



Detection and quantification of capsular polysaccharide of  
*Burkholderia pseudomallei* in melioidosis patient urine samples

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## *Disclosure statement*

I currently, and have in the past, collaborated with InBios International, Inc. (Seattle WA). My university licenses technologies to InBios that are produced by my laboratory.



- ❖ Biomarker discovery
- ❖ Hybridoma facility
- ❖ mAb analysis
- ❖ BSL3/ABSL3
- ❖ Lateral flow fabrication

### Research areas

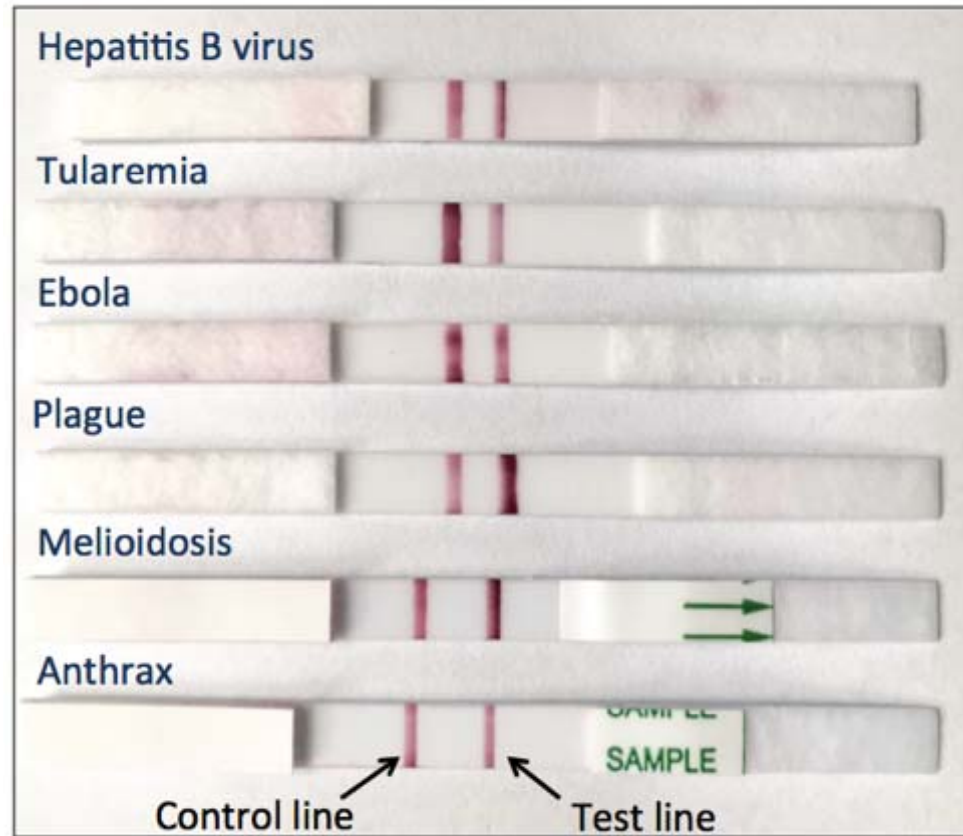
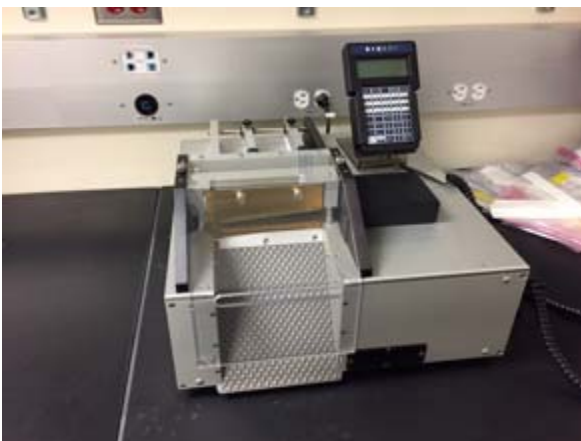
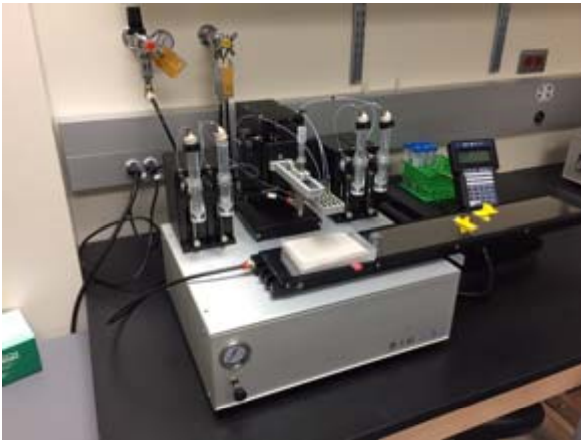
- Melioidosis - *Burkholderia pseudomallei*
- Anthrax - *Bacillus anthracis*
- Tularemia - *Francisella tularensis*
- Plague - *Yersinia pestis*
- Whooping cough - *Bordetella pertussis*
- Leptospirosis - *Leptospira spp.*
- Lyme disease - *Borrelia burgdorferi*




- ❖ University startup
- ❖ On campus
- ❖ STTR/SBIR funding

- Ebola
- Chikungunya virus
- Lassa Fever Virus
- Hantavirus
- Hepatitis B
- VEEV

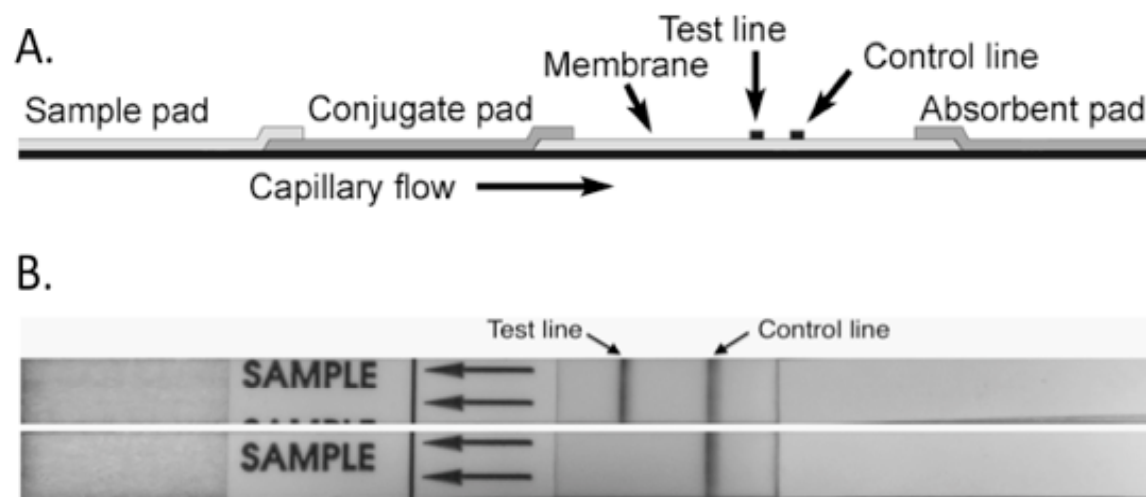
# LFI fabrication



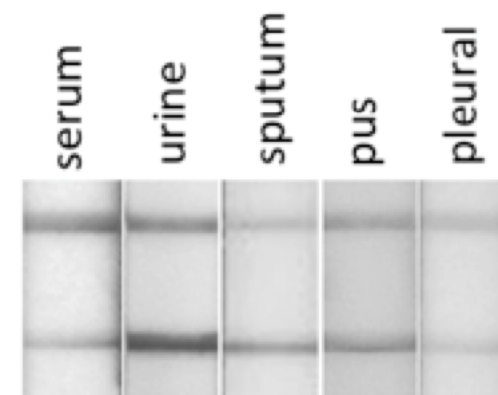
## Development of a Prototype Lateral Flow Immunoassay (LFI) for the Rapid Diagnosis of Melioidosis

Raymond L. Houghton, Dana E. Reed, Mark A. Hubbard, Michael J. Dillon, Hongjing Chen, Bart J. Currie, Mark Mayo, Derek S. Sarovich, Vanessa Theobald, Direk Limmathurotsakul, Gumphol Wongsuvan, Narisara Chantratita, Sharon J. Peacock, Alex R. Hoffmaster, Brea Duval, Paul J. Brett, Mary N. Burtnick, David P. AuCoin  [ [view less](#) ]

Published: March 20, 2014 • <https://doi.org/10.1371/journal.pntd.0002727>



### Melioidosis patient samples

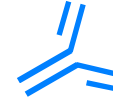


Pre-submission to the FDA January 2019

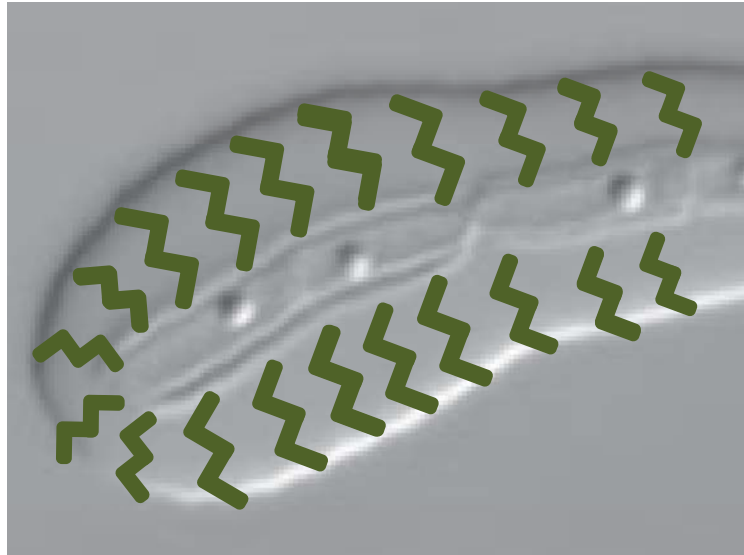
DETECTION



DETECTION



DETECTION



DETECTION



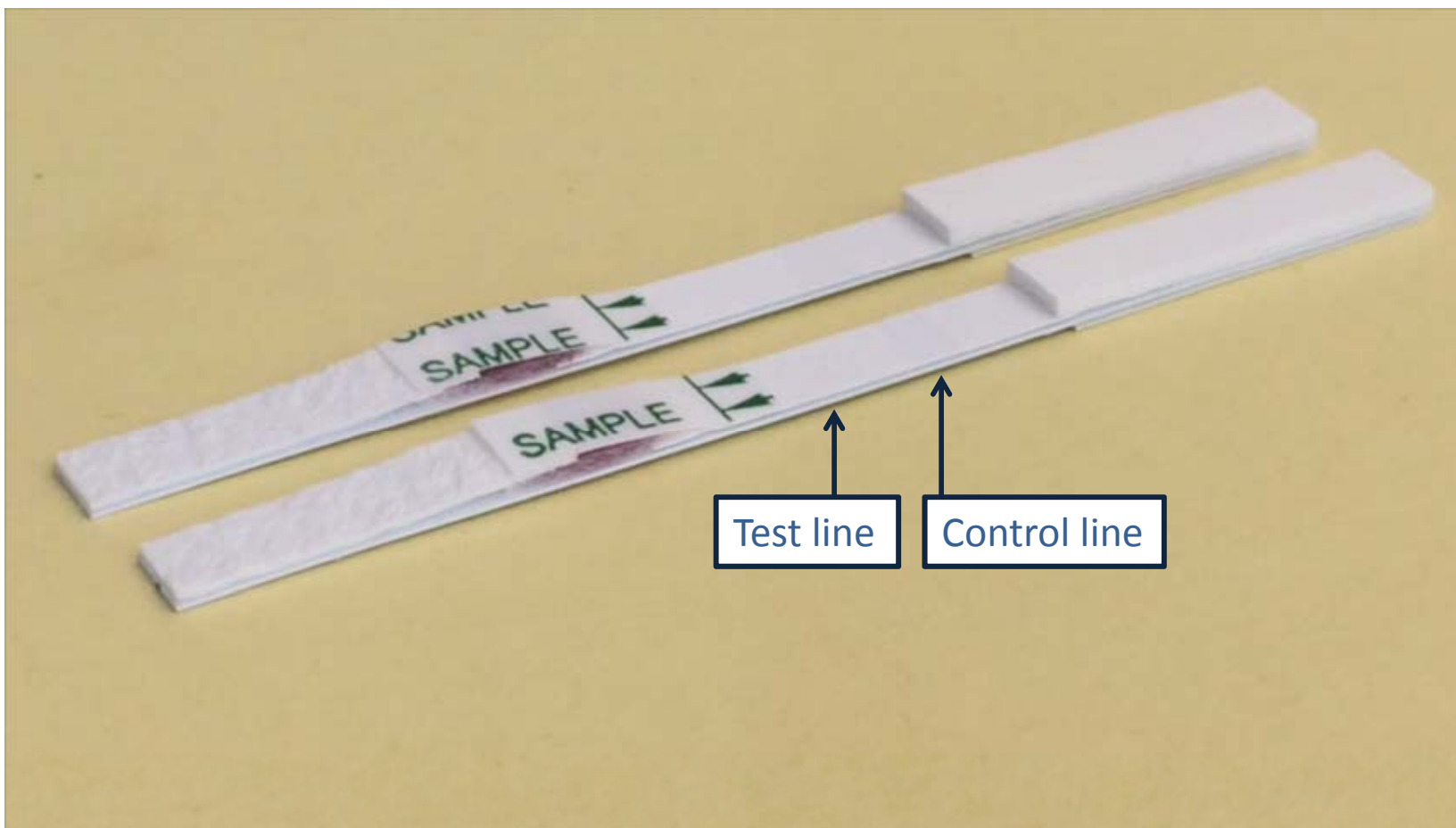
CPS – capsular polysaccharide



Monoclonal antibody (mAb)



DETECTION



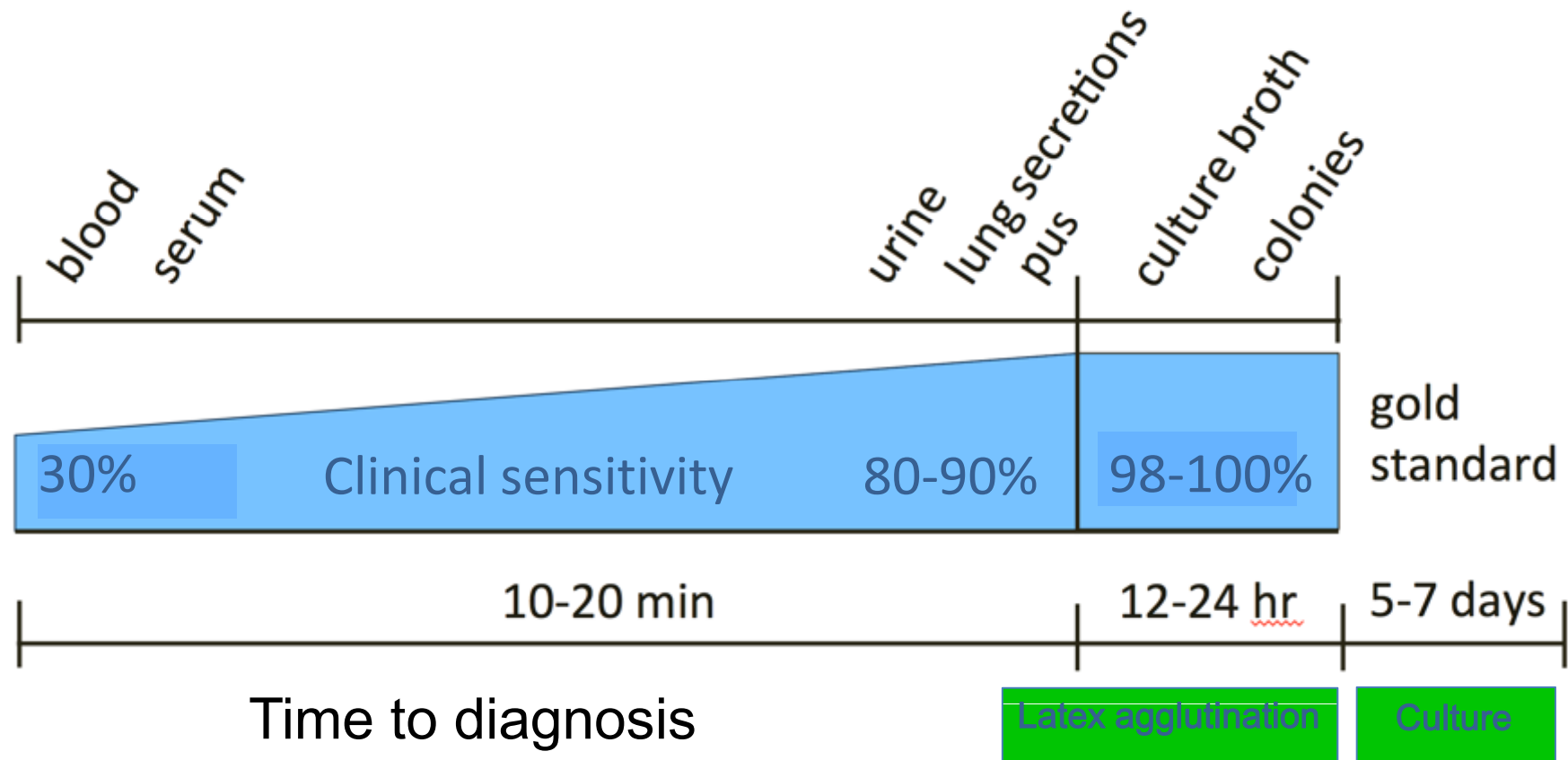


CPS





# AMD LFI sensitivity estimation





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## Journal of Clinical Microbiology

### Evaluation of a rapid diagnostic test for the detection of *Burkholderia pseudomallei* in the Lao People's Democratic Republic

Kate L Woods, Latsaniphone Boutthasavong, Caoimhe NicFhogartaigh, Sue J Lee, Viengmon Davong, David P AuCoin, David AB Dance

- 814 cases since 1999 at LOMWRU
- ~100 cases/year
- mortality 40-50%
- 412 blood cultures tested with AMD LFI
- Compared to latex agglutination and IFA



- AMID LFI was 99% (99/100) sensitive and 100% (308/308) specific on turbid blood culture bottles





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## Journal of Clinical Microbiology

### Evaluation of a rapid diagnostic test for the detection of *Burkholderia pseudomallei* in the Lao People's Democratic Republic

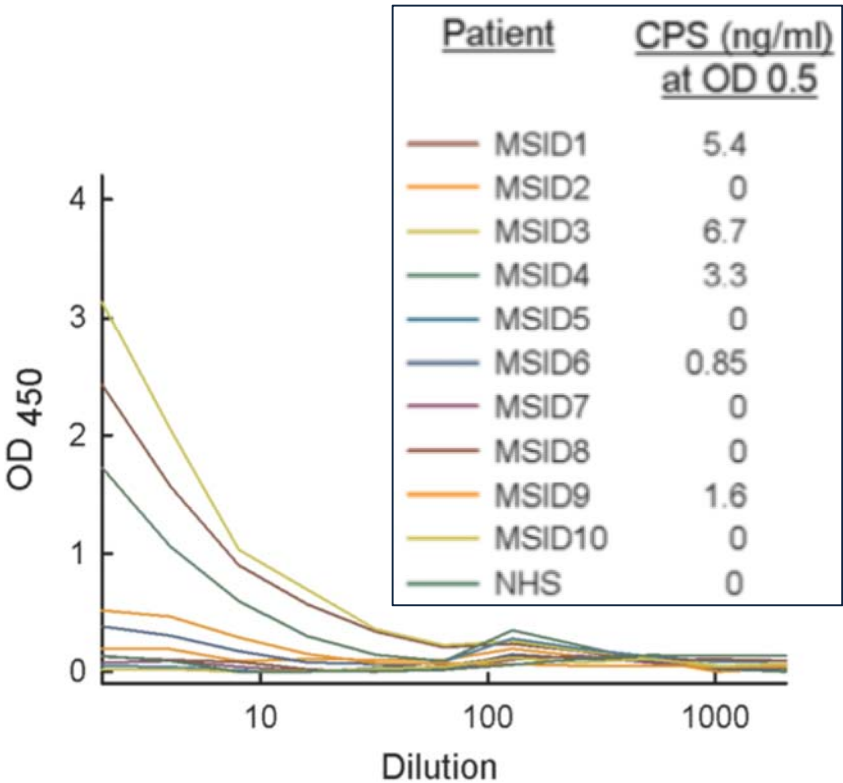
- Urine AMD had a positive predictive value of 94% (32/34) for diagnosing melioidosis in this cohort
- AMD sensitivity on stored sera from melioidosis cases during this study, was 13.9% (5/36) when compared to blood culture samples taken on the same day
- In conclusion, the AMD is an excellent tool for rapid diagnosis of melioidosis from turbid blood cultures, and maintains specificity across all sample types
- It is a promising tool for urinary antigen detection, which could revolutionise diagnosis of melioidosis in resource-limited settings
- Further work is required to improve sensitivity on non-blood culture samples

# Detection of CPS in filtered melioidosis patient samples

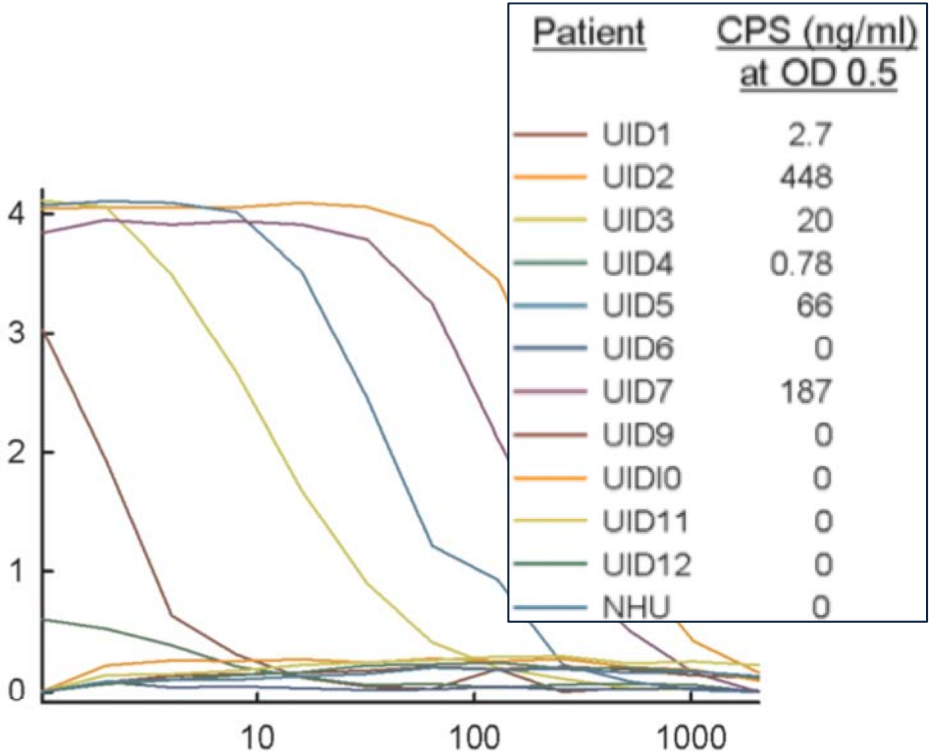


## Quantitative antigen-capture ELISA

SERUM



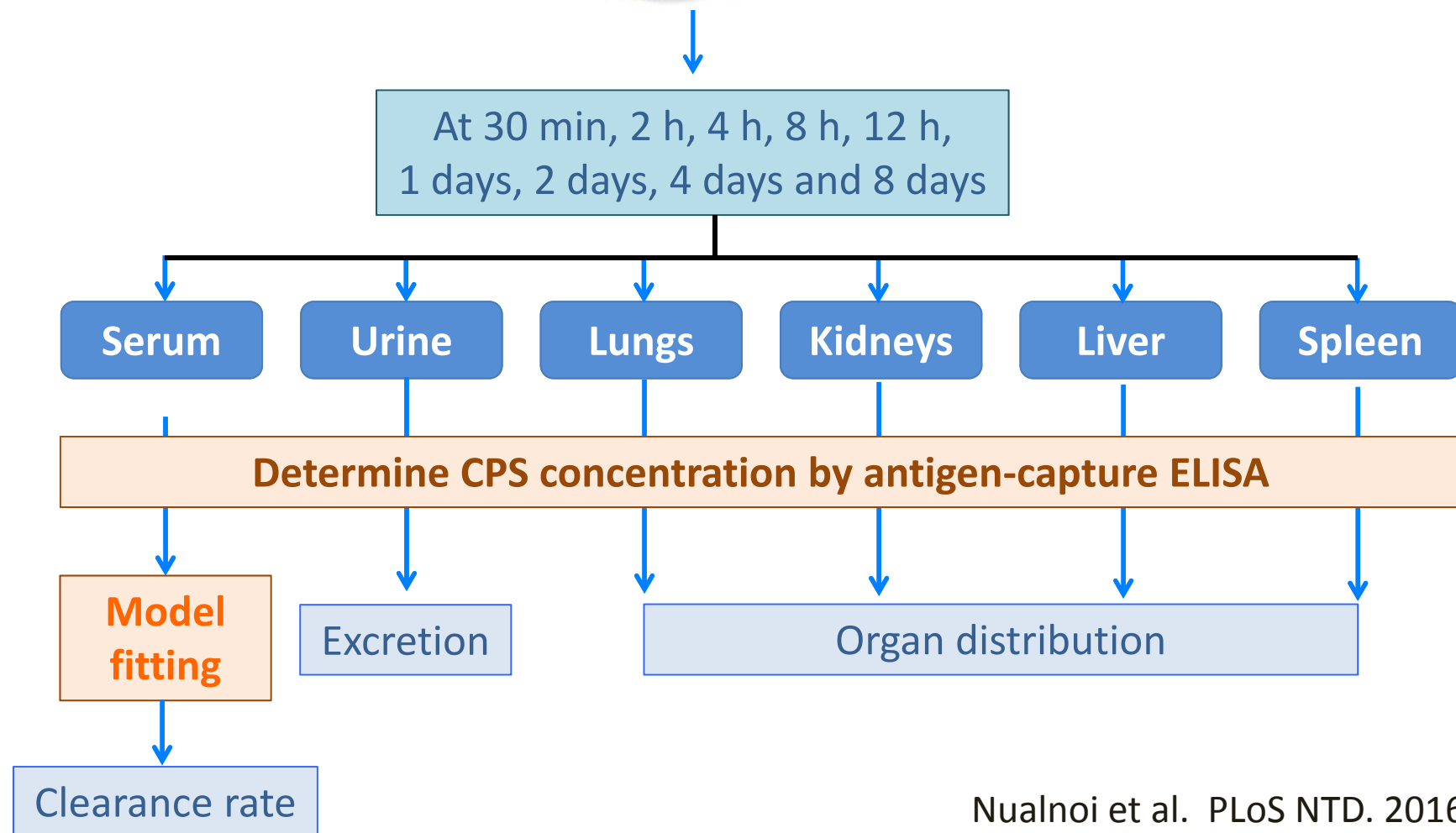
URINE



# CPS clearance study

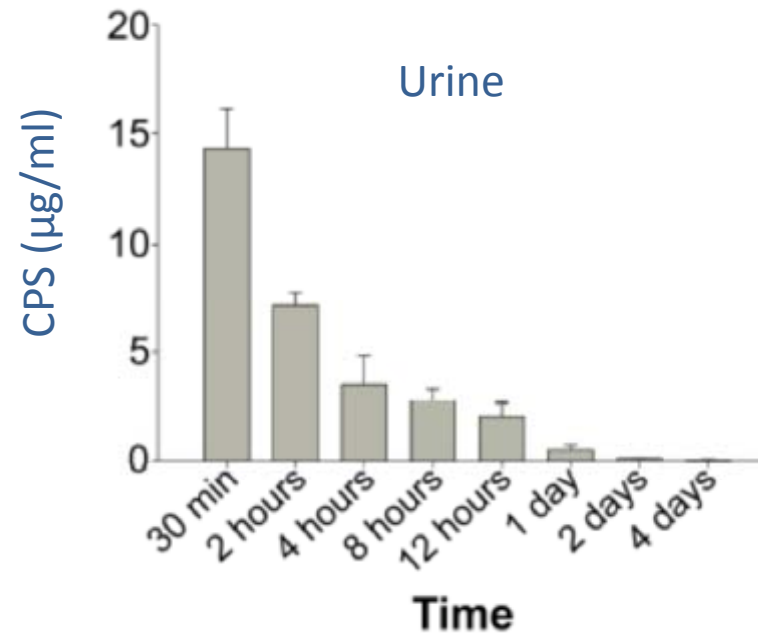
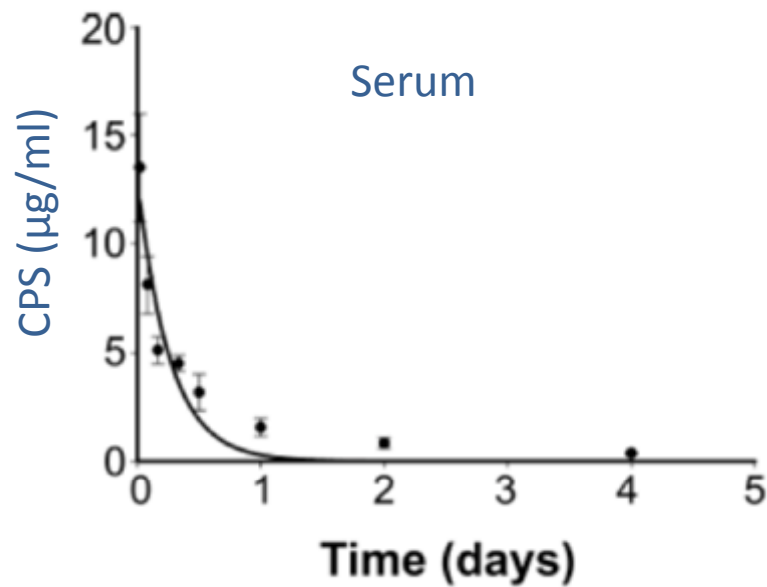


**Intravenous tail injection**  
4  $\mu\text{g}$ , 20  $\mu\text{g}$ , and 100  $\mu\text{g}$  of purified CPS



# CPS clearance

- Mice intravenously injected with purified CPS
- Not detected in major organs

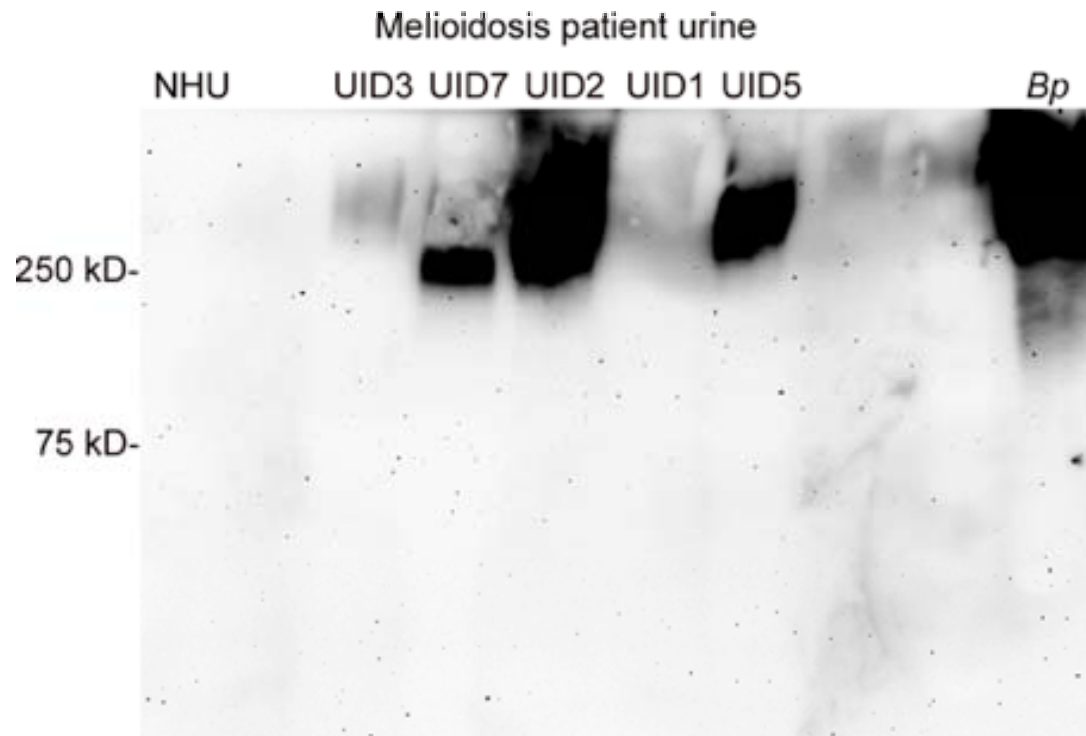


CPS is cleared rapidly from serum with a short half-life of 2.9 – 4.4 hours

# Detection of CPS in melioidosis patient urine

## CPS does not appear to be degraded

Western blot probed with anti-capsule mAb



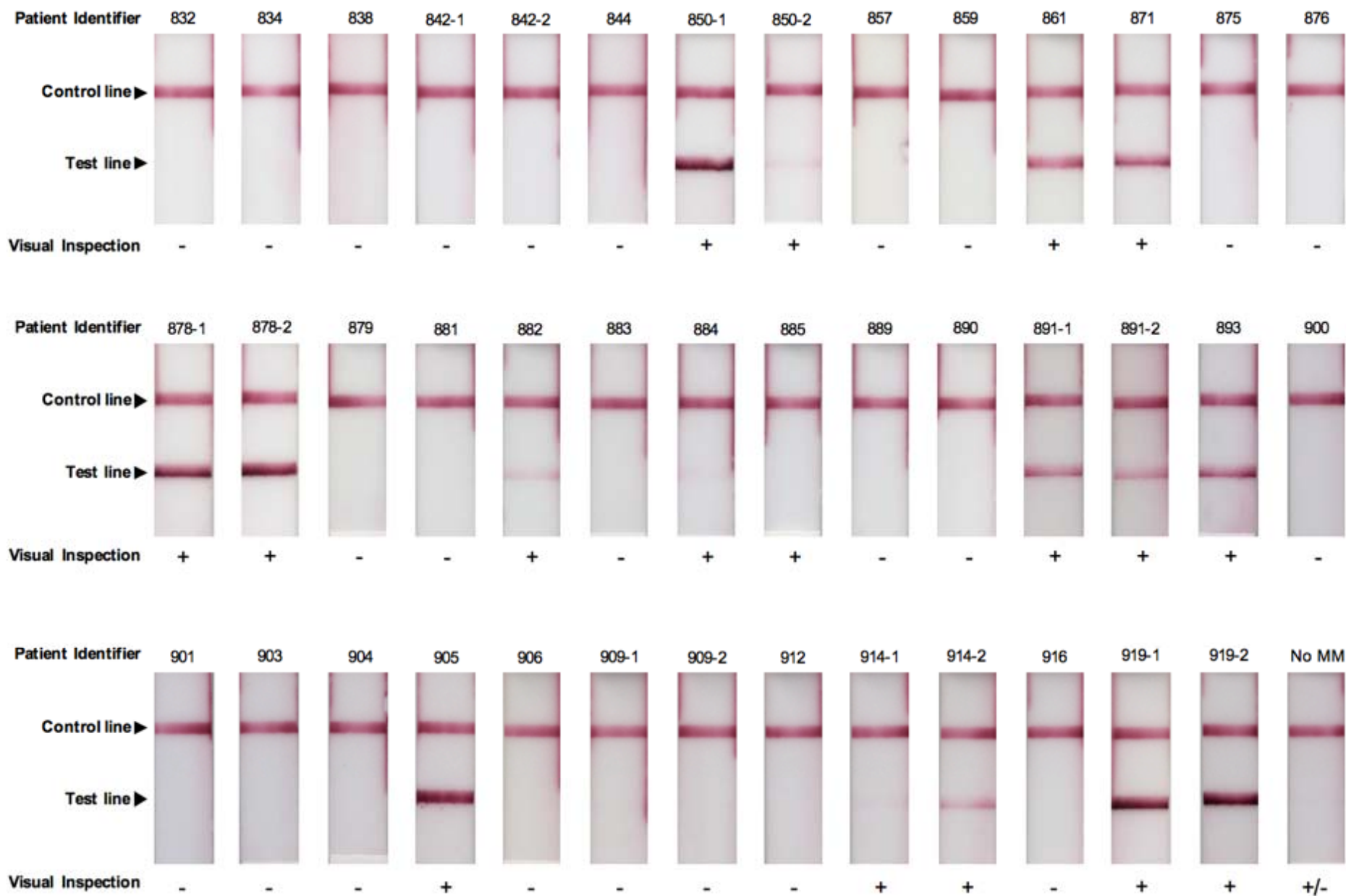
- Filtered melioidosis patient urine samples
- No bacteria in sample
- CPS high molecular weight antigen



# Urine samples - Laos

- Received 42 urine samples from Lao-Oxford-Manhosot Hospital-Wellcome Trust Research Unit (LOMWRU) courtesy of Dr. David Dance
- Most were melioidosis positive patients - samples blinded
- Samples were 0.2  $\mu\text{m}$  sterile filtered in a biosafety level 3 laboratory
- Verified for sterility using a validated procedure and brought to a biosafety laboratory 2 for further testing
- LFI read by 3 blinded readers

# LFI results – Laos urine samples



# Quantitative ELISA – CPS concentration

- Melioidosis culture positive patient samples were tested for the presence of CPS
- Quantitative antigen-capture ELISA (analytical sensitivity – LOD is ~7 pg/ml)

Patient Identifier	CPS (ng/ml)	Vol (ml) BSL-3	Patient Identifier	CPS (ng/ml)	Vol (ml) BSL-3	Patient Identifier	CPS (ng/ml)	Vol (ml) BSL-3
905*	1247	3	914-1*	0.522	3	857	0	1.3
850-1*	535.1	0	909-1	0.195	2.7	875	0	1.7
919-1*	517.7	3	909-2	0.0918	1	876	0	3
919-2*	487	3	885	0.0732	2	879	0	12
878-2*	336.3	3	No MM	0.062	3	881	0	0
878-1*	178.1	3	906	0.0589	3	883	0	8
861	64.5	0	842-2	0.0362	2	889	0	13
871	41.7	0	904	0.0349	17	890	0	15
893*	21.5	11	859	0.0197	0	900	0	18
891-1*	14.6	0	842-1	0.017	3	901	0	1.5
891-2*	5.53	0	832	0	4	903	0	18
914-2*	5.11	0	834	0	4	912	0	2
882	3.13	1	838*	0	18	916	0	4
884	0.814	1	844	0	3			

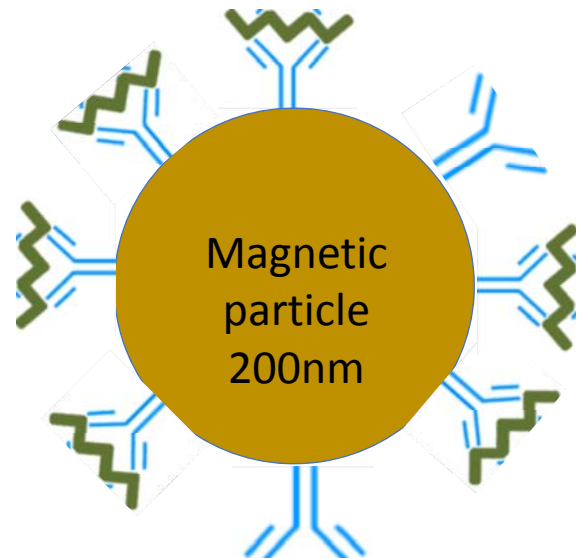
\* Urine culture positive

Green: LFI positive

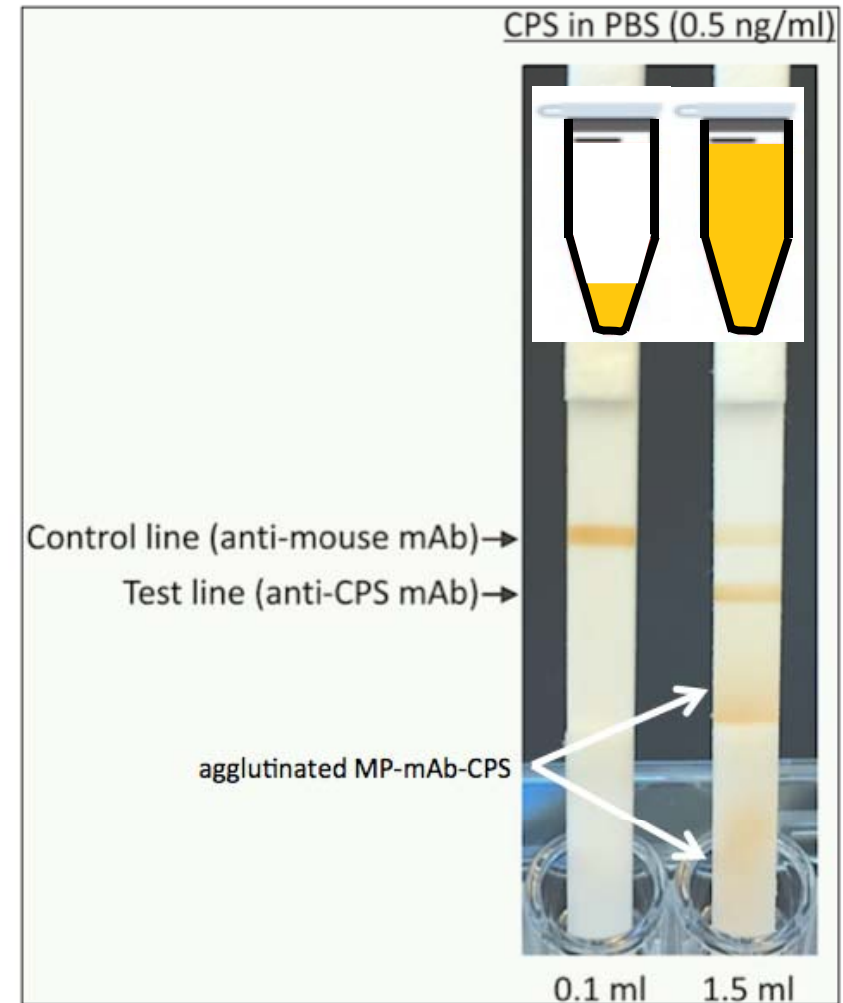
Black: LFI negative



# mAb-magnetic particle (MAG) LFI

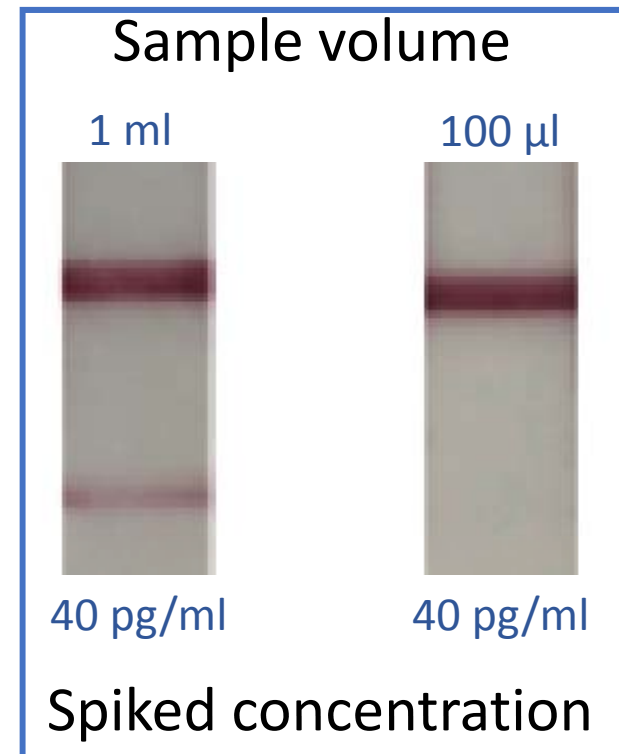


- Add particles to 1 – 5 ml urine sample
- Incubate for 5 minutes
- Isolate particles with magnet
- Apply magnetic particles to LFI
- Boost in sensitivity vs. low volume/ no mag enrichment

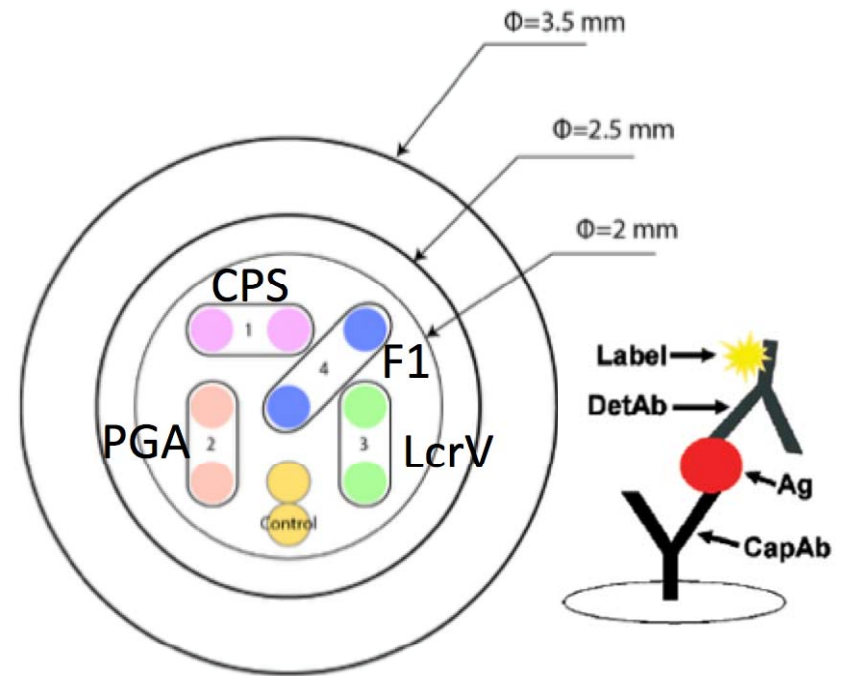
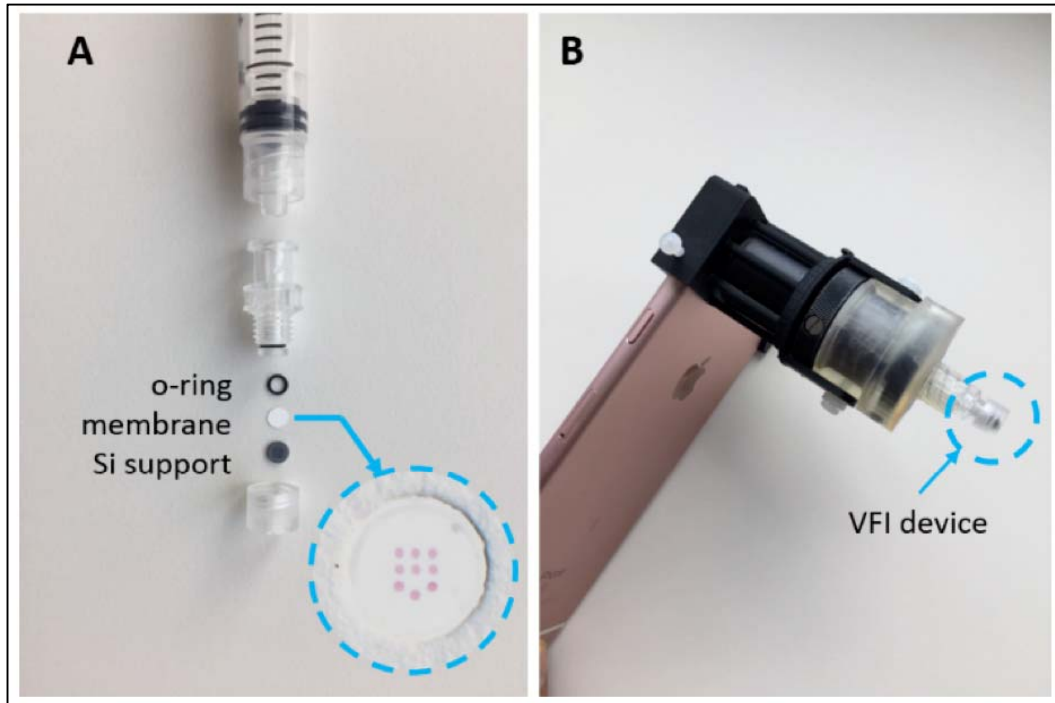


# mAb-magnetic particle LFI

- 1 ml urine spiked with 40 pg/ml CPS
- Add magnetic particles
- Incubate for 5 minutes
- Isolate particles only with magnet
- Disassociate CPS from mAb – magnet
- Add directly to AMD LFI
- Boost in sensitivity vs. standard volume



# Vertical Flow Immunoassay



- Assay larger volume of serum, urine, or other sample
- Increased analytical and clinical sensitivity
- Much easier to multiplex
- Connectivity



**MEDICINE**

AuCoin Laboratory

- Haley Kinney
- Teerapat Nualnoi
- Kate Pflughoeft
- Marcie Hollingsworth
- Michael Dillon
- Sujata Pandit
- Dana Reed
- Derrick Hua
- Emily Hannah
- Jacob Sorenson

University of Nevada

- Paul Brett
- Mary Burtnick



- Chad Roy



- Jasmine Ozsurekci



- Bart Currie
- Derek Serovich
- Mark Mayo
- Vanessa Theobald



- Direk Limmathurotsakul
- Narisara Chantratita
- Gumphol Wongsuvan



- Paul Keim
- Jason Sahl



- Frederic Zenhausern
- Jian Gu
- Peng Chen



- Raymond Houghton
- Syamal Raychaudhuri
- Jean Chen
- Aarthy Vallur



- David Dance
- Kate Woods
- Caoimhe NicFhogartaigh
- Latsaniphone Boutthasavor



Public Health Agency of Canada

- Xianggo Qiu, Ph.D.
- Shihua He, Ph.D.







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Clinical Microbiology

## Evaluation of a rapid diagnostic test for the detection of *Burkholderia pseudomallei* in the Lao People's Democratic Republic

- 241 urine samples
- 15/241 urine samples were *B. pseudomallei* culture positive
- 13/15 of which were LFI positive
- 21/226 urine culture negative samples were LFI positive
- 19/21 melioidosis confirmed by culture from another site/sample
- suggesting that these were not “false positive” LFI results
- The positive predictive value of LFI on urine for correctly diagnosing melioidosis in this cohort was therefore 94.1% (32/34; 79.7 – 98.5%) with a disease prevalence of 35.7% (86/241)

# Melioidosis diagnostic summary

- Suspected melioidosis: diabetics with sepsis or fever; prostate abscess; or deep abscess in lung, liver or spleen
- Should be useful to directly test samples from suspected melioidosis cases so recommended antibiotics can be administered sooner
- AMD LFI sensitivity when testing blood directly is low (20-40%)
- However, higher sensitivity with with urine and pus when testing samples from suspected melioidosis cases
- Following microbiological culture *B. pseudomallei* may be dismissed as a culture contaminant
- Can be misidentified as *Pseudomonas* spp. or other organisms by API 20NE and automated bacterial identification systems
- AMD LFI or the latex assay should be used for testing all Gram-negative, oxidase positive bacilli that are isolated from blood culture and that cannot be simply identified as *Pseudomonas aeruginosa*