DNA & PROTEIN DETECTION BASED ON MICROBEAD AGGLUTINATION

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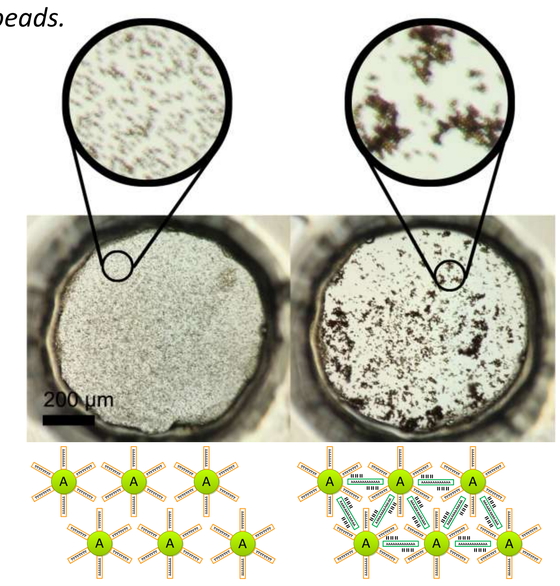
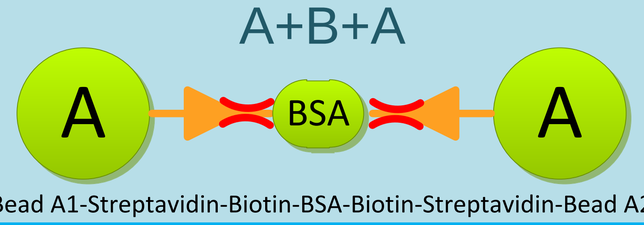
Introduction

We report a simple and rapid room temperature assay for point-of-care (POC) testing that is based on specific agglutination. Agglutination tests are based on aggregation of microparticles in the presence of a specific analyte thus enabling the macroscopic observation. Agglutination-based tests are most often used to explore the antibody-antigen reactions [1]. Agglutination has been used for mode protein assays [2] using a biotin/streptavidin two-component system, as well as a hybridization based two-component assay [3]; however, as our work shows, two-component systems are prone to self-termination of the linking analyte and thus have a lower sensitivity. Three component systems have also been used with DNA hybridization [4], as in our work; however, their assay requires 48 hours for incubation, while our assay is performed in 5 minutes making it a real candidate for POC testing. We demonstrate three assays: a two-component biotin/streptavidin assay, a three-component hybridization assay using single stranded DNA (ssDNA) molecules and a stepped three-component hybridization assay. The comparison of these three assays shows our simple stepped three-component agglutination assay to be rapid at room temperature and more sensitive than the two-component version by an order of magnitude. An agglutination assay was also performed in a PDMS microfluidic chip where agglutinated beads were trapped by filter columns for easy observation.

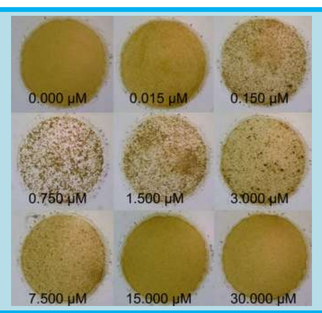
Example of microbeads with no agglutination present (left) and agglutination (right). Diagrams at bottom illustrate links between beads.



Two-component system A+B+A

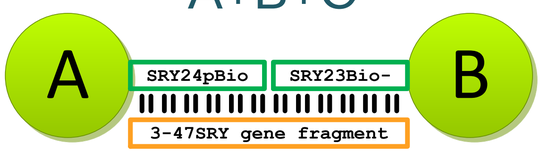


Bead A1-Streptavidin-Biotin-BSA-Biotin-Streptavidin-Bead A2

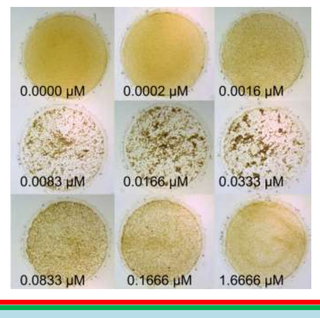


Titration of biotinylated BSA protein concentration for the agglutination assay with streptavidin coupled beads in 2 µl solution (A + B + A). Sequence of photographs, each labeled with its µM final concentration of biotinylated BSA protein.

Three-component system A+B+C

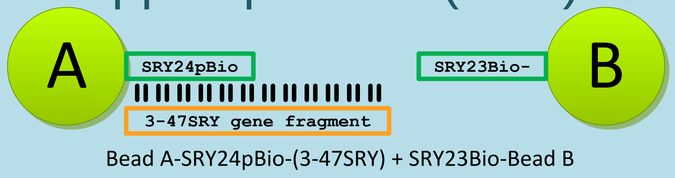


Bead A-SRY24pBio-(3-47SRY)-SRY23Bio-Bead B

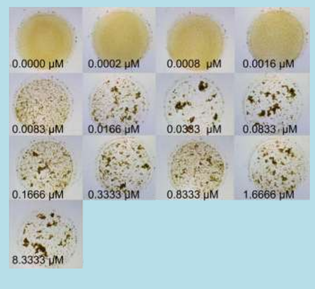


SRY gene hybridization based agglutination example. Target DNA (3-47SRY) concentration titration by mixing with oligo SRY24pBio, SRY23Bio-functionalized microbeads in 2 µl solution (A + B + C). Sequence of photographs, each labeled with its µM final concentration of (3-47SRY) oligonucleotide.

Three-component system Stepped process (A+C)+B

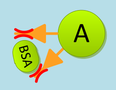


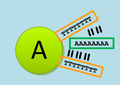
Bead A-SRY24pBio-(3-47SRY) + SRY23Bio-Bead B



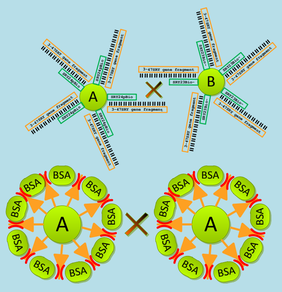
Same SRY gene hybridization based agglutination example. Beads functionalized with oligo SRY24pBio were mixed with various concentrations of target DNA (3-47SRY). After hybridization and washing away non-hybridized DNA, SRY23Bio-functionalized microbeads were introduced ((A + C) + B) with following agglutination.

Assay Comparison



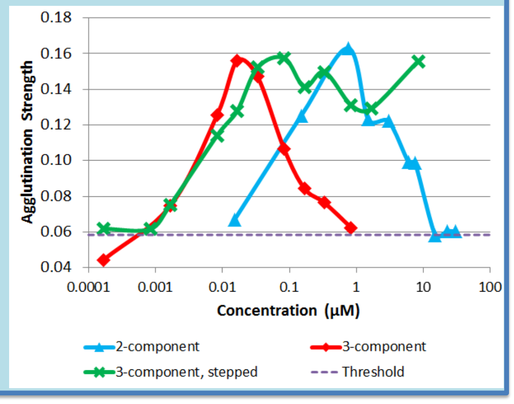


*The ligand (biotinylated BSA or oligo) in the **two-component** system assay can link two sites on same bead, therefore not aiding in agglomeration. This results in higher concentrations of analyte required to produce agglutination.*

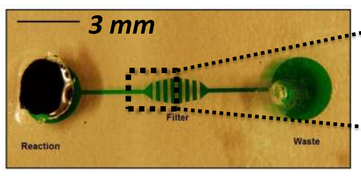


***Non-stepped** assays are prone to active site saturation and loss of agglomeration at high analyte concentrations.*

***Stepped three-component** system assay is an order of magnitude more sensitive than two component system and maintains positive signal at high analyte levels.*



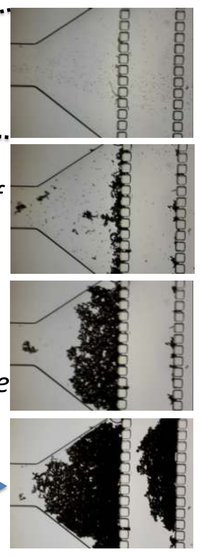
Agglutination detection in PDMS microfluidic chip



The PDMS microfluidic device consists of

- *reaction chamber where beads are pipetted*
- *the filter with 10 µm spaced 50 µm high columns*
- *waste output where negative pressure is applied*

The flow direction is from left to right



Non-bound beads are flowing freely through the columns in microfluidic device

Agglutinated beads preferentially get trapped at the first row of columns

The agglutinated "colored" beads are visible by naked eye

Higher flow rates move some agglutinated beads through columns, however they are captured by subsequent rows of columns

Summary

- *We developed a rapid (5 minute) room temperature assay, which is based on microbead agglutination.*
- *Our three-component assay solves the linker self-termination issue allowing an order of magnitude increase in sensitivity over two–component assays.*
- *Our stepped version of the three-component assay solves the issue with probe site saturation thus enabling a wider range of detection.*
- *Detection of the agglutinated beads with the naked eye by trapping in microfluidic channels has been shown.*

References

1. Bains, W. and P. Noble, *Sensitivity limits of latex agglutination tests.* American clinical laboratory, 1993. 12(3): p. 14, 16-7.
2. Moser, Y., T. Lehnert, and M.A. Gijs, *On-chip immuno-agglutination assay with analyte capture by dynamic manipulation of superparamagnetic beads.* Lab on a chip, 2009. 9(22): p. 3261-7.
3. Vollenhofer-Schrumpf, S., R. Buresch, and M. Schinkinger, *A simple nucleic acid hybridization/latex agglutination assay for the rapid detection of polymerase chain reaction amplicons.* Journal of microbiological methods, 2007. 68(3): p. 568-76.
4. Rogers, P.H., et al., *Selective, controllable, and reversible aggregation of polystyrene latex microspheres via DNA hybridization.* Langmuir: the ACS journal of surfaces and colloids, 2005. 21(12): p. 5562-9.