Challenges and future solutions for detection of *Clostridioides difficile* in adults

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Abstract

There are no established standards for the diagnosis of *Clostridioides difficile* infection (CDI), even though the importance of this infection in humans is well known. The effectiveness of the commercially available techniques, which are all standardized for use with human feces, is also limited in terms of the accuracy of the tests. Furthermore, the current approach lacks a point-of-care diagnosis with an acceptable range of sensitivity and specificity. This article reviews the challenges and possible future solutions for the detection of CDI in adults. Existing diagnostic methods, such as enzyme-linked immunoassays and microbial culturing for the detection of toxins A and B, appear to work poorly in samples but exhibit great sensitivity for glutamate dehydrogenase. Real-time polymerase chain reaction and nucleic acid amplification tests have been investigated in a few studies on human samples, but so far have shown poor turnaround times. Thus, developing a multiplex point-of-care test assay with high sensitivity and specificity is required as a bedside approach for diagnosing this emerging infection.

Keywords Clostridioides difficile, diagnosis, point-of-care device

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Introduction

Most cases of pseudomembranous colitis and 25-30% of cases of antibiotic-associated diarrhea are caused by Clostridioides difficile (C. difficile) [1]. As C. difficile continues to cause significant morbidity and mortality, patients who present with diarrhea after being hospitalized for 3 or more days should be tested [2]. With an attributable mortality rate of 6-15%, C. difficile-associated diarrhea poses a serious hazard to health with rising prevalence and severity. A drastic shift in the epidemiology of C. difficile infection (CDI), with substantial fatality rates, has been documented globally since 2003 [3,4]. For instance, a rare but highly infectious genotype BI/NAP1/027 has emerged as a potent cause of infection in healthcare settings [5,6]. The clinical diagnosis of C. difficile has improved over the last few decades, with rapid and advanced detection techniques developed during the past 20 years. According to the UK recommendations, laboratories should test specimens using either a neutralized cell cytotoxicity assay or an enzyme immunoassay (EIA) that can detect both C. difficile toxins (CDT) A and B [7]. A survey found that, so far, no single "gold standard" test has been developed for the diagnosis of CDI [8].

There is clearly a need to develop a detection kit for CDI that should be user-friendly and pocket friendly with good efficacy. Therefore, we wrote this review to emphasize the development of a rapid point-of-care assay with improved accuracy that can also be a standalone test for detection of CDI. A literature survey was performed in Google Scholar and PubMed using search terms including "Clostridium difficile AND lateral flow assay". Initially a total of 19,615 records were found. A filter of "year 2017 to 2022" was then applied to look into the recent developments in the field of point-of-care testing and the number of publications came down to 14,900. Duplicate data from 15 papers were removed from the total. The criteria were clearly maintained to include all articles that gave precise details about lateral flow assays used, with significant information on the sensitivity and specificity, and eventually yielded 13 articles that met our requirements as shown in Fig. 1 [9-19]. Information was collected regarding the author, year of publication, study site, study setting, test performed, target, sensitivity, specificity, and commercial kit/indigenous kit used. Table 1 displays the 13 selected relevant studies. The full-length original articles were obtained to screen the laboratory-based data and compare the accuracy of the diagnostics tests. We also aimed to determine the point-of-care device being used for managing CDI.

Epidemiology of CDI

CDI has been identified as a significant source of nosocomial infections, primarily in industrialized nations [20-22]. Most



Figure 1 Studies included and excluded from the review

of the countries in North America, Europe, Australasia and many parts of Asia have observed a significant increase in the prevalence of the infection [20,23,24]. According to the Centers for Disease Control and Prevention (CDC; Atlanta, GA), at least 250,000 people in the United States contract CDI each year, and a mortality of 14,000 has been reported.

According to a previous estimate, there were 453,000 new cases of CDI in 2011, resulting in 29,000 deaths, making it the most common infectious disease in the US, with an incidence rising from 4.5 to 8.2 CDIs per 1000 persons between 2000 and 2010 [25]. Between 2003 and 2005, the United Kingdom saw its first-ever hospital epidemics, followed by several nations, including Ireland, The Netherlands, Belgium, Finland, France, Luxemburg and Switzerland [26,27]. Although CDI was initially identified in Western Europe and North America, it is now a hazard to worldwide health. Multiple outbreaks of CDI, with significant fatality rates, have been caused by changes in antibiotic usage patterns and the introduction of hypervirulent strains that generate toxins; these outbreaks can only be managed by enhancing infection control procedures and early detection [28,29].

Ribotypes commonly found in Asia

The common emerging strains from studies performed in Asia indicated that the most prevalent toxinogenic ribotypes in Asia were 017, 018, 014, 002 and 001. In particular, ribotype 017 is widespread in Asia; the lack of toxin A production by these strains demonstrates the importance of using tests that detect both toxins A and B, not only toxin A (now true for most CDT tests in commercial use) [30-32]. However, a study in a Thailand hospital found that ribotypes 014/020 (A+B+) and 017 (A-B+) were more toxigenic [31,33]. In 2015, a study of hospitalized inpatients in Chandigarh, India, by Vaishnavi et al found the ribotypes of the toxinogenic strains yielded in culture to be 001, 017 and 106 [31,34]. The CDC discovered a strain known as North American pulsed-field gel electrophoresis type 1 (NAP1), also known as the restriction endonuclease analysis group BI/polymerase chain ribotype 027 strain, which was resistant to the antibiotic fluoroquinolone and had a greater propensity to produce toxins and spread. As a result, CDI epidemics in clinical settings were caused by this strain [9,21]. As a result of rare hypervirulent B1/NAP1/027 strains, the incidence of CDI rose in the United Kingdom during the 1990s, rising from 18,354 cases in 1999 to 28,819 cases by 2002. Furthermore, unlike ribotypes 027 and 078, which are only found in North America and Europe, CDI epidemics generated by ribotypes 017, 018, 014, and 002 are prevalent in many Asian nations, primarily Korea, Japan, Taiwan, Singapore, Hong Kong, Indonesia, Malaysia, India and Bangladesh [10]. Western Australia experienced a very high incidence of CDI in the 1980s (2.09 cases per 1000 discharges), which later reduced to 0.89 cases per 1000 discharges in 1998-1999 as a result of the cessation of cephalosporin medications [29].

| No | Author | Year [ref.] | Study site | Study setting | Test performed | Target | Sensitivity | Specificity | Commercial kit/ Indigenous |
|----|--------------------------------------|----------------|--------------|-------------------------------------|---|--|-------------|-------------|----------------------------------|
| 1 | Hae-Sun Chung, <i>et al</i> | 2017 [44] | Seoul, Korea | Tertiary care hospital | <i>C. diff</i> Quik Chek Complete | GDH antigen Toxin A/B | 97.60% | 93.90% | Commercial kit |
| 2 | Ja Young | 2017 | Incheon, | Tertiary care | C. diff Quik | GDH, toxin | 97.10% | 99.40% | Commercial |
| 2 | Seo, <i>et al</i> | [45] | Korea | hospital | Chek Complete | A/B | 510/ | 0.20/ | kit |
| 3 | John L. Vaughn, <i>et al</i> | 2018 [46] | USA | University | C. <i>aff</i> Quik Chek Complete | GDH | /1% | 83% | kit |
| 4 | Maria C | 2019 | Argonting | Modical | C diff Quilt | Toxin A/B | 29% | 100% | Commercial |
| 4 | Legaria, <i>et al</i> | [47] | Aigentina | laboratories, Hospitals (n=9) | Chek Complete | GDH T i A/D | 50.2004 | 70.9270 | kit |
| 5 | Emily I | 2019 | Dhiladalahia | Childron's | C diff Quilt | LOXIN A/B | /8.30% | 94.41% | Commercial |
| 5 | Gomez, <i>et al</i> | [48] | USA | hospital | Chek Complete | GDH | 0070 | 9270 | kit |
| | T X7 X7 | 2010 | 0 1 77 | | | Toxin A/B | 21% | 99% | |
| 6 | In Young Yoo, et al | 2018 [49] | Seoul, Korea | center | C. <i>diff</i> Quik Chek Complete | GDH | 95.70% | 92.50% | kit |
| - | | 2010 | 0 1 | TT · ·, | | Toxin A/B | 60% | 94.40% | 0 1 |
| / | Marcela Krutova, <i>et al</i> | 2019 [50] | Republic | hospital | MariPOC CDI | GDH | 96.40% | 95.21% | kit |
| | | | | | | Toxin A/B | 66.67% | 99.24% | |
| | | | | | <i>C. diff</i> Quik Chek Complete | GDH | 95.68% | 97.60% | kit |
| 0 | Deser | 2020 | Finland | II | Musipoc odi | Toxin A/B | 55.56% | 99.81% | Communial |
| δ | Roosa Savolainen, <i>et al</i> | 2020 [51] | Finiand | Hospital & Healthcare company | MariPOC CDI | GDH | 95.20% | 98.30% | kit |
| | | | | | | Toxin A/B | 81.60% | 100% | |
| | | | | | <i>C. diff</i> Quik Chek Complete | GDH | 100% | 100% | Commercial kit |
| | | | | TT 1 1 | | Toxin A/B | 71.10% | 99.20% | |
| 9 | Hatice Yazisiz, <i>et al</i> | 2020 [52] | Turkey | University, hospital | <i>C. diff</i> Quik Chek Complete | GDH | 94.40% | 97.70% | Commercial kit |
| | | | | | | Toxin A/B | 92.30% | 100% | 0 1 |
| | | | | | Ioxin A + B (<i>Clostridium</i> <i>difficile</i>) DUO kit | Ioxin A/B | 53.80% | 87.80% | kit |
| 10 | Hoda Jaffal, <i>et al</i> | 2020 [53] | Lebanon | University | <i>C. diff</i> Quik Chek Complete | GDH | 96% | 81% | Commercial kit |
| | | | | | | Toxin A/B | | | |
| 11 | Soo jin Yoo, et al | 2020 [54] | Seoul, Korea | University, hospital | <i>C. diff</i> Quik Chek Complete | GDH | 90.50% | 92.90% | Commercial kit |
| 10 | X47 1 1 4 | 2025 | 1 117 | TT 1 1. | D | Toxin A/B | 51.40% | 100% | т 1. |
| 12 | Waleed A. Hassanin, <i>et al</i> | 2021 [55] | UK | University | Raman scattering-based lateral flow assay (SERS -based LFA) | surface layer protein A (SlpA) and toxin B (ToxB) | NA | NA | Indigenous kit |
| 13 | Hope Adamson, <i>et al</i> | 2022 [56] | UK | University | NanoBiT Split -Luciferase Assay | GDH and Toxin B | NA | NA | Indigenous kit |

Table 1 Summary of characteristics of all studies included for reviewing the accuracy of tests for detecting Clostridium difficile

GDH, glutamate dehydrogenase; UK, United Kingdom; LFA, lateral flow assay

Pathogenesis and risk factors

There are 2 prerequisites for developing *C. difficile*associated diarrhea: disruption of the normal gastrointestinal flora and diminished colonization caused by acquiring the organism from an exogenous source [11]. The main virulent factors associated with the occurrence of CDI are the potent toxins produced by this anaerobe. The pathogenesis of these toxins initiates the catalyzing of the glycosylation, and hence inactivation of Rho-GTPases and small regulatory proteins of the eukaryotic actin cell cytoskeleton. This leads to the disorganization of the cell cytoskeleton and cell death [12].

Many pathogenic *C. difficile* strains produce not only toxin A and toxin B, but also binary CDT. This toxin was detected in 17-25% of *C. difficile* strains under routine diagnostic conditions. Very few studies in the literature mention the pathogenicity of toxin A over toxin B, and some mention the presence and virulence of toxin B, as observed in most of the outbreaks. This third type, the binary toxin, also seems to play a vital role in causing disease in many patients [13-15].

Apart from this formidable internal mechanism of causing fatal CDI, inflammatory bowel disease (IBD) has been found to be a major underlying comorbidity in severe cases. The most common cause of nosocomial diarrhea and colitis associated with antibiotic usage is CDI. These infections are more common in people with IBD and have worse outcomes, including greater rates of colectomy and death, and thereby more recurrences. It is still unclear, nevertheless, whether C. difficile contributes to IBD or results from the inflammatory conditions of the gut. Moreover, the presence of all toxigenic genes has been observed in cases of IBD with long-term hospitalization. A gene knockout by Sarah et al showed the importance of both toxin A and B, and highlighted the need to continue considering both toxins in the development of diagnostic tests and effective countermeasures against C. difficile [13]. Nevertheless, there still seems to be controversy with regard to selecting the best diagnostically important toxin. Therefore, there is a need to develop a multiplexing kit that targets the maximum possible number of virulent factors in a single test cassette.

Diagnosis of C. difficile

The most commonly practiced diagnostic method for infection caused by *C. difficile* includes endoscopy. This invasive technique plays a therapeutic function in the management of CDIs that are resistant to oral and parenteral antibiotic therapy, though these treatments are effective in the majority of CDI patients. This modern-day phenomena includes invasive procedures which might delay the diagnosis. Laboratory-based diagnosis, on the other hand, seems affordable and user friendly [35]. The commonly observed targets mentioned are glutamate dehydrogenase (GDH) and the toxins. It was observed that 11 of 13 [36-46] studies were conducted using the commercially available diagnostic kit, C. diff Quik Check Complete. For these 13 studies, the maximum

sensitivity was observed to range from 71-100%, while the specificity ranged from 76.92-100%. Many of the studies using Quik Check Complete reported poor sensitivity and specificity [38,40]. However, the others lack either sensitivity or specificity [36,37,39,41,42,44-46]. One study, by Savolainen et al, yielded a 100% sensitivity and specificity, but only for detection of the antigenic GDH [39]. Two previous studies, by Hassanain et al and Adamson et al, mention using indigenously developed protocols [43,44]. Hassanain et al mention 2 specific biomarkers, surface layer protein A and toxin B, using a surfaceenhanced Raman scattering-based lateral flow assay (SERSbased LFA). The use of a handheld device in this SERS-based LFA, rather than a benchtop machine, paves the way for rapid, selective, sensitive and cheap clinical evaluation of CDI at the point of care, with minimal sample backlog. The commercial kit mostly used is the C. diff Quik Chek Complete. Adamson et al mention targeting affimers (13 kDa non-immunoglobulin binding proteins) for C. difficile biomarkers GDH and toxin B, which can be used in diagnostic assays for CDI. They mention point-of-care testing (POCT) using NanoBiTBiP assays for GDH and Toxin B [44]. The 2 studies by McDonald et al and Barbut et al are observed to have targeted similar genes as Quik Chek Complete, i.e., GDH and Toxin A & B. However, this kit has unfortunately been seen to struggle with its accuracy. A few former studies mention that watery stool, stomach cramping or tenderness are some of the common diarrheal symptoms, but the same symptomatic speculation associated with antibiotic therapy turns the focus to a dysbiosis caused by a sporeforming anaerobic bacterium, C. difficile [45]. Diagnosis of organism-specific diarrhea has always been debatable because of the lack of a sustainable test [46]. A mini-review by G. A. Rolden mentions a world scenario of the burden of C. difficile across the globe, where its prevalence is said to vary drastically because of varying testing facilities and lack of awareness [31]. C. difficile is now known to be the cause of approximately 10-35% of all cases of antibiotic-associated diarrhea and the most common infectious cause of nosocomial diarrhea, associated with substantial morbidity and mortality [47,48].

Earlier diagnosis of CDI used latex agglutination, which had a sensitivity of 58-68% and a specificity of 89-99%. Most commonly, CDT A and CDT B were diagnosed using an EIA, which provides a turnaround time of about 1-2 h, with 75-85% sensitivity and 95-100% specificity. This is the most costeffective and easy-to-use technique, so it is the most popular test in all laboratories. Tests detecting *C. difficile* antigens are based on the detection of GDH and are characterized by ease of use and rapid turnaround time, as well as a specificity of almost 100%. A major drawback of GDH-based assays is their lack of differentiation between the toxigenic strains (specificity of 59%) [49,50].

In 2009, with more advances in science and technology, tests that use amplification of nucleic acid (nucleic acid amplification test [NAAT]) were introduced. They are based on either a PCR method or isothermal amplification. NAAT was seen to have a higher sensitivity (80-100%) and specificity (87-99%) compared to an EIA test, and so these 2 traditional testing methods needed to be improved in terms of rapidity and cost-effectiveness (Fig. 2). Some cases showed a specificity reaching



Figure 2 A 2-step diagnostic test algorithm for detecting *C. difficile IBD, inflammatory bowel disease; GDH, glutamate dehydrogenase; NAAT, nucleic acid amplification technique; CDI, Clostridiodes difficile* (*C. difficile) infection*

95% with a negative result. In such conditions, another cause of diarrhea should be considered [51-55]. This high-detection molecular technique has some limitations: namely, high cost and non-user-friendly application. NAAT detects the presence of a toxin-encoding gene, thus confirming the presence of the CDT-producing strain, but it does not necessarily mean that the strain is producing any toxins at the moment. If the diarrhea is of another origin, detection of such strain would be misleading, as it would shift further treatment towards CDI. Persistent and often ineffective treatment of only colonized patients does not improve their clinical situation. When dealing with such diagnostic difficulties, a comprehensive diagnostic evaluation of other potential diarrhea-causing disorders is required. A cytotoxic assay test is not routinely used in microbial culture, because of its slow turnaround time and lack of standardization (48-72 h) [55-57]. According to the guidance of the European Society of Clinical Microbiology and Infectious Diseases, no single test is suitable as a standalone test confirming CDI.

According to the available literature, no standard lab test for CDI diagnosis has been marked as standalone in India. As regards the current status, there is a dearth of literature on the subject. Many articles suggest various protocols for the detection and confirmation of the *C. difficile* pathogen and its signature. In India, it is still considered an emerging hospitalassociated infection, as the mortality rate reported is in the nearnegligible range compared to western countries [48]. A few studies mention combining 2 tests in the algorithm, in order to combine a high negative predictive value (either a GDH EIA or NAAT) with a high positive predictive value (toxin A/B EIA) [58]. The question remains: will even such improvised conceptualities provide an accurate and affordable bedside diagnosis?

POCT

Point-of-care rapid diagnostics adopted with appropriate linkage to health systems have led to faster test-to-result turnaround times, reducing losses to follow up, and enabling prompt case management in resource-constrained settings where lack of access to laboratories constitutes a critical gap in delivering healthcare services [59]. LFAs, which are primarily qualitative diagnostic procedures to ascertain the presence or absence of a target analytes within a non-invasive sample such as whole blood or urine [60], are among the most well-known point-of-care formats. A patient may have multiple coinfections in such circumstances, where lateral flow point-of-care tests have an advantage over more complex but laboratory-confined diagnostics [61]. In addition, infectious diseases of concern, such as tuberculosis, malaria and parasitic helminths, may exhibit symptoms similar to those of viral and/or bacterial illnesses. As a result, several public health initiatives have frequently come under fire for being overly disease-focused and failing to consider an individual's total health. Indeed, sustainable control, including disease elimination, will require integrated strategies using more versatile diagnostic tools, rather than single-disease, single-test approaches that do not take into account the overall health needs of people living in resource-constrained settings [62].

Although there is a lack of current evidence, it can be argued that the larger commercial relevance and possible costeffectiveness of such tools have enhanced the motivation for scale-up. However, more critically, tests that can identify or rule out major infections in a single point-of-care interaction would make it easier to manage the frequently complex nature of patient morbidity more effectively. Such testing would undoubtedly be appealing, even in low-income counties, given the financial restrictions encountered by health programs around the world, as well as the growing desire for decentralized healthcare. To quickly distinguish between an endemic infectious disease and the start of an epidemic, improved case management of diseases with common symptoms is especially important in places with dense populations (such as refugee camps) where explosive epidemics may emerge [63].

Achieving acceptable levels of sensitivity for active infections has always been a challenge in serological screening, and may necessitate the detection of multiple biomarkers, such as combining the detection of antigen-specific antibodies and antigens [64,65]. The necessity for multiplexed diagnostics has increased as a result of these requirements. Because of the tiny size of the micro-systems and the capacity of the microarray, microfluidic biosensors offer a suitable platform for multiplexed diagnostics [66]. There have been several reports of various tests that may accurately identify several analytes on lab-on-achip devices [67]. Difficulties in mass production, particularly the high cost of sensor fabrication, mean that technologies in the lab cannot yet be scaled up to reach the end-user market, which restricts their translation into POCT. This article aims to answer the question of whether the ostensible simplicity of membrane-based LFA may be adaptable to multiplexed formats, given the demand for multiplexed testing and the prevailing advantages of the LFA over alternative rapid diagnostic platforms for resource-constrained settings [68,69].

Discussion

Today's world, with its modern life style, faces a new age of health hazards, because of which a huge mass of people get hospitalized annually. This review addresses an issue mainly observed in patients admitted to intensive care units. A very common phenomenon observed is gut dysbiosis, which usually occurs in patients with long hospital stays. This could possibly be due to time-consuming diagnostic assays, and so we propose to develop a rapid and accurate multiplexing diagnostic panel as a futuristic solution to the new age of diagnosis. Critical patients admitted for a long tenure are also bombarded with heavy doses of antibiotics. This practice leads to unfavorable conditions in the internal epithelial lining of the stomach and causes alterations in the pattern of gut microbiota. The bacterium of interest in this article, C. difficile, is then found to be dominating the gut. This leads to fatal diarrhea, making this infection a critical one to diagnose.

The field of laboratory medicine today is known for its POCT and is quickly changing in terms of its analytical capabilities and therapeutic applications. Through this study, we have tried to add to the existing recommendations for diagnostic utility for medical practitioners. POCT has reduced the turnaround time usually required for clinical decisionmaking about subsequent testing and therapies. In addition, the lower costs and better medical outcomes have proved to be a boon for the medical fraternity, as near-patient testing is a useful complement to conventional laboratory analyses.

The mainstay of C. difficile diagnostic techniques is the identification of its toxins. GDH, a constitutive enzyme generated in significant levels by all strains of C. difficile regardless of toxigenicity, is another antigenic component that the organism is known to produce [70]. However, many scientists believe that the only pathogenic and diagnostically significant strains are those that produce toxins [71]. Toxigenic culturing has been observed in many laboratories across the globe as one of the best techniques available, according to George et al [72]. The identification of C. difficile culture is primarily done on cycloserine cefoxitin egg yolk agar and cycloserine cefoxitin fructose agar, in combination with sodium taurocholate, allowing spores to germinate [73]. This approach is regarded as the gold standard, so holds a key position in the diagnosis [74]. During study, it is critical to successfully isolate C. difficile from stool samples, especially when there may be few spores or vegetative cells present [75]. Since oxygen levels in the atmosphere can vary greatly, C. difficile is extremely sensitive to them. These methods demand the use of an anaerobic chamber, because C. difficile manipulation and maintenance in the lab require a controlled, anaerobic environment. Increased recovery and isolation of obligatory anaerobes have come about as a result of the use of anaerobic chambers, but the issue with the turnaround time still persists [74,75].

To overcome such limitations, more quick procedures have largely supplanted this traditional approach. Particularly for developing nations, it is extremely important to take into account the complex setup and maintenance costs. The tissue cytotoxic test is typically the gold standard, although it is difficult to perform, time-consuming, and requires at least 48 h before results are available.

Because of their simplicity, and the ability to deliver results in just 2 h, EIAs are used by the majority of diagnostic laboratories, despite their low sensitivity. Even though anaerobic culture of *C. difficile* is more sensitive, the use of culturing techniques is limited by the amount of effort required, the lengthy turnaround time, and the inability to distinguish between strains that produce toxins and those that do not. These drawbacks are overcome in some laboratories that use more sensitive toxigenic cultures and an EIA to distinguish between the strains obtained from the plate culture as being toxin positive or negative. In summary, the infection caused by *C. difficile* has been observed to be difficult to manage and so there is a need for an affordable diagnosis.

Future perspectives

Diagnosis and detection of *C. difficile* has become the need of the hour, as the associated diarrhea is creating a threat in today's world. However, the detection of this bacterium is very difficult, and existing diagnostic tests fail to meet modern medical needs, including multiplexing, rapidity and affordable diagnosis. We therefore propose the development of point-ofcare devices that, when made and implemented, could be a boon for medical practitioners.

For the past 60 years, lateral flow immunoassay (LFIA)-based technologies have been employed successfully in the diagnosis of numerous illnesses and ailments. These diagnostic platforms are becoming more and more common in hospitals, especially those with limited funds and staffing, as well as in homes for personal health monitoring. These inexpensive tools have advantages over contemporary laboratory-based analyzers, due to their accessibility, capacity for quick detection and simplicity of use. The portable diagnostic devices are attractive because they offer high analytical sensitivity and specificity, as well as a simple visual readout of data. As a result, there has been increasing acceptance of LFIA in underdeveloped nations when used in small hospitals during times of emergency, where screening and health status monitoring are critical, both for patient self-testing and overall. These tools have stood the test of time, and today's LFIA test systems are completely in line with the modern idea of POCT in the world, finding use in a variety of fields, including human medical, ecology, veterinary medicine and agriculture. The numerous opportunities offered by LFIA aid in the ongoing growth and advancement of this technology, as well as the development of new-generation formats [76].

The construction of test systems in a multiplex (multipurpose) format, which allows the detection of numerous bacterial or viral targets simultaneously in a single test, is one



Figure 3 Flowchart showing the central concept signifying the points to consider when developing a multiplex lateral flow assay (LFA) for translational research [79]

of the important themes in the development of adaptable LFIA technology. This technique is a cutting-edge diagnostic method that offers numerous opportunities for the verification of causal factors [77]. LFIAs are based on inducing a sample stream along a strip that contains the target analytes. A membrane and a few functional pads, such as a sample pad, conjugate pad and adsorbent pad, are the main components of the strip. The immobilized nanoparticles on the conjugate pad are used for binding, and to show that the target molecules have successfully trapped the antibody on the nitrocellulose membrane surface. A favorable outcome is indicated by the emergence of a line of conjugated reporter particles [61].

Concluding remarks

Healthcare diagnosis has always been a challenging task for medical scientists, as detection kits assays with high accuracy and rapidity are the first choice of healthcare practitioners. In this world of advanced medical practices, there is constant pressure to move towards better and more rapid diagnosis. However, culturing has numerous drawbacks, including a slow turnaround time and the inability to identify the presence of toxins [74, 78]. To compensate for this and manage the shift in epidemiological trends, point-of-care diagnostics are used for infectious diseases, food safety and many other applications. Given the diagnostic limitations, LFAs are rapidly taking over the traditional diagnostic methods and developing high utility diagnosis which could then be taken as a concept of capacity building for innovative business ideas and scale up for developing countries (Fig. 3). Better LFA sensitivity and innovative capabilities are possible, thanks to the distinctive optical, electrical and chemical features of nanoparticles that result from their nanostructure and material properties. Nonspecific adsorption, protein denaturation and steric hindrance are a few unfavorable side-effects that can occur when interacting with nanomaterials in complicated biological contexts. In LFAs, where there are numerous types of inorganicbiological interactions, frequently of a complex nature, these problems are even more serious. Therefore, it is essential to take advantage of the special qualities of nanomaterials for LFAs in a way that addresses these interface difficulties [75,78].

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