TRU LEGIONELLA®

(US Patent No. US D560,281S; US D561,344S; US 744,9775 B2)

A Rapid Immunoassay for the Detection of *Legionella pneumophila* Serogroup 1 Antigens in Human Urine Specimens

REF **751930**

IVD

Rx Only

INTENDED USE
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.

SUMMARY AND EXPLANATION OF THE TEST
The genus *Legionella* was first described in 1976 after an outbreak of severe pneumonia at an American Legion Convention in Philadelphia.[3] Since then over 50 species within the *Legionella* genus have been described, with over 24 species associated with human disease.[1] Human disease caused by *Legionella* can range from severe pneumonia (Legionnaires' disease or LD) to a milder influenza-like syndrome (Pontiac Fever). *Legionella* spp. are important causes of community-acquired, nosocomial and travelers-associated disease and can occur both sporadically and in outbreak settings. Over 90% of the cases of LD are attributed to *Legionella pneumophila* serogroup 1 when the organism is isolated in culture.[2] The most common reservoirs for human infection with *Legionella* are man-made water systems. Person to person transmission of *Legionella* has never been demonstrated.[1] The pneumonia associated with LD cannot readily be distinguished from pneumonia caused by other microorganisms based solely on clinical parameters, hence clinicians rely on laboratory tests for the detection of LD. Laboratory tests available for the detection of LD include bacterial culture, DFA, serology and the urine antigen assay. Urine antigen tests are highly specific, moderately sensitive, rapid and can direct antibiotic therapy. They have become the most common assay used for the detection of LD.[4]

BIOLOGICAL PRINCIPLES
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 and polyclonal donkey anti-chicken IgY control 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 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.

REAGENTS/MATERIALS PROVIDED
The maximum number of tests obtained from this test kit is listed on the outer box.
1. **Test Strip:** A test strip attached to a plastic holder enclosed in a foil pouch with desiccant. The test strip carries anti-*Legionella pneumophila* serogroup 1 capture antibody for the test line. The holder is used to stopper the Conjugate Tube. The strip frame portion of the holder indicates where test and control lines should appear. Store the pouch at 2-27 C when not in use. Do not use a device if the desiccant indicator (line in center of desiccant) has changed from blue to pink.
2. **Conjugate Tube:** A capped plastic tube containing a conjugate bead. The tube is enclosed in a foil pouch. The conjugate consists of gold-conjugated anti-*Legionella pneumophila* serogroup 1, which serves as the detector antibody and polyclonal donkey anti-chicken IgY control detector antibody. Store the foil pouch at 2-27 C when not in use. Do not store in the freezer. Do not remove the cap until just before use.
3. **Sample Diluent/Negative Control:** A buffered protein solution provided in a plastic vial. Sodium azide (0.094%) added as a preservative. Use as supplied. Store at 2-27 C when not in use.
4. **Positive Control:** Inactivated *Legionella pneumophila* serogroup 1 in a buffered diluent containing sodium azide (0.094%) as a preservative. The reagent is supplied ready to use. Store at 2-27 C when not in use.
5. **Plastic transfer pipettes** (with 100, 200 and 300 µL volume marks).

MATERIALS NOT PROVIDED
1. Disposable latex gloves (urine samples are considered potentially hazardous biological material.)
2. Vortex for suspending the specimen in the Sample Diluent (optional)
3. Interval timer
4. Marking pen
5. Optional: Urine preservative containing boric acid, such as the BD Vacutainer® C&S Preservative Plus Urine Tube

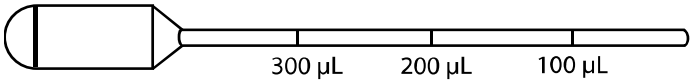
PRECAUTIONS
1. All reagents are for in vitro diagnostic use only.
2. DO NOT interchange the Test Strips, Conjugate Tubes and Positive Control reagents from different kit lot numbers. The Sample Diluent/Negative Control can be interchanged between kits providing the reagents are within their expiration periods. Do not use any reagent beyond its labeled expiration date.
3. Do not use components that lack a label, lot number, or an expiration date.
4. Do not use any reagent if it is discolored or turbid. Discoloration or turbidity may be a sign of microbial contamination.
5. Allow reagents to warm to 19-27 C before use.
6. All reagents should be mixed gently before use.
7. The Positive Control reagent vial should be held vertically when dispensing drops to ensure consistent drop size and delivery.
8. The transfer pipettes provided with the kit must be used for specimen preparation and transfer. Use one per specimen.
9. Any deviation below or above set incubation times may affect sensitivity and specificity and should be avoided.
10. Test Strips and Conjugate Tubes are packaged in foil pouches that exclude moisture during storage. Inspect each foil pouch before use. Do not use Test Strips or Conjugate Tubes in pouches that have holes or where the pouch has not been completely sealed. Do not use the Test Strip if the desiccant indicator has changed from blue to pink. The change in the desiccant color is an indicator the Test Device has been exposed to moisture. False-negative reactions may result if Test Strips or Conjugate Tubes are exposed to moisture.
11. All urine samples must be mixed thoroughly before testing to ensure a representative sample prior to pipetting.
12. *Legionella pneumophila* serogroup 1 antigens may be relatively unstable. Care should be taken to store samples as indicated in this procedure. Even when samples are stored in the frozen state, the rate at which antigen deterioration occurs varies from sample to sample and cannot be predicted.
13. Sample Diluent/Negative Control must be added to the Conjugate Tube within one minute after removing the cap from the tube.

WARNINGS
1. Patient specimens may contain infectious agents and should be handled and disposed of as potential biohazards.
2. Dispose of all used test materials in an appropriate container. Treat waste as a potential biohazard.
3. Positive Control contains inactivated *Legionella pneumophila* serogroup 1 antigen but should be handled as if it were potentially infectious. This reagent contains 0.094% sodium azide. Sodium azide is a skin irritant. Avoid skin contact. Disposal of reagents containing sodium azide into drains consisting of lead or copper plumbing can result in the formation of explosive metal oxides. Eliminate build-up of oxides by flushing drains with large volumes of water during disposal.

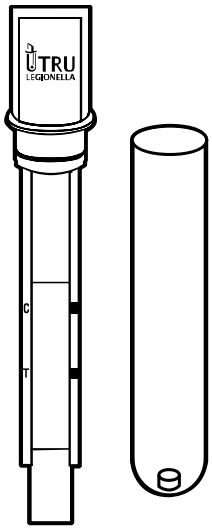
HAZARDS and PRECAUTIONARY STATEMENTS
There are no known hazards associated with this product.

SHELF LIFE AND STORAGE
The kit expiration date is indicated on the kit label. Store the kit at 2-27 C.

PROCEDURAL NOTES
The TRU LEGIONELLA transfer pipette is diagrammed below.



The TRU LEGIONELLA Test Strip and Conjugate Tube are diagrammed below.



SPECIMEN COLLECTION AND PREPARATION
Human urine samples, unpreserved: Samples should be received in an airtight transport container and stored at 2-8 C prior to testing. Samples should be tested as soon as possible, but may be held up to seven days at 2-8 C. Samples that will not be tested within seven days should be frozen immediately upon receipt and stored at ≤ -20 C until tested. Samples may be frozen and thawed up to two times after storage at ≤ -20 C prior to testing.

Human urine samples, boric acid preserved: Samples should be received in an airtight Boric Acid Urine Tube and stored at 2-8 C prior to testing. Samples should be tested as soon as possible, but may be held up to seven days at 2-8 C. (See SPECIMEN PREPARATION section for instructions on boric acid preserved urine.) Samples that will not be tested within seven days should be frozen immediately upon receipt and stored at ≤ -20 C until tested. Samples may be frozen and thawed up to two times after storage at ≤ -20 C prior to testing.

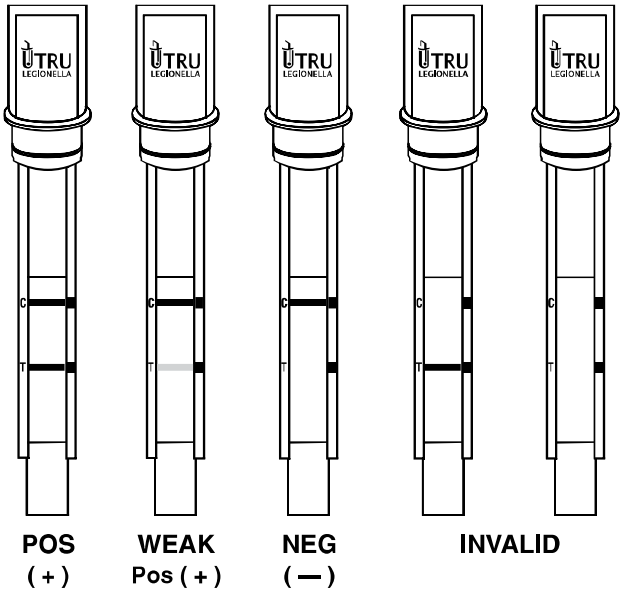
SPECIMEN PREPARATION
Mix urine thoroughly prior to pipetting. Bring specimens and reagents to room temperature (19-27 C) before testing.

1. **Human urine samples, unpreserved and preserved:**
   a. Remove one Conjugate Tube from its foil pouch and discard the pouch. Label the tube with the patient's name.
   b. Remove the cap from the Conjugate Tube and discard the cap.
   c. Using a transfer pipette supplied with the kit, immediately add 100 µL (first mark from the tip of the pipette) of Sample Diluent/Negative Control to the Conjugate Tube. Dispense directly into the center of the tube. Vortex or swirl the contents of the Conjugate Tube for 10 seconds.*
   d. Mix patient sample thoroughly. Use one of the transfer pipettes supplied with the kit to mix the sample gently but thoroughly by squeezing the pipette bulb three times. Alternatively, mix for at least 10 seconds using a vortex mixer.
   e. Using the same pipette, draw 100 µL of specimen (first mark from the end of the pipette) and add it to the Conjugate Tube.
   f. Using the same pipette, mix the sample and conjugate thoroughly but gently by squeezing the pipette bulb three times. Alternatively, mix for at least 10 seconds using a vortex mixer. Discard the pipette.
   ***Warning: Dilution errors may affect test performance. Failure to add sufficient urine sample to the Sample Diluent/Negative Control may result in falsely negative tests. Failure to add the full amount of Sample Diluent/Negative Control may result in falsely positive tests. Addition of too much sample may result in invalid test results due to the inhibition of proper sample flow.**

TEST PROCEDURE
1. Remove the Test Strip from its foil pouch and discard the pouch. Visually inspect Test Strip. Do not use Test Strip if a green line is present.
2. Insert the narrow end of the Test Strip into the Conjugate Tube and firmly press down on the cap to close the tube.
3. Incubate at 19-27 C in an upright position in a rack for 20 minutes.
4. Read the results on the test strip within 1 minute. Do not read results beyond this period. (NOTE: Remove the Test Strip from the Conjugate Tube if Test or Control Lines are difficult to read. Recap the Conjugate Tube with the Test Strip holder and discard when testing is completed.)

EXTERNAL CONTROL TESTS
1. Bring all test components, reagents and samples to room temperature (19-27 C) before testing.
2. Use 1 Conjugate Tube and 1 Test Strip for Positive Control testing and 1 Conjugate Tube and 1 Test Strip for Sample Diluent/Negative Control testing.
3. Remove the Conjugate Tubes from their foil pouches and label accordingly. Discard the pouches.
4. Remove the caps from the Conjugate Tubes and discard the caps.
5. Add exactly 5 drops of the Positive Control reagent to the Conjugate Tube marked for the Positive Control. The drops should be dispensed directly into the center of the tube.
6. Using 1 of the transfer pipettes supplied with the kit, add 200 µL (second mark from the end of the pipette tip) of Sample Diluent/Negative Control to the Conjugate Tube marked for the Negative Control. Add directly to the center of the tube.
7. Vortex or swirl the contents of the tubes for 10 seconds.
8. Remove 2 Test Strips from their foil pouches and discard the pouches.
9. Insert the narrow end of a Test Strip to each Conjugate Tube and firmly press down on the caps to close each tube.
10. Incubate both tubes at 19-27 C for 20 minutes.
11. Read the results on the test strip within 1 minute. Do not read results beyond this period. (NOTE: Remove the Test Strip from the Conjugate Tube if test or control lines are difficult to read. Recap the Conjugate Tube with the Test Strip holder and discard when testing is completed.)

INTERPRETATION OF RESULTS



Negative test: A PINK-RED band at the Control Line position. No other bands are present.
Positive test: PINK-RED band of any color intensity at the Control and *Legionella* Test Line positions. The color of the Test Line can be lighter than that of the Control Line. Test Lines may appear strongly visible or may appear less strongly visible.
Invalid test results:
1. No band at the designated position for the Control Line. The test is invalid since the absence of a control band indicates the test procedure was performed improperly.
2. A PINK-RED band appearing at the Test Line position of the device after 21 minutes of incubation, or a band of any color other than PINK-RED. Falsely positive results may occur if tests are incubated too long. Bands with colors other than PINK-RED may indicate reagent deterioration.

If any result is difficult to interpret, the test should be repeated with the same sample to eliminate the potential for error. Obtain a new sample and retest when the original sample repeatedly produces unreadable results.

QUALITY CONTROL
This test should be performed per applicable local, state, or federal regulations or accrediting agencies.
At the time of each use, kit components should be visually examined for obvious signs of microbial contamination, freezing or leakage. Do not use contaminated or suspect reagents.

Internal procedural controls: Internal procedural controls are contained within the Test Strip and therefore are evaluated with each test.
1. A PINK-RED band appearing at the Control Line serves as a procedural control indicates the test has been performed correctly, and that proper flow occurred.
2. A clean background around the Control or Test Lines also serves as a procedural control. Control or Test Lines that are obscured by heavy background color may invalidate the test and may be an indication of reagent deterioration, use of an inappropriate sample or improper test performance.

External Control reagents should be tested according to the requirements of the laboratory or applicable local, state or accrediting agencies.
1. See section EXTERNAL CONTROL TESTS for instructions on performing these control tests.
2. The reactivity of each new lot and each new shipment of TRU LEGIONELLA should be verified on receipt using external Positive and Negative Control reagents. The number of additional tests performed with external controls will be determined by the requirements of local, state or federal regulations or accrediting agencies.
3. The external controls are used to monitor reagent reactivity. Failure of the controls to produce the expected results can mean that one of the reagents or components is no longer reactive at the time of use, the test was not performed correctly, or that reagents or samples were not added. If the Positive or Negative External Controls fail, do not report test results to the clinician.
4. The results expected with the Controls are described in the section on INTERPRETATION OF RESULTS.
5. The kit should not be used if control tests do not produce the correct results. **If the expected control reactions are not observed, repeat the control tests as the first step in determining the root cause of the failure. If the control failures are repeated please contact Meridian's Technical Services Department at 1-800-343-3858 (US) or your local distributor.**
6. Positive and Negative Control reagents manufactured for this assay are prepared in the matrix of the Sample Diluent/Negative Control, which may not mimic test specimens. If control materials that are identical in composition to test specimens are preferred, the user can prepare those by diluting known positive and negative specimens in Sample Diluent/ Negative Control according to the SPECIMEN PREPARATION section of this insert.
   Note: The positive and negative controls reagents (Cat. No. 7949 and 7950) provided with the TRU LEGIONELLA kit have been internally validated. The manufacturer is not responsible for results obtained using this device with any other not validated control.

EXPECTED VALUES
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.[2, 3]

LIMITATIONS OF THE PROCEDURE
1. The test is qualitative and no quantitative interpretation should be made with respect to the intensity of the positive Test Line when reporting the result.
2. TRU LEGIONELLA cannot be used as the sole means of determining *Legionella pneumophila* infection. Test results must be used in conjunction with information available from the patient clinical evaluation and other diagnostic procedures.
3. Overincubation of tests may lead to an increase in false-positive test results. Conversely, incubation for periods less than those defined in this insert can result in an increase in false-negative tests. Follow incubation times defined in this insert.
4. TRU LEGIONELLA detects both viable and non-viable *Legionella pneumophila* serogroup 1. The appearance of TRU LEGIONELLA positive tests depends on the antigen load in the specimen; therefore a TRU LEGIONELLA positive test may not always correlate with the results of bacterial culture.
5. The antibodies used in the test may not detect all antigenic variants or new strains of *Legionella pneumophila* serogroup 1.
6. A negative test result does not exclude infection with *Legionella pneumophila* serogroup 1 nor does it rule out other microbial-caused respiratory infections or disease caused by serogroups of *Legionella pneumophila* other than serogroup 1. A positive test result does not rule out coinfection with other microbes.
7. The TRU LEGIONELLA assay is prescription use only.
8. The TRU LEGIONELLA is intended for use with urine specimens. Use of this assay is not recommended with other specimens.
9. A positive results implies the presence of antigen to *Legionella pneumophila* serotype 1, however, all test results should be reviewed in light of other clinical data by the physician.
10. The TRU LEGIONELLA assay is not intended for monitoring therapy.
11. Performance characteristics are not established for patients younger than 21 years of age.
12. The performance of the TRU LEGIONELLA assay has only been established for manual reading and/or visual result determination.

SPECIFIC PERFORMANCE CHARACTERISTICS

Performance characteristics for the TRU LEGIONELLA assay were established in 2011. A total of 350 qualified patient samples were evaluated; 227 specimens were retrospective frozen samples and 123 specimens were prospective samples. One hundred and forty two (142) retrospective frozen samples were from a well-characterized specimen panel, collected from patients with confirmed Legionnaires Disease and previously confirmed non-*Legionella* cases; the remaining retrospective samples were from patient samples that had been previously submitted for *Legionella* testing. Samples were collected from males (63.4%) and females (36.6%). No differences in test performance were observed based on gender. Specimens were collected from patients with an age range from 23 years to 96 years. Table 1 compares the performance of the TRU LEGIONELLA assay for all specimens to the predicate assay. Table 2 compares the performance of the TRU LEGIONELLA assay to the predicate assay for retrospective specimens only. Table 3 compares the performance of the TRU LEGIONELLA assay to the predicate assay for prospective specimens only.

Table 1: Percent agreement of TRU LEGIONELLA to Predicate Assay for Retrospective and Prospective Specimens from Patients > 21 years of age

	Predicate Assay		
TRU LEGIONELLA	**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%
Overall Agreement	345/350	98.8%	97.3 – 99.5%

Table 2: Percent agreement of TRU LEGIONELLA to Predicate Assay for Retrospective Specimens from Patients > 21 years of age

	Predicate Assay		
TRU LEGIONELLA	**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%
Overall Agreement	222/227	97.8%	94.9 – 99.1%

Table 3: Percent agreement of TRU LEGIONELLA to Predicate Assay for Prospective Specimens from Patients > 21 years of age

	Predicate Assay		
TRU LEGIONELLA	**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%
Overall Agreement	123/123	100.0%	97.0 – 100.0%

REPRODUCIBILITY

Assay precision, intra-assay variability, and inter-assay variability were assessed with a reference panel prepared from pools of negative samples spiked with *L. pneumophila* antigen. The reproducibility panel consisted of moderately positive (n=3), low positive (n=3), high negative (n=3), and negative (n=1) specimens. The low positive and high negative specimens were prepared near the assay cutoff. Each sample was evaluated twice per day for five days by three different laboratories. Reproducibility was 100% with no inter-assay or intra-assay variability for samples prepared above or below the limit of detection for this assay.

CROSSREACTIVITY STUDIES

Crossreactivity studies were performed with positive and negative urine specimens inoculated with bacterial or fungal organisms to a final concentration of 1.2×10^8 CFU/mL or viral concentration greater than 1×10^5 $TCID_{50}$/mL. None of the following organisms in urine reacted with TRU LEGIONELLA:

Alcaligenes faecalis, Bacillus cereus, Bacillus subtilis, Candida albicans, Candida glabrata, Candida parapsilosis, Citrobacter freundii, Enterobacter aerogenes, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae, Klebsiella pneumoniae, Legionella bozemanii, Legionella dumoffii, Legionella feeleii, Legionella gormanii, Legionella longbeachae, Legionella micdadei, Legionella pneumophila serogroup 2 Togus, *Legionella pneumophila* serogroup 3 Bloomington, *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* groups A, B, D, F, G, *Streptococcus pneumoniae*, Adenovirus, Coxsackievirus, Influenza A, Influenza B, Parainfluenza Virus, Respiratory Syncytial Virus A, Respiratory Syncytial Virus B.

ASSAY SPECIFICITY

The following *L. pneumophila* stock cultures from different sources were tested and produced positive reactions at a minimum concentration of 4.8×10^6 CFU/mL with the TRU LEGIONELLA assay:

Pontiac subgroup: CCUG 13395; NCTC 12024, Allentown 1; NCTC 12006, Benidorm; CCUG 33058, Knoxville; NCTC 12007, France.

Non-Pontiac subgroup: NCTC 12008, OLDA; NCTC 12098, Camperdown; NCTC 12025, Heysham; NCTC 12009, Oxford.

ANALYTICAL SENSITIVITY

The analytical sensitivity of the TRU LEGIONELLA assay was assessed using two strains of *L. pneumophila*. The organisms tested and the analytical sensitivities are listed in the table below.

Strain ID	Limit of Detection (LoD)
Philadelphia strain (Pontiac subgroup; ATCC 33152)	3.76×10^5 CFU/mL
Bellingham strain (non-Pontiac subgroup; NCTC 11404)	5.2×10^5 CFU/mL

TESTS FOR INTERFERING SUBSTANCES

The following substances, at the specified saturated solvent/diluent concentrations, do not interfere with the test results in the final concentrations listed:

Amphotericin B (0.22 mg/mL), Antihistamine [Diphenhydramine HCl (0.22 mg/mL)], Ascorbic acid (1.0 mg/mL), Beet Juice Powder (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/flu tablets [Acetaminophen (50 mg/mL), Chlorpheniramine maleate (0.2 mg/mL), Phenylephrine hydrochloride (0.5 mg/mL)], Cough drops [Menthol (0.22 mg/mL)], Cough syrup [Dextromethorphan HBr (0.20 mg/mL), Guaifenesin (2.0 mg/mL)], Decongestant [Phenylephrine HCl (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)], Pseudoephedrine [Pseudoephedrine HCl (0.01 mg/mL)], Rifampicin (0.09 mg/mL), Tobacco (0.4%), Urea (20 mg/mL), Vaginal contraceptive gel containing 4% Nonoxynol-9 (5% v/v), Water-based personal lubricant (K-Y®) (5% v/v), White blood cells (10%), and Whole blood (10%).