Jackson Heart Study Visit 4

Manual 12

Quality Assurance and Quality Control

Version 2

October 31, 2023

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Version History

Version #	Version date	Revisions
1	10/20/2020	n/a
2	10/31/2023	• Removed sagittal abdominal diameter measurement • Added ankle-brachial index measurement • Added pain sensory measurement • Revised repeated QC measurement for anthropometry • Added analysis of digital preference • Added sensory study reliability QC protocol • Added creatinine calibration protocol • Added methylation QC protocol • Revised personnel certification form

1. Quality Assurance and Quality Control Procedures

Quality assurance procedures include the following activities:

1) Detailed protocol development. A clear description of the study design, training, certification, and the various data collection activities provides the blueprint for the study. Each protocol is a written reference for staff and researchers. Procedures for handling the routine, as well as the exceptional, are given. Those protocols constitute the JHS Visit 4 Manuals of Operation.

2) Training. Training is the transfer of the study plans in the protocol to the research staff. The process has resulted in clarification and revision of the protocol. Special materials for this purpose have been developed for JHS and are the basis for continuing education during the study.

3) Certification. Criteria to examine the adequacy of an individual's training have been established. Individuals meeting these criteria are qualified to execute a protocol or a segment of it. Certification indicates that an acceptable performance standard has been mastered or an adequate knowledge of material has been achieved. The Coordinating Center (CC) monitors the study to ensure that the research staff performs only those functions for which they are certified.

Quality control procedures involve monitoring data collection by observation (directly and by audio or video tape recording) and quantitative assessment (using repeated measurements and statistical analysis of study data). Monitoring is performed both by personnel within the Field Center and when necessary by monitoring visits from the CC.

1) Observation monitoring. Over-the-shoulder observations of staff by supervisors are made to identify techniques that need improvement and points where the protocol is not being followed. Also, periodic monitoring visits by CC staff are made to observe clinic activities. Immediate feedback is given on issues related to protocol adherence, and recommendations for improvements are given to the Field Center Principal Investigator (PI) for action.

2) Quantitative monitoring. Repeat measurements taken by the same and different technicians are used as quality control tools. Randomly re-doing a fraction of an individual's work may not only stimulate better overall quality of data, but also allows estimation of measurement reliability. At the time of reporting the results of the study, it is important to establish that the “error” in the data is not so large as to threaten the validity of conclusions. In addition, descriptive statistics and graphical representation of study variables by technician and month are monitored to identify differences among technicians or trends over time.

2. Monitoring of Data Quality and Implementing Corrective Action

A Quality Control Subcommittee (QCSC) is designated by the JHS Steering Committee to coordinate and direct the quality control activities. The QCSC has representation from the CC, the Field Center, reading centers, laboratories and NHLBI. The QCSC is charged with establishing the content of the quality control reports and reviewing them with specific attention given to deviation from protocols, recurrent problems and temporal trends.

The QCSC conducts monthly conference calls to discuss issues that arise and review QC reports. The QCSC prepares recommendations to the Steering Committee (SC) to advise them of a problem and to discuss the mechanism for correction.

The CC is responsible for preparation and dissemination of QC reports. The laboratory and reading centers are responsible for providing the CC with reports of the quality of specimens and images collected for incorporation into the QC reports.

The QC reports are distributed to the Field Center PI, study coordinators, the QCSC and the SC. The following individuals should respond to the reports as follows:

1) Field Center PI, study coordinators: Review each QC report including technician-specific performance measures; identify a solution to each problem; implement corrective action; report corrective action to the QCSC.

2) Quality Control Subcommittee: Review each QC report with attention to deviation from protocol, recurrent technician or Field Center problems, and temporal trends; contact Field Center, reading center, or laboratory investigators to review data quality problems and ensure solutions are proposed; monitor the implementation of corrective action.

3) Steering Committee: Review QC summary reports; monitor data quality trends; direct the QCSC in areas needing special attention; propose changes to protocol when necessary.

3. Certification Procedures

All Exam Center staff must attend the JHS training meeting. This training covers all aspects of the study protocol and is led by individuals with specific expertise in the given exam component. In addition, staff must be certified in the certain areas in order to begin data collection. Specific criteria and requirements for training and certification are summarized below in **Table 1**.

Table 1. Training and Certification Criteria for JHS Exam 4 Interviews and Procedures

Component	Certification requirement	Recertification requirements	Additional observations	Certifier or reviewer
Interviews				
General Interview	Adequate technique on 5 observations or taped interviews	Annually, 1 taped participant interview included in round robin review	Two recordings in the first month of the study Quarterly observations thereafter	Exam Center Manager, Interviewer Team Leader
Medical Interview	Adequate technique on 5 observations or taped interviews	Annually, 1 taped participant interview included in round robin review	Two recordings in the first month of the study Quarterly observations thereafter	Exam Center Manager, Interviewer Team Leader
Food Frequency Questionnaire	Adequate technique on 5 observations or taped interviews	Annually, 1 taped participant interview included in round robin review	Two recordings in the first month of the study Quarterly observations thereafter	Exam Center Manager, Interviewer Team Leader
Informant Interview	Adequate technique on 5 observations or taped interviews	Annually, 1 taped participant interview included in round robin review	Two recordings in the first month of the study Quarterly observations thereafter	Exam Center Manager, Interviewer Team Leader
Six Item Screener	Adequate technique on 5 observations or taped interviews	Annually, 1 taped participant interview included in round robin review	Two recordings in the first month of the study Quarterly observations thereafter	Exam Center Manager, Interviewer Team Leader
Procedures				

Component	Certification requirement	Recertification requirements	Additional observations	Certifier or reviewer
Informed Consent	Adequate technique on 5 consents	Semi-annually (January/July) 2 taped consents	n/a	Exam Center Manager, Director of Data Acquisition
Anthropometry Height Weight Waist Hip	Two replicate measures Agreement with trainer: Height: within 0.25 cm Waist: within 2.2 cm Hip: within 2.2 cm Weight: within 0.2 kg	Semi-annually (January/July), results sent to CC annually Annual recertification for lead technician	Twice for the first month following certification	Exam Center Manager, Lead Technician
Blood Pressure, Sitting	Two observations Cuff size agreement with trainer and follow BP measurement protocol	Semi-annually (January/July), results sent to CC annually Annual recertification for lead technician	Quarterly	Exam Center Manager, Lead Technician, Director of Data Acquisition
Neurocognitive Test	Three audio-taped neurocognitive assessments and a PDF of each Neurocognitive Booklet (and relevant scoring materials)	As needed	Five observations after certification. QC recording once every two months	Neurocognitive Consultant
Physical Function	Review MOP, training videos and an in-person/web-conference training session with AS team	Annual review of MOP and training videos	Quarterly	Exam Center Manager, Lead Technician
Pulse Wave Velocity	Successful demonstration of calibration checks, blood pressure cuff and neckband placement, care and maintenance of the equipment, and data transfer	As needed	Continuous monitoring of PWV quality by AS Investigator Frequency of reports to CC monthly	Ancillary Study Investigator
ZioPatch	Successfully complete the registration of a participant on the iRhythm website	Semi-annually (January/July)	n/a	Ancillary Study Investigator/ iRhythm Technician
Electrocardiography	Three ECGs reviewed by Reading Center	As needed	Continuous monitoring of ECG quality by Reading Center Monthly reports to CC	ECG Reading Center, Senior certified Technician

Component	Certification requirement	Recertification requirements	Additional observations	Certifier or reviewer
Echocardiogram	Two ECHOs reviewed by Reading Center	As needed	Continuous monitoring of image quality by Reading Center Weekly reports to CC	Echo Reading Center
Brain MRI	Scan a phantom using the electronically loaded JHS Human Scan protocols	As needed when there is a scanner software and/or hardware upgrades	Continuous monitoring of image quality by Reading Center	MRI Reading Center
Ankle brachial blood pressure	Perform at least three live readings. Conduct exam according to protocol as demonstrated by a QC checklist	Semi-annually (January/July) Annual recertification via direct observation	n/a	Ancillary study investigator
Sensory (pain) test	Complete training requirements, pass algometer calibration test, conduct exam on two volunteers according to protocol as demonstrated by a QC checklist	As needed	n/a	Ancillary study investigator
Biospecimen Collection, Processing and Shipping				
Venipuncture	Two acceptable draws, processing and shipping	Semi-annually (January/July)	Quarterly	Lead Lab Technician or Exam Center Manager
Urine	Two acceptable collections, processing and shipping	Semi-annually (January/July)	Quarterly	Lead Lab Technician or Exam Center Manager
Results Reporting				
Results Reporting	Adequate technique on 5 reports	Annually review of examples of results reports	n/a	Exam Center Manager, Director of Data Acquisition
Letters sent to Participants with Reports of Exam Results	Two batches of accurate letters and reports	Annual review of methods	n/a	Exam Center Manager, Director of Data Acquisition
Exam Visit Review				
Data Inventory	Accurate review of data inventory	Annually review of methods	n/a	Exam Center Manager, Director of Data Acquisition

Component	Certification requirement	Recertification requirements	Additional observations	Certifier or reviewer
Referrals	Adequate technique on 5 referrals	Annually review of referral guidelines	n/a	Exam Center Manager, Director of Data Acquisition
Exit Interview	Accurate interview technique	Annual review of methods	n/a	Exam Center Manager, Interviewer Team Leader
Other				
Participant Safety	Local review of safety procedures	Annual safety review	n/a	Exam Center Manager
Medication coding	N/A	Annually; coding a set of selected medication names and blinded recoding of 10% of medications recorded during the previous year	n/a	CC

Study coordinators will submit a Certification Form (Appendix 1) to the CC to document that a staff member has completed the necessary requirements for certification. The Certification Request Form documents how, when and which procedures/interviews were certified. The CC will assign a staff code number upon receipt of this form. Should staff learn more procedures and interviews for certification since the initial certification request, a re-submission of the form is needed to update those new areas of certification.

The CC will continually update records of all certifications at the Field Center, and staff code numbers will be compared against the data collection forms to ensure that only certified staff perform data collection on the specific procedures/interviews to which they have been assigned. Additional training and supervision will be carried out as individually needed at the Field Center. Continued supervision will be the responsibility of the study coordinator. If the QCSC finds that the Field Center is lacking in certification requirements or the quality of data collection, the Field Center will be notified.

4. Minimum Frequency of Procedures and Interviews to Maintain Certification

Table 2 shows the minimum frequency of procedures and interviews performed by study technicians to maintain certification.

Table 2. Minimum frequency of procedures and interviews to maintain certification

Procedure/Interview (Associated Forms)	Min. # / Month Primary Technicians	Min. # / Month Designated Back- up Techs
General Interview (AEI, FSQ, LSQ, PAC, PSI, SES, SLE, STS, QOL)	4	2
Medical Interview (DQF, HBF, LLE, MHX, MSR, NHX, PFH, PFX, RSQ)	4	2
Food Frequency Questionnaire (FFQ)	4	2

Procedure/Interview (Associated Forms)	Min. # / Month Primary Technicians	Min. # / Month Designated Back- up Techs
Six Item Screener (SIS)	4	2
Anthropometry (ANT)	6	2
Blood collection & processing (BIO)	4	4
Seated BP (SBP)	4	2
Physical Function (TMW)	4	2
Physical Ability (PAQ)	4	2
Neuropsychiatric Inventory (NPI)	4	2
Clinical Dementia Rating (CDP, CDI, CDS)	4	2
Neurocognitive + Mini-Mental State (MME) + Summary (NCS)	4	2
Depressive Symptoms Form (CES)	2	1
Pulse Wave Velocity (PWV)	6	6
Electrocardiogram (ECG)	3	3
Echocardiogram (EPC)	4	4
MRI (MPC)	4	4
Zio®XT Patch (AFC)	4	2
Ankle Brachial Blood Pressure (ABI)	4	4
Sensory Test (PST)	4	4

5. Frequency for Observation and Equipment Checks

The frequency of observations of interviewers and technicians and associated forms to be completed are summarized in **Table 3**. The frequency of equipment checks and the associated forms to be completed are summarized in **Table 4**.

Summary of observation and equipment checklists is submitted to the CC quarterly using the Summary of Observation and Equipment Checklists (Appendix 2).

Table 3. Frequency of Observations of Interviewers and Technicians

Frequency	Observation and Equipment Checks	Forms to be Completed
Twice during the first month	1. Anthropometry technicians observed twice for the first month following certification	Checklist for Observation of Anthropometry Measures (Appendix 3)
	2. Interviewers audio-recorded. Two recordings in the first month of the study a. General interviewers b. Medical interviewers c. Food frequency questionnaire interviewers d. Informant interviewers e. Neurocognitive interviewers	Checklist for Observation of Interview Technique (Appendix 4)

Frequency	Observation and Equipment Checks	Forms to be Completed
Once every two months	1. Neurocognitive interviewer audio-recorded. One recording every other month	Evaluation by Neurocognitive Consultant
Quarterly	1. Interviewer observed	Checklist for Observation of Interviewing Technique (Appendix 4)
	2. Blood pressure technicians observed	Checklist for Observation of Blood Pressure Measurements (Appendix 5)
	3. Biospecimen collection, processing observed	Checklist for Observation of Biospecimen Collection and Processing (Appendix 6)
	4. Physical function technicians observed	Checklist for Observation of Physical Function (Appendix 7)

Table 4. Frequency of Equipment Checks

Frequency	Observation and Equipment Checks	Forms to be Completed
Daily	1. Anthropometry scales balanced to read zero	Anthropometry Equipment Calibration Log (Appendix 8)
	2. Temperature check in refrigerators, freezers, etc.	Equipment Temperature Log (Appendix 9)
	3. ECG machine	ECG Maintenance Sheet (Appendix 10)
	4. Availability of all sizes of blood pressure cuffs and measuring tapes	Supply checklist (Appendix 11)
Weekly	1. Anthropometry scales calibrated	Anthropometry Equipment Calibration Log (Appendix 8)
	2. Check the Zio®XT Patch inventory to ensure devices not expired	Supply checklist (Appendix 11)
Monthly	1. Pulse wave velocity	Pulse Wave Velocity Equipment Maintenance Log (Appendix 12)
	2. ECG machine	ECG Maintenance Sheet (Appendix 10)
Quarterly	1. OMRON BP monitor	OMRON BP Monitor Maintenance and Calibration Log (Appendix 13)
Semi-annually	1. Grip strength dynamometer calibrated	Grip Strength Dynamometer Calibration Log (Appendix 14)
Annually	1. Tanita scale professionally calibrated and serviced	Summary of Observation and Equipment Checklists (Appendix 2)
	2. Checking of the actual speed of the centrifuge with a tachometer	Summary of Observation and Equipment Checklists (Appendix 2)
	3. Calibration and professional cleaning of pipettes	Summary of Observation and Equipment Checklists (Appendix 2)
As needed	1. Anthropometry scales calibrated when moved	Anthropometry Equipment Calibration Log (Appendix 8)

6. Quality Control Repeated Measurements for Anthropometry

To estimate the reliability of anthropometry measures, 5% of the participants will have anthropometric measurements repeated by a second technician at the same visit.

Descriptive statistics of repeated measurements and the differences of repeated measurements will be calculated. Bland-Altman plots of the differences vs. the means of the repeated measurements will be presented. Variability of the repeated measurements will be analyzed statistically to track variability overtime for trends.

Additionally, the proportion of repeated measurements with a difference outside of ± 2 repeatability standard deviations (RSD) based on QC repeated measurements collected during Visit 3 and the beginning of Visit 4 (7/21-11/21) (**Table 5**) will be calculated. The staff ID's associated with these differences will be analyzed to identify potential needs for staff re-training.

Table 5. Pooled Repeatability Standard Deviations (RSD) of QC Repeated Measurements Collected during Visit 3 and Visit 4

Anthropometry measure	N	RSD	2 x RSD
Height (cm)	228	0.2	0.4
Waist (cm)	269	1.1	2.2
Hip (cm)	269	1.2	2.4
Weight (kg)	234	0.2	0.4

7. Analysis of Digit Preference

Digit preference will be monitored for blood pressure and anthropometric measures. A Pearson chi-square goodness-of-fit test will be done to test the null hypothesis that all possible final digits (0, 1, 2, 3, 4, 5, 6, 7, 8, 9) are observed with equal frequency, N/k, where N is total number of observations and k is 10 for all possible final digits.

8. Reliability Testing for Quantitative Sensory Testing (QST) Measures

Metronome reliability testing

The technicians should perform a series of PPT readings using an algometer on a hard surface such as table or desk. During the tests, a timer will be set to 5 and 10 seconds, and the technician will apply pressure with the algometer while recording the readings. When the timer is set to 5 seconds, the reading should be approximately 2.5 kg and when set to 10 seconds, the readings should be close to 5 kg. This test should be done once a week to maximize consistency across the technicians.

Intra-rater reliability testing (completed by primary technician)

The primary technician will perform intra-rater reliability testing monthly. The primary technician will perform pain pressure threshold test (PPT) 1 (includes 3 PPT trials) and PPT 2 (includes 3 PPT trails with BP cuff applied and pain rating of 4 or more) on a participant or volunteer. The participant or volunteer will be asked to return at minimum 2 hours and maximum 48 hours to repeat the procedure by the primary technician.

Inter-rater reliability testing (completed by primary technician and backup technician)

The primary technician and back up technician will perform inter-rater reliability testing monthly. The two technicians, out of each other's view, will perform one set of PPT 1 and PPT 2 measurements on a participant or volunteer. The technicians should perform the assessments on the same participant or volunteer with at least a 30-minute break between the two technicians.

A detailed protocol is included in Appendix 15.

9. Creatinine Calibration

A common method to determine incident kidney disease is to look at changes in eGFR, a calculated value based on serum creatinine. Since serum creatinine measured in Visits 1, 3 and 4 were all done in different labs using different instruments/methods, the ability to harmonize data over visit years and lab sites is crucial to using these data longitudinally. A detail protocol for creatinine calibration is included in Appendix 16.

10. Methylation Data Quality Control

The JHS Genetics Working Group will perform additional quality control on existing methylation data collected using Visit 1 specimens. These quality control processes using current best practices will be followed consistently in the expected next batch of JHS methylation data created by the TOPMed program. A quality control plan proposed by the working group is included in Appendix 17.

11. Analysis of Study Data for Quality Control Purposes

The methods to monitor the quality of the data collection process include analyses of the study data itself, overall and by technician. There will be periodic reporting by the CC on:

1) Completeness of data collection
2) Specimens collection (blood and urine)
3) Analysis of repeated measure
4) Analysis of digit preference
5) Analysis of out of range values
6) Data transfer from reading centers and central lab
7) Quality of specimens and images collected
8) Quality of food frequency questionnaire collected
9) Descriptive statistic

A QC report template in included in Appendix 18.

Appendices

Appendix 1 Certification Form

CERTIFICATION FORM

Instructions: This form outlines the certification components for the JHS visit and various ancillary studies. This form facilitates the communication of training completion and certification to the Coordinating Center (CC). The list of certification components is separated into those components that require the site supervisor to certify successful completion of training and those components that require either a central trainer or CC staff to certify successful completion of training.

Table 1 documents which procedures/interviews a staff member is certified to perform per the site supervisor. The completed form is submitted by the **site supervisor** to *jhsccrc@umc.edu* at the CC for final evaluation and assignment of staff code number. Table 2 lists procedures/interviews for which a specified trainer or CC staff member must certify training completion. In these cases, **the trainer/certifier or CC staff member** should email *jhsccrc@umc.edu* and include the study staff member's name and/or staff code, component(s) certified, and the date certification completed.

Date: _____

Staff Name: _____ or code number: _____ (if already assigned)

Supervisor: _____

Specify which procedure/interviews the staff member has been certified to collect.

Table 1. Training certified by site supervisor

Measurement/Procedure/Interview	Method of Certification*	Date Certified
REDCap		
Informed Consent (ICT)		
Participant Safety (PSA)		
General Interview (AEI, FSQ, GCS, LSQ, PAC, PAQ, PCE, PSI, QOL, SES, SLE, TOB)		
Medical Interview (DQF, LIT, MSA, MSB, NHX, PHX, RSQ, WHX)		
Food Frequency Questionnaire (FFQ)		
Anthropometry (ANT)		
Sitting Blood Pressure (SBP)		
Physical Function (PFX, TMW)		
Lower Extremity Exam (LEE)		
Exit Interview (PFF)		
Results Reporting (RAR)		

*Codes for method of certification:

1 = Attended JHS training meeting
2 = Certified by central trainer
3 = Direct observation by the local certified lead staff member in specified area
4 = Completed written exam
5 = Completed practice. Specify how many sets of practice were performed, and the differences of the measurements compared to the local trainer's for local certification.
6 = Other (specify)
7 = N/A (not applicable to the staff member)
8 = Attended webinar

The following should be reported via email to *jhsccrc@umc.edu* by the Trainer/Certifier listed or CC staff member.

Table 2. Training certified by the trainer or applicable CC staff.

Measurement/Procedure/Interview	Trainer/Certifier
Specimen collection and processing (BIO, BIP)	Central laboratory
Neurocognitive test, including neurologic interview, informant interview	Tiffany Owens
Pulse wave velocity	Hirofumi Tanaka
Ankle brachial blood pressure	Keith Diaz/ABI device rep
Electrocardiography	ECG Reading Center
Echocardiogram	Echo Reading Center
Ziopatch (ZRE, ZPC)	James Floyd
Sensory Study	Tuhina Neogi
Platelet Study	Andrew Johnson
ABPM	Marwah Abdallah/Spacelab rep
24H-ACT	Kelley Gabriel
Stand-up	Keith Diaz
Mindfulness	Tanya Spruill
Resistant hypertension	Rikki Tanner

Coordinating Center Use Only

Assigned staff code number: _____

Appendix 2 Summary of Observation and Equipment Checklists

Instructions: This form should be completed quarterly and sent to the Coordinating Center (CC).

Date:

Quarterly reporting period:

A. Observation Checklist

	Technician ID	Supervisor ID	Date (mm/dd/yy)
General interview			
Medical interview			
Food frequency questionnaire			
Informant interview			
Neurocognitive assessments			
Anthropometry			
Sitting blood pressure			
Biospecimen collection			
Physical function			

B. Equipment Checklist

	Frequency	No. times assessed	No. times within calibration
Anthropometry			
1) Tanita scale read zero	Daily		
2) Balance beam read zero	Daily		
3) Wheelchair scale read zero	Daily		
4) Tanita scale calibrated	Weekly		
5) Balance beam calibrated	Weekly		
6) Wheelchair scale calibrated	Weekly		
7) Tanita scale professional calibrated	Annually		
Blood Pressure			
1) Checks for the OMRON BP machine	Quarterly		
Physical Function			
1) Grip strength dynamometer	Semi-Annually		
Biospecimen collection			
1) Refrigerators, freezers, room temp	Daily		
2) Speed of centrifuge	Annually		
3) Calibration and professional cleaning of pipettes	Annually		
Echo machine	Quarterly		
ECG machine	Quarterly		
Ziopatch	Quarterly		
PWV			
1) Maintenance Procedure (submit Maintenance Sheet to CC with this checklist)	Monthly		

Comments:

Appendix 3 Checklist for Observation of Anthropometry Measurement

Instructions: This checklist documents observation of anthropometry technicians by supervisors. Quarterly checklists and logs are summarized onto the **Summary of Observation and Equipment Checklists** (Appendix 2).

Tech ID: Supervisor ID: Date:

	Yes	No	Comments
1. Anthropometry is done BEFORE the snack			
2. If the participant is wearing any nylon hose other than knee highs, the participant is instructed to remove hose			
3. Participant is wearing light-weight, non-constricting underwear			
4. Participant is wearing a light clothes or scrub suit			
5. Participant has removed shoes			
6. Participant has emptied bladder			

Weight Measurement

	Yes	No	Comments
A. Equipment			
1. Scale firm on floor			
2. 10 kg standard weight available			
3. Anthropometry Equipment Calibration log up-to-date			
B. Procedure			
1. Participant prepared and procedure explained			
2. Participant is bare-foot			
3. Position of participant on center of scale			
4. Balance achieved			
5. Recordings completed			

Technician's measurement of participant weight: _____ kg

Supervisor's measurement of participant weight: _____ kg

Waist Measurement

	Yes	No	Comments
1. Procedure is explained to participant			
2. Participant stands erect, yet relaxed, with weight equally distributed on both feet, and feet together			
3. Place the tape horizontally at the level of the umbilicus (navel)			
4. Participant takes a normal breath and gently exhales, holding breath in a relaxed manner at the end of exhalation			
5. Tape is horizontal and snug, but not tight enough to compress tissue. [Invert tape, if needed, to ensure reading edge of tape is snug to skin for measurement.]			
6. Reading is recorded to the nearest centimeter, rounding down			

Technician's measurement of participant waist: _____ cm

Supervisor's measurement of participant waist: _____ cm

Hip Measurement

	Yes	No	Comments
1. Procedure is explained to participant			
2. Participant stands erect, yet relaxed, with weight equally distributed on both feet, and feet together			
3. Place the tape horizontally at the maximal protrusion of the gluteal muscles			
4. Tape is horizontal and snug, but not tight enough to compress tissue. [Invert tape, if needed, to ensure reading edge of tape is snug to skin for measurement.]			
5. Reading is recorded to the nearest centimeter, rounding down			

Technician's measurement of participant hip: _____ cm

Supervisor's measurement of participant hip: _____ cm

Arm Measurement

	Yes	No	Comments
1. Procedure is explained to participant			
2. Have the participant remove his/her upper garment or clear the upper arm area so that an unencumbered measurement may be made			
3. Have the participant stand, with the right/left arm hanging and bending the elbow so that the forearm is horizontal (parallel) to the floor			
4. Measure arm length from the acromion (bony protuberance at the shoulder) to the olecranon (tip of the elbow), using the tape measure			
5. Mark the midpoint on the dorsal surface of the arm			
6. Have the participant relax arm alongside of the body			
7. Draw the tape snug around the arm at the midpoint mark. Keep the tape horizontal. Tape should not indent the skin			
8. Reading is recorded to the nearest centimeter, rounding down			

Technician's measurement of participant arm: _____ cm

Supervisor's measurement of participant arm: _____ cm

Comments:

Appendix 4 Checklist for Observation of Interviewing Technique

Interviewer ID: Supervisor ID: Date:

Proficiency in Interview technique

	Yes	No	Comments
1. Introduces her/himself at beginning of the interview			
2. Thanks participant at the end of the interview			
3. Explains purpose of interview when appropriate, e.g., reads introductions or transition statements when included on form			
4. States questions exactly as written, stressing time frame and key elements			
5. Demonstrates familiarity with content, flow, definitions, and skip patterns			
6. Uses standardized tone of voice with supportive, non-judgmental statements			
7. Paces interview in response to participant's level of comprehension/comfort			
8. Trains participant in response patterns when appropriate			
9. Refrains from probing except to clarify ambiguous, unclear, or inconsistent responses			
10. Uses standardized definitions when asked for clarification			
11. Repeats questions stressing portions of question which were misunderstood			
12. Interviewer demonstrates knowledge of participant's tracing information			
13. Selects appropriate type of probe			
14. Accurately records participant's responses			

Comments:

Appendix 5 Checklist for Observation of Blood Pressure Measurements

Instructions: This checklist documents observation of technicians certified to perform blood pressure by supervisors. Quarterly checklists and logs are summarized onto the **Summary of Observation and Equipment Checklists** (Appendix 2).

Tech ID: Supervisor ID: Date:

	Yes	No	Comments
1. Checks function settings on OMRON unit (ENTER, 3 inflations, 30)			
2. Checks Mode and P-setting on OMRON unit			
3. Makes sure AC adapter for OMRON unit is securely connected (tends disconnect from unit)			
4. Checks AC adapter cord and tubing for cracks			
5. Cleans all the equipment			
6. Allows subject to rest for five full minutes			
7. Performs arm measurement and cuff selection properly			
8. Identified brachial pulse location properly			
9. Proper cuff placement			
10. Attaches cuff to monitor			
11. Uses proper settings on OMRON unit			
12. Turns monitor on with ON/OFF button			
13. Sets MODE selector to AVG			
14. Sets P-SET knob to AUTO			
15. Pushes START button			
16. Records 1st, 2nd, 3rd and average systolic and diastolic BP readings and average heart rate			
17. Instructions to participant are clear			
18. Holds arm vertically for 5 seconds between readings			
19. Informs participant of average readings			

Comments:

Appendix 6 Checklist for Observation of Biospecimen Collection and Processing

JHS Exam 4 Phlebotomy - Supervisor Checklist

DATE: ☐☐ **mo** ☐☐ **day** ☐☐ **year**

Technician Name/ID: _____

Supervisor: _____

Please check the appropriate box if technician performance is satisfactory for each line item. Please note any comments or remedial action taken in 'Comments' section if performance was not satisfactory.

Preparation:

1. ☐ Phlebotomy area properly prepared and stocked with supplies (tube rocker, ice bucket, extra draw tubes & labels, etc.).
2. ☐ Blood draw tubes in correct order and correctly labeled.
3. ☐ Checked Phlebotomy Form has correct participant ID.
4. ☐ Questions on Phlebotomy Form asked and answers recorded.

Venipuncture:

5. ☐ Non-permeable lab coat, gloves, and face shields used.
6. ☐ Correct preparation of venipuncture site.
7. ☐ Venipuncture smoothly executed.
8. ☐ Tubes filled in correct draw tube priority order.
9. ☐ Any replacement tubes correctly labeled.
10. ☐ Tourniquet released within 2 minutes; time noted on Phlebotomy form.
11. ☐ Proper appropriate care of venipuncture site after needle is removed.
12. ☐ Needle & tubing appropriately disposed.

Handling of filled draw tubes:

13. ☐ The correct tubes inverted and placed on the rocker for the time limits specified in the protocols.
14. ☐ Filled tubes placed in the correct racks - on ice or at room temperature – ASAP per protocol.
15. ☐ EDTA, serum tubes < ½ full discarded.

P/P Form:

16. ☐ Correct sample ID labels on both pages of Phlebotomy/Processing form.
17. ☐ Venipuncture starts and end times legibly recorded on the Phlebotomy form.
18. ☐ Elapsed tourniquet time noted on form.
19. ☐ Form completely filled out, and any comments recorded in the Comments section.

Urine:

20. ☐ Urine collection container correctly labeled and urine section on Phlebotomy Form completed.

Comments: _____

JHS Exam 4 Laboratory Processing - Supervisor Checklist

DATE: ☐ ☐ ☐ **Technician Name/ID:** _____
mo **day** **year**

Supervisor: _____

Please check the appropriate box if technician performance is satisfactory for each line item. Please note any comments or remedial action taken in 'Comments' section if performance was not satisfactory.

Preparation:

1. ☐ Aliquot racks organized and cryovials checked that they are correctly labeled
2. ☐ Non-permeable lab coats, gloves, and face shields (if desired) used.

Stage 1:

3. ☐ Time checked to ensure tubes are processed within the correct time limits post venipuncture per protocol.
4. ☐ Equipment is checked to ensure all tubes requiring centrifuging are centrifuged at the correct temperature and speed.
5. ☐ EDTA plasma from 10 mL tubes pooled before aliquoting into correctly labeled and color-coded cryovials.
6. ☐ New pipet tip used for each sample type and aliquots kept on ice during aliquoting.
7. ☐ Packed red blood cells from each tube transferred to correctly labeled freezing tube.
8. ☐ Filled cryovials checked off on the Processing Form and frozen upright @ -80 ^{o}C within 10 minutes

Stage 2:

9. ☐ Time monitored to ensure serum tubes remain at room temperature for > 40 minutes and < 90 minutes.
10. ☐ Serum from 10 mL tubes pooled before aliquoting into correctly labeled and color-coded cryovials.

Processing Completion:

11. ☐ Urine is kept refrigerated until aliquoting into correctly labeled tubes (#25-32)
12. ☐ Processing area and equipment is cleaned with appropriate disinfectant.
13. ☐ Processing Form completely filled out, including recording all blood and urine aliquots obtained and if any are less than the required volume. Any comments noted in comment section.

Comments: _____

_____

_____

Supervisor Signature _____

JHS Exam 4 Laboratory Refrigerated Shipping - Supervisor Checklist

DATE: ☐ ☐ ☐ **Technician Name/ID:** _____
mo **day** **year**

Supervisor: _____

Please check the appropriate box if technician performance is satisfactory for each line item. Please note any comments or remedial action taken in 'Comments' section if performance was not satisfactory.

Preparation:

1. ☐ Shipping packs have been stored at -20ºC for at least 24 hours.
2. ☐ All packing materials are available and in good condition
3. ☐ Shipping list printed of samples to be shipped

Shipping:

4. ☐ Line Styrofoam shipper(s) with absorbent material (i.e. absorbent pads).
5. ☐ Place 2 FROZEN ice packs on the bottom of the mailer. Place packing material on top of the ice packs. This can be more absorbent material, several small pieces of cardboard, etc. This prevents direct contact of the frozen ice packs with the refrigerated samples.
6. ☐ Collect the EDTA blood draw Tube #5 samples to be shipped, and check the participant ID numbers against the Processing Forms and Shipping Forms for that shipment.
7. ☐ Place each blood draw tube in an individual slot in the adsorbent blood draw sleeves.
8. ☐ Lay the tubes (wrapped in the absorbent sleeves) on their side in a freezer box. If there are 12 or less tubes, use a 5 x 5 x 2 inch freezer box. If there are more than 12 tubes use a 5 x 5 x 3 inch box or multiple 5x5x2 inch boxes.
9. ☐ Put lid in place and secure with a rubber band.
10. ☐ Place each freezer box in a leak-proof zip top plastic bag with absorbent pad, then carefully place these bagged boxes in the shipping container. The rubber band helps prevent freezer boxes from opening and spilling contents; the zip top bag serves as an additional form of containment, and the absorbent material is essential in the event of a thaw and spill.
11. ☐ Place another layer of absorbent material on top of the bagged freezer boxes containing the samples.
12. ☐ Add 2 more FROZEN ice packs on top of this last layer of absorbent material in the shipping container.
13. ☐ Place the Processing Forms for all the samples included in the shipment, along with a copy of the corresponding Shipping Form(s), in a zip top bag, then place the zip top bag **on top** of the Styrofoam lid before securely taping the outer cardboard sleeve closed.

14. ☐ The outside of all packages must be marked with the following:
    - A UN3373 Biological Substance Category B label
    - A completed Fed-Ex Airbill for Fedex ***Priority Overnight***
    - The shipper and recipient's name, address, and phone number

15. ☐ E-mail notification of the shipment, including the FedEx airbill number(s) and number of participant sample sets shipped, the day samples are packaged to: Elaine.Cornell@uvm.edu and Rebekah.Boyle@uvm.edu

16. ☐ All packages will be shipped to:

Rebekah Boyle/ JHS
University of Vermont,
Laboratory for Clinical Biochemistry Research
360 South Park Drive,
Colchester, VT 05446
Attn: Rebekah Boyle JHS
(802) 656-8938

Comments: _____

_____

_____

Supervisor Signature _____

JHS Exam 4 Laboratory Frozen Shipping - Supervisor Checklist

DATE: ☐ ☐ ☐ **Technician Name/ID:** _____

mo **day** **year**

Supervisor: _____

Please check the appropriate box if technician performance is satisfactory for each line item. Please note any comments or remedial action taken in 'Comments' section if performance was not satisfactory.

Preparation:

1. ☐ Ensure sufficient dry ice is available for shipping.
2. ☐ All packing materials are available and in good condition
3. ☐ Shipping list printed of samples to be shipped

Shipping:

4. ☐ Line Styrofoam shipper(s) with absorbent material (i.e. absorbent pads).
5. ☐ Place approximately ½ the dry ice (~5-7 lbs) on the bottom of the shipping container.
6. ☐ Place another layer of absorbent material on top of the dry ice – so it will be between the dry ice and the zip top plastic bag enclosing freezer boxes containing samples.
7. ☐ Collect the freezer boxes containing samples to be shipped, and check the participant ID numbers against the Processing Forms and Shipping Forms for that shipment.
8. ☐ For the 2" size freezer boxes, place an absorbent strip inside the freezer box on top of the cryovials. Replace the freezer box lid and secure closed with a rubber band. Place each freezer box in a leak-proof zip top plastic bag, ensuring the bag is properly zipped closed, then carefully place these bagged boxes in the shipping container. The rubber band helps prevent freezer boxes from opening and spilling contents; the zip top bag serves as an additional form of containment, and the absorbent material is essential in the event of a thaw and spill.
9. ☐ Place another layer of absorbent material on top of the bagged freezer boxes containing the samples.
10. ☐ Add remaining dry ice on top of this last layer of absorbent material in the shipping container.
11. ☐ Place the Processing Forms for all the samples included in the shipment, along with a copy of the corresponding Shipping Form(s), in a zip top bag, then place the zip top bag **on top** of the Styrofoam lid before securely taping the outer cardboard sleeve closed.
12. ☐ Affix shipping label(s) to the shipping container. Make certain to ship Fedex **priority overnight** [NOTE this is ***not*** first overnight] (Package samples as close to time of FedEx pick-up as possible to minimize the length of time on dry ice.)
13. ☐ E-mail notification of the shipment, including the FedEx airbill number(s) and number of participant sample sets shipped, the day samples are packaged to: Elaine.Cornell@uvm.edu and Rebekah.Boyle@uvm.edu

Mailing Address:

Rebekah Boyle/ JHS
University of Vermont,
Laboratory for Clinical Biochemistry Research
360 South Park Drive,
Colchester, VT 05446
Attn: Rebekah Boyle JHS
(802) 656-8938

Comments: _____

_____

_____

Supervisor Signature _____

Appendix 7 Checklist for Observation of Physical Function

Instructions: This checklist documents observation of technicians responsible for physical function by the lead supervisor. Quarterly checklists and logs are summarized onto the **Summary of Observation and Equipment Checklists** (Appendix 2). Copies of this log may be requested by the CC.

Tech ID: Supervisor ID: Date:

Chair Stands

	Satisfactory Yes/No	Comments
1. Back of chair against a wall		
2. Script correctly and clearly delivered		
3. Correctly demonstrates single stand, emphasizing keeping arms tight across chest		
4. Correctly demonstrates two stands, emphasizing full stand and return to complete sit		
5. Says “ready? Go” for each test		
6. Counts each chair stand and record final time when participant comes to a full standing position on the fifth stand		
7. Records and explains unusual values		
8. If task was not performed, codes and explains reasons		

Standing Balance/Side-by-side Stand

	Satisfactory Yes/No	Comments
1. Script correctly and clearly delivered		
2. Correctly demonstrates position		
3. Timing started coincident with participant release and stopped when participant takes a step or holds on		
4. If task was not performed, codes and explains reasons		

Semi-tandem Stand Standing Balance/Side-by-side

	Satisfactory Yes/No	Comments
1. Script correctly and clearly delivered		
2. Correctly demonstrates position		
3. Timing started coincident with participant release and stopped when participant takes a step or holds on		
4. If task was not performed, codes and explains reasons		

Tandem Stand

	Satisfactory Yes/No	Comments
1. Script correctly and clearly delivered		
2. Correctly demonstrates position		
3. Timing started coincident with participant release and stopped when participant takes a step or holds on		
4. If task was not performed, codes and explains reasons		
5. Repeats second trial, if necessary		

Short Walks, *Usual Pace*

	Satisfactory Yes/No	Comments
1. Script correctly and clearly delivered		
2. Correctly demonstrates		
3. Toes touching start line		
4. Timing started coincident with participant's first movement		
5. Time stopped when the first foot crosses imaginary plane extending vertically up from the ending line/tape		
6. Repeats second trial		

Grip Strength

	Satisfactory Yes/No	Comments
1. Asked participant about recent surgery on hands		
2. Asked participant about pain and arthritis in hands		
3. Recording dial reset to zero after sub maximal practice		
4. Appropriate hand placement and grip adjustment if needed		
5. Forearm resting on table, elbow bent to approximate right angle		
6. Standard encouragement (motivation and feedback) offered to participant		
7. Recording dial (peak hold needle) reset to zero after first trial		

Comments:

Appendix 8 Anthropometry Equipment Calibration Log

Instructions: This checklist documents the daily, weekly and monthly calibration of anthropometry measurement equipment. Quarterly checklists and logs are summarized onto the **Summary of Observation and Equipment Checklists** (Appendix 2). Copies of this log may be requested by the CC. There should be one such log done each week though the monthly portion will be filled out only in the weeks that it is needed. If there is more than one piece of equipment used for a particular function indicate the checks for each piece on the same log.

Week of [Monday's Date]: Field Center: Tech ID:

Daily Checks

Scales read zero

M	T	W	Th	F	Sa	Su
___	___	___	___	___	___	___

Weekly Checks

A. Reading of scale with 10 kg weight (if reading outside 9.5 to 10.5 range, scale should be serviced).

Date: Reading:

Date service REQUESTED:

Date RECALIBRATD by service technician:

B. Repeat calibration because of moving scales

Date: Reading:

Date: Reading:

Appendix 9 Equipment Temperature Log

JHS Exam 4 Equipment Temperature Log

Equipment_____

Equipment ID#_____

Year_____

Month			
Date	Temp °C	Tech	Comments
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			
21			
22			
23			
24			
25			
26			
27			
28			
29			
30			
31			

Month			
Date	Temp °C	Tech	Comments
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
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26			
27			
28			
29			
30			
31			

Appendix 10 ECG Maintenance Sheet

Instructions: This form is completed by the ECG technician during the maintenance procedure on the ECG system.

Week of [Monday's Date]:

Tech ID:

1. Daily Inspection of ECG System

Perform a visual inspection of all equipment and peripheral devices daily.

	Mon	Tue	Wed	Thu	Fri	Sat	Sun
1. Check the case and display screen for cracks or other damage							
2. Inspect all plugs, cords, cables, and connectors for fraying or other damages							
3. Verify that all cords and connectors are securely seated							
4. Inspect keys and controls for proper operation							
5. Toggle keys should not stick in one position							
6. Knobs should rotate fully in both directions							

2. Monthly Cleaning and Disinfecting Exterior Surfaces

Clean and disinfect the exterior surfaces of all equipment and peripheral devise monthly or more frequently if needed. Complete this section during the first week of the month.

Date ECG machine cleaned:

Appendix 11 Supply Checklist

Week of [Monday's Date]:

Tech ID:

	Mon	Tue	Wed	Thu	Fri	Sat	Sun
1. Availability of all sizes of blood pressure cuffs and measuring tapes							
2. Check the Zio®XT Patch inventory to ensure devices not expired (weekly)							

Appendix 12 Pulse Wave Velocity Equipment Maintenance Log

Instructions: The machine is inspected once a month by the technician. These inspections include appearance and condition of all sensors, tubing and fittings. The equipment unit is cleaned if inspection indicates that it is needed or at least once a month. The unit is cleaned by wiping them with a cloth moistened with a diluted neutral detergent. The blood pressure cuff and neckband need to be gently wiped with a mild soap or antiseptic solution daily. ***Do not use alcohol or solvents.*** Apply pressure to the neckband pad as little as possible. Quarterly checklists are summarized onto the **Summary of Observation and Equipment Checklists** (Appendix 2).

Month of: _____ [Monday's Date]

Tech ID:

MONTHLY CHECKS

	Satisfactory Yes/No	Comments
1. Appearance		
2. Condition of sensors		
3. Tubing		
4. Fittings		
5. Cleanness		

Send detailed record of equipment problems to the PWV team promptly (Dr. Hirofumi Tanaka: htanaka@austinutexas.edu and Dr. Michelle Meyer: mlmeyer@unc.edu)

Appendix 13 OMRON BP Monitor Maintenance and Calibration Log

Instructions: This checklist documents the quarterly checks for the OMRON BP machine. There should be one such log done every quarter. If there is more than one BP monitor used indicate the checks with a separate log for each monitor. Quarterly checklists are summarized onto the **Summary of Observation and Equipment Checklists** (Appendix 2).

Tech ID: Date:

Blood Pressure Measurement **OMRON unit #:**

	Yes/No	If YES, action
Cracking		
Holes		
Worn outer cloth of Velcro		
Leakage of cuff bladder		
Calibration Check with Pressure-Vacuum Meter		
Smooth descent of OMRON LED mm Hg from 280 to 100 mm Hg		
Observed pressure values 250 to 20 mm Hg, in approximant decrements of 20 mm Hg	**OMRON (mm Hg)**	**Pressure-Vacuum Meter (mm Hg)**
Measurement Number 1		
Measurement Number 2		
Measurement Number 3		
Measurement Number 4		
Measurement Number 5		
Measurement Number 6		
Measurement Number 7		
Measurement Number 8		
Measurement Number 9		
Measurement Number 10		
Measurement Number 11		
Measurement Number 12		

Appendix 14 Grip Strength Dynamometer Calibration Log

Instructions: This log documents the semi-annual calibration of the grip strength dynamometer. There should be one such log done every six months. The calibration checks are summarized onto the **Summary of Observation and Equipment Checklists** (Appendix 2).

Tech ID: Date:

Weight	Measurement 1	Measurement 2	Measurement 3	Average of 3 measurements	The average within ± 2 kgs
4.5 kg					
Initial trial					Y N
Repeat trial*					
1					Y N
2					Y N
10 kg					
Initial trial					Y N
Repeat trial*					
1					Y N
2					Y N

Comments:

*If the average of the initial trial is outside of the acceptable range (± 2 kgs), remove the weight and turn the dynamometer position to zero. Repeat the procedure for that weight, and verify if the result is in the acceptable range. Check the positioning of the weight on the dynamometer. The position of the weight can affect the reading. If the result is still outside of the acceptable range, take all the weights off the handle, turn the dynamometer off and then notify the PI or designated person. If readings within the acceptable range still cannot be obtained, a backup device, if available, must be used and the malfunctioning device sent to the company for repair or must be replaced. A dynamometer cannot be used if it does not calibrate properly.

Appendix 15 Protocol for Reliability Testing for Quantitative Sensory Testing (QST) Measures

JHS-Sensory Study

Reliability Testing for QST Measures

Metronome Reliability Testing

The technicians should perform a series of PPT readings using an algometer on a hard surface such as table or desk. During the tests, a timer will be set to 5 and 10 seconds, and the technician will apply pressure with the algometer while recording the readings. When the timer is set to 5 seconds, the reading should be approximately 2.5 kg and when set to 10 seconds, the readings should be close to 5 kg. This test should be done once a week to maximize consistency across the technicians.

Intra-rater reliability (Completed by all technicians)

Each month two research technicians will perform intra-rater reliability on a participant or volunteer (For eg, Tech A and Tech B in one month; Tech C and Tech D in the following month and so on). The technician will record these readings on a shared excel sheet that the PI and project manager can review. The primary technician will perform PPT 1 (includes 3 trials) and PPT 2 (includes 3 trails with BP cuff applied and pain rating of 4 or more) on the participant. The technician will record the date of measurement, technician ID, volunteer ID number, time of measurement, time of cuff inflation, final pain rating, and PPT readings in the excel sheet. The participant or volunteer will be asked to return at minimum 2 hrs and maximum 48 hrs to repeat the procedure. When the volunteer returns the primary technician will perform PPT1 (includes 3 trials) and PPT 2 (includes 3 trails with BP cuff applied and pain rating of 4 or more). The technician will again record the date of measurement, volunteer ID number, time of measurement, time of cuff inflation, final pain rating, and PPT readings in the excel sheet.

Inter-rater reliability (Completed by all technicians)

Two research technicians will perform inter-rater reliability on a participant or volunteer monthly (For eg, Tech A and Tech B in one month; Tech C and Tech D in the following month and so on). The technicians will record these readings on a shared excel sheet that the PI and project manager can review. The two technicians, out of each other's view, will perform one set of PPT1 (3 PPT trials) and PPT2 (3 PPT trails with BP cuff applied and pain rating of 4 or more) measurements on the same participant. The technician assessments (including PPT1 and PPT2) should be performed on the volunteer with at least a 30-minute break between the two technicians. The technicians will record the date of measurement, technician ID, volunteer ID number, time of measurement, time of cuff inflation, final pain rating, and PPT readings in the excel sheet.

Appendix 16 Protocol for Creatinine Calibration

Jackson Heart Study

Creatinine Calibration Study Protocol

Version 1.0

April 5, 2023

Contents

1. Background

It has become *de rigour* in research of chronic kidney disease to calibrate serum creatinine measurements against a gold standard. Calibration is important for analyses that present the prevalence of chronic kidney disease. The visit 1 (2000-2004) serum creatinine was originally analyzed at the University of Mississippi Medical Center using a multipoint enzymatic spectrophotometric assay (Vitros CREA dry reaction slides on a Vitros 5,1 Ortho-Clinical Diagnostics analyzer, Raritan, NJ) (1). In 2006, the National Institute of Standards and Technology (NIST) created a creatinine reference material, certifying its concentration by ion dilution mass-spectrometry (IDMS) (https://pubmed.ncbi.nlm.nih.gov/17660272/). IDMS is the gold standard method for determining creatinine concentration for the NIST reference material. The creatinine measurements from visit 1 in the Jackson Heart Study (JHS) were harmonized in 2006 using a simple random sample of 206 JHS participants, so the visit 1 creatinine could be traceable to the NIST reference material (2). For this harmonization assay, serum creatinine was re-measured using the enzymatic method on a Roche Modular P Chemistry Analyzer (Roche Diagnostics Corporation, Indianapolis, IN 46250) at the University of Minnesota. This method is calibrated using a NIST standard, traceable to reference material SRM 909b (IDMS). The JHS serum creatinine measurements on specimens from visit 3 (2009-2012) were also measured at the University of Minnesota using the Roche Modular P Chemistry analyzer. Serum creatinine was also measured in an ancillary study (JHS Sleep) in a subset of participants (n=908) collected in 2013-2016, using the IDMS traceable enzymatic assay on the Roche Cobas Integra 400 at the University of Vermont. For visit 4, creatinine is being measured at the University of Vermont Medical Center (UVMMC)'s CLIA certified laboratory using an IDMS-traceable, enzymatic two-point rate assay on the Ortho Clinical Diagnostics Vitros 5600.

Reason for harmonization

A common method to determine incident kidney disease is to look at changes in eGFR, a calculated value based on serum creatinine. Since serum creatinine measured in visit 1, 3, and 4 were all done in different labs using different instruments/methods, the ability to harmonize data over visit years and lab sites is crucial to using this data longitudinally.

2 Study Design

The proposed study is an analytical study to harmonize serum creatinine measurements in the JHS from visits 1, 3 and 4. Creatinine levels will be re-measured using an IDMS traceable method in a random sample of stored serum specimens selected from visit 1 and visit 3 using the visit 4 (ongoing) method used at the UVMMC hospital lab.

2.1 Study Samples

A random sample of 150 stored serum specimens from visit 1 and visit 3, for a total of 300 samples, will be selected and tested using and IDMS traceable method at the JHS central laboratory at the UVMMC. Previously thawed samples will be used first when available, as creatinine stability to freeze/thaw and various temperatures has been well documented (3,4).

Study samples will be selected such that the samples selected are distributed evenly across the entire range of creatinine levels at each visit. Sampling will be performed using simple random sampling within each quartile of creatinine levels; 50, 25, 25 and 50 samples will be selected from 1st, 2nd, 3rd and 4th quartiles, respectively.

2.2 Blinding

All samples submitted for assay are identified only by a lab ID with no descriptive information.

2.3 Sample Sequence

Samples will be assayed in a random fashion with no pre-specified order.

2.4 Study Procedures

2.4.1 Sample Handling. Requested samples will be pulled from the biorepository.

2.4.2 Sample Preparation. Samples are thawed in a 37^{o}C water bath for 5-7 minutes, vortexed and aliquoted into appropriate tube for the diagnostic equipment. Samples are refrigerated until taken by courier to the UVMMC lab for testing. Sample stability at refrigerated temperatures has been documented (4).

2.4.3 Sample Testing. Creatinine testing is performed using the Ortho Clinical Diagnostics Vitros 5600, an Enzymatic two-point rate assay utilizing creatinine amidohydrolase, creatine amidohydrolase, and sarcosine oxidase, that is IDMS-traceable.

2.4.4 Testing Location. Testing is performed at the UVMMC laboratory using CLIA approved testing procedures and QC protocols.

2.4.5 Data Collection. A data report containing the following:

- Blood ID number
- Assay Code
- Assay result
- Units
- Quality code
- Assay date
- Comments

2.4.6 Criteria for Sample and Run Acceptance

QC issues are resolved for any point >2s from the current set mean for the hospital lab, as well as evaluated for 4_{1s} and 10_{X} trends. All rejected data will be carefully documented. A record of rejected data will be retained along with any discovered causes and problems.

2.4.7 Data Management

The JHS central laboratory will retrieve assay data from the UVMMC laboratory using a secure data transfer network. The JHS central laboratory will review the data and it will be sent by secure file transfer to the JHS coordinating center.

2.5 Operator Training Requirements

All tests are performed by CLIA trained hospital lab personnel.

2.6 Data Management Quality Control

Ongoing inspection of data collected during the study will be performed by the study coordinator to ensure timely detection of errors (e.g., outliers) and to conduct investigations and corrective actions, if necessary. Should any discrepancies be found once data has been sent to the JHS coordinating center, the coordinating center will be notified by email of the discrepancy and an updated data file with an explanation of the changes will be sent by secure file transfer.

Study data will be uploaded into a study database and will be conducted via secure internet file transfer protocol (FTP) to a server located at JHS coordinating center. Administration of the server and database will be managed by authorized JHS coordinating center personnel, or designees. Only the database manager has the authority to edit the database under the guidance of the study coordinator.

2.7 Statistical Analysis

2.7.1 Sample Size Justification

The sample size is determined to meet or exceed the recommendations of CLSI guidance EP09-A3 (5). EP09-A3 recommends a minimum of 100 clinical samples be tested in a measurement procedure comparison study. One-hundred-fifty (150) stored clinical samples from visit 1 and visit 3 for a total of 300 samples will be used in the proposed study.

2.7.2 Outliers

Prior to analyzing data, all serum creatinine values and creatinine value differences will be screened for outliers using box plots and histograms. All data analyses will be performed two ways: one includes outliers and one exclude outliers.

2.7.3 Data Analysis

We will compare the original serum creatinine measurements (i.e., calibrated measures measurements from visit 1 and actual measurements from visit 3) to the re-measured measurements at visit 1 and visit 3, separately. The following analyses will be performed:

- Exploratory data analysis: scatter plots and difference plots will be used to inspect underlying characteristics of the measures being compared and to identify anomalous results.
- Regression analysis: measures will be compared using four potential regression models, including simple linear regression, quadratic regression, piecewise linear regression, and Deming regression.
- If a calibration equation is needed, an appropriate training dataset, a validation dataset, test dataset, and 10-fold cross validation will be used to select an appropriate calibration equation, estimate true error, and also assess performance of the final selected calibration equation.
- Summary statistics will be computed for the original and IDMS traceable serum creatinine as mean and standard deviation. Differences between IDMS and original creatinine measurements will be calculated and compared using paired t-test to account for the within subject correlation. Prior to the formal statistical analysis, exploratory data analysis will be conducted to check for outliers and model assumptions. Inferential statistical method will be employed to fit a calibration equation. For this purpose, the dataset will be split in to three independent datasets as a training dataset, a validation dataset and a test dataset. Of the total sample size, 75% will be for model selection and true error estimation and the remaining 25% will be used for testing. Following the exploratory data

analysis, four potential candidate models will be considered: simple linear regression, quadratic regression, piecewise linear regression and Deming regression. The best equation that fits the data well will then be selected using statistical criteria such as mean square error and coefficient of multiple determination. Bland-Altman plot will also be used to assess the agreement between the measured and calibrated serum creatinine values. P-value <0.05 will be considered statistically significant based on two-tailed tests.

3 Documentation and Handling of Materials

Re-measurement of serum creatinine would use 100 ul of serum.

4 References

1. MA Carpenter, R Crow, M Steffes, et al. Laboratory, Reading Center, and Coordinating Center Data Management Methods in the Jackson Heart Study. Am J Med Sci 2004;328(3):131-144.
2. W Wang, BA Young, T Fulop, IH de Boer, et al. Effects of Serum Creatinine Calibration on Estimated Renal Function in African Americans: the Jackson Heart Study. Am J Med Sci 2015:349(5):379-384.
3. Gislefoss RE, Lauritzen M, Langseth H, Mørkrid L. Effect of multiple freeze-thaw cycles on selected biochemical serum components. Clin Chem Lab Med. 2017 Jun 27;55(7):967-973. doi: 10.1515/cclm-2016-0892. PMID: 27987362.
4. Donnelly JG, Soldin SJ, Nealon DA, Hicks JM. Stability of twenty-five analytes in human serum at 22 degrees C, 4 degrees C, and -20 degrees C. Pediatr Pathol Lab Med. 1995 Nov-Dec;15(6):869-74. doi: 10.3109/15513819509027023. PMID: 8705197.
5. CLSI. Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline–Third Edition. CLSI document EP09-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2013.

Appendix 17 Methylation Data Quality Control

JHS Genetics WG QC Plan
07.03.2023

Some quality control (QC) has already been done on existing JHS methylation data by both Dr. Horvath and Dr. Lange's groups. However, standards for methylation data QC have continued to evolve, and there are some newer best practices that are hard to implement without going back to the idat files. This will be relevant as we start to think about how to handle the second batch of methylation data that will be coming in from visit 1 from TOPMed this year… we'll want methods to be very consistent, and the minfi processing done multiple years ago now is likely not all that we would want to do. This is very much an evolving field, and there is not one set of best practices for QC (people have different opinions and new papers are coming out all the time). In particular, removal of probes at idat file stage has not been clearly documented in the current datasets according to current best practices; this requires further work for both Dr. Zannas's proposal and others in future. Redoing the QC will further allow us to better identify the most important technical variables contributing to batch effects (to include in downstream models), and having the idat files we can also leverage the array control probes to adjust for batches (and reduce inflation). These steps are important because use of ComBat and reported batch/plate information alone has been shown to inadequately adjust for batch effects. One thing to note is that many of these steps are not as important when limiting analyses to epigenetic age; however, they are important – according to current standards – for epigenome-wide association analyses. All QC report and processes will be discussed with Genetics working group members, and analysts can decide which set of QC processes they'd like to adopt.

New QC of DNA methylation data will be primarily performed using the CHAMP package in RStudio (Tian et al., 2017). ChAMP is an integrated analysis pipeline that filters low-quality probes and samples, adjusts for Infinium I and Infinium II probe design, and corrects for technical batch effects. The following steps are tentative and may be adapted during data exploration in order to maximize data yield and quality. Starting from raw data (idat files), we will first remove: (i) probes with mean detection $p > 0.01$ or with beadcount < 3 in at least 1% of samples; and (ii) samples with mean detection $p > 0.01$ across all probes. Raw signal intensities will be normalized with subset quantile normalization available in the minfi package (Aryee et al., 2014), and normalized values will then be converted into beta values, which will be used in subsequent steps. Technical batch effects will be captured by calculating principal components (PCs) based on the EPIC array control probes and the 1000 least variable CpG sites across all samples as previously (Rahmani et al., 2017). Resulting PCs will be available for technical correction in future analyses. In addition, data will be visually inspected by singular value decomposition and remaining batch effects will be removed using ComBat (Johnson, Li, & Rabinovic, 2007; Leek, Johnson, Parker, Jaffe, & Storey, 2012). According to standard quality control procedures, the following probes will further be removed: i) CpG probes in cross-reactive regions and those containing single nucleotide polymorphisms with minor allele frequency above 1% within 10 bp as retrieved from (Chen et al., 2013); and (ii) probes located on the X and Y chromosomes. White blood cell type proportions will be estimated from DNA methylation following the Houseman reference method (Houseman et al., 2012). Once run successfully, we will share with the coordinating center both the raw beta (including all beta values with sufficient intensity signals) and the ComBat-corrected (after removal of probes as above) dataset versions as well as the technical correction PCs, along with detailed documentation of all performed QC steps. The same QC steps will be followed consistently in the expected next batch of JHS methylation data when available.

Appendix 18 QC Report Template

Contents

1. Forms completion as of date

Form Name	Expected N	Completed N (%)	Pending completion N (%)	Permanently missing N (%)
Anthropometry Form				
Biospecimen Collection Form				
Depressive Symptoms				
Medication Survey Form A				
Medication Survey Form B				
Sitting Blood Pressure				
Form A				
Form B				
Form C				
Etc.				

2. Items completion as of date

Form Name	Total # of forms completed N	Forms with all essential items* N (%)
Anthropometry Form		
Biospecimen Collection Form		
Depressive Symptoms		
Medication Survey Form A		
Medication Survey Form B		
Sitting Blood Pressure		
Form A		
Form B		
Form C		
Etc.		

*Include here definitions of essential items for each form.

3. Specimens collection – Blood as of date

	N
Consented for specimen collection	
Blood specimens collected	
Reason blood specimen not collected	
Reason 1	
Reason 2	
Etc.	

4. Specimens collection – Urine as of date

	N
Consented for specimen collection	
Urine specimens collected	
Reason urine specimens not collected	
Reason 1	
Reason 2	
Etc.	

5. Evidence of digit preference as of date

Technician	SBP	DBP	Weight	Height	Waist Circumference	Hip Circumference
1	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N
2	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N
3	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N
4	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N

6. Out of range values (outside of the soft rules for range checks in REDCap) as of date

Measurement	Range observed in Visit 4	DMS range check values	Observations outside of DMS range	
			N	%
Height (cm)				
Weight (kg)				
Waist circumference (cm)				
Hip circumference (cm)				
Systolic blood pressure (mm Hg)				
Systolic blood pressure (mm Hg)				
Pulse (bpm)				

7. Data transfer from reading centers as of date

	Collected N	Results at CC N	Backlog (> 45 days after exam date) N
Chemistry			
Urinalysis			
CRP/Cystatin-C[1]			n/a
ECG			
Echo			
Brain MRI[2]			n/a
FFQ[3]			n/a
ECG (Local Read)			

[1]Batched analysis at the Central Lab. The latest data transfer to the CC was on mm/dd/yyyy.

[2]Quarterly delivery of results from Reading Center to CC. The latest delivery was on mm/dd/yyyy.

[3]Quarterly delivery of FFQ data from CC to UMass-Lowell for processing (QC and calculations of nutrients). The last data transfer from the CC was on mm/dd/yyyy.

8. Food Frequency questionnaire QC as of date (report from UM Lowell)

Staff ID	Mon-Year			Mon-Year			Mon-Year		
	Total N	Invalid* N	Rate %	Total N	Invalid* N	Rate %	Total N	Invalid* N	Rate %
1									
2									
3									
Total									

*Defined as total energy intake < 600 kcal or > 4800 kcal.

9. MRI QC report as of September 28, 2023 (report from MRI reading center)

	# Exams N	Comments
(0) Fail		
(1) Pass		

10. Echo QC report – percentages of missing data as of date (report from Echo reading center)

Measure type		Volume		Linear		Doppler & tissue Doppler	
Echos Analyzed N	LVEF N (%)	Simpson LVEF N (%)	LAV N (%)	LVEDD N (%)	LVWTm N (%)	E wave N (%)	TDI e' septal N (%)

LVEF – LV ejection fraction; LAV – left atrial volume; LVEDD – LV end-diastolic diameter; LVWTm – LV mean wall thickness

11. ECG QC report as of date

	Quality Grade				
Time Period	0 (best) N (%)	1 N (%)	2 N (%)	5 (poorest) N (%)	Total
mm/dd/yyyy – mm/dd/yyyy					
mm/dd/yyyy – mm/dd/yyyy					
mm/dd/yyyy – mm/dd/yyyy					
Etc.					
Total					

Notes: Results are based on data received on mm/dd/yyyy from ECG Reading Center. Not including N visual read ECG's (N with best quality, N with average quality).

12. Specimen QC report as of date

Assay Name	Normal result N	Poor quality - with result N	Out of range – low N	No result - poor quality N	Out of range – high N	No result – other N	No result – QNS N	No result - no sample N
Serum Glucose (mg/dl)								
Hemoglobin A1c (%)								
Cholesterol (mg/dl)								
High Density Lipoprotein (mg/dl)								
Low Density Lipoprotein (mg/dl)								
Triglycerides (mg/dl)								
Cholesterol to HDL ratio (%)								
Creatinine (mg/dl)								
Urinary Albumin (mg/dl)								
Urinary Creatinine (mg/dl)								
Albumin Creatinine Ratio (ug/mg)								
Potassium (mEq/L)								
C-reactive Protein (ug/mL)								
Cystatin-C (mg/L)								

Note: Results are based on data received on mm/dd/yyyy from Central Lab.