

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY**

A. 510(k) Number:

K113190

B. Purpose for Submission:

To obtain substantial equivalent determination for this new device

C. Measurand:

Legionella pneumophila serogroup 1 antigen

D. Type of Test:

Rapid lateral flow immunoassay

E. Applicant:

Meridian Bioscience, Inc

F. Proprietary and Established Names:

TRU Legionella assay

G. Regulatory Information:

1. Regulation section:

21CFR 866.3300, Haemophilus spp. Serological Reagents

2. Classification:

Class II

3. Product code:

MJH: Legionella, spp., ELISA

4. Panel:

83 Microbiology

H. Intended Use:

1. Intended use(s):

The TRU Legionella assay is an in vitro, rapid, lateral-flow immunoassay for the qualitative detection of *Legionella pneumophila* serogroup 1 antigen in human urine specimens. It is designed to test specimens from patients with symptoms of pneumonia. Test results are to be used as an aid in diagnosis of *Legionella pneumophila* serogroup 1 infection. A negative result does not preclude infection with *Legionella pneumophila* serogroup 1. Test results are to be used in conjunction with information obtained from the patient's clinical evaluation and other diagnostic procedures.

2. Indication(s) for use:

The TRU Legionella assay is an in vitro, rapid, lateral-flow immunoassay for the qualitative detection of *Legionella pneumophila* serogroup 1 antigens in human urine specimens. It is designed to test specimens from patients with symptoms of pneumonia. Test results are to be used as an aid in diagnosis of *Legionella pneumophila* serogroup 1 infection. A negative result does not preclude infection with *Legionella pneumophila* serogroup 1. Test results are to be used in conjunction with information obtained from the patient's clinical evaluation and other diagnostic procedures.

3. Special conditions for use statement(s):

For Prescription Use only

4. Special instrument requirements:

Not applicable

I. Device Description:

TRU Legionella is a single use capture immunoassay to detect *Legionella pneumophila* serogroup 1 in human urine specimens. The test consists of a Conjugate Tube, a Test Strip, and Sample Diluent/Negative Control. The Conjugate Tube contains a lyophilized bead of colloidal gold-linked polyclonal antibody to *Legionella pneumophila* serogroup 1 detector antibody. The Test Strip carries a nitrocellulose membrane with dried capture antibodies placed at a designated Test Line for *Legionella*. The Test Strip holder caps the Conjugate Tube during testing and subsequent disposal to reduce exposure to potential pathogens.

The conjugate bead is first rehydrated in the Conjugate Tube with the Sample Diluent/Negative Control. Patient sample is then added, the contents mixed and the Test Strip added. *Legionella pneumophila* serogroup 1 antigens, if present, bind to the

antibody-colloidal gold conjugate. When the sample migrates up the Test Strip to the Test Line, the antigen-conjugate complex is bound to the capture antibody, yielding a pink-red line. When no antigen is present, no complexes are formed and no pink-red line appears at the Test Line. An internal control line helps determine whether the test has been executed properly, if kit reagents are performing appropriately and that adequate flow has occurred through the Test Strip during a test run. A visible pink-red line at the Control position of the Test Strip should be present each time a specimen or control is tested. If no pink-red control line is seen, the test is considered invalid.

J. Substantial Equivalence Information:

1. Predicate device name(s):

Binax NOW® Legionella Urinary Antigen Test

2. Predicate 510(k) number(s):

K982238

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
	TRU Legionella	Binax NOW® Legionella
Qualitative/Quantitative	Qualitative	Qualitative
Target Antigen	<i>Legionella pneumophila</i> serogroup 1	<i>Legionella pneumophila</i> serogroup 1
Specimen Type	Human urine, preserved and unpreserved	Human urine, preserved and unpreserved
Detection Antibody	Colloidal gold conjugated Rabbit polyclonal antibody	Colloidal gold conjugated Rabbit polyclonal antibody
Reading Method	Visual	Visual
Results Interpretation	Negative: A single pink-red band at the Control Line position. Positive: Pink-red bands at both the Control and Legionella Test Line positions. Invalid: No band at the Control Line position, a pink-red band appearing after 21 minutes of incubation, or a band of any color other than pink-red	Negative: Single pink to purple colored Control Line visible in the top half of the window. Positive: Two pink to purple colored lines. Invalid: No line at the Control Line position or no lines at the Control or Sample Line positions.

Differences		
Item	Device	Predicate
Test Format	Rapid lateral flow immunoassay. Insert test strip into tube containing prepared sample.	Rapid immunochromatographic membrane assay. Insert sample swab into test device well.
Reagents/Components	Test Strip, Conjugate Tube, Sample Diluent/Negative Control, Positive Control, Plastic transfer pipettes with 100, 200 and 300 μ L marks	Test Device, Reagent A, Positive Control Swab, Negative Control Swab, Swabs
Urine Sample Preparation	1. Add 100 μ L of Sample Diluent to Conjugate Tube. Vortex for 10 seconds. 2. Add 100 μ L of thoroughly mixed urine sample to the Conjugate Tube. 3. Mix sample and conjugate thoroughly	1. Dip swab into the urine sample and then insert swab into the bottom hole of the Test Device. 2. Add 2 drops of Reagent A to the bottom hole.
Conjugate format	Lyophilized bead of conjugate antibody in the Conjugate tube.	Conjugate antibody dried onto fibrous conjugate pad.
General Laboratory Equipment required	Vortex, Disposable latex gloves	Urine collection container, BinaxNOW® Legionella Urinary Antigen Control Swab Pack
Testing Time	Approximately 20 minutes	Approximately 15 minutes

K. Standard/Guidance Documents Referenced:

- User Protocol for Evaluation of Qualitative Test Performance (EP12-A2)
- User Verification of Performance for Precision and Trueness (EP15-A2)
- Guidance on Informed Consent for In Vitro Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable - Guidance for Sponsors, Institutional Review Boards, Clinical Investigators and FDA Staff
- Guidance for Industry and FDA Staff - Statistical Guidance on Reporting

Results from Studies Evaluating Diagnostic Tests

L. Test Principle:

The conjugate bead is first rehydrated in the Conjugate Tube with the Sample Diluent/Negative Control. Patient sample is then added, the contents mixed and the Test Strip added. *Legionella pneumophila* serogroup 1 antigens, if present, bind to the antibody-colloidal gold conjugate. When the sample migrates up the Test Strip to the Test Line, the antigen-conjugate complex is bound to the capture antibody, yielding a pink-red line. When no antigen is present, no complexes are formed and no pink-red line appears at the Test Line. An internal control line helps determine whether the test has been executed properly, if kit reagents are performing appropriately and that adequate flow has occurred through the Test Strip during a test run. A visible pink-red line at the Control position of the Test Strip should be present each time a specimen or control is tested. If no pink-red control line is seen, the test is considered invalid.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Reproducibility was assessed at three clinical laboratories using a blind coded sample panel. The panel consisted of three contrived moderately positive samples, three low positive samples at a concentration just above the limit of detection, three high negative samples at a concentration just below the limit of blank, and one natural negative sample. The panels were tested in two runs at each site by two operators each day for five non-consecutive days. Reproducibility was 100%.

b. *Linearity/assay reportable range:*

Not applicable

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

Sample storage

A sample storage study was conducted to verify the sample preservation, handling, and storage criteria included in the TRU Legionella package insert. Aliquots of unpreserved and boric acid preserved positive and negative samples were stored at the following temperatures to determine the worst case storage time for each temperature range: room temperature (20-26 °C) up to

eight days, refrigerated (2-8 °C) up to eight days, conventional freezer (-16 to -28 °C) up to 31 days, and ultralow freezer (-66 to -84 °C) up to 31 days. Ten contrived samples were tested: four negatives, four positives, a high negative sample, and a limit of detection positive. Samples were tested at various times during storage. All samples produced the expected results. The data supports test performance with preserved and unpreserved samples that are stored for up to seven days at 2-8 °C and 20-26 °C, and/or stored for up to 30 days at ≤ -16 °C.

Fresh versus frozen samples

A fresh versus frozen specimen study was conducted to verify that frozen samples can be thawed multiple times without affecting the detection of the measurand. Ten contrived samples were tested: four negatives, four positives, a high negative sample, and a limit of detection positive. Unpreserved and boric acid preserved specimens were tested. Baseline tests were run, and then aliquots were tested every cycle for three freeze thaw cycles each at -16 to -28 °C and at -66 to -84 °C. All samples produced the expected results. The data supports test performance with preserved and unpreserved samples that are frozen and thawed up to two times.

Kit stability

Reagent stability was provisionally established using accelerated (stressed) testing methods. TRU Legionella kits were stored at 3-7 °C, 21-25 °C, 40-44 °C, and 48-52 °C. Test performance was evaluated at various time points for up to 65 or 104 days using a sample panel containing a negative sample, low positive sample, reference standard, and both positive and negative controls. The 2-8 °C temperature range was used as T1 (initial or baseline temperature) when calculating stability using the Q rule, with $Q_{10} = 3$. The 40-44 °C and 48-52 °C ranges were used as T2. Real-time stability studies are currently ongoing with testing performed at quarterly intervals and will be completed at the end of 27 months.

All samples produced the expected result with kits stored at the different temperatures and times. The data supports final product storage at 2-8 °C for 18 months.

High-dose hook effect

L. pneumophila Philadelphia and Bellingham strains were spiked into negative donor urine and then serially diluted. A false negative result obtained with a very high level of target antigen would indicate a high-dose hook effect.

Determination of a high-dose hook effect with *L. pneumophila*
Philadelphia strain ATCC 33152

Sample	Replicate	Result
Positive Control	Positive	Pass
Negative Control	Negative	Pass
Negative Sample Control	Negative	Pass
Dilution 1: 2.0×10^8 CFU/mL	Positive	Pass
Dilution 2: 5.0×10^7 CFU/mL	Positive	Pass
Dilution 3: 1.25×10^7 CFU/mL	Positive	Pass
Dilution 4: 3.1×10^6 CFU/mL	Positive	Pass
Dilution 5: 7.8×10^5 CFU/mL	Positive	Pass
Dilution 6: 2.0×10^5 CFU/mL	Positive	Pass

Determination of a high-dose hook effect with *L. pneumophila* Bellingham strain NCTC 11404

Sample	Replicate	Result
Positive Control	Positive	Pass
Negative Control	Negative	Pass
Negative Sample Control	Negative	Pass
Dilution 1: 4.2×10^8 CFU/mL	Positive	Pass
Dilution 2: 1.0×10^8 CFU/mL	Positive	Pass
Dilution 3: 2.6×10^7 CFU/mL	Positive	Pass
Dilution 4: 6.5×10^6 CFU/mL	Positive	Pass
Dilution 5: 1.6×10^6 CFU/mL	Positive	Pass
Dilution 6: 4.1×10^5 CFU/mL	Negative	Pass*

*A negative result is not unexpected as Dilution 6 was at a concentration below the claimed limit of detection.

L. pneumophila Philadelphia and Bellingham strains did not produce a high-dose hook effect when tested at high concentrations with the TRU Legionella assay.

d. *Detection limit:*

The analytical limit of detection (LoD) was determined for *L. pneumophila* Philadelphia and Bellingham strains diluted in a negative human urine matrix. The *L. pneumophila* antigen was serially diluted two-fold and 45 replicates of each dilution were tested to determine the LoD in the TRU Legionella assay.

Strain ID	Serogroup	Subgroup	Limit of Detection (LoD) CFU/mL
<i>L. pneumophila</i> Philadelphia strain	1	Pontiac	3.76 x 10 ⁵
<i>L. pneumophila</i> Bellingham strain	1	Non-Pontiac	5.2 x 10 ⁵

e. *Analytical specificity:*

Analytical specificity was demonstrated by testing the following potentially cross-reactive bacteria, fungi, and viruses: *Acaligenes faecalis*, *Bacillus cereus*, *Bacillus subtilis*, *Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Citrobacter freundii*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Enterococcus faecalis* (Group D Streptococcus), *Enterococcus faecium*, *Escherichia coli*, *Haemophilus influenza*, *Klebsiella pneumoniae*, *Legionella bozemanii*, *Legionella dumoffii*, *Legionella feelii*, *Legionella gormanii*, *Legionella longbeachae*, *Legionella micdadei*, *Legionella pneumophila* serogroup 2, *Legionella pneumophila* serogroup 3, *Legionella pneumophila* serogroup 4, *Legionella pneumophila* serogroup 5, *Legionella pneumophila* serogroup 6, *Morganella morganii*, *Moraxella osloensis*, *Mycoplasma pneumoniae*, *Nocardia asteroides*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Serratia liquefaciens*, *Serratia marcescens*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Streptococcus pyogenes* (Group A), *Streptococcus agalactiae* (Group B), *Streptococcus anginosus* (Group F), *Streptococcus dysgalactiae equisimilis* (Group G), *Streptococcus pneumoniae*, Adenovirus, Coxsackievirus, Influenza A, Influenza B, Parainfluenza virus, Respiratory Syncytial Virus A, and Respiratory Syncytial Virus B.

Bacteria, fungi (at a final concentrations of 1.2 x 10⁸ CFU/ml), or viruses (at concentrations greater than 1 x 10⁵ TCID₅₀/ml) were added to negative urine samples or to urine samples spiked with *L. pneumophila* serogroup 1 at its limit of detection. None of the tested microorganisms cross-reacted with the TRU Legionella assay.

Analytical reactivity

TRU Legionella was tested for its reactivity with Pontiac and non-Pontiac strains of *L. pneumophila*. Aliquots of five Pontiac and four non-Pontiac strains of *L. pneumophila* were adjusted to visually match a MacFarland Standard 4.0 (approximately 1.2 x 10⁹ CFU/mL) and then spiked into a natural negative urine matrix to a final concentration of 1.2 x 10⁶ CFU/mL. Three non-Pontiac strains did not react at the concentration of 1.2 x 10⁶ CFU/mL and were retested at a higher concentration of 4.8 x 10⁶ CFU/mL.

Analytical reactivity of *L. pneumophila* strains at 1.2×10^6 CFU/mL

<i>L. pneumophila</i> strain	Source	Designation	CFU/mL	Test Sample	Result
Positive Control	N/A	N/A	N/A	Positive	Pass
Negative Control	N/A	N/A	N/A	Negative	Pass
Dilution Control	N/A	N/A	N/A	Negative	Pass
13395	CCUG	Pontiac	1.2×10^6	Positive	Pass
12024, Allentown 1	NCTC	Pontiac	1.2×10^6	Positive	Pass
12006, Benidorm	NCTC	Pontiac	1.2×10^6	Positive	Pass
12008, OLDA	NCTC	Non-Pontiac	1.2×10^6	Positive	Pass
33058, Knoxville	CCUG	Pontiac	1.2×10^6	Positive	Pass
12007, France	NCTC	Pontiac	1.2×10^6	Positive	Pass
12098, Camperdown	NCTC	Non-Pontiac	1.2×10^6	Negative	Fail*
12025, Heysham	NCTC	Non-Pontiac	1.2×10^6	Negative	Fail*
12009, Oxford	NCTC	Non-Pontiac	1.2×10^6	Negative	Fail*

*Analytical reactivity of *L. pneumophila* strains at 4.8×10^6 CFU/mL

<i>L. pneumophila</i> strain	Source	Designation	CFU/mL	Test Sample	Result
Positive Control	N/A	N/A	N/A	Positive	Pass
Negative Control	N/A	N/A	N/A	Negative	Pass
Dilution Control	N/A	N/A	N/A	Negative	Pass
12098, Camperdown	NCTC	Non-Pontiac	4.8×10^6	Positive	Pass
12025, Heysham	NCTC	Non-Pontiac	4.8×10^6	Positive	Pass
12009, Oxford	NCTC	Non-Pontiac	4.8×10^6	Positive	Pass

Results indicate that TRU Legionella will detect Pontiac and non-Pontiac strains.

f. Assay cut-off:

Not applicable

g. Interfering substances:

The following substances were tested for interference with the TRU Legionella assay:

Amphotericin B (0.22 mg/mL), Antihistamine (0.22 mg/mL), Ascorbic acid (1.0 mg/mL), Beet root (0.01%), Bilirubin (0.2 mg/mL), Boric acid (2.63 mg/mL), Caffeine, purified (0.4%), Chlorophyll (0.81 mg/mL), Ciprofloxacin (0.22 mg/mL), Cold and flu tablets (50 mg/ml), Cough drops (0.22 mg/mL), Cough syrup (0.20 mg/mL), Decongestant (0.22 mg/mL), Erythromycin (0.067 mg/mL), Glucose (20 mg/mL), Itraconazole (0.22 mg/mL), Miconazole (5%), Oxalic acid (0.01%), Prednisone (0.22 mg/mL), Protein (BSA) (5 mg/mL), Rifampicin (0.09 mg/mL), Tobacco, purified (0.4%), Urea (20 mg/mL), Vaginal contraceptive gel with nonoxynol-9, 4% (5%), Water-

based personal lubricant (KY Jelly) (5%), White blood cells (10%), Whole blood (10%).

The substances were added to three negative samples and three samples spiked with *L. pneumophila* antigen at the limit of detection. These substances did not interfere with the results of the TRU Legionella assay.

2. Comparison studies:

a. *Method comparison with predicate device:*

Clinical performance of the TRU Legionella assay was demonstrated by comparison to the predicate Binax NOW® *Legionella* assay. Results were reported as percent agreement between the two assays. The method comparison study was performed independently at three sites in the US and one site in The Netherlands using three lots of TRU Legionella. 123 prospective and 227 retrospective human urine specimens were tested from patients with symptoms of pneumonia or for whom *Legionella* testing had been ordered. Retrospective samples were randomized and masked prior to testing.

Percent agreement of TRU Legionella to Predicate Assay for Retrospective and Prospective Specimens from Patients >21 years of age.

TRU Legionella	Predicate Assay		
	Positive	Negative	Total
Positive	106	0	106
Negative	5	239	244
Total	111	239	350
			95% CI
Positive Agreement	106/111	95.5%	89.9 – 98.1%
Negative Agreement	239/239	100.0%	98.4 – 100.0%

Percent agreement of TRU Legionella to Predicate Assay for Retrospective Specimens from Patients >21 years of age

TRU Legionella	Predicate Assay		
	Positive	Negative	Total
Positive	101	0	101
Negative	5	121	126
Total	106	121	227
			95% CI
Positive Agreement	101/106	95.3%	89.4 – 98.0%
Negative Agreement	121/121	100.0%	96.9 – 100.0%

Percent agreement of TRU Legionella to Predicate Assay for Prospective Specimens from Patients >21 years of age

TRU Legionella	Predicate Assay		
	Positive	Negative	Total
Positive	5	0	5
Negative	0	118	118
Total	5	118	123
			95% CI
Positive Agreement	5/5	100.0%	56.6 – 100.0%
Negative Agreement	118/118	100.0%	96.8 – 100.0%

b. *Matrix comparison:*

Not applicable

3. Clinical studies:

a. *Clinical Sensitivity:*

Not applicable

b. *Clinical specificity:*

Not applicable

c. Other clinical supportive data (when a. and b. are not applicable):

See method comparison with predicate device.

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

Legionella pneumophila serogroup 1 is an important cause of travel, community and hospital acquired pneumonia worldwide. Legionnaires' disease (LD) is known to occur sporadically and in outbreak settings. Recent data indicates that 0.5-5.0% of adults hospitalized for pneumonia has LD. Approximately 8,000-18,000 cases of LD occur each year in the USA. With this assay, 111 positive samples were tested, 5 were misidentified as negative; and 239 negative samples were tested, all were correctly identified.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.