INTENDED USE
Oxoid Xpect™ Legionella test kit is a rapid in vitro immunochromatographic test designed for the direct, qualitative detection of Legionella pneumophila serogroup 1 and 6 antigens in human urine. It is intended to aid in the presumptive diagnosis of Legionella infection caused by L. pneumophila serogroups 1 and 6 in conjunction with culture and other methods.

SUMMARY AND EXPLANATION
The Centers of Disease Control and Prevention first identified Legionella pneumophila in 1977 as the cause of an outbreak of pneumonia that caused 34 deaths at the 1976 American Legion Convention in Philadelphia. The organism was found to be breeding in the cooling tower of the hotel air conditioning system and subsequently spread through the entire building.

Legionellosis varies from a mild, febrile illness to a potentially fatal form of pneumonia and is caused by Legionella pneumophila (up to 90%) and other Legionella species. Legionellosis has two distinct clinical syndromes: Legionnaires' disease and Pontiac fever. Pontiac fever is an acute, self-limiting influenza-like illness that typically lasts two to five days. Legionnaires' disease can have symptoms including a high fever, chills, coughing, muscle aches and headaches. Many patients experience diarrhoea, nausea, vomiting, and abdominal pain. Changes in mental status, such as disorientation, confusion and hallucinations, also occur in about a quarter of cases.

L. pneumophila serogroup 1 is the most frequent among human isolates. L. pneumophila serogroup 6 is the second most common serogroup according to the frequency of isolation from clinical samples. Legionnaires' disease is an important cause of travel, community and hospital-acquired pneumonia. Outbreaks are usually seen in the summer and early autumn but may occur year-round. In untreated patients, the mortality rate may be as high as 80%. Marston et al. reported the likelihood of death was increased in patients who were elderly or male; those with hospital-acquired infection, renal disease, malignancy, or immuno-suppression; and those from whom L. pneumophila serogroup 6 was isolated. Legionella are poorly staining, aerobic, Gram-negative bacilli. They are found naturally in the environment and thrive in warm water and warm damp places. Laboratory diagnosis is based on the results of culture growth, direct immunofluorescence, serological testing, and/or antigen detection in urine. Current consensus guidelines on the management of community-acquired pneumonia (CAP) in adults, recommended by the Infectious Diseases Society of America and the American Thoracic Society, include a urinary antigen test for L. pneumophila as part of the diagnostic test regimen for patients with severe CAP. Urinary antigen tests permit early diagnosis and initiation of appropriate antibiotic therapy, and lead to the recognition of outbreaks, allowing a rapid epidemiological investigation.

PRINCIPLE
Xpect Legionella is a rapid immunochromatographic assay designed for the qualitative detection of L. pneumophila serogroup 1 and 6 soluble antigens in human urine. To perform the test, the patient specimen is added to the sample well of the device and moves along the membranes by capillary action. If present, L. pneumophila serogroup 1 and/or 6 antigens bind the immobilised anti-L. pneumophila serogroup 1 and 6 antibody. A visible line forms as a complex of antibody-antigen-antibody coated particles in the test region (TEST). Antibody coated coloured particles not bound at the test line are later captured by the antibody in the control region (CTRL). A visible line will always appear in the control region indicating that the test is working properly. The presence of a control line combined with the absence of a visible test line is interpreted as a negative test result.

STORAGE
The product should be stored at room temperature or refrigerated (2-25°C) until the expiration date printed on the box. Do not freeze or overheat. If stored refrigerated, allow components to come to room temperature before use.

PRECAUTIONS
1. For In Vitro Diagnostic Use.
2. Standard precautions should be taken against the dangers of biological hazards by properly sterilising specimens, containers, and test devices after use. Consult appropriate references where necessary. Directions should be read and followed carefully.
3. Do not use test devices beyond the printed expiration dates.
4. Do not reuse test devices.

SPECIMEN COLLECTION, STORAGE AND TRANSPORTATION
- Specimens should be collected in clean, leak-proof containers.
- Specimens may be stored at room temperature if testing will occur within 24 hours of collection.
- Alternatively, specimens may be stored at 2-8°C for up to 14 days or at -20°C or below for longer periods before testing.
- Boric acid should not be used as a preservative.
- Allow specimens to equilibrate to room temperature prior to testing.

REAGENTS AND MATERIALS SUPPLIED
2. Disposable transfer pipettes (20): Pipettes with marked graduations at approximately 0.1ml increments.
3. Positive Control (1ml): Control material containing L. pneumophila antigen with preservative.
4. Negative Control (1ml): Buffered solution with preservative.
5. Instructions for Use (IFU) (1).

MATERIALS REQUIRED BUT NOT SUPPLIED
1. Specimen collection containers.
2. Timer.

PROCEDURE
1. Mix specimens thoroughly prior to testing.
2. Remove the test device from the foil pouch when ready to perform the test and place it on a flat surface.
3. Label the device with patient or control identification.
4. Use a transfer pipette to dispense 0.2ml (second graduated mark from tip of pipette) of specimen into the centre of the sample well of the test device.
5. Read and record the test results visually at 45 minutes according to the INTERPRETATION section. (Strong positive results may be apparent within 15 minutes. Do not interpret results as negative until 45 minutes have elapsed.)

INTERPRETATION OF RESULTS
Positive Test (antigen present): A positive test is indicated by two black lines: one in the TEST region and one in the control (CTRL) region. A complete, black, clearly visible test band of any intensity should be interpreted as positive. A positive test indicates the presence of L. pneumophila serogroup 1 and/or 6 antigen in the sample.
Negative Result (antigen not detected):
A negative test is indicated by only one black line in the control (CTRL) region. A negative test indicates that *L. pneumophila* serogroup 1 and 6 antigens are absent or below the detection limit of the test.

Invalid Result:
An invalid test occurs when the test line is partial or incomplete, or the control line is absent or incomplete. Lines that appear in the TEST area after 45 minutes should be ignored.

Quality Control
Internal: A procedural control is included in the test. A black coloured line appearing in the control band (CTRL) region is considered an internal positive procedural control, indicating proper performance, reactive reagents, a functionally active control line and that adequate capillary flow has occurred. A clear background in the results area is considered an internal negative control. If the test has been performed correctly and reagents are working properly, the background will clear to give a discernible result.

External: The Positive and Negative Controls provided with the kit should be run with each shipment and new kit lot number received. The Positive Control is used to verify reactivity of the reagents associated with the assay and is not intended to ensure precision at the analytical assay cut-off. Each laboratory should follow their local requirements. If aberrant quality control results are noted, patient results should not be reported.

To use controls provided in the kit, hold the dropper bottle vertically and dispense seven (7) drops of the Positive or Negative Control directly into the sample well.

**LIMITATIONS**
1. A negative test does not exclude the possibility of infection from *L. pneumophila* serogroups 1 and 6. The antigen concentration may fall below the detectable limit of the test. It is recommended that culture be used for patients with suspected pneumonia to determine causative agents other than *L. pneumophila* serogroups 1 and 6.

2. In order to make an accurate diagnosis for *L. pneumophila* infection, culture results, serology testing and antigen detection methods should be used in conjunction with clinical findings.

3. Sensitive immunoassays may demonstrate positive results with specimens containing heterophilic antibodies. If the qualitative interpretation is inconsistent with clinical findings, then further testing by an alternate method should be performed.

4. Excretion of *Legionella* antigen may be seen as early as three days after the onset of symptoms and may continue up to one year afterwards. A positive test result may indicate current or past infection. Culture results, serology testing and antigen detection methods should be used in conjunction with clinical findings to make an accurate diagnosis.

**PERFORMANCE DATA**

**Clinical Accuracy:**
One hundred and thirty seven (137) frozen urine specimens were evaluated with Xpect Legionella by an independent laboratory. Fifty-one (51) samples were from patients with Legionnaires' disease proven by a positive result in at least two of the following methods: modified urine antigen test, seroconversion in an IgM and/or IgG assay, a positive culture on a lower respiratory tract sample (LRTS), and/or PCR on a LRTS. Eighty-six (86) urine samples were from patients with respiratory tract infections other than *Legionella*; primarily community-acquired pneumonia due to *Streptococcus pneumoniae*.

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<td>TOTAL</td>
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Relative sensitivity: 94.1% (95% CI = 77.4–95.8%)
Relative specificity: 100% (95% CI = 95.8–100%)
Agreement: 97.8% (95% CI = 90.9–98.4%)
Positive Predictive Value: 100% (95% CI = 92.6–100%)
Negative Predictive Value: 96.6% (95% CI = 86.3–97.6%)

**Clinical Cross-Reactivity:**
One hundred and fifty eight urine specimens from patients diagnosed with other etiological respiratory tract infections (86) or urinary tract infections (72) were tested using Xpect Legionella. The results yielded a specificity of 100%.

**Cross-Reactivity:**
To confirm the analytical specificity of Xpect Legionella, bacterial cultures likely to be found in the urinary or respiratory tract were tested. Bacterial cultures were tested at 1 x 10^7 cfu/ml. All yielded negative results.

**Reproducibility:**
The reproducibility of Xpect Legionella was evaluated in-house with two operators. Xpect Legionella was tested against a panel of four samples, which included three levels of positives and a negative. The overall reproducibility for Xpect Legionella was 100%.

**BIBLIOGRAPHY**
PACKAGING
REF R24680, Xpect Legionella test kit......................... 20 Tests/Kit

Symbol Legend

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IFU 24680, Revised August 21, 2008 Printed in U.S.A.