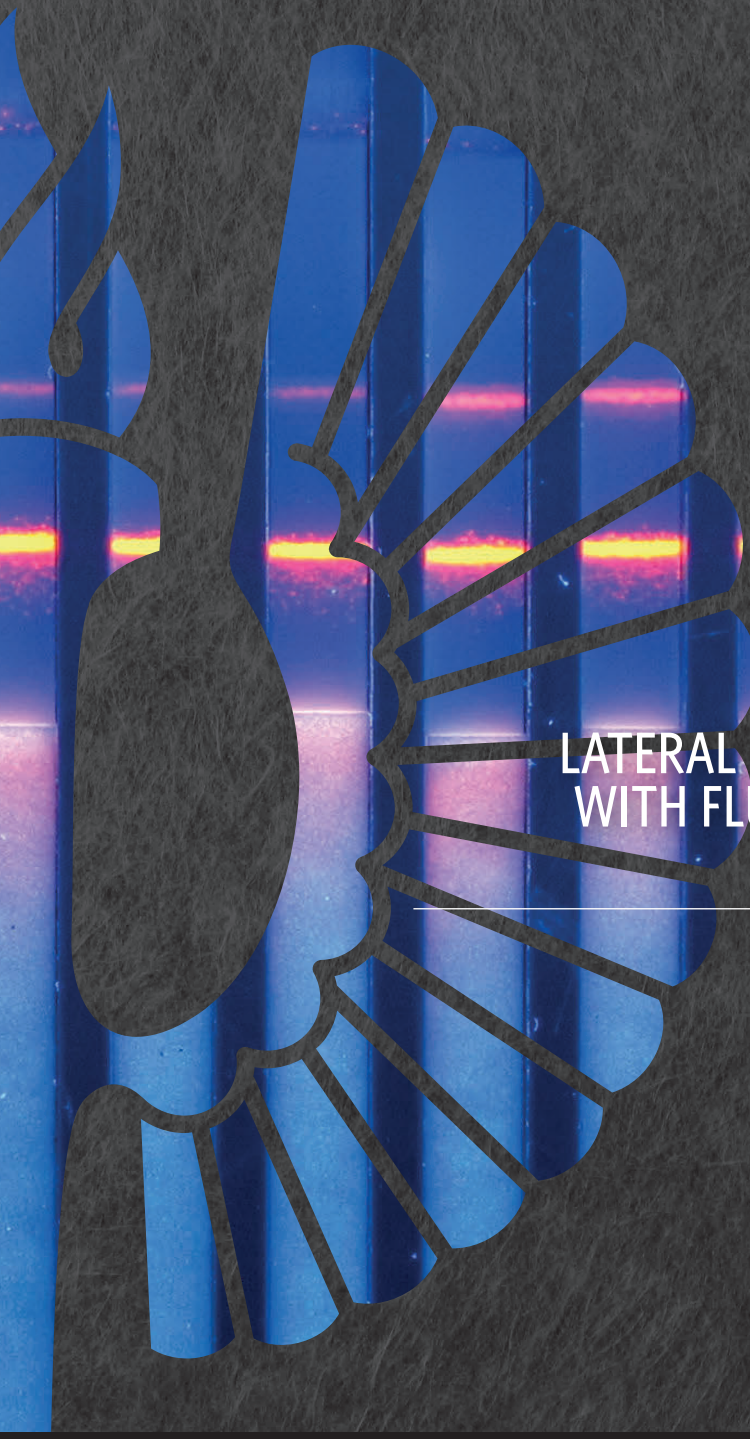
Turun yliopisto
University of Turku



LATERAL FLOW IMMUNOASSAYS WITH FLUORESCENT REPORTER TECHNOLOGIES

Etvi Juntunen

TURUN YLIOPISTON JULKAISUJA - ANNALES UNIVERSITATIS TURKUENSIS
Sarja - ser. AI osa - tom. 575 | Astronomica - Chemica - Physica - Mathematica | Turku 2018

Turun yliopisto
University of Turku

LATERAL FLOW IMMUNOASSAYS WITH FLUORESCENT REPORTER TECHNOLOGIES

Etvi Juntunen

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
Sarja - ser. A I osa - tom. 575 | Astronomica - Chemica - Physica - Mathematica | Turku 2018

University of Turku

Faculty of Science and Engineering
Department of Biochemistry
Molecular Biotechnology and Diagnostics
Doctoral Programme in Molecular Life Sciences

Supervised by

Professor Kim Pettersson, PhD
Department of Biochemistry
Molecular Biotechnology and Diagnostics
University of Turku
Turku, Finland

Professor Tero Soukka, PhD
Department of Biochemistry
Molecular Biotechnology and Diagnostics
University of Turku
Turku, Finland

Reviewed by

Adjunct Professor
Petri Ihalainen, PhD
MetGen Oy
Kaarina, Finland

Senior scientist
Aart van Amerongen, PhD
Wageningen University & Research
Wageningen, Netherlands

Opponent

Professor Richard O'Kennedy, Ph.D.
Vice president for research
Hamad Bin Khalifa University
Doha, Qatar

Cover image by author

The originality of this thesis has been checked in accordance with the University of Turku quality assurance system using the Turnitin OriginalityCheck service.

ISBN 978-951-29-7126-8 (PRINT)
ISBN 978-951-29-7127-5 (PDF)
ISSN 0082-7002 (PRINT)
ISSN 2343-3175 (ONLINE)
Painosalama Oy - Turku, Finland 2017

“If you hit a wrong note, it's the next note that you play that determines if its's good or bad.”

–Miles Davis

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications, referred to in the text by their Roman numerals (I-IV).

I Juntunen, E., Myyryläinen, T., Salminen, T., Soukka, T., & Pettersson, K. Performance of fluorescent europium(III) nanoparticles and colloidal gold reporters in lateral flow bioaffinity assay. *Anal. Biochem*. **428**, 31–38 (2012)

II Juntunen, E., Arppe, R., Kalliomäki, L., Salminen, T., Talha, S.M., Myyryläinen, T., Soukka, T. & Pettersson, K. Effects of blood sample anticoagulants on lateral flow assays using luminescent photon-upconverting and Eu(III) nanoparticle reporters. *Anal. Biochem*. **492**, 13–20 (2016)

III Juntunen, E., Salminen, T., Talha, S.M., Martiskainen, I., Soukka, T., Pettersson, K. & Waris, M. A lateral flow immunoassay with upconverting nanoparticle-based detection for measurement of interferon response by the level of MxA. *J. Medical Virol.* **89**, 598–605 (2016)

IV Salminen, T., Juntunen, E., Lahdenranta, M., Martiskainen, I., Talha, S.M. & Pettersson, K. Microparticle–based platform for point-of-care immunoassays. [manuscript]

ABBREVIATIONS

ASSURED	Affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, delivered
bio-BSA	Bovine serum albumin conjugated with biotin-isothiocyanate
BSA	Bovine serum albumin
CAS	Chemical abstracts service
CMOS	Complementary Metal Oxide Semiconductor
COP	cyclo-olefin polymer
CRP	C-reactive protein
cTnI	Cardiac troponin I
DPP	Dual Path Platform (technology by Chembio Diagnostic Systems Inc.
EDC	*N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride
ELISA	Enzyme-linked immunosorbent assay
FDA	(U.S.) Food and Drug Administration
fPSA	Free prostate-specific antigen
HBsAg	Hepatitis B surface antigen
hCG	human chorionic gonadotropin
HIV	Human immunodeficiency virus
HRP	Horse radish peroxidase
HRP-2	histidine rich protein (malaria antigen)
IR	Infrared
IgG	Immunoglobulin G
LED	light-emitting diode
LF	Lateral flow
LFA	Lateral flow assay
LFIA	Lateral flow immunoassay
LSPR	Localized surface plasmon resonance
mAb	Monoclonal antibody
MEMS	Microelectromechanical system
MxA	Myxovirus resistance protein A
µPMT	miniaturized photomultiplier tube
NAAT	Nucleid acid amplification test
NHS	*N*-hydroxysuccinimide
NIR	near-infrared
NT-proBNP	N-terminal prohormone of brain natriuretic peptide
PCT	Procalcitonin
PMT	photomultiplier tube
POC	Point-of-care
PSA	Prostate-specific antigen
PVC	polyvinyl chloride

RDT	Rapid diagnostic test
RF	Radio-frequency
SERS	Surface-enhanced Raman spectroscopy
TB	Tuberculosis
TSA	Tris saline azide buffer
TPP	Target product profile
UCNP	Upconverting nanoparticle
UV	Ultraviolet
VIS	Visible
WHO	World health organization

ABSTRACT

Lateral flow assays (LFAs) are user-friendly diagnostic test devices most commonly known from the home pregnancy tests. Since their appearance in the market in 1980's, LFAs have become well-established and products have been developed for various applications, but the most commonly sold LFAs still have the same basic features as the early products. Compared to other rapid diagnostic test (RDT) platforms, the main benefits of LFAs include inexpensive manufacturing costs, relatively fast assay development process, and the stand-alone capability of the test to be used without any instrumentation.

The analytical membrane that provides the solid support for the bioassay reagents and allows the liquids to migrate through the binder lines by capillary force is almost exclusively manufactured of nitrocellulose. As the nitrocellulose remains the most widely used material, its optical properties, mechanical robustness, and chemical stability are not optimal for the RDT development. However, the established status of the nitrocellulose membrane in the RDT industry and the continuous product development suggests that the material will remain in LFAs for years to come.

Typically, in LFAs, the coloured reporter particles form visible lines on the analytical membrane depending on the presence or absence of the analyte of interest. The visible lines can be interpreted visually without any instrumentation. However, the visual assessment of the assay read-out is prone to subjectivity in interpretation and can be affected by poor lighting conditions. Moreover, the visual read-out can only be used to generate a qualitative or a semi-quantitative result.

The versatility of the lateral flow technology can be improved by using efficiently quantifiable reporter technologies such as fluorescent nanoparticles. However, the drawback of pursuing high analytical sensitivity and quantitative results by fluorescent reporter is the apparent need for a reader instrument. With fluorescent reporters, the optical properties of the assay membranes and sample fluids must be considered in order to achieve minimal interference to the detection of the reporters. Autofluorescence originating from the assay materials can be avoided by using the upconverting nanoparticle (UCNP) detection technology. Nevertheless, the non-analyte specific background signal can still occur from non-specific binding of the reporter particles.

The aim of the thesis is to explore the opportunities arising from the use of different fluorescent reporter particles to improve the analytical sensitivities of LFAs, and to evaluate the feasibility of fluorescent reporter particles as a substitute for common visually detectable reporters. Exploiting the increased detectability of the reporter particles to improve the assay sensitivity requires careful re-optimization of the assay conditions.

TIIVISTELMÄ

Lateraalivirtausmääritykset ovat helppokäyttöisiä diagnostisia testilaitteita, jotka ovat tulleet tunnetuksi kotona tehtävistä raskaustesteistä. Tultuaan markkinoille 1980-luvulla lateraalivirtausmääritykset ovat saavuttaneet vakiintuneen aseman ja tuotteita on kehitetty monenlaisiin sovelluksiin. Useimmissa kaupallisissa lateraalivirtausmäärityksissä hyödynnetään kuitenkin edelleen samoja perusominaisuuksia kuin varhaisimmissa kaupallisissa tuotteissa. Merkittävimpinä etuina lateraalivirtaustesteissä muihin pikatestikonsepteihin verrattuna on edulliset valmistuskustannukset, nopea määritysten kehitysprosessi sekä mahdollisuus käyttää määrityksiä ilman erillisiä laitteita.

Analyyttinen kalvo toimii biologisen määrityksen sitojamolekyylien kiintokantajana ja mahdollistaa määrityksen liuosten kulkemisen sitojamolekyyliviivojen läpi. Analyyttinen kalvo valmistetaan yleisimmin nitroselluloosasta, vaikka sen optiset ominaisuudet, mekaaninen kestävyys ja kemiallinen pysyvyys eivät ole optimaalisia pikatestien kehittämistä varten. Nitroselluloosan vakiintuneen aseman pikatestiteollisuudessa ja nitroselluloosakalvojen valmistusprosessien kehittymisen perusteella nitroselluloosan korvautumista muilla materiaaleilla ei ole odotettavissa lähivuosina.

Tyypillisesti lateraalivirtausmäärityksissä käytettävät värilliset leimapartikkelit muodostavat silmin nähtävät viivat analyyttiselle kalvolle riippuen määritettävän analyytin läsnäolosta. Tuloksen tulkintaan ei tällaisessa määrityksessä tarvita erillistä mittalaitetta. Määritystuloksen visuaalinen tarkastelu on kuitenkin subjektiivista ja lisäksi huonot valaistusolosuhteet voivat aiheuttaa virheellisiä tuloksia. Visuaalisen tuloksen tarkastelun puutteena on myös kvantitoitavuuden puute, sillä silmämääräisesti tarkasteltavat tulokset voidaan tulkita ainoastaan kvalitatiivisesti tai puolikvantitatiivisesti.

Lateraalivirtausmääritysten käyttökelpoisuutta erilaisiin sovelluksiin voidaan parantaa käyttämällä kvantitatiivisia leimateknologioita, kuten fluoresoivia nanopartikkelileimoja. Korkean herkkyyden ja kvantitatiivisten tulosten tavoittelemisessa fluoresoivien partikkelien haittapuolena on kuitenkin tarve käyttää erillistä mittalaitetta. Fluoresoivia nanopartikkelileimoja käytettäessä on myös huomioitava kalvomateriaalin ja näyteliuoksen optiset ominaisuudet mittaustarkkuutta heikentävien häiriöiden minimoimiseksi. Näytteestä ja kalvomateriaaleista johtuvaa autofluoresenssia voidaan välttää käyttämällä leimoina käänteisviritteisiä nanopartikkeleita.

Autofluoresenssin lisäksi epäspesifistä signaalia määrityksessä aiheuttaa myös analyyttiselle kalvolle juuttuneen leimapartikkelit.

Tämän väitöstyön tavoitteena oli tutkia erilaisten fluoresoivien leimapartikkeleiden tuomia mahdollisuuksia lateraalivirtausmääritysten analyyttisen herkkyyden parantamiseksi sekä arvioida mahdollisuuksia korvata tavallisia visuaalisesti tarkasteltavia leimapartikkeleita fluoresoivilla leimapartikkeleilla. Fluoresoivien partikkeleiden hyvän mitattavuuden tuoman edun hyödyntäminen vaatii kuitenkin määritysolosuhteiden huolellista optimointia.

1 INTRODUCTION

During the late 1980s, the rapid test for pregnancy became available for home testing [1-3]. The pregnancy test's distinctive appearance with a self-contained assay device with visual lines appearing on a blank membrane became well-known. The rapid diagnostic tests (RDTs) based on such structure are commonly known as lateral flow assays (LFAs). Since the introduction of LFA for pregnancy testing, numerous other RDT applications have been developed mostly for clinical applications but also for other market segments such as food safety[4] and environmental diagnostics[5] using the lateral flow (LF) platform[6].

The most typical variant of LFAs is the lateral flow immunoassay (LFIA), also known as immunochromatographic assay or sol particle immunoassay[7]. In LFIA, antibodies are used to bind the target analyte from the sample fluid and detect a signal output generated by the antibody–antigen interaction. Immunoassay, in the original format that was first described in 1959, had mostly been restricted to laboratory use due to the requirements of precise liquid handling capability and sophisticated instruments.[8] Furthermore, the latex agglutination test from 1959[9] can be considered an another predecessor of the LFA.

However, following the home pregnancy test the trend to perform clinical diagnostics in point-of-care (POC) conditions has become more apparent. In addition to the pregnancy test, the most prominent simple POC assays include amperometric blood glucose tests[10], colour-indicator urine dipsticks[11], and lateral flow assays for human immunodeficiency virus (HIV)[12]. Owing to the simple usability, very low manufacturing costs[13], and rapid prototyping process[14], the LF-platform is the most popular format used in rapid immunoassays. The technology has gained wide acceptance in resource-poor conditions where the possibilities to perform clinical laboratory diagnostics is limited due to the lack of facilities, machinery, and trained staff.

Despite the many advantages of the technology[15], the applicability of LFIAs is limited by certain shortcomings. Assays requiring high analytical sensitivity or quantitative results may not be possible to produce by using the traditional reporter and detection technology relying on the visual interpretation of the test line intensity. Most of the currently available LFAs are usually capable of qualitative or semi-quantitative analysis with visually detectable reporter particles.[6] These limitations are logical considering the signal detection mechanism of the assay. The result is commonly inspected by the formation of visible test lines on the membrane, and the amount of the reporter particles retained on the test line should be sufficient to generate detectable decrease in light

reflection. The visual interpretation of the assay read-out does not require any instrumentation, but the capability to produce quantitative data can only be achieved by using reader instruments.

This thesis examines the potential of luminescent reporter particles in extending the capabilities of lateral flow assays. Special focus is placed on the upconverting phosphor nanoparticle (UCNP) technology[16] due to its unique feature of excluding all non-reporter-specific luminescence from the measurement[17]. The following literary review will focus on sandwich immunoassays in LF-platform with optically detectable reporters, providing an overview of the current technologies and the emerging opportunities with technological improvements.

2 REVIEW OF THE LITERATURE

2.1 Lateral flow assays

The concept of point-of-care (POC) testing has been defined in multiple ways, depending on the focus of the literature in question. Despite the differences, the consensus is as follows: "patient specimens assayed at or near the patient with the assumption that test results will be available instantly or in a very short timeframe to assist caregivers with immediate diagnosis and/or clinical intervention".[18]

The conditions where POC tests are used are very diverse. Simple and rapid tests are useful in very resource-limited conditions as well as in well-equipped primary care centres and hospital wards[19]. In modern healthcare settings in industrialized countries, the point-of-care tests are used by the primary care clinicians to support making the diagnosis[20]. The POC tests are valued for their promptly available results in both sophisticated medical care settings and developing countries where they are even more significant. In conditions where centralized laboratory diagnostics is not available to the public due to the lack of logistics, facilities and trained laboratory personnel, the rapid diagnostics tests (RDTs) have been successful in providing information of the causative agents of relevant infectious diseases[21-23].

Because of their low manufacturing costs, relatively quick assay development time, and user-friendly operating procedure, lateral flow assays (LFAs) have become a widely used format of affordable RDTs[6]. The World Health Organization's (WHO) list of prequalified diagnostic tests (updated 17 March 2017), which has been maintained since 2010, contains altogether 67 products, of which 31 are lateral flow assays with either antibodies or antigens used as binder molecules. The prequalification status indicates that the test performance matches the criteria defined by WHO.[24]

Despite being an established platform technology for the RDTs, most of the LFAs still rely on visual examination of the formed signals. In many cases, especially with occasionally used over-the-counter tests, a dedicated LFA reader device is not desirable by the consumer. This limits the use of LFAs to assays which do not require high analytical sensitivity or quantitative results. The selection of potential LFA applications can be broadened by implementing a reader device and a compatible reporter technology that upgrades the assays with higher analytical sensitivity and quantitative or semi-quantitative results.

LFIA technology as a research subject has become more popular after the turn of the millennium. Articles mentioning lateral flow immunoassay or immunochromatography were searched for on PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) using search terms "immunochromatograph*" and '"lateral flow" assay'. A total of 2745 publications mentioned immunochromatography in 1984–2015 and 1211 mentioned lateral flow assay in 1978–2016. A significant incline in the annual number of articles has taken place after the year 2000 (figure 1). Rarely used terms "sol particle immunoassay" and "sol particle assay" yielded only 21 distinct publications.

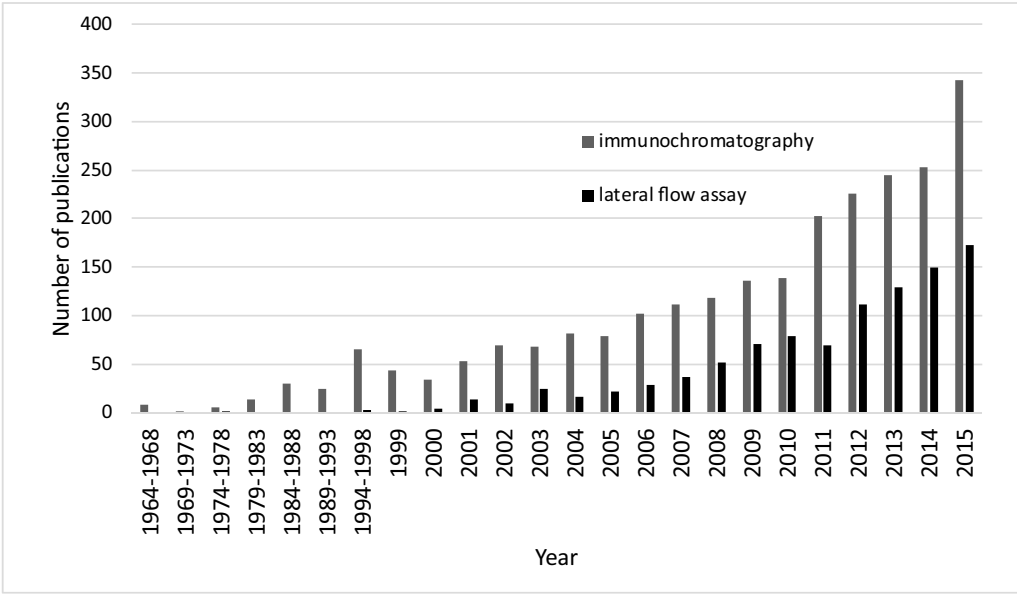


Figure 1. Numbers of research publications mentioning sol particle immunoassay, immunochromatography or lateral flow immunoassay from 1964 to 2016.

A database search for patent applications using Espacenet patent search (https://fi.espacenet.com/) with search terms '"lateral flow" assay' yielded a total of 541 hits and "immunochromatograph*" yielded 1382 hits. The annual numbers of patent applications follow the growing trend of increasing number of research publications (figure 2). First patent application mentioning immunochromatography was filed in 1982[25]. In 2007, a major increase in filed patent applications coincided with the expiration a of patent entitled "Solid phase assay with visual readout"[1]. Another fundamental patent covering the use of monoclonal antibodies in immunochromatography expired in 2003[26].

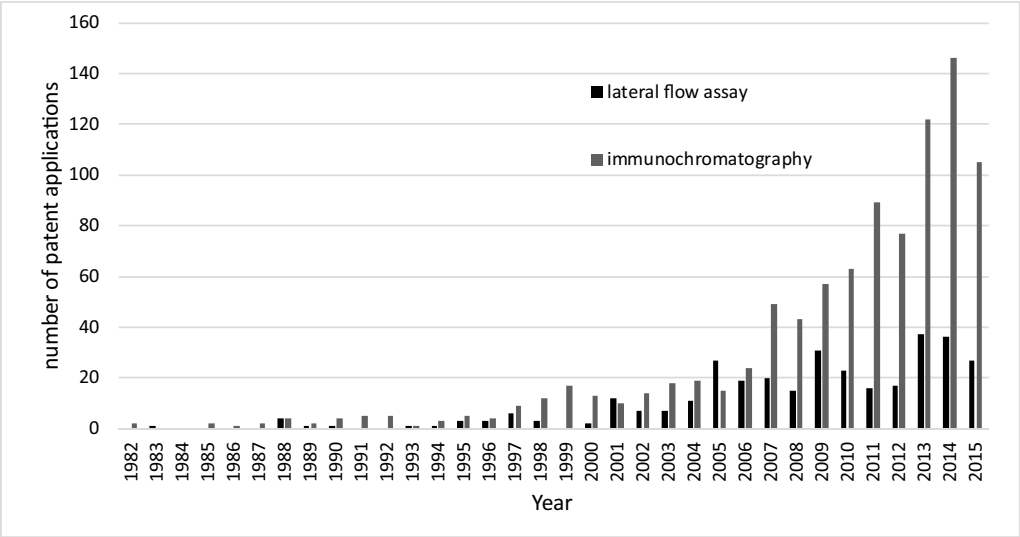


Figure 2. Annual numbers of patent applications mentioning immunochromatography or lateral flow assay from 1982 to 2016.

2.1.1 From discovery of capillary action to biochemical assays

According to J.C. Poggendorff, the capillary action was first described by Leonardo Da Vinci[27]. However, the understanding of the phenomenon started to emerge along the scientific revolution with the paradigm shift from Aristotelian mechanics to classical mechanics. In 1661, Robert Hooke presented a finding stating that the narrower the tube, the higher water rise in it[28]. Hooke was guided and employed by Robert Boyle who believed capillary action had been discovered by "some inquisitive Frenchmen"[29]. Boyle later became famous for the Boyle's law describing the relation of gas pressure and volume. The fundamental mechanics in capillary action are based on two phenomena: cohesion and adhesion[30]. Cohesion in this context refers to the intramolecular non-covalent forces attracting molecules towards each other to form a droplet. Adhesion in the capillary action means the non-covalent attractive forces between the liquid droplet and an external surface. The mechanisms of intramolecular interactions were practically unknown at the time when the significance of cohesion and adhesion were described in capillary action. Especially in water, the hydrogen bonding by electrostatic attraction of the water molecule dipoles contributes significantly to the cohesive forces.[31-33] Quantitative explanation of capillary action was published by Carl Friedrich Gauss in 1830 based on the research of Thomas Young, who developed the qualitative theory of surface tension, and Pierre-Simon Laplace, who presented the mathematical description of the phenomenon. The Young-Laplace equation (1) has a fundamental significance in

the quantification of the capillary force as it can be used to calculate the capillary pressure across the interface of liquid and air.[34]

$$\Delta p = \gamma \left(\frac{1}{R_1} + \frac{1}{R_2} \right) \qquad (1)$$

In the equation Δp is the pressure difference across the interface, γ is the surface tension as R_1 and R_2 are the radii of curvature. In 1959, Yalow and Berson presented the first immunoassay. The target analyte insulin was quantified by measuring the presence of I^{131}-labeled insulin which competed of binding with endogenous insulin.[8] The exploitation of the capillary action in immunoassays has revolutionized the rapid diagnostic testing by providing a concept of mass-producible, affordable and robust test kits. The first application of lateral flow immunoassay (LFIA) technology that gained popularity, and still remains the most popular over-the-counter test kit for human chorionic gonadotropin (hCG), was used for testing for pregnancy. Following the pregnancy home tests, various other test kits utilizing LFIA have established their position on the market. Especially the LFIAs for HIV and malaria have been recognized to have a major impact on global healthcare[22, 35-38].

2.1.2 Lateral flow market segments

The largest segment of the LFA market is currently in clinical diagnostics with the pregnancy tests still being the source of most revenue covering 89 % of the total revenue in 2010. Within the clinical diagnostics segment, the test kits for infectious diseases are the second and the test kits for cardiac markers the third most sold POC applications after pregnancy tests. The second largest market segment is the veterinary health diagnostics with approximately 9 % of the revenue. The rest of the market segments covering a total of 3 % of the total market revenue consists of food and beverage manufacturing, pharmacological quality control, environment diagnostics, and water utilities.[39, 40]

Various potential application segments for LFAs are currently emerging. For example, WHO has identified that counterfeit medicines are a serious issue especially in the developing countries.[41] Testing for such counterfeits, simple-to-use rapid tests could be provided to ensure the originality of the drug. For personal medicine, a rapid test for monitoring the levels of anti-TNF-α monoclonal antibodies used in treating autoimmune disease symptoms has been reported.[42] In veterinary health diagnostics, the most common LFAs include cow-side milk progesterone tests[43], feline leukemia

virus tests[44], and rapid tests for canine parvovirus[45]. In agriculture, rapid diagnostics for detecting plant pathogens are being developed.[46]

Furthermore, interest in the use of LFAs in food-safety testing is also growing[47, 48] as multiple tests are commercially available for food processing contaminations and international regulations for the testing of contaminations have been implemented[49]. For example, a rapid test can be used for detecting ochratoxin in wine[50] or aflatoxin M1 in milk[51]. In food industry, certain specific cross-contaminations, such as peanut and hazelnut, can be tested with a simple LFA[52]. The use of rapid diagnostic assays for chemical and biowarfare agents is also a significant application area of LFAs[53-55], although it is intended for a more specific professional use rather than a large consumer business.

The total estimated value of the global POC market was $19.3 billion in 2016, with the U.S. market covering $9.7 billion and Europe $4.8 billion[56].

2.1.3 Principle structure and function

Most commonly available LFA test kits have an assay cassette (figure 3) that contains the assay strip and, in some cases, a desiccant tablet. The plastic cassette allows the liquid to be applied to the assay strips without the need to dip the strip into the liquid. In addition to providing mechanical durability, the cassette also contains the biological fluids tightly inside and reduces the user's exposure to potentially hazardous fluids. In all the assay strip membranes, the structural strength needs to be sufficient to withstand the mechanical stress of the reel-to-reel assembly process. Especially the sample pad and conjugate release pad must retain the tensile strength even when wet from the blocking solutions[57].

Structure and function of a typical lateral flow assay strip

A common feature in all lateral flow assays is that the capillary action drives the liquid movement. The strip typically contains two printed capture zones, a test line and a control line, where the immunoassay reporters form detectable lines depending of the presence or absence of the analyte.[3] Although the exact details on the material compositions and manufacturing processes are proprietary trade secrets of the companies, the basic principles of the LFA composition are in the public domain. The binder lines are typically dispensed using a contact- or non-contact dispensers. The contact dispensing units such as BioDot Frontline HR™ or Imagene Technology IsoFlow™ Low Contact Pressure nozzle typically have a syringe pump-actuated liquid dispensing mechanism and nozzle capillaries dragged on the analytical membrane.

Non-contact dispensers spray the binder solutions through a solenoid-actuated nozzle like in BioDot BioJet HR™ dispenser or piezo-actuated nozzles like in Scienion sciFLEXARRAYER dispensers. Multiple strategies for controlling the liquid flow and introducing sequential flow of multiple reagents by different liquid barriers, on/off-switches and membrane configurations have been reported[58, 59]. Currently such gating functions are rare in commercially available LFAs.

Some variations of the standard LFA-structure have been published with different cross-flow configurations[60]. For example, Dual Path Platform (DPP) by Chembio Diagnostic Systems Inc. (Medford, NY, USA) has successfully entered the market and has an established status in RDTs for infectious diseases[24].

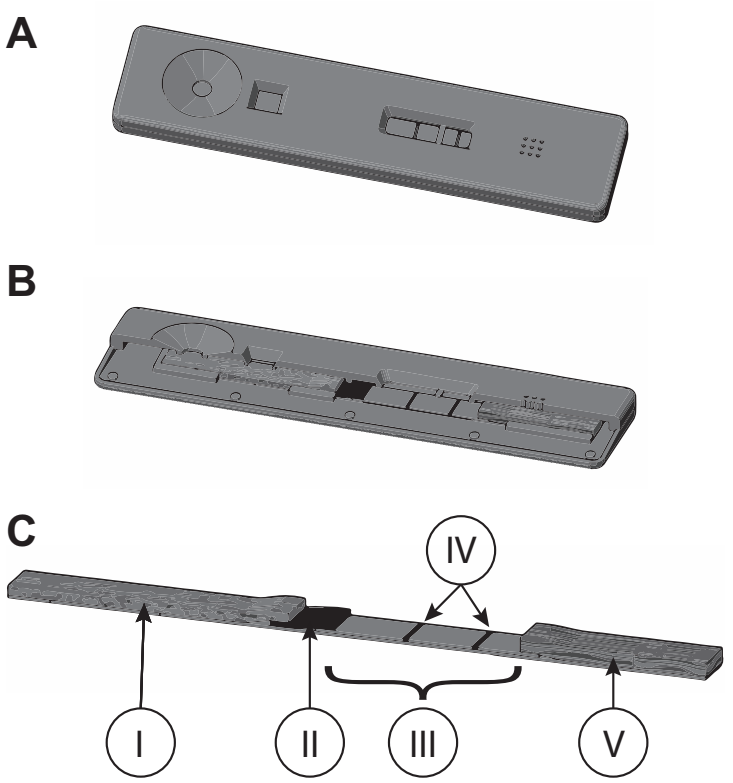


Figure 3. A typical assay cassette used in LFAs. A) A top view of a cassette with separate inlets for sample fluid and washing buffer. B) A view of the cassette with the top cover part sliced. C) The membrane assembly strip contained inside the cassette comprising sample pad (I), conjugate pad (II), nitrocellulose membrane (III), printed test and control lines (IV) and wicking pad (V).

Competitive and non-competitive immunoassays

Both, competitive- and non-competitive formats of immunoassays have been successfully used in the LF-platform. Competitive LF immunoassays have been developed for low molecular weight analytes such as vitamin B12[61], progesterone[62], cortisol[63], testosterone[64] food toxins[50, 65] and multiple drugs of abuse[66, 67]

In the two formats of competitive immunoassays the analyte of interest either competes of (a) solid-phase binding sites with a reported-conjugated analyte analogue or (b) blocks the binding sites of reporter-conjugated antibodies.[6] In the latter format the binding of antibody-conjugated reporters to the immobilized analyte analogue is prevented by the abundance of the measured analyte. Thus, the abundance of the analyte in the sample is observed by the decrease of the reporter-specific signal.[6, 57] Non-competitive immunoassays (also referred to as sandwich immunoassays or direct immunoassays) are typically preferred when the analyte has multiple immunogenic sites and a sufficient size to react with two binding antibodies.[39] Generally, non-competitive immunoassays allow wider dynamic range than competitive immunoassays due to the reason that the concentration of competing analyte analogue must be in a range where analyte-induced signal inhibition is measurable. However, competitive immunoassays can be used for analytes too small for non-competitive immunoassays.[68]

Binder molecules

The binder molecules commonly used in LFAs include monoclonal or polyclonal antibodies, and recombinant or native antigens. Most commonly, the antibodies are used as the analyte-binding molecules in LFIAs. In both assay formats, competitive and non-competitive immunoassay, the use of antibodies is required. The exception is the assays detecting antibodies against specific pathogenic molecules which use antigens as the binding reagents. For the test line printed on to the nitrocellulose, monoclonal antibodies of Immunoglobulin G-class (IgG) are most commonly directly adsorbed on to the nitrocellulose matrix. As the adsorption of the protein to the nitrocellulose matrix may not be efficient enough or the protein functionality may be compromised in the adsorption process, there are strategies to modify the antibodies or use generic tag-binding proteins such as streptavidin[69] to achieve better compatibility with the adsorption on to the nitrocellulose.[70]

Analytical membrane

Analytical membrane is the porous matrix through which the liquid passes onto the absorbent pad. Currently, the analytical membranes are almost exclusively made of nitrocellulose (figure 4). However, alternative materials have been reported. Instead of an actual membrane, the capillary flow can be achieved by using micropillar surface manufactured of hot-embossed cyclo-olefin polymers[71]. Such technology was patented by Åmic AB, Uppsala, Sweden[72, 73]. Currently, the micropillar technology has not been adopted in many products with the exception of the Meritas POC concept by Trinity Biotech, which includes a rapid test for cardiac troponin I[74].

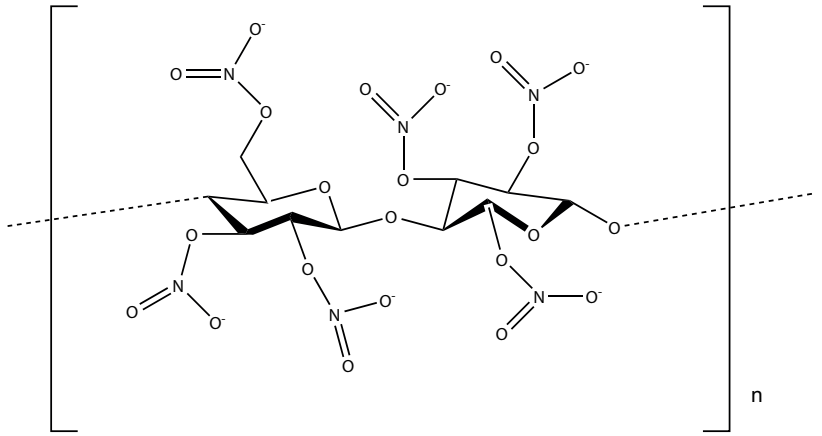


Figure 4. Chemical composition of a nitrocellulose polymer subunit.

Nitrocellulose membranes with different liquid flow rates (also referred to as "wicking rate" or "capillary rise time") are manufactured commercially (Table 1). The flow rate is dependent of the pore size of the membrane and the surfactants used in the manufacturing. Therefore, the flow rate is considered a more comparable parameter in LFA-development process than the actual pore size. The flow rate is defined as time in seconds that it takes for the fluid front to move 40 mm.[57] The flow rate, however, is not constant throughout the length of the strip. The flow becomes slower as the fluid proceeds through the porous matrix[75]. During the wetting of the membrane, the liquid flow can be approximated with the simplified Washburn equation (2)[76, 77].

$$L^2 = \frac{\gamma d_p t \cos(\theta)}{4\mu} \qquad (2)$$

In the equation, L is the length of the fluid column in the porous matrix, γ is the fluid surface tension, d_p is the pore diameter, θ is the contact angle of the liquid on the capillary material, μ is the dynamic fluid viscosity, and t is the elapsed time. Steepness of the liquid contact angle is dependent on the hydrophobicity of the matrix surface as a more hydrophobic surface tends to minimize the contact area between the fluid and the surface. Hydrophobicity is defined by IUPAC as "the association of non-polar groups or molecules in an aqueous environment which arises from the tendency of water to exclude non-polar molecules"[78] and in this context refers to the lack of attractive forces between polar and non-polar substances.

After full wetting, the liquid flow can be approximated with Darcy's law (3) for flow in porous media.[79, 80]

$$Q=-\frac{\kappa wh}{\mu L}\Delta P \qquad (3)$$

In the equation, Q is the volumetric flow rate, κ is the permeability of the matrix, wh is the cross-sectional matrix area, μ is the dynamic fluid viscosity and ΔP is the pressure drop over the length of the whole matrix.

In both equations, simplifying assumptions have been made to allow mathematical modelling of a complex system. Neither of the presented equations used to approximate the flow characteristics in porous media take into account the ambiguous pore structure and possible structural changes of the porous matrix due to wetting. The Washburn equation (2) assumes that the capillary matrix consists of a multitude of uniform capillaries having a circular cross-section, the effect of gravity or air resistance is negligible and the pressure difference over the interface of liquid and air follows the Young-Laplace equation (1).[77] The equation (3) derived from Darcy's law assumes that the liquid flow is laminar (i.e. no turbulence occurs in the flow), the liquid is incompressible, the permeability and the cross-sectional area of the matrix are constant throughout the system. The Reynold's number describing the turbulence properties of the water flow is approximately 10^{-3} in nitrocellulose membranes. Thus, the flow is considered laminar and the approximation is applicable.[75]

The manufacturers generally recommend smaller pore size and slow flow rate membranes for LFAs requiring high analytical sensitivity, and larger pore size membranes for shorter assay time and less-demanding sensitivity requirements. The trade-off situation between assay sensitivity and liquid flow results in a compromise of the assay time and analytical sensitivity[81].

Table 1. Commercially available analytical membranes intended for use in LFAs.

GE Healthcare Life Sciences	Merck	mdi Membrane Technologies	Nupore Filtration Systems	Pall Corporation	Sartorius
Immunopore RP	HI-Flow™ Plus HF75	CNPH-N SS40 70	LFNC-C 15 µm	Vivid™ 90	UniSart® CN95
FF80HP	HI-Flow™ Plus HF90	CNPH-N SS40 90	LFNC-C 12 µm	Vivid™ 170	UniSart® CN110
FF120HP	HI-Flow™ Plus HF180	CNPH-N SS60 150	LFNC-C 10 µm		UniSart® CN140
FF170HP	HI-Flow™ Plus HF135	CNPH-N SS60 200	LFNC-C 8 µm		UniSart® CN150
	HI-Flow™ Plus HF120	CNPF SN12 10 µm	LFNC-H 15 µm		
		CNPF SN12 8 µm	LFNC-H 12 µm		
		CNPF SN12 5 µm	LFNC-F 90		
		CNPF SS12 15 µm	LFNC-F 130		
		CNPF SS12 12 µm	LFNC-F 150		
		CNPF SS12 10 µm	LFNC-F 170		

Nitrocellulose membrane is typically manufactured by casting the nitrocellulose in a solution of organic solvents on to a polyester or polyethylene terephtalate backing in to 100 m master rolls[82, 83]. The porous structure is formed as the solvent evaporates from the cast nitrocellulose sheet[84]. As the thickness of nitrocellulose membranes typically used in LFAs is approximately 100 to 150 µm[82] and the evaporation occurs only from the air side, the porosity of the membrane is smaller at the side of the backing (figure 5).

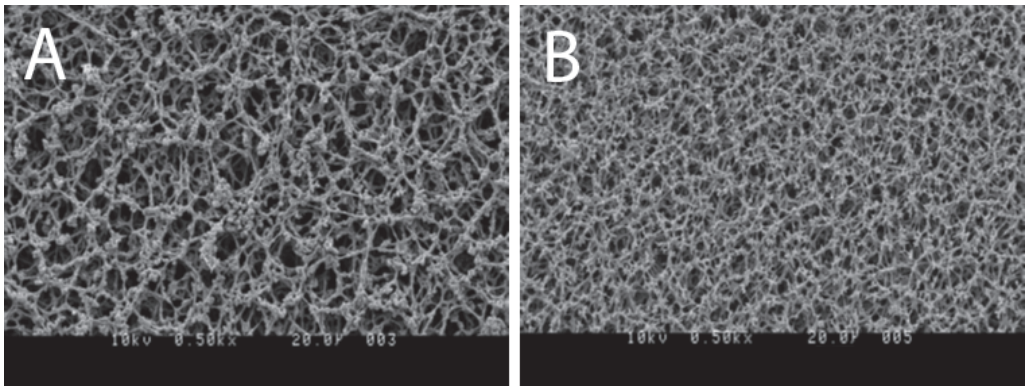


Figure 5. Scanning electron micrograph of an AE 100 unbacked nitrocellulose membrane (GE Healthcare Life Sciences). A) air (top) side and B) belt (bottom) side. A significant asymmetry can be observed between the two sides due to the evaporation of the solvent. Images courtesy of Brendan O'Farrell, DCN Diagnostics.

Considering the anisotropy of the porous nitrocellulose matrix with the smaller pore size on the backing side of the membrane and the liquid flow equations (1–3), the liquid flow is expected to be slower at the backing side of the nitrocellulose membrane.

Absorbent pad

Absorbent pad, also known as wicking pad, serves as the sink to where the liquid passes through the analytical membrane and is absorbed to maintain the flow capacity by capillary action. The absorbent pad is usually manufactured of cellulose (table 2), and its size is estimated so that it is able to absorb all the liquid applied to the assay strip or a limiting capacity can be used to stop the liquid flow at a desired time.[57]

Conjugate release pad

Conjugate release pad contains the reporter particles that are released by the liquid flow to interact with the analyte and to bind to the test or control line. The pad is usually manufactured from glass fibre or polyester (table 2) and treated with impregnation solution typically containing polymers, surfactants, and proteins to achieve effective conjugate release properties and rapid wettability. The reporter conjugate is typically deposited to the release pad by a mechanical liquid dispenser or by immersion[57, 85].

Sample pad

The sample pad is the first material on the LF strip assembly to contact the sample liquid. The main function of the membrane is to absorb the sample fluids and release the fluids to the analytical membrane via conjugate release pad. The sample pad is commonly manufactured of glass fibre (table 2), but also cellulose or rayon have been used[86]. The sample pad can also contain dried reagents such as buffering components, salt or inert proteins to adjust the reaction conditions, such as pH and salinity, without adding separate buffer solutions.[57]

Table 2. Commercially available absorbent, blood separator, conjugate release and sample pad materials intended for use in LFAs.

	Ahlstrom-Munksjö		GE / Whatman		Merck		mdi Membrane Technologies		Nupore		Pall Corporation	
	Product name	material	Product name	material	Product name	material	Product name	material	Product name	material	Product name	material
Absorbent	222	cotton	CF4	cotton	SureWick® C048	cellulose	AP045	cellulose	AP-08	cellulose		
	226	cotton	CF5	cotton	SureWick® C068	cellulose	AP080	cellulose				
	237	cotton	CF6	glass fibre / cellulose	SureWick® C083	cellulose	AP110	cellulose				
	238	cotton	CF7	cotton	SureWick® C248	cellulose	AP120	cellulose				
	319	cotton										
	320	cotton										
	601	cotton										
Blood Separator	CytoSep® HV	not disclosed	Fusion 5	fused glass			WFR1	polyester composite	RBS		Vivid™ GF	Asymmetric polysulfone
	CytoSep® 1660	not disclosed	GF/DVA	glass fibre			FR1 (0.35)	not disclosed			Vivid™ GX	Asymmetric polysulfone
	CytoSep® 1662	not disclosed	LF1	glass fibre			FR1 (0.6)	not disclosed			Vivid™ GR	Asymmetric polysulfone
	CytoSep® 1663	not disclosed	MF1	glass fibre			FR2 (0.7)	not disclosed				
			VF2	glass fibre								
Conjugate release	ReliaFlow™	Polyester	Fusion 5	fused glass	SureWick® GFDX	glass fibre	PT-R1	polyester	PT1-05			
	8964	glass fibre	Standard 14	glass fibre	SureWick® G027	glass fibre	PT-R5	polyester				
	8950	glass fibre	Standard 17	glass fibre	SureWick® G041	glass fibre	PT-R6	polyester				
	8951	glass fibre					PT-R7	polyester				
	6613	Polyester										
	6615	Polyester										
Sample pad	601	cotton	CF1	cotton	SureWick® C048	cellulose	GFB-R4	glass fibre	GP-04	glass fibre		
	238	cotton	CF3	cotton	SureWick® C068	cellulose	GFB-R7L	glass fibre				
	222	cotton	CF4	cotton	SureWick® C038	cellulose						
	226	cotton			SureWick® C248	cellulose						

Backing laminate

The components of LFA are assembled on the backing laminate manufactured of polyester, polystyrene or polyvinylchloride (PVC), or mixtures of the previous. The laminate is covered with pressure-sensitive adhesive to enable the binding of the assay components. Commonly, a thicker backing laminate is recommended for assays done in the dipstick format, as the strips used inside a plastic cassette are usually manufactured with thinner laminates to reduce the wearing of the strip cutter blades. The adhesive used in the backing laminate must be soft enough to provide sufficient adhesion, but hard enough to avoid the migration of the adhesive into the assay membranes. Adhesives typically used in the backing laminate, such as KN-2211 (Kenosha C.V., Netherlands), do not adhere instantly when the membranes are attached, but form a strong bonding within 24 hours.[87]

Auxiliary components

A plastic case is often included to provide a sample inlet serving as a reservoir for the sample fluid. The rigid structure of the plastic case also provides mechanical stability for the assay strip assembly and often contains desiccant that absorbs moisture to extend the product's shelf-life[82]. The plastic cassette must hold the assay firmly in a correct position without choking the liquid flow by applying pressure to the membranes.

Especially in the LFAs used in the dipstick format, the membranes are typically covered by a transparent adhesive film printed with assay identification markings and sample application point indicators. The film provides structural integrity to the strip, prevents liquid evaporation, and protects the analytical membrane from damage.

2.1.4 Reporter particles

Reporter bioconjugation

The inherent protein-binding feature of the colloidal offers a convenient way to produce a reporter bioconjugate. The optimal amount of protein used in the conjugation can be determined by the amount of protein that prevents the visible aggregation of gold particles.[88] For reporter particles with carboxyl-modified surface a popular method for covalent linking of the biomolecule and the reporter is done by carbodiimide linkage using N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) as a stabilizing agent.[89] In LFAs, the covalent protein

conjugation with activated latex is a common method that also enables controlled orientation of the antibody in the conjugation.[39]

Visually detectable reporters

Gold particles, also known as colloidal gold, are the most commonly used reporter technology. The inherent physical characteristics of gold also enables the measuring of the colloidal gold in LFAs with surface-enhanced Raman spectroscopy (SERS)[90] or localized surface plasmon resonance (LSPR)[91]. A reporter particle specially optimized for SERS detection and consisting of gold core in a silver shell has been reported to yield analytical sensitivity higher than that provided by chemiluminescent detection technology[92]. The typical analytical sensitivities achieved using visual detection of reporters in LFAs ranges from to 3 pM to 10 μM.[93]

Various methods for improving the assay sensitivity with visually detectable reporters have been described. The detectability of colloidal gold can be improved up to 400-fold by using an electrokinetic pre-concentration method[94] or up to 500-fold by using a silver enhancement method to increase the light absorptivity of colloidal gold[95, 96].

Visually detectable reporters also include the enzymatic reactions used to generate a visible substance at the test line where the immunocomplex formation occurs. The presence of analyte can be indicated by tracer antibodies having the colour-reaction catalysing enzymes conjugated with antibodies or alternatively enzymes produced as fusion proteins. Such technology has been described most commonly using horse-radish peroxidase (HRP) as the catalyst enzyme.[60, 97, 98] Enzyme-coupled tracer antibodies are not only used as visually detectable reporters, but also used in electrochemical detection in LFAs[64, 99].

Carbon nanoparticles have been reported as a reporter technology well suitable for rapid diagnostic assays[100, 101]. The main benefits of the technology over the colloidal gold are better contrast on the white nitrocellulose membrane and low-cost manufacturing process.[102] The carbon nanoparticles have been reported to yield better analytical sensitivities compared to colloidal gold or latex in a variety of LFIAs[93, 103]. An analytical sensitivity of 31 pg/mL in a rapid LFA for mouse and rat urinary proteins demonstrates that sensitivities comparable to fluorescent reporters can be achieved using carbon nanoparticles.[104] In addition to carbon nanoparticles used as visually detectable reporters there are also fluorescent carbon nanoparticles[105] that have been used for cell imaging.[106] Currently, there are no publications of the fluorescent carbon nanoparticles being used in LFAs.

Fluorescent reporters

In his article describing the conversion of incident ultraviolet light to an emission of blue light by fluorite, Sir George Gabriel Stokes named the phenomenon fluorescence[107]. The shift of wavelength from shorter to longer in a fluorescence process, named after Sir Stokes as the Stokes' shift, is a fundamental feature of fluorescent molecules used as reporters in biochemical assays [108, 109]. Generally, fluorescence refers to a process leading to the emission of light from a material that has absorbed electromagnetic radiation.[78] This differs from the normal reflection of light by the occurrence of intramolecular energy transfer processes that convert the radiation energy absorbed by a material to an emission of photons at different wavelengths characteristic to the energy transitions within the material.

Europium(III) nanoparticles were described as an LFA reporter for the first time in 2007 in an assay for blood eosinophils and neutrophils[110]. A subsequent publication on Eu(III) chelate loaded silica shells described a method for imaging the LFAs with Eu(III) reporters by using a digital camera.[111] Near-infrared (NIR) dyes have been reported to be feasible for LFA-applications due to the low-degree of autofluorescence originating from the assay materials and sample fluids at the near-infrared range.[112]

Furthermore, it is possible to exploit the quenching capability of fluorescent reporters to generate analyte-specific assay response. In a recent article, quantum dot reporters were conjugated with analyte-specific antibodies and immobilized to the test line of the LFA strip. Graphene oxide was used to reveal the antigen binding to the quantum dot particle, as graphene oxide was only able to quench the fluorescence from quantum dots not masked by the bound antigen.[113] Such a method could potentially be advantageous for multiplex assays, as there would be no need to dry multiple different reporter bioconjugates on the conjugate pad.

Non-optical reporters

The amount of reporter particles retained on the test line can be measured also with a magnetic field. The magnetic field induces a measurable modulation in the radio frequency (RF) signal in close proximity to the test line.[114] The magnetic detection has shown potential for the production of high detection sensitivity. With superparamagnetic particles, an LFA for cTnI has been reported with an analytical sensitivity of 0.01 ng mL^{-1}.[115]

Different electrochemical detection technologies have also been reported with LFAs. The change of electric conductivity or the generated current in the presence of certain reporters can be measured with simple electrodes attached to the membrane.[116] The electronic current generated by a redox reaction catalysed by HRP was used as a signal-generating part of the reporter bioconjugate.[64] An LFIA for cardiac troponin I (cTnI) using an electrochemical reporter system with β-galactosidase-conjugated (IgG)[99] has been described with an analytical sensitivity of 0.1 pg mL^{-1}, and another LFIA for the measurement of cortisol from saliva with glucose oxidase-conjugated reporters has been reported with a dynamic range of 1–10 ng/mL.[117]

2.1.5 The advantages and limitations of the LF-platform

Liquid movement by capillary action

A cornerstone of the popularity of the lateral flow platform is the simple and robust mechanism of agitating the liquid flow over the bioreactive surface. The surface is manufactured on a membrane with capillary flow wicking properties. Typically the surface is manufactured from nitrocellulose, but alternative technologies, such as micropillars embossed on cyclo-olefin polymers (COP), have been envisioned to overcome the limitations of the nitrocellulose[71, 118]. Other, very affordable and mechanically robust materials, for instance, plain cellulose paper[119] and even cotton threads, have been investigated[120]. It seems that the liquid flow by capillary action enables the tests to be used without any instrumentation, but certain drawbacks, such as limited immunoassay kinetics, originate from the fixed flow rate determined by the analytical membrane.

Controlling the protein binding

Typically, the antibodies on the test line are printed without any attempts to produce covalent binding to the surface. The proteins are allowed to dry completely and bind on the membrane by the non-covalent interactions of the protein and nitrocellulose. Therefore, a certain degree of the protein-binding feature in the nitrocellulose is desirable. However, as the reporter particles have typically been conjugated with an antibody or an antigen, the same protein-binding feature tends to bind the bioconjugated reporter particles.

The binding of proteins to the test line relies on the affinity of the protein and the nitrocellulose membrane. The non-covalent affinity has been reported to be thermodynamically driven[75, 121] but it is still disputed, whether the affinity originates

from dipole–dipole attractions or hydrophobic interactions.[75] After dispensing, the proteins are typically dried in 35–40 ° C for 1–2 hours.[57, 122, 123] In the in-line production system, the nitrocellulose with the dispended protein travels through a dry tower as, for example, in the RR120 Web Handling Platform (BioDot Inc, Irvine, CA, USA).

Multiplexing

Having assays for multiple analytes can be done by having multiple assay strips inside a single cassette. In this kind of a cassette, the reaction conditions, such as binder molecule density, can be optimized individually and analytical membranes of different wicking rates can be selected. Producing multiple test lines for each analyte on the same strips is very common in commercially produced LFAs. For example, the SD Bioline HIV-1/2 3.0 (Standard Diagnostics) rapid test for anti-HIV antibodies has two test lines: one for HIV-1 and another for HIV-2. The drawback of having multiple immunoreactions on the same strip is that the different reporter particle bioconjugates may not work as optimally as in single-plex assays.

Multiplex immunoassays on the LF-platform can also be achieved by printing an array of binder molecules on the analytical membrane in multiple spots. Such lateral flow microarray immunoassays (LMIAs) have been described for use in biomarker screening[124] and amplicon detection NALFIAs[102].

In late 2016, Sartorius presented analytical membranes for multiplex LFAs by printing hydrophobic barriers into the nitrocellulose membranes. The Unisart StructSure® membranes (Sartorius AG, Germany) are custom produced according to the specifications of the assay design. Similar strategies have been reported by printing hydrophobic barriers with polydimethylsiloxane (PDMS)[125] or molten wax[126].

Assay kinetics

The time available for the binder molecules to interact with the analyte is practically limited by the time in which the analytes pass the test line. Furthermore, the time that it takes to bind the analyte with the reporter particle is another variable that affects the degree of immunocomplex binding obtained in the sandwich immunoassay.[127] As the width of the typical test line is approximately 1 mm, the liquid front passes the reactive surface area in 2–5 seconds depending on the flow rate of the nitrocellulose. A method for increasing the reaction time of the reporter particles and the binder surface has been described with wax micropillars printed on nitrocellulose[128].

Single-step immunoreaction

In the typical format of LFIAs, the sample fluid is applied to the sample inlet of the cassette and allowed to absorb. After the sample application, a wash buffer is added in some cases to rinse the membrane of non-bound reporters and residues of the sample fluid. In such a procedure, the sample fluids first come into contact with the dried reporter particles on the conjugate pad and the immunoreaction starts with the binding of the analyte by the reporter particles and is followed by the binding of the formed analyte–reporter complexes by the binder molecules immobilized on the test line.

The major drawbacks of this immunoassay format include the lack of an effective washing step, which limits the use of anti-human antibodies for the detection of humoral responses for infections. In addition, the non-related antibodies contained in the sample fluid bind to the reporter particle and the disease-related antibodies cannot be effectively detected.

The typical LFIA-format is also susceptible to the high-dose effect that relates to the saturation of immunoassay binding sites, also known as "hook" or prozone effect, in which a high concentration of an analyte partially prevents the formation of the immunocomplex. This is problematic for assays with a wide concentration range, such as hCG or malaria HRP-2. The hook-effect may lead to misinterpretation of the test result if this possibility is not considered.[129-131]

2.2 Fluorescent reporter particles

2.2.1 Common characteristics

All fluorescent reporters have the common feature of having the excitation and emission light occurring at different wavelengths. Fluorescence in naturally occurring compounds always transform the excitation light into emitted light with a longer wavelength. This shift of wavelength from shorter to longer is known as Stokes' shift.[107] For the sake of simplicity, the common term of fluorescence is used in this context although the upconversion photoluminescence in the UCNP technology is not commonly referred to as fluorescence due to the anti-Stokes' shift of the excitation photons to emitted photons. Still, according to the definition of fluorescence by IUPAC[78], upconversion photoluminescence is fluorescence.

Exclusion of non-reporter-specific fluorescence

In the fluorescence measurement, the excitation light must be excluded from reaching the emission light sensor by using optical filters that block the non-reporter-specific light.[132] This is known as spectral resolution. In an optimal situation, the emission band of the reporter occurs far from the excitation light wavelength that allows the optical filters to block the excitation light efficiently while the emission light is transmitted without significant loss. Moreover, some lanthanide-ions have a feature of sustaining the emission for up to 1 ms. Reporters with such compounds can be measured by using a pulsed excitation light and measuring the emitted light after a delay time of 50–500 µs. The method is known as time-resolved measurement[133, 134] and it is typically used with optical filters that enable spectral resolution.[135]

Quantum yield

According to IUPAC, the term quantum yield is defined as the number of defined events occurring per photon absorbed by the system.[78] In the context of fluorescence, the term more specifically refers to the ratio of emitted photons and incident photons. Thus, the quantum yield is a parameter of the reporter molecule and it significantly contributes to the detectability of the reporter. Particles with high quantum yield require less excitation energy to emit photons. For upconverting nanoparticles, the quantum yield has been reported to be dependent on the particle size. Micron-sized bulk particles have been reported with a quantum yield of 3 %, whereas particles with 100-nm diameter have a quantum yield of 0.3 %.[136] Compared to a commonly used fluorescence reporter molecule fluorescein that has a quantum yield of 85 %[137], the quantum yield of the UNCPs is relatively low. However, several strategies, including the synthesis of core-shell particles, for increasing the quantum yield are under intensive research.[138] A maximum quantum yield of 31 %[139] has been reported for Eu(III)-nanoparticles and a maximum quantum yield of 73 % for quantum dots [140].

Photostability

Organic fluorophores, such as eosin, fluorescein and cyanine dyes, are sensitive to light and may permanently lose the fluorescence activity in an irreversible process called photobleaching.[141-143] The photostability of the reporter must be considered in the LFA development to avoid photobleaching of the reporters.[144] Even if the reporters remain functional in proper storage conditions, the excitation light in the measurement may induce photobleaching. The excitation-induced photobleaching demonstrated with Eu(III)-nanoparticles was rapid enough to have a practical effect on the immunoassay,

and thus remeasurements of the assays may be prone to decreased fluorescence activity.[145]

In addition to photobleaching, the environment-dependent intermittency of the fluorescence known as blinking can occur with organic fluorophores but also with quantum dots.[146, 147] The strategies for improving the stability of organic fluorophores include implementing oxygen depletion, stabilizing additives, and self-healing fluorophores.[148] The UCNP reporters have exceptional photostability characteristics since they do not bleach[145] or blink[149]. However, the YF_4-lattice commonly used in UCNPs has been reported to dissolve in aqueous solutions with low fluoride content.[150]

2.2.2 Quantitative lateral flow assays

Quantitative, semi-quantitative or qualitative results

Although LFAs were originally intended for rapid qualitative testing using visual interpretation of the formed colour on the test and control lines, the need for quantitative results (figure 6A) and the trend towards LFA readers[151] has led to a growing number of attempts to produce quantitative or semi-quantitative LFAs.[57]

The semi-quantitative assays refer to assays that produce more informative results than just a positive or negative readout. In a typical semi-quantitative analysis, the analyte concentration is reported as "negative", "low positive" or "high positive" (figure 6B). Such information can be useful, for example, in CRP testing where the high positive result can be interpreted as an acute bacterial inflammation and low-positive result may occur from various other conditions[152]. The semi-quantitative results are typically not comparable with assays done with other methods. In a quantitative assay, the results are reported numerically (figure 6A) and they should be universally comparable with reference assays with different methodologies.

In LFAs, semi-quantitative results can be produced by separate test lines indicating high and low levels of the analyte. For example, the FebriDX assay (Rapid Pathogen Screening Inc. Sarasota, FL, USA) for MxA and CRP uses this approach with a semi-quantitative CRP measurement combined with a qualitative (figure 6C) MxA-measurement.[152] Another common strategy is to convert numerical result acquired with a reader device into a semi-quantitative result by calibrating the numerical readouts with reference values of relevant analyte concentrations[50].

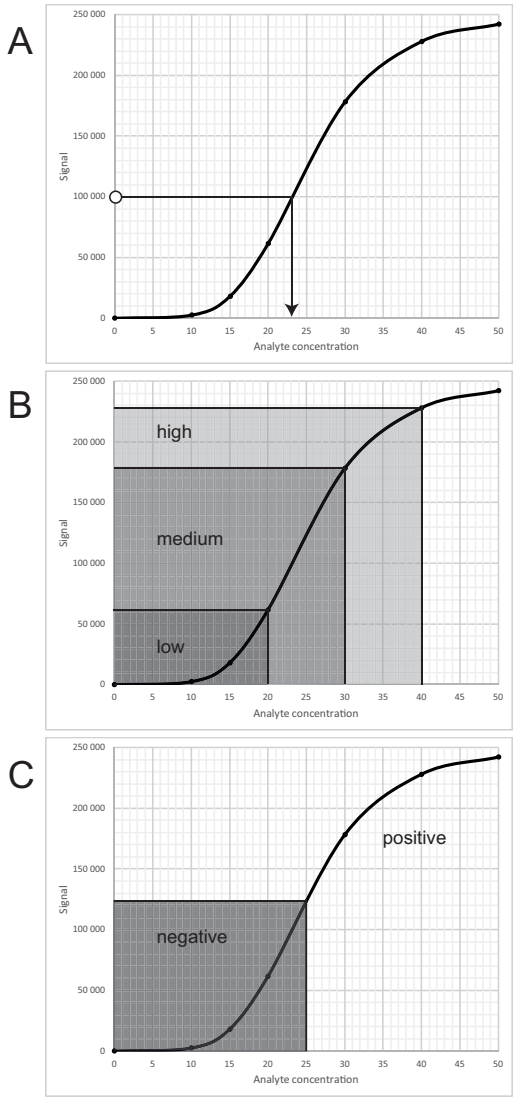


Figure 6. A schematic illustration of A) quantitative, B) semi-quantitative and C) qualitative result interpretations. The numerical output of the assay result can be interpreted according to the need of quantification and the precision of the assay results.

As several quantitative LFAs with reflectance-based detection of visually detectable reporters have been developed[153, 154], there is a growing trend to develop quantitative LFAs using fluorescent reporters[110, 155-159]. The benefit of fluorescent reporters over visually detectable reporters is a better discrimination of reporter-specific signal from the non-reporter-specific background signal. Whereas fluorescent reporter signal is measured directly by the intensity of the emitted light at a defined wavelength range, the

reflectance-based measurement cannot exclude non-reporter-specific background signals by spectral resolutions because the image artefacts originating from dust-speckles, scratches or uneven lighting conditions are not limited at any specific wavelength range. In addition, higher analytical sensitivities have been reported with fluorescent reporters when compared to visually detectable reporters[93, 123, 160]

The need for quantitative LFAs

In clinical diagnostics, the need for quantification is evident in analytes that are constantly present in variable concentrations and in situations where the clinical condition is related to abnormal biomarker levels. A potential use for a quantitative LFA could be in the testing of hormones[62, 63, 161, 162], inflammation markers[152, 163-166] or monitoring biomarkers of non-communicable diseases in resource-limited settings[167].

In the agriculture industry, the ability to quantify the plant pathogens in order to optimize the usage of plant protection products would be extremely valuable.[46] For environmental diagnostics, the need for quantitative results is less obvious. For example, a quantitative LFA for detecting microcystins in water can detect the analyte at concentrations far below the limit recommended by the WHO for drinking water[168]. However, as the result is eventually considered as 'acceptable' or 'not acceptable' based on the maximum acceptable level of the analyte, the benefit of quantitative result would be useful in scenarios with variable acceptable threshold limits.

2.2.3 Calibration

To achieve quantitative results, it is necessary to have a calibration method that translates the output of the reader device sensor into a comprehensible readout. For this purpose, a calibration procedure can be used to generate a regression model of the dependency of the numerical output of the test device and the corresponding, measured amount of analyte.

Optical reference standards can be used to calibrate the reader devices in order to achieve uniform performances among the reader devices. Lot-specific calibrations of the assays must be done to compensate for the differences occurring in the manufacturing process. Commonly, the calibration is done by measuring a set of assay lot-specific calibrator samples with the reader device and generating the calibration curve based on the measured results. The Sofia Analyzer for LFAs with fluorescent reporters (Quidel Corp. CA, US) is shipped with an analyzer calibration cassette which is used to re-calibrate the reader device monthly.[169] Another approach for the reader device

calibration is an in-cassette index line containing only printed reporter particles that can be used as reference to compensate for the reader-to-reader variations[170].

One method to compensate for variable signal intensities, presented by Response Biomedical Corp., called "Ramp ratio", is to present the intensity ratio of the test line and control line. This patented method comprises a separate internal control antibody conjugated with reporter particles, which enables the binding of the reporter particles to the control line.[171] This method compensates for the variable amount of reporter particles released from the conjugate pad. [172]

The fitting of the calibration curve from the data points have generally not been disclosed by the LFA manufacturers. However, the dose-response of the LFAs is similarly dependent of the ligand-binding interactions as are other formats of immunoassays. The resulting calibration curve has a sigmoidal relationship with the measured signal and analyte concentration[173]. The fitting with four-parametric logistic fit (4-PL) is generally recommended for ligand binding assays [174, 175].

2.2.4 Working range, signal variation and errors

The working range, also referred to as the reportable range, means the analyte concentration ranges between the lower limit of quantification LLOQ and upper limit of quantification ULOQ. It can be determined by using a precision profile (figure 7) which is a plot of CV% to represent a back-calculated variation of analyte concentrations[176]. The term dynamic range is often used as a synonym for the working range but is not as strictly defined.[177]

Fluorescent reporters have been reported to produce wider dynamic range of the assay than colloidal gold in LF-format.[178]

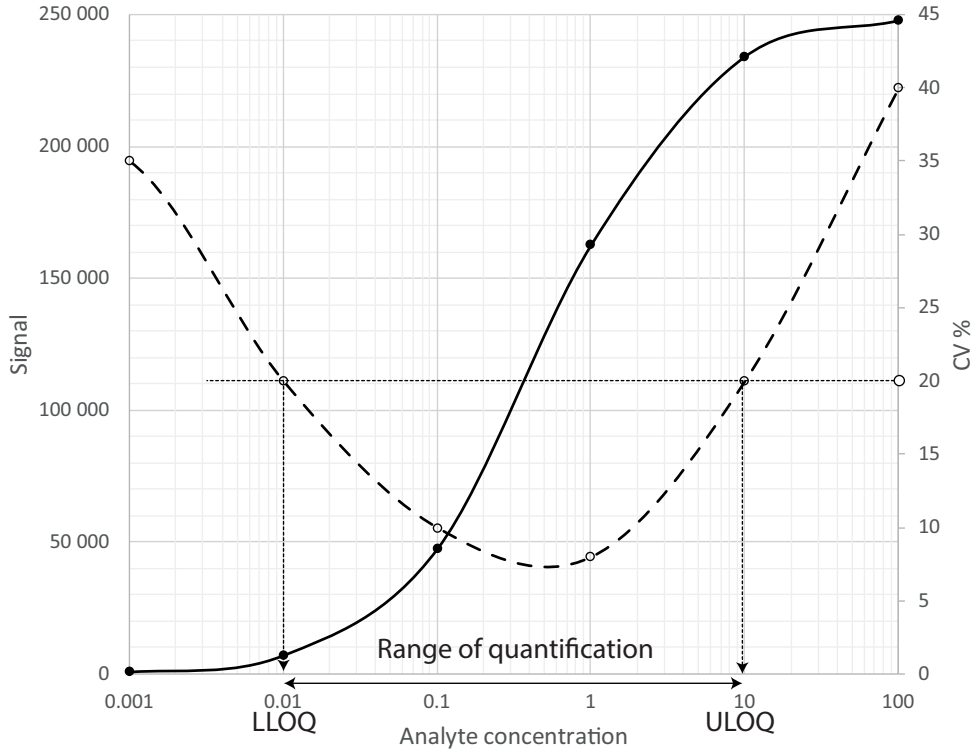


Figure 7. A schematic illustration of the range of quantification in the precision profile with a threshold CV of 20 %. The lower limit of quantification (LLOQ) is at analyte concentration 0.01 and the upper limit of concentration (ULOQ) is at concentration 10.

According to IUPAC, the precision is defined as follows: "The closeness of agreement between independent test results obtained by applying the experimental procedure under stipulated conditions."[78] In the case of immunoassays, the precision of the assay is determined by the standard deviation of calculated analyte concentration in replicate reactions. With LFAs, the precision is typically limited by factors such as imprecise sample volume[6] and inaccurate result readout timing[82]. Furthermore, strip position errors may occur because of the imprecision of the binder line dispensing, strip cutting, and the test cassette assembly. Depending on the reader device, the positioning error can critically affect the test result.[179]

In addition to these error sources, the signal intensity variation can be caused by various factors, such as storage time of the assay cassettes, reader device calibration errors, and a variable amount of reporters released from the conjugate pad. These variations can be at least partially compensated for by using the test line / control line intensity ratio. LFAs

with fluorescent reporter have been reported with a CV % less than 10 in assays for PSA[155] and ractopamine[157], and 2–17 in an assay for human neutrophil lipocalin[110].

However, in good conditions (a very controlled environment), a relatively small signal variation can be achieved even without compensating the test line signal variation with the control line intensity. An example of such a case with a CV % less than 10 has been achieved in an assay for HBsAg.[111] With a reflectometric measurement of colloidal gold particles in an assay for PSA, a CV% of 12 to 17 has been reported.[180]

2.3 Commercial LFAs with fluorescent reporters

Although all the RDTs with a WHO prequalified status use visually detectable reporters, there are numerous products with fluorescence-based detection systems for assays requiring better sensitivity or quantification. In the United States, the Food and Drug Administration (FDA) regulates the selling of medical devices. The *in vitro* diagnostics category covers all the tests that can detect diseases and the conditions of an infection, including companion diagnostics, laboratory developed tests, tests used in clinical care, home use tests, blood glucose monitoring devices, and drugs abuse tests.[181] Therefore, practically all LFAs cleared for sale in the U.S. have 510(k) documentation that discloses the relevant features of the products.

2.3.1 Assays with FDA 510(k)-clearance

The i-CHROMA CRP (k062981) and iFOB (k132167) tests (Boditech Med, South Korea) are among the few LFAs with fluorescent reporter particles that have received the FDA 510(k) clearance. The tests are intended to be used with the i-chroma reader that has been reported to detect Alexa Fluor 647 reporters with laser excitation at 637 nm wavelength.[182, 183] The Quidel Corporation (CA, USA) manufactures LFAs with fluorescent UV-excitable reporters for influenza A&B (k153012) to be used with Sofia reader that has been re-branded from the cPOC-reader platform by Esterline Technologies Corp. The more sophisticated Sofia 2 reader has a touchscreen user interface and a built-in CMOS camera instead of a scanning optical sensor configuration.[184] Assays for RSV (k162911) and influenza A&B (k162438) are available for the Sofia 2 platform.

The UPLink test system was the first commercial LFA platform using UCNP-reporters. The system included an FDA cleared test kit for the detection of opiates in saliva[67] (k020371). Test kits were produced for the detection of infectious diseases, such as

respiratory syncytial virus[185]. However, the UPLink test system was discontinued in 2005 due to lack of commercial success[186].

2.3.2 Assays without FDA 510(k) clearance

Fluoro-Check™ tests with Eu(III) particles as reporters are available for cTnI, PCT and NT-proBNP by Nano-Ditech Corp. (NJ, USA).[187] The tests, however, have not received FDA clearance and thus are not sold in the United States. The VerOFy® platform with LIAM™ reader by Oasis Diagnostics Corporation (WA, USA) is LFA-based point-of-care system especially intended for saliva samples. The platform uses Eu(III) nanoparticles as reporters[188], but the capability of time-resolved detection of the reporters has not been disclosed.

UCNP-LF assays for 40 different analytes are being manufactured by Hotgen Biotech Co. (Beijing, China). The assays for detecting biowarfare agents, e.g. *Bacillus anthracis* and *Francisella tularensis*, have been reported to be suitable for use in field conditions.[189, 190] In China, the sales of medical devices and IVD products is regulated by China Food and Drug Administration (CFDA). The approval status of the Hotgen Biotech Co. rapid diagnostic kits is not available.

SRI International is developing a biodosimeter by LFAs with UCNP reporters for the triage diagnosis of an exposure to ionizing radiation.[191] The technology is being developed for the Biomedical Advanced Research and Development Authority (BARDA) of the United States[192] and thus it is unlikely that it will be marketed as a commercial product.

2.3.3 Reporter technologies

Despite numerous publications describing the use of quantum dots in LFAs[160, 193-195], no known commercial manufacturers of LFAs with quantum dots were identified. The use of upconverting reporter particles with a non-covalently linked biomolecule was patented by SRI International[196] in 1995, but the commercial exploitation of the technology in now less restricted as the patent has already expired.

LFAs with UCNP-technology are currently being manufactured by Hotgen Biotech (Beijing, China) and SRI International (CA, USA). The Hotgen UPT-3A LFA Reader has been reported to be used for detecting $NaYF_4$:Yb^{3+}, Er^{3+} UCNP reporters with a diameter of 50 nm.[197] The diameters of $(YYbEr)_2O_2S$ and $(YYbTm)_2O_2S$ UCNPs manufactured by SRI International in 2001 have been reported to be 480 nm.[198] The

UCNP reporters of two different sizes and morphologies manufactured by SRI International are sold under Sunstone® brand by Sigma-Aldrich. Smaller, rod-shaped 10x60 nm nanocrystals are manufactured with a $NaYF_4$ crystal host lattice and the larger, <150 nm diamond-shaped nanocrystals are manufactured with $LiYF_4$-lattice.[199]

UV-excitable Eu(III) nanoparticles are sold by Thermo Fisher Scientific under the brand name Fluoro-Max™ [200] and by Innova Biosciences Ltd. as a rapid conjugation kit with 200-nm diameter Eu(III) particles[201]. Alexa Fluor™ reporters are different from most reporters used in LFAs as they are not particles but organic molecules.[202] Alexa Fluor™ 647 is one of the largest molecules of the Alexa-series with molecular weight of 1155 g/mol[203].

2.4 Reader devices for lateral flow assays

An internet search for commercially available LF readers revealed a total of 67 devices (Table 3). From those, 27 have the capability of fluorescence detection and 37 are based solely on colorimetric reflectance measurement of the formed test and control lines. Only three devices (Magnia Reader, MIAtek® and MICT® analyzer) use non-optical detection technology. Six of the devices have been designed to be used with mobile devices, such as smart phones or tablets.

Table 3. Commercially manufactured reader devices for LFAs. Re-branded products manufactured by known subcontractors not included.

Manufacturer	Product	Detection technology*
Abcam PLC	Abcam dipstick reader	REF
Abingdon Health Ltd.	ADxLR5	REF/FLU
Alere Inc.	Alere Reader	REF
Alexeter Technologies LLC	Defender TSR	REF
Alexeter Technologies LLC	Guardian	REF
America Diagnosis, Inc.	Biohit Quick Test Reader (QTR)	REF
ANP Technologies Inc.	NIDS® Stand-Alone Reader III reader	REF
Axxin Pty Ltd.	AX-2X	REF/FLU
BBI Group (Novarum DX)	Novarum Reader	REF‡
Becton Dickinson & Co. Ltd.	BD Veritor System	REF†
Bioassay Works LLC	CUBE Lateral-Flow Reader	REF
Bioassay Works LLC	Multiple Line Reader	REF
Boditech Med Inc.	ichroma D	FLU
Boditech Med Inc.	iCHROMA II	FLU
Boditech Med Inc.	ichroma reader	FLU
Cellmic LLC	HRDR-200: Chromatographic Reader	REF/FLU‡
Changchun L.T.H Technology Development Co. Ltd.	Rapid Immuno Assay Reader	REF
Changchun L.T.H Technology Development Co. Ltd.	Rapid Immuno Assay Reader POCT	REF
Charm Sciences Inc	Charm EZ Lite	REF
DCN Diagnostics Inc.	DCN Fluorescent Assay Visualizer	FLU
Detekt Biomedical LLC	Chameleon Mini	REF
Detekt Biomedical LLC	RDS-1500 PRO	REF/FLU

Manufacturer	Product	Detection technology*
Esterline Technologies Corp. (LRE Medical GmBh)	cPOC	REF/FLU
Fio Corporation	DEKI Multiparametric POC reader	REF
Hamamatsu Photonics K.K.	C10066-10 Immunochromato-Reader	REF
Hangzhou Allsheng Instruments Co. Ltd.	TSR-100 Test Strip Reader	REF/FLU
Hangzhou Allsheng Instruments Co. Ltd.	TSR-200	REF/FLU
Helmut Hund GmbH	LFT 100 Lateral Flow Tester	REF
Hotgen Biotech Co. Ltd.	UPT-3A	UCP
Humasis Co. Ltd.	HUBI-QUANpro	REF
Hybridmojo LLC	Q-Reader	REF/FLU
i-calQ LLC	i-calQ	REF‡
iSTOC Oy	IDA	REF‡
JD Biotech Corp.	Immuno gold Q-reader P.O.C.T	REF
LifeAssays AB (MagnaSense Technologies)	Magnia Reader	MAG
Maccura Biotechnology Co. Ltd.	R01	REF/FLU
Magna Biosciences LLC	MICT® analyzer	MAG
Magnisense SE	MIAtek®	MAG
Maxwell Sensors Inc.	FS-Scanner™	FLU
Maxwell Sensors Inc.	MicroDx™ 1000	FLU
Medisensor Inc.	aQcare TRF	FLU°
Merck KGaA	RQflex® 10 Reflectoquant®	REF
Merck KGaA	RQflex® plus 10 Reflectoquant®	REF
Micropoint Bioscience Inc.	mLabs Immunometer	FLU
Mobile Assay Inc.	Mobile Diagnostic Lateral Flow Test Strip Reader	REF‡
Nano-Ditech Corp.	Fluoro-Checker TRF Reader	FLU°
Nano-Ditech Corp.	Nano-Checker 710 Reader	REF
Neogen Corp.	Accuscan Pro	REF
NG Biotech	NG-Test Reader	REF‡
Oasis Diagnostics® Corporation	LIAM™	REF/FLU
Otsuka Electronics Co., Ltd.	Fluorescent Immunochromato Reader DiaScan α	FLU
opTricon GmbH	Cube-reader	REF
opTricon GmbH	Optrilyzer Med	REF/FLU
QIAGEN Lake Constance GmbH (ESEQuant)	LFR	REF/FLU/UCP
QIAGEN Lake Constance GmbH (ESEQuant)	LR3	REF/FLU
Response Biomedical Corp.	RAMP 200	FLU
Response Biomedical Corp.	RAMP reader	FLU
Securetec Detektions-Systeme AG	DrugRead	REF
Shanghai Kinbio Tech Co. Ltd.	Strip Reader DT1032	REF
Shanghai Kinbio Tech Co. Ltd.	Strip Reader DT2032	REF
Skannex AS	SkanFlexi	REF
Skannex AS	SkanSmart	REF
Taiwan Unison Biotech Inc.	Uniscan	REF/FLU
Vedalab S.A.	Easy Reader Plus	REF
Wuhan J.H. Bio-Tech Co., Ltd	Quantitative rapid test reader	REF
Zhejiang Chemtrue Bio-Tech Co. Ltd.	KD-I	REF
Zhejiang Chemtrue Bio-Tech Co. Ltd.	KD-IV	REF

*reflectance (REF), fluorescence (FLU), fluorescence upconversion (UCP) or magnetic (MAG) detection
°capability of time-resolved measurement
†proprietary enhanced reporters
‡ used with mobile devices

2.4.1 Requirements for reader devices

The specifications of an ideal LFA reader for use in point-of-care diagnostics has many similarities with the ASSURED criteria that describes the features of ideal point-of-care diagnostic test kits.[179, 204, 205] Summarized, the following features are desirable for a device:

Affordable: The requirement of affordability is highly ambiguous and dependent on the target product's profile of the intended use. Generally, the system should have a positive impact on reducing the total costs of health care services.

Sensitive: The detection sensitivity of the reader device must be adequate enough to detect the low analyte concentrations at the range specified for the assay. The performance of the detecting system should be stable and consistent.

Specific: False positive results must not occur due to instrumentation errors such as improper calibration or electromagnetic interference.

User-friendly: The device must be safe to use. The possibilities of electric shock, exposure to harmful radiation, harmful chemicals, or biohazardous materials should be minimized. The user-interface should be as intuitive as possible with graphical guidance.

Rapid and robust: The device should be ready for use with minimal start up time and should be able to process the assay strips at a rate of which patients are incoming for testing. The device should tolerate high temperatures and humidity, as for example disaster medical assistance teams may have operate in conditions with temperature up to +40 °C and relative humidity up to 100 %[206, 207]. Furthermore, a method for preventing fungal growth[208] on the optical surfaces should also be considered. The device should have warning systems for errors that will prevent the use of the device in unacceptable conditions or in cases of electronic or mechanical failures.

Equipment-free: An ideal LFA reader system can be operated with only minimal accessories required. Practically, the assay kit and the reader device must contain all the accessories and functions needed for the testing. Some disposable single-use POC devices with simple electronic circuits are already on the market. For example, the Clearblue Digital test is comprised of a plastic housing with a lateral flow strip and a small battery-operated optical system with a LED-light and photodiodes for measuring the signal from the test and control lines. More futuristic technology including printed electronics with RF powering and even hydrogen fuel-cell technology have been demonstrated in applications for POC diagnostics. [209]

Delivered: The device is portable and can be transported to remote locations, for example, with a motorcycle. The device must be battery-operated and have the power capacity to be operational for the working shift of the user.

2.4.2 Principles of optical measurement

As errors originating from variable lighting conditions and subjective interpretation of the test result are well-known problems of the visual interpretation of the LFA test results[210], the reader devices used to generate the result of the LFA are not affected by such errors. The reflectometric measurement, which is the most common detection technology in the LF reader devices, measures the decrease of reflected light at a certain wavelength area. The light absorbance of the coloured reporter particle appears as a darker band in the measurement and the decrease of light is measured by the optical sensor.[179]

For fluorescent reporters, two measurement strategies are used for the signal detection and quantification. In a scanning measurement, the length of the assay strip moves in relation to the optical sensor. In a static imaging measurement, a multi-pixel image is generated. Due to the high excitation power requirement, only scanning measurements are used in LFAs with UCNP reporters[136]. The main benefit of the scanning measurement over the static imaging methods includes higher detectability of the reporter, as the multi-pixel sensors used in the imaging method are not as sensitive as PMT or μPMT modules.[211, 212]

The static imaging measurement has the capability to analyse two-dimensional images. This can be used to recognize errors originating from imaging artefacts, but more importantly, it enables the analysis of multiplex arrays printed on the LFA strips[102]. The first publications describing the method utilized flatbed scanners for the image acquisition.[101, 153] The apparent trend of utilizing the built-in CMOS cameras of smart phones for LFA result analysis[61, 178, 213] emphasizes the benefits of compact sensor and image processing technology with low power consumption.

Most of the optical measurement methods operate in epi-illumination configuration, in which the assay strip is illuminated from the same side as the light sensor. The illumination and detection can be either in a confocal or off-axis setting. In the confocal setting, the illumination beam and the light sensor are perpendicular to the assay strip, whereas in the off-axis setting, the illumination beam and light sensor are in an angle and have separate light pathways.[179] The trans-illumination configuration is commonly used in gel electrophoresis applications with transparent agarose or polyacrylamide gels[214]. In LFAs, the trans-illumination is less common, but it is used in the Clearblue Digital system[215] and also demonstrated with Eu(III) nanoparticles[216].

2.4.3 Sensor technologies

Optical sensors suitable for use in the LFA readers currently include photodiodes, charge-coupled devices (CCD), complementary metal oxide semiconductors (CMOS), photomultiplier tubes (PMT), and micro PMTs (μPMTs)[211, 212, 217].

Single sensor photodetectors

Photodetectors having only one sensor unit are called single sensor photodetectors. These include PMTs, μPMTs and different types of photodiodes. Optical plate readers used for various formats of immunoassays typically contain a PMT that is used for measuring the light modulated by the sample fluids. In the photon multiplier tube, the incoming photons activate a cascade of electrons induced by the photoelectric effect occurring at the photocathode component of the tube, and the electron flow is amplified by dynodes set at incremental voltages.[218]

As PMTs are relatively complex devices to manufacture, the physical dimensions and the cost of the sensor modules have limited the feasibility of the PMTs in point-of-care diagnostics. Microelectromechanical systems in manufacturing have enabled the automatized production of more affordable miniaturized versions of PMTs called μPMTs[212]. A μPMTs has the same basic structure containing a photocathode, focusing electrodes, dynodes, and the anode as a traditional PMT. With a μPMT sensor, the detection module can be manufactured practically as small as a standard photodiode sensor (figure 8). Additionally, the requirement of high-voltage circuitry for the gain stages of the PMT contributes to the cost of the sensor modules.

Standard p-n and p-i-n photodiodes are the most affordable photosensors used in diagnostic assay readers[211, 216]. Silicon photodiodes commonly used in smoke detectors have also been demonstrated as highly affordable and feasible for a reflectance-based LFA reader[219]. For example, Clearblue digital is a very affordable self-contained LFA device sold for pregnancy testing. The test device contains strip assembly with transparent backing laminate, LEDs under the strip, and photodiodes on top of the strip.[215] The ESEQuant LFA reader for UCNP reporters also contains a photodiode sensor[220, 221]. However, the reporter particles used in the assays for ESEQuant LFA have a very large, and therefore high, emission intensity[136].

Avalanche photodiodes (APD) used in Geiger-mode are also known as single-photon APD (SPAD). The SPAD technology has been reported to have the potential to detect single photons as PMTs.[222] The technology has also been demonstrated for time-

resolved fluorometry[223], but the usefulness of the technology, as that of the most avalanche photodiode sensors, is limited by excessive signal noise level and the temperature dependency of the signal gain[224]. The dynamic range of the APDs used in Geiger-mode is limited by the recovery time after each avalanche pulse.[222] In theory, a reported 1 MHz pulse frequency[225] would limit the maximum rate of detected photons to one million counts per second.

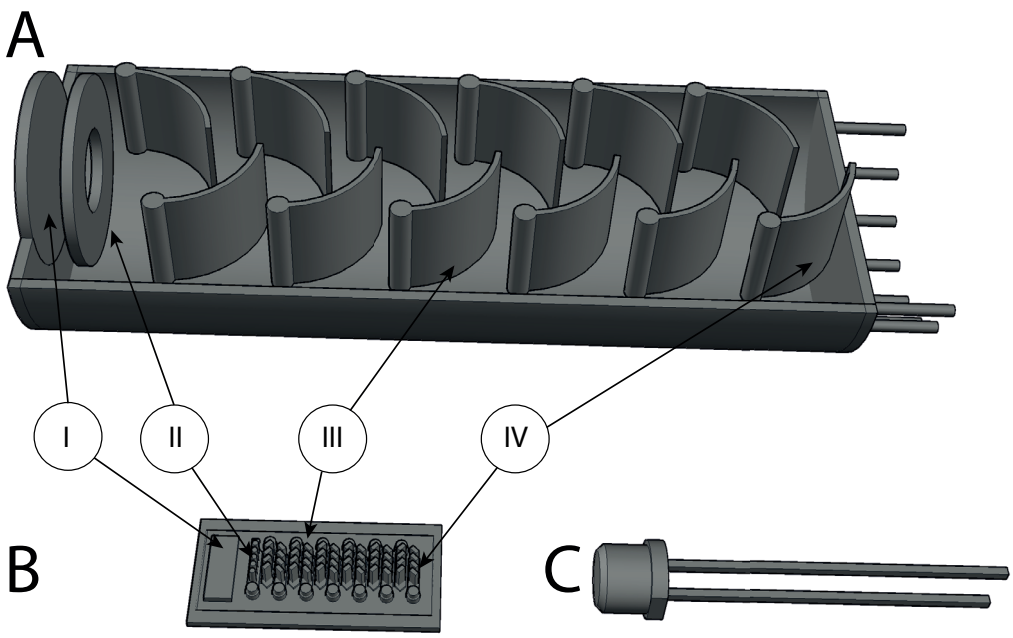


Figure 8. A schematic representation of A) the PMT and B) μPMT (bottom), and C) the photodiode sensors showing I) photocathode, II) focusing electrode, III) dynodes, and IV) anode.

Multipixel photodetectors

As single sensor photodetectors quantify the total number of photons reaching the photoreactive surface of the sensor during a defined time, the multipixel photosensors commonly used in imaging applications can provide a two-dimensional intensity distribution map. The basic principle of photoreactive surface triggering an electron is similar to single sensor photodetectors. However, multipixel photosensors combine a large number of miniaturized photoreactive units commonly referred to as pixels.

Two main types of multipixel photodetector charge-coupled devices (CCD), complementary to metal oxide semiconductors (CMOS), are used in various fields of industry from consumer electronics to space research[226, 227]. Cooled CCD camera modules are well-suitable for use in high-sensitivity bioimaging applications[228], whereas the CMOS technology has replaced the older CCD technology in compact imaging devices[226]. CCD photodetectors have successfully been used in LFA readers for detecting quantum dot reporters[229] and bioluminescent reporters[230].

Current camera units in mobile phones are almost exclusively based on complementary metal oxide semiconductors (CMOS) and these applications are discussed in the next chapter. Multipixel photon counter (MPPC) is one of the most recent photon counting devices. It consists of multiple APD units used in Geiger-mode which enables high-sensitivity imaging applications in solid-state devices.[231] However, no rapid testing applications of the MPPC photodetectors have been identified so far.

2.4.4 Mobile device applications

As smart phones have gained popularity and become common household commodities, the ability to use them for diagnostic testing has been under active study recently. Although there are still significant issues with the new point-of-care technologies, the capabilities of smart phones can greatly benefit POC testing[232, 233]. In addition to the rapid image processing capability that generates a diagnostic test result, the geolocation functionality and wireless data transmission of a smart phone application can provide spatio-temporal information for disease monitoring, which can be extremely helpful in tracking and controlling epidemics.[213, 234]

As the built-in CMOS camera and flash LED are suitable for reflectance-based analysis of visible test and control lines, the capabilities of smart phone-based diagnostic devices can be expanded by using different extensions containing the needed optics, electronics, and cassette holders. The phone-attached modules can be used to provide excitation light for fluorescence measurement of the LFAs. Such a system has been reported to achieve superior sensitivity and detection range over visually detectable colloidal gold.[178]

In addition to high-resolution imaging capability and sophisticated connectivity features, software algorithms can also be used to improve the performance of the smartphone-based image analysis efficiency[235]. The management of the test cassettes can be integrated into the smart phone application by reading QR codes or RFID tags from the cassette to ensure that a correct cassette is being used and the expiry date of the test has not passed.[179, 189, 236]

In food-safety applications, the extremely portable smart phone-based applications capable of quantitative analysis have been reported to be highly advantageous with the apparent limitations set by the sample pretreatment requirements.[47] Home-testing with a smart phone application also provides an opportunity for conveniently measuring the circadian fluctuation of hormones, such as cortisol [63, 161, 162].

2.5 Feasibility for rapid diagnostic testing

2.5.1 Characteristics of the point-of-care conditions

The ASSURED criteria of the WHO outline the desired characteristic of an RDT intended for use in resource-poor settings. The acronym stands for "affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, delivered".[237, 238] In terms of evaluating the impact of POC tests on the healthcare, the ASSURED criteria may set an artificial barrier for adopting POC tests. Instead of defining a POC test based on the used technology, using criteria based on the usefulness of the test in POC settings is justified. In different target product profiles (TPP), the requirements for the diagnostic product are defined in regard to the characteristics of the user environment.[239]

In TPP1 (home), the product is used by a lay person in a household environment. In TPP2, the tests are done by a minimally trained health worker with no special equipment at their disposal. The TPP3 is the most basic level where dedicated instruments are used and the tests are done by professional clinical staff. The TPP4 includes the peripheral laboratories with lab technicians capable of most routine analysis (e.g. microscopy and ELISA). TPP5 is the most sophisticated user environment where in-patients are monitored in hospitals.[19]

Over-the-counter products sold to lay users, such as pregnancy tests, belong to the TPP1 which is the only target product profile for products intended for non-professional users. The current trend suggests that more products for various analytes will be available for home testing.[240, 241] Home testing is not only a matter of convenience but can be considered a prerequisite for the clinical diagnostic testing in the most resource-limited settings. The preference of home testing of malaria over having the diagnostic test done by a professional at a clinical facility was abundant among rural populations due to the distance to the clinical facilities.[242]

Although many of the ASSURED criteria can be fulfilled by LFAs, the shortcomings of the technology include the limitations of multiplexing[57] and throughput capacity[6], and the lack of clinical sensitivity with clinically important analytes. For example, there is currently only two rapid assays for HCV and no rapid assays for hepatitis-B with a WHO prequalification status.[24]

2.5.2 Sample matrices

Various sample materials have been used in clinical LFAs including urine, whole blood, serum, plasma, and saliva.[6] In point-of-care assays, the ideal sample pretreatment should be minimal to achieve a rapid and user-friendly workflow. Therefore, the most preferable sample materials for rapid testing would be the ones that require minimal effort to prepare for use in LFAs. Capillary whole blood that can easily be collected from a finger prick sample, whereas urine and saliva[243] are collected using non-invasive sampling methods.

Saliva is a sample type that can be collected by swabs or expectoration.[161] Saliva samples are popular in LFAs detecting drug abuse[66] because of the ease of sampling, for example, in roadside testing conditions. Analysis of hormones from saliva samples[161] has also been investigated intensively, as the small molecular weight hormones are secreted in the saliva and can reflect the concentration of the hormone in plasma[244]. The significance of oral fluid sampling for global healthcare is especially emphasized in the detection of HIV-antibodies from gingival fluid swabs which has been reported to be a cost-effective and non-invasive method in areas with high prevalence of HIV.[245]

Urine has been used with LFAs since the appearance of home pregnancy tests. The non-invasive sampling method and abundance of the sample are obvious factors why urine is used in rapid testing. Drawbacks of the urine sampling are the high fluctuation of the sample volume that may lead to diluted analyte concentrations and the need for concentration pre-treatment before performing the LFA[246] in addition to the common requirement of collecting the first urine in the morning for the analysis.

LFAs can also be very useful in scenarios where complicated and invasive sampling methods are required. For example, amniotic fluid samples can be taken in emergency room scenarios to identify the risk of intra-amniotic inflammation in cases of preterm delivery.[164] Cerebrospinal fluid collected with a needle from a lumbar puncture can be used for diagnosing cryptococcal meningitis.[247] For rapid diagnosis of rabies, samples of the brains of suspected rabid animals can be tested.[248] Venous blood samples require a

skilled phlebotomist, sterile needles and the sample collection tubes which in many cases may not be available.

2.5.3 Easy assay procedure

Sample collection methods impact the assay success in a remarkable way.[249] Separate bottles of wash buffer may get lost when performing the assay and a temptation to substitute the missing buffers with other fluids has been reported to produce false positive results[250]. To avoid problems arising from separate buffer bottles, some examples of buffer compartments integrated into the assay cassette have been demonstrated.[159, 251, 252] Integrated in-cassette sample pre-treatment functions have been reported in addition to conventional use of blood filter membranes. For example, a method for in situ lysis of blood cells in the LFA-cassette has been patented by Rapid Pathogen Screening Inc.[253] Furthermore, a sophisticated method using stimulus-activated iron oxide and gold nanoparticle system for sample enrichment and purification for malarial HRP2-protein has been reported.[254]

Assays requiring multiple steps with additions of different fluids in a specific order are generally not accepted in POC conditions. In the WHO prequalification guidelines for HIV-antibody assays, the ease of performance is scored according to the relevant parameters constituting the assay procedure. These parameters include a number of steps in the procedure, clarity of kit instructions, kit and packaging labelling, a number of reagents that are needed to prepare, stability of diluted reagents after opening, and a number of required items not provided in the kit.[255]

Currently, embedded on-cassette flow control mechanisms are more complicated to produce than conventional LFA strips, as special liquid distribution functions are required of the assembly line. A relatively inexpensive and robust technology for sequential introduction of fluids into an LFA strip has been described by using simple one or two-dimensional fluid pathways[256, 257] for capillary-driven assay devices, but the patenting of the technology[258] restricts its commercial use.

The ASSURED criteria do not state the time for completing the assay. However, all the RDTs for HIV antibodies or malaria having the WHO prequalification status can be completed within 10 to 31 minutes [255, 259]. This can be considered as a benchmark for other RDTs for infectious diseases, as the use of RDTs for HIV and malaria have been successfully scaled up in the most demanding conditions[35].

The total time from sample to results consists of sample pre-treatment and the incubation of the initiated immunochromatographic reaction. The incubation time of the reaction is defined by the liquid flow rate in the assay membranes and the total volume of the fluids passing through the membranes as described in chapter 2. Although a major impact on the global HIV diagnostics has been achieved by rapid point-of-care tests hurdles, the standardization, regulatory affairs and human resource management have been recognized to limit the cost-effectiveness of the HIV testing programmes.[260]

2.5.4 RDTs and biosafety

As blood samples are often used in LFAs, the capability of the assay cassette to contain the assayed fluids without leakages is essential to avoid the exposure to biohazardous material. Safety must also be considered in the sample collection procedure. As the blood transfer devices must have sufficient volumetric accuracy, the unintentional release of blood must be minimized in order to avoid healthcare workers' exposure to blood.[261]

When discarding the used assay cassettes and or expired test kits, potential harmful contents must be considered. For example, cadmium in the quantum dots may present a potential health and environmental hazard[194]. Sodium azide (CAS nr. 26628-22-8) often used in buffer solutions is classified as harmful to the environment and classified as hazardous waste by US Environmental Protection Agency[262]. Concerns have also been generally expressed on nanoparticle waste in the environment. The potential toxicity of nanoparticles relates to their small size which allows them to migrate through biological structures, such as cell nuclei or blood–brain barrier.[263-266]

2.5.5 Cost of the assays

According to a survey conducted in Tamil Nadu, India, people in the poorest, "Base of the Pyramid" socio-economic population group are willing to pay 50–100 INR (1.3 €) for a diagnostic test.[267] Currently, only simple LFAs and flow-through RDTs can be provided for the POC market within this price range. An example of the lack of affordability, which is an unmet ASSURED criterion, is the POC testing for tuberculosis (TB). At the moment, the only POC test with sufficient sensitivity for managing multidrug-resistant TB epidemics is the Xpert MTB/RIF (Cepheid, Sunnyvale, CA, USA) nucleic acid amplification test (NAAT)[268]. The currently available LFAs for TB are sensitive enough to be used only with a growth medium culture[269]. In this case, the LFAs fail to meet the sensitivity requirement of the ASSURED criteria, as the Xpert MTB/RIF is considered too expensive with the $9.98 USD price for assay cartridge within eligible

countries.[270] According to a simulation model, a cheap rapid test suitable for mass screening would have a significant impact on curbing drug-resistant TB epidemics. [271]

Quantitative LFA technologies have been reported to be affordable enough for manufacturing in low-income settings. The UCNP technology with a small stand-alone reader has been demonstrated to be feasible for use in remote, low-resource locations for the testing of *Schistosoma* circulating anodic antigen.[220] Another UCNP-based LFA for anti-HBV antibodies has been described as affordable and reported to have analytical sensitivity better than ELISA, which demonstrates that the technology is well-suited for serological testing.[272] The usefulness of such a serological test would be most apparent in monitoring antibody formation after a HBsAg-vaccination.

The production of a typical LFA test kit with a visually interpretable result has been estimated to cost $0.38 USD in total.[13] Depending on the additional price components of the products, such as logistics, personnel, marketing, and profit margins, the price of the LFAs can match the needs of low-income settings. Even though the assay kits with a disposable immunochromatographic strip are valuable because of the stand-alone capability, they do not have the versatility enabled by quantification. In another approach to permanent integrated instruments that require maintenance, significant complications are expected with the maintenance and up-keep. Considering the drawbacks of these strategies, a strategy with disposable assay cassettes and a simple reader device appears to be promising for low-income settings.[273]

3 AIMS OF THE STUDY

The overall aim of this thesis was to examine the use of Eu(III) nanoparticles and UCNPs as reporters in LFAs, and to evaluate the potential of improving assay sensitivity and the capability to produce quantitative LFAs.

More specifically, the aims were:

I To evaluate the potential of improving the analytical sensitivity of LFAs by replacing the colloidal gold reporter particles with fluorescent Eu(III)nanoparticles and compare time-resolved, pulse-excited, and continuous detection modes.

II To examine the compatibility of blood samples containing different anticoagulants with an LFA using Eu(III) nanoparticles or UCNPs as reporters.

III To demonstrate the quantification potential of an LFA with UCNP reporters with a clinically relevant analyte, and compare the assay performance against a conventional ELISA using real patient samples.

IV To compare the LFA platform with a point-of-care assay platform with microparticles used as the solid phase.

4 SUMMARY OF MATERIALS AND METHODS

A brief summary with some additional information is presented here. A more detailed description of the materials and methods used in this study can be found in the original publications (**I**-**IV**).

4.1 Reporter particles

4.1.1 Bioconjugation of reporter particles with binder molecules

Upconverting nanoparticles and Eu(III) nanoparticles were covalently bioconjugated with monoclonal antibodies using NHS-EDC chemistry.

Colloidal gold was passively conjugated with monoclonal antibodies by the inherent protein-binding feature of the gold particles.

4.1.2 Upconverting nanoparticles

The upconverting nanoparticles used in the assays for PSA and cTnI (**II**) were synthesized in-house, coated with silica layer, and reactive carboxylic acid groups were induced on the particle surface. The particles (figure 9) used in the MxA assay (**III**) were manufactured by Kaivogen Oy (Turku, Finland).

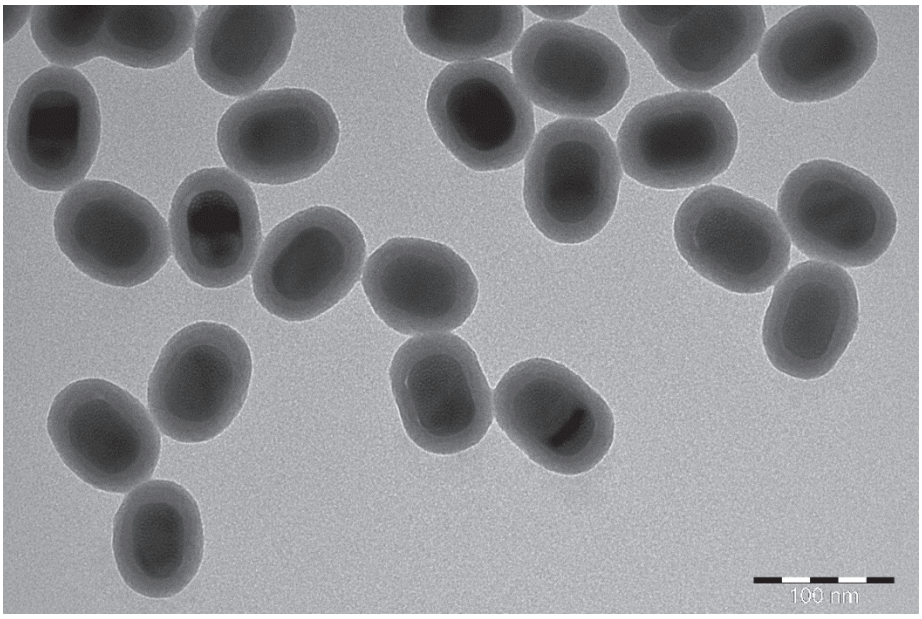


Figure 9. Transmission electron micrograph of the NaYF4:Yb3+, Er3+ UCNPs manufactured by Kaivogen Oy. JEM-1400+ TEM, 80 kV (JEOL, Tokyo, Japan). Image courtesy of Kaivogen Oy, Antti Pihlava.

The carboxyl-activated UCNPs were bioconjugated with mAbs using 2 mM EDC and 30 mM sulfo-NHS as described earlier by Kuningas *et al*.[274]

For the PSA-assays (**II**) mAb-5A10[275] was conjugated with upconverting nanoparticles using 83 µg of mAb per 1 mg of UCNPs in the reaction. For the cTnI-assays (**II**), mAb-8I7 (International Point of Care Inc., Toronto, ON, Canada) was conjugated using 83 µg of mAb per 1 mg of UCNPs. For the MxA assay (**III**), mAb-AFI7B3 (Institute of Clinical Medicine, University of Eastern Finland) was conjugated using 41.5 mg of mAb per 1 mg of UCNPs.

4.1.3 Eu(III) nanoparticles

The Eu(III) nanoparticles used in the assays (**I-II**) were polystyrene spheres containing Eu(III) chelates with product name OptiLink (Seradyn, Indianapolis, IN, USA). The particles were shipped with activated carboxyl groups on the surface. The bioconjugations were done as described earlier by Kokko et al.[276] and Soukka et al.[277]. For the cTnI assay, mAb-8I7 and, for the PSA assay, mAb-5A10 were conjugated using

mAb concentration of 0.5 g/L in the reaction at pH 6.1. For the bio-BSA assay, streptavidin was conjugated at a concentration of 2 g/L.

4.1.4 Colloidal gold

The colloidal gold used in the assay for biotinylated bovine serum albumin (bio-BSA) (**I**) was streptavidin-conjugated colloidal gold particles with a diameter of 40 nm and an optical density of 10.3 AU at 520 nm manufactured by British Biocell International (Cardiff, UK).

For the PSA-assays (**I-II**), mab-5A10 was diluted in 20 mM sodium borate buffer pH 9.0 to a total volume of 1 mL with concentration of 0.188 g/L. and added to 5 mL of colloidal gold solution (Ani Biotech, Vantaa, Finland) with pH previously adjusted to 9.0.

4.2 Preparation of LFIA strips

4.2.1 Strip membrane assembly

A 25 mm wide nitrocellulose membrane HF180 Hi-Flow plus (Merck, Ireland) (figure 10) was pasted on a 70 mm wide plastic adhesive laminate (G&L Precision Die Cutting) previously cut into 20 cm cards. The feed pad was 8 (**I**) or 16 mm (**II**) wide GFCP203000 glass fibre membrane (Merck) or pre-blocked 10 mm wide polyester membrane PT-R1 (mdi Membrane Technologies, Ambala, India). The feed pad was pasted on to the backing laminate with a 2 mm overlap with the nitrocellulose membrane.

Wicking pad of CFSP223000 cellulose membrane (Merck) cut to the width of 27 mm was pasted on to the distal end of the backing laminate overlapping 2 mm with the nitrocellulose membrane.

Dedicated blood filter membranes or sample pads were not used in the experiments as unprocessed whole blood was not used, and the assays were done by dipping the strips in microtiter wells instead of using plastic strip holder cassettes. The fluorescence spectra of the HF180 nitrocellulose membrane (Merck) were scanned using Varian Cary Eclipse fluorescence spectrometer (Varian Scientific Instruments, Mulgrave, Australia). A scan representing a typical fluorescence measurement conditions with an assumed 20 nm Stokes' shift of a reporter was done using synchronical emission measurement at excitation wavelength + 20 nm. Another fluorescence scanning was done with a static

excitation at 350 nm and a scanning emission measurement at range 400–650 nm using a 5 nm slit.

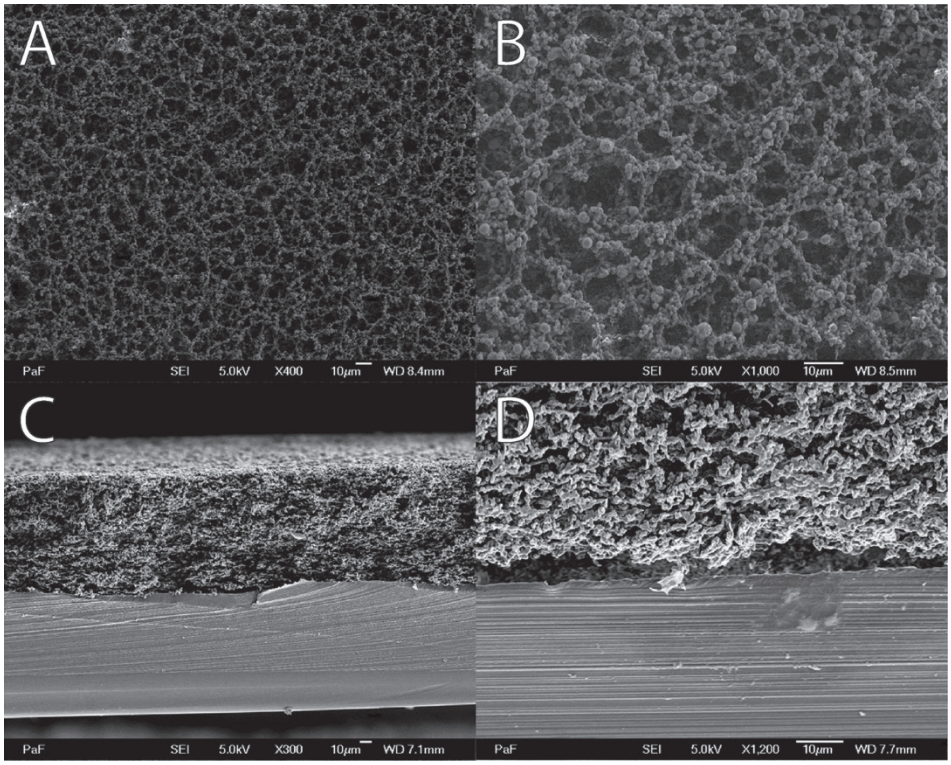


Figure 10. Scanning electron micrographs of nitrocellulose membrane HF180 Hi-Flow plus (Merck, Ireland). A) Porous side, top view, 400-fold magnification; B) porous side, top view, 1000-fold magnification; C) side cut view, 300-fold magnification; D) side cut view; 1200-fold magnification. In pictures C and D, the backing film can be seen under the porous nitrocellulose matrix. The horizontal lines visible in the backing film indicate the cutting direction of the membrane sample. Due to the cutting direction the porous material has not been collapsed in the cutting process. In picture D the asymmetric structure of nitrocellulose matrix is visible. The pore size is smaller on the side of the backing than on the air side. Images courtesy of prof. Martti Toivakka (Center for Functional Materials, Åbo Akademi University).

4.2.2 Dispensing of binder lines and strip cutting

Monoclonal antibodies were used as capturing reagents on the test line in each of the published LFIAs with the exception of the bio-BSA assay. The control lines were

dispensed using 0.6 g/L rabbit anti-mouse polyclonal antibodies (Dako, Denmark) in 10 mM citrate-phosphate buffer pH 5.0 using Linomat 5 line dispenser (Camag, Switzerland) with a flow rate of 250 nL s^{-1} and dispensing rate of 1 µL cm^{-1}.

For the bio-BSA assays, the test line was dispensed with 4 g/L streptavidin (Bioreba, Reinach, Germany) in 10 mM in citrate-phosphate buffer pH 5.0 and the control line was dispensed with 10 mg/L bio-BSA diluted in the same buffer. For the PSA assay, mAb-H117 (University of Turku) was dispensed in concentration 1.5 g/L (**I**) or 0.45 g/L (**II**)

For the cTnI assays, the test lines were dispensed with 0.6 g/L mAb-4T21-19C7 (Hytest) for the single capture format or with a mixture of mAb-4T21-19C7 and mAb-4T21-MF4 (Hytest), each in concentration of 0.3 g/L. For the MxA assay (**III**), the test line was printed with 0.6 g/L mAb-AFI10G10 (Institute of Clinical Medicine, University of Eastern Finland). All the monoclonal antibodies were diluted in 10 mM Tris-HCl pH 8.0 for dispensing.

After dispensing the binder lines, the membranes were incubated in +35 °C for two hours. The membrane cards were cut into the 4 mm (**I**) or 5 mm wide (**II-III**) strips using a desktop paper cutter (**I-II**) (Ideal 105, Krug & Priester, Balingen, Germany) and a dedicated CM4000 guillotine cutter (**III**) (Biodot Inc., Irvine, CA, US) before performing the assays.

4.3 Sample materials

4.3.1 Buffer-based calibrators

All the published assays were initially optimized using the respective analyte molecules spiked in 7.5 % BSA in tris-saline-azide (TSA) buffer pH 7.4. In PSA assays (**I-II**), the calibrators were prepared by diluting recombinant prostate-specific antigen produced in a baculovirus expression system[278]. In the cTnI assays (**II**), calibrator solutions were diluted from purified human cTnI-T-C complex (Hytest).

4.3.2 Serum and plasma

For serum samples (**I-II**), the blood was collected in tubes containing separation gel and clotting activator (VenoSafe VF-108SAS, Terumo). For plasma samples (**II**), the blood was collected in K_3EDTA tubes (Venoject VT-100STK, Terumo) or Li-heparin tubes

(VenoSafe VF-109SHL, Terumo). The samples were produced with centrifugation according to the user manuals of the blood collection tubes. Calibrator solutions for PSA assays (**II**) were prepared by diluting recombinant prostate-specific antigen and, for cTnI assays (**II**) by diluting human cTnI-T-C complex in serum and plasma.

4.3.3 Whole blood

In the LFIA for MxA whole blood (**III**), the clinical samples were collected in non-specified Li-heparin tubes and diluted to 1:20 in hypotonic buffer solution to lyse hemocytes and used in the assay without further preparation steps. A calibrator solution was prepared by diluting recombinant MxA[279] into diluted whole blood.

4.4 LFIA procedure

4.4.1 Fluid absorption sequence

The fluids were absorbed into the LFA strips from polypropene microtiter plate wells (Figure 11). In the assays without pre-incubation of reporter bioconjugates and sample fluid **(I),** the first well contained only the sample fluid and reaction buffer. After absorption of the sample fluid, the strips were transferred into wells containing the reporter bioconjugate and, after complete absorption, into wells with wash buffer solution. In the assays with pre-incubation step, the first well contained a mixture of reporter bioconjugate, sample fluid and reaction buffer **(II, III)**. After absorbing the fluid, the strips were transferred into wells with wash buffer solution. The compositions of the used buffers are presented in table 4.

Table 4. The buffer solutions used in the LFAs.

Assays	Reaction buffer	Wash buffer
bio-BSA & PSA	10 mM HEPES, pH 7.4, 270 mM NaCl, 1 % BSA, 0.5 % Tween-20	same as reaction buffer
cTnI	10 mM Na_2HPO_4/NaH_2PO_4, pH 7.4, 135 mM NaCl, 1 % BSA, 0.5 % Tween-20, 0.06 % Bovine γ-globulin	same as reaction buffer
MxA	0.9 M NaCl, 60 mM K_2HPO_4, 12 mM KH_2HPO_4, pH 7.4, 3 % BSA, 0.3 % NaN_3, 0.3 % Tween-20	10 mM Tris-HCl, pH 8.5, 270 mM NaCl, 1 % BSA, 0.5 % Tween-20

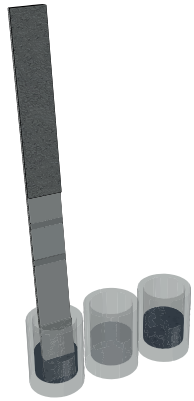


Figure 11. A schematic presentation of the fluid absorption procedure by dipping the assay strip into microtiter wells.

4.4.2 Measurements and data interpretation

The signal intensities of the assay strips were measured either by using a scanning reader device or by a photographic procedure. The assays with Eu(III) nanoparticles (**I** and **II**) were scanned with VictorX4 plate reader (Perkin Elmer, Waltham, MA, USA) using a 340-nm excitation filter and 615-nm emission filter. The delayed, time-resolved measurements were done using a 400-µs delay time, a 400-µs counting window and a duty cycle of 1000 µs. The instant detection measurement was otherwise similar but without the 400-µs delay time.

In the assays with Eu(III) nanoparticles used as reporters, the signal of the test and control lines was calculated from a single data point and, as with the VictorX4 plate reader, the measurement area covered by a single scanning point was approximately 3 mm in diameter. Therefore, the whole width of the test line was covered by a single scanning point making the integration of multiple scanning points unnecessary.

Fluorescence imaging of the Eu(III) nanoparticles (**I**) was done by using a Canon Powershot SX130 IS digital camera (Canon) attached to a laboratory stand and clamp, and taking the picture through a D615/25 bandpass filter while illuminating the assay strips with a handheld ultraviolet lamp (Spectroline, Westbury, VT, USA).

The signals produced by visually detectable colloidal gold particles were measured using reflectometric scanning methods. A USB flatbed scanner (Canoscan 9900F, Canon, Tokyo, Japan) was used to generate a photograph of the assay strips and the reflectance intensity profiles were generated with ImageJ software (National Institutes of Health,

Bethesda, MD, USA) (**I**). Another method of reflectometric measurement of colloidal gold (**II**) was demonstrated using ChemiDoc MP gel imaging device (Bio-Rad, Hercules, CA, USA) and the proprietary Image Lab software.

Assay strips with upconverting nanoparticles were scanned using a modified[274, 280] Plate Chameleon (Hidex Oy, Turku, Finland) (**II**) or UPCON™ Reader (Labrox Oy, Turku, Finland)(**III**). The area covered by a single scanning point was smaller than the width of the binder lines, thus the signals obtained from the location of the test line were summed from a scanning range of 1.5 mm.

The calibration of the assay was done by fitting the data points obtained with the calibrator solutions with a four-parametric (4-PL) logistic fit (4), where A_1 is the initial y-axis value, A_2 is the final y-axis value, x_0 is the centre, and p is the power.

$$y = \frac{A_1 - A_2}{1 + (x/x_0)^p} + A_2 \qquad (4)$$

The control line signal was used to define the location of the test line printed at 6 mm distance from each other. A scanning area of 1.5 mm at the location was used to calculate the average signal of the binder line area (figure 12).

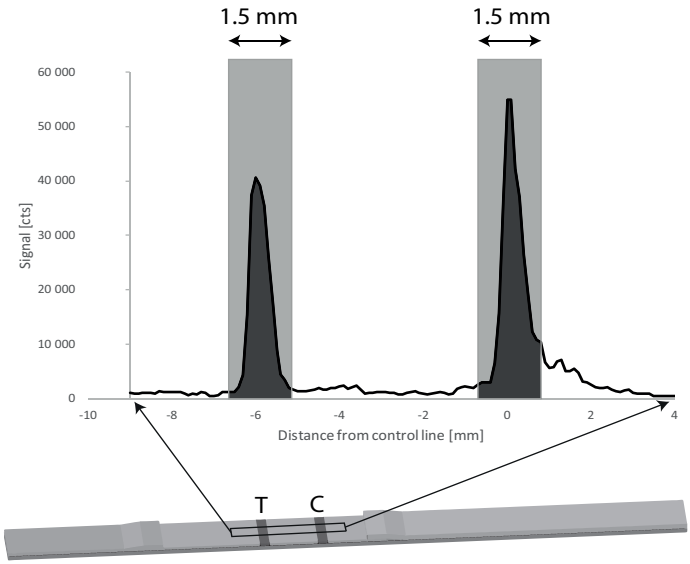


Figure 12. Schematic illustration of the peak integration of the test and control line signals. The control line (C) peak is used to locate the test line (T). The total area of the peak is integrated from the 1.5 mm scanning area.

4.5 Microparticle-based assay platform

To study simple alternatives for nitrocellulose-based LFAs, a simple and affordable platform was developed for point-of-care conditions. Instead of having the capture antibodies bound on a nitrocellulose membrane, the capture antibodies were conjugated with 140 µm polystyrene microparticles that first react with soluble immunoreagents and are then collected on a filter mesh to be measured.

4.5.1 Assay cassette

A simple syringe-operated assay cassette (figure 13) was designed using AutoCAD software (Autodesk Inc., CA, US). Initial prototype stages of the design were produced with stereolithographic 3D printing. The finalized design was produced by injection moulding. The cassette body was moulded from polypropene and the revolving optical cap was moulded from poly(methyl methacrylate) (PMMA). A gasket ring to support the filter mesh was injection moulded from transparent thermoplastic urethane (TPU). A cover cap for the reaction chamber was manufactured by casting polydimethyl siloxane (PDMS) Sylgard 184 (Dow Corning, Midland, MI, US).

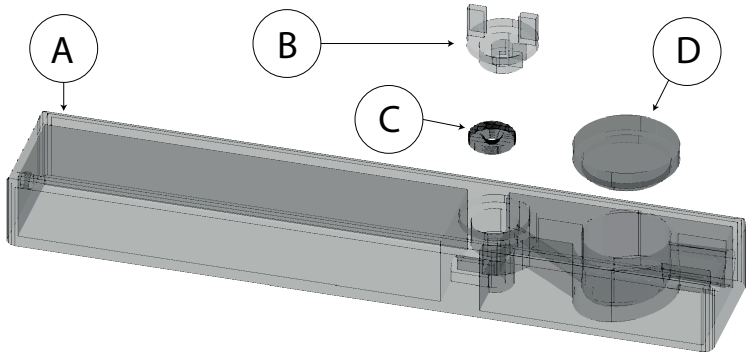


Figure 13. A schematic illustration of the main parts of the assay cassette. (A) Cassette body, (B) revolving optical cap, (C) gasket ring to support the filter mesh, and (D) reaction chamber cap.

4.5.2 Capture microparticle bioconjugation

Dynoseeds TS140 (Microbeads AS, Skedsmokorset, Norway) polystyrene microparticles were passively coated with crosslinked streptavidin. The crosslinking of the streptavidin was done by glutaraldehyde treatment to produce multimers with

increased stability and binding capacity[281]. The streptavidin-coated microparticles were incubated in a mixture of biotin-linked anti-cTnI antibodies mAb4T21-19C7, mAb4T21-MF4, and mAb-4T21-916 to produce capture particles with minimal interference occurring from anti-cTn autoantibodies[282]. After the conjugation with biotinylated antibodies, the non-conjugated antibodies were washed off of the sedimentation of particle by centrifugation and resuspending in 8 % NaCl solution.

4.5.3 Assay procedure

First, 140 µl of sample fluid, 100 µl of assay buffer, 2100 capture microparticles in 14.5 µl volume, and 500 ng of mAb-8I7-UCNPs in 10 µl volume were added to the reaction chamber and incubated for one hour in 500 rpm shaking. After the incubation, the reactions were washed by opening the rotating optical cap to allow liquids to pass through the filter mesh. Negative pressure was applied to the suction channel orifice by a syringe while a pre-filled wash buffer pouch with 3 ml of wash buffer was attached to the buffer inlet of the cassette.

4.5.4 Signal detection

The UCNP signals from the immune complexes captured on the microparticles were measured from the detection chamber using a portable custom fluorometer instrument (DesignInnova, New Delhi, India).

5 SUMMARY OF RESULTS AND DISCUSSION

5.1 Reporter particles

5.1.1 Colloidal gold versus luminescent reporters

The detectability of the optical reporter particles (**I**) used in LFAs contributes to the achievable assay sensitivity with two major factors: 1) The ability to generate enough signal to be detectable by the optical sensor and 2) the ability to exclude non-reporter-specific light emission from the detection. These two features, however, do not define the sensitivity of LFAs using fluorescent reporters, as the non-specifically bound reporter particles commonly cause a background signal that cannot be excluded by optical detection systems.

With colloidal gold, the optical detection is based on reflectance measurement, in which the intensity of the coloured lines on the assay strips are measured by the decrease of the reflected light. The reflectance of the coloured line is relative to the reflectance of the surrounding area on the nitrocellulose membrane. An 8-bit grayscale image allows each pixel to have 256 intensities, of which value 255 represents white and value 0 black. When the blank assay strip signal is calibrated into being bright white, a completely non-reflective area on the strip would result in a grayscale value 0. Simplified, the reflectometric measurement detects non-reporter-specific background light, from which the decrease of reflected light is translated into the reporter-specific signal.

Reflectometric measurement is prone to errors due to dust speckles, remnants of coloured sample fluids, or uneven illumination of the strip surface. Such factors decrease the amount of reflected light and can be interpreted as reporter-specific signals. With luminescent reporters, the non-reporter-specific signals commonly originate from the autofluorescence of the used material or the inefficiency of the optical detection system in blocking the light of the excitation light source.

5.1.2 Measurement mode of Eu(III)-nanoparticles

The luminescence of the Eu(III) nanoparticles can be measured in a time-resolved manner, which further reduces the residual non-reporter-specific fluorescence that is not excluded by the spectral resolution achieved by the Stokes' shift. The effect of the measurement mode on the analytical sensitivity was shown to affect the amount of non-specific fluorescence originating from the assay materials (**I**).

A significant advantage of the Eu(III) nanoparticles is the long Stokes' shift that enables very efficient spectral resolution of the excitation and emission light. The excitation light was filtered to pass only a 40-nm range with a centre wavelength of 340 nm in measurements done with a fluorescence reader.

The significance of the Stokes' shift was even more evident in the fluorescence photography procedure. A continuous, non-filtered UV excitation by a handheld UV lamp with a peak wavelength at 365 nm (figure 14) was used with bandpass emission filter passing only a 25 nm range with a centre wavelength of 615 nm. Most non-reporter-specific fluorescence was excluded by the spectral resolution, but a measurable fluorescence was still observed from the nitrocellulose membrane. Thus, the detectability of the reporter particle signal was not as high as by using pulsed excitation measurement.

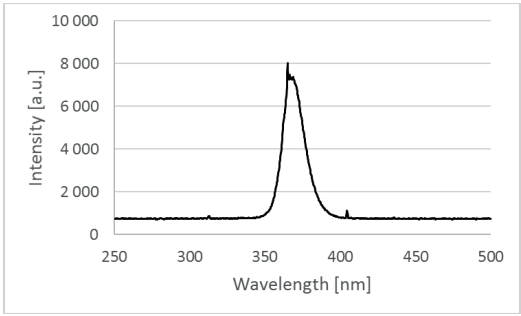


Figure 14. Emission spectrum of the handheld UV-lamp (Spectroline ENF-280C/F) with a peak wavelength at 365nm. Measured with Ocean Optics HR4000 UV-VIS spectrophotometer.

In addition to the spectral resolution, the non-reporter-specific background signal could be further decreased with time-resolved measurement. The effect of time-resolution was tested with three detection modes: 1) Delayed time-resolved measurement, 2) instant-detection time-resolved measurement and 3) continuous excitation imaging.

The instant-detection time-resolved measurement mode resulted in analytical sensitivities (PSA assay 0.08 µg/L, bio-BSA assay 0.03 µg/L) that were very close to the sensitivities achieved with delayed time-resolved measurement mode (PSA assay 0.07 µg/L, bio-BSA assay 0.02 µg/L) (**I**). This suggests that the non-reporter-specific fluorescence constitutes only a small portion of the total fluorescence of the reporter during the time of the measurement cycle between the excitation pulses. Thus, the use

of delay time gate after an excitation pulse provides only a minor improvement in the detectability of the Eu(III) nanoparticles in LFAs.

The practical implication of this result encourages the consideration of using Eu(III) nanoparticles with small portable reader devices equipped with pulsed UV-LED excitation, as the complicated mechanism for the temporal gating of the emission signal is not needed. The spectral resolution combined with pulsed excitation enables the utilization of the large Stokes' shift and the long lifetime of fluorescence in the Eu(III) nanoparticles.

5.1.3 Measurement of UCNP-reporters

A major advantageous feature of the UCNP detection was the capability to exclude non-reporter-specific fluorescence from the assay measurements (**II** & **III**). The scanning profiles of the assays with UCNPs used as reporters indicated that the background fluorescence originates from the reporter particles that have been retained on the nitrocellulose matrix. Therefore, the mobility of the UCNPs in the nitrocellulose was identified as a key factor for achieving high-sensitivity LFAs.

As the upconversion process requires the absorption of two or more low-energy photons to emit a single photon of higher energy, the emitted energy increases with a non-linear dependency on the excitation light. Therefore, the capability of the UCNPs to generate sufficient signal to be detected by an optical sensor is dependent on the intensity of the IR excitation.[283] The estimated excitation power densities achieved by the measurement instruments used in the experiment were approximately 50–100 W/cm^2. Similar excitation power densities have been reported also for larger, 0.48 μm UCNPs.[198] The reported saturation level of the excitation power dependency on the quantum yield is above 150 W/cm^2,[136] which suggests that, with a higher laser diode output and more accurately focused optics, higher reporter signal intensities can be achieved. However, with an excitation power density of 100 W/cm^2, the strips were likely to burn if there were dust speckles or red colour from the blood sample in the assay strip.

The potential of the UCNPs for achieving quantitative LFAs was observed by correlating UCNP-LFA for MxA results with an ELISA test as a reference (**III**). Interpreting the assay result by using the test line per control line (T/C) intensity ratio instead of the test line signal alone compensated for variations in UCNP amounts migrating in the analytical membrane. This is especially helpful in LFAs where the release of the reporter particles from the conjugate pad is inconsistent.

5.1.4 Performance of lateral flow assays

Analytical sensitivity

The analytical sensitivities achieved in the study by using colloidal gold, Eu(III) nanoparticles, and UCNPs in assays for bio-BSA, PSA, cTnI and MxA are presented in table 5. Based on the results, the fluorescence-based reporter technologies, Eu(III) nanoparticles, and UCNPs produced assays with a higher sensitivity than assays with colloidal gold (**I-II**). Whether the Eu(III) nanoparticles or the UCNPs will generally result in higher analytical sensitivity remains inconclusive (**II**), as the assay sensitivity was limited by a background signal originating from non-specifically bound reporters.

Table 5. The analytical sensitivities of the LFAs performed in the study.

Analyte	Reporter	Sample matrix	Analytical sensitivity [µg/L]
bio-BSA	colloidal gold	Buffer	6.10
bio-BSA	Eu(III) nanoparticles	Buffer	0.02
PSA	colloidal gold	Buffer	0.44
PSA	Eu(III) nanoparticles	Buffer	0.196
PSA	upconverting nanoparticles	Buffer	0.556
cTnI	Eu(III) nanoparticles	Serum	2.039*
cTnI	upconverting nanoparticles	Serum	0.041
MxA	upconverting nanoparticles	Buffer	1.669**

*High variation in no-analyte (blank) reactions

** rMxA concentration of the diluted sample fluid

As the LFA procedures used for the studies were optimized for use with batches of 30–100 assay strips handled simultaneously, these results do not necessarily reflect the actual performance of a single, stand-alone assay cassette in a commercialized diagnostic assay kit with dried reporters and a plastic strip housing. Furthermore, as the assays were performed in strictly controlled laboratory conditions, the performance of the assay in realistic point-of-care conditions may be affected by factors such as humidity and heat.

Clinical sensitivity and specificity of the MxA-assay

The UCNP-LFA platform was demonstrated to be feasible in an actual clinical application for the indirect measurement of IFNβ therapy response monitoring in multiple sclerosis (MS) patients (**III**). The quantitative results of the assay were

interpreted by using a MxA cut-off level of 100 µg/L to identify treatment responders and non-responders. Tested with 36 clinical patient samples, the clinical sensitivity of the UCNP-LFA for MxA was 96 % with the specificity of 89 % when a routine ELISA procedure was used as a reference.

The UCNP-LFA for MxA was seen as feasible for use in routine clinical laboratory settings where ELISAs are typically done. The main benefit achieved with the UCNP-LFA was a significant decrease in the performing time of the assay procedure. The ELISA procedure is carried out in two days with an overnight incubation where the UCNP-LFA can be completed within two hours. The MxA assay was the only assay in the study that considers pre-treatment procedure and its effect on the analytical sensitivity.

5.2 Assay platforms

5.2.1 Lateral flow assay platform

As the lateral flow assay platform is practically dependent on the nitrocellulose membrane, the membrane characteristics are very significant factors that define the limitations of LFAs. As stated by the Washburn equation and the Darcy's law, the liquid flow speed in nitrocellulose depends on the pore diameter of the nitrocellulose matrix, viscosity of the sample, hydrophobicity of the matrix surface (contact angle), and the surface tension of the fluid. For this reason, the liquid flow speed in the LFAs can only be adjusted by selecting nitrocellulose membranes rated with different flow speeds or by introducing barriers to restrain the liquid flow. The HF180 nitrocellulose membranes (Merck) used in the studies were among the slowest membranes available with a wicking rate of 180 s / 4 cm.

A slow nitrocellulose membrane was selected to achieve maximal time for forming of the immunocomplexes on the capture lines, thus assuming increased assay sensitivity (**I-III**). Furthermore, the printing of the capture lines resulted in thinner capture lines, as the biomolecules used for the capture line could not disperse in the nitrocellulose as much as in a faster membrane before the solvent had evaporated.

The steps of the LFA procedures in this study included dispensing the sample fluid, reporter solution, and wash buffer into the microtiter wells. Subsequently, the assay strips were dipped in the fluids in sequential order and allowed to absorb the fluids completely. The process constituted of approximately 5–10 minutes of hands-on time, as the strips had to be allowed to absorb the fluids completely before moving them into

the consecutive microtiter well. When the assay strips were in the wash buffer, the assay was left unattended until the measurement step which was performed after the strips had dried completely (i.e. at least two hours). For the measurement step, the assay strips were attached to a strip holder tray and measured with a reader compatible with the reporter technology. The total required time for the assays performed in the study was typically 2–4 hours. The limited liquid capacity and increased assay time set a limit for the wash buffer used in the assay. Therefore, non-specifically-bound reporters retained at the assay strip may limit the assay sensitivity.

Nitrocellulose membrane characterization (previously unpublished additional data)

The fluorescence characteristics of the HF180 nitrocellulose membrane indicated that the membrane has its most significant short Stokes' shift fluorescence peaks at wavelengths shorter than 520 nm (figure 15A). With a static excitation at 350 nm, the scanning indicated that the autofluorescence with UV-excitation spans throughout the whole scanning range (figure 15B). The substantial autofluorescence peak observed at 550 nm has also been reported by Swanson et al.[112]. These observations confirmed that a significant part of the autofluorescence originating from the nitrocellulose is not eliminated by the spectral resolution as seen in the fluorescence photography of Eu(III) nanoparticles. The autofluorescence of the nitrocellulose can be problematic, especially with reporter technologies having insufficient Stokes' shift to efficiently resolve the reporter-specific signal from non-specific fluorescence by optical filters.

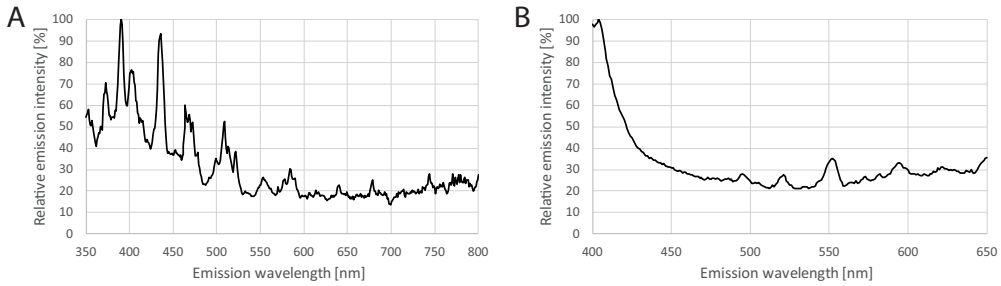


Figure 15. Fluorescence spectrum of the HF180-nitrocellulose membrane. A) Emission detected synchronically at excitation wavelength + 20 nm. B) Emission scanning with constant excitation at 350 nm.

The size distribution observed with the Hg porosimetry analysis (figure 16) indicated that the average pore size was 4 µm, but a significant portion of the pores have a diameter smaller than 4 µm. Combining this result with the observation that smaller pores are

located at the bottom of the nitrocellulose matrix (figures 5&10), it is likely that the liquid flow speed is not constant throughout the depth of the matrix. A situation where the liquid flow would be slower at the bottom of the matrix would be likely to cause reporter particles to be retained in the nitrocellulose membrane.

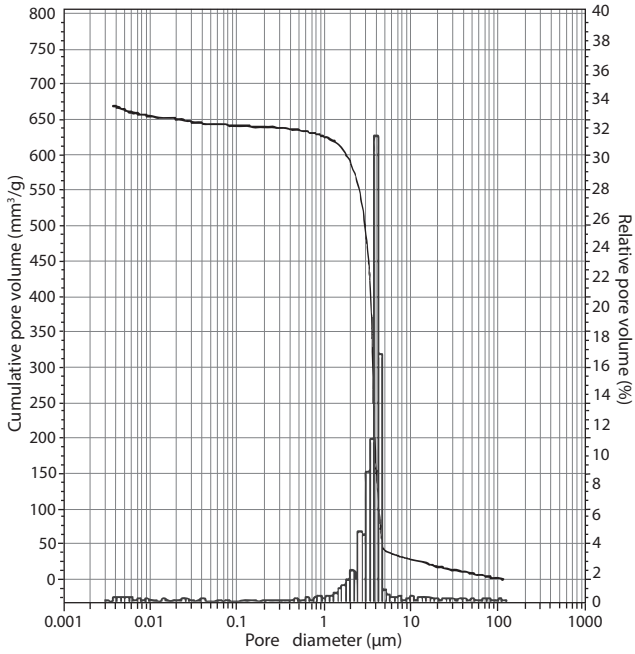


Figure 16. Mercury porosimetry analysis graph of the Hi-Flow Plus HF180 nitrocellulose (Merck). Histogram indicating the size distribution of the nitrocellulose pores (left y-axis). Cumulative pore volume indicated with a curve (right y-axis)

5.2.2 Microparticle-based assay platform

As in the LFAs, the formed immunocomplexes are bound to a stationary location in the binder lines and the capture microparticles are free to move in the reaction mixture of the microparticle-based assay. (**IV**) The collection of the capture microparticles on the filtration mesh for the reporter signal measurement could efficiently collect the capture microparticles (figure 17). This feature enables favourable reaction conditions that allow the immunocomplex to be formed for a longer time on to the capture surface, and mixing to improve reaction kinetics. However, the variation of the amount, orientation, and location of the capture microparticles in the detection chamber may present variation in the assay results.

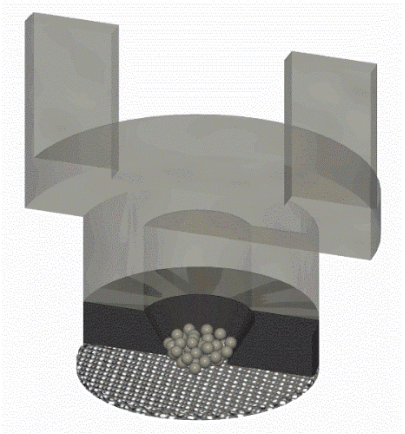


Figure 17. A schematic illustration of capture microparticles collected in the measurement chamber to be measured through an optical cap.

The performance of the microparticle-based assay platform was demonstrated with a cTnI assay done with cardiac troponin ITC complex diluted in assay buffer. The assay included a mixing of the analyte solution, capture microparticle solution and mab-8I7-UCNP solution, incubation for one hour, and a washing step prior to the measurement. With a total assay time of less than 90 minutes and a hands-on time of less than five minutes, a limit of detection of 19.7 ng/L was achieved.

As the manufacturing process of the microparticle-based platform requires specific injection-moulded cassettes, nylon filters, optical caps, gasket rings, buffer pouches, and separate syringes to drive the wash step, significant assembly is required for manufacturing complete test kits. Therefore, the manufacturing price is unlikely to be competitive with highly automatized LFA manufacturing lines. The microparticle-based assay, however, may have a niche of products requiring more efficient immunocomplex capturing, improved assay kinetics, and more efficient washing step than in LFA, while the assay would still be affordable and capable of working in point-of-care conditions.

6 CONCLUSIONS

The rapidly increasing number of publications and patents with LFAs indicates that the technology is becoming increasingly popular, although the basic concept has been in commercial production since the 1980s. The simplicity of the LFAs' basic structure makes the assays relatively cost-effective to manufacture in automatized production lines. The development process for new products is relatively rapid and can be set in motion with relatively small initial costs, as the basic instrumentation required for small-scale prototype production include only a strip cutter and a line dispenser in addition to standard laboratory equipment.

For a rapid diagnostic test to be suitable for use in the most resource poor conditions, the test kits must be stable in ambient temperatures for at least several months. The test procedure must be very simple to perform in order to minimize the risk of operator errors. The clinical sensitivity and specificity must be sufficient to provide helpful information for the medical practitioners. All these criteria must be fulfilled without sacrificing the affordability of the product. Although clinical diagnostics is the most dominant market segment of LFAs, the assay platform has become increasingly popular in applications for environmental monitoring, food safety and pharmaceutical industry.

The main conclusions based on the original publications are presented below.

I Up to 300-fold improvement of analytical sensitivity can be achieved by using Eu(III) nanoparticles as reporters in LFAs compared to colloidal gold reporters. Time-resolved detection for the elimination of non-reporter specific background signal can be efficiently reduced by using a pulsed excitation light source.

II Blood anticoagulants used in venipuncture tubes have variable compatibility with LFAs. The blood samples collected in tubes containing K_3EDTA as the anticoagulant resulted in significant interference by decreased reporter particle mobility. An LFA for measuring cardiac troponin in a serum was demonstrated with an analytical sensitivity of 41 ng/L.

III A quantitative and user-friendly LFA for diluted whole blood samples can be developed using upconverting nanoparticles. Interfering sample matrix effects can be mitigated by diluting the sample fluid while still retaining sufficient analytical sensitivity to achieve clinically feasible diagnostic assays. The

agreement of results obtained from real patient was demonstrated by a linear regression analysis of the UNCP-LFA and ELISA (n: 36, R^2: 0.86).

IV A microparticle-based immunoassay platform suitable for point-of-care use was developed. The potential of achieving high analytical sensitivities was based on a less limited immunocomplex formation time than in LFAs, as the capture microparticles could be incubated with the reaction mixture as long as required. UCNP reporters can successfully be used in challenging measurement conditions where the plastic materials have significant autofluorescence properties.

As the stand-alone capability of the LFAs to be used without any reader device makes the platform very convenient for diagnostic assays that are needed only occasionally, the need for higher analytical performance with LFAs indicates a growing trend of using reader devices with LFAs.[151] Along with different reader instruments, different reporter technologies can be expected to gradually appear to the market. The UCNP technology, Eu(III) nanoparticles and Alexa Fluor reporters have already been included in commercial products, but a breakthrough by any particular reporter technology is yet to happen. For achieving optimal UCNP particles for LFAs, the balance of the size-dependent quantum yield and the mobility of the particles in the nitrocellulose matrix is vital.

ACKNOWLEDGEMENTS

This work was carried out at the Department of Biotechnology of the University of Turku in 2009–2016. During that time, a lot of water passed in the Aura river and doctoral programmes have come and gone. However, I believe a slow maturation process may generally be beneficial for brewing insightful researchers.

I express my deep gratitude for Professor Kim Pettersson, who, as a paragon of science, has ignited my inspiration to work in the field of diagnostics. His kindness and benign attitude has been a very important support during the times when my frustration with the laboratory work has been overwhelming. My sincerest thanks to Professor Tero Soukka, who has been an inexhaustible resource of advice regarding anything related to science. Many thanks to Professor Emeritus Timo Lövgren, who has laid a solid foundation for the education and research of biotechnology and diagnostics in Turku. His valuable insights into what it is worth for pursuing research are greatly appreciated. I want to thank Dr. Urpo Lamminmäki for all the help with the administrative tasks related to various projects, inspiring lectures during my M.Sc. studies, and great and fun company on the work trips. I also wish to express my heartfelt thanks to Assistant Professor Dr. Gaurav Batra, who has seamlessly collaborated with our projects despite the six-hour flight distance. A special thanks to Professor Dr. Navin Khanna, who is ever so encouraging and pleasant to meet, and Dr. Dinesh Kumar who keeps impressing everyone with his brilliant engineering and artistic mindset. I also wish to thank Professor Martti Toivakka for providing very valuable information of the nitrocellulose membrane by electron microscopy and Hg porosimetry.

I have been privileged to work in a team with enthusiastic and skilled people who have been crucial for achieving the publications. As more than ten years have passed during my stay as a university employee, the sheer number of new friends from that time makes it practically impossible to mention you all by name in this context. As we know, nothing can be achieved without teamwork. Therefore, the co-authors Teppo Salminen, Tiina Myyryläinen, Sheikh Talha, Riikka Arppe, Merja Lahdenranta, Laura Kalliomäki and Iida Martiskainen deserve praises for their valuable contribution to laboratory work, discussions, and occasional bleeding. For Mikko Pyykkö, Anttoni Korkiakoski and Joni Juvonen, I present my sincerest thanks for all the help regarding the computer programming, soldering and the various miscellaneous undefined tasks. For Dr. Matti Waris I am grateful for getting the great opportunity to work on the MxA-assay. For the great work in getting rid of the spelling and grammar errors in my thesis I sincerely thank Mari Ratia.

Without the help of the regular staff at the Department of Biotechnology, it would have been practically impossible to get any laboratory work done. Therefore, I wish to thank Jani Koskinen, Teija Luotohaara, Pirjo Pietilä, Hannele Heinonen, Sanna Laitinen, Sari Heikkilä, and those already retired from active duty: Pirjo Laaksonen and Mirja Jaala.

Additional special thanks to Riikka Arppe, who has been a co-author, co-worker, bandmate, and a friend since my first biotechnology lab course. All the students and summer workers during all these years have contributed much to the three-week works, summer works and all the random scientific efforts we have had. I wish to thank you all.

Outside work and family-time, my time for unwinding is mostly occupied by music. In my opinion, soloists can play with themselves but a band playing together adds up to more than the sum of its components. I want to thank all my current and past bandmates: Jari-Matti Sirkka, Timo Paukkio, Riina Palmroth, Eero Lehtonen, Veikko Wahlroos, Tommi Alho, Tommi Siivonen and Antti Janka-Murros for making the time spent in rehearsals and performances very pleasant.

Naturally, I am very grateful to mom and dad for the encouraging upbringing and to my sister Inkeri and her family for many enjoyable moments. For the most fundamental support for the efforts needed in completing this work, I express my humble gratitude to my dear wife Maija and to my daughters Alma and Selma. Thank you for bringing love and such enormous joy in my life.

In Turku,

15. January 2018

REFERENCES

1. Campbell, R.L., Wagner, D.B., and O'connell, J.P., Solid phase assay with visual readout. US4703017 (A) (1987).
2. Rosenstein, R., Solid phase assay., EP0284232 (A1) (1988).
3. May, K., Prior, M., and Richards, I., Immunoassays and devices therefor., EP0291194 (B1) (1988).
4. Dzantiev, B.B., Byzova, N.A., Urusov, A.E., and Zherdev, A.V., Immunochromatographic methods in food analysis. *Trends Anal. Chem.* **55**: p. 81-93 (2014).
5. Koets, M., Sander, I., Bogdanovic, J., Doekes, G., and van Amerongen, A., A rapid lateral flow immunoassay for the detection of fungal alpha-amylase at the workplace. *J. Environ. Monit.* **8**(9): p. 942-946 (2006).
6. Posthuma-Trumpie, G.A., Korf, J., and van Amerongen, A., Lateral flow (immuno)assay: its strengths, weaknesses, opportunities and threats. A literature survey. *Anal. Bioanal. Chem.* **393**(2): p. 569-82 (2009).
7. Leuvering, J.H., Thal, P., Waart, M.v.d., and Schuurs, A., Sol particle immunoassay (SPIA). *J. Immunoassay.* **1**(1): p. 77-91 (1980).
8. Berson, S.A. and Yalow, R.S., Quantitative aspects of the reaction between insulin and insulin-binding antibody. *J. Clin. Invest.* **38**(11): p. 1996 (1959).
9. Singer, J.M. and Plotz, C.M., The latex fixation test. *Am. J. Med.* **21**(6): p. 888-892 (1956).
10. Newman, J.D. and Turner, A.P.F., Home blood glucose biosensors: a commercial perspective. *Biosens. Bioelectron.* **20**(12): p. 2435-2453 (2005).
11. Luntz, G., A Simple Quick Test for Glucose in Urine. *BMJ.* **1**(5017): p. 499-500 (1957).
12. Greenwald, J.L., Burstein, G.R., Pincus, J., and Branson, B., A rapid review of rapid HIV antibody tests. *Curr. Infect. Dis. Rep.* **8**(2): p. 125-131 (2006).
13. Bangs Laboratories Inc. *Tech notes 303: Lateral flow tests.* 2008; Available from: http://www.bangslabs.com/sites/default/files/imce/docs/TechNote%20303%20Web.pdf.
14. Volkov, A., Mauk, M., Corstjens, P., and Niedbala, R.S., Rapid prototyping of lateral flow assays, in Biosensors and Biodetection: Methods and Protocols: Electrochemical and Mechanical Detectors, Lateral Flow and Ligands for Biosensors, A. Rasooly and K.E. Herold, Editors. 2009, Springer. p. 217-235 (2009).
15. Sajid, M., Kawde, A.-N., and Daud, M., Designs, formats and applications of lateral flow assay: A literature review. *J. Saudi Chem. Soc.* **19**(6): p. 689-705 (2015).
16. DaCosta, M.V., Doughan, S., Han, Y., and Krull, U.J., Lanthanide upconversion nanoparticles and applications in bioassays and bioimaging: A review. *Anal. Chim. Acta.* **832**: p. 1-33 (2014).
17. Auzel, F., Upconversion and anti-stokes processes with f and d ions in solids. *Chem. Rev.* **104**(1): p. 139-174 (2004).
18. Ehrmeyer, S.S. and Laessig, R.H., Point-of-care testing, medical error, and patient safety: a 2007 assessment. *Clin. Chem. Lab. Med.* **45**(6): p. 766-73 (2007).
19. Pai, N.P. and Pai, M., Point-of-care diagnostics for HIV and tuberculosis: landscape, pipeline, and unmet needs. *Discov. Med.* **13**(68): p. 35-45 (2012).
20. Howick, J., Cals, J.W., Jones, C., Price, C.P., Plüddemann, A., Heneghan, C., Berger, M.Y., Buntinx, F., Hickner, J., and Pace, W., Current and future use of point-of-care tests in

primary care: an international survey in Australia, Belgium, The Netherlands, the UK and the USA. *BMJ open*. **4**(8): p. e005611 (2014).

21. Yager, P., Domingo, G.J., and Gerdes, J., Point-of-care diagnostics for global health. *Annu. Rev. Biomed. Eng.* **10**: p. 107-44 (2008).
22. Peeling, R.W. and Mabey, D., Point-of-care tests for diagnosing infections in the developing world. *Clin. Microbiol. Infect.* **16**(8): p. 1062-9 (2010).
23. Pruett, C.R., Vermeulen, M., Zacharias, P., Ingram, C., Tayou Tagny, C., and Bloch, E.M., The Use of Rapid Diagnostic Tests for Transfusion Infectious Screening in Africa: A Literature Review. *Transfus. Med. Rev.* **29**(1): p. 35-44 (2015).
24. World Health Organization. *WHO list of prequalified in vitro diagnostic products*. 2017 Aug 16. [cited 2017 September 7.]; Available from: http://www.who.int/diagnostics_laboratory/evaluations/170816_prequalified_product_list.pdf.
25. Zuk, R.F. and Litman, D.J., Immunochromatographic assay with support having bound "MIP" and second enzyme. US4435504 (A) (1984).
26. David, G.S. and Greene, H.E., Immunometric assays using monoclonal antibodies. US4376110 (A) (1983).
27. Poggendorff, J.C., Wiedemann, E., and Wiedemann, G.H., *Annalen der Physik*. Vol. 23. 1884: JA Barth (1884).
28. Hooke, R., *Micrographia*. 1665: Royal Society of London (1665).
29. Boyle, R. and Birch, T., The Works of the Honourable Robert Boyle: In Six Volumes. To which is Prefixed the Life of the Author. 1772: J. and F. Rivington (1772).
30. Young, T., An essay on the cohesion of fluids. *Philos. Trans. R. Soc. Lond.* **95**: p. 65-87 (1805).
31. Langmuir, I., Monolayers on solids. *J. Chem. Soc.*: p. 511-543 (1940).
32. Eberhart, M., Donovan, M., MacLaren, J., and Clougherty, D., Towards a chemistry of cohesion and adhesion. *Prog. Surf. Sci.* **36**(1): p. 1-34 (1991).
33. Jorgensen, W.L. and Madura, J.D., Temperature and size dependence for Monte Carlo simulations of TIP4P water. *Mol. Phys.* **56**(6): p. 1381-1392 (1985).
34. Gauss, C.F., Principia generalia theoriae figurae fluidorum in statu aequilibrii, in Werke. 1877, Springer. p. 29-77 (1877).
35. World Health Organization. *Universal access to malaria diagnostic testing – An operational manual*. 2013 [cited 2017 07.04.2017]; Available from: http://www.who.int/malaria/publications/atoz/9789241502092/en/.
36. World Health Organization, *HIV assays: operational characteristics (Phase 1)*. HIV assays: operational characteristics (Phase 1). 2004, Geneva, Switzerland: World Health Organization (2004).
37. Sharma, S., Zapatero-Rodríguez, J., Estrela, P., and O'Kennedy, R., Point-of-care diagnostics in low resource settings: present status and future role of microfluidics. *Biosensors*. **5**(3): p. 577-601 (2015).
38. Mbanya, D., Use of quality rapid diagnostic testing for safe blood transfusion in resource-limited settings. *Clin. Microbiol. Infect.* **19**(5): p. 416-421 (2013).
39. O'Farrell, B., Lateral Flow Immunoassay Systems: 2.4 Evolution from the Current State of the Art to the Next Generation of Highly Sensitive, Quantitative Rapid Assays, in The Immunoassay Handbook: Theory and applications of ligand binding, ELISA and related techniques, D. Wild, Editor. 2013, Elsevier: Amsterdam, Netherlands. p. 89 (2013).

40. Rosen, S., Market trends in lateral flow immunoassays, in Lateral Flow Immunoassay. 2009, Springer. p. 1-15 (2009).

41. Nayyar, G.M., Breman, J.G., Newton, P.N., and Herrington, J., Poor-quality antimalarial drugs in southeast Asia and sub-Saharan Africa. *Lancet Infect. Dis.* **12**(6): p. 488-96 (2012).

42. Corstjens, P.L.A.M., Fidder, H.H., Wiesmeijer, K.C., de Dood, C.J., Rispens, T., Wolbink, G.-J., Hommes, D.W., and Tanke, H.J., A rapid assay for on-site monitoring of infliximab trough levels: a feasibility study. *Anal. Bioanal. Chem.* **405**(23): p. 7367-7375 (2013).

43. Ingenhoff, L., Hall, E., and House, J.K., Evaluation of a cow-side milk progesterone assay and assessment of the positive predictive value of oestrus diagnosis by dairy farmers in New South Wales. *Aust. Vet. J.* **94**(12): p. 445-451 (2016).

44. Liu, J., O'Connor, T., Beall, M., Chandrashekar, R., and Lappin, M., Evaluation of rapid diagnostic test kits for feline leukemia virus infection using samples from naturally infected cats. *JFMS Open Rep.* **2**(2): p. 2055116916667757 (2016).

45. Schmitz, S., Coenen, C., Matthias, K., Heinz-Jürgen, T., and Neiger, R., Comparison of Three Rapid Commercial Canine Parvovirus Antigen Detection Tests with Electron Microscopy and Polymerase Chain Reaction. *J. Vet. Diagn. Invest.* **21**(3): p. 344-345 (2009).

46. Danks, C. and Barker, I., On-site detection of plant pathogens using lateral-flow devices*. *EPPO Bulletin*. **30**(3-4): p. 421-426 (2000).

47. Tripathi, P., Upadhyay, N., and Nara, S., Recent advancements in lateral flow immunoassays: a journey for toxin detection in food. *Crit. Rev. Food Sci. Nutr.*: p. 00-00 (2017).

48. Singh, J., Sharma, S., and Nara, S., Evaluation of gold nanoparticle based lateral flow assays for diagnosis of enterobacteriaceae members in food and water. *Food Chem.* **170**: p. 470-483 (2015).

49. Schubert-Ullrich, P., Rudolf, J., Ansari, P., Galler, B., Fuhrer, M., Molinelli, A., and Baumgartner, S., Commercialized rapid immunoanalytical tests for determination of allergenic food proteins: an overview. *Anal. Bioanal. Chem.* **395**(1): p. 69-81 (2009).

50. Anfossi, L., Giovannoli, C., Giraudi, G., Biagioli, F., Passini, C., and Baggiani, C., A lateral flow immunoassay for the rapid detection of ochratoxin A in wine and grape must. *J. Agric. Food Chem.* **60**(46): p. 11491-7 (2012).

51. Anfossi, L., Baggiani, C., Giovannoli, C., Biagioli, F., D'Arco, G., and Giraudi, G., Optimization of a lateral flow immunoassay for the ultrasensitive detection of aflatoxin M 1 in milk. *Anal. Chim. Acta.* **772**: p. 75-80 (2013).

52. Röder, M., Vieths, S., and Holzhauser, T., Commercial lateral flow devices for rapid detection of peanut (Arachis hypogaea) and hazelnut (Corylus avellana) cross-contamination in the industrial production of cookies. *Anal. Bioanal. Chem.* **395**(1): p. 103-109 (2009).

53. Lane, H.C., Montagne, J.L., and Fauci, A.S., Bioterrorism: A clear and present danger. *Nat. Med.* **7**(12): p. 1271-1273 (2001).

54. Bahadır, E.B. and Sezgintürk, M.K., Applications of commercial biosensors in clinical, food, environmental, and biothreat/biowarfare analyses. *Anal. Biochem.* **478**: p. 107-120 (2015).

55. Bartholomew, R.A., Ozanich, R.M., Arce, J.S., Engelmann, H.E., Heredia-Langner, A., Hofstad, B.A., Hutchison, J.R., Jarman, K., Melville, A.M., Victry, K.D., and Bruckner-Lea, C.J., Evaluation of Immunoassays and General Biological Indicator Tests for Field Screening of Bacillus anthracis and Ricin. *Health. Secur.* **15**(1): p. 81-96 (2017).

56. Evans, J. *Point of Care Diagnostics - HLC043E*. Market Research Reports 2016 [cited 2017 25.1.2017]; Available from: http://www.bccresearch.com/market-research/healthcare/point-of-care-diagnostics-report-hlc043e.html.

57. O'Farrell, B., Evolution in lateral flow–based immunoassay systems, in Lateral Flow Immunoassay. 2009, Humana Press. p. 1-33 (2009).

58. Byrnes, S., Thiessen, G., and Fu, E., Progress in the development of paper-based diagnostics for low-resource point-of-care settings. *Bioanalysis*. **5**(22): p. 2821-2836 (2013).

59. Urusov, A.E., Petrakova, A.V., Zherdev, A.V., and Dzantiev, B.B., "Multistage in one touch" design with a universal labelling conjugate for high-sensitive lateral flow immunoassays. *Biosens. Bioelectron.* **86**: p. 575-579 (2016).

60. Cho, J.H., Paek, E.H., Cho, I.H., and Paek, S.H., An enzyme immunoanalytical system based on sequential cross-flow chromatography. *Anal. Chem.* **77**(13): p. 4091-7 (2005).

61. Lee, S., O'Dell, D., Hohenstein, J., Colt, S., Mehta, S., and Erickson, D., NutriPhone: a mobile platform for low-cost point-of-care quantification of vitamin B12 concentrations. *Sci. Rep.* **6**: p. 28237 (2016).

62. Posthuma-Trumpie, G.A., Korf, J., and van Amerongen, A., Development of a competitive lateral flow immunoassay for progesterone: influence of coating conjugates and buffer components. *Anal. Bioanal. Chem.* **392**(6): p. 1215-1223 (2008).

63. Zangheri, M., Cevenini, L., Anfossi, L., Baggiani, C., Simoni, P., Di Nardo, F., and Roda, A., A simple and compact smartphone accessory for quantitative chemiluminescence-based lateral flow immunoassay for salivary cortisol detection. *Biosens. Bioelectron.* **64**: p. 63-68 (2015).

64. Inoue, K., Ferrante, P., Hirano, Y., Yasukawa, T., Shiku, H., and Matsue, T., A competitive immunochromatographic assay for testosterone based on electrochemical detection. *Talanta*. **73**(5): p. 886-892 (2007).

65. Johnson, S., Harrison, K., and Turner, A.D., Application of rapid test kits for the determination of Amnesic Shellfish Poisoning in bivalve molluscs from Great Britain. *Toxicon*. **117**: p. 76-83 (2016).

66. Goessaert, A.S., Pil, K., Veramme, J., and Verstraete, A., Analytical evaluation of a rapid on-site oral fluid drug test. *Anal. Bioanal. Chem.* **396**(7): p. 2461-8 (2010).

67. Niedbala, R.S., Feindt, H., Kardos, K., Vail, T., Burton, J., Bielska, B., Li, S., Milunic, D., Bourdelle, P., and Vallejo, R., Detection of analytes by immunoassay using up-converting phosphor technology. *Anal. Biochem.* **293**(1): p. 22-30 (2001).

68. Davies, C., 2.1 Principles of Competitive and Immunometric Assays (Including ELISA), in The Immunoassay Handbook: Theory and applications of ligand binding, ELISA and related techniques, D. Wild, Editor. 2013, Elsevier: Amsterdam, Netherlands(2013).

69. Chaiet, L. and Wolf, F.J., The Properties of Streptavidin, a Biotin-Binding Protein Produced by Streptomycetes. *Arch. Biochem. Biophys.* **106**: p. 1-5 (1964).

70. Holstein, C.A., Chevalier, A., Bennett, S., Anderson, C.E., Keniston, K., Olsen, C., Li, B., Bales, B., Moore, D.R., Fu, E., Baker, D., and Yager, P., Immobilizing affinity proteins to nitrocellulose: a toolbox for paper-based assay developers. *Anal. Bioanal. Chem.* **408**(5): p. 1335-1346 (2016).

71. Jönsson, C., Aronsson, M., Rundstrom, G., Pettersson, C., Mendel-Hartvig, I., Bakker, J., Martinsson, E., Liedberg, B., MacCraith, B., Ohman, O., and Melin, J., Silane-dextran chemistry on lateral flow polymer chips for

immunoassays. *Lab Chip*. **8**(7): p. 1191-1197 (2008).

72. Jönsson, C. and Melin, J., Capillary driven assay device and its manufacture. EP2281632 (B1) (2011).

73. Öhman, P.O. and Mendel-Hartvig, I., Micro fluidic structures. WO03103835 (A1) (2003).

74. Amundson, B.E. and Apple, F.S., Cardiac troponin assays: a review of quantitative point-of-care devices and their efficacy in the diagnosis of myocardial infarction. *Clin. Chem. Lab. Med.* **53**(5): p. 665-76 (2015).

75. Fridley, G.E., Holstein, C.A., Oza, S.B., and Yager, P., The evolution of nitrocellulose as a material for bioassays. *MRS Bulletin*. **38**(4): p. 326-330 (2013).

76. Washburn, E.W., The Dynamics of Capillary Flow. *Phys. Rev.* **17**(3): p. 273-283 (1921).

77. Oliver, J.F., *Wetting and Penetration of Paper Surfaces*, in *Colloids and Surfaces in Reprographic Technology*, M. Hair and M.D. Croucher, Editors. 1982, American Chemical Society: Washington DC. p. 435-453 (1982).

78. McNaught, A.D. and McNaught, A.D., *Compendium of chemical terminology*. Vol. 1669. 1997: Blackwell Science Oxford (1997).

79. Darcy, H., Les fontaines publiques de la ville de Dijon: exposition et application. 1856: Victor Dalmont (1856).

80. Osborn, J.L., Lutz, B., Fu, E., Kauffman, P., Stevens, D.Y., and Yager, P., Microfluidics without pumps: reinventing the T-sensor and H-filter in paper networks. *Lab Chip*. **10**(20): p. 2659-2665 (2010).

81. Henderson, K. and Stewart, J., Factors influencing the measurement of oestrone sulphate by dipstick particle capture immunoassay. *J. Immunol. Methods.* **270**(1): p. 77-84 (2002).

82. Mansfield, M.A., Nitrocellulose membranes for lateral flow immunoassays: a technical treatise, in Lateral Flow Immunoassay. 2009, Springer. p. 1-19 (2009).

83. Baker, R.W., *Membrane technology*. 2000: Wiley Online Library (2000).

84. Pinnau, I. and Freeman, B.D., Formation and Modification of Polymeric Membranes: Overview, in Membrane Formation and Modification. 1999, American Chemical Society. p. 1-22 (1999).

85. Tisone, T.C. and O'Farrell, B., Manufacturing the next generation of highly sensitive and reproducible lateral flow immunoassay, in Lateral Flow Immunoassay, R. Wong and H. Tse, Editors. 2009, Humana Press. p. 131-156 (2009).

86. Aki, M., Nagai, S., Saito, N., and Imoarai, T., Immunochromatographic test device. (2008).

87. Ponti, J.S., Material platform for the assembly of lateral flow immunoassay test strips, in Lateral Flow Immunoassay. 2009, Springer. p. 1-7 (2009).

88. Horisberger, M. and Rosset, J., Colloidal gold, a useful marker for transmission and scanning electron microscopy. *J. Histochem. Cytochem.* **25**(4): p. 295-305 (1977).

89. Dent, A.H., Chapter 3.4 Conjugation methods, in The immunoassay handbook: theory and applications of ligand binding, ELISA and related techniques, D. Wild, Editor. 2013, Elsevier: Amsterdam, Netherlands(2013).

90. Doering, W.E., Piotti, M.E., Natan, M.J., and Freeman, R.G., SERS as a foundation for nanoscale, optically detected biological labels. *Adv. Mater.* **19**(20): p. 3100-3108 (2007).

91. Tanaka, R., Yuhi, T., Nagatani, N., Endo, T., Kerman, K., Takamura, Y., and Tamiya, E., A novel enhancement assay for immunochromatographic test strips using gold nanoparticles. *Anal. Bioanal. Chem.* **385**(8): p. 1414-20 (2006).

92. Blanco-Covián, L., Montes-García, V., Girard, A., Fernández-Abedul, M.T., Perez-Juste, J., Pastoriza-Santos, I., Faulds, K., Graham, D., and Blanco-López, M.C., Au@ Ag SERRS tags coupled to a lateral flow immunoassay for the sensitive detection of Pneumolysin. *Nanoscale*, (2017).

93. Gordon, J. and Michel, G., Analytical Sensitivity Limits for Lateral Flow Immunoassays. *Clin. Chem.* **54**(7): p. 1250 (2008).

94. Moghadam, B.Y., Connelly, K.T., and Posner, J.D., Two orders of magnitude improvement in detection limit of lateral flow assays using isotachophoresis. *Anal. Chem.* **87**(2): p. 1009-17 (2015).

95. Rodríguez, M.O., Covián, L.B., García, A.C., and Blanco-López, M.C., Silver and gold enhancement methods for lateral flow immunoassays. *Talanta*. **148**: p. 272-278 (2016).

96. Shyu, R.H., Shyu, H.F., Liu, H.W., and Tang, S.S., Colloidal gold-based immunochromatographic assay for detection of ricin. *Toxicon*. **40**(3): p. 255-8 (2002).

97. Pal, A. and Dhar, T.K., An analytical device for on-site immunoassay. Demonstration of its applicability in semiquantitative detection of aflatoxin B1 in a batch of samples with ultrahigh sensitivity. *Anal. Chem.* **76**(1): p. 98-104 (2004).

98. Parolo, C., de la Escosura-Muñiz, A., and Merkoçi, A., Enhanced lateral flow immunoassay using gold nanoparticles loaded with enzymes. *Biosens. Bioelectron.* **40**(1): p. 412-416 (2013).

99. Akanda, M.R., Joung, H.-A., Tamilavan, V., Park, S., Kim, S., Hyun, M.H., Kim, M.-G., and Yang, H., An interference-free and rapid electrochemical lateral-flow immunoassay for one-step ultrasensitive detection with serum. *Analyst*. **139**(6): p. 1420-1425 (2014).

100. van Dam, G.J., Wichers, J.H., Ferreira, T.M., Ghati, D., van Amerongen, A., and Deelder, A.M., Diagnosis of schistosomiasis by reagent strip test for detection of circulating cathodic antigen. *J. Clin. Microbiol.* **42**(12): p. 5458-61 (2004).

101. Lönnberg, M. and Carlsson, J., Quantitative Detection in the Attomole Range for Immunochromatographic Tests by Means of a Flatbed Scanner. *Anal. Biochem.* **293**(2): p. 224-231 (2001).

102. Posthuma-Trumpie, G.A., Wichers, J.H., Koets, M., Berendsen, L.B.J.M., and van Amerongen, A., Amorphous carbon nanoparticles: a versatile label for rapid diagnostic (immuno)assays. *Anal. Bioanal. Chem.* **402**(2): p. 593-600 (2012).

103. Linares, E.M., Kubota, L.T., Michaelis, J., and Thalhammer, S., Enhancement of the detection limit for lateral flow immunoassays: evaluation and comparison of bioconjugates. *J. Immunol. Methods*. **375**(1-2): p. 264-70 (2012).

104. Koets, M., Renstrom, A., Zahradnik, E., Bogdanovic, J., Wouters, I.M., and van Amerongen, A., Rapid one-step assays for on-site monitoring of mouse and rat urinary allergens. *J. Environ. Monit.* **13**(12): p. 3475-3480 (2011).

105. Sun, Y.P., Fluorescent carbon nanoparticles. US7829772 (B2) (2010).

106. Bhunia, S.K., Saha, A., Maity, A.R., Ray, S.C., and Jana, N.R., Carbon Nanoparticle-based Fluorescent Bioimaging Probes. *Sci. Rep.* **3**: p. 1473 (2013).

107. Stokes, G.G., On the change of refrangibility of light. *Philos. Trans. R. Soc. Lond.* **142**: p. 463-562 (1852).

108. Hemmilä, I., Lanthanides as probes for time-resolved fluorometric immunoassays. *Scand. J. Clin. Lab. Invest.* **48**(5): p. 389-399 (1988).

109. Soini, E. and Hemmilä, I., Fluoroimmunoassay: present status and key problems. *Clin. Chem.* **25**(3): p. 353 (1979).

110. Rundstrom, G., Jonsson, A., Martensson, O., Mendel-Hartvig, I., and Venge, P., Lateral flow immunoassay using Europium (III) chelate microparticles and time-resolved fluorescence for eosinophils and neutrophils in whole blood. *Clin. Chem.* **53**(2): p. 342-8 (2007).

111. Xia, X., Xu, Y., Zhao, X., and Li, Q., Lateral flow immunoassay using europium chelate-loaded silica nanoparticles as labels. *Clin. Chem.* **55**(1): p. 179-82 (2009).

112. Swanson, C. and D'Andrea, A., Lateral flow assay with near-infrared dye for multiplex detection. *Clin. Chem.* **59**(4): p. 641-8 (2013).

113. Morales-Narváez, E., Naghdi, T., Zor, E., and Merkoçi, A., Photoluminescent lateral-flow immunoassay revealed by graphene oxide: highly sensitive paper-based pathogen detection. *Anal. Chem.* **87**(16): p. 8573-8577 (2015).

114. Laitinen, M.P.A., Salmela, J., Gilbert, L., Kaivola, R., Tikkala, T., Oker-Blom, C., Pekola, J., and Vuento, M., Method and apparatus using selected superparamagnetic labels for rapid quantification of immunochromatographic tests. *Nanotechnol. Sci. Appl.* **2**: p. 13-20 (2009).

115. Xu, Q., Xu, H., Gu, H., Li, J., Wang, Y., and Wei, M., Development of lateral flow immunoassay system based on superparamagnetic nanobeads as labels for rapid quantitative detection of cardiac troponin I. *Mater. Sci. Eng.* **29**(3): p. 702-707 (2009).

116. Kim, J.-H., Cho, J.-H., Cha, G.S., Lee, C.-W., Kim, H.-B., and Paek, S.-H., Conductimetric membrane strip immunosensor with polyaniline-bound gold colloids as signal generator. *Biosens. Bioelectron.* **14**(12): p. 907-915 (2000).

117. Yamaguchi, M., Matsuda, Y., Sasaki, S., Sasaki, M., Kadoma, Y., Imai, Y., Niwa, D., and Shetty, V., Immunosensor with fluid control mechanism for salivary cortisol analysis. *Biosens. Bioelectron.* **41**: p. 186-191 (2013).

118. Li, J.J., Ouellette, A.L., Giovangrandi, L., Cooper, D.E., Ricco, A.J., and Kovacs, G.T., Optical scanner for immunoassays with up-converting phosphorescent labels. *IEEE Trans. Biomed. Eng.* **55**(5): p. 1560-71 (2008).

119. Teerinen, T., Lappalainen, T., and Erho, T., A paper-based lateral flow assay for morphine. *Anal. Bioanal. Chem.* **406**(24): p. 5955-65 (2014).

120. Zhou, G., Mao, X., and Juncker, D., Immunochromatographic assay on thread. *Anal. Chem.* **84**(18): p. 7736-43 (2012).

121. Van Oss, C.J., Good, R.J., and Chaudhury, M.K., Mechanism of DNA (southern) and protein (western) blotting on cellulose nitrate and other membranes. *J. Chromatogr. A.* **391**(Supplement C): p. 53-65 (1987).

122. Zhang, G.P., Wang, X.N., Yang, J.F., Yang, Y.Y., Xing, G.X., Li, Q.M., Zhao, D., Chai, S.J., and Guo, J.Q., Development of an immunochromatographic lateral flow test strip for detection of β-adrenergic agonist Clenbuterol residues. *J. Immunol. Methods.* **312**(1): p. 27-33 (2006).

123. Juntunen, E., Myyryläinen, T., Salminen, T., Soukka, T., and Pettersson, K., Performance of fluorescent europium(III) nanoparticles and colloidal gold reporters in lateral flow bioaffinity assay. *Anal. Biochem.* **428**(1): p. 31-8 (2012).

124. Gantelius, J., Bass, T., Sjöberg, R., Nilsson, P., and Andersson-Svahn, H., A Lateral Flow Protein Microarray for Rapid and Sensitive Antibody Assays. *Int. J. Mol. Sci.* **12**(11): p. 7748-7759 (2011).

125. Bruzewicz, D.A., Reches, M., and Whitesides, G.M., Low-cost printing of poly (dimethylsiloxane) barriers to define microchannels in paper. *Anal. Chem.* **80**(9): p. 3387-3392 (2008).

126. Carrilho, E., Martinez, A.W., and Whitesides, G.M., Understanding wax printing: a simple micropatterning process for paper-based microfluidics. *Anal. Chem.* **81**(16): p. 7091-7095 (2009).

127. Qian, S. and Bau, H.H., A mathematical model of lateral flow bioreactions applied to sandwich assays. *Anal. Biochem.* **322**(1): p. 89-98 (2003).

128. Rivas, L., Medina-Sánchez, M., de la Escosura-Muñiz, A., and Merkoçi, A., Improving sensitivity of gold nanoparticle-based lateral flow assays by using wax-printed pillars as delay barriers of microfluidics. *Lab Chip*. **14**(22): p. 4406-4414 (2014).

129. Sturgeon, C.M. and McAllister, E.J., Analysis of hCG: Clinical Applications and Assay Requirements. *Ann. Clin. Biochem.* **35**(4): p. 460-491 (1998).

130. Gillet, P., Mori, M., Van Esbroeck, M., Ende, J.V.d., and Jacobs, J., Assessment of the prozone effect in malaria rapid diagnostic tests. *Malar. J.* **8**(1): p. 271 (2009).

131. Akhtar, K., Sherwani, R.K., Sofi, L.A., Sharma, A., and Singh, P., Hook's effect—a rare presentation in HBsAg screening. *Indian J. Hematol. Blood Transfus.* **25**(1): p. 27-29 (2009).

132. Olson, R.A., Rapid scanning microspectrofluorimeter. *Rev. Sci. Instrum.* **31**(8): p. 844-849 (1960).

133. Badea, M.G. and Brand, L., [17] Time-resolved fluorescence measurements. *Methods Enzymol.* **61**: p. 378-425 (1979).

134. McGown, L.B., Fluorescence lifetime filtering. *Anal. Chem.* **61**(14): p. 839A-847A (1989).

135. Soini, E. and Kojola, H., Time-resolved fluorometer for lanthanide chelates--a new generation of nonisotopic immunoassays. *Clin. Chem.* **29**(1): p. 65-68 (1983).

136. Boyer, J.-C. and Van Veggel, F.C., Absolute quantum yield measurements of colloidal NaYF 4: Er3+, Yb3+ upconverting nanoparticles. *Nanoscale*. **2**(8): p. 1417-1419 (2010).

137. Zhang, X.F., Zhang, J., and Liu, L., Fluorescence properties of twenty fluorescein derivatives: lifetime, quantum yield, absorption and emission spectra. *J. Fluoresc.* **24**(3): p. 819-26 (2014).

138. Nadort, A., Zhao, J., and Goldys, E.M., Lanthanide upconversion luminescence at the nanoscale: fundamentals and optical properties. *Nanoscale*. **8**(27): p. 13099-13130 (2016).

139. Zhang, L., Tian, L., Ye, Z., Song, B., and Yuan, J., Preparation of visible-light-excited europium biolabels for time-resolved luminescence cell imaging application. *Talanta*. **108**: p. 143-149 (2013).

140. Grabolle, M., Spieles, M., Lesnyak, V., Gaponik, N., Eychmüller, A., and Resch-Genger, U., Determination of the fluorescence quantum yield of quantum dots: suitable procedures and achievable uncertainties. *Anal. Chem.* **81**(15): p. 6285-6294 (2009).

141. Masashi, I. and Masao, K., Irreversible Photobleaching of the Solution of Fluorescent Dyes. I. Kinetic Studies on the Primary Process. *Bull. Chem. Soc. Jpn.* **28**(2): p. 117-124 (1955).

142. Levitus, M. and Ranjit, S., Cyanine dyes in biophysical research: the photophysics of polymethine fluorescent dyes in biomolecular environments. *Q. Rev. Biophys.* **44**(1): p. 123-151 (2011).

143. Song, L., Hennink, E.J., Young, I.T., and Tanke, H.J., Photobleaching kinetics of

fluorescein in quantitative fluorescence microscopy. *Biophys. J.* **68**(6): p. 2588-2600 (1995).

144. Chun, P., *Colloidal gold and other labels for lateral flow immunoassays*, in *Lateral Flow Immunoassay*, R. Wong and H. Tse, Editors. 2009, Humana Press: New York, USA. p. 75-93 (2009).

145. Ukonaho, T., Rantanen, T., Jämsen, L., Kuningas, K., Päkkilä, H., Lövgren, T., and Soukka, T., Comparison of infrared-excited up-converting phosphors and europium nanoparticles as labels in a two-site immunoassay. *Anal. Chim. Acta.* **596**(1): p. 106-15 (2007).

146. Kuno, M., Fromm, D.P., Hamann, H.F., Gallagher, A., and Nesbitt, D.J., Nonexponential "blinking" kinetics of single CdSe quantum dots: A universal power law behavior. *J. Chem. Phys.* **112**(7): p. 3117-3120 (2000).

147. Orrit, M., Chemical and physical aspects of charge transfer in the fluorescence intermittency of single molecules and quantum dots. *Photochem. Photobiol. Sci.* **9**(5): p. 637-642 (2010).

148. Zheng, Q., Juette, M.F., Jockusch, S., Wasserman, M.R., Zhou, Z., Altman, R.B., and Blanchard, S.C., Ultra-Stable Organic Fluorophores for Single-Molecule Research. *Chem. Soc. Rev.* **43**(4): p. 1044-1056 (2014).

149. Wu, S., Han, G., Milliron, D.J., Aloni, S., Altoe, V., Talapin, D.V., Cohen, B.E., and Schuck, P.J., Non-blinking and photostable upconverted luminescence from single lanthanide-doped nanocrystals. *Proc. Natl. Acad. Sci. U.S.A.* **106**(27): p. 10917-21 (2009).

150. Lahtinen, S., Lyytikäinen, A., Päkkilä, H., Hömppi, E., Perälä, N., Lastusaari, M., and Soukka, T., Disintegration of Hexagonal NaYF4:Yb3+,Er3+ Upconverting Nanoparticles in Aqueous Media: The Role of Fluoride in Solubility Equilibrium. *J. Phys. Chem.* **121**(1): p. 656-665 (2017).

151. Mak, W.C., Beni, V., and Turner, A.P.F., Lateral-flow technology: From visual to instrumental. *Trends. Analyt. Chem.* **79**: p. 297-305 (2016).

152. Sambursky, R. and Shapiro, N., Evaluation of a combined MxA and CRP point-of-care immunoassay to identify viral and/or bacterial immune response in patients with acute febrile respiratory infection. *Eur. Clin. Respir. J.* **2**: p. 28245 (2015).

153. Van Amerongen, A., Wichers, J., Berendsen, L., Timmermans, A., Keizer, G., Van Doorn, A., Bantjes, A., and Van Gelder, W., Colloidal carbon particles as a new label for rapid immunochemical test methods: quantitative computer image analysis of results. *J. Biotechnol.* **30**(2): p. 185-195 (1993).

154. Fu, X., Xie, R., Wang, J., Chen, X., Wang, X., Sun, W., Meng, J., Lai, D., Zhou, L., and Wang, B., Development of Colloidal Gold-Based Lateral Flow Immunoassay for Rapid Qualitative and SemiQuantitative Analysis of Ustiloxins A and B in Rice Samples. *Toxins*. **9**(3): p. 79 (2017).

155. Yoo, J., Jung, Y.M., Hahn, J.H., and Pyo, D., Quantitative analysis of a prostate-specific antigen in serum using fluorescence immunochromatography. *J. Immunoassay Immunochem.* **31**(4): p. 259-65 (2010).

156. Li, Z., Wang, Y., Wang, J., Tang, Z., Pounds, J.G., and Lin, Y., Rapid and sensitive detection of protein biomarker using a portable fluorescence biosensor based on quantum dots and a lateral flow test strip. *Anal. Chem.* **82**(16): p. 7008-7014 (2010).

157. Hu, L.-M., Luo, K., Xia, J., Xu, G.-M., Wu, C.-H., Han, J.-J., Zhang, G.-G., Liu, M., and Lai, W.-H., Advantages of time-resolved fluorescent nanobeads compared with fluorescent submicrospheres, quantum dots, and colloidal gold as label in lateral flow assays

for detection of ractopamine. *Biosens. Bioelectron.* **91**: p. 95-103 (2017).

158. Choi, S., Choi, E.Y., Kim, D.J., Kim, J.H., Kim, T.S., and Oh, S.W., A rapid, simple measurement of human albumin in whole blood using a fluorescence immunoassay (I). *Clin. Chim. Acta.* **339**(1-2): p. 147-56 (2004).

159. Cho, J.H., Kim, M.H., Mok, R.S., Jeon, J.W., Lim, G.S., Chai, C.Y., and Paek, S.H., Two-dimensional paper chromatography-based fluorescent immunosensor for detecting acute myocardial infarction markers. *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.* **967**: p. 139-46 (2014).

160. Foubert, A., Beloglazova, N.V., and De Saeger, S., Comparative study of colloidal gold and quantum dots as labels for multiplex screening tests for multi-mycotoxin detection. *Anal. Chim. Acta.* **955**: p. 48-57 (2017).

161. Miočević, O., Cole, C.R., Laughlin, M.J., Buck, R.L., Slowey, P.D., and Shirtcliff, E.A., Quantitative Lateral Flow Assays for Salivary Biomarker Assessment: A Review. *Front. Public Health.* **5**: p. 133 (2017).

162. Choi, S., Kim, S., Yang, J.-S., Lee, J.-H., Joo, C., and Jung, H.-I., Real-time measurement of human salivary cortisol for the assessment of psychological stress using a smartphone. *Sens. Biosensing Res.* **2**: p. 8-11 (2014).

163. Juntunen, E., Salminen, T., Talha, S.M., Martiskainen, I., Soukka, T., Pettersson, K., and Waris, M., Lateral Flow Immunoassay With Upconverting Nanoparticle-Based Detection for Indirect Measurement of Interferon Response by the Level of MxA. *J. Med. Virol.* **89**(4): p. 598-605 (2017).

164. Chaemsaithong, P., Romero, R., Docheva, N., Chaiyasit, N., Bhatti, G., Pacora, P., Hassan, S.S., Yeo, L., and Erez, O., Comparison of Rapid MMP-8 and Interleukin-6 Point-of-Care Tests to Identify Intra-amniotic Inflammation/Infection and Impending Preterm Delivery in Patients With Preterm Labor and Intact Membranes. *J. Matern. Fetal Neonatal. Med*: p. 1-42 (2017).

165. Leung, W., Chan, C.P., Leung, M., Lehmann, K., Renneberg, I., Lehmann, M., Hempel, A., Glatz, J.F., and Renneberg, R., Novel "Digital-Style" Rapid Test Simultaneously Detecting Heart Attack and Predicting Cardiovascular Disease Risk. *Anal. Lett.* **38**(3): p. 423-439 (2005).

166. Qi, X., Huang, Y., Lin, Z., Xu, L., and Yu, H., Dual-Quantum-Dots-Labeled Lateral Flow Strip Rapidly Quantifies Procalcitonin and C-reactive Protein. *Nanoscale Res. Lett.* **11**(1): p. 167 (2016).

167. Novak, M.T., Kotanen, C.N., Carrara, S., Guiseppi-Elie, A., and Moussy, F.G., Diagnostic tools and technologies for infectious and non-communicable diseases in low-and-middle-income countries. *Health Technol.* **3**(4): p. 271-281 (2013).

168. Kim, Y.M., Oh, S.W., Jeong, S.Y., Pyo, D.J., and Choi, E.Y., Development of an Ultrarapid One-Step Fluorescence Immunochromatographic Assay System for the Quantification of Microcystins. *Environ. Sci. Technol.* **37**(9): p. 1899-1904 (2003).

169. Quidel Corporation. *Sofia User Manual.* [cited 2017 August 26]; Available from: http://www.quidel.com/sites/default/files/product/documents/sofia_user_manual_ef12031 01en00_0.pdf.

170. Corstjens, P.L.A.M., Zuiderwijk, M., Nilsson, M., Feindt, H., Sam Niedbala, R., and Tanke, H.J., Lateral-flow and up-converting phosphor reporters to detect single-stranded nucleic acids in a sandwich-hybridization assay. *Anal. Biochem.* **312**(2): p. 191-200 (2003).

171. Brooks, D.E., Harris, P.C., Olal, A.D., Xie, Z.C., and Richards, B.G., Compensation for non-specific signals in quantitative immunoassays. US6509196 (B1) (2003).

172. Cloney, L.P., Spiller, L.J., Fong, W.K., Harris, J.E., and Harris, P.C., RAMP®: High Accuracy from Immunochromatographic Assays by the Use of Internal Control Ratios. *Clin. Chem.* **49**(10): p. 1775-1777 (2003).

173. Juntunen, E., Arppe, R., Kalliomaki, L., Salminen, T., Talha, S.M., Myyrylainen, T., Soukka, T., and Pettersson, K., Effects of blood sample anticoagulants on lateral flow assays using luminescent photon-upconverting and Eu(III) nanoparticle reporters. *Anal. Biochem.* **492**: p. 13-20 (2016).

174. Findlay, J.W.A. and Dillard, R.F., Appropriate calibration curve fitting in ligand binding assays. *AAPS J.* **9**(2): p. E260-E267 (2007).

175. Rodbard, D. and Frazier, G.R., [1] Statistical analysis of radioligand assay data. *Methods Enzymol.* **37**: p. 3-22 (1975).

176. Ekins, R., Radioimmunoassay and related procedures in medicine. *IAEA, Vienna*: p. 6 (1978).

177. Lee, J.W., Devanarayan, V., Barrett, Y.C., Weiner, R., Allinson, J., Fountain, S., Keller, S., Weinryb, I., Green, M., Duan, L., Rogers, J.A., Millham, R., O'Brien, P.J., Sailstad, J., Khan, M., Ray, C., and Wagner, J.A., Fit-for-purpose method development and validation for successful biomarker measurement. *Pharm. Res.* **23**(2): p. 312-28 (2006).

178. Lee, L., Nordman, E., Johnson, M., and Oldham, M., A Low-Cost, High-Performance System for Fluorescence Lateral Flow Assays. *Biosensors*. **3**(4): p. 360 (2013).

179. Faulstich, K., Gruler, R., Eberhard, M., Lentzsch, D., and Haberstroh, K., *Handheld and portable reader devices for lateral flow immunoassays*, in *Lateral Flow Immunoassay*, R. Wong and H. Tse, Editors. 2009, Humana Press: New York, USA. p. 157-183 (2009).

180. Fernandez-Sanchez, C., McNeil, C.J., Rawson, K., Nilsson, O., Leung, H.Y., and Gnanapragasam, V., One-step immunostrip test for the simultaneous detection of free and total prostate specific antigen in serum. *J. Immunol. Methods*. **307**(1-2): p. 1-12 (2005).

181. U.S. Food and Drug Administration. *In Vitro Diagnostics*. 2017 Jan 13. 2017 [cited 2017 July 3.]; Available from: https://www.fda.gov/MedicalDevices/ProductsandMedicalProceduresInVitroDiagnostics/.

182. Kim, T.K., Oh, S.W., Hong, S.C., Mok, Y.J., and Choi, E.Y., Point-of-Care Fluorescence Immunoassay for Cardiac Panel Biomarkers. *J. Clin. Lab. Anal.* **28**(6): p. 419-427 (2014).

183. Kim, T.K., Oh, S.W., Mok, Y.J., and Choi, E.Y., Fluorescence Immunoassay of Human D-dimer in Whole Blood. *J. Clin. Lab. Anal.* **28**(4): p. 294-300 (2014).

184. Brehm, E.C. *FDA 510(k) Premarket Notification: Substantial equivalence determination decision summary*. 2017 [cited 2017 June 28.]; Available from: https://www.accessdata.fda.gov/cdrh_docs/reviews/K162911.pdf.

185. Mokkapati, V.K., Sam Niedbala, R., Kardos, K., Perez, R.J., Guo, M., Tanke, H.J., and Corstjens, P.L., Evaluation of UPlink–RSV. *Ann. N. Y. Acad. Sci.* **1098**(1): p. 476-485 (2007).

186. OraSure Technologies, I. *Press release: OraSure Announces Record Second Quarter Financial Results; Increases Full-Year Earnings Guidance*. 2005 [cited 2017 Apr 18. 2017]; Available from: http://phx.corporate-ir.net/phoenix.zhtml?c=99740&p=irol-newsArticle&ID=738696.

187. Nano-Ditech Corp. *NANO-DITECH*. 2017 [cited 2017 June 26. 2017]; Available from: http://www.nanoditech.com/.

188. Shirtcliff, E.A., Buck, R.L., Laughlin, M.J., Hart, T., Cole, C.R., and Slowey, P.D., Salivary Cortisol Results Obtainable Within Minutes of Sample Collection Correspond With

Traditional Immunoassays. *Clin. Ther.* **37**(3): p. 505-514 (2015).

189. Zhang, P., Liu, X., Wang, C., Zhao, Y., Hua, F., Li, C., Yang, R., and Zhou, L., Evaluation of up-converting phosphor technology-based lateral flow strips for rapid detection of Bacillus anthracis Spore, Brucella spp., and Yersinia pestis. *PLoS One*. **9**(8): p. e105305 (2014).

190. Hua, F., Zhang, P., Zhang, F., Zhao, Y., Li, C., Sun, C., Wang, X., Yang, R., Wang, C., Yu, A., and Zhou, L., Development and evaluation of an up-converting phosphor technology-based lateral flow assay for rapid detection of Francisella tularensis. *Sci. Rep.* **5**: p. 17178 (2015).

191. Cooper, D.E., Balog, R., Chang, P., Shaler, T.A., Lin, H., D'Andrea, A., Harrison, T., Schmidt, R.H., Swanson, C., and Mendoza, E., Biomarker panel for dose assessment of radiation injury and micro plasma filter. US2016109437 (A1) (2016).

192. SRI International. 2016; Available from: https://www.sri.com/work/projects/biodosimeter-triage-exposure-ionizing-radiation.

193. Yang, Q., Gong, X., Song, T., Yang, J., Zhu, S., Li, Y., Cui, Y., Li, Y., Zhang, B., and Chang, J., Quantum dot-based immunochromatography test strip for rapid, quantitative and sensitive detection of alpha fetoprotein. *Biosens. Bioelectron.* **30**(1): p. 145-50 (2011).

194. Beloglazova, N.V., Sobolev, A.M., Tessier, M.D., Hens, Z., Goryacheva, I.Y., and De Saeger, S., Fluorescently labelled multiplex lateral flow immunoassay based on cadmium-free quantum dots. *Methods*, (2017).

195. Berlina, A.N., Taranova, N.A., Zherdev, A.V., Vengerov, Y.Y., and Dzantiev, B.B., Quantum dot-based lateral flow immunoassay for detection of chloramphenicol in milk. *Anal. Bioanal. Chem.* **405**(14): p. 4997-5000 (2013).

196. Zarling, D.A., Rossi, M.J., Peppers, N.A., Kane, J., Faris, G.W., Dyer, M.J., Ng, S.Y., and Schneider, L.V., Up-converting Reporters for Biological and Other Assays Using Laser Excitation Techniques. U.S. Patent 5698397 (1997).

197. Zhao, Y., Liu, X., Wang, X., Sun, C., Wang, X., Zhang, P., Qiu, J., Yang, R., and Zhou, L., Development and evaluation of an up-converting phosphor technology-based lateral flow assay for rapid and quantitative detection of aflatoxin B1 in crops. *Talanta*. **161**: p. 297-303 (2016).

198. Hampl, J., Hall, M., Mufti, N.A., Yao, Y.M., MacQueen, D.B., Wright, W.H., and Cooper, D.E., Upconverting phosphor reporters in immunochromatographic assays. *Anal. Biochem.* **288**(2): p. 176-87 (2001).

199. Sigma-Aldrich. *Sunstone® Luminescent UCP Nanocrystals*. Available from: http://www.sigmaaldrich.com/technical-documents/articles/biology/upconverting-ucp-nanocrystals.html.

200. Thermo Fisher Scientific. *Fluoro-Max™ Fluorescent Carboxylate-Modified Particles*. [cited 2017 August 27]; Available from: https://www.thermofisher.com/order/catalog/product/93470350010150.

201. Innova Biosciences Ltd. *Europium Conjugation Kit*. [cited 2017 August 27]; Available from: https://www.innovabiosciences.com/europium-conjugation-kit/europium-conjugation-kit.html.

202. Panchuk-Voloshina, N., Haugland, R.P., Bishop-Stewart, J., Bhalgat, M.K., Millard, P.J., Mao, F., Leung, W.-Y., and Haugland, R.P., Alexa Dyes, a Series of New Fluorescent Dyes that Yield Exceptionally Bright, Photostable Conjugates. *J. Histochem. Cytochem.* **47**(9): p. 1179-1188 (1999).

203. Esteban, A., Popp, M.W., Vyas, V.K., Strijbis, K., Ploegh, H.L., and Fink, G.R., Fungal recognition is mediated by the association of

dectin-1 and galectin-3 in macrophages. *Proc. Natl. Acad. Sci. U.S.A.* **108**(34): p. 14270-14275 (2011).

204. Kost, G.J., Tran, N.K., and Louie, R.F., Point-of-Care Testing: Principles, Practice, and Critical-Emergency-Disaster Medicine, in Encyclopedia of Analytical Chemistry. 2006, John Wiley & Sons, Ltd(2006).

205. Fu, E., Yager, P., Floriano, P.N., Christodoulides, N., and McDevitt, J.T., Perspective on diagnostics for global health. *IEEE Pulse.* **2**(6): p. 40-50 (2011).

206. Kost, G.J., Tran, N.K., Tuntideelert, M., Kulrattanamaneeporn, S., and Peungposop, N., Katrina, the Tsunami, and Point-of-Care TestingOptimizing Rapid Response Diagnosis in Disasters. *Am. J. Clin. Pathol.* **126**(4): p. 513-520 (2006).

207. Louie, R.F., Sumner, S.L., Belcher, S., Mathew, R., Tran, N.K., and Kost, G.J., Thermal stress and point-of-care testing performance: suitability of glucose test strips and blood gas cartridges for disaster response. *Disaster Med. Public Health Prep.* **3**(1): p. 13-17 (2009).

208. Dowling, K., Morgan, F., Mueller, G., Lys, I., and Tullman, M., Ultraviolet light emitting diode systems and methods. (2002).

209. Esquivel, J.P., Buser, J.R., Lim, C.W., Domínguez, C., Rojas, S., Yager, P., and Sabaté, N., Single-use paper-based hydrogen fuel cells for point-of-care diagnostic applications. *J. Power Sources.* **342**: p. 442-451 (2017).

210. Wong, R.C. and Harley, Y.T., Quantitative, false positive, and false negative issues for lateral flow immunoassays as exemplified by onsite drug screens, in Lateral Flow Immunoassay. 2009, Humana Press: New York, USA. p. 185-203 (2009).

211. Yotter, R.A. and Wilson, D.M., A review of photodetectors for sensing light-emitting reporters in biological systems. *IEEE Sensors J.* **3**(3): p. 288-303 (2003).

212. Szczęśniak, T., Grodzicka, M., Moszyński, M., Szawłowski, M., and Baszak, J., microPMT–A New Photodetector for Gamma Spectrometry and Fast Timing? *IEEE Trans. Nucl. Sci.* **61**(5): p. 2687-2693 (2014).

213. Mudanyali, O., Dimitrov, S., Sikora, U., Padmanabhan, S., Navruz, I., and Ozcan, A., Integrated rapid-diagnostic-test reader platform on a cellphone. *Lab Chip*. **12**(15): p. 2678-2686 (2012).

214. Garcia, G., Transilluminator having light emitting diode (LED) array. (2006).

215. Nazareth, A.R., Delahanty, F.T., Bandru, G.M., Wieck, H.J., and Synakowski, S.R., Electronic analyte assaying device. US8623635 (B2) (2014).

216. Song, X. and Knotts, M., Time-resolved luminescent lateral flow assay technology. *Anal. Chim. Acta.* **626**(2): p. 186-192 (2008).

217. Fleming, W., Buck, R., Miracle, R., Morrow, D., Dietz, L., and Myrick, S., Optical reader systems and lateral flow assays. (2016).

218. Hamamatsu Photonics, K. *Photomultiplier tubes: Basics and applications*. Edition 3a 2007 [cited 2017; Available from: http://www.hamamatsu.com/resources/pdf/etd/PMT_handbook_v3aE.pdf.

219. Kim, S. and Park, J.-K., Development of a test strip reader for a lateral flow membrane-based immunochromatographic assay. *Biotechnol. Bioprocess Eng.* **9**(2): p. 127-131 (2004).

220. van Dam, G.J., Claudia, J., Lewis, M., Deelder, A.M., van Lieshout, L., Tanke, H.J., van Rooyen, L.H., and Corstjens, P.L., A robust dry reagent lateral flow assay for diagnosis of active schistosomiasis by detection of Schistosoma circulating anodic antigen. *Exp. Parasitol.* **135**(2): p. 274-282 (2013).

221. Qiagen Lake Constance GmbH. [cited 2017 August 28]; Available from: https://www.qiagen.com/us/products/oem-services/ese-instruments/esequant-lateral-flow-reader/.

222. Renker, D., Geiger-mode avalanche photodiodes, history, properties and problems. *Nucl. Instrum. Methods. Phys. Res. A*. **567**(1): p. 48-56 (2006).

223. Stoppa, D., Mosconi, D., Pancheri, L., and Gonzo, L., Single-Photon Avalanche Diode CMOS Sensor for Time-Resolved Fluorescence Measurements. *IEEE Sensors. J.* **9**(9): p. 1084-1090 (2009).

224. Ker, P.J., David, J.P., and Tan, C.H., Temperature dependence of gain and excess noise in InAs electron avalanche photodiodes. *Opt. Express*. **20**(28): p. 29568 (2012).

225. Zappa, F., Giudice, A., Ghioni, M., and Cova, S. Fully–integrated active–quenching circuit for single–photon detection. in Solid-State Circuits Conference, 2002. ESSCIRC 2002. Proceedings of the 28th European. 2002. IEEE (Year).

226. S. Carlson, B. Comparison of modern CCD and CMOS image sensor technologies and systems for low resolution imaging. in Proceedings of IEEE Sensors. 2002. (Year).

227. Mendis, S.K., Kemeny, S.E., Gee, R.C., Pain, B., Staller, C.O., Kim, Q., and Fossum, E.R., CMOS active pixel image sensors for highly integrated imaging systems. *IEEE J. Solid-State Circuits*. **32**(2): p. 187-197 (1997).

228. Ylihärsilä, M., Valta, T., Karp, M., Hattara, L., Harju, E., Hölsä, J., Saviranta, P., Waris, M., and Soukka, T., Oligonucleotide array-in-well platform for detection and genotyping human adenoviruses by utilizing upconverting phosphor label technology. *Anal. Chem.* **83**(4): p. 1456-61 (2011).

229. Gui, C., Wang, K., Li, C., Dai, X., and Cui, D., A CCD-based reader combined with CdS quantum dot-labeled lateral flow strips for ultrasensitive quantitative detection of CagA. *Nanoscale Res. Lett.* **9**(1): p. 57 (2014).

230. Mirasoli, M., Buragina, A., Dolci, L.S., Guardigli, M., Simoni, P., Montoya, A., Maiolini, E., Girotti, S., and Roda, A., Development of a chemiluminescence-based quantitative lateral flow immunoassay for on-field detection of 2,4,6-trinitrotoluene. *Anal. Chim. Acta.* **721**: p. 167-172 (2012).

231. Gomi, S., Hano, H., Iijima, T., Itoh, S., Kawagoe, K., Kim, S.H., Kubota, T., Maeda, T., Matsumura, T., Mazuka, Y., Miyabayashi, K., Miyata, H., Murakami, T., Nakadaira, T., Nakaya, T., Otono, H., Sano, E., Shinkawa, T., Sudo, Y., Takeshita, T., Taguchi, M., Tsubokawa, T., Uozumi, S., Yamaoka, M., Yamazaki, H., Yokoyama, M., Yoshimura, K., and Yoshioka, T., Development and study of the multi pixel photon counter. *Nucl. Instrum. Methods. Phys. Res. A*. **581**(1): p. 427-432 (2007).

232. Vashist, S.K., Luppa, P.B., Yeo, L.Y., Ozcan, A., and Luong, J.H., Emerging technologies for next-generation point-of-care testing. *Trends Biotechnol.* **33**(11): p. 692-705 (2015).

233. Syedmoradi, L., Daneshpour, M., Alvandipour, M., Gomez, F.A., Hajghassem, H., and Omidfar, K., Point of care testing: The impact of nanotechnology. *Biosens. Bioelectron.* **87**: p. 373-387 (2017).

234. Nicchio, M.V.C., Araujo, S., Martins, L.C., Pinheiro, A.V., Pereira, D.C., Borges, A., Antunes, D.E., Barreto, J.G., and Goulart, I.M.B., Spatial and temporal epidemiology of Mycobacterium leprae infection among leprosy patients and household contacts of an endemic region in Southeast Brazil. *Acta Trop.* **163**: p. 38-45 (2016).

235. Kim, H., Jung, Y., Doh, I.-J., Lozano-Mahecha, R.A., Applegate, B., and Bae, E., Smartphone-based low light detection for bioluminescence application. *Sci. Rep.* **7**: p. 40203 (2017).

236. Quesada-González, D. and Merkoçi, A., Nanoparticle-based lateral flow biosensors. *Biosens. Bioelectron.* **73**: p. 47-63 (2015).

237. Huppert, J., Hesse, E., and Gaydos, C.A., What's the Point? How Point-of-Care STI Tests Can Impact Infected Patients. *Point Care*. **9**(1): p. 36-46 (2010).

238. Kettler, H., White, K., and Hawkes, S.J. *Mapping the landscape of diagnostics for sexually transmitted infections: key findings and recommendations*. 2004 [cited 2017 August 27]; Available from: http://apps.who.int/iris/bitstream/10665/68990/1/TDR_STI_IDE_04.1.pdf.

239. Tebbey, P.W. and Rink, C., Target Product Profile: A Renaissance for its Definition and Use. *J. Of Med. Marketing.* **9**(4): p. 301-307 (2009).

240. Ying, R., Sharma, M., Celum, C., Baeten, J.M., van Rooyen, H., Hughes, J.P., Garnett, G., and Barnabas, R.V., Home testing and counselling to reduce HIV incidence in a generalised epidemic setting: a mathematical modelling analysis. *Lancet HIV*. **3**(6): p. e275-e282 (2016).

241. Ibitoye, M., Frasca, T., Giguere, R., and Carballo-Diéguez, A., Home Testing Past, Present and Future: Lessons Learned and Implications for HIV Home Tests. *AIDS Behav*. **18**(5): p. 933-949 (2014).

242. Ranasinghe, S., Ansumana, R., Lamin, J.M., Bockarie, A.S., Bangura, U., Buanie, J.A., Stenger, D.A., and Jacobsen, K.H., Attitudes toward home-based malaria testing in rural and urban Sierra Leone. *Malar. J.* **14**(1): p. 80 (2015).

243. Streckfus, C. and Bigler, L., Saliva as a diagnostic fluid. *Oral Dis.* **8**(2): p. 69-76 (2002).

244. Vining, R.F. and McGinley, R.A., The measurement of hormones in saliva: Possibilities and pitfalls. *J. Steroid Biochem.* **27**(1): p. 81-94 (1987).

245. Zachary, D., Mwenge, L., Muyoyeta, M., Shanaube, K., Schaap, A., Bond, V., Kosloff, B., de Haas, P., and Ayles, H., Field comparison of OraQuick® ADVANCE Rapid HIV-1/2 antibody test and two blood-based rapid HIV antibody tests in Zambia. *BMC Infect. Dis.* **12**(1): p. 183 (2012).

246. Corstjens, P.L., Nyakundi, R.K., de Dood, C.J., Kariuki, T.M., Ochola, E.A., Karanja, D.M., Mwinzi, P.N., and van Dam, G.J., Improved sensitivity of the urine CAA lateral-flow assay for diagnosing active Schistosoma infections by using larger sample volumes. *Parasit. Vectors*. **8**(1): p. 241 (2015).

247. Kabanda, T., Siedner, M.J., Klausner, J.D., Muzoora, C., and Boulware, D.R., Point-of-Care Diagnosis and Prognostication of Cryptococcal Meningitis With the Cryptococcal Antigen Lateral Flow Assay on Cerebrospinal Fluid. *Clin. Infect. Dis.* **58**(1): p. 113-116 (2014).

248. Eggerbauer, E., de Benedictis, P., Hoffmann, B., Mettenleiter, T.C., Schlottau, K., Ngoepe, E.C., Sabeta, C.T., Freuling, C.M., and Müller, T., Evaluation of Six Commercially Available Rapid Immunochromatographic Tests for the Diagnosis of Rabies in Brain Material. *PLoS Negl. Trop. Dis.* **10**(6): p. e0004776 (2016).

249. Harvey, S.A., Jennings, L., Chinyama, M., Masaninga, F., Mulholland, K., and Bell, D.R., Improving community health worker use of malaria rapid diagnostic tests in Zambia: package instructions, job aid and job aid-plus-training. *Malar. J.* **7**(1): p. 160 (2008).

250. Gillet, P., Mori, M., Van den Ende, J., and Jacobs, J., Buffer substitution in malaria rapid diagnostic tests causes false-positive results. *Malar. J.* **9**(1): p. 215 (2010).

251. Chen, D., Mauk, M., Qiu, X., Liu, C., Kim, J., Ramprasad, S., Ongagna, S., Abrams, W.R., Malamud, D., Corstjens, P.L.A.M., and Bau,

H.H., An integrated, self-contained microfluidic cassette for isolation, amplification, and detection of nucleic acids. *Biomed. Microdevices*. **12**(4): p. 705-719 (2010).

252. Liu, C., Qiu, X., Ongagna, S., Chen, D., Chen, Z., Abrams, W.R., Malamud, D., Corstjens, P.L., and Bau, H.H., A timer-actuated immunoassay cassette for detecting molecular markers in oral fluids. *Lab Chip*. **9**(6): p. 768-776 (2009).

253. VanDine, R.W., Babu, U.M., and Sambursky, R.P., In situ lysis of cells in lateral flow immunoassays. US 8614101 B2 (2013).

254. Nash, M.A., Waitumbi, J.N., Hoffman, A.S., Yager, P., and Stayton, P.S., Multiplexed enrichment and detection of malarial biomarkers using a stimuli-responsive iron oxide and gold nanoparticle reagent system. *ACS Nano*. **6**(8): p. 6776-6785 (2012).

255. World Health Organization. *HIV assays: Laboratory performance and other operational characteristics*. 2015 [cited 2017 07.04.2017]; Available from: http://www.who.int/diagnostics_laboratory/publications/15032_hiv_assay_report18.pdf.

256. Dharmaraja, S., Lafleur, L., Byrnes, S., Kauffman, P., Buser, J., Toley, B., Fu, E., Yager, P., and Lutz, B. Programming paper networks for point of care diagnostics. in Proceedings of SPIE, 8615, Microfluidics, BioMEMS, and Medical Microsystem XI. 2013. (Year).

257. Fu, E., Liang, T., Spicar-Mihalic, P., Houghtaling, J., Ramachandran, S., and Yager, P., Two-dimensional paper network format that enables simple multistep assays for use in low-resource settings in the context of malaria antigen detection. *Anal. Chem.* **84**(10): p. 4574-9 (2012).

258. Bishop, J., Buser, J., Byrnes, S., Dharmaraja, S., Fu, E.S., Houghtaling, J., Kauffman, P.C., Kumar, S., Lafleur, L., and Liang, T., Sequential delivery of fluid volumes and associated devices, systems and methods. US2015361487 (A1) (2015).

259. World Health Organization. *Public reports of WHO prequalified IVDs*. 2017; Available from: http://www.who.int/diagnostics_laboratory/evaluations/pq-list/malaria/public_report/en/.

260. Stevens, W., Gous, N., Ford, N., and Scott, L.E., Feasibility of HIV point-of-care tests for resource-limited settings: challenges and solutions. *BMC Med.* **12**(1): p. 173 (2014).

261. Hopkins, H., Oyibo, W., Luchavez, J., Mationg, M.L., Asiimwe, C., Albertini, A., González, I.J., Gatton, M.L., and Bell, D., Blood transfer devices for malaria rapid diagnostic tests: evaluation of accuracy, safety and ease of use. *Malar. J.* **10**(1): p. 30 (2011).

262. US Environmental Protection Agency. *Resource Conservation and Recovery Act (RCRA) Regulations*. 2012 [cited 2017 06.04.2017]; Available from: https://www.epa.gov/rcra/resource-conservation-and-recovery-act-rcra-regulations.

263. Mueller, N.C. and Nowack, B., Exposure Modeling of Engineered Nanoparticles in the Environment. *Environ. Sci. Technol.* **42**(12): p. 4447-4453 (2008).

264. Bear, J., Charron, G., Fernández-Argüelles, M.T., Massadeh, S., McNaughter, P., and Nann, T., *In vivo applications of inorganic nanoparticles*, in *BetaSys*. 2011, Springer. p. 185-220 (2011).

265. Mohanraj, V. and Chen, Y., Nanoparticles-a review. *Trop. J. Pharm. Res.* **5**(1): p. 561-573 (2006).

266. Gnach, A., Lipinski, T., Bednarkiewicz, A., Rybka, J., and Capobianco, J.A., Upconverting nanoparticles: assessing the toxicity. *Chem. Soc. Rev.* **44**(6): p. 1561-1584 (2015).

267. Rimpiläinen, T., Opportunity Identification at the Base of the Pyramid. Department of

Management and International Business, Aalto Univesity. M.Sc. thesis, Archive nr. 13142 (2013).

268. Boehme, C.C., Nicol, M.P., Nabeta, P., Michael, J.S., Gotuzzo, E., Tahirli, R., Gler, M.T., Blakemore, R., Worodria, W., and Gray, C., Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study. *The Lancet*. **377**(9776): p. 1495-1505 (2011).

269. Bekmurzayeva, A., Sypabekova, M., and Kanayeva, D., Tuberculosis diagnosis using immunodominant, secreted antigens of Mycobacterium tuberculosis. *Tuberculosis*. **93**(4): p. 381-388 (2013).

270. Albert, H., Nathavitharana, R.R., Isaacs, C., Pai, M., Denkinger, C.M., and Boehme, C.C., Development, roll-out and impact of Xpert MTB/RIF for tuberculosis: what lessons have we learnt and how can we do better? *Eur. Respir. J.* : p. ERJ-00543-2016 (2016).

271. Uys, P.W., Warren, R., van Helden, P.D., Murray, M., and Victor, T.C., Potential of rapid diagnosis for controlling drug-susceptible and drug-resistant tuberculosis in communities where Mycobacterium tuberculosis infections are highly prevalent. *J. Clin. Microbiol.* **47**(5): p. 1484-1490 (2009).

272. Li, L., Zhou, L., Yu, Y., Zhu, Z., Lin, C., Lu, C., and Yang, R., Development of up-converting phosphor technology-based lateral-flow assay for rapidly quantitative detection of hepatitis B surface antibody. *Diagn. Microbiol. Infect. Dis.* **63**(2): p. 165-72 (2009).

273. Yager, P., Edwards, T., Fu, E., Helton, K., Nelson, K., Tam, M.R., and Weigl, B.H., Microfluidic diagnostic technologies for global public health. *Nature*. **442**(7101): p. 412-8 (2006).

274. Kuningas, K., Rantanen, T., Ukonaho, T., Lövgren, T., and Soukka, T., Homogeneous assay technology based on upconverting phosphors. *Anal. Chem.* **77**(22): p. 7348-55 (2005).

275. Pettersson, K., Piironen, T., Seppälä, M., Liukkonen, L., Christensson, A., Matikainen, M., Suonpää, M., Lövgren, T., and Lilja, H., Free and complexed prostate-specific antigen (PSA): in vitro stability, epitope map, and development of immunofluorometric assays for specific and sensitive detection of free PSA and PSA-alpha 1-antichymotrypsin complex. *Clin. Chem.* **41**(10): p. 1480-1488 (1995).

276. Kokko, L., Lövgren, T., and Soukka, T., Europium (III)-chelates embedded in nanoparticles are protected from interfering compounds present in assay media. *Anal. Chim. Acta.* **585**(1): p. 17-23 (2007).

277. Soukka, T., Härmä, H., Paukkunen, J., and Lövgren, T., Utilization of kinetically enhanced monovalent binding affinity by immunoassays based on multivalent nanoparticle-antibody bioconjugates. *Anal. Chem.* **73**(10): p. 2254-2260 (2001).

278. Rajakoski, K., Piironen, T., Pettersson, K., Lövgren, J., and Karp, M., Epitope mapping of human prostate specific antigen and glandular kallikrein expressed in insect cells. *Prostate Cancer Prostatic Dis.* **1**(1): p. 16-20 (1997).

279. Kochs, G., Haener, M., Aebi, U., and Haller, O., Self-assembly of human MxA GTPase into highly ordered dynamin-like oligomers. *J. Biol. Chem.* **277**(16): p. 14172-6 (2002).

280. Soukka, T., Kuningas, K., Rantanen, T., Haaslahti, V., and Lövgren, T., Photochemical characterization of up-converting inorganic lanthanide phosphors as potential labels. *J. Fluoresc.* **15**(4): p. 513-28 (2005).

281. Välimaa, L., Pettersson, K., Vehniäinen, M., Karp, M., and Lövgren, T., A High-Capacity Streptavidin-Coated Microtitration Plate. *Bioconjug. Chem.* **14**(1): p. 103-111 (2003).

282. Savukoski, T., Engström, E., Engblom, J., Ristiniemi, N., Wittfooth, S., Lindahl, B., Eggers, K.M., Venge, P., and Pettersson, K., Troponin-Specific Autoantibody Interference in Different Cardiac Troponin I Assay Configurations. *Clin. Chem.* **58**(6): p. 1040-1048 (2012).

283. Pollnau, M., Gamelin, D.R., Lüthi, S.R., Güdel, H.U., and Hehlen, M.P., Power dependence of upconversion luminescence in lanthanide and transition-metal-ion systems. *Phys. Rev. B.* **61**(5): p. 3337-3346 (2000).

Annales Universitatis Turkuensis

Turun yliopisto
University of Turku

Painosalama Oy, Turku, Finland 2018

ISBN 978-951-29-7126-8 (PRINT)
ISBN 978-951-29-7127-5 (PDF)
ISSN 0082-7002 (PRINT) | ISSN 2343-3175 (ONLINE)