# Point-of-Care Sensors for Salivary Endotoxin Detection

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*Abstract*— Porphyromonas gingivalis is a key biomarker responsible for chronic periodontitis and closely related to systemic diseases. Early and accurate detection of its endotoxin LPS is critical for effective oral health as well as whole body wellness. Here, we present different methodologies of using a point-of-care lateral flow assay for detecting P. gingivalis LPS. Conventional colorimetric LFAs using antibody-based sandwich assay achieved limit of detections of ~22 ng/mL in water and ~46.5 ng/mL in saliva samples, with excellent selectivity. Saliva samples were pretreated with syringe filtration and potato starch to improve the assay performances by inhibiting α-amylase, abundant in saliva. To further enhance sensitivity under the physiological range, SERS detection method was integrated into LFA using SERS-sensitive silver-coated gold nanostars, achieving an LOD < 10 ng/mL. Preliminary results of thermal detection using more affordable IR camera is also presented.

***Keywords—biomarkers, saliva, endotoxin, P. gingivalis, LPS, lateral flow assay, antibody, SERS***

## I. Introduction

The presence of endotoxin biomolecules, such as bacterial lipopolysaccharide (LPS) and others, affect the homeostasis of the body.[1, 2] The concentrations of these molecules in the body are an indication of its state, hence the use of the term biomarker. Evaluation of the level of biomarkers is important to diagnose physical diseases and to evaluate mental conditions from stress, depression, etc. Historically, biomarker detection was accomplished by laboratory analysis after proper sampling of biological fluids, such as whole blood, plasma, sweat, urine, and saliva.[3] Low cost and highly sensitive diagnostic devices, are becoming a powerful option for this purpose, as experienced during the COVID-19 Pandemic. It is not only useful for detecting personal disease but also helpful for preventing the community-wide spread of the disease. Although the conventional laboratory-based detection and diagnosis approach is still the routine procedure, demand is increasing for rapid and/or continuous monitoring of biomarkers. Demand is also increasing for the simultaneous evaluation of multiple biomarkers in order to provide a more global understanding of related symptoms.

## II. Biomarkers in Bodily Fluids

Routine assessments of biomarkers using PoC devices is increasingly important. Monitoring biomarkers can provide the insight of the human health in both mental and physical aspects. Most biomarkers can be detected from various bodily fluids with a variety of sensor types, including electrical, chemical, and optical detection, as illustrated in Fig. 1.

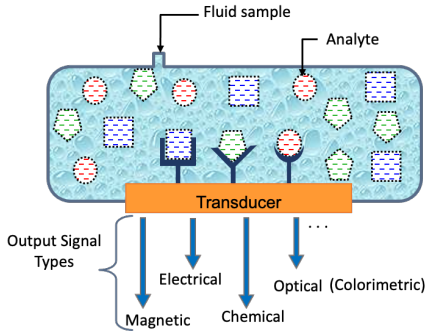


Fig. 1 Transducer types for detecting biomarkers in various bodily fluids. Reprinted with permission from ref.[4] Copyright 2018 Amer. Chem. Soc.

Molecular weight and size of biomarkers are critical factors in selecting the appropriate detection methodology.[4] While blood testing is often considered as the "gold standard" for quantifying a wide variety of biomarkers varieties, it typically needs invasive sampling in controlled environment, which can be stressful and inappropriate for frequent sampling. Moreover, some biomarkers are found at higher concentration in other bodily fluids. For example, dopamine is most concentrated in urine, while NPY and BDNF concentrations in saliva are higher than that in blood plasma.[5] Similarly, endotoxin LPS concentration in blood is ~1pM, whereas in saliva it ranges widely from <1fM to >1nM. Considering targeted biomarkers and their abundance, specific bodily fluids can be selected. For example, saliva can be used as the medium for detecting cortisol and endotoxin LPS. Saliva is very versatile and attractive biofluid for PoC applications because of its non-invasiveness,

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stress-free collection, and excellent availability.[6] It contains a complex mixture of proteins, peptides, DNA, cell debris, etc., which can serve as biomarkers for many medical conditions, such as infections, drug use, and other toxic compounds.[7] Especially, bacterial lipopolysaccharides (LPS) found in saliva, is an important macromolecule that are released after death and lysis of Gram-negative bacteria. Endotoxins mainly consist of lipid and polysaccharide parts as shown in Fig. 2a. Lipid A is the most toxic part and the polysaccharide has diverse configurations depending on different species and strains of the bacteria.[8] Once LPS is released, the toxic lipid A part is exposed to the host's immune system. LPS elicits strong defense reactions, such as immune responses, various inflammations, life-threatening sepsis, and possibly septic shock.[9]

*P. gingivalis* is a key Gram-negative pathogenic bacterium that is responsible for chronic periodontitis, which presents a chronic inflammation caused by bacterial infection, leading to gum degradation/disease and eventual tooth loss.[10] LPS derived from *P. gingivalis* (PG LPS) elicits strong immune responses in gingival tissue by stimulating the production of inflammatory biomarkers, such as interleukin (IL)-1β, IL-6, IL-8, interferon-γ and tumor necrosis factor alpha (TNF-α) in gingival tissue.[11] High level of *P. gingivalis* LPS in saliva is strongly related to a chronic periodontitis disease. Moreover, recent studies have found that P. gingivalis is closely linked to the systemic diseases such as cardiovascular diseases[12], rheumatoid arthritis[13], and neurodegenerative (Alzheimer's) disorders [14] (Fig. 2b). Therefore, quantitative detection of PG LPS is increasingly important not only for oral health, but also for systemic health monitoring. The Litmus Amebocyte Lysate (LAL)[15], a current gold-standard for LPS detection, and other methods such as ELISA, electrochemical (EC) sensing[16], electrochemical impedance spectroscopy (EIS), have been developed.[17] However, they often face limitations such as specificity and portability for the POC use.

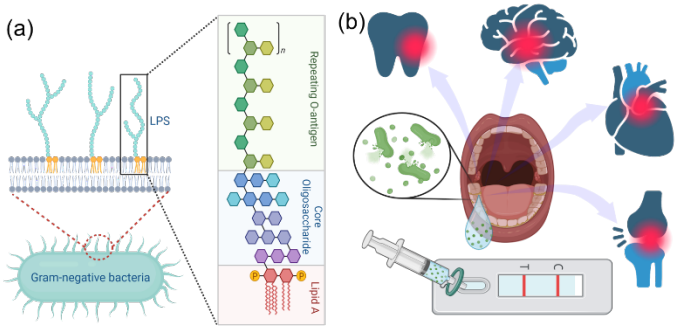


Fig. 2 Biomarkers present in saliva indicate and affect multiple aspects of human health. P. gingivalis bacterium releases lipopolysaccharide toxin. Modified from ref. [18] Copyright 2023 The Royal Society of Chemistry.

## III. LATERAL FLOW ASSAY DETECTION

The lateral flow assay (LFA) platform is widely used and commercialized for PoC applications for pregnancy, infectious diseases and for other health conditions.[19, 20] During the COVID-19 pandemic, LFA-based test kits have achieved a nearly universal use.[21] In LFA operation, the liquid sample under test is dispensed onto a structure consisting of a stack of porous membranes. The capillary force in a porous membrane allows the fluid sample to flow without external pumps. The label and recognition molecules are contained in various regions of the LFA stack and sample solution interacts with the relevant molecules as it flows through the device. LFAs are known as robust, cost-effective, and user-friendly POC systems. Colorimetric test lines formed on the membrane indicate the presence of the target biomarkers. The selection of the recognition element (antibody or aptamer) is based on its ability to bind to the target analyte with high affinity and selectivity. The structure of an LFA biosensor is shown schematically in Fig. 3. The colorimetric detection is caused by a color change of the test and control lines due to the accumulation of label molecules, such as gold nanoparticles (AuNP). The most common approach is the use of antibody as biorecognition molecule. The simplest approach uses direct detection of the analyte captured by the biorecognition molecules immobilized in the test and control lines. Reporter (or label) molecules are introduced that will bind to the analyte and generate a measurable optical signal. However, the signal and specificity produced in the direct detection method is too low. Improvements in LOD and specificity are obtained using the "sandwich" detection approach.

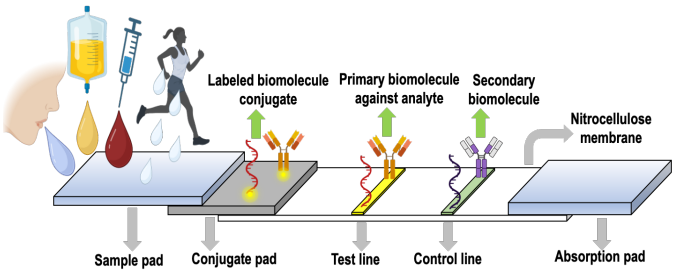


Fig. 3 Biomarker detection using lateral flow assays.

Using antibody-based sandwich LFIA, we have successfully detected *P. gingivalis* LPS in human saliva. Fig. 4a illustrates the basic design of the sandwich LFA assay. LPS is suitable for sandwich assay due to its large molecular weight (> 10 kDa) with multiple binding epitopes. When the saliva sample containing LPS is dispensed on the sample pad, the LPS binds to the antibody (Ab)-conjugated AuNPs at the conjugate pad. Resulting LPS-Ab-AuNP conjugated complex migrates along the nitrocellulose membrane and is captured at the test line by an immobilized 2nd antibody that can bind to different binding epitope of the LPS. Sufficient accumulation of these complexes at the test line generates the visible red color line on the membrane, indicating a positive result. The control line appears regardless of the LPS presence by detecting the antibody conjugated AuNPs, validating the assay's functionalities. The colorimetric intensity of the test line increases with LPS concentration (Fig. 4b), enabling the semi-quantitative analysis.

The selectivity of the sandwich assay was evaluated against other LPS and proteins. The LFA demonstrated excellent selectivity for *P. gingivalis* LPS (Fig. 4c). Other LPS resulted in minimal or no test line formation. *P. pallens* LPS formed a weak test line, this is not an issue for for oral health evaluation because *P. pallens* LPS is also closely related to oral disease, especially for gingivitis. Mucin, which is the most abundant protein in saliva, and *E. coli* LPS did not produce a test line signal, confirming the high selectivity of the assay.

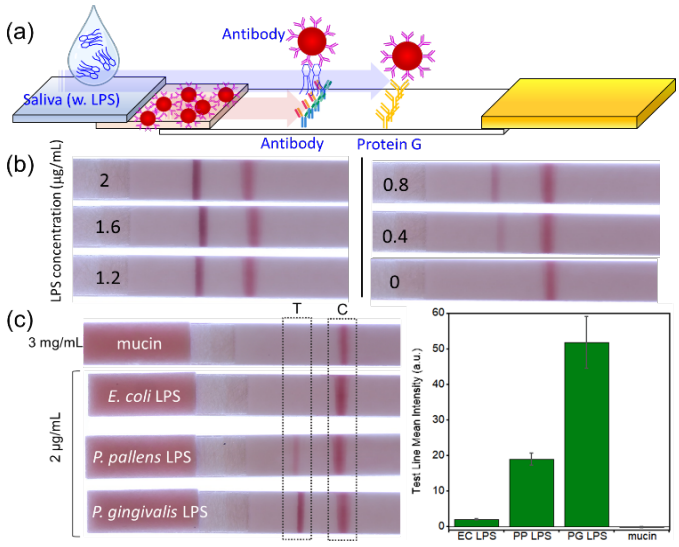


Fig. 4 Antibody-based sandwich LFIA for *P. gingivalis* LPS detection: (a) basic LFA mechanism and implementations; (b) LFA test line intensities vs LPS conc; (c) Selectivity of P. gingivalis vs other salivary endotoxins. Modified from ref. [18] Copyright 2023 The Royal Society of Chemistry.

## IV. SERS-BASED OPTICAL DETECTION

To enhance the LFA sensitivity, we report on the use of surface-enhanced Raman scattering (SERS) technique for detecting *P. gingivalis* LPS.[22] Fig. 5a illustrates the schematic of the SERS and thermal detection mechanisms on the LFA strip. In the presence of target molecules (PG LPS), antibodies conjugated on nanoparticles and a Raman reporter first bind to the LPS, and then bind to the 2nd antibody immobilized on the nitrocellulose membrane of LFA strip. Upon laser excitation, Raman reporter molecules absorbed on the surface of metal NPs generate strong Raman scattering output through the SERS effect. The enhanced electromagnetic field on metal nanoparticles also generates the localized heating, which can be detected by a low-cost infrared (IR) camera. Experimental

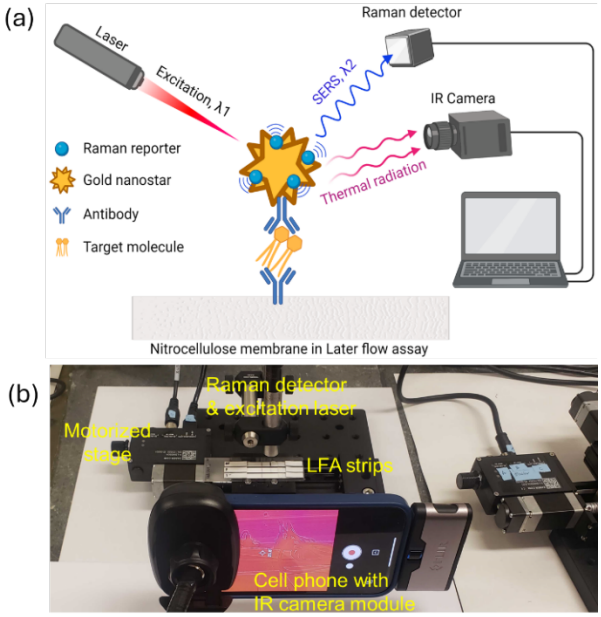


Fig. 5 Measurements of SERS and thermal imaging detection on LFA test strips: (a) schematic of working mechanisms, (b) experimental configuration.

setup of both SERS and thermal imaging (Fig. 5b) shows the LFA strip mounted on a motorized stage to scan the targeted strip area. Vertically aligned laser and Raman detector are located above the LFA strips. During SERS measurement, thermal imaging is simultaneously taken using the IR camera module connected to the cell phone.

Fig. 6 shows the results of thermal imaging and SERS measurements on LFA test strips. The plasmonic photothermal properties of Au nanostars, generating temperature increases, are a straightforward and sensing methodology using an affordable IR camera. Temperature changes by plasmonic photothermal effects on LFA test strip are shown in Fig. 6a. For the negative case (no LPS), localized heating is only observed in the control line, increasing up to ~41 °C, while the test line exhibits only minimal temperature change (no test line formation). On the other hand, the positive test strip presented the localized heating for both control and test lines (both lines are formed). All temperature measurements were taken after 10 s of laser exposure. The SERS test line intensity is compared with the corresponding colorimetric intensity measurements (using ImageJ) in Fig. 4b. Colorimetric detection had LODs in the 20-100 ng/mL range, while SERS achieved LOD < 10 ng/mL, ~ 10x enhancement of the LFA sensitivity.

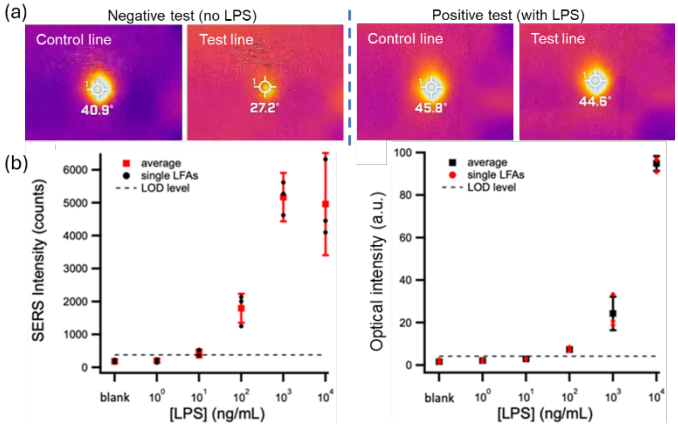


Fig. 6 Measurements of thermal imaging and SERS on LFA test strips: (a) thermal images taken by IR camera for negative and positive test results on LFA; (b) comparison of SERS and colorimetric signal vs LPS concentration. Modified from ref. [22] Copyright 2024 The Royal Society of Chemistry.

## V. FUTURE PROSPECT

The COVID-19 pandemic clearly proved the value of cost-effective and user-friendly LFA-based PoC platform to prevent community-wide spreading of contagious disease. In the future, biomarker sensors can be combined with actuators and control electronics to provide timely "Smart" treatments corresponding to detected biomarkers in an active or passive mode. For example, different stages of wound recovery can be recognized by detecting related biomarker levels, triggering the release of the proper drug or stimulation of the wound to accelerate the recovery process.

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