

# VITASSAY

## Strep. pneumoniae + Legionella

Rapid test for the simultaneous qualitative detection of *Streptococcus pneumoniae* and *Legionella pneumophila* in human urine samples.

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For professional *in vitro* diagnostic use only.

### INTENDED USE

**Vitassay Strep. pneumoniae + Legionella** is a rapid one step immunochromatographic assay for the simultaneous qualitative detection of *Streptococcus pneumoniae* and *Legionella pneumophila* in human urine samples.

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

### INTRODUCTION

*Streptococcus pneumoniae* is a potent human pathogen. Infections leads to common diseases such as otitis media, meningitis and pneumonia, which affect several million people and is responsible for significant infant death in developing countries. Worldwide each year, there are over 14 million serious *Streptococcus pneumoniae* infections in children <5 years of age leading to over 800.000 deaths.

There are at least 95 capsular serotypes, but only a few causes the majority of disease. The organism produces a range of colonization and virulence factors, including the polysaccharide capsule, surface proteins and the toxin pneumolysin (PLY).

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 Strep. pneumoniae + Legionella** is a qualitative immunochromatographic assay for the detection of *Streptococcus pneumoniae* and/or *Legionella pneumophila* (*L. pneumophila*) in human urine samples.

**Strip A:** The test line zone of the nitrocellulose membrane is pre-coated with monoclonal antibodies against *Streptococcus pneumoniae*.

**Strip B:** 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 *Streptococcus pneumoniae* (strip A) and/or *L. pneumophila* (strip

B), forming conjugates. The mixture moves upward on the membrane by capillary action. If the sample is *Streptococcus pneumoniae* positive, antibodies present on the membrane (test line) capture the conjugate complex and a red line will be visible in the strip A, and if the sample is *L. pneumophila* positive, antibodies present on the membrane (test line) capture the conjugate complex and a red line will be visible in strip B. Although the sample is positive or negative, the mixture continues to move across the membranes and the blue control line always appears (for both strips).

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 **Strep. pneumoniae + 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 (2-30°C/36-86°F).

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
<ul style="list-style-type: none"> <li>25 Tests/kits <b>Vitassay Strep. pneumoniae + Legionella.</b></li> <li>Instructions for use.</li> <li>25 Plastic pipettes.</li> <li>25 Testing tubes.</li> <li>Reagent (sample and controls diluent).</li> <li>Positive Control: Inactivated Strep. pneumoniae swab + testing tube + pipette.</li> <li>Positive Control: Inactivated <i>L. pneumophila</i> swab + testing tube + pipette.</li> <li>Negative Control: Negative swab + testing tube + pipette.</li> </ul>	<ul style="list-style-type: none"> <li>Specimen collection container.</li> <li>Disposable gloves.</li> <li>Timer.</li> </ul>

## SPECIMEN COLLECTION

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 (35.6-46.4°F) for up to 14 days or at -10°C to -20°C (14°F to -4°F) for longer periods before testing.

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

## SPECIMEN PREPARATION

Allow all specimens to equilibrate to room temperature before testing.

## PROCEDURE

Allow tests, urine samples, reagent and controls to reach room temperature (15-30°C/59-86°F) 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 6 drops of urine sample. (figure 1).
- Add 2 drops of Reagent into the testing tube or vial and mix (figure 2). Homogenize the sample.
- Remove the **Vitassay Strep. pneumoniae + Legionella** from its sealed bag just before using it.
- Use a separate pipette and device for each sample or control. Dispense 3 drops from the testing tube in the circular window

marked with the letter A- Strep. pneumoniae (figure 3) and 3 drops, using the same testing tube, in the circular window marked with the letter B-Legionella (figure 4).

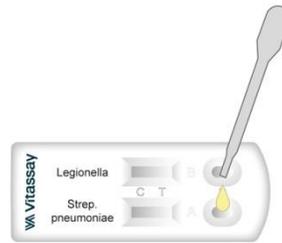
- Read the results at **15 minutes**. Do not read the test results later than 15 minutes.



Add 6 drops of urine sample.



Add 2 drops of Reagent and mix.



Dispense 3 drops in the circular window marked with the letter A- Strep. pneumoniae.



Dispense 3 drops in the circular window marked with the letter B- Legionella.

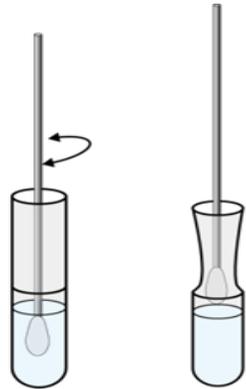
### Positive Swabs controls:

- Hold Reagent vertically. Add slowly 13 free falling drops of Reagent into the testing tube (figure 1a).
- Remove the Vitassay Strep. pneumoniae Positive Control Swab from the pouch and put the swab into the testing tube with the reagent, 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 2a).
- Remove the **Vitassay Strep. pneumoniae + 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 A- Strep. pneumoniae (figure 3a).
- Read the results at **15 minutes**. Do not read the test results later than 15 minutes.



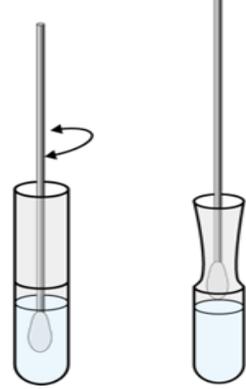
Add 13 drops of Reagent into a testing tube.

# 2a



Put the swab into the tube, rotating 1 minute and extract the liquid.

# 2b



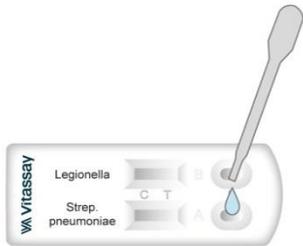
Put the swab into the tube, rotating 1 minute and extract the liquid.

# 1



Add 13 drops of Reagent into a testing tube.

# 3a



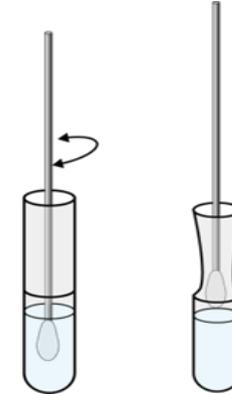
Dispense 3 drops in the circular window marked with the letter A- Strep. pneumoniae.

# 3b



Dispense 3 drops in the circular window marked with the letter B- Legionella.

# 2



Put the swab into the tube, rotating 1 minute and extract the liquid.

Repeat the procedure for Positive Control Swab using Vitassay Legionella Positive Control Swab (figure 1b and 2b) to dispense 3 drops from the testing tube, into the circular window marked with the letter B-Legionella (figure 3b).

### Negative control Swab:

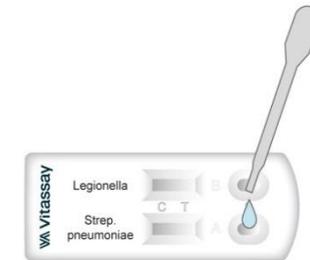
- Hold Reagent vertically. Add slowly 13 free falling drops of Reagent into the testing tube (figure 1).
- Remove the Vitassay Negative Control Swab from the pouch and put the swab into the testing tube with the reagent, mix 1 minute and extract as much liquid possible from the swab, squeezing the sides of the tube as the swab is withdrawn. Discard the swab (figure 2).
- Remove the **Vitassay Strep. pneumoniae + 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 A- Strep. pneumoniae (figure 3) and other 3 drops from the same testing tube into the circular window marked with the letter B- Legionella (figure 4).
- Read the results at **15 minutes**. Do not read the test results later than 15 minutes.

# 1b

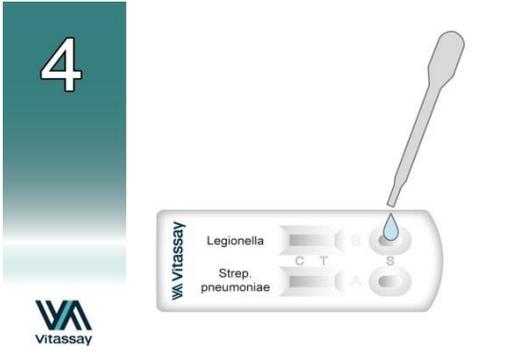


Add 13 drops of Reagent into a testing tube.

# 3



Dispense 3 drops in the circular window marked with the letter A- Strep. pneumoniae.



Dispense 3 drops in the circular window marked with the letter B- Legionella.

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

#### INTERPRETATION OF THE RESULTS

RESULTS	Strip A Strep. pneumoniae	Strip B Legionella	INTERPRETATION
	Negative BLUE	Negative BLUE	There is no <i>Streptococcus pneumoniae</i> or <i>L. pneumophila</i> presence. No infection caused by <i>Streptococcus pneumoniae</i> or <i>L.pneumophila</i> . Negative control result.
	Positive BLUE - RED	Positive BLUE - RED	There is <i>Streptococcus pneumoniae</i> and <i>L. pneumophila</i> presence. Infection caused by <i>Streptococcus pneumoniae</i> and <i>L.pneumophila</i> . Positive controls result.
	Positive BLUE - RED	Negative BLUE	There is <i>Streptococcus pneumoniae</i> presence. Infection caused by <i>Streptococcus pneumoniae</i> . Strep. pneumoniae Positive control result.

	Negative	Positive	
	BLUE	BLUE - RED	There is <i>L. pneumophila</i> presence. Infection caused by <i>L. pneumophila</i> . Legionella Positive control result.
<b>ANY OTHER RESULTS</b>			Invalid result either A or B, we recommend repeating the assay using the sample with another test. <b>Note:</b> Wrong procedural techniques or deterioration of the reagents are the main reasons of control line failure. If the symptoms or situation still 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 the **Vitassay Strep. pneumoniae + Legionella**. Blue lines 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

- the **Vitassay Strep. pneumoniae + 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 *Streptococcus pneumoniae* and/or *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 in the urine sample is lower than the detection limit value. If symptoms or situation

still persist, a *Streptococcus pneumoniae* and/or *L. pneumophila* detection should be carried out from a culture or other methods.

- Excretion of *Streptococcus pneumoniae* and *Legionella pneumophila* antigen in urine may vary depending on the individual patient. For Legionella 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

*Streptococcus pneumoniae* is a common cause of invasive disease and respiratory tract infections in more and less developed countries. Risk groups for diseases caused by pneumococci such as meningitis, sepsis and pneumonia; include young children, elderly people and patients with immunodeficiencies. Each year, 1 million children younger than 5 years old die from pneumonia and invasive diseases. In USA, the annual number of fatal pneumococcal infections is 40000.

*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 *Legionella 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 the **Vitassay Strep. pneumoniae + Legionella** is: 0.25 ng/mL of CWPS for strip A (Strep. pneumoniae) and 12.5 ng/mL (pool of several serovars of *L. pneumophila*) for strip B (Legionella).

##### Clinical sensitivity and specificity

An evaluation was performed comparing the **Vitassay Strep. pneumoniae + Legionella** and another commercial test (BinaxNOW® *Streptococcus pneumoniae* Antigen Card, Alere) for strip A.

Results were as follows:

		BinaxNOW® <i>Streptococcus pneumoniae</i> Antigen Card (Alere)		
		Positive	Negative	Total
Vitassay Strep. pneumoniae+ Legionella	Positive	33	1	34
	Negative	4	73	77
Strep. pneumoniae	Total	37	74	111

Vitassay Strep. pneumoniae+ Legionella vs BinaxNOW® <i>Streptococcus pneumoniae</i> Antigen Card		
	Mean Value	95% confidence interval
Sensitivity	89.2%	74.6-97.0%
Specificity	98.6%	92.7-100.0%
PPV	97.1%	84.7-99.9%
NPV	94.8%	87.2-98.6%

Another evaluation was performed, with urine samples, comparing the **Vitassay Strep. pneumoniae + Legionella** and another commercial test (BinaxNOW® *Legionella* Urinary Antigen Card, Alere) for strip B.

Results were as follows:

		BinaxNOW® <i>Legionella</i> Urinary Antigen Card (Alere)		
		Positive	Negative	Total
Vitassay Strep. pneumoniae+ Legionella	Positive	31	1	32
	Negative	0	117	117
Legionella	Total	31	118	149

Vitassay Strep. pneumoniae+ Legionella vs BinaxNOW® <i>Legionella</i> Urinary Antigen Card		
	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 Strep. pneumoniae + Legionella** has a high sensitivity and specificity to detect *Streptococcus pneumoniae* and *L. pneumophila*.

#### Cross reactivity

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

<i>Legionella</i> (strip A)	<i>Streptococcus pneumoniae</i> (Strip B)
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#### Reproducibility Study

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

#### REFERENCES

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2. MITCHELL, A.M.; MITCHELL, T.J. "Streptococcus pneumoniae: Virulence factors and variation". *CLINICAL MICROBIOLOGY AND INFECTION* 2010; 16(5): 411-418.
3. MIERNYK, KAREN M.; BULKOW, LISA R.; CASE, SAMANTHA L.; ET AL. "Population structure of invasive *Streptococcus pneumoniae* isolates among Alaskan children in the conjugate vaccine era, 2001 to 2013". *DIAGNOSTIC MICROBIOLOGY AND INFECTIOUS DISEASE* 2016; 86(2): 224-230.
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7. M. S. ANBUMANI; A. CHAUDHURY; A. GURURAJKUMAR. "Clinical Prevalence of *Legionella*, Associated Risk and Clinical Features". *Biol Med Res.* 2014; 5(4): 4582-4585.

#### SYMBOLS FOR IVD COMPONENTS AND REAGENTS

	in vitro diagnostic device		Keep dry
	Consult instructions for use		Temperature limitation
	Use by		Manufacturer
	Batch code		Contains sufficient for <n> test
	Sample diluent		Catalogue number
	Positive Control		Negative Control



