PINWHEEL ASSAY VIA A 'PIPET, AGGREGATE AND BLOT' (PAB) APPROACH ON FILTER PAPER

Jingyi Li[1, 5], Hussein Alshammari[1], Beeta Ehdaie[2], KimberlyA. Kelly[2], James P. Landers[1, 3, 4, 5]

Departments of [1]Chemistry, [2]Biomedical Engineering, [3]Mechanical and Aerospace Engineering, [4]Pathology, [5]Center For Microsystems For The Life Sciences, University of Virginia, Charlottesville, VA 22904, USA

ABSTRACT

We present a new version of the pinwheel assay for nucleic acid and cell quantitation with improved simplicity and portability. The whole process includes four steps: pipetting, aggregating, blotting and scanning. The sensitivity and limit of detection is determined, and an application of bacteria detection is demonstrated. The protocol requires minimal instrumentation, and thus is suitable for more cost-effective point-of-care applications.

KEYWORDS: pinwheel, nucleic acid, quantitation, superparamagnetic beads, point-of-care testing

INTRODUCTION

Detection of nucleic acids (NA), viruses, and cells plays a fundamentally important role in biological science and applications, but the complexity and cost of conventional techniques limit their prevalence in microfluidic devices and hinders development of point-of-care testing (POCT). In MicroTAS 2010, we presented the 'pinwheel assay' as a novel label-free optical technique to quantitate NA, viruses and cells by monitoring aggregation of superparamagnetic beads induced by NA in a rotating magnetic field (RMF). In order to develop this new technique towards POCT, we have evolved a simple 'pipet, aggregate, and blot' (PAB) approach of the pinwheel assay, which requires minimal instrumentation and is a promising low-cost alternative of conventional methods towards various biomedical applications in the field.

EXPERIMENTAL

The PAB approach consists of four steps (Figure 1): (1) draw up sample into a pipet tip containing superparamagnetic beads and mix adequately, (2) briefly expose the tip to a magnetic field to induce aggregation, (3) expel (blot) the beads onto an adsorbent material (filter paper) to lock the aggregate formation, and (4) scan and analyze the resultant images to quantitate analytes. The entire process took less than 10 minutes, requiring simply a pipettor, pipet tips, a magnet, filter paper, and a document scanner.

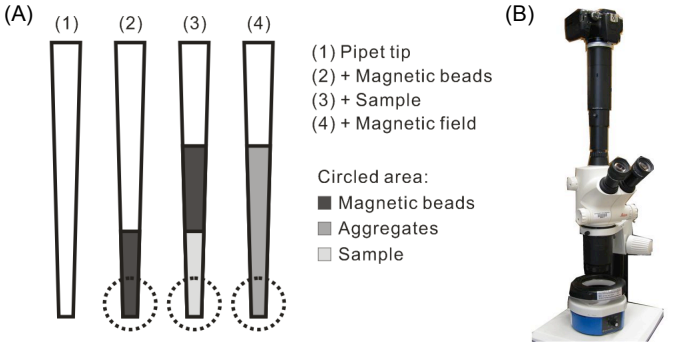

Figure 1. (A) Experimental procedure of PAB-pinwheel approach. DNA and superparamagnetic beads were directly mixed in a pipet tip, and aggregation was induced by magnetic field. Dispersed beads and/or aggregates were clearly visualized after blotted on filter paper and were further analyzed quantitatively. (B) Previous pinwheel setup with a camera on a microscope [1]. The simplicity and portability of instrumentation is significantly improved in the PAB-pinwheel assay.

RESULTS AND DISCUSSION

We have studied the sensitivity and limit of detection (LOD) of the PAB-pinwheel assay, and demonstrated an application for food safety testing. Figure 2 shows that smaller beads were more prone to aggregate with DNA providing higher

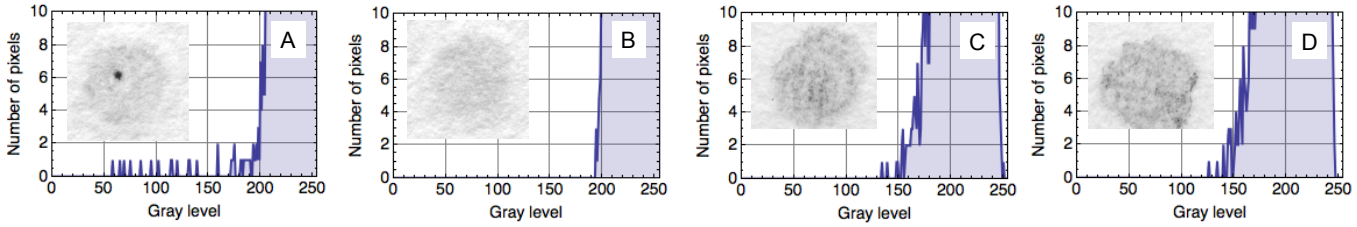

Figure 2. Smaller beads result in higher sensitivity. 1 μL λ-DNA (0.25 ng/μL) caused distinct aggregates (A) of 1 μm (diameter) beads compared to a negative control (B, 1 μm w/o DNA), while 10x higher DNA concentration (2.5 ng/μL) did not cause aggregation of 8 μm beads (C) compared to its negative control (D, 8 μm w/o DNA).

sensitivity. For DNA quantitation, more concentrated DNA caused tighter aggregates on filter paper (Figure 3A). Correspondingly in histogram, pixels representing beads moved towards lower gray level for high DNA concentration (Figure 3B). A gray level = 120 was defined as the threshold to discriminate aggregates versus dispersed beads and

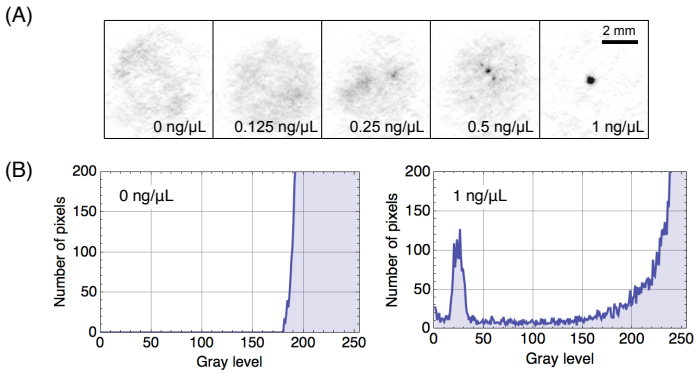

Figure 3. Aggregation dependence on DNA concentration. (A) 2 μL beads (1 μm) were mixed with various concentrations of λ-DNA, resulting in different degrees of aggregation. (B) As DNA concentration increased, more pixels were distributed at low gray levels in the histograms, which provided a means to quantitatively characterize bead aggregation. Gray level 120 (dashed line) was selected to distinguish aggregates (<120) from dispersed beads and background (>120).

background, and the total number of pixels (i.e., dark area) under 120 represented bead aggregation. Figure 4 illustrates that the dark area values increased with higher DNA concentrations, denoting more aggregation, with an LOD of ~250 pg/μL readily achievable. For the same aggregates on filter paper, the sensitivity of PAB approach also depends on image resolution (Figure 5). Although higher resolution resulted in better sensitivity, more time and disk space were needed, so a compromise is required for specific applications.

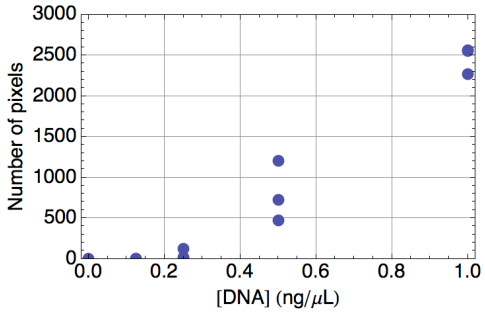

Figure 4. Determination of LOD. The total number of pixels below 120 (i.e. dark area) was calculated for each DNA concentration (n =3). Down to ~250 pg/μL was detectable in current protocol.

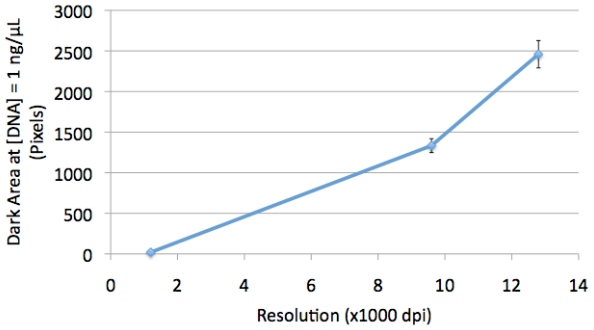

Figure 5. Higher image resolution (dpi = dots per inch) provides better sensitivity for the same aggregates on filter paper.

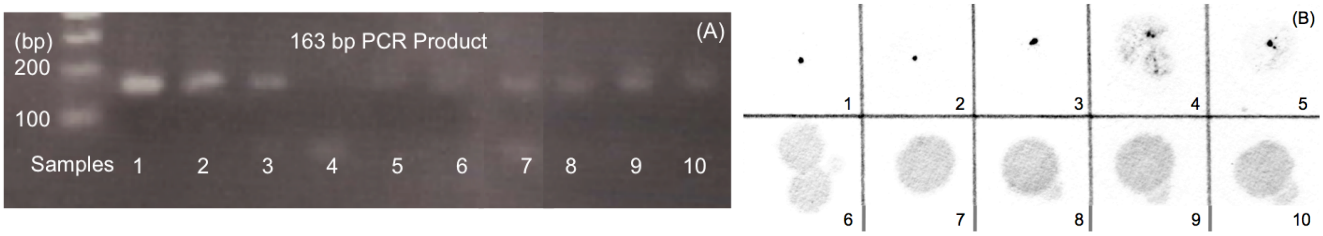

Figure 6. Simple and Inexpensive Detection of E. coli in apple juice. E. coli with different concentrations were spiked into apple juice samples and then infected by M13 phage. After 5 hours of incubation, amplified phage were isolated and detected using conventional PCR/gel electrophoresis and PAB-pinwheel assay separately, and both indicated the presence and concentration of E. coli in original samples. The result of PAB-pinwheel assay (B) correlated well with PCR/gel electrophoresis but required less time and lower cost. E. coli concentrations (cfu/mL): (1) $3.47x10^8$, (2) $3.47x10^7$, (3) $3.47x10^6$, (4) $3.47x10^5$, (5) $3.47x10^4$, (6) $3.47x10^3$, (7) $3.47x10^2$, (8) 3.47x10, (9) 3.47, (10) 0.

Figure 6 shows an application of the PAB-pinwheel assay to detect *E. coli* in apple juice through phage amplification [2]. We contaminated apple juice samples with different concentrations of E. coli, and spiked in M13 phage, which infects *E. coli*

specifically and replicates rapidly [3]. After 5 hours of incubation, the resultant number of phages was quantified using the PAB-pinwheel assay and compared against data from PCR. PAB-pinwheel assay generated similar results to PCR/gel electrophoresis, but provided a significant reduction of experimental time, cost and effort.

CONCLUSION

In summary, we demonstrate a simple and rapid alternative to the pinwheel assay that requires minimal instrumentation but is applicable to *E. coli* detection. Current efforts include optimizing the PAB protocol and developing potable PAB-pinwheel scanner systems for more cost-effective point-of-care applications.

REFERENCES

[1] J. Li, D.C. Leslie, D.M. Haverstick, K.A. Kelly, N.S. Barker, and J.P. Landers, *Pinwheel Assay: A Visual and Label Free Method for DNA Quantitation*, Proc. Micro Total Analysis Systems Conferences, 61-63 (2010).

[2] S.M. Wilson, Z. Al-Suwaidi, R. McNerney, J. Porter, and F. Drobniewski, *Evaluation of a new rapid bacteriophage based method for the drug susceptibility testing of Mycobacterium tuberculosis*, Nat Med, 3(4), 465-468 (1997).

[3] D. a. Marvin, Filamentous phage structure, infection and assembly, Current opinion in structural biology, 8(2), 150-158 (1998).