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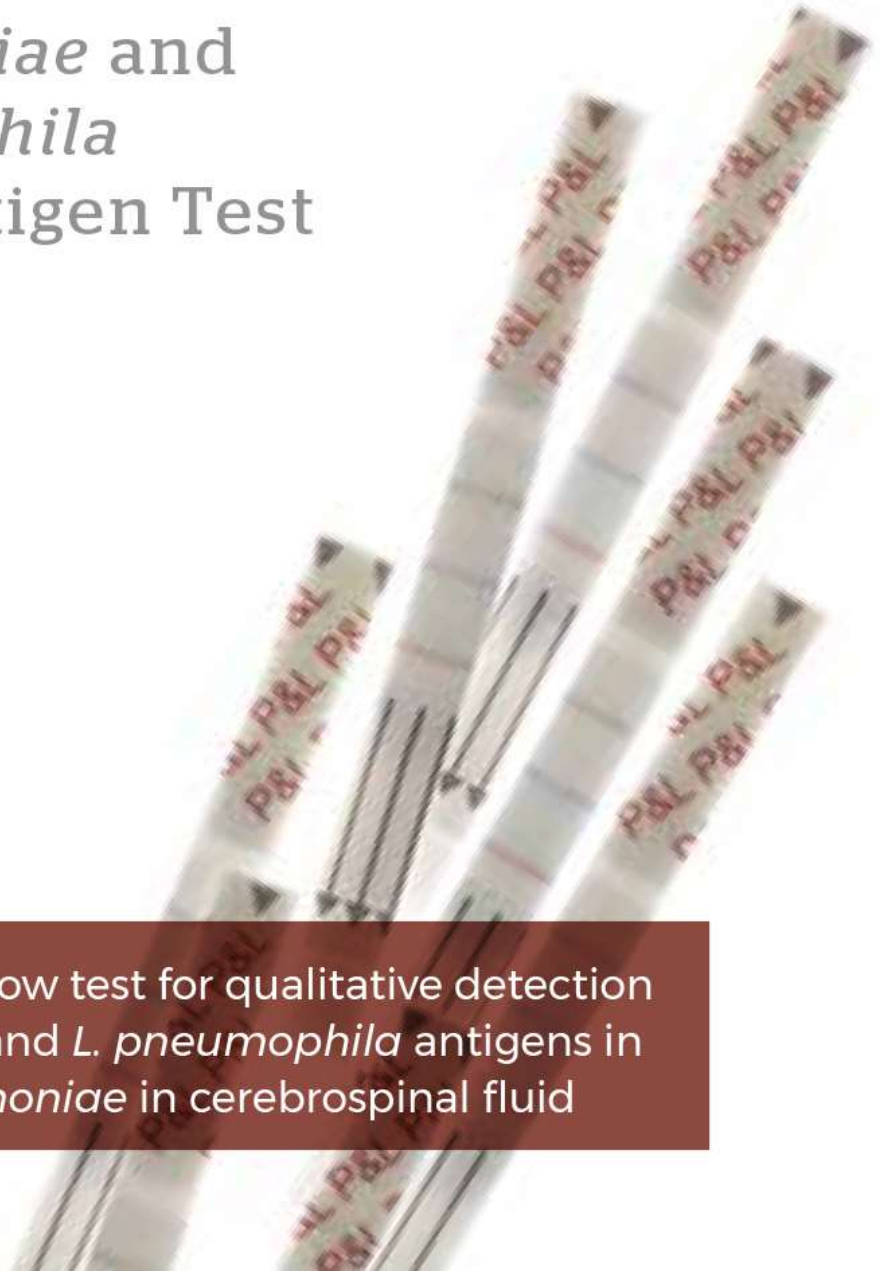
IMMUVIEW®

S. pneumoniae and *L. pneumophila* Urinary Antigen Test



ENGLISH

Combined lateral flow test for qualitative detection of *S. pneumoniae* and *L. pneumophila* antigens in urine and *S. pneumoniae* in cerebrospinal fluid

IMMUVIEW® S. PNEUMONIAE AND L. PNEUMOPHILA URINARY ANTIGEN TEST

For *in vitro* diagnostic use

Intended use

The ImmuView® *S. pneumoniae* and *L. pneumophila* Urinary Antigen Test is intended for diagnosis of *Streptococcus (S.) pneumoniae* and *Legionella (L.) pneumophila* infections by detection of urinary antigens for either or both *S. pneumoniae* and *L. pneumophila* serogroup 1. The assay is furthermore intended for diagnosis of *S. pneumoniae* infections by detection of *S. pneumoniae* antigen in cerebrospinal fluid (CSF). The test is a lateral flow test also known as a lateral flow immunochromatographic assay.

Description

ImmuView® *S. pneumoniae* and *L. pneumophila* Urinary Antigen Test is a rapid lateral flow test for qualitative detection of *S. pneumoniae* in human urine and CSF samples and *L. pneumophila* serogroup 1 antigens in human urine samples.

The test is effective in presumptive diagnosis of pneumococcal pneumonia caused by *S. pneumoniae* or *Legionella* pneumonia (Legionnaires' disease) caused by *L. pneumophila* serogroup 1, in conjunction with culture and other methods. Correct and early treatment is vital for the prognosis of both diseases and therefore quick methods to confirm both diseases in the initial phase are very important in order to initiate the proper antibiotic treatment as soon as possible.

Principle

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

Limitations

- ImmuView® *S. pneumoniae* and *L. pneumophila* Urinary Antigen Test has not been validated to be used with urine samples from children under 8 years.

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

- The sensitivity of ImmuView® *S. pneumoniae* and *L. pneumophila* Urinary Antigen Test when testing CSF samples has only been validated for *S. pneumoniae*.

- The diagnosis of a *S. pneumoniae* or *L. pneumophila* infection cannot be based on clinical or radiological evidence alone.

- A negative result does not exclude a *Legionella* infection, as it can be caused by other serogroups and *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.

- A negative result does not exclude a *S. pneumoniae* infection. The result of this test as well as culture, serology or other antigen detection methods should be used in conjunction with clinical findings to make an accurate diagnosis.

- Test of urine samples from patients that have been *S. pneumoniae* vaccinated during the last 6 days may cause false positive results in ImmuView® *S. pneumoniae* and *L. pneumophila* Urinary Antigen test.

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

- 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 *S. pneumoniae* and *L. pneumophila*
- 0.5 mL combined negative control for *S. pneumoniae* and *L. pneumophila*
- 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 8.

Materials Required but not Provided

- Timer
- Sterile standard urine or CSF collection containers/ transport tubes.

Sample Collection

Collect the urine sample in a sterile standard container (with or without boric acid as 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 (-20˚C) for at least 2 weeks. Make sure that samples always reach room temperature before testing. CSF samples should be tested as soon as possible after sampling or be stored frozen until testing is possible.

Procedure

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

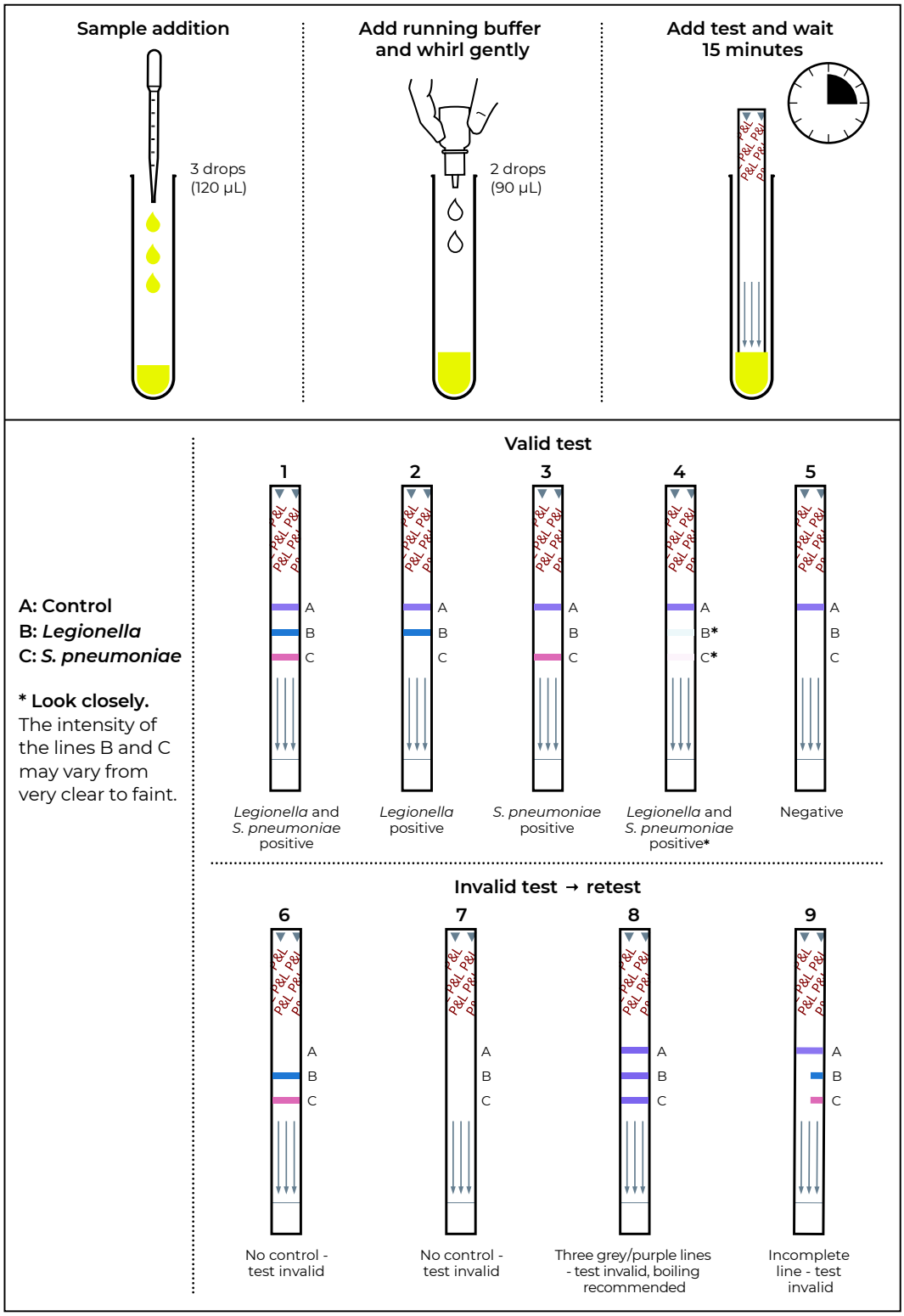
1. Bring the patient urine or CSF sample to room temperature. Whirl thoroughly prior to testing.*
2. Apply a test tube in the cardboard holder.
3. Fill the transfer pipette with urine or CSF and add 3 drops (120 µL) of sample to the test tube (hold the pipette vertically). **
4. Add 2 drops (90 µL) of 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 10 minutes.

** The test has also been validated for using only 10 µL CSF adding 200 µL running buffer.

*** 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: Legionella
C: S. pneumoniae
* 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*
Legionella and S. pneumoniae positive
Legionella positive
S. pneumoniae positive
Legionella and S. pneumoniae 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 purple/grey, but can also be more blue or red depending on whether the sample is positive for either *S. pneumoniae* or *L. pneumophila* serogroup 1. Only a full line indicates a positive result - dots do not indicate a positive result (see test result number 9, page 8).

A **positive sample for both *Legionella* and *S. pneumoniae*** will show a pink/red line in the bottom half of the test for *S. pneumoniae* positive followed by a blue line in the middle for *L. pneumophila* serogroup 1 positive, and at the top of the test a purple/grey control line will appear (see test result number 1, page 8).

A **positive sample for *Legionella*** will show a blue line for *L. pneumophila* serogroup 1 positive, and at the top of the test a purple/grey control line will appear (see test result number 2, page 8).

A **positive sample for *S. pneumoniae*** will show a pink/red line for *S. pneumoniae* positive, and at the top of the test a purple/grey control line will appear (see test result number 3, page 8).

Look closely. Even if there is a very faint line for either *Legionella* or *S. pneumoniae* 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.

A **negative sample** will show a single purple/grey control line in the top of the test (see test result number 5, page 8). A negative result does not exclude a *S. pneumoniae* or *Legionella* infection, see limitations.

Note: Three grey/purple test lines do not indicate a positive result.
(see test result number 8, page 8).

If three grey lines are observed the result can be confirmed by boiling the urine sample for approx. 10 minutes. Boiling can also be used for confirmation of a positive result as *Legionella* and *S. pneumoniae* antigens are heat stable. Remember to let the urine sample cool down to room temperature before retesting the sample.

If no control line is observed the test is **invalid** and the sample should be retested (see test results number 6 and 7, page 8).

Clinical Sensitivity and Specificity for urine

The clinical sensitivity of the *S. pneumoniae* test line was obtained by testing retrospective urine samples from patients with a blood culture positive sample for *S. pneumoniae*[1].

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 criterias[1].

The clinical specificity of the *S. pneumoniae* and *L. pneumophila* test lines was obtained by testing urine samples from patients with urinary tract infections and blood culture negative samples. Furthermore, no cross-reaction between *S. pneumoniae* and *L. pneumophila* serogroup 1 urine samples was detected.

Sensitivity values were calculated using Wilson confidence interval 95%.

Confirmed *S. pneumoniae* cases (71 samples)		
ImmuView®	positive	60
	negative	11
ImmuView® Sensitivity		85% (CL: 74-91%)
Comparator	positive	55
	negative	16
Comparator Sensitivity		77% (CL: 66-86%)

Confirmed *L. pneumophila* cases (99 samples)		
ImmuView®	positive	88
	negative	11
ImmuView® Sensitivity		89% (CL: 81-94%)
Comparator	positive	71
	negative	28
Comparator Sensitivity		72% (CL: 62-80%)

	Specificity
S. pneumoniae	99% (75/76 CL: 93-100%)
L. pneumophila	100% (76/76 CL: 95-100%)

Positive agreement with other UAT

S. pneumoniae positive agreement was made in a sample population containing blood culture positive samples. *L. pneumophila* positive agreement was made in a sample population containing culture and/or PCR positive samples. The positive agreement was calculated in accordance with Wilson confidential 95%, by diving the number of positive ImmuView® samples with the number of positive samples by the comparator.

S. pneumoniae **Blood culture positive samples**		Comparator positive	negative	Total
ImmuView®	positive	58	5	63
	negative	2	11	13
Total		60	16	76
Positive agreement		97% (58/60 CL: 89-99%)		

L. pneumophila **culture and/or PCR positive**		Comparator positive	negative	Total
ImmuView®	positive	64	10	74
	negative	1	8	9
Total		65	18	83
Positive agreement		98% (64/65 CL: 92-100%)		

Negative agreement with other UAT

S. pneumoniae negative agreement was made in a sample population containing non-pneumococcal cases (n = 90 bacteremic samples and n =6 non-bacteremic.)[2].

L. pneumophila negative agreement was made in a sample population prescreened with urinary antigen EIA. All samples included in the pool showed a negative result when using the comparator (urinary antigen EIA)[3].

96 non-pneumococcal cases		Comparator positive	Comparator negative	Total
ImmuView®	positive	3	0	3
	negative	0	93	93
Total		3	93	96
Positive agreement		100% (93/93 CL: 96-100%)		

Negative urinary antigen EIA directed at *L. pneumophila* sg. 1		Comparator positive	Comparator negative	Total
ImmuView®	positive	0	0	0
	negative	0	456	456
Total		0	456	456
Positive agreement		100% (456/456 CL: 96-100%)		

Analytical Sensitivity and Specificity for urine samples

To determine the analytical sensitivity and specificity of the ImmuView® *S. pneumoniae* and *L. pneumophila* Urinary Antigen Test a panel of the 92 *S. pneumoniae* serotypes, the 8 subgroups of *L. pneumophila* serogroup 1, 16 *L. pneumophila* non-serogroup 1, 4 *Legionella* species, and a panel of 116 potential cross-reactants (see table on page 15) were tested. No cross-reactions were detected. The panel of 116 potential cross-reactants was spiked in negative urine at a concentration of 10^7 CFU/mL.

The analytical test performance is:
Sensitivity (n = 100) 100%. Specificity (n = 116) 100%

Acinetobacter (4)	*L. catenaforme*	*S. mutans*
B. subtilis	*L. rhamnosus*	*S. parasanquis*
B. pertussis	*L. monocytogenes*	*S. sanquis*
B. catarrhalis	*M. morganii*	*S. saprophyticus*
C. albicans (4)	*M. olsoensis*	*S. thomson*
C. aquaticum (2)	*N. cineria*	*S. typhimurium*
Corynebacterium sp.	*N. gonorrhoeae (3)*	*S. marcescens*
E. cloacea (4)	*N. lactamica*	*S. aureus (6)*
E. coli (10)	*N. meningitidis*	*S. epidermidis (5)*
E. faecalis (5)	*N. polysak*	*S. saprophyticus*
E. faecium	*P. mirabilis (2)*	*S. maltophilia*
E. durans	*P. vulgaris (2)*	*Streptococcus group A (2)*
G. vaginalis	*Pseudomonas (2)*	*Streptococcus group B (10)*
H. influenzae (11)	*Ps. aeruginosa (4)*	*Streptococcus group C*
H. parainfluenzae	*Ps. stutzeri*	*Streptococcus group F*
K. oxytoca (2)	*S. bredeney*	*Streptococcus group G*
K. pneumoniae (3)	*S. epidermidis*	*Streptococcus group L*
Lactobacillus	*S. glostrup*	

Clinical Sensitivity and Specificity for CSF

The sensitivity of the *S. pneumoniae* test line was obtained by testing 12 CSF samples which were culture positive *S. pneumoniae* and 15 CSF samples spiked with *S. pneumoniae*. The specificity of the *S. pneumoniae* test line was obtained by testing 170 negative CSF samples from negative donors.

	Sensitivity	**Specificity**
S. pneumoniae	100% (27/27)	98.8% (168*/170)
L. pneumophila	N/A	100% (170/170)

* 2 samples were tested positive and confirmed positive with both another lateral flow test for *S. pneumoniae* and ImmuLex *S. pneumoniae* Omni. It was not possible to culture any bacteria from the samples, which can be caused by too many times of freezing and thawing of the sample.

The sensitivity of the *L. pneumophila* test line was not validated as only one case of *Legionella* meningitis has been reported. The specificity of the *L. pneumophila* test line was 100% (170/170).

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.

Presafe Denmark A/S

Quality System DS/EN ISO 13485

CE

REF 95389

References

1. Jørgensen, Uldum, Sørensen, Skovsted, Otte, Elverdal. (2015) "Evaluation of a new lateral flow test for detection of *Streptococcus pneumoniae* and *Legionella pneumophila* urinary antigen." J Microbiol Methods. 116 (2015): 33-36.

2. Athlin, Iversen, Özenci. (2017) "Comparison of the ImmuView and the BinaxNOW antigen tests in detection of *Streptococcus pneumoniae* and *Legionella pneumophila* in urine". Eur J Clin Microbiol Infect Dis. 2017 Jun 6. Epub 2017 Jun 6.

3. D. Lindsay et al. 2014, Poster on ESGLI 2016: Evaluation of the ImmuView® urinary antigen test for the detection of *Legionella pneumophila* and *Streptococcus pneumoniae*.

Information and Ordering

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