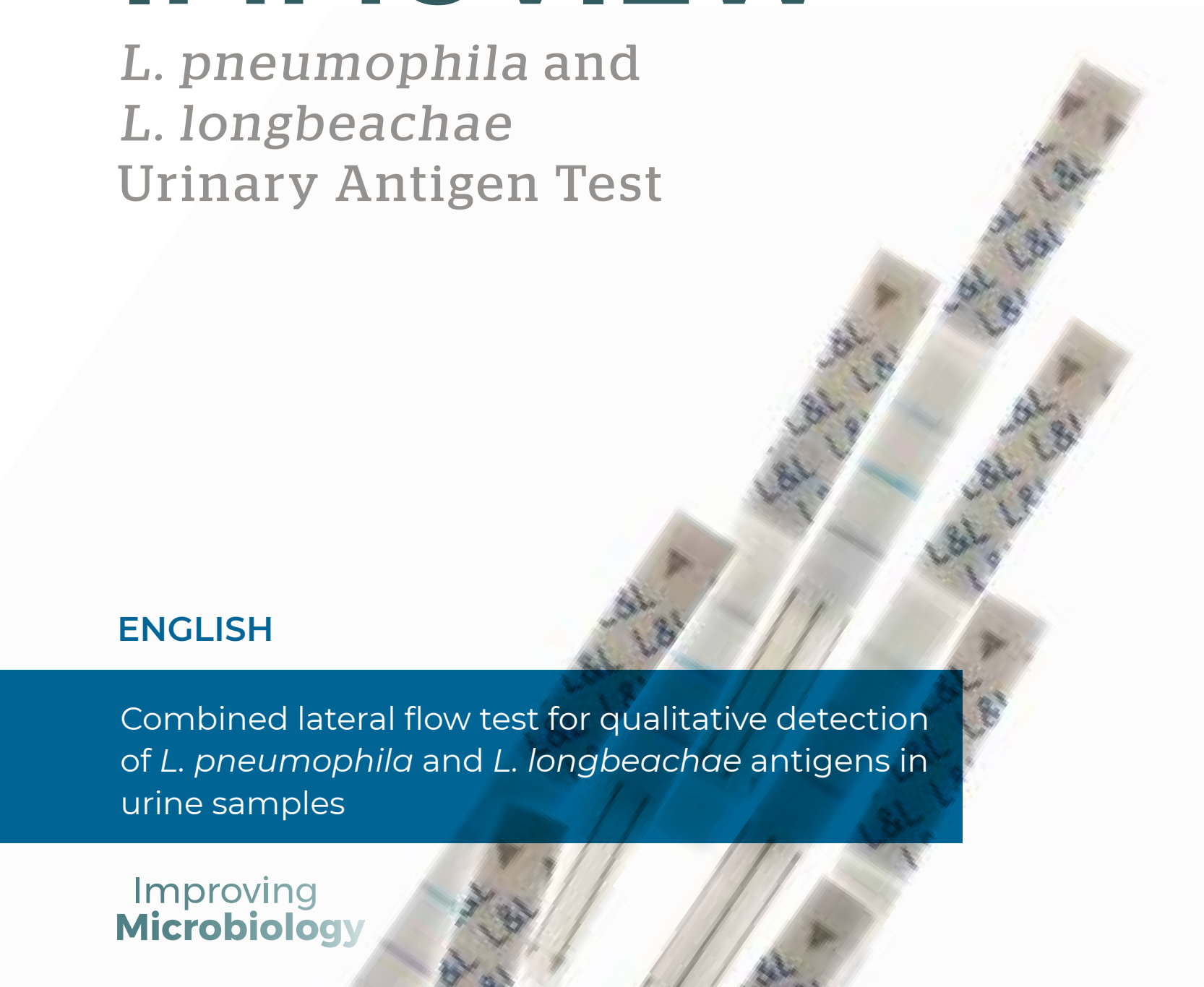
SSI DIAGNOSTICA

IMMUVIEW®

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



ENGLISH

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

Improving **Microbiology**

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.

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 a *L. pneumophila* 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, as it can be caused by other *L. pneumophila* serogroups or *Legionella* species. 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.

- Reading test results before 15 minutes or after 20 minutes may give incorrect results.

- When a patient have a strong *L. pneumophila* line, a faint lines for *L. longbeachae* can occour.

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

Materials Provided

- 1 tube with 22 test strips
- 0.5 mL combined positive control for *L. pneumphila* 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

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

Sample Collection

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 24 hours it can be stored at room temperature. Alternatively, the sample can be stored at 2-8°C for 1 week or frozen (-20°C) for at least 2 weeks. Make sure that samples always reach room temperature before testing.

Procedure

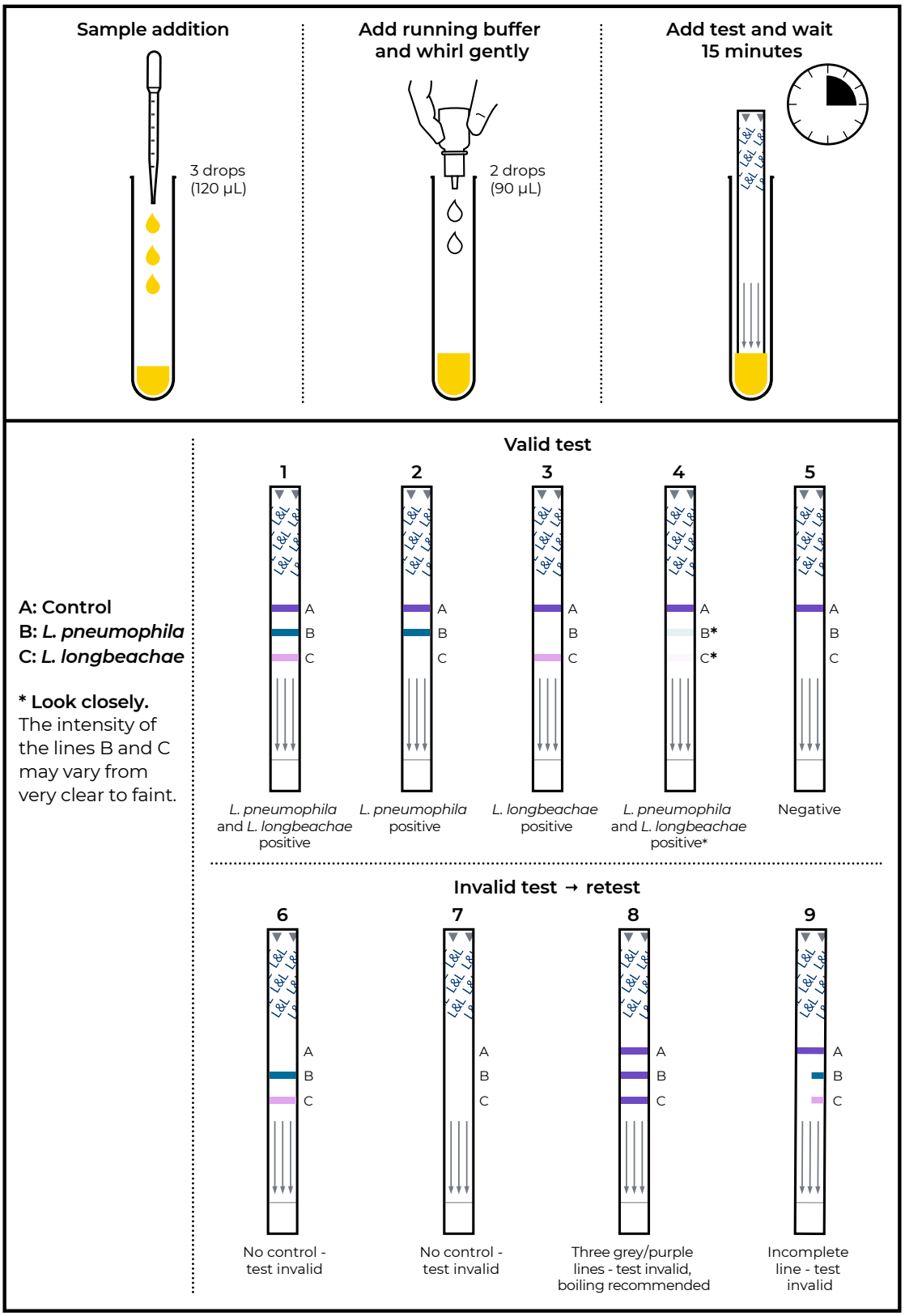
The positive and negative controls should follow the same procedure as if it was a urine sample. The positive control should be visible at the control test line and the *L. pneumophila* and *L. longbeachae* test line. The negative control should only be visible at the control line.

1. Bring the patient urine sample to room temperature. Whirl thoroughly 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 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.
Valid test
1
2
3
4
5
A
B
C
B*
C*
L. pneumophila and L. longbeachae positive
L. pneumophila positive
L. longbeachae positive
L. pneumophila and L. longbeachae positive*
Negative
Invalid test → retest
6
7
8
9
No control - test invalid
No control - test invalid
Three grey/purple lines - test invalid, boiling recommended
Incomplete line - test invalid

Interpretation of results

The control test line in the top will appear bluish/grey, but can also be more blue or purple depending on whether the sample is positive for either *L. pneumophila* and *L. longbeachae*. Only a full line indicates a positive result - dots do not indicate a positive result (see test result number 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 bluish/grey control line will appear (see test result number 1, page 7).

A **positive sample for *L. pneumophila*** will show a blue line, and at the top of the test a bluish/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 bluish/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 bluish/grey control line at the top of the test. A negative result does not exclude a *L. pneumophila* or a *L. longbeachae* infection (see test result number 5, page 7 and limitations).

Note: three bluish/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 8, page 7).

If no control line is observed the test is invalid and the sample should be retested (see test results number 6 and 7, page 7).

Clinical Sensitivity and Specificity for urine

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

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

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 influenza, Moraxella catarrhalis, Staphylococcus aureus, Escherichia coli, Acinetobacter baumannii, Streptococcus pyogenes, Mycobacterium tuberculosis, Pneumocystis jirovecii,* and other pathogens. 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*.

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

Confirmed *Legionella longbeachae* cases (culture N= 15, PCR N=43)		
Culture	positive negative	10 5
ImmuView® Sensitivity		67% (CL: 42-85%)
PCR	positive negative	23 20
Comparator Sensitivity		54% (CL: 39-68%)

Negative *Legionella* cases (48 retrospective and 195 prospective samples)		
Culture	positive negative	0 243
ImmuView® Specificity		100% (CL: 98-100%)
Comparator	positive negative	0 243
Comparator Specificity		100% (CL: 98-100%)

Positive agreement with other UAT

L. pneumophila positive agreement was made in a sample population containing 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.

Legionella pneumophila SG1, PCR, UAT and/or culture positive		Comparator positive	Comparator negative	Total
ImmuView®	positive negative	48 0	0 2	48 2
Total		48	0	48
Positive agreement			100% (48/48 CL: 93-100%)	

Negative agreement with other UAT

The negative agreement was calculated on a total of 243 samples negative for *L. pneumophila*. 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.

***L. pneumophila* negative samples (48 retrospective & 195 prospective samples)**		**Comparator positive**	**negative**	**Total**
ImmuView®	positive	0	0	0
	negative	0	243	243
Total		0	243	243
Negative agreement			100% (243/243 CL: 98-100%)	

Analytical Sensitivity and Specificity for urine samples

To determine the analytical sensitivity of the ImmuView® *L. pneumophila* and *L. longbeachae* Urinary Antigen Test a panel of the following were tested:

- 8 subgroups of *L. pneumophila* serogroup 1
- 16 *L. pneumophila* non-serogroup 1
- 2 subgroups of *L. longbeachae*
- 2 *Legionella* species

To determine the analytical specificity of the ImmuView® *L. pneumophila* and *L. longbeachae* Urinary Antigen Test a panel of 120 potential cross-reactants (see table on page 12) was tested, all spiked in negative urine at a concentration of 10^7 CFU/mL.

	Overall analytical test performance
L. pneumophila SG1 (n=8)	100%
L. pneumophila non-SG1 (n=16)	100%
L. longbeachae (n=2)	100%
Other *Legionella* species (n=2)	100%
Specificity (n=120)	100%

Acinetobacter (4)	*H. parainfluenzae*	*S. mutans (2)*
B. subtilis	*K. oxytoca (2)*	*S. parasanquis*
B. pertussis	*K. pneumoniae (3)*	*S. sanquis*
B. catarrhalis	*L. catenaforme*	*S. thomson*
C. albicans (4)	*L. rhamnosus*	*S. typhimurium*
C. aquaticum (2)	*Lacto. sp*	*S. glostrup*
Corynebactvrium sp.	*L. monocytogenes*	*S. marcescens*
E. cloacea (4)	*M. morganii*	*S. Aureus (6)*
E. coli (10)	*M. olsoensis*	*S. epidermis (6)*
E. faecalis (7)	*Mycoplasma sp.*	*S. saprophyticus (2)*
E. faecium	*N. cineria*	*S. maltophilia*
E. durans	*N. gonorrhoeae (3)*	*Streptococcus gr. A*
G. vaginalis	*N. lactamica*	*Streptococcus gr. A (colindale)*
H. influenzae a	*N. meningitidis*	*Streptococcus gr. B (10)*
H. influenzae b	*N. polysak*	*Streptococcus gr. C*
H. influenzae c	*P. mirabilis (2)*	*Streptococcus gr. F*
H. influenzae d	*P. vulgaris (2)*	*Streptococcus gr. G*
H. influenzae e	*Pseudomonas (2)*	*Streptococcus gr. L*
H. influenzae f	*Ps. aeruginosa (4)*	
H. influenzae non caps	*Ps. stutzeri*	
H. influenzae (4)	*S. bredeny*	

Limit of Detection (LOD)

Species	Pure antigen
L. pneumophila SG 1 Philadelphia	10 ng/mL
L. pneumophila SG 1 Knoxville	10 ng/mL
L. pneumophila SG 1 Olda/Oxford	10 ng/mL
L. pneumophila SG 1 Allentown/France	10 ng/mL
L. longbeachae SG 1	1 ng/mL
L. longbeachae SG 2	1 ng/mL

Interference

An internal validation of interfering substances was performed and included; blood, plasma, protein, glucose, pH, caffeine, bilirubin levels, hemoglobin and ascorbic acid.

Each substance was tested individually and in combination at high, medium and low levels. None of the substances influenced the test results.

Two urine preservatives were tested, 25 mM PIPES (piperazine-N, N'-bis 2-ethane sulfonic acid) and Boric acid, respectively. None of these influenced the test results.

Storage and Shelf Life

Store at room temperature. Expiry date is printed on the package.

Quality Certificate

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

98749

Article number

98749

References

1. R. Podmore, M. Schousboe, D. Murdoch, Evaluation of an ImmuView *Legionella longbeachae* urinary antigen test for the diagnosis of pneumonia. International *Legionella* congress in Rome 2017, poster 26.

Publications ongoing.

Information and Ordering

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