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Assessment and validation of a device for measuring hydrogen peroxide in exhaled breath condensate in COPD

IRAS Project ID: 303156

Study Management Group

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Clinical Queries

Clinical queries should be directed to Dr Omar Usmani who will direct the query to the appropriate person

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:

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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 Chief 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.

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TITLE	Assessment and validation of a device for measuring hydrogen peroxide in exhaled breath condensate in COPD
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 individuals with COPD & healthy volunteers. (ii) Qualitative evaluation of the usability of the device and the collection protocols from participants. (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 COPD & healthy participants. This will be assessed by comparison with the gold standard fluorometry and mass spectrometry methods.
POPULATION	COPD & Healthy subjects
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 μM compared to healthy control 0.2 μM[7-9]). Even higher H_2O_2 concentrations are reported for patients with exacerbation, 0.6 μM 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

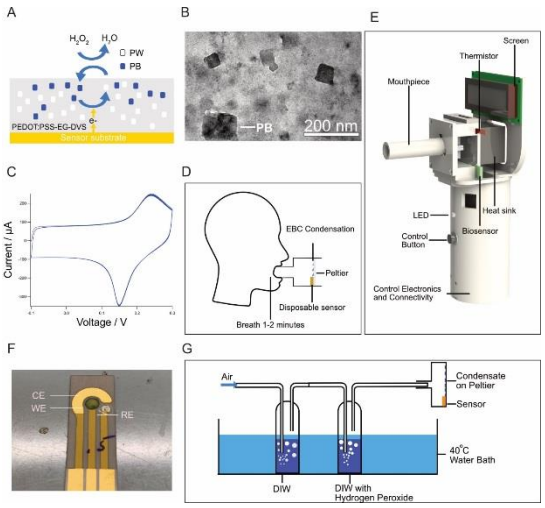


Figure 1: Schematic of the catalytic biosensor mechanism **B** TEM image of the electrode modifier **C** Reproducible i-V behaviour of the composite **D** condenser mechanism **E** Device schematic **F** disposable biosensor strip **G** Generation of test specimens for laboratory evaluation

Figure 2

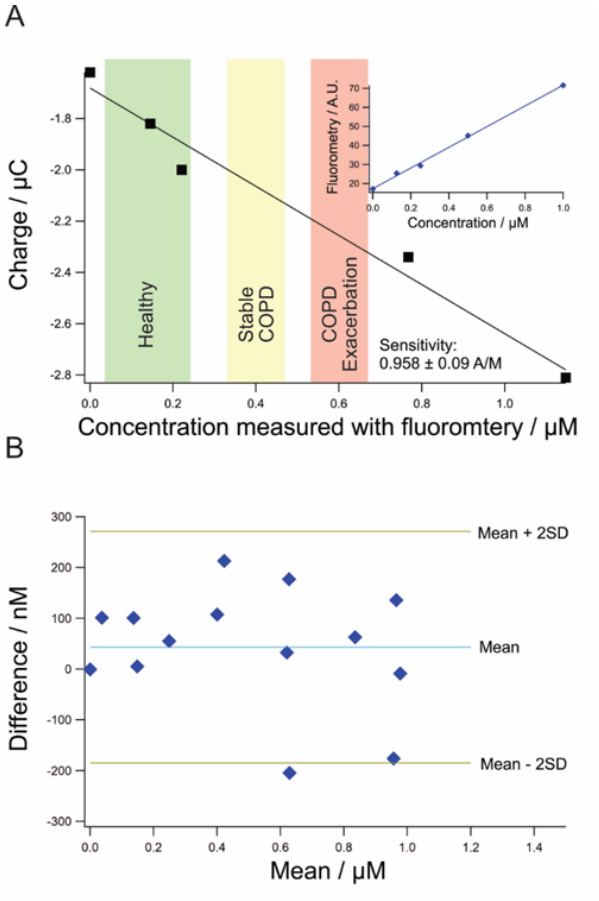


Figure 2: A Biosensor coulometry data measured with PEDOT:PSS-PB-EG-DVS modified sensor against fluorometry data measured with HRP and Amplex Red. The colour strips illustrate reported physiological H_2O_2 in EBC in various patient groups [7-9, 11]. The inset demonstrates the calibration standard for 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 and a clinical assessment in healthy volunteers (1st Clinical Evaluation) we now want to evaluate the accuracy and precision of the EBC device in patients with COPD (2nd Clinical Evaluation). We want to evaluate the reproducibility of 3 measurements a month apart in real breath samples in the same manner as the previous clinical evaluation with 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 substantially reduce these variations. Collecting replicate samples from patients with COPD will allow us to evaluate this approach in this patient group.

2. STUDY OBJECTIVES 2

The questions to answer in clinical evaluation:

1. Volume collectable in 3-5 minutes,
2. Concentration of hydrogen peroxide in COPD and healthy subjects,
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),
6. 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 further clinical evaluations.

3. STUDY DESIGN

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

We aim to recruit 15 patients with COPD and 15 healthy volunteers from our database of volunteers at the royal Brompton Hospital. Our purpose with this study is to evaluate the analytical performance of the device and the data processing and consistency of measurements over a 3-month period.

Three successive measurements will be made at monthly intervals in each patient with COPD and with each healthy volunteer requiring them to visit the Hospital on three occasions. The first visit also includes screening which will take up to 1 hour.

Firstly, each potential participant will be sent a participant information sheet.
If they decide that they wish to take part in the research study, they will be asked to come to the Clinical Research Facility (CRF) at the Royal Brompton Hospital, for screening where the

research team will further explain the study to the participant. 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 which they will be asked to sign before any procedures are made.

As part of screening, they will have a baseline screening questionnaire including a check of medications they are currently taking.

If the participant satisfies the screening criteria, they can then enter the study. After the consent form has been signed, participants will perform spirometry measurements prior to the following the breath collection procedures. These procedures will be performed on each study visit.

An exclusion questionnaire will be presented at the appointment.

Sample collection protocol

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 breathe 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.
It will not be possible to identify the participant from the sample labelling.
The individual results of the analysis will not be made available to the participants.

The flow chart of the sample collection is shown in Figure 3 (A&B) below, C & D shows the collection device.

Participants will also be asked to fill in a short questionnaire on visit 1 related to the use of portable medical devices.

Figure 3

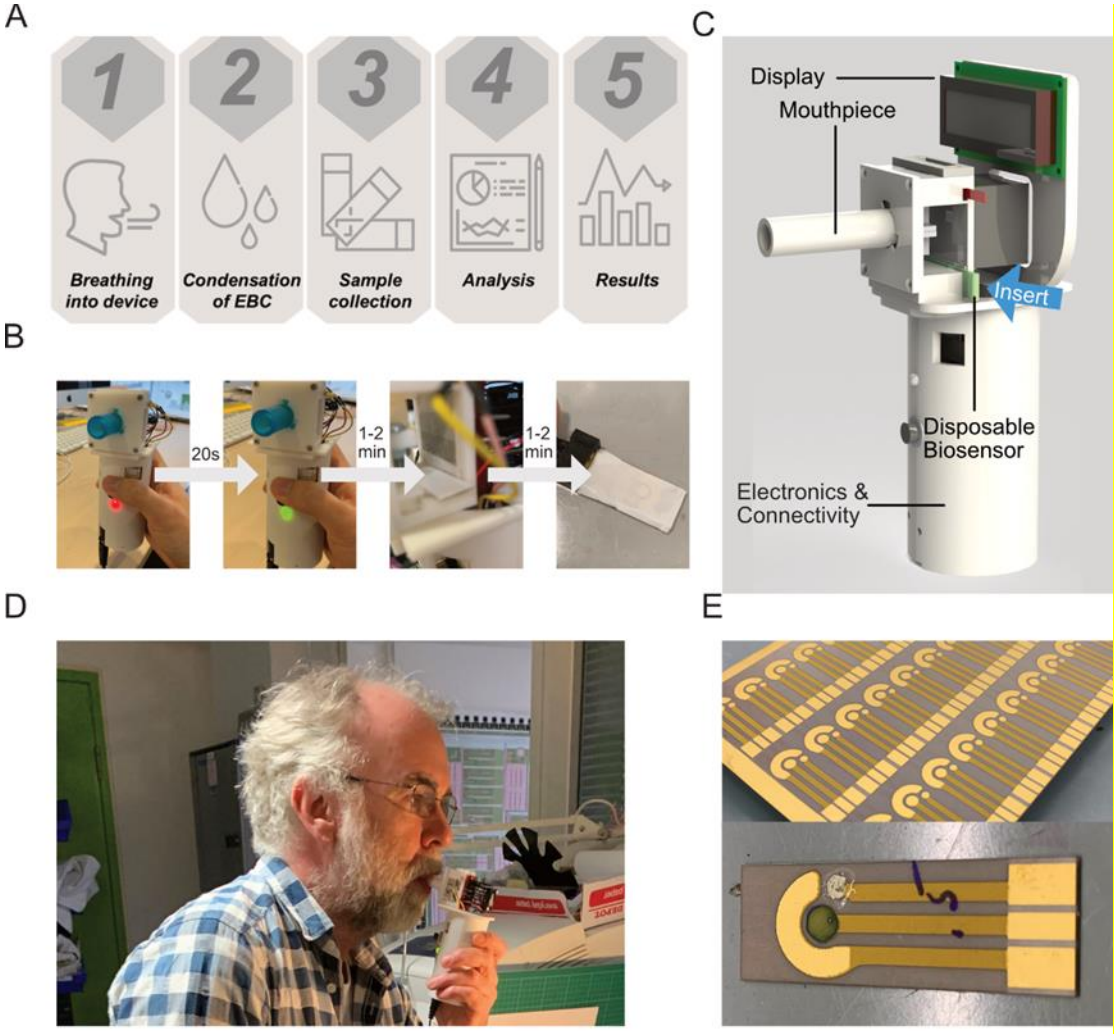
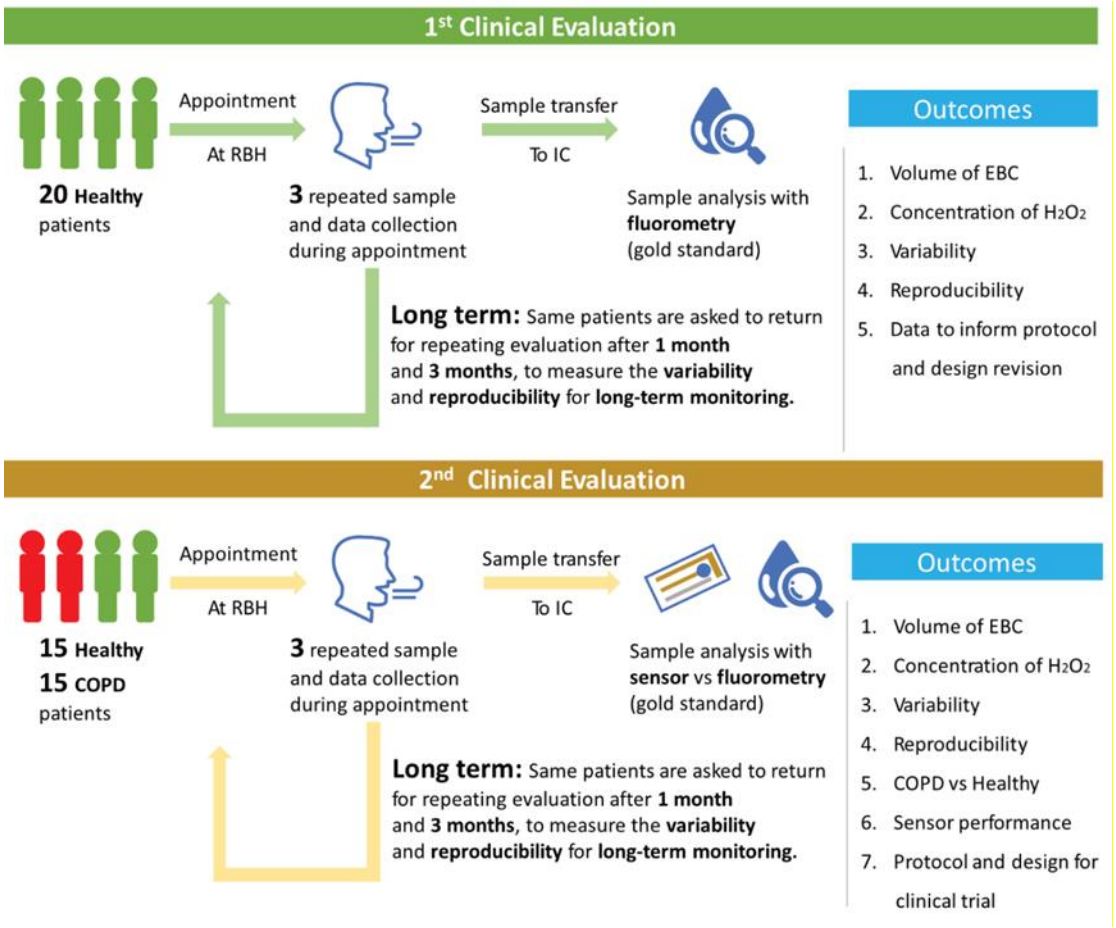

A
1
2
3
4
5
Breathing into device
Condensation of EBC
Sample collection
Analysis
Results
B
20s
1-2 min
1-2 min
C
Display
Mouthpiece
Insert
Disposable Biosensor
Electronics & Connectivity
D
E

Figure 4 Showing 1st and 2nd Clinical Evaluation



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 participant:

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:

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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

Potential participants 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 by email or post or in person.

4.2 Inclusion Criteria

COPD participants:

- A diagnosis of COPD.
- Not current smokers
- Stable COPD (no chest infection requiring antibiotics and/or oral steroids in the past 1 month).
- Able to give written informed consent prior to participation in the study including all of its procedures.
- Male or female subject aged 18 years and above 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

Healthy subjects:

- non-smokers
- Healthy individuals, free of significant disease.
- Able to give written informed consent prior to participation in the study including all of its procedures.
- Able to comply with the requirements and restrictions listed in the consent form.
- Male or female subject aged 18 years and above 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

COPD Participants:

- We will not recruit subjects who lack the capacity to consent.
- Current or past diagnosis of asthma.
- History of any chronic respiratory diseases other than COPD.
- History of another medical condition, which in the opinion of the Unit Physician, contraindicates his/her participation in the study.

- Unstable respiratory disease in the last four weeks prior to the screening visit (indicated by any change in their maintenance inhaled therapy or who have had a lower respiratory tract infection in the previous four weeks).
- Evidence of a respiratory exacerbation requiring emergency room treatment and/or hospitalisation within four weeks before screening.
- Use of systemic (oral or intravenous) steroids 4 weeks prior to inclusion (injectable depot steroids 6 weeks) or more than 3 periods during the last 12 months.
- Patients who have evidence of alcohol or substance abuse.
- Participation in another clinical trial with an investigational drug in the four weeks preceding the screening visit.
- 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.

Healthy subjects:

- 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.

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 unless this is explicitly specified by the participant. 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 medical occurrence in a patient or clinical study subject.

Serious Adverse Event (SAE): any untoward medical 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, or prolongation of existing inpatients' hospitalisation**
- **Results in persistent or significant disability or incapacity**
- **Is a congenital anomaly or birth defect**

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Medical judgement should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

5.2. Reporting Procedures

All adverse events should be reported. Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the Chief 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 Chief Investigator within 24 hours. However, relapse and death due to pre-existing conditions , and hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs.

All SAEs should be reported to the **South Central - Berkshire Research Ethics Committee** where in the opinion of the Chief Investigator, the event was:

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

Reports of related and unexpected SAEs should be submitted within 15 days of the Chief Investigator becoming aware of the event, using the NRES SAE form for non-IMP studies. The Chief Investigator must also notify the Sponsor of all related and unexpected SAEs.

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

Contact details for reporting SAEs
RGIT@imperial.ac.uk
CI email (and contact details below)
Please send SAE forms to: o.usmani@imperial.ac.uk
Tel: 0207 3518051 (Mon to Fri 09.00 – 17.00)

6. ASSESMENT AND FOLLOW UP

There is no planned follow up. Participants will not be informed of the results obtained from their samples. Any incidental findings made during the course of the study will be reported to the PI who will inform the participant's GP.

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7. STATISTICS AND DATA ANALYSIS

There was no formal size calculation as this is a preliminary study looking for consistency of result.

The data will be evaluated using Bland Altman plots. Performance will be evaluated in comparison with gold standard reference methods fluorimetry and mass spectrometry

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.

8. REGULATORY ISSUES

7.1 Ethics approval

The Study Coordination Centre has obtained approval from the South Central - Berkshire 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. After the participant has entered the study the clinician remains free to give alternative treatment to that specified in the protocol at any stage if he/she feels it is in the participant's best interest, but the reasons for doing so should be recorded. In these cases the participants remain within the study for the purposes of follow-up and data analysis. All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment.

7.3 Confidentiality

The Chief Investigator will preserve the confidentiality of participants taking part in the study and is registered under the Data Protection Act.

Data will be /pseudonymised

7.4 Indemnity

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

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7.5 Sponsor

Imperial College London will act as the main Sponsor for this study. Delegated responsibilities will be assigned to the NHS trusts taking part in 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.

9. STUDY MANAGEMENT

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

10. 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.

11. REFERENCES

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