remel

LEGIONELLA POLY-ID TEST KIT

INTENDED USE

Remel Legionella Poly-ID Test Kit is recommended for use in qualitative procedures to aid in the identification of *Legionella* bacteria in clinical specimens, cultures, and environmental water samples. This fluorescent antibody (FA) procedure is highly specific and sensitive for 33 strains of *Legionella* but should be used in conjunction with cultural isolation and biochemical characterization.

SUMMARY AND EXPLANATION

Legionella bacteria are ubiquitous gram-negative bacilli, occurring naturally in aquatic habitats.[1,2] Two distinct clinical syndromes have been attributed to *Legionella* spp. Legionnaires' Disease (LD) is an acute severe pneumonia, distinguishable by its lack of a clinical response to β-lactam and aminoglycoside antibiotics. *Legionella pneumophila* has been reported to account for 5% of community-acquired pneumonias and tends to occur seasonally (summer and fall). LD is also associated with nosocomial infections and, because of associated host factors, carries a significant risk of mortality. Pontiac Fever is an acute febrile illness caused by *L. pneumophila* but, unlike LD, it is not complicated by pneumonia. Cases of Pontiac Fever tend to have short incubation periods, last 36 to 48 hours, occur in clusters, and have not been reported as a cause of death.

POLYVALENT FLUORESCENT ANTIBODY REAGENTS

Legionella Poly-ID Test Kit is recommended for use with culture smears and clinical specimens such as, fresh lung tissues, respiratory tract fluids, scrapings of formalin-fixed tissue, paraffin-embedded tissue, sputum, transtracheal aspirates, bronchial washings, and pleural fluids.[3-9] The Polyvalent Anti-Legionella Serum simultaneously identifies the 33 strains of *Legionella* listed below:

Serogroup	Strain	Serogroup	Strain
L. pneumophila			
1	Bellingham	*L. gormanii* 1	LS-13
1	Knoxville	*L. micdadei* 1	TATLOCK
1	Philadelphia-1	*L. wadsworthii* 1	81-716
2	Togus 1	*L. oakridgensis*	Oak Ridge 10
3	Bloomington 2	*L. feeleii* 1	WO-44C-C3
4	Los Angeles 1	*L. sainthelensi* 1	Mt. St. Helens 4
5	Dallas 1	*L. jordanis* 1	BL-540
6	Chicago 2	*L. anisa* 1	WA-316-C3
7	Chicago 8	*L. spiritensis* 1	Mt. St. Helens 9
8	Concord 3	*L. hackeliae* 1	Lansing 2
9	IN-23-G1-C2	*L. maceachernii*	PX-1-G2-E2
10	Leiden 1	*L. jamestowniensis*	JA-26-G1-E2
L. dumoffii 1	NY-23	*L. cherrii* 1	ORW
L. longbeachae 1	Long Beach 4	*L. steigerwaltii* 1	SC-18-C9
L. longbeachae 2	Tucker 1	*L. parisiensis*	PF-209C-C2
L. bozemanii 1	WIGA	*L. rubrilucens* 1	WA -270AC2
		L. erythra 1	SE-32A-C8

PRINCIPLE

In the FA technique, the test sample is fixed on a microscope slide and covered with Polyvalent Anti-Legionella Serum which binds with *Legionella* bacteria in the test sample. FITC-Conjugate w/ Counterstain is added to the slide and, following incubation, the smear is examined microscopically with ultraviolet-blue light. Excitation of ultraviolet light by bound FITC-Conjugate emits wavelengths in the yellow-green portion of the color spectrum. *Legionella* cells are seen as brilliant yellow-green or apple-green bacilli.

MATERIALS SUPPLIED

Polyvalent Anti-Legionella Serum: (2.0 ml x 1) Lyophilized rabbit anti-*Legionella* IgG, bovine serum albumin (BSA), and Evans blue counterstain in phosphate buffered saline (PBS) with preservative

FITC Conjugate w/ Counterstain: (4.0 ml x 1) Lyophilized FITC-labeled goat anti-rabbit IgG, BSA, PBS, and rhodamine counterstain with preservative

Negative Control Serum: (2.0 ml x 1) contains lyophilized normal rabbit IgG, BSA, and Evans blue counterstain in PBS with preservative

Legionella Control Antigens: (1.0 ml x 1) Polyvalent pool of formalin-killed *Legionella* antigens resuspended in PBS with 0.05% sodium azide

Buffer Powder: (9.9 g x 1) Each packet contains powdered salts for one liter of PBS (pH 7.6)

Mounting Medium: (3.0 ml x 1) Glycerol in phosphate buffer with preservative (pH 9.0)

Instructions for Use (IFU)

PRECAUTIONS

This product is for *In Vitro* diagnostic use and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, media, and test materials after use. Carefully read the entire procedure prior to performing any tests.

1. Sodium azide may react with copper or lead plumbing to form highly explosive metal azides. Upon disposal of reagent into sink, flush with large amounts of water to prevent azide build-up.
2. Refer to Material Safety Data Sheet for additional information on reagent chemicals.
3. Do not mix reagents of different kit lot numbers.

REAGENT PREPARATION

1. Buffer Solution (PBS): Add the contents of the Buffer Powder packet to a 1 liter volumetric flask containing about 500 ml demineralized water. Swirl the flask to completely dissolve the powder before adding the remaining water, bringing the solution to one liter. Once rehydrated, the buffer solution is stable for 6 months when stored at 2-8°C.
2. FITC-Conjugate Solution: Add 4.0 ml demineralized water to the lyophilized FITC-Conjugate. Allow 1 to 5 minutes for complete dissolution. Mix gently before use.
3. Negative Control Serum: Add 2.0 ml demineralized water to the lyophilized Negative Control. Allow 1 to 5 minutes for complete dissolution. Mix gently before use.
4. Polyvalent Anti-Legionella Serum: Add 2.0 ml demineralized water to the lyophilized Polyvalent Anti-Legionella Serum. Allow 1 to 5 minutes for complete dissolution. Mix gently before use.

STORAGE

Store product in its original container at 2-8°C in the dark. After rehydration and if stored at 2-8°C, the Conjugate and Anti-Legionella Serum are stable for 90 days or until the expiration date on the kit, whichever comes first. Store Control Antigens at 2-8°C in the dark until the expiration date on the kit. After rehydration, the buffer solution is stable for six months when stored at 2-8°C. Store mounting medium at room temperature.

PRODUCT DETERIORATION

This product should not be used if (1) the color has changed, (2) the expiration date has passed, or (3) there are other signs of deterioration.

SPECIMEN COLLECTION, STORAGE, AND TRANSPORT

Specimens should be collected and handled following recommended guidelines.[1,2]

MATERIALS REQUIRED BUT NOT SUPPLIED

(1) Loop sterilization device, (2) Inoculating loop, collection containers, tissue grinder, (3) Biological safety cabinet, centrifuge, incubator, (4) Supplemental media, (5) Quality control organisms (optional), (6) Clean FA microscope slides with two 1.5 cm wells, 2N NaOH, cover slips (#1), (7) Coplin jars, (8) Calibrated pipette, 25 µl, (9) Sterile water, (10) One liter volumetric flask, (11) Sterile petri dishes, scalpel, forceps, (12) Sterile swabs, applicator sticks, (13) Neutral formalin (1 or 10%), acetone, (14) Xylol, absolute ethanol, 95% ethanol, (15) Moist chamber, (16) Fluorescent microscope with suitable filter system*, (17) Immersion oil, (18) McFarland Turbidity Standards (No. 1 or 2).

*Maximum excitation and wavelength = 490 nm; emission wavelength = 520 nm; objectives capable of 400 – 500 X magnification and 630 – 1000 X magnification.

PROCEDURE

Sample Slide Preparation:

Prior to slide preparation, wash FA microscope slides in 2 N NaOH for 10 minutes and rinse well with demineralized water. Allow slides to air dry completely before using. Prepare duplicate wells for each test sample and control antigen. One well is for the test (Polyvalent Anti-Legionella Serum), the other well for the control (Negative Control Serum). Quality control should be included with each test run of patient specimens.

1. **Formalin-fixed tissue scrapings:**
   a. Select one or more areas in the lung which are dense grey or reddish areas of consolidation.
   b. Transfer tissue block to a sterile petri dish.
   c. With a sharp scalpel, cut through these areas to produce new tissue faces for scraping.
   d. Grasp the tissue with forceps and hold the scalpel at a right angle to the tissue face. Scrape the tissue to produce a fine puree of tissue particles.

e. Smear tissue particles and fluids onto both wells of a clean FA microscope slide. Using the scalpel blade, smear the specimen over the well.
f. Allow the smears to air dry and gently heat-fix.

2. **Fresh-frozen tissue:** Process in a safety cabinet. If the tissue needs to be cultured, process the culture before making imprints.
   a. Use sterile instruments. With a scalpel, cut a fresh face of tissue. With forceps, press the tissue against a slide. Blot excessively moist tissue with sterile gauze so smear will not be too thick.
   b. Allow the smears to air dry. Gently heat-fix the slides.
   c. Fix the smears by covering with 1-10% neutral formalin for 10 minutes. Place slides in a moist chamber until fixation is complete.
   d. Drain off formalin and rinse with a gentle stream of demineralized water. Rinse slides in PBS for 10 minutes, followed by a dip in demineralized water.
   e. Allow the smears to air dry and gently heat-fix.

3. **Tissue sections:** Legionella bacteria maintain their serologic integrity through histopathological processing. Cut tissue sections as thin as possible (4 mu or less). Note: For paraffin-embedded tissue, fix sections for approximately 15 minutes at 58-60°C. Remove paraffin by passing through 2 rinses of xylol followed by two passages each through absolute ethanol, 95% ethanol, and demineralized water.

4. **Lung Exudates:** Process in a safety cabinet. Sputum, transtracheal aspirates, bronchial washings, and other lower respiratory tract specimens are acceptable for testing. However, such specimens may be very viscous and tenacious and may or may not be purulent.
   a. If possible, select a purulent area of the specimen and prepare smears of moderate thickness. Spread the material over the wells using a sterile swab. Allow the smears to air dry and gently heat-fix.
   b. Fix slides as described in steps 2.c. and 2.d.
   c. Allow the smears to air dry and gently heat-fix.

5. **Pleural Fluid:** Process in a safety cabinet. If the specimen needs to be cultured, process culture before making the smears.
   a. Prepare thin smears, air dry, and heat fix.
   b. Process as described for lung exudates.

   Note: Pleural fluids tend to form a fibrin clot on the slide. Unless handled carefully, the entire film may be dislodged during processing.

6. **Culture Smears:** Process in a safety cabinet.
   a. Make a suspension of the culture in 1% neutral formalin with turbidity equal to a #1 or #2 McFarland.
   b. Centrifuge the suspension at 3000 rpm for 10 minutes. Resuspend the bacterial cells in PBS.
   c. Transfer a drop of the inoculum to each of two wells of a slide.
   d. Allow the smears to air dry and gently heat-fix.

7. **Environmental water samples:**
   a. Concentrate water sample by centrifugation or filtering.
   b. Transfer inoculum to each of two wells on a slide.
   c. Allow the smears to air dry. Gently heat fix.

8. **Quality Control Slide:**
   a. Resuspend the vial of Legionella Control Antigens by inversion to ensure a homogeneous suspension.
   b. Transfer 25 µl of the antigen suspension to each of two wells on a clean slide and spread over the well with an applicator stick. Allow the smears to air dry.
   c. Gently heat-fix or fix each slide individually in acetone for one minute.

FA Staining:
Note: Process each slide separately to ensure organisms are not transferred from one slide to another.

1. **Test Well**: Dispense 25 µl of Polyvalent Anti-Legionella Serum to the **first** well of each slide.
2. **Control Well**: Dispense 25 µl of Negative Control Serum to the **second** well of each slide.
3. Place slides in a moisture chamber and incubate at room temperature for 30 minutes.
4. Rinse slides with a gentle stream of PBS without aiming directly at the smears. Immerse in PBS for 10 minutes.
5. Rinse with a stream of demineralized water. Remove excess moisture by draining slides against absorbent paper.
6. Apply a drop of FITC Conjugate w/ Counterstain to each test and control well.
7. Incubate slides for 30 minutes at room temperature in a moisture chamber.
8. Rinse slides with a gentle stream of PBS. Immerse in PBS for 10 minutes.
9. Rinse with a stream of demineralized water. Remove excess moisture by draining slides against absorbent paper. Allow smears to air dry.
10. Add one or two drops of mounting medium and a coverslip.

Examination of Stained Slides:
Examine slides under low-power objective (10 X) using a fluorescent microscope. In strongly positive preparations the bacteria will be visible as uniformly sized dots. Switch to the high-power objective for rapid screening. *Legionella* organisms are single, short rods or small intra- or extracellular clumps of organisms with strong peripheral staining and darker centers. Use the oil immersion objective (100 X) to confirm.

Reading of Final Reaction:
Use the following numerical notations to describe the observed intensity of fluorescence:

4+ = Brilliant apple-green staining
3+ = Bright apple-green staining
2+ = Definite but dull apple-green staining
1+ = Dim apple-green staining
- = No apple-green staining

Note: Each sample plus Negative Control Serum (Control Well) must stain with an intensity of less than 3+ or the test is considered invalid. The Negative Control Serum must be used with every test sample and positive control slide to ensure that positive reactions obtained are specific.

Stained slides are stable for 24 hours when stored in the dark at 2-8°C. Read slides within 24 hours of preparation.

QUALITY CONTROL
1. **Negative Control:** The Legionella Control Antigen and Negative Control Serum, used to verify proper performance of the antisera and conjugate, must stain with an intensity of less than 3+.
2. **Positive Control:** The Legionella Control Antigen and Polyvalent Anti-Legionella Serum must stain with an intensity of 3+ or 4+. The test is considered invalid if the staining intensity is less than 3+.

INTERPRETATION OF RESULTS
1. *Legionella* bacteria are pleomorphic bacilli. Organisms in culture are usually longer rods than those seen in tissues. In older cultures, long filaments, swollen rods, and other pleomorphic forms may be seen.
2. The following criteria established by CDC are recommended to interpret and report the test results of clinical specimens:

Report:	Result:
FA positive -	>25 typical bacilli with 3+ to 4+ cell-wall fluorescence per smear
Number of stained cells, only -	<25 typical bacilli with 3+ to 4+ cell-wall fluorescence per smear
FA negative -	No typical bacilli with 3+ to 4+ cell-wall fluorescence (i.e., bacteria of atypical morphology which stain brightly with either immune or negative control conjugate or any bacteria which stain 2+ or less)

Note: In sputum, organisms are seldom numerous. The observation of 5 or more morphologically typical bacilli with 3+ or 4+ fluorescence is considered a positive result.

3. Cultural isolates that are morphologically typical and brightly fluorescent (3+ to 4+) are considered positive.
4. FA-positive results should be reported as presumptive *Legionella* spp. A positive test indicates one or more of the strains listed in the IFU are present.

LIMITATIONS
1. Legionella Poly-ID Test Kit is only part of the overall scheme for identification of *Legionella*. Final diagnosis should be made by a physician familiar with the patient's symptomology and physical examination in combination with the results of all laboratory testing.
2. A negative result indicates the sample is negative for the *Legionella* serogroups listed in this IFU.
3. Microscopic interpretation is straightforward with specimens such as, tissue scrapings, formalin-fixed lung tissue, fresh lung impressions, pleural fluids, and culture smears. However, white blood cells may be highly auto-fluorescent and some bacteria may fluoresce due to a nonspecific reaction with IgG.[8]

4. A strain of *Pseudomonas fluorescens* has been reported which is brightly and specifically stained by the *Legionella* conjugate. The morphology and staining characteristics of the organisms must be carefully evaluated before reporting as positive for *Legionella*.[10,11]
5. *Legionella* organisms will grow on charcoal yeast extract medium, but will not grow on conventional 5% blood agar plates
6. Lower respiratory tract specimens contain few *Legionella* organisms, when positive. A thorough 5-minute examination of the smear is required before calling such specimens negative.[1,2]
7. Particles obtained by scraping formalin-fixed lung tissue may be lost from the slide during processing, however free bacteria and tissue cells usually remain on the slide and provide a good substrate. If the lung tissue is friable (i.e., rubbery or spongy), a positive result is unlikely.

PERFORMANCE CHARACTERISTICS

Legionella Poly-ID Test Kit was tested on 139 organisms (i.e., 68 of which were gram-negative bacilli, 51 *Enterobacteriaceae* and 17 *Pseudomonas*) with no fluorescence observed. Thirty-one serogroups of *Legionella* were tested with Legionella Poly-ID Antiserum and all were positive following the interpretative criteria in this IFU. A currently available commercial product was found to fluoresce 7/7 different *Legionella* antigens tested. Legionella Poly-ID Test Kit yielded positive results on 7/7 different commercial antigens tested.[12]

The sensitivity of Legionella Poly-ID Test Kit FA test was 100% (39/39) and the specificity was 100% (139/139).

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PACKAGING

REF R24400, Legionella Poly-ID Test Kit ..... 50 Tests/Kit

Symbol Legend

REF	Catalog Number
IVD	In Vitro Diagnostic Medical Device
LAB	For Laboratory Use
	Consult Instructions for Use (IFU)
	Temperature Limitation (Storage Temp.)
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)

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