DISCUSSION 1

Does collecting fluid in impingers affect collection/survival of Chlamydia and if so what collecting fluid should be used?

CLOSURE 1

Collecting fluid in impingers can affect survival of C. psittaci. Normal saline will be detrimental to the collected organisms. I recommend using SPG (sucrose-phosphate-glutamate) medium. The formula for preparing SPG is contained in the manuscript. Rosebury [18] found that viral extract broth was comparable to 10% sheep serum in distilled water for collecting C. psittaci. He also showed poor survival in glycerin solutions and in distilled water.

DISCUSSION 2

In regard to airborne Chlamydia:

A) is drying of the organisms a problem when sampling with filter membranes;

B) is there a published method of sampling;

C) could you elaborate on interpretation of sampling results; and

D) what is dose/effect or infection threshold, if any?

CLOSURE 2

A) Drying should not be considered a problem when sampling for C. psittaci using filter membranes. The organism survives well in the dried state as evidenced by its infectivity via the aerosol route.

B) There has not been a published method for the routine sampling of C. psittaci in the indoor environment. Recommendations for monitoring come from aerobiological studies conducted in test chambers, such as those of Rosebury [18].

C) Any evidence of viable C. psittaci collected by aerosol sampling is significant. No quantitative evaluation of airborne particles needs to be demonstrated.

D) The human infectious dose of C. psittaci, that is, the number of viable organisms necessary to produce serological conversion, has not been accurately determined. It must be remembered that infection does not always result in disease. The development of clinical disease depends to a very large extent on the susceptibility of the host. Thus the highest rates of mortality from psittacosis occur in the elderly whose immune systems are often compromised by aging, immunosuppressive therapy, and/or other chronic and debilitating illnesses.

DISCUSSION 3

Can pet birds carry Chlamydia and be asymptomatic or must they be overtly ill to have the organism?

CLOSURE 3

A major characteristic of virtually all chlamydial infection is latency [11]. Infected pet birds might appear to be healthy, but can develop clinical symptoms and overt disease when stressed. Healthy carriers normally shed fewer chlamydiae than those that are physically ill.

DISCUSSION 4

A) If Chlamydia is transmitted by aerosol, why isn't it a Class III agent?

B) Why do you feel that a dust/mist mask with PF of 10, which is not a HEPA filter material, is sufficient respiratory protection?

C) What is the infectious dose by inhalation of each Chlamydia?

D) What is the minimum particle size collected by the AGI-30 and does efficiency of collection increase with AGIs in series?

E) What is the filter media used in cassette sampling?

F) Elaborate on the PCR analytical technique.

CLOSURE 4

A) Although Chlamydia is transmitted by the aerosol route it normally requires only Biosafety Level 2 practices. These include the use of a Class I or II biological safety cabinet and centrifuge safety precautions. Additional primary containment and personnel precautions, such as those recommended for Biosafety Level 3 may be indicated for activities with high potential for droplet or aerosol production.

B) I do not feel that a dust/mist mask is sufficient protection when monitoring for Chlamydia. I recommend the use of a respirator with HEPA filter cartridges.

C) The human infectious dose or number of viable organisms necessary to produce serological conversion, has not been accurately determined for any of the Chlamydia species.

D) Viruses, smaller than the chlamydiae, have been satisfactorily collected in the AGI-30 prior to assay in tissue culture [Akers, et al, Applied Microbiology, Vol. 14, 1966, pp. 361-364; Akers, et al, Journal of Immunology, Vol. 97, 1966, pp. 379-385]. Some particles, however, of less than 0.3 μm may be carried by the high velocity of the jet air stream through the

impinger fluid without being trapped [20]. Efficiency of collection would not necessarily be significantly improved with AGI-30s in series.

E) For cassette sampling, the filters used are made of mixed esters of cellulose.

F) Polymerase chain reaction (PCR) technology is a highly acclaimed technique for rapidly multiplying desired segments of genetic information. It has broad applications in basic research, diagnostics, and forensics. It is anticipated that PCR technology for the detection of Chlamydia will be widely utilized in the future.

DISCUSSION 5

Why operate AGIs for 30 minutes? Doesn't the sampling volume depend on the expected concentration and required sampling sensitivity?

CLOSURE 5

A thirty minute sampling time should be sufficient to collect airborne Chlamydia, levels of which could be "high" or "low." Continued sampling beyond 30 minutes might result in the steady decline in viability of collected organisms. Additionally, 30 minutes is the sampling time recommended by the American Conference of Governmental Industrial Hygienists [Guidelines for the Assessment of Bioaerosols in the Indoor Environment, ACGIH, Cincinnati, 1989].

DISCUSSION 6

A) Has any study been done to determine how long an aerosol of Chlamydia can survive in the environment, in particular the indoor environment, and still be pathogenic?

B) Would you consider doing air testing in an office setting where conditions seemed appropriate for Chlamydia and if so what criteria should be followed?

CLOSURE 6

A) The survival of aerosolized microorganisms is dependent upon temperature, humidity, airflow, length of time airborne, exposure to ultraviolet radiation, intrinsic resistance to desiccation, and the size of the aerosol cloud. I am not aware of data indicating maximum environmental resistance of airborne Chlamydia psittaci and the ability to cause disease. In addition, the number of C. psittaci required to produce infection (as opposed to disease) in man has not been accurately determined and may vary from strain to strain.

B) Bioaerosol sampling for C. psittaci in an office environment should be considered when the attack rate is significant, symptoms are consistent with psittacosis, and airborne exposure to a bird population has been implicated.