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Introductory Chapter: Rapid Test - Advances in Design, Formats, and Detection Strategies

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1. Rapid tests

The term “rapid test” is used to indicate a series of analytical methods that generally share common features, such as being easy to operate, quick, and cheap, and above all enable on-site application. Among several variants, the most explicative synonym of such kind of analytical methods is represented by the locution “point-of-care test” (“point-of-need test” to extend to fields of applications other than medicine), which highlights the key feature of rapid tests, i.e., the ability to provide a response to an analytical demand exactly where the demand is posed. The point-of-need testing approach bases on simplifying and shortening the analytical process by cutting most of the steps required by traditional, laboratory-based analysis (**Figure 1**). This strategy allows for multiple benefits to be reached, besides saving time and money, namely, (i) enabling timely decisions and intervention, (ii) encompassing issues associated to sample transportation and storage, (iii) enlarging the access to control especially in low-resource settings, and (iv) allowing for multiple measures over time, thus increasing the efficiency of monitoring programs.

The story of rapid tests originates in the medical field where they were developed for providing portable diagnostic tools that can be operated directly by physicians or even patients themselves. In the clinical context, a timely response means superior benefits for the patient, who immediately access the treatment, and for the society as a whole. In fact, the point-of-care testing strategy enables efficiently controlling the spreading of infectious diseases and also generally reducing healthcare costs.

The first rapid test dates back to 1962, when a new method to measure glucose in blood was developed [1]. The next milestone was reached in 1976 with the approval of three home pregnancy tests by the US Food and Drug administration (FDA). These tests were placed on

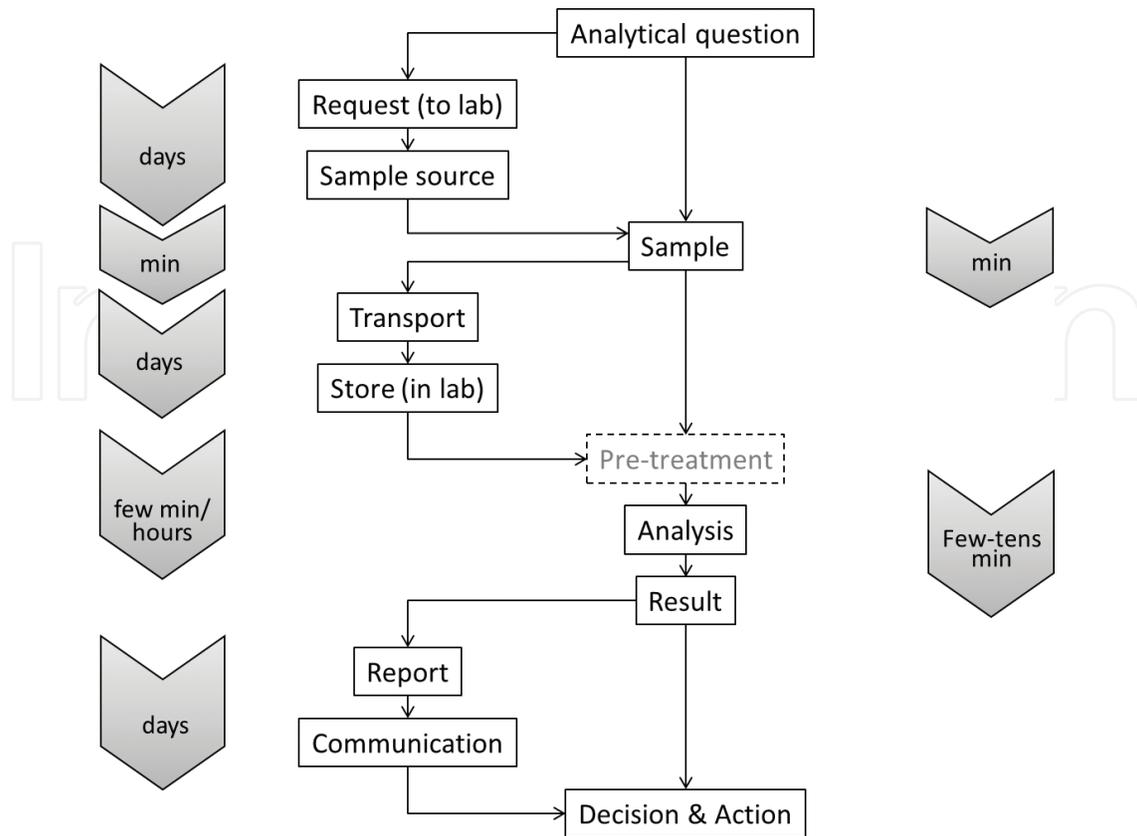


Figure 1. Schematic workflow for laboratory-based (left) and for point-of-need testing (right). The time delay within the analytical question and the decision (action) consequent to the obtainment of the response (result) falls from several days to few minutes.

the market the following year, and since then, sales have been continuously growing. The huge success of home clinical testing boosted the research in the field that, at least initially, was completely driven by industrial interests.

In the late 1990s, a scenario mutated under the influence of two new incentives: on the one hand, point-of-need testing started to attract the interest of scientists and academics, which meant a rapid evolution of technologies, materials, and analytical strategies, and on the other hand, new fields of applications were opened. The transition is clearly perceivable when considering the trends of scientific publications describing the development and applications of rapid tests plotted by time and by subject area (**Figure 2**). The picture is partial, as the number of techniques exploited for point-of-need testing and, in parallel, the ways used to indicate them are growing. However, the general trend is still increasing and is expected to further expand in the next decade.

According to the prevalent use of medicine, rapid test definition has been furnished by the World Health Organization (WHO) as follows: “rapid tests are diagnostic tools designed for use where a preliminary screening test result is required” [3]. Paradoxically, the rapidity is just one (and not the primary) of a lists of features that contribute to define this kind of analytical methods (**Table 1**). In particular, simplicity, portability, and inexpensiveness, together with

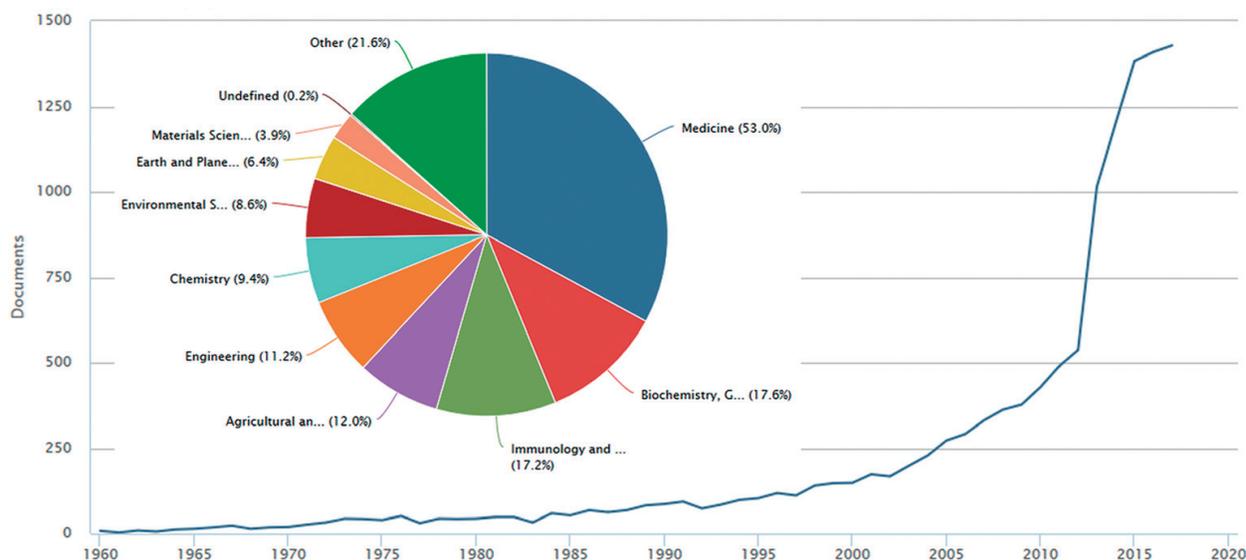


Figure 2. Trends of scientific documents sorted by year and by subject area. Search conducted by keywords “rapid test” or “lateral flow.” (Source: Scopus [2]).

Requirement	Technology	Intended for
<ul style="list-style-type: none"> • Easy to use • Quick (10 min–2 h) • Require little or no additional equipment • Economical • Possibility to store at room temperature for extended period of time 	<ul style="list-style-type: none"> • Agglutination • Immuno-dot • Immunochromatographic • Immuno-filtration 	<ul style="list-style-type: none"> • Use with individual or a limited number of samples • Use in resource-poor settings • Enabling timely treatment interventions • Emergency testing

Table 1. Characteristics of rapid tests (for infectious disease diagnosis) according to the WHO [3].

speediness, are all unavoidable requirements. Techniques and technologies able to meet these requirements are various and numerous.

Although biosensors can be considered as rapid tests based on the abovementioned definition (as they are designed for enabling fast and in situ analysis), usually are not included in the category of point-of-care testing. Principally, this is due to the fact that the ideal rapid test does not require any equipment, while biosensors include a transducer device by definition. However, the boundary is uncertain and destined to be overcome by advances in both fields: rapid tests are increasingly more sophisticated, while biosensors are turning to operational simplification.

At the state-of-the art, most rapid tests are based on the lateral flow, flow-through, and paper-based microfluidic assays. A lateral flow assay (LFA), also known as immunochromatographic assay or strip test, relies on the use of a porous membrane that enables the movement of samples and reagents through the capillary force. Flowing along the membrane, the sample encounters first a labeled recognition element (the probe), which is pulled along the membrane by the sample itself and, then, some capturing (bio)reagents that are anchored onto the membrane in well-defined zones called “lines.” The result of the sample migration is the formation of complexes (involving the analyte and the probe) in correspondence of the lines, which

provides a signal related to the analyte presence/amount (**Figure 3A**). The most popular LFA uses antibodies as capturing reagents and as recognition elements, while colored nanoparticles (typically gold nanoparticles) are employed as labels. Additional membranes can be included in the stand-alone device in order to mitigate the interference of the sample composition and heterogeneity (sample pad), hold the dried probe for long-term stability (probe pad), and help sample and probe flowing to reduce background signal (adsorbent pad) [4, 5].

Flow-through assays are also based on a series of layered membranes (**Figure 3B**). The core of the device is represented by the reactive membrane where capturing reagents are fixedly bound. The sample is passed through the reactive membrane by gravity or by applying a positive (negative) pressure. Subsequently, the labeled recognition element is fluxed to reveal the formation of the complexes between the analyte and the capturing reagent. A typical flow-through assay employs antibodies as capturing reagents and recognition elements, again. Different from LFAs, labels are usually enzymes that enable signal amplification and, therefore, increased sensitivity. However, the use of enzymes requires an additional step to introduce the enzyme substrate, and often rinse steps are also required to avoid sample interference on the enzymatic activity and to decrease background signals. Additional membranes, such as the sample and adsorbent membranes, are included in the device. The role is parallel to those above described for the corresponding parts of LFAs [6].

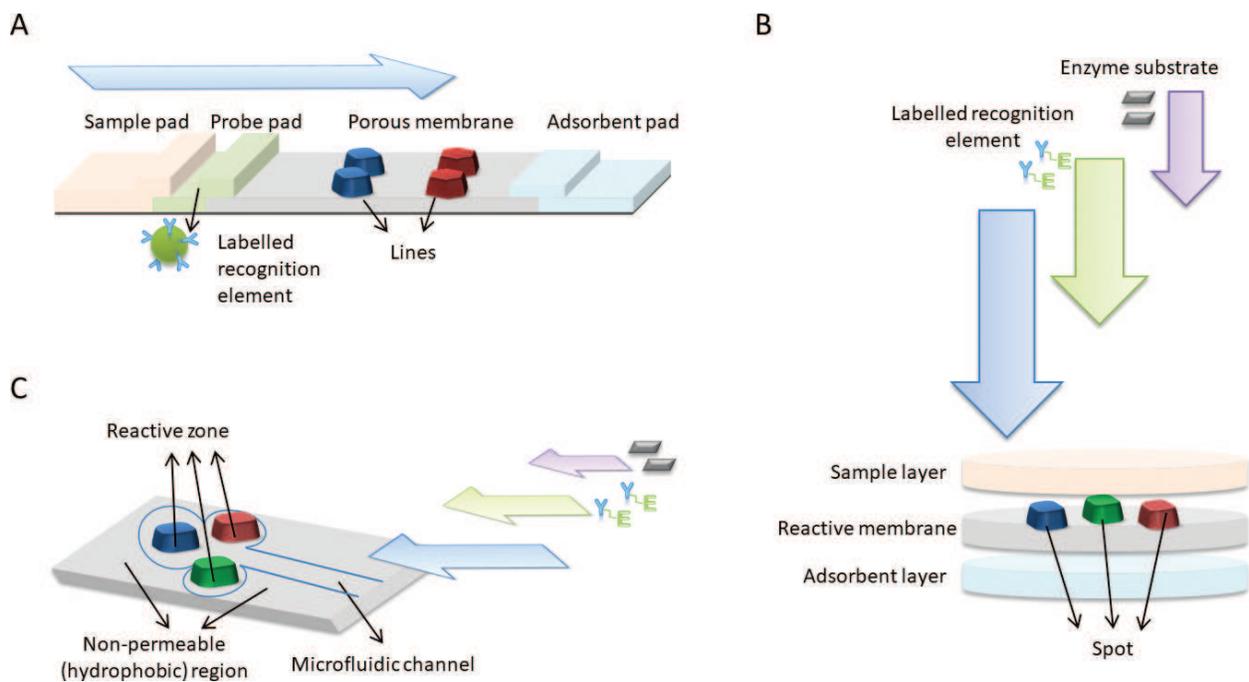


Figure 3. Schematic picture of generic lateral flow (A), flow-through (B), and paper-based (C) rapid tests. In the LFA architecture, the sample flows and pulls the labeled recognition element along the porous membrane. Capturing reagents are anchored onto the membrane to form lines. The color produced by accumulation of the label (usually colored nanoparticles) at the lines is indicative of the presence (amount) of the analyte. Flow-through and paper-based microfluidic assays use enzymatic labels that are added to the device following the sample. The analyte modulates the binding of the labeled recognition element to the capturing reagent immobilized onto the reactive membrane as spots or the reaction zones. Upon the further addition of the enzyme substrate, the color develops and indicates the presence (amount) of the analyte. Different capturing reagents disposed as spots in array format (or in different reactive zones) allow for multiple detection.

Paper-based microfluidic assays have been introduced more recently. In fact, the lateral flow and the flow-through assays can be also regarded as “paper-based” assays, since the porous/reactive membrane is made up of cellulosic materials [7, 8]. However, the paper-based microfluidic assay definition strictly applies to devices that use filter papers (of various grades) and a single cellulose-based material for playing the role of the sample, reactive and adsorbent material. This is obtained by folding a single sheet of paper so that paper-based devices are described as origami devices. The paper surface is modified with hydrophobic materials (typically wax) to fabricate hydrophilic microfluidic channels that convey the sample toward reactive zones where capturing reagents are immobilized. The principle of the assay relies on capillary forces as in the LFAs, while detection is accomplished by the enzyme-mediated production of the signal. Therefore, as for flow-through assays, several sequential steps to apply the sample, the probe, and the enzyme substrate are required [7, 8]. Usually, the device furnished an electrochemical output and requires to be coupled to microelectrodes. In such a way, paper-based microfluidic assays can be regarded as a bridge between equipment-free rapid tests and biosensors.

Flow-through and paper-based microfluidic assays are also available in array form, where multiple capturing reagents are immobilized on the same support and eventually multiple probes are used to allow the simultaneous detection of several analytes in one sample, therefore reaching high-throughput analysis.

Independently on the assay architecture, the detection of rapid tests relies upon the generation of a colored output that allows for the visual inspection and does not require any reading system. In this way, the result is partially subjective and usually provides a bare yes/no response, according to the level of the analyte being above or below a cutoff value.

2. Advances in “point-of-need” testing

Nowadays, rapid tests are widely applied as an efficient screening method for conducting on-site analysis for several applications, such as food, forensic, and environmental analysis.

Still, the medical field represents the largest for a number of available systems and a variety of applications. The availability of specific and sensitive diagnostic tools that can be operated almost everywhere and by no-trained operators is strongly appreciated in low-resource settings and is regarded as a feasible way for improving access to care.

In this field, some lines of future developments can be traced.

Provided that rapid tests are intended for self-testing and also for frequent testing, the preferential specimen should be repeatedly and noninvasively collected. Moreover, noninvasive collection enables sampling children and non-collaborative patients (i.e., for testing abuse drugs, sport doping, adherence to therapy, etc.). Therefore, diagnostic devices capable of working with specimens other than blood (i.e., urine, saliva, hair, and feces) are expected to expand. Parallel to the evolution of the materials and device architecture required to achieve this task, also a robust confirmation of the clinical relevance of detecting a biomarker in an unconventional specimen is needed.

Furthermore, we are entering the era of personalized medicine that is giving new impulses to the development of diagnostic tests enabling each patient to watch over himself/herself.

Beside clinical diagnostics, rapid tests are expanding to several other fields of applications. The role played by these analytical tools in veterinary, forensic investigations, and food safety controls is worth mentioning. Application to veterinary diagnostics is the natural continuation of the human diagnostics and takes advantage especially of the development toward exploitation of unconventional biological matrices (e.g., saliva, hair) that can be collected easily from animals by the veterinarian.

The same specimens are also useful in forensic investigation as they represent samples that can be collected from non-collaborative subjects. Other aspects that are of utmost relevance for the forensic use of rapid tests are their on-site usability and speediness of response that allows for implementing actions of controls at the street level and timely intervening in dangerous situations.

Food safety controls represent a vast area of applications for rapid tests, mainly because of international regulations that are becoming more and more severe about the quality of food (i.e., the need to assess the absence of contaminants, to discover frauds, to trace production and transformation chain, to ascertain freshness, to confirm origin, etc.) and because of the globalization of trades. Key features of rapid tests that especially meet requirements in ascertain food safety are cheapness and rapidity. The first feature enables self-testing carried out by producers, transformers, and sellers, thus assuring the safety across the whole food chain. The second feature is of paramount relevance when dealing with perishable goods. It should be noticed in this context that the designing of rapid tests for food analysis implies facing a number of diverse sample typologies, each deserving an appropriate study of materials and reagents.

Synchronous to the extension of applications and thanks to the increasing scientific activity on the subject, the research on rapid tests is being drawn from the urgency of immediate marketability. Therefore, new and most efficient probes to generate the signal have been proposed, such as those based on fluorescence, chemiluminescence, and fluorescence quenching detection and on magnetic nanoparticles. Several signal enhancement strategies have been described for improving sensitivity. Besides, artificial recognition elements with increased stability and robustness compared to antibodies have been incorporated in rapid test devices. In particular, aptamer-based LFAs are the new frontiers in this area.

Finally, test design and device architecture are becoming more flexible to adapt to novel demands, such as the hyphenation with PCR amplification to detect molecular markers and the increase of multiplexing capacity.

Conflict of interest

Authors declare no conflict of interest.

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