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Legionella Latex Test

REF DR0800M.....  50 **EN**

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 *Legionellosis* or from environmental sources. The Oxoid Legionella Latex Test allows a separate identification of *Legionella pneumophila* serogroup 1 and serogroups 2–14 and detection of seven other *Legionella* species which have been implicated in human disease.

2. INTRODUCTION

Legionnaires' disease, named after the outbreak in 1976 at the American Legion Convention in Philadelphia, is caused by *Legionella pneumophila* and other *Legionella* species. It is characterised as an acute febrile respiratory illness ranging in severity from mild illness to fatal pneumonia. Since that time, it has been recognised that the disease occurs in both epidemic and endemic form and that the sporadic cases are not readily differentiated from other respiratory infections by clinical symptoms.

It is estimated that worldwide about 25,000 cases of *Legionella* infections occur annually. Known risk factors include immunosuppression, cigarette smoking, alcohol consumption and concomitant pulmonary disease. The mortality rate, which can be as high as 25% in untreated immunosuppressed patients, can be lowered if the disease is diagnosed rapidly and appropriate antimicrobial therapy started earlier.

Legionella pneumophila has been shown to be a major cause of both pneumonia and an acute self limiting febrile disease called Pontiac Fever. *L. pneumophila* strains and other *Legionella* species are isolated from patients with pneumonia and from the environment (mainly water).

Rare isolations have also been made in cases other than pneumonia, such as wound abscesses. The major reservoir of *Legionella* species appears to be fresh water sites, air-conditioning units and various water plumbing fixtures.

L. pneumophila is the most common cause of Legionnaires' disease. At present, 14 different serotypes exist of which *L. pneumophila* serogroup 1 accounts for 90% of cases.

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}

3. COMPONENTS OF THE KIT

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.

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. Each kit contains sufficient reagent for 50 tests.

DR0803 Legionella species Test Reagent

Consist of a suspension of blue polystyrene 'latex' particles sensitised with specific rabbit antibody reactive with the following species and serotypes:

L. longbeachae 1 and 2
L. bozemanii 1 and 2
L. dumoffii
L. gormanii
L. jordanis
L. micdadei
L. anisa

Each kit contains sufficient reagent for 50 tests.

DR0804 Positive Control Suspension

A polyvalent suspension of *Legionella* cells in buffer, sufficient for 25 tests.

DR0805 Negative Control Suspension

A suspension of cells in buffer non-reactive with the test reagents, sufficient for 25 tests.

DR0806 Control Latex

Consists of a suspension of blue polystyrene 'latex' particles sensitised with non-reactive rabbit globulin. Each kit contains sufficient reagent for 50 tests.

DR0807 Suspension Buffer X 2

A phosphate buffered saline solution. pH 7.3.

DR0500 Reaction Cards

There are 50 disposable reaction cards provided in the kit.

Instructions for use.

4. MATERIALS REQUIRED

The following materials are required but not provided in this kit: Microbiological loop and bunsen burner. 0.85% saline (for optional tube method). Suitable laboratory disinfectant e.g. Sodium hypochlorite solution >1.3% w/v.

5. PRECAUTIONS

IVD This product is for *in vitro* diagnostic use only.

Do not freeze.

Reagents contain 0.1% sodium azide as a preservative.

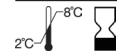
Sodium azide may react with lead or copper plumbing to produce metal azides which are explosive by contact detonation. To prevent azide accumulation in plumbing flush with copious amounts of water immediately after waste disposal.

Specimen materials may contain pathogenic organisms, handle with appropriate precautions.

Aerosol formation should be avoided, particular care should be taken during vortexing.

Please refer to the manufacturer's safety data sheet and the product labelling for information on potentially hazardous components.

6. STORAGE



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

7. CONTROL PROCEDURES

The control suspensions provided should be used to check the correct working of the latex reagents each day before routine tests are performed.

The positive control suspension (DR804) must show agglutination with the latex reagent within one minute. The negative control suspension (DR805) must show no agglutination within one minute.

Do not use the test if reactions with the control suspensions are incorrect.

8. IMPORTANT PROCEDURE NOTES

Do not allow the reagents to become contaminated by letting the dropper tip touch the specimens on the reaction card. Ensure that the caps are securely fitted after use to prevent contamination and drying out of reagents. After use, return the kit to the refrigerator ensuring that the bottles are stored in an upright position.

9. SPECIMEN COLLECTION AND PREPARATION

Isolates derived from environmental and clinical samples may be cultured on standard non-selective or selective *Legionella* culture media. Typical isolation schemes are given in references 3 and 4. *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 (*Legionella* Agar without cysteine CM655 + SR175). This confirmation may be performed prior to or after the latex test.

The following Oxoid media and supplements may be used for the culture of *Legionella* before performing the latex test.

BCYE (CM655 + SR110), BPMA α (CM655 + SR110 + SR111), MWY (CM655 + SR110 + SR118), GVPC (CM655 + SR110 + SR152).

For further details of these products please consult your local distributor.

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.

10. 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

- 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.
- Dispense 1 drop of each of the latex reagents onto 4 circles within and close to the edge of a circle on a reaction card.
- Add 1 drop of diluent buffer suspension to each of the 4 test circles. Ensure that the latex and buffer do not mix at this stage.

- 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 buffer. For optimal results ensure that the suspension is smooth. Repeat for similar colonies with each of the other reagents.
- Mix the latex reagents and suspensions together and spread to cover the reaction areas using the loop. Flame the loop.
- 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.
- When finished, dispose of the reaction card into a suitable disinfectant.
- Recap the bottles and return to the refrigerator.

(b) Tube Method

- 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.
- Label test tubes appropriately and dispense 0.4 ml of 0.85% saline into each tube.
- Select 4–10 colonies of a similar colonial appearance with a loop and emulsify in the saline.
- Vortex the cell suspension for 5 seconds. (Refer to Precautions Section.)
- Dispense 1 drop of each latex reagent (3 test reagents and the control reagent) onto 4 circles on the reaction card. Place them close to the edge of the circle.
- Using a Pasteur pipette add 1 drop of cell suspension to each of the 4 circles, and mix this into the latex reagents. Spread to cover the reaction areas.
- 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.
- When finished, dispose of the reaction card into a suitable disinfectant.
- Recap the bottles and return to the refrigerator.

11. 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 control circle. 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.

Uninterpretable Result

The test is uninterpretable if the control reagent shows agglutination. This indicates that the culture causes autoagglutination.

Granular or Stringy Reactions

Occasional granular or stringy reactions may be seen due to the particulate nature of the test material. When such reactions are seen to occur they should be interpreted using the following criteria:

The result is positive when there is noticeable clearing of the blue background in the test reagents.

12. LIMITATIONS

1. The latex agglutination test is presumptively diagnostic. Confirm positive results using biochemical tests.⁵
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 *Legionella pneumophila* serogroups 1 through to 14 nor *L. longbeachae* 1 and 2, *L. bozemanii* 1 and 2, *L. dumoffii*, *L. gormanii*, *L. jordanis*, *L. micdadei*, *L. anisa*.⁶
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 Legionellae (e.g. *L. parisiensis*, *L. sainthelensi*, *L. steigerwaltii*, *L. wadsworthii*, *L. santicrucis*, *L. tusconensis*, *L. gratiana*, *L. cincinnatiensis*.⁶)
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 cysteine-deficient media.

13. 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. cherri
L. birminghamensis
L. rubrilucens
L. maceachernii *L. oakridgensis* *L. erythra*
L. feelei
L. fairfieldensis
L. brunensis
L. spiritensis
Pseudomonas fluorescens
Pseudomonas cepacia
Pseudomonas aeruginosa
Aeromonas hydrophila
L. hackeliae
L. israeliensis
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.⁷

L. pneumophila serogroup 15 has not currently been isolated from clinical or environmental samples in Europe⁸ 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).^{12,13}

Additional internal data has been collected which demonstrates that the Oxoid Legionella Latex Kit (DR0800M, DR0801M, DR0802M and DR0803M) 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.

	Oxoid Legionella Number	Latex Kit/ Serology %
<i>Legionella pneumophila</i> serogroup 1	59/59	100
<i>Legionella pneumophila</i> serogroups 2–14	134/134	100
Other legionellae included in the kit	63/65	97
Other legionellae not included in the kit	0/93	100
Other organisms	0/10	100

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









WARNING: This product contains sodium azide. Harmful if swallowed.

14. REFERENCES:

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15. SYMBOL LEGEND

	Catalogue Number
	<i>In Vitro</i> Diagnostic Medical Device
	Consult Instructions for Use (IFU)
	Temperature Limitations (Storage temp.)
	Contains sufficient for <N> tests
	Batch Code (Lot Number)
	Use By (Expiration Date)
	Manufactured by



IFU X5057D Revised May 2016

Printed in the UK



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For all inquiries contact your local distributor.