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Virus Detection Using Filament-Coupled Antibodies

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Abstract

Two attractive features of ELISA are the specificity of antibody–antigen recognition and the sensitivity achieved by enzymatic amplification. This report describes the development of a non-enzymatic molecular recognition platform adaptable to point-of-care clinical settings and field detection of biohazardous materials. This filament-antibody recognition assay (FARA) is based on circumferential bands of antibody probes coupled to a 120 μm diameter polyester filament. One advantage of this design is that automated processing is achieved by sequential positioning of filament-coupled probes through a series of 25–60 μL liquid filled microcapillary chambers. This approach was evaluated by testing for the presence of M13KO7 bacterial virus using anti-M13KO7 IgG₁ monoclonal antibody coupled to a filament. Filament motion first positioned the antibodies within a microcapillary tube containing a solution of M13KO7 virus before moving the probes through subsequent chambers, where the filament-coupled probes were washed, exposed to a fluorescently labeled anti-M13KO7 antibody, and washed again. Filament fluorescence was then measured using a flatbed microarray scanner. The presence of virus in solution produced a characteristic increase in filament fluorescence only in regions containing coupled antibody probes. Even without the enzymatic amplification of a typical ELISA, the presence of 8.3×10^8 virus particles produced a 30-fold increase in fluorescence over an immobilized negative control antibody. In an ELISA comparison study, the filament-based approach had a similar lower limit of sensitivity of $\sim 1.7 \times 10^7$ virus particles. This platform may prove attractive for point-of-care settings, the detection of biohazardous materials, or other applications where sensitive, rapid, and automated molecular recognition is desired.

Keywords

molecular recognition; virus detection; ELISA

INTRODUCTION

The enzyme linked immunosorbent assay (ELISA) is one of the most common antibody based molecular recognition assays in the laboratory, but the requirements of many clinical applications, where speed and throughput are critical parameters, have exceeded the capability of traditional plate-based ELISA. Automated systems have overcome these limitations in large hospital settings and can process hundreds of samples per hour (Owen and Roberts, 2003; Stern et al., 2001). However, large integrated systems are not practical for point-of-care clinical settings where cost is a prohibitive factor. In these settings, samples often are sent off-site for analysis, adding a significant time lag before results are in hand. In many situations, the need for quick diagnosis and treatment make onsite testing very desirable. Many viruses are more easily treated when diagnosed early, so quick and reliable diagnosis that can be performed

while a patient waits could allow treatment to begin immediately and, thus, improve the efficacy of treatment. In addition to clinical testing, many field applications exist where rapid identification of a hazardous material is required to formulate an appropriate response.

The generation of monoclonal antibodies and the advent of recombinant DNA technology have significantly increased both speed and sensitivity of molecular based assays (Iqbal et al., 2000; Reischl, 1996). A number of schemes based on the sandwich immunoassay have been reported in the literature. For example, several recent applications are based upon changes in electrical properties as antigen binds to an immobilized probe (Balasubramanian et al., 2004; Baselt et al., 1996; Mubammad-Tahir and Alocilja, 2003; Ronkainen-Matsuno et al., 2002) or based on the interaction of evanescent light waves with surface-bound antibodies (Ligler et al., 2003; Plowman et al., 1999; Rowe et al., 1999). Many of these approaches are either labor intensive or require complex microfluidic devices and are not well suited for point-of-care clinical diagnostics or for field testing for biohazardous materials. A simpler antibody-based detection assay would be very desirable.

This report uses a model bacteriophage/monoclonal antibody system to demonstrate a simple sandwich immunoassay that is based on immobilized antibody probes along a polyester monofilament. M13K07 virus and anti-M13K07 IgG provide a simple test system for initial studies. M13K07 is a harmless phage, so experiments can be performed with minimal safety concerns. In addition, highly specific antibodies to M13K07 are commercially available, and binding between virus and antibody is well-characterized. The advantages of this approach include a reduction in reagent volumes and incubation times compared to standard ELISA, along with automation of the reaction process and elimination of the enzymatic amplification required in ELISA. This study describes the initial studies using this design and compares its sensitivity to traditional plate-based ELISA.

METHODS

Reagents

Anti-M13 monoclonal IgG₁ (anti-M13) and anti-E tag monoclonal IgG₁ (anti-E) were obtained from Amersham Biosciences (Piscataway, NJ) and fluorescently labeled with Alexa Fluor 647 (AF647), Alexa Fluor 555 (AF555) (Molecular Probes, Eugene, OR), Cy3, or Cy5 (Amersham Biosciences). Labeling procedures were performed according to the manufacturers' instructions. The concentration of the labeled antibody along with the degree of labeling was calculated from the absorbance at 280 nm and the peak label absorbance. A working solution of labeled antibody was stored at 4°C, and aliquots were stored long term at -20°C.

M13K07 Virus (M13 virus) was obtained from the Vanderbilt Molecular Recognition and Screening facility in a stock concentration of 3.3×10^{11} virions/mL and was diluted in phosphate buffered saline containing 0.1% Tween-20 (PBS-T) to working concentrations immediately prior to experiments.

Antibody Immobilization on Filament

A 120 µm diameter clear polyester filament (440 yd spool, Sulky Invisible, Punta Gorda, FL) was obtained from webofthread.com (Kenmore, WA). Glass, polyester, nylon, PVDF, and silk filaments were tested and rated based on autofluorescence at common wavelengths, flexibility, chemical composition, and strength. The polyester filament provided the best combination of strength and flexibility and exhibited less intrinsic autofluorescence than the other polymer filaments. In addition, polyester provided an excellent substrate upon which to passively adsorb antibodies. The filament was wound around the ends of a PhastGel sample applicator (Amersham Biosciences) and placed within the concave teeth as shown in Figure 1. Combs

were cleaned with a nearly saturated solution of NaOH in approximately 60% ethanol prior to winding the filament. The comb/filament apparatus was washed in 70% ethanol, rinsed, washed in 10% HCl, and rinsed again. The filament was allowed to dry completely, and 0.75 μ L of antibody solution was applied to each tooth of the comb. In some assays, a low concentration of cytochrome C labeled with Cy3 protein label was added to the antibody solution ($\sim$ 2 μ g/ μ L) as an indicator of antibody distribution. The comb and filament were incubated in a humidified box for 45–60 min to allow time for adequate adsorption of antibody to the filament. Following incubation, the comb and filament were immediately rinsed in PBS-T to remove unbound antibody from the filament. Filaments were used within 2 h of this final rinse.

Automated Virus Detection In Microcapillary Chambers

A rotary stage and control system from Yaskawa (Waukegan, IL) were used to control the movement of the filament through five processing chambers. The system of micro-reaction chambers was attached to the top of a horizontal stage using LegoTM building blocks. This simple modular design allowed chambers to be easily repositioned or replaced. Three sets of chambers were placed in parallel onto the horizontal stage. After each filament was threaded through the chambers, they were attached to a single rotating spindle on one end and small weights on the other. In this manner, three filaments could be controlled in parallel with each other using a single interface. The rotary stage was controlled through a LabView Virtual Instrument (National Instruments, Austin, TX) that wound and unwound the filament to maneuver the probe segments sequentially through each chamber as depicted in Figure 2. The LabView interface controlled the speed of the filament through the chambers, oscillatory movements within the chambers, and total residence time within each chamber. Filaments were oscillated 2 cm at a velocity of 0.5 cm/s for the requisite time within each chamber before moving to the next. Overall travel of the filament from the first chamber through the final was approximately 40 cm. Table I summarizes the contents and purpose of each chamber.

Chambers are separated by a distance of 1 cm, but contamination between chambers is a possibility. Cross-contamination between microcapillary chambers was tested by placing labeled anti-M13 in one chamber followed by a chamber containing only PBS-T. Mock processing was performed using typical filament motion parameters, and solutions from each capillary were collected and spotted onto glass slides for fluorescence scanning.

To test for the consistency of virus detection, filaments were prepared with four distinct anti-M13 segments as described above. The specificity of virus detection was tested by adding negative control segments to each filament. Filaments were prepared with an alternating pattern of anti-M13 and anti-E that was not specific for M13 virus, for a total of four segments on each filament. Labeled cytochrome C was not used as a positive control marker in these assays. Reaction conditions for both experiments are summarized in Table II.

To test the diffusion dependence of this system, virus incubation time was varied to examine the dependence of virus detection on antigen diffusion. Filaments were prepared with a negative control anti-E tag segment adjacent to five anti-M13 segments. Cy3-labeled cytochrome C was added to the spotting solution prior to filament preparation for a final concentration of approximately 2 μ g/mL, serving as a positive control, so that spotted regions could easily be determined after scanning. Virus incubation time course experiments were done statically, and filaments were not oscillated within the virus chamber, but oscillation within other chambers was similar to previous experiments. Table II summarizes the other reaction parameters for this assay.

The effect of filament oscillation on virus detection was also tested. A negative control segment was adjacent to four anti-M13 segments on each filament. One set of filaments was oscillated

within the virus chamber similar to previous experiments, while a second set was incubated using static conditions. Additional reaction conditions are summarized in Table II.

The sensitivity of virus detection was tested using larger capillary reaction chambers (1 mm ID, 60 μ L). In these experiments, filaments had a negative control segment adjacent to four anti-M13 segments. Segments were incubated with 1.7×10^6 , 1.7×10^7 , 1.7×10^8 , 1.7×10^9 , or 1.7×10^{10} virus particles in 60 μ L PBS-T for 100 min. The number of virus particles used in these experiments corresponded to the number of particles that was found to be detectable using plate ELISA. The total assay time of 175 min was 45 min less than the equivalent ELISA time. Table II summarizes the other reaction conditions.

ELISA

A standard criss-cross serial dilution analysis was performed to determine the optimal concentrations of immobilized capture antibody and detecting antibody (anti-M13 conjugated to HRP) that yielded the highest sensitivity to virus. Fifty microliters of PBS was added to each well of a 96-well ELISA plate. Next, 50 μ L of unlabeled anti-M13 (50 μ g/mL in PBS) was pipetted into column 1 and serially diluted 1:2 through column 11, leaving column 12 with only PBS. The plate was incubated for 1 h at room temperature in a humidified box, rinsed three times with PBS-T, and blocked with 50 μ L PBS-T for 15 min. After blocking, 50 μ L of virus solution (3.3×10^{10} virions/mL) was added to each well, and the plate was incubated for another hour in a humidified box, followed by six rinses with PBS-T. Fifty microliters of PBS-T was added again to each well of the plate. HRP-conjugated anti-M13 was diluted 1:500 in PBS-T, and 50 μ L was added to each well in the first row of the plate. The antibody was then serially diluted 1:2 through row G, leaving row H with PBS-T only. The plate was then incubated for 1 hour in a humidified box and rinsed. To develop the plate, 50 μ L of the HRP substrate (50 mM sodium citrate buffer, pH 4.2, containing 90 mM ABTS and 0.05 mM H_2O_2) was added to each well and left at room temperature. Absorbance readings were taken using a microplate reader (405 nm) at both 20 and 75 min, and the signal to noise ratios were calculated by dividing the raw values with the corresponding negative control values in column 12. Optimal concentrations for capture antibody and HRP-conjugated detecting antibody were found to be 1.56 μ g/mL and 1:2,000 dilution, respectively.

ELISA experiments using the optimized values for primary and detecting antibody were performed to determine the sensitivity of this assay to M13 virus. The ELISA plates were processed as described above, using different virus concentrations. Additional wells containing virus of each concentration were added in duplicate into the wells of column 7 for use as negative controls. Signal to noise ratios were determined as described above.

Reagent Testing

Preliminary experiments and optimization of immobilized antibody were performed using antibody-filament reactions in 2 mL Eppendorf tubes in place of capillary reaction chambers. After blocking in PBS-T for 10 min, the filaments were transferred to a virus solution (3.3×10^{10} virions/mL) and gently rocked on an orbital shaker for 30 min. After a 5 min wash in PBS-T, the filaments were again removed and placed in a solution of anti-M13 labeled with either Alexa Fluor 647 or Alexa Fluor 555 protein label (~ 10 μ g/mL in PBS-T) and were gently rocked for an additional 30 min. After a final wash of 10 min in PBS-T, the filaments were scanned for fluorescence. Due to the relatively large volume used for Eppendorf processing, the amount of virus used was approximately 50 times greater than automated, capillary chamber experiments.

Detection and Analysis

Following all experiments, filaments were removed after the final wash, taped to glass microscope slides, and scanned in a GenePix 4000B microarray scanner (Axon Instruments, Union City, CA). Due to the three dimensionality of the filament, a series of scans on a single filament was obtained at different focal depths to determine the scan depth for maximum fluorescence. A 75 μm depth of focus yielded the strongest signal, so all subsequent filaments were scanned at this depth. Filaments were scanned using both the 532 and 635 nm wavelengths of the scanner, and segment fluorescence and background fluorescence were quantified (Image Pro 4.0, Media Cybernetics, San Diego, CA) by taking the average pixel fluorescence over the segment area. Fluorescence images from the microarray scanner were artificially rendered green or red by the scanning software to better distinguish the 532 and 635 nm wavelengths of the device. To determine the signal to noise ratio of each experiment, fluorescence of anti-M13 segments was divided by the background fluorescence or negative control fluorescence if a negative control was present. Analysis of variance of fluorescence signals and background signals was used to determine statistical significance ($P < 0.05$).

RESULTS

Figure 3 shows a typical fluorescence pattern after detection of M13 virus during reagent testing experiments. This experiment shows little or no detectable cross reactivity between M13 virus and a non-specific anti-E antibody. Immobilized segments one and two (left side of Fig. 3) are each labeled with a different fluorescent tag and serve as positive controls for each wavelength. These segments should fluoresce at their corresponding wavelengths under all conditions. Segment three is an anti-E tag monoclonal antibody that is not specific for M13 virus and serves as a negative control. This region should not bind virus or detecting antibody and, therefore, should not fluoresce under any conditions. Segments four, five, and six contain unlabeled M13 Ab and should fluoresce only if virus is present.

Holding all other parameters constant, filament oscillation within the virus chamber was also tested to determine whether it increases segment fluorescence over a static virus incubation. Using a static incubation, the average signal to noise ratio for each of three filaments (each with four segments) is 1.4, 1.2, and 2.0 (avg = 1.5). Filament oscillation increased the average signal to noise ratio nearly five times to 5.6, 6.6, and 8.8 (avg = 7.0). Signal to noise was defined as the average fluorescent signal of anti-M13 regions divided by average fluorescence of negative control regions. When a negative control was not present, signal to background was used to judge the success of the experiment. In these circumstances, average fluorescent signal of anti-M13 regions was divided by average fluorescence of empty regions on the filament that were processed in the same manner as the anti-M13 regions. These experiments suggest that filament oscillation leads to an increase in virus interaction with the filament-bound antibody.

After appropriate reaction conditions were identified, the effectiveness of automated filament movement through microcapillary chambers was evaluated. To determine detection repeatability, four filaments, each with four immobilized anti-M13 segments, were prepared, threaded through the system of capillary chambers, and filament motion was programmed into the LabView virtual instrument. The top panel in Figure 4 shows both the fluorescence pattern obtained from the filament scan of one filament and the average segment fluorescence of each segment. Comparison of four filaments, each with four segments, yielded an overall average for all sixteen segments of $10,542 \pm 1,374$ (mean $\pm$ SD) and an overall coefficient of variation (CV) of 0.13. Intra-assay CV for individual filaments varied from 0.05 to 0.4.

Detection specificity was also evaluated. For these experiments, three filaments were prepared with patterns of four alternating segments of unlabeled anti-M13 and unlabeled anti-E as a negative control. The bottom panel in Figure 4 shows the fluorescence pattern and the average

segment intensities from one of these experiments. In this experiment, signal from anti-M13 segments is approximately 40 times greater than the negative control anti-E segments. The overall average results from all three filaments in this set were ~30 times greater for anti-M13 segments ($16,264 \pm 5,396$) than negative control segments (573 ± 278). Overall CV for anti-M13 and negative control signals was 0.33 and 0.48, respectively. Intra-assay CV calculations were not possible since only two segments of each type were present on each filament. A direct fluorescence comparison between reproducibility and specificity graphs is not possible since all parameters were not the same for each test. These experiments provide strong evidence that this system consistently and specifically detects virus in solution with a high signal to noise ratio compared to a non-specific antibody.

Since immobilized antibody concentration significantly effects sensitivity of standard ELISA, an experiment was performed to determine the optimal spotting concentration of anti-M13 for the filament based system. Anti-M13 of varying concentrations was spotted and immobilized on a filament and then processed through capillary reaction chambers to determine which concentration gave the strongest signal. A negative control anti-E segment was adjacent to increasing concentrations of anti-M13 spotting solution, and the filament was processed through capillary reaction chambers using parameters similar to previous virus detection experiments. As Figure 5 illustrates, the data shows an increase in signal intensity up to 600 $\mu\text{g/mL}$, after which the signal begins to drop, a trend that is consistent with the literature for antibodies immobilized on other polymers (Schramm and Paek, 1992; Schramm et al., 1993).

Antigen-antibody sensitivity was determined using a traditional ELISA. Three ELISAs were run to determine the lower limit of detection of M13 virus with anti-M13 antibody. The number of virus particles ranged from 1.7×10^4 to 1.7×10^9 , and each assay included eight trials for each condition. The lowest number of virus particles that yielded a statistically significant signal was 1.7×10^7 ($P < 0.05$) (Fig. 6).

Experiments were performed to determine the lower limit of sensitivity of this filament-antibody recognition assay (FARA) approach. Five filaments, each containing four distinct M13 Ab segments and one negative control anti-E segment, were incubated with five different virus copy numbers comparable to virus numbers detected with standard ELISA. Incubation times for virus and detecting antibody were 100 and 50 min, respectively, so that total assay time for automated filament based detection (~175 min) was 20% less than the ELISA (~220 min). These approximate assay times include all washing steps and ELISA developing time but do not include filament or ELISA preparation time. As shown in Figure 6, the filament based assay detected similar amounts of virus with similar signal to noise ratios as standard ELISA.

Diffusion of the virus to the filament surface may be a major factor that limits detection sensitivity with the FARA approach. Figure 7 shows results from a time course of virus incubation time. Fluorescence intensity increases with virus incubation time, which is consistent with the hypothesis that diffusion/delivery of virus is an important factor. However, our data suggests that other factors contribute as well, since signal intensity is not proportional to the square root of the incubation time. Nevertheless, virus incubation time appears to be a critical parameter.

DISCUSSION

These data demonstrate that anti-M13 antibody can be passively adsorbed to a polyester filament, and the antibody remains functional. Direct binding is shown by the hydrophobic attachment of fluorescently-labeled control antibody (Fig. 3). Indirect evidence suggests that binding sites on filament-immobilized antibodies remain active and capture virus out of

solution (Figs. 3 and 7). The addition of Tween-20 to the wash solution after initial capture antibody attachment appears to effectively block further non-specific filament-antibody interaction. This is supported by the observation that the fluorescently-labeled second antibody does not directly interact with the filament, as shown by the non-fluorescent regions between immobilized segments.

The lower limit of sensitivity of this assay was similar to a traditional plate ELISA. The limit of sensitivity of the FARA approach is 1.7×10^7 particles (~400 pg), which is similar to the limit of traditional ELISA (Fig. 6). This level of sensitivity was achieved in a shorter assay without the required amplification step for ELISA and with automation of the reaction process. The detection area for one spot using the FARA approach is approximately $7.5 \times 10^5 \mu\text{m}^2$ ($d = 120 \mu\text{m}$, $L = 2,000 \mu\text{m}$). FARA detects 1.7×10^7 particles, which means that at the lower limit of sensitivity, FARA detects 22.5 virus particles/ μm^2 . A similar calculation shows that a single well in an ELISA with a much larger detection area of approximately $3.8 \times 10^6 \mu\text{m}^2$ ($d = 0.7 \text{ cm}$) has a detection sensitivity of only 0.44 virus particles/ μm^2 . Using this measure, FARA is approximately 50 times more sensitive per unit area than ELISA.

Exact calculation of mass transfer coefficients for each system can not be made, but the ratio of the coefficients can be estimated by making some assumptions. The total amount of virus transferred per unit time can be calculated by multiplying the mass transfer coefficient (k), the detector surface area (A), and the virus concentration (C). The total amount of virus transferred can be calculated by multiplying the product kAC by the total virus incubation time (T). From Figure 6, both assays produced similar signal to noise ratios using 10^7 – 10^8 virus particles. If the assumption is made that equal numbers of virus particles are transferred to the surface within this range of virus particles, the ratio of the mass transfer coefficients is estimated as: $k_F/k_E = c_E A_E T_E / c_F A_F T_F$, where the subscripts E and F denote ELISA and FARA. Since concentration, area, and assay times are known values, this ratio is approximately 3. Several assumptions were made in this calculation that need to be further verified.

Although FARA sensitivity is similar to ELISA, adding an amplification step may increase sensitivity even further. Precipitating substrates used in ELISA may be an attractive amplification method in this system, as well, but it is not clear whether the precipitant could be confined to each spotted region as filament processing continued. If precipitants became dissociated from specific regions, detection of individual regions would not be possible.

The microcapillary geometry of the FARA approach may account for the high sensitivity observed. The linear geometry of the microcapillary chambers combined with filament motion reduces the diffusion distance required for target molecules to encounter immobilized probes. In this system, probes can be slowly oscillated within a reaction chamber, allowing the immobilized probes to query the entire axial length of target solution. As a result, the limiting diffusion distance is the radial distance from the filament to the edge of the chamber. The current size of the capillary chambers is approximately 700 or 1,000 μm ID. Since the filament diameter is 120 μm , the radial diffusion distance is either 290 or 440 μm , which is an order of magnitude less than the diameter of a 96-well ELISA plate. Based on values reported in the literature for large proteins, the virus diffusion coefficient was estimated to be on the order of $10^{-8} \text{ cm}^2/\text{s}$ (Arrio-Dupont et al., 2000; Eimer and Pecora, 1991). Assuming the simple diffusion relationship of $d = 2Dt$, diffusion of 290 μm would still take nearly 700 min. Much smaller capillaries are available that could reduce this distance to well under 100 μm , a distance over which diffusion could occur much more quickly. Although this distance is still too great for target molecules to completely diffuse under reasonable incubation times, it is much smaller than the distance required in a plate based assay.

A possible complication arising from the filament motion and open geometry of the capillary reaction chambers is fluid contamination between chambers. However, cross-contamination experiments showed that contamination is minimal and is less than 4% between chambers. The addition of extra wash chambers is an easy solution, which could be used to further reduce carry over between adjacent chambers.

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Figure 1. Filament was wrapped around the ends of a gel loading comb and then placed across the concave teeth for antibody spotting.

Figure 2.

Capillary reaction chambers were arranged sequentially on a horizontal stage. A filament with antibody segments immobilized at known locations was threaded through the chambers. One end was attached to the shaft of a precision rotary stage, and a small fishing weight was attached to the other. The rotary stage controlled the movement of the antibody segments through each reaction chamber by winding and unwinding the filament. [Color figure can be seen in the online version of this article, available at www.interscience.wiley.com.]

Figure 3.

Immobilized anti-M13 detects the presence of M13 virus in solution. Segments 1 and 2 are fluorescently labeled positive controls and segment 3 shows the level of cross-reactivity with a non-specific negative control antibody. Segments 4–6 show virus detection in solution.

Figure 4.

Typical fluorescence patterns and average fluorescence of immobilized probe segments, showing repeatability (**top**) and specificity (**bottom**). Fluorescence varies by approximately 5% between segments (top), and fluorescence from anti-M13 segments is approximately 40 times greater than negative control anti-E segments (bottom). [Color figure can be seen in the online version of this article, available at www.interscience.wiley.com.]

Figure 5. Fluorescence intensity increases with spotting solution concentration up to about 600 $\mu\text{g}/\mu\text{L}$. At concentrations above this point, intensity begins to decrease.

Figure 6.

Detection and calibration curve for both FARA (closed circles) and ELISA (open circles). Both methods have a lower limit of sensitivity of $\sim 1.7 \times 10^7$ virus particles. Data points represent SNR obtained for different virus copy numbers (mean $\pm$ SD, $n = 3$ experiments for ELISA and 4 spotted regions for FARA); * indicates significantly increased signal over negative controls.

Figure 7. Signal strength increases with virus incubation time (mean $\pm$ SD, $n = 3$).

Table I

Contents and purpose of each reaction chamber.

	Solution	Purpose
Chamber 1	PBS-T	Rehydrate probes, block non-specific binding
Chamber 2	M13 virus	Expose immobilized antibody to antigen
Chamber 3	PBS-T	Wash away unbound antigen
Chamber 4	Labeled anti-m13 antibody	Expose bound virus to labeled antibody
Chamber 5	PBS-T	Wash away unbound labeled antibody

Table II

Summary of reaction parameters used in each experiment.

	Experiment			
	Consistency of virus detection	Specificity of virus detection	Diffusion dependence	Effects of filament oscillation
Chamber 1	10–15 min	10–15 min	10–15 min	10–15 min
Chamber 2	35 min	45 min	25–990 min	100 min
Chamber 3	5 min	5 min	5 min	5 min
Chamber 4	35 min	45 min	45 min	50 min
Chamber 5	10 min	10 min	10 min	10 min
Oscillation	2 cm, 0.5 cm/s	2 cm, 0.5 cm/s	None in virus chamber	2 cm, 0.5 cm/s
Chamber volume	25 μ L	25 μ L	25 μ L	60 μ L
Virus particles	8.3×10^8	8.3×10^8	8.3×10^8	1.7×10^7 – 1.7×10^9
Labeled antibody	40–50 μ g/mL AF555 anti-m13	40–50 μ g/mL AF555 anti-m13	40–50 μ g/mL AF647 anti-m13	40–50 μ g/mL AF647 anti-m13