Disclaimer: Executive Summary with full independent lab results available under NDA.

Determination of the BioCloud Analyzer Efficiency in Aerosolized Pathogen Capture and SARS CoV-2 Detection

Study conducted and data review by: ***The University of Western Ontario***
Date: ***August 19, 2021***
Study Duration: ***8-month test and data review***

ABSTRACT

Viruses that effect humans, animals and plants are often dispersed by aerosols and transmitted through airborne routes of infection. This study will characterize the Kontrol BioCloud air analyzer efficiency of capturing aerosolized pathogens, and detection of specific viral components of SARS CoV-2. The Kontrol BioCloud air analyzer was designed to continually monitor ambient air within the surrounding environment, collecting and trapping various viruses, bacteria, and fungi. Once collected, the trapped pathogens are then analyzed to detect the specific pathogen of choice (SARS CoV-2). Pathogen detection is reliant on optical scintillation using infrared spectroscopy, and fluorescence detection. This study evaluated the efficiency of detection specifically for the SARS CoV-2 virus. The testing and data generated was conducted under contract to the University of Western Ontario.

The efficiency of the Kontrol BioCloud air analyzer viral capture system was assessed by aerosolized MS2 phage, by an independent level 4 lab. The initial test data average was generated by way of aerosolizing the MS2 phage to a calculated average concentration of $4.1x10^6$ and an average calculated capture of $3.85x10^6$ determined by RT PCR test. The initial test data indicated an average capture efficiency of the 93.9 %. More testing will be conducted to further aggregate capture efficiency percentages.

The study data indicated validation of detection by the Kontrol BioCloud air analyzer for SARS CoV-2 virus. The study data spanned an eight-month test routine that included validation of principle of isolation and capture of the SARS CoV-2 virus specifically, the consistent and repeatable reaction of the BioCloud antibody solution to the captured SARS CoV-2 virus, and the validation of optical detection of the SARS CoV-2 virus by the infrared excitation and emission detector. The study included the review of separate independent tests validating the Kontrol BioCloud antibody response to all tested variants of the SARS CoV-2 virus.

Disclaimer: Executive Summary with full independent lab results available under NDA.

SUMMARY

The study objectives of the Kontrol BioCloud pathogen detection system for the detection of SARS CoV-2 virus, the causative agent of the current global COVID pandemic. The following summary of test and data findings were conducted under contract to The University of Western Ontario in level 3 and 2 laboratories. Specific study milestones were achieved, and experiments performed demonstrated the following:

- **Proof of principle experiments showed that recombinant SARS CoV-2 spike protein could be captured from solution *in vitro* and in a BioCloud prototype device**
- **Recombinant human ACE2 could be used to capture recombinant SARS CoV-2 spike protein**
- **Recombinant human ACE2 could be used to capture active SARS CoV-2 virus**
- **Antibody that can be used immunodetect recombinant SARS Cov-2 spike protein and active SARS CoV-2 virus**
- **The utility of the BioCloud in making infrared fluorescence-based measurements was established**
- **Determination of capture efficiency of pathogens in capture medium**

The immediate application of the BioCloud is to monitor environments for the presence of SARS CoV-2. Here we devised and analyzed proof of concept experiments that support the development of the BioCloud for this application. Note, all validation tests preformed through the University of Western Ontario were conducted anywhere between 3-30 times for repeatability and accuracy.

Disclaimer: Executive Summary with full independent lab results available under NDA.

OVERVIEW

The BioCloud air analyzer is designed to survey the ambient air environment for SARS CoV-2, additionally the device can potentially be adapted for the detection of a variety of microorganisms. The BioCloud technology requires that microorganisms in the air be drawn into the device by way of a piston style pump. The microorganisms are then collected and trapped in the liquid collection medium by way of titration reaction, condensing / conglomeration, and impact / collision.

The trapped microorganisms are then transported and isolated and trapped within the devices membrane target specifically tuned to capture SARS CoV-2 virus. Detection of a given microorganism occurs when a solution containing a primary antibody targeted toward the microorganism passes over the target within the BioCloud. Successful detection also requires the use of a secondary antibody that is conjugated to an infrared fluorophore that emits at a specific frequency in the infrared range. After microorganism labeling, the BioCloud detection system will excite the target and if fluorescence signal is measured above background, the system will indicate the presence of the target microorganism. Therefore, the BioCloud is reliant on effective immunodetection of microorganisms and is based on methods routinely employed in molecular biology.

BioCloud Study Test Results

Test #1: Validated Capture of SARS CoV-2 Spike Protein with Human ACE2 Protein

Commercially available recombinant human ACE2 was spotted onto the nitrocellulose membrane. Recombinant Spike-RBD-Dc was also spotted on to the nitrocellulose membrane directly. The dried membrane was blocked with 5% (M/V) BSA in tris-buffered saline solution and then incubated with either 0.5 ∝g or 0.05 ∝g of the soluble Spike-RBD in TBS. The membrane was washed and detected with goat anti-human HRP conjugated secondary antibody chemiluminescent HRP detection substrate.

Test #2: Validation of BioCloud Analyzer Capture of SARS CoV-2 RBD-Spike Protein

As the objective of this endeavor was to develop a viral capture and detection device it was next determined whether membrane-bound recombinant ACE2 protein could “capture” recombinant RBD-Spike protein from solution using a simplified BioCloud viral detection device. The data demonstrates that recombinant human ACE2 captured the RBD-Spike recombinant protein from the solution that was percolating over the ACE2 bearing target in the BioCloud prototype. These observations validate the design concept indicating the BioCloud could be used to capture virus from the environment.

Disclaimer: Executive Summary with full independent lab results available under NDA.

Test #3: Validated Capture of active (live) SARS CoV-2 with Human ACE2 Protein

Given the ACE2 protein could be used to bind recombinant SARS CoV-2 RBD-Spike protein using a BioCloud prototype it was next determined whether intact whole SARS CoV-2 virus could also be "captured" via ACE2 protein. To determine whether this could be established experiments using ***active SARS Cov-2 were performed by qualified personnel in the level III biocontainment facility at the University of Western Ontario***. In addition, a commercially available anti-SARS Cov-2 antibody directed towards the viral spike protein was purchased for the purpose of whole virus detection.

Figure #1: Validated Capture of active (live) SARS CoV-2 with Human ACE2 Protein

Test #4: Validated Viral Capture and Detection of Infrared Labelled Antibody

To simplify detection of virus binding to ACE2 the utility of Infrared Label conjugated secondary antibody was evaluated. The use an infrared dye would eliminate the need for chemical substrates which would simplify the detection of primary antibody binding to captured SARS CoV-2.

To investigate this, active SARS CoV-2 was spotted directly onto a nitrocellulose membrane. Furthermore, to evaluate the lower limit of virus detection samples of virus that had been subject to ten-fold serial dilution were also spotted onto the membrane. The membrane carrying SARS CoV-2 was blocked and detected using the rabbit anti-SARS CoV-2 spike neutralizing antibody followed by a goat-anti rabbit Infrared Label conjugated secondary antibody.

Disclaimer: Executive Summary with full independent lab results available under NDA.

Test #5: Validation of BioCloud Antibody Solution Detection of SARS CoV-2 Virus

Continued testing of the SARS CoV-2 spike neutralizing antibody where performed. It was noted that consideration to the detection and long-term monitoring for variants of the SARS CoV-2 virus would be of great significance to the success of the BioCloud unit.

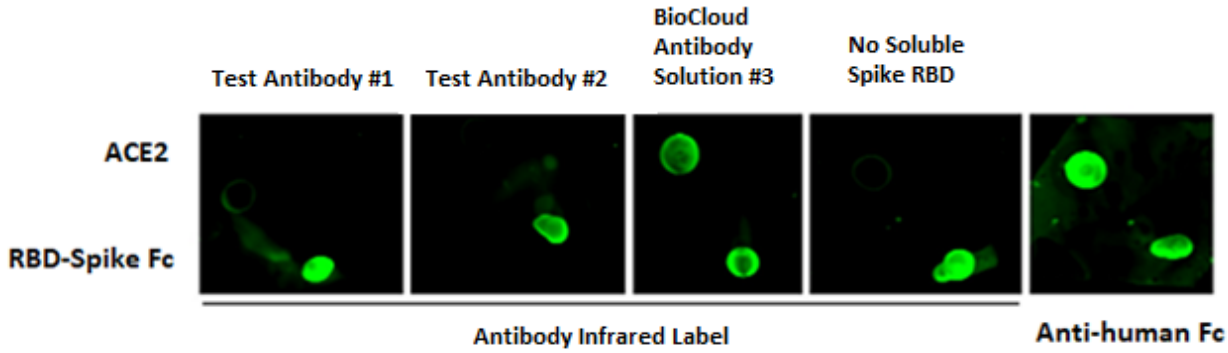

Figure #2: Validation of BioCloud Antibody Solution Detection of SARS CoV-2 Virus

Given the positive result using the recombinant RBD-spike protein we next tested whether the monoclonal antibody BioCloud antibody solution #3 could also detect active SARS CoV-2 virus. Here 2 μg of recombinant human ACE2 was spotted onto nitrocellulose membranes to capture SARS CoV-2 form solution. In addition, rabbit IgG was spotted onto the membrane to serve as a positive control. After blocking ~$5.3x10^6$ virus particles were incubated with the blocked membrane and blotting with the anti-SARS CoV-2 antibody BioCloud antibody solution #3 resulted in positive detection of active virus specifically where ACE2 protein was located **(Figure 3)**.

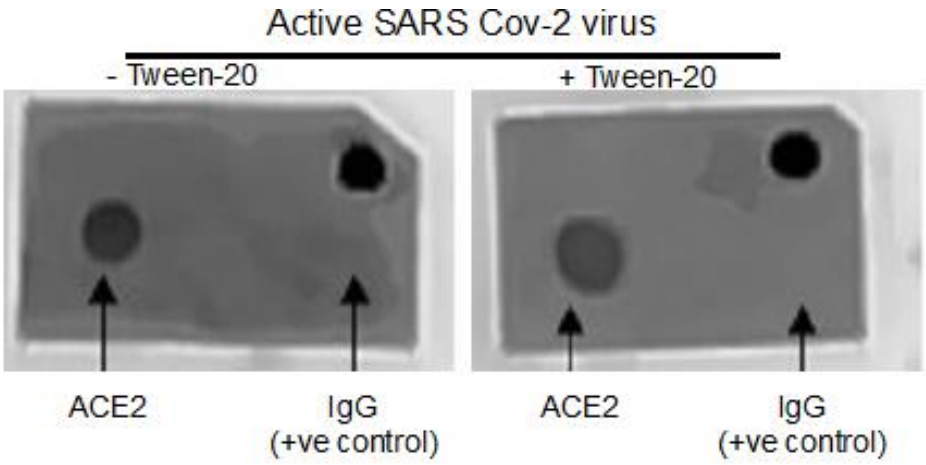

Figure #3: positive detection of active virus specifically where ACE2 protein was located

The IgG control also yielded a strong positive result. The impact of using the detergent 0.1% (v/v) Tween-20 in the presence of virus was evaluated. The results were indistinguishable with respect to virus detection in the presence and absence of tween. Given these data we concluded that the monoclonal antibody BioCloud antibody solution #3 could detect active SARS CoV-2 virus.

Test #6: Validation of BioCloud Antibody Solution Detection of SARS CoV-2 Virus Variants

The study then focused on the ability of the BioCloud antibody solution #3 to respond to know variants of the SARS CoV-2 virus. An independent third-party laboratory conducted tests on BioCloud antibody solution #3 with the procedure and data as follows.

The data was produced by soluble binding using an Octect HTX. The value is based on the interference pattern of white light that is reflected from a layer of biomolecules immobilized on the surface of a sensor tip (bio-layers) in real time and in solution. In this experiment the Bio sensor tip is pre-loaded with the monoclonal antibodies to be tested and then the sensor tip is dipped into a well containing the virus of interest and the binding reaction is measured. The increase interference pattern correlates with antibody binding to virus particles.

	S1-His(D614G)	S1-His (HV69-70del, N501Y, D614G)	UK Variant	South African Variant	RBD-His(N439K)	RBD-His(S477N)
BioCloud antibody solution #3	**1.00**	**1.19**	**1.18**	**1.23**	**0.89**	**1.02**

Figure #4: Validation of BioCloud Antibody Solution Detection of SARS CoV-2 Virus Variants

In this experiment the binding was normalized to the D614G analyte. So yellow indicates a relative slight decrease, while red would indicate a significant decrease in binding interaction, while green indicates the uniform or better. The BioCloud antibody solution #3 performs well against all variants, with slight decrease in N439K. In the experiments conducted on the BioCloud antibody solution #3, there was no show of perturbation of binding as indicated in ***Figure 4.***

Disclaimer: Executive Summary with full independent lab results available under NDA.

Test #7: Validation of BioCloud Analyzer Detection of Inactivated SARS CoV-2 Virus

To characterize the ability of the BioCloud to detect virus on nitrocellulose targets a series of experiments were performed on the BioCloud device. As a proof of principle experiment purified rabbit IgG was spotted onto nitrocellulose targets. To detect the rabbit IgG deposited onto nitrocellulose using the BioCloud a detection solution consisting of secondary antibody with infrared label was allowed to flow over the target according to the pre-programmed BioCloud detection cycling parameters. Rabbit IgG was employed for these tests as a positive control as it would be expected that the secondary antibody would bind to the IgG on the surface of the nitrocellulose membrane. As a control, targets without IgG were used which would be expected to reveal background levels of fluorescence.

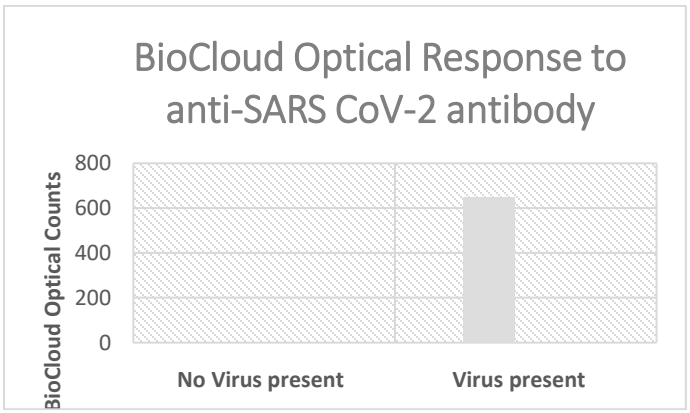

Figure #5: Validation of BioCloud Analyzer detection of Inactivated SARS CoV-2 Virus

This analysis revealed that targets in the BioCloud detection solution that demonstrated increased fluorescence intensity as compared to negative control targets **(Figure 5)**. Image analysis of these targets confirmed the presence of Infrared labelled antigens and revealed that targets appeared to have the optimal signal.

Test #8: Validation of BioCloud Analyzer Microorganism Capture and Efficiency

BioCloud capture efficiency initial test was conducted by an independent level 4 laboratory. The aerosolization was derived from spiking 6 mls of PBS with 10 logs of MS2 phage and aerosolized 4.5 mls using a dry fogger, this was used instead of the nebuliser as the system is used extensively for creating small aerosols that are dry and hang in the air for extensive periods into approximately 623 L^3 (max volume) of air in the class 3 cabinet (does not include the volume of the BioCloud nor the glove volume).

Disclaimer: Executive Summary with full independent lab results available under NDA.

Sampling was preformed for using the normal sampling protocol and bubbled into 30mls PBS with BSA and 0.05% Tween 20. The bubbler unit was removed, liquid aspirated, RNA extracted and Quantified.

The standard was a calculated amount of RNA based on amount of RNA present and the genome length.

For our calculations:

- In 623 L^3 of space it was calculate at $2.11x10^7$ MS2/L^3
- The sampler was run at 2L/min sampling for a total of 120L of air in the cabinet, this calculated to be an average test concentration of $4.1x10^6$ MS2 in 30mls of the bubbler

Average RT PCR Results
Average Bubbler sampler = $3.85x10^6$ within 30mls of the bubbler

Based on initial averaged test data the BioCloud aerosolized microorganism capture technique appears to have a greater than 93.9% recover. Additional testing will be conducted to further aggregate capture efficiency percentages.

SUMMARY OF STUDY FINDINGS

The BioCloud pathogen detection device has demonstrated the ability to detect the SARS CoV-2 virus through the infrared labelled BioCloud antibody solution #3 that consistently reacts with the SARS CoV-2 virus. In the development of this device a reliable antibody solution (BioCloud Antibody Solution #3) that targets the SARS CoV-2 spike protein was identified. The BioCloud Antibody Solution #3 antibody was selected as the spike protein is displayed at the surface of the SARS CoV-2 virion and because the spike protein is well conserved and critical to viral entry into susceptible host cells. The detection of target associated antigens and the in vitro experiments reveal that inactivated SARS CoV-2 can be detected in the BioCloud detection solution, comprised of primary and secondary antibody. The BioCloud Antibody Solution #3 also showed a consistent reaction for all currently tested variants of the SARS CoV-2 virus. It was also demonstrated that a secondary antibody conjugated to infrared fluorescent dye label was detected by the BioCloud system. The initial collection and capture efficiency data of the BioCloud unit was reviewed and validated to be 93.9% based on findings from independent laboratory testing. Additional testing will be conducted to further aggregate capture efficiency percentages.