

Evaluation of 16 urinary antigen tests for *Legionella pneumophila* serogroup 1 LPS detection

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INTRODUCTION

Legionnaires' disease (LD), caused by *Legionella pneumophila* represent 2–8% of community-acquired pneumonia with a fatality rate around 10%. Today, urinary antigen tests (UATs) detecting *Legionella pneumophila* serogroup 1 (Lp sg 1) lipopolysaccharide (LPS) account for 70-80% of LD diagnosis in Europe and United States. These methods, based on immunochromatographic (ICT), immunofluorimetric (IFT) lateral flow assays or enzyme immunoassays (EIA) are widely used in European labs. Many tests are commercialized, with overall sensitivity of 70-90% and specificity >95%. Some works showed different performances when comparing one to another. However, no study has been conducted to compare a large panel of UATs on the same samples.

AIMS

- Detection comparison of 9 *Legionella pneumophila* serogroup 1 (Lp1) LPS subgroups in surcharged urine samples (US) by 16 UATs
- Cross-reactivity evaluation with Lp sg 2-14 LPS

This work is part of a global study, led by ESGLI, aiming to compare the performance of 16 UATs on US in 9 European National Reference Centers for *Legionella*.

METHOD

LPS were chemically extracted from reference Lp1 Pontiac and non-Pontiac strains:
 - Pontiac: Philadelphia, Benidorm, Knoxville and France/Allentown
 - non-Pontiac: OLDA, Oxford, Heysham, Bellingham and Camperdown
 LPS from Lp2-14 were also purified
 Fixed amounts of LPS between 0.03 ng/mL and 30 ng/mL for Lp1 and 3, 30 or 3000 ng/mL for Lp2-14 were added to a pool of sterile urine (US) that tested negative with the 16 UATs. The tested concentration were chosen according to published data (1). LPS concentration was defined by 2-keto-3-deoxyoctonate (KDO) determination. They were assessed in one run or triplicates
 Tested UATs are listed on Table

Lateral flow assays		
Automatic Reader	Detection	Name of the test
YES	Immuno-chromatographic (ICT)	- BinaxNOW® <i>Legionella</i> Urinary Antigen Card (Abbott ARD) - ImmuView S. pneumoniae and L. pneumophila Urinary Antigen Test (SSI Diagnostica) - Operon, Simple/Stick Legio pneumo ou Simple/Stick Strep pneumo-Legio
	Immuno-fluorescence (IFT)	- Sofia <i>Legionella</i> FIA (Quidel / Eurobio Scientific) - Standard F <i>Legionella</i> Ag FIA (SD Biosensor, Orgentec)
NO	Immuno-chromatographic (ICT)	- Biosynex L. PNEUMOPHILA (Biosynex) - CerTest Biotec - Urising legionella color (servibio) - Immunocatch™ <i>Legionella</i> (EIKEN CHEMICAL CO., LTD) - Nadal® <i>Legionella</i> test 552022 (nal von minden) - Legionella Monlab Test (Monlab) - Legionella vitassay, single or with pneumococcus - Legionella K-Set (Coris Bioconcept) - TRU <i>Legionella</i> (Meridian Bioscience)
		Enzyme Immunoassays
NO	Spectrophotometric	- Binax™ <i>Legionella</i> Urinary Antigen EIA (Alere) - RIDASCREEN <i>Legionella</i> ELISA (R-biopharm)

RESULTS

AUTOMATIC vs OPTICAL READING

- Results for 0.03 ng/mL LPS concentration
- Detection of ≥ 5/8 LPS by 4/5 ICT or IFT with reader (Binax now, Immuvue, Operon and SD Biosensor)
 - Detection of ≥ 1/8 LPS by 3/9 ICT with optical reading (Certest, Monlab and Immunocatch)
 - No detection by 7/8 ICT with optical reading (Biosynex Vitassay Servibio Nadal, K-set Coris, Tru)
 → better performance for UAT with automatic reader

ICT and IFT vs EIA

- Results for 0.03 ng/mL LPS concentration
- Detection of ≥ 1/8 LPS by 2/2 EIA
 → EIA not more sensitive than ICT with reader

PONTIAC VS NON-PONTIAC LPS DETECTION

- Results for 0.3 ng/mL and 0.03 LPS concentration
- Detection by 13/16 UATs of ≥ 4/5 non-Pontiac strains at 0.3 ng/mL (4/16 for 0.03 ng/mL concentration)
 - Detection by 15/16 UATs of ≥ 2/4 Pontiac strains at 0.3 ng/mL (2/16 for 0.03 ng/mL concentration)
 → non-Pontiac LPS are well detected as previously shown (1)
 → Philadelphia and Benidorm better detection among Pontiac LPS

Lp sg 2 – 14 CROSS REACTIVITY

- Only 4 LPS were detected at 30 ng/mL concentration:
- Lp7 was detected by 10/16 UATs - Lp9 was detected by 2/16 UATs
 - Lp12 was detected by 4/16 UATs - Lp4 was detected by 1/16 UATs

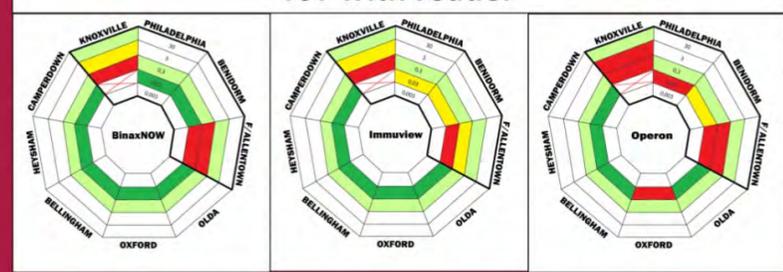
Other Lp2-14 LPS were detected at 3000 ng/mL concentration or non detected

CONCLUSIONS

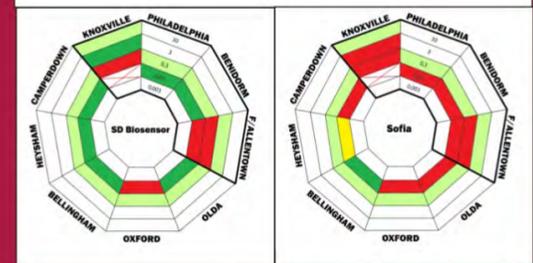
This study describes an easy method based on extracted LPS to compare UATs. ICTs with a reader were more sensitive, with the best sensitivity obtained for ICT with reader and one IFT. The two ELISA kits didn't provide a gain of sensitivity. Non-Pontiac LPS are globally detected at lower concentration than Pontiac LPS. Only 4 Lp2-14 LPS were detected at a concentration 100 times higher than Lp1 subgroups.

However, our results do not reflect the real LPS concentration in clinical US. That limitation should be acknowledged with the comparison of the 16 UATs in LD patients US.

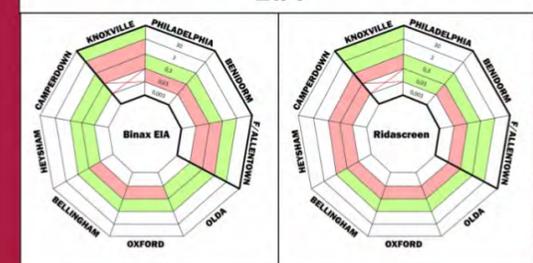
ICT with reader



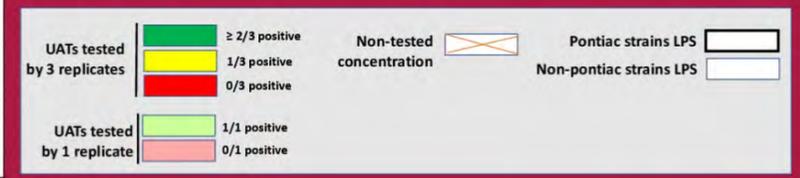
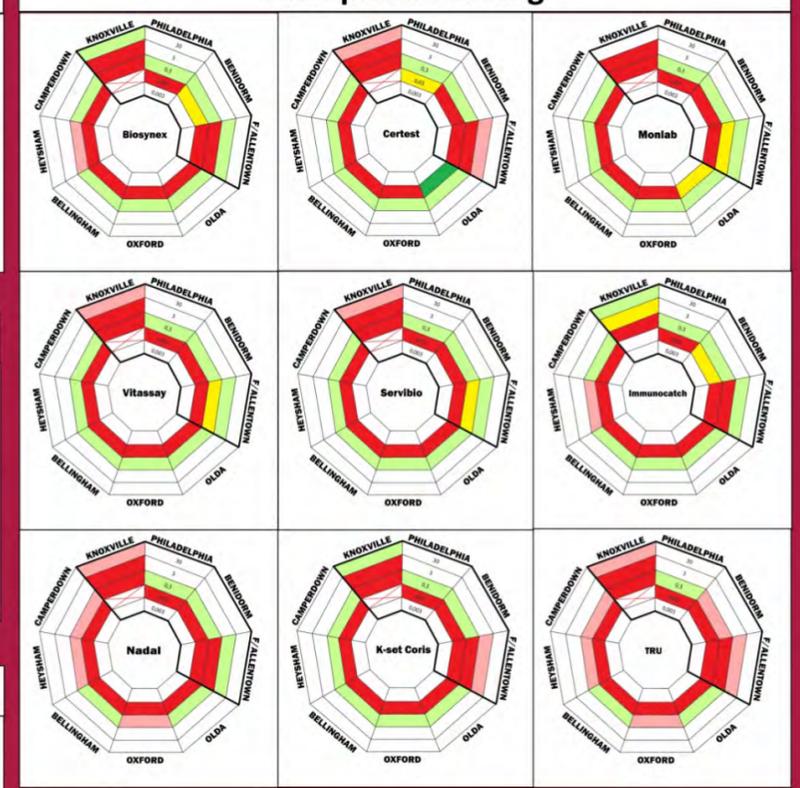
IFT



EIA



ICT optical reading



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