course chemotherapy in Hong Kong was collected directly into glass universal containers, packed in sealed containers, and transported to London by air.

SPUTUM SPECIMENS FROM NON-TUBERCULOUS PATIENTS

Specimens of sputum, collected in plastic universal containers, were received for routine (non-tuberculous) bacteriological examination from patients admitted to, or attending, the Hammer-smith Hospital.

DETECTION OF TUBERCLE BACILLI ON CONTAINER SURFACES

During a three-month period (June–August 1981) 317 pretreatment sputum specimens were examined for the presence of *Mycobacterium tuberculosis* on the outside of the container. Packages containing the sputum containers were opened in a class I microbiological safety cabinet and the containers removed. Each sputum container was inverted at an angle of approximately 45° over an open bottle containing 10 ml selective Kirchner medium (SK).³ The neck of the container, to a depth approximately 10 mm below the cap, was washed with about 6 ml of SK medium using a Pasteur pipette. The washing medium was allowed to drain back into the original medium container, incubated at 37°C, and examined

macroscopically at weekly intervals for evidence of growth. Positive cultures were subcultured onto two Löwenstein-Jensen (LJ) slopes which were incubated until sufficient growth was available for further testing. Cultures were identified as *M tuberculosis* by the production of niacin and failure to grow on medium containing 500 mg/l 4-nitrobenzoate. Negative cultures were discarded after six weeks' incubation. Sputum specimens were also cultured on LJ medium using a modified Petroff decontamination technique.²

DETECTION OF SPUTUM CONTAMINATION ON NON-TUBERCULOUS SPECIMEN CONTAINERS

As few specimens containing *M tuberculosis* were available, from local patients, sputum specimens from 300 non-tuberculous patients were examined for evidence of sputum contamination of the outside of the container. Specimen containers were swabbed using a cotton wool swab moistened with sterile nutrient broth. Using a rotating motion the neck of the container was swabbed from below the cap to a depth of approximately 10 mm. Swabs were cultured on blood agar medium incubated aerobically at 37°C for 48 h. Containers were regarded as contaminated with sputum when the swab culture yielded a moderate or heavy mixed growth of organisms usually found in sputum.

EVALUATION OF DISINFECTION TECHNIQUE

Glass universal containers were deliberately infected with smear positive tuberculous sputum to simulate maximum contamination of the container. Sterile water (5 ml) was added to each bottle to provide a weight similar to the usual sputum content. A swab stirred in sputum was used to coat the outside of each container from the rim to an area approximately 10 mm below the shoulder. Two containers were coated with sputum from each of 101 specimens. The caps were replaced and sputum allowed to dry on the containers for 30 min. One container was immersed for 30 min in a solution of Clearsol (Tenneco Organics Ltd, Bristol) disinfectant diluted 1/45 with cold tap water in accordance with the manufacturer's recommendation. After exposure containers were removed from disinfectant and allowed to dry for 30 min. Each container was then washed with 6 ml SK medium as previously described except that the total volume of culture medium, into which the washings were collected was increased to 50 ml in order to dilute trace amounts of disinfectant which may have been introduced during washing. To confirm that inhibitory concentrations of disinfectant were not present cultures which were negative after 6 weeks' incubation were inoculated with 0.2 ml of a 10 day Tween albumin culture

of a marked strain of *M tuberculosis*. The culture used was a drug sensitive strain made resistant to rifampicin by heavy inoculation onto LJ medium containing 64 mg/l rifampicin. Reinoculated cultures were incubated at 37°C for up to 4 wk. Growth was subcultured onto LJ medium containing 64 mg/l rifampicin in order to detect the marked strain. The second container was washed with SK medium and used as a positive growth control. This growth was identified as *M tuberculosis* and inoculated onto LJ medium containing 64 mg/l rifampicin to ensure that the test strains differed from the marked strain.

DETECTION OF DISINFECTANT INSIDE SPECIMEN CONTAINERS

Although the glass universal container has an adequate seal and does not leak, when tested by the method described in British Standard 5213,⁴ it is necessary to ensure that disinfectant does not enter containers during immersion. The test method (BS 5213) was modified in order to detect disinfectant within the containers. Water containing sodium fluorescein at a concentration of 0.1 g/l was used to dilute Clearsol disinfectant to the recommended strength. In each of three separate tests 35 glass universal containers containing 5 ml water were immersed in dye-labelled Clearsol solution for 30 min. After immersion bottles were rinsed in cold tap water, to remove disinfectant from the outside of the containers, then allowed to drain for 15 min. Containers were then exposed to a source of short wave ultraviolet light to detect penetration of dye-labelled disinfectant.

Results

TUBERCLE BACILLI ON CONTAINER SURFACES

Using a modified Petroff technique *M tuberculosis* was isolated from 279 (88%) of the 317 pretreatment sputum specimens examined. Negative or contaminated cultures (12%), and corresponding SK cultures, were excluded from the investigation. The SK washing technique resulted in *M tuberculosis* being isolated from the surface of 18 (6.5%) of 279 culture-positive specimens.

SPUTUM CONTAMINATION OF NON-TUBERCULOUS SPECIMEN CONTAINERS

Of the 300 containers examined, using the moist swab technique, 41 (14%) were considered to be contaminated with sputum according to the criteria described. Cultures prepared from uncontaminated containers were usually sterile or yielded a few colonies of micrococci.

EVALUATION OF DISINFECTION TECHNIQUE

Of 101 control sputum-coated containers washed with SK medium 100 yielded organisms identified as *M tuberculosis*. All isolates were sensitive to rifampicin 64 mg/l. The remaining culture was contaminated and was excluded from the investigation. Immersion of infected containers in disinfectant was highly successful; none of the 100 containers exposed to Clearsol solution yielded growth in SK medium. There was no evidence to suggest carry-over of disinfectant during the washing procedure, all reinoculated cultures yielded growth identified as the marked strain of *M tuberculosis* demonstrating that they were capable of supporting growth and not therefore significantly contaminated with disinfectant.

PENETRATION OF DISINFECTANT

Tests were carried out on three occasions. Although traces of fluorescein were detectable on the outside of containers, there was no evidence of dye within the 105 containers tested.

Discussion

It is clear from the results of this investigation that contamination of specimen container surfaces occurred during the collection of sputum. Viable tubercle bacilli were detected on containers, even after several days in transit, and bacteria indicative of sputum contamination were cultured from containers when specimens were collected locally.

Two widely used manuals give advice on the procedures to be followed when collecting sputum. The United States Department of Health⁵ recommends that patients hold the container to their lower lip and gently release the sample into the container. The International Union against tuberculosis⁶ advises holding the container close to the lips during collection. When this advice is followed the chance of sputum contaminating the container is considerable irrespective of the size of container used. Complex disposable sputum containers have been designed to prevent spillage and contamination, but the cost of such containers prevents their use in many countries.

It has been recommended that all sputum specimens should be processed in a microbiological safety cabinet.⁷ However, this and a previous investigation,⁸ in which tubercle bacilli were cultured from heat-fixed sputum smears, show that laboratory staff

may unknowingly handle dried infected sputum without the protection of a safety cabinet. Although droplet nuclei from liquid sputum present the greatest danger to laboratory staff⁹ dried material in which infective units become concentrated may also present a hazard.¹⁰

In laboratories situated in areas with a low incidence of pulmonary tuberculosis decontamination of sputum containers would seem to be unnecessary, provided sputum specimens are transported to the laboratory in sealed plastic bags and kept separate from the request form. Laboratory techniques used for the examination of sputum from known or suspected tuberculosis patients usually involve considerable handling of the specimen container. It is recommended that a method of containment is used during transportation of high risk specimens and that containers are decontaminated on arrival in the laboratory.

An alternative would be to transfer high risk specimens to a fresh container before commencing laboratory examination, however, this would increase the cost of each investigation and add yet another potentially hazardous manipulation.

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