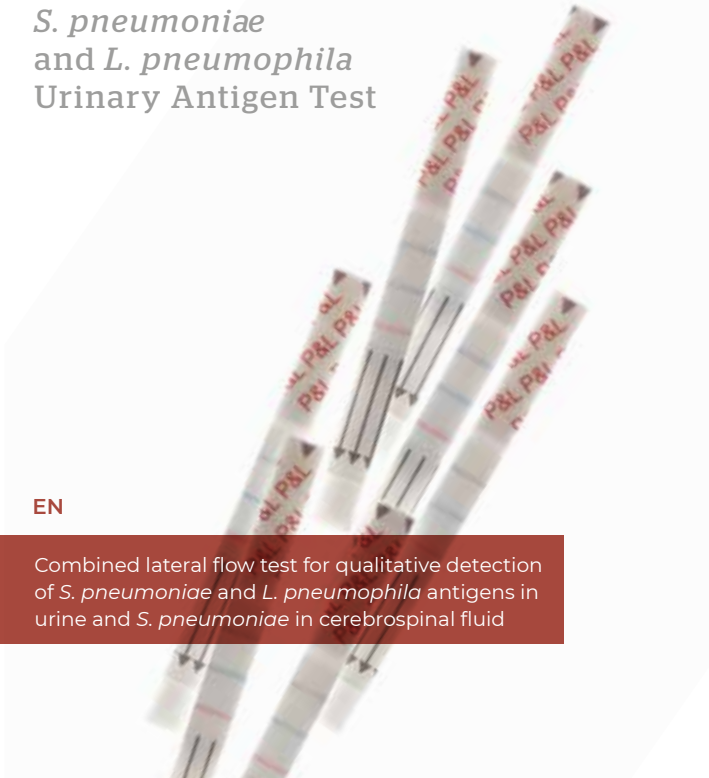
SSI DIAGNOSTICA

Instructions for use

IMMUVIEW®

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



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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 an *in vitro* rapid lateral flow test, also known as a lateral flow immunochromatographic assay, intended for the qualitative detection of *Streptococcus pneumoniae* and *Legionella pneumophila* antigens in urine specimens from patients with symptoms of pneumonia. The assay is intended to aid in diagnosis of *S. pneumoniae* and *L. pneumophila* serogroup 1 infections. The assay is further intended to aid in the diagnosis of *S. pneumoniae* infections by detection of *S. pneumoniae* antigen in cerebrospinal fluid (CSF). Results from the ImmuView® *S. pneumoniae* and *L. pneumophila* Urinary Antigen Test should be interpreted in conjunction with the patient's clinical evaluation and other diagnostic methods.

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* (primarily 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*, 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.

Precautions

- The presence of partial lines and dots represent **invalid** test results. The patient sample should be re-tested.
- Ensure that the test running buffer is added to all the test tubes and verified as present. **False positive results can occur if no running buffer is added to the test tubes**.
- 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

CONTROL + · 0.5 mL combined positive control for *S. pneumoniae* and *L. pneumophila*

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

RUN BUFF · 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 seven.

Materials required but not provided

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

Storage and stability

Please find the information on the box and labels.

Preservatives

The use of boric acid or piperazine-N,N'-bis(2-ethanesulfonic acid) (PIPES) do not interfere with the ImmuView® *S. pneumoniae* and *L. pneumophila* Urinary Antigen Test and can be used.

Sample collection and storage

Collect the urine sample in a sterile standard container (with or without boric acid 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). 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. Follow your laboratory procedures for long term storage of CSF samples.

Quality control

The positive and negative controls provided with ImmuView® *S. pneumoniae* and *L. pneumophila* 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 the kit is used 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 the procedure described in this IFU.

Procedure

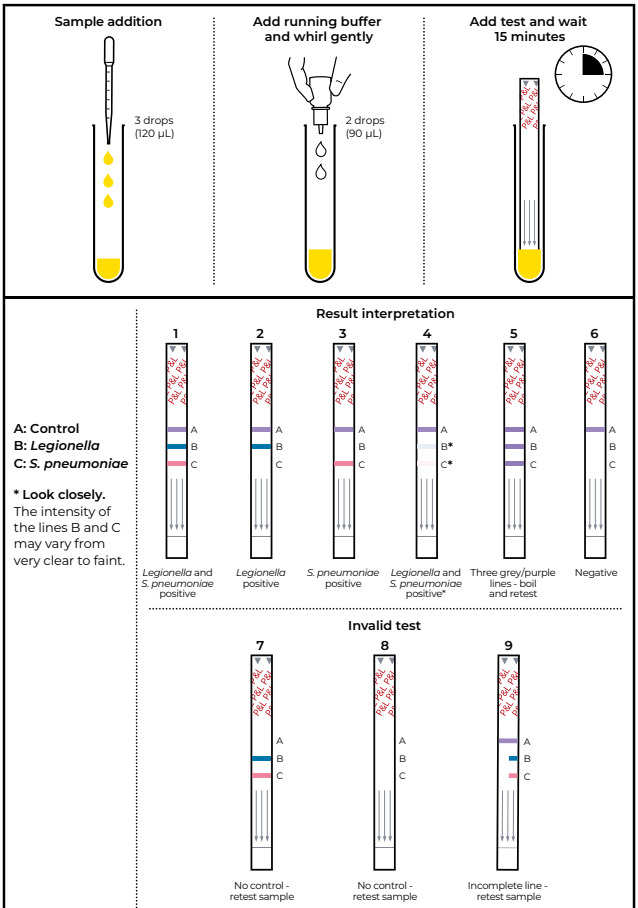
The positive and negative controls should follow the same procedure as if it was a urine or a CSF sample. The positive control should be visible at the control test line and both the *S. pneumoniae* and *L. pneumophila* test lines. The negative control should only be visible at the control line.

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 container with test, 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[1,2] the sample for 5 minutes 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: Legionella
C: S. pneumoniae
* 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*
Legionella and S. pneumoniae positive
Legionella positive
S. pneumoniae positive
Legionella and S. pneumoniae positive*
Three grey/purple 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 purple/grey but can also be more blue or red depending on whether the sample is positive for either *S. pneumoniae* or *L. pneumophila*.

A positive sample for *S. pneumoniae* and *L. pneumophila*

A pink/red line for *S. pneumoniae* and a blue line for *L. pneumophila* and a purple/grey control line (see test results number 1 and/or number 4, page 7).

A positive sample for *L. pneumophila*

A blue line and a purple/grey control line at the top of the test (see test result 2, page 7). A positive result for *L. pneumophila* in CSF should be investigated further, if repeatedly positive for *Legionella*.

A positive sample for *S. pneumoniae*

A pink/red line and a purple/grey control line at the top of the test (see test result 3, page 7).

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 7). The enclosed "Scorecard" can help to determine if the test result is positive or negative.

Note: Three grey/purple test lines do not indicate a positive result (see test result number 5, page 7). There can be colour differences between line A (the control line) and B/C (*S. pneumoniae/L. pneumophila*). If line B and C are the same colour, boil for approximately five (5) minutes and retest. Remember to let the urine sample cool down to room temperature before retesting the sample.[1, 2]

Boiling can also be used for confirmation of a positive result as *Legionella* and *S. pneumoniae* antigens are heat stable.

A negative sample

A purple/grey control line at the top of the test (see test result 6, page 7).

Invalid sample

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

Only a full line indicates a positive result - dots and half lines do not indicate a positive result (see test result number 9, page 7).

Disposal

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

Limitations

- Current American Academy of Pediatrics (AAP) Red Book recommendations note that detection of *S. pneumoniae* is not useful in children because asymptomatically colonised children may have positive test results.

- ImmuView® *S. pneumoniae* and *L. pneumophila* Urinary Antigen Test has been validated using urine and CSF specimens only. Other specimens (e.g. serum, plasma or other body fluids) can cause false results and should **not** be tested.

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

- A negative result does not exclude the possibility of 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 an *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.

- *S. pneumoniae* vaccine may cause false positive results up to 10 days after vaccination.

- Administration of antibiotics might influence the test results for *S. pneumoniae*.

- The ImmuView® *S. pneumoniae* and *L. pneumophila* Urinary Antigen Test is only validated for *S. pneumoniae* detection in CSF samples. A positive result for *L. pneumophila* in CSF should be investigated further if the CSF sample is repeatedly positive for *Legionella*.

- False results may occur from highly basic (pH≥9) urine and give false positive *S. pneumoniae* results. Water-based personal lubricant might result in false positive or grey *L. pneumophila* lines when found in the sample at high levels.

Clinical sensitivity and specificity for urine samples (retrospective study)

To determine the sensitivity of the ImmuView® *S. pneumoniae* and *L. pneumophila* Urinary Antigen Test, 100 frozen urine samples from patients originally determined to be infected with ***S. pneumoniae*** were tested. All 100 urine samples came from Europe, and all were from blood culture positive patients; forty-eight (48) samples were from Sweden[3] and fifty-two (52) samples were from Denmark.

To determine the sensitivity of the ImmuView® *S. pneumoniae* and *L. pneumophila* Urinary Antigen Test, 98 stored frozen urine samples from patients with a culture confirmed ***Legionella*** infection were tested. A total of 55 urine samples came from Europe. The remaining 43 urine samples came from the United States (U.S.), and these were also determined to be previously positive in a urinary antigen test.

The clinical specificity of the ImmuView® *S. pneumoniae* and *L. pneumophila* test lines was obtained by testing **known negative** (culture confirmed negative) urine samples collected from 3 sites, one in the U.S. and two in Europe.

Table 1

***S. pneumoniae* culture verified vs ImmuView®**		
	Culture positive	Culture negative
ImmuView® positive	78	4
ImmuView® negative	22	217
Total	100	221
ImmuView® sensitivity	78.0%	95%CI (69.0-85.0%)
ImmuView® specificity	98.1%	95%CI (95.4-99.3%)

Table 2

***L. pneumophila* culture verified vs ImmuView®**		
	Culture positive	Culture negative
ImmuView® positive	86	1
ImmuView® negative	12	239
Total	98	240
ImmuView® sensitivity	87.8%	95%CI (79.8-92.9%)
ImmuView® specificity	99.6%	95%CI (97.7-99.9%)

Table 3

Sensitivity (urine) based on culture vs comparator		
	ImmuView®	Comparator
S. pneumoniae (blood culture only)	78% (78/100) (CI 67-85%)	80% (76/95[a]) (CI 71-87%)
L. pneumophila Sg 1 (U.S.)	97.7% (42/43) (CI 88-100%)	100% (43/43) (CI 92-100%)
L. pneumophila Sg 1 (Europe)	80.0% (44/55) (CI 68-88%)	66.7% (36/54[b]) (CI 53-78%)
Specificity (urine) based on culture vs comparator		
	ImmuView®	Comparator
S. pneumoniae (Europe)	98.2% (217/221[c]) (CI 95-99%)	97.8% (218/223) (CI 95-99%)
L. pneumophila (U.S.)	100% (19/19) (CI 83-100%)	100% (19/19) (CI 83-100%)
L. pneumophila (Europe)	99.5% (220/221[d]) (CI 97-100%)	99.6% (223/224) (CI 98-100%)

a: 5 samples were QNS (quantity not sufficient) for testing, b: 1 sample was QNS for testing, c: 3 samples were QNS for testing, d: 3 samples were QNS for testing

S. pneumoniae sensitivity (Europe) increased to 81/100 or 81% for ImmuView® *S. pneumoniae* and *L. pneumophila* Urinary Antigen Test compared with comparator that after boiling had 76/95 or 80%. *L. pneumophila* sensitivity (Europe) changed to 41/55 or 74.6% for ImmuView® and remained 36/54 or 66.7% for the comparator. The specificity (Europe) increased to 98.6% (218/221) and 100% (221/221) for *S. pneumoniae* and *L. pneumophila* respectively after boiling when using ImmuView®. The comparator did not change after boiling. *L. pneumophila* sensitivity (U.S.) increased to 43/43 or 100% (95%CI 91.8-100%) in the ImmuView® Test for *L. pneumophila* after boiling[1,2]. *L. pneumophila* specificity (U.S.) did not change after boiling for either test.

Positive and negative percent agreement for urine samples (prospective study)

In a prospective study three-hundred-six (306) prospective collected urine samples from two different sites (Spain and Denmark) were tested with both the ImmuView® *S. pneumoniae* and *L. pneumophila* Urinary Antigen Test and the Comparator tests. Fresh* urine samples were from patients (all comers) at risk of having community acquired pneumonia. The results were compared with other lateral flow urine antigen tests (comparator).

Table 4

Prospective samples positive agreement *S. pneumoniae*			
ImmuView®	Comparator positive	Comparator negative	Total
Positive	72	6	78
Negative	3	225	228
Total	75	231	306
Positive agreement	96.0%	95% CI (88.9%-98.6%)	
Negative agreement	97.4%	95% CI (94.5%-98.8%)	
Prospective samples positive agreement *L. pneumophila* SG1			
ImmuView®	Comparator positive	Comparator negative	Total
Positive	3	0	3
Negative	0	303	303
Total	3	303	306
Positive agreement	100.0%	95% CI (43.9%-100%)	
Negative agreement	100.0%	95% CI (98.8%-100%)	

* Of the 306 samples, a total of 92 had to be frozen before testing could be performed.

The positive agreement for *S. pneumoniae* was 72/75 or 96% (88.9-98.6%). The negative agreement for *S. pneumoniae* was 226/232 or 97.4% (94.5-98.8). The positive agreement for *L. pneumophila* was 3/3 or 100% (43.9-100%). Negative agreement for *L. pneumophila* was 304/304 or 100% (98.8-100%).

After boiling[1,2] the positive and negative agreement for *S. pneumoniae* and *L. pneumophila* remained the same.

Analytical studies-urine

Specificity (cross-reactivity)

To determine the analytical specificity of the ImmuView® *S. pneumoniae* and *L. pneumophila* Urinary Antigen Test for cross-reactivity with urines spiked with whole cell bacteria and different inactivated viruses (N=143). The whole cell bacterial panel was tested in a 10^7 CFU/mL diluted from a stock solution. The viral panel had a concentration of 10^5 TCID50/mL. The panel was also tested in negative urine.

Table 5

Organisms tested for interference	
Acinetobacter spp. (4)	*Morganella morganii*
Adenovirus 2	*Moraxella catarrhalis*
Bacillus subtilis	*Moraxella osloensis*
Bordetella pertussis	*Mycoplasma genitalium*
Candida albicans (4)	*Neisseria gonorrhoeae* (3)
Chlamydia trachomatis	*Neisseria lactamica*
Chlamydophila pneumoniae (2)	*Neisseria meningitidis*
Citrobacter freundii	*Neisseria polysaccharea*
Corynebacterium sp.	Parainfluenza virus 1,2,3 (3)
Corynebacterium urealyticum	*Proteus mirabilis* (2)
Cytomegalovirus	*Proteus vulgaris*
Escherichia coli (10)	*Pseudomonas aeruginosa* (4)
Enterobacter cloacae (3)	*Pseudomonas* spp. (2)
Enterococcus durans	*Pseudomonas stutzeri*
Enterococcus faecalis (7)	Respiratory Syncytial Virus A
Enterococcus faecium	*Salmonella* Bredeney
Enterovirus D68	*Salmonella* Glostrup
Gardnerella vaginalis	*Salmonella* Thompson
Haemophilus influenzae type a-f and non-caps (11)	*Salmonella* Typhimurium

Haemophilus parainfluenzae	*Serratia marcescens*
Herpes Simplex Virus 1,2	*Staphylococcus aureus* (6)
Influenza A (H1N1 and H3N2) virus	*Staphylococcus epidermidis* (5)
Influenza B virus	*Stenotrophomonas maltophilia*
Klebsiella oxytoca (2)	*Streptococcus* gr. A, B, C, F, L and G (16)
Klebsiella pneumoniae (3)	*Streptococcus mitis*
Lactobacillus catenaformis	*Streptococcus mutans* (2)
Lactobacillus rhamnosus	*Streptococcus parasanguinis*
Lactobacillus sp.	*Streptococcus sanguinis*
Listeria monocytogenes	*Staphylococcus saprophyticus* (3)

All of the above bacterial isolates were negative when using ImmuView® *S. pneumoniae* and *L. pneumophila* Urinary Antigen Test. The only potential cross-reactivity was 1 of 3 isolates of *E. cloacae* which was positive for *L. pneumophila*. This was confirmed on re-testing of that one isolate.

A total of 19 urinary tract infections from patients were tested. Previously culture results had shown that eight (8) of them were infected with *Escherichia coli*, five (5) with *Staphylococcus aureus*, five (5) with *Streptococcus agalactiae* gr. B and one (1) with *Candida albicans*. None showed any cross reactions with the ImmuView® test.

Sensitivity (limit of detection (LOD))

The limit of detection (LOD) for the ImmuView® *S. pneumoniae* and *L. pneumophila* Urinary Antigen Test is 62.5 pg/mL for purified *S. pneumoniae* CWPS antigen (native). For LPS specific for *L. pneumophila* SG1 (Philadelphia) the LOD is 25 ng/mL. Whole cell *S. pneumoniae* bacteria can be detected at an LOD at 10^5 CFU/mL and *L. pneumophila* SG1 (Philadelphia) has a LOD at 10^4 CFU/mL. Boiling or urine preservatives did not change these results.

Table 6

Stock solution	LOD
S. pneumoniae antigen	62.5 pg/mL
L. pneumophila SG 1 (Philadelphia) antigen	0.025 µg/mL
L. pneumophila SG 1 (Bellingham) antigen	0.5 µg/mL
S. pneumoniae (serotype 1)	10^5 CFU/mL
L. pneumophila SG1 (Philadelphia)	10^4 CFU/mL
L. pneumophila SG 1 (Bellingham)	10^5 CFU/mL

Strain reactivity

Isolates from different *S. pneumoniae* serotypes were also positive tested with the ImmuView® assay including serotype three (3), five (5), and thirty-seven (37). Different species of *L. pneumophila* were also found to be positive using the assay. Within serogroup one (1) these includes Philadelphia, Knoxville, OLDA/Oxford, Allentown/France, and Benidorm-Strain Lens. Additional studies have found other *Legionella* serogroups to be positive such as serogroup 3, 6, 8, 10 and 12.

Table 7

***Streptococcus pneumoniae* in urine**		
Serotype	Antigen concentration (µg/mL)	Whole organism Concentration (CFU/mL)
1	ND*	10^4
3	0.001	10^4
5	0.010	10^5
37	0.0001	ND*

***Legionella pneumophila* in urine**				
Serogroup	Pontiac/ non-pontiac	Species	Concentration (µg/mL)	Concentration (CFU/mL)
1	Pontiac	Knoxville	0.100	10^5
1	Pontiac	Allentown/France	0.005	ND*
1	Pontiac	Benidorm	ND	10^4
1	Pontiac	Philadelphia	0.010	10^4
1	Non-Pontiac	OLDA/Oxford	0.001	ND
1	Non-Pontiac	Camperdown	0.315	ND
1	Non-Pontiac	Heysham	1.250	ND
3			250	ND
6			250	ND
8			250	ND
10			250	ND
12			7.8	ND

*ND=Not done

Interfering substances

ImmuView® *S. pneumoniae* and *L. pneumophila* Urinary Antigen Test was tested with forty-seven (47) interfering agents at different concentrations in urine samples.

Table 8

Agent	Concentration
Acetaminophen	0.1mg/mL
Acetylsalicylic acid	0.1mg/mL
Amantadine	0.03mg/mL
Amoxicillin	0.075mg/mL
Amphotericin B	0.22mg/mL
Antihistamine	0.22mg/mL
Ascorbic acid (C-Vitamin)	1mg/mL
Augmentin (Amoxicillin Clavulanate)	0.22mg/mL
Azithromycin	0.012mg/mL
Beet root	20%
Beet root	1.17%
Beet root	0.01%
Bilirubin	0.2mg/mL
Bromhexine/cough drops/cough syrup	0.22mg/mL
Caffeine	15mg/mL
Chlorophyll	0.11mg/mL
Chlorophyll	0.04mg/mL
Chlorophyll	0.01mg/mL
Ciprofloxacin	0.22mg/mL
Corticosterone (Corticosteroids)	0.015mg/mL
Decongestant	0.22mg/mL
Erythromycin	0.067mg/mL
Glucose (H)	20mg/mL
Glucose (M)	10mg/mL
Glucose (L)	3mg/mL
Hemoglobin	5mg/mL
Human albumin	35mg/mL
Human red blood cells 10% Washed pooled cells	10%
Ibuprofen	0.1mg/mL
Itraconazole	0.22mg/mL

Agent	Concentration
Leukocytes	>250 cells/µL
Miconazole	5%
Mix (pH, whole blood, protein and glucose) (H)	
Mix (pH, whole blood, protein and glucose) (M)	
Mix (pH, whole blood, protein and glucose) (L)	
Mucin	0.086mg/mL
Oseltamivir (Tamiflu)	0.03mg/mL
Oxalic acid	0.01%
pH (acidic)	4
pH (neutral)	7
pH (basic)	9
Plasma	90%
Plasma	50%
Plasma	10%
Prednisone	0.22mg/mL
Protein (albumin) (H)	10mg/mL
Protein (albumin) (M)	5mg/mL
Protein (albumin) (L)	0.6mg/mL
Pyridium	1mg/mL
Rifampicin	0.09mg/mL
Spinach	1%
Tobacco purified	0.4mg/mL
Triglycerides	4mg/mL
Urea	20mg/mL
Vaginal contraceptive gel	5%
Vancomycin	0.1mg/mL
Water-based personal lubricant	5%
White blood cells	10%
Whole blood	10%
Whole blood	15%

High concentration of plasma in urine may result in grey test lines. Additionally, basic (pH≥9) conditions in urine can give false positive *S. pneumoniae* lines. Water-based personal lubricant might result in false positive or grey *L. pneumophila* lines, however, this outcome seems dose-related.

Clinical sensitivity and specificity - CSF

The sensitivity of the *S. pneumoniae* test line was obtained by testing leftover CSF specimens from patients suspected of meningitis, see table 9. The test was furthermore, evaluated testing negative CSF samples spiked with *S. pneumoniae* at LoD.

Table 9

ImmuView®	Culture positive	Culture negative
Positive	13	7
Negative	1	162
Total	14	169
Sensitivity	92.9% (13/14)	95% CI (68.5%-98.7%)
Specificity	96.0% (162/169)	95% CI (91.7%-98.0%)

U.S.A Laboratory testing

Of the samples tested at the two U.S. labs, 9 were known positive for *S. pneumoniae* meningitis. One-hundred-thirteen (113) were negative human CSF samples. These samples were blinded, and the testing of the ImmuView® test was performed by three operators on different days to prevent test bias.

European Laboratory testing

Of the samples tested within Europe, 5 were known to be positive for *S. pneumoniae*. Of the total samples, 56 were negative CSF samples. These samples were blinded and the testing with the ImmuView® test was performed by one operator on different days to prevent test bias.

The sensitivity of ImmuView® *L. pneumophila* test line was not validated in this study. *Legionella* does not usually cause meningitis.

Spiked CSF testing

Additional human CSF samples were spiked at the LOD with *S. pneumoniae*

(N=50) and additional unspiked negative CSF samples (N=10) were tested with the Immuview® test and the comparator test. The sensitivity for both the ImmuView® test and the comparator test was 50/50 (100%) and the additional negative CSF samples used for blinding of the testing were negative 10/10 (100%) in both the ImmuView® test and the comparator test.

Table 10

60 real human CSF samples 50 spiked with *S. pneumoniae*			
ImmuView®	Comparator		
	Positive	Negative	Total
Positive	50	0	50
Negative	0	10	10
Positive agreement	100%	95% CI (92.9%-100%)	
Negative agreement	100%	95% CI (72.2%-100%)	

Analytical studies - CSF

Specificity (cross-reactivity)

ImmuView® *S. pneumoniae* and *L. pneumophila* Urinary Antigen Test was tested with a panel of 24 potential cross-reacting agents. No cross-reactions were detected for the *S. pneumoniae* or the *L. pneumophila* test lines.

Table 11

Organisms not affecting test performance in CSF	
E. coli (5)	*Neisseria meningitidis* Gr. B, D and W135 (3)
Haemophilus influenzae type a-f and non-caps (7)	*Staphylococcus aureus*
Listeria monocytogenes	*Streptococcus* Gr A
Measles	*Streptococcus agalactiae* (GBS) sg Ia, Ib, II, III (4)
	Streptococcus mitis

Sensitivity (limit of detection (LOD)) in CSF

ImmuView® *S. pneumoniae* and *L. pneumophila* analytical sensitivity was determined by limit of detection. Two different operators performed the dilutions and the testing. The dilutions were made with whole cell bacteria spiked in human CSF.

Table 12

CSF	LOD
S. pneumoniae	10^3 CFU/mL

Interference agents

ImmuView® *S. pneumoniae* and *L. pneumophila* Urinary Antigen Test was tested with forty-seven (47) interfering agents at different concentrations in artificial CSF either negative or spiked with either CWPS or *S. pneumoniae* 10^7 CFU/mL.

Table 13

Agent in CSF	Concentration	Agent	Concentration
Whole *S. pneumoniae* (Type 1)		**Negative Artificial CSF**	
Glucose (H)	1mg/mL	Glucose (H)	1mg/mL
Glucose (M)	0.5mg/mL	Glucose (M)	0.5mg/mL
Glucose (L)	0.1mg/mL	Glucose (L)	0.1mg/mL
Red blood cells (H)	15%	Red blood cells (H)	15%
Red blood cells(M)	10%	Red blood cells(M)	10%
Red blood cells (L)	5%	Red blood cells (L)	5%
Protein (H)	60mg/mL	Protein (H)	60mg/mL
Protein (M)	30mg/mL	Protein (M)	30mg/mL
Protein (L)	10mg/mL	Protein (L)	10mg/mL
White blood cells	$10.6x10^6$/mL	White blood cells	$10.6x10^6$/mL
White blood cells	$5.3x10^6$/mL	White blood cells	$5.3x10^6$/mL
White blood cells	$2.7x10^6$/mL	White blood cells	$2.7x10^6$/mL
White blood cells	$1.8x10^6$/mL	White blood cells	$1.8x10^6$/mL
White blood cells	$0.9x10^6$/mL	White blood cells	$0.9x10^6$/mL
		Bilirubin	
Antigen		Bilirubin	
Bilirubin	15%	Bilirubin	
Bilirubin	10%	Plasma	
Bilirubin	5%	Plasma	
Plasma	15%	Plasma	
Plasma	10%		
Plasma	5%		

Red blood cells may give false positive shadows on the *S. pneumoniae* line due to excessive red color. The other agents in the panel did not interfere with the test.

Reproducibility study

The ImmuView® *S. pneumoniae* and *L. pneumophila* Urinary Antigen Test demonstrated excellent overall reproducibility with 1,068 correct results out of 1,072 test results (99.6%), when tested with 10 members of real positive *S. pneumoniae* or *L. pneumophila* urine samples and negative urine samples; and artificial CSF positive spiked with *S. pneumoniae* isolates as well as negative artificial CSF samples. The ImmuView® Positive Control and Negative Control were also tested as blinded/masked panel members. The testing was performed for 5 days with a different kit lot at each site, two in the U.S. and one in Europe.

Table 14

Description	Correct results	Agreement
S. pneumoniae, moderate positive urine	90/90 positive	100.0%
S. pneumoniae, moderate positive CSF	89/89[1] positive	100.0%
S. pneumoniae, low positive spiked in artificial CSF	89/90[2] positive	98.9%
S. pneumoniae, low positive urine	90/90 positive	100.0%
L. pneumophila, moderate positive urine 2A	90/90 positive	100.0%
L. pneumophila, moderate positive urine 2B	88/89[3] positive	98.9%
L. pneumophila, low positive urine 1A	89/89[4] positive	100.0%
L. pneumophila, low positive urine 1B	89/90[5] positive	98.9%
Negative pooled urine	90/90 negative	100.0%
Negative artificial CSF	90/90 negative	100.0%
ImmuView® Pos Control	89/90[6] positive	98.9%
ImmuView® Neg Control	85/85[7] negative	100.0%
Summary	**1068/1072 correct**	**99.6%**

A total of 3 different lots were tested. Each site, using two operators (A and B) performed a total of 360 reproducibility tests and a grand total of 1,072 reproducibility results out of a total of 1,080 tests in the study using 6 operators. A total of 8 test results (0.7%) were determined to be invalid and were excluded and not re-tested. The panel members were blinded by changing of the panel member numbers and identity daily. The reading and interpretation of the reproducibility panels was performed visually. There were no statistical differences in reproducibility by lot, by site, by time or by operator.

1. Operator did not see a positive control band, so one sample was invalid as the package insert states that this is necessary before interpreting the result. The sample was not re-tested.
2. A visual *L. pneumophila* band was seen.
3. Operator interpreted band as *S. pneumoniae* positive instead of *L. pneumophila* positive. One sample was invalid due to dot (incomplete band) on the strip per the package insert and was not re-tested.
4. One sample was invalid due to an incomplete band in *S. pneumoniae* according to the package insert.
5. No *L. pneumophila* band present.
6. Operator interpreted *S. pneumoniae* band result as negative even though band was present.
7. Five samples excluded due to the presence of dots and incomplete bands. The samples were not re-tested.

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.

References

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3. Athlin S, Iversen A, Özenci V. 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;36(10):1933-1938. doi:10.1007/s10096-017-3016-6

Information and ordering

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2nd edition · February 2023 · 2498

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