

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.

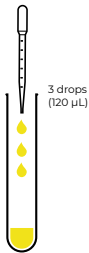
1. Bring the patient urine or CSF sample to room temperature. Whirl thoroughly prior to testing.*
2. Apply a test tube in the cardboard holder.
3. Fill the transfer pipette with urine or CSF and add 3 drops (120 μ L) of sample to the test tube (hold the pipette vertically).
4. Add 2 drops (90 μ L) of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

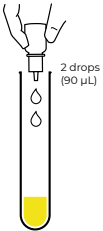
Quick Guide

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.


Valid test

1




Legionella and S. pneumoniae positive

2




Legionella positive

3




S. pneumoniae positive

4



Legionella and S. pneumoniae positive*


5



Negative


Invalid test → retest

6




No control - test invalid

7




No control - test invalid

8



Three grey/purple lines - test invalid, boiling recommended

9



Incomplete line - test invalid

Agent	Concentration
Leucocytes	>250 cells/ μ L
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 gray test lines. Additionally, basic (pH \geq 9) conditions in urine can give false positive *S. pneumoniae* lines. Water-based personal lubricant might result in false positive or gray *L. pneumophila* lines, however, this outcome seems dose-related.

ImmuView[®] test and the comparator test. The sensitivity for the 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 <i>S. pneumoniae</i>			
ImmuView [®]	Comparator		Total
	Positive	Negative	
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 were 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	
<i>E. coli</i> (5)	<i>Neisseria meningitidis</i> Gr. B, D and W135 (3)
<i>Haemophilus influenzae</i> type a-f and non-caps (7)	<i>Staphylococcus aureus</i>
<i>Listeria monocytogenes</i>	<i>Streptococcus</i> Gr A
Measles	<i>Streptococcus agalactiae</i> (GBS) sg Ia, Ib, II, III (4)
	<i>Streptococcus mitis</i>

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
<i>S. pneumoniae</i>	10 ³ CFU/mL

Interference agents

ImmuView[®] *S. pneumoniae* and *L. pneumophila* Urinary Antigen Test were tested with forty-seven (47) interfering agents at different concentrations in artificial CSF either negative or spiked with either CWPS or *S. pneumoniae* 10⁷ CFU/mL.

Agent in CSF	Concentration	Agent	Concentration
Whole <i>S. pneumoniae</i> (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
<i>S. pneumoniae</i> , moderate positive urine	90/90 Positive	100.0%
<i>S. pneumoniae</i> , moderate positive CSF	89/89 ¹ Positive	100.0%
<i>S. pneumoniae</i> , low positive spiked in artificial CSF	89/90 ² Positive	98.9%
<i>S. pneumoniae</i> , low positive urine	90/90 Positive	100.0%
<i>L. pneumophila</i> , moderate positive urine 2A	90/90 Positive	100.0%
<i>L. pneumophila</i> , moderate positive urine 2B	88/89 ³ Positive	98.9%
<i>L. pneumophila</i> , low positive urine 1A	89/89 ⁴ Positive	100.0%
<i>L. pneumophila</i> , 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 pack 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.

References

1. Rota MC, Fontana S, Montaña-Remacha C, et al. Legionnaires' disease pseudoepidemic due to falsely positive urine antigen test results. *J Clin Microbiol.* 2014;52(6):2279-2280. doi:10.1128/JCM.00493-14
2. Briones ML, Blanquer J, Ferrando D, Blasco ML, Gimeno C, Marín J. Assessment of analysis of urinary pneumococcal antigen by immunochromatography for etiologic diagnosis of community-acquired pneumonia in adults. *Clin Vaccine Immunol.* 2006;13(10):1092-1097. doi:10.1128/CVI.00090-06
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

Quality Certificate

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



R_x Only



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