Improving Viral Diagnostic Methods: A MATLAB Single Virion Counting System Utilizing Gold Nanoparticle Virus Aggregation and Plasmonic Nanobubble Detection

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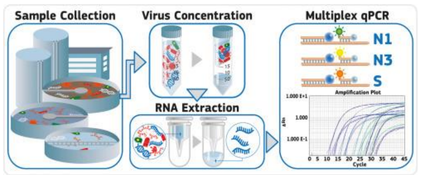
ISSUE

One of the largest issues plaguing the field of medicine is the lack of an accurate and efficient form of viral diagnosis. For example, our two most commonly utilized test diagnosis systems, the PCR and rapid test, sacrifice either accuracy or speed to achieve the other. Another issue currently present is the issue of viral quantification, the counting of virions within a nasal sample. These provide doctors with crucial information in treating infections; however, our current methods are under-developed, unstandardized, too slow, or too inaccurate.

RSV Test Accuracy:
PCR: 94% accuracy, avg 2 hours
Rapid Test: 75% accuracy, avg 15 mins
LAMP Test: 91% accuracy, avg 50 mins



Demonstration of the Cycle Threshold Viral Quantification Mechanism. *Figure created by Bryson Tiller.*

ENGINEERING GOALS

I. Design a virus detection mechanism that rapidly and accurately detects the Human Respiratory Syncytial Virus (RSV).

II. Create a virus quantification system that that determines the viral load using an individual virion counter.

III. Code an computer algorithm that takes in the virion count, age, and other factors as an input and outputs values such as the viral prognosis timeline and the severity of the infection in order to provide healthcare workers with a better interpretation of the virion count.

MATERIALS

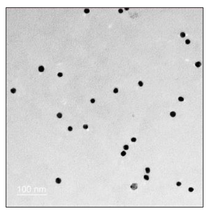
CHEMICAL:
1) Deionized Water (dH_2O), 195 mL, resistivity of 18.0 MΩ·cm
2) Gold nanoparticle seeds. 0.338 mL, 2.23 nM
3) Tetra chloroauric (III) acid trihydrate ($HAuCl_4 \cdot 3H_2O$, 16961-25-4, 99.9%), 1.997 mL, 25 mM
4) Sodium Citrate tribasic dihydrate ($Na_3CA \cdot 2H_2O$, 6132-04-3, ≥99%), 1.997 mL, 112.2 mM
5) 3,3'-Dithiobis (sulfosuccinimidyl propionate) (DTSSP, 21578), 50 mg, 5 mM
6) Borate Buffer, 2 Mm

BIOLOGICAL:
1) Palivizumab/Synagis
2) Purified Human Respiratory Syncytial Virus strain A2

EQUIPMENT:
1) Amicon™ ultra centrifugal filter units
2) Centrifuge machine
3) 250 mL Erlenmeyer flask
4) Magnetic hot plate
5) Red HeNe 633 nm laser
6) Ekspla 28 ps, 532 nm, ultrafast pump laser
7) 200 µm micro-capillary
8) MATLAB software for data collection
9) Synergy 2 BioTek plate reader
10) Malvern ZetaSizer Nano ZS DLS Machine
11) JEOL JEM-2010 Transmission electron microscope
12) Thorlabs photodetector for data collection
13) LeCroy WaveRunner oscilloscope for data collection
14) Syringe pump
15) Tkinter Python Interface Software
16) 10K MWCO Dialysis Cassette
17) 20, 200, 500, and 1000 µL pipette

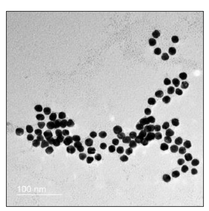
METHODOLOGY

AuNP Synthesis- 15nm AuNPs were synthesized using the Plech Turkevich method. The deionized water, gold nanoparticle seeds, tetra chloroauric acid, and sodium citrate were mixed together in a 250 mL Erlenmeyer flask on a magnetic hot plate. Repeat this twice.



Unconjugated 15nm AuNPs

AuNP Virus Conjugation- One batch of the 15nm AuNPs were incubated in a 2 Mm borate buffer while the DTSSP crosslinkers were incubated with the Synagis antibodies for 15 mins. Eventually the DTSSP-Synagis were combined with the AuNPs in the borate buffer. Finally, sucrose-purified A2 RSV strains were incubated with the AuNPs for 10 mins, and then cleaned using the Amicon centrifugal units, creating RSV-conjugated AuNPs.



RSV-conjugated 15nm AuNPs

Laser Detection Setup- The 28 ps, 532 nm, pulsating laser beam and the red HeNe laser beam were focused together through the center of the cross-section of the 200 µm micro-capillary. A syringe pump pumps the AuNP solution at a rate of 6 µL/minute. The laser beams were focused onto a photodetector and an **oscilloscope.**

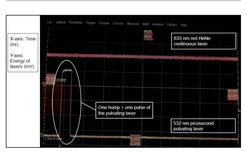
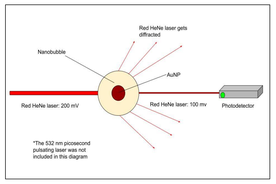


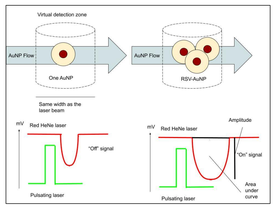
Diagram of the oscilloscope

METHODOLOGY

Virion Counting Mechanism- The picosecond laser will hit the AuNPs causing it to generate small **nanobubbles**. When the red HeNe laser hits the nanobubble, some of its energy will be **diffracted**, causing a decrease of laser energy. This decrease of energy is represented by the oscilloscope as a small dip in the laser energy. Furthermore, the size of the nanobubble also affects the amount of laser energy being diffracted. Unconjugated AuNPs will generate smaller nanobubbles, causing a smaller dip; while RSV-conjugated AuNPs will generate larger nanobubbles, causing it to diffract more laser energy and generate a larger dip.

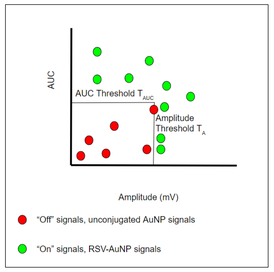


Laser diffraction by Gold nanoparticle



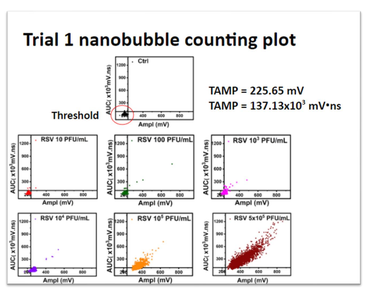
Oscilloscope dip generated by **unconjugated vs conjugated** AuNP

Threshold calculation- One batch of the **unconjugated** AuNPs was pumped through the system. The **amplitude** and **area-under-the-curve (AUC)** was recorded for every nanobubble signal. Since the values for amplitude and AUC are normally distributed, the **threshold** was calculated to be **μ+5σ.** Finally, the batch of RSV-conjugated nanoparticles was pumped through, and the **amplitudes** and **AUC** were recorded for every single value. If either the values for amplitude **OR** AUC were greater than its respective threshold value, then an ON signal is counted, indicating 1 RSV virion. The virion count along with other values were inputted into a coding algorithm.

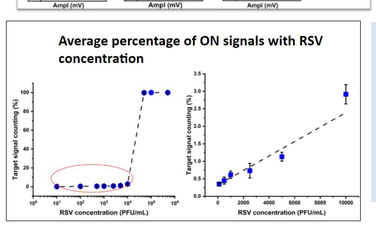


Threshold values

RESULTS

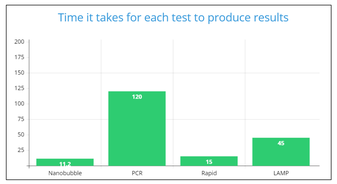


TRIAL 1:
0 PFU mL: **0 virions**
TAMP: 225.65 mV, TAUC: 137.13x10^3 mV·ns
10 PFU mL: **3 virions**
100 PFU mL: **10 virions**
10^3 PFU mL: **156 virions**
10^4 PFU mL: **895 virions**
10^5 PFU mL: **4.321x10^4 virions**
5x10^5 PFU mL: **2.4787x10^5 virions**

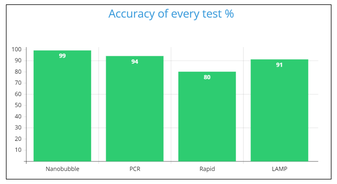


AVERAGE PERCENTAGE OF ON SIGNALS:
10 PFU mL: **0.452%**
100 PFU mL: **0.530%**
10^3 PFU mL: **0.712%**
10^4 PFU mL: **2.792%**
10^5 PFU mL: **97.621%**
5x10^5 PFU mL: **98.172%**

RESULTS



SPEED OF DIAGNOSIS (mins) :
Nanobubble: **11.2**
PCR: **120**
Rapid: **15**
LAMP: **45**



ACCURACY OF RESULTS (%) :
Nanobubble: **99**
PCR: **94**
Rapid: **80**
LAMP: **91**

CONCLUSIONS

I. The results indicate that the **nanobubble** test reported an average speed of diagnosis that is around **4 minutes faster** than our current fastest means of diagnosis and reported an average sensitivity that is **5% higher** than our current most sensitivity means of diagnosis. Thus, concluding that our **engineering goal I** has been successfully accomplished.

II. Using Poisson Statistics to cross-validate the theoretical vs experimental results, we concluded that our virion counting mechanism had an accuracy rate of **97.8%.** This indicates that our **engineering goal II** has successfully been accomplished. However, we found that the **μ+5σ** threshold value created too many type II errors. **In future research,** we will work to minimize both type I and type II errors.

III. Our virion counting algorithm was compared to many clinical studies of individuals with virion counts. Since the outputs of our python coding algorithm were all qualitative data, statical analysis was not viable. We did conclude that our virion coding algorithm produced around an **88%** accuracy result, indicating that our **engineering goal III** was successful accomplished. However, more work to calibrate and incorporating other factors into our system is necessary for **future research**.

IMPACTS

I. Scientists- Scientists would be able to use our technology to allow them to understand the spread of pandemics and viruses, potentially saving millions of lives as they are able to better prevent and diagnosis viruses.

II- Healthcare Workers- Healthcare workers could utilize our virion counting mechanism and our coding algorithm to personalize treatments for individual patients. For example, different amounts of viral load with different severities could be prescribed different kinds of medication and different types of monitoring. Notably, a baby with a high virion count could be prescribed to be monitored more carefully than a healthy adult with a low virion count.

With pandemics jeopardizing the lives of billions across this planet, it's up to hands of scientists to develop technology that promises to save lives.