Microparticle–based platform for point-of-care immunoassays

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Running title: Novel platform for rapid immunoassays

Subject category: Immunological procedures

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Abstract

There is a need for quantitative and sensitive, yet simple point-of-care immunoassays. We have developed a point-of-care microparticle-based immunoassay platform which combines the performance of a microtiter well-based assay with the usability of a rapid assay. The platform contained a separate reaction and detection chambers and microparticles for the solid-phase. Photoluminescent up-converting nanoparticles (UCNPs) were used as labels. The platform was tested with a cardiac troponin I assay, and a limit of detection of 19.7 ng/L was obtained. This study demonstrates the feasibility of developing point-of-care assays on the new platform for various analytes of interests.

Keywords: point-of-care, microparticle, fluorescence, cardiac troponin, nanoparticle, up-conversion

There is a continuous quest for improved (immuno)assays for known biomarkers and additional assays for newly discovered biomarkers. Apart from the assays that can only be run in laboratory environment, there is a huge and ever-increasing demand for simple-to-operate rapid assays that can be used at point-of-care, in decentralized resource-limited laboratories, doctor's office, emergency medicine departments and ambulances. Examples of rapid point-of-care assay platforms are lateral flow, dipstick, flow-through devices, agglutination–based tests, microfluidics devices, micropillar–based devices and all-in-one (Aio!) dry-reagent assays [1,2]. These assay platforms may or may not require automation and/or reader instrument. The most commonly used point-of-care platform is the lateral flow immunoassay, which is well-accepted, low-cost and simple. However, problems limiting the sensitivity and usefulness of lateral flow assays include irregularities in the complex multi-membrane components, short reaction times, and subjective reading of visually read test lines [3]. New research is overcoming many of the problems of lateral flow assay mainly by using nanoparticle-reporters providing increased sensitivity and quantification together with portable optical or electrochemical reader instrumentation [4,5]. Microfluidic platforms have also been used to automate high-sensitivity immunoassays for example for cardiovascular diagnostics [6,7].

In this study, a syringe-driven microparticle–based robust assay platform was developed which combines the performance of a microtiter well–based assay with the usability of a rapid assay. In order to have control over the reaction incubation time, a separate reaction chamber and a valve mechanism was designed in the assay cassette. The assay cassette was manufactured by injection molding. The reagents to capture the analyte are immobilized on the microparticles and the detection reagents are coated on the up-converting nanoparticles (UCNPs). The capture and tracer reagents and the sample are added to the reaction chamber. The analyte, if present in the sample, is sandwiched in between capture and tracer reagents forming an immune complex. When the reaction incubation is over, all the reaction materials flow to the detection chamber through the open valve due to the negative pressure applied through a syringe. The microparticles and the immune complex attached to it are trapped on the filter mesh in the detection chamber and the rest of the materials are washed away to the waste chamber. UCNP signals are measured from the detection chamber. A cardiac troponin I (cTnI) assay was developed on the new platform and its performance was evaluated resulting into an LoD of 19.7 ng/L.

The assay cassette was conceptualized by keeping in mind several parameters, namely, geometry of the reaction chamber, suitable optical cap allowing label-specific excitation and emission wavelengths, large waste chamber, and practical buffer inlet and suction channel orifice for maintaining

undisrupted liquid flow, easy liquid handling, and injection moldable design for mass production. Initial designs of the cassette were drawn with AutoCAD software (Autodesk Inc., CA, US). 3D-printed cassette prototypes were built according to CAD-drawings at Alphaform, Rusko, Finland. After several reiterations, injection moldable design of the cassette was finalized. Emphasis was also given to identify optimal materials for manufacturing the assay cassette, optical cap, sealing gasket and filtration mesh. Assay cassette was manufactured using polypropene. Most suitable material for optical caps was Lucite Diakon CLG356 Clear 0011 (Lucite International Holland BV, The Netherlands) which can be used with a wide variety of fluorescent labels including UV-excitable ones. For gasket ring which seals optical cap, thermoplastic polyurethane was compatible. For microparticle filtration mesh, NY1H nylon membrane (Merck Millipore, Darmstadt, Germany) with pore size 100 µm was selected for laboratory use because of its robustness and affordability. Finally, injection molded cassettes were manufactured using optimal materials at Scaletec Oy, Turku, Finland. Vertical cross-section schematics of the cassette are shown in Fig. 1A.

The potential of achieving high analytical sensitivity with newly developed platform has been demonstrated using cTnI as a model analyte. Analyte-specific reagents were prepared and the assay was set up. Streptavidin (SAv; SPA, Milan, Italy) was crosslinked using glutaraldehyde as described before [8]. Dynoseeds TS140 polystyrene microparticles (140 µm diameter; Microbeads AS, Skedsmokorset, Norway) were passively coated with crosslinked SAv. Briefly, 200 µg of crosslinked SAv was mixed with 100 mg of microparticles in 1 mL coating buffer (10 mM Tris-HCl, pH 8), and incubated for overnight at 4°C in rotation. After the incubation, crosslinked SAv coated microparticles (microparticle-SAv) were washed 4-times with 1.25 mL wash buffer (Kaivogen Oy, Turku, Finland) supplemented with 0.05% Tween-20. Then the microparticle-SAv were incubated with 1.25 mL surface saturation buffer (50 mM Tris-HCl, pH 7, 150 mM NaCl, 0.05% NaN3, 0.2% BSA and 6% sorbitol) for 2 h at RT in rotation. Finally, the microparticle-SAv were washed 2-times with 1.25 ml of TSA (50 mM Tris-HCl, pH 7.75, 154 mM NaCl, 0.05% NaN3), resuspended in 8% NaCl, and stored at 4°C. Anti-cTnI mAbs MF4, 19C7 and 916 (Hytest Ltd., Turku, Finland) were biotinylated using biotin isothiocyanate as described earlier [9]. 200 ng of each of the bio-mAbs were incubated with 2100 microparticles-SAv in the presence of 6% NaCl for 1 h with rotation. After the incubation, microparticles-SAv coupled with bio-mAbs were washed twice with wash buffer (Kaivogen Oy) and resuspended in 8% NaCl. Anti-cTnI mAb 8I-7 (International Point of Care Inc., Toronto, Canada) was coated on silanized and carboxyl-modified Upcon™ up-converting nanoparticles (UCNP; Kaivogen) utilizing EDC-NHS chemistry and stored in storage buffer (5 mM Tris pH 8.5, 0,05% NaN_3, 0,05% Tween-80, 0,5% BSA) as described before [10]. A two-fold dilution series (6400 – 25 ng/L) of human cardiac troponin ITC complex (Hytest) was prepared in 7.5% BSA in TSA. The dilution series including blank was tested in assay cassettes in

five replicates. The revolving optical cap with the gasket ring was mounted to the cassette in close position of the valve, which does not allow liquid flow from the reaction chamber to the detection chamber. In order to set up the reaction, the reaction chamber cap was opened, 140 µl of troponin ITC complex dilution, 500 ng of mAb 8I-7-UCNP in 10 µl storage buffer, 100 µl assay buffer (Kaivogen) and 2100 microparticles-SAv coupled with bio-mAbs in 14.5 µl of 8% NaCl were added to the reaction chamber of each cassette. The reaction chamber caps were closed and cassettes were incubated for one hour with shaking (500 rpm) on a plate shaker and then washed. For washing, wash pouch prefilled with 3 ml wash buffer (Kaivogen) was attached to the buffer inlet of the cassette by removing a peel-off strip from the inlet, optical cap was turned to the open position of the valve, and a negative pressure was applied through a syringe from the suction channel orifice. The outer assembly of the cassette including syringe driven suction channel and wash buffer pouch is visualized in Fig. 1B. All the reaction materials flowed from the reaction chamber to the waste container via detection chamber, except the microparticles that were entrapped in the detection chamber due to filter mesh. Since the liquid which comes out through the filter mesh contains biohazardous waste, absorbent material (Sodium polyacrylate) was placed inside the waste chamber in order to absorb it. The UCNP signals from the immune complexes bound to the microparticles (Fig. 1C) were measured from dry detection chamber using a portable Fluoro-I instrument (DesignInnova, New Delhi, India).

The average of UCNP signals from five replicate cassettes was calculated for each concentration of cTnI and blank, and a dose-response curve was plotted (Fig. 2). The limit of detection (LoD) of cTnI was determined by the cutoff value, which was calculated by adding the three times standard deviation to the average of blank signal values. An LoD of 19.7 ng/L was obtained for cTnI with the new platform. Current high sensitivity cardiac troponin I assays have an LoD of <10 ng/L [11], whereas point-of-care assays have LoD of 10 – 50 ng/L [1]. The LoD of microparticle–based cTnI assay is within the range of point-of-care cTnI assays. However, comparison between different immunoassays is complicated by the use of different binding antibodies and different label technologies, in addition to the differences in the assay platforms. With regards to the UCNP reporters, a well-based immunoassay for cTnI with UCNP reporters achieved a sensitivity of 3.1 ng/L [12], while a lateral flow assay with UCNP reporters had a sensitivity of 41 ng/L in serum [13]. Current study was a proof-of-concept study to demonstrate the performance of microparticle–based platform in a highly challenging and demanding application viz. cTnI. However, more complex sample matrices like serum, plasma and whole blood should be tested in order to truly realize the potential of the developed platform.

The developed assay platform offers a robust design which obviated the need to use complex microfluidics and multi-membrane lateral flow platforms. The assay platform is designed to have a

dedicated reaction chamber enabling absolute control over the reaction incubation time. The design also permits efficient washing which may help in producing better specificity. The use of UCNP in the assay in combination with reader instrument can not only provide better sensitivity than visual label, but also eliminates the problem of subjectivity. The UCNP-detection can eliminate the autofluorescence produced from assay components and sample matrix, and thus whole blood samples can also be used. Additionally, quantitative or semi-quantitative assay development is also possible [14]. Present study is also an example of a quantitative assay.

In conclusion, a proof-of-concept robust syringe-driven assay platform was developed that incorporates reaction incubation and waste containment. The platform uses fluorescent UCNPs as label that may provide better assay sensitivity than conventional visual labels. A cTnI assay was fabricated on the developed platform and showed to achieve an LoD which is comparable to the best point-of-care cTnI assays. The developed assay can be further simplified by drying all the assay-specific reagents in the reaction chamber, facilitating minimal liquid handling by the end-user. This study demonstrates the feasibility of developing point-of-care assays utilizing microparticle–based platform for a variety of analytes that call for high-sensitivity e.g. cancer biomarkers in biological samples, infectious disease biomarkers in human/veterinary samples, contaminants/toxins in food/environmental samples etc.

Acknowledgements

This study was supported by grants from the Finnish Funding Agency for Technology and Innovation (TEKES), Finland, and Department of Biotechnology (DBT), Government of India. The authors declare no conflict of interest.

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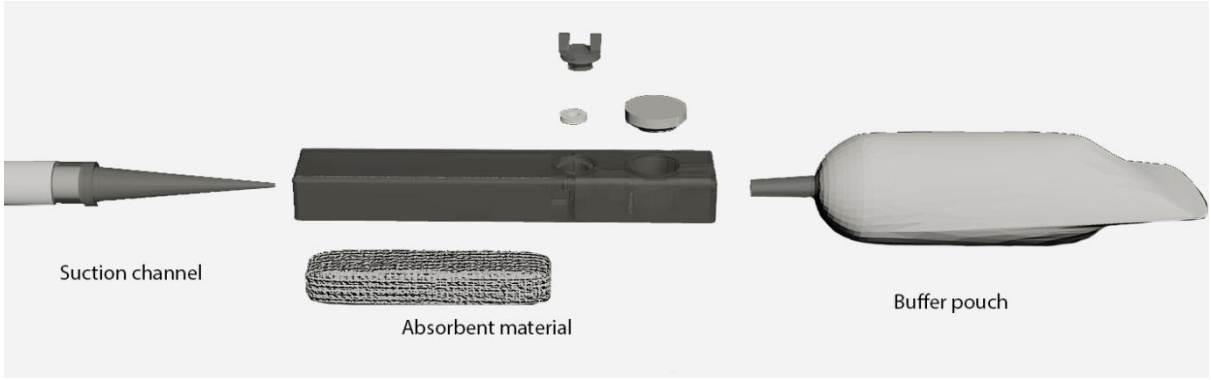

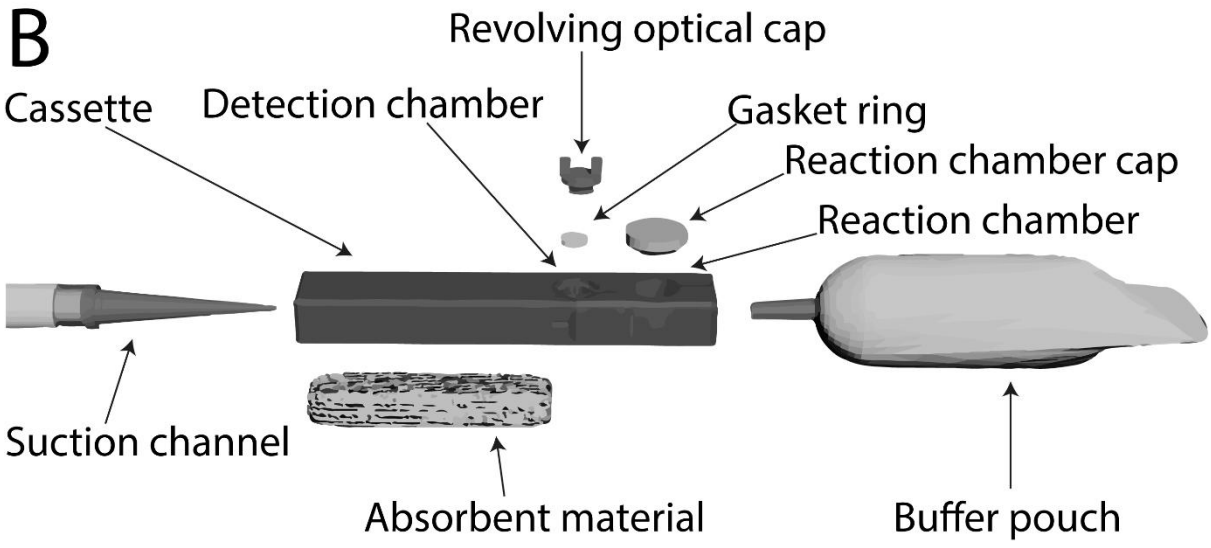

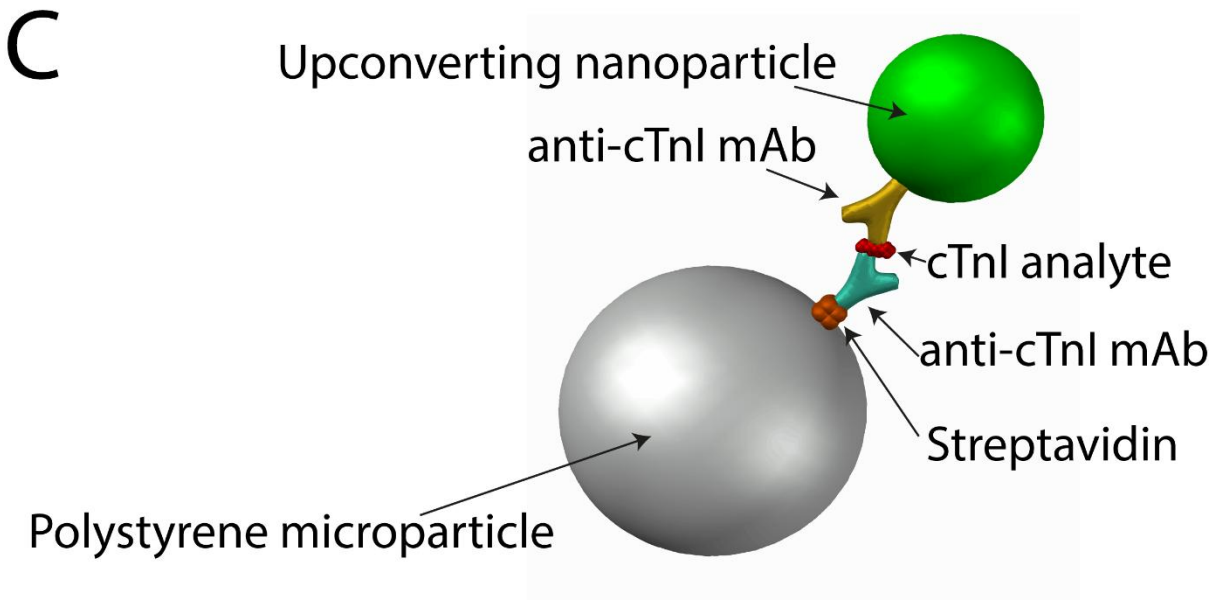

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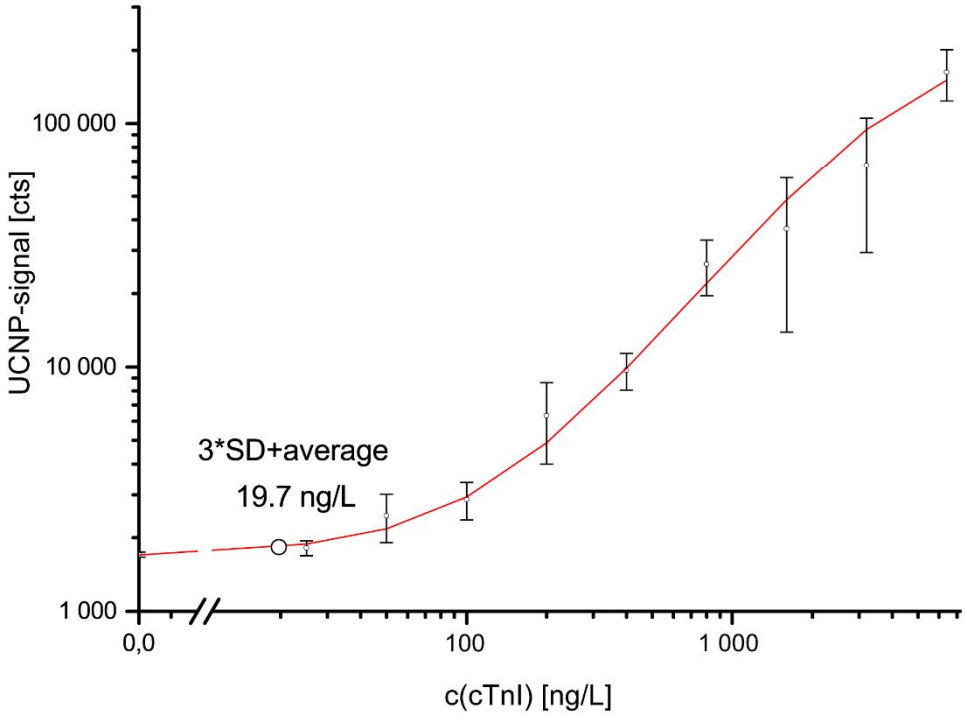
185 *Fig. 1. Design of the assay cassette. (A) Vertical cross-section of the assay cassette showing various*
186 *components. (B) Outer assembly of the cassette. Additional components like buffer pouch for washing*
187 *and syringe driven suction channel are also shown. Absorbent material is placed inside the waste*
188 *chamber in order to absorb and retain the liquid. (C) A schematic of the immune complex formed on*
189 *the microparticle in an analyte positive assay. An immune complex is formed when cTnI is sandwiched*
190 *in between a streptavidin-coated microparticle coupled with biotinylated capture antibodies and*
191 *antibody coated UCNP labels. Unbound sample materials and label are washed away, while the large*
192 *microparticles are entrapped on the filter.*



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194 *Fig. 2. A dose-response curve of cTnI-assay using purified human cardiac troponin ITC-complex spiked*
195 *in assay buffer as calibrator*

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