Supplement

Rapid and specific detection of intact viral particles using functionalized microslit silicon membranes as a fouling-based sensor

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Calculations

Calculation S.1: Pore Occlusion and Membrane Resistance

In order to determine how the resistance of a porous membrane changes while being occluded, the analysis below was completed on a microfluidic device that is similar to the µSiM-DX which used a one window, 0.5 µm slit pore membrane. In each analysis, shown in Figure S.1, all dimensions (height, width, length) but one are held constant to observe how the unconstrained dimension individually affects the total membrane resistance. In doing so, trends are observed on how the total membrane resistance is affected by each dimension. The length dimension is not analysed since it is the least likely to clog in actual capture. Instead, total pore occlusion via the number of available pores is analysed. The resistance through each slit is modelled using the Hagen-Poiseuille equation for a rectangular shaped channel. Resistance values are determined via the following calculations with their results shown in Figure S.1. The height and width analyses assume uniform pore occlusion across every slit in the porous window, while the pore occlusion analysis assumes 100% occlusion of pores. While no analysis is perfect, together they suggest that total membrane occlusion is not necessary in order to trigger a resistance switch.

Dimensions:
Top channel dimensions: L = 15000 µm, w = 1000 µm, and h = 100 µm
Dimensions of a single slit in the membrane: L = 0.4 µm, w = 50 µm, and h = 0.5 µm
The viscosity of water at 25 °C (µ) = 8.9x10-4 Pa•s

Number of pores in a window
Number of pores in 1 window = (Percent Porosity • Membrane Area) / Area of a single pore
= 10% • (2100000 μm^2) / 25 μm^2
= 8400 pores

Channel to the indicator port resistance (remains constant for every analysis)
Indicator port channel resistance = (12 • µ • L) / w • h^3
= 12 • 8.9E-4 Pa•s • 15000 µm / 1000 µm • (100 µm)^3
= 1.602E-7 Pa•s/μm^3

Membrane resistance (representing the minimum membrane resistance)
Resistance of a pore: R_p = (12 µL / (1-(0.63(h / w))) w • h^3
= (12 • 8.9E-4 Pa•s • 0.4 µm) / (1-(0.63(0.5 µm / 50 µm))) • (0.5 μm^3)
= 6.9E-4 Pa•s/μm^3
Membrane resistance: $R_m = R_p$ / (total number of pores)
= (6.9E-4 Pa•s/μm^3) / 8400 pores
= 8.189E-8 Pa•s/μm^3

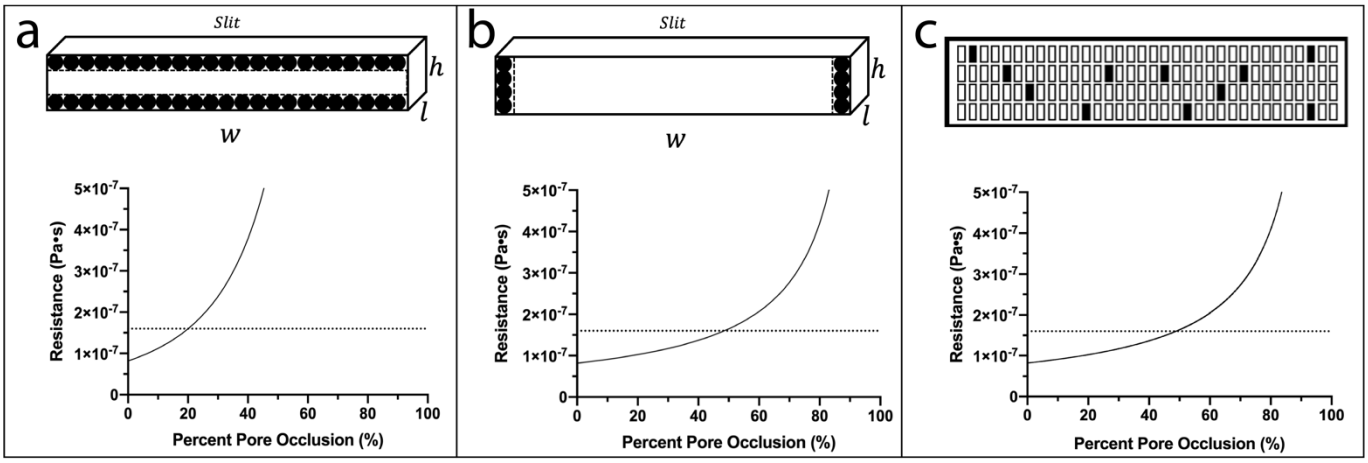


Figure S.1: Pore occlusion in a microfluidic device using a silicon nanomembrane. Three different analyses are done by decreasing the size/number of each individual pore with respect to height (a), width (b), and open pore number (c). Each is shown with an illustration of their pore occlusion and the corresponding analysis. The dashed line on each analysis represents the resistance of the channel to the indicator port.

Height Analysis

Decreased the height dimension when calculating the resistance of a pore using 0.01 μm steps. Calculated and plotted the membrane resistance as a function of percent pore occlusion. The membrane resistance crosses and becomes greater than the resistance of the indicator port channel at ~20% pore occlusion of all of the pores within the porous window.

Width Analysis

Decreased the width dimension when calculating the resistance of a pore with 1 μm steps. Calculated and plotted the membrane resistance as a function of percent pore occlusion. The membrane resistance crosses and becomes greater than the resistance of the indicator port channel at ~50% pore occlusion of all of the pores within the porous window.

Open Pore Number Analysis

Decreased the number of pores within the membrane when calculating the resistance of the membrane with 1 pore steps. Calculated and plotted the membrane resistance as a function of percent pore occlusion. The membrane resistance crosses and becomes greater than the resistance of the indicator port channel at ~50% pore occlusion of all of the pores within the porous window.

Calculation S.2: Biotin-avidin affinity-based capture calculations

The following calculations were done to determine the amount of time an avidin bound molecule would have to stay within the pore of a silicon nanomembrane in order to have the chance to interact and bind to a biotin coating on the wall of the pore.

Fluid flow rate

Injection time = 8 sec

Sample volume = 40 μL ≡ 4E10 μm^3

Fluid flow rate = 4E10 μm^3/ 8 sec

= 5E9 μm^3/sec

Fluid flow rate within a pore

Pore area = 1 μm • 50 μm

= 50 μm^2

Number of pores in 1 window = (Percent Porosity • Membrane Area) / Area of a single pore

= 11% • (2100000 μm^2) / 50 μm^2

= 4620 pores

Number of pores in 2 windows = 4620 pores • 2

= 9240 pores

Total pore area across 2 windows = 9240 pores • 50 μm^2

= 462000 μm^2

Fluid flow rate within a pore = (5E9 μm^3/sec) / 462000 μm^2

= 10822.5 μm/sec ≡ 10.8 mm/sec

Residency time within a pore
Membrane thickness = 0.4 μm
Residence time within a pore = 0.4 μm / (10822.5 μm/sec)
= 3.7E-5 sec ≡ 37 μsec

Streptavidin conjugated antibody concentration
Streptavidin conjugated antibody concentration = 0.185 μL * 1.06E-4 μg/μL
= 1.961E-5 μg ≡ 1.961E-11 g
= 1.961E-11 g / 150000 g/mol
= 1.307E-16 mol
Sample injection volume = 0.04 L
Streptavidin conjugated antibody concentration = 1.307E-16 mol / 0.04 L
= 3.268E-15 M

Kode™ molecule concentration across 2 windows
Solid surface area of a single pore = 2(1 μm • 0.4 μm) + 2(50 μm • 0.4 μm)
= 40.8 μm^2
Total pore solid surface area across 2 windows = 40.8 μm^2 • 9240 pores
= 376992 μm^2 ≡ 3.76992E11 nm^2
Kode™ Biotin Spatial area (assumption) = 1 nm^2
Number of Kode™ molecules within the pores of 2 windows = 3.76992E11 Kode™ molecules
Molecular mass of Kode™ Biotin = 2057.34 g/mol ≡ 3.4175E-21 g/Kode™ molecule
Mass of Kode™ molecules within the pores of 2 windows = 3.76992E11 Kode™ molecules • 3.4175E-21 g/Kode™ molecule
= 1.288E-9 g
Moles of Kode™ molecules within the pores of 2 windows = 1.288E-9 g / 2057.34 g/mol
= 6.26E-13 mol
Kode molecule size[1, 2] = ~7 nm = 0.007 μm
Volume within the vicinity of Kode™ molecules in a single pore = 2(1 μm • 0.4 μm • 0.007 μm) + 2(50 μm • 0.4 μm • 0.007 μm)
= 0.2856 μm^3
Total volume within the vicinity of Kode™ molecules across 2 windows = 0.2856 μm^3 • 9240 pores
= 2638.944 μm^3 ≡ 2.639E-12 L
Kode™ molecule concentration across 2 windows = 6.26E-13 mol / 2.639E-12 L
= 0.237 M

Concentration of biotin-avidin complexes
Streptavidin-Biotin affinity constant[3] (K_a) = 2.5E13 M^{-1}
K_a = [AB] / [A] • [B] ≡ [streptavidin-biotin complexes] / [streptavidin conjugated antibody] • [Kode™ biotin molecules]
2.5E13 M^{-1} = [AB] / 3.268E-15 M • 0.237 M
2.5E13 M^{-1} = [AB] / 7.755E-16 M^2
[AB] = 0.0194 M

Time required for a streptavidin-biotin complex to form
Streptavidin-Biotin on rate[3] (k_{on}) = 1.3E8 M^{-1} • sec^{-1}
Time required for a streptavidin-biotin complex to form = 1.3E8 M^{-1} • sec^{-1} • 0.0194 M
= 2520354.7 sec^{-1} = 3.968E-7 sec ≡ 0.3968 μsec

Calculation S.3: Antibody-antigen affinity-based capture calculations (assuming similar conditions as above)

These calculations are a follow-up to Calculation S.2 which were done in order to determine the amount of time a protein antigen would have to stay within the pore of a silicon nanomembrane in order to have the chance to interact and bind to an antibody coating on the wall of the pore.

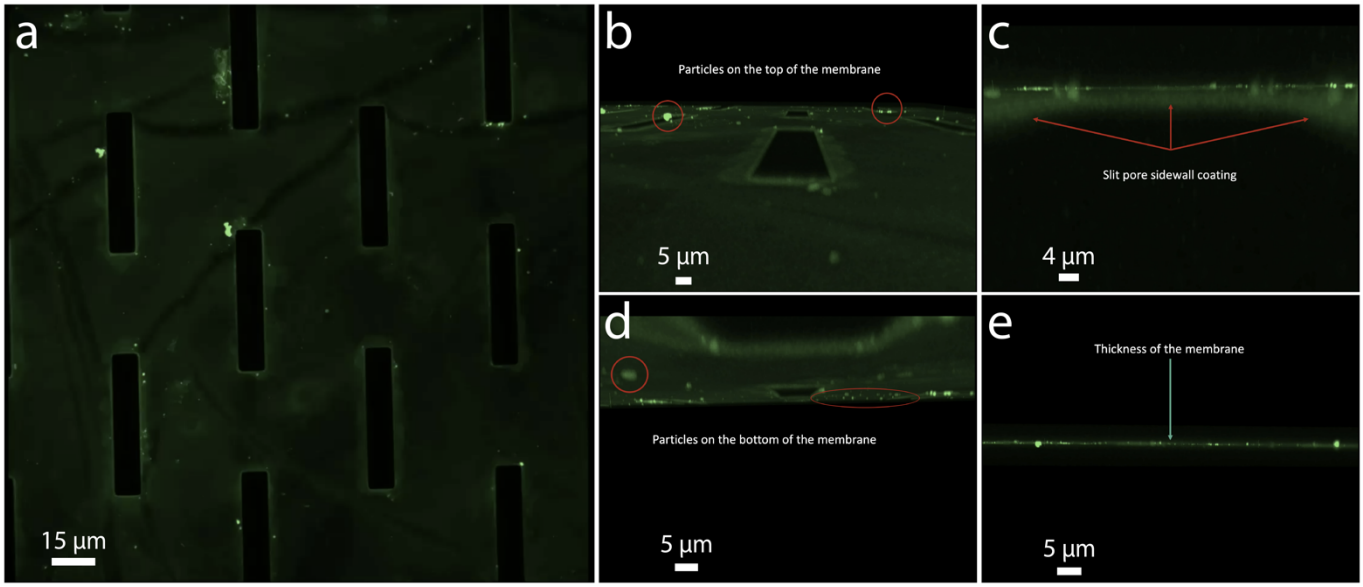
Concentration of antibody-antigen complexes
Median antibody-antigen affinity constant[4] (K_a) = 66E12 M^{-1}
K_a = [AB] / [A] • [B] ≡ [antibody-antigen complexes] / [antigen] • [antibody]
66E12 M^{-1} = [AB] / 3.268E-15 M • 0.237 M
66E12 M^{-1} = [AB] / 7.755E-16 M^2
[AB] = 0.0512 M

Time required for an antibody-antigen complex to form
Average antibody-antigen on rate[4] (k_{on}) = 1E5M^{-1} • sec^{-1}
Time required for a protein-antibody complex to form = 1E5 M^{-1} • sec^{-1} • 0.0512 M
= 5118.3 sec^{-1} = 1.954E-4 sec ≡ 195.4 μsec

Data

Video S.1: Kode™ FITC Conformal Coating

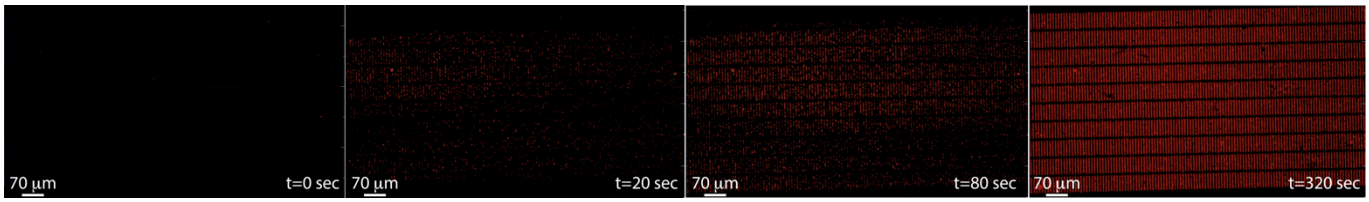
To determine whether or not Kode™ molecules were conformally coating the membranes, we coated an 8 μm slit pore membrane with Kode™ FITC molecules (Kode™ molecules with a terminal FITC group). In combination with a confocal microscope, we were able to gather a z-stack which definitively showed fluorescent coating on the top of the membrane, within the pores of the membrane, and on the bottom of the membrane.



Video S.1: An 8 μm slit pore membrane coated with Kode™ FITC fluorescent molecules, imaged with an Andor Dragonfly Spinning Disc Confocal microscope. A conformal coating of the fluorescent molecules is shown in an orientation perpendicular to the membrane (a). A closer look provides evidence for conformal coating on the top of the membrane (b), the walls of the pores (c), and the bottom of the membrane (d). The thickness of the membrane can also be shown when imaging parallel to the side of the membrane (e).

Video S.2: Bead Capture Video

Visualization of bead capture on silicon nanomembranes was done using 1.2 μm fluorescent (637 nm/700 nm exc/em) polystyrene beads and 1 μm slit pore membranes. A tangential flow through device[5] was setup and beads were injected using a syringe pump at 25 μL/min. Capture was completed over 5 minutes. Despite having a different setup, capture in this setup should be analogous to capture within a μSiM-DX.



Video S.2: 1.24 μm bead capture on a 1 μm slit pore membrane is imaged on a confocal microscope. Fluorescence intensity increases over time as more beads are captured on the membrane.

Figure S.2: Single Virus Capture Analysis

Figure S.2 shows a number of viruses captured on a microslit membrane. It is recorded under a confocal microscope using a camera (2048 x 2048 pixel with a pixel pitch of 6.5 μm) and a 60X/1.2NA microscope objective. This configuration makes each pixel size approximately 108 nm since it is equal to the camera pixel pitch per the magnifying power of the objective. The dimension of a single virus is roughly 300 nm, and each virus emits at a wavelength of 633 nm. According to Abbe's diffraction limit, the spatial resolution of the optical system is almost 186 nm which is less than the virus size. In other words, each virus occupies an area of approximately 3 x 3 pixels on the image plane. These parameters can be used to detect single viruses on the membrane. To detect single virus, the recorded image is normalized and a threshold operation is applied. The threshold value can be empirically estimated by sweeping it between zero and one and then sequentially monitoring single viruses on the image. It is observed that the threshold value of 0.7 provides better results and detects single viruses near the clustered ones. Once the threshold operation is performed, the particles with an area of less than 3 x 3 pixels and higher than 4 x 4 pixels should be eliminated since a single virus roughly covers an area between these values. Figure S.2 marks the single viruses in blue boxes. A total number of 544 single viruses on the membrane are detected.

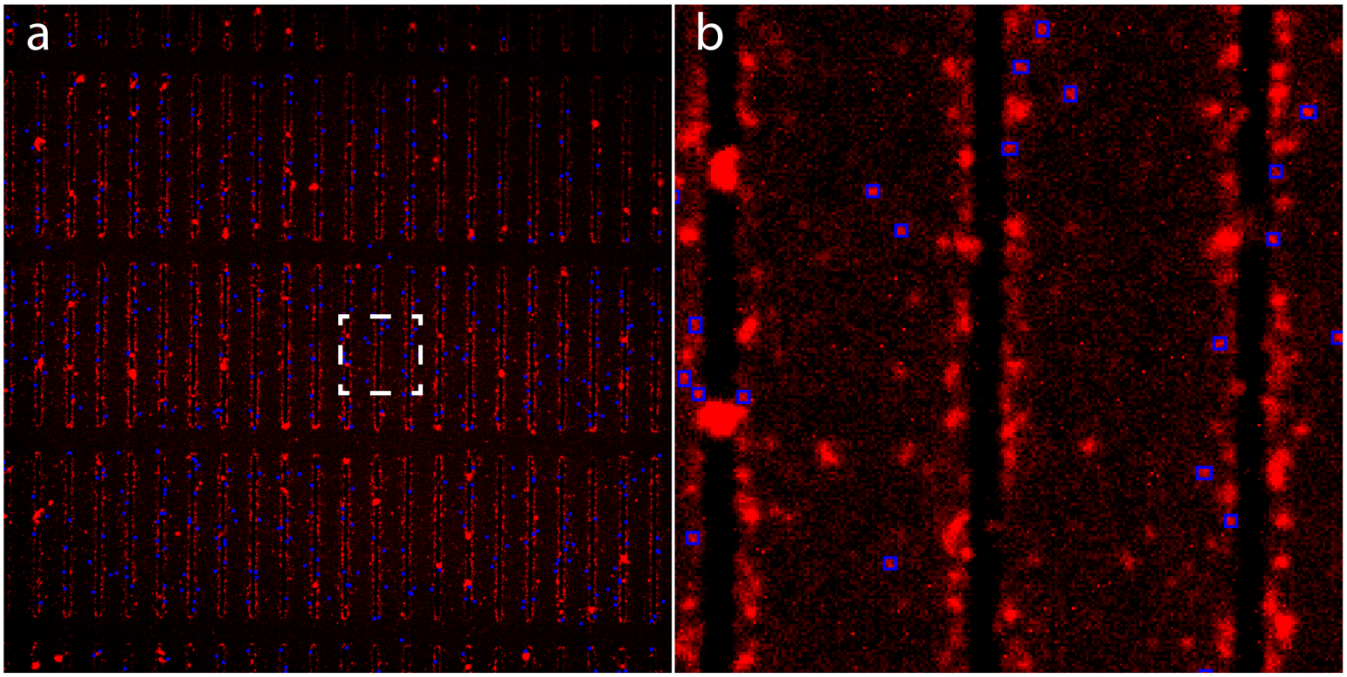


Figure S.2: The vaccinia virus that was captured from an injection of 40 μL of a 3E9 virus/mL solution in a 1 μm slit pore μSiM-DX is analysed for the presence of single virus. Marked in blue boxes, there are 544 single viruses found in the entire image (a). The white box represents the zoomed in area shown in panel b (b).

Figure S.3: μSiM-DX Vaccinia Virus Dynamic Range

The dynamic range of 2 window and 3 window 1 μm slit pore μSiM-DX according to diagnostic sensitivity and specificity is represented on a vertical bar plot in Figure S.3. As in Figure 4, this shows that the 2 window μSiM-DX has a lower dynamic range than the 3 window μSiM-DX. Despite this, the dynamic range of both versions of the μSiM-DX are complimentary to each other.

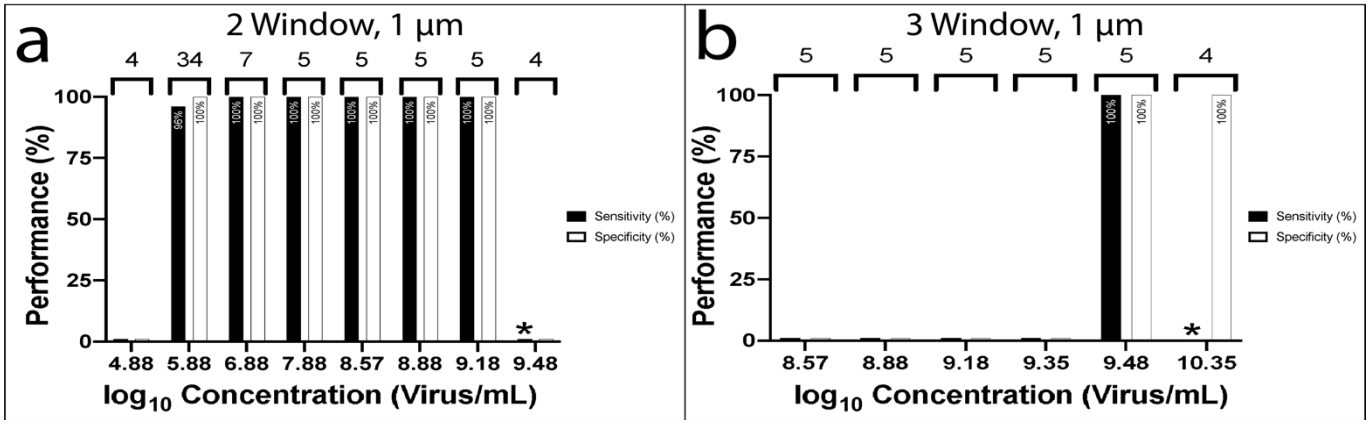


Figure S.3 The dynamic range of 2 window and 3 window 1 µm slit pore µSiM-DX are represented using diagnostic sensitivity and specificity (a, b). N values are shown above each data set. Asterisks denote undefined values due to a lack of true positives and false negatives.

Figure S.4: DLS Size Distribution by Number for Intact and Sonicated Vaccinia Virus

Figure S.4 shows the size distribution by number for intact and sonicated vaccinia virus after analysis via DLS, plotted on a semi-logarithmic scale. Three technical replicates are analysed and shown for both sample types.

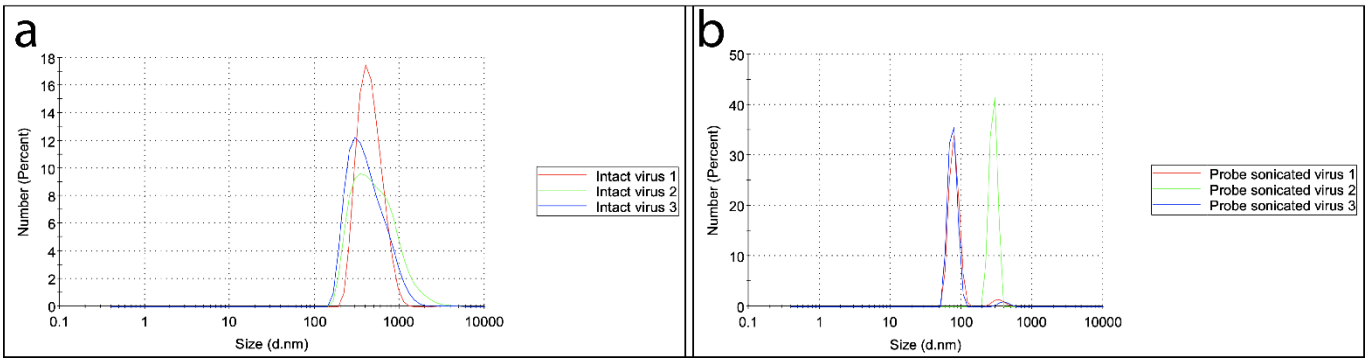


Figure S.4: Vaccinia virus is analysed using DLS and the resulting size distribution by number is shown for intact virus (a) and sonicated virus (b). Technical replicates are measured for each sample type. Size is displayed on a logarithmic scale along the x-axis according to the diameter of the measured particles.

µSiM-DX Version	Target	PBS (Switch/Total Tests)	Target capture (Switch/Total Tests)	False Positive (FP)	False Negative (FN)	True Positive (TP)	True Negative (TN)	N value	Sensitivity (TP/(TP+FN)	Sensitivity (%)	Specificity (TN/TN+FP)	Specificity (%)
0.5 µm	Steric Beads	2/3	8/10	2	2	8	1	13	0.80	80%	0.33	33%
	Affinity Beads		5/8	2	3	5	1	11	0.63	63%	0.33	33%
0.5 µm with a tube	Steric Beads	4/13	13/13	4	0	13	9	26	1.00	100%	0.69	69%
	Affinity Beads		8/10	4	2	8	9	23	0.80	80%	0.69	69%
3 slot, 1 µm	Steric Beads	0/7	5/7	0	2	5	7	14	0.71	71%	1.00	100%
	Affinity Beads		2/3	0	1	2	7	10	0.67	67%	1.00	100%
2 slot, 1 µm	Steric Beads	0/3	7/7	0	0	7	3	10	1.00	100%	1.00	100%
	Affinity Beads		7/7	0	0	7	3	10	1.00	100%	1.00	100%

Table S.1: Figure 2 Raw Data

log Concentration (virus/mL)	PBS - (Switch/Total Tests)	Vaccinia - (Switch/Total Tests)	Vaccinia + Vaccinia Ab - (Switch/Total Tests)	Vaccinia + Rabbit Ab - (Switch/Total Tests)	False Positive (FP)	False Negative (FN)	True Positive (TP)	True Negative (TN)	N value	Sensitivity (TP/(TP+FN)	Sensitivity (%)	Specificity (TN/TN+FP)	Specificity (%)
3 slot, 1 μm													
8.57	0/3	0/1	0/1	NA	0	1	0	4	5	0.00	0%	1.00	100%
8.88		0/1	0/1	NA	0	1	0	4	5	0.00	0%	1.00	100%
9.18		0/1	0/1	NA	0	1	0	4	5	0.00	0%	1.00	100%
9.35		0/1	0/1	NA	0	1	0	4	5	0.00	0%	1.00	100%
9.48		0/1	1/1	NA	0	0	1	4	5	1.00	100%	1.00	100%
10.35		0/1	NA	NA	0	0	0	4	4	Undefined	Undefined	1.00	100%
2 slot, 1 μm													
4.88	0/3	NA	0/1	NA	0	1	0	3	4	0.00	0%	1.00	100%
5.88		0/3	24/25	0/3	0	1	24	9	34	0.96	96%	1.00	100%
6.88		0/1	3/3	NA	0	0	3	4	7	1.00	100%	1.00	100%
7.88		0/1	1/1	NA	0	0	1	4	5	1.00	100%	1.00	100%
8.57		0/1	1/1	NA	0	0	1	4	5	1.00	100%	1.00	100%
8.88		0/1	1/1	NA	0	0	1	4	5	1.00	100%	1.00	100%
9.18		0/1	1/1	NA	0	0	1	4	5	1.00	100%	1.00	100%
9.48		1/1	NA	NA	1	0	0	3	4	Undefined	Undefined	0.75	75%

Table S.2: Figure 4 Raw Data

PBS - (Switch/Total Tests)	Vaccinia + Vaccinia Ab - (Switch/Total Tests)	Sonicated Vaccinia + Vaccinia Ab - (Switch/Total Tests)	False Positive (FP)	False Negative (FN)	True Positive (TP)	True Negative (TN)	N value	Sensitivity (TP/(TP+FN)	Sensitivity (%)	Specificity (TN/TN+FP)	Specificity (%)
0/3	3/3	0/10	0	0	3	13	16	1.00	100%	1.00	100%

Table S.3: Figure 5 Raw Data

PBS - (Switch/Total Tests)	Vaccinia - (Switch/Total Tests)	Vaccinia + Vaccinia Ab - (Switch/Total Tests)	Vaccinia + Rabbit Ab - (Switch/Total Tests)	Sonicated Vaccinia + Vaccinia Ab - (Switch/Total Tests)	False Positive (FP)	False Negative (FN)	True Positive (TP)	True Negative (TN)	N value	Sensitivity (TP/(TP+FN)	Sensitivity (%)	Specificity (TN/TN+FP)	Specificity (%)
0/6	0/10	32/33	0/3	0/10	0	1	32	29	62	0.97	97%	1.00	100%

Table S.4: Total Vaccinia Test Data*

*Data compiled from tests done within the dynamic range of the devices

References

1. E. Korchagina, A. Tuzikov, A. Formanovsky, I. Popova, S. Henry and N. Bovin, *Carbohydrate research*, 2012, **356**, 238.
2. E. Williams, K. Barr, E. Korchagina, A. Tuzikov, S. Henry and N. Bovin, *International journal of molecular sciences*, 2016, **17**, 118.
3. L. Deng, E. N. Kitova and J. S. Klassen, *Journal of the American Society for Mass Spectrometry*, 2013, **24**, 49-56.
4. J. P. Landry, Y. Ke, G.-L. Yu and X. D. Zhu, *Journal of immunological methods*, 2015, **417**, 86-96.
5. M. Dehghani, K. Lucas, J. Flax, J. McGrath and T. Gaborski, *Advanced materials technologies*, 2019, **4**, 1900539.