Study Title: Development of a breath analysis test

NCT number: 04341012

Protocol version number and date: Version 1.2, March 18, 2020

Research Question and Aims

Hypothesis:
The analysis of breath contents can be used to detect human diseases

Aims, purpose, or objectives:

The purpose of the study is to develop a test based on breath analysis. The study will seek to collect and analyze breath samples from a broad range of patients to develop reference ranges and to refine the testing methods.

Aim 1: To establish breath sample collection and analysis techniques

Aim 2: To establish reference ranges for selected clinical populations, and to assess correlations between breath analysis and presence or absence of clinical or pathological evidence of disease.

The study will provide the basis for the use of breath analysis for future research studies to develop biomarkers of disease.

Background (*Include relevant experience, gaps in current knowledge, preliminary data, etc*.):

Breath analysis for the detection of volatile organic compounds has promise for use in diagnostic tests. These compounds in breath are the end product of metabolic processes in the body that are modulated by a variety of diseases. The best known example perhaps is the exhalation of acetone by diabetic patients during ketoacidosis. Analysis of these volatile compounds may be useful for monitoring, diagnosis and prognosis of disease. However, their analysis is complex and involves the use of specialized laboratory techniques. We will collect breath samples for analysis using Field Asymmetric Ion Mobility Spectrometry. If this technology can be used to reliably detect volatile compounds in breath, it may be possible to use breath analysis to develop new biomarkers of disease. This study will collect breath samples for use in developing analysis methods and to establish reference ranges for selected clinical and demographic groups of people.

Study Design and Methods

Methods: *Describe in lay terms, completely detailing the research activities that will be conducted by Mayo Clinic staff under this protocol*.

Subjects who are undergoing evaluation at Mayo Clinic in Florida will be candidates. A group of subjects who have had recent COVID-19 testing and have known test results will also be candidates. These subjects will be approached and invited to participate. Subjects may be identified from appointment calendars at Mayo Clinic Florida and approached about participation. All ethnic and racial groups are eligible for inclusion in this protocol. Attempts will be made to ensure that the racial and ethnic distribution among participants is reflective of the underlying eligible populations from which these participants are drawn.

Subjects will be approached about participating in the study during routine clinical visits and given sufficient time to ask questions and consider consenting. The study coordinator or PI will obtain consent electronically or on a paper form. Subjects will receive a copy of the consent document and a copy will be scanned into their medical chart.

Documentation of informed consent/HIPAA authorization will involve the use of the Research Participant Tracking (PTrax) Digital Signature Capture technology for research informed consent forms/HIPAA authorization forms. This is an institutionally approved process for obtaining consent/HIPAA authorization only while the subject, and/or the subject's representative, is in the physical presence of the person authorized to obtain consent. The study team may print a copy of the signed consent form/HIPAA authorization form for the subject or their representative. The consent form/HIPAA authorization form will also be available to the subject via the patient portal.

Note: If the subject or their representative prefers not to use the Digital Signature Capture technology, the study team will provide a paper consent form/HIPAA authorization form for signature.

If informed consent/HIPAA authorization waiver is requested, justify the waiver: N/A

Specimen collection
Subjects will have one breath sample collection during a single visit. There will be no follow-up procedures or visits. Breath samples will be collected during normal tidal breathing for a period up to fifteen minutes in the clinic area or in the exam rooms in the Clinical Studies Unit. Breath samples will be collected in sample containers and taken to the processing laboratory in Griffin 1 for analysis. The sample contents will be analyzed and any residual breath samples will be discarded.

Data will be abstracted from the patients existing medical record . Data collection will include demographics, medical history, current medications, smoking and alcohol history, and clinical, laboratory or pathological data from any recent visit. For the group of patients who have had COVID-19 testing, the results of the testing (either positive or negative) will be recorded.

Breath samples will be collected using BioVOC-2 breath samplers and Bio-monitoring thermal desorption (TD) tubes (Markes International). These tubes contain a porous polymer and carbon adsorbents that trap organic molecules. Prior to use, tubes are preconditioned and undergo a leak test using a Unity Thermal Desorption unit. Each BioVOC-2 sampler and separable disposal mouthpiece(s) will be single use per patient, and disposed after collection.

Study participants will be asked to exhale normally into the BioVOC-2 to capture ~100 mL of exhaled alveolar (end-tidal) air. Participants will be asked to repeat this process three separate times per sample tube. After each breath, the sample will be expunged onto a TD tube. The tubes will be capped using brass end caps, and stored at 4° C.

For analysis, the caps will be removed and the tubes will be placed directly into a thermal desorption unit without any further manipulation. The tubes will be heated in the unit to 40 ℃ for 2 minutes, then ramped up at 5 ℃ per minute to 120 ℃, maintained at this temperature for 2 minutes and then further ramped up at 8 ℃ per minute to 200 ℃ and maintained at this temperature for 6 minutes. This evaporates the contents of the tube and the vapors emitted are then directly collected and analyzed using gas chromatography and FAIMS, within a closed system. At these temperatures it is expected that all virus particles will be degraded.

Of note, the infectivity of SARS Coronavirus was lost after heating at 56 ℃ (1,2) and thermal desorption is used for the clean-up and removal of organic contaminants from environmentally contaminated superfund sites.

1. Rabenau HF, et al. Stability and inactivation of SARS coronavirus. Med Microbiol Immunol. 2005 Jan;194(1-2):1-6.
2. Chan KH et al. The Effects of Temperature and Relative Humidity on the Viability of the SARS Coronavirus. Adv Virol. 2011;2011:734690.

Specimen and Data Storage

Specimens will be stored at 4C in Griffin 1 after collection, and until the time of analysis. Specimens will be labeled with a unique study ID number and will not include any patient identifiers. All data abstracted for study participants will be entered into the Research Electronic Data Capture (REDCap) database which is secure and web-based. Describe how the database is maintained and secured: The database is only accessible from the internal Mayo Clinic server and is password protected. The database will be maintained by study staff.

Data will be entered directly from existing medical records into the database. Ideally, data will be entered directly into REDCap and data collection forms will not be used. This should be possible for data abstracted from medical records, radiology reports, laboratory reports, etc. It may be necessary logistically, however, to collect some data on forms and then enter them into REDCap. Data collected on data collection forms will be stored in the patients research chart kept in a secure location. The database is password protected and accessible only by the PI and study staff. The records will contain only the minimum amount of PHI needed for data collection.

If a subject withdraws consent, the specimen and data will be destroyed and excluded from further use.

Risks

The risks to subjects will be minimal. The breath sample collection will be done using a loose fitting mask with attached sample collection containers. Patients will be asked to breathe in and out as they would normally. The collection will be continuously monitored, and stopped once the collection containers are full, which can take upto ten minutes. For patients with suspected respiratory tract infections, breath collection will be done by using disposable collection devices. The samples will be collected by trained staff, with the use of personal protective equipment as appropriate.

Policies/procedures to manage risks to subjects:
Subjects will be identified using a unique identifier. Data will be store in a web-based, password protected data management system that is only accessible to study staff.

Potential risks to privacy and confidentiality:
Access to data will be restricted and will not become part of the medical record. Subjects will be assigned a unique identifier. Data and specimens will be restricted to the PI and study staff. Unique IDs will be used and minimal patient identifiers will be used to prevent invasion of privacy or breaches of confidentiality. Data will be stored on a secure, web-based, and password protected databased. Information about current or future studies will not be documented in the medical record. The database is password protected and accessible only by the PI

and study staff. Subjects will be identified by a randomly assigned study ID number. The records will contain only the minimum amount of PHI needed.

The information generated will be used for development of the test profiles. No information will be returned back to the patient or become part of the medical record.

Subject Information

Target accrual is the proposed total number of subjects to be included in this study at Mayo Clinic. A "Subject" may include medical records, images, or specimens generated at Mayo Clinic and/or received from external sources.

Target accrual: 300

Subject population (children, adults, groups): Persons aged 18 years or older

Inclusion Criteria:
a) Subjects 18 years or older
b) Able to provide informed consent

Exclusion Criteria:
None

Biospecimens

Collection of blood samples. When multiple groups are involved copy and paste the appropriate section below for example repeat section b when drawing blood from children and adults with cancer.

a. **From healthy, non-pregnant, adult subjects who weigh at least 110 pounds**. For a minimal risk application, the amount of blood drawn from these subjects may not exceed 550ml in an 8 week period and collection may not occur more frequently than 2 times per week.
   Volume per blood draw: _____ml
   Frequency of blood draw (e.g. single draw, time(s) per week, per year, etc.) _____

b. **From other adults and children considering age, weight, and health of subject.** For a minimal risk application, the amount of blood drawn from these subjects may not exceed the lesser of 50 ml or 3 ml per kg in an 8 week period, and collection may not occur more frequently than 2 times per week.
   Volume per blood draw: _____ml
   Frequency of blood draw (e.g. single draw, time(s) per week, per year, etc.) _____

Prospective collection of biological specimens other than blood: _____

Review of medical records, images, specimens

Check all that apply (data includes medical records, images, specimens).

☐ Only data that exists before the IRB submission date will be collected.

Date Range for Specimens and/or Review of Medical Records:
Examples: *01/01/1999 through 12/31/2015*, or all records through *mm/dd/yyyy*.

Note: The Date Range must include the period for collection of baseline data, as well as follow-up data, if applicable.

☐ The study involves data that exist at the time of IRB submission **and** data that will be generated after IRB submission. Include this activity in the Methods section.
Examples

- The study plans to conduct a retrospective chart review and ask subjects to complete a questionnaire.
- The study plans to include subjects previously diagnosed with a specific disease and add newly diagnosed subjects in the future.

☐ The study will use data that have been collected under another IRB protocol. Include in the Methods section and enter the IRB number from which the research material will be obtained. *When appropriate, note when subjects have provided consent for future use of their data and/or specimens as described in this protocol*.

Enter one IRB number per line, add more lines as needed

☐ Data ☐ Specimens ☐ Data & Specimens _____

☐ Data ☐ Specimens ☐ Data & Specimens _____

☐ Data ☐ Specimens ☐ Data & Specimens _____

Data Analysis

Power analyses may not be appropriate if this is a feasibility or pilot study, but end-point analysis plans are always appropriate even if only exploratory. Provide all information requested below, or provide justification if not including all of the information.

Power Statement:
Not applicable

Data Analysis Plan:
The samples will be used to generate a profile of breath constituents.

Endpoints
Primary: Detection of a breath constituent profile