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In vitro diagnosis based on SERS–LFIA: research hotspots, increase sensitivities, combined detection, multimodal detection and related patents†

Yongwei Zhang, ‡[a] Qian Zhang,‡[a] Ziyue Li,[a] Xuelei Zhou,[g] Chunyan Liu,[f] Hefei Zha,[f] Xin Zhang,[f] Hui Zhao,[e] Guodong Lü,[d] Jiutong Li*[ag] and Xinxia Li *[abc]

In recent years, the SERS–LFIA platform has gained significant traction in *in vitro* diagnostics. However, a comprehensive review of its advancements and applications is still lacking. This review first employing a bibliometric approach to analyze research trends. It then outlines strategies to enhance sensitivity, focusing on Raman reporter molecules, SERS tags, coupling methods, detection instruments. Additionally, the review explores the use of SERS–LFIA for diagnosing multiple disease biomarkers, highlighting its potential to improve diagnostic accuracy. The review also synthesizes the application of multimodal SERS–LFIA technology, integrating signals such as colorimetric, magnetic, photothermal, fluorescent, and catalytic modalities. This approach enhances detection versatility and broadens diagnostic capabilities. Furthermore, it examines the current patent landscape, providing insights into the technology's commercial and technological progress. Lastly, the review discusses ongoing challenges, including stability and reproducibility and quantitative detection, while suggesting directions for future research. In summary, this review consolidates the latest advancements in SERS–LFIA technology for *in vitro* diagnostics over the past decade. Anticipated to furnish a robust scientific foundation and theoretical underpinning for the advancement of SERS–LFIA technology, this endeavor aims to enhance its efficacy in clinical diagnostics.

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1. Introduction

In the current context, the incidence of various diseases, including tumors, cardiovascular diseases, and infectious diseases, is continuously rising. Traditional clinical detection methods in the medical field often exhibit certain limitations in the timely detection and precise treatment of these conditions. Some detection methods may lack sufficient sensitivity to identify subtle changes in biomarkers during the early stages of disease, or they may demonstrate poor specificity, leading to false-positive or false-negative results.[1] The advent of Surface-Enhanced Raman Scattering – Lateral Flow Immunoassay (SERS–LFIA) provides a novel approach to address these limitations.[2] This technique enables the detection of biomarkers, including tumor markers, pathogen antigens or antibodies, and disease-related biomolecules, with high sensitivity and accuracy in clinical specimens such as blood, urine, saliva, nasal swabs, and throat swabs. SERS–LFIA is anticipated to play a pivotal role in various clinical applications, including early disease screening, diagnostic staging, and disease monitoring during treatment, thereby significantly enhancing patient treatment outcomes and prognoses. Consequently, a comprehensive review of the applications of SERS–LFIA in clinical detection is of paramount importance.

SERS utilizes the Raman scattering effect to achieve ultrasensitive detection through the enhancement of Raman signals

[a] College of Pharmacy, Xinjiang Medical University, Urumqi, 830054, China. E-mail: lxx6668@xjmu.edu.cn, 2330617483@qq.com
[b] Key Laboratory of High Incidence Disease Research in Xingjiang (Xinjiang Medical University), Ministry of Education, Urumqi, 830054, China
[c] Xinjiang Key Laboratory of Biopharmaceuticals and Medical Devices, Urumqi, 830013, China
[d] State Key Laboratory of Pathogenesis, Prevention, and Treatment of Central Asian High Incidence Diseases, Clinical Medical Research Institute, The First Affiliated Hospital of Xinjiang Medical University, Urumqi, 830054, China
[e] Department of Clinical Laboratory, The First Affiliated Hospital of Xinjiang Medical University, Urumqi, 830054, China
[f] Department of Clinical Laboratory, Hospital of Xinjiang Production and Construction Corps, No. 232, Qingnian Road, Tianshan District, Urumqi, 830002, China
[g] Xinjiang Xingyi Bio-Science Co., Ltd, Urumqi, 830011, China

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‡ Yongwei Zhang and Qian Zhang made equal work to this paper.

by specialized metal nanostructures, which can amplify the signals of target substances by a factor of 10^6 or greater.[3] A distinctive feature of SERS is its spectral fingerprinting capability, wherein different molecules exhibit unique Raman spectra, thereby providing additional identification information for precise detection.[4] SERS holds significant application value in medical diagnostics, enabling the detection of disease biomarkers associated with tumors, cardiovascular diseases, and infectious diseases, which facilitates early diagnosis.[5] Furthermore, SERS can rapidly and accurately identify pathogens, including bacteria, viruses, and fungi, by determining their species, quantities, and drug sensitivity profiles.[6] Additionally, SERS can be employed for cell and tissue imaging through labeling techniques, aiding in the study of cellular physiological and pathological processes as well as pathological diagnosis.[7]

LFIA is a rapid diagnostic technique based on the specific antigen–antibody interaction. Its principle involves combining immunological reactions with chromatographic techniques, allowing for the rapid detection of target analytes on substrates.[8,9] LFIA offers several unique advantages. First, its operation is straightforward and does not require complex instrumentation, making it suitable for point-of-care testing, bedside applications, and resource-limited settings. Medical personnel or even minimally trained individuals can perform the detection, significantly reducing the diagnostic turnaround time and enabling timely intervention.[10] LFIA has demonstrated broad application potential in clinical fields, including infectious disease diagnosis, tumor marker detection, and pregnancy testing.[11] However, traditional LFIA relies on visual inspection or simple optical instruments to detect colloidal gold aggregation on the test line (T line), which limits its sensitivity and may fail to generate detectable signals for low-concentration targets.

The integration of SERS with LFIA combines the specificity of LFIA's antigen–antibody reaction with the spectral fingerprinting capability of SERS, thereby enhancing the overall specificity and recognition ability of the detection system for target biomarkers.[12–14] LFIA provides an immune-based platform for SERS, while SERS contributes a powerful signal amplification capability to LFIA, significantly improving the sensitivity of the system.[15] Compared to other LFIA technologies, such as colorimetric, fluorescence, and photothermal methods, SERS–LFIA offers one distinct advantage: SERS exhibits no photobleaching or autofluorescence, ensuring accurate and reproducible assay results.[16–18] As an emerging detection technology, SERS–LFIA is increasingly demonstrating its unique advantages and potential in medical diagnostics and trace analysis of clinical samples.[19,20]

In this review, we comprehensively summarize the advancements in SERS–LFIA technology for clinical sample diagnostics over the past decade. This includes an analysis of research trends, strategies for sensitivity enhancement, the development of multimodal and combined detection approaches, and an overview of the international patent landscape (Fig. 1). Our aim is to provide researchers and practitioners in this field with a thorough and insightful scientific foundation for further exploration and application.

2. Analysis of research hotspots

CiteSpace is a bibliometric analysis tool that enables precise identification of research hotspots and frontier directions within scientific fields.[21] By analyzing document keywords and co-cited references, it can detect frequently studied topics and emerging research trends.[22] This capability provides valuable guidance for researchers to understand current research dynamics, select innovative topics, and align their work with disciplinary frontiers, thereby avoiding redundant studies and enhancing the novelty and impact of their research.[23]

The temporal distribution of publications related to SERS and LFIA from 2015 to 2024 reveals the developmental

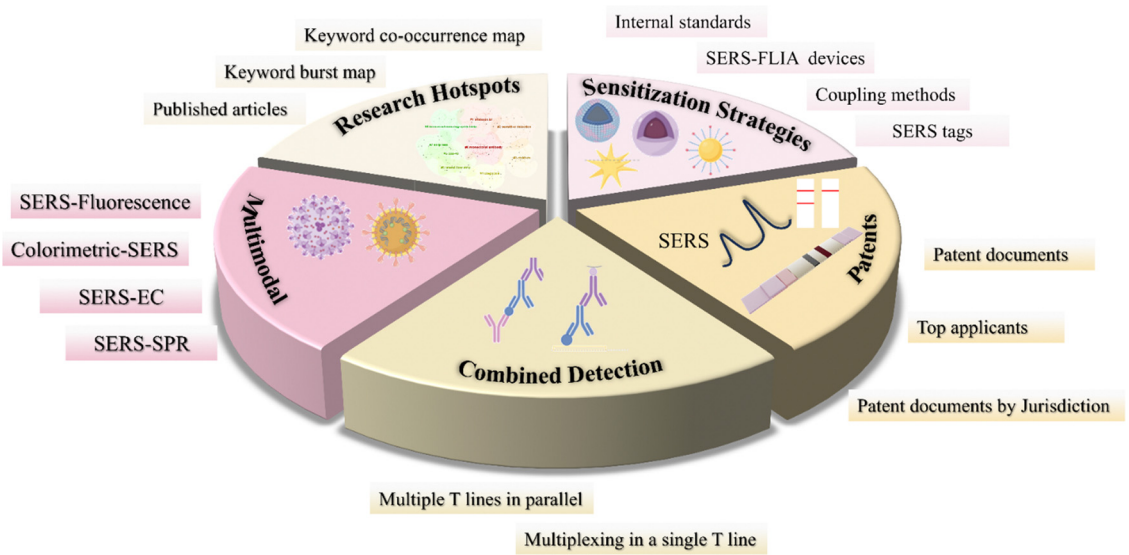

Fig. 1 The schematic overview of *in vitro* diagnosis based on SERS–LFIA.

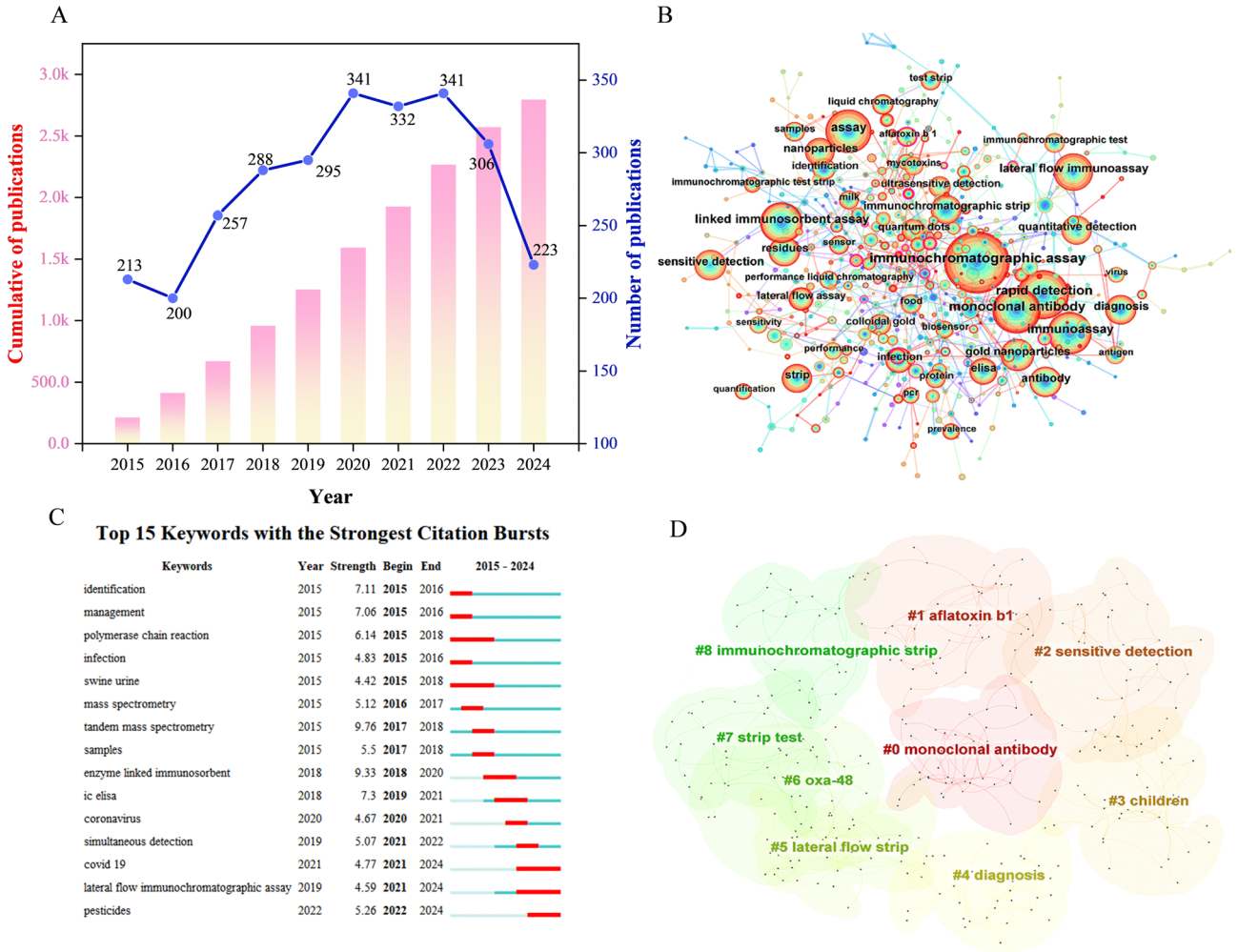

Fig. 2 (A) The number of published articles and the cumulative number of published articles on SERS and LFIA. (B) Keyword co-occurrence map. (C) Keyword burst map. (D) Keyword clustering map.

trajectory and trends in this field. Fig. 2A illustrates the annual and cumulative number of English-language publications on SERS and LFIA. Notably, the number of publications exhibited a consistent upward trend during this period, reflecting the growing interest of the international research community in these technologies.

A keyword co-occurrence analysis was performed to identify research foci, and the resulting co-occurrence network is presented in Fig. 2B. Among the prominent keywords, ''immunochromatographic analysis'' was most frequent, followed by ''monoclonal antibody,'' ''sensitivity detection,'' ''quantitative detection,'' ''qualitative detection,'' ''colloidal gold,'' ''diagnosis,'' and ''rapid detection.'' From 2015 to 2024, keywords such as ''infection,'' ''lateral flow immunoassay,'' ''identification,'' ''combined detection,'' and ''sample'' gained prominence, indicating a shift toward rapid detection research, particularly in the context of clinical infectious diseases like COVID-19 (Fig. 2C). This trend underscores the expanding application potential of LFIA in clinical infectious disease diagnostics. Cluster analysis (Fig. 2D) generated nine distinct clusters, primarily focusing on topics such as monoclonal antibodies, aflatoxin B1, sensitivity detection, and combined diagnosis in pediatric populations.

These findings demonstrate that since 2015, research efforts have increasingly concentrated on the diagnostic applications of LFIA, as evidenced by the high-frequency keywords. Researchers have explored various possibilities of immunochromatographic technology to achieve more accurate and efficient diagnostic outcomes, driving advancements in medical diagnostics across multiple dimensions, including technological innovation and clinical implementation.

3. Strategies for enhancing the sensitivity of SERS–LFIA

3.1 SERS tags

SERS tags typically consist of three key components: Raman reporter molecules, metal nano substrates, and protective shells or linking molecules. The metal nano substrates can be

either single-sized and single-shaped nanoparticles or complex architectures composed of multiple nanoparticles. Variations in the shape and size of these nano substrates significantly influence their localized surface plasmon resonance (LSPR) properties, thereby affecting the Raman enhancement efficiency. Additionally, the distribution and arrangement of Raman reporter molecules on the metal nano substrates play a critical role in determining the overall performance of SERS tags. These molecules can be uniformly adsorbed onto the nanoparticle surfaces or precisely anchored at specific sites through targeted chemical bonding strategies. Furthermore, the functionalization of SERS tags represents a pivotal approach for broadening their application scope and enhancing their performance. A summary of clinical sample detection using SERS tags in conjunction with the SERS–LFIA method is provided in Table S1 (ESI†).

3.1.1 Raman reporting molecule. Raman reporter molecules are defined as molecules capable of generating specific Raman signals in spectroscopic analysis.[24] These molecules provide detectable and characteristic Raman spectral signals, enabling the identification and quantitative analysis of target substances.[25–29] Typically, their structures contain unsaturated bonds, such as alkenes and alkynes, or specific functional groups, which exhibit distinct Raman scattering peaks under defined conditions. By analyzing the positions and intensities of these peaks, the identification and quantification of clinical samples can be achieved. A summary of commonly used Raman reporter molecules and dye molecules in SERS–LFIA for *in vitro* diagnostics is provided in Table S2 (ESI†).

Among the most frequently used Raman reporter molecules in SERS–LFIA-based *in vitro* diagnostics are 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) and 4-mercaptobenzoic acid (4-MBA). DTNB features two nitrobenzoic acid groups connected by a disulfide bond, while 4-MBA contains a mercapto group and a benzoic acid group. These structural components confer unique Raman spectral characteristics, making them ideal for SERS applications.

For instance, Yan *et al.* developed SERS tags by conjugating a specific antibody for *Escherichia coli* O157:H7 to a core–shell nanostructure coated with DTNB as the Raman reporter. The corresponding SERS–LFIA test strips exhibited a characteristic Raman peak at 1335 cm^{-1} on the test line.[30] Similarly, Wu *et al.* synthesized 4-mercaptophenylboronic acid (4-MPBA)-modified gold nanoparticles and applied them in an antibody-independent LFIA method. This approach demonstrated higher sensitivity compared to the traditional double-antibody sandwich method, with a visual detection limit as low as 10^3 CFU per mL.[31] Additionally, to ensure strong Raman signals, some researchers have employed 4-nitrobenzenethiol (4-NBT), which has the largest Raman cross-section among mercapto aromatic derivatives, as the Raman reporter.[15]

Raman dyes are dye molecules capable of generating characteristic Raman signals during spectroscopic detection.[32] These signals can be used to identify the dyes themselves, and their interactions with other substances can provide valuable information for target detection. Many Raman dye molecules contain extensive conjugated double-bond systems, and some possess reactive functional groups, such as thiol (–SH) and amino ($–NH_2$) groups, which facilitate interactions with target analytes.[33] For example, Zhang *et al.* prepared SERS tags using methylene blue (MB), 4-nitrobenzoic acid (NBA), and rhodamine 6G (R6G) to generate three distinct SERS nano labels. Through an established SERS–LFIA platform, they achieved simultaneous quantitative detection of three cardiac biomarkers.[34]

In summary, high-quality Raman reporter molecules should exhibit the following characteristics: (1) a high Raman scattering cross-section, (2) stability under Raman laser irradiation, (3) stability in diverse chemical environments, (4) ease of conjugation with target molecules, and (5) the ability to generate stable and reproducible Raman signals under varying experimental conditions.

3.1.2 Biorecognition molecules. Biorecognition molecules are specialized molecules capable of identifying and binding to specific target molecules in clinical samples. These molecules exhibit high specificity and play critical roles in diagnostic and sensor applications. Common examples include antibodies, antigens, ligands, enzymes, nucleic acids (DNA and RNA), aptamers, and proteins.[35,36]

Ilhan *et al.* compared the performance of phages and antibodies as analytical reagents on LFIA test strips. They found that phage-based systems significantly enhanced sensitivity and exhibited most characteristics of an ideal pathogen detection assay.[37] Additionally, aptamers offer advantages over antibodies, including smaller size, higher specificity, superior chemical stability, and ease of synthesis. For instance, Li *et al.* developed a multivalent aptamer for detecting *Staphylococcus aureus*, which increased binding affinity from 135.9 nM to 16.77 nM, an improvement of more than eightfold. The detection system achieved a limit of detection (LOD) of 2 CFU per mL and completed analysis within 30 min.[38] Similarly, Pang *et al.* integrated ultrasensitive SERS tags with CRISPR-Cas12a for nucleic acid detection. Without pre-amplification, they directly quantified HIV-1 double-stranded DNA with an LOD of 0.3 fM, nearly four orders of magnitude lower than that of colorimetric methods.[39]

When developing SERS–LFIA methods for clinical sample analysis, the selection of biorecognition molecules should adhere to the following criteria: (1) appropriate affinity to ensure effective capture of low-concentration targets, thereby enhancing sensitivity; (2) batch-to-batch consistency to ensure reliable and reproducible detection results; and (3) compatibility with the SERS–LFIA platform to maintain optimal performance.

3.1.3 Metal SERS tags. SERS nanotags can generate highly intense fingerprint signals, making them suitable replacements for colorimetric methods and other sensors as ultrasensitive indicators.[40] A typical SERS tag consists of noble metal nanoparticles, which serve as plasmonic enhancement substrates, coupled with Raman reporter molecules attached to the nanostructures.[41]

Initially, single-metal nanoparticles, such as gold nanoparticles (AuNPs), gold nanosheets, and silver nanoparticles

(AgNPs), were widely adopted in the detection of medical disease markers due to their strong plasmonic properties and straightforward synthesis processes.[42] For example, Zeng *et al.* developed polydopamine (PDA)-functionalized gold nanoparticles (Au@PDA) as SERS tags. The incorporation of PDA enhanced the light absorption of AuNPs and provided abundant functional groups for conjugation. This approach enabled the quantitative detection of *Staphylococcus aureus* and *Escherichia coli* with a LOD of 100 CFU per mL.[43] Despite their widespread use in clinical detection, single-metal nanoparticles face significant challenges, including susceptibility to environmental interference and limitations in sensitivity and stability.[44,45]

To address these issues, advanced SERS tags with superior Raman signals have been developed, including hollow gold nanospheres (HGNs), gold nanorods (Au NRs), and gold nano stars (Au NSs), which aim to enhance the sensing capabilities of SERS–LFIA. For instance, a research team developed Au NRs with significantly enhanced SERS activity, achieving an LOD of 9.2 pg mL^{-1} for alpha-fetoprotein detection (Fig. 3A).[46] Su *et al.* employed core–shell Au/Au nanostructures as multifunctional markers to establish a colorimetric/SERS dual-modal analytical method for detecting clenbuterol hydrochloride. This method achieved an LOD of 0.05 ng mL^{-1}, which is 200 times lower than that of conventional LFIA (Fig. 3B).[47] Similarly, Wu *et al.* utilized AuNF-PMBA as multifunctional SERS tags for *Escherichia coli* detection, achieving a visual LOD of 10^3 CFU per mL, surpassing the performance of traditional methods (Fig. 3C).[48]

However, SERS tags with Au or Ag shells exhibit limitations such as low chemical stability, poor biocompatibility, and a tendency for nonspecific aggregation in high-salt or low-pH environments, which can compromise the precision and robustness of SERS–LFIA methods.[50]

3.1.4 Metal–nonmetal spherical SERS tags. The integration of metals with non-metallic materials, such as SiO_2, has emerged as a key strategy in the development of SERS–LFIA for detecting disease markers in the medical field.[51,52] These composite materials primarily function as label supports to enhance signal intensity, thereby improving the detection efficiency, and chemical stability of the system.[53] Specifically, gold or silver nano shells can be deposited onto SiO_2 microspheres *via* chemical reduction methods, followed by the formation of nanocomposite microspheres through electrostatic adsorption.[54,55]

Recent studies by Wang *et al.* have demonstrated that SiO_2-based nanocomposite LFIA test strips exhibit excellent stability and high accuracy, making them suitable for clinical infectious disease screening.[56–58] Additionally, research teams led by Liu and Pang have developed SiO_2-based SERS nanocomposites (*e.g.*, SiO_2@Ag and SiO_2@Au) as alternatives to traditional colloidal SERS tags. These SiO_2-based SERS tags demonstrate superior sensitivity and stability in detecting target proteins (IgG/IgM) and HIV dsDNA using LFIA (Fig. 3D).[39,49] However, the application of SiO_2 nanocomposites in SERS–LFIA requires specialized sample buffer solutions to ensure recognition precision, which complicates the preparation process. Furthermore, the relatively weak signals generated by SERS tags and the limited loading capacities of Au and Ag nanoparticles constrain the potential for signal enhancement.

Magnetic nanomaterials (MNPs) offer a promising solution to address interference issues in SERS–LFIA. Due to their ability to capture and separate analytes, remove impurities, and amplify signals, MNPs have been widely employed in the construction of biosensors for clinical samples.[59,60] For instance, Wang *et al.* developed Fe_3O_4@Ag nanoparticles as magnetic SERS tags for a sensitive and quantitative SERS–LFIA-based test strip capable of simultaneously detecting H1N1 virus and human adenovirus (HAdV). The LODs for H1N1 and HAdV were 50 and 10 pfu per mL, respectively, with a sensitivity 2000 times greater than that of Au nanoparticle-based methods.[61]

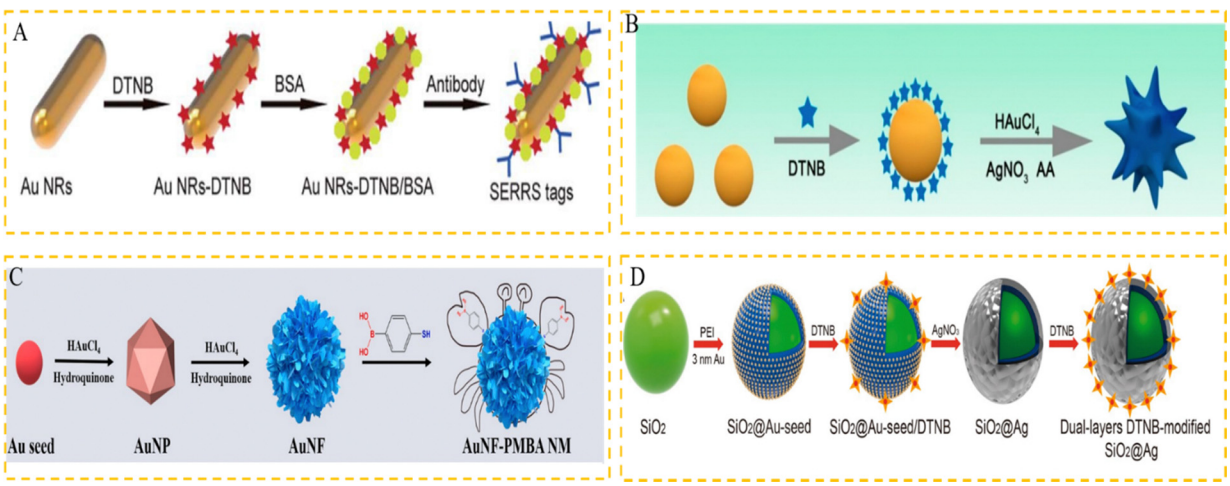

Fig. 3 Metal and metal–nonmetal spherical SERS tags for detecting clinical samples based on the SERS–LFIA method. (A: reproduced from ref. 46 with permission from Royal Society of Chemistry, copyright 2020; B: reproduced from ref. 47 with permission from American Chemical Society, copyright 2021; C: reproduced from ref. 48 with permission from Elsevier, copyright 2023; D: reproduced from ref. 49 with permission from Elsevier, copyright 2021).

Despite their advantages, the preparation of magnetic nanomaterials *via* coating methods involves a series of chemical reduction reactions, which often result in challenges such as low reproducibility, non-uniform nanostructures, unstable SERS activities, and uncontrollable hot spots.

3.1.5 Film-like SERS tags. The SERS tags fabricated from traditional spherical nanomaterials exhibit significant limitations, including a tendency for simple agglomeration, inadequate carrying capacity, and poor fluidity. These limitations have emerged as critical bottlenecks in the advancement of SERS–LFIA technology. In contrast, recent studies have demonstrated that both 2D and 3D thin-film nanoparticles possess exceptional electronic and optical properties, rendering them highly suitable for biosensor applications.[62]

In comparison to spherical tags, GO@Au nanomaterials exhibit a greater number of binding sites owing to their substantially enhanced surface area. This unique characteristic facilitates more efficient capture of target pathogens while simultaneously improving the uniformity and fluidity of immune complex distribution on test strip surfaces. Li *et al.* developed 2D GFe-DAu-D/M tags with universal bacterial capture capability through a layer-by-layer assembly process, incorporating one layer of small-sized Fe_3O_4 NPs and two layers of 30-nm AuNPs. This configuration created a 0.5 nm built-in nanogap on single-layer GO, followed by co-modification with 4-MPBA and DTNB. The resulting GFe-DAu-D/M tags demonstrated rapid enrichment of multiple bacterial species through MPBA-mediated capture, enabling quantitative analysis at the test line (Fig. 4).[63]

Despite their high sensitivity, membranous SERS tags present several technical challenges: (1) their typical size exceeds 500 nm, (2) the incorporation of numerous gold or silver nanoparticles within the membrane structure significantly increases the mass and volume of 2D/3D SERS tags, and (3) these factors collectively contribute to compromised fluidity and increased susceptibility to clogging on both the sample pad and nitrocellulose membrane.[64]

In summary, an ideal SERS tag should demonstrate the following essential characteristics: high SERS activity: capable of significantly enhancing Raman signals for highly sensitive molecular detection. Chemical stability and biocompatibility: maintaining consistent SERS performance without interference or degradation during sample processing and analysis. Facile surface functionalization: enabling the attachment of specific recognition molecules or functional units through chemical or biological modification strategies.

3.2 Conjugation method

3.2.1 Non-covalent binding. In medical detection applications utilizing SERS–LFIA technology, the physical adsorption method is predominantly employed for conjugating SERS tags with antibody or antigen molecules. This approach involves labeling detection molecules, including antibodies, proteins, and peptides, onto SERS tags through various intermolecular interactions, such as hydrophobic interactions, electrostatic interactions, hydrogen bonding, and van der Waals forces.[65]

Fig. 5A illustrates the physical adsorption of antibodies on citrate-stabilized AuNP surfaces under varying pH conditions, demonstrating the effect of pH on antibody adsorption.[66] Hattori *et al.* demonstrated that antibody immobilization on gold nanoparticles *via* physical adsorption reached optimal efficiency within the pH range of 7.0–9.0.[67] Similarly, Wiriyachaiporn *et al.* successfully employed the physical adsorption method to immobilize antibodies on nanobead-strung carbon nanoparticles for rapid influenza A virus detection, achieving a LOD of 350 $TCID_{50}$ per mL.[68]

3.2.2 Covalent bonding. Fig. 5B mainly illustrates the functional group-mediated conjugation processes on nanoparticle surfaces, including three representative strategies: (i) antibody conjugation to carboxyl-modified nanoparticles *via* EDC/NHS chemistry, (ii) antibody coupling with amino-modified nanoparticles through EDC/NHS activation, and (iii) glutaraldehyde-facilitated antibody binding to hydroxyl-modified nanoparticles.[66,70] Fig. 5C demonstrates two antibody conjugation approaches through chemical modification, offering versatile strategies for linking detection molecules to labeling probes: (A) thiol-functionalized antibody conjugation to gold nanoparticles,

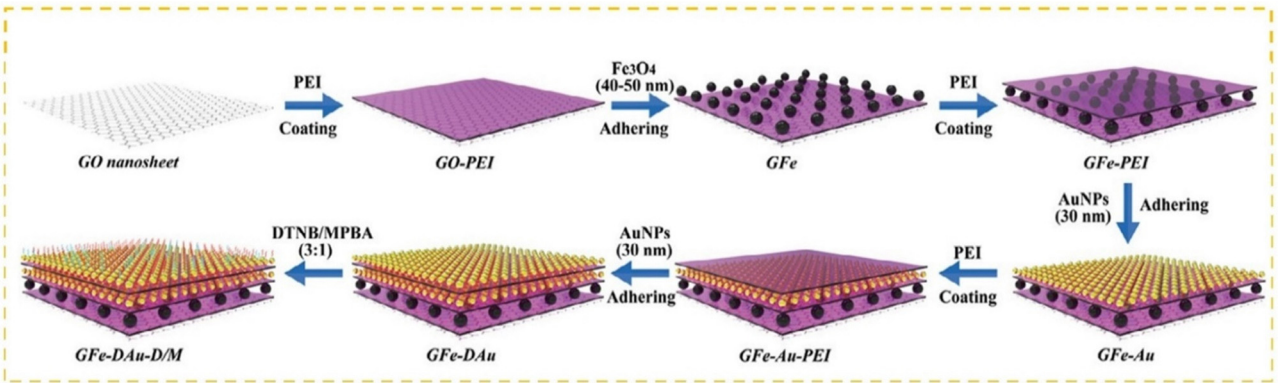

Fig. 4 Film-like SERS tags for detecting clinical samples based on the SERS–LFIA method. Reproduced from ref. 63 with permission from Wiley, copyright 2024.

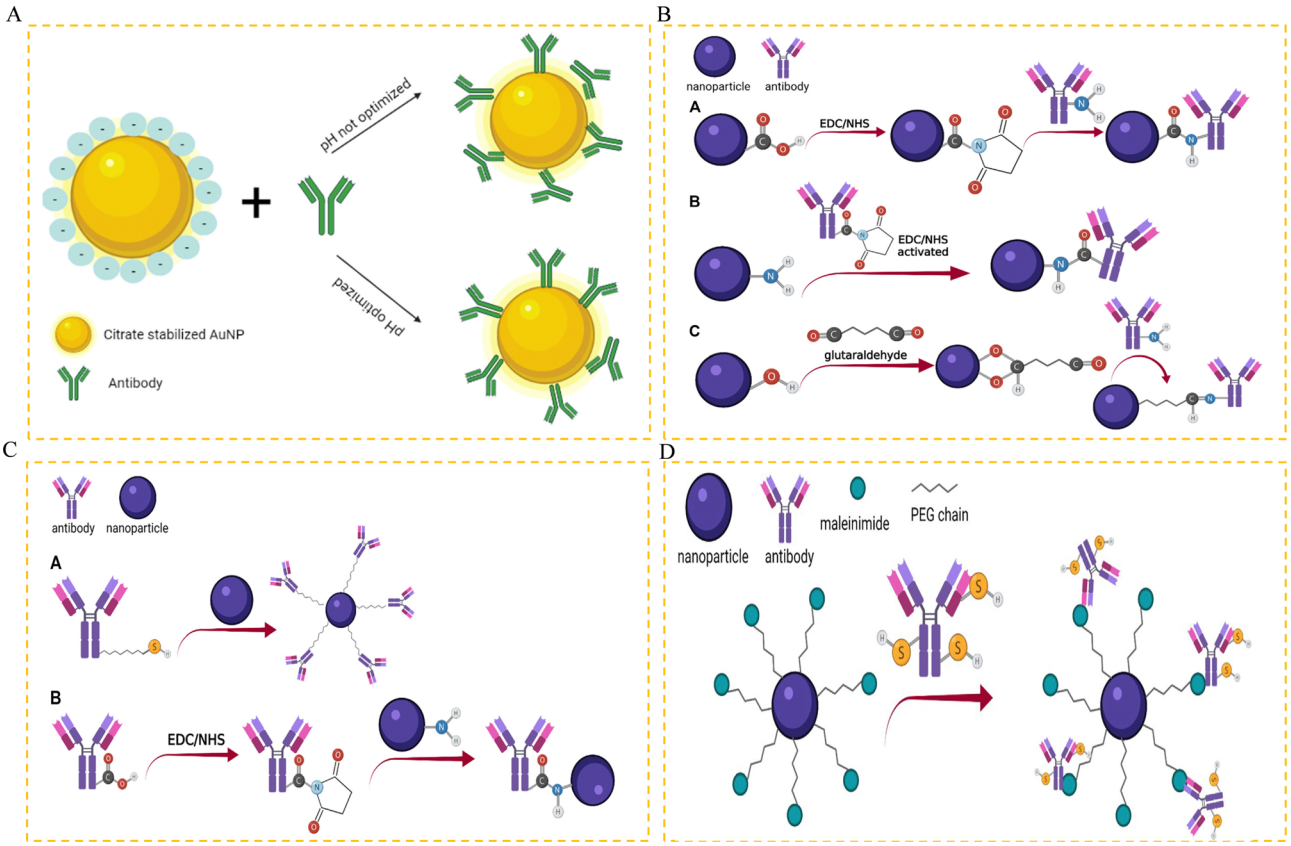

Fig. 5 Common processes of the conjugation between SERS tags and antibodies. Reproduced from ref. 69 with permission from Frontiers, copyright 2022.

and (B) EDC/NHS-facilitated coupling of antibody carboxyl groups with amino-modified nanoparticles.[70,71]

To enhance conjugate stability and reproducibility, researchers have developed advanced coupling strategies between chemically modified antibodies and SERS tags. Fig. 5D illustrates the conjugation strategy employing chemically functionalized detection molecules and labeling probes. Through specific chemical modifications applied to detection molecules (such as antibodies) and labeling probes (including nanoparticles), this approach facilitates enhanced molecular binding efficiency, consequently improving detection efficacy.[72,73]

In SERS–LFIA detection systems, the conjugation process represents a critical and sensitive component that significantly impacts assay reproducibility and sensitivity. Currently, covalent binding methods are predominantly employed due to their superior stability and enhanced functionalization capabilities compared to physical adsorption approaches.

3.3 Raman detection equipment

The current landscape of Raman detection instrumentation can be systematically categorized into three primary classifications: (1) portable Raman spectrometers, characterized by their compact, handheld designs for field applications; (2) laboratory-grade systems, including benchtop configurations optimized for precision measurements; and (3) high-throughput analytical platforms designed for large-scale sample processing. Modern Raman spectrometers have achieved substantial advancements in detection sensitivity through several technological innovations: enhanced signal acquisition capabilities, sophisticated background noise reduction algorithms, implementation of multimodal data analysis protocols, and integration of automated, intelligent operational systems.[74]

Xiao *et al.* engineered a portable SERS–LFIA detection system incorporating multi-channel LFIA reaction columns, enabling simultaneous analysis of multiple samples or biomarkers. This innovative device achieved a remarkable LOD of 0.01 ng mL^{-1}, representing a three-order-of-magnitude improvement over conventional visual signal detection. The system addressed critical limitations of previous detection platforms, particularly their high cost and operational complexity, while establishing a robust foundation for advancing multiplexing, automation, and high-sensitivity applications of Raman technology in LFIA (Fig. 6A).[75]

In a separate development, Joung *et al.* designed a SERS–LFIA reader leveraging the local surface plasmon effect, which demonstrated superior sensitivity compared to traditional detection methodologies and commercial LFIA systems (Fig. 6B).[76] Recognizing the limitations of conventional portable Raman instruments in SERS–LFIA strip analysis, Jia *et al.* developed a specialized portable SERS–LFIA detector for automated, high-sensitivity detection of West Nile virus (WNV)

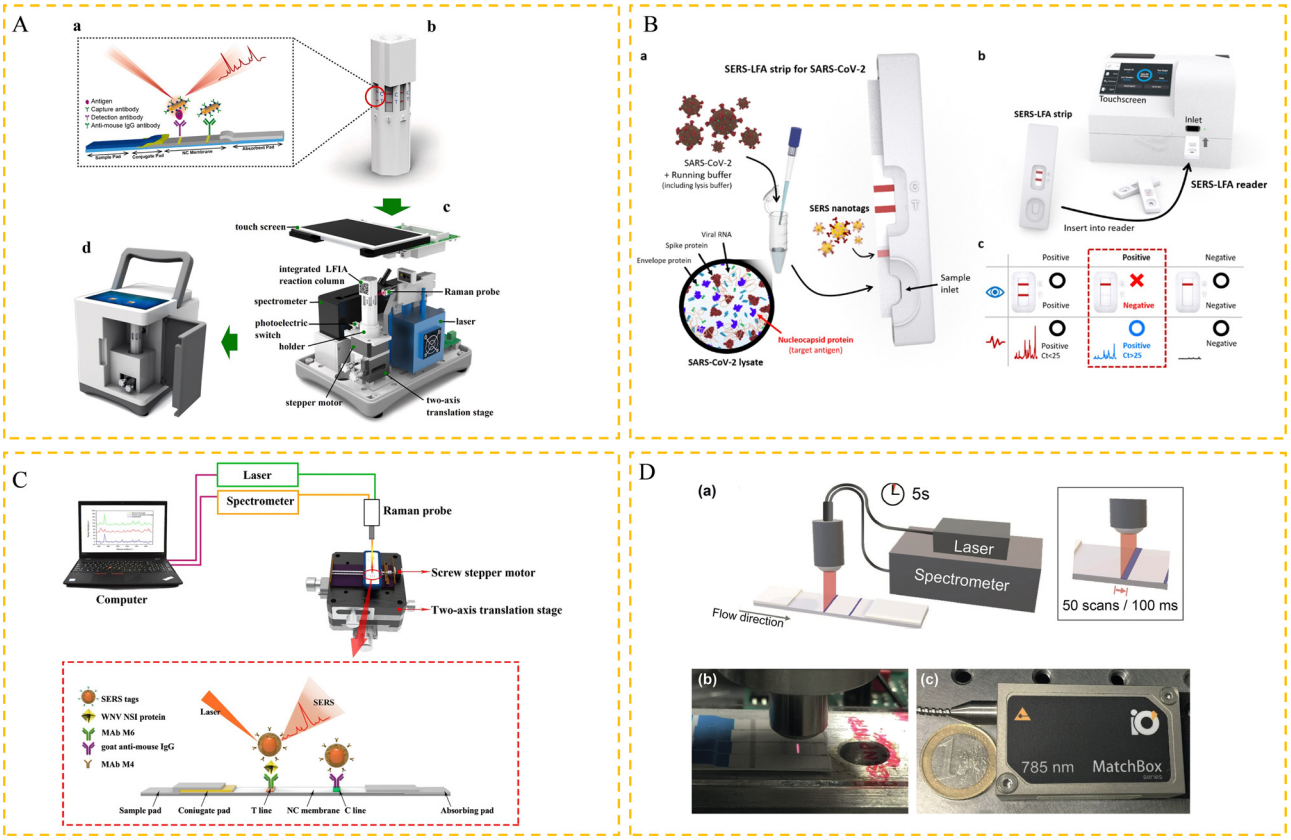

Fig. 6 (A) Xiao *et al.* designed a multichannel portable SERS–LFIA detector. Reproduced from ref. 75 with permission from Elsevier, copyright 2020. (B) SERS–LFIA detector designed by Joung *et al.* Reproduced from ref. 76 with permission from American Chemical Society, copyright 2022. (C) Portable SERS–LFIA detector designed by Jia *et al.* Reproduced from ref. 77 with permission from Springer, copyright 2021. (D) Portable SERS–LFIA detector designed by Tran *et al.* Reproduced from ref. 78 with permission from Wiley, copyright 2019.

non-structural protein 1 (NS1). Their system achieved an LOD of 0.1 ng mL^{-1} for WNV NS1 protein, representing a 100-fold sensitivity enhancement over visual detection. For inactivated WNV, the detector demonstrated an LOD of 0.2×10^2 copies per μL, with sensitivity comparable to RT-qPCR (Fig. 6C).[77]

Tran *et al.* advanced the field further by introducing a portable SERS–LFIA card reader incorporating a customized fiber optic probe. This system enabled rapid detection of human chorionic gonadotropin (HCG) within 2–5 seconds, achieving an LOD of approximately 1.6 mIU per mL. The acquisition time was reduced by several orders of magnitude compared to conventional Raman instruments, while demonstrating 15-fold greater sensitivity than traditional LFIA methods (Fig. 6D).[78]

While contemporary handheld Raman spectrometers offer promising capabilities for on-site ultrasensitive, rapid, and quantitative analysis, their widespread adoption remains constrained by the high cost of high-performance Raman detection equipment.

3.4 SERS–LFIA platform with internal standard

Functioning as a reference substance, the internal standard undergoes simultaneous analysis within the same detection system as the target analyte. This approach enables effective correction of systematic errors through comparative analysis of signal intensities between the target analyte and internal standard. Consequently, the integration of internal standards has emerged as an essential strategy for optimizing the detection performance and reliability of SERS–LFIA platforms.

Chen *et al.* developed an innovative approach by co-immobilizing 4-MBA as an internal standard with aptamer DNA on a gold nanocore substrate. They utilized the characteristic Raman peak intensity at 1075 cm^{-1} as the internal reference signal to mitigate measurement variability.[79] In a separate advancement, Fan *et al.* addressed signal standardization challenges by integrating silver–gold alloy nanoparticles functionalized with Raman reporter molecules into the nitrocellulose membrane as an internal reference. This plasmonic internal standard LFIA (PIS-LFIA) platform enabled simultaneous measurement of plasmonic immune probes and internal standard (IS) signals, with signal normalization through intensity ratio calculation. This approach demonstrated significant improvements, achieving 1.8-fold greater uniformity and 3.2-fold lower LOD for carcinoembryonic antigen detection compared to conventional SERS–LFIA (Fig. 7).[80]

However, excessive internal standard concentrations may generate overwhelming Raman signals that obscure target analyte detection, while insufficient quantities fail to provide

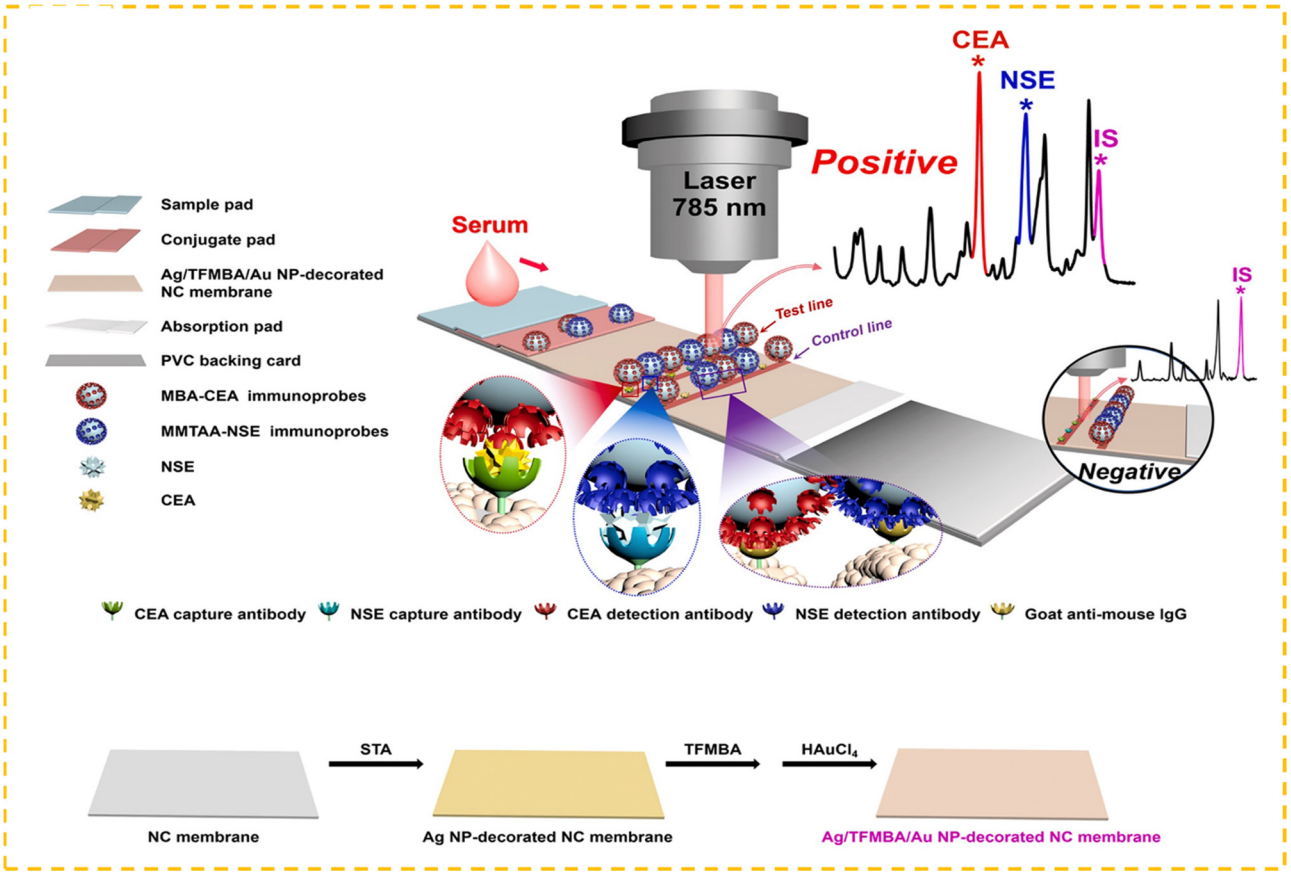

Fig. 7 Decorated with Raman reporter molecules on the nitrocellulose membrane as an internal standard. Reproduced from ref. 80 with permission from Elsevier, copyright 2023.

adequate calibration. Therefore, precise optimization of internal standard concentration is essential to maintain an appropriate signal balance between the reference and target analytes.

4. Combined detection

The SERS–LFIA system demonstrates remarkable multiplex detection capabilities, enabling simultaneous analysis of multiple disease biomarkers.[50] This combined detection approach primarily manifests in two distinct configurations: (1) incorporation of multiple T lines on a single strip, with each line specifically designed for a unique target analyte; and (2) utilization of differentially modified SERS tags, allowing for discrimination of distinct Raman reporter molecules on a single T line.[81] Multiplex detection is used in the diagnosis of SERS–LFIA (Table S3, ESI†).[82]

4.1 A single T line

In immunochromatographic technology, the principle of achieving multiplex detection on a single T line is based on immobilizing multiple specific capture molecules (such as antibodies or antigens) on the same T line, thereby enabling the simultaneous detection of multiple target substances in a sample.[83] During analytical detection, the immunocomplexes formed between target analytes, their corresponding antibodies, and SERS tags are simultaneously captured by specific capture reagents immobilized on a single test line. The unique Raman spectral fingerprints generated by different SERS nanomaterials, characterized by specific peak positions and intensities, enable precise discrimination and quantification of multiple target analytes through characteristic spectrum.

Shen *et al.* developed a novel approach utilizing two Raman probes (DTNB/4-MBA) for the ultrasensitive simultaneous detection and quantification of H1N1 and *Streptococcus pneumoniae* on a single T line. The assay demonstrated remarkable detection limits of 29 pfu per mL for H1N1 and 16 cells per mL for *S. pneumoniae*, representing approximately 172-fold and 312-fold improvements in sensitivity compared to conventional Au NP-LFIA (Fig. 8).[84] In a related study, Zhang *et al.* employed Raman microspheres with distinct characteristic peaks, conjugated to IgG and IgM antibodies respectively, as immunological probes. By immobilizing Brucella-specific antigens on the T line to capture immune complexes, they successfully established a detection system for Brucella-specific IgG and IgM antibodies. Clinical validation of this method revealed exceptional diagnostic performance, achieving both sensitivity and specificity of 100%.[1]

Despite these advancements, multiplex detection on a single T line presents technical challenges, particularly regarding

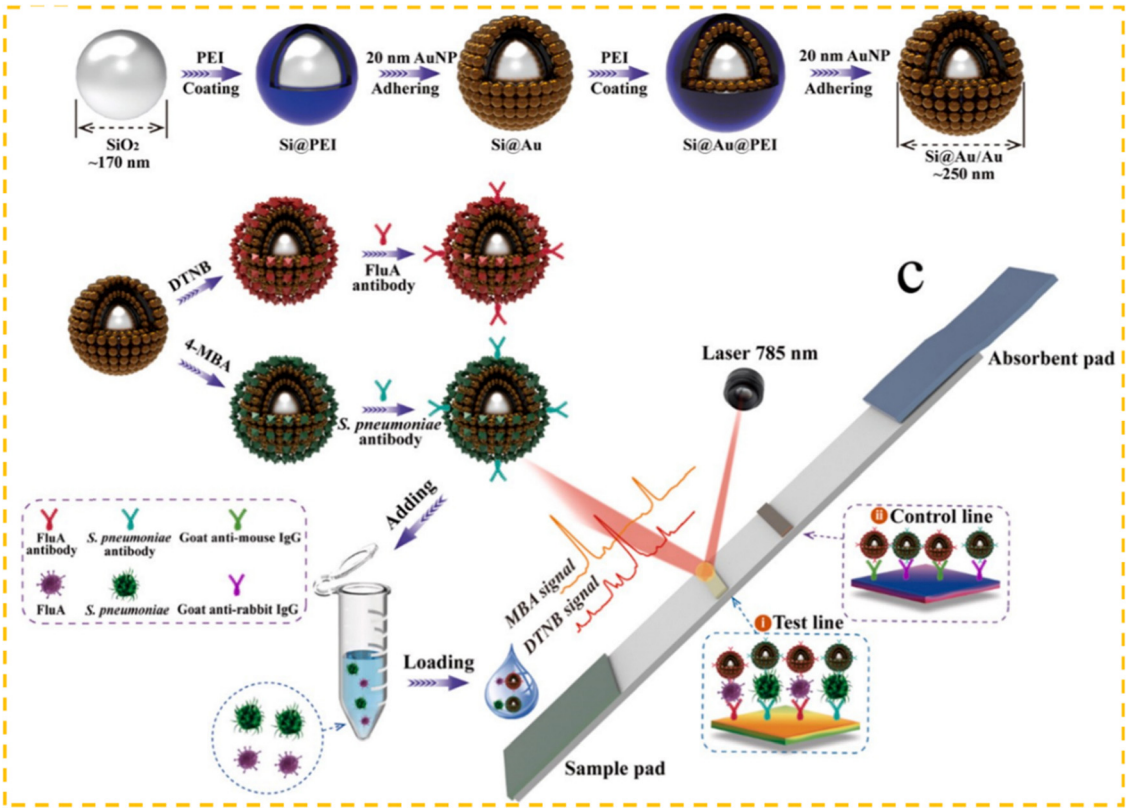

Fig. 8 Shows simultaneous detection and monitoring of H1N1 and *Streptococcus pneumoniae* on one T line. Reproduced from ref. 84 with permission from Elsevier, copyright 2024.

potential sensitivity reduction. This limitation primarily stems from competitive binding among multiple target analytes for the finite binding sites available. Furthermore, structural homology between different analytes may lead to cross-reactivity when present simultaneously in test samples, potentially compromising antibody binding specificity.

4.2 Multiple T lines in parallel

The implementation of multiple T lines in SERS–LFIA offers significant advantages for clinical diagnostics. This innovative approach enables simultaneous multiplex detection, facilitating high-throughput analysis while significantly enhancing diagnostic precision. Additionally, this methodology provides practical benefits by reducing both sample volume requirements and overall testing costs.

Shen *et al.* developed a multiplex diagnostic platform by conjugating capture antibodies against three tumor biomarkers (CEA, AFP, and PSA) with Au NP tags, establishing three distinct detection systems on a single test strip. This innovative approach enabled simultaneous quantification through SERS signal analysis at corresponding test lines, achieving remarkable detection limits of 1.43, 1.92, and 3.84 pg mL^{-1} for CEA, AFP, and PSA, respectively (Fig. 9).[85] In a separate study, Li *et al.* engineered a lateral flow assay by precisely depositing streptavidin (2.0 mg mL^{-1}) on the C line, while immobilizing anti-Staphylococcus aureus antibody (1.5 mg mL^{-1}) and anti-Salmonella typhimurium antibody (0.8 mg mL^{-1}) as T1 and T2 lines, respectively, on the nitrocellulose membrane. This configuration demonstrated a detection limit of 10 cells per mL.[63]

Critical technical considerations must be addressed in multi-line test strip design: (1) the minimum distance between adjacent test lines and the reaction zone should exceed 2 millimeters to ensure sufficient interaction between analytes and detection tags, thereby guaranteeing detection accuracy; (2) while multiple test lines allow simultaneous immobilization of distinct antibodies at specific locations for targeted antigen capture, this configuration may increase the risk of antibody cross-reactivity, potentially compromising assay specificity.

5. Multimodal detection in SERS–LFIA

Multimodal SERS–LFIA integrate multifunctional nanomaterials capable of generating diverse detection signals, including but not limited to colorimetric,[86] electrochemical,[87] magnetic,[88] photothermal,[89] fluorescent,[90–95] and Raman signals.[47] Notably, the colorimetric approach has emerged as a predominant choice for multimodal detection platforms due to its inherent advantages in result interpretation and user-friendly readout.[96,97]

Huang *et al.* pioneered the development of multifunctional sea urchin-shaped Au–Ag@Pt nanoparticles exhibiting colorimetric-SERS-photothermal-catalytic (CM/SERS/PT/CL) properties, which were subsequently integrated with LFIA for multiplex detection and specific identification of pathogenic bacteria in blood samples. Using *Staphylococcus aureus* as a model organism, the study

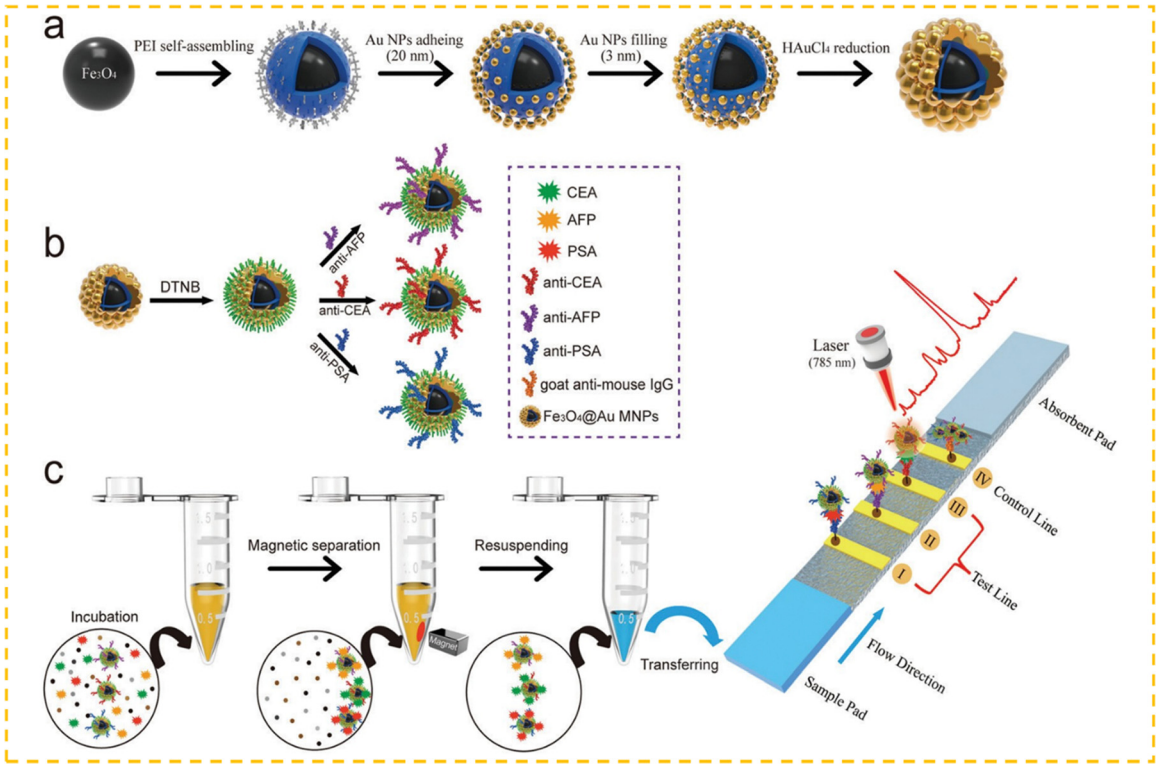

Fig. 9 Demonstrates the simultaneous detection of CEA, AFP, and PSA viruses on three T lines, respectively. Reproduced from ref. 85 with permission from Royal Society of Chemistry, copyright 2020.

demonstrated remarkable detection sensitivities: the LOD for SERS–LFIA reached 3 CFU per mL, while PT-LFIA and CL-LFIA achieved LODs of 27 CFU per mL and 18 CFU per mL, respectively. These sensitivities represented significant improvements, being 330-fold, 37-fold, and 55-fold higher than conventional visual LFIA (Fig. 10).[98] In a related advancement, Wu *et al.* employed AuNF-PMBA as detection tags, demonstrating dual colorimetric-Raman-photothermal functionality with superior bacterial capture efficiency. Their methodology achieved a visual LOD of 10^3 CFU per mL for *Escherichia coli* detection, representing a three-order-of-magnitude improvement over traditional LFIA. Quantitative analysis revealed LODs of 10^3 CFU per mL in colorimetric mode, 10^2 CFU per mL in SERS mode, and 10^2 CFU per mL in photothermal mode.[48]

Despite these technological advancements, the multimodal detection approach presents certain limitations. The increased complexity of the detection process necessitates additional operational steps, thereby requiring operators to possess enhanced technical expertise. Furthermore, the simultaneous utilization of multiple detection modalities may lead to signal cross-interference, potentially affecting measurement accuracy and reliability.

6. Patents related to SERS–LFIA

To comprehensively assess the developmental trajectory of SERS–LFIA technology, a systematic patent analysis was conducted using the Lens.org database with "SERS–LFIA" as the primary search term, yielding 390 relevant patents. Temporal analysis of the patent landscape reveals a consistent upward trend in SERS–LFIA-related patent filings over the past decade (Fig. 11A), reflecting the growing research interest and technological potential in this field.

Analysis of the global patent distribution demonstrates substantial engagement from diverse research entities, including academic institutions, healthcare organizations, and industrial enterprises, in SERS–LFIA technology development and intellectual property protection. The leading patent assignees are Massachusetts General Hospital (44 patents), Brown University (35 patents), and Boston University (25 patents). This predominance of U.S.-based institutions in patent filings suggests not only robust academic exploration but also effective translation of research outcomes into practical applications. The strong performance of the United States in this domain can be attributed to several factors: substantial investment in scientific research, a well-established intellectual property protection framework, and an innovation-conducive environment that attracts top-tier research talent and fosters technological advancement.

Geographical distribution analysis reveals that the United States, World Intellectual Property Organization (WIPO), and European patents collectively account for 99.7% of total filings, with respective shares of 68%, 25%, and 6.7%. In contrast, Chinese patents constitute only 0.26% of the total (Fig. 11B). This disparity may stem from China's relatively late entry into this research field and insufficient technological accumulation.

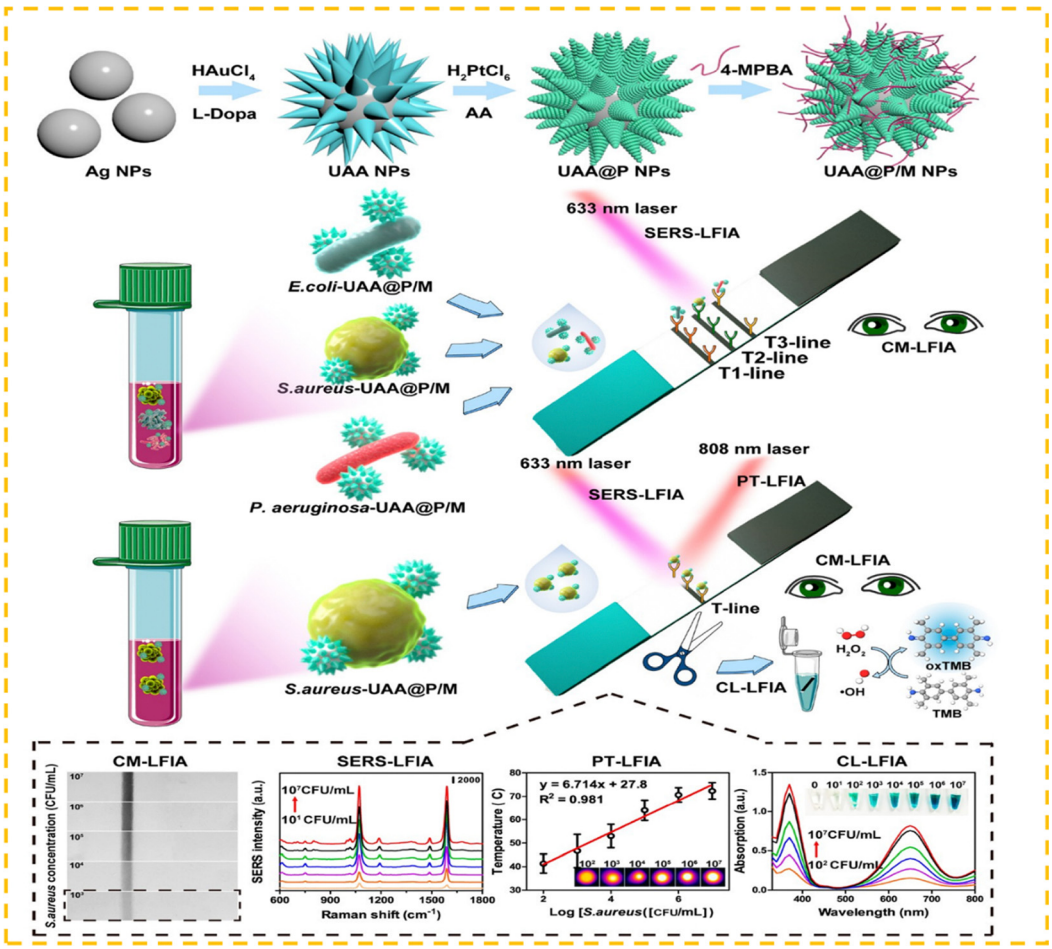

Fig. 10 Shows multimodal detection of clinical multiple bacterial infections. Reproduced from ref. 98 with permission from American Chemical Society, copyright 2023.

Detailed information on recent SERS–LFIA-related patents is provided in Table S4 (ESI†).

The current patent landscape suggests that SERS–LFIA technology remains in its nascent stage of development, with limited global patent activity. While the United States maintains a leadership position in SERS–LFIA research, followed by WIPO and European entities, China demonstrates substantial potential for growth in this field. To enhance its competitiveness, China should prioritize increased research investment, innovation capacity building, and strengthening of intellectual property protection mechanisms.

7. Challenges

7.1 Stability and reproducibility

To comprehensively address the challenges of stability and reproducibility in SERS–LFIA systems, a multi-faceted approach integrating advanced fabrication techniques, rigorous quality control, innovative stabilization strategies, robust data analysis methods, and standardization efforts is essential. Advanced substrate fabrication techniques, such as atomic layer deposition (ALD), DNA-directed assembly, and microfluidic-based synthesis, have emerged as promising solutions to achieve precise control over nanostructure dimensions, highly ordered nanoparticle arrangements, and uniform nanoparticle production, respectively. These methods lay the foundation for consistent and reliable SERS substrates. Quality control measures, including standardized characterization techniques (*e.g.*, SEM, TEM, AFM) to ensure batch-to-batch consistency, statistical process control during substrate preparation, and the implementation of reference materials for performance validation, further enhance the reproducibility of SERS–LFIA systems.

In parallel, emerging stabilization strategies have been developed to address issues such as nanoparticle aggregation and hotspot variability. For instance, protective coatings (*e.g.*, silica, alumina), molecular spacers, and polymer encapsulation techniques have shown significant potential in maintaining the structural integrity and functionality of SERS substrates over time. Additionally, advanced data normalization and analysis methods, including spectral processing algorithms for background subtraction, machine learning-based normalization, and multivariate analysis, play a critical role in improving data interpretation and reducing variability in SERS signal quantification.

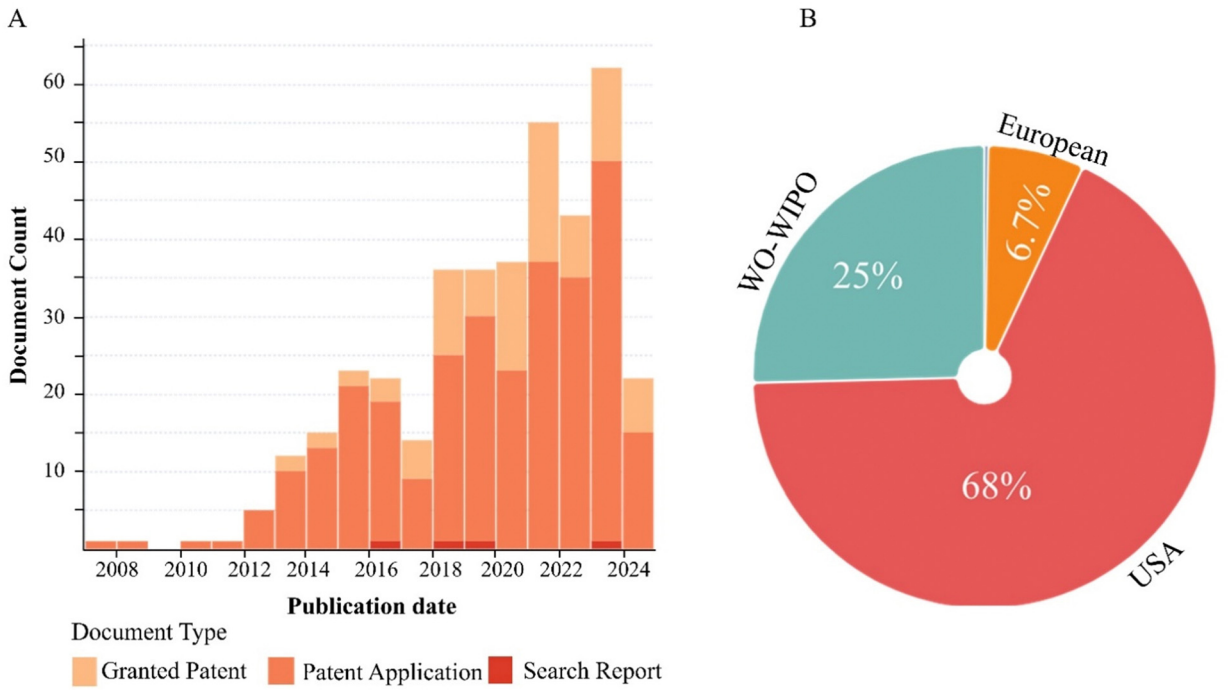

Fig. 11 (A) Patent documents in recent ten years. (B) Patent documents by Jurisdiction.

Finally, standardization efforts, such as the development of reference measurement procedures, establishment of performance evaluation criteria, and creation of certified reference materials, are crucial for ensuring the consistency and reliability of SERS–LFIA systems across different laboratories and applications.

7.2 Quantitative detection

Quantitative detection in SERS–LFIA systems faces significant challenges related to interference from marker-substrate interactions, environmental factors, and the need for precise calibration and data processing. To address these issues, first, minimizing interference from marker-substrate interactions is critical, which can be achieved through surface modification techniques to optimize binding specificity, the incorporation of spacer molecules to reduce non-specific interactions, and the development of novel substrate materials with inherently low background signals. Second, environmental factors, such as temperature and humidity fluctuations, can be mitigated by implementing controlled measurement environments, utilizing protective coatings to shield substrates, and designing robust nanostructures resistant to environmental variations.

Third, emerging calibration methods have significantly improved the accuracy and reliability of quantitative analysis. These include internal reference-based calibration using embedded Raman reporters, machine learning-assisted calibration for handling complex sample matrices, and multi-point calibration strategies to enhance linearity and dynamic range. Fourth, advanced data processing techniques, such as multivariate analysis for interference correction, deep learning algorithms for spectral deconvolution, and advanced baseline correction methods, have been employed to improve signal interpretation and reduce noise.

Together, these strategies address the key challenges in quantitative SERS–LFIA detection, enabling more reliable and precise analysis in practical applications, particularly in clinical and diagnostic settings where accuracy is paramount. This holistic approach not only enhances the performance of SERS–LFIA systems but also paves the way for their broader adoption in quantitative *in vitro* diagnostics.

8. Conclusions

In summary, this review systematically examines the recent advancements in SERS–LFIA technology for *in vitro* diagnostics over the past decade, with particular emphasis on research trends, sensitivity enhancement strategies, multiplex detection approaches, multimodal detection systems, and patent landscape analysis. First, surface functionalization of nanomaterials has emerged as a crucial strategy for enhancing target molecule binding affinity, thereby increasing the density of Raman signal molecules and improving detection sensitivity. Various SERS tags, including SiO_2 nanoparticles, Fe_3O_4 nanoparticles, and both 2D and 3D film structures, have demonstrated exceptional performance in complex clinical sample analysis.

Second, multiplex detection strategies, whether through multiple test lines or a single test line, offer substantial advantages in clinical applications. These include simultaneous multi-analyte detection, high-throughput analysis, improved diagnostic precision, enhanced early disease

detection sensitivity, dynamic disease monitoring capabilities, and reduced sample volume requirements and testing costs.

Third, the development of multimodal SERS–LFIA systems has expanded quantitative analysis capabilities and established diverse detection standards for clinical applications. Fourth, the current patent landscape reveals: the United States maintains a dominant position in both patent applications and technology translation, while the World Intellectual Property Organization and European also demonstrate significant influence in this field.

9. Future perspectives and emerging trends

The future of SERS–LFIA is poised for transformative growth, driven by emerging research trends and integration with advanced technologies. First, the design of SERS tags with high signal intensity, excellent stability, rich functionality, and ease of storage is critical for meeting the demands of complex sample analysis. Such as the use of 2D materials like MXenes and graphene derivatives, offer promising pathways to enhance signal amplification and substrate stability. Second, the issue of cross-interference in multimodal detection must be addressed through innovative approaches in detection principles, signal marker selection, and equipment design to improve the accuracy and reliability of results. Third, the combination of SERS–LFIA with ''5G cloud'' technology further underscores its potential to enter the ''big data era,'' enhancing monitoring and prevention capabilities for public health challenges. These advancements collectively highlight the transformative potential of SERS–LFIA in reshaping diagnostics and beyond.

Looking ahead, the long-term vision for SERS–LFIA includes the development of wearable devices for continuous health monitoring, the creation of global databases for disease surveillance, and its application in personalized medicine and therapeutic monitoring. By addressing these research directions and fostering interdisciplinary collaborations, SERS–LFIA technology can achieve its full potential as a robust analytical tool, ultimately contributing to improved healthcare outcomes and societal well-being.

Author contributions

Yongwei Zhang: conceptualization, software, writing – original draft; editing. Qian Zhang, Ziyue Li, Xuelei Zhou: supervision, conceptualization. Chunyan Liu, Hefei Zha, Xin Zhang: funding acquisition, resources. Jiutong Li, Guodong Lü, Xinxia Li: conceptualization, funding acquisition, supervision.

Data availability

No primary research results, software or code have been included and no new data were generated or analysed as part of this review.

Conflicts of interest

The author declares that there are no relationships affecting the interests or personal aspects of this work.

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