DRG® Legionella pneumophila Ag (urine) (EIA-4205)

Revised 1 Feb. 2011 rm (Vers. 4.1) **RUO in the USA**

Please use only the valid version of the package insert provided with the kit.

This kit is intended for Research Use Only.

This kit is not intended for diagnostic purposes.

Intended Use

This Legionella Urinary Antigen test is intended for the detection of Legionella pneumophilia antigen in human urine.

Principle of Procedure

This assay is a double antibody (sandwich) ELISA using an anti-Legionella pneumophilia antibody to capture the antigen from the urine. A second antibody, conjugated to horseradish peroxidase (HRP), is then added which binds to the complex. This reaction is visualized by the addition of the chromogen tetramethylbenzidine (TMB). The resulting blue color development indicates the presence of Legionella pneumophilia antigens being bound by the anti-Legionella pneumophilia antibodies. Addition of a stop solution ends the reaction and turns the blue color to yellow.

Reagents

Item	Description	Symbol
Test Strips	Microwells containing purified anti- *Legionella pneumophilia* antibodies.	MT PLATE
Enzyme Conjugate	One (1) bottle containing 11 ml of anti-*Legionella pneumophilia* antibodies conjugated to horseradish peroxidase with red dye and Thimerosal.	CONJ
Positive Control	One (1) vial containing 2 ml of diluted *Legionella pneumophilia* antigen in buffer with Thimerosal.	CONTROL +
Negative Control	One (1) vial containing 2 ml of buffer with Thimerosal.	CONTROL −
Chromogen	One (1) bottle containing 11 ml of tetramethylbenzidine (TMB) and peroxide.	SUBS TMB
Wash Concentrate (20X)	One (1) bottle containing 25 ml of concentrated buffer with surfactant and Thimerosal.	WASH BUF
Stop Solution	One (1) bottle containing 11 ml of 5% phosphoric acid solution .	SOLN

Warnings/Precautions

- **Do not deviate from the specified procedures when performing this assay.** All specimen dilutions, incubation times/temperatures and washings have been optimized for the best performance characteristics. Deviations from the specified procedures may affect the sensitivity and specificity of the assay.
- Do not interchange reagents between kits with different lot numbers.
- Do not use reagents that are beyond their expiration dates. Expiration dates are on each reagent label. Use of reagents beyond their expiration dates may affect results.
- Unused microwells should be stored in the desiccated pouch to protect them from moisture.
- Do not use solutions if they precipitate or become cloudy.
  Exception: Wash concentrate may precipitate during refrigerated storage, but will dissolve upon warming.
- Do not add azides to the samples or any of the reagents.
- Controls and some reagents contain thimerosal as a preservative, which may be irritating to skin, eyes and mucous membranes. In case of contact, flush eyes or rinse skin with copious amounts of water.
- Treat all reagents and samples as potentially infectious materials. Use care to prevent aerosols and decontaminate any spills of samples.
- Stop solution is a 5% solution of phosphoric acid in water. If spilled on the skin, wash with copious amounts of water. If acid gets into the eyes, wash with copious amounts of water and seek medical attention.
- Persons who are color blind or visually impaired may not be able to read the test visually and should use spectrophotometric readings to interpret results.

Storage Conditions

Reagents, strips and bottled components should be stored at 2-8 °C.
Squeeze bottle containing diluted wash buffer may be stored at room temperature (15-25 °C).

Preparation

Before use, bring all reagents and samples to room temperature (15-25 °C) and mix.

(20X) Wash Concentrate may precipitate during refrigerated storage, but will go back into solution when brought to room temperature (15-25°C) and mixed. **Ensure that (20X) wash concentrate is completely in solution before diluting to working concentration**.
To dilute (20X) wash concentrate to working dilution, remove cap and add contents of one bottle of Wash Concentrate to a squeeze bottle containing 475 ml of DI water. Swirl to mix. Squeeze bottle should have a narrow tip to optimize washings.

Specimen Collection and Handling

Urine specimens should be collected in standard sterile containers, stored at room temperature or refrigerated (2-8 °C) and assayed within 24 hours of collection.
Alternatively, specimens may be stored at 2-8 °C for up to 14 days or frozen (-20 °C) for longer periods before testing.

Whenever possible, urine specimens should be shipped at 2-8 °C or frozen
Urine specimens containing excess urates, phosphates, or other dissolved salts may develop salt crystals when stored from 2-8 °C or lower. Ensure all samples are at room temperature before performing the assay.

Procedure

Materials Provided

Legionella Urinary Antigen Detection Microwell ELISA Kit (see reagents in chapter 4)

Materials Required But Not Provided

- Squeeze bottle for washing strips (narrow tip is recommended) or microplate washer
- Distilled or deionized grade water and graduated cylinder
- Transfer Pipettes
- Absorbent paper towel

Suggested Equipment

ELISA plate reader capable of reading bichromatically at 450/620-650 nm.

Proper Temperature

All incubations are at room temperature (15 °C to 25 °C)

Test Procedure

Notes:

- Ensure all samples and reagents are at room temperature (15-25 °C) before use. Frozen samples must be thawed completely before use.
- When running the assay, try to avoid the formation of bubbles in the wells. Bubbles may affect overall performance and reading of end results. Slapping the wells out on a clean absorbent towel after each wash step should help to minimize bubbles in the wells.
- Controls must be included each time the kit is run. Controls are provided prediluted. DO NOT dilute further.

- Break off the required number of wells needed (number of samples plus 2 for controls) and place in well holder. Return all unused strips to the pouch and reseal the zip lock closure.
- Add **100 µl** of negative control to well # 1
- Add **100 µl** of positive control to well # 2.
- Add **100 µl** of the urine samples to each test well.
- Incubate for **30 minutes** at room temperature (15-25 °C), then wash.* After last wash, slap the wells out on a clean absorbent towel to remove excess wash buffer.
- Add **2 drops** of Conjugate to each well.
- Incubate for **10 minutes**, then wash*. After last wash, slap the wells out on a clean absorbent towel to remove excess wash buffer.
- Add **2 drops** of Chromogen to each well. Incubate for **5 minutes.** DO NOT WASH after this step.
- Add **2 drops** of Stop Solution to each well. Mix wells by gently tapping the sides of the plate with index finger.
- Read results visually or using an ELISA plate reader (see instructions below).

**** Washings consist of vigorously filling each well to overflowing and decanting contents three (3) separate times. When possible, avoid formation of bubbles in the wells as this may affect the end results.***

References

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