Troponin I Paper-based Microfluidic Assay for the Diagnosis of AMI: A Systematic Review

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ABSTRACT

Acute Myocardial Infarction (AMI) remains a major cause of morbidity and mortality worldwide, necessitating rapid and accurate diagnostic strategies for timely clinical intervention. Cardiac Troponin I (cTnI) is the most specific and sensitive biomarker for myocardial injury and is the current gold standard for AMI diagnosis. However, conventional laboratory-based cTnI assays require sophisticated instrumentation, trained personnel, and longer turnaround times, limiting their application in emergency and resource-constrained settings. Paper-based microfluidic and point-of-care (POCT) platforms emerged as promising alternatives for a rapid, low-cost, and decentralized cTnI detection. This systematic review critically evaluated recent studies published between 2019-2025 on paper-based microfluidic assays for cTnI detection in AMI categorically based by method of detection, including: Colorimetry, Lateral Flow Assay (LFA), Chemiluminescent Assay, Electrochemical Biosensor, Surface-Enhanced Raman Scattering (SERS)-based platforms, and Sandwich Immunoassay. Such were assessed on key parameters including limit of detection (LoD), sample volume, test duration, device design, portability, and suitability for point-of-care use. Among the reviewed studies, the lowest LoD values were reported by Han et al., (0.2 pg/mL in 2024; 0.84 pg/mL in 2020; 0.92 pg/mL in 2019), Li et al. (0.3 pg/mL), Wang et al. (1.0 pg/mL), Gao et al. (2.94 pg/mL), and Fu et al. (4.6 pg/mL). The smallest sample volumes were observed in Vasantham et al., (2 µL) and Li et al., (2.5 µL). In terms of test duration, Song et al., and Vasantham et al., achieved rapid detection times of ~1 minute, while Poosinuntakul et al., reported a longer duration of 60 minutes. Overall, while no single device is optimal across all parameters, paper-based µPADs show substantial potential for transforming AMI diagnostics. Future research should prioritize large-scale clinical validation, device stability, reproducibility, and mass production feasibility to facilitate real-world implementation.

Key words: Paper-based microfl uidics, µPAD, cardiac troponin I (cTnI), acute myocardial infarction (AMI), point-of-care testing (POCT)

In recent years, Acute Myocardial Infarction (AMI) has remained the leading cause of mortality worldwide.[39] Cardiac troponin I (cTnI) is widely regarded as the gold standard biomarker for detecting acute myocardial infarction (AMI) due to its high sensitivity, cardiac specificity, and strong clinical relevance. Unlike other biomarkers such as creatine kinase-MB (CK-MB) and myoglobin, cTnI is expressed exclusively in cardiac muscle, which minimizes false-positive results from skeletal muscle injury.[15,29] After myocardial damage, cTnI levels begin to rise within 3 to 4 hours, peak around 18 to 24 hours, and remain elevated for 7 to 10 days, allowing for both early and late detection.[1]

In contrast, myoglobin rises earlier but lacks cardiac specificity, while CK-MB may also be elevated in skeletal muscle trauma and returns to baseline more quickly.[32] Serial cTnI testing achieves nearly 100% sensitivity and specificity for AMI when measured 6–12 hours after symptom onset.[21] Furthermore, international guidelines, including the

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Universal Definition of Myocardial Infarction, define AMI based primarily on dynamic changes in cardiac troponin levels, emphasizing the critical diagnostic role of cTnI.[29] Its unique expression in cardiac tissue, extended diagnostic window, and prognostic value make cTnI the most reliable and clinically relevant biomarker for AMI diagnosis. As the incidence of AMI continues to rise, there is an urgent need for a rapid and accurate diagnostic method to enhance patient outcomes. In 2006, World Health Organization developed the ASSURED (Affordable, Sensitive, Specific, User-friendly, Rapid, Robust, Equipment-free and Deliverable to end users) criteria for point-of-care tests (POCTs).

Microfluidic paper-based analytical devices (μPADs) represent a new generation of point-of-care diagnostic tools designed to meet ASSURED criteria. Microfluidic paper-based analytical devices (μPADs) were first introduced in 2007 by Martinez, Phillips, and Whitesides, who demonstrated a low-cost method for creating diagnostic assays using photolithographically patterned paper. This innovation marked the beginning of μPADs as viable platforms for point-of-care (POC) testing. The following year, the technology was advanced further with the creation of three-dimensional μPADs, enabling multiplexed analysis by stacking patterned paper and adhesive layers.[18] Over time, simpler and more accessible fabrication techniques such as wax printing and inkjet patterning accelerated their development.[28] Although μPADs were initially used for general diagnostics, their application in acute myocardial infarction (AMI) detection began emerging in the late 2010s. Lim et al., as cited in Campu et al., developed a wax-printed μPAD capable of detecting multiple AMI biomarkers—creatine kinase-MB, glycogen phosphorylase BB, and cardiac troponin T—on a single paper-based platform.[2] More recently, Fu et al. (2023) designed an origami-style μPAD integrating electrochemical detection and plasma separation to measure cardiac troponin I, BNP, and D-dimer from a finger-prick blood sample.[9] These advancements highlight the growing potential of μPADs in cardiac diagnostics, transitioning from simple paper tests to sophisticated POC tools capable of detecting critical cardiac biomarkers rapidly and affordably. Several systematic reviews and guidelines have explored platforms and biosensor technologies for cTnI detection. While promising, many of these methods have limitations such as high manufacturing costs, complex fabrication processes, and insufficient sensitivity and specificity at the point-of-care level. Moreover, few studies have fully optimized paper-based microfluidic systems to combine affordability, ease of use, and diagnostic accuracy in a single platform. These gaps highlight the need for further research to design a practical, reliable, and scalable paper-based assay for AMI diagnosis.

This study hypothesized that a paper-based microfluidic assay can be engineered to sensitively and specifically detect troponin I levels, enabling rapid diagnosis of AMI at the point-of-care. The primary aim is to evaluate and synthesize a systematic review on current evidence showcasing the advantages and disadvantages of each device design in terms of cost, easy-to-use paper-based microfluidic applicability for the early detection of AMI to determine the most effective assay assessing devices with the Limit of detection, Materials used, Duration of test, Sample volume needed and Type of sample.The significance of this work lies in its potential to democratize access to cardiac diagnostics, especially in low-resource settings, ultimately contributing to faster clinical decision-making and improved patient survival rates.

Microfluidic Devices

A. Colorimetry

Colorimetry is a detection method that utilizes the observable color change due to the chemical or enzymatic reaction to determine the concentration of a targeted analyte, like cardiac troponin I. Due to its simple operation and straightforward interpretation, pads that use colorimetry have been developed many times. One of the traditional colorimetric immunoassays is the enzyme-linked immunosorbent assay that uses enzyme-coupled antibodies.[3,6]

According to the study by Yuan et al. (2021), the detection of troponin I, manual and 3D-printed portable centrifuges called “Handfuge” was based on a hand fan that the user can use to rotate the hand crank to generate airflow as the blades will spin as it a portable centrifuge–generating a high separation efficiency by a simple rotation. The hand blades are engineered to a platform where the blood samples can be placed. Pure plasma can yield > 47.1% with as high as 99% purity within 3 minutes (150 seconds) without electricity. Furthermore, the pure plasma obtained can be used for the detection of troponin-I along with the use of paper-based enzyme-linked immunosorbent assay (ELISA), having a detection limit of as low as

30 pg/mL. Additionally, the whole detection process only consumes 10 μL of blood, which is an advantage and similar to the volume acquired in a clinical finger prick, thus a good point-of-care testing device.[41]

Using the colorimetric principle, Wang et al. (2021), made a microfluidic paper based on the coffee-ring effect detecting the biomarker cTnI. The device is based on the principle that a sandwich complex formed when cTnI is caught by a DNA probe-decorated detection antibody may hybridize with H1 probes to create a G-quadruplex DNAzyme. The G-quadruplex DNAzyme, acting as a peroxidase mimic, catalyzed a colorimetric reaction, producing colored products. These products were then directed by evaporation to the end edge of the paper, where they formed a visible colored band in 4 minutes, the visible colored band will then be measured with a ruler. Thus, the total assay time is 44 minutes (preparation, incubation, and reaction). This microfluidic paper achieves a 1.0 pg/mL detection limit using serum to determine cardiac troponin I with the use of 4 μL sample volume. Through this, the length of colored bands and concentration of cTn1 is proportional to each other.[36]

Low et al. (2023) introduced a paper-based multiplexed colorimetric biosensor with a 12.1 pg/mL detection limit for cTnI, integrating machine learning algorithms to improve AMI diagnosis and prognosis. Likewise, the materials used were paper microfluidics and gold nanoparticles conjugated with colorimetric dyes. The device allowed less than 6 minutes of the test after sample application with a minimum sample of 5 μL (per analyte zone) using serum. By employing electrophoresis, this device efficiently eliminates non-specific adsorption for rapid and resource-limited settings.[17]

B. Lateral Flow Assay

Lateral Flow Immunoassay relies on the principle of either a sandwich or a competitive assay wherein a liquid will contain the target analyte coupled with a label to be detected. The target analyte will migrate into a capillary membrane wherein capture molecules are embedded. This interaction of the target analyte and molecule will be detected, and a signal will be given once the specific analyte is detected by what is targeted by the device. Due to its inexpensiveness and simplicity, this analytical method has been widely used and integrated in detecting various vital proteins to indicate the presence and absence of a disease, such as in diagnosing AMI.[34]

As stated in the research of Han et al. (2019), the quantitative results of cTnI in human serum samples of 10 μL were obtained within 20 minutes over a wide detection range of over 6 orders of magnitude, LoD of 0.84 pg mL-1 (595-fold higher than the conventional LFA using colloidal AuNP label), and Coefficient of Variance of (CV) <10% at and below the 99th percentile level, comparable to the performance of the hs-cTnI laboratory equipment and acceptable under clinical guidelines. It employs polyHRP to be used in AuNP conjugation that enables the conjugate preparation with high analytical sensitivity, reproducibility, and robustness by enhancing the HRP content on each AuNP.[14]

Another study conducted by Natarajan et al. (2021) proved that the Lateral Flow Assay (LFA) provides a quantitative, fluorescent-based detection specific to cardiac marker troponin I (cTnI). Three LFA types were used in this study, wherein the cellulose filter paper was developed into an analytical strip. Cellulose paper is standard for absorbent pads like LFA. It gives an excellent background with no auto-fluorescence, which is adequate for detecting fluorescent lines. At the same time, NC strips need to be functionally improved by layering carbon nanofibers (CNF) on cellulose. As a result, the Nitrocellulose (NC) material exhibited a limit of detection (LOD) of 1390 pg/mL. In comparison, the cellulose material could detect cTnI concentrations as low as 1280 pg/mL. The Cellulose CNF demonstrated the lowest LOD at 1400 pg/mL. The serum samples were mixed with cardiac troponin I in the study, and 50 μL of each was placed into the LFA cartridge. Within 15 minutes, the entire study was completed with great sensitivity and specificity.[20]

Another μPAD that utilizes lateral flow is a device integrated with Nitrocellulose (NC), conjugate pad, sample pad, and absorbent pad. The reagents are Zinc nitrate hexahydrate ($Zn(NO_3)2 \cdot 6H_2O$), 2-methylimidazole (2-MIM), and sodium hydroxide (NaOH) which were purchased from J&K Scientific Ltd. Monoclonal anti-cardiac troponin I antibody, polyclonal anti-cardiac troponin I antibody, bovine serum albumin (BSA), chloroauric acid (HAuCl4), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), N-hydroxysuccinimide (NHS), and silver nitrate ($AgNO_3$) were purchased from Sigma Aldrich (Darmstadt, Germany). Deionized (DI) water was used in all the experiments. It has a 9 pg/mL LOD with 20 μL of human serum within 10 minutes.[19]

A microfluidic device made by Han et al., (2020) engineered a paper/soluble polymer hybrid-based

lateral flow biosensing platform with a smartphone-based reader and used polyvinyl alcohol as a fluid regulator. In this device, they incorporated polymeric barriers that automate progressive reactions through a polymer dissolving mechanism. The smartphone-based reader has a simple optomechanical interface that allows a stable framework for accurate results. Additionally, it is coupled with colorimetric/catalytic gold-ion amplification. The sample used is serum. It produces a result within 20 minutes, a detection limit of 0.92 pg/mL with 50 µL serum. It has a coefficient of less than 10% variation, making it equivalent to the quality of a high-performance analyzer. A test strip was used–a hybrid structure that consists of a typical test strip subpart with advanced functional subparts such as intermediate film, buffer pad, reagent pad, and NC membrane with a PVA barrier, and shares raw materials and processes with a framework of a typical LFA, reducing the cost. The result was transferred to a computer or server through Wi-Fi or Bluetooth to quantify the target concentration. Additionally, cTnI clinical sample tests indicated r=0.981, which was more correlated with the contemporary analyzers. Ultimately, this innovative approach provides a cost-effective way to conduct high-performance POCT, is portable, user-friendly, and is universally practical for programming multistep reactions in miniature architecture or paper-based diagnostic devices.[13]

Recent advancements introduced by Han et al., (2024) with high-sensitivity vertical flow assays (hs-VFAs) as a rapid, cost-effective alternative to traditional diagnostic methods for quantitative cardiac troponin I (cTnI) measurement. Simple operation and short turnaround times are the advantages of VFAs in point-of-care settings. Likewise, the nanoparticles provide a larger surface for target binding and signal generation, and their sensitivity was further enhanced with nanoparticle amplification. The device utilizes time-lapse imaging and computational algorithms for digital assay validation and outlier detection using 50 µL of serum within 15 minutes per test (detection of 0.2 pg/ml).[12]

The study of Chen et al. (2022) argued that cTnI is in low concentration in the blood, so only those with high sensitivity can detect it accurately. To overcome this, they established a lateral flow strip POC based on lanthanide-doped nanoparticles and used Eu3+-doped vanadate nanoparticles as luminescent probes to quantify the captured cTnI. This lateral flow strip can be completed within 15 minutes with a detection limit of 17 pg/mL and 75 µL of serum sample, showing a rapid and sensitive detection of cTnI determination. It also showed the LFA platform's high reproducibility, specificity, and stability.[5]

Additionally, a chemiluminescent LFIA based on the PEGylation of gold nanoparticles (AuNPs) was proposed by Ren et al. (2023) for the detection of cardiac troponin I (cTnI). The device's efficient non-specific binding and aggregation reduction improved the assay's sensitivity and specificity. With 5–10 µL of serum for 20 minutes, this LFIA showed a limit of detection (LOD) of 10 pg/mL, clinically significant for point-of-care diagnostics, particularly for the early identification of acute myocardial infarction (AMI), as it enhances test performance and reduces false-positive outcomes.[23]

C. Chemiluminescence

Chemiluminescence is a detection technique that uses photons produced by a particular substrate redox or hydrolysis reactions. This method improves the signal-to-noise ratio because it is independent of external excitation light. It broadens the dynamic range, making it a valuable tool for various research applications.[33]

An innovative invention by Li et al. (2019) developed a 3D microfluidic paper-based analytical device (µPAD) for the multiplexed detection of AMI biomarkers using a chemiluminescence immunoassay enhanced by multifunctionalized gold nanoparticles. The assay achieved detection limits (LOD) of 0.3 pg/mL for cTnI, with an ultra-wide dynamic range. The primary materials used were Whatman chromatography paper, gold nanoparticles, and chitosan. The test lasted approximately 30 minutes, with a minimum of 2.5 µL serum sample required per biomarker. The device operated using serum samples and demonstrated high accuracy, with 94% and 108% recovery rates. Designed for point-of-care testing (POCT), the paper-based assay (µPADS) employed a chemiluminescence sandwich immunoassay method with temporal signal separation enabled by sequential H_2O_2 flow. Integrating a dual-signal amplification strategy using Ab1-GNPs and Co(II)-Ab2-luminol-GNPs significantly enhanced sensitivity.[16]

D. Electrochemical Biosensors

Electrochemical biosensors are analytical devices that transform biochemical interactions into electrical signals, facilitating the precise

detection of early cardiac biomarkers, particularly cardiac troponin I (cTnI). These biosensors feature a biorecognition element, typically an antibody or aptamer, immobilized on the surface of an electrode. Electrochemical biosensors can be divided into several types, including electrochemical impedance spectroscopy, electrochemical luminescence, field-effect transistors, potentiometric, and voltammetric sensors.[8,26]

Based on the label-free, electrochemical impedance spectroscopy (EIS) immunoassay, the μPAD was presented. It promises rapid and sensitive testing through carbon electrodes decorated with semiconductor zinc oxide nanowires. The essential components of an electrochemical impedance spectroscopy immunoassay are integrated on a single chip in this all-in-one designed electrochemical μPAD. This device presents a limit of detection of 4.6 pg/mL for cardiac troponin, which was detected from a drop of finger-pricked whole blood human plasma sample within 46 minutes.[9]

Furthermore, an electrochemical Impedance Spectroscopy (EIS) was used to assess the paper-based sensing platforms that were developed by combining carboxyl group functionalized multi-walled carbon nanotubes (MWCNT) with antibodies of the cardiac troponin I (anti-cTnI) biomarker. In the study of Vasantham et al. (2019), different cTnI and anti-cTnI concentrations were examined to monitor changes in impedance. A spiking cTnI in blood serum samples demonstrated the applicability of the suggested immunosensor, in which the limit of detection (LOD) was determined to be 50 pg/mL and 2 μL volume sample. Its sensitivity was approximately 1.85 mΩ/ng/mL with a response time of ~1 min. Likewise, EIS aids the need for a portable assay kit that provides a rapid response, low detection limit, and is cost-effective in monitoring early diagnosis of acute myocardial infarction.[35]

Likewise, the combination of thin adhesive films and paper folding paved the way for the development of three-dimensional (3D) microfluidic paper-based analytical devices (μPADs) made possible by Wu et al. (2019). This matching inhibited complex binding sequences and the use of cellulose powders. Similarly, by enabling dual colorimetric and electrochemical detection, the 3D μPADs were made to perform more tests on a smaller footprint to show their importance and capabilities. The device demonstrated a wide dynamic detection range with a detection limit of 350 pg/mL using Troponin I as a model in standard serum samples within 20 minutes with the use of 60 μL volume sample. As a result, several assays can be performed inexpensively on a single 3D device, owing to the invented platform.[40]

E. Surface-Enhanced Raman Spectroscopy (SERS)

Surface-enhanced Raman Spectroscopy (SERS) was recognized as a highly sensitive and specific method for detecting cardiac biomarkers, particularly cardiac troponin I (cTnI). This technique enhances Raman scattering signals through the interaction of analytes with nanostructured metallic surfaces, making it a viable option for point-of-care testing (POCT). SERS-based platforms are gaining attention due to their rapid detection capabilities, low sample requirements, and potential for miniaturization.[24]

Tu et al. (2020b) developed a paper strip based on an aptamer assay using surface-enhanced resonance Raman spectroscopy (SERS) to detect cTnI. The gold nanoparticles were modified with malachite green isothiocyanate, a Raman reporter molecule. It was encapsulated using a silica shell and used a handheld Raman system with an excitation wavelength of 638 nanometers to provide signal. Furthermore, the device used a primary and secondary aptamer of cardiac troponin I, which was applied in the sandwich assay format to bind to a test line paper fluidic platform. Concentration can also be determined while measuring the SERRS signal in the test line. Under its detection range, it has a detection limit of 0.016 to 0.1 ng/mL and an LOD of 15 pg/mL, making it a good selection for detecting cTnI with good stability for 10 days and performance using the serum sample matrix. A 25-μL sample was implemented in the sample pad of the paper strip and resuspended with the silica/aptamer/MGITC/AuNPs. The initial flow took 10 minutes, while complete absorption for increased sensitivity required 40 minutes.[31]

The study of Song et al. (2023) presented a Surface-Enhanced Raman Scattering (SERS)-based Lateral Flow Assay (LFA) capable of detecting Cardiac Troponin I at concentrations as low as 20 pg/mL (LOD) in 20 μL human serum. The device represented a 78-fold increase in sensitivity within 30 minutes. Not only this, three optimizations were conducted: AuNP sizes (20, 50, 80, 100nm) for quantitative assay, the flow of fluid and LFA components were investigated with SERS technology, and different wavelengths and powers of laser for SERS-based LFA were evaluated for the analysis

of cTnI. Also, they utilized 50 nm AuNPs, which yielded the strangers in terms of SERS intensity with a fine-tuning laser wavelength and power setting, and can adjust the reaction time and buffer components. Thus, it offered a quantitative assay with higher sensitivity than the visual detection in the naked eye for quantifying cTnI.[27]

In an additional study by Tu et al. (2020a), the researchers developed a Surface-enhanced Raman spectroscopy (SERS) that is an example of a sensitive optical technique and used an aptamer to recognize cardiac troponin I in samples. Using the SER's readout signal, the paper-based sensing assay detected cTnI in 0-0.5 ng/mL with a limit of detection of 50 pg/mL in 15 minutes. In the paper-based SERS assay analysis, the solutions were tested in different concentrations (0, 0.1, 0.5, 1 ng/mL) in PBS with a pH of 7.4. In its first attempt, 50 μL of cTnI solution and 5 μL of probe 1 were mixed and incubated for 10 minutes; the second dipstick with probe 2 was inserted into the solution with the blocking agent, and 100 ul of washing buffer was added to remove excess reagents, then SERS signal was measured using a portable Raman spectrometer. In evaluating 10% human serum with different concentrations of cTnI, a total of 1.56ng/ml LOD was achieved, a 78-fold higher sensitivity than with the optical sensing.[30]

In the work of Gao et al. (2022), a pump-free hybrid microfluidic chip was developed for the simultaneous detection of cardiac markers cTnI using a SERS-based immunoassay. The device achieved limits of detection (LOD) of 2.94 pg/mL for cTnI. The primary materials included patterned filter paper with in-situ synthesized AuNPs and PDMS microchannels. The total test duration was approximately 30 minutes, requiring only a small volume (~20 μL per inlet). The assay used serum samples and demonstrated high accuracy with excellent reproducibility and selectivity. This design supports point-of-care testing (POCT) through a paper-based assay (μPADS) architecture driven by capillary forces without external pumps. The microfluidic detection method was a surface-enhanced Raman scattering (SERS) sandwich immunoassay, enabling sensitive, multiplexed biomarker quantification critical for rapid AMI diagnosis.[10]

On the other hand, SERS-based immunochromatography test strip (SERS-ICTS) using AuNPs-Tribipyridine Ruthenium(II) nano aggregates as a signal amplifier for detecting cTnI with a detection limit of 60 pg/mL using 10-50 μL of human serum for 5 minutes in the study of Shao et al. (2024). The study was focused on enhancing the sensitivity and speed of traditional lateral flow assays by incorporating surface-enhanced Raman scattering (SERS) signal amplification technology. The device achieved excellent sensitivity and rapid detection time and strongly agreed with traditional chemiluminescence assays for point-of-care settings.[25]

Guo et al. (2019) made a paper-based fluorogenic immune device for detecting multiplexed cardiac troponin I (cTnI). The device used zinc oxide nanowires (ZnO NWs) to enhance fluorescence signals with a 1000 pg/mL detection limit using 15 μL serum for sample collection, completing the test within 5 minutes. This development improved the early diagnosis of acute myocardial infarction and monitoring by detecting multiple cardiac biomarkers. It provided evidence of its efficacy in point-of-care settings with high sensitivity and specificity.[11]

F. Sandwich Immunoassay

Sandwich immunoassay is a detection method that has been widely used and is one of the most commonly used assay designs in detecting cardiac troponin-I in recent years. It involves targeting a particular analyte by using a capture antibody to bind with the target analyte and a detection antibody that the target analyte binds to at a different site to amplify the signaling of the detection process. This method uses a surface to immobilize the capture antibody using materials like microwells, a nanoparticle, an electrode, or a paper surface like cellulose or nitrocellulose.[37]

In the study by Chen et al. (2024), a Troponin I paper-based microfluidic assay was developed to diagnose Acute Myocardial Infarction (AMI), featuring an automated μPAD integrated with a rotary valving system. The assay achieved a detection limit (LOD) of 200 pg/mL for cTnI. The primary materials used included Whatman No.1 chromatography paper and PMMA plates. The test duration was 30 minutes, requiring only a 20 μL serum sample. The assay demonstrated high accuracy, recovery rates between 89.8% and 106.3%, and low relative standard deviations (RSDs). Designed for point-of-care testing (POCT), this paper-based assay (μPAD) utilized a colorimetric sandwich immunoassay method. The platform's innovation lies in its incubation control through an on-chip rotary valve, enhancing sensitivity and flexibility for multiplex biomarker detection, which is critical for rapid AMI diagnosis.[4]

The study of Poosinuntakul et al. (2022), developed a dip strip-based sandwich immunoassay integrated with silver-enhanced colloidal gold nanoparticles and smartphone-based colorimetric detection for higher sensitivity and rapid and straightforward detection of cardiac troponin I in serum samples. Three sample volumes from 40 µL, 60 µL, and 80 µL, wherein 60 µL was selected as the optimum volume for the test trip for its ability to bring the highest color intensity, signifying its most sensitive detection for CTnI. This device demonstrated a limit of detection of 500 pg/mL, which could be observed by the naked eye at 0.5 ng/mL, and the pre-incubation of the AuNP-Ab conjugate with the sample required around 45 minutes to produce this high sensitivity. This pre-incubation not only increases the sensitivity but also its reproducibility and accuracy. Afterward, the dip strip was dipped into the sample and the silver enhancement solution. The estimated analysis time for this was around 15 minutes, so the overall testing time was 60 minutes.[22]

MATERIALS AND METHODS

Research Question

Detecting cardiac troponin I (cTnI), a highly sensitive and cardiac-specific biomarker, is essential to detect myocardial injury, and a paper-based microfluidic assay was used for its detection.[9,20] Despite having such a tool, it still had underlying challenges, including costly complex production techniques, limited cTnI sensitivity, and poor point-of-care implementation. To overcome these limitations, an affordable, practical, and accurate diagnostic platform should be developed aligned with the ASSURED criteria. Moreover, the development should also focus on having it more accessible to areas with limited resources, most especially for crisis situations. Hence, this study was designed to assess and verify a low-cost paper-based microfluidic assay that aided in the diagnosis of acute myocardial infarction by detecting cardiac troponin I at the point of care.

Literature and Search Strategy

This systematic review utilized search engines such as Google Scholar to choose and gather professional and known websites from Research Gate, PubMed, Science Direct, Springer Link, ACS Publications, SPIEL Digital Library, Wiley Online Library, RSC Publication, and Biosensors. All materials searched were limited only to studies from 2019 to 2020. The methodology and reporting followed the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) 2020 guidelines, which displayed various stages of literature selection, ensuring the transparency and reproducibility in the inclusion of relevant studies.

The following terms were used for searching research articles based on the study objectives:"AMI," "Acute Myocardial Infarction," "AMI Biomarker," "Biomarker," "Microfluidic Paper-Based Analytical Devices," "µPADs," "POCT," "Point of Care Test," "Troponin I," "TnI," "Cardiac Troponin I," "cTnI." The Boolean operator and phrase searches were used, alongside the use of truncation to capture word variations and nesting to structure search terms, to develop comprehensive and precise retrieval of relevant studies.

In accordance with the PRISMA 2020 guidelines, the study selection process consisted of three primary stages: Identification, Screening, and Inclusion. The identification phase was about preliminary searches using predetermined keywords used to retrieve relevant studies from selected databases and other sources. The screening phase involved scrutinized studies that were based on their relevance to the objectives of the systematic review, with exclusions applied to non-paper-based microfluidic devices, non-POCT devices, and studies that did not address troponin I detection for AMI. Finally, in the inclusion phase, eligible studies that met the criteria were systematically compiled for data synthesis and analysis within the systematic review.

Data Extraction and Quality Assessment

The criteria used for the study to be included are: (1) the diagnosis of acute myocardial infarction (AMI) using microfluidic paper-based analytical devices; (2) usage of point-of-care devices for AMI diagnosis; (3) involving synthetic and human samples: serum, plasma, and whole blood; (4) the studies detected the Troponin I; (5) peer-reviewed original research and case studies from 2019-2025; and (6) studies written in the English language.

On the other hand, the exclusions include: (1) review articles that are not based on primary research; and (2) articles with inadequate data, such as no sensitivity and specificity reporting, because

these relevant informations were necessary for the comparison and contrast of the results.

In gathering relevant studies, there were three (3) screening processes performed based on the PRISMA diagram (Figure 1). The first screening included the consideration of such factors as the range of publication, study design type, intervention used, and the specified inclusions. Followed by the retrieval of sought reports from the compiled papers. Then, the QUADAS-2 implementation with four (4) domains (patient selection, index test, reference standard, and flow and timing) was used for bias risk assessment. Finally, the screened related papers were analyzed with the use of objectives for this review.

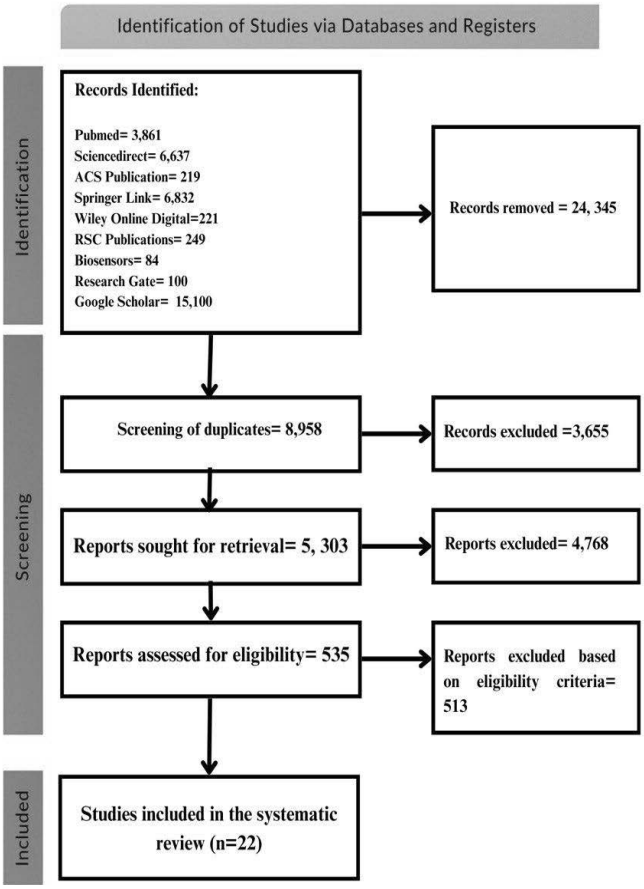

Figure 1. Selection of studies using PRISMA 2020 flow diagram

In synthesizing, the qualitative summarization of the findings was used, wherein the Google Scholar became the primary search engine for the retrieval of related studies. Duplicated studies of different databases or sites were excluded. Related literatures, which were not accessible and available screened, as well as the studies which reported not in compliance to the identified eligibility criteria of the systematic review were also ruled out. The screening and identification phases of the systematic review involved the comparison and contrast of the characteristics of each cTnI detection method identified from the studies screened based on their analytical performance, design and fabrication, point-of-care usability, and cost and accessibility, hence 22 studies showed adherence and in relatability by the end of the assessment.

Eligibility Criteria

In alignment with the PRISMA guidelines, the PICO (Participant, Intervention, Comparison, Outcome) framework was employed to structure the review question systematically (Figure 2). Participants included patients or assays associated with Troponin I detection to diagnose acute myocardial infarction (AMI) at the point of care. The intervention under investigation involved paper-based microfluidic devices. Comparisons were conducted based on detection methodologies, including turnaround time, limit of detection (LOD), material types, and sample volumes. Among the results of interest are low implementation costs, portability, operational effectiveness, diagnostic sensitivity, specificity, accuracy, overall performance, dependability, and possible uses. The systematic nesting of parentheses and logical operators were employed as part of a complete Boolean search strategy to improve the literature search procedure's transparency, reproducibility, and thoroughness.

Inclusion and Exclusion Criteria

This review included studies that specifically focused on microfluidic paper-based analytical devices (μPADs) for Troponin I detection in the diagnosis of Acute Myocardial Infarction (AMI), with particular attention to Point-of-Care (POC) devices. Studies involved human biological and clinical samples such as serum, plasma, and whole blood. Only peer-reviewed original research articles and case studies written in English were considered. The selected studies were assessed based on analytical performance parameters such as limit of detection (LOD), sensitivity, specificity, precision, accuracy, and repeatability. Additionally, design and fabrication aspects were evaluated, including the type of paper

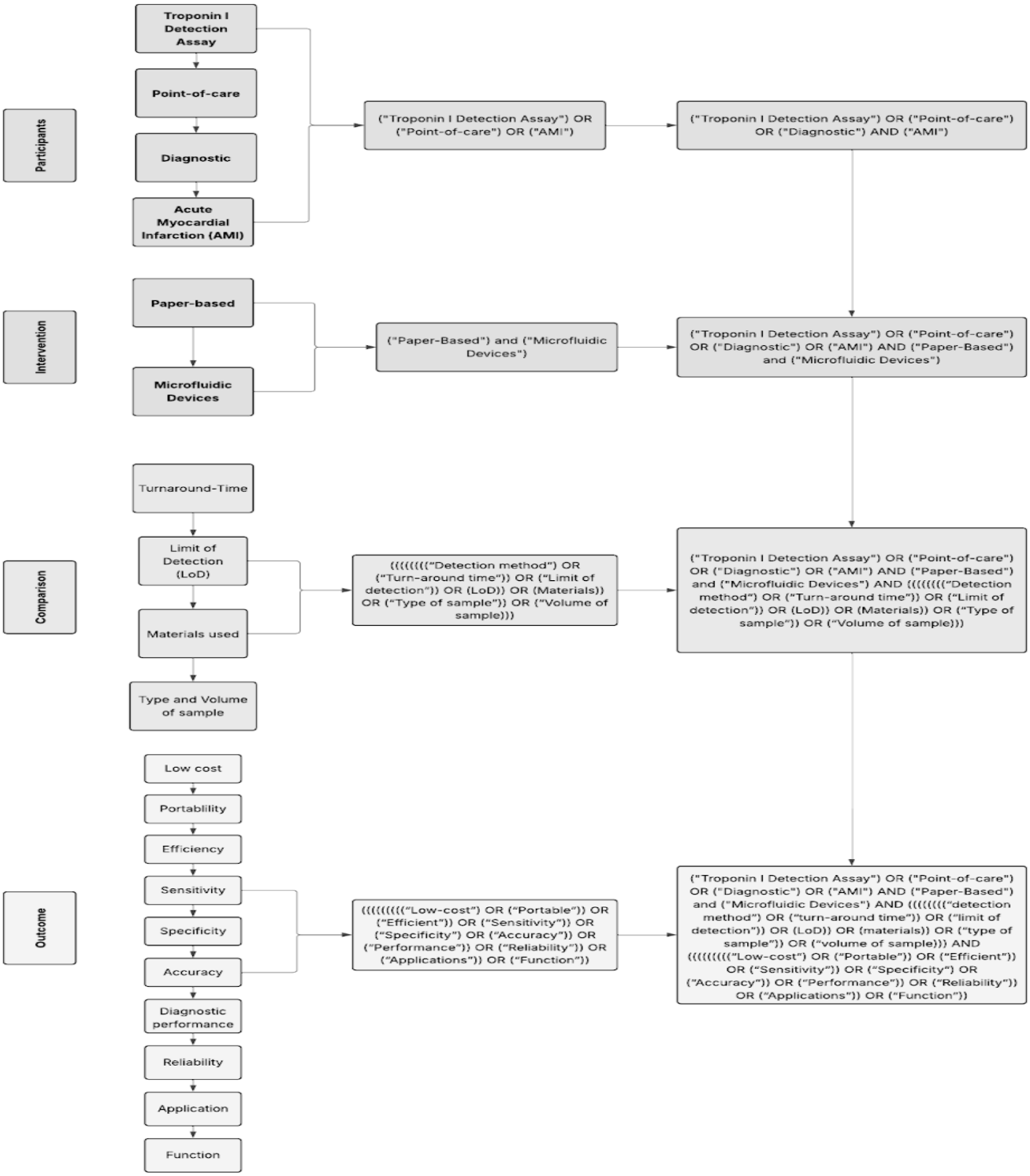

Figure 2. PICO (Participant, Intervention, Comparison, Outcome) framework.

used, microfluidic mechanisms (e.g., capillary flow, wax printing), and biosensor integration (e.g., nanoparticles, colorimetric or fluorescence-based detection).

The review also compared detection methodologies and operational principles across various device types. Point-of-care usability is another primary criterion, encompassing turnaround time, user-friendliness, portability, and overall miniaturization. Economic considerations such as production cost, commercial accessibility, and affordability were reviewed. References were limited to studies published between 2019 and 2025 and retrieved from reputable databases, including ResearchGate, Elsevier, PMC, Google Scholar, PubMed, ScienceDirect, SpringerLink, ACS Publications, SPIE Digital Library, Wiley Online Library, RSC Publications, Royal Society of Chemistry, Springer Nature, ResearchGate, and Biosensors.

Research that does not target Troponin I or POC applications were omitted. Furthermore, review articles that are not based on primary research and studies that lack essential data, such as sensitivity and specificity, were excluded from the scope of this review.

Inclusion

a. What type of studies are included?
- Studies focusing on microfluidic paper-based analytical devices for Troponin-I in Acute Myocardial Infarction Diagnosis.
- Studies about Point-of-Care Devices used in AMI.
- Articles or clinical trials that involve synthetic and real human samples: serum, plasma, and whole blood.
- Studies written in English
- Peer-reviewed original research and case studies.

b Create criteria: Include points of comparison from specific objectives in selecting RRL:

Analytical Performance
- Limit Of Detection (LOD)
- Sensitivity and Specificity
- Precision, Accuracy, and Repeatability
- Advantages

Design and Fabrication
- Composition of Material (e.g. type of paper)
- Microfluidic mechanism (e.g. capillary flow, wax printing, etc.)
- Integration of biosensors (e.g. nanoparticles, colorimetric, fluorescence-based detection)

Detection Methodology
- Differences or comparison of all types of devices based on their principle/mechanism.

Point-of-Care Usability
- Turnaround time (TAT)
- Ease of use
- Portability and miniaturization
- Smartphone application

c. References Duration
- Studies published between 2019 to 2025

d. What are the websites where RRLs are retrieved?
- Research Gate
- Google Scholar
- PubMed
- Science Direct
- Springer Link
- ACS Publication
- SPIEL Digital Library
- Wiley Online Library
- RSC Publication
- Biosensors

Exclusion

1. What are excluded from the scope/ criteria when selecting RRL?
   a Articles/Research that focuses on non-paper-based microfluidic devices.
   b. Studies that do not detect Troponin I.
   c. Review articles that are not based on primary research.
   d. Study devices that do not use Point-Of-Care devices.
   e. Articles with inadequate data (no full access), such as no sensitivity and specificity reporting.

Summary of Evidence

The following studies of Point-of-Care (POC) devices, specifically microfluidic paper-based

analytical devices (μPADs), for detection of Troponin I were screened strictly based on English only and peer-reviewed original research articles and case studies. The chosen studies were then selected based on the assessment of QUADAS-2 Tool (Quality Assessment of Diagnostic Accuracy Studies) for screening and bias assessment as well as the consideration limit of detection (LOD), sensitivity, specificity, precision, accuracy, and repeatability.

Moreover, the domains gained from the QUADAS-2 Tool are Patient selection, Index test, Reference standard, and Flow and timing for the Risk of Bias which were then characterized as low, high, or unclear risk of bias. Consequently, there were also domains such as Patient Selection, Index Test, and Reference Standard for the Applicability of Concern which were then characterized as low, high, or ambiguous concern.[2] In addition, more information was considered which included the assay's detection limit, time to result, minimum sample, and the type of sample used.

Table 1. Summary of evidence for colorimetry.

Materials	Detection Limit (pg/mL)	Time (within an hour)	Sample	Sample Volume (μL)	Authors
3D-printed Handfuge, Whatman cellulose chromatography paper, wax printing	30 pg/mL	14 minutes	Whole Blood (plasma separated)	10 ul	Yuan et al.[41]
Paper Strip (starch-iodide), anti-cTnI ab, DNA oligonucleotides, hemin, laminating film, sealed chamber, silica gel dessicant	1.0 pg/mL	44 minutes	Serum	4 ul	Wang et al.[36]
Paper microfluids, capture & detect antibodies with colorimetric tags, 3D-electrophoretic cassette, gold nanoparticles, tetraethyl orthosilicate, Nitrocellulose membrane	12.1 pg/mL	< 6 min	Serum	5 ul	Low et al.[17]

Table 2. Summary of evidence for lateral flow assay.

Materials	Detection Limit (pg/mL)	Time (within an hour)	Sample	Sample Volume (μL)	Authors
AuNP linked to polyHRP and antibodies	0.84 pg/mL	20 minutes	Serum or plasma	10 μL	Han et al.[14]
Alexa Fluor 647–labeled anti-cTnI antibody	Cellulose-LFA: 1280 pg/mL NC-LFA: 1390 pg/mL Cellulose-CNF-LFA: 1400 pg/mL	Cellulose-LFA and Cellulose-CNF-LFA: 15 minutes NC-LFA: 10 minutes	Serum	50 μL	Natarajan et al.[20]

ZIF-8@BSA Au/Ag nanoclusters conjugated with monoclonal anti-cTnI antibody	9 pg/mL	10 minutes	Serum, plasma, or whole blood	20 µL	Mirzaeizadeh et al.[19]
AuNP-Ab conjugate	0.92 pg/mL	20 minutes	Serum or plasma	50 µL	Han et al.[13]
AuNPs conjugated with monoclonal anti-cTnI antibodies	0.2 pg/mL	15 minutes	Serum	50 µL	Han et al.[12]
$GdVO_4:Eu^{3+}$ lanthanide-doped vanadate nanoparticles	17 pg/mL	15 minutes	Serum	50 µL	Chen et al.[5]
HRP-conjugated anti-cTnI detection antibody linked to AuNPs	10 pg/mL	CL-LFIA: 20 minutes Multiplexed CL-LFIA: 15 minutes	Serum	10 µL	Ren et al.[23]

Table 3. Summary of evidence for chemiluminescence.

Materials	Detection Limit (pg/mL)	Time (within an hour)	Sample	Sample Volume (µL)	Authors
AuNPs with Ab1and Co(II)-Ab2-luminol-GNPs	0.3 pg/mL	30 minutes	Serum	2.5 µL	Li et al.[16]

Table 4. Summary of evidence: Electrochemical biosensors.

Materials	Detection Limit (pg/mL)	Time (within an hour)	Sample	Sample Volume (µL)	Authors
Carbon electrodes decorated with semiconductor zinc oxide nanowires	4.6 pg/mL	46 minutes	Whole blood (plasma)	20 µL	Fu et al.[19]
Carboxyl group functionalized multi-walled carbon nanotubes (MWCNT)	50 pg/mL	1 minute	Serum	2 µL	Vasantham et al.[35]
Polymethyl methacrylate/ paper hybrid chip, polystyrene support, PMMA/paper hybrid chip	350 pg/mL	20 minutes	Serum	60 µL	Wu et al.[40]

Table 5. Summary of evidence for surface-enhanced raman scattering (SERS).

Materials	Detection Limit (pg/mL)	Time (within an hour)	Sample	Sample Volume (µL)	Authors
AuNPs with MGITC	15 pg/mL	40 minutes	Serum	25 µL	Tu et al.[31]
50 nm AuNPs with MGITC, fine-tuning laser wavelength, and power setting	20 pg/mL	30 minutes	Serum	1 drop of blood	Song et al.[27]
AuNP with MGITC, silica coating, and core–shell nanotags	50 pg/mL	15 minutes	Serum (clinical sample)	50 µL	Tu et al.[30]
In-situ synthesized AuNPs with patterned filter paper and PDMS microchannels	2.94 pg/mL	30 minutes	Serum	20 µL	Gao et al.[10]
AuNPs with Tribipyridine Ruthenium(II) nano aggregates	60 pg/mL	5 minutes	Serum	10-50 µL	Shao et al.[25]
Zinc oxide nanowires (ZnO NWs) integrated	1000 pg/mL	5 minutes	Serum	15 µL	Guo et al.[11]

Table 6. Summary of evidence for sandwich immunoassay.

Materials	**Detection Limit (pg/mL)**	**Time (TAT)**	**Sample**	**Sample Volume (µL)**	**Authors**
Gold nanoparticles, nitrocellulose membrane, sample & absorbent pad, silver nitrate + hydroquinone in citrate buffer, smartphone RGB analysis	200 pg/mL	30 minutes	Serum	20 uL	Chen et al.[4]
Chromatography paper, PMMA plates, servo motor, Arduino controller, RGB sensors, 3D printed housing	500 pg/mL	60 minutes	Serum	60 ul	Poosinuntakul et al.[22]

All 22 screened studies were evaluated as low risk in all four domains of the QUADAS-2 Tool for the risk of bias. Additionally, the applicability of concern included in the QUADAS-2 Tool had marked all 22 studies as low concern. Therefore, the studies screened have shown a low risk and concern; thus, all studies were considered for this systematic review.

RESULTS AND DISCUSSION

Comparison of Devices in Colorimetry

Colorimetric detection is a widely used assay format in point-of-care diagnostics because the presence of a target analyte produces a visible color change that can be read by eye or simple imaging. In cardiac biomarker testing, traditional colorimetric immunoassays like ELISA have set high sensitivity benchmarks. These advances illustrated how microfluidic paper devices couple simple color chemistry with novel fluid control (electrophoresis or manual centrifugation) to enable sensitive, rapid troponin assays.

A comparison of the three paper-based microfluidic assays presented herein for cardiac troponin I, cTnI, served to illustrate how differences in material composition, signal amplification strategies, and fluid-handling mechanisms directly impacted sensitivity, turn-around time, and point-of-care suitability.

In comparing three paper-based colorimetric platforms for Troponin I detection, the coffee-ring effect paper strip with DNAzyme-based signal amplification, as conducted by Wang et al. (2021), demonstrated the highest sensitivity. Achieving a limit of detection as low as 1.0 pg/mL, and showing strong specificity for Trop I, with less than 2.6% interference from other serum proteins. The increased sensitivity was due to the natural concentration of analytes at the ring boundary during the evaporation of solvent and the inclusion of DNAzyme-based signal amplification. Both allowed chromogenic molecules to accumulate and intensify the signal. The coffee-ring effect paper strip system enabled chromogenic molecules to accumulate at the border of the cellulose paper ring during the evaporation period. Cellulose paper, DNAzyme amplification, and chromogenic substances were used to facilitate this process. The coffee ring paper strip naturally raised local analyte density and amplified signal intensity, allowing for an ultralow detection limit of 1.0 pg/mL due to its material-driven concentration. However, its drawback was a longer assay time of 44 minutes, due to the drying required for color formation. A longer relation time was needed since the system depended on the solvent evaporation and ring formation. This limited its use in clinical scenarios requiring urgent action, such as AMI assessment, notwithstanding its very good sensitivity.[36]

In contrast, the multiplexed biosensor developed by Low et al. (2023), offered the fastest detection, under 6 minutes, by removing nonspecific proteins using electrophoresis in the sample matrix, thereby eliminating multiple washing steps. This was done with the use of hydrophobic wax barriers and patterned chromatography paper, and the most important of all, the incorporation of several immobilized antibodies with electrophoretic washing zones. This approach allowed the researchers to simultaneously measure cardiac and lipid biomarkers, such as HDL and LDL. Although the fastest among the three and achieving a high sensitivity, its reliance on electrophoretic transport required a sample volume of 5 μL of serum and more complex materials to maintain the continuous flow across numerous biomarker detection zones. Furthermore, its application to minimally processed samples was further restricted by its increasing complexity and several reagent layers.[23]

Meanwhile, the Handfuge-based paper device by Yuan et al. (2021), struck a balance between practicality and simplicity. The researchers used simple cellulose chromatography paper (Whatman Grade 1 and 3MM) that was patterned with wax to create hydrophilic wells using the handfuge-based paper device. Lower binding surface area and weaker signal amplification, possible with unmodified cellulose fibers compared to DNAzyme-enhanced materials, limited the researchers' work to attain the detection limit of 30 pg/mL. It required only a 10 μL finger-prick whole blood sample with the help of Handfuge, which was made of 3D-printed plastic that extracted plasma from the blood sample without the need for electricity. This then provided results within 13–15 minutes due to the entire experiment's simplicity. The simplicity, low material cost, and electricity-free nature of this device made it particularly well-suited to resource-constrained settings.[41]

In all, results showed that no single platform outperformed all others for every performance parameter. Rather, each device has a different strong suit: the coffee-ring device excelled in sensitivity, the multiplexed system provided the fastest results, and the Handfuge assay was best suited for point-of-care testing due to its low cost, minimal sample requirement, and compatibility with whole blood. These differences underscored the importance in selecting a diagnostic approach based on the clinical setting-whether sensitivity, speed, or operational simplicity is the major need. Further development

that combined these strengths, such as enhancing the sensitivity of simpler devices or reducing sample requirements for fast platforms, may facilitate the advance of paper-based troponin testing toward broader clinical use.

Table 7. Comparison of selected colorimetric assay based on criteria.

Detection Limit (Sensitivity)	Reference LoD	Sample Volume	Test Duration	Device Design	Practicability/ Portability	Analytical Robustness	Authors
1.0 pg/mL *(highest sensitivity)*	1.0-30 pg/mL	4 ul (Serum)	44 minutes	Coffee-ring distance-based paper assay	Moderate (Simple, no complicated equipment, highly sensitive to humidity)	Excellent (Very low LoD and fast generation for signals)	Wang et al.[36]
12.1 pg/mL	1.0-30 pg/mL	500 ul (Serum)	< 6 minutes	Paper-based multiplexed electrophoretic µPAD	High (Battery operated, needs electrophoresis cassette, large sample volume)	High (Good precision, strong linearity, LoD clinically relevant for nTnI)	Low et al.[17]
30 pg/mL	1.0-30 pg/mL	10 ul (Whole Blood - plasma separated)	14 minutes	3D-printed Manual "Handfuge" and paper ELISA	Excellent (Ectricity-free, low-cost, finger-prick samples can be used)	High (Good LoD for cTnI and high plasma purity)	Yuan et al.[41]

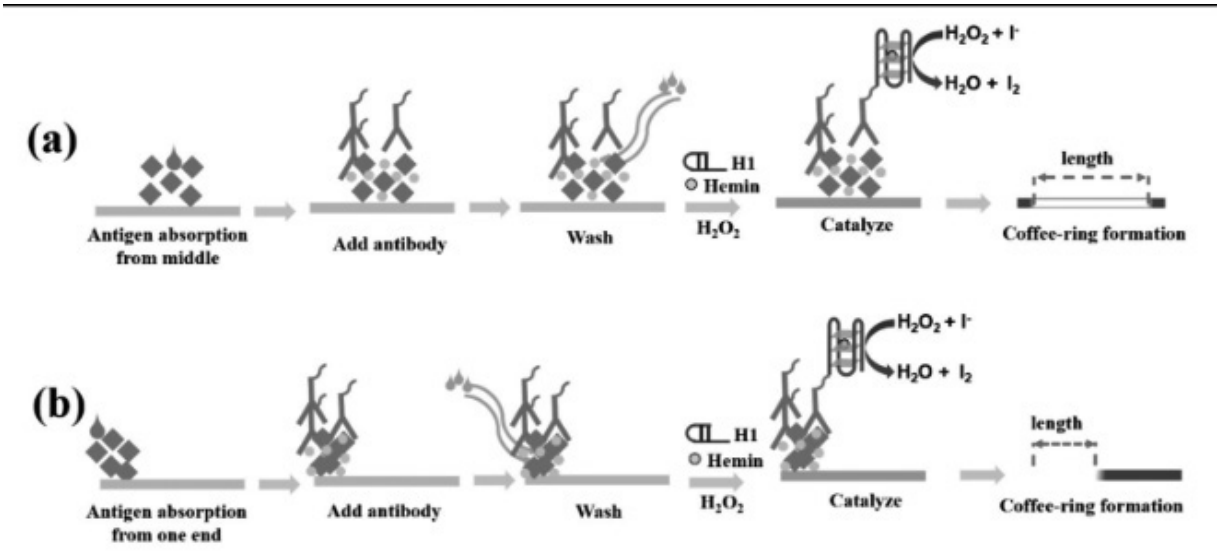

Figure 3. The Coffee-ring paper strip uses a sequence of binding, washing, and signal amplification leading to a visible color formation. For both configurations (a) sample introduced at the center and (b) at one end of the paper strip, both processes begin with the application of sample containing cTnI. A sandwich complex was then created when the antigen and captured antibodies interacted. The washing step was done to remove unbound substances. DNA probes (H1) and the sandwich complex hybridizes forming a G-quadruplex DNAzyme with hemin. With the addition of Hydrogen peroxide (H_2O_2), a colorimetric reaction was catalyzed that produced iodine, yielding in a visible colored product. The colored particles were then transported toward the paper's edges thru capillary and evaporation-driven flow, creating a "coffee-ring" pattern. In the configuration (a), the sample was distributed in two directions, creating two borders. While in configuration (b), the flow was unidirectional, creating a more distinct single band. The length of the colored zone, that is proportional to the quantity of cTnI, served as a straightforward, ruler-based quantification. The figure was retrieved from Wang et al. (2021).[36]

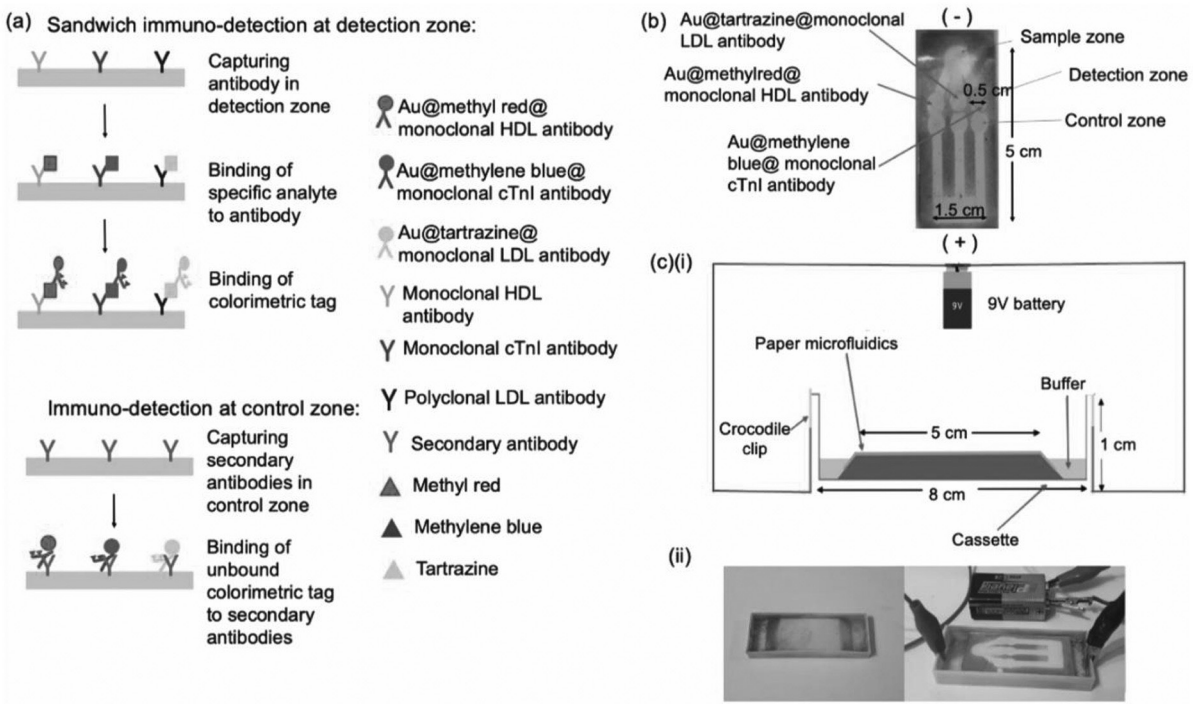

Figure 4. The dimensions and design of microfluidics in the paper (a) A schematic illustration of the immunodetection mechanisms in the detection and control zones.(b) Paper microfluidics with cTnI (blue), LDL (yellow), and HDL (red) detection and control zones. (c) (i) The electrophoretic biosensor configuration (i) The 3D-printed cassette (left) and the paper-based electrophoretic setup (right). The figure was retrieved from Low et al. (2023).[17]

Comparison of Devices of Lateral Flow Assays

A lateral flow immunoassay (LFA) is a simple, rapid, and cost-effective method used to detect a target analyte, such as cardiac troponin I, in a liquid sample through capillary action along a membrane strip, where binding with labeled antibodies produces a visible colored line. Advances such as gold nanoparticles, nanoclusters, europium-doped particles, enzyme-linked conjugates, and Au-ion amplification have enhanced sensitivity, with most assays delivering faster results. These improvements highlighted LFAs as practical and reliable point-of-care testing tools for early AMI diagnosis, particularly in emergency and resource-limited settings.

A lateral flow immunoassay is a method that determines the presence or absence of a target analyte in a liquid sample. A target analyte undergoes capillary action through a membrane in a linear strip format. The target analyte, if present, binds with an antibody label, which then accumulates at the test line, producing a colored product as an indication of a positive result. Recognizing that Acute Myocardial Infarction (AMI) still remains as a primary concern of death. This immunoassay is one of the most sensitive, inexpensive, and simplest methods available in the detection of small concentrations of Troponin I in the blood.

Table 8 describes the different materials used, detection limit (pg/mL), time, sample, and sample volume (uL). Among the six materials the ZIF-8@ BSA Au/Ag nanoclusters (NCs) LFA demonstrated the fastest turnaround time of 10 minutes. All of the devices primarily utilized serum. As referenced in the table cited in this paragraph and focusing on sensitivity, the data showed that the High-Sensitivity Vertical Flow Assay (hs-VFA) has the lowest detection limit at 0.2 pg/mL. Lastly, in terms of needing a small sample volume PEGylated AuNP Chemiluminescent LFIA needs only 5-10ul of the serum sample while others had utilized bigger sample volumes. Overall, ZIF-8@BSA Au/Ag nanoclusters (NCs) LFA was considered the most practical over all devices since it demonstrated having the best balance between speed, volume and sensitivity.

Significantly, the lateral flow assays for detection of cardiac troponin I achieved remarkably low

detection limits. In some cases, reaching the single-digit pg/mL range (e.g, 9 pg/mL for ZIF-8@BSA Au/Ag nanoclusters). However, there were some types of tests that reached lower detection limits, such as demonstrated by the use of Au-ion amplification, which reached as low as 0.92 pg/mL. In addition, the typical range in turnaround time is 10-20 minutes by using a small volume of serum, which is simply represented by its relevance in rapid point-of-care testing. The test's sensitivity was increased by adding gold nanoparticles, nanoclusters, europium-doped particles, and enzyme-linked conjugates. In addition, vertical flow assays (VFAs) have ultrasensitive detection down to 0.2 pg/mL, and the usage of PEGylated AuNPs in chemiluminescent LFAs enhanced its reliability by decreasing non-specific binding.

The detection of cardiac troponin I is important for the diagnosis of Acute Myocardial Infarction (AMI). Therefore, the usage of instruments to detect it with a less tedious procedure can lessen the death cases caused by AMI. The lateral flow assays are a good tool for POCT because of their short turnaround time and low demand in the volume of the specimen. The enhancements to these instruments strengthen the relevance of LFAs as practical POCT for early AMI triage, especially in emergency, remote, or resource-limited settings.

Table 8. Comparison of selected lateral flow assay based on criteria.

Detection Limit (Sensitivity)	Reference LoD	Sample Volume	Test Duration	Device Design	Practicability/ Portability	Analytical Robustness	Authors
0.2 pg/mL *(highest sensitivity)*	0.2-17 pg/mL	50 µL (serum)	15 minutes	Vertical-flow layer stack + nanoparticle amplification + digital image analysis	Very fast, minimal manual steps, suited for POC	Excellent (Digital validation, outlier detection; extremely sensitive)	Han et al.[12]
0.84 pg/mL	0.2-17 pg/mL	10 µL (serum)	20 minutes	AuNP linked to PolyHRP and Ab, Supporting film, water-swellable polymer, flow-control film, adhesive film	Highly sensitive, automated, universal, and manufacturable	High (CV of 2.9-8.5%; strong correlation ($R^2 = 0.991$))	Han et al.[14]
0.92 pg/mL	0.2-17 pg/mL	50 µL (serum)	20 minutes	Hybrid paper–polymer strip, PVA barrier, multistep automated reaction, smartphone reader	Highly portable, smartphone-enabled, cost-effective	High (<10% CV; excellent clinical correlation ($R^2 = 0.981$))	Han et al.[13]
9 pg/mL	0.2-17 pg/mL	*20* µL *(*serum)	10 minutes	µPAD / NC-based LFA with MOF-encapsulated NC labels	Portable, low-volume, fast; feasible for POC	Excellent (High sensitivity; stable nanoclusters; suitable for real serum)	Mirzaeizadeh et al.[19]

10 pg/mL	0.2-17 pg/mL	5-10 µL (serum)	20 minutes	Chemiluminescent LFA using PEGylated AuNPs to reduce nonspecific binding	Very low sample volume; high sensitivity; clinically relevant	High (Reduced false positives; high sensitivity & specificity)	Ren et al.[23]
17 pg/mL	0.2-17 pg/mL	50 µL (serum)	15 minutes	Standard LFA strip using Eu-doped luminescent probes	Rapid, sensitive, reproducible; requires fluorescence reader	High (High stability, specificity, reproducibility)	Chen et al.[5]
1280–1400 pg/mL	0.2-17 pg/mL	50 µL (serum)	15 minutes	Cellulose and NC membranes with CNF enhancement; fluorescence detection	Low-cost materials, fluorescence-based; moderate ease of use	Moderate (Lower sensitivity (ng/mL range); consistent and specific)	Natarajan et al.[20]

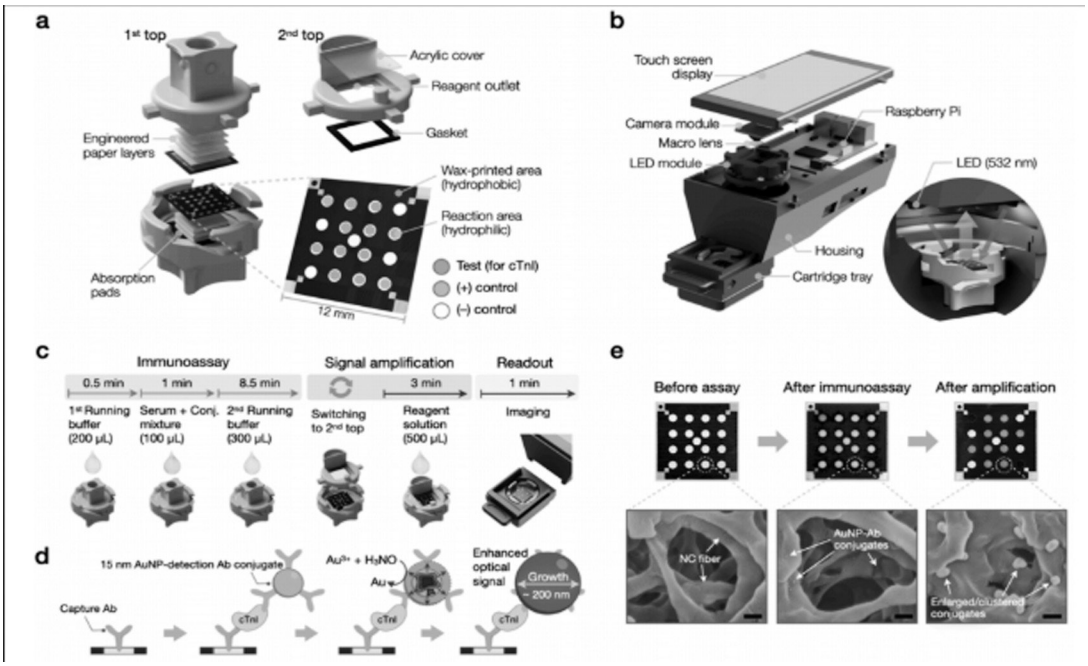

Figure 5. The high-sensitivity vertical flow assay (hs-VFA) was designed with a layered paper-based immunoassay where the serum sample (50 ul) flows vertically through an engineered membrane that contains immobilized capture antibodies. cTnI antigen will bind to gold nanoparticle (AuNP)-conjugated detection antibodies; this antigen–antibody complex will remain at specific test zones on the sensing membrane. Afterwards, the AuNP conjugates will act as nucleation sites for signal amplification via gold ion chemistry, where Au^{3+} was reduced from $HAuCl_4$ and will be added into the nanoparticle surface for intensifying the visible color signal. The features to control fluid flow and localization included hydrophobic wax-patterned paper, absorption pads, and reaction areas. For visualization, a portable reader with LED illumination and a camera captures time-lapse images of the signal development, which were eventually processed using computational algorithms to quantify cTnI concentrations. The figure was retrieved from Han et al. (2024).[12]

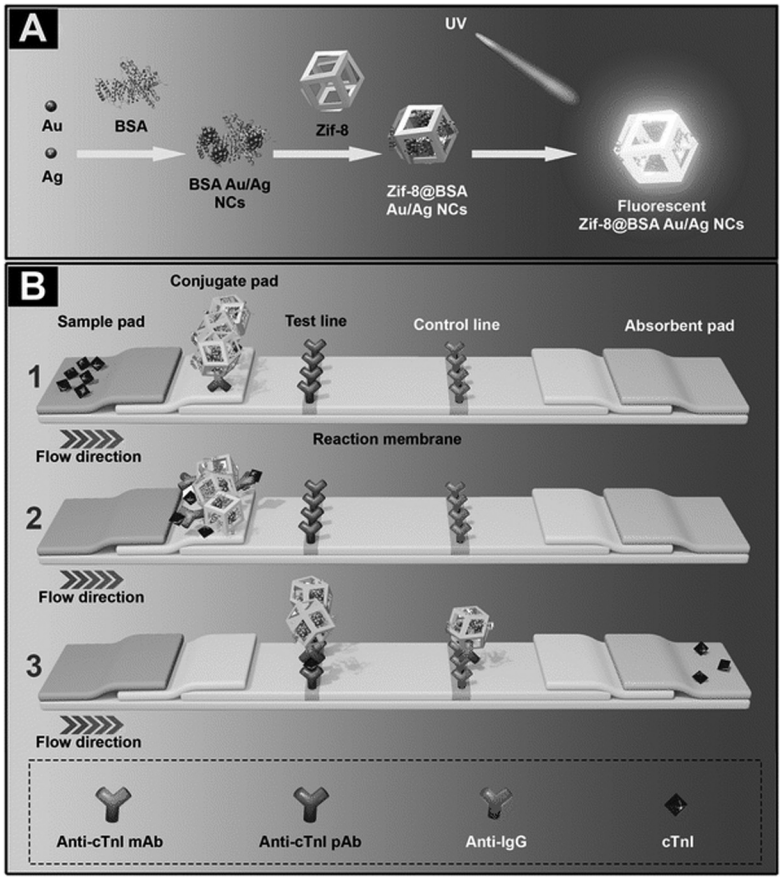

Figure 6. Schematic representation of ZIF-8@BSA Au/AgNCs wherein (A) nanoclusters were synthesized using bovine serum albumin capped Au/Ag nanoclusters encapsulated in ZIF-8 as fluorescent probes. (B) A cTnI-containing serum will complex with the monoclonal cTnI antibodies (mAb) on the conjugate pad, which is captured by the polyclonal cTnI antibodies (pAb) on the test line. The mAb will be captured at the control line via anti-IgG to indicate a negative test. This figure is retrieved from Mirzaeizadeh et al. (2024).[19]

Comparison of Devices in Chemiluminescence

A chemiluminescence assay is a way to find and measure certain substances in the laboratory by using a chemical reaction that imparts light. Using this method, a chemiluminescent reagent reacts with the target analyte and gives off energy in the form of light. With this, a sensitive detector measures the light that arises out, and the amount of the substance in the sample is directly related to the intensity of the light, enabling accurate quantification. Furthermore, chemiluminescence is commonly used in clinical diagnostics, especially in immunoassays that look at how antigens and antibodies work together. It is also used in biochemical research and environmental analysis. It is valued for its high sensitivity, specificity, quick signal generation, and ability to find analytes at very low concentrations.

Under the principle of Chemiluminescence, only the study of Li et al. (2019), applied a paper-based microfluidic assay and troponin I in detecting acute myocardial infarction.[16] The materials utilized for the study include gold nanoparticles (AuNPs) conjugated to a primary antibody (Ab1), a secondary type of gold nanoparticle (GNPs) that has been attached with a secondary antibody (Ab2), and luminol molecules, achieving a detection limit (LOD) of 0.3 pg/mL using just 2.5 μL of serum, demonstrating high sensitivity. However, its 30-minute turnaround time is relatively slow for point-of-care testing (POCT), where more rapid results are typically required. The device demonstrated moderate practicability due to its multi-step operation, while analytical robustness was high, evidenced by its excellent sensitivity and accuracy achieved through dual-signal amplification.[16]

Table 9. Comparison of selected chemiluminescence based on criteria.

Detection Limit (Sensitivity)	Reference LoD	Sample Volume	Test Duration	Device Design	Practicability/ Portability	Analytical Robustness	Authors
0.3 pg/mL	0-0.3 pg/mL	2.5 µL	30 minutes	AuNPs with Ab1 and Co(II)-Ab2-luminol-GNPs	Moderate (requires multiple steps and external detection, limiting ease of use)	Excellent (high sensitivity and accuracy with dual-signal amplification)	Li et al.[16]

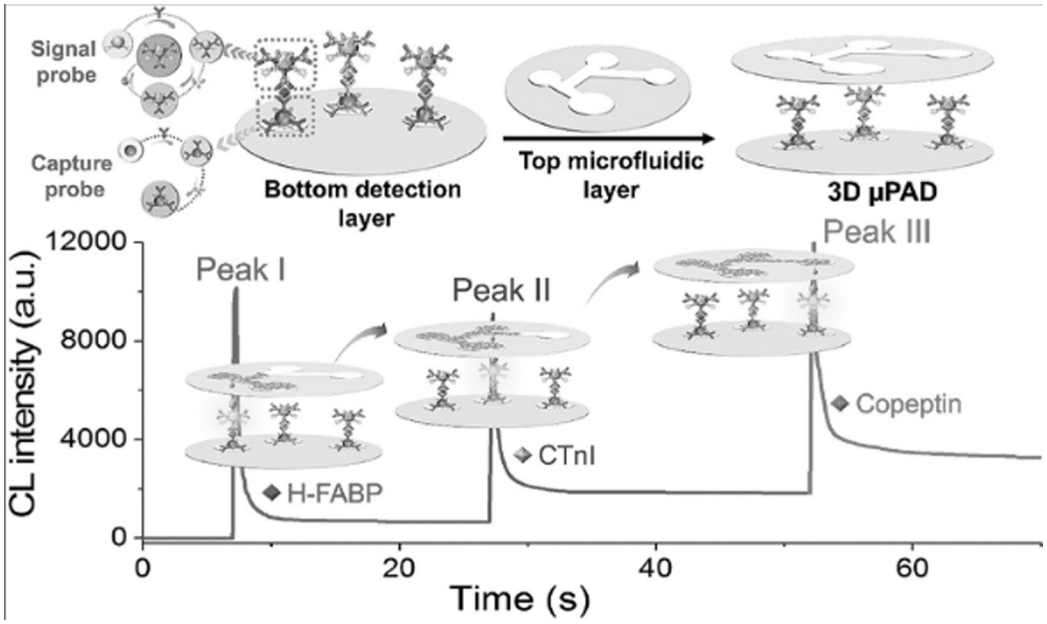

Figure 7. Three-dimensional microfluidic paper-based device that uses chemiluminescence. A minimal serum volume (2.5 µL) moves through layered channels of Whatman chromatography paper by capillary action, allowing the target biomarkers (H-FABP, cTnI, and copeptin) to sequentially bind to their specific capture probes. The primary gold nanoparticle (AuNP)-labeled antibodies for cTnI, which were attached to the paper surface via chitosan, while Bovine serum albumin (BSA) improves the assay by preventing non-specific binding. The Secondary-labeled AuNP antibodies amplified the signal, using cobalt ions to catalyze the luminol reaction. An alkaline environment created by diluted hydrogen peroxide (H_2O_2) in sodium hydroxide (NaOH), creating chemiluminescent emission. Three distinct emission peaks (I, II, and III) correspond to each biomarker, and the signal intensity is directly correlated with the analyte concentration, enabling both qualitative and quantitative analysis of acute myocardial infarction biomarkers. The Figure was retrieved from Li et al. (2019).[16]

Comparison of Devices in Electrochemical Biosensors

Electrochemical biosensors are devices that transform biochemical interactions into electrical signals, one that Vasantham et al. (2019) utilized in their study, facilitating the precise detection of early cardiac biomarkers, particularly cardiac troponin I (cTnI).

The study by Fu et al. (2023) presented an all-in-one nanobiosensor origami µPAD. It is ZnO-NW-coated WE which can have a uniform distribution of the sample. Additionally, 3 different samples were used as a comparison for the detection of cTnI; 20 µL of spiked human blood samples, 20 µL of commercial human blood, and as well as 3 µL of spiked artificial blood samples. The 3 µL of ABP was able to detect 4.6 pg/mL of cTnI making the study have the highest sensitivity. Though, it has a test duration of 46 minutes. In contrast, the test had an excellent practicability since it was finger-prick compatible and has a high analytical robustness. The analytical

robustness was due to the integration of nanowires for enhanced conductivity and multiplex potential.[9]

Meanwhile, Vasantham et al., (2019) had come up with multi-walled carbon nanotube anti-cTnI conjugates on a μPAD as an impedimetric immunosensor. The test was placed as the fastest test duration with an approximate of 1 minute and least volume requirement of 2 μL of serum sample. This was due to the minimal power required, the addition of MWCT on the μPAD, and the lower cost of the paper-based electrodes used, making the incubation time shorter. Despite the low cost of paper-based electrodes used, it has a sensitivity of 50 pg/mL with a low sample volume requirement.[35]

Lastly, Wu et al. (2019) has shown a moderate comparison compared to the previous electrochemical biosensors.[40] The need of 60 μL of serum sample but has a test duration of 20 minutes, which is a shorter duration than Fu et al. (2023), and yet a detection limit of 350 pg/mL.[9] This is because of the use of 3D μPADs with thin adhesive films and electrochemical detection. It required multiple steps due to a combination colorimetric assay and electrochemical detection. The focus of this study circled around low-cost materials that can detect the clinically significant presence of cTnI. Finally, emphasizing the analytical robustness, the test may have a dual-mode detection, yet it has a seven times higher limit of detection than the study of Vasantham et al. (2019).[35]

To summarize, the study of Vasantham et al., (2019) excelled the most. With a reference range of 4.6 pg/mL to 350 pg/mL, its only weakness was that it had a higher sensitivity than the study of Fu et al. (2023), but when it comes to the test duration, sample volume, design, and practicability it satisfied the turn-around-time and the majority of the criteria among the selected electrochemical biosensors.[9]

Comparison of Devices in Surface-Enhanced Raman Scattering

Recent advances in SERS illustrate commonalities and differences in sensitivity, multiplexing, portability, and practical deployability. Despite the challenges among intricate and unique instrumentation, when SERS was incorporated in microfluidic devices for the detection of cTnI developed a hybrid path for future diagnostic platforms for AMI detection.

The study conducted by Tu et al., (2020b) used primary amine-functionalized and secondary immobilized aptamers in the test line, and malachite green isothiocyanate (MGITC) as the Raman reporter molecule (RRM), which matched the excitation wavelength of the Raman spectrometer. The researchers introduced an aptamer-based surface-

Table 10. Comparison of selected electrochemical biosensors based on criteria.

Detection Limit (Sensitivity)	Reference LoD	Sample Volume	Test Duration	Device Design	Practicability/ Portability	Analytical Robustness	Authors
4.6 pg/mL *(highest sensitivity)*	4.6 to 350 pg/ml	20 μL (whole blood)	46 minutes	All-in-one origami μPAD; ZnO decorated carbon electrodes	Excellent (self-contained, finger-prick compatible)	High (integrates nanowires for enhanced conductivity and multiplex potential	Fu et al.[9]
50 pg/ml	4.6 to 350 pg/ml	2 μL (serum) *(least volume)*	~1 minute *(fastest)*	Simple 2D paper with MWCNT anti-cTnI conjugates	Excellent (low cost, minimal power)	Moderate (Excellent rapid response, but lower sensitivity)	Vasantham et al.[35]
350 pg/mL	4.6 to 350 pg/ml	60 μL (serum)	20 minutes	3D μPAD with dual colorimetric electrochemical readout	Moderate (requires multiple layers, more steps)	Moderate (dual-mode detection but higher LOD)	Wu et al.[40]

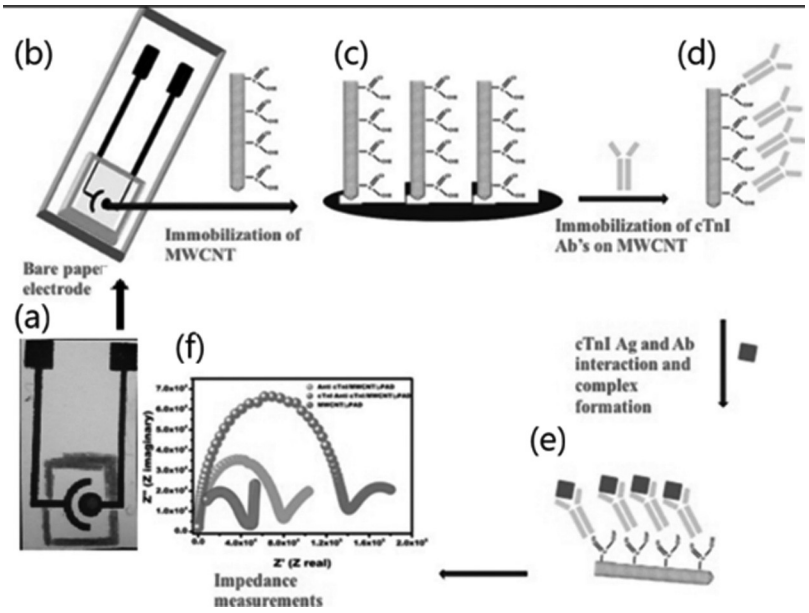

Figure 8. The model works by starting with the construction of (a) the electrochemical immunosensor with carboxyl-functionalised MWCNT (b) 2 µL of 1mg/mL MWCNT is placed on the electrode. (c) By allowing it to dry for 20 minutes, the MWCNT is immobilized. (d) 50 ng/mL of anti-cTnI is added for it to be immobilized. (e) 10 µL of activated antibody is added for a complex interaction of cTnI antigen and antibody. (f) impedance measurement is done with a Nyquist plot and is differentiated as pink for MWCNT, orange for anti-cTnI and MWCNT, and green for cTnI and MWCNT. Concisely, even by not reaching its highest sensitivity compared to Fu et al. (2023), it has reached its points for the criteria of point-of-care by achieving a duration of 1 minute, and the need of minimal power and its low-cost for paper-based electrodes. The Figure is retrieved from Vasantham et al. (2019).[35]

enhanced resonance Raman scattering (SERRS) assay on a paper fluidic strip, which is superior for molecular recognition and increased stability compared to antibody-conjugated tags. The test demonstrated, potentially, high specificity due to the sandwich mechanism in the test area, in which the researchers used primary and secondary aptamers to minimize false positives. The primary aptamer captures the sample containing cTnI to stabilize it. The secondary aptamer was conjugated to SERRS-active nanoparticles, which are the gold nanoparticles (AuNP) coated in silica and tagged with the MGITC. Any signal detected by the Raman spectrometer correlates with the concentration of cTnI, wherein higher intensities indicated higher concentrations.[31]

Despite this, the study also had several limitations. The assay's detection range (0.016-0.1 ng/mL) is rather narrow compared to other SERS-based assays included in this review. This may limit the assay's capability to capture a spectrum of clinical presentations in clinical settings, particularly in early or severe myocardial infarction cases, or generally any cardiac events. The study also lacked broader interference testing since it only used C-reactive protein (CRP), heart-type fatty acid-binding protein (h-FABP), and B-type natriuretic peptide (BNP). The reason why this is important is to determine the tendencies of cross-reactivity with other clinically relevant biomarkers. Although the use of aptamers increases the specificity of this assay, it is important to show data on its reactions with other serum constituents to ensure that the assay is selective only to cTnI. In connection with this, although serum was used in the study, it was tested using spiked serum samples and not actual patient samples, which limited the interpretation of its diagnostic accuracy. Lastly, the measurement of stability was only observed after storage for 10 days, which made long-term storage stability uncertain and unlikely to be correlated with actual clinical settings.

The use of antibody-conjugated nanoparticles is common in multiple lateral flow assays, which is the mechanism used by Song et al. (2023).[27] Its similarity with Tu et al. (2020b) is that they use the

same RRM, which is MGITC, as well as the formation of a sandwich complex.[31] In this study, AuNPs are conjugated with MGITC as the RRM and a detection antibody that binds specifically to cTnI. In the case that a sample containing cTnI is introduced to the assay, it will bind to the AuNP, forming a complex. As the complex travels to the test line, it will be stabilized by capturing antibodies that bind to a different epitope of the cTnI. The Raman spectrometer excites the MGITC molecules and detects the intensity of the signals, which correlates with the concentration of cTnI.[27]

It is important to note that aptamers are more stable than antibodies in terms of their use as recognition elements. This poses as the main limitation in this study, not to mention the high costs involved in its usage, as well as its limited thermal stability. In addition to this, long-term stability is also uncertain due to the lack of investigation initiated in this study.[27] Although this study shares similarities with the study of Tu et al. (2020b), in that it used potential interferences[31], the specific interferences employed and the assay's sensitivity to cTnI cannot be compared between the two studies because a different panel was used. This study uses CRP, myoglobin, Tau, and bovine serum albumin (BSA). The limitation in this study was the lack of a broader interference testing setup, similar to the previous study. Another similarity in limitations with the previous study was the use of spiked serum samples instead of actual patient samples, which, as mentioned, is fundamental in determining the feasibility of this assay to be used in real-world point-of-care testing. A distinguishable contrast between this study and the previous was the wider detection range, which started at 0.01 ng/mL and extends to 100 ng/mL, with a low limit of detection at 0.02 ng/mL. Finally, the susceptibility of the nitrocellulose membrane to damage due to higher laser excitation signals affected the operational flexibility of the assay.

The assay produced in the study conducted by Tu et al. (2020a), has a similar mechanism to the assay produced by Tu et al. (2020b). However, as the study by Tu et al. (2020a), is more focused on standard SERS rather than SERRS, it requires the use of multiple peaks to determine which produces a strong signal and has a low variability. On the other hand, the same sandwich assay format was used in Tu et al. (2020b), but since this study does not involve the matching of the laser wavelength to the absorbance of MGITC, weaker and more unstable signals were produced. The use of multiple peaks allowed the researchers to select which signal was most suitable for the assay.

However, this assay (Tu et al. 2020a) demonstrated multiple limitations. Firstly, the assay was tested on buffer solutions only, unlike the previous studies that used spiked serum samples. To add to this, the absence of interference testing left the specificity of this test uncertain, leaving the possibility of false positives and cross-reactivity. Moreover, the stability of the assay was also unassessed. This made shelf life and reproducibility uncertain, which is important in assessing its applicability in clinical settings.

The use of standard SERS instead of SERRS is, in itself, a limitation. Since resonance enhancement was absent in this study, signals are weaker and more variable, which required the researchers to conduct tests on multiple Raman peaks to determine the strongest and most stable signal. This can lead to potential inconsistencies, especially if the assay was used on actual serum samples, since the presence of noise exacerbated by other serum constituents can cause the assay's sensitivity to decline. It is also important to note that the detection range of this assay is 0-0.5 ng/mL, which is relatively narrow and more suitable for detecting early elevations in cTnI. This explains why the signal plateaus at higher cTnI concentrations (>0.5 ng/mL), which may compromise quantitative accuracy in real-world cases of severe myocardial injury or cardiac events (Tu et al. 2020a).

The first work by Guo et al. (2019), introduced a paper-based fluorogenic immunodevice that harnesses zinc-oxide nanowires (ZnO NWs) to amplify fluorescence signals. By using ZnO NWs, the authors reported roughly a 5-fold increase in fluorescence relative to bare paper, which enables simultaneous detection of three cardiac biomarkers — FABP, cTnI, and myoglobin — on a single device. The limits of detection (LODs) are in the low ng/mL range: 1.36 ng/mL for FABP, 1.00 ng/mL for cTnI, and 2.38 ng/mL for myoglobin. The assay is rapid (approximately 5 minutes), and readout relies on a simple setup: a homemade chamber combining a smartphone camera and a UV lamp — highlighting its potential for low-cost, decentralized diagnostics.[11]

In contrast, the second study by Shao et al. (2024), shifted to surface-enhanced Raman scattering (SERS), employing electrostatic nanoaggregates composed of negatively charged gold nanoparticles (AuNPs) and positively charged [Ru(bpy)3_33]$^{2+}$ complexes as SERS tags. These nanoaggregates exhibited

exceptionally strong SERS responses because each Ru complex carries multiple Raman-active ligands and the aggregated geometry creates abundant "hot spots". When incorporated into an immunochromatographic test strip (ICTS) format, the SERS-based assay achieves cTnI detection down to ~60 pg/mL, with a total assay time of ~5 minutes. The rapid formation of SERS tags (<3 min) and the translation into a lateral-strip format suggests a path toward practical, sensitive point-of-care tests.[25]

Building on the advantages of SERS and microfluidics, the third paper by Gao et al. (2022), implemented a pump-free hybrid microfluidic chip where paper-based microchannels were integrated with a PDMS layer. This design allowed capillary-driven fluid flow without external pumps. Within this device, the authors performed a "sandwich" immunoassay for two key myocardial biomarkers—cTnI and CK-MB—using magnetic beads bound with SERS nanoprobes; after immunoreaction, non-bound reagents are washed out, and the bead–nanoprobe complexes are captured magnetically in a detection chamber. The device achieved LODs as low as 0.01 ng/mL (10 pg/mL) for both biomarkers, with a total assay duration of about 30 minutes and no need for external pumping hardware. When compared side-by-side, it becomes clear that each approach trades different strengths against limitations.[10]

These contrasts reflected deeper mechanistic differences. ZnO nanowires improved fluorescence by enhancing excitation and photon yield and providing high surface area for antibody immobilization — but fluorescence is still limited by background fluorescence, photobleaching, and scattering from paper or biological media. SERS nanoprobes relied on localized surface plasmon resonance and electromagnetic hot-spot formation, which dramatically boosts signal and supports very low LOD; but SERS performance is sensitive to nanoparticle aggregation, hot-spot reproducibility, and colloidal stability — factors that complicated manufacturing and long-term storage.

From a translational perspective, each method offers a niche: the ZnO-fluorescent paper immunodevice is well suited for broad screening, multiplex biomarker panels, low-cost distributed diagnostics, especially in low-resource settings. The SERS-based methods (both lateral-flow and microfluidic) are better suited for high-sensitivity confirmatory testing, clinical diagnostics, or scenarios demanding accurate quantitation near clinical thresholds.

A promising real-world workflow would therefore adopt a two-tier strategy: use the ZnO-paper fluorescent device as an initial screen, followed by a SERS-based assay for confirmation or quantitation when screening yields positive or borderline results. This hybrid workflow balances cost, accessibility, and diagnostic accuracy.

Nonetheless, moving toward practical deployment requires addressing critical challenges. For ZnO-paper devices: batch-to-batch reproducibility (uniform nanowire deposition), long-term stability of the fluorescence signal under varying storage/transport conditions, and minimizing background signals in real biological samples. For SERS-based assays: robust, reproducible nanoparticle synthesis and tag-stability under storage; development of cheap, portable, and user-friendly Raman readers; and manufacturing scalable microfluidic or strip-based devices with consistent performance. Finally, both approaches would benefit from rigorous clinical validation on real patient samples (serum or plasma), with blinded comparison to gold-standard assays, to evaluate sensitivity, specificity, reproducibility, and interference robustness.

In conclusion, the three studies together mapped a spectrum of design trade-offs in modern biomarker immunoassays — from low-cost multiplexed screening to high-sensitivity quantitation — illustrating that no single "perfect" platform yet exists, but rather a toolbox of complementary techniques, each suitable for different diagnostic needs. Future diagnostic platforms may benefit from hybrid designs that combine the affordability and accessibility of paper-based fluorescence assays with the sensitivity of SERS-based microfluidic or lateral-flow systems, thereby maximizing both reach and clinical relevance.

Guo et al. (2019), offers a paper-based fluorogenic immunodevice where ZnO nanowires to amplify fluorescence signals on paper using a homemade chamber with a smartphone and an ultraviolet lamp. The reported to obtain 1000pg/mL within 5 minutes.[11] On another design for SERS microfluidic device, Shao et al. (2024), showcased a Raman reporter strategy using an electrostatically designed as SERS tags with nanoaggregates of AuNPs + $[Ru(bpy)_3]^{2+}$ (AuNPs-Tribipyridine Ruthenium(II) to amplify signal.[25] This is further constructed in a way by which lateral-flow assay or the utilization of immunochromatographic test strips is incorporated for detection of cTnl from serum up to 60 pg/mL within 5 minutes. Gao et al.,

(2022) developed a pump-free hybrid microfluidic chip designed for the detection of CK-MB and cTnI cardiac markers in simultaneous SERS-based immunoassay. The capillary-fabricated flow of the device gave a 2.94 pg/mL LOD within 30 minutes.[10] When sensitivity matters, the devices made from Shao et al. (2024) and Gao et al. (2022) have more complex instrumentation but can provide quantitative clinical accuracy with the incorporation of SERS provided lower LOD than ZnO-fluorescence paper devices in Guo et al. (2019), a low-cost smartphone readout device. Guo et al. (2019) and Shao et al. (2024), offer faster detection duration. These three papers, although use serum, vary with the amount of sample needed for detection. Respectively, Shao et al. (2024), only needs at least 10uL, Guo et al. (2019), with 15uL, and Gao et al. (2022), with approximately 20uL per inlet. Gao et al. (2022), and Shao et al. (2024), characteristically have multiplexing capacity and the instrumentation such as pump-free chip and lateral flow format reduce fluidic complexity. Arguably, all of the designs in SERS assays have capabilities for rapid detection using serum samples but in design and detection time where the assays compete.

Table 10. Comparison of selected surface-enhanced raman scattering based on criteria.

Detection Limit (Sensitivity)	Reference LoD	Sample Volume	Test Duration	Device Design	Practicability/ Portability	Analytical Robustness	Authors
15 pg/mL	2.94-1000 pg/mL	25µL (whole blood)	40 minutes	AuNPs were encapsulated in a silica shell and provided a SERRS signal using a handheld Raman system	Excellent (simple operation for end-users; Good selectivity to cTnI compared with other potential interferents)	High (Good selectivity; strong and stable SERRS signal at a 638-nm excitation wavelength)	Tu et al.[31]
20 pg/mL	2.94-1000 pg/mL	10µL (serum) *(least volume)*	~1 minute *(fastest)*	Simple 2D paper with MWCNT anti-cTnI conjugates	Excellent (low cost, minimal power)	Moderate (Excellent rapid response, but lower sensitivity)	Song et al.[27]
50 pg/mL	2.94-1000 pg/mL	50µL (serum)	15 minutes	Aptamer for cTnI detection with spectral processing techniques	Excellent (handheld Raman spectrometer)	Excellent (Produce stable and repeatable results)	Tu et al.[30]
2.94 pg/mL	2.94-1000 pg/mL	~20 µL per inlet	~30 minutes	Pump-free hybrid microfluidic chip; patterned filter paper with in-situ synthesized AuNPs and PDMS microchannels	Excellent (Pump-free type of SERS, portable, low-cost, useful for the simultaneous detection of cardiac markers)	Moderate (Rapid detection of markers for accurate AMI diagnosis; high sensitivity but low specificity)	Gao et al.[10]

60 pg/mL	2.94-1000 pg/mL	10-50 µL	5 minutes	Electrostatic nanoaggregates of negatively charged Au nanoparticles and positively charged trisbipyridine ruthenium(II) ions	Moderate (Portable but the applicability may be affected in the occurrence of multiple factors)	Moderate (Only poses potential applications but still shows risks of error and interference real complex biological samples)	Shao et al.[25]
1000 pg/mL	2.94-1000 pg/mL	15 µL	5 minutes	Zinc-oxide nanowires (ZnO NWs) integrated in the enhancement of fluorescence signal intensity.	Excellent (Rapid and low-cost instrumentation)	Moderate (Reagent storage degradation limiting application to Resource-constrained area; validation to clinical applications is restricted to small selected sample size)	Guo et al.[11]

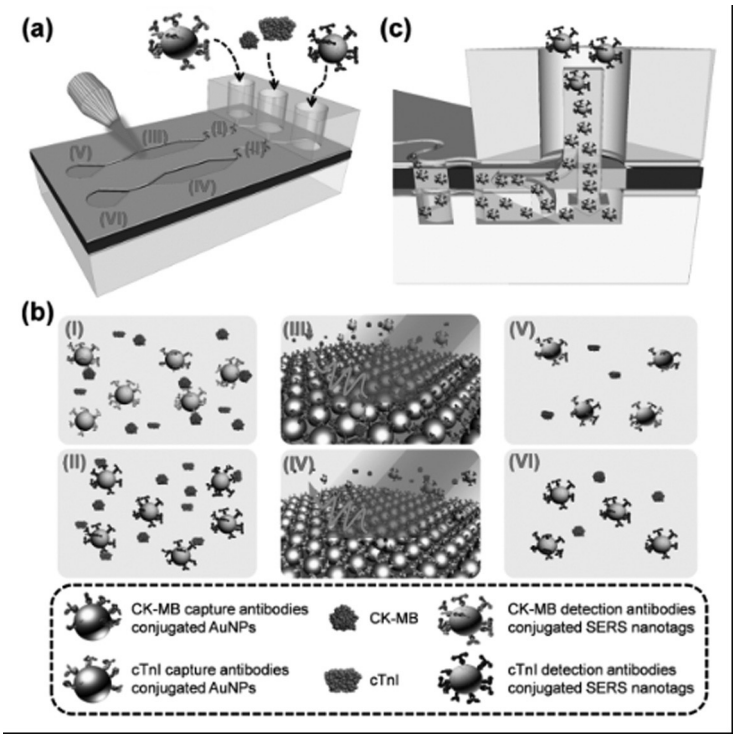

Figure 9. The SERS-based immunoassay device consists of (a) three round inlets, which were used to introduce CK-MB capture antibodies, spiked antigens, and cTnI capture antibodies. (c) Capillary action via the paper substrate and PDMS channels allows the samples to continuously flow. Winding microchannels (b I-II) function as mixing and reaction zones, facilitating immunoreactions between antigens and nanotags. Hexagonal capture chambers (b III-IV) coated with capture antibodies formed sandwich immunocomplexes. Oscillation of the paper substrate and SERS nanotags shows enhanced Raman signals due to these complexes. Unbound material was washed using a PBS buffer (b V-VI), directing the flow to the outlets. The figure was retrieved from Gao et al. (2022).[10]

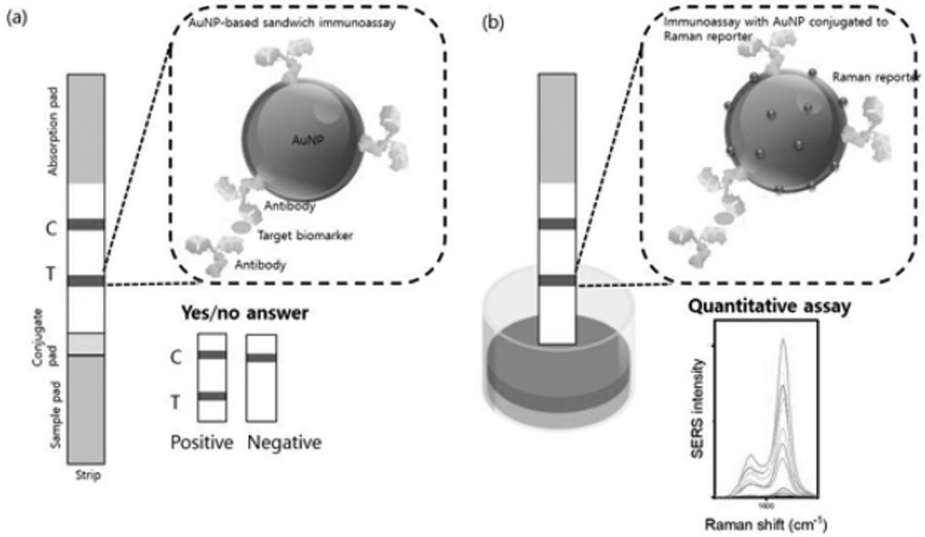

Figure 10. (a) Qualitative readings form sandwich immunoassay structures with the target biomarker on the test line using Au SERS tags. This indicates a positive result. In the absence of the target biomarker, the unbound Au SERS tag is captured at the control line. (b) Positive qualitative tests can be measured quantitatively using the Raman reporter MGITCs conjugated to Au nanoparticles. This generates SERS signals upon excitation, which are directly proportional to the concentration of the target biomarker. The figure was retrieved from Song et al. (2023).[27]

Comparison of Devices in Sandwich Immunoassay

Detection of cTnl is targeted through "sandwiching" two antibodies where a capture antibody is traditionally immobilized in a solid surface and a labeled antibody attached to a different epitope of the target antigen both significant to enable detection. It has been noted to provide excellent sensitivity and specificity best used in POCT settings integrated in strip detection, and multi-marker detection designed with distinct labels or unique signal tags . This has shown to provide easy-to-use, portable, and promising benefits for the invention of devices in the early diagnosis and on-site testing.

The present study developed and evaluated a sandwich immunoassay, specifically a paper-based microfluidic device (uPAD) for rapid detection of Troponin I, and highlighted its analytical performance relative to existing platforms. In Table 4, Poosinuntakul et al. (2022), reported a limit of detection (LOD) of 500 pg/mL, a total assay time (TAT) of 60 minutes, and the use of a 60 µL serum sample along with materials such as gold nanoparticles, nitrocellulose membrane, absorbent pad, and silver nitrate. The study demonstrated practicality by simplifying the microfluidic configuration and eliminating lengthy pre-incubation or silver enhancement, thereby improving usability in low-resource settings.[22]

In comparison, Chen et al. (2024), developed a multiplex uPAD with motor-driven rotary valves and RGB detection, achieving a lower LOD of 200 pg/mL, a shorter TAT of 30 minutes, and requiring only a 20 µL serum sample. While the platform in Chen et al. (2024), increases sensitivity and speed, it required additional hardware, making it less simple to operate than the present design, which remains more suitable for low-resource environments. In summary, with a reference range of 200 pg/mL to 500 pg/mL, the assay by Chen et al. (2024), demonstrated the highest sensitivity, fastest, and low sample volume, offering superior portability and analytical robustness under sandwich immunoassay platforms.[4]

Implication of the Parameters

Twenty two studies using different detection methods had been evaluated. Among all, the studies of Han et al. (2024), Low et al. (2023), and Mirzaeizadeh et al. (2024), were found to portray excellent performances which results relatively align with each parameter mentioned such as Materials used, Limit of Detection (LoD), Sample Type, Test

Table 11. Comparison of selected sandwich immunoassay based on criteria.

Detection Limit (Sensitivity)	Reference LoD	Sample Volume	Test Duration	Device Design	Practicability/ Portability	Analytical Robustness	Authors
500 pg/mL	200-500 pg/mL	60 µL (serum)	60 minutes	Gold nanoparticles, nitrocellulose membrane, sample & absorbent pad, silver nitrate + hydroquinone in citrate buffer, smartphone RGB analysis	Excellent (improves usability in low-resource settings, easy to deploy).	High (integrates silver enhancement, high linearity, and quantitation.)	Poosinuntakul et al.[22]
200 pg/mL	200-500 pg/mL	20µL (serum)	30 minutes	Chromatography paper, PMMA plates, servo motor, Arduino controller, RGB sensors, 3D printed housing	Excellent (compact, integrated and user-friendly)	Excellent (Multi-marker detection with independent optimization and strong)	Chen et al.[4]

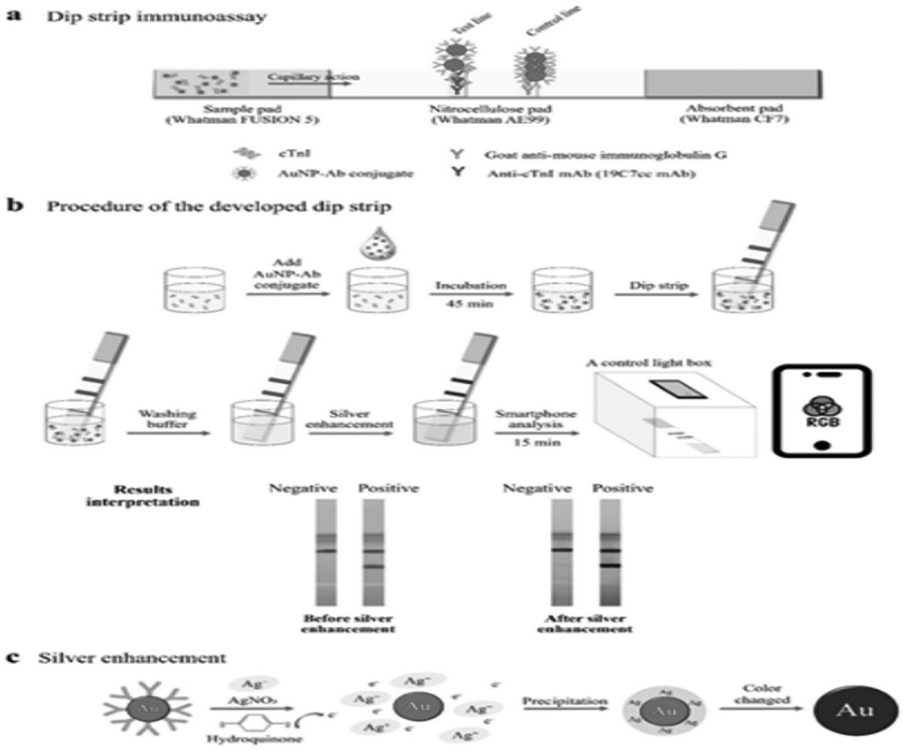

Figure 11. Dip strip based on sandwich immunoassay that starts with the sample pre-incubated with gold nanoparticle-conjugated antibodies (AuNP-Ab) to allow the formation of a cTnI–AuNP-Ab complex. The dip strip was then immersed in the mixture and enabled capillary-driven migration of the complex along the membrane, where immobilized anti-cTnI antibodies at the test line captured the complex, and the excess conjugates were bound at the control line to validate the assay. cTnI was trapped between a detection antibody, which was attached to gold nanoparticles (AuNPs), and a capture antibody creating a stable complex of antibody-antigen-antibody. The presence of AuNPs produces a visible color change at the test line, and the intensity of this color is directly related to the amount of cTnI present. The signal was strengthened by depositing silver onto the AuNPs, which intensified the color and improved the sensitivity of the detection. The Figure is retrieved from Poosinuntakul et al. (2022).[22]

Duration, and Sample Volume in the requirement for POCT application.

A. Materials Used

In evaluating microfluidic paper-based analytical devices, "materials used" was one of the most important factors since it can directly influence the assay analytical properties, functionality, and application efficiency at points-of-care. The choice of materials, such as cellulose paper, nitrocellulose membranes, gold nanoparticles, and nanocomposites, can determine the limit of detection (LoD) because the sensitivity of the signal amplification and recognition process were greatly impacted by certain materials. Han et al. (2024) was able to get 0.2 pg/mL in 15 minutes. This can bind cTnI antigen with its gold nanoparticle (AuNP)-conjugated detection antibodies forming antibody-antigen complexes at specific zones. The (AuNP)-conjugates served as nucleation sites for amplification signals where gold ions (Au^{3+}) was reduced into $HAuCl_4$ added in the nanoparticle to intensify color signal visibility. Low et al. (2023) was a lateral flow assay designed with Hydrophobic wax barriers and patterned chromatography paper, and the incorporation of several immobilized antibodies with electrophoretic washing zones. Multiple washing steps were eliminated by the removal of nonspecific proteins using electrophoresis in the sample matrix. With this, an LoD of 12.1 pg/mL under 6 minutes was achieved. Another study with unique materials were used in the device of Mirzaeizadeh et al. (2024) gaining 9 pg/mL LoD. It used a Schematic representation of ZIF-8@BSA Au/AgNCs where nanoclusters were synthesized using bovine serum albumin capped Au/Ag nanoclusters encapsulated in ZIF-8 as fluorescent probes.

It can be said that given that the speed of reaction depends on specific fluidic qualities like capillary force, porosity, and permeability. The choice of material was crucial in deciding the amount of time required to complete the test. Additionally, materials determined how much sample was required because some materials are highly absorbent, require little input, or are made specifically for a given sample. The material used can minimize interference and maximize test result specificity by enabling appropriate interaction with serum, plasma, and whole blood based on the unique sample. Therefore, the materials utilized were the key component that integrated several criteria in a single system, such as sensitivity, speed, and other elements that define a test's efficiency—ultimately determining whether the device meets the ASSURED criteria for reliable, accessible, and effective point-of-care diagnostics.

B. Limit of Detection (LoD)

Limit of Detection (LoD) is a parameter of an assay that can measure an analyte to its minimum extent of level on a certain given amount of time. This constituted a threshold of an assay in terms of analyte sensitivity. In paper-based microfluidic devices for detecting troponin I in AMI, a lower limit of detection (LoD) was generally associated with higher diagnostic sensitivity. The 22 studies were analyzed and each was able to detect troponin I but with different ranges of LoD.

In respect to other parameters, Han et al., (2024) was the most sensitive achieving 0.2 pg/mL, followed by Low et al. (2023) with 12.1 pg/mL and Mirzaeizadeh et al. (2024) with 9 pg/mL. The systematic review selected these three as most effective despite a lower LoD such as 0.3 pg/mL was achieved by Li et al. (2019), 2.94 pg/mL by Gao et al. (2022), Han et al. (0.84 pg/mL in 2019, 0.92 pg/mL in 2020), or 1.0 pg/mL by Wang et al. (2021) because other parameters were also taken into consideration. Although Li et al. (2019) has 0.3 pg/mL which was more sensitive than Low et al. (2023) with 12.1 pg/mL and Mirzaeizadeh et al. (2024) with 9 pg/mL, Li et al. (2019) needed 30 minutes to prove detection. Other studies portrayed good LoD but POCT devices should necessarily be fast, feasible and available as early AMI may present with very low troponin levels, and devices with low LoD must detect these small concentrations, reducing false negatives. Conversely, higher LoDs may miss early elevations, resulting in lower sensitivity. Thus, across studies, improved LoD through better assay design directly enhances the test's ability to identify AMI accurately.

C. Sample Type

Like other parameters, sample type also played an important role in the accurate detection of cardiac troponin I (cTnI). Serum was regarded as the most commonly used sample because it was cleaner than plasma and whole blood, reducing possible interferences and providing better sensitivity and more reliable results. Although it may require clotting time, which can delay turnaround, it ensures

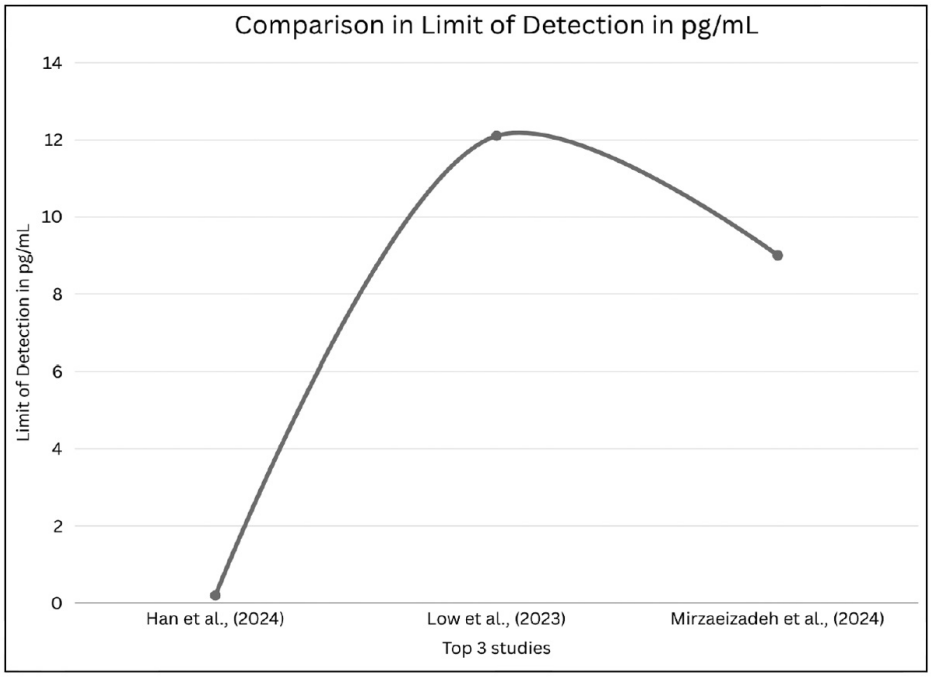

Figure 12. Comparison of Limit of Detection (LoD) in pg/mL where Han et al. (2024) with 0.2 pg/mL, Low et al. (2023) with 12.1 pg/mL and Mirzaeizadeh et al., (2024) with 9 pg/mL LoD. Han et al., (2024) was the most sensitive, while Low et al. (2023) and Mirzaeizadeh et al. (2024) also provided sensitive detection of Troponin I

consistency in testing. On the other hand, plasma allows faster processing since it does not need to clot, making it useful in emergency situations; however, the presence of anticoagulants and other factors may affect test results. Lastly, whole blood was mainly used in bedside or point-of-care testing because only minimal preparation was required, but it was not preferred due to possible interferences from blood cells and a higher risk of hemolysis, which can lead to less accurate results.

Overall, testing with serum and plasma offered the lowest (best) detection, a shorter test duration, minimal sample volumes, and only required the simplest material design due to having a relatively cleaner matrix. Most studies evaluated use serum such as Han et al. (2024), Low et al. (2023) and Mirzaeizadeh et al. (2024). Yuan et al. (2021) used whole blood or plasma, Tu et al. (2020b) and Fu et al. (2023) used whole blood. In contrast, while whole blood may be the easiest to handle and to process in rural areas, it was less recommended because its viscosity and cellular components can interfere with testing, resulting in a higher detection limit and a more complex test design. Hence, serum and plasma were generally preferred to achieve an absolute analytical precision in the diagnosis of acute myocardial infarction.

D. Test Duration

Test duration was critical for the detection of Troponin I using paper-based microfluidic assays because rapid results enable timely diagnosis of Acute Myocardial Infarction, which was essential for early treatment and improved patient outcomes. Shorter assay times enhanced point-of-care capability, allowing faster clinical decision-making in emergency settings, as demonstrated by rapid assays such as those reported by Vasantham et al. (2019) with ~1 minutes, Low et al. (2023) with <6 minutes, and Yuan et al. (2021) with 14 minutes. The duration of the test was also a key parameter that was correlated with other assay characteristics, including limit of detection (LoD), material design, sample volume, and sample type. Longer assay durations can improve reaction completeness and signal development, as seen in studies like Poosinuntakul et al. (2022) where extended pre-incubation contributed to improved sensitivity, although this relationship was not absolute and depends on the detection method and overall assay design.

If most effective devices were evaluated, Figure 17 showcased Han et al., (2024) with 15 minutes, Low et al., (2023) with less than 6 minutes and Mirzaeizadeh et al., (2024) with 10 minutes were chosen in consistency of results with other parameters associated. The effect of duration was further influenced by the materials used, as components such as gold nanoparticles and PEGylated surfaces, reported in studies like Ren et al. (2023), improved sensitivity and reduced non-specific interactions, allowing reliable performance even at shorter assay times. Additionally, assays using small sample volumes required highly efficient binding to maintain consistency, as shown in devices such as Li et al., (2019) with 2.5 μL and Vasantham et al., (2019) with 2 μL, which still achieved low detection limits. Moreover, sample type plays a critical role, as complex matrices like whole blood often require longer processing times compared to cleaner samples such as serum, which generally yield higher specificity and more stable signals. Overall, test duration acts as a balancing factor between speed and analytical performance, while interacting closely with the assay's materials, sample requirements, and overall design.

E. Sample Volume

Sample volume with the other parameters (Limit of Detection, Test Duration, Materials used, and Type of Sample) were all evaluated with one another in using microfluidic paper-based assays. More volume was theoretically known to be more concentrated with the known analyte.

In POCT and resource-limited areas, allowable volume of sample should be at least small volume, represented in uL, or enough necessary to account for different situations such as insufficient draw or for hard to extract patients, and also benefiting to lessen patients' pains during venipuncture. Thus, the device design must be able to specifically and sensitively detect Troponin I in those circumstances. However, the detection of analytes may still be affected by the whole design of the microfluidic devices used because sensitivity and specificity of detection of Troponin I depended on how the antigen-antibody complexes, or analytes captured, were generated. Devices operating at lower volumes require enhanced signal amplification strategies (i.e. cellulose paper, nitrocellulose membranes, gold nanoparticles, and nanocomposites).

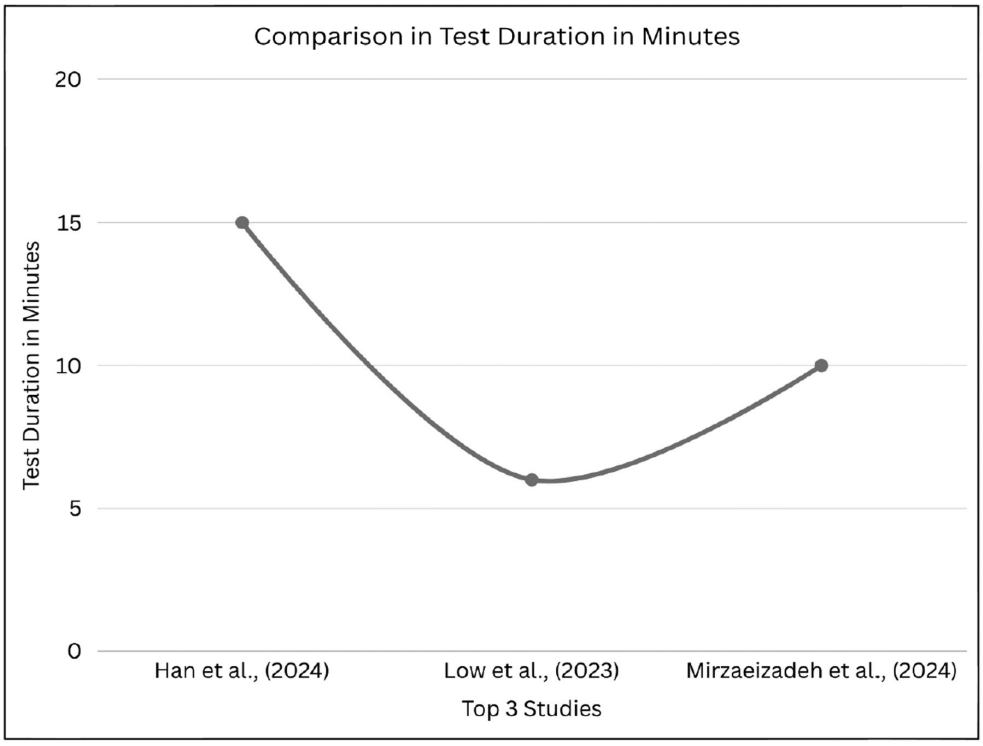

Figure 13. Comparison of Test Duration in Minutes where Han et al. (2024) with 15 minutes, Low et al. (2023) with less than 6 minutes and Mirzaeizadeh et al. (2024) with 10 minutes.

In Figure 18, Han et al. (2024) used gold nanoparticle (AuNP)-conjugates which enabled the design to get 0.2 pg/mL even if the sample volume needed was 50 uL. Comparatively, an LoD of 12.1 pg/mL was retrieved by Low et al. (2023) under 6 minutes requiring only 5 uL which was a smaller volume than Han et al. (2024), using Hydrophobic wax barriers with patterned chromatography paper, and the incorporation of several immobilized antibodies with electrophoretic washing zones. Additionally Li et al. (2019) required only 2.5 uL to detect 0.3 pg/mL (30 minutes) while Wang et al. (2021) utilized 4 uL of serum to achieve 1.0 pg/mL (44 minutes). More of the studies offer such cases where small volumes require longer detection time for a lower detection limit. In POCT standard, although Han et al. (2024) used 50 uL, it has showcased promising results in all parameters with 50 uL volume, allowable to compensate for the reduced analyte availability, while also enabling faster assay times and compatibility with real-world usability.

Still, the LoD and materials used in the assays offer significant pronunciation of a good paper-based microfluidic device's sensitivity and the design of capture and detection portrayed excellent specificity results of Troponin I. In consideration of all the 5 parameters reviewed in each 22 studies under Colorimetry, Lateral Flow Assay (LFA), Chemiluminescent Assay, Electrochemical Biosensor, Surface-Enhanced Raman Scattering (SERS)-based platforms, and Sandwich Immunoassay, Han et al. (2024), Low et al. (2023) and Mirzaeizadeh et al. (2024) provided the most effective Microfluidic Paper-based Analytical Devices (uPADS) in point-of-care test settings for detecting Troponin I in the diagnosis of Acute Myocardial Infarction.

SUMMARY AND LIMITATIONS

Various microfluidic assays and devices were assessed and compared in this systematic review for the detection of cardiac troponin (cTnI) in the diagnosis of acute myocardial infarction (AMI) for point-of-care testing (POCT). Assays in this study included lateral flow assays, chemiluminescence, electrochemical biosensors, surface-enhanced Raman scattering (SERS), sandwich immunoassays, and colorimetry. In Lateral flow assays, seven different studies were analyzed. The shortest turnaround time of 10 minutes was achieved with the ZIF-8@BSA

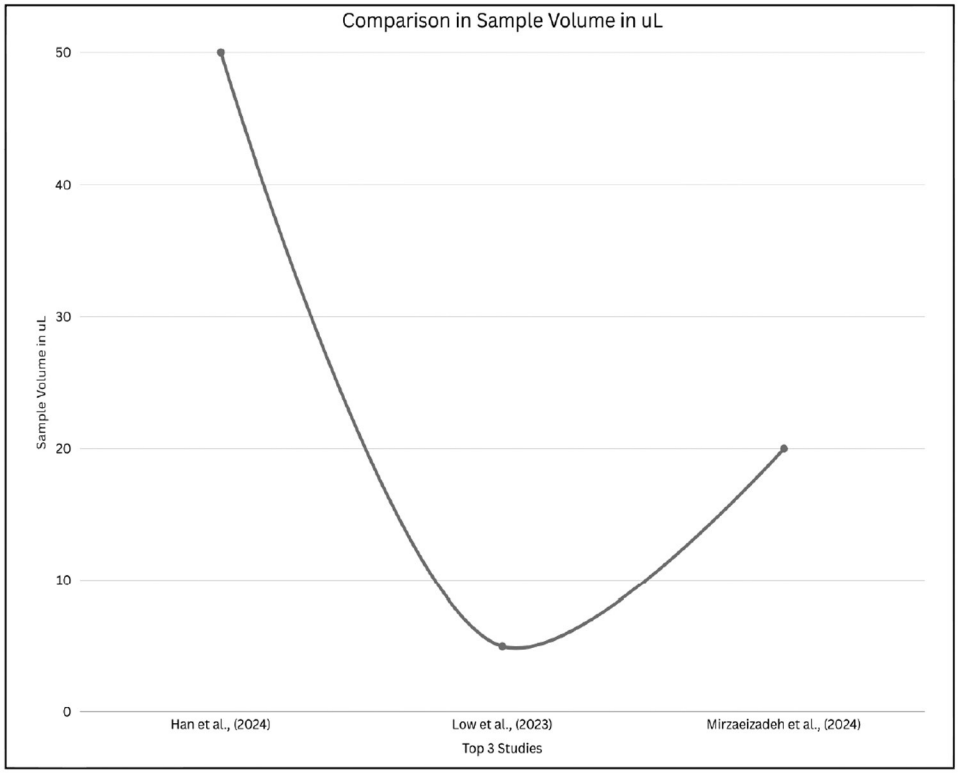

Figure 14. Comparison of test duration in microliter (uL) where Han et al. (2024) with 50 uL, Low et al. (2023) with 5 uL and Mirzaeizadeh et al. (2024) with 20 uL.

Au/Ag nanoclusters; this also supported the use of different sample types, including serum, plasma, and whole blood. The lowest detection limit among all of the methods were shown by AuNPs linked to polyHRP and antibodies, a detection limit of 0.84 pg/mL was shown, showcasing a possibility for a suitable sensitive point-of-care testing (POCT). As for the chemiluminescence-based study by Li et al. (2019), it demonstrated remarkable 0.3 pg/mL sensitivity with minimal sample requirements; however, due to its multi-step procedure and 30-minute test duration, it was limited for quick diagnosis.[16] In the field of electrochemical biosensors, three studies were reviewed. Fu et al. (2023), reported the highest sensitivity, with a detection limit of 4.6 pg/mL.[9] The fastest assay was done by Vasantham et al. (2019), with a test duration of about 1 minute.[35] For SERS-based assays, six studies were evaluated. Gao et al. (2022), portrayed the highest sensitivity, with a detection limit of 2.94 pg/mL.[10] While Song et al. (2023), portrayed the fastest test duration out of all the six.[27] Aptamer-based designs demonstrated better stability than antibody-conjugated devices. The use of Hybrid microfluidic chips and electrostatic nanoaggregates also enabled quick, pump-free detection for the SERS-based devices. In Sandwich immunoassays, two studies were compared and evaluated. Between the two, Chen et al. (2024), outperformed Poosinuntakul et al. (2022), achieving a 200 pg/mL detection limit with a 30-minute assay time that requires only 20 µL of sample.[4,22] Lastly, in colorimetric assays, Wang et al. (2021) demonstrated the highest sensitivity among the three devices, as well as the smallest sample volume required, using DNAzyme amplification.[36] On the other hand, Low et al. (2023), reported the fastest results in less than 6 minutes.[17] Yuan et al. (2021), enabled electricity-free finger-prick-compatible testing.[41]

Among all the assays reviewed, surface-enhanced Raman scattering (SERS), particularly the devices designed by Song et al. (2023), and Gao et al. (2022), was the most efficient for point-of-care testing (POCT) detection of cardiac troponin (cTnI) based on the inclusion criteria.[23,10] The most effective and practical between the two devices is from Song et al. (2023).[23] It demonstrated low detection limits, a fast turnaround time, low cost, and an easy-to-use operation, outperforming other assays. The researchers used a Simple 2D paper with MWCNT anti-cTnI conjugates, which provided high sensitivity with a detection limit of 20 pg/mL via the formation of a sandwich complex, conjugated to AuNPs with MGITC as the RRM. With a detection time of about 1 minute, a required sample volume of 10 µL, and no complex equipment or steps, its portability is ideal for emergencies. Gao et al. (2022), on the other hand, was considered the second-best due to its pump-free design despite a longer detection time of about 30 minutes.[10] Although it has offered strong analytical robustness, shown a good portability, and was useful for detecting other cardiac markers, it was not as practical as Song et al. (2023), in urgent situations.

This systematic review has several limitations that need to be considered in interpreting the findings. First, the studies reviewed have widely varying assay designs, principles of detection, materials, and device configurations. Such a wide variation made comparisons between platforms difficult. Variations in the composition of biosensors, for instance, nanoparticles, DNAzymes, antibodies, paper substrates, or methods of signal amplification, brought methodological heterogeneity into account. It thus naturally compromised the consistency of reported limits of detection and turnaround times across the different studies. In effect, conclusions regarding the most effective assay were influenced by the intrinsic diversity of the included methodologies.

Second, although the review tested devices on human biological samples such as serum, plasma, and whole blood, a large number of the included papers conducted experiments on small or restricted sample populations, often using spiked or laboratory-prepared samples rather than real patient populations that presented with clinical symptoms of AMI. The external validity was limited. Of the studies reviewed, only a subset actually evaluated the performance of devices using actual patient-derived samples, and fewer still considered interindividual variation in biomarker expression or physiological differences between diverse populations. Most devices of different methods of detection have used serum despite the need of additional centrifugal activity prolonging testing duration, but in the end, the results promising LoD. The use of Plasma and Whole blood lessened testing duration but only few utilized the said samples thus narrowing comparison which is best for POCT setting test for early detection of cTnl.

Another important limitation is that the studies reviewed did not examine or account for possible interindividual biological variation of cTnI, such as age, sex, renal function, comorbidities, and baseline troponin levels of healthy or chronically

ill populations. Even the kinetics of troponin during AMI differ from individual to individual, depending on ischemic time, infarct size, reperfusion status, and metabolic clearance. Such biological variations can significantly alter biomarker concentration in body fluids and may therefore have an impact on the validity and reliability of the paper-based assay under real conditions. The absence of data showing how these devices will function in diverse physiological and demographic backgrounds remains a knowledge gap that deserves further attention.

Second, many assays showed impressive detection performance in controlled laboratory settings but have yet to be clinically validated, field-tested, or trialed in resource-limited environments on a large scale. This is particularly important because the paper-based POC devices are destined for use in decentralized and thus potentially high-stress medical contexts. Various environmental and operational factors, including temperature and humidity, variable sample quality, or operator-dependent inconsistencies, might affect assay performance but were not considered in most studies.

Finally, while an extensive search strategy has been adopted for this review, limiting to the period between 2019 and 2025 excluded older foundational studies and unpublished data that may have relevance. English-language publications were used exclusively, which might have introduced some form of language bias.

CONCLUSION

This systematic review has shown that μPADs hold considerable potential as point-of-care diagnostic tools for the detection of cardiac troponin I in patients suspected of AMI. Throughout the 22 studies assessed, μPAD platforms consistently displayed quick turnaround times and low sample volume requirements, along with highly sensitive detection capability down to picogram limits of detection in some instances. Such features fulfilled many of the requirements under the ASSURED criteria set forth by the World Health Organization, emphasizing suitability not only for clinical settings but also for resource-limited environments. In terms of sensitivity, the lowest LoD accounts to the reliable value that cTnI was detected despite the background noises and interferences, in this case notably portrayed by studies in each method of detection such as Wang et al. (2021), with 1.0 pg/mL under colorimetry; Han et al. (2024), with 0.2 pg/mL, Han et al. (2020), with 0.84 pg/mL, and Han et al. (2019), with 0.92 pg/mL under Lateral Flow Assay; Li et al. (2019), with 0.3 pg/mL under Chemiluminescence; Fu et al. (2023), with 4.6 pg/mL under Electrochemical Biosensor; Gao et al. (2022), with 2.94 under SERS; And, Chen et al. (2024), with 200 pg/mL under sandwich immunoassay. However, differences of the materials in designing each microfluidic device greatly affect the test duration by which studies like Poosinuntakul et al. (2022), Tu et al. (2020b), Fu et al. (2023), and Wang et al. (2021), by which all required more than 30 minutes test duration due to additional steps such as incubation of reagents and samples, and separation of blood components which is important in retrieving the preferred sample type either whole blood, plasma, or serum. Additionally, required sample volume is practically as important because the success of phlebotomy is variable in availability and in some cases, skill of the personnel thus lower the need for sample volume, the better in POCT settings

Furthermore, as some studies utilized synthetic samples or blood-based spiked with cTnI samples, further clinical trials of real human samples should be implemented to fulfill gaps that acknowledge the diverse physiological characteristics and demographic background of each patient. With the provided data, the appropriate detection method is not only limited in choosing 1 per operation, rather the studies like Song et. al. and Wu et al. also proved that combination of the methods may improve sensitivity and specificity of detection of cTnI in the early diagnosis of AMI. Other assays also showed promising results and potential in POCT settings for the detection of cTnL in the diagnosis of AMI. However, practicality and robustness will be an issue for some assays due to complex instrumentation; scarcity of materials in selected areas; too high volume of sample needed; too long detection time and too high LOD; and lastly, complicated steps in processing which makes standardization difficult.

Although all the detecting methods: colorimetry, lateral flow assays, chemiluminescence, electrochemical biosensing, SERS, and sandwich immunoassays-presented certain strengths, collectively, they demonstrated the potential of paper diagnostics for cardiac testing decentralization and the facilitation of timely clinical decisions. The studies reviewed demonstrated a low risk of bias and high applicability across all QUADAS-2 domains, reinforcing the reliability of the synthesized evidence.

Overall, this systematic review confirmed that μPAD-based assays have reached a technological maturity that they are well-positioned to complement and, conceivably even transform, traditional laboratory-based diagnostics for AMI. Their affordable cost, portability, and ease of operation may fill critical gaps in emergency and low-resource health environments, moving diagnostic innovations closer toward enabling early detection, timely interventions, and better patient outcomes. Ultimately, this will further strengthen their position within cardiac diagnostic frameworks of the future through continued innovation and validation.

RECOMMENDATIONS

a. Future research should focus on wide-scale implementation of microfluidic devices for the rapid detection of cTnI to diagnose AMI using real biological samples to assess physiological diversity.

b. Future research should expand to include recently published studies and include studies under different languages for appropriate and potential use of data.

c. Future research should explore other methods of detection integrated in paper-based microfluidic devices for the rapid detection of cTnI in the diagnosis of AMI.

d. Future researchers may use data from this systematic review to revolutionize AMI diagnosis by designing paper-based microfluidic devices exploring the integration of more than 1 method of detection to optimally satisfy the needed parameters.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest regarding the conduct, analysis, or publication of this systematic review. No financial, personal, or professional affiliations have influenced the selection of studies, interpretation of their findings, or the presentation of results. All included data were identified through publicly available, peer-reviewed scientific literature, and the review was carried out independently following ethical and methodological standards.

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