OPTEEV™
Making Your World Safer

222 East Thomas Avenue
Baltimore, MD 21225

Main: 443-457-1370
Fax: 443-773-9072

www.opteev.com

July 22, 2022

Dear Sir/Ma'am,

This letter of introduction is in relation to a Pre-EUA application for our proposed first-line personal COVID screening device which is detailed in the additional attachment.

Opteev Technologies, Inc., the developer of this screening device, was born from its sister companies that have extensive experience in sensor technology for various engineering applications. When the pandemic hit in early 2020, the companies mobilized their knowledge and efforts in a variety of ways including this work on a biosensor to Covid-19, but also including temperature scanning devices and room virus detection. Opteev took advantage of semiconductor-based biosensors and the inherent nature of viruses as charge carriers to develop label-free SARS-CoV-2 and other respiratory virus screening devices. In particular, Opteev developed biosensors for the screening of COVID-19 based on a specially designed liquid non-polar medium for personal space monitoring and introduced the device in late 2021 after testing throughout the world.

Using many of the same characteristics described above, Opteev set out to adapt this technology to an individual easy-to-use breath analyzer device, trade named BrefX, that is based on an electrical biosensor made of polypyrrole (PPy) conductive fiber grown on a substrate silk fiber. BrefX uses exhaled breath as the input sample which is non-invasive and easy for most individuals to provide. BrefX is a pocket-sized portable device that can be used multiple times until a positive viral infection is detected. BrefX is variant agnostic and detects currently circulating variants as well as future variants. BrefX has a very quick result time with positive/negative results being displayed in under one minute. Because of its portability, ease-of-use, and rapid result time, we believe BrefX provides a unique opportunity to improve the ongoing pandemic situation by providing earlier information on probable infection with COVID-19, especially in protecting vulnerable communities such as the elderly, the immunocompromised and those afflicted with co-morbidities.

With this in mind, Opteev Technologies would like to apply for an Emergency Use Authorization for our breath analyzer device, BrefX. However, due to the novel nature of the technology and its intended application as a first-line screening device to be used to advance additional confirmation testing and treatment, we believe that there is no similar FDA/EUA template for this submission of relevant data for the EUA application. As a result, we are submitting the attached pre-EUA application information and data and are requesting FDA guidance in the process of the Emergency Use Authorization application.

We have included a document describing the principle of the test, the intended use, demonstrative laboratory analytical results and a product description of BrefX in this application. At present, Opteev has generated the laboratory based analytical data necessary for the EUA application. Further clinical evaluation studies are also underway within several medical centers, including a noted Hospital Medical Center in New Jersey.

Our rigorous laboratory testing demonstrates that BrefX has outstanding performance. In multiple separate analytical validation experiments we have measured the accuracy of BrefX at different viral concentrations ranging from 100k to as low as 5k viral particles. Our results have shown that BrefX has a sensitivity above 95% and a specificity of 90%. Detailed information on the performance of BrefX and the experimental results are included within the main document.

We believe strongly, especially since the pandemic is seemingly going through yet another wave of infections bolstered by ongoing mutant variants, that BrefX, our first line screening device, which is fully electronic, affordable, pocket-sized, easy to use and gives results in under a minute, can significantly strengthen the national test-to-treat policy by encouraging people to more readily use a personal screening device followed up by specific testing and treatment. BrefX will allow potential infections to be identified earlier, thus allowing people to isolate earlier, which will serve to protect those vulnerable communities from unintended exposure. Given the outstanding performance of our device and the central role it can play in helping to mitigate the pandemic, an expedited process to the EUA could save lives that could have otherwise been lost to the ongoing pandemic.

We are more than happy to provide any additional information to expedite this process.

Sincerely,

Conrad M. Bessemer, Chief Executive Officer
(cbessemer@opteev.com)

Dr. Biplab Pal, Chief Technology Officer
(bpal@opteev.com)

Dr. Mesfin Meshesha, Virologist
Chief Medical Science Officer/Vice President
(mmeshesha@opteev.com)

Pre-EUA Application

Applicant information:

- Applicant Company Name: Opteev Technologies
- Applicant Address: 222 Thomas Ave, Baltimore, MD 21225
- Applicant Contact Person: Conrad Bessemer
- Applicant Contact Phone#: 443 851 -4999 (cell), 702 296 4510

Applicant Contact Email: cbessemer@opteev.com

PROPRIETARY AND ESTABLISHED NAMES

Proprietary Name – *BrefX by Opteev*

Established Name - *BrefX*

Structural Background and Need for Consumer based COVID-19 Screening Test

The COVID-19 pandemic is now seemingly going through the fifth wave in the US unabated. Even though several vaccines are now available, and the majority of the US population is fully vaccinated, and a considerable number of people have been boosted, rate of re-infections is still very high (Rahman. S, 2022). Current variants of SARS-CoV-2 virus are increasingly capable of evading neutralizing antibodies limiting the effectiveness of vaccines to prevent new infections (Garcia-Beltran WF, 2021, Yiska Weisblum et.al.2020). What makes the current wave of infections unique is that mask mandates and other restrictions are lifted and direct measurement of the burden of infections is not adequately available due to declining number of PCR testing and reporting. And the obvious conclusion that at best only very symptomatic individuals are testing leaving some 40 – 60% of potentially infectious people unknowing as their condition. This is further supported by recent statements by officials at the University of Washington who tested blood samples to assess the true level of infections, and have estimated that only 14% of the COVID-19 cases are being reported. This is also borne out by the high positive rate as we speak with some states reporting as high as 25% positivity with those that are testing.

Although many healthy children and adults mercifully have strong immune systems that can help combat COVID-19 infection, many others in the United States are not so lucky. Particularly, the United States have a very large population of vulnerable communities including the elderly whose immune system is breaking down with age, many immunocompromised individuals, never mind the heavy population of morbidly obese, high diabetes or hypertensive individuals in the US all of which are more severely affected by the COVID-19 infection.

Indeed, this is exactly why there is such a need for classic screening test for COVID-19. Screening have often been described as “test that look for disease before you have symptoms” and screenings can often pinpoint the illness early when treatment is easier.

At the early stages of the pandemic, Infrared thermometers were used as first-line screening devices in many places including building entrances, in hospitals and doctor’s offices to screen for possible contagious people through elevated body temperature detection (Reviewed in Facente, S.N et al, 2021). However, such devices suffer from low sensitivity since most infections are asymptomatic and among individuals who show symptoms only a fraction of them have fever. As a consequence, the United States Food and Drug Administration (FDA) has released a statement in June 2020 noting that non-contact temperature assessment devices “are not effective if used as the only means of detecting a COVID-19 infection” (FDA, reference 5). Antigen tests have a good degree of sensitivity and specificity and can serve as first line screening devices. However, such methods are complex and multistep for many users resulting in high false negative results (Lindner AK. et al., 2021; reviewed by Kepczynski, C. M et al, 2021; Arshadi M et al., 2022). Turnaround time for results on average takes 15 to 20 minutes and are also expensive for continuous self-monitoring as each time would require the use of a new test kit and the average consumer cannot easily and repeatably re-test with the high number of current exposures. Therefore, to fill this gap in screening, the ideal consumer screening test would have the following criteria:

- It must be very simple to use so that anyone can do it correctly. It should not be a multistep procedure where an untrained user could make a mistake at any of the

steps such as procuring a sample through a nasal swab or creating a component of the test by adding a buffer or other reagents.

- It should also be portable, small, and accessible for use spontaneously, such as something you can carry in your pocket or handbag or even a wearable device. An exposed person can start breathing out viruses at any time given enough incubation period. Access to such a portable personal device can provide superior advantage to self-monitor and protect others.
- Another important feature of a personal screening device is that it should be designed to be used multiple times as needed without the need to replace the detection media so that continuous self-monitoring is possible. The results should also be available as quickly as possible, such as within a minute or two so that people will not be discouraged to continuously monitor because of longer waiting time for the results.
- It should be able to capture multiple variants and possibly new ones. The ability for a screening device to be variant agnostic is very important as new mutations are frequently occurring. And the accuracy of the results shouldn't change significantly with the new variants
- Technology of the device should be simple and suitable for mass manufacturing
- It should also be affordable so that everyone can have access to it which consequentially benefits efforts to control transmission of the virus in at a community level or beyond.

However, developing a personal screening device that meets the above criteria is challenging following the usual methods of detection of pathogens based on amplification of nucleic acids, serologic antibody detection methods or enzymatic reaction based immuno-assays owing to the amount of time and complexity they require to perform. Therefore, it is necessary to explore new and novel approaches to address these challenges.

In the last decade, electrical biosensors have attracted a significant amount of attention for electrical detection of charged biomolecules by their intrinsic charge. Electrochemical biosensors enable label-free detection of viruses, are highly sensitive, selective, cost-effective, and have fast response time, in addition to their portability that makes point-of-

care testing possible (Souf, 2016), hence are suitable for developing personal screening devices.

Using this known history, Opteev Technologies has developed a breakthrough breath analyzer type personal first line screening device for COVID-19 (BrefX) based on a biosensor made of polypropyline (PPy) conductive polymer grown on silk fiber. BrefX uses exhaled breath as input sample. Breath is one of the most appealing non-invasive sample types for diagnosis of infectious and non-infectious diseases that is recently attracting a lot of attention. Exhaled breath is very easy to provide and is less prone to user errors. Breath contains a number of biomarkers associated with different ailments that include volatile organic compounds (VOCs), viruses, bacteria, antigens, and nucleic acid. A number of studies have demonstrated detection of respiratory viruses including SARS-CoV-2 in exhaled breath (Tellier et al,.2019; Leung, N. H. L. et al.2020; Grassin-Delyle, S.et al.2020) and also reviewed in detail in (Davis, C. E. et al.2021).

BrefX is small, fits in the palm of a hand, is portable and can be used multiple times as required until a positive signal is detected. The conductive polymer in BrefX biosensor is functionalized with an organic layer that immobilizes viruses and, upon binding, alters the electrical property of the conductive polymer. Changes in electrical properties of the biosensor upon interaction with the virus can be sensed with simple electronic circuits and analyzed using algorithms to decide threshold values and make a positive or negative call. BrefX generally detects respiratory viruses in human exhaled breath and is not specific to COVID-19. However, in the current situation where there is high prevalence of SARS-CoV-2 and historically low prevalence of other respiratory viruses (Avolio M, et al, 2022, Olsen SJ., et al. 2021) it is expected that the overwhelming majority of virus detection by BrefX and associated symptoms are of SARS-CoV-2 which require further confirmatory molecular test.

Intended use

BrefX is breath analyzer type of device based on conductive polymer chemiresistive biosensing test for the in vitro qualitative detection of SARS-CoV-2 and other respiratory viruses. BrefX detects whole virus in exhaled breath from individuals 5 years and older, with or without symptoms or other epidemiological reasons to suspect COVID-19. Results are for the detection of SARS-CoV-2, Influenza virus, common cold viruses and other respiratory

viruses based on the interaction of charge carried on viral surface proteins and the biosensor.

BrefX does not differentiate between SARS-CoV-2 and other common respiratory viruses such as Influenza virus and common cold viruses in human exhaled breath. Positive results indicate the presence of viruses in breath specimen, but clinical correlation with patient history and other diagnostic information is necessary to determine SARS-CoV-2 infection status. Positive results should be treated as presumptive and confirmed with a molecular assay. Positive results do not rule out bacterial infection or infection with other viruses.

Negative results do not rule out SARS-CoV-2 infection and should not be used as the sole basis for treatment or patient management decisions, including infection control decisions. Negative results should be considered in the context of a patient's recent exposures, history, and the presence of clinical signs and symptoms consistent with COVID-19 and should undergo follow up testing if necessary for patient management. Individuals who suspect possible exposure can continuously monitor their status with BrefX for detection of virus in exhaled breath.

BrefX is intended for first line personal screening test as non-prescription home use for individuals 14 years old or above. Children below the age of 14 can still use BrefX but needs to be monitored by their parents or adults above the age of 21.

Principle of the test

BrefX, a silk-based chemiresistive sensor made with in-situ grown conducting polymer has been developed for the detection of SARS-Cov-2 like viruses. For the fabrication of this sensor, silk thread extracted from *Bombyx mori* cocoon has been chosen as a protein-based support. This protein backbone provides suitable active sites for the growth of the conducting polymers on it by virtue of its electrostatic attraction as well as hydrogen bonding capability. The very thin width of the silk fibre limits the number of the conducting paths. Therefore, any small perturbation in any of those conducting paths can lead to significant change in overall conductivity and resistance thereby increasing the sensitivity. As the conducting polymer, chloride doped polypyrrole ($PPy^{+}Cl^{-}$) [in this document $PPy^{+}Cl^{-}$ will be referred to as PPy here onwards], has been chosen for its p-type semiconducting nature which at the same time gives good binding on the silk surface as well as ample

electrophilic hole (h^+) density to interact with the negatively charged surface proteins of virus or a spike proteins (S-protein) in case of SARS-Cov-2 like viruses. The PPy was grown on the silk thread substrate using radical polymerization method. Upon polymerization, the surface of the silk turns into a semiconducting surface as opposed to a non-conducting surface prior to polymerization.

Furthermore, the PPy surface has been functionalized with 2% Terephthaldehyde (TA) solution. The TA molecule has -CHO at its para position. One end of TA molecule is attached to the PPy by means of hydrogen bonding between the aldehydic oxygen and the positively charged centre of PPy. This leaves a -CHO (aldehyde site) open which acts as an anchor for the amine residues of surface proteins of the virus. Viruses such as SARS-CoV-2 have alternate layers of capacitive positive and negative charge. When the virus come in contact with the surface of the sensor through bonding with TA as well as electrostatic interaction between the h^+ dense sites, it causes re-arrangement of electronic configuration on the PPy due to the oxidative nature of the virus. A transient shoot in the conductivity of the PPy based sensor occurs instantaneously as it encounters the virus.

Overall, when the aerosolized virus particle encounters the surface of the sensor, the virus gets anchored to the TA end of the surface and interact with the high positively charged sites of the p-type semiconductor surface. By virtue of the oxidative property of the virus there occurs a transient increase in the electron flux leading to a sharp shoot in conductivity of the semiconductor surface. This reduces the resistance of the PPy surface.

A low power micro-controller based electronic circuit has been developed to monitor the impedance of the silk-based TA-coated PPy sensor across a specified distance in terms of frequency of an RC circuit. The frequency is inversely proportional to the resistance. A differential change in the conductivity is measured and the positive jump in frequency output indicates exposure to the viral particles. This phenomenon is exclusively observed for the virus with spike proteins.

BrefX device is first calibrated by capturing frequency data from multiple experiments in laboratory environment with aerosolized pseudo virus particles. These experiments are conducted with different concentration of nebulized virus particles, and without virus particles (control media) as well. As the experiments are conducted with multiple devices,

the frequency readings obtained are normalized against the baseline frequency of each device. Normalized frequency data from multiple “virus” and “no-virus” experiments are tabulated and through statistical analysis a threshold is calculated where the test statistic value gives a good separation between “true positive” (virus) and “true negative” (no-virus) cases with lesser number of “false” values. The threshold value, thus obtained from the laboratory experiments, are programmed back into the micro-controller, which then uses it for classification of the human breath during blow tests. There could be also few cases where the result is indeterminate. As a demonstration of these concept, some results from laboratory analytical experiments are shown below.

Experimental Results

Analytical experiments with the BrefX are conducted in a controlled environment with pseudovirus (SARS-COV-2) particles of different concentrations (5K, 50K, 100K in 1µl). The specified dose of viral particles is applied on the sensor in the form of droplets in 1µl nuclease free water. Nuclease free water is also used as control medium.

The addition of virus in the sensor generates a time varying electrical signal as an output. The output signal shows different patterns for different viral load as evident in Figure 1. It is clearly observable that output signals for virus and control are distinguishable. It is clearly observable that the output signal of control media does not considerable change in conductivity.

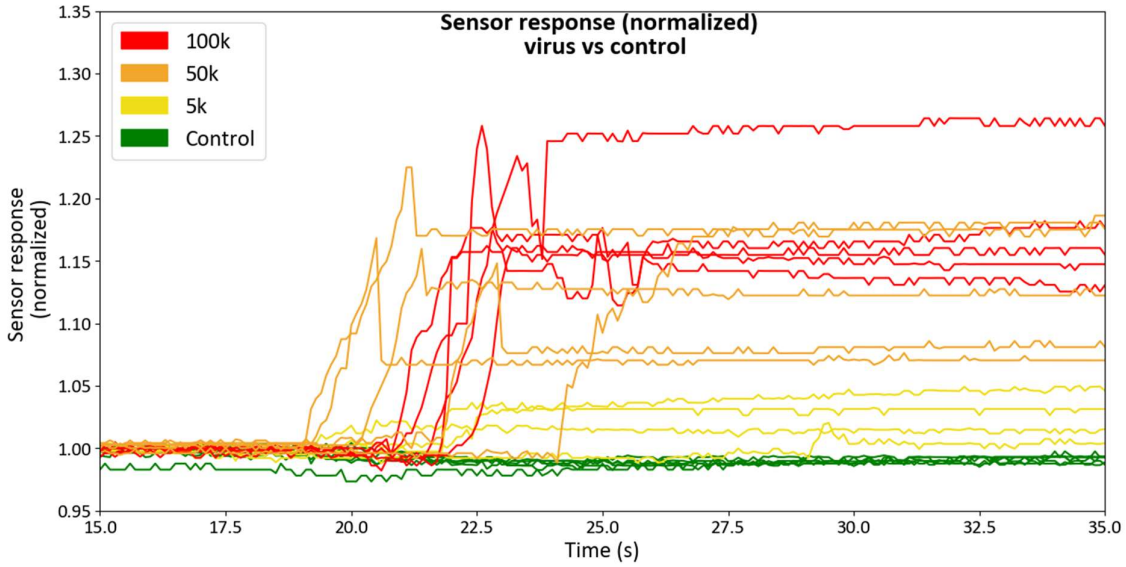


Figure 1: Sensor output as a time series data for different virus concentrations and control. Data is presented after normalization of signals to baseline values before sample application on the sensor.

In order to make a positive or negative call to a specific input sample, first, different statistical features were extracted, and analysis was performed to determine a statistical feature that provides clear separation of signals due to virus and control. In this manner, "Rate of Change in Normalized Signal" was selected, and threshold value is drawn above background noise. Based on this threshold value, positive and negative results are determined (Figure 2).

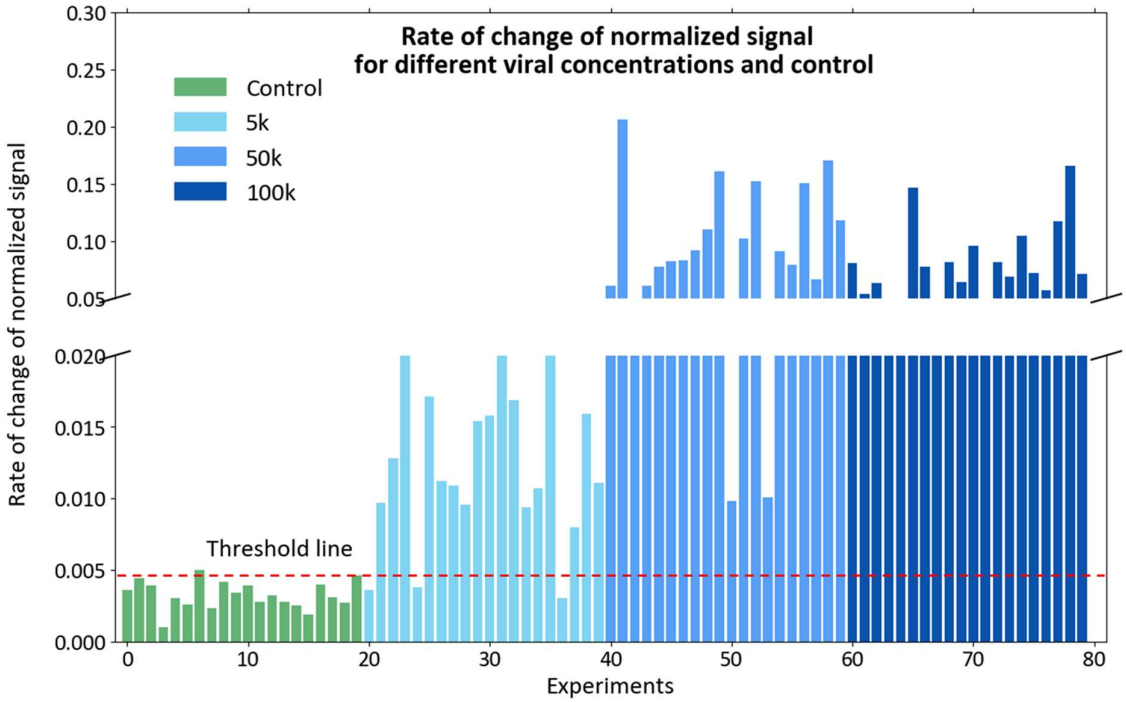


Figure 2: BrefX response to different concentrations of viral particles. the values of statistical feature (Rate of Change in Normalized Signal) corresponding to each experiment (virus & control) is shown. The threshold value separating the virus & control signal is in red dotted line. 20 replicates of each viral concentration are considered in the analysis.

Based on the above analysis positive and negative results are identified and accuracy of BrefX is calculated at each viral concentration. Above 50,000 viral particles, BrefX could detect 100% of the cases and at 5000 viral particles, BreFX detected 90% of the cases. BrefX has also identified 90% of True negatives (Table 1). Based on these results, the sensitivity of BrefX is above 95% and its specificity is 90%.

Virus Concentration (in 1 µl)	True Positive	False Negative	True Negative	False Positive
5k	90%	10%	N/A	N/A

50k	100%	0%	N/A	N/A
100k	100%	0%	N/A	N/A
No Virus (Control)	N/A	N/A	90%	10%

Table 1: Detection accuracy of the sensor for various virus concentration

BrefX Product Description

Opteev BrefX current prototype is a small pocket size device that can be easily carried as a personal object. Device is operated using a ON/OFF button and a display LED communicates the status of the Device to the user. The electronic components and biosensor components are housed inside the same Device. Device has a replaceable battery and doesn't need to be connected to an external power source.

Figure 3: Fully assembled BrefX Device

The Bref-X Device has three separable parts as shown in Figure 4. These are:

(A) **Printed Circuit Board (PCB) Chamber:** This contains the controller electronic circuit embedded in a PCB, Battery, LED indicator and Push Button.

(B) **Biosensor Chamber:** This contains the Sensor PCB connected to the Controller PCB in the other chamber. This chamber also contains a HEPA filter through which the exhaled air passes before exiting the Device. The Biosensor chamber is replaceable. Used Biosensor chamber should be disposed off following specific protocols once the Device detects a virus or after the Biosensor needs replacement

(C) **Mouthpiece:** A separate mouthpiece attaches snugly to the "Air Chamber" through which the user breathes into the Device.

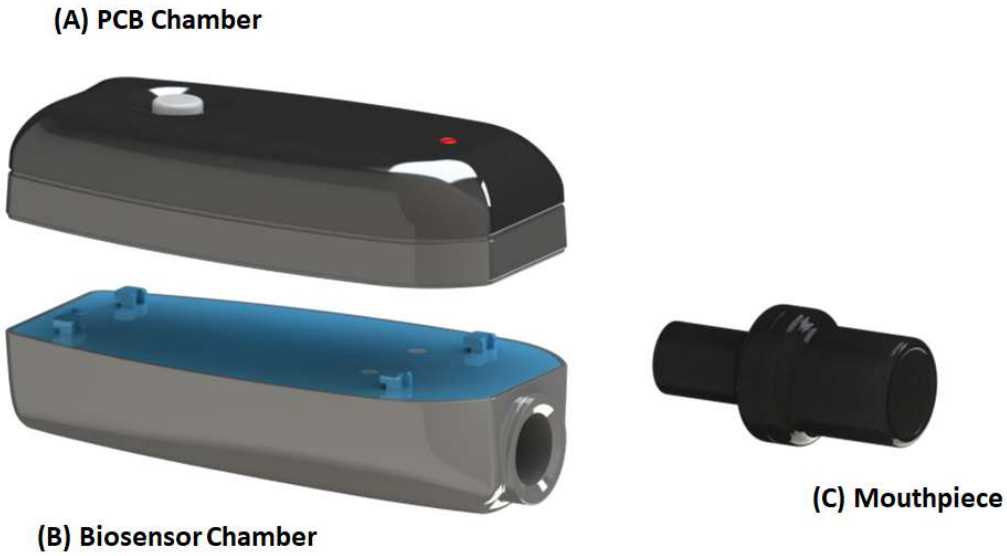


Figure 4: Three separable parts of the BrefX Device

How BrefX Device Works

A person willing to take a breath test using BrefX is required to take a deep breath and blow into the mouthpiece three to five times. The exhaled breath passes through the biosensor chamber where it comes in contact with the biosensor. The biosensor senses the presence of virus and passes the signal to the electronic circuit. The exhaled air further passes through a HEPA filter that traps any residual virus in the air. The filtered air, devoid of any virus particle, is then released out through the air vents. Figure 5 shows a cross section of the Device and the chambers through which the exhaled air passes.

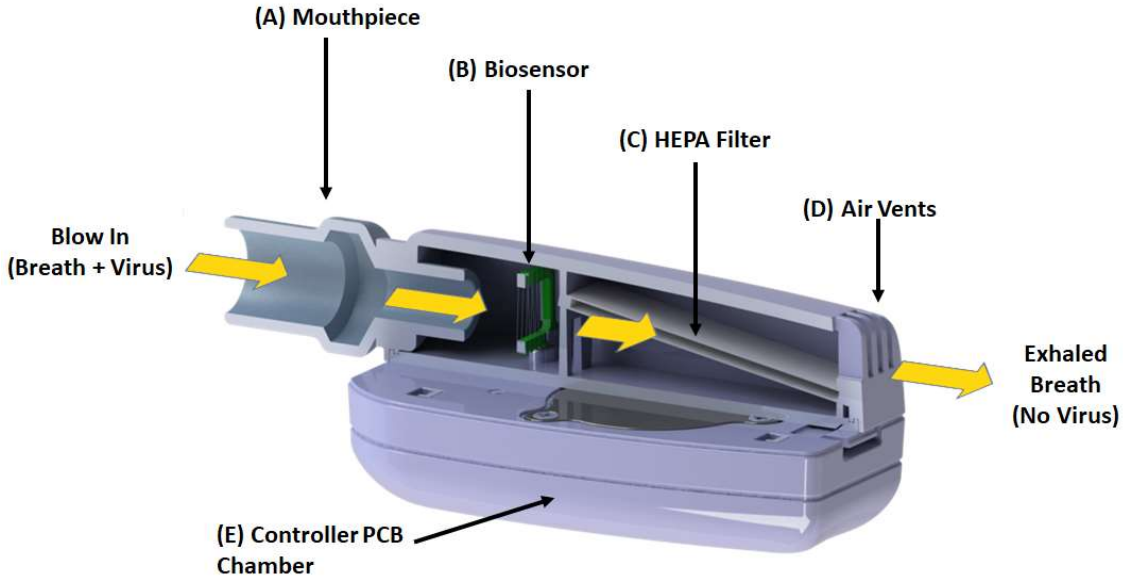


Figure 5: Cross section of the Device showing the passage of exhaled breath through the Biosensor chamber and HEPA filter

Device Dimensions

When fully assembled, dimensions of the BrefX Device (including mouthpiece) are 80 mm (Length) x 30 mm (Width) x 28 mm (Height)

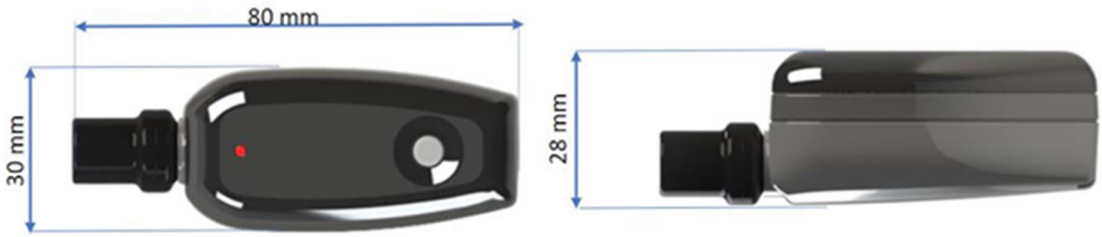
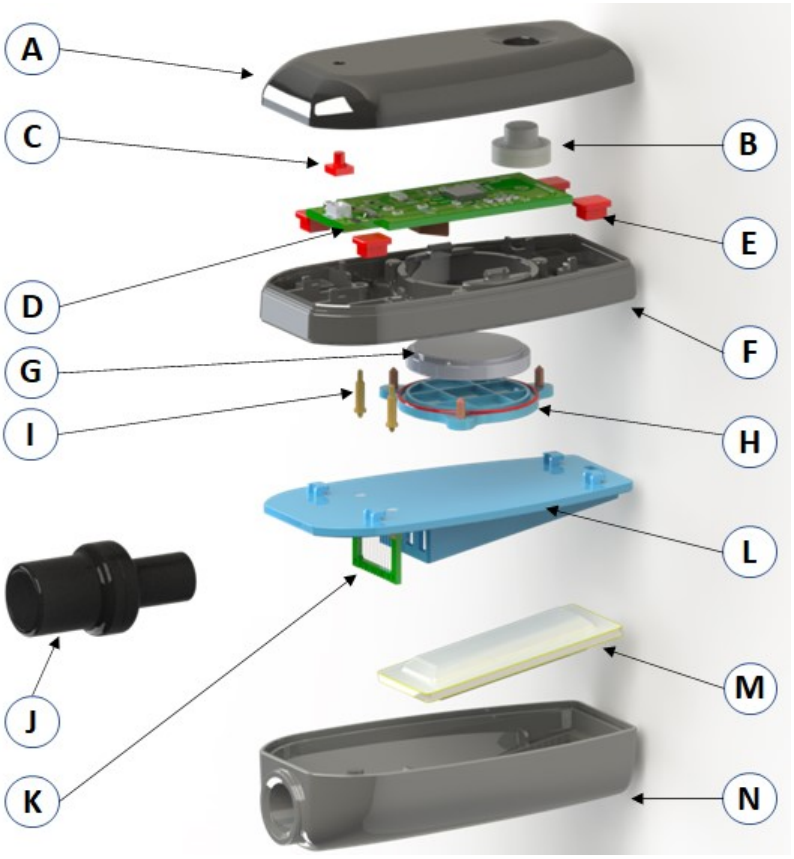


Figure 6: BrefX Device dimensions

Mechanical Components



A. PCB Chamber Cover
B. Silicone Button
C. LED Cap
D. PCB Board
E. Bung
F. PCB Holder
G. Battery
H. Battery Cover
I. Connector Pins
J. Mouthpiece
K. Biosensor
L. Back Cover
M. HEPA Filter
N. Biosensor Chamber Cover

Figure 7: Mechanical Components of BrefX Device

Electronic Components

BrefX Device is a low power handheld electronic device that runs on a coin cell battery, and does not emit any significant electromagnetic radiation. The Device consists of a low-power microcontroller unit connected to a timer and the proprietary biosensor developed by Opteev. Figure 8 below shows the electronic components used in the BrefX Device:

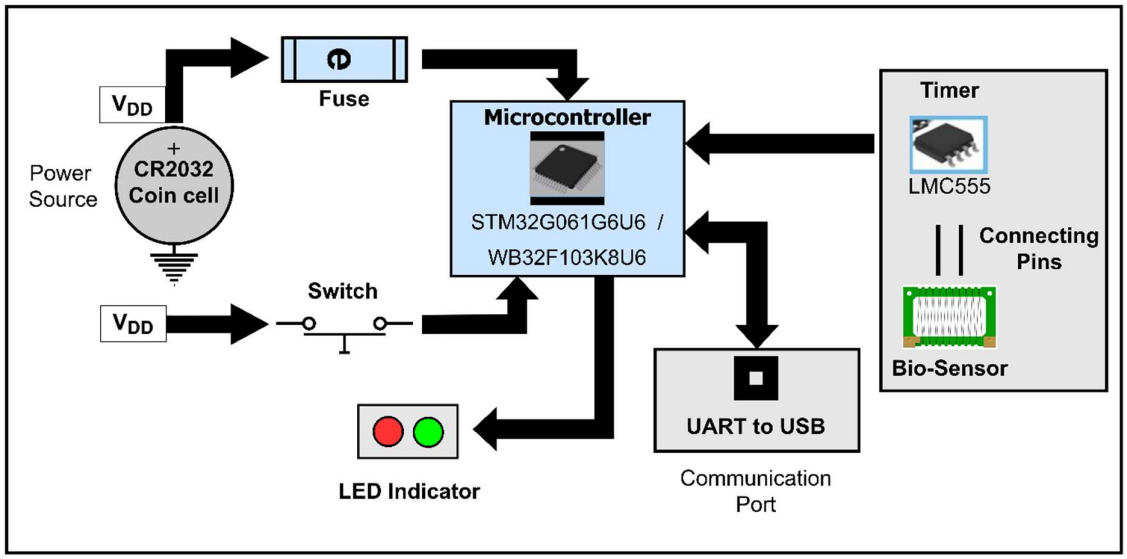


Figure 8: Schematic diagram of the electronic circuit in BrefX

Part Specifications

Following table shows the electronic components and their specifications

	Component	Technical Name	Purpose	Power Specification	Detail Spec Link
1	Battery	CR2032 (220 mAh)	To power the circuit (Power Source)	2.5v-3V, 220mAh	Link
2	Microcontroller	STM32G061G6U6 OR WB32F103K8U6	To run the virus detection algorithm	1.8V-3.3V, <500uA, <2mW	Link Or Link
3	Timer	TLC555I OR LMC555	Low power timer for chemo-resistivity detection through frequency	2.5V-3V, <350uA, 1mW (Typ.)	Link Or Link
4	Bicolor LED	LED GREEN/RED CLEAR SMTL4-BC	To indicate power status, battery status and virus detection status	7mA (Max), 2.1W(Max)	Link
5	Micro-USB Port	CP2102 – UART to USB converter	This is only for data download purpose to be used during trial. Production version will not have this port	1.5mW	Link
6	Power Button	- NA -	Silicon rubber pad (carbon-pill at the bottom) is used for external trigger		Link

7	SMD Fuse	USFF 1206 FUSE 50MA FF SMT (1206)	To break the circuit if an internal fault in the Device causes too much current to flow.		Link

Table 2: Component list with technical details

Power Consumption Guide

	Component	Maximum power	Total power
A	Microcontroller	2 mWatt	< 7 mWatt (A+B+C+D) < 5.5 mWatt (A+B+C)
B	Timer	1 mWatt	
C	LED	2.1 mWatt	
D	UART to USB	1.5 mWatt	

Table 3: Power consumption of BrefX device

Intrinsic Safety (IS)

Maximum power consumption of the BrefX Device is ≤ 7mWatt. The circuit contains a fast-interrupting SMD fuse at the input line to provide protection against fire hazards due to internal short circuit.

As described earlier that the intended use of BrefX Device will be of personal in nature; hence there is less chance of the Device getting exposed into explosive environment.

As per IEC 60079-11: intrinsic safety "i" is concerned, the Device consumes very little power (only 7 mWatt as shown in Table 3) compared to IS specified limit of 1.3 Watt.

As per IEC60950-1, basic requirements of safety standards have been provided.

- V_{oc} (Open Circuit Voltage): 3V max
- I_{sc} (Short Circuit Current): ≤ 200mA (limited by passive (R-L) components).
- Protection: Fast interrupting fuse.
    - UL 248-14 certified
    - IEC 60068-1 certified
    - IEC 60068-2-14 certified

FCC and Emission Guidelines

As the Device is operated with a non-rechargeable coin cell (CR2032) battery, Conducted Emission (CE) as per FCC part 15 subpart A or subpart B is not applicable as the Device is not to be connected to AC supply line.

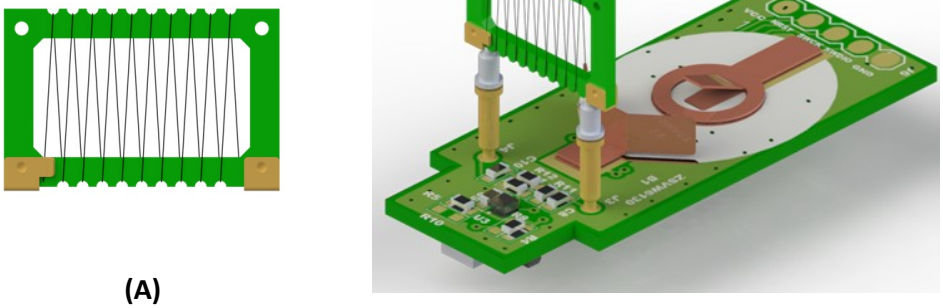
The external oscillator used in the circuit (RC oscillation based on LMC555/TLC555) can generate a maximum of 100kHz. Unintended Radiated Emission (RE) can occur due to 32 MHz internal crystal of the microcontroller on which the microcontroller runs.

Frequency of radiation	Source of emission	Type of emission	Comment
100kHz	External RC oscillator	Intended	Not Applicable under FCC part 15 subpart A or subpart B
32MHz	Internal crystal of microcontroller	Un-intended	Maximum power consumed by BrefX Device will be ~ 2 mWatt, which converts to an estimated Radiated Emission of < 5 dBm, which is significantly less than 39dBm (limit as specified in FCC part 15 subpart A)

Table 4: Sources of Emission

Biosensor PCB

The Biosensor is the heart of the BrefX Device. The biosensor consists of a conducting polymer-coated silk thread, wrapped around in crisscross fashion across a PCB frame. The silk thread is held together by a gold-plated metallic clip that acts as an electrode and connects to the main controller circuit through connecting pins.



(A) (B)

Figure 9: Illustration of the biosensor PCB. (A) Biosensor PCB with metallic electrodes (B) The biosensor PCB is connected to the main controller PCB through a press-fit mechanism as shown in the schematic diagram

Preparation of the Sensors:

Polypyrrole was grown on the silk thread substrate using radical polymerization method. Upon polymerization, the surface of the silk turns into a semiconductor surface as compared to a non-conducting surface prior to polymerization. The PPy surface has been functionalized with 2% Terephthaldehyde solution.

Product Safety

The outer casing of BrefX is moulded using FDA approved plastic for medical use. The Device is a low power coin battery operated device with radiation limits within the control. The biosensor used in the Device contains silk, which is an organic material and non-toxic in nature. The sections below describe the detailed safety specifications of the components and materials used in BrefX.

Safety Data Sheet of Chemical components of the biosensor

Chemicals used in preparing the biosensor are generally sourced from Sigma-Aldrich. The product safety data sheet links are provided in the table below. None of these are known to be toxic or hazardous in nature for human:

Sl#	Material	Product Safety Data Sheet Link or Equivalent
1	Polypyrrole	Link
2	Terephthalaldehyde	Link
3	Silk Fiber	Link

Table 5: Link to Safety Data Sheets of the chemicals used

Restriction of Hazardous Substances Directive 2002/95/EC (RoHS) Compliance:

The RoHS compliance of the BrefX Device is pending evaluation. However, the following components used in the electronic circuit are RoHS compliant as certified by their manufacturers:

Sl #	Component Part#	Description	RoHS Information	Reference
1	STM32G061G6U6	Microcontroller	RoHS Compliant	Link
2	TLC555I	Timer	RoHS & Green	Link
3	LMC555	Timer	RoHS & Green	Link
4	CR2032	Battery	RoHS Compliant	Link
5	CP2102	USB to UART	RoHS Compliant	Link
6	USFF 1206	Fuse	RoHS Compliant	Link
7	SMTL4-BC	LED	RoHS Compliant	Link

Table 6: ROHS compliance of the components used

References

1. Rahman, S., Rahman, M.M., Miah, M. *et al.* COVID-19 reinfections among naturally infected and vaccinated individuals. *Sci Rep* **12,** 1438 (2022). https://doi.org/10.1038/s41598-022-05325-5
2. Garcia-Beltran WF, Lam EC, St Denis K, et al. Multiple SARS-CoV-2 variants escape neutralization by vaccine-induced humoral immunity [published correction appears in Cell. 2021 Apr 29;184(9):2523]. *Cell*. 2021;184(9):2372-2383.e9. doi:10.1016/j.cell.2021.03.013
3. Yiska Weisblum, Fabian Schmidt, Fengwen Zhang, Justin DaSilva, Daniel Poston, Julio CC Lorenzi, Frauke Muecksch, Magdalena Rutkowska, Hans-Heinrich Hoffmann, Eleftherios

Michailidis, Christian Gaebler, Marianna Agudelo, Alice Cho, Zijun Wang, Anna Gazumyan, Melissa Cipolla, Larry Luchsinger, Christopher D Hillyer, Marina Caskey, Davide F Robbiani, Charles M Rice, Michel C Nussenzweig, Theodora Hatziioannou, Paul D Bieniasz (2020) Escape from neutralizing antibodies by SARS-CoV-2 spike protein variants eLife 9:e61312 https://doi.org/10.7554/eLife.61312

4. Facente, S.N., Hunter, L.A., Packel, L.J. *et al.* Feasibility and effectiveness of daily temperature screening to detect COVID-19 in a prospective cohort at a large public university. *BMC Public Health* **21,** 1693 (2021). https://doi.org/10.1186/s12889-021-11697-6
5. U.S. Food and Drug Administration. Non-contact Temperature Assessment Devices During the COVID-19 Pandemic. 2020. https://www.fda.gov/medica l-devices/coronavirus-covid-19-and-medical-devices/non-contact-tempera ture-assessment-devices-during-covid-19-pandemic. Accessed 7 Sept 2020.
6. Lindner AK, Nikolai O, Kausch F, Wintel M, Hommes F, Gertler M, Krüger LJ, Gaeddert M, Tobian F, Lainati F, Köppel L, Seybold J, Corman VM, Drosten C, Hofmann J, Sacks JA, Mockenhaupt FP, Denkinger CM. Head-to-head comparison of SARS-CoV-2 antigen-detecting rapid test with self-collected nasal swab *versus* professional-collected nasopharyngeal swab. Eur Respir J. 2021 Apr 15;57(4):2003961. doi: 10.1183/13993003.03961-2020. PMID: 33303544; PMCID: PMC7736752.
7. Kepczynski, C. M., Genigeski, J. A., Koski, R. R., Bernknopf, A. C., Konieczny, A. M., & Klepser, M. E. (2021). A systematic review comparing at-home diagnostic tests for SARS-CoV-2: Key points for pharmacy practice, including regulatory information. *Journal of the American Pharmacists Association : JAPhA*, *61*(6), 666–677.e2. ttps://doi.org/10.1016/j.japh.2021.06.012
8. Arshadi M, Fardsanei F, Deihim B, Farshadzadeh Z, Nikkhahi F, Khalili F, Sotgiu G, Shahidi Bonjar AH, Centis R, Migliori GB, Nasiri MJ, Mirsaeidi M. Diagnostic Accuracy of Rapid Antigen Tests for COVID-19 Detection: A Systematic Review With Meta-analysis. Front Med (Lausanne). 2022 Apr 7;9:870738. doi: 10.3389/fmed.2022.870738. PMID: 35463027; PMCID: PMC9021531.
9. Tellier, R.; Li, Y.; Cowling, B. J.; Tang, J. W. Recognition of Aerosol Transmission of Infectious Agents: A Commentary. BMC Infect. Dis. 2019, 19 (1), 1−9.
10. Leung, N. H. L.; Chu, D. K. W.; Shiu, E. Y. C.; Chan, K. H.; McDevitt, J. J.; Hau, B. J. P.; Yen, H. L.; Li, Y.; Ip, D. K. M.; Peiris, J. S. M.; et al. Respiratory Virus Shedding in Exhaled Breath and Efficacy of Face Masks. Nat. Med. 2020, 26 (5), 676−680.

11. Grassin-Delyle, S.; Roquencourt, C.; Moine, P.; Saffroy, G.; Carn, S.; Heming, N.; Fleuriet, J.; Salvator, H.; Naline, E.; Couderc, L.-J.; et al. Metabolomics of Exhaled Breath in Critically Ill COVID-19 Patients: A Pilot Study. EBioMedicine 2021, 63, 103154.
12. Davis, C. E.; Schivo, M.; Kenyon, N. J. A Breath of Fresh Air – the Potential for COVID-19 Breath Diagnostics. EBioMedicine 2021, 63, 2020–2021.
13. Avolio M, Venturini S, De Rosa R, Crapis M, Basaglia G. Epidemiology of respiratory virus before and during COVID-19 pandemic. Infez Med. 2022 Mar 1;30(1):104-108. doi: 10.53854/liim-3001-12. PMID: 35350252; PMCID: PMC8929737.
14. Olsen SJ, Winn AK, Budd AP, Prill MM, Steel J, Midgley CM, Kniss K, Burns E, Rowe T, Foust A, Jasso G, Merced-Morales A, Davis CT, Jang Y, Jones J, Daly P, Gubareva L, Barnes J, Kondor R, Sessions W, Smith C, Wentworth DE, Garg S, Havers FP, Fry AM, Hall AJ, Brammer L, Silk BJ. Changes in Influenza and Other Respiratory Virus Activity During the COVID-19 Pandemic - United States, 2020-2021. MMWR Morb Mortal Wkly Rep. 2021 Jul 23;70(29):1013-1019. doi: 10.15585/mmwr.mm7029a1. PMID: 34292924; PMCID: PMC8297694.