Industry Day Theme # Sustainable Medical Technologies

IIT DELHI INDUSTRY DAY 2019

Design and Development of Point-of-care Strategies for Effective Management of Bloodstream Gram-positive Bacterial Infections

Pramod Jagtap, Rohini Singh, Rituraj Mishra, Shruti Khanna, Bhanu Mittal, Pratibha Kumari, Karuna Deepika, Hemant K. Kashyap, Venkataraman Sritharan and Shalini Gupta*

* shalinig@chemical.iitd.ac.in

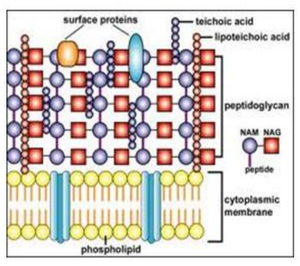
Chemical Engineering

MOTIVATION

- Bacterial infections are the world's leading cause of premature deaths and the third in line after cancer and cardiovascular related mortalities.
- The absence of an early conclusive diagnostic proof, clinicians empirical antibiotic therapy and the excessive use of antibiotics results in risk of toxicity , super infections and selection of multiple drug resistant strains.
- Pathogen associated molecular patterns (PAMPs) such as lipoteichoic acid (LTA) can serve as early indicators of Gram-positive bacterial infection.
- We are developing strategies for efficient management of bloodstream Gram-positive bacterial infections. The objective are as follows;

I. A point-of-care assay for early and rapid diagnosis of Gram-positive bacteremia

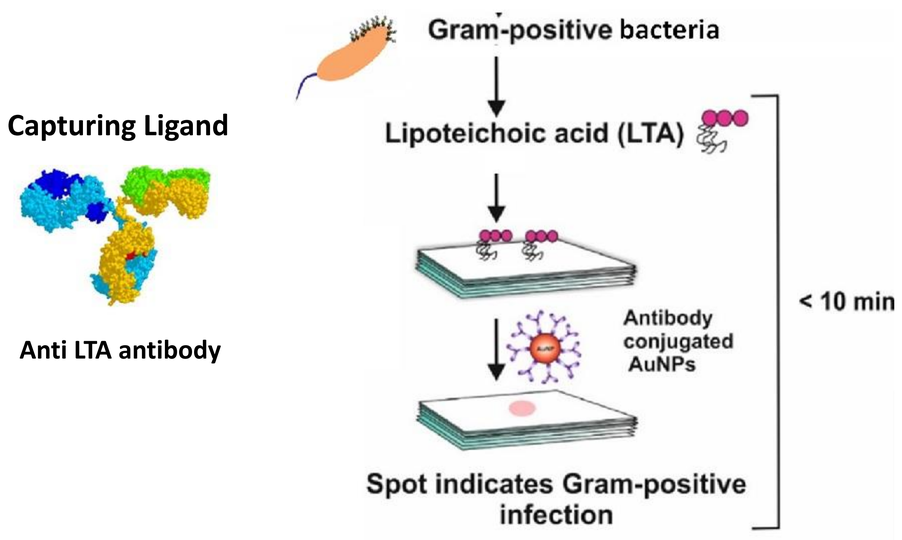
II. Identify small molecule as a potential ligands for *in vitro* diagnosis



J. Cohen, *Nature,* **2002**, *420*, 885–891

Gram-positive contribute 60% of total bacterial infection cases

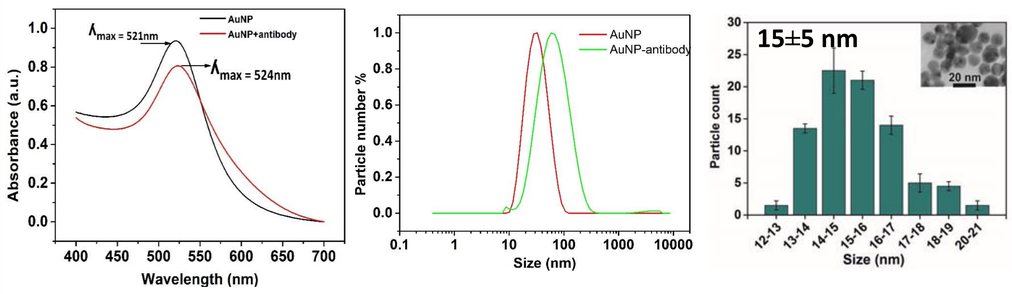
I. Septiflo-P technology



Similar system was developed for Gram-negative infection detection (Septiflo-N)

Characterization of Gold (AuNP) Nanobioconjugates

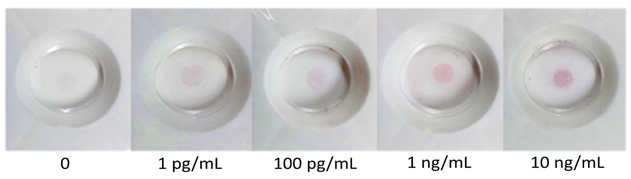
UV-Vis spectroscopy **Dynamic light scattering** **TEM**



Peak shift in wavelength (λ_{max}) suggests successful conjugation of ligand to the AuNP surface

Barford assay showed Abs: AuNPs conjugation ratio ~ 6:1 (on average)

Color gradient based on LTA concentrations



Color Chart		
Color	LPS level	Symbol
	> 1 ng/mL	++++
	500 - 1000 pg/mL	+++
	100 - 500 pg/mL	++
	1 - 100 pg/mL	+
	< 1 pg/mL	±

Lower limit of detection (LOD) ~ 1 pg/mL by eye, <10 min

Categorization of endotoxin levels with severity of infection

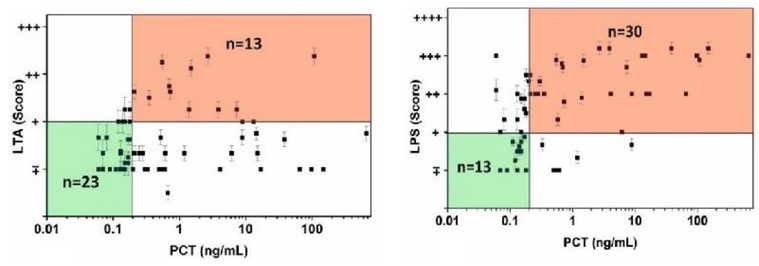
LPS level (pg/mL)	Severity of sepsis	Death risk
6-100	Bacteremia with low susceptibility risk to sepsis; treatable	0 %
100 - 200	Elevated risk for severe sepsis but can be managed with standard treatment	10-20 %
200 - 1000	Early stages of sepsis	15-30 %
1000 - 7500	Sepsis with risk for severe sepsis	30-60 %
≥ 7500	Septic shock	40-80 %

Gram-negative endotoxin (lipopolysaccharide (LPS)) levels are correlated to severity of infection however no such data exists for LTA but should be similar to LPS due to their structural similarity

Clinical validation of Septiflo-P results

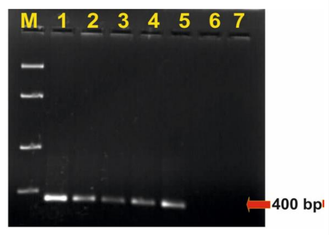
*Blind reviewing (n = 8)

Procalcitonin (PCT) is an approved biomarker for sepsis, indicates inflammatory response in host whose levels correlate with sepsis severity **n = 60**



- Clinical validation of the assay shows that it cover ~ 80% of all bloodstream bacterial infections and could be used for pre-symptomatic identification of sepsis
- No instrumentation, naked eye detection and cost effective
- Better performance than benchtop ELISA

PCR data



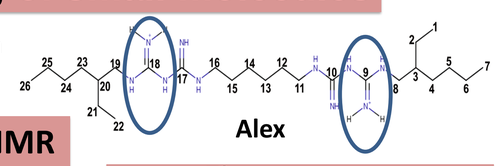
Statistical Anova analysis

Assay	Area under curve (AUC) value
Septiflo-N	0.88554**
Septiflo-P	0.9271**

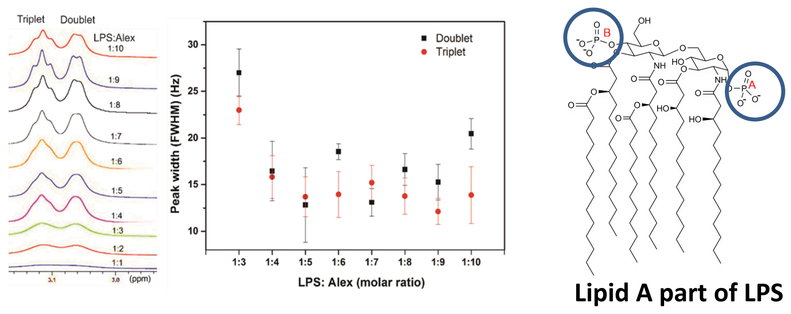
**Statistically excellent data

II. Mechanistic understanding of small molecules

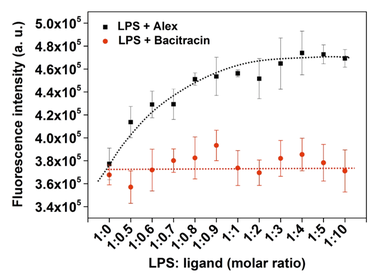
✓ Alexidine (alex) selected as a model ligand for LPS interaction using experimental and *in silico* approaches



Peak broadening in LPS-alex mixture using ^{1}H NMR



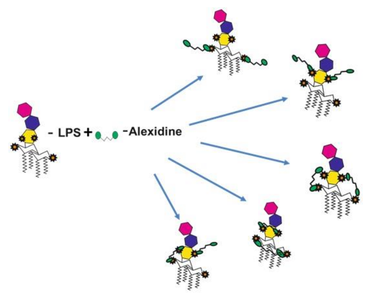
Fluorescence spectroscopy results



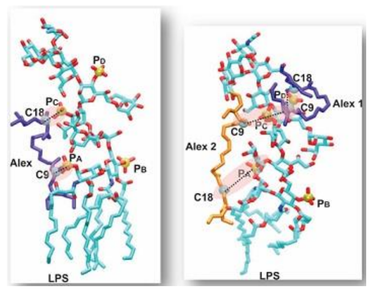
Variation in peak width caused by increasing concentration of alex suggestive of LPS-alex interaction

>1: 1 (LPS:alex) stochiometry

Proposed model of LPS-alex interaction

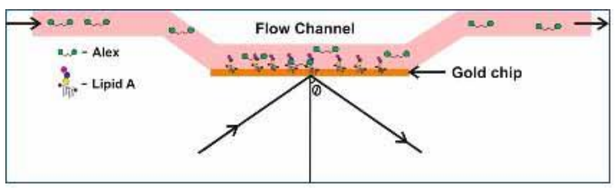


Single alex system Two alex system

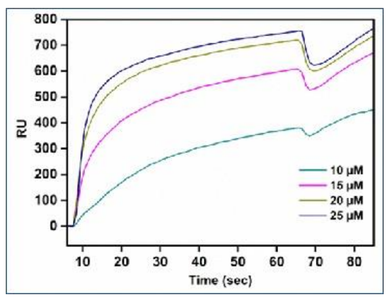


Alex interacts with LPS in a monodentate/bidentate manner with an overall stoichiometry between 1:2 and 1:4

Kinetics of LPS-alex binding using surface plasmon resonance (SPR) spectroscopy



Alex affinity (K_D) = 38 ± 5.6 µM



Association-dissociation profile

Industrial significance

- High throughput, simple, handy and affordable stratification device which can be utilized at patients bedside
- Currently no bedside device clinically approved for stratification of Gram status in blood samples
- The technology can be translated to commercial scale as the components are simple and cost effective
- In future antibody ligands can be replaced with small molecules to reduce overall cost of the device: the work is underway
- **Current status: clinical trials are running at Government Medical Hospitals, New Delhi**

Publications

Jagtap & Singh et al, Flow through assay for rapid, bedside stratification of bacteremia in critically ill patients: a pilot study, **Journal of Clinical Microbiology 2018,** 56 (9):e00408-18

Jagtap et al, Mechanistic Evaluation of Lipopolysaccharide-Alexidine Interaction Using Spectroscopic and in Silico Approaches. **ACS Infect Dis 2018** Nov 9;4(11):1546-1552

Acknowledgements

BIRAC, DST, CSIR , GYTI for funding, #Global Hospitals Hyderabad for providing septicemia patient's clinical samples