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Current and emerging trends in point-of-care urinalysis tests

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Abstract

Introduction—The development of point-of-care testing (POCT) has made clinical diagnostics available, affordable, rapid, and easy to use since the 1990s. The significance of this platform rests on its potential to empower patients to monitor their own health status more frequently in the convenience of their home so that diseases can be diagnosed at the earliest possible time-point. Recent advances have expanded traditional formats such as qualitative or semi-quantitative dipsticks and lateral flow immunoassays to newer platforms such as microfluidics and paper-based assays where signals can be measured quantitatively using handheld devices.

Areas Covered—This review discusses: (1) working principles and operating mechanisms of both existing and emerging POCT platforms, (2) urine analytes measured using POCT in comparison to the laboratory or clinical “gold standard,” and (3) limitations of existing POCT and expectations of emerging POCT in urinalysis.

Expert Opinion—Currently, a variety of biological samples such as urine, saliva, serum, plasma, and other fluids can be applied to POCT for quick diagnosis, especially in resource-limited settings. Emerging platforms will increasingly empower individuals to monitor their health status through frequent urine analysis even from their homes. The impact of these emerging technologies on healthcare is likely to be transformative.

Keywords

dipstick; lateral flow assay; microfluidic paper-based analytical device; point-of-care testing; urinalysis

1. Introduction

This review strictly includes point-of-care diagnostic devices which are defined as low-cost, rapid, portable, and easy to use for sample introduction and interpretation. For all POCT platforms included in this paper, sample preparation, sample introduction, and interpretation can all be performed by a doctor, nurse, care-giver, patient, or customer, or can be completed

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Declaration of interest

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by simply following the instructions provided or by attending a one-time training session before usage.

1.1 Detection of urine analytes

The detection of urine analytes discussed here include proteins, metabolites, small molecules, nucleic acids, pathogens, and exosomes. Urine serves as an important source of biomarkers for analysis, since it is easy to collect, readily available, has fewer proteins, and it directly reflects on-going pathology in the kidney [1]. The analysis of urine, or urinalysis, is commonly used as a routine medical exam for early disease detection [2]. In fact, early signs of certain diseases can be detected by monitoring specific urine biomarkers both qualitatively and quantitatively: For example, early involvement of the kidneys in diseases such as diabetes and lupus can be detected through increasing urine protein levels. Besides detecting of early signs of developing disease, urinalysis can also be used to detect drug abuse, such as morphine overdose or food safety violations, such as antibiotic residues in livestock. Commonly-used POCT in these scenarios include paper-based dipsticks and lateral flow assays; however, microfluidic paper-based analytical devices (μ PADs) have the potential to transcend currently popular point-of-care devices due to its reliable control of fluid flow in reaction and detection zones and quantitative analysis with or without a portable reader [3,7,9].

1.2 Characteristics of point-of-care technologies

Point-of-care technologies are widely used in physicians' offices, clinics, and homes without the help of central laboratory devices or advanced expertise [4]. The turnaround time from the moment of sample introduction into the device to the final readout by naked eye or electronic reader can range from minutes to hours. However, as rapid and convenient point-of-care technologies are, their sensitivity and specificity must not be compromised, thus requiring further research to optimize this tradeoff.

1.3 Overview of paper

This paper begins with the well-established and most commonly used type of dipstick (urine glucose assay) and lateral flow assay (urine pregnancy test stick) in order to explain the conventional working principles of paper-based POCT. Next, we discuss prototypes of microfluidic paper-based analytical devices (μ PADs) and different types of urinalysis formats, categorized based on the analyte detected – proteins, metabolites and drugs and food safety, infectious diseases, etc. Finally, a critical review of the limitations of current POCT and the expectations of next-generation POCT are discussed.

2. Dipstick applications in urinalysis

In the 1950s, single glucose urinalysis was made possible using a paper-based dipstick through enzymatic oxidation of chromogen, followed by commercial introduction in the 1960s [1,4]. Today, commercially available dipsticks have evolved to be a highly efficient tool for investigating, detecting, and screening diseases with rapid, high-quality results, all while maintaining ease of use. The reaction between glucose and oxygen is catalyzed by glucose oxidase and the initially produced hydrogen peroxide reacts with the chromogen

substrate, which is later catalyzed by peroxidase to yield a final colored product. This colored product, which is proportional to glucose concentration in the sample, can be semi-quantitatively determined by comparing the color of the detection pad on the strip with the color scale on the test strip vial or quantitatively determined using an analyzer [14].

2.1 Glucose urinalysis

Currently, the multiplex dipstick has dominated the market by achieving detection of up to ten parameters in a standard dipstick strip with the same simplistic operation. In urinalysis, these strips are available for simultaneous detection of glucose, leukocyte, ketone, nitrite, pH, total protein, specific gravity, urobilinogen, and other metabolites or proteins [1]. Multiplexed detection is achieved through the use of several reagent pads, each of which corresponds to the detection of one specific analyte. Structurally, a plastic carrier foil sits at the base to support the various layers of reagent pads. Finally, a nylon mesh is sealed onto the strip to secure all the reagent pads and ensure urine penetrates homogeneously [11].

As described above, the colorimetric reaction for glucose detection happens in the glucose pad after dipping the test strip in urine for several seconds, resulting in color change [14]. However, there are limitations to the urine glucose test. Compared to urinalysis, blood glucose assayed using a glucose meter with an electrochemical sensor can better reflect physiological glucose levels because it quantitatively assays glucose in real time. Moreover, qualitative (yes or no) and semi-quantitative urine glucose tests may not accurately reflect low levels of glucose and may thus yield negative results [34]. In addition, with devices such as Urisys 1100® by Roche Diagnostics, Cobas® U 411 analyzer also by Roche Diagnostics, and the LAURA® Semi-Automated Urine Strip Reader by Erba®, a colorimetric optical signal can be quantitatively analyzed, but the high-cost (\$1000+) and bulky size of the analyzer may not be ideal for point-of-care diagnostics or at home testing.

2.2 Protein urinalysis

Besides the determination of glucose, urine protein detection is also an important indicator of renal disease and can readily be assayed using dipstick tests. The common method is based on color change in the presence of protein, where tetrabromophenol blue changes from yellow to blue [14]. A recent work reported a better indicator of diabetes or renal disease based on the determination of albumin to creatinine ratio (ACR) in the urine [70,71]. In these reports, with the use of a reagent cartridge (DCA 2000® Microalbumin/Creatinine Kit), quantitative measurement of urine albumin by immunoturbidimetry (using polyclonal goat antiserum) and urine creatinine by spectrophotometry (using 3,5-dinitrobenzoic acid at alkaline pH) was achieved. Considering that the quantitative readout required a DCA2000® analyzer, this method is likely limited to a doctor's office or clinic.

Another report used a digital sensing platform, termed Albumin Tester, that has an attachment mechanically installed on the existing camera unit of a smart-phone [72]. Fluorescence-based detection occurs in a test tube and the acquired fluorescent images are digitally processed within a second using an Android application running on the same smart-phone. It takes around five minutes for sensitive and specific detection of albumin in urine with a limit-of-detection (LoD) of 5 to 10 µg/ml, which is three times lower than the

clinically accepted normal range. Beyond these colorimetric and fluorescent detection methods, other platforms commercially available and actively in research include an electrochemical urine dipstick probe biosensor that used molybdenum electrodes on nanoporous polyamide substrate for the quantitative multiplexed detection of inflammatory factors C-reactive protein (CRP) and Interleukin-6 (IL-6) in urine [74]. The portable electronics platform demonstrated results within five minutes with a LoD of 1 pg/ml for both CRP and IL-6 in human and synthetic urine.

2.3 Other dipstick applications in urinalysis

Other dipstick-based urinalysis tests are widely used as well. Reaction between sodium nitroprusside, acetoacetic acid, and acetone can yield a violet color, which can be used to detect ketones. To detect nitrite, the reaction between it and p-Arsanilic acid is used to form a diazonium compound, which couples with a quinolone compound to produce a pink color. Color change from orange to turquoise is indicative of a pH shift. Lastly, the Ehrlich Aldehyde reaction and a bilirubin and diazonium salt reaction are used to yield an azo red compound, which can be used to detect urobilinogen and bilirubin in the urine [14].

Currently available urine dipsticks are generally rapid, low-cost, and highly convenient, as discussed further below in the Tables. However, many of the current dipsticks are still limited by low sensitivity, specificity, and accuracy compared to the respective “gold standard” laboratory methods or are not quantitative. Moreover, not all proteins or metabolites can readily be detected using conventional dipstick-based approaches, although newer platforms aim to surmount this limitation.

3. Lateral flow (immuno) assay applications

Compared to the dipstick, the lateral flow assay (LFA) represents the simplest and widely used rapid diagnostic testing platform, which was originally derived from the latex agglutination test developed by Singer and Plotz in 1956. There is some confusion in distinguishing the dipstick from the lateral flow assay because they are similar in appearance and employ the same simplistic operation. However, in the lateral flow assay, the sample of interest is first introduced to the sample pad, then flows through the membrane, and finally reaches the absorbent pad at the end of the strip, all without the assistance of external force, but rather by internal capillary forces from the membrane and the absorbent pad, which also prevents backflow of the liquid [1,3,4,7,10,19,25]. Compared to a lateral flow assay, in a dipstick test the urine analyte makes instantaneous contact with the reagent pad, with little need for capillary flow.

A brief working principle and general structure of the LFA is summarized below and detailed elsewhere [1,4,7]. A typical lateral flow test strip is composed of four overlapping elements that are mounted on an adhesive backing. The first element is the sample pad, which is typically made of cellulose or glass fiber to consistently deliver the sample of interest to the second element, which is the conjugate pad. The conjugate pad can be made of cellulose, glass fiber, or polyesters depending on the labeled conjugates and the sensitivity of the assay. The labeled biomolecules are held in the conjugate pad and should bind to the analyte in the sample of interest when the sample of interest reaches the conjugate pad. The

analyte-conjugate complex then flows through the third element, which is the nitrocellulose membrane where specific biological compounds (typically antibody, protein, or nucleic acids) are immobilized at pre-defined lines. The analyte, analyte-conjugate complex, and conjugates should react specifically to the compounds dispensed on the membrane. Lastly, any remaining sample of interest should be absorbed by the fourth element, which is the absorbent pad.

Depending on the analyte of interest, lateral flow assays can be classified into two categories: one is lateral flow immunoassay (LFIA) where antibodies are used as recognition elements to detect proteins, the other is nucleic acid lateral flow assay (NALFA) where nucleic acids are used as recognition elements to detect amplicons, or results of amplification reactions like polymerase chain reaction (PCR) or recombinase polymerase amplification (RPA) [7/32/33/42]. Furthermore, depending on the size and number of antigenic targets, LFIA can be subclassified as sandwich assay for protein and nucleic acid detection, and competitive immunoassay mainly for protein detection [54].

3.1 Sandwich format LFIA tests

A sandwich format is where the target analyte is immobilized between two complementary antibodies and a positive test line indicates the existence of such a target analyte. Typically, the labeled conjugates (enzymes, colloidal nanoparticles, latex microspheres, fluorescent dyes, antibody or aptamer) are dried onto a conjugate pad and will bind to the target analyte when the sample of interest (in the urine) reaches the conjugate pad. The primary antibody or aptamer is immobilized onto the test line and binds to the analyte-conjugate complex to form a conjugate-analyte-primary antibody or aptamer complex. On the control line, an immobilized secondary antibody or probe against the conjugate demonstrates the successful release of dried conjugates. Signal intensity captured at the test line by naked eye or optical reader is generally proportional to the analyte concentration in the test sample.

One excellent example of a commercial LFIA is the ubiquitous pregnancy test [10]. Most pregnancy test strips use a sandwich immunoassay format to detect human chorionic gonadotropin (hCG) in a qualitative way (yes/no), specifically through color appearance at the test line, generated from colloidal gold nanoparticles. One reason that pregnancy test strips have been remarkably effective is the relatively high analyte level - hCG levels could rise from 20 IU/ml to 50 IU/ml (around 1.3 ng/ml to 3.3 ng/ml) in early pregnant women and double every two to three days. With such abundance, it is easy to attain strong signals quickly, whereas it could be challenging with other LFA tests where the target of interest is low in concentration (less than 100ng/ml). One concern with the use of the pregnancy test strip is the false-negative results derived from predominant hCG variant hCG-beta-core-fragment (hCG-beta-cf). Eleven pregnancy test products were surveyed and screened for susceptibility to inhibition by hCG-beta-cf and results showed that BC Icon 20 and Alere brand pregnancy products were the least affected. Another interesting work reported a star-pattern multiplex (up to 10 analytes) lateral flow test strip detecting glucose and albumin simultaneously, fabricated by two-dimensional cut-outs through polyester-backed nitrocellulose membranes [4]. Compared to traditional LFAs, this star-pattern multiplex LFA

was able to draw a sample containing analytes across multiple capture zones and can thus reduce operator error associated with lateral flow tests.

3.2 Competitive format LFIA tests

This format is suitable for small molecules or molecules with limited antigen determinants that make sandwich assays challenging to construct. There are three major formats used for the competitive format, and all of them demonstrate the absence of target analyte with a signal appearance at the test line, which is the opposite of the sandwich assay. The first format holds the conjugated antibody (specific to the analyte) in the conjugate pad, the analyte of interest immobilized at the test line, and secondary antibody (specific to the conjugated antibody) at the control line. When the sample is introduced, “little to no” analyte will not react with the impregnated conjugate, hence, the free conjugate later binds to analytes immobilized at the test line and control line to yield signals.

The second format carries the conjugated analytes in the conjugate pad and primary antibody (specific to the analyte) immobilized at the test line. When the sample is introduced, “little to no” analyte will not compete with the labeled analyte, thus the labeled analyte can bind at the test line to yield a signal.

The last format contains three lines: the test line where the primary antibody is immobilized, the antigen line where the target analyte is immobilized, and the control line where the secondary antibody is immobilized. In the conjugate pad, the conjugated antibody is dispensed. When the sample is introduced, “little to no” analyte allows the conjugated antibody free to bind to the immobilized analyte at the test line and to the secondary antibody at the control line, demonstrating a negative result. If there is enough analyte in the sample, a sandwich complex will form at the test line, and the remaining conjugated antibody will bind at the control line, to yield a positive result.

3.3 Detection systems for LFA

Compared to the dipstick, where the reagent pad instantaneously contacts the sample upon dipping into the urine, analyte detection in LFA is more challenging. Multiplexed detection of analytes in LFA can be realized through separate detection sites in a single strip (architecturally similar to the dipstick) or strips in an array where each strip is used for the detection of a single analyte, or through the use of different reporters that offer different signals for each analyte of interest. The development of multiplexed detection in LFA is also compounded by decrease/increase of signal for test lines away from sample pad because of the influence of variations in salt concentration, pH, and non-specific binding of the reporter.

Most lateral flow assays use visual inspection (colorimetric-based) for a quick qualitative answer (yes/no) or for a quick semi-quantitative answer where an estimated concentration of the analyte is determined by comparing the color intensity to a provided color chart. Although both qualitative and semi-quantitative analysis have been very useful in terms of screening and diagnosis, more attention is now drawn to quantitative analysis because it provides more accurate and almost- immediate diagnostic results, particularly with respect to evolving disease status [14]. For quantitative assays, colorimetric and luminescent systems often require an optical strip reader in combination with a camera or charge-coupled

device (CCD) imaging technology. Also, an integrated image-processing algorithm is required to convert the intensity of the lines to the corresponding analyte concentrations. It is reported that the test line to control line ratio can better reflect analyte levels, correcting for operator error or individual product variance. On the other hand, electrochemical and chemiluminescent systems often use amperometric immunosensors where counter electrodes, working electrodes, and reference electrodes are assembled, to measure the current (flow of electrons) arising from the analyte of interest reacting with the working electrode.

Common detection systems used for quantification in LFAs can be classified into six types as detailed elsewhere and summarized below [14].

3.31 Colorimetric detection for LFA

This is the most commonly used detection system and includes gold nanoparticles, carbon nanoparticles, selenium nanoparticles, silver nanoparticles, latex beads, and magnetic particles. For POC use, qualitative or semi-quantitative results can be visually read while quantitative analysis can only be achieved using a handheld reader or smartphone. In fact, multiple detection systems could be used together to achieve higher sensitivity, as exemplified by the use of silver enhancement for gold nanoparticles, enzyme-loaded gold nanoparticles, or fluorescent dye/colored dye/magnetic components-coupled latex beads [10].

3.32 Fluorescent detection for LFA

Fluorescent detection includes quantum dots, up-converting phosphors, organic fluorophores, and textile dyes. This detection system requires an optical reader for quantification. Importantly, fluorescent labels demonstrate a much higher sensitivity than traditional gold nanoparticles.

3.33 Electrochemical detection for LFA

Initial applications of electrochemical sensors for LFA were reported for glucose, lactate, uric acid, and other metabolites. Electrochemical biosensors are well suited for urinary diagnostics due to their excellent sensitivity, low-cost, and ability to detect a wide variety of target molecules, but its requirement for detection equipment has made its at-home POC application very limited. A custom-made electrochemical reader for \$90 for simultaneous detection of glucose, lactate, and uric acid has recently been reported, making the POC application at home more promising [23].

3.34 Electrochemiluminescent detection for LFA

Electrochemiluminescence (ECL) combines the strength of luminescence and electrochemical sensing. Basically, the analyte of interest is sandwiched by biotinylated capture antibody and ruthenium-labeled detection antibody. Then, streptavidin-coated paramagnetic beads bind to the sandwich complex through biotin and the completed amino acid complex moves to one side of the measuring cell under the influence of a magnet. Next, with the introduction of TPrA (Tripropylamine), which serves as a reductant, ruthenium returns to its base state, where it emits light and therefore enables readouts without the

requirement of a photodetector. However, to calculate the concentration of analyte, a relatively costly photomultiplier is required.

3.35 Chemiluminescent detection for LFA

A chemiluminescent (CL) detection system is best exemplified by a glow stick, where the emission of light is generated by peroxide reacting with a phenyl oxalate ester. Likewise, for quantification, an optical reader is required [8].

3.36 Enzymatic detection for LFA

In a typical enzymatic detection system, horseradish-peroxidase-labeled detection antibody reacts with a substrate to generate a colored product, which can be optically read.

For all of the above-mentioned detection methods and LFA formats, mass production is a major obstacle. There are limits to the commercialization of lateral flow assays due to potentially high coefficient-of-variation (greater than 20%). In LFA POC test systems, unit operations and overall system specifications that control one element could affect all other aspects of the test. Elements include but not limited to are sample conditioning (inaccurate sample preparation and introduction), recognition ligand conditioning (less active and consistent antibody, probe, and the conjugates), amplification (inaccurate operation of components required for signal amplification), measurement (visual prejudice or inaccurate operation of strip reader), and environmental conditioning (temperature, humidity, light, etc.). Looking at the currently available LFA tests (as discussed further below in the Tables), some limitations include the lack of quantitation, limited sensitivity, and assay times that are longer than those for dipstick tests. These limitations need to be surmounted before this platform can be widely used for POC testing.

4. Microfluidic paper-based analytical device (μ PAD) applications

μ PADs have emerged as a simple yet powerful platform for low-cost analytical tests and have witnessed phenomenal use in urinalysis due to its efficiency, affordability, and non-invasive methodology [9]. Undoubtedly, one major advantage of μ PADs is its cost-effectiveness: they are easy to mass-produce, transport, store, implement, dispose, and do not require excessive equipment to move liquid like other devices [11]. However, there are also disadvantages: they are relatively new, which means issues such as flow rate control, mixing, and interaction times between sample and reagents have not yet been perfected. The advent of POC testing has led to the development of a variety of microfluidic devices, which can be categorized based on their underlying working principle: capillary driven (similar to LFA), pressure driven, centrifugal, electrokinetic and acoustic. Currently, in the field of urinalysis, the majority of μ PADs are designed to assay glucose, protein, or uric acid.

Several approaches for the fabrication of paper-based microfluidic devices have been published in recent literature with an emphasis on working principles, the materials, sample processing, microfluidic elements (such as valves, pumps, and mixers), market requirements, strengths, and limitations [1,9,11,14,17,33,47,53]. Most of them utilize the hydrophilic properties of the paper substrate and some form of hydrophobic ink deposited as a barrier. In fact, fabrication techniques can be broken down into analog and digital methods. Analog

methods, which require the use of a pre-fabricated mask or plate, include photolithography, flexographic printing, plasma treatment, wax patterning, screen printing, and wet etching. On the contrary, digital methods, which allow for near-instantaneous changes to the pattern, include laser patterning, inkjet printing, and pen plotting. All in all, the ability to dynamically alter the pattern through digital means is a significant advantage, especially for low-cost point-of-care diagnostic devices.

Paper-based microfluidic devices can be manufactured in both two-dimensional (2D) and three-dimensional (3D) formats. 2D paper-based microfluidic analytical devices typically operate by capillary action through a cellulose-based paper substrate. When a hydrophilic sample is placed on the paper, it navigates through hydrophilic paper structures between the hydrophobic barriers formed using a patterning and deposition process. As a result, fluids can be transported and manipulated through horizontal and vertical patterns tailored to the complexity and requirements of the given analytical application. An example of a 2D μ PAD is explained in [74], as mentioned above.

3D μ PADs, made from stacking layers of 2D patterned paper, offer potential advantages for multiplexing and more advanced detection systems. 3D format μ PADs have been reported for colorimetric detection of glucose and total protein using devices engineered from wax printing fabrication techniques and basic principles of origami [76]. The amounts of glucose and protein in the sample was found to be proportional to the color change and could be quantified using Adobe Photoshop. Recently, another work used electrochemical detection methods for multiplexed detection of glucose, lactate and uric acid through enzymatic oxidation of chromogen [93]. In that report, the authors custom-made a \$90 electrochemical reader (potentiostat) in lieu of a \$1000 benchtop potentiostat, for quantitative analysis. This electrochemical biosensor array yielded results within two minutes after sample introduction and achieved LoD values of 0.35mM, 1.76mM, and 0.52mM for glucose, lactate, and uric acid, respectively. Fortunately, the measured LoD values for all three metabolites were either within or below the clinically-relevant ranges, enabling the acquisition of high-density, statistically meaningful diagnostic information at the point-of-care, in a rapid and cost-efficient way. Likewise, another μ PAD report demonstrated the multiplexed detection of glucose and uric acid using GOD/HRP and UAO/HRP bienzyme colorimetric reactions [92]. The results can be analyzed using a camera, making it suitable for home testing. Microfluidic devices could potentially dominate the next generation of point-of-care diagnostics and address challenges faced with the dipstick, LFA, and current laboratory or clinical platforms.

5. Application of rapid diagnostics in urinalysis

Urinalysis is a useful tool for diagnosing disease, detecting organ failure or malfunction, and testing for drug abuse or exposure to toxins. Published works on POCTs that provide rapid diagnostics in urinalysis can be categorized into devices for the detection of disease-relevant protein biomarkers, metabolites and drugs, infectious diseases, or cells and exosomes. Tables 1–4 summarize these 4 broad categories of applications, respectively.

Fig. 1 summarizes the literature search algorithm that was executed. Out of the initial sources retrieved from database searches and supplemental sources linked from review paper references, those that did not focus on urine or did not clearly describe a point-of-care device were excluded. In addition, further filtering was done after extensively reading the text, and excluding sources which described a point-of-care urinalysis test that performed worse than the gold standard. The remaining sources are referenced throughout this paper, and in Tables 1–4.

5.1 Detection of disease-relevant protein biomarkers in urine

Protein biomarkers have served a very important role as early harbingers of disease, tools for disease monitoring, and tracking treatment response. Although the accurate quantification of various disease biomarkers in clinical diagnostics constitutes the gold standard method, the long turnaround time, relatively high cost, and requirement of expertise make the applicability of such biomarker detection methods impractical or challenging in developing countries or resource-limited settings. However, with the development of POCT urinalysis, disease-relevant biomarkers can be detected in a more rapid, easy, and affordable way, even at the patient's home. These applications are summarized in Table 1.

5.11 Renal disease biomarkers in the urine

Cystatin C, kidney injury molecule 1 (KIM-1), and transforming growth factor beta 1 (TGF- β -1) are proteins that have been reported to be renal disease-relevant biomarkers. Urine Cystatin C and KIM-1 were simultaneously detected and quantitatively analyzed using a semi-automated microchip where analytes of interest were semi-automatically mixed in with capture microbeads, detection antibodies, and magnetic nanoparticles [75]. (Table 1) Some disadvantages of this platform include the need to measure T2 relaxation using a magnetic resonance device and the long duration of incubation steps within the semi-automated microchip. Although these limitations hinder the device's applicability in a home environment, its high sensitivity makes this micro-urine nanoparticle detection (μ UNPD) system suitable for a clinical setting. However, compared to this μ UNPD platform, the more commonly used LFA platform demonstrates significant ease of use and quicker assay times, thus favoring the LFA as an at-home test over the μ UNPD system [81]. This work using a sandwich immunoassay colorimetric detection system, developed using gold nanoparticles conjugated to detection antibodies, displayed a LoD of 0.8 ng/ml and an assay duration of 15 minutes. In a recent work, urine TGF- β -1, another renal disease biomarker, was detected using an amperometric immunosensor where a sandwich immunoassay was first performed using a capture antibody, analyte, and biotinylated antibody [86] (Table 1). Then, the current was measured through the enzymatic reaction between streptavidin-HRP and hydrogen peroxide. This appears to be highly sensitive with a LoD of 10 pg/ml. However, this immunoassay has a long incubation time thus restricting its use at home. Urine albumin, a biomarker of kidney damage, has also been quantitatively analyzed using a microfluidics cartridge based on a competitive lateral flow immunoassay (LFIA) [80] (Table 1). The signal from a chemiluminescent interaction between HRP and its substrate is automatically measured using a photosensor and displayed using an electronic readout console integrated within the device.

5.12 Cancer biomarkers in the urine

Recent bladder, colorectal, and ovarian cancer biomarker discovery works have reported the detection of various urine biomarkers using POCT. Nuclear matrix protein 22 (NMP22) and nuclear mitotic apparatus protein have been reported to be biomarkers for bladder cancer, and gold nanoparticle-based detection of these proteins have been described using LFIA test strips [82,83] (Table 1). Another work reported the detection of human epididymis protein 4 (HE4), a biomarker of ovarian cancer, using a microchip ELISA format where a smartphone is employed to quantitate the colorimetric reaction [79]. (Table 1) Instead of tracking a naturally-found biomarker, a synthetic substrate designed for specific proteases have been used in LFAs for disease diagnostics [84]. Following injection of a nanoworm-conjugated reporter, the reporter was freed by specific proteases in diseased body fluid and urine. The advantages of this reported technology are the use of disease-tailored “biomarkers” and the lack of a need for a normalizer. However, the need for the additional injection step makes this technique somewhat cumbersome.

Other diseases like acute pancreatitis have been diagnosed using urinary trypsinogen-2 (UT-2) as a biomarker, and colorimetry as the analyte detection system [87] (Table 1).

5.2 Detection of metabolites and drugs in urine

Food safety monitoring from processing to transportation and the detection of foodborne pathogens and toxins have been a long-standing focus. Urine antibiotic residues from food-producing animals have been assayed using POCT [90,99]. To test for drug abuse, rapid, low-cost, and convenient point-of-care technologies are desired. Additionally, metabolites related to kidney damage have been assayed using LFAs, as summarized in Table 2.

5.21 Applications in food safety monitoring

The most common POCT platform used in food safety administration is the LFA. Clenbuterol, a representative of the class of synthesized β -adrenergic agonists, is reported to reside in meat and liver for a long time as a result of its long half-life. One study described using a competitive LFIA to detect clenbuterol by capturing its fluorescence, which happens when analytes bind to fluorescent nanosilica conjugated-antibodies, which then bind to staphylococcal protein-A to form a sandwich-like complex only at the control line [90]. Readouts were achieved qualitatively through visual inspection under UV light and quantitatively through an ESE-Quant LFA reader. Sulphamethazine (SMZ), an antimicrobial, has been qualitatively analyzed using competitive LFIA using colorimetric carbon nanoparticles, as listed in Table 2 [99].

5.22 Testing for drug abuse and over-prescribed drugs

Drug abuse or over-prescribed drugs can result in a wide spectrum of complications, depending on the abused drug. One report described the profile of 10 different drugs in the urine using an (immunoassay) dipstick and compared it to a liquid chromatography tandem mass spectroscopy (LC/MS/MS) reference test [88] (Table 2). Those commercial dipsticks for drug testing exhibited good correlation to the gold standard, although there is a lack of POC tests with lower detection cutoff levels. Several POCT urinalysis tests, all of which used colorimetric detection, tested buprenorphine, morphine, and oxycodone [89,95,97]

(Table 2). Compared to the gold standard methods, such as gas or liquid chromatography (GC, LC), MS, or enzyme-linked immunosorbent assay (ELISA), the POCT exhibited high sensitivity, high specificity, and good correlation to the gold standard. Rapid assay time, ease of operation, and low cost were the advantages of these POCT.

5.23 Metabolite biomarkers of health and disease

Creatinine serves an important role as a urine metabolite that can be used to normalize urine biomarker levels. One study used an amperometric immunoassay to detect an oxidative current from a copper–creatinine complex on a copper electrode without redox reactions or enzymes [91] (Table 2). Other molecules like human growth hormones (hGH), oral direct Xa inhibitors, and oral direct thrombin inhibitors have also been assayed in the urine [94,96] (Table 2).

5.3 Detection of infectious disease biomarkers in urine

Although several formats of LFAs are available for the detection of infectious agents, the majority of LFAs utilize a sandwich immunoassay with a colorimetric gold nanoparticle detection system (Table 3). Examples include the detection of cryptococcal antigen (CRAG), lipoarabinomannan (LAM), and schistosome circulating cathodic antigen (CCA), as listed in Table 3 [104,105,107,108,109,110,111]. Detection of bacteria seeks the presence of bacterial nucleic acids (DNA or RNA), which necessitates the purification and amplification of these molecules in order to reach the sufficient sensitivity. The detection of *B. anthracis*, *C. trachomatis*, *S. haematobium*, and *E. coli* using LFAs required PCR (polymerase chain reaction) or RPA (recombinase polymerase amplification) for amplification [102,103,112] (Table 3). However, an interesting work showcased a PCR-free, chip-based sensing system for the detection of *E. coli* and provided bacterial sensing at clinically relevant levels [36].

5.4 Detection of cells and exosomes in urine

For macrohematuria diagnosis, commercial lateral flow assays for erythrocytes are available. They employ gold nanoparticles as a label; qualitative analysis involves visual detection while quantitative analysis requires a strip reader [113]. A recent study utilized an integrated double-filtration microfluidic device to isolate and enrich extracellular vesicles (EVs) with a size range of 30–200 nm from the urine, and subsequently quantified the EVs via a microchip ELISA, as listed in Table 4 [114]. This integrated device has the potential to be used in conjunction with urine cytology and cystoscopy to improve clinical diagnosis of bladder cancer in clinics and at point-of-care (POC) settings. Compared to conventional ultracentrifugation for isolation of EVs, this technique is much faster. Further validation of this technology and application are awaited.

6. Conclusion

In conclusion, this review overviews the working principles and operation mechanisms underlying both existing and emerging POCT. Limitations of existing POCT and projected growth areas of next-generation POCT were briefly discussed. Undoubtedly, significant growth is anticipated in the field of POCT urinalysis, both in terms of the technologies employed as well as the range of analytes detected. The continuing explosion on all OMICs

fronts, including genomics, proteomics, and metabolomics, coupled with rapid progress in cell-phone technology and global connectivity, will soon make personalized POCT a reality and revolutionize next-generation urinalysis.

7. Expert opinion

The lateral flow assay is the most widely used POCT that dominates the market of central laboratory, clinical diagnostics, and at-home testing. Typical LFA tests embody most of the desired characteristics of POCT, including but not limited to rapid assay time, low cost, limited sample volume needs, wide applicability for a variety of analytes, product stability, convenient storage conditions, and customizable qualitative / semi-quantitative / quantitative assays. However, its limitations cannot be ignored. Drawbacks include inaccurate sample introduction and preparation, limited sensitivity, potentially high cross-reactivity, low reproducibility, lack of appropriate biorecognition elements including non-specific antibodies / nucleic acids, antibody unavailability, inhomogeneous membrane pores, and inconsistent conjugate release.

Enhanced strip biosensor using oligonucleotide-functionalized gold nanoparticles as an enhancer probe (AuNP-DNA) for rapid and sensitive detection have been reported [115]. The latter exhibited significant signal enhancement within 15 minutes with a LoD 10-fold and 15-fold lower than the conventional strip biosensor and western blot, respectively. A recent study [124] reported that assaying early morning urine could improve the sensitivity of lateral flow lipoarabinomannan assay in hospitalized TB-HIV co-infected patients, due to higher concentration of analytes in the morning. This finding joins a growing number of analytes that exhibit diurnal variation in urine concentration, and underscores the importance of factoring in this variable when comparing competing technologies and/or studies in this field.

With respect to the dipstick, false positive, false negative, and overall low sensitivity and specificity metrics are commonly recognized drawbacks. The urine dipstick does not seem to be an appropriate tool in the diagnosis of urinary tract infection among elderly patients. It has low reliability and it cannot differentiate between a urinary tract infection and asymptomatic bacteriuria [117]. On the other hand, paper-based microfluidics, although in early development, has shown promise to be the next-generation POCT, based on recent research studies [33,43,76,92,93]. Currently, 2D and 3D microfluidic paper-based devices for the detection of glucose, uric acid, and other metabolites have been reported. Significant efforts have also been taken to integrate μ PADs with electrochemical devices such as enzyme-free amperometric biosensors. Vertical flow assays (VFA) have been explored as well. In comparison with the lateral flow assay, the VFA sensor is more rapid but with comparatively lower sensitivity partially due to the limited contact time between the sample and detection ligand. However, higher sensitivity and limited hook effect have been reported for the VFA [41,116]. As only one vertical flow cartridge is currently available commercially, more research is warranted in this area.

Another growth area is integration of POCT with cell-phone based imaging and analysis. Examples of cell-phone based devices include the Holomic Rapid Diagnostic Reader

(HRDR-200, a light-weight opto-mechanical attachment and a smartphone application) for lateral flow assay, cell-phone-based electrochemical sensor, angle-resolved surface plasmon resonance (a label-free biosensing tool that measures adsorption of material onto gold or silver particles), cell-phone-based fluorescent microscopy platform, cell-phone fluorimeter where an optical cradle is installed on the phone, and cell-phone dongle for POCT diagnosis of infectious diseases [118,119,120,121,122,123]. Of note, fully automated Lab-on-the-Chip platforms paving the way for the next generation of POCT, although improvements are needed with respect to sample preparation and reagent addition [17,32]. One promising example is the LabDisk centrifugal microfluidic platform which simultaneously integrates sample preparation, mixing, reaction and detection in a disk-like platform [125].

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Reference

*articles of importance that detail the principles underlying the dipstick, lateral flow assay, vertical flow assay, microfluidics, and next generation of point-of care diagnostics.

**articles of considerable importance that report innovative advancements in recent years in quantitative point-of-care diagnostics.

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Figure 1:
Literature search and algorithm for identification of manuscripts for inclusion

Table 1:

Currently available Point-of-Care Tests for Proteins in Urine

Analyte (ref)	Type of test (r/c)	Detection Method	Site of Use	Indications	Time	LoD / working range	Advantages	Disadvantages
Albumin to creatinine ratio—ACR (70)	DCA 2000™ desktop microalbumin system (c)	Immunoturbidimetric for albumin & colorimetric for creatinine	Laboratory Clinic office	Type 2 diabetes (70)	7 min	1 to 25 mg/mmol	Rapid; Low-cost; Quantitative	DCA 2000 analyzer required
Albumin to creatinine ratio—ACR (71)				Renal disease microalbuminuria (71)		NA		
Albumin (72)	Albumin Tester (r)	Fluorescence	Laboratory Clinic office Home	Chronic disease	5 min	5 to 10 µg/ml	Rapid; Quantitative; High TPR & TNR; Light camera attachment for analysis	Training required for sample introduction
Albumin (73)	Sandwich LFA (r)	Protein error of indicator	Laboratory Clinic office Home	NA	4 to 8 min	0 to 75 µM	Rapid; Low-cost; Multiplex detection; Easy operation	Semi-quantitative
Glucose (73)		Enzymatic oxidation of chromogen						
C-reactive protein—CRP (74)	Dipstick (r)	Electrochemical	Laboratory Clinic office	Inflammation	5 min	1 pg/ml	Rapid; Multiplex detection; Quantitative; Portable electronic sensor for analysis	Training required for operation
Interleukin 6—IL-6 (74)								
Cystatin C (75)	Magnetic immuno-sandwich assay (r)	Micro-urine nanoparticle detection—µUNPD	Laboratory Clinic office	Kidney injury	2 hours	20 ng/ml 0.1 ng/ml	Quantitative; High TPR, µNMR read-out for analysis	Training required for sample preparation and reagent addition; Separate step required for measuring T2 relaxation, Slow procedure
Kidney Injury Molecule-1—KIM-1 (75)								
Glucose (76)	3D paper-based microfluidic device—3D µPADs (r)	Colorimetric	Laboratory Clinic office	Diabetes	50 min	0.25 to 8 mg/dL 6.3 to 10.4 mg/dL	Rapid; Low-cost; Multiplex detection; Portable	Semi-quantitative
Total protein (76)								
Glucose (77)	Dipstick—Combur 10 TestM, ChoiceLine 10, Combur 10 TestUX, CombuStik 10M, CombuStik 11M, CombiScreen 11SYS, CombiScreen 10SL, Combina 13, Combina 11S, Combina 10M, UriGnost 11, Multistix 10SG (c)		Laboratory Clinic office Home	NA	Less than 10 min	1.43 to 20.4 mmol/L NA NA NA	Rapid; Low-cost; Multiplex detection; Quantitative; Easy operation	Semi-quantitative; Disagreement between observers using color scale; Analyzer required for quantitative analysis
Leukocyte (77)								
Ketone (77)								
Nitrite (77)								

Analyte (ref)	Type of test (r/c)	Detection Method	Site of Use	Indications	Time	LoD / working range	Advantages	Disadvantages
pH (77)						NA		
Total protein (77)						0.18 to 1.26 g/L		
Specific gravity (77)						NA		
Urobilinogen (77)						NA		
Human chorionic gonadotropin—hCG (78)	Sandwich LFIA (c)	GNP- colorimetric	Laboratory Clinic office Home	Pregnancy	5 min	NA	Rapid; Low-cost; Easy operation;	Qualitative; False-negative possibility due to variant hCG- β ef
Human epididymis protein 4—HE4 (79)	Microchip ELISA (r)	HRP-TMB- colorimetric	Laboratory Clinic office	Ovarian cancer	5 hours	19.5 ng/ml	Multiplex detection; Quantitative; Cell phone/CCD-based analysis, High TPR & TNR	Training required for reagent addition; Slow
Human serum albumin—HSA (80)	LFA (r)	Competitive chemiluminescence	Laboratory Clinic office	Kidney damage	32 min	2.5 mg/ml	Rapid; Low-cost; Quantitative; Photosensor array and electronic readout board	Training required for microfluidic cartridge operation and reagent addition
Kidney Injury Molecule-1—KIM-1 (81)	Sandwich LFIA (r)	GNP- colorimetric	Laboratory Clinic office Home	Acute kidney injury	15 min	0.8 ng/ml	Rapid; Easy operation; Low-cost	Semi-quantitative;
Nuclear matrix protein 22—NMP 22 (82)	LFA—NMP22 BladderChek Test (c)	GNP- colorimetric	Laboratory Clinic office Home	Bladder cancer	30 to 50 min	10 U/ml	Rapid; Easy operation; ow-cost; Higher TNR than cytology reference	Lower TPR than cytology reference; Qualitative
Nuclear mitotic apparatus protein (83)	LFA—NMP22 BladderChek (c)	GNP- colorimetric	Laboratory Clinic office Home	Bladder cancer	30 to 50 min	NA	Rapid; Easy operation; Low-cost	Qualitative
Synthetic reporter DNP (84)	Nanoworm-based sandwich LFIA (r)	GNP - colorimetric	Laboratory Clinic office	Colorectal cancer (5) Thrombosis (5)	30 min to 1 hour	1 nM	Rapid; Low-cost; Multiplex detectio; Easy operation	Semi-quantitative; Intravenous injection
Synthetic reporter TMR (84)								
Transferrin—Tf (85)	Competitive LFIA (r)	Radiolabeled antibody & Dextran-coated GNP—colorimetric and radioactive	Laboratory Clinic office	NA	20 min	NA	Rapid	Training required for operation; Cobra Auto-Gamma counter required for radioactivity measurement and analysis

Analyte (ref)	Type of test (r/c)	Detection Method	Site of Use	Indications	Time	LoD / working range	Advantages	Disadvantages
Transforming growth factor beta 1—TGF-β1 (86)	Sandwich amperometric enzyme-based immunoassay (r)	Electrochemical	Laboratory Clinic office	Renal disease	3 hours	10 pg/ml	Quantitative	Training required for sample preparation and reagent addition; Slow
Urinary trypsinogen-2—UT-2 (87)	LEA urine trypsinogen (UT) POC test—Actim Pancreatitis (c)	Blue latex- colorimetric	Laboratory Clinic office Home	Acute pancreatitis	3 min	NA	Rapid; Low-cost; High TPR & TNR	Qualitative

Abbreviations: (c): Available for commercial use; CCD: Charge-coupled device; GNP: Gold nanoparticle; HRP-TMB: Horseradish peroxidase- 3,3',5,5'-tetramethylbenzidine; LEA: Lateral flow assay; LoD: Limit of detection; μNMR: Miniaturized nuclear magnetic resonance; POC: Point-of-care; (r): Available for research use; TNR: True negative rate; TPR: True positive rate

Table 2:

Currently available Point-of-Care Tests for Metabolites and Drugs in Urine

Analyte (ref)	Type of test (r/c)	Detection Method	Site of Use	Indications	Time	LoD / working range	Advantages	Disadvantages
Amphetamine (88)	Dipstick immunoassay (c)	Colorimetric	Laboratory Clinic office Home	Drug abuse	NA	1000 ng/ml	Rapid; Low-cost; Easy operation	Qualitative; Lack of lower-cutoff-level-based POC tests
Barbiturates (88)						200 ng/ml		
Benzodiazepines (88)						200 ng/ml		
Cocaine (88)						300 ng/ml		
Codeine (88)						300 ng/ml		
Heroin (88)						300 ng/ml		
Marijuana (88)						20, 50 100 ng/ml		
Methadone (88)	CEDIA® Buprenorphine Assay (c)	Homogeneous enzyme immunoassay	Laboratory Clinic office Home	Drug abuse	NA	300 ng/ml	Rapid; Low-cost; Easy operation; High TPR & TNR	Modular P800 instrument required for semi-quantitative analysis
Methamphetamine (88)						1000ng/ml		
Phencyclidine (88)						25 ng/ml		
Buprenorphine (89)						5 ng/ml		
Clenbuterol (90)	QuikPac II OneStep Buprenorphine Test (c)	Drug-conjugated competitive colorimetric immunoassay	Laboratory Clinic office Home	Food administration	5 min	10 ng/ml	Rapid; Quantitative; Easy operation	Semi-quantitative
	QuikStrip One Step Buprenorphine Test (c)				5 min	10 ng/ml		
Clenbuterol (90)	Competitive LFIA (r)	Fluorescent	Laboratory Clinic office	Food administration	8 min	0.037 ng/ml	Rapid; Quantitative; Easy operation	Observation by eye requires UV light; Quantitative analysis requires ESE-Quant Lateral Flow Reader
Creatinine (91)	Amperometric immunoassay (r)	Electrochemical	Laboratory Clinic office	Kidney function	20 min	6.8 µg/dl	Rapid; Quantitative; Enzyme-free assay; Easy operation	Training required for sample preparation
Glucose (92)	Microfluidic paper-based analytical devices—µPADs (r)	Colorimetric-GOD/HRP	Laboratory Clinic office	NA	30 min	0.213 mM	Rapid; Low-cost; Quantitative; Multiplex detection;	Gel documentation systems required for quantification;

Analyte (ref)	Type of test (r/c)	Detection Method	Site of Use	Indications	Time	LoD / working range	Advantages	Disadvantages
Uric acid (92)		Colorimetric- UAO/HRP				0.287 mM	Easy operation	Higher LoD from camera use
Glucose (93)	Paper-based microfluidic— μPADs (r)	Electrochemical	Laboratory Clinic office	NA	2 min	0.35 mM	Rapid; Low-cost; Quantitative; Multiplex detection; Easy operation; Electrochemical reader (potentiostat); Custom-made;	Training required for at-home diagnosis; Fresh enzymes needed
Lactate (93)						1.76 mM		
Uric acid (93)						0.52 mM		
Human growth hormone—hGH (94)	Ultrasensitive bimodal waveguide biosensor (r)	Bimodal waveguide interferometer—BiMW	Laboratory Clinic office	Growth disorders	8 min	10 pg/ml	Rapid; Low-cost; Quantitative; High TPR; Chip carries optical sensor for quantitative analysis	Training required for sample preparation
Morphine (95)	Competitive LFA (r)	GNP- colorimetric	Laboratory Clinic office	Drug abuse	10 min	2.5 ng/ml	Rapid; Easy operation; High TPR	Semi-quantitative
Oral direct factor Xa inhibitors (96)	Ministrip (r)	Colorimetric	Laboratory Clinic office Home	Venous thromboembolism & embolism in atrial fibrillation	15 min	NA	Rapid; Low-cost; Easy operation; High TPR & TNR, NOAC not required	Qualitative
Oral direct thrombin inhibitors (96)						NA		
Oxycodone (97)	LFA (c)	NA	Laboratory Clinic office	Drug abuse	5 min	50 to 500 ng/ml	Rapid; Low-cost; Easy operation; Few false positives and indeterminate results	Qualitative
P-aminophenol—PAP (98)	Test strip (r)	Tb ³⁺ @1 luminescence	Laboratory Clinic office Home	Phenylamine chemical in human body	5 min	5 μg/ml	Rapid; Low-cost; Easy operation; Quantitative; High TPR & selectivity, Good reusability, Smartphone used for imaging	Smartphone-compatible colorimetric analysis is still in development
Sulphamethazine—SMZ (99)	Competitive LFA (r)	Colloidal carbon-colorimetric	Laboratory Clinic office	Food administration	15 to 20 min	6.3 ng/ml	Rapid; Low-cost; Easy operation; Lyophilized urine; High TPR	Qualitative; Some false positives

Abbreviations: (c): Available for commercial use; GNP: Gold nanoparticle; GOD/HRP: Glucose oxidase enzyme- horseradish peroxidase; LFA: Lateral flow assay; LoD: Limit of detection; NOAC: New oral anticoagulant; (r): Available for research use; TNR: True negative rate; TPR: True positive rate; UAO/HRP: Uric acid degradation bifunctional protein- horseradish peroxidase

Table 3:

Currently available Point-of-Care Tests for Infections in Urine

Analyte (ref)	Type of test (r/c)	Detection Method	Site of Use	Indications	Time	LoD / working range	Advantages	Disadvantages
Bacteria (100)	Urine culture kit—UK Flexicult™ SSI urinary kit (c)	Colorimetric & counting colony numbers and semi-confident growth	Laboratory Clinic office	Urinary tract infection	Overnight	500 cfu/ml	High TPR & TNR, Few false positives (3%)	Semi-quantitative; Long procedure time
Bacterial nucleic acid—I6S rRNA (101)	Sandwich amperometric enzyme-based immunoassay (r)	Electrochemical	Laboratory Clinic office	Urinary tract infection (UTI)	2 hours	NA	Quantitative; Multiplex detection; Automated reader for analysis	Need expertise; Training required for sample preparation
Lactoferrin—LTF (101)						145 pg/ml		
<i>B. anthracis</i> RNA (102)	Sandwich lateral flow microarray—LFM (r)	Colorimetric- dyed microspheres	Laboratory Clinic office	Anthrax	2 min	250 amol	Rapid; Easy operation; Quantitative; High TPR	1-hour nucleic acid sequence-based amplification—NASBA required
<i>C. trachomatis</i> —CT amplicons (103)	Sandwich nucleic acid LFA— PCR from Abingdon Health (c)	Anti-FAM GNP- colorimetric	Laboratory Clinic office	Chlamydia trachomatis	20 min	5 to 12 pathogens	Rapid; Easy operation; Quantitative; High TPR & TNR	Semi-quantitative; RPA required
Cryptococcal antigen—CRAG (104)	Sandwich LFA— Immunononologies Inc. (c)	GNP- colorimetric	Laboratory Clinic office Home	Meningitis	10 min	NA	Rapid; Low-cost; Easy operation;	Semi-quantitative
Cryptococcal antigen—CRAG (105)	Semi-quantitative sandwich LFA (c) Quantitative sandwich LFA (r)	GNP- colorimetric	Laboratory Clinic office Home	Meningitis	10 min	5 ng/ml	Rapid; Low-cost; Easy operation; Quantitative; High TPR & TNR, Shelf stable at RT	Laser and infrared camera required for quantitative analysis
<i>E. coli</i> mRNA (106)	Nanostructured microelectrodes— NMEs (r)	Electrochemical reporters (Ru/Fe)	Laboratory Clinic office	Various infections	30 min	1 cfu/ul	Rapid; Low-cost; Quantitative; High TPR & TNR, PCR-free	Training required for sample introduction; sample preparation; and reagent addition
Lipoarabinomannan—LAM (107)	Sandwich LFIA (c)	GNP- colorimetric	Laboratory Clinic office Home	Tuberculosis	25 min	OD: 0.681	Rapid; Low-cost; Easy operation; High TPR & TNR	Semi-quantitative
Lipoarabinomannan—LAM (108/109)	Sandwich LFIA (r)	GNP- colorimetric	Laboratory Clinic office Home	Tuberculosis	NA	NA	Low-cost; Easy operation;	Low TPR; Works only in HIV patients
Schistosoma circulating cathodic antigen—CCA (110/111)	Dipstick— Rapid Medical Diagnostics (c)	Colorimetric	Laboratory Clinic office Home	Schistosomiasis mansoni	20 minutes	NA	Rapid; Low-cost; High TPR	Qualitative

Analyte (ref)	Type of test (r/c)	Detection Method	Site of Use	Indications	Time	LoD / working range	Advantages	Disadvantages
<i>S. haematobium</i> DNA (112)	Sandwich LFA (r)	GNP- colorimetric	Laboratory Clinic office	Schistosomiasis	1 hour	100 fg	Low-cost; Easy operation	Semi-quantitative; RPA required

Abbreviations: (c): Available for commercial use; GNP: Gold nanoparticle; LFA: Lateral flow assay; LoD: Limit of detection; (r): Available for research use; RPA: Recombinase polymerase amplification; RT: Room temperature; TNR: True negative rate; TPR: True positive rate

Table 4:

Currently available Point-of-Care Tests for Cells and Exosomes in Urine

Analyte (ref)	Type of test (r/c)	Detection Method	Site of Use	Indications	Time	LoD / working range	Advantages	Disadvantages
Erythrocytes (113)	LFA— UBC rapid (c)	Colorimetric	Laboratory Clinic office	Macrohematuria	10 min	NA	Rapid; Quantitative; Easy operation	POC reader concile @ Ω100 required for quantitative analysis
	LFA— NMP22 BladderChek (c)				30 min	NA	Rapid; Quantitative; Easy operation	POC reader concile @ Ω100 required for quantitative analysis
	LFA— BTA stat (c)				5 min	NA	Rapid; Easy operation	Qualitative
Exosomes (114)	Microchip ELISA (r)	Colorimetric	Laboratory Clinic office	Bladder cancer	2.5 hours	Exosome size range of 30 to 200 nm	Quantitative; High TPR & TNR, Smartphone used for imaging	Laptop required for quantitative analysis

Abbreviations: (c): Available for commercial use; LFA: Lateral flow assay; LoD: Limit of detection; (r): Available for research use; TNR: True negative rate; TPR: True positive rate