Legionella Latex Test

1. INTENDED USE
The Oxoid Legionella Latex Test is a latex agglutination test for the identification of predominant Legionella species grown on plate media from patients with suspected Legionnaires' disease or from environmental sources. The Oxoid Legionella Latex Test uses antibody sensitised blue polystyrene 'latex' particles which will agglutinate in the presence of specific Legionella cell wall antigens to form visible clumps. This provides a fast and simple screening procedure for predominant pathogenic Legionella species and serotypes.1

2. COMPONENTS OF THE KIT
This kit contains one of the following test reagents:

- DR0801 Legionella pneumophila serogroup 1 Test Reagent consists of a suspension of blue polystyrene 'latex' particles sensitised with specific rabbit antibody reactive with Legionella pneumophila serogroup 1 antigen. Each kit contains sufficient reagent for 50 tests. OR
- DR0802 Legionella pneumophila serogroups 2-14 Test Reagent consists of a suspension of blue polystyrene 'latex' particles sensitised with specific rabbit antibody reactive with Legionella pneumophila serogroups 2–14 antigen and is also able to detect L. pneumophila serogroup 1 and serogroup 2–14 isolates. Each kit contains sufficient reagent for 50 tests.

3. MATERIALS REQUIRED
A phosphate buffered saline solution. pH 7.3.

4. PRECAUTIONS
These reagents are for in vitro diagnostic use only. Do not freeze. Reagents contain 0.1% sodium azide as a preservative. Sodium azide may react with lead or copper plumbing to produce metal azides which are explosive by contact detonation. To prevent azide accumulation in plumbing flush with copious amounts of water immediately after waste disposal. Please refer to the manufacturer's safety data sheet and the product labelling for information on potentially hazardous components.

5. STORAGE
2°C to 8°C

This kit must be stored 2-8°C. Under these conditions the reagents will retain their reactivity until the expiry date shown on the kit box.

6. SPECIMEN COLLECTION AND PREPARATION
Isolates derived from environmental and clinical samples may be cultured on standard non-selective or selective Legionella culture media. Legionella species on primary isolation have an absolute requirement for L-cysteine hydrochloride. To ensure that an isolate is a Legionella it is necessary to show that it cannot grow on any medium which does not contain L-cysteine hydrochloride. This confirmation may be performed prior to or after the latex test. Cultures may be tested at any stage of growth providing that the colonies are of sufficient size. Older cultures, however, may produce stringy reactions making interpretation more difficult.

7. TEST METHODS
There are two test methods which may be used. Both direct and tube methods give reliable results. If an isolate has a stringy consistency it is recommended that the tube method is used.

(a) Direct Test
1. Bring the latex reagents to room temperature. Make sure the latex suspensions are mixed by vigorous shaking. Expel any latex from the dropper pipette for complete mixing.
2. Dispense 1 drop of each of the latex reagents within and close to the edge of a circle on a reaction card.
3. Add one drop of diluent buffer to each circle. Ensure that the latex and buffer do not mix at this stage.
4. Using a loop, pick off a colony of at least 1 mm (use 2 or more if the colonies are smaller) and carefully emulsify in the first drop of buffer. For optimal results ensure that the suspension is smooth. Repeat for similar colonies with the second drop of buffer.
5. Mix the latex reagents and suspensions together and spread to cover the reaction areas using the loop. Flame the loop.
6. Gently rock the card in a circular motion and look for agglutination. Do not rock the card for more than 1 minute and do not use a magnifying glass to aid reading the result.
7. When finished, dispose of the reaction card into a suitable disinfectant.
8. Recap the bottles and return to the refrigerator.

(b) Tube Method
1. Bring the latex reagents to room temperature. Make sure the latex suspensions are mixed by vigorous shaking. Expel any latex from the dropper pipette for complete mixing.
2. Label test tubes appropriately and dispense 0.4 ml of 0.85% saline into each tube.
3. Select 4-10 colonies of a similar colonial appearance with a loop and emulsify in the saline.
4. Vortex the cell suspension for 5 seconds.
5. Dispense 1 drop of the Test latex within and close to the edge of a circle on a reaction card and repeat for the Control latex reagent.
6. Using a Pasteur pipette add 1 drop of cell suspension to each of the 2 circles, and mix this into the latex reagents. Spread to cover the reaction areas.
7. Gently rock the card in a circular motion and look for agglutination. Do not rock the card for more than 1 minute and do not use a magnifying glass to aid reading of the result.
8. When finished, dispose of the reaction card into a suitable disinfectant.
9. Recap the bottles and return to the refrigerator.

8. CONTROL PROCEDURES
Quality control testing should be run with each shipment and new lot number received. Each laboratory should follow their state and local requirements. In addition, known stock cultures of Legionella should be used periodically as controls.

9. READING AND INTERPRETATION OF RESULTS
Positive Results
A result is positive if agglutination of the blue polystyrene 'latex' particles occurs within 1 minute and with no agglutination in the background in the test reagent. A positive reaction indicates that antigens to that serogroup of Legionella species have been detected in the sample.

Negative Results
A negative result is obtained if no agglutination occurs and a smooth blue suspension remains after 1 minute in the test circles.

10. LIMITATIONS
The latex agglutination test is presumptively diagnostic. Confirm positive results using biochemical tests.2

2. A negative latex agglutination test does not mean that the culture is not a Legionella species. It only indicates that the culture is not a Legionella species detected by the test reagent used.

3. A cross reaction may occur between L. pneumophila serogroup 1 and serogroup 9 due to naturally occurring group antigens. If both the L. pneumophila serogroup 1 and 2-14 reagents agglutinate with the isolate then this cross reaction should be suspected.

4. Cross reactions with the Legionella Species Test Reagent have been reported to occur occasionally with certain serotypes of other Legionella (e.g. L. parisiensis, L. sainthelenis, L. steigerwalti, L. wadsworthii, L. santricini, L. tuscensis, L. grattiana, L. cincinnatiensis).3

5. The test is designed to differentiate between different species and serotypes of Legionella. Cultures should be confirmed as Gram-negative rods which do not grow on cystine-deficient media.

For further information please refer to instruction leaflet in the Legionella Latex Test Kit (DR0800M).

11. PERFORMANCE CHARACTERISTICS
The reagents in the Oxoid Agglutination Legionella Test Kit have been tested for cross-reactivity against a panel of organisms listed below. No cross-reactivity was observed with any of the organisms.

L. chéri
L. birminghensis
L. rubricens
L. macecharinii L. ozarkidgensis L. erythra
L. feei
L. fielderensis
L. brunnens
L. spiritens
Pseudomonas fluorescens
Pseudomonas cepacia
Pseudomonas aeroginosa
Aeromonas hydrophila
L. hackeliae
L. icalenensis
L. jamestowniensis
L. quinlivanii
L. moravica
Stenotrophomonas maltophilia
Bacillus subtilis
Citrobacter freundii
Escherichia coli
Serratia marcescens

The Oxoid Legionella Latex Test was evaluated in a clinical and an environmental laboratory. A total of 40 clinical isolates and 279 environmental isolates were tested, covering Legionella pneumophila serogroups 1–14 and non-pneumophila Legionella. Each isolate was confirmed by serology. The performance of the Oxoid kit was also compared against other commercially available Legionella Latex Reagent Kits. The results of the trial are summarised opposite.4

L. pneumophila serogroup 15 has not currently been isolated.
from clinical or environmental samples in Europe\(^8\) and has only
been isolated once in the USA.\(^8\)

This most recent serogroup to be designated contains only one
strain (Lansing-3 (ATCC® 35251). A 16th serogroup was proposed
from studies of the Jena-1 isolate\(^10,11\) until further analysis showed
that the strain did not form a unique serogroup but was a member
of serogroup 4 L. pneumophila (monoclonal group Portland 1).\(^11,11\)

Additional internal data has been collected which demonstrates that the Oxoid Legionella Latex Kit (DR0800M and DR0802M) is
able to detect the Legionella pneumophila serogroup 15 (ATCC®
35251) as well as detecting L. pneumophila 1-14 and other
pathogenic non-L. pneumophila, Legionella strains. However,
as only one serogroup 15 strain is available for testing, it is not
thought appropriate to rename the serogroup 2-14 latex reagent
based on the results from a single isolate.

The Oxoid Legionella kits benefit the user by allowing
discrimination of samples into three groups: L. pneumophila
serogroup 1, L. pneumophila serogroups 2-15 (with the 2-14
reagent) and other Legionella species in a fast and simple
screening procedure.

<table>
<thead>
<tr>
<th>Oxoid Legionella Latex Kit/Serology</th>
<th>Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Legionella pneumophila Serogroup 1</td>
<td>59/59</td>
<td>100</td>
</tr>
<tr>
<td>Legionella pneumophila Serogroup 2-14</td>
<td>134/134</td>
<td>100</td>
</tr>
<tr>
<td>Other Legionellae included in the kit</td>
<td>63/65</td>
<td>97</td>
</tr>
<tr>
<td>Other Legionellae not included in the kit</td>
<td>0/93</td>
<td>100</td>
</tr>
<tr>
<td>Other Organisms</td>
<td>0/10</td>
<td>100</td>
</tr>
</tbody>
</table>

The overall sensitivity of the Oxoid Legionella Latex Kit was 99%.
The overall specificity of the Oxoid Legionella Latex Kit was 100%.

12. REFERENCES
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