Letters

The Table shows results obtained with 24 strains of ONPG negative H influenzae. Using the heavy inoculum, results were identical for tube and API-10S strips, except for one strain that was positive for ornithine decarboxylase in the tube but negative on the strip (strain 8). The Table clearly shows the inadequacy of the smaller inoculum. The numbers of positive tube tests for urease, ornithine decarboxylase, and indole formation were 18, 11, and 16, respectively, for the heavy inoculum compared with 16, 1, and 12 for the smaller inoculum. Inoculum size seems to be especially critical for detection of ornithine decarboxylase. A series of replicate tests showed that reproducible results could be obtained with either tube or API-10S strips with the heavy inoculum, but all three tests gave variable results with smaller inocula. A series of viable counts performed on inocula prepared according to the API recommendations gave values ranging from 1.3 x 10^5 to 3.0 x 10^5 colony forming units, depending on colony size. We found that a minimum inoculum size of 1.25 x 10^6 colony forming units is required for reliable detection of urease and indole formation and 1.25 x 10^8 for ornithine decarboxylase production.

References

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Identification of Legionella pneumophila with commercially available immunofluorescence test

Legionella pneumophila is recognised in the clinical laboratory by its cultural and biochemical characteristics. Confirmation of identity is either by immunofluorescence or slide agglutination with known antisera. In addition, strains may be examined further for fatty acid content, ubiquinone content and, in the case of new serogroups, by DNA homology to confirm their identity. A monoclonal antibody has recently been produced, which reacts with a protein with a molecular weight of 29,000 present in Legionella pneumophila but which was not detected in other legionellas. This antibody does not cross react with a wide range of other bacterial species. A commercially available kit has been produced using this monoclonal antibody conjugated with fluorescein isothiocyanate (Genetic Systems Corporation, Seattle, Washington, United States) that does not require specimens to have special pretreatment with detergent and edetic acid, as described previously. This antibody can be used to examine either preparations of bacterial antigens or clinical specimens in a direct fluorescence antibody test (DFAT). The Table shows the strains of legionellas and other organisms examined. Legionellas were grown on buffered charcoal yeast extract agar and suspensions in 1% formal saline or, in the case of some legionellas, aqueous suspensions of organisms heated at 100°C for 15-30 minutes were used. A suspension made to the turbidity of McFarland No 1 standard is recommended. One drop of the suspension was placed in a well of a 12 well Teflon coated slide (Hendley, Essex). Suspensions were air dried and then lightly heat fixed. The minimum amount of the fluorescein conjugated monoclonal antibody necessary to cover the well of the slide was applied and the slides incubated at 36°C for 20 minutes in a moist chamber. After rinsing and mounting slides were examined using a x 10 eye piece and a x 50 water immersion objective in a Leitz microscope with a Plömpak incident light fluorescence attachment. Formal saline suspension of L pneumophila of all serogroups fluoresced apple green under examination by ultraviolet light, some strains fluorescing more brilliantly than others. Some heated suspensions of L pneumophila did not fluoresce, but this may have been due to deterioration of the antigen on

Table: Effect of varying inocula in tube tests

<table>
<thead>
<tr>
<th>Strain</th>
<th>Inoculum</th>
<th>Ur</th>
<th>ODC</th>
<th>Ind</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.25 x 10^8 cfu</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>1.25 x 10^6 cfu</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>1.25 x 10^4 cfu</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Ur = urease
ODC = ornithine decarboxylase
Ind = indole

Strains of organisms examined with monoclonal antibody

L. anisa
L. bozemanii (serogroups 1 and 2)
L. cherrillii
L. damoffii
L. erythra
L. felelei (serogroup 1)
L. gormanii
L. hackellae
L. jamaestw忸ans
L. jordanis
L. longbeachae (serogroups 1 and 2)
L. maceachernii
L. micdadei
L. odderidgensis
L. parisiensis
L. pneumophila (serogroup 1, 5 strains) (serogroups 2, 3, 6–10, and undesignated strain P183)
L. rubriiucens
L. sainthelensi
L. santneriacs
L. spiritoiensis
L. steigervaitii
L. wadsworthi

Undesignated strains 1267, 1466
“Species 1” organisms (Los Angeles 1, Dallas IE)
“Species 2” organisms U7W, U8W and MIC-UB
Brucella abortus
Bacteroides fragilis
Pseudomonas aeruginosa
long storage. Legionellas of “species 1 and 2” reacted in the test but the other legionellas examined did not, nor did Brucella abortus, Bacteroides fragilis, or Pseudomonas aeruginosa.

The test was easy to use, with very clear instructions, and was found to be extremely helpful in the preliminary sorting out of strains of legionellas (characterised initially as Gram negative rods growing on buffered charcoal yeast extract agar, with typical colonies showing a “cut glass appearance” and failing to grow in the absence of added cysteine and iron in the medium), which had been isolated both from clinical and environmental sources. As reported the monoclonal antibody also reacted strongly with legionellas in smears made from lungs from which L pneumophila serogroup 1 had been isolated and also from lung from which serogroup 6 had been isolated. Economical use of the expensive reagent can be made by using a 2 mm platinum wire loop to add reagent to the wells in the slides (care being taken to ensure the reagent does not dry on the slide during incubation), and it was found that it was much easier to locate the stained film of organisms, or tissue, using the white coated Teflon slides in routine use in this laboratory (Hendley, Essex) than with the grey Teflon coated slides supplied with the kit. We find PVA mountant more easy to work with in this laboratory than the buffered glycerol supplied with the kit.

This kit obviously has many applications in the preliminary identification of possible legionellas before further examination by reference laboratories, as well as in the examination by the Dfat of specimens for the presence of L pneumophila. Where it is essential that the organism isolated and characterised should be differentiated from “species 1 and 2,” however, such cultures will have to be examined further to determine their precise identity.

I acknowledge the provision of the Genetic Systems kit by American Hospital Supplies and the secretarial help of Mrs EP Law in preparing the manuscript for publication.

Stains of legionellas other than those isolated in this department were provided by the Centers for Disease Control, Atlanta, Georgia, United States (Drs Bibb, McKinney, Tsai, and Wilkinson), Mr PJ Dennis, Drs HJ Koornhoff, AD Macrae, PL Meenhorst, J O’H Tobin, and S Toma.

RJ FALLONN
Department of Laboratory Medicine,
Ruchill Hospital,
Bilsland Drive,
Glasgow G20 9NB

References


Normocellular bacterial meningitis

Case reports

CASE 2
A two year old girl was admitted with a three day history of mild diarrhoea and anorexia with occasional vomiting. Four hours after admission she developed a widespread purpuric rash, and a lumbar puncture was performed. The cerebrospinal fluid was acellular with protein and glucose contents of 0·16 g/l and 3·9 mmol/l (70·2 mg/g/100 ml), respectively. (Adult cerebrospinal fluid reference ranges 0·1–0·4 g/l protein and 2·2–3·3 mmol/l (39·6–59·4 mg/g/100 ml) glucose.) A Gram film was not examined. Despite treatment with intravenous benzyl-penicillin she died three hours later. Neisseria meningitidis group B was subsequently grown from both cerebrospinal fluid and blood.

CASE 2
An eight month old girl was admitted with a one day history of lethargy and occasional vomiting. There was minimal neck stiffness, and purpuric spots were noted over her back, legs, and buttocks. The cerebrospinal fluid was acellular with protein and glucose contents of 0·25 g/l and 2·1 mmol/l (37·8 mg/g/100 ml), respectively. No organisms were seen in the Gram film of a spun deposit. N meningitidis group B type 8 was subsequently grown from both the cerebrospinal fluid and blood. She made a complete recovery with intravenous benzyl-penicillin.

CASE 3
An 84 year old woman who had received a splenectomy was admitted with a 24 hour history of fever and confusion. Although no signs of meningism were present, a lumbar puncture was performed. The cerebrospinal fluid cell count was: polymorphs 2·0×10⁶/l, lymphocytes 2·0×10⁶/l, red cells 1·0×10⁶/l; protein and glucose contents were 0·25 g/l and 5·3 mmol/l (37·8 mg/g/100 ml), respectively. Numerous Gram positive diplococci were seen in the Gram film of a spun deposit, and Streptococcus pneumoniae serotype 12 was grown from both the cerebrospinal fluid and blood. She was treated with intravenous benzyl-penicillin and made a full recovery.

CASE 4
A 28 year old woman was admitted with a one day history of sore throat and generalised arthralgia. Minimal neck stiffness and a widespread purpuric rash were noted. The cerebrospinal fluid cell count was: polymorphs 1·0×10⁶/l, red cells 3·0×10⁶/l; and protein and glucose contents were 0·39 g/l and 4·8 mmol/l (86·4 mg/g/100 ml), respectively. Numerous Gram negative diplococci were seen in the Gram film of a spun deposit and N meningitidis group B type 2b was grown from the cerebrospinal fluid and blood. She was treated with intravenous benzyl-penicillin and made a full recovery.

Discussion

Normal cerebrospinal fluid white cell counts
Identification of Legionella pneumophila with commercially available immunofluorescence test.

R J Fallon

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