Analytica Chimica Acta 853 (2015) 682–688

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Acoustic trapping as a generic non-contact incubation site for multiplex bead-based assays

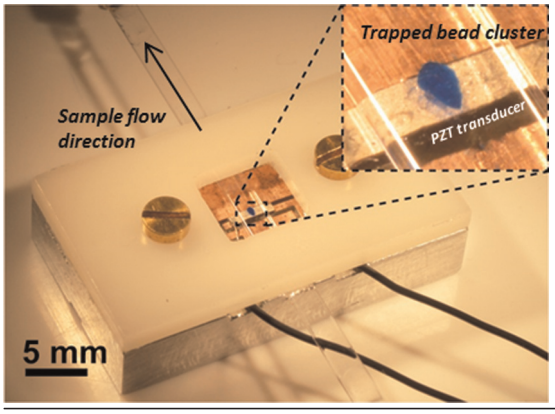
Maria Tenje [a,*], Hongyan Xia [b,c], Mikael Evander [a], Björn Hammarström [a], Axel Tojo [a], Sándor Belák [b,c,d], Thomas Laurell [a,e], Neil LeBlanc [c,d]

[a] Department of Biomedical Engineering, Lund University, Sweden
[b] Department of Biomedical Sciences and Veterinary Public Health (BVF), Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden
[c] The World Organization for Animal Health (OIE) Collaborating Centre for the Biotechnology-based Diagnosis of Infectious Diseases in Veterinary Medicine, Uppsala, Sweden
[d] National Veterinary Institute, Uppsala, Sweden
[e] Dongguk University, Seoul, Republic of Korea

HIGHLIGHTS

- Multiplex Luminex assay performed in acoustic trap.
- Bead recovery increased to more than 75%.
- Assay time can be reduced by more than 50%.
- Maintained assay sensitivity in microfluidic format.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 3 March 2014
Received in revised form 25 June 2014
Accepted 2 July 2014
Available online 14 July 2014

Keywords:
Acoustic trapping
Immuno-based assay
Multiplex
Luminex

ABSTRACT

In this study, we show a significantly reduced assay time and a greatly increased bead recovery for a commercial Luminex-based multiplex diagnostic immunoassay by performing all liquid handling steps of the assay protocol in a non-contact acoustic trapping platform.
The Luminex assay is designed for detecting antibodies in poultry serum for infectious bursal disease virus, infectious bronchitis virus, Newcastle disease virus and avian reovirus. Here, we show proof-of-concept of a microfluidic system capable of being fully automated and handling samples in a parallel format with a miniature physical footprint where the affinity beads are retained in a non-contact levitated mode in a glass capillary throughout the assay protocol. The different steps are: incubation with the serum sample, secondary antibodies and fluorescent reporters and finally washing to remove any non-specifically bound species. A Luminex 200 instrument was used for the readout. The flow rates applied to the capillary during the initial trapping event and the wash steps were optimised for maximum bead recovery, resulting in a bead recovery of 75% for the complete assay. This can be compared to a bead recovery of approximately 30% when an automatic wash station was used when the assay was performed in the conventional manual format.

* Corresponding author. Tel.: +46 222 93 70; fax: +46 222 45 27.
E-mail address: maria.tenje@bme.lth.se (M. Tenje).

http://dx.doi.org/10.1016/j.aca.2014.07.008

The time for the incubation steps for a single assay was reduced by more than 50%, without affecting assay performance, since intermediate wash steps became redundant in the continuously perfused bead trapping capillary. We analyzed seven samples, in triplicates, and we can show that the readout of the assay performed in the acoustic trap compared 100% to the control ELISAs (positive or negative readout) and resulted in comparable *S*/*P* values as the conventional manual protocol. As the acoustic trapping does not require the particles to have magnetic properties, a greater degree of freedom in selecting microparticles can be provided. In extension, this can provide an opportunity to develop cheaper and more effective microparticles.

1. Introduction

Microbead-based assays using Luminex xMAP® technology for the detection of both human and animal pathogens and antibodies have proliferated in recent years [1–4]. This technology allows multiplex detection in the same assay by having color-coded microbeads that can be distinguished in the Luminex flow cytometer. With an open architecture format that allows various bio-molecules, such as nucleic acids and proteins, to be used for capturing targets, Luminex has become an industry leader in multiplex technology. For an antibody detection assay, each bead set is coupled by a chemical reaction with a specific antigen and multiplexing is achieved by matching the bead color with the antigen. The Biovet Luminex-based Flock Monitor assay is designed to detect the presence of antibodies against infectious bursal disease virus (IBDV), avian infectious bronchitis virus (IBV), Newcastle disease virus (NDV) and avian reovirus (REO). These viruses are the underlying causes of respiratory diseases and lead to substantial economic loss in poultry industry [5]. The assay has been approved for use in veterinary diagnostics by the United States Department of Agriculture and is available commercially from Biovet Inc. (Québec, Canada).

Acoustic trapping is a method to control and position particles on the micrometer scale at distinct positions in microfluidic networks without physical contact [6]. Compared with other non-contact trapping techniques, such as electrical or optical, the acoustic trapping has several advantages, e.g., a simple integration with micro fluidic networks since no electrodes are used in contact with the fluids. The complete set-up is also highly suitable for miniaturization and does not require large and bulky equipment. Another advantage is that the technique is largely unaffected by particle charge, pH and ionic strength of the solution. The only requirements are that the density and the compressibility of the particles to be trapped must differ from the surrounding media. All micrometer-sized affinity beads in aqueous solutions meet the requirements for successful trapping, including the magnetic Luminex beads used for the Flock Monitor assay.

Here, we present for the first time the transfer of all incubation steps in a commercial bead-based assay onto a microfluidic platform utilising acoustic trapping. Acoustic trapping has previously been presented as a tool to perform bead-based assays [7,8]. Our work is however the first demonstration where all incubation steps of a commercial bead-based assay are integrated in an acoustic bead trapping platform. Performing the assay in an acoustofluidic system has advantages such as avoiding cross-contamination, increased bead recovery and reduced incubation time offering the potential for a fully automated system in a microscale format.

Antigen coated beads were aspirated into the glass capillary based microchannel and were trapped at the position of the local acoustic standing wave field, where after sample and reagents sequentially were aspirated across the trapped bead cluster. Subsequently, the bead cluster was released directly into a 96-well-plate from the micro channel for analysis in the Luminex instrument. The assay was performed on seven different serum samples, in triplicates. The performance of the microfluidic assay protocol showed a higher reliability than when the assay was performed conventionally, according to manufacturer's instructions.

In this work we show that several advantages (time for a single assay, bead recovery, lower sample volume, assay reliability and assay time) are gained when acoustic trapping in a glass capillary is integrated with the fluid handling sequence in the incubation steps of a multiplex bead-based assay, prior to readout in a standard Luminex 200 instrument. In this work, we have only operated a single glass capillary to show proof-of-concept, but the technology lends itself well to up-scaling through simple engineering.

2. Materials and methods

A glass capillary (i.d. 0.2 mm × 2 mm, Vitrocom) was used as the micro channel, total volume 20 μL in our set-up [9]. Glass syringes (1 ml and 5 ml, Hamilton) were mounted in syringe pumps (neMESYS, Cetoni GmbH) to control the volumetric flow rates in the capillary, either in aspirate or dispense mode of the syringes. All microfluidic parts were connected with Tygon® tubing (i.d. 0.254 mm, Saint-Gobain). Beads, sample and reagents were introduced into the capillary from Eppendorf tubes (0.2 ml, Axygen) in aspirate mode and the bead cluster was washed in dispense mode. A two-position-valve (VICI Instruments) was used to switch the connections between the waste syringe and the wash buffer syringe during the assay. A function generator (HP 33,120A, Agilent) actuated the piezoelectric transducer (PZ26, Meggitt A/S) and an oscilloscope (TDS1002, Tektronix) was used to monitor the frequency and amplitude of the signal. The transducer was driven at 4 MHz at 17 V_{pp}. The frequency was manually tuned for optimal trapping efficiency during tests runs prior to starting the assay.

The trapping efficiency was measured at different flow rates by following the fluorescence intensity of the bead cluster building up when 50 μL MagPlex beads were introduced into the glass capillary. A Mercury lamp (HBO, Osram, Sweden) was used to illuminate the trapping site and the fluorescence signal was collected through a 670 nm filter (AxioImager HQ-Cy5). The bead solution was prepared by diluting MagPlex stock solution to 1:10 in Milli-Q water with 0.025% Tween (Sigma–Aldrich). Images were captured with a camera (ORCA-1394 ER, Hamamatsu) at 500 ms time intervals. A MatLab script (MatLab R2012a, MathWorks) was used to measure the mean fluorescence intensity in the images, which is directly related to the number of the fluorescent beads in the cluster. As microscopy was performed in a dark system, background compensation could be achieved by subtracting the first (empty stage) image from every image in the measurement series. All pixels of the images were used for the analysis. To track the cluster growth, the mean fluorescence intensity was plotted over time, where an increase in the mean fluorescence intensity is directly related to an increase of the cluster size. The same method was used to follow the cluster size when evaluating the flow rates for the wash step.