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Rapid-VIDITEST *Legionella* Card

IVD

One step Legionella Card test for the detection of Legionella pneumophila antigen from urine specimen.

Instruction manual

Producer: VIDIA spol. s r.o., Nad Safinou II 365, Vestec, 252 42 Jesenice, Czech Republic,
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INTENDED USE:

An *in vitro* rapid immunochromatographic assay for the qualitative detection of *Legionella pneumophila* serogroup 1 antigen (*L. pneumophila* serogroup 1 antigen) in urine specimens from patients with symptoms of pneumonia. It is intended to aid in the presumptive diagnosis of *Legionella* infection (Legionnaires' Disease) caused by *L. pneumophila* serogroup 1 in conjunction with culture and other methods.

INTRODUCTION:

Legionnaires' disease is a serious form of pneumonia that carries with it a mortality rate in the order of 10-15% in otherwise healthy individuals. Symptoms include a flu-like illness, followed by a dry cough and frequently progress to pneumonia. Approximately 30% of people infected may also present with diarrhoea and vomiting and around 50% may show signs of mental confusion. The incubation period normally ranges from 2-10 days with 3-6 days the typical illness onset time after exposure. Legionnaires' disease may present as an outbreak of two or more cases following a limited temporal and spatial exposure to a single source, as a series of independent cases in an area in which it is highly endemic or as sporadic cases without any obvious temporal or geographical grouping. Outbreaks have occurred repeatedly in buildings such as hotels and hospitals.
The Rapid-VIDITEST *Legionella* Card allows for early diagnosis of *Legionella pneumophila* serogroup 1 infection through detection of a specific soluble antigen present in the urine of patients with Legionnaires' Disease. *Legionella pneumophila* serogroup 1 antigen has been detected in urine as early as three days after the onset of symptoms. The test is rapid, giving a result within 15 minutes, and utilizes a urine specimen which is convenient for collection, transport, and subsequent detection of early, as well as later, stages of disease.

PRINCIPLE:

The Rapid-VIDITEST *Legionella* Card is an immunochromatographic membrane assay to detect *Legionella pneumophila* serogroup 1 soluble antigen in human urine. Anti-*Legionella pneumophila* serogroup 1 antibody, the test line, is adsorbed onto nitrocellulose membrane. Antibodies of the control line were adsorbed onto the same membrane as a second band. Anti-*Legionella pneumophila* serogroup 1 antibodies are conjugated to visualizing particles that are dried onto an inert absorbent support.

During testing the sample is allowed to react with the conjugate which was pre-adsorbed on the strip test. The mixture then moves upward on the membrane by capillary action. As the sample flows through the test membrane, the coloured particles migrate. In the case of a positive result the specific antibodies present on the membrane will capture the coloured conjugate. *L. pneumophila* serogroup 1 urinary antigen captured by immobilized anti- *L. pneumophila* serogroup 1 antibody reacts to bind conjugated antibody. The other immobilized antibodies also captures visualizing conjugate, forming the control line. A positive test result is read visually in 10-15 minutes or less depending on the concentration of antigen present in the urine specimen. A negative *Legionella* Card result, read in 15 minutes, indicates that *L. pneumophila* serogroup 1 antigen was not detected in the urine sample.

The test is interpreted by the presence or absence of visually detectable redish colored lines. A positive result will include the detection of both a test and a control line, while a negative assay will produce only the control line. Failure of the control line to appear, whether the test line is present or not, indicates an invalid assay.

MATERALS PROVIDED:

- Rapid-VIDITEST *Legionella* Card tests
- Instructions for use
- Reagent A
- Positive Control Swab: Inactivated *L. pneumophila* swab + Reagent Control (+) vial + testing tube
- Negative Control Swab: *L. pneumophila* negative swab + Reagent Control (-) vial + testing tube
- Testing tubes or vials 2 ml

MATERIALS REQUIRED BUT NO PROVIDED:

- Specimen collection container
- Disposable gloves
- Timer

SPECIMEN COLLECTION AND PREPARATION:

Urine specimens should be collected in standard containers. The samples can be stored at room temperature (15-30°C/59-86°F) if assayed within 24 hours of collection. Alternatively, specimens may be stored at 2-8°C for up to 14 days or at -10°C to -20°C for longer periods before testing. Boric acid may be used as a preservative.
When necessary, urine specimens should be shipped in leak-proof containers at 2-8°C or frozen. Allow all specimens to equilibrate to room temperature before testing.

PROCEDURES:
Procedure for Patient Samples (and liquid urine controls).
Do not remove Rapid-VIDITEST *Legionella* Card test from pack until test sample has reached room temperature. Bring patient urine and/or liquid urine control(s) to room temperature (15-30ºC/59-86°F).

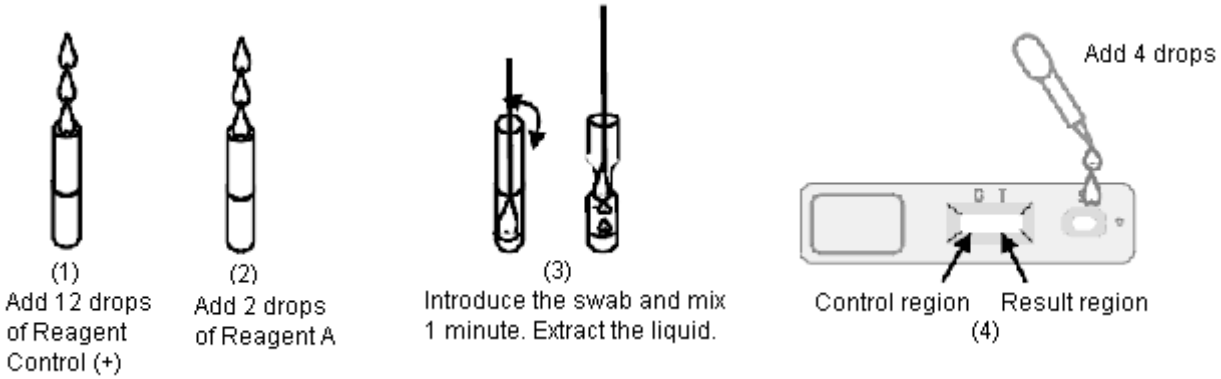
1. Use a separate testing tube or vial for each sample. Add 350 µL (7 drops) of urine sample or liquid urine control (1).
2. Add 50 µL (1 drop) of Reagent A into the testing tube or vial and mix (2).
3. Remove the test from its pack just before use. Use a separate test for each sample.

4. Place the test on a flat surface. Use separate pipette and device for each sample or control. Dispense exactly 4 drops or 100 µL from the testing tube, into the circular window marked with an arrow (4). Start the timer.
5. Read the result at 15 minutes.

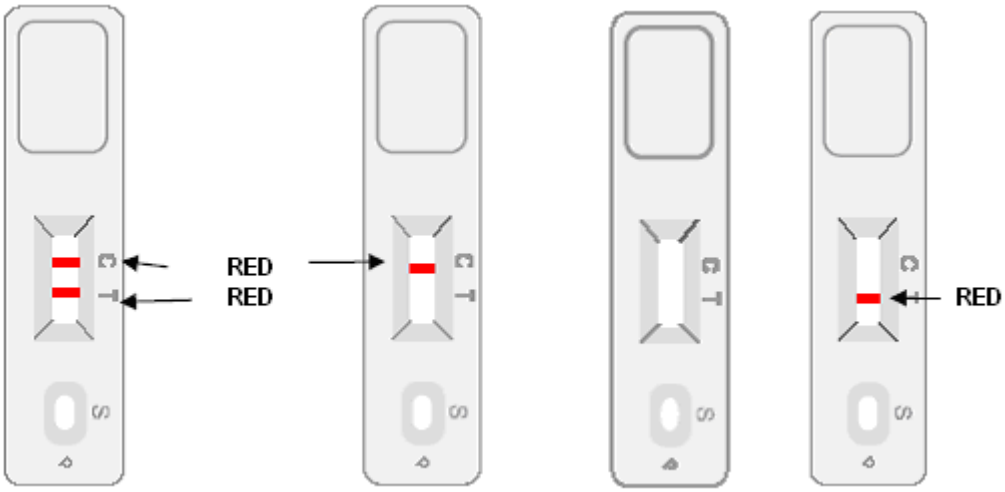
Procedure for Positive and Negative Swab Controls.
Remove Rapid-VIDITEST *Legionella* Card tests from the pack just before use. Run test as follows:

1. Hold Reagent Control (+) vial vertically. Add slowly 12 free falling drops of Reagent Control (+) into the testing tube (1).
2. Add 2 free falling drops of Reagent A (2).
3. Immediately remove swab control from the pouch and put the swab into the testing tube with reagents, mix 1 minute and extract as much liquid possible from the swab, squeezing the sides of the tube as the swab is withdrawn. Discard the swab. (3)
4. Remove the Rapid-VIDITEST *Legionella* Card test from its sealed bag just before using.
5. Place the test on a flat surface. Use a separate pipette and device for each sample or control. Dispense exactly 4 drops or 100 µL from the testing tube, into the circular window marked with and arrow (4). Start the timer.
6. Read the result at 15 minutes.

Repeat the procedure for Negative Swab Control using the Reagent Control (-) instead the Reagent Control (+).



INTERPRETATION OF RESULTS:



POSITIVE ***NEGATIVE*** ***INVALID***

POSITIVE: A positive sample or positive control swab will give two reddish lines across the central window, in the result line region (test line marked with the letter T) and in the control line region (control line marked with the letter C). This means that antigen was detected.

Recommended report: Presumptive positive for *L. pneumophila* serogroup 1 antigen in urine, suggesting current or past infection.

NEGATIVE: A negative sample or negative control swab will give only one reddish band across the control line region marked with the letter C (control line). The control line means that the detection part of the test was done correctly, but no *L. pneumophila* serogroup 1 antigen was detected.

Recommended report: Presumptive negative for *L. pneumophila* serogroup 1 antigen in urine, suggesting no recent or current infection. Infection due to *Legionella* cannot be ruled out since other serogroups and species may cause disease, antigen may not be present in urine in early infection, and the level of antigen present in the urine may be below the detection limit of the test.

INVALID: If no lines are seen, or if just the test line is seen, the assay is invalid. Invalid tests should be repeated. If the problem persists, discontinue using the test kit and contact you local distributor.

QUALITY CONTROL:

Daily Quality Control:

The Rapid-VIDITEST *Legionella* Card contains built-in control features. The manufacturer's recommendation for daily quality control is to document these controls for each sample run.

Positive Procedural Control The reddish line at the Control line region can be considered an internal positive procedural control. If capillary flow has occurred, this line will always appear.

Negative Procedural Control The clearing of background color in the result window provides a negative background control. The background color in the window should be light pink to white within 10-15 minutes and should not interfere with the reading of the test result.

External Positive and Negative Controls:

Good Laboratory Practice recommends the use of positive and negative controls to assure functionality of reagents and proper performance of assay procedure. Positive and negative control swabs that will monitor the entire assay are provided in the kit.

To use liquid urine controls, simply process as you would a patient sample.

Positive and negative controls should be tested once for each new test kit opened and as otherwise required by your laboratory's standard quality control procedures.

LIMITATIONS:

1. The Rapid-VIDITEST *Legionella* Card has been validated using urine samples only. Other samples (e.g. plasma, serum or other body fluids) that may contain *Legionella* antigen have not been evaluated. The test cannot be used on environmental samples (i.e. potable water).
2. This test will not detect infections caused by other *L. pneumophila* serogroups and by other *Legionella* species. A negative antigen result does not exclude infection with *L. pneumophila* serogroup 1. Culture is recommended for suspected pneumonia to detect causative agents other than *L. pneumophila* serogroup 1 and to recover *L. pneumophila* serogroup 1 when antigen is not detected in urine.
3. The diagnosis of Legionnaires' disease cannot be based on clinical or radiological evidence alone. There is no single satisfactory laboratory test for Legionnaires's disease. Therefore, culture results, serology and antigen detection methods should be used in conjunction with clinical findings to make an accurate diagnosis.
4. Excretion of *Legionella* antigen in urine may vary depending on the individual patient. Antigen excretion may begin as early as 3 days after onset of symptoms and persist for up to 1 year afterwards. A positive *Legionella* Card result can occur due to current or past infection and therefore is not definitive for infection without other supporting evidence.

Performance of the Rapid-VIDITEST *Legionella* Card on diuretic urine has not been evaluated. The Rapid-VIDITEST *Legionella* Card has been evaluated on hospitalized patients only. An outpatient population has not been tested.

EXPECTED VALUES:

The disease occurs in both epidemic and endemic forms and sporadic cases are not easily differentiated from other respiratory infections by clinical symptoms. An estimated 25,000 to 100,000 cases of *Legionella* infection occur in the United States annually. The resulting mortality rate, ranging from 25% to 40%, can be lowered if the disease is diagnosed rapidly and appropriate antimicrobial therapy is instituted early.

PERFORMANCE CHARACTERISTICS:

Sensitivity and specificity

The Rapid-VIDITEST *Legionella* Card was highly specific (>99%) and also sensitive (>99%) compared with the results of the Binax NOW® *Legionella* Urinary Antigen Test.
The Rapid-VIDITEST *Legionella* Card test was used to evaluate 63 frozen archived patient urine specimens and 9 fresh specimens from a French hospital. Five of these patients were positive for *Legionella pneumophila* serogroup 1 infection as determined by the Binax NOW® *Legionella* Urinary Antigen Test.
Overall agreement of the Rapid-VIDITEST *Legionella* Card with the Binax NOW® *Legionella* Urinary Antigen Test was >99%. Sensitivity and specificity were each >99%.

STORAGE AND STABILITY:

Store as packaged in the sealed pack either at refrigerated or room temperature (2-30ºC/36-86ºF). The test and reagents are stable until the expiration date printed on their packaging and external kit. Do not use the kit beyond its labelled expiration date.

PRECAUTIONS:

- For professional *in vitro* diagnostic use only.
- Do not use components past its expiration date.
- Do not mix components from different kit lots.
- Solutions used to make the control swabs are inactivated using standard methods. However, patient samples, controls, and Card tests should be handled as though they could transmit disease. Observe established precautions against microbial hazards.

The test should be discarded in a proper biohazard container after testing.

REFERENCES:

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SYMBOLS FOR IVD COMPONENTS AND REAGENTS:

IVD	*In vitro* diagnostic device	LOT	Batch code
	Use by		Manufacturer