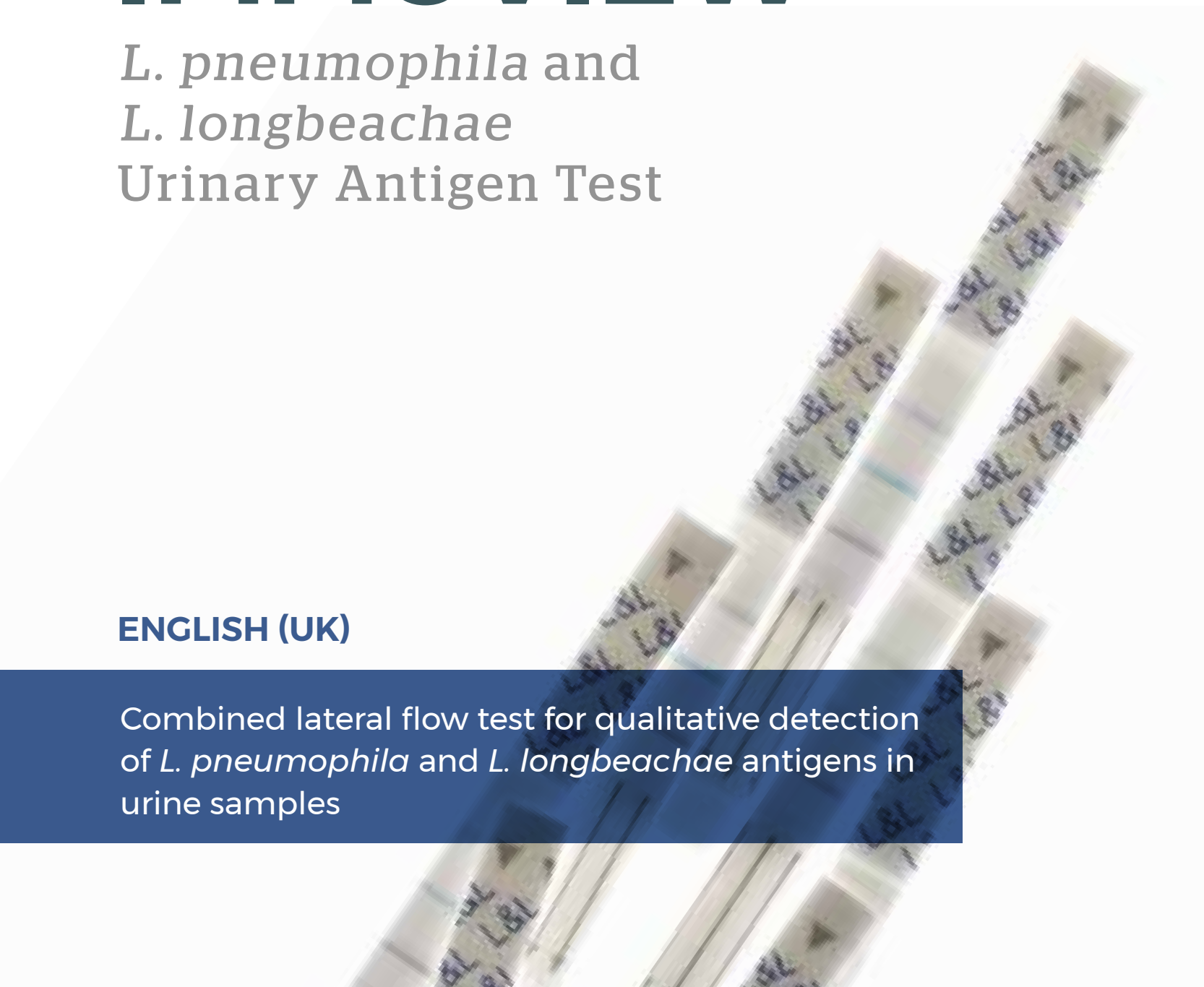
SSI DIAGNOSTICA

IMMUVIEW®

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



ENGLISH (UK)

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 intended for diagnosis of *Legionella* infections by detection of urinary antigens for either or both *L. pneumophila* and *L. longbeachae*. The test is a lateral flow test also known as a lateral flow immunochromatographic assay.

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 antibiotic treatment as soon as possible.

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 represent INVALID test results. The patient sample should be re-tested.

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

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

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

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

Materials Provided

- 1 tube with 22 test strips
- 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
- Scorecard

Quick guide can be found on the inside of the box and on page 7.

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.

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 to be tested within 48 hours, it can be stored at room temperature. Alternatively, the sample can be stored at 2-8˚C for a week. 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. Follow your local or state requirements for frequency of quality control testing. Before using a new lot of a kit, or a new shipment of the same lot or by a new operator, please perform quality control testing before testing of clinical samples. The positive and negative controls within the kit are tested according to procedure described in this IFU.

Procedure

The positive and negative controls should follow the same procedure as if it was a urine sample. The positive control should be visible at the control test line and the *L. pneumophila* and *L. longbeachae* test lines. The negative control should only be visible at the control line.

1. Bring the patient urine sample to room temperature.*

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 test strips needed, and close it firmly afterwards.

7. Insert the test strip into the test tube.

8. Wait 15 minutes.

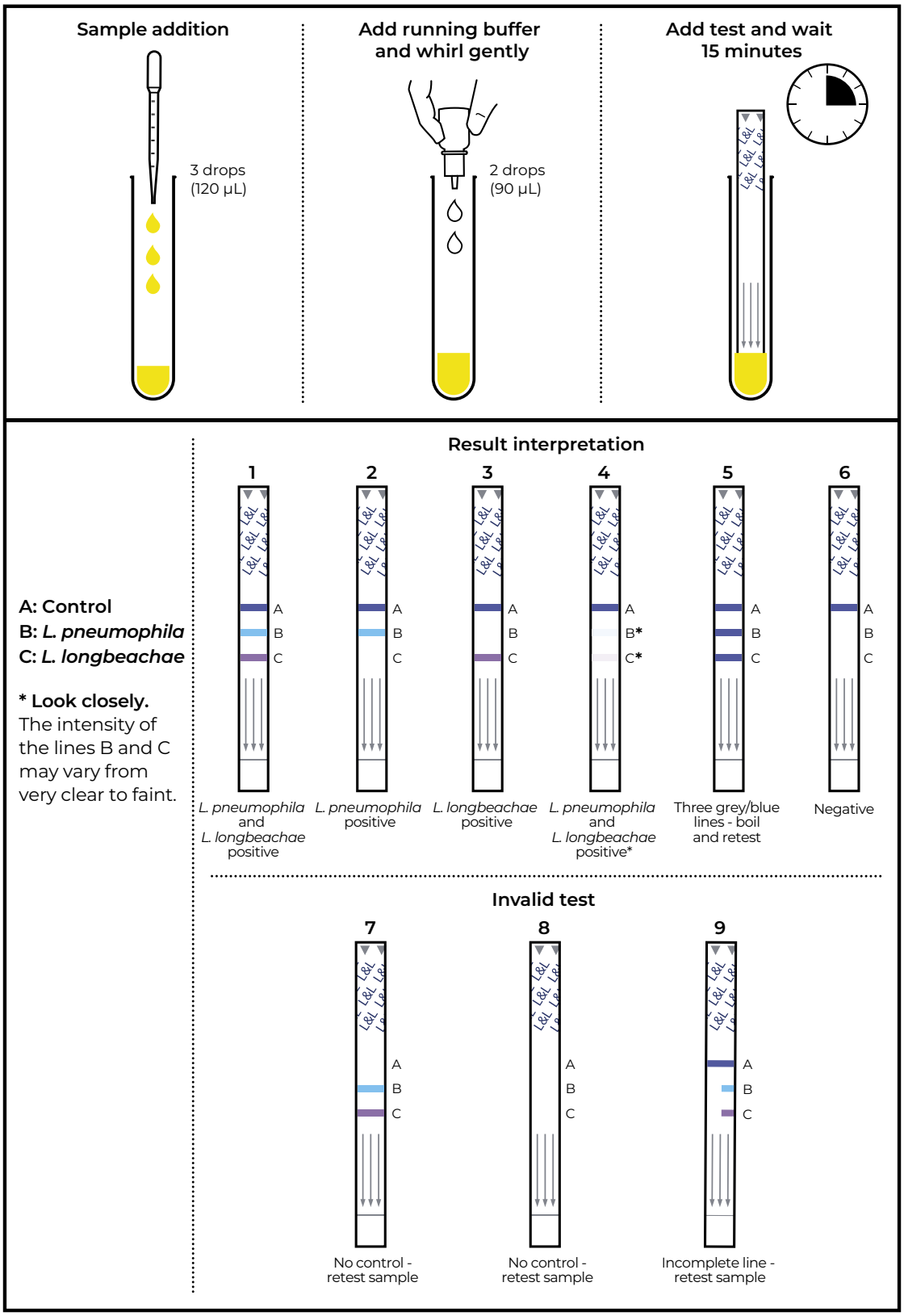
9. Lift the test strip out of the test tube. Read the result within 5 minutes.**

10. Discard the test strip after interpretation of the result.

* If the urine sample contains visible blood, please confirm a positive result by boiling the sample for 5 minutes.

** 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
A
B
C
L. pneumophila and L. longbeachae positive
2
A
B
C
L. pneumophila positive
3
A
B
C
L. longbeachae positive
4
A
B*
C*
L. pneumophila and L. longbeachae positive*
5
A
B
C
Three grey/blue lines - boil and retest
6
A
B
C
Negative
Invalid test
7
A
B
C
No control - retest sample
8
A
B
C
No control - retest sample
9
A
B
C
Incomplete line - retest sample

Interpretation of results

The control test line in the top 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*.

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 7).

A **positive sample for both *L. pneumophila* and *L. longbeachae*** will show a purple line in the bottom half of the test for *L. longbeachae* positive followed by a blue line in the middle for *L. pneumophila* positive, and at the top of the test a blue/grey control line will appear (see test result number 1 and 4, page 7).

A **positive sample for *L. pneumophila*** will show a blue line, and at the top of the test a blue/grey control line will appear (see test result number 2, page 7).

A **positive sample for *L. longbeachae*** will show a purple line, and at the top of the test a blue/grey control line will appear (see test result number 3, page 7).

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 7).

A **negative sample** will show a single blue/grey control line at the top of the test. A negative result does not exclude an *L. pneumophila* or an *L. longbeachae* infection (see test result number 6, page 7 and limitations).

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. 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. (see test result number 5, page 7).

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.

- The diagnosis of an *L. pneumophila* and/or *L. longbeachae* infection cannot be based on clinical, radiological evidence or laboratory test alone. Therefore, culture results, serology, or antigen detection methods should be used in conjunction with clinical findings to make an accurate diagnosis.

- 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 used in conjunction with clinical findings to make an accurate diagnosis.

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

- The test is not intended to replace PCR or culture.

- Personal lubricant can cause three identical (blue/grey) test lines both with and without antigen present.

Clinical Sensitivity and Specificity for urine

The clinical sensitivity of the *L. pneumophila* test line was determined by testing 50 retrospective urine samples from patients with a confirmed Legionnaires' disease according to positive culture, PCR, and/or urinary antigen test.[1]

The clinical sensitivity for *L. longbeachae* was determined by testing 43 prospective urine samples from patients with a presumptive Legionnaires' Disease. All 43 cases were confirmed with PCR. Fifteen samples were confirmed with culture.

The clinical specificity was determined by testing 48 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*, *Strep tococcus pyogenes*, *Mycobacterium tuberculosis*, *Pneumocystis jirovecii*, and other pathogens.[1] Furthermore, 195 prospective negative samples were included in the calculation. 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*.[1]

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 other UAT

L. pneumophila positive agreement was made in a sample population containing fifty (50) culture, UAT, and/or PCR positive samples. 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% confidential interval. None of these samples were shown as *L. longbeachae* positive using the ImmuView Test.

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

Negative agreement with other UAT

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% confidential interval.

Negative *Legionella* cases (48 retrospective samples and 195 prospective samples)		**Comparator**	
		Positive	**Negative**
ImmuView®	Positive	0	0
	Negative	0	243
Total		48	2
Positive agreement		243/243	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)
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))

The limit of detection (LOD) for the ImmuView® *L. pneumophila* and *L. longbeachae* Urinary Antigen Test is 10 ng/mL for *L. pneumophila* SG1 Philadelphia purified antigen. *L. longbeachae* purified antigen was detected at 2.5 ng/mL. Whole cell *L. pneumophila* SG1 (Philadelphia) and *L. longbeachae* could both be detected at 0.25 x 10^4 CFU/mL. Boiling or urine preservatives did not change these results.

Strain	LOD
L. pneumophila SG 1 (Philadelphia) antigen	10 ng/mL
L. longbeachae antigen	2.5 ng/mL
L. pneumophila SG 1 (Philadelphia)	0.25 x 10^4 CFU/mL
L. longbeachae	0.25 x 10^4 CFU/mL

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.

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

Serogroup	Species	Subspecies	Strain name	ATCC
14	*Legionella*	*pneumophila*	1169-MN-H	43703
15	*Legionella*	*pneumophila*	Lansing 3	35251
***L. longbeachae* positive**				
N/A	*Legionella*	*bozemanii*	-	-
N/A	*Legionella*	*micdadei*	-	-
1	*Legionella*	*longbeachae*	A	-
2	*Legionella*	*longbeachae*	A	-

Interference 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	Sample content	Concentration
Plasma	15%	pH 6	-
Plasma	10%	pH 7	-
Plasma	5%	pH 8	-
Protein	15%	pH 9	-
Protein	10%	pH 10	-
Protein	5%	Ascorbic acid	1 mg/mL
Glucose	1 mg/mL	Personal lubricant	5%
Glucose	0.5 mg/mL	Personal lubricant	1%
Glucose	0.1 mg/mL	Personal lubricant	0.1%
Human red blood cells	15%	Ciproflaxin	0.22 mg/mL
Human red blood cells	10%	Urea	20 mg/mL
Human red blood cells	5%	Amphotericin	0.22 mg/mL
pH 4, blood 5%, glucose 0.5 mg/mL	-	Chlorofyll	0.09 mg/mL
pH 7, blood 10%, glucose 5 mg/mL	-	Beet root	0.01%
pH 9, blood 15%, glucose 10 mg/mL	-	Caffeine	15 mg/mL
Whole blood	15%	Bilirubin	0.2 mg/mL
Whole blood	10%	Oseltamivir (Tamiflu)	0.03%
Whole blood	5%	Antihistamine	0.22 mg/mL
pH 4	-	Ibuprofen	0.1 mg/mL
pH 5	-		

Protein, plasma, mix 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

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 (AM/PM) and operator (A/B).

Quality Certificate

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

Presafe Denmark A/S

Quality System DS/EN ISO 13485

REF 98749

IVD

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References

1. 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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