Journal of Medical Devices Technology
jmeditec.utm.my

Review Article

Lateral Flow Biosensors (LFBs) for Rapid Detection of Infectious Diseases and Saliva as a Diagnostic Biofluid

Norjihada Izzah Ismail[1,2*], Pavithiran Sathis Kumar[1], Najib Abdul Salam L. William[1], Nur Natasha Amyra Ahmad Azizi[1], Tham Chun Hwa[1], Abdullah Basam Halak[1], Ibrahim Adel Mohammed Qahteh[1]

[1]Department of Biomedical Engineering and Health Sciences, Faculty of Electrical Engineering, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia.
[2]Medical Devices and Technology Centre, Institute of Human Centered Engineering, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia.

ARTICLE INFO

Article History:
Received 24 November 2024
Accepted 29 December 2024
Available online 30 December 2024

Keywords:
Lateral flow biosensor,
Infectious disease,
Pathogen,
Saliva,
Antibodies

ABSTRACT

This review highlights the importance and recent development of lateral flow biosensors (LFBs) for rapid detection of infectious diseases. The LFBs could provide better disease prognosis, diagnosis, community surveillance and continuous care of patients and useful for point-of-care (POC) testing. This review also discusses the alternative sampling using saliva for sensitive and specific detection of infectious agents, indicating the effectiveness of saliva as a noninvasive diagnostic biofluid for diagnosis of infectious diseases.

INTRODUCTION

A biosensor is a device that is commonly used to measure biological or chemical interactions. The signals produced by biosensor are proportional to the concentration of an analyte in the reaction. The components of a typical biosensor include analyte, bioreceptor, transducer, electronics and display (Bhalla et al., 2016). Biosensors are used in a variety of applications, including environmental monitoring, food safety testing as well as disease diagnosis and monitoring for detection of pathogenic microorganisms, and disease markers in physiological fluids such as blood, urine, saliva, and sweat (Lin et al., 2021; Tang et al., 2022).

Lateral flow biosensors (LFBs) are a form of flow devices that has the recognition layer on the surface of a porous membrane such as nitrocellulose (NC) membrane. The membrane generates and maintains the flow of the sample and reagents by capillary action, and it contains specialized recognition elements in the specified zones of the membrane known as reactive zones or detection sites (Anfossi et al., 2018; Tang et al. 2022). A typical LFBs or also called lateral flow device (LFD), lateral flow test strip (LFTS), lateral-flow immunoassay (LFIA) or immunochromatographic assay (ICA) consists of four sections known as the sample pad, conjugate pad, nitrocellulose pad and absorbent pad (Huang et al., 2020). There are at least two reaction sites presence on the detecting membranes where selective antibodies are aligned to produce the test and control lines. Because of its cheaper cost, fast detection, adaptability for use by unskilled workers, portability, multiplex capability and easy analytical procedures, the LFBs have received a lot of interest as a rapid detection approach for biological investigation and clinical diagnostics (Liu et al., 2018).

The human oral cavity serves as a home and a significant entrance site for microorganisms, many of which dwell and grow there including bacteria, fungi, viruses, and protozoa. While some of the oral microbiome plays important roles for oral health, the opportunistic or pathogenic microorganisms may compromise the microbiome environment, affecting systemic health and leading to disease development (Lu et al., 2022; Tian et al., 2024). The bacteria are the dominant component of oral

* Norjihada Izzah Ismail (norjihada@utm.my)

MEDITEC, Institute of Human Centered Engineering, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia.

microbiota with those belonging to the phyla *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, *Fusobacteria*, *Proteobacteria*, and *Spirochetes* are the most prevalent (Lu et al., 2022; Tian et al., 2024). Viruses such as Epstein-Barr virus (EBV), human papillomavirus (HPV), and herpes simplex viruses (HSV-1) in the oral cavity have been linked to various types of oral conditions such as ulcers, oral tumors, classical oral infectious diseases, and periodontitis (Asai and Nakashima, 2018).

Oral sampling can be divided into invasive and noinvasive sampling. Noninvasive oral sampling such as tongue and buccal swabs, saliva, and oral rinses are simple, reliable, and versatile options that might considerably improve high-throughput sampling in community settings since such technique does not require specialist training and imposes little or no pain to the patients (Azzi et al., 2020; Valinetz and Cangelosi, 2021). The noninvasive oral sampling is not only reliable for detection of oral diseases but also infectious agents such as respiratory virus SARS-CoV-2 (Azzi et al., 2020; Valinetz and Cangelosi, 2021). Using saliva as a testing medium is more convenient to users and saliva collection is considered as a potential alternative to nasopharyngeal swab or sputum sampling because of its technical simplicity (Azzi et al., 2020).

LFBS FOR DETECTION OF INFECTIOUS DISEASES

An infectious disease is an illness that originates from live pathogens such as bacteria, viruses and parasites or its toxic product. This illness which is capable of rapid transmission arises through inoculation, airborne or waterborne transmission from an infected person or animal, or a contaminated inanimate object to a susceptible host (van Seventer and Hochberg, 2017; Wang et al., 2021a). Development of rapid, sensitive andaccurate detection methods to control infection source is the most important management strategies of infectious disease. Therefore, the development of point-of-care (POC) diagnostics which provides real-time, accurate, rapid and on-site detection for timely prevention and control of the pandemic is of utmost priority (Wang et al., 2021a).

LFB is one of the most commonly used biosensors especially for POC testing, commercially available due to its low-cost, long storage time, usage simplicity, rapid results, and high sensitivity (Lin et al., 2021; Guliy and Dykman, 2024). LFBs can be used to detect the presence of target analytes in the various samples taken from human body including saliva, urine, serum, plasma and blood (Liu et al., 2018; Bjerrum et al., 2019; Yrad et al., 2019; Lin et al., 2021; Yang et al., 2021). For detection of pathogenic virus or bacteria in a sample, some studies have combined LFBs with other techniques, allowing detection of corresponding infections based on the binding of analytes such as antibodies, antigens, RNA or DNA presence in body's sample to the bioreceptor attached to the membrane test line. The results of LFBs are interpreted as either positive or negative by visual observation of colour changes (colorimetric) or accompanied by more accurate analytical methods such as the magnetic, electrochemical, fluorescent, and surface-enhanced Raman scattering (SERS) assays which can be measured using devices (Lin et al., 2021; Huang et al., 2020). Table 1 presents the different types of analytes detected using LFBs for diagnosis of infectious diseases

Among all methods, gold nanoparticle-based lateral flow biosensors (AuNPs-LFB) are widely used due to their relative stability, ease of synthesis, unique optical properties, and visual analysis (Guliy and Dykman, 2024). Besides, AuNPs has been used due to their highly sensitive and selective detection of target molecules such as DNA, proteins and small molecules, attributed by their surface plasmon resonance (SPR) properties (Ferrari, 2023). These nanoparticles can act as anchors for different antibodies. The AuNP-conjugated antibodies can rapidly detect biomolecule and gives colorimetric results from the binding (Guliy and Dykman, 2024). The appearance of a band at the test line following the capture and detection of AuNP-conjugated antibodies is as shown in Figure 1. As shown in Figure 1(a), the gold spheres in the sample that represent the target analytes will bind to the AuNP-conjugated antibodies that are located in the conjugate pad. The sample moves along the test strip towards the absorbent pad via lateral diffusion due to the capillary forces, and specific immune complexes are formed at the test and control lines which appeared as coloured bands (Huang et al., 2020; Guliy and Dykman, 2024). The signals are visible to the naked eye as reddish bands as shown in Figure 1(b) due to the use of AuNP (Martinez-Liu et al., 2022).

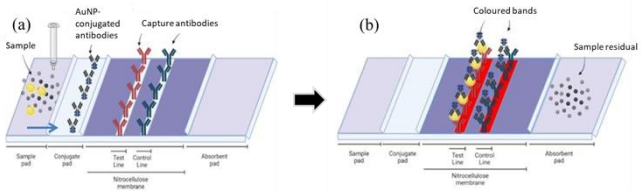

Fig. 1 A schematic diagram of a gold nanoparticle-based LFB (AuNP-LFB).

The reverse transcription-loop-mediated isothermal amplification (RT-LAMP) is another technique that has been paired with LFB for rapid detection of pathogens including hepatitis C (Shi et al., 2024) and HIV type 1 virus (Chen et al., 2023). The LAMP is a cost-effective and highly sensitive nucleic acid amplification which takes 20 minutes to complete and does not require complex equipment (Yan et al., 2020; Chandrasekaran et al, 2022). By using the RT-LAMP, amplification of DNA can be made without requiring RNA extraction. The viral RNA is converted to DNA substrates using a reverse transcriptase (Chandrasekaran et al, 2022). Besides, recombinase polymerase amplification lateral flow strip (RPA-LFS) is also a promising type of LFBs for detection of infectious pathogens due to its simplicity, rapidity and readability by naked eye (Srisrattakarn et al., 2022). Similar to LAMP, RPA is an isothermal method for DNA amplification and serves as an alternative to PCR. This technique is highly sensitive, rapid, and more robust than the PCR method in which the amplification process of target gene can be done across a temperature range from 25 to 45 °C within 3 to 20 min as well as in the presence of inhibitors (Srisrattakarn et al., 2022).

Table 1. Analytes detected using LFBs for infectious diseases rapid detection.

Human sample	Pathogen	Detected Analyte(s)/ Component(s)	Method(s)	Diagnosis	References
-	*Mycobacterium tuberculosis*	TB antigens (ESAT-6 and CFP-10)	Gold nanoparticle- based lateral flow immunoassay (AuNP-based LFIA)	Tuberculosis (TB)	Seele et al. (2023)
Urine	*Mycobacterium tuberculosis*	Mycobacterial antigen lipoarabinomann-an (LAM)	Lateral flow urine lipoarabinomannan (LF-LAM)	Tuberculosis (TB)	Bjerrum et al. (2019)
-	Methicillin-resistant *Staphylococcus aureus* (MRSA)	Peptidoglycan and penicillin-binding protein 2a (PBP2a)	Gold nanoparticle- based multiplex lateral flow immunoassay (AuNP-based multiplex LFIA)	Pneumonia	Amini et al. (2023)
Blood culture	Methicillin-resistant *Staphylococcus aureus* (MRSA)	nuc- and mecA-RPA products	Recombinase polymerase amplification lateral flow strip (RPA-LFS)	Pneumonia	Srisrattakarn et al. (2022)
Nasal swab	*Bordetella pertussis*	DNA	Multiple cross displacement amplification (MCDA)-LFBs	Pertussis	Li et al. (2020)
Serum	*Clostridium tetani*	Tetanus antibody	Quantum dots (QD)-based lateral flow test strips (LFTS)	Tetanus	Wang et al. (2019)
Whole blood	*Clostridium tetani*	Tetanus antibody	AuNP-based LFTS	Tetanus	Liu et al. (2018)
Stool	*Salmonella typhi and Salmonella paratyphi*	DNA amplicons	Multiplex PCR-lateral flow biosensor (mPCR-LFB)	Typhoid fever	Amalina et al. (2021)
Serum	Dengue-1 virus (DENV-1)	RNA	Nucleic acid sequence-based amplification (NASBA)-LFBs	Dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS)	Yrad et al. (2019)
-	Dengue-1,2,3,4 virus	Nonstructural protein 1 (NS1) antigen	Fluorescent nanodiamond (FND)-based spin-enhanced lateral flow immunoassay (SELFIA)	Dengue fever (DF) and Dengue hemorrhagic fever (DHF)	Le et al. (2022)
Isolated DENV sample from positive serum	Dengue virus	Domain II of protein E	AuNP-based LFIA	Dengue	Martinez-Liu et al. (2022)
-	Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2 virus)	Nucleocapsid (N) and spike (S) antigens	FND-based SELFIA	2019 Coronavirus disease (COVID-19)	Hsiao et al. (2022)
Nasop-harynge-al swabs	SARS-CoV-2 virus	DNA amplicons	Reverse transcription loop-mediated isothermal amplification (RT-LAMP) lateral flow assays (LFA)	2019 Coronavirus disease (COVID-19)	Tang et al. (2024)
Serum	SARS-CoV-2 virus	SARS-CoV-2 IgM and IgG antibodies	SARS-CoV-2 IgM/IgG antibody test kit (colloidal gold method)	2019 Coronavirus disease (COVID-19)	Wang et al. (2021b)
Serum	Human immunodeficiency virus type one (HIV-1)	DNA amplicons	Reverse transcription loop-mediated isothermal amplification (RT-LAMP) AuNP-based LFA	Acquired immunodeficiency syndrome (AIDS)	Chen et al. (2023)
Plasma	Hepatitis B virus (HBV)	DNA	Polymerase spiral reaction (PSR)-LFBs	Hepatitis B	Lin et al. (2021)
Serum	Hepatitis B virus (HBV)	DNA amplicons	LAMP-AuNPs-LFB	Hepatitis B	Chen et al. (2021)
Serum	Hepatitis C virus (HCV)	DNA amplicons	RT-LAMP-AuNPs-LFB	Hepatitis C	Shi et al. (2024)

Table 2 Salivary biomarkers for specific infectious agents.

Biomarkers	Type	Pathogen	Detection method(s)	Reference
E and RdRP genes of SARS-CoV-2	RNA	SARS-CoV-2 virus	Real-time reverse transcriptase polymerase chain reaction (rRT-PCR)	Yoon et al. (2020)
IgG	Antibody	SARS-CoV-2 virus	Enzyme-linked immunosorbent assay (ELISA)	MacMullan et al. (2020)
IgA	Antibody	SARS-CoV-2 virus	Enzyme-linked immunosorbent assay (ELISA)	Varadhachary et al. (2020)
ORF1ab and N genes of SARS-CoV-2	RNA	SARS-CoV-2 virus	Colorimetric reverse transcription loop-mediated isothermal amplification (RT-LAMP)	Yang et al. (2021)
N2 gene of SARS-CoV-2 and human ACTB gene	RNA	SARS-CoV-2 virus	Multiplex reverse transcription loop-mediated isothermal amplification lateral flow biosensor (RT-LAMP LFB)	Naranbat et al. (2024)
IgA, IgG, and IgM	Antibodies	Human immunodeficiency virus (HIV)	Enzyme-linked immune assay (ELISA)	Vohra et al. (2020)
UL23 gene (thymidine kinase gene of HSV-1)	DNA	Herpes simplex viruses 1 (HSV-1)	Polymerase chain reaction (PCR) and agarose gel electrophoresis	Robinson et al. (1992)
-	DNA	Herpes simplex viruses 1 (HSV-1)	Real-time quantitative PCR	Kaufman et al. (2005)
-	DNA	Varicella Zoster virus (VZV) also known as human herpesvirus 3 (HHV-3)	Real-time quantitative PCR	Park et al. (2018)
5′ untranslated (5′UTR) region of HCV	RNA	Hepatitis C virus (HCV)	Reverse transcription polymerase chain reaction (RT-PCR) and agarose gel electrophoresis	Hermida et al. (2002)
5′ untranslated (5′UTR) and non-structural protein 5B (NS5B) regions of HCV	RNA	Hepatitis C virus (HCV)	RT-PCR and agarose gel electrophoresis	Zitha et al. (2022)
Insertion sequence (IS) 6110 and IS1081	DNA	*Mycobacterium tuberculosis* complex (MTBC)	Quantitative PCR (qPCR)	Ayalew et al. (2024)
-	DNA	*Neisseria meningitidis*	Quantitative PCR (qPCR)	Miellet et al. (2021)
IgG	Antibody	*Trypanosoma cruzi*	Indirect enzyme linked immunosorbent assay (ELISA)	Pinho et al. (1999)

SALIVA AS A DIAGNOSTIC TOOL OF INFECTIOUS DISEASES

Saliva is a complex biofluid produced by the salivary glands, containing water as its primary content and a wide range of other compositions such as minerals, electrolytes, enzymes, cytokines, immunoglobulins, sugars, lipids, polypeptide proteins, hormones, vitamins, organic acids and inorganic ions as well as shed cells, food residues, and microbes (Cui et al., 2022; Lu et al., 2022; Tian et al., 2024). pH of human saliva naturally varies between 6.8 and 7.4 (Yang et al., 2021). The presence of nutrients and specific ions are essential for specific bacteria growth (Tian et al., 2024). In healthy adults, approximately 600 – 1500 mL of saliva is produced daily that covers oral cavity areas including mucosa, teeth, gingiva, tongue, and palate. The flow rate of saliva decreases during sleeping, gradually increases after waking up in the morning and remain high during the day (Cui et al., 2022; Lu et al., 2022). Saliva play several roles including provides lubrication, promotes chewing and digestion, and acts as a buffer for acidic foods. It also contains numerous substances with antibacterial, antifungal and/or antiviral properties such as immunoglobulins, defensin and thiocyanate, hence affecting oral microbiota in multiple ways (Cui et al., 2022).

Saliva samples are easier to obtain via self-collection and serve as important source of infection biomarkers such as viral DNA and RNA, antigens and antibodies (Zhang et al., 2016; Yang et al., 2021). Table 2 presents the salivary biomarkers of infectious disorders. It has been revealed that saliva samples contain comparable levels of SARS-CoV-2 viral load as the

nasopharyngeal swabs, indicating its potential as a diagnostic tool (Yang et al., 2021). A higher viral load was detected in saliva than in the oropharynx during the early stage of COVID-19 and saliva exhibited consistently high SARS-CoV-2 viral load (Yoon et al., 2020). Other than that, enzyme-linked immunosorbent assay (ELISA) technique can be utilized to detect HIV specific antibodies in saliva (Vohra et al., 2020). As the saliva composition including the biomarkers can also be found in plasma, its use for clinical diagnosis and monitoring offers simplicity and may reduce dependency on invasive procedure of blood sampling (Cui et al., 2022). Saliva has been used as diagnostic fluid for detection of viruses, bacteria and parasites including SARS-CoV-2 virus, HIV, HCV, HSV-1, HHV-3, *Mycobacterium tuberculosis* complex (MTBC), *Neisseria meningitidis* and *Trypanosoma cruzi* as shown in Table 2.

CONCLUSION

Emerging and remerging infectious diseases remain global health threats, therefore LFBs play crucial role in providing rapid, sensitive and accurate detection of infectious agents in order to control infection within community as well as prevent transmission to new populations or geographic areas. Saliva has been proven as a suitable substitute for blood, has simple collection procedure and can provide reliable results, which are important for infectious disease diagnosis, prognosis and population surveillance.

ACKNOWLEDGEMENT

This research was financially supported by the Ministry of Higher Education (MoHE) of Malaysia through Fundamental Research Grant Scheme (FRGS/1/2023/SKK06/UTM/02/2).

REFERENCES

Amalina, Z.N., Khalid, M.F., Rahman, S.F., Ahmad, M.N., Ahmad Najib, M., Ismail, A., & Aziah, I. (2021). Nucleic acid-based lateral flow biosensor for *Salmonella typhi* and *Salmonella paratyphi*: A detection in stool samples of suspected carriers. *Diagnostics* (Basel). 11(4), 700.

Amini, M., Pourmand, M.R., & Faridi-Majidi, R. (2023). Development of a high sensitive multiplex lateral flow immunoassay (lfia) system for rapid detection of methicillin-resistant *Staphylococcus aureus* (MRSA). *Avicenna Journal of Medical Biotechnology*, 15(2), 100-107.

Anfossi, L., Di Nardo, F., Cavalera, S., Giovannoli, C., & Baggiani, C. (2018). Multiplex lateral flow immunoassay: An overview of strategies towards high-throughput point-of-need testing. *Biosensors*, 9(1), 2.

Asai, D., & Nakashima, H. (2018). Pathogenic viruses commonly present in the oral cavity and relevant antiviral compounds derived from natural products. *Medicines* (Basel). 5(4), 120.

Ayalew, S., Wegayehu, T., Wondale, B., Kebede, D., Osman, M., Niway, S., Tarekegn, A., Tessema, B., Berg, S., Ashford, R.T., & Mihret, A. (2024). Detection of Mycobacterium tuberculosis complex in saliva by quantitative PCR: A potential alternative specimen for pulmonary tuberculosis diagnosis. *Tuberculosis*, 148, 102554.

Azzi, L., Carcano, G., Gianfagna, F., Grossi, P., Gasperina, D.D., Genoni, A., Fasano, M., Sessa, F., Tettamanti, L., Carinci, F., Maurino, V., Rossi, A., Tagliabue, A., & Baj, A. (2020). Saliva is a reliable tool to detect SARS-CoV-2. *Journal of Infection*, 81(1), e45-e50.

Bhalla, N., Jolly, P., Formisano, N., & Estrela, P. (2016). Introduction to biosensors. *Essays in Biochemistry*. 60(1), 1-8.

Bjerrum, S., Schiller, I., Dendukuri, N., Kohli, M., Nathavitharana, R.R., Zwerling, A.A., Denkinger, C.M., Steingart, K.R., & Shah, M. (2019). Lateral flow urine lipoarabinomannan assay for detecting active tuberculosis in people living with HIV. *Cochrane Database of Systematic Review*, 10(10), CD011420.

Chandrasekaran, S.S., Agrawal, S., Fanton, A. Jangid, A.R., Charrez, B., Escajeda, A.M., Son, S., Mcintosh, R., Tran, H., Bhuiya, A., de León Derby, M.D., Switz, N.A., Armstrong, M., Harris, A.R., Prywes, N., Lukarska, M., Biering, S.B., Smock, D.C.J., Mok, A., Knott, G.J., Dang, Q., Van Dis, E., Dugan, E., Kim, S., Liu, T.Y., Consortium, I.T., Moehle, E.A., Kogut, K., Eskenazi, B., Harris, E., Stanley, S.A., Lareau, L.F., Tan, M.X., Fletcher, D.A., Doudna, J.A., Savage, D.F., & Hsu, P.D. (2022). Rapid detection of SARS-CoV-2 RNA in saliva via Cas13. *Nature Biomedical Engineering*, 6, 944-956.

Chen, X., Du, C., Zhao, Q., Zhao, Q., Wan, Y., He, J., & Yuan, W. (2023). Rapid and visual identification of HIV-1 using reverse transcription loop-mediated isothermal amplification integrated with a gold nanoparticle-based lateral flow assay platform. *Frontiers in Microbiology*, 14, 1230533.

Chen, X., Wang, S., Tan, Y., Huang, J., Yang, X., & Li, S. (2021). Nanoparticle-based lateral flow biosensors integrated with loop-mediated isothermal amplification for the rapid and visual diagnosis of Hepatitis B virus in clinical application. *Frontiers in Bioengineering and Biotechnology*, 9, 731415.

Cui, Y., Yang, M., Zhu, J., Zhang, H., Duan, Z., Wang, S., Liao, Z., & Liu, W. (2022). Developments in diagnostic applications of saliva in human organ diseases. *Medicine in Novel Technology and Devices*, 13, 100115.

Ferrari, E. (2023). Gold nanoparticle-based plasmonic biosensors. *Biosensors* (Basel), 13(3), 411.

Guliy, O.I. & Dykman, L.A. (2024). Gold nanoparticle–based lateral-flow immunochromatographic biosensing assays for the diagnosis of infections. *Biosensors and Bioelectronics*: X, 17, 100457.

Hermida, M., Ferreiro, M.C., Barral, S., Laredo, R., Castro, A., & Dios, P.D. (2002). Detection of HCV RNA in saliva of patients with hepatitis C virus infection by using a highly sensitive test. *Journal of Virological Methods*, 101(1-2), 29-35.

Hsiao, W.W.-W., Sharma, N., Le, T.-N., Cheng, Y.-Y., Lee, C.-C., Vo, D.-T., Hui, Y.Y., Chang, H.-C., & Chiang, W.-H. (2022). Fluorescent nanodiamond-based spin-enhanced lateral flow immunoassay for detection of SARS-CoV-2 nucleocapsid protein and spike protein from different variants. *Analytica Chimica Acta*, 1230, 340389.

Huang, Y., Xu, T., Wang, W., Wen, Y., Li, K., Qian, L., Zhang, X., & Liu, G. (2020). Lateral flow biosensors based on the use of micro- and nanomaterials: a review on recent developments. *Microchima Acta*, 187, 70.

Kaufman, H.E., Azcuy, A.M., Varnell, E.D., Sloop, G.D., Thompson, H.W., & Hill, J.M. (2005). HSV-1 DNA in tears and saliva of

normal adults. *Investigative Ophthalmology & Visual Science*, 46, 241-247.

Le, T.-N., Hsiao, W.W.-W., Cheng, Y.-Y., Lee, C.-C., Huynh, T.-T., Pham, D.M., Chen, M., Jen, M.-W., Chang, H.-C., & Chiang, W.-H. (2022). Spin-enhanced lateral flow immunoassay for high-sensitivity detection of nonstructural protein NS1 serotypes of the dengue virus. *Analytical Chemistry*, 94(51), 17819-17826.

Li, S., Liu, C., Liu, Y., Ma, Q., Wang, Y., & Wang, Y. (2020). Establishment and application of a multiple cross displacement amplification combined with nanoparticles-based biosensor method for the detection of *Bordetella pertussis*. *BMC Microbiology*, 20(1), 263.

Lin, L., Guo, J., Liu, H., & Jiang, X. (2021). Rapid detection of Hepatitis B virus in blood samples using a combination of polymerase spiral reaction with nanoparticles lateral-flow biosensor. *Frontier in Molecular Biosciences*, 7, 578892.

Liu, J., Wang, J., Li, Z., Meng, H., Zhang, L., Wang, H., Li, J., & Qu, L. (2018). A lateral flow assay for the determination of human tetanus antibody in whole blood by using gold nanoparticle labeled tetanus antigen. *Microchima Acta*, 185(2), 110.

Lu, H., Zou, P., Zhang, Y., Zhang, Q., Chen, Z., & Chen, F. (2022). The sampling strategy of oral microbiome. *iMeta*. 1(2), e23.

MacMullan, M.A., Ibrayeva, A., Trettner, K., Deming, L., Das, S., Tran, F., Moreno, J.R., Casian, J.G., Chellamuthu, P., Kraft, J., Kozak, K., Turner, F.E., Slepnev, V.I., & Le Page, L.M. (2020). ELISA detection of SARS-CoV-2 antibodies in saliva. *Scientific Reports*, 10(1), 20818.

Martinez-Liu, C., Machain-Williams, C., Martinez-Acuña, N., Lozano-Sepulveda, S., Galan-Huerta, K., Arellanos-Soto, D., Meléndez-Villanueva, M., Ávalos-Nolazco, D., Pérez-Ibarra, K., Galindo-Rodríguez, S., Garza-Juarez, A.d.J. & Rivas-Estilla, A.M. (2022). Development of a rapid gold nanoparticle-based lateral flow immunoassay for the detection of dengue virus. *Biosensors*, 12, 495.

Miellet, W.R., Mariman, R., Pluister, G., de Jong, L.J., Grift, I., Wijkstra, S., van Logchem, E.M., van Veldhuizen, J., Immink, M.-M., Wijmenga-Monsuur, A.J., Rots, N.Y., Sanders, E.A.M., Bosch, T., & Trzciński, K. (2021). Detection of *Neisseria meningitidis* in saliva and oropharyngeal samples from college students. *Scientific Reports*, 11, 23138.

Naranbat, D., Murphy, J., & Tripathi, A. (2024). An automated syringe-based PoC RT-LAMP LFB platform for infectious disease detection from saliva. *Frontiers in Lab on a Chip Technologies*, 3, 1450891.

Oliveira Neto, N.F.d., Caixeta, R.A.V., Zerbinati, R.M., Zarpellon, A.C., Caetano, M.W., Pallos, D., Junges, R., Costa, A.L.F., Aitken-Saavedra, J., Giannecchini, S., & Braz-Silva, P.H. (2024). The emergence of saliva as a diagnostic and prognostic tool for viral infections. *Viruses*, 16, 1759.

Park, S.Y, Kim, J.Y, Kim, J.A., Kwon, J.S., Kim, S.M., Jeon, N.Y., Kim, M.C., Chong, Y.P., Lee, S.O., Choi, S.H., Kim, Y.S., Woo, J.H., & Kim, S.H. (2018). Diagnostic usefulness of Varicella-Zoster virus real-time polymerase chain reaction analysis of DNA in saliva and plasma specimens from patients with herpes zoster. *Journal of Infectious Disease*, 217, 51-57.

Pinho, R.T., Pedrosa, R.C., Costa-Martins, P., & Castello-Branco, L.R. (1999). Saliva ELISA: a method for the diagnosis of chronic Chagas disease in endemic areas. *Acta Tropica*. 72(1), 31-8.

Robinson, P.A., High, A.S., & Hume, W.J. (1992). Rapid detection of human Herpes Simplex virus type 1 in saliva. *Archives of Oral Biology*. 37(10), 797-806.

Seele, P.P., Dyan, B., Skepu, A., Maserumule, C., & Sibuyi, N.R.S. (2023). Development of gold-nanoparticle-based lateral flow immunoassays for rapid detection of TB ESAT-6 and CFP-10. *Biosensors (Basel)*. 13(3), 354.

Shi, Y., Zhou, Q., Dong, S., Zhao, Q., Wu, X., Yang, P., Zeng, X., Yang, X., Tan, Y., Luo, X., Xiao, Z. & Chen, X. (2024). Rapid, visual, label-based biosensor platform for identification of hepatitis C virus in clinical applications. *BMC Microbiology*, 24, 68.

Srisrattakarn, A., Panpru, P., Tippayawat, P., Chanawong, A., Tavichakorntrakool, R., Daduang, J., Wonglakorn, L., & Lulitanond, A. (2022). Rapid detection of methicillin-resistant Staphylococcus aureus in positive blood-cultures by recombinase polymerase amplification combined with lateral flow strip. *PLoS ONE*, 17(6), e0270686.

Tang, J., Zhu, J., Wang, J., Qian, H., Liu, Z., Wang, R., Cai, Q., Fang, Y. & Huang, W. (2024). Development and clinical application of loop-mediated isothermal amplification combined with lateral flow assay for rapid diagnosis of SARS-CoV-2. *BMC Infectious Diseases*, 24, 81.

Tang, R., Xie, M.Y., Li, M., Cao, L., Feng, S., Li, Z., & Xu, F. (2022). Nitrocellulose membrane for paper-based biosensor. Applied Materials Today, 26, 101305.

Tian, S., Ding, T., & Li, H. (2024). Oral microbiome in human health and diseases. *mLife*. 3, 367-383.

Valinetz, E.D., & Cangelosi, G.A. (2021). A look inside: oral sampling for detection of non-oral infectious diseases. *Journal of Clinical Microbiology*, 59, e02360-20.

van Seventer, J.M., & Hochberg, N.S. (2017). Principles of infectious diseases: transmission, diagnosis, prevention, and control. *International Encyclopedia of Public Health*, 6, 22-39.

Varadhachary, A., Chatterjee, D., Garza, J., Garr, R.P., Foley, C., Letkeman, A., Dean, J., Haug, D., Breeze, J., Traylor, R., Malek, A., Nath, R., & Linbeck, L. III (2020). Salivary anti-SARS-CoV-2 IgA as an accessible biomarker of mucosal immunity against COVID-19. *medRxiv*. 2020.08.07.20170258.

Vohra, P., Belkhode, V., Nimonkar, S., Potdar, S., Bhanot, R., Izna, Tiwari, R.V.C. (2020). Evaluation and diagnostic usefulness of saliva for detection of HIV antibodies: A cross-sectional study. *Journal of Family Medicine and Primary Care*, 9(5), 2437-2441.

Wang, C., Liu, M., Wang, Z., Li, S., Deng, Y., & He, N. (2021a). Point-of-care diagnostics for infectious diseases: From methods to devices. *Nano Today*, 37, 101092.

Wang, H., Li, X., Li, T., Wang, L., Wang, L., Lin, J., Zhang, S., Xu, Y., & Wei, W. (2021b). Development of a SARS-CoV-2 rapid antibody detection kit and study on dynamic changes in antibodies in infected patients. *Clinical Respiratory Journal*, 15(5), 499-505.

Wang, J., Meng, H.-M., Chen, J., Liu, J., Zhang, L., Qu, L., Li, Z., & Lin, Y. (2019). Quantum dot-based lateral flow test strips for highly sensitive detection of the tetanus antibody. *ACS Omega*, 4(4), 6789-6795.

Yan, C., Cui, J., Huang, L., Du, B., Chen, L., Xue, G., Li, S., Zhang, W., Zhao, L., Sun, Y., Yao, H., Li, N., Zhao, H., Feng, Y., Liu, S., Zhang, Q., Liu, D., & Yuan, J. (2020). Rapid and visual detection

of 2019 novel coronavirus (SARS-CoV-2) by a reverse transcription loop-mediated isothermal amplification assay. *Clinical Microbiology and Infection*, 26(6), 773-779.

Yang, Q., Meyerson, N.R., Clark, S.K., Paige, C.L., Fattor, W.T., Gilchrist, A.R., Barbachano-Guerrero, A., Healy, B.G., Worden-Sapper, E.R., Wu, S.S., Muhlrad, D., Decker, C.J., Saldi, T.K., Lasda, E., Gonzales, P., Fink, M.R., Tat, K.L., Hager, C.R., Davis, J.C., Ozeroff, C.D., Brisson, G.R., McQueen, M.B., Leinwand, L.A., Parker, R., & Sawyer, S.L. (2021). Saliva TwoStep for rapid detection of asymptomatic SARS-CoV-2 carriers. *elife*. 10, e65113.

Yoon, J.G., Yoon. J., Song. J.Y., Yoon, S.Y., Lim, C.S., Seong, H., Noh, J.Y., Cheong, H.J., Kim, W.J. (2020). Clinical significance of a high SARS-CoV-2 viral load in the saliva. *Journal of Korean Medical Science*, 35(20), e195.

Yrad, F.M., Castañares, J.M., & Alocilja, E.C. (2019). Visual detection of dengue-1 RNA using gold nanoparticle-based lateral flow biosensor. *Diagnostics*, 9, 74.

Zhang, C.Z., Cheng, X.Q., Li, J.Y., Zhang, P., Yi, P., Xu, X., & Zhou, X.-D. (2016). Saliva in the diagnosis of diseases. *International Journal of Oral Science*, 8, 133–137.

Zitha, T., Chen, C.Y., Mudawi, H., Hussein, W., Mukhtar, M., Shigidi, M., Yousif, M.E.A., Ali, M.A., Glebe, D., & Kramvis, A. (2022). Molecular characterization and genotyping of hepatitis C virus from Sudanese end-stage renal disease patients on haemodialysis. *BMC Infectious Diseases*. 22(1), 848.