VITASSAY

# Legionella

Rapid test for the qualitative detection of *Legionella pneumophila* in human urine samples.

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CE IVD EN

Vitassay

**For professional *in vitro* diagnostic use only.**

## INTENDED USE

**Vitassay Legionella** is a rapid one step immunochromatographic assay for the qualitative detection of *Legionella pneumophila* in human urine samples.

Simple, non-invasive and highly sensitive screening assay to make a presumptive diagnosis of legionelosis in infected humans.

## INTRODUCTION

Legionella species cause 2 clinical syndromes, known as Legionnaires disease and Pontiac fever. Legionnaire disease is an acute, serious, and sometimes lethal pneumonia, whereas Pontiac fever is generally a self-limited, non-pneumonic, influenza-like condition.

*Legionella pneumophila* has been increasingly recognized as a significant cause of sporadic and epidemic community acquired pneumonia in all age groups and in both healthy and immunosuppressed hosts.

## PRINCIPLE

**Vitassay Legionella** is a qualitative immunochromatographic assay for the detection of *Legionella pneumophila (L. pneumophila)* in human urine samples.

The test line zone of the nitrocellulose membrane is pre-coated with polyclonal antibodies against *L. pneumophila.*

During the process, the sample reacts with the antibodies against *L. pneumophila*, forming conjugates. The mixture moves upward on the membrane by capillary action. If the sample is positive, antibodies present on the membrane (test line) capture the conjugate complex and a red line will be visible. Although the sample is positive or negative, the mixture continues to move across the membranes and the blue control line always appears.

The presence of this blue line (in the control zone (C)) indicates that sufficient volume is added; proper flow is obtained and serves as an internal control for the reagents.

## PRECAUTIONS

- For professional *in vitro* use only.
- Do not use after expiration date.
- Do not use the test if its pouch is damaged.
- Specimens should be considered as potentially hazardous and handle in the same manner as an infectious agent. A new test must be used for each sample to avoid contaminations errors. Single use device.
- Tests should be discarded in a proper biohazard container after testing.
- Reagents contain preservatives. Avoid any contact with the skin or mucous membrane. Consult safety data sheet, available on request.
- Components provided in the kit are approved for use with the **Vitassay Legionella**. Do not use any other commercial kit component.
- Follow Good Laboratory Practices, wear protective clothing, use disposal gloves, goggles and mask. Do not eat, drink or smoke in the working area.

## STORAGE AND STABILITY

Store as packaged in the sealed pouch either at refrigerated or room temperature (2-30ºC/36-86ºF).

The test is stable until the expiration date printed on the sealed pouch.

The test must remain in the sealed pouch until use.

Do not freeze.

## MATERIALS

| MATERIAL PROVIDED | MATERIAL REQUIRED BUT NOT PROVIDED |
|---|---|
| ▪ 25 Tests/kits **Vitassay Legionella.**<br>▪ Instructions for use.<br>▪ 25 Plastic pipettes.<br>▪ 25 Testing tubes.<br>▪ Reagent (sample and controls diluent).<br>▪ Positive Control: Inactivated *L. pneumophila* swab + testing tube + pipette.<br>▪ Negative Control: *L. pneumophila* negative swab + testing tube + pipette. | ▪ Specimen collection container.<br>▪ Disposable gloves.<br>▪ Timer. |

## SPECIMEN COLLECTION

Urine specimens should be collected in standard containers. The samples can be stored at room temperature (15-30ºC/59-86ºF) if assayed within 24 hours of collection. Alternatively, specimens may be stored at 2-8ºC (35.6-46.4ºF) for up to 14 days or at -10ºC to -20ºC (14ºF to -4ºF) for longer periods before testing.

When necessary, urine specimens should be shipped in leak-proof containers at 2-8ºC (35.6-46.4ºF) or frozen.



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## SPECIMEN PREPARATION

Allow all specimens to equilibrate to room temperature before testing.

## PROCEDURE

Allow tests, urine samples, reagent and controls to reach room temperature (15-30ºC/59-86ºF) prior to testing.

Do not open pouches until the performance of the assay.

**Patient samples:**

- Use a separate testing tube or vial for each sample. Add 3 drops of urine sample. (figure 1).
- Add 1 drop of Reagent into the testing tube or vial and mix (figure 2). Homogenize the sample.
- Remove the **Vitassay Legionella** from its sealed bag just before using it.
- Use a separate pippette and device for each sample or control. Dispense 3 drops from the testing tube in the circular window marked with the letter S (figure 3).
- Read the results at **15 minutes**. Do not read the test results later than 15 minutes.

1

Add 3 drops of Urine Sample.

2

Add 1 drop of Reagent and mix.

3


Dispense 3 drops in the circular window marked with the letter S.

**Positive and negative Swabs controls:**

- Hold Reagent vertically. Add slowly 13 free falling drops of Reagent into the testing tube (figure 1b).
- Remove the Positive Control swab from the pouch and put the swab into the testing tube with the reagent (figure 2b), 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 (figure 3b).
- Remove the **Vitassay Legionella** from its sealed bag just before using.
- Use a separate pipette and device for each sample or control. Dispense 3 drops from the testing tube, into the circular window marked as the letter S (figure 4b).
- Read the results at **15 minutes**. Do not read the test results later than 15 minutes.

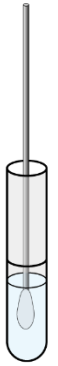
Repeat the procedure for Negative Control swab using the Reagent, same used for sample dilution and for Positive Control swab.

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.

1b

Add 13 drops of Reagent into a testing tube.

2b



Put the swab into the testing tube with Reagent.

3b

Mix and extract as much liquid possible from the swab.

4b


Dispense 3 drops in the circular window marked with the letter S.



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## INTERPRETATION OF THE RESULTS

| | | |
|---|---|---|
|  | **NEGATIVE**<br>Only one blue line in the control zone **(C)** | There is no *Legionella pneumophila* presence. No infection caused by *Legionella pneumophila*.<br>Negative control result. |
|  | **POSITIVE**<br>In addition to the blue line (**control line C**), another red line appears, **test line(T)** | There is presence of *Legionella pneumophila.* Infection caused by *Legionella pneumophila*.<br>Positive control result. |
| **ANY OTHER RESULTS** | | Invalid result, we recommend repeating the assay using the sample with another test. **Note:** Wrong procedural techniques, deterioration of the reagents or insufficient specimen volume are mostly the main reasons for control line failure. If the symptoms or situation persist, discontinue using the test kit and contact your local distributor. |

**Notes:** The intensity of the red colored test line in the result line zone (T) will vary depending on the concentration of antigens in the specimen.

## QUALITY CONTROL

Internal procedural control is included in **Vitassay Legionella**. Blue line appearing in the results window is an internal control, which confirms sufficient specimen volume and correct procedural technique.

External Positive and Negative Controls are included in the kit. The use of positive and negative controls is recommended to assure functionality of reagents and proper performance of assay procedure.

## LIMITATIONS

- **Vitassay Legionella** must be carried out within 2 hours of opening the sealed bag.
- The use of other samples different from human urine samples has not been established.
- Positive results determine the presence of *L. pneumophila* (mainly serogroup 1 but other serogroups could also be detected) in urine samples; nevertheless, a positive result should be followed up with additional laboratory techniques to confirm the results. A confirmed infection should only be made by a physician after all clinical and laboratory findings have been evaluated and must be based in the correlation of the results with further clinical observations.
- Negative results should not be considered as conclusive; it is possible that the concentration of antigens is lower than the detection limit value. If symptoms or situation still persist, a *L. pneumophila* detection should be carried out from a culture.
- 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 result can occur due to current or past infection and therefore is not definitive for infection without other supporting evidence.
- The test is compatible with acid boric used as a preservative up to a percentage of 2%.

## EXPECTED VALUES

*L. pneumophila* is particularly frequent among patients with community acquired pneumonia who require admission to an intensive care unit. Therefore, *L. pneumophila* continues to be an important public health problem worldwide.

True prevalence of *L. pneumophila* remains unclear because it remains unrecognized and empirical treatment for respiratory tract infection leading to recovery. Death rates are therefore difficult to assess. It is estimated that about 10% to 15% of patients with Legionella pneumonia die, with the higher mortality occurring in untreated nosocomial cases.

## PERFORMANCE CHARACTERISTICS

### Analytical sensitivity (detection limit)

Detection limit value of **Vitassay Legionella** is: 12.5 ng/mL (pool of *L. pneumophila* several serovars of *Legionella pneumophila*).

### Clinical sensitivity and specificity

An evaluation, with urine samples, was performed comparing the results obtained by **Vitassay Legionella** and another commercial test (BinaxNOW® *Legionella* Urinary Antigen, Alere).

Results were as follows:

| | | **BinaxNOW® *Legionella* Urinary Antigen (Alere)** | | |
|---|---|---|---|---|
| | | **Positive** | **Negative** | **Total** |
| **Vitassay Legionella** | **Positive** | 31 | 1 | 32 |
| | **Negative** | 0 | 117 | 117 |
| | **Total** | 31 | 118 | 149 |

| **Vitassay Legionella vs BinaxNOW® *Legionella* Urinary Antigen** | | |
|---|---|---|
| | **Mean Value** | **95% confidence interval** |
| **Sensitivity** | 100.00% | 88.8-100.0% |
| **Specificity** | 99.20% | 95.4-100.0% |
| **PPV** | 96.90% | 83.8-99.9% |
| **NPV** | 100.00% | 96.9-100.0% |

The results showed that **Vitassay Legionella** has a high sensitivity and specificity to detect *L. pneumophila*.

### Cross reactivity

No cross reactivity was detected against other pathogens that are occasionally present in urine:

*Streptococcus pneumoniae*

### Reproducibility Study

Evaluation studies were performed to determine reproducibility of the **Vitassay Legionella** including inter-day, inter-laboratory, inter and intra lot, showing high reproducibility in all cases.

## REFERENCES


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2. GARCIA-VIDAL C, LABORI M, VIASUS D, SIMONETTI A, GARCIA-SOMOZA D, DORCA J, GUDIOL F, CARRATALA J. "Rainfall Is a Risk Factor for Sporadic Cases of Legionella pneumophila Pneumonia". PLoS ONE, 2013, 8(4): e61036. doi:10.1371/journal.pone.0061036.

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## SYMBOLS FOR IVD COMPONENTS AND REAGENTS

| | | | |
|---|---|---|---|
| IVD | *in vitro* diagnostic device | | Keep dry |
| | Consult instructions for use | | Temperature limitation |
| | Use by | | Manufacturer |
| LOT | Batch code | Σ n | Contains sufficient for <n> test |
| DIL | Sample diluent | REF | Catalogue number |
| Control + | Positive Control | Control - | Negative Control |

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