Journal of Pharmaceutical and Biomedical Analysis 219 (2022) 114889

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.journals.elsevier.com/journal-of-pharmaceutical-and-biomedical-analysis

Review

A review on urinary tract infections diagnostic methods: Laboratory-based and point-of-care approaches

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ARTICLE INFO

Keywords:
Urinary tract infections
UTI-diagnosis devices
Point-of-care detection
Biosensors
UTI-self-testing kits

ABSTRACT

Urinary tract infections (UTIs) are among the most common infectious diseases worldwide. This type of infections can be healthcare-associated or community-acquired and affects millions of people every year. Different diagnostic procedures are available to detect pathogens in urine and they can be divided into two main categories: laboratory-based and point-of-care (POC) detection techniques. Traditional methodologies are often time-consuming, thus, achieving a rapid and accurate identification of pathogens is a challenging feature that has been pursued by many research groups and companies operating in this area. The purpose of this review is to compare and highlight advantages and disadvantages of the traditional and currently most used detection methods, as well as the emerging POC approaches and the relevant advances in on-site detection of pathogens´ mechanisms, suitable to be adapted to UTI diagnosis. Lately, the commercially available UTI self-testing kits and devices are helping in the diagnosis of urinary infections as patients or care givers are able to perform the test, easily and comfortably at home and, upon the result, decide when to attend an appointment/Urgent Health Care Unit.

1. Introduction

One of the most common types of infectious diseases is urinary tract infections (UTIs), being responsible for about 150–250 million cases per year, worldwide [1,2]· Typically, these infections are caused by bacteria belonging to the host endogenous microbiota, such as *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus* spp., *Enterobacter* spp., etc. [2,3]. *E. coli* is the main responsible for UTIs in ambulatory and hospitalized patients, representing 75% and 65% of the cases, respectively. *K. pneumoniae* is the second responsible for UTIs, representing approximately 6–8% of the cases [1]. In addition to the patient health condition, the costs associated with hospitalization and treatment of acquired urinary infections are extremely high [2,4].

The "Gold standard" methods for the detection/identification of pathogenic bacteria in urine are based on cultural enrichment, bacteria isolation and growth to increase cell number to detectable levels, followed by subsequent biochemical and serological tests and determination of antibiotic susceptibility profile [5]. The overall turnaround times of these tests are long, typically 48–72 h [6]· Serious infections are time sensitive and often their treatment must proceed without test results. In the case of less serious infections, delaying treatment while waiting for these results leads to infectious disease spreading or associated complications. Over the past two decades, solutions that have been pursued to shorten the time-consuming methods include the development of advanced molecular-level assays and nanotechnologies. Rather than waiting for bacteria to multiply, these approaches are based on molecular and proteomic technologies that can reduce the time required for analysis from days to hours, creating an impact on the effectiveness of infectious disease management [6].

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https://doi.org/10.1016/j.jpba.2022.114889
Received 9 March 2022; Received in revised form 18 May 2022; Accepted 11 June 2022
Available online 14 June 2022

2. Infection associated to urinary catheterization

Urinary catheters are crucial tools in different clinical scenarios, although the UTIs associated with their use represent 30–80% of the total acquired infections (varying among hospitals and countries). Urinary catheterization is a very common procedure in which a long tube (catheter) is inserted from the urethra to the bladder to drain or collect urine [7]. Catheter-associated urinary tract infections (CAUTI) are very common among patients with indwelling urinary catheters, with an incidence of around 80% and are responsible for the deterioration of the health status of already debilitated patients, increasing the length of hospital stay, cost of treatment, morbidity and mortality [1,8].

The catheterization period is associated with the clinical context, varies from patient to patient and can go up to several months, in long-term situations. However, it has been reported that most catheters have to be removed earlier than expected due to the occurrence of bacterial colonization that cause catheter obstruction and medical complications [7]. The longer the catheter remains in the patient, the more likely it is to cause this type of infections: the risk of infection is 1–2% for a single day catheterization, with this percentage increasing by approximately 10% each additional day in women, and approximately 3–4% in men [9]. Although necessary and extremely useful in treatment and recovery of patients, urinary catheters weaken the barriers that prevent the colonization of bacteria naturally existent in the urinary tract, leading to biofilm formation. Thus, catheterized patients are more susceptible to pathogenic bacteria, increasing their predisposition to acquire UTIs [10].

The percutaneous nephrostomy catheters are a specific type of catheters that are inserted directly into the kidneys and are indicated for urinary tract obstructions [11]. The occurrence of infections in these situations (with incidence up to 19%) is even more serious due to the risk of pyelonephritis, kidney damage and loss of renal function [12].

3. Relevance of earlier and accurate UTI diagnosis

In the context of UTIs, antibiotics administration is the most effective way to treat advanced infections. However, they are consistently used in unnecessary situations and often misused as preventive tools. Consequently, its overuse has increased the prevalence of resistant pathogens, a major concern of the World Health Organization, which is working on a global action plan to tackle this problem [13].

Despite the existence of international guidelines for antibiotic prescription, a US study has revealed that their misuse is quite common, representing about 30% of all prescriptions in primary care [2,14]. Another study by Durkin et al. reported that in most antibiotic prescriptions for uncomplicated UTIs, treatment agents not listed in the guidelines are being used [15]. They also concluded that, in many cases, the duration of treatment is longer than recommended. Therefore, clinical practice guidelines are not having the proper impact or being clinically relevant in hospitals procedures.

As a result, in the past years, resistance to fosfomycin, ampicillin, fluoroquinolones, trimethoprim-sulfamethoxazole, and other broad-spectrum antibiotics used in this type of treatment, has increased substantially in outpatients, leading to decreased efficacy when they are administered [2,16,17].

Common symptoms of UTIs include severe back pain; inflammation or burning sensation while urinating; cloudy, dark, bloody or bad-smelling urine; and fever, which can ultimately result in pyelonephritis [3]. However, when these symptoms are detected, it is no longer possible to prevent the infection and the only option is to proceed with the treatment, which can be more or less aggressive, depending on the severity of the infection. This fact, allied with the development of bacterial resistance as consequence of the exposure to antibiotics, are extremely important to understand the need to detect UTIs at an early stage. This way, prophylactic measures can be taken in a timely manner, preventing the progression of infections and reducing the antibiotic administration.

4. Classical and modern methods for UTI diagnosis

The UTI diagnosis is usually supported by systemic or localized symptoms, along with a positive urine culture demonstrating numbers of uropathogens above a given threshold (bacteriuria), although, in some cases, symptoms and bacteriuria can occur independently [18,19]. UTIs are caused by a high concentration of specific bacteria, and symptomatic patients generally present values $\geq 10^5$ of colony-forming units (CFU) of bacteria per mL in their urine samples [20].

The diagnostic methods currently available to detect pathogens in urine fall into two categories: laboratory methods that require prior sample processing, and the point-of-care (POC) tools that perform an on-site direct analysis (Fig. 1).

4.1. Laboratory detection methods

One of the most commonly used methods to detect an UTI is the urine examination by collecting a midstream urine sample or catheter specimens and subsequent microbiological evaluation (urine culture) for pathogen isolation, identification and antimicrobial susceptibility testing (AST) [3]. This type of diagnosis usually takes 2–3 days, delaying treatment and allowing the infectious disease to spread. Due to this drawback, empirical decisions are commonly made and unnecessary antibiotics are prescribed [6,21].

Separation and filtration techniques (chemical, physical and antibody-based techniques) can be used to minimize the testing period, helping to recover and concentrate bacteria. However, these methods are not ideal due to the use of aggressive chemical reagents that can cause cell damage and interfere with the surface properties of microorganisms during their recovery and concentration [22].

There are also different immunoassays based on the specific interaction between an antigen and an antibody in order to measure its concentration in a solution. The Enzyme-Linked Immunosorbent Assay (ELISA) is a biochemical assay that use antibodies and enzyme-mediated color change to detect the presence of antigens, antibodies and proteins in a sample [23]. This method is used in different diagnoses and is also the base of the urinary Uristal test (Shield Diagnostics Ltd.) to detect antibodies specific for antigens of common pathogens [23,24]. However, ELISA assays have been reported as not sufficiently sensitive and specific to be used in urine routine diagnoses [24,25].

New developed approaches such as polymerase chain reaction (PCR), fluorescence in situ hybridization (FISH), matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) and forward light scattering have been used in UTIs diagnosis [26–31]. These techniques exhibit higher sensitivity and specificity than culture-based assays and represent a significative reduction in detection time. They can also be combined, allowing an accurate identification and precise evaluation of the pathogen's antibiotic susceptibility. However, these procedures require a clinical microbiology laboratory, expensive reagents and equipment, as well as highly trained technicians to perform the tests [32,33].

Table 1 summarizes some methods available to perform urine analysis in a hospital environment, as well as their features, advantages and limitations.

4.2. Point-of-care detection methods

POC diagnostic tools have been identified as a promising way to overcome the disadvantages of the previously mentioned methods. In this type of procedure, it is possible to perform an on-site analysis, without requiring transport of sample to laboratory, time-consuming sample processing and/or specialized equipment [32,33]. POC have the potential of improving patients' care, since they significantly reduce the time needed to obtain results and, consequently, lead to faster and

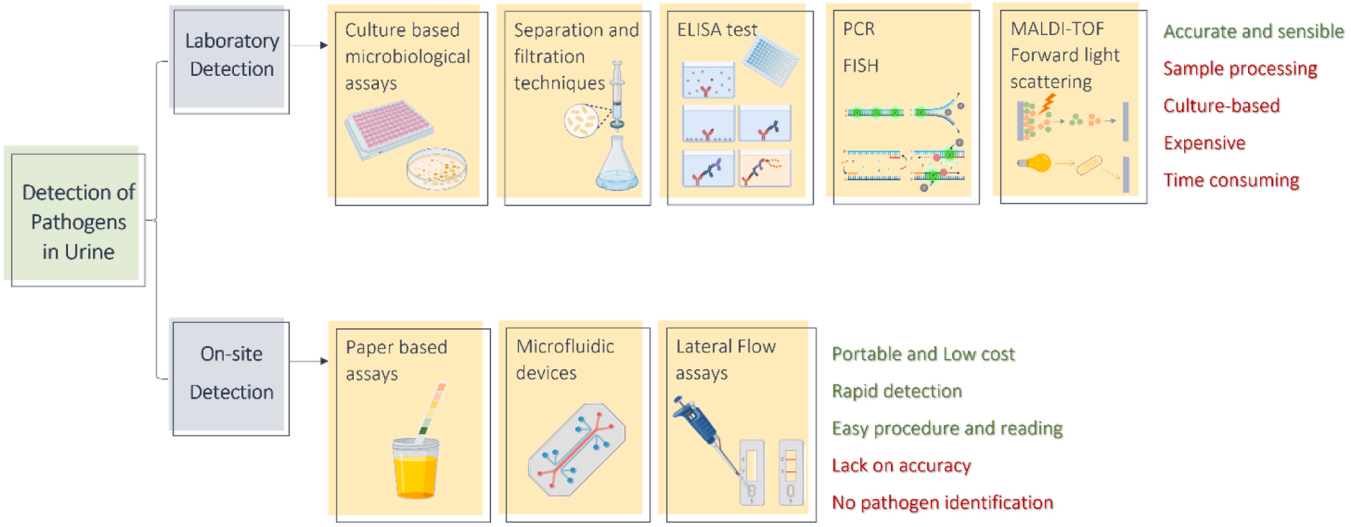

Fig. 1. Detection of pathogens: Laboratory and on-site detection methods, advantages and limitations.

Table 1
Methods for UTI diagnosis. Advantages and disadvantages based on method principle and ability to test for associated antimicrobial sensitivity (AST).

Detection method	Operating principle	Analysis time	AST	Advantages	Disadvantages	Ref.
Culture	Urine culture on agar plates for growth, concentration, identification and isolation of pathogens	2–3 days	Yes	Sensitive, inexpensive, gold standard for UTI diagnosis	Time consuming; limited to clinical laboratories	[21]
Microscopic urinalysis	Microscopic examination of centrifuged urine to observe bacteria, parasites and cells	10 min	No	Fast	Poor sensitivity and specificity; limited to clinical laboratories	[34,35]
Uristal ELISA test	Indirect qualitative colorimetric detection of pathogens based on specific antigen-antibody combinations	2–3 h	No	Fast	Extensive processing, insufficient sensitivity and specificity	[23–25]
PCR	Amplification of specific genes (known to be specific from certain bacteria) from the total genomic DNA extracted from urine samples	5–6 h	Resistance gene probes available	Sensitive, specific	Requires specific probes for all pathogens; extensive initial processing; lack on quantitative data	[21,26]
MALDI-TOF	Charged molecules are created by ionization, separated based on the mass/charge ratio and detected and measured using the TOF mass analyzer.	1–3 h	Developing	Fast, sensitive, specific	Expensive; difficult samples preparation; complex interpretation; limited to clinical laboratories	[28,29]
FISH	Microscopic detection of microorganisms using fluorescently labeled nucleic acid probes hybridized to complementary targets	20 min	Developing	Fast, sensitive, specific	Requires specific probes for all pathogens	[27]
Forward light scattering	Detection of bacterial growth based on changes in forward light scattering over time	90 min	Developing	Inexpensive, small amount of sample	No pathogen identification	[30]

more informed responses [21]. However, POC methods commercially available, such as the dipstick testing strips and lateral flow assays, lack diagnostic accuracy (limited quantitative precision and detection range) and pathogen identification and are mostly used in routine qualitative analysis [6,31,36].

Several companies are also developing new products that combine the readiness of POC whole-urine samples analysis with the accuracy of the techniques that require time-consuming processing to identify the uropathogen antibiotic susceptibility [6]. Roche is currently testing SeptiFast®, a multiplex real-time PCR test already approved for identification of pathogens in blood, to be used on whole-urine samples [37]. FilmArray, developed by bioMérieux, is an integrated PCR platform that identifies the pathogen and its antibiotic susceptibility in 1 h, using a whole-sample, and is feasible to adapt to UTIs detection [38,39]. Cepheid uses a multiplex-PCR (GeneXpert) for a simple and quick identification of pathogens in blood samples and can also be adapted to whole-urine samples [6,40]. Although very promising, these devices are only able to give a qualitative result but not the pathogens' concentration, which is a critical parameter to define the best treatment. Integrated biosensor-microfluidic platforms have also great potential as they promote direct urine analysis, covering all stages of the test in a compact device [6]. However, in most cases, microfluidic technology is still expensive and requires a power source to drive de sample in the microchannels and a detector to get the results of the procedure [41].

Moreover, some innovative and non-invasive devices capable of performing POC UTI tests in an easy and fast way are now commercially available. However, some of these POC tests are culture-based and still take 16–24 h to reveal any results. Even so, they can be advantageous, as they represent a more economical option and are able to give information such as antibiotic resistance, identification of the pathogen or even its concentration in the sample (examples in Table 2). In addition, some of these tests can be easily performed in the comfort of the user's home, facilitating the entire process that usually requires scheduling an appointment, performing the analysis and communicating the results. Table 2 describes some examples of these single-use tests and their detection mechanism.

5. Promising approaches to point-of-care detection of pathogens

In addition to the traditional methods and the aforementioned

Table 2
Point-of-care innovative devices available in the market for UTI diagnosis.

Device/ Manufacturer	Operating principle	Analysis time	Ref.
Dip UTI (Healthy.io, England)	Dip UTI test is composed by a dipstick, a urine collection cup and a color-board. An app with a virtual nurse guides the user through the test. The test measures the number of leukocytes, nitrites and blood in urine by color change in the test strip.	3 min	[42]
Flexicult™ (SSI Diagnostica, Denmark)	Culture based approach using an agar plate divided into six segments: one with culture medium alone and the others with different antibiotics commonly used in UTI treatment. The sample of urine is placed in the plate and after 24 h incubation the results of bacterial growth and antibiotic resistance can be analyzed by a nurse/clinician.	24 h	[43, 44]
Uricult® Trio (Orion Diagnostica, Finland)	Uricult® Trio is a dip-slid method that detects bacteria and yeast in urine samples. The test contains three different media: CLED, MacConkey and *E. coli* agar. CLED medium is non-selective where all UTI bacteria and yeasts grow. MacConkey inhibits gram-positive bacteria, and the *E. coli* selective medium specificity detects gram-negative, β-glucuronidase-producing organisms (as *E. coli*).	16–24 h	[45]
DipStreak®/ ChromoStreak (Novamed, Israel)	DipStreak® urine culture device is a semi-quantitative screening method for inoculating and transporting urine sample, also detecting and identifying specific bacteria in urine. The detection is performed after incubation (in chromogenic agar UTI selective and MacConkey agar) by comparison with the Colony Density Chart to determine the CFU/mL in the sample.	18–24 h	[46]
DiaSlide® (Novamed, Israel)	DiaSlide® urine culture device is similar to DipStreak® containing two types of media: CLED and MacConkey agar or UTI selective and MacConkey agar, allowing the differentiation of enteric microorganisms by color changes.	24 h	[46]
TestCard UTI Test Kit (TestCard Ltd, UK)	This urine self-test kit uses test strips and a smartphone app to deliver a rapid and accurate result on the existence of a UTI. The dipstick is immersed in a urine sample, and, with a smartphone camera, the user is able to scan the colorimetric results (leukocytes, pH and nitrates), which can later be shared with a healthcare professional.	2 min	[47]
UriScreen™ (Savyon® Diagnostics Ltd, Israel)	UriScreen is a rapid and sensitive screening test used to detect bacteriuria and somatic cells in urine. The sample is first mixed with the test reagent powder in the test tube, enabling catalase detection. Then a small amount of hydrogen peroxide solution (provided in a 10 mL dropper bottle) is added. The formation of foam indicates a positive result for UTI.	2 min	[48]
Uroshield (Ideal Medical Solutions Ltd., UK)	UroShield is an external medical device that uses an ultrasound nanotechnology to prevent CAUTI in long-term catheterized patients. The ultrasonic waves reduce the formation of bacterial biofilm, reducing catheter encrustations and blockages and the use of antibiotics.	Not applicable	[49]

recently developed technologies, it is also important to highlight the improvements and new approaches that have emerged in this research field, with potential to be implemented in the future. Still within the scope of POC methods and to achieve rapid and selective detection of pathogenic bacteria without requiring advanced equipment or skilled personnel, promising strategies have been reported in the literature in recent years. These technologies rely, in most cases, on substrates that provide a naked-eye visible sign, easy to detect and suitable to be used as POC diagnostic tools. In this section, some different bacterial detection mechanisms reported by researchers, including hydrogels, lateral flow assays, stack pad assays and paper-based tests, will be summarized. These methods have mainly been applied in food and water analysis, but are also suitable to be considered for urine analysis.

5.1. Paper-based tests

Paper matrixes are very promising approaches to bacteria detection devices. They are light weighted, inexpensive and have interesting properties such as porosity and hydrophilicity, allowing absorption and transport of liquids through capillary forces [50]. Other advantages are their biocompatibility, high surface area, flexibility and easy functionalization, storage, transportation, and disposal [22]. Whatman papers are the most used matrices in these paper-based test devices (Fig. 2).

Shih et al., used Whatman Fusion 5™ hydrophilic paper to prepare a paper-based test to rapidly detect *E. coli* DH-5α [16]. In this device, colorimetric ELISA reactions were tested by placing samples from 10^5 to 10^9 CFU/mL on the paper surface (Fig. 2-A), followed by the addition of the 1st antibody (anti-*E. coli* biotin conjugate) to bound with the sample bacteria. After 1 h of blocking, the washing step was carried out, followed by the addition of a secondary antibody (horseradish peroxidase (HRP) conjugated streptavidin). Finally, a colorimetric reaction took place, through the addition of chromogenic reagent 3,3′,5,5′-Tetramethylbenzidine (TMB). To evaluate the performance of this paper-based ELISA, the reactions with samples with different cell concentrations were photographed after 40, 80 and 120 s, and the intensity of the blue color was compared (Fig. 2-A). The paper test proved to detect *E. coli* within 5 h with a limit of detection (LOD) of 10^5 CFU/mL.

Other low-cost, portable and compact three-dimensional paper-based sensor was developed by Kim et al. to be used in foodborne pathogen detection [51]. This test was assembled using wax-printed Whatman filter paper grade 4 and four different chromogenic substrates: 5-bromo-6-chloro-3-indoxyl-β-D-galactopyranoside (Magenta-β-gal), 5-bromo-4-chloro-3-indoxyl-β-D-glucuronide sodium salt (X-GlcA), Aldol® 518 myoinositol-1-phosphate (Aldol-MIP), and 5-bromo-4-chloro-3-indoxyl-β-D-glucopyranoside (X-Glc).

Paper sensors were first produced using the photolithography method, in which the paper was patterned with photoresists by ultraviolet (UV) light curing. Each layer was designed with a 4 mm diameter black circle pattern in a transparent square (150 mm × 150 mm), to selectively permit passage of ultraviolet light (Fig. 2-B-(1)). The reagents (lysis, oxidizing and chromogenic agents) were previously deposited on paper channels (Fig. 2-B-(2)) and each chromogenic substrate reacted with specific bacterial enzymes (β-D-galactosidase, β-glucuronidase,

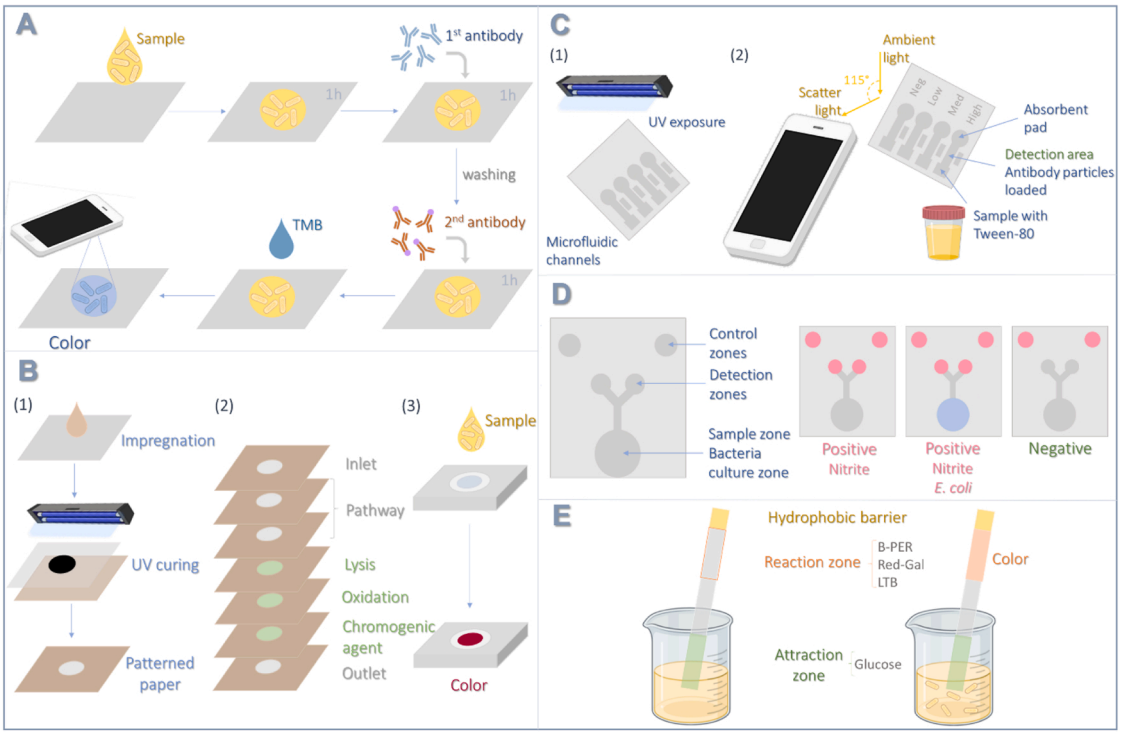

Fig. 2. Paper-based bacteria detection tests. A - Paper-based colorimetric ELISA test; B - 3D paper device from fabrication to color observation: (1) Photolithography, (2) Paper layers assembly, (3) Sample analysis and color change; C - Microfluidic paper analytical device: (1) Pattern of microfluidic channels by UV exposure, (2) Functioning of the device; D - Nitrite and *E. coli* detection of the paper-based device; E - Schematic representation of the functioning of DipTest paper strips.

myoinositol monophosphatase and β-D-glucosidase). Finally, the paper layers were inserted between two plastic holders. To perform the analysis, a drop of sample was placed in the device, flowing through the several layers, and producing a specific color signal: sky blue, burgundy, light pink or strong blue (Fig. 2-B-(3)), resulting from the enzymatic reaction. The multiple reagent layers excluded steps as pre-lysis and pH regulation, reducing analysis time. When combined with enrichment, 10^1 CFU/mL of pathogens were successfully detected within 4–8 h. For milk samples, the analysis took 12 h.

The authors also studied a similar paper-based microbiological diagnostic system able to detect fecal-indicating *E. coli* and highly pathogenic *E. coli* O157:H7, with a specificity of 90% and 10 CFU/mL LOD, in just 4 h [52]. The device's assembly was similar to the previous study, with the improvement of being multifunctional and simultaneously detect two *E. coli* strains. The two chromogenic substrates (6-Chloro-3-indoxyl-β-D-galactopyranoside (Sal-β-gal) and X-GlcA) added to this device allowed two-in-one selective analysis.

Cho and co-workers developed a microfluidic paper analytical device (μPAD) to simultaneously detect *E. coli* and *Neisseria gonorrhoeae* (responsible for causing gonorrhea) in urine samples [53]. The microfluidic channels were patterned on chromatography paper using UV exposure lithography technique. Thus, four channels were obtained: negative control, low detection, middle detection and high detection (Fig. 2-C-(1)). *E. coli* and *N. gonorrhoeae* antibodies conjugated to submicron particles (Ab-particles) were added to the center of each microfluidic paper channel. The urine samples were incubated with 1% Tween®80 for 5 min and then were introduced to the inlet of the microfluidic channel through which they flowed by capillary force. Target antigens in the sample induced immunoagglutination of Ab-particles, causing an increase in light scattering, which was quantified by the angle-specific Mie dispersion under ambient lighting conditions, using a smartphone camera as a detector (Fig. 2-C-(2)). The detection limit obtained for this device was 10 CFU/mL for both *E. coli* and *N. gonorrhoeae*, with a time of analysis inferior to 30 s

Other authors reported a paper analytical device (PAD) based on Whatman no.1 filter paper to grow bacteria in situ and rapidly detect the presence of nitrite on the same device [54]. The PAD developed by Noiphung and Laiwattanapaisal was prepared by creating a hydrophobic area on the paper through wax printing technique [54]. Then, it was combined with a cotton sheet in order to support bacterial culture. To allow a rapid screening of Gram-negative bacteria, the nitrite detection is based on the principle of Griess reaction, wherein nitrite reacts with sulfanilamide, producing a red-pink color (Fig. 2-D). Simultaneously, a biochemical test is also performed on the PAD using the ability of *E. coli* to specifically produce the enzyme β-glucuronidase, responsible for converting the previously immobilized colorless salt 5-bromo-4--chloro-3-indolyl-β-D-glucuronide (X-GlcA), into a blue-colored compound (Fig. 2-D). The device reached a selective detection of *E. coli* with a range of 10^4-10^7 CFU/mL within 6 h.

A μPAD for detection of *Staphylococcus aureus* and *E. coli* and their antibiotic resistant strains was also fabricated on Whatman filter paper and using wax printing [50]. The paper was printed with 7 mm diameter and 0.5 mm thick circles, wrapped in aluminum foil and heated to melt the wax and allow it to pass through the paper and to create a hydrophobic barrier. The impregnation was carried out with solutions of different chromogenic substrates: chlorophenol-β-D-galactopyranoside red (CPRG), p-toluidine salt of 5-bromo-6-chloro-3-indolyl phosphate (BCIP), nitrocefin and chromogenic cephalosporin (HMRZ −86). The detection principle of this test was based on the reaction between enzymes, produced specifically by each bacterium, and the chromogenic agents. This way, different colored compounds are produced, depending on the bacteria present in the sample. Colorimetric responses may be further evaluated visually and using a smartphone camera for semi-quantitative analysis. The LOD of the two bacteria tested in milk samples was 10^6 CFU/mL, with enrichment in selective medium for 12 h. The sensitivity and selectivity of this procedure was 90% and 100%, respectively, when compared to the sophisticated laboratory technique of polymerase chain reaction (PCR).

Gunda et al. reported a method of detecting *E. coli* in water samples using Whatman blotting paper to produce a paper-based test – DipTest

[55]. In this already patented process (WO2018045449A1), *E. coli* cells present in the sample are attracted to the D-glucose adsorbed on paper strips [56]. Then they migrate along with water by capillarity and flow to the other end of the strip until they reach the hydrophobic barrier. There, the cells accumulate and react with the immobilized compounds: the enzyme extracting reagent and Red-Gal chromogenic agent, responsible for the detection through the color change (Fig. 2-E). The β-galactosidase enzyme of *E. coli* hydrolyzes the chromogenic reagent and produces a pinkish red color. The LOD was 2×10^5 CFU/mL for a 75 min analysis and 200 CFU/mL for a > 180 min analysis. The patent also mentions other possible uses of the paper strip, for instance, as a method of treating contaminated water, with a neutralization zone containing the cationic protein Moringa oleifera (MOCP), responsible for killing *E. coli*.

Table 3 summarizes the referred paper-based studies and some other examples reported in the literature.

5.2. Lateral flow assays

The lateral flow assays (LFA) are the most common POC tests and are based on the migration of a liquid sample through different membranes placed in a backing card: sample pad (usually made of cellulose acetate or glass fiber), conjugate pad, reaction membrane (usually made of nitrocellulose (NC)), and absorption pad (Fig. 3(A)) [62,63]. When a sample is dropped in the sample pad, it flows by capillary force to the conjugation pad, where labeled biorecognition elements bond to the analyte [62–64]. Then, depending on the presence or absence of the target pathogen, the control and test lines composed by biorecognition elements fixed on the reaction membrane, produce a detectable signal. The absorption pad is responsible for stopping the flow of the remaining fluid [62]. The label elements used in this type of devices to obtain direct or indirect detectable signals can be gold nanoparticles (GNPs), polymeric latex, selenium nanoparticles, silver nanoparticles, magnetic particles, quantum dots (QD), carbon-based materials, electrochemical active tags, fluorescent and luminescent materials, textile dyes, liposomes, enzymes amongst others [63–65].

LFA tests can be direct or competitive: in the direct type, the assays are in double-antibody sandwich format and the result observed in the test line is directly proportional to the concentration of the analyte. In competitive assays, the inhibitive antibody bounds to the analyte, and the response in the test line is inversely proportional to the analyte concentration (Fig. 3(B)) [36]. Although being generally used for single target detection, LFA tests can also be used in multiplex format, enabling the detection of several targets simultaneously [63].

This type of tests is already used in urine analysis such as in the case of pregnancy tests, or in food and water analysis. However, there are some limitations regarding their sensitivity. Recent efforts have been made to improve LFAs and make them more sensitive and reliable [66]. In this section, some examples of LFA for the detection of *E. coli* recently developed and reported by researchers will be presented.

A rapid, sensitive and real-time LFA test for visual detection of *E. coli* O157-H7 was developed by Jiang and co-workers [67]. This device relied on the amplification of peroxidase like activity of Pt-Au bimetal nanoparticles (NPs) that catalyzed the oxidation with the chromogenic reagent TMB, leading to the production of blue-colored bands that allow *E. coli* detection. The system was designed as a sandwich immunoreaction device composed by a sample pad, a conjugation pad, an absorption pad, and a NC membrane. Once the analyte sample was added to the sample pad, it began to flow and react with the captured antibodies (Ab1) covalent bounded with Pt-Au NPs (Pt-Au-Ab1), forming a complex at the conjugation pad. This complex migrated through the NC membrane and reached the test area, where the detection antibodies (Ab2) were found. After the addition of TMB to the test line, the blue color sign could be observed.

Song and co-workers also developed a simple and economical LFA for pathogen detection in food samples, using fluorescein isothiocyanate (FITC) labeled antigen and antibody for dual FITC-LFIA [68]. The device had a similar sandwich assembly to other systems previously described. In this system, the FITC was mixed with the sample culture medium which, after incubation in the presence of *E. coli* O157:H7, causes them to emit a yellow-green fluorescence, creating a fluorescent antigen probe. This antigen probe was added to LFIA containing fluorescent *E. coli* O157:H7 monoclonal antibodies (McAb-FITC) in the conjugate pad. Other *E. coli* O157:H7 McAb constituted the test line, and the goat anti-mouse IgG antibody (IgGAb) was placed on the NC membrane as a control line. For a positive result, both test line and control line should be visible. The detection results could then be observed in a black box equipped with UV light and an optical filter and recorded with a digital camera. In addition to the naked eye observation, the fluorescence intensity of the test line could be assessed using a semi-quantitative analysis with a scanning reader. This strip method showed an LOD of 10^5 CFU/mL and 10^4 CFU/mL for a qualitative (naked-eye observation) and semi-quantitative detection, respectively.

Another study compared the sensitivity of LFA immunoassays based on colloidal palladium nanoparticles (PdNPs) with the one of a conventional colloidal gold nanoparticles (AuNPs) in milk samples [69]. In this system, the sample pad contained antibodies labeled with colloidal PdNPs. When the sample was added, they were responsible for forming a complex that then migrated to the test area, where the colored line appeared. Palladium catalyzes the oxidation of TMB and 3,3'-diaminobenzidine (DAB) substrates that are also recognized by the enzyme HRP. The oxidation of DAB forms a dark brown precipitate and the

Table 3
Paper-based POC tests for bacteria detection.

Analyte	Source	Revealing agent	Reading Signal	LOD [CFU/mL]	Ref.
E. coli DH-5α	–	TMB	Color	10^5	[16]
E. coli, E. coli O157:H7, *L. monocytogenes* and *V. vulnificus*	Milk	Magenta-β-gal, X-GlcA, Aldol-MIP, X-Glc	Color	10^1	[51]
E. coli O157:H7	Milk	Sal-β-gal and X-GlcA	Color	10	[52]
E. coli O157:H7 and *N. gonorrhoeae*	Urine	–	Light scattering	10	[53]
E. coli O157:H7 and Nitrite	Urine	Sulfanilamide and X-GlcA	Color	10^4-10^7	[54]
E. coli	Water	Red-Gal	Color	200 – 2×10^5	[55]
E. coli O157:H7, *Salmonella* Typhimurium, and *L. monocytogenes*	Food	CPRG, X-InP[a] and Magenta caprylate	Color	10^1	[57]
E. coli BL21, *E. coli* O157:H7, *Bacillus subtilis* and *Salmonella enterica*	Food and beverages	X-GlcA and CPRG	Color	5–20	[58]
E. coli and *S. aureus*	Milk	BCIP, CPRG, Nitrocefin and HMRZ-86	Color	10^6	[50]
E. coli O157:H7	Vegetables	TMB	Color	10^4	[59]
E. coli O157:H7	Food	TMB	Color	30.8	[60]
E. coli, S. aureus, Enterococcus faecalis, Streptococcus mutans and Salmonella pullorum	Ascites samples from mice	GOX catalyzed reaction and glucose metabolism	Color	10^4	[61]

[a] X-InP- 5-bromo-4-chloro-3- indolyl-myo-inositol phosphate; Magenta caprylate- 5-bromo-6-chloro-3- indolyl caprylate; GOX- Glucose oxidase.

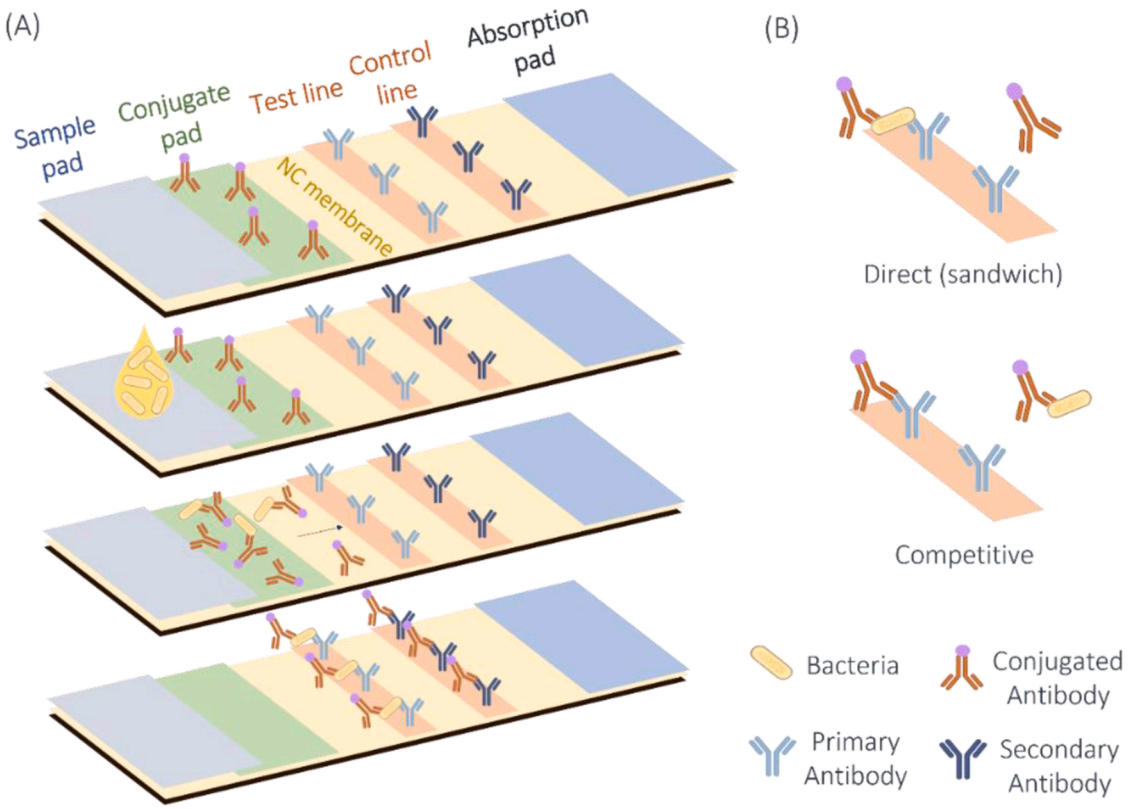

Fig. 3. Lateral Flow Assay: (A) Basic structure and functioning of a direct LFA; (B) Direct and competitive types of LFA.

aggregation of colloidal PdNPs results in a black precipitate. These two different mechanisms of formation of colored complexes increase the sensitivity of the test, when compared to those of AuNPs. Unlike what was observed for AuNPs, the oxidation of TMB in PdNPs assays developed by Tominaga and colleagues, demonstrated to be time and concentration dependent. The sensitivity of the tests performed with PdNPs-HRP LFTS resulted in a detection 5–10-fold higher than that of AuNPs based assays.

Liu et al., developed a label-free bidirectional LFA based on antigen-antibody reaction and "lac dye" coloration, without resorting to the use of nanomaterials and double antibody sandwiches, and reducing the difficulty and cost of the test [70]. Lac dye is a natural dye extracted from lac insect secretions and was firstly found by this research group to have the ability to dye *E. coli* O157:H7. In this work, the authors reported the preparation of a LFA based on one sample pad, one conjugation pad and two absorption pads, as shown in Fig. 4. The main difference between the bidirectional LFA and traditional lateral flow immunoassay strip is that the sample pad is placed in the middle, dividing the control and test zones. To form the control line (C) and test line (T), anti-*E. coli* O157:H7 McAb were dispersed on the NC membranes. On the conjugation pad, placed in the control side of the bidirectional LFA, pre-stained *E. coli* O157:H7 were assembled. The results could be detected on the test and control lines few minutes after dropping a sample solution (after 12 h culture and addition of lac dye) in the sample pad. When a sample containing *E. coli* is dropped in the sample pad, the bacteria migrate to the NC membrane, being captured by the antibodies on the test line. *E. coli* cells previously placed in the conjugation pad also migrate to the control line. For a test result to be considered positive, the C and T black lines should appear. If only the C line is present, the test result is negative (Fig. 4). Successful and rapid results were obtained in pre-incubated samples of milk, bread, and jelly, with a LOD of 10^6 CFU/mL.

Table 4 summarizes the LFA studies mentioned above, among other examples of this type of devices recently reported in the literature. All

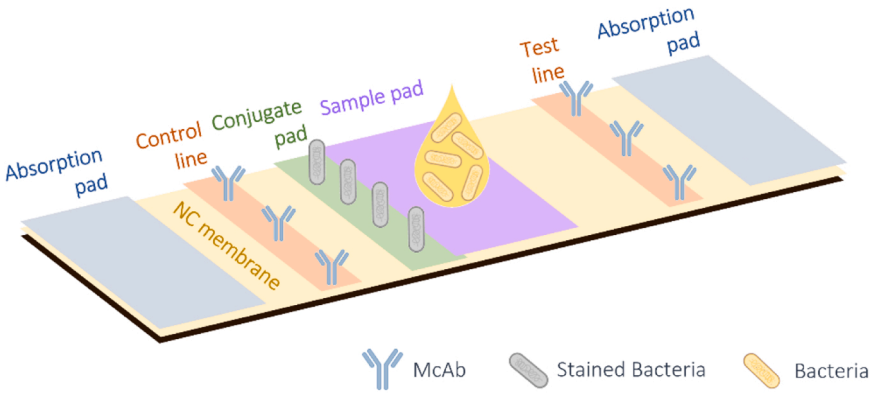

Fig. 4. Schematic representation of bidirectional lateral flow immunoassay strip device.

Table 4
Original lateral flow assays and the latest newly reported.

Analyte	Source	Label element/ Revealing agent	Reading Signal	LOD [CFU/mL]	Ref.
E. coli O157:H7	–	Pt-Au NPs / TMB	Color	10^2	[67]
E. coli O157:H7	Milk	Pd-Pt NPs / TMB	Color	10^2	[71]
E. coli O157:H7 and *Salmonella Enteritidis*	Food	Pt-Pd NPs / TMB	Color	20–34	[72]
E. coli O157:H7	Food	FITC	Fluorescence	10^4-10^5	[68]
E. coli O157:H7, *L. monocytogenes* and *Y. enterocolitica*	Milk	AuNPs, PdNPs /TMB, DAB	Color	10^6-10^7	[69]
E. coli O157:H7	Food	- / Lac dye	Color	10^6	[70]
E. coli O157:H7	Milk	GNP, QD, FNP and EuNP	Color, Absorbance, Fluorescence	2.5×10^4, 5×10^3, 10^3, and 5×10^2	[73]
E. coli O157:H7 and Shiga Toxins/ Food	Food	CGNPs	Color	10^5	[74]
E. coli O157:H7	Milk and water	GO, rGO	Color	10^5-10^6	[65]
E. coli O157:H7, *Staphylococcus aureus, Salmonella Typhimurium, and Bacillus cereus*	Fresh lettuce	AuNPs	Color	10^5-10^6	[75]
E. coli O157:H7	Juice	GNPs /BPEI-loaded liposomes	Color	600	[76]
E. coli O157:H7	Milk	FMNBs	Fluorescence	2.4×10^2-2.5×10^3	[77]
E. coli O157:H7	Fecal samples	CG	Color	10^4	[78]
E. coli O157:H7	Milk	rGO	Color, photothermal effect	5×10^5	[79]
E. coli O157:H7	Milk	CdTe/CdS QDs	Fluorescence	10^4	[80]
E. coli O156:H7	Milk	MNPs	Color	6.4×10^4	[81]
Klebsiella group	Food	PdNPs	Color	10^4	[82]

*FNP- Fluorescent nanoparticles; EuNP- Europium (III) chelate nanoparticles; CGNPs- Colloidal gold nanoparticles; GO- Graphene oxide; rGO- Reduced graphene oxide; BPEI- Branched polyethylenimine; FMNBs- Fluorescent magnetic nanobeads; CG- Colloidal gold; CdTe/Cds QD- CdTe/CdS core shell quantum dots; MNPs- Carboxyl group coated Fe_3O_4 magnetic nanoparticles.

examples use antibodies as biorecognition element.

5.3. Stack pad assays

Stack pad assays are a new concept of POC device, similar to the lateral flow immunoassays. The main difference in these tests is the fact that the membranes are stacked onto each other instead of in the common lateral setup [33,83].

Eltzov and Marks developed a colorimetric stack pad immunoassay for bacterial identification (Fig. 5), where the liquid sample diffuses by capillarity from a series of different membranes. Between each two active layers, separation pads were added to direct the flow to the center of the pads, giving space and time for immunoreactions to occur [33].

The liquid containing the analyte is added to the bottom layer (sample pad) further starting migration to the next layer (conjugation pad) where the analyte bounds with anti-analyte antibodies conjugated with HRP. Then, the analyte/antibody complex diffuses to the blocking layer composed by a modified NC membrane with immobilized DH5-α

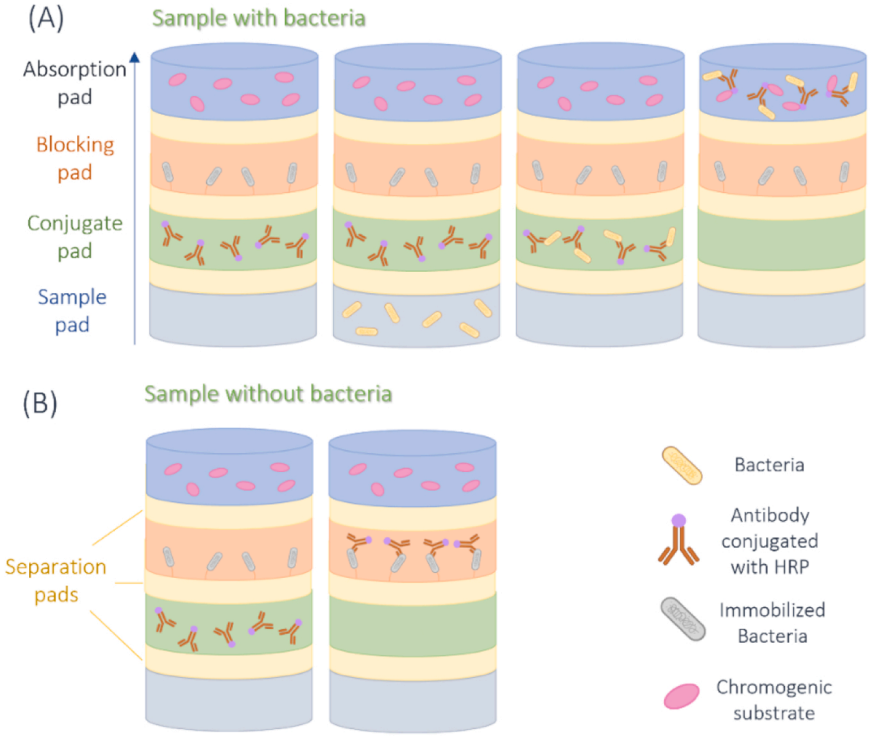

Fig. 5. Illustration of the device assembly and operation in the presence of a contaminated and uncontaminated sample.

E. coli DH5-α (chemically competent cells engineered by Douglas Hanahan). Here free/unbound antibodies are conjugated, interrupting their migration to the next layers. The bacteria conjugated to the antibodies do not interact with the immobilized bacteria, being free to flow to the upper layer. In this top layer (absorption pad), the HRP bounded to the antibodies will react with the enzymatic substrate (TMB) and produce a colorimetric signal for positive detection. This way, the color change will be observed only when target bacteria are present. After the optimization, with an assembly containing a sample pad, a conjugation pad, six blocking layers and an absorption pad, this technology allowed an affordable, portable and simple measurement in less than 5 min, with a sensitivity of 10^2 cells/mL, 1000-folds higher than ELISA test.

Recently, the same research group developed another colorimetric antibody-based stack pad system composed of multiple membranes with an assembly similar to the previous study: a sample pad, a conjugation pad (with anti-*E. coli* antibodies conjugated with HRP), six *E. coli* strain DH5α bacteria-blocking NC layers (blocking pad), an absorption pad with dry substrate and separation pads between the active layers [83]. In this system the sample migrates from the upper to the bottom layer and the blocking layer efficiency is enhanced by using several hydrophobic modified target-capturing NC layers. This way, they prevent the unbound HRP-conjugated target antibodies from flowing to the substrate layer, allowing the color observation only when target bacteria are present in the sample. Assembly, using 6 blocking layers, produced the best results. This simple, rapid and low-cost diagnostic device was tested using different milk samples (with and without *E. coli* DH5α) and it was found to be more sensitive than standard ELISA protocol up to 1000-fold.

5.4. Hydrogels

Hydrogels are hydrophilic polymers with properties such as biocompatibility and permeability and widely used in biomedical applications as sensors, drug delivery systems and tissue engineering scaffolds. These polymers are able to form 3D networks with high water sorption capacity and can be designed to be sensitive to pH, temperature and light [84].

One of the most reported type of hydrogels to be used as matrix in bacteria detection devices are chitosan-based hydrogels. Chitosan is a well-established biodegradable and biocompatible polysaccharide with wound-healing activity, widely used in numerous tissue engineering applications and identification systems [85].

A chitosan hydrogel functionalized to detect in situ the presence of *E. coli*, in a rapid and selective way, was produced by Ebrahimi et al. [85]. In this work, chitosan hydrogel films were functionalized by covalent immobilization of the fluorogenic substrate 4-methylumbelliferyl-β-D-glucuronide (MUG) and the chromogenic substrate 4-nitrophenyl-β-D-glucuronide (PNPG), via amide bond formation. In the presence of β-glucuronidase (β-GLU, enzyme secreted by >98% of *E. coli* strains), the fluorogenic substrate MUG suffered hydrolysis and the coumarin derivative 4-methylumbelliferone (4-MU) was cleaved from the glucuronide unit. Due to the deprotonation in the hydrogel matrix, its fluorescence emission changed. The chromogenic substrate PNPG, also susceptible to enzymatic cleavage when in contact with β-GLU, produced a detectable color signal (from colorless to yellow), as a result of 4-nitrophenol (4-NP) deprotonation. The described approach is summarized in Fig. 6. The enzymatic reaction was studied for both types of substrates using pure enzyme solution and bacterial supernatant. These studies revealed the apparent reaction kinetics, allowing to determine the concentration of β-GLU in the supernatant and the limit of detection. Under optimized conditions, the MUG and PNPG functionalized hydrogels reported the presence of β-GLU within 15 min with a limit of detection of < 1 nM and 40 nM, respectively. Also, the study revealed that the covalent immobilization to the hydrogels did not hinder the enzymatic reaction.

Ebrahimi and research group also developed a portable optical fiber setup with a self-reporting chitosan hydrogel as a coating on the glass fiber tip for *E. coli* detection [86]. The setup (Fig. 7) consisted of a fiberglass lighting with a tungsten halogen lamp as a light source, a single-mode detection fiber and a mini spectrometer. Spectra Suite software was used to analyze the recorded data and the detection was performed by a thin film of chitosan hydrogel coating the tip of the detection fiber. In this study, chitosan was functionalized (via amidation) with the chromogenic substrate 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (X-GlcA). The

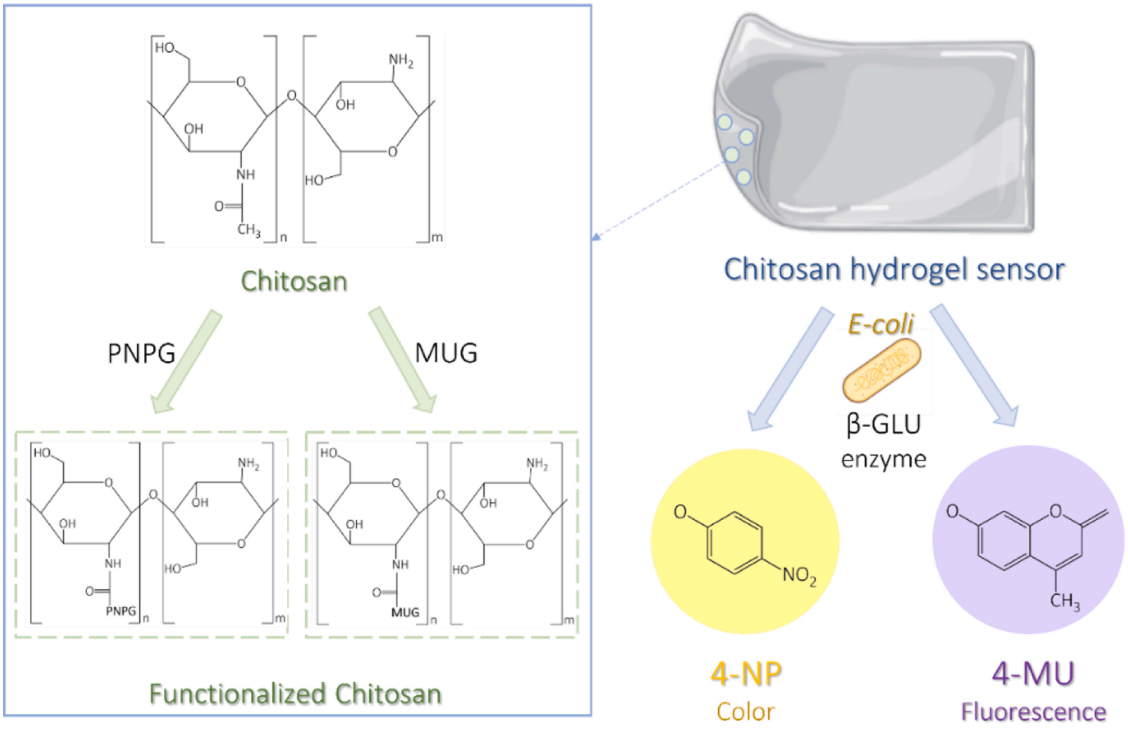

Fig. 6. Schematic representation of chitosan hydrogel functionalization and enzymatic reaction.

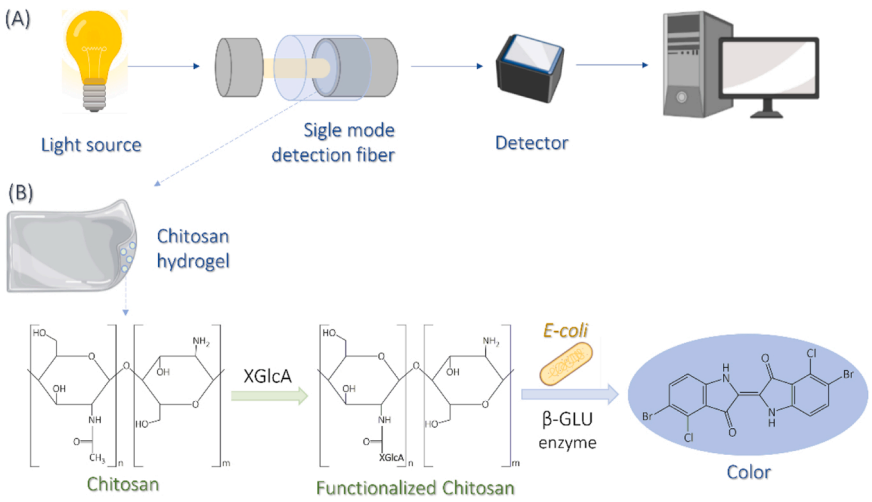

Fig. 7. (A) Schematic representation of the optical fiber with functionalized chitosan hydrogel coating for the remote detection of *E. coli*; (B) Chitosan functionalization and selective enzymatic reaction.

substrate reaction with the β-GLU enzyme promoted the release of a non-leaching dimerized indigo dye, with an intense and easily detectable blue color (Fig. 7 (B)). Under optimized conditions, the sensor detected the presence of β-GLU with a detection limit of < 40 nM within 5 min.

Two similar studies from Jia et al. also reported chitosan functionalized hydrogels for bacteria detection [87]. In the first study the matrixes were functionalized with three different agents: PNPG, MUG and X-GlcA for β-GLU *E. coli* enzyme detection. In less than 80 min, the color changes were detectable by naked eye, leading to a reliable sign and minimizing false positives. Also, this strategy is feasible to be used in multiplex bacteria detection or to distinguish pathogenic from non-pathogenic bacteria. This approach was further developed in a second study using different chromogenic agents (X-GlcA and X-Gal) to detect β-GLU enzyme from *E. coli* K12 and β-GAL from pathogenic enterohemorrhagic *E. coli* O157:H7 [88].

A chitosan-based sensing hydrogel-coated paper was developed to perform a quantitative detection of *E. coli* using a smartphone camera [89]. The chitosan hydrogel functionalized with a chromogenic substrate was deposited in paper substrates, filling the micropores of the matrix. The chromogenic agent X-GlcA was responsible for developing a blue color when β-GUS enzyme from bacteria contacted with the coated paper. This method, studied by Kaur and co-workers, also allows the quantification using smartphone camera photographs to perform the analysis based on color intensity. The limit of detection using a *E. coli* suspension was 5.8×10^7 CFU/mL within 6 h.

Gunda et al. formulated another hydrogel-based matrix for a rapid detection of *E. coli.* The chemical compounds were encapsulated in an agarose hydrogel, and a plunger-tube assembly containing the matrix was used posteriorly to perform the analysis [90]. While preparing the hydrogel matrix, the chemical reagents (Bacteria Protein Extraction Reagent, nutrient medium Lauryl Tryptose Broth and the chromogenic agent 6-Chloro-3-indolyl-β-D-galactopyranoside, Red-Gal) were added. The dried hydrogel was then placed in a plunger-tube assembly with a built-in filter. This way, *E. coli* in contaminated water concentrated in the headspace between the bottom of the tube and the filter contacting with the hydrogel impregnated with the color change compounds (Fig. 8). This POC device allowed a detection of 4×10^6 to 4×10^5 CFU/mL in only 5 min and 4×10^4 to 400 CFU/mL in 60 min.

All POC tests mentioned in this section represent a significant evolution in the detection of pathogens field. They can be very helpful tools in water and food analysis and can also be adapted for urinary infection diagnosis and assist in the rapid identification of uropathogens. Comparing the different POC strategies mentioned, it is possible to find some advantages and disadvantages. Stack pad and lateral flow based

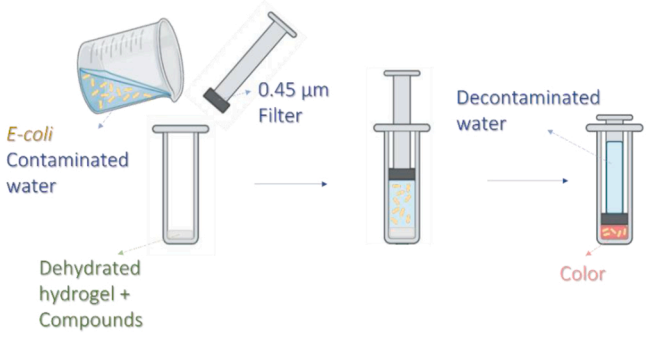

Fig. 8. Detection of *E. coli* in contaminated eater using the plunger-tube device.

methods are promising approaches, achieving results in minutes with LOD up to 10^2 and 20 CFU/mL, respectively. However, these tests have a complex preparation, rely on antibodies assays and, in some cases, require sample preparation prior to testing and/or additional equipment to detect/analyze the results. As for chitosan hydrogels, they have the advantage of being functionalizable with different chromogenic agents, being able to distinguish different bacteria in the same test and revealing results in just a few minutes. However, the methods that stand out are those based on paper matrices, in particular the DipTest. Amongst the cited examples, this is possibly the cheapest and simplest method (both in preparation and in use) and can be more easily implemented commercially. It allows a slightly slower detection (75–180 min) than some of the tests mentioned, but is able to detect up to 200 CFU/mL, presenting a good compromise between simplicity, cost and effectiveness and being able to distinguish between different bacteria with just one test.

However, as well as the home test kits presented in Table 2 (Section 4.2), despite the POC test that we consider, this type of analysis will always be single-use and will only be performed when there is evidence of a possible UTI.

6. Conclusion

The crescent need for faster and more reliable techniques for pathogenic bacteria detection, namely in UTI diagnosis has driven the search for new alternatives. However, there is still a way to go in order to find solutions that can replace the traditional diagnostic procedures. The early detection of a UTI is a key factor in infection control and spread.

The fact that all procedures and devices available or under development are dependent on patient's symptomatology to trigger the need to perform an analysis, compromises that ideal early-stage detection.

The compilation of different type of methodologies and devices here presented, and the alignment of corresponding advantages and disadvantages exposed some needs that are still missing to cover. Based on that, new technologies that allow to monitor and predict an UTI before the CFU achieve infection levels, should be the next generation of diagnostic tools. This way, it should be possible to be continuously informed about the bacteria concentration level in patient's urine and take measures in a timely manner, detecting asymptomatic bacteriuria, reducing the complicated UTI and antibiotic administration and shortening the hospital stay and the costs associated to the patient's treatment.

CRediT authorship contribution statement

Marta Santos: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. **Marcos Mariz:** Conceptualization, Writing – original draft, Funding acquisition. **Igor Tiago:** Conceptualization, Writing – original draft, Funding acquisition. **Jimmy Martins:** Writing – original draft, Funding acquisition. **Susana Alarico:** Conceptualization, Resources, Writing – original draft, Writing – review & editing, Supervision, Funding acquisition. **Paula Ferreira:** Conceptualization, Resources, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: P. Ferreira, M. Santos, M. Mariz, S. Alarico, I. Tiago reports financial support was provided by Foundation for Science and Technology. P. Ferreira, M. Santos, M. Mariz, S. Alarico, I. Tiago, J. Martins reports financial support was provided by La Caixa Welfare Projects.

Acknowledgments

This work was supported by Fundación Bancaria Caixa d'Estalvis i Pensions de Barcelona, "la Caixa", through CaixaImpulse non-profit-making program, which supported the project referenced as CI19-0100 TimeUp with an economic Grant.

The project was also supported by FCT (Fundação para a Ciência e a Tecnologia | Portuguese Foundation for Science and Technology) through COMPETE 2020–Operational Program for Competitiveness and Internationalization (POCI), PORTUGAL 2020 through projects UIDB/00102/2020 (authors from CIEPQPF), UIDB/04539/2020, UIDP/04539/2020 and LA/P/0058/2020 (authors from CNC-UC) and for PhD grant DFA/BA/5675/2020 (to M. S.) and contract SFRH/BPD/108299/2015 (to S. A.).

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