Lateral Flow Assays and their applications

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Abstract— Lateral flow assay is a simple and inexpensive test with a huge potential, not only for home tests as the well-known pregnancy test but for clinical diagnosis at point-care and hospitals. Recently, experts in this field have been developing lateral flow assays to detect different analytes increasing their applications. These tests were designed to improve our life and undoubtedly, they could become in applications that would improve our daily life, probably thanks to our own smartphone.

Keywords— Lateral Flow assay, Pregnancy test, Prostate Specific Antigen, smartphone

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1. INTRODUCTION

Lateral Flow Assays (LFA) are simple tests based on a device able to detect an analyte. Traditionally, they have given a positive/negative result, as the well-known home pregnancy test. However, there are experts improving these tests adding them quantitative abilities. For this aim, the coloured particles that we could see are being replaced by magnetic beads and magnetic sensors. [1]

Many experts in the field are trying to introduce these LFA in the daily routine of Hospitals and even at home due to its easy of use, rapidity and its price. The number of these tests in the market has increased a lot, just as their applications. Recent works have described that they can be used to detect several biological entities as allergens in the food, human hormones, biomarkers of diseases as hearth damage or cancer and bacteria. Here, we are going to discuss the characteristics of LFA and some of its applications. [1],[2]

2. SELECTION OF LATERAL FLOW IMMUNOASSAYS AS A GOOD DIAGNOSTIC TEST

A general selection criteria for a good diagnostic test is the ASSURED criteria. It means that a test should be Affordable, Sensitive, Specific, User-friendly, Rapid and Robust, Equipment-free and Deliverable to those who need it. Of course, this is a general criteria and each case needs to be studied because not always a test for a specific issue can be simplified to the ASSURED criterio. [2]

3. DIFFERENT APLICATIONS FOR LATERAL FLOW ASSAYS

3.1. Pregnancy test

The first pregnancy test appeared in 1976 what is really recent. However, in less than 50 years this test has become the diagnosis test most used at home. Its success relies on the analyte chosen to be studied, which is the human chorionic gonadotropin (hCG) because it increases in early pregnancy and it's easy and rapid to see this increase just using urine as sample. Nowadays, as a result of its success, there are many brands that commercialize these tests and there are different types of them. [3]
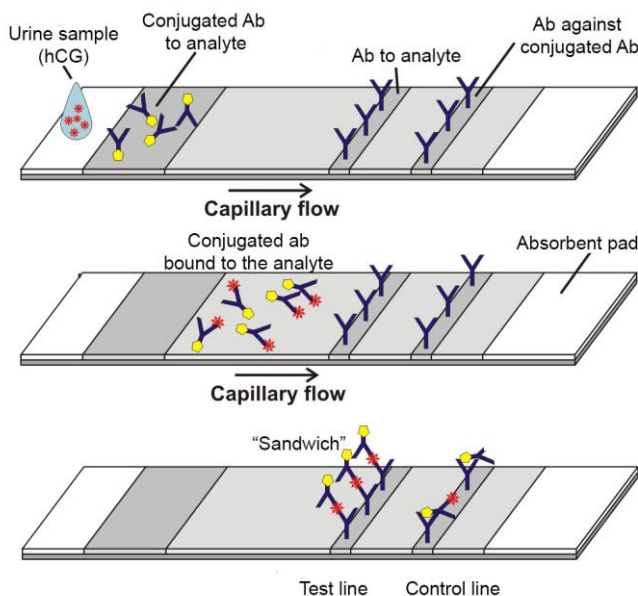

Fig. 1. Basis of the LFA of the pregnancy test. It is posible to see the flow of the analyte bound to the conjugated antibody along the strip, arising both, the test and the control line. Image adapted from [5].

The simplest basis of this device consists on a strip where the sample, in this case urine, travels by capillarity through it. The sample is placed in one end of the strip (sample pad) that contains buffers and salts that allows the analyte, if it is present, to be recognized. The next step in this movement is the recognition of the analyte by a conjugated or labelled antibody specific for this analyte. These antibodies can be conjugated with coloured, fluorescent or gold particles that allows its visual detection. The antibodies conjugated bound or not to the analyte migrates along the strip and they are going to find two detection lines. The first one is the test/detection line for the analyte under study and it has immobilized specific antibodies against the analyte. If a "sandwich" of the analyte between two antibodies appears in the test line

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means pregnancy. The second one is the control line and it has immobilized specific antibodies for the antibody conjugated used in the test. So, it has to be always positive if the test has worked properly. At the end of the strip, an absorbent material is placed to ensure the flow of the sample and to avoid the backflow. In Figure 1 it is possible to see a strip and the distribution of the elements described according to the direction flow. [4][5]

3.2. Prostate Specific Antigen as a biomarker for the detection of prostate cancer

LFA is considered as a good aplication not only for home tests but also for hospitals. LFAs are easy and quick tests that wouldn't need even qualified professionals. So, it would be a relevant and low cost-implementation to the rapid diagnosis of different diseases. [6]

In this article, we are going to focus in a test to detect the Prostate Specific Antigen (PSA) wich is a generally accepted biomarker for the detection of prostate cancer. In fact, it has been studied that when serum PSA concentration is above 4 ng/mL correlates with the possible presence of prostate cancer with a 25% of probability. In this situation, further analysis would be needed to check the disease. If the concentration of PSA is above 20 ng/ml the probability of suffering prostate cancer arise until 80% and it is almost 100% when is higher than 30ng/mL. [7][8]

PSA can be measured through LFA in strip being this time the sample serum, instead of urine and PSA the analyte recognized. The conjugated antibodies are often labelled with gold nanoparticles whose gold is reduced with sodium citrate giving a red color that allows a visual and qualitative detection, as we have seen in pregnancy test. [8][9]

However, to know other type of LFA used to determine PSA concentration in serum, we are going to introduce one that use paramagnetic particles (PMPs). The analyte PSA is recognized for an antibody conjugated to PMPs that are particles whose behavior is magnetic when they are in a magnetic field. These PMPs act as as labels that are detected thanks to their magnetic properties. When the antibody and the PMPs are in the test and/or the control line, they are measured by a device called resonant coil magnetometer (RCM). RCM will measure PMPs in the antibodies along both lines and not only those visible on the surface but all of them. That's why this is a truly quantitave analysis. PMPs have advantages like they allow a quantitative test and they are stable over long period of time, so it is posible to reanalyze the strip. In figure 2 we can see all the elements described. [7]
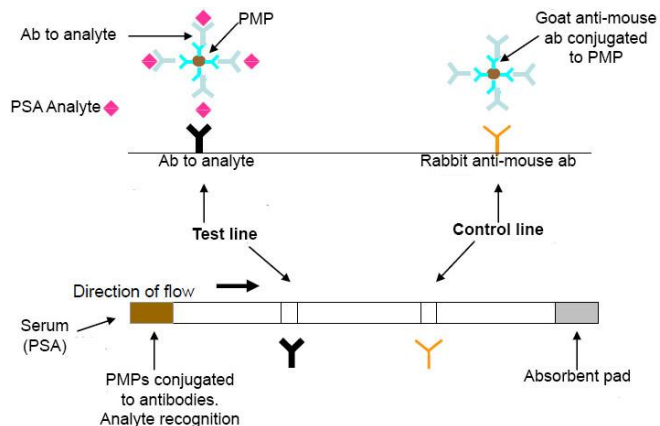

Fig.2. Basis of the LFA using PMPs to detect PSA. It is possible to see the direction flow of the elements and above the strip we can see in detail the structure recognized in both, test and control lines. Adapted from [7]

The measurement of PSA is controversial because of the indolent and non-invasive nature of most prostate cancer. A severity biomarker is needed to treat the disease just in the cases that really need a treatment. PSA and other severity biomarkers could be measured in the same strip at the same time using PMPs. [7],[10]

3.3. Smartphones in LFAs

A recent advance in LFAs is the use of smartphones to read the outcomes of the strips. It allows to transform ordinary qualitative test into quantitative test without having to use expensive or complex equipment. [11]

To use your smartphone as a LFA reader, it would be necessary to download the app, run your LFA measuring the analyte desired and read the result with your new app. Besides, it has other advantage which is the storage of your result with the possibility of sharing them with for example your doctor or other people or synchronize them with other devices. In figure 3 it's posible to see this workflow.
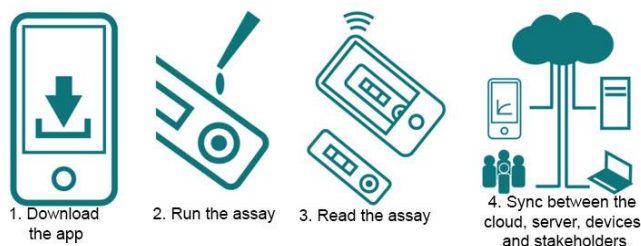

Fig. 3. Workflow using a smartphone as LFA reader. [12]

This app can be used at innumerable fields, for instance, in agriculture to check the presence of pathogens preventing devasting losses. Moreover, the data collected can be storage and linked to their geographic data helping other producers to control plagues. The same can be done with enviromental contaminants that can affect water quality as microorganisms, heavy metal, pollutants, etc. All these

alements can be geotagged and time stamped creating a map useful for travellers to avoid infections among others. [11]

This smartphone reader is also useful to healthcare. It can empower patients to manage their conditions at home saving and/or sharing the results of their LFAs with their doctor. It would be possible to meassure many analytes that need to be under control but one of them that has been described is the cortisol. Cortisol is a biomarker of physiological stress which is one of the major causes of psychiatric diseases. Cortisol would be the analyte that can be measured in the sample of saliva and the colourimetric signal from the LFA would be readed using a smartphone, obtaining a quantitative result. These real-time measurements allow a patient's situation monitoring and an easy data record that can can improve the treatment and the patient life. [13],[14]

Related to healthcare, LFAs have been really attractive for doctors that are working in difficult areas like poor countries, war regions or after a catastrophe because they are simple and low-cost test that can provide valuable information and with this application for the smartphone the result can be quantitative without any bulky equipment. [11],[13]

CONCLUSIONS

Lateral Flow assay is understood as a technique affordable and rapid to evaluate different analytes. LFA is a versatile test that results interesting in clinical enviroments, in different industries and at home. Their applications are in exponential growth, even more with the introduction of the smartphone as a reader, and in this article have been reviewed only few of these applications.

REFFERENCES

[1] D. Lago-Cachóna, M. Rivasa, J.C. Martínez-García, M. Oliveira-Rodríguez, M.C. Blanco-López, J.A. García, "High frequency lateral flow affinity assay using superparamagnetic nanoparticles", Journal of Magnetism and Magnetic Materials, vol. 423, pp. 436–440, Feb 2017, doi: https://doi.org/10.1016/j.jmmm.2016.09.106

[2] C.S. Kosack, A-L. Page and P.R. Klatser, "A guide to aid the selection of diagnostic tests," Bull World Health Organ, vol. 95, pp. 639-645, Jun 2017, doi: http://dx.doi.org/10.2471/BLT.16.187468

[3] C. Gnoth and S. Johnson, "Strips of hope: accuracy of home pregnancy tests and new developments", Geburtsh Frauenheilk, vol. 74, pp. 661-669, May 2014, doi: http://dx.doi.org/10.1055/s-0034-1368589

[4] K.M. Koczula and A. Gallotta, "Lateral flow assays", Essays in Biochemistry, vol. 60, pp. 111-120, June 2016, doi: 10.1042/EBC20150012

[5] L.G. Lee, E.S. Nordman, M.D. Johnson and M.F. Oldham, "A low-cost, high-performance system or fluorescence lateral flow assays", Biosensors, vol. 3, pp. 360-373, October 2013, doi: 10.3390/bios3040360

[6] E.B. Bahadir and M.F. Sezgintürk, "Lateral flow assays: principles,designs and labels", Trends in analytical chemistry, vol. 82, pp. 286-306, September 2016, https://doi.org/10.1016/j.trac.2016.06.006

[7] J.M. Barnett, P. Wraith, J. Kiely, R. Persad, K. Hurley, P. Hawkins and R. Luxton, "An inexpensive, fast and sensitive quantitative lateral flow magneto-immunoassay for total prostate specific antigen", Biosensors, vol. 4, pp. 204-220, July 2014, doi:10.3390/bios4030204

[8] I.P. Andreeva, V.G. Grigorenko, A.M. Egorov and A.P. Osipov, "Quantitative lateral flow immunoassay for total prostate specific antigen in serum", Analytical Letters, vol. 94, pp. 579-588, September 2015, https://doi.org/10.1080/00032719.2015.1075130

[9] E.B. Bahadir and M.K.Sezgintürk, "Lateral flow assays: principles designs and labels, Trends in analytical chemistry, vol. 82, pp. 286-306, September 2016, https://doi.org/10.1016/j.trac.2016.06.006

[10] T. Grozescu, F. Popa, C. Davila, "Prostate cancer between prognosis and adequate/proper therapy", Journal of medicine and life, vol.10, pp.5-12, October 2016.

[11] Web of Mobile assay. https://mobileassay.com/applications

[12] Web of Abingdon health. https://www.abingdonhealth.com/contract-services/smartphone-lateral-flow-reader-customisation/

[13] Web of Novarum. https://www.novarumdx.com/

[14] S. Choi, S. Kim, J.S. Yang, J.H. Lee, C. Joo and H.I. Jung, "Real-time measurement of human salivary cortisol for the assessment of psychological stress using a smartphone", Sensing and bio-sensing research, vol. 2, pp. 8-11, December 2014, https://doi.org/10.1016/j.sbsr.2014.08.001

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