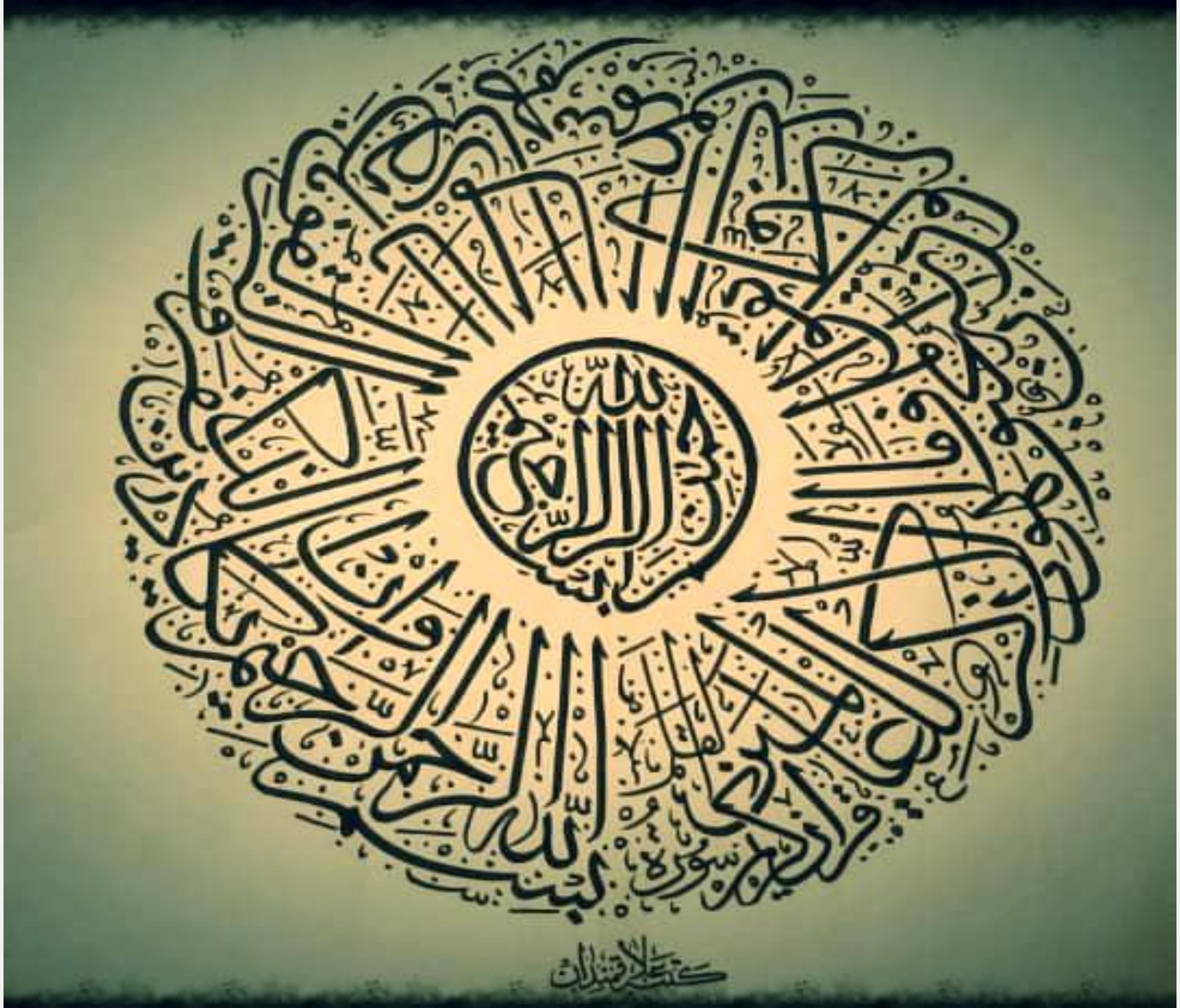

Direct detection of bacteremia by exploiting host-pathogen interactions of lipoteichoic acid and lipopolysaccharide

Azadeh Taraghian
Phd. Isfahan university of medical science

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Direct detection of bacteremia by exploiting host-pathogen interactions of lipoteichoic acid and lipopolysaccharide

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Jessica Z. Kubicek-Sutherland[1], Dung M. Vu[1], Aneesa Noormohamed[1], Heather M. Mendez[2], Loreen R. Stromberg[1,2], Christine A. Pedersen[1], Astrid C. Hengartner[1], Katja E. Klosterman[1], Haley A. Bridgewater[1], Vincent Otieno[3], Qiuying Cheng[4], Samuel B. Anyona[5], Collins Ouma[6], Evans Raballah[7], Douglas J. Perkins[3,4], Benjamin H. McMahon[8] & Harshini Mukundan[1]

Los Alamos National Laboratory

Introduction

- Bacteremia is a leading cause of death especially in childhood
- invasive non-Typhi *Salmonella* (iNTS) serovars responsible for sepsis in under 5 children
- The early diagnosis of bacteremia and of treatment saves lives, especially in high-disease burden areas

- There is an urgent need for a rapid method for detecting bacteremia

- The innate immune system rapidly identifying all pathogen associated molecular patterns (PAMPS) via pattern recognition receptors, such as TLRS.

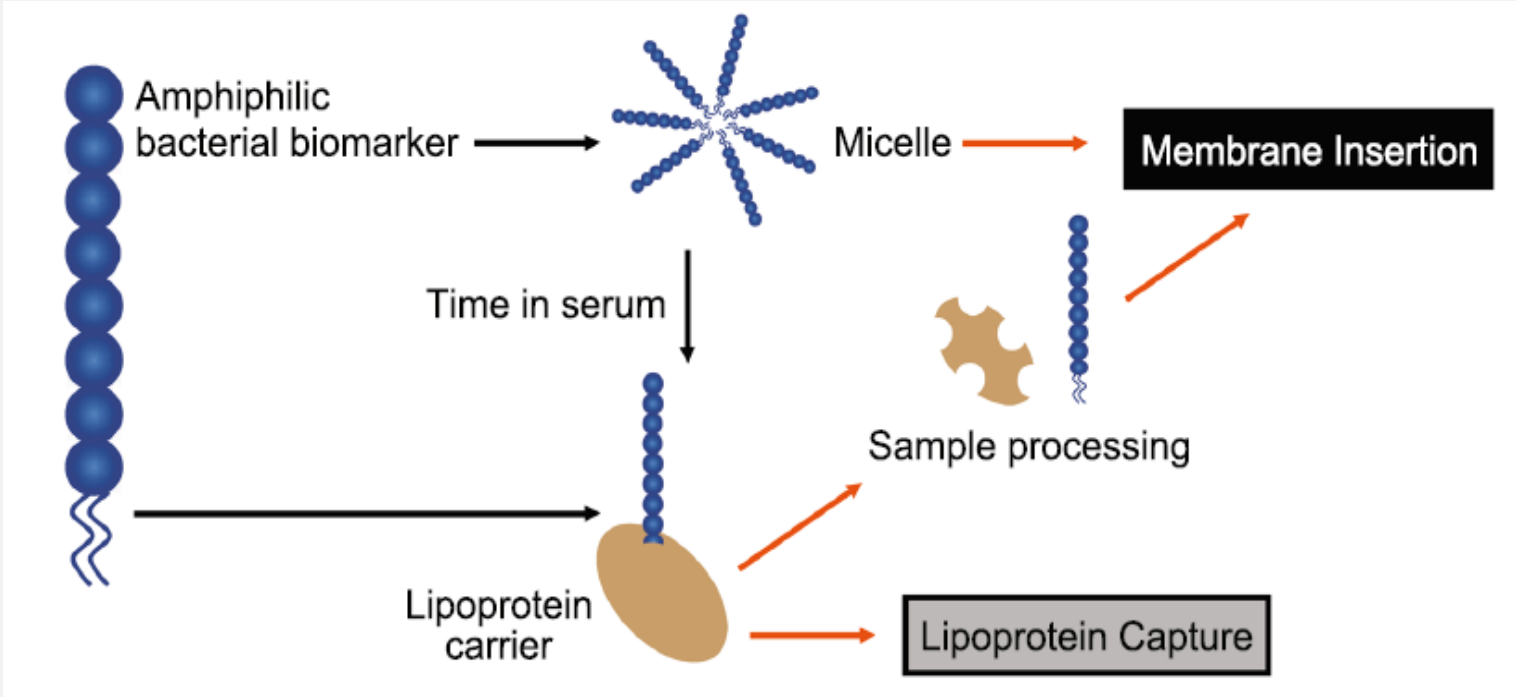
- Many bacterial PAMPS are amphiphilic molecules

- Amphiphilic pathogen biomarkers such as LPS and LTA with host lipoprotein carriers in blood

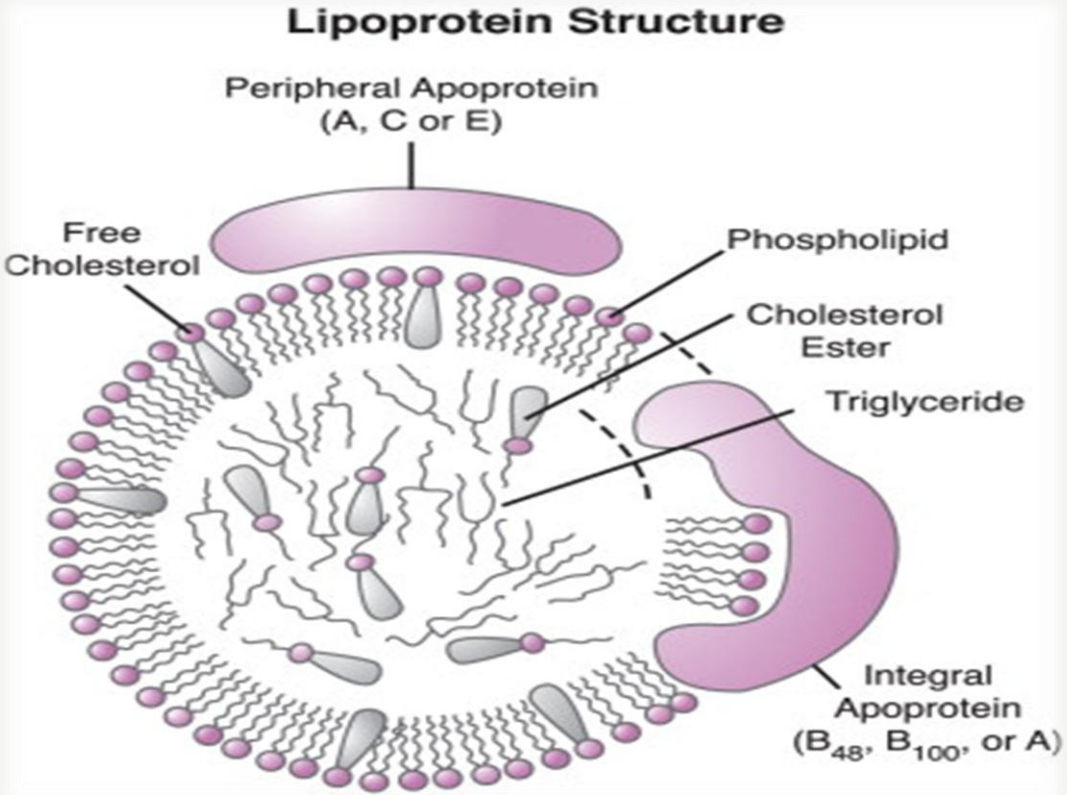
- In this manuscript, developed a clinically **novel method for the direct detection of amphiphilic pathogen biomarkers** indicative of bacteremia, directly in **aqueous blood**, by mimicking innate immune recognition.

Lipopolysaccharide structures and conformations

- LPS is a pathogen-specific biomarker, it is an indicator of acute infection
- Approximately 62 pg of LPS per cell
- Lipid A is the conserved portion and consists of six, fatty acid tails, gives the hydrophobic properties
- In aqueous solutions, amphiphiles like LPS can present in a micellar conformation

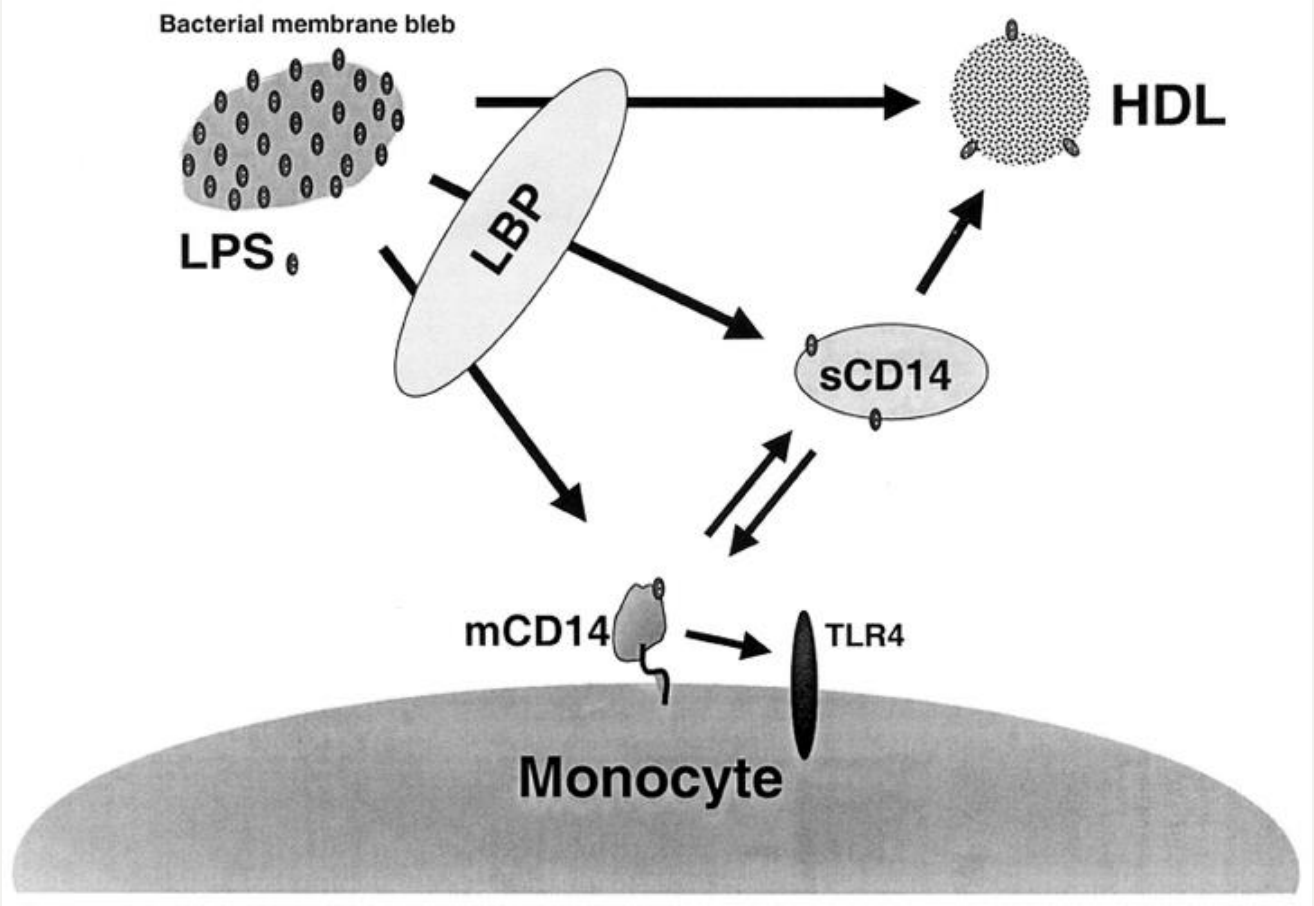


- shapes for LPS micelles include cubic, lamellar, and hexagonal
- Variation in LPS micelles modifies presentation of o-ag-specific epitopes to antibodies, making detection challenging
- HDL and LDL are composed of a core nanodisc lipidated structure that associates with the lipid moieties of LPS, LTA and lipoarabinomannan from *mycobacterium tuberculosis*.



- The majority of the LPS and LTA are bound to HDL. This inhibits the ability of LPS and LTA to interact with TLR and activate macrophages

- The interaction of LPS and LTA with host lipoproteins interferes with traditional methods for detecting these bacterial biomarkers directly in patient blood



While many methods for LPS detection exist, most of them are not optimized for amphiphilic detection in physiological samples. An ideal measurement for LPS should be sensitive enough to detect low concentrations of the amphiphile in aqueous physiological milieu

Herein, present tailored ultra-sensitive assays for the direct detection of a suite of amphiphilic bacterial PAMPs– LPS for Gram-negative pathogens

Biosensors

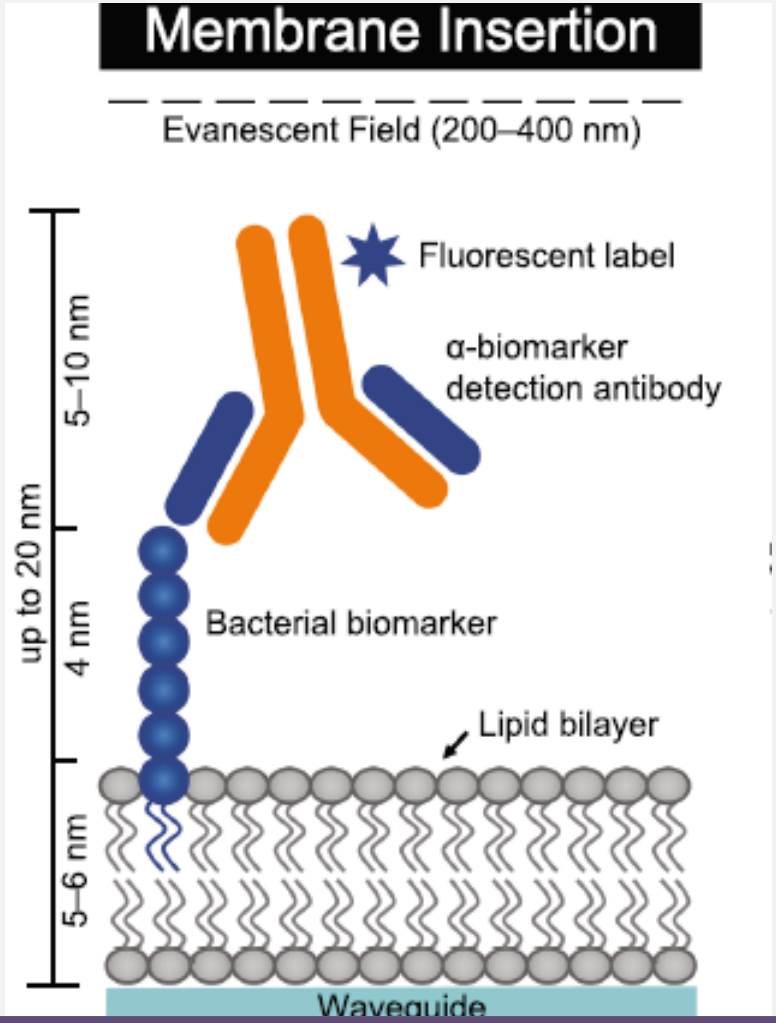
- ❑ biosensors recognize target molecules and produce a measurable signal.

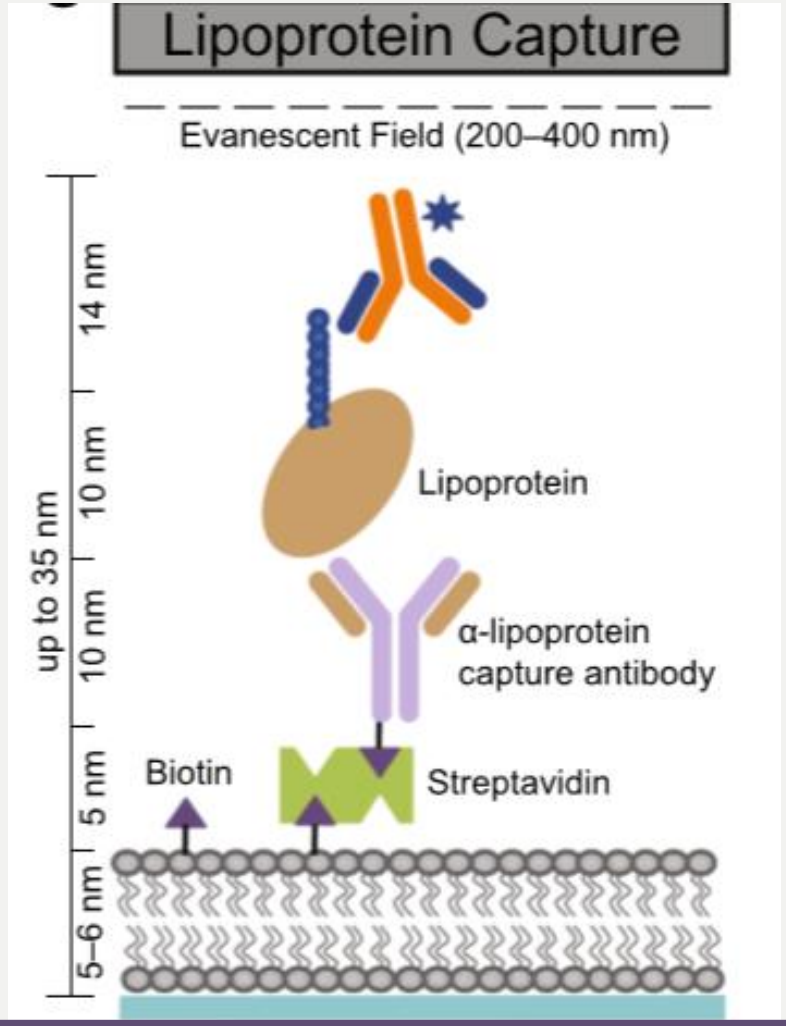
1. Optical
2. electrochemical
3. mechanical

- ✓ have been adapted for the detection amphiphilic biomarkers, using a variety of assay methodologies which can be categorized as:

- ■ (a) labeled
- ■ (b) label-free

- two novel assay methodologies

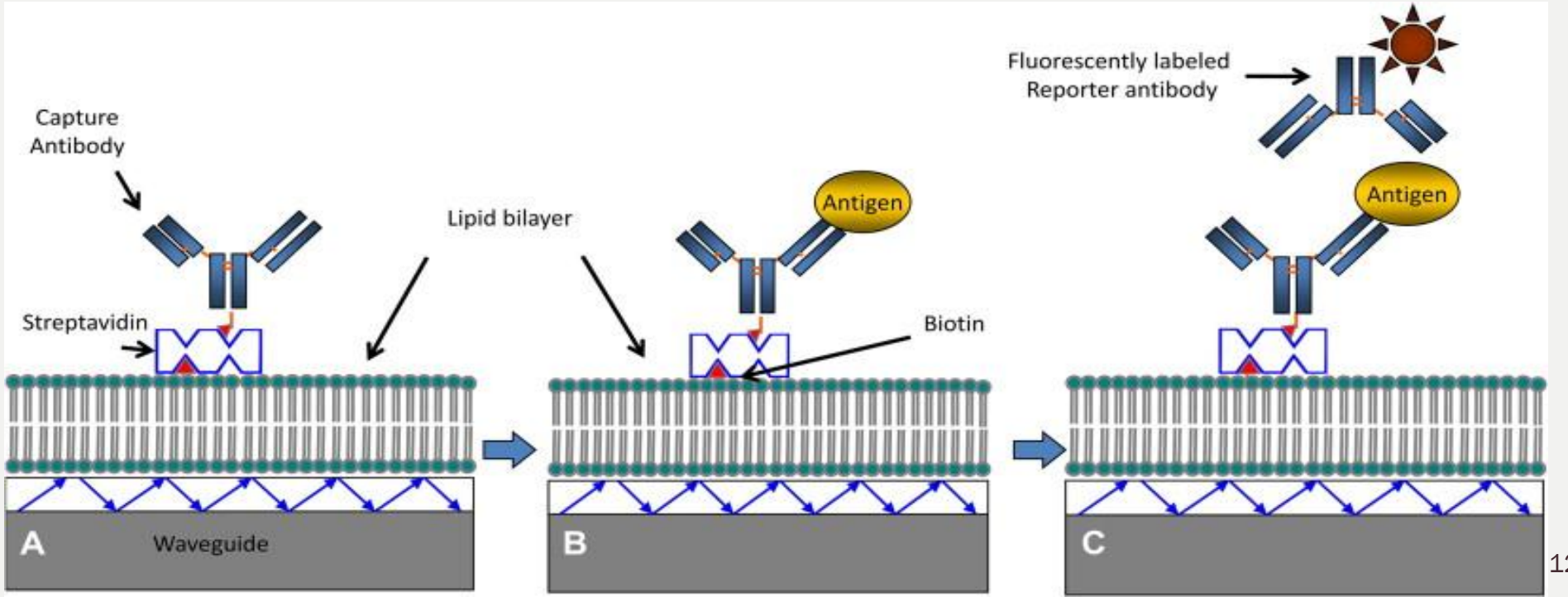




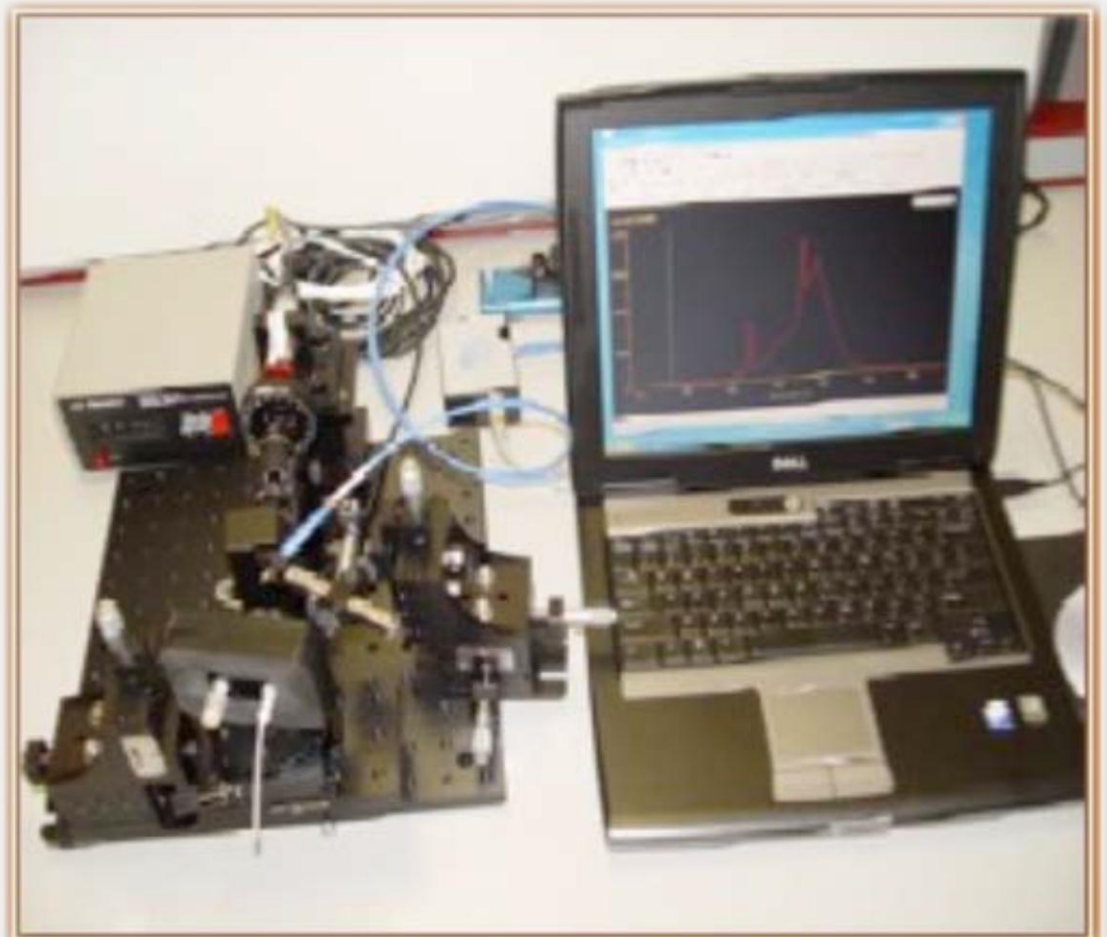
1- membrane insertion:utilizes the interaction between an amphiphilic biomarker and a lipid bilayer to capture the biomarker directly on the biosensor surface with a dye-conjugated antibody

2- lipoprotein capture exploits the interaction between amphiphilic biomarkers and host carrier lipoproteins in blood, an antibody targeting the lipoprotein carrier is used to capture a host-biomarker complex

- material and methods
- **Waveguide-based optical Biosensor:**
- **Waveguide preparation and Flow Cell Assembly:**
- **Lipid Micelle preparation:**
- 2 mM DOPC and 1% cap biotinyl were combined.
- Once the lipids are stabilized, the addition of biotin allows for the bilayer integrity to be evaluated during immunoassay experiments by streptavidin conjugate.

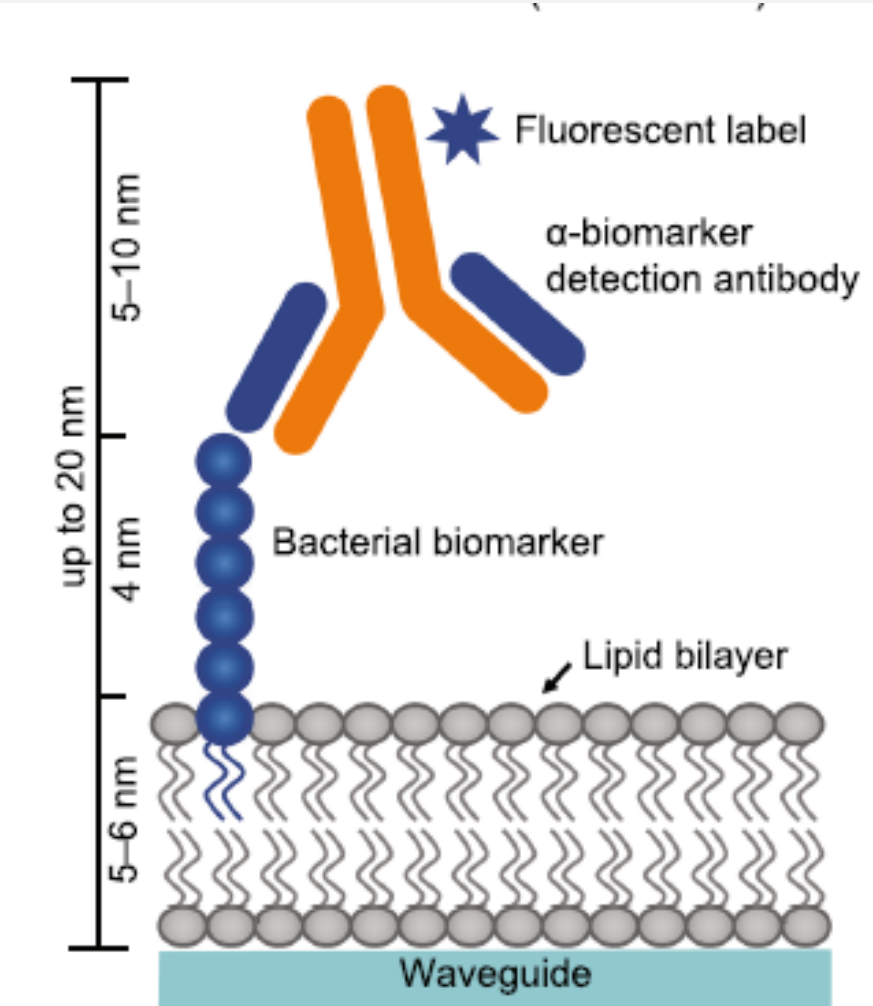


- **Waveguide-based Assays:**
- All incubations occurred at room temperature.
- Incident light from a 635 nm laser



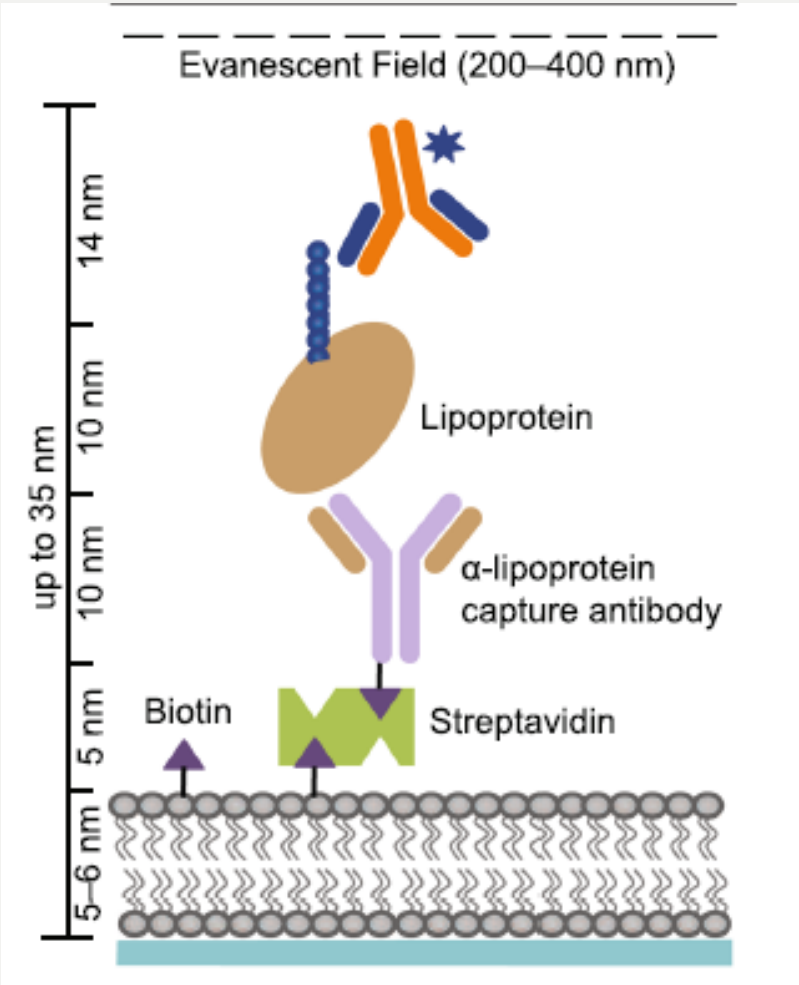
❖ Membrane Insertion Assay:

- ❑ The non-specific signal between the detection antibody and the lipid bilayer was determined by incubation of detection antibody.
- ❑ Then LPS or LTA was incubated allowing for association with the lipid bilayer.
- ❑ detection antibody was incubated specific signal associated with the antibody and LPS or LTA associated with the lipid bilayer was measured.

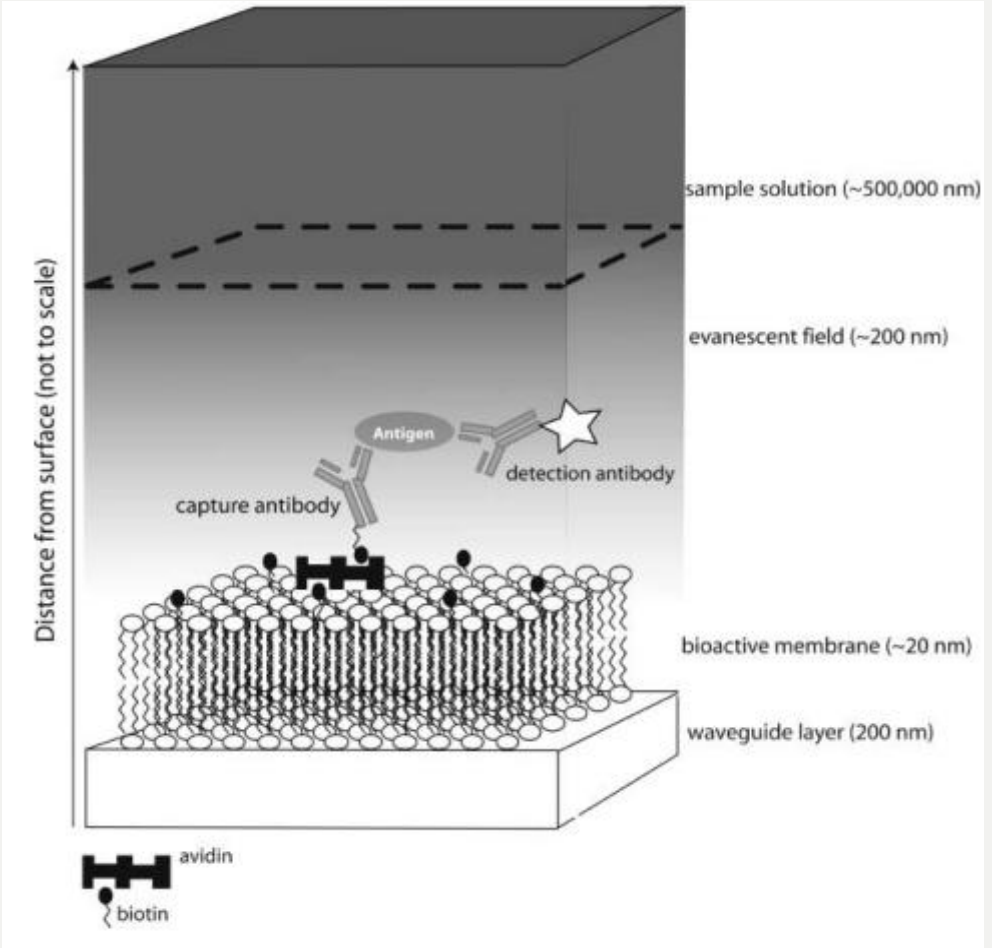


❖ Lipoprotein Capture Assay.

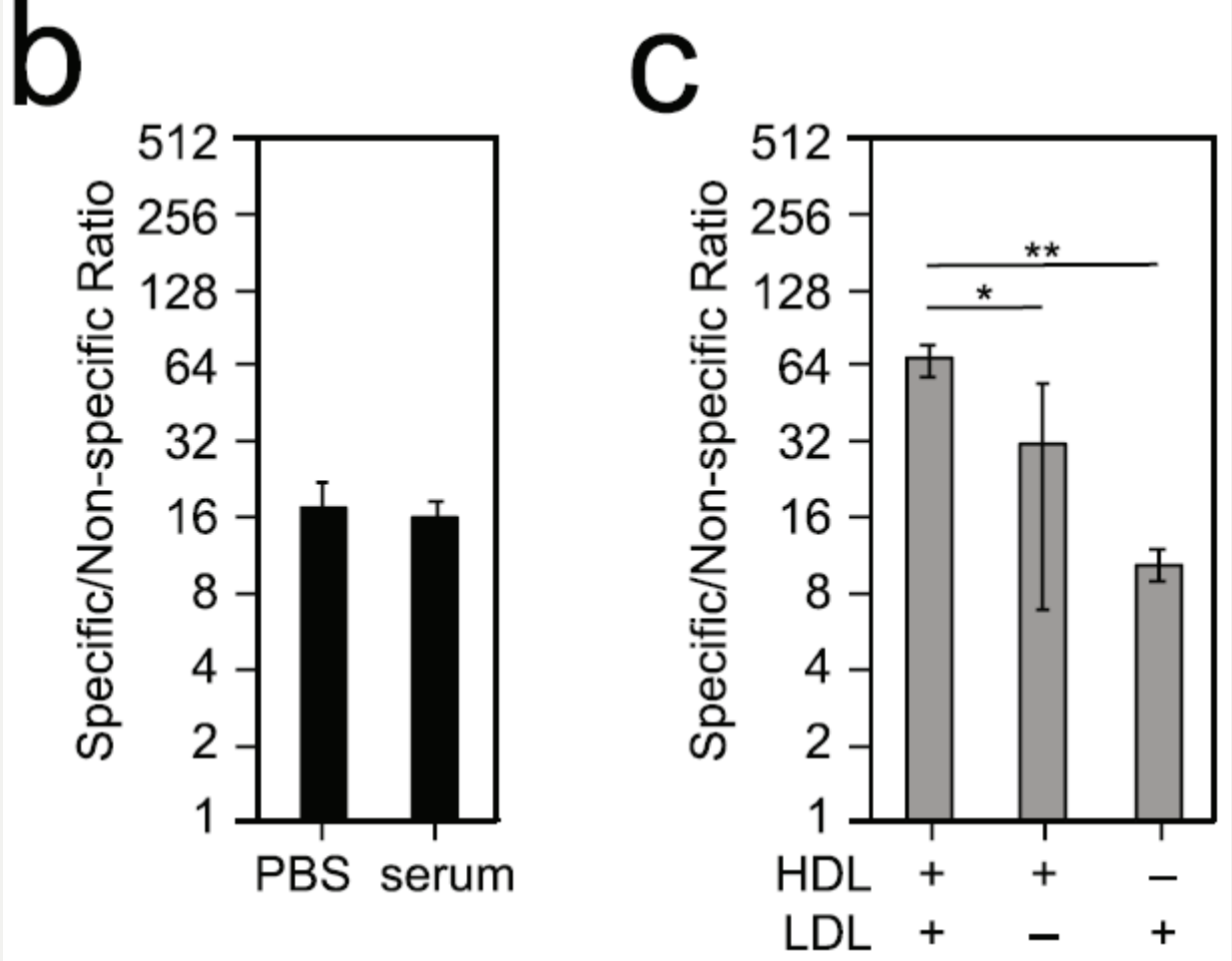
- Utilized capture antibodies directed against the coat proteins of HDL and LDL nanodiscs (α-apoa1 and α-apob).
- Streptavidin was added and incubated for 10 min to saturate the biotin embedded in the lipid bilayer.



- For assays capturing both HDL and LDL, each was combined and injected.
- The non-specific signal was determined by association of the biomarker-lipoprotein complex with the capture antibody.
- specific signal was measured following the addition of the detection antibody.



- **LPS Assay.** The α-Salmonella pAb antibody was used as the LPS detection antibody
- LPS was diluted to the desired concentration in either PBS or human serum overnight at 4 °C to allow for association with lipoproteins in serum.
- The LPS sample was then injected into the flow cell and incubated for 2h.



b:When 50 ng/mL LPS was detected by membrane insertion, S/N ratio of 16 regardless of whether LPS was diluted in PBS or incubated in human serum

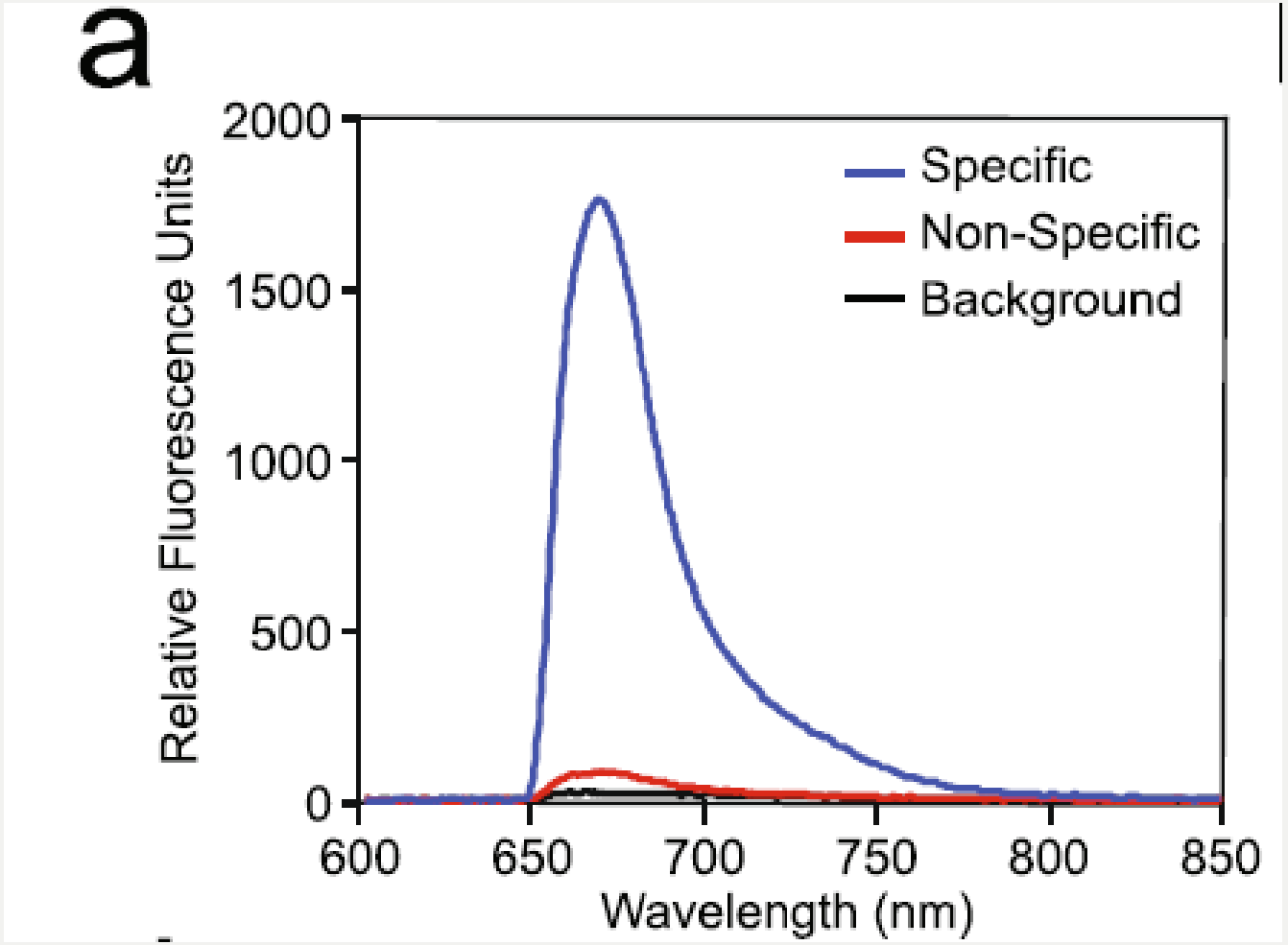
C: In contrast, the LPS S/N ratio significantly increased by 4-fold when using the lipoprotein capture assay

- **sample processing:** biomarker-containing solution was used as the biomarker sample for immunoassays.
- In the case of clinical samples, 40 µL of patient serum
- **ELISA.**
- **Lipoprotein Capture:** LPS and LTA were measured in ELISA format

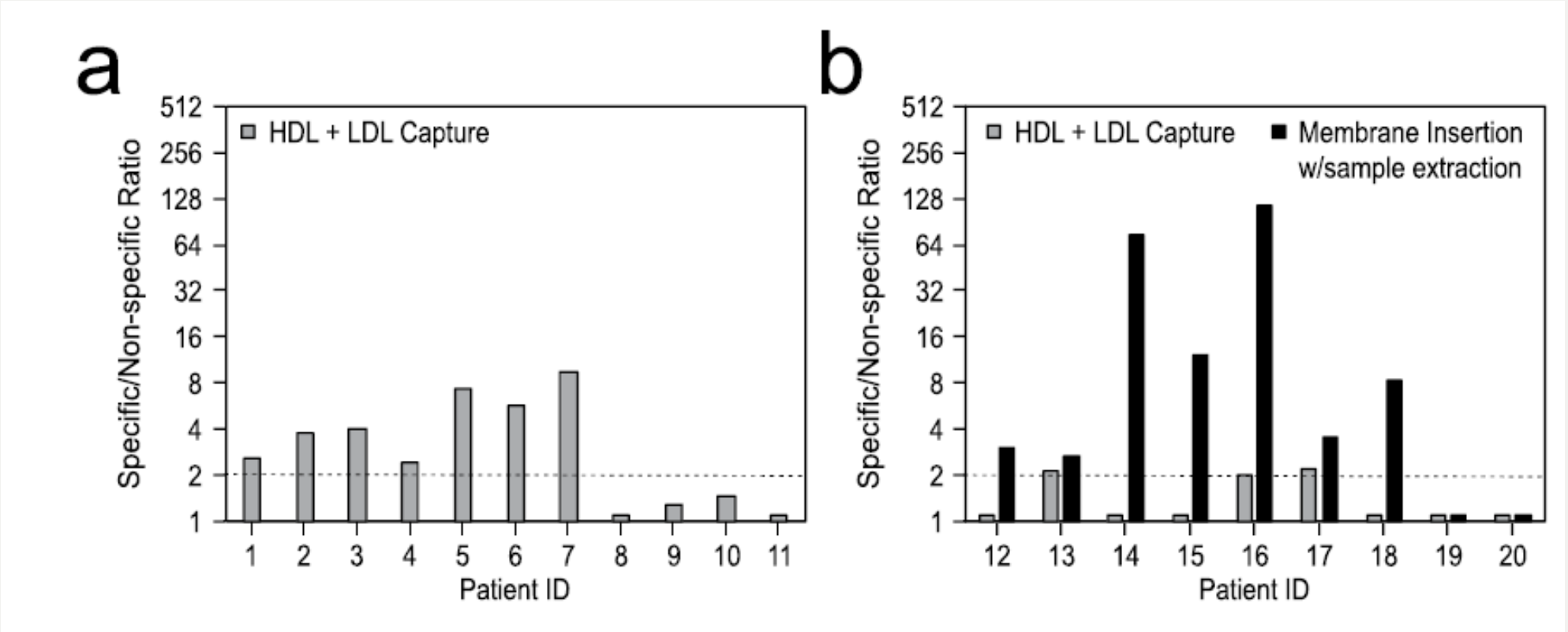
Results

- detection of LPS using the lipoprotein capture assay in the waveguide-based biosensor displayed a limit of detection of **4 ng/mL**, over **100-fold increase in sensitivity**

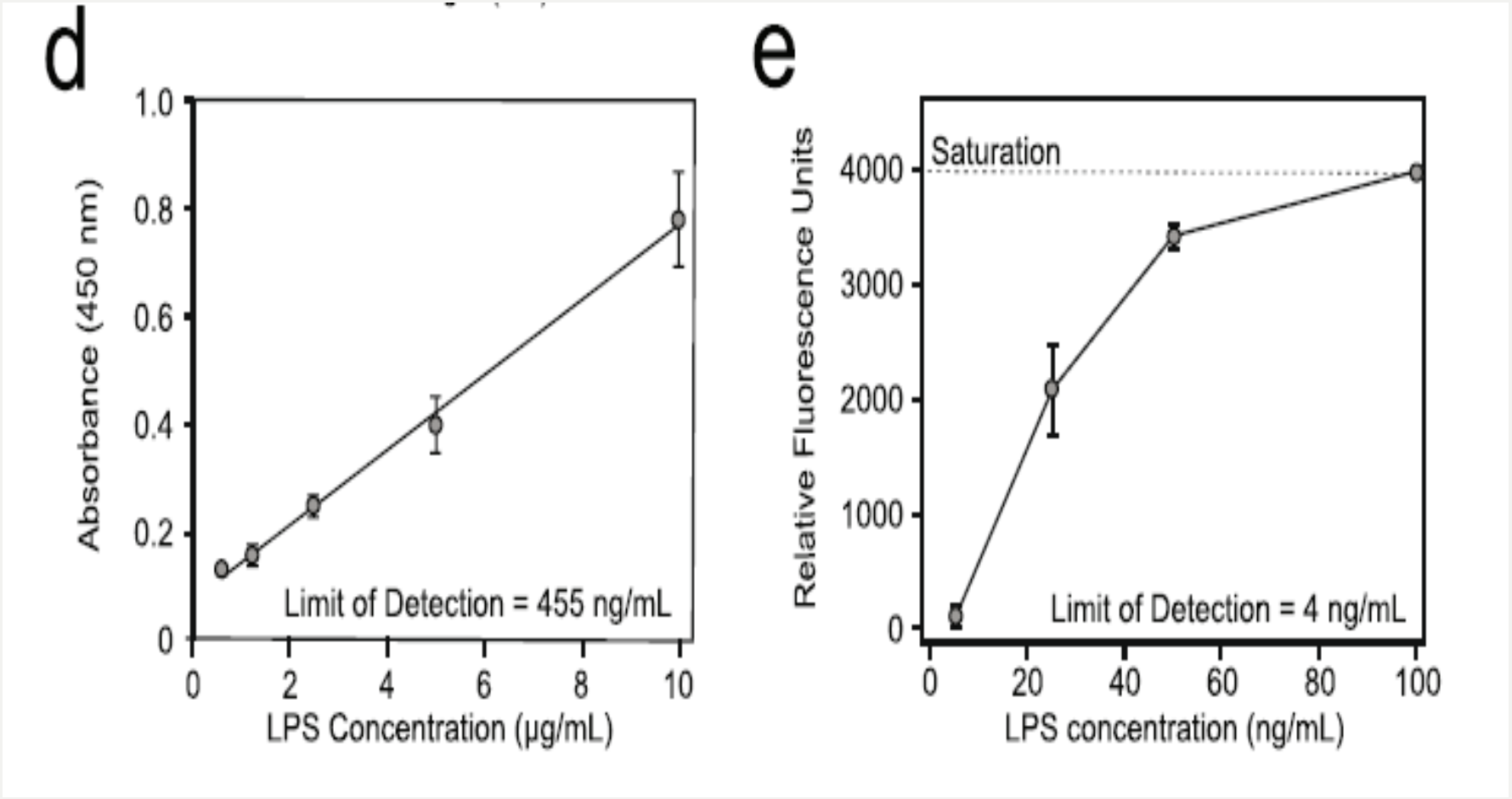
- previously reported over **1000-fold increase in sensitivity** for the detection of **LAM from *M. tuberculosis*** in serum on the biosensor platform as compared to ELISAs



measurement of LPS (25 ng/mL) incubated overnight at 4 °C in control human serum, with the specific signal (RFU) from the detection α-LPS antibody as a function of emission wavelength (nm). The background and nonspecific signals are measured before the addition of LPS.



Detection of LPS and LTA directly in pediatric patient serum samples. Data are presented as the S/N ratio with a value above 2 indicating a positive result. (a) Detection of LPS in clinical serum samples using the lipoprotein capture assay. (b) Detection of LTA in clinical serum samples using both the lipoprotein capture (grey bars) and membrane insertion (black bars) assays.



d) Concentration dependent detection of S. Typhimurium LPS using lipoprotein capture with 25 nM of the α-LPS antibody in ELISA format measured in absorbance at 450 nm. (e) Concentration dependent detection of S. Typhimurium LPS using lipoprotein capture in the LANL waveguide-based biosensor.

- Measurements are extremely rapid (3 seconds),
- Can be adapted to the simultaneous detection of a variety of fluorescent labels.
- Previously validated the application of this technology for the detection of anthrax, influenza, breast cancer and tuberculosis

Discussion

- ELISAs for LPS detection suffer from low sensitivity and reproducibility
- Blood culture technologies which include BACTEC take hours, if not days, to detect bacteremia.
- The majority of childhood sepsis deaths occur in low-income countries where malnutrition, poor sanitation, and co-morbidities increase
- Most polyclonally antibodies targeting LPS, demonstrate cross-reactivity to the antigen from a variety of gram-negative bacteria. Such antibodies can be further used for broadly sensitive assay for sepsis
- No point of care methods for the direct and sensitive measurement of LPS from blood, other than the one presented in this manuscript

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