Expert Opinion

Nucleic acid lateral flow tests for molecular diagnosis: an update

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Lateral flow tests for molecular diagnosis have enormous potential in the fields of clinical practice, food safety, genotyping and environmental contamination. This paper describes the basic features of nucleic acid lateral flow (NALF) tests and some recent evolutions in this domain. The paper intentionally does not report on the accuracy of NALF tests, a largely under-investigated aspect of these tests. As for lateral flow immunoassays, NALF tests are intended to simplify detection of an analyte. Thus, designing NALF tests that are less dependent on or independent of sophisticated equipment remains a major challenge.

Keywords: dipstick, lateral flow, molecular diagnosis, nucleic acid

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1. Background on nucleic acid lateral flow tests

Molecular diagnosis is a general term for the detection of nucleic acid sequences of interest in a specimen under examination. The type and origin of the specimen can vary largely, for example, geological sediment, waste water, food product, biopsy material, and so on. Molecular diagnosis is widely applied in human and veterinary medical research and diagnostic practice aimed at explaining the health condition of a person or an animal by the presence of a pathogenic organism or a particular genotype. Starting with Southern blot 40 years ago, the technology behind molecular diagnosis has evolved tremendously [1]. Some of these technologies are well established and terms such as polymerase chain reaction (PCR), real time PCR, fluorescent *in situ* hybridisation (FISH) and loop-mediated isothermal amplification (LAMP) seldom need explanation in publications.

For applications such as monitoring of pandemic influenza, high-throughput sample preparation and testing is necessary and can now be carried out with fully or semi-automated laboratory equipment such as the GeneXpert® system of Cepheid. At the other end of the spectrum, some researchers and companies invest in the development of simple, integrated systems, sometimes called 'lab-on-chip' (LOC), which should be appropriate for testing low sample numbers and that preferably are applicable out of the laboratory. An example of LOC that is able to extract and detect bacterial DNA and viral RNA in saliva has been described by Chen and co-workers [2]. Another example is the GeneSTAT® system, commercialised by DxNA, including kits for the detection of avian influenza RNA that are now being tested by the International Atomic Energy Agency (IAEA) in some tropical countries [3]. Whether LOC will revolutionise the domain of molecular diagnostics remains, however, an open question.

Still, the demand for point-of-care (POC) molecular diagnostics, not necessarily in the form of an integrated system, is high, particularly under conditions where the molecular biology laboratory infrastructure is basic or almost absent. What exactly

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can be called a molecular POC test depends on its application environment. The technological environment in a European hospital where a patient should be tested for methicillin-resistant *Staphylococcus aureus* is rather different from the situation in a health centre in sub-Saharan Africa where a patient is to be tested for *Trypanosoma brucei gambiense*, the parasite causing sleeping sickness. Yet, for both applications, PCR-based molecular tests have been developed that make use of a lateral flow device for the simplified visualisation of the amplification product [4,5]. Lateral flow devices, also called 'dipstick', were first developed for immunoassays and have only recently been introduced in molecular diagnosis [6]. In the field of immunoassays, lateral flow tests occupy a considerable segment of the market. The same trend is now visible in molecular diagnosis, as exemplified by the ever growing number of different nucleic acid lateral flow tests (NALF) on the market, particularly in the domain of infectious diseases (human, animal, plant), genotyping, food safety and environmental contaminants. Obvious advantages of NALF tests over lateral flow immunoassays are that the target sequence, whether DNA or RNA, can be amplified before detection, and the tests can be applied in infections when antibodies are absent (early infection, HIV) or when antibodies persist after cure. Also, developing specific primer or hybridisation sequences is far easier than generating specific immunoreagents such as antigens or antibodies. On the other hand, NALF tests are in general more expensive than most lateral flow immunoassays and actually depend on rather sophisticated equipment for prior target amplification and/or for reading of the reaction.

Anyone interested in NALF tests should be aware of the rather confusing variety of denominations in use, sometimes also referring to the amplification method preceding the amplicon detection step, for example, LAMP-lateral-flow dipstick [7], PCR-oligochromatography [8], cycling probe technology-strip assay [9], PCR-immunochromatography [10], lateral flow-DNA test [11], dipstick-type biosensor [12], NALF immunoassay [13], line blot hybridization [14] and speed-oligo assay [15].

The general outline of a NALF device is similar to a lateral flow test strip used in immunoassays and consists of a backing membrane covered with a migration membrane on which several capture ligands are coated in the form of lines or dots. An excellent, although a bit outdated, review for laypersons is given by Seal *et al.* [6]. As an example, the generic PCR-oligochromatography strip developed by Coris BioConcept is represented in **Figure 1**. A liquid sample containing the target nucleic acid strand is applied on a 'conjugate' or 'sample' pad of absorbent material on one end of the strip and will migrate by capillary force along the membrane to the other end of the strip where it is eventually absorbed by another absorbent 'waste' pad. During the migration, the target sequences and control reagents are immobilised and concentrated by the capture ligand lines. Some NALF strips are functionalised on both sides, allowing for more controls. In some cases, the strips are encapsulated in a plastic housing with apertures for sample application and for reading of the result. A variety of systems are available to capture the nucleic acid sequence of interest onto the lateral flow membrane. This can be achieved by means of non-covalent interactions between antigen and antibody or biotin and avidin or probe hybridisation and their combinations. Target nucleic acid sequences in the sample can be 'tagged' during amplification or hybridisation steps before or after application on the strip. In principle, colorimetric enzyme reactions can be used to visualise the captured target sequence and controls, but enzyme reactions are not appropriate for simple and fast POC testing. Most of the NALF tests now on the market make use of nanoparticle detection signals. Nanoparticles can be colloidal gold or silver, coloured polystyrene microspheres, paramagnetic particles, carbon nanotubes, dye-charged liposome nanovesicles, quantum dots (QDots), up-converting phosphor (UCP) reporter particles, and so on. Colloidal gold, carbon nanotubes, coloured polystyrene microspheres and lyposome nanovesicles generate coloured lines on the NALF test strip that allow reading of the result by the naked eye. Fluorescent microspheres and Qdots emit visible light when excited with ultraviolet light, and UCP reporter particles become visible when excited with infrared light. NALF tests based on these light-emitting nanoparticles have the disadvantage that they can be read only with the help of special instrumentation. On the other hand, these readers, some handheld, allow detection of very low concentrations of target nucleic acid in a sample. Of special interest is upcoming technology that allows the output of a NALF reader to be transmitted to a central laboratory by means of a mobile phone [3].

An interesting characteristic of NALF tests is the possibility of simultaneous testing for multiple conditions. A well-established example is the human papillomavirus genotyping INNO-LiPA® test developed by Innogenetics. Recently, NALF tests have been miniaturised and transformed into a microarray format, thus increasing the potential information capacity and decreasing the amount of test sample needed [16]. Miniaturisation is a general trend in technology development with a lot of advantages, but in the field of diagnosis it has a disadvantage: not only the volume of sample but also the amount of target analyte are decreased. To solve this dilemma, as well as to overcome the need for previous enzymatic amplification of target nucleic acid sequences, researchers continuously investigate the signal enhancement capacities of new materials and chemistry to increase the sensitivity of nucleic acid biosensors in detecting nucleic acid hybridisation events. An extensive review on nanomaterial-assisted hybridisation signal enhancement has been published by Liu *et al.* and reports, among others, on direct electrical detection of DNA facilitated by gold nanoparticles [17]. It may be expected that these investigations will allow further simplification of NALF tests to become as easy to use and affordable as lateral flow immunoassays.

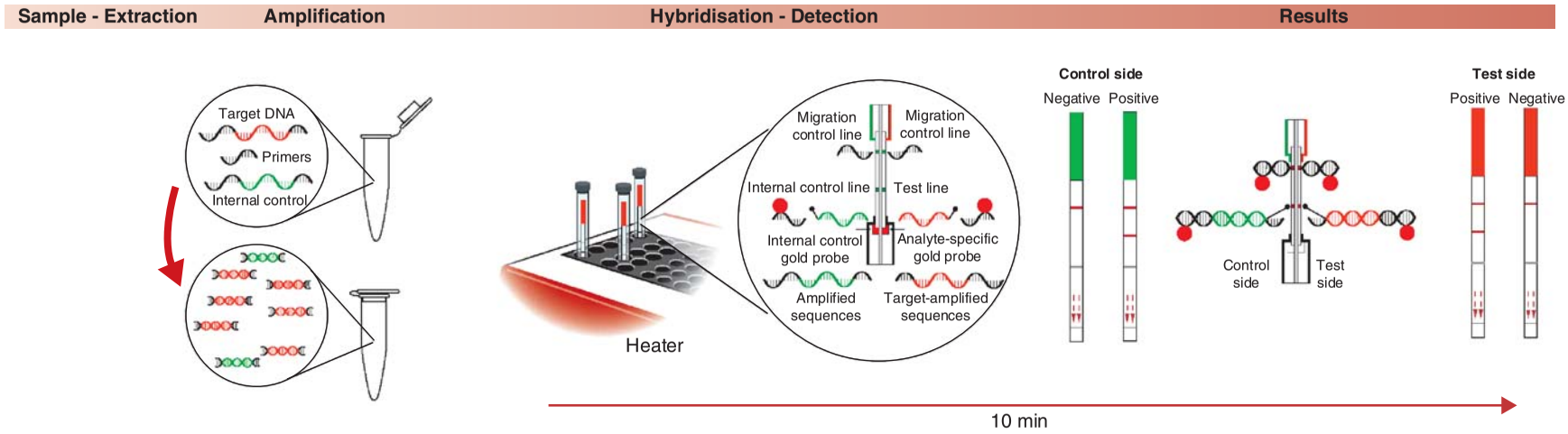

Figure 1. Coris BioConcept PCR-oligochromatography test as an example of a NALF test with previous PCR amplification of the target sequence and with one control and one test side.

Used with permission from Thierry Leclipteux, CEO of Coris BioConcept.

NALF: Nucleic acid lateral flow; PCR: Polymerase chain reaction.

2. Expert opinion

Molecular diagnostics is a rapidly evolving domain, and in particular the development of POC tests has attracted a lot of interest from researchers and developers in the public and private sectors. As for immunoassays, the lateral flow devices allow rapid and sensitive detection of nucleic acid sequences in a liquid sample. Several systems exist to capture and concentrate the target nucleic acid sequences on the lateral flow membrane for subsequent detection. Whether new molecules such as aptamers will ever replace the capturing antibodies, avidin or nucleic acid probes is uncertain. Also uncertain is the future of LOC systems integrating enzymatic amplification of target sequences, for reasons of technological requirements, small spectrum applicability and price. NALF test formats, preferably generic, that are independent of previous amplification reactions will probably be more attractive for the end-user. In this respect, the challenges are high and a reasonable equilibrium between test specimen volume and analytical sensitivity of the detection system will determine the applicability of the system. Whether the tests will need a reading instrument or not is of less importance, except when a quantitative result is expected; but readers should be robust, easy to use and, for remote use, chargeable with a solar panel. Sending reader output to a central laboratory by means of a mobile phone is a sexy topic, but the author is not convinced about the real demand. More may be expected from research into signal enhancement, multiplex and microarray formats. Again, designers of multiplex and microarray molecular diagnostics and in particular NALF tests should find a balance of what technically is feasible and what the eventual application and price of the test will be. Also in the domain of diagnostic immunoassays, only a few multiplex tests have reached the market. It may also be expected that in the near future NALF tests will also be useful to assay the expression of a particular gene instead of its mere presence. In this respect, research into detection of micro-RNAs for diagnosis and monitoring of several cancers (breast, prostate, lung) and inflammation conditions (sepsis, lupus, rheumatoid arthritis) is noteworthy. Micro-RNAs are short (19 – 23 nucleotides) non-coding RNAs controlling gene expression by interfering with protein synthesis. Usually, their concentrations in blood or other body fluids are very low, thus making their detection extremely challenging [18].

Notwithstanding all the positive aspects of NALF tests, stakeholders and particularly end-users should keep in mind that a test readout still has to be interpreted for its significance. For example, the presence of DNA from a pathogenic organism in the blood of a person does not necessarily mean that this person needs treatment. Thus, designing new NALF test applications should be preceded with careful investigation of the niche it is expected to occupy.

As a final note, the author wishes to express his concern about the general lack of independent studies investigating the accuracy of commercially available NALF tests. International organisations such as the World Health Organization and the Food and Agriculture Organization could play an important role in this domain, to safeguard not only the interests of end-users, but also the interests of diagnostic test developers.

Declaration of interest

The author states no conflict of interest and has received no payment in preparation of this manuscript.

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