# Assessment and validation of a device for measuring hydrogen peroxide in exhaled breath condensate in Healthy Volunteers

IRAS Project ID: 289549

## Study Management Group

**Principal Investigator:** Dr Omar Usmani, National Heart & Lung Institute, Imperial College

**Co-investigator:** Dr Danny O'Hare, Department of Bioengineering, Imperial College

**Sponsor:** Imperial College London is the main research Sponsor for this study. For further information regarding the sponsorship conditions, please contact the Head of Research Governance and Integrity at r.nicholson@imperial.ac.uk .

**Funder:** Innovate UK Smart Grants: October 2019

This protocol describes the **Assessment and validation of a device for measuring hydrogen peroxide in exhaled breath condensate** and provides information about procedures for entering participants. Every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the study. Problems relating to this study should be referred, in the first instance, to the Principal Investigator.

This study will adhere to the principles outlined in the UK Policy Frame Work for Health and Social Care Research It will be conducted in compliance with the protocol, Data Protection Act 2018 and General Data Protection Regulations (Europe) and other regulatory requirements as appropriate.

Imperial College London

# TABLE OF CONTENTS

Imperial College London

| | |
|---|---|
| TITLE | Assessment and validation of a device for measuring hydrogen peroxide in exhaled breath condensate in Healthy Volunteers |
| DESIGN | Assessment of accuracy and precision of a novel instrument for collecting exhaled breath condensate (EBC) and analysing the levels of hydrogen peroxide. |
| AIMS | (i) Assess consistency in three successive measurements at monthly intervals in individual healthy volunteers.<br>(ii) Qualitative evaluation of the usability of the device and the collection protocols from volunteers and from clinical staff operating the device.<br>(iii) To collect usability feedback, in terms of how easy to use the device and to hold to breathe through the device. |
| OUTCOME MEASURES | (i) Precision and accuracy of the EBC device in healthy subjects |
| POPULATION | Healthy volunteers |
| DURATION | 12 months |

# 1. INTRODUCTION

## 1.1 Background

Current assessments of lung inflammation for asthmatics and chronic obstructive pulmonary disease (COPD) are mainly based on patient symptoms and simple spirometry tests. Unfortunately, these features are not directly correlated to eosinophilic lung inflammation. Consequently, clinicians cannot always predict the degree of lower airway inflammation in COPD and asthmatic patients; hence it can be difficult to provide appropriate treatment. [1, 2]

More than 70% of COPD patients are underdiagnosed [3] and the 2-year mortality rate for severe COPD patients is about 50% because of exacerbation, which on average occurs 2-4 times per year [4]. Early diagnostics for COPD could not only reduce direct healthcare costs but also reduce the probability of exacerbation and mortality [5] and lead to improved patient care. COPD is a progressive disease with potential acute exacerbations; therefore, long-term monitoring must be in place to track patient condition and such monitoring requires out-patient or GP surgery visits [6].

Exhaled Breath Condensate (EBC) serves as simple and non-invasive sampling which gives access to lung chemistry and which can potentially inform clinical decision-making. Significantly higher hydrogen peroxide ($H_2O_2$) concentration in EBC is found in COPD patients, (typically 0.46 $\mu$M compared to healthy control 0.2 $\mu$M[7-9]). Even higher $H_2O_2$ concentrations are reported for patients with exacerbation, 0.6 $\mu$M in comparison with stable COPD patients and corticosteroid-treated patients. [10, 11] Due to the limited range between stable COPD patients and COPD exacerbation, an exceptionally sensitive sensor is required for reliable diagnosis.

Most previous approaches to EBC collection have prioritised the collecting of more condensate in a shorter time and this has led to the use of a lower temperature condensation surface. However, previous EBC collector designs have not considered how EBC sample composition depends on temperature and flow rate, due to widely varying absolute values and temperature dependences of the Henry's law constants for different analytes and the enthalpy of condensation. A failure to consider quantitatively the impact of collection conditions on EBC composition has led to large reported variations between different researchers for same health condition[7-15] which has impeded progress in this field. Here we report an approach to EBC collection aimed at optimising target analyte concentration by exploiting the differential thermodynamic and kinetic parameters involved. This model-based approach is qualitatively borne out by experimental data which we have collected by blowing compressed air through Dreschel bottles of normal saline in a water bath.

The published literature largely attributes variations in EBC analyte concentration to difference in patient physiological conditions. However, Loyola et al. [16] demonstrated different acetone concentrations in EBC samples at difference condensation temperatures. Zamuruyev *et al.* also showed how the amount of analyte collected varies with condensation temperature.[17]

Ferric ferrocyanide (Prussian blue (PB)) modified electrodes are well-established for both $H_2O_2$ and oxygen sensing [18]. Karyakin *et al.* [19] demonstrated Prussian blue $H_2O_2$ catalysis exhibits a significantly higher kinetic constant compared to a platinum electrochemical sensor and that PB is comparable to horseradish peroxidase. PB modified sensors were found to have good linear range between 0.1 μM to 100 μM, with sensitivity of 0.6 A $M^{-1}c\ m^{-2}$ and detection limit of 0.1 μM. [20]

$H_2O_2$ electrochemical sensor sensitivity and stability commonly involve the use of nanoparticles of PB [21], sometimes combined with polymer reagent blends [22, 23], or conducting polymers [24-26].

We prepared an easy-to-use water dispersible poly(3,4-ethylenedioxythiophene)-poly(styrenesulfonate) potassium ferric ferrocyanide composite, cross linked with ethylene glycol and divinyl sulfone (PEDOT:PSS-PB-EG-DVS) for electrochemical $H_2O_2$ and oxygen detection purposes. The calibration plot shows a good linear response which easily covers the concentration ranges reported for EBC (0.1 μM to 25.6 μM) and which has a detection limit of 103 nM. We have manufactured disposable biosensors based on this technology.

Performance in artificial samples has been compared to the laboratory-based fluorometric analysis using Amplex Red, which is well-established as the gold standard for hydrogen peroxide determination in low concentration biological samples and show good agreement.

**Figure 1**

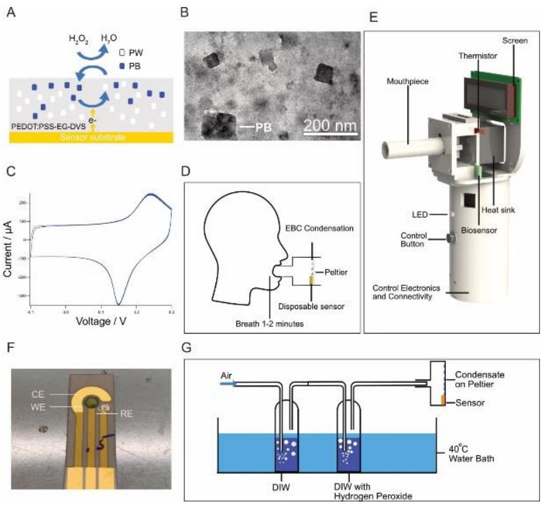



**Figure 1:** Schematic of the catalytic biosensor mechanism **B** TEM image of the electrode modifier **C** Reproducible i-V behaviour of the composite **D** condenser mechanism **E** Device schematic **F** disposable biosensor strip **G** Generation of test specimens for laboratory evaluation

**Figure 2**

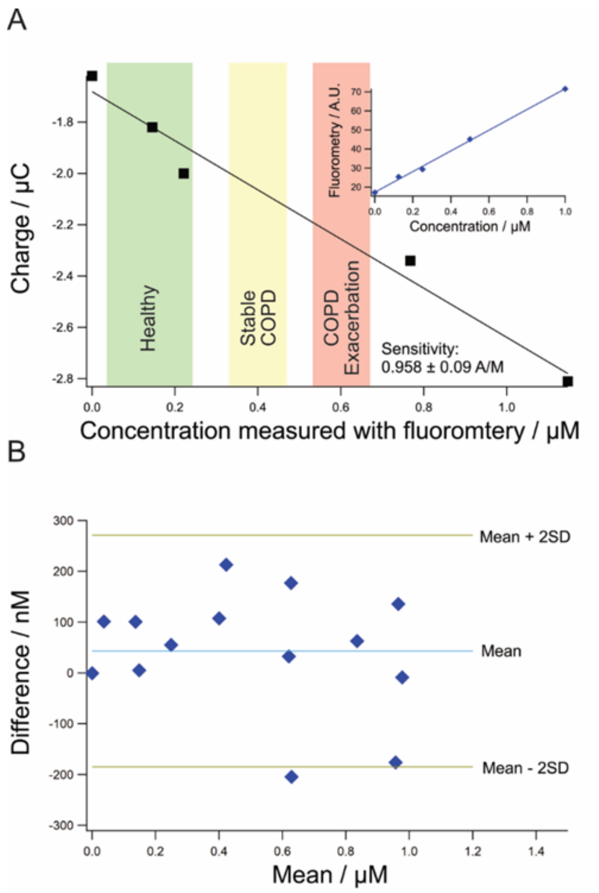



**Figure 2**: A Biosensor coulometry data measured with PEDOT:PSS-PB-EG-DVS modified sensor against fluorometry data measured with HRP and Amplex Red. The colour strips illustrate reported physiological $H_2O_2$ in EBC in various patient groups [7-9, 11]. The inset demonstrates the calibration standard for HRP and Amplex Red against $H_2O_2$ concentration. **B.** Bland Altman analysis comparing electrochemical sensor results and the Amplex Red assay

### 1.2 Study Rationale

Following a successful pilot study to evaluate the accuracy and precision of the EBC device, its biosensor element and the algorithm for corrections arising from variations in temperature and flow the device will be evaluated in real breath samples in healthy volunteers. This will take place at the Clinical Research Facility (CRF) at the Royal Brompton Hospital. Real biological specimens are complex and there is the potential for losses of precision and accuracy. This can only be evaluated by comparison with established methodology. Secondly, whilst many reports can correctly classify COPD patients, there are substantial variations in absolute levels between researchers. We have shown with artificial samples that correcting for variations in heat and mass transport can substantial reduce these variations. Collecting replicate samples from healthy volunteers will allow us to evaluate this approach in real samples.

## 2. STUDY OBJECTIVES 2

The questions to answer in 1st evaluation:

1. Volume collectable in 3-5 minutes
2. Concentration of hydrogen peroxide in healthy volunteer
3. Variability between 3 samples collected in 1 day
4. Variability between 3 tests collected in 1st month, 2nd month and 3rd month.
5. Usefulness of standardisation method, data including breath temperature entering and leaving the device as well as breath flow rate would be collected in-situ (sensors already on device, no naked wires).

Usability feedback, in terms of how easy to use, to hold and to breathe through the device

The outcomes of this study will inform the design of a clinical evaluation.

## 3. STUDY DESIGN

We aim to recruit 20 healthy volunteers from our database of volunteers at the royal Brompton Hospital and also by advertising. Our purpose with this study is to evaluate the analytical performance of the device and the data processing and consistency of measurement over 3 measurements a month apart.

I participants wish to take part in the study they will be invited to the Clinical Research Facility (CRF) at the Royal Brompton Hospital where you will meet the research team face to face. They will explain the study to the participant, and they will have the opportunity to ask any questions related to the study. They will then be asked to read and sign a consent form if they would like to participate.

Volunteers will be sent the participant information by email, or in person, prior to any appointment.

The participant will then be screened to see if they are suitable for the study. The Screening procedure will take approximately one hour and involve the researcher going through a list of questions related to your health and any medications you are currently taking.

A hard copy of the participant information sheet will be provided at the appointment and participants will be encouraged to ask questions.

Participants will then be presented with a consent form.

Venue: This will be performed in the Clinical Respiratory Facility located at the Royal Brompton Hospital.

Sample collection protocol

After the consent form has been signed, the breath collection protocol can then begin.
The handheld breath collection apparatus will be switched on by the researcher and allowed to achieve the correct temperature (1-2 minutes). A sterilised disposable plastic mouthpiece will be fitted. The participant will be asked to hold the breath collection device and breath normally into the plastic mouthpiece for up to 5 minutes (Figure 3).
The researcher will take the instrument and remove the condensed breath sample, place it in a numbered vial and remove it for analysis in the laboratory. Ideally, three samples per participant will be collected, but the participant may withdraw consent at any point.
All samples will be pseudonymised to protect participant confidentiality.
The individual results of the analysis will be made available to the participants. If requested.
The flow chart of the sample collection is shown in Figure 3 (A&B) below, C & D shows the collection device.

Three successive measurements will be made at monthly intervals in each individual healthy volunteer requiring them to visit the Hospital on three occasions.

In addition, participants will be asked to fill in a short questionnaire on visit 1 related to the use of portable medical devices.

Figure 3

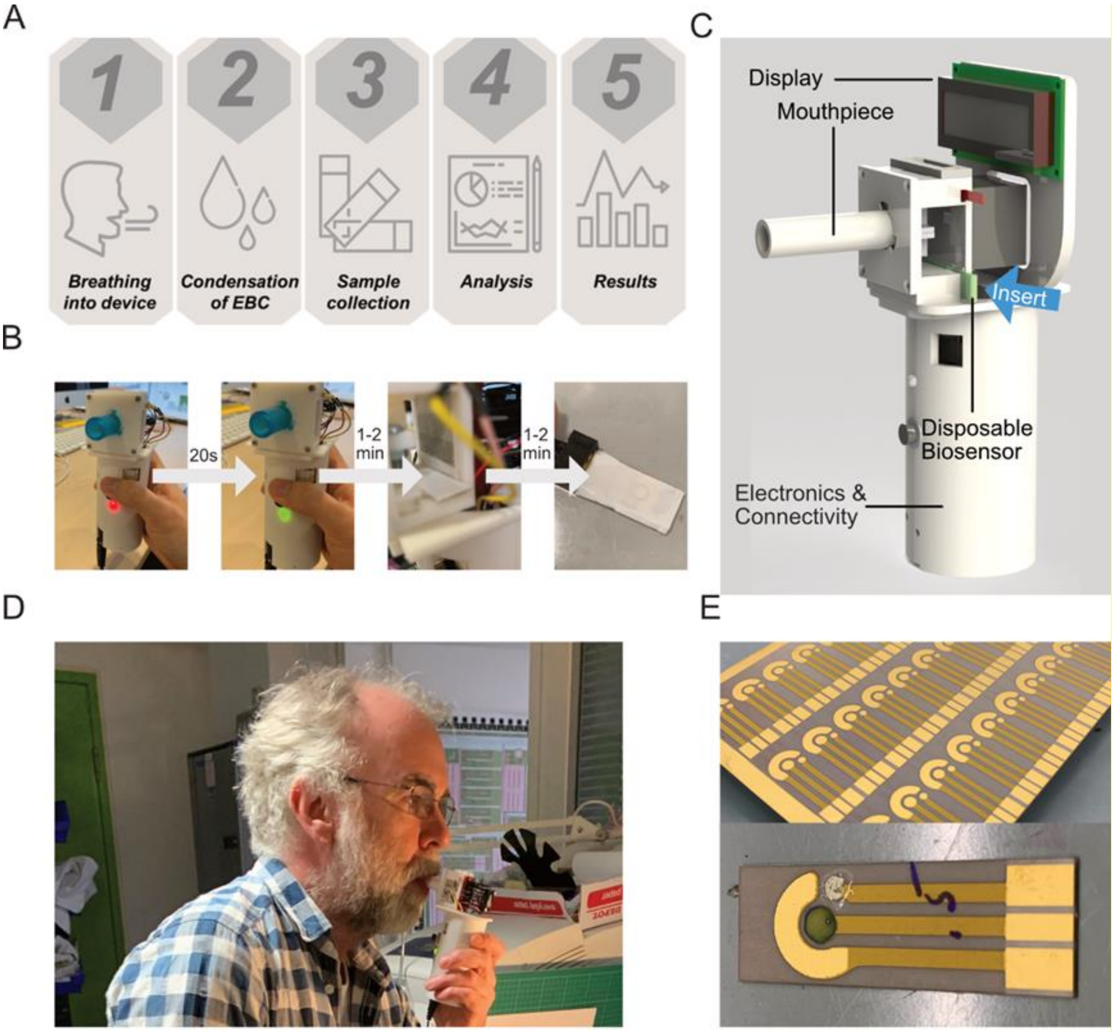
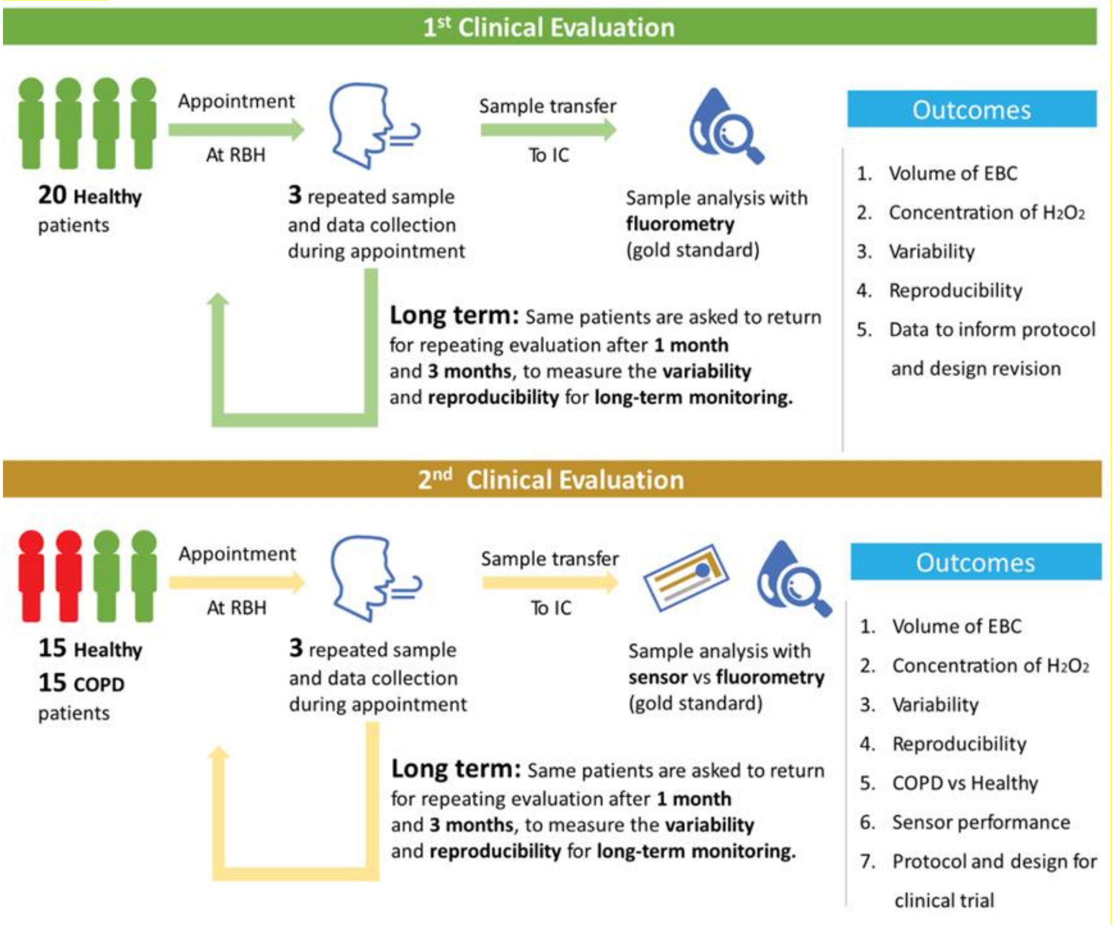

A
1
2
3
4
5
Breathing into device
Condensation of EBC
Sample collection
Analysis
Results
B
20s
1-2 min
1-2 min
C
Display
Mouthpiece
Insert
Disposable Biosensor
Electronics & Connectivity
D
E

Figure 4




## STUDY TESTS

Apparatus during sample collection: 1 pipette, a bag of pipette tips, numbered Eppendorf vials, sterilised mouthpiece, and exhaled breath condensate (EBC) collector, 75%(v/v) aqueous ethanol solution for sterilisation of the mouthpiece and apparatus.

The following process is repeated 3 times in total to collect 3 EBC samples per patient:

1. A researcher will control device operation and sample collection.
2. The researcher switches on the device and waits until the Peltier temperature reaches 20°C, typically 1-3 minutes
3. Install the sterilised PET mouthpiece.
4. Volunteer holds the device and breathes through the PET mouthpiece normally for a maximum of 5 minutes.
5. After EBC condensation, the volunteer is to pass the device to the researcher.
6. The researcher transfers the EBC sample with pipette to vials for further analysis.
7. The EBC collector is wiped with 75% aqueous ethanol to remove remaining EBC.

Samples will be taken the Biosensor Laboratory (Bessemer 105) for analysis according to the following protocol:

Protocol for EBC analysis – electrochemical sensor and Amplex red. To take place in Bessemer 105, Biosensor Laboratory, researchers only.

All samples will be analysed. No samples will be retained.

1. Potassium phosphate monobasic, potassium phosphate dibasic, and potassium chloride salts are added to 200 µl of EBC to make pH 7.4 0.1 M phosphate buffer with 0.1 M potassium chloride.
2. 30 µl of buffered EBC is dropped onto electrochemical sensor for hydrogen peroxide analysis.
3. 48 µl of buffered EBC is withdrawn into 96-well plates with addition of 1 ul 5 U/ml horse radish peroxidase (HRP) and 1µl 2.5mM Amplex red solution. The 96-well plates are than incubated for 30 minutes at room temperature with platform shaker before measurement with fluorometer. The excitation and absorption wavelength of the fluorometer are set to 535 and 587 nm respectively.
4. The electrochemical analysis results are compared with the Amplex red assay. Paired *t* tests, correlation, regression and Bland-Altman analysis will be used for comparative purposes, following standard practise in analytical sciences.

PET mouthpiece sterilisation:
1. Mouthpiece is wiped with 75% ethanol before use and safely disposed of following use.

## Cleaning of the Equipment & Transport of Sample

We will be following the Local Imperial College Bioengineering departmental protocol for transporting exhaled breath samples and disinfection of equipment in the document of BioSOP026:

### Transport and receipt of Materials

a) Human tissue must be transported in UN3373 compliant packaging. This packaging consists of three components.
    1. A primary receptacle: the tube, vial or other container that is in direct contact with the specimen.
    2. A secondary packaging that fully encapsulates the primary receptacle. Adsorbent material should be placed inside the secondary packaging in case the primary receptacle leaks.
    3. An outer packaging for shipping or transit.

Designated couriers should transport the tissue to Imperial College. For example, CitySprint can courier human tissue

### Cleaning and decontamination

Following all procedures work surfaces (which should be in spill trays or biological safety cabinets) must be cleaned with 1% Virkon solution followed by soap and water, and finally 70% ethanol. Whenever practicable, small articles that have been contaminated with human tissue or body fluids should be submerged in disinfectant at the appropriate working dilution for several hours before washing. Other contaminated surfaces (e.g floor, cupboards and walls) should be washed down with disinfectant. Bench surfaces should be washed down with disinfectant at the end of every experimental session. A discard bin containing freshly prepared disinfectant should be within easy reach on each working bench and should be clearly labelled with the type, strength and usage of the disinfectant.

### Duration of Study

Overall, the study will last 12 months from advertising to the end of data collection.

## 4. PARTICIPANT RECRUITMENT

### 4.1 Pre-recruitment evaluations

Volunteers expressing an interest will be contacted directly by the research team by email, phone, or in person and invited to the screening meeting. A copy of the participant information leaflet and study outline will be sent to the volunteer by email or post or in person.

### 4.2 Inclusion Criteria

**Healthy subjects:**
non-smokers
Healthy individuals, free of significant disease.

•Given written informed consent prior to participation in the study including all of its procedures.
•Comply with the requirements and restrictions listed in the consent form.
•Male or female subject aged 18-65 years at screening.
•Able to complete the study and all measurements.
•Able to read, comprehend, and write at a sufficient level to complete study related materials.

### 4.3 Exclusion Criteria

***Exclusion criteria for all subjects:***
Subjects will not be eligible if any of the following apply: -

•A history of recreational drug use or allergy which in the opinion of the investigators contra-indicates their participation.
•Participation within 3 months in any other study testing a new molecular entity or drug or involving invasive procedures.
•Those, in the opinion of the investigator, who may prove non-compliant with study procedures.

•History of an upper or lower respiratory infection (including coryza) within 3 weeks of baseline assessments (assessments and entry could be deferred.

### 4.4 Withdrawal Criteria

Participants can withdraw at any point of the study without providing a reason for this during the study. In order to do this, they should contact the recruiting researcher in person, or by phone or email. Data collected up to point of study withdraw will still be included in result analysis.. Participants may stop the sample collection at any point without providing a reason. They can do this by informing the researcher. The EBC device will be held by the participants during sample collection.

## 5. ADVERSE EVENTS

## 5.1 Definitions

Adverse Event (AE): any untoward occurrence in a participant.

Serious Adverse Event (SAE): any untoward and unexpected occurrence or effect that:

- Results in death
- Is life-threatening – *refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe*
- Requires hospitalisation
- Results in persistent or significant disability or incapacity

### 5.2. Reporting Procedures

All adverse events should be reported. Any questions concerning adverse event reporting should be directed to the Principal Investigator in the first instance.

#### 5.2.1 Non serious AEs

All such events, whether expected or not, should be recorded.

#### 5.2.2 Serious AEs

An SAE form should be completed and emailed to the Principal Investigator within 24 hours.

All SAEs should be reported to the London - Surrey Research Ethics Committee where in the opinion of the Principal Investigator, the event was:

- 'related', i.e. resulted from the administration of any of the research procedures; and
- 'unexpected', i.e. an event that is not listed in the protocol as an expected occurrence

Local investigators should report any SAEs as required by their Local Research Ethics Committee, Sponsor and/or Research & Development Office.

# 6. ASSESMENT AND FOLLOW UP

There is no planned follow up. Participants will only be informed of the results obtained from their samples if requested. It is unlikely that there will be any incidental findings however should there be any the PI will inform the participant.

# 7. REGULATORY ISSUES

## 7.1 Ethics approval

The Study Coordination Centre has obtained approval from the London – Surrey Research Ethics Committee (REC) and Health Research Authority (HRA). The study must also receive confirmation of capacity and capability from each participating NHS Trust before accepting participants into the study or any research activity is carried out. The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions.

## 7.2 Consent

Consent to enter the study must be sought from each participant only after a full explanation has been given, an information leaflet offered and time allowed for consideration. Signed participant consent should be obtained. The right of the participant to refuse to participate without giving reasons must be respected. All participants are free to withdraw at any time.

## 7.3 Confidentiality

The Principal Investigator will preserve the confidentiality of participants taking part in the study and fulfil transparency requirements under the General Data Protection Regulation for health and care research. Data and all appropriate documentation will be stored for a minimum of 10 years after the completion of the study, including the follow-up period.

## 7.4 Indemnity

Imperial College London holds negligent harm insurance policies which apply to this study.

## 7.5 Sponsor

Imperial College London will act as the main Sponsor for this study

## 7.6 Funding

The study is funded by a grant from Innovate UK. Participants will be reimbursed £30 per visit.

## 7.7 Audits

The study may be subject to inspection and audit by Imperial College London under their remit as sponsor and other regulatory bodies to ensure adherence to GCP and the UK Policy Framework for Health and Social Care Research.

# 8. STUDY MANAGEMENT

The day-to-day management of the study will be co-ordinated through Dr Omar Usmani

# 9. PUBLICATION POLICY

The methods and data from the evaluation of the EBC device, biosensor and algorithm will be presented at conferences to analytical sciences specialists. Data collected in this study will be presented at seminars and meetings with potential clinical collaborators.

The results of this study will be published in a peer-review analytical sciences journal to enable wider engagement of researchers and ensure dissemination of findings.

# 10. REFERENCES


[1] R. H. Green, C. E. Brightling, S. McKenna, B. Hargadon, D. Parker, P. Bradding, A. J. Wardlaw, and I. D. Pavord, "Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial," (in English), *Lancet,* Article vol. 360, no. 9347, pp. 1715-1721, Nov 2002.

[2] E. Andreeva, M. Pokhaznikova, A. Lebedev, I. Moiseeva, O. Kuznetsova, and J. M. Degryse, "Spirometry is not enough to make clinical diagnosis of COPD," (in English), *European Respiratory Journal,* Meeting Abstract vol. 48, p. 2, Sep 2016.

[3] N. Diab, A. S. Gershon, D. D. Sin, W. C. Tan, J. Bourbeau, L. P. Boulet, and S. D. Aaron, "Underdiagnosis and Overdiagnosis of Chronic Obstructive Pulmonary Disease," (in English), *American Journal of Respiratory and Critical Care Medicine,* Review vol. 198, no. 9, pp. 1130-1139, Nov 2018.

[4] F. ANDERSSON, S. BORG, S. JANSSON, A. JONSSON, A. ERICSSON, C. PRUTZ, E. R. RONMARK, and B. LUNDBACK, "The costs of exacerbations in chronic obstructive pulmonary disease (COPD)," *RESPIRATORY MEDICINE,* vol. 96, pp. 700-708, 2002.

[5] K. Larsson, C. Janson, B. Stallberg, K. Lisspers, P. Olsson, K. Kostikas, J. B. Gruenberger, F. S. Gutzwiller, M. Uhde, L. Jorgensen, and G. Johansson, "Impact of COPD diagnosis timing on clinical and economic outcomes: the ARCTIC observational cohort study," (in English), *International Journal of Chronic Obstructive Pulmonary Disease,* Article vol. 14, pp. 995-1008, 2019.

[6] W. T. Liu, C. H. Wang, H. C. Lin, S. M. Lin, K. Y. Lee, Y. L. Lo, S. H. Hung, Y. M. Chang, K. F. Chung, and H. P. Kuo, "Efficacy of a cell phone-based exercise programme for COPD," (in English), *European Respiratory Journal,* Article vol. 32, no. 3, pp. 651-659, Sep 2008.

[7] D. Nowak, M. Kasielski, A. Antczak, T. Pietras, and P. Bialasiewicz, "Increased content of thiobarbituric acid-reactive substances and hydrogen peroxide in the expired breath condensate of patients with stable chronic obstructive pulmonary disease: no significant effect of cigarette smoking," (in English), *Respiratory Medicine,* Article vol. 93, no. 6, pp. 389-396, Jun 1999.

[8] A. A. De Benedetto F, Dragani B, Spacone A, Formisano S, Cocco R, Sanguinetti CM, "Validation of a new technique to assess exhaled hydrogen peroxide: results from normals and COPD patients," *Monaldi Arch Chest Dis.,* vol. 55, no. 3, pp. 185-8, 2000.

[9] K. Kostikas, G. Papatheodorou, K. Psathakis, P. Panagou, and S. Loukides, "Oxidative stress in expired breath condensate of patients with COPD," (in English), *Chest,* Article vol. 124, no. 4, pp. 1373-1380, Oct 2003.

[10] I. Horvath, J. Hunt, P. J. Barnes, K. Alving, A. Antczak, B. Balint, E. Baraldi, G. Becher, W. J. C. van Beurden, A. Blomberg, M. Corradi, R. Dekhuijzen, R. A. Dweik, T. Dwyer, R. Effros, S. Erzurum, J. Freels, B. Gaston, C. Gessner, M. Goldman, A. Greening, L. P. Ho, J. M. Hohfeld, Q. Jobsis, S. A. Kharitonov, F. Kelly, D. Laskowski, C. Lehmann, A. Lindstrom, S. Loukides, D. Marlin, P. Montuschi, A. C. Olin, A. E. Redington, P. Reinhold, E. L. J. van Rensen, R. Robbins, I. Rubinstein, P. Silkoff, H. J. Smith, W. R. Steinhaeusser, W. G. Teague, K. Toren, G. Vass, J. Vaughan, C. Vogelberg, H. Wirtz, and A. E. T. F. E. Breath,

"Exhaled breath condensate: methodological recommendations and unresolved questions," (in English), *European Respiratory Journal,* Review vol. 26, no. 3, pp. 523-548, Sep 2005.
[11] P. N. R. Dekhuijzen, K. K. H. Aben, I. Dekker, L. Aarts, P. Wielders, C. L. A. vanHerwaarden, and A. Bast, "Increased exhalation of hydrogen peroxide in patients with stable and unstable chronic obstructive pulmonary disease," (in English), *American Journal of Respiratory and Critical Care Medicine,* Article vol. 154, no. 3, pp. 813-816, Sep 1996.
[12] I. Horvath, L. E. Donnelly, A. Kiss, S. A. Kharitonov, S. Lim, K. F. Chung, and P. J. Barnes, "Combined use of exhaled hydrogen peroxide and nitric oxide in monitoring asthma," (in English), *American Journal of Respiratory and Critical Care Medicine,* Article vol. 158, no. 4, pp. 1042-1046, Oct 1998.
[13] A. W. Dohlman, H. R. Black, and J. A. Royall, "EXPIRED BREATH HYDROGEN-PEROXIDE IS A MARKER OF ACUTE AIRWAY INFLAMMATION IN PEDIATRIC-PATIENTS WITH ASTHMA," (in English), *American Review of Respiratory Disease,* Article vol. 148, no. 4, pp. 955-960, Oct 1993.
[14] Q. Jobsis, H. C. Raatgeep, P. W. M. Hermans, and J. C. deJongste, "Hydrogen peroxide in exhaled air is increased in stable asthmatic children," (in English), *European Respiratory Journal,* Article vol. 10, no. 3, pp. 519-521, Mar 1997.
[15] A. Antczak, Z. Kurmanowska, M. Kasielski, and D. Nowak, "Inhaled glucocorticosteroids decrease hydrogen peroxide level in expired air condensate in asthmatic patients," (in English), *Respiratory Medicine,* Article vol. 94, no. 5, pp. 416-421, May 2000.
[16] B. R. Loyola, A. Bhushan, M. Schivo, N. J. Kenyon, and C. E. Davis, "Temperature changes in exhaled breath condensate collection devices affect observed acetone concentrations," (in English), *Journal of Breath Research,* Article vol. 2, no. 3, p. 7, Sep 2008, Art. no. 037005.
[17] K. O. Zamuruyev, E. Borras, D. R. Pettit, A. A. Aksenov, J. D. Simmons, B. C. Weimer, M. Schivo, N. J. Kenyon, J. P. Delplanque, and C. E. Davis, "Effect of temperature control on the metabolite content in exhaled breath condensate," (in English), *Analytica Chimica Acta,* Article vol. 1006, pp. 49-60, May 2018.
[18] K. Itaya, N. Shoji, and I. Uchida, "CATALYSIS OF THE REDUCTION OF MOLECULAR-OXYGEN TO WATER AT PRUSSIAN BLUE MODIFIED ELECTRODES," (in English), *Journal of the American Chemical Society,* Article vol. 106, no. 12, pp. 3423-3429, 1984.
[19] A. A. Karyakin, E. E. Karyakina, and L. Gorton, "The electrocatalytic activity of Prussian blue in hydrogen peroxide reduction studied using a wall-jet electrode with continuous flow," (in English), *Journal of Electroanalytical Chemistry,* Article vol. 456, no. 1-2, pp. 97-104, Sep 1998.
[20] A. A. Karyakin and E. E. Karyakina, "Prussian Blue-based 'artificial peroxidase' as a transducer for hydrogen peroxide detection. Application to biosensors," (in English), *Sensors and Actuators B-Chemical,* Article; Proceedings Paper vol. 57, no. 1-3, pp. 268-273, Sep 1999.
[21] S. S. Kumar, J. Joseph, and K. L. Phani, "Novel method for deposition of gold-prussian blue nanocomposite films induced by electrochemically formed gold nanoparticles: Characterization and application to electrocatalysis," (in English), *Chemistry of Materials,* Article vol. 19, no. 19, pp. 4722-4730, Sep 2007.
[22] V. Hornok and I. Dekany, "Synthesis and stabilization of Prussian blue nanoparticles and application for sensors," (in English), *Journal of Colloid and Interface Science,* Article vol. 309, no. 1, pp. 176-182, May 2007.
[23] T. Uemura and S. Kitagawa, "Prussian blue nanoparticles protected by poly(vinylpyrrolidone)," (in English), *Journal of the American Chemical Society,* Article vol. 125, no. 26, pp. 7814-7815, Jul 2003.
[24] A. A. Karyakin and M. F. Chaplin, "POLYPYRROLE PRUSSIAN BLUE FILMS WITH CONTROLLED LEVEL OF DOPING - CODEPOSITION OF POLYPYRROLE AND PRUSSIAN BLUE," (in English), *Journal of Electroanalytical Chemistry,* Note vol. 370, no. 1-2, pp. 301-303, Jun 1994.
[25] S. N. Sawant, N. Bagkar, H. Subramanian, and J. V. Yakhmi, "Polyaniline-Prussian blue hybrid: synthesis and magnetic behaviour," (in English), *Philosophical Magazine,* Article vol. 84, no. 20, pp. 2127-2138, Jul 2004.
[26] D. Raffa, K. T. Leung, and F. Battaglini, "A microelectrochemical enzyme transistor based on an N-alkylated poly(aniline) and its application to determine hydrogen peroxide at neutral pH," (in English), *Analytical Chemistry,* Article vol. 75, no. 19, pp. 4983-4987, Oct 2003.