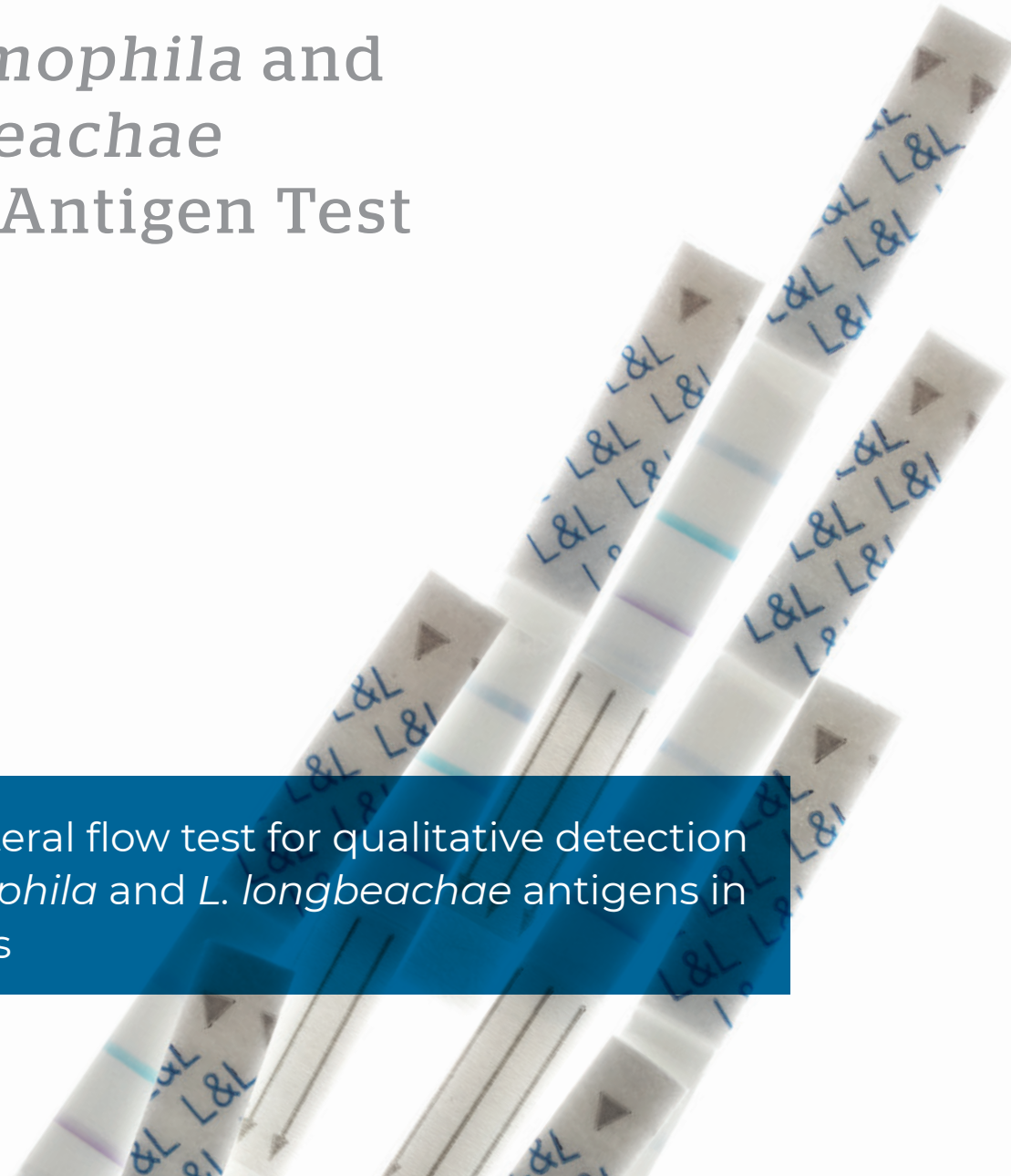
SSI DIAGNOSTICA

Instructions for use

IMMUVIEW®

L. pneumophila and *L. longbeachae* Urinary Antigen Test



EN

Combined lateral flow test for qualitative detection of *L. pneumophila* and *L. longbeachae* antigens in urine samples

IMMUVIEW® *L. PNEUMOPHILA* AND *L. LONGBEACHAE* URINARY ANTIGEN TEST

For *in vitro* diagnostic use

Intended use

The ImmuView® *L. pneumophila* and *L. longbeachae* Urinary Antigen Test is an *in vitro* rapid lateral flow test, also known as a lateral flow immunochromatographic assay. The assay is intended for qualitative detection of *Legionella* infections by detection of antigens in urine specimens from patients with symptoms of pneumonia. The ImmuView® *L. pneumophila* and *L. longbeachae* Urinary Antigen Test can be read visually.

The assay is intended to aid in diagnosis of *Legionella (L.) pneumophila* serogroup 1 and *L. longbeachae* infections. Results from the ImmuView® *L. pneumophila* and *L. longbeachae* Urinary Antigen Test should be interpreted in conjunction with the patient's clinical evaluation and other diagnostic methods.

Description

ImmuView® *L. pneumophila* and *L. longbeachae* Urinary Antigen Test is a rapid lateral flow test for qualitative detection of *L. pneumophila* and *L. longbeachae* antigens in human urine samples. The test is effective in presumptive diagnosis of *Legionella* pneumonia (Legionnaires' disease) caused by *L. pneumophila* or *L. longbeachae*, in conjunction with culture or other methods. Correct and early treatment is vital for the prognosis of Legionnaires' disease and therefore quick methods to confirm the disease in the initial phase are very important in order to initiate the proper treatment as soon as possible. ImmuView® *L.*

pneumophila and *L. longbeachae* Urinary Antigen Test is for use by laboratory professionals and/or healthcare professionals only.

Principle

ImmuView® *L. pneumophila* and *L. longbeachae* Urinary Antigen Test is a rapid lateral flow test for detection of *L. pneumophila* and *L. longbeachae* using the same test.

Precautions

- The presence of partial lines and dots represents invalid test results. The patient sample should be re-tested.

- If a test shows a strong *L. pneumophila* line, a faint line for *L. longbeachae* can occur.

- Test results should be read within the recommended reading time.

- Let the kit components equilibrate to room temperature before testing.

- Three blue/grey lines indicate plasma/protein/mix (pH, blood, glucose)/ whole blood present in the urine. The sample should be boiled for 5 minutes before retesting the sample.

- Water-based personal lubricant might result in false positive or grey *L. pneumophila* lines.

- The intensity of a test line is not related to the antigen level in the sample.

- Do not mix the components of the specific kit lot with components from other kits.

- Do not use the test after the kit lot or components' expiry date.

- Inspect the tests and vials before use to ensure they are intact. Any damaged vials/tests should be discarded.

Materials provided

- 1 tube with 22 tests
- 0.5 mL combined positive control for *L. pneumophila* and *L. longbeachae*
- 0.5 mL combined negative control for *L. pneumophila* and *L. longbeachae*
- 2.5 mL running buffer
- 1 tweezer
- 22 transfer pipettes
- 22 test tubes
- 1 cardboard test tube holder
- 1 scorecard

Quick guide can be found on the inside of the box and on page 8.

Materials required but not provided

- Timer
- Sterile standard urine collection containers/transport tubes

Storage and stability

Please find the information on the box and labels. This product does not require any additional storage conditions. Do not freeze the product.

Preservatives

The use of boric acid does not interfere with the ImmuView® *L. pneumophila* and *L. longbeachae* Urinary Antigen Test.

Sample collection and storage

Collect the urine sample in a sterile standard container (with or without boric acid as the preservative). If the sample is run within 24 hours, it can be stored at room temperature. Alternatively, the sample can be stored at 2-8 °C for 1 week or frozen at -20 °C (avoid multiple freeze/thaw circles). Make sure that samples always reach room temperature before testing.

Quality control

The positive and negative controls provided with ImmuView® *L. pneumophila* and *L. longbeachae* Urinary Antigen Test function as the kit quality control. The positive and negative controls should follow the same procedure as if they were urine samples. The positive control should be visible at the control test line and both the *L. pneumophila* and *L. longbeachae* test lines. The negative control should only be visible at the control line.

Before use check the vials to ensure there is no damage and/or leak. In case of damage or leak discard the vial.

Before using a new lot of a kit, or a new shipment of the same lot, or if the test is performed by a new operator, please perform quality control testing before testing clinical samples. Follow your local or state requirements for frequency of quality control testing.

Procedure

1. Bring the patient urine sample to room temperature.
   Whirl sample prior to testing.*

2. Apply a test tube in the cardboard holder.

3. Fill the transfer pipette with urine and add 3 drops (120 µL) of sample to the test tube (hold the pipette vertically).

4. Add 2 drops (90 µL) of running buffer to the test tube (hold the buffer bottle vertically).

5. Whirl the test tube gently.

6. Take the test container, open it and take out the number of tests needed, and close it firmly afterwards.

7. Insert the test into the test tube.

8. Wait 15 minutes.

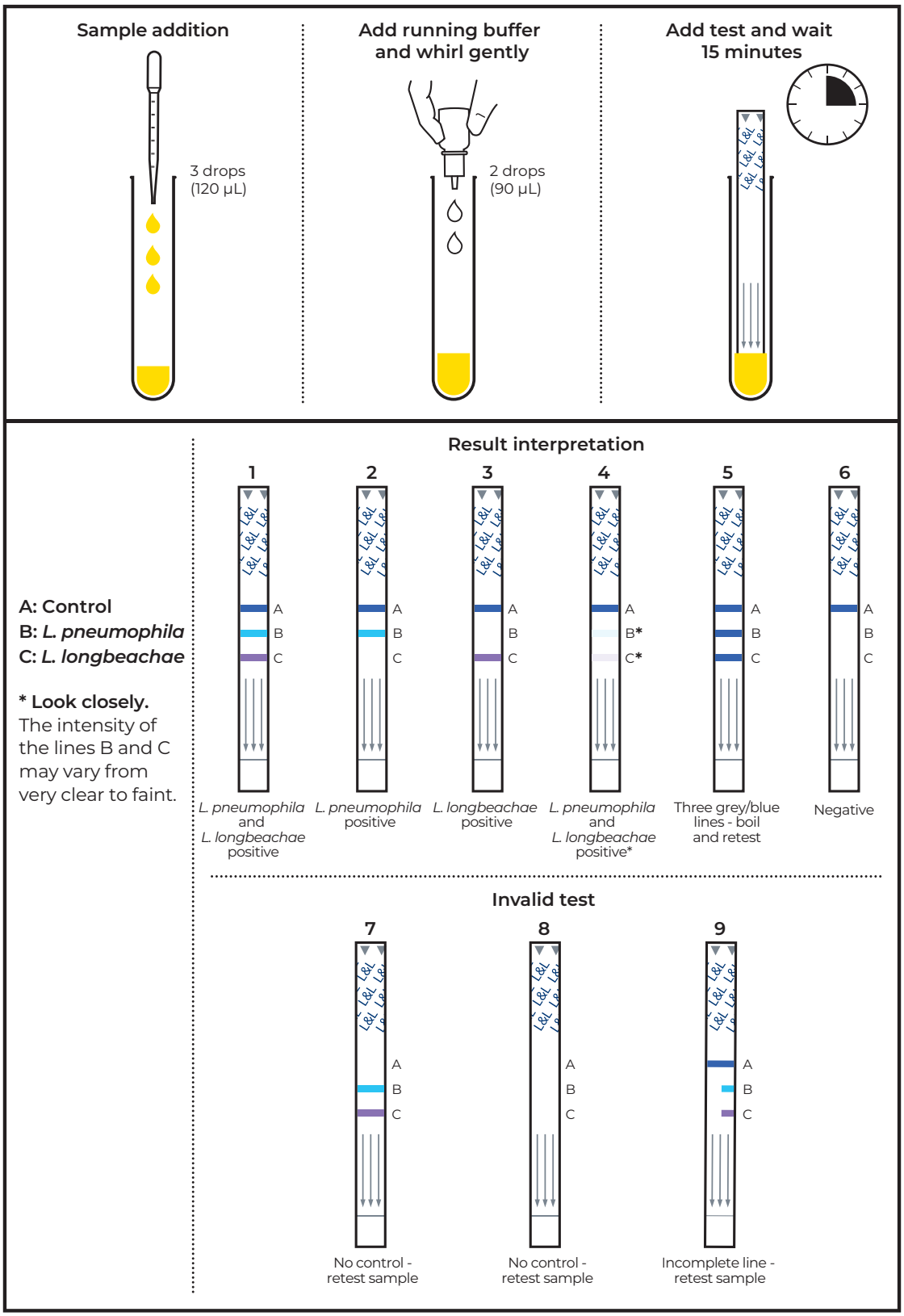
9. Lift the test out of the test tube. Read the result within 5 minutes.**

10. Discard the test after interpretation of the result.

* If the urine sample contains visible blood, please confirm a positive result by boiling[1,2] the sample at ≥ 95 °C for 5 minutes (e.g. heating block) and retest.

** Otherwise, the test result may be inaccurate.

Quick guide


Sample addition
3 drops
(120 µL)
Add running buffer
and whirl gently
2 drops
(90 µL)
Add test and wait
15 minutes
A: Control
B: L. pneumophila
C: L. longbeachae
* Look closely.
The intensity of the lines B and C may vary from very clear to faint.
Result interpretation
1
2
3
4
5
6
A
B
C
B*
C*
L. pneumophila and L. longbeachae positive
L. pneumophila positive
L. longbeachae positive
L. pneumophila and L. longbeachae positive*
Three grey/blue lines - boil and retest
Negative
Invalid test
7
8
9
No control - retest sample
No control - retest sample
Incomplete line - retest sample

Interpretation of results

The control line in the top of the test will appear blue/grey but can also be more blue or purple depending on whether the sample is positive for either *L. pneumophila* or *L. longbeachae*.

A positive sample for both *L. pneumophila* and *L. longbeachae*

A purple line in the bottom half of the test for *L. longbeachae* and a blue line in the middle for *L. pneumophila*, and a blue/grey control line (see test results number 1 and 4, page 8).

A positive sample for *L. pneumophila*

A blue line, and at the top of the test a blue/grey control line (see test result number 2, page 8).

A positive sample for *L. longbeachae*

A purple line at the bottom and a blue/grey control line at the top (see test result number 3, page 8).

Look closely. Even if there is a very faint line for either *L. pneumophila* or *L. longbeachae* or both, the test result is positive (see test result number 4, page 8). The enclosed "Scorecard" can help to determine if the test result is positive or negative.

Note: three blue/grey lines do not indicate a positive result. If three grey lines are observed the result can be confirmed by boiling the urine sample for approx. 5 minutes (see test result number 5, page 8). Boiling can also be used for confirmation of a positive result as *Legionella* antigens are heat stable.

Remember to let the urine sample cool down to room temperature before retesting the sample.

A negative sample
A blue/grey control line at the top of the test.

A negative result does not exclude infection with *L. pneumophila* or *L. longbeachae* (see test result number 6, page 8 and limitations).

Invalid sample
If no control line is observed and/or incomplete test lines are present the test is invalid and the sample should be retested (see test results number 7, 8, and 9, page 8).

Disposal

Follow your local procedures and/or guidelines from national authorities for disposal of biological materials.

Limitations

- ImmuView® *L. pneumophila* and *L. longbeachae* Urinary Antigen Test has been validated using urine specimens only. Other specimens (e.g. serum or other body fluids) that may contain antigen have not been validated.

- A negative result does not exclude a *Legionella* infection. There is no single satisfactory laboratory test for Legionnaires' disease. Therefore, culture results, PCR, serology, and/or antigen detection methods should be considered in conjunction with clinical findings to make an accurate diagnosis.

Clinical sensitivity and specificity for urine samples

The clinical sensitivity of the *L. pneumophila* test line was obtained by testing retrospective urine samples from patients with a confirmed Legionnaires' disease according to the ECDC criteria.[1]

The clinical sensitivity for *L. longbeachae* was determined by testing prospective urine samples from patients with a presumptive Legionnaires' disease. All cases were confirmed with PCR. Fifteen samples were confirmed with culture.

The clinical specificity was determined by testing retrospective urine samples. The samples came from patients with suspected lower respiratory tract infections other than *Legionella* infections. These infections included *Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, Staphylococcus aureus, Escherichia coli, Acinetobacter baumannii, Streptococcus pyogenes*, *Mycobacterium tuberculosis, Pneumocystis jirovecii*, and other pathogens.[1] Furthermore, prospective negative samples were included in the validation. The specificity is a total for both *L. pneumophila* and *L. longbeachae* for ImmuView®. The result for the comparator is only based on *L. pneumophila*.[2]

Sensitivity values were calculated using a two-sided Wilson 95% confidence interval.

Confirmed *Legionella pneumophila* SG1 cases (50 samples)		
ImmuView®	Positive Negative	48 2
Sensitivity		96% (CL: 87-99%)
Comparator	Positive Negative	48 2
Sensitivity		96% (CL: 87-99%)

Confirmed *Legionella longbeachae* cases culture verified (15 samples)		
ImmuView®	Positive Negative	10 5
Sensitivity		67% (CL: 42-85%)
Confirmed *Legionella longbeachae* cases PCR verified (43 samples)		
ImmuView®	Positive Negative	23 20
Sensitivity		54% (CL: 39-68%)

Negative *Legionella* cases (48 retrospective and 195 prospective samples)		
ImmuView®	Positive Negative	0 243
L. pneumophila specificity *L. longbeachae* specificity		100% (CL: 98-100%) 100% (CL: 98-100%)
Comparator	Positive Negative	0 243
L. pneumophila specificity *L. longbeachae* specificity		100% (CL: 98-100%) N/A

Positive agreement with another urinary antigen test

L. pneumophila positive agreement was made in a sample population containing fifty (50) culture, urinary antigen test, and/or PCR positive samples. Thus, the agreement is based on *L. pneumophila* and not *L. longbeachae* as the comparator was not able to detect *L. longbeachae*. The positive agreement was calculated as the total number of common positive samples, divided by the total number of positive samples found by the comparator using a two-sided Wilson 95% confidence interval. None of these samples were shown as *L. longbeachae* positive using the ImmuView *L. pneumophila* and *L. longbeachae* Urinary Antigen Test.

Confirmed *Legionella pneumophila* SG1 cases (50 samples)		**Comparator**		
		Positive	**Negative**	**Total**
ImmuView®	Positive	48	0	48
	Negative	0	2	2
Total		48	2	50
Positive agreement				100% (CI: 93-100%)

Negative agreement with another urinary antigen test

The negative agreement was calculated on a total of 243 samples negative for *Legionella* (48 retrospective and 195 prospective samples). Thus, the agreement is based on *L. pneumophila* and not *L. longbeachae* as the comparator was not able to detect *L. longbeachae*. The negative agreement was calculated as the total number of common negative samples, divided by the total number of negative samples found by the comparator using a two-sided Wilson 95% confidence interval.

Negative *Legionella* cases (48 retrospective samples and 195 prospective samples)		Comparator Positive	Comparator Negative	Comparator Total
ImmuView®	Positive	0	0	0
	Negative	0	243	243
Total		0	243	243
Negative agreement				100% (CI: 98-100%)

Analytical studies

Specificity (cross reactivity)

To determine the analytical specificity of the ImmuView® *L. pneumophila* and *L. longbeachae* Urinary Antigen Test for cross-reactivity with urines spiked with whole cell bacteria (N=120), the whole cell bacterial panel was tested in a 10^7 CFU/mL diluted from a stock solution. The panel was also tested in negative urine.

Organisms tested for interference (10^7 CFU/mL)	
Acinetobacter ssp. (4)	*Neisseria cineria*
Bacillus subtilis	*Neisseria gonorrhoeae* (3)
Bordetella pertussis	*Neisseria lactamica*
Moraxella catarrhalis	*Neisseria meningitidis*
Candida albicans (4)	*Neisseria polysaccharea*
Cornyebacterium sp.	*Proteus mirabilis* (2)
Cornyebacterium uralyticum	*Proteus vulgaris*
Enterobacter cloacae (3)	*Pseudomonas aeruginosa* (4)
Escherichia coli (10)	*Pseudomonas stutzeri*
Enterococcus faecalis (7)	*Pseudomonas* spp. (2)

Organisms tested for interference (10^7 CFU/mL)	
Enterococcus faecium	*Salmonella* Bredeney
Enterococcus durans	*Salmonella* Thompson
Gardnerella vaginalis	*Salmonella* Typhimurium
Haemophilus influenzae type a-f and non-caps (11)	*Serratis marcescecns*
	Staphylococcus epidermidis
Haemophilus parainfluenzae	*Salmonella* Glostrup
Klebsiella oxytoca (2)	*Streptococcus mutans* (2)
Klebsiella pneumoniae (3)	*Streptococcus parasanguis*
Lactobacillus catenaforme	*Streptococcus sanguinis*
Lactobacillus rhamnosus	*Streptococcus aureus* (6)
Lactobacillus sp.	*Streptococcus epidermidis* (5)
Listeria monocytogenes	*Streptococcus saprophyticus* (3)
Morganella morganii	*Stenotrophomonas maltophilia*
Moraxella osloensis	*Streptococcus* Gr. A, B, C, F, L and G (16)
Mycoplasma sp.	

None of the tested organisms interfered with the ImmuView® *L. pneumophila* and *L. longbeachae* Urinary Antigen Test.

Sensitivity (limit of detection (LoD))

Strain	LoD
L. pneumophila SG 1 (Philadelphia) antigen	10 ng/mL
L. longbeachae antigen	2.5 ng/mL
L. pneumophila SG 1 (Philadelphia) whole cells	0.25×10^4 CFU/mL
L. longbeachae whole cells	0.25×10^4 CFU/mL

Boiling of urine preservatives did not change these results.

Strain reactivity

Isolates from different *Legionella* species and serogroups were tested. 12 different serogroups were detected within the *L. pneumophila* species and both known serogroups for *L. longbeachae* species were detected. Furthermore, *L. bozemanii* and *L. micdadei* were detected on the *L. longbeachae* test line.

Species	Subspecies	Serogroup	Strain name	ATCC
***L. pneumophila* positive**				
Legionella	*pneumophila*	1	OLDA/Oxford	-
Legionella	*pneumophila*	1	Knoxville	-
Legionella	*pneumophila*	1	Philadelphia	33152
Legionella	*pneumophila*	1	Benidorm	-
Legionella	*pneumophila*	1	Allentown/France	-
Legionella	*pneumophila*	1	Bellingham	43111
Legionella	*pneumophila*	1	Heysham	-
Legionella	*pneumophila*	1	Camperdown	-
Legionella	*pneumophila*	2	Togus-1	33154
Legionella	*pneumophila*	5	Dallas 1E	33216
Legionella	*pneumophila*	5	Cambridge	-
Legionella	*pneumophila*	6	Chicago-2	33215
Legionella	*pneumophila*	7	Chicago-8	33823
Legionella	*pneumophila*	8	Concord-3	35096
Legionella	*pneumophila*	9	IN-23-G1-C2	35289
Legionella	*pneumophila*	10	Leiden-1	43283
Legionella	*pneumophila*	12	570-CO-H	43290
Legionella	*pneumophila*	13	U7W	43703
Legionella	*pneumophila*	14	1169-MN-H	43703
Legionella	*pneumophila*	15	Lansing 3	35251

Species	Subspecies	Serogroup	Strain name	ATCC
***L. longbeachae* positive**				
Legionella	*bozemanii*	-	-	-
Legionella	*micdadei*	-	-	-
Legionella	*longbeachae*	1	-	-
Legionella	*longbeachae*	2	-	-

Interfering substances

Eighteen (18) different interfering organisms were validated at different concentrations and combinations. Every agent was artificially spiked into urine and tested.

Sample content	Concentration
Plasma	15%
Plasma	10%
Plasma	5%
Protein	15%
Protein	10%
Protein	5%
Glucose	1 mg/mL
Glucose	0.5 mg/mL
Glucose	0.1 mg/mL
Human red blood cells	15%
Human red blood cells	10%
Human red blood cells	5%
pH 4, blood 5%, glucose 0.5 mg/mL	-
pH 7, blood 10%, glucose 5 mg/mL	-
pH 9, blood 15%, glucose 10 mg/mL	-
Whole blood	15%
Whole blood	10%
Whole blood	5%
pH 4	-
pH 5	-

Sample content	Concentration
pH 6	-
pH 7	-
pH 8	-
pH 9	-
pH 10	-
Ascorbic acid	1 mg/mL
Personal lubricant	5%
Personal lubricant	1%
Personal lubricant	0.1%
Ciproflaxin	0.22 mg/mL
Urea	20 mg/mL
Amphotericin	0.22 mg/mL
Chlorofyll	0.09 mg/mL
Beet root	0.01%
Caffeine	15 mg/mL
Bilirubin	0.2 mg/mL
Oseltamivir (Tamiflu)	0.03%
Antihistamine	0.22 mg/mL
Ibuprofen	0.1 mg/mL

Protein, plasma, mix (pH, blood, glucose) and whole blood resulted in three identical blue/grey lines. These disappeared by boiling the samples for 5 minutes. Personal lubricant also resulted in three blue/grey lines. These did not disappear with boiling.

Repeatability and reproducibility

The repeatability is 100/100 or one hundred percent (100%) (CI 95% 95-100%) using ImmuView® *L. pneumophila* and *L. longbeachae* Urinary Antigen Test. Visual interpretation is independent of time and operator.

The reproducibility is 50/50 or one hundred percent (100%) (CI 95% 95-100%) using ImmuView® *L. pneumophila* and *L. longbeachae* Urinary Antigen Test.

Incident reporting

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the member state in which the user and/or patient is established.

Quality certificate

SSI Diagnostica's development, production and sales of *in vitro* diagnostics are quality assured and certified in accordance with ISO 13485.

Presafe
Presafe Denmark A/S

Quality System
DS/EN
ISO 13485

REF
98749

IVD

CE

References

1. Jørgensen CS, Uldum SA, Sørensen JF, Skovsted IC, Otte S, Elverdal PL; Evaluation of a new lateral flow test for detection of *Streptococcus*

pneumoniae and *Legionella pneumophila* urinary antigen; Journal of Microbiological Methods; 2015.

2. Badoux P, Euser SM, Bruin JP, Mulder PPG, Yzerman EPF. Evaluation of the bioNexia *Legionella* test, including impact of incubation time extension, for detection of *Legionella pneumophila* serogroup 1 antigen in urine. Journal of Clinical Microbiology. 2017;55(6):1733-1737. doi:10.1128/JCM.02448-16.

Information and ordering

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IFU's in other languages

https://ssidiagnostica.com/ifu/immuview-legionella-pneumophila-and-legionella-longbeachae/

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