HEI

ADDITIONAL MATERIALS AVAILABLE ON THE HEI WEB SITE

Research Report 186

Ambient and Controlled Particle Exposures as Triggers for Acute ECG Changes

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Additional Materials 1. Quality Assurance Procedures

Additional Materials may appear in a different order than in the original Investigators' Report, and some remnants of their original names may be apparent. HEI has not changed the content of these documents, only their numeric identifiers.

Additional Materials 1 was originally Appendix U.

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Appendix U. Quality Assurance Procedures

As part of this study, we re-analyzed ECG recordings for the Rochester REHAB, UPCON, and UPDIABETES studies. To do these analyses, holter monitors were annotated first automatically and then annotated by a trained technician using standard procedures. See the Standard Operating Procedure for this beat annotation (Standard Operating Procedure #1 "WI for H Scribe 4 32"). Next, RR intervals were exported to a custom made HRV program which produced the ECG outcomes used in our analyses (Standard Operating Procedure #2 "Super ECG").

We also assayed total antioxidant capacity in blood samples from both the Augsburg Panel Study and the Rochester REHAB Study. The Standard Operating Procedure (SOP) for those analyses is presented below (Standard Operating Procedure #3).

Last, our data management procedures both with the Augsburg data and Rochester data are also provided below (Standard Operating Procedure #4). However, we do not provide SOP's for data collection or data management procedures from the original work from these 4 studies, as they are already completed and data collection was not part of this analysis/study. We do discuss the data management and data checking procedures used with the new ECG data and total antioxidant analyses data generated for the study.

Standard Operating Procedure #1
"WI for H Scribe 4 32"

University of Rochester – Heart Research Follow-up
Mortara H Scribe Scanner
HRFUP NIG

Purpose (1.0) High speed, PC based, Mortara Holter scanner

Scope (2.0) Mortara H- Scribe Scanner Version 4.32

Requirements (3.0)
   H-Scribe Version 4.32 software loaded on your computer

Prerequisites (3.1)

1) Flash card with a valid recording
2) Means of identifying the subject

Acquiring data from the flash card (3.2)
1. Insert the flash card into the flash card reader
2. Select patient screen will appear with lines 1-99
3. Select the working location, O:\LQTS\LQT3NC
4. Select the next Empty line to work on
5. Left Click on the "Connect To" button located near the bottom of screen
6. A box will appear, click "Acquire"
7. The data will automatically load in the Patient line you selected

Patient Information Screen (3.3)
1. Verify the subject ID matches
2. Left Click on the scan criteria, turn on the Long RR/ Pause to Detect Normal to Normal, set your SVPS Prematurity, Turn off the A-Fib detect and Left click on OK and this screen will close
3. Left click on the Auto Scan button to begin, a profile screen will appear when uploading is complete.

Edit Templates (3.4)
1. Left click on Templates box
2. Templates with the same morphology will appear
3. To edit left click of the incorrectly labeled beat
4. Right click on the same and select proper classification

Profile Summary Screen Editing (3.5)
1. Left click the mouse on the desired event and you will be moved into the rhythm screen, right click on the desired beat and the menu will allow you to re-label that beat.
2. Confirm "minimum rate", "minimum RR", "maximum rate", Longest RR, pauses, supraventricular and ventricular ectopies.

Selecting Strips (3.6)
      1. From the Profile Screen select ECG
      2. From the ECG screen select the Select Strip icon from the tool bar
      3. Move the red box over the desired beats and left click the mouse
      4. The edit strip box appears, label the strip

Making Annotations and Printing Report (3.7)
      1. From the pull down menu along the top of screen Select View, Select Report Preview, Select Report Option, Select Override, here you can type the conclusion of the analyzes (rhythm, heart rates, supraventricular and ventricular ectopies, AV block, Bundle Branch blocks, ST elevation/depression, QT measurements). Left click OK button.
      2. Close the Report Options Screen
      3. Left Click Print Button
      4. You need to print on the Mortara PDF and also need a paper printed report to keep on the MexDM1 study binder.

Post requisites (3.8)
      1. The physician will give a final interpretation
      2. Annotated Data should be moved to the safe server

Revisions History and Approvals

Reason for Revision (3.9)

Implementation (4.0)

Approvals (4.1)

Document Owner   _____   Date _____
Name:
Title:

Management
Representative   _____ Date _____
Name:
Title:

Standard Operating Procedure #2
"SOP Super ECG"

University of Rochester – Heart Research Follow-up
Running Super ECG and Spectrum
HRFUP NIG

Purpose (1.0) Super ECG and Spectrum Programs

Scope (2.0) Running Super ECG and Spectrum

Requirements (3.0)
Super ECG Version 4.0 on your computer
Spectrum Version 2.0 on you computer

Prerequisites (3.1)

1) A folder made on the C drive labeled superecgdata
2) Existing Mortara annotated data found on server or loaded data into a USR/Pat #

Super ECG Program (3.2)

Open Super ECG Program:
Select Process Database
Map the pathway to the Annotated Holter Data you want to run, into the Source   Path field and click the OK box.
Wait for program to stop running
The data you requested has been exported to the your computer folder   C:\superecgdata

Spectrum Program (3.3)

Open the Spectrum Program after you have completed running Super ECG   program on the Annotated Holter data.
Select Process Database
Map the pathway to the Super ECG Output data folder on your computer called
C:\superecgdata, in the Database Path field and click the OK box.
The data you requested has been exported to the your computer folder   C:\superecgdata

Moving Data (3.4)
The data should be moved to server location for this study under Additional Programs Super ECG

Revisions History and Approvals

Reason for Revision (3.5)

Implementation (3.6)

Approvals (3.7)

Document Owner   _____   Date _____
Name:
Title:

Management
Representative   _____ Date _____
Name:
Title:

Standard Operating Procedure #3
"Total Antioxidant Capacity"

OxiSelect™ Total Antioxidant Capacity (TAC) Assay Kit

Cell Biolabs, Inc. STA-360

The Cell Biolabs OxiSelect™ Total Antioxidant Capacity (TAC) Assay Kit is used to measure total antioxidant capacity within a sample. The assay is based upon the reduction of copper (II) to copper (I) by antioxidants such as uric acid. Upon reduction, the copper (I) ion further reacts with a coupling chromogenic reagent that produces a color with a maximum absorbance at 490 nm. The results for unknown samples are compared to a standard curve prepared from known uric acid concentrations. Results are expressed as "µM Copper Reducing Equivalents" or "mM Uric Acid Equivalents". The copper assay method detects all classes of antioxidants, including thiols, with marginal radical interference.

Plasma Sample Preparation:

Collect blood in heparin or citrate tube and centrifuge at 1500 g for 10 minutes at 4°C. Remove the plasma and aliquot samples for testing. Samples can be run immediately or stored at -80°C for later analysis. EDTA can interfere with the TAC assay and should not be present in any sample.

Preparation of reagents:

1. 1X reaction buffer: dilute the reaction buffer 1:100 with 1X PBS. Mix to homogeneity. Store at 4°C for up to three months.
2. 1X copper ion reagent: dilute the copper ion reagent 1:100 with deionized water.   Mix to homogeneity. Store at 4°C for up to three months.
3. 1X stop solution: Dilute the stop solution 1:10 with deionized water.   Mix to homogeneity. Store at 4°C for up to three months.

Preparation of uric acid standard curve:

1. Prepare fresh uric acid standards by weighing out the uric acid powder for a 5 mg/ml solution in 1N NaOH. This 5 mg/ml is equivalent to a concentration of 30 mM/. Use the 30 mM uric acid solution to prepare a 2mM solution of uric acid (e.g. add 100 µl of the 30 mM uric acid standard to 1.400 ml of deionized water).
2. Prepare a series of the remaining uric acid standards according to the table below.

Tubes	2mM Uric Acid Antioxidant Standard (µl)	Deionized Water (µl)	Resulting Uric Acid Concentration (mM)
1	1000	0	2
2	500 of tube #1	500	1
3	500 of tube #2	500	0.5
4	500 of tube #3	500	0.25
5	500 of tube #4	500	0.125
6	500 of tube #5	500	0.0625
7	500 of tube #6	500	0.03125
8	0	500	0

Assay protocol:

Each uric acid standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

1.   Add 20 µl of the diluted uric acid standards or samples to a 96-well microtiter plate.
2.   Add 180 µl of the 1X reaction buffer to each well using a multichannel pipette. Mix thoroughly.
3.   Obtain an initial absorbance by reading the plate at 490 nm.
4.   To initiate the reaction, add 50 µl if the 1X copper ion reagent into each well. Incubate 5 minutes on an orbital shaker.
5.   Add 50 µl of 1X stop solution to each well to terminate the reaction.
6.   Read the plate again at 490 nm.

Calculation of the results:

1.   Calculate the net absorbance by subtracting the initial absorbance readings for samples and standards from the final readings taken for each.
2.   Plot the net absorbance against the uric acid concentration for the uric acid standard curve.
3.   Calculate the antioxidant capacity of unknown samples by comparing the net OD 490 nm values of samples to the uric acid standard curve. Result : **mM uric acid equivalents**.

Standard Operating Procedure #4
Data Management

A. AUGSBURG PANEL STUDY

We only included the examinations of participants with valid ECG recordings and blood withdrawals as well as the baseline examination of these subjects. Of the initial 112 individuals who participated in the add-on program, two subjects had no valid ECG recording. One further participant with ECG recordings had no valid blood withdrawals. These three individuals were excluded. Hence, 109 subjects with ECG recordings and blood withdrawals were available for analysis. One baseline visit of a subject with ECG recordings and blood withdrawals was missing (N=108 blood withdrawals at baseline). By mistake five blood samples were not sent to Rochester for determination of total antioxidant capacity and are therefore missing. Overall, 459 examinations of 109 subjects were available for analyses.

All TAC and other study data were checked for plausibility and validated before preparing datasets used for analyses. In general, checks included:
- check for uniqueness (no ID twice) and completeness,
- check of panel classification
- check of differences between visit dates and times,
- check of number of visits per participant,
- check of extreme values of continuous variables,
- check of values of categorical variables,
- check of "if yes, then…" variables,
- check of variables being contradictory,
- check for missing values.

Changes to and corrections of the data have been documented.

Ambient air pollution and meteorology measurements

Hourly means of air pollutants and meteorological variables were measured at a fixed monitoring station in the urban background of Augsburg, Germany. Missing values of meteorological variables and particulate air pollutants were not replaced since <1% of 24h-averages were missing (meteorological variables, ozone, $PM_{2.5}$, PM_{10}) or no parallel measurement with other devices (UFP) was available. For the TAC analyses, and each participant and visit, we calculated individual 24h-average concentrations of each pollutant in the 24 hours preceding the clinical examination (0-23h) and up to the previous 4 days (24-47h, 48-71h, 72-95h, 96-119h) if more than two-thirds of the hourly measurement were available.

Data Backup

During the *field phase* electronic data that were made anonymous were stored regularly on the server of the Helmholtz Zentrum München where data are saved daily by the computing service center. In addition, data were saved twice to DVD. Paper/pencil forms were filed in folders which were stored in lockable rooms of the Helmholtz Zentrum München. After *completion of data management* all electronic data (e.g. correspondence per email, miscellaneous documents, and final datasets) were stored on an external hard drive. All paper/pencil forms will be stored for 10 years.

B. ROCHESTER REHAB, UPCON, AND UPDIABETES STUDIES

For the REHAB Study, we started with 1489 ECG recordings from 76 subjects. However, 3 subjects had inadequate ECG recordings to conduct 1 hour analyses, leaving 73 subjects and n=657 blood samples to measure total antioxidant capacity as described above.

For the UPCON Study, we started with n=20 subjects. However, 1 subject did not have adequate ECG recordings for either the UFP exposure or clean air exposure, and three subjects had ECG recordings from only 1 exposure, resulting in 19 subjects and 35 recordings (24 hours in length) available for any 1 hour or 5 minute analysis.

For the UPDIABETES Study, we started with n=19 subjects. Again, 1 subject did not have ECG recordings available for either exposure, and two subjects had ECG recordings from only 1 exposure (17 subjects with an UFP exposure and 17 subjects with a clean air exposure), resulting in 18 subjects and 34 recordings available (48 hours in length) for any 1 hour or 5 minute analysis described below.

All ECG and TAC data were checked for plausibility and validated before preparing datasets for analysis. Similar to the data checks listed above, our checks of these ECG and TAC data included:
- check of differences between visit/exposure dates and times and ECG recording times,
- check of number of visits/exposures per study subject,
- check of extreme values and outliers of continuous variables,
- check of values of categorical variables,
- check for missing values.

Changes to and corrections of any data were documented by study staff. All statistical SAS code includes *comments* to describe what each section of code is intended to do. All SAS code creating study analysis results presented in tables and figures of the HEI Final Report were reviewed and checked for errors by a second study staff member, independent of the programmer creating the code.

Ambient air pollution and meteorology measurements

For the Rochester REHAB Study, hourly means of air pollutants and meteorological variables were measured at a fixed monitoring station in Rochester, New York. We did not replace missing values of any meteorological variable or particulate air pollutant during the study period. For the TAC analyses, for each participant and visit, we calculated individual 24h-average concentrations of each pollutant in the 24 hours preceding the clinical examination (0-23h) and up to the previous 4 days (24-47H, 48-71h, 72-95h, 96-119h) if at least 75% of the hourly values were non-missing. For all other analyses, we used just the 1 hour values.

Data backup

All datasets (including original datasets received from the coordinators or data managers of each Rochester study, the new ECG data, ambient air pollution data, new TAC data, and the analysis datasets used in the study), as well as all SAS code, SAS output, and other files related to this study are stored on password protected computers and servers in the Department of Public Health Sciences, Biostatistics and Computational Biology, Pulmonary and Critical Care Medicine, and the Heart Research Follow-up Program at the University of Rochester. They are accessible only to study personnel. These files are backed up nightly as part of regular university IT protocols.