FRAMINGHAM HEART STUDY

INFLAMMATORY MARKERS MANUAL

EXAMINATION 1

GENERATION 3 OMNI GENERATION 2

The FHS inflammatory marker manual prepared by:
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Inflammatory Marker Measurement Funding

N01-HC 25195 (PI: Philip A. Wolf, M.D.) 02/01/02 - 09/30/08
NIH/NHLBI
The Framingham Heart Study, Physical Examination, Testing and Surveillance
Specific Aims: Provide resources and personnel for examination and surveillance of cohort and offspring; recruit a third generation cohort; maintain surveillance on all participants. In addition, contract personnel are responsible for performing statistical analyses, writing reports and manuscripts, and for dissemination of results.

1RO1 HL64753 (PI, Benjamin Emelia J, M.D., Sc.M.) 07/01/00 - 06/31/04
NIH/NHLBI
Inflammation: Correlates and Prognosis in Framingham
Specific Aims: The objectives are to determine the relations between systemic markers of inflammation and cardiovascular disease risk factors, endothelial dysfunction, and subclinical disease; and relate markers of inflammation to prevalent and incident cardiovascular disease events adjusting for standard risk factors.

1 R01 HL76784 (PI: Emelia J. Benjamin, MD, ScM) 06/01/04 - 05/31/09
NIH/NHLBI
Framingham: Inflammation, Genes & Cardiovascular Disease
Specific Aims: To examine the environmental determinants of systemic inflammation in the community; To investigate the genetic determinants of systemic inflammation (heritability, linkage, and genotyping known polymorphisms in 60 inflammatory candidate genes); To identify the inflammatory phenotypic and genetic determinants of subclinical CVD; To determine the contribution of inflammatory phenotype versus genotype to prevalent and incident CVD and to incident hypertension.

1R01 AG028321 (PI, Emelia J. Benjamin, MD, ScM) 07/01/06 - 06/30/11
NIH/NIA
Aging and Inflammation: Longitudinal Markers and Genetics in the Framingham Study
Specific Aims: To examine the risk factors related to 7 year progression of systemic inflammation (between Offspring exams 7 and 8, and Omni exams 2 & 3) in the community: To investigate the genetic factors associated with systemic inflammation and to examine the relation between inflammatory SNPs/haplotypes and frailty and declining physical function; To study the relation of changes in inflammatory markers to progression of blood pressure and subclinical disease including ankle brachial index, arterial tonometry, echocardiographic left ventricular structure and function and carotid intimal medial thickness; To identify the relations between changes in inflammatory biomarkers to frailty and progression of declining physical function over 7 years of follow-up.

Grant in response to RFA AG 05-011

Please see acknowledgements for actual grant specific aims and key personnel.

Specimen Collection, Storage, Distribution and Measurement Procedures

FHS blood and urine collection/processing
Blood was drawn from participants after a 12 hour fast. Specimens were centrifuged at 2400 g for 22 minutes in a refrigerated centrifuge, and aliquoted. Participants are asked to leave a random urine specimen during the clinic exam. Specimens are stored at -80 until assay. All specimens are labeled with bar-coded labels that include the Framingham ID number, draw date and sample type. The stability of specimens that have been stored at -80^{o}C for years has been verified for CRP.

FHS timing of phlebotomy and urine collection
Some biomarkers potentially could be affected by time of day or food; samples are collected in the morning, typically between 7 and 9 am after an overnight fast, shortly after clinic arrival. Some of the Omni exam 2 specimens were obtained in the afternoon and were not fasting.

Procedures for assays performed at the Framingham Heart Study laboratory.
Prior to analysis, samples are thawed to room temperature and mixed well. All assays are performed using commercially available kits, following manufacturer's instructions.
CRP was measured on serum, using an immunoturbidometric method on a Roche cobas 501, with a Roche high sensitivity assay. A subset of the specimens was measured in duplicate (8%). Reproducibility was assessed using these duplicate measures as well as blinded phantom specimens.

Fibrinogens were measured on citrated plasma, using a method based on clot detection on a Diagnostica Stago STart4 Analyzer with Diagnostica Stago reagents. Fibrinogens were run in duplicate and averaged. Testing was repeated if there was >5% discrepancy between replicates.

Urine creatinines were measured using a modified Jaffe reaction on Roche Hitachi 911, using Roche reagents.

Procedures for ELISA markers measured in John F. Keaney, Jr, MD's laboratory.
For analysis, samples are thawed at room temperature, vortexed vigorously, and the specimens (serum or plasma) are measured using commercially available enzyme-linked immunosorbent assay kits (ELISA) according to the manufacturer's instructions (see appendix for ELISA kit pdfs). Standards and samples are run in duplicates and OD is read using microplate reader (Molecular Devices VersaMax). Duplicates that are not within CV <95th percentile are rerun.

FHS lab assigns a dummy ID number to about 4% of randomly chosen duplicate phantom specimens. If possible the lab attempts to order and use 1 lot for each specific ELISA assay. If not possible, we examine the variability secondary to lot. If lot accounts for a significant amount of variability, lot is adjusted for in analyses (e.g. isoprostanes).

Table 1. Inflammatory Biomarker Quality Control (QC) Protocol			
Element	**Frequency**	**Procedure**	**Statistics**
Control Samples		• When available commercial control is run with each ELISA plate together with internal control (75% plates are run with both controls) • Pooled plasma (internal control) is run on 75% of plates. Whole blood for pooled plasma is drawn from 1-10 healthy volunteers. Blood is centrifuged and aliquots are frozen at -80C.	• The OD of each plate is read using Molecular Devices VERSAmax plate reader. The results are calculated using SOFTmax Pro. Data are sent to biostatistician including subject ID, position on plate, result, mean result, Std.Dev., dilution factor and final result.
Reproducibility	Each ELISA Assay	• ***All calibrators controls and participant specimens are run in duplicate*** • ***Duplicates* with CV >95th percentile rerun.**	• Mean $\pm$ sd $\lvert y - y \rvert$ • Range $\lvert y - y \rvert$ • CV%
	Daily	**Phantom variability** • 4% specimens assigned a dummy ID and rerun as phantoms	• $\lvert y_1 - y_2 \rvert$ compared to assay specific table • Correlations; CV%
Data cleaning	Quarterly	• Generated by data management staff	• Out of range data; Missing data
QC reports	Bi-annually	• Reproducibility statistics included in reports • Review by lab, co-investigators, consultant	• Descriptive statistics; Data cleaning • Analyte means by quarter
Lab meetings	Weekly	• Dr. Keaney meets weekly with lab staff to review issues • Review QC reports as they are available	
sd = standard deviation; **All exam 8 kits for each specific ELISA will be from same manufacturer's LOT**			

C-reactive protein (CRP)

1. Funding Source/Lab

Framingham specimens	PI: Emelia J. Benjamin, MD, ScM & PAW emelia@bu.edu;
Grant #	**RO1 HL076784, 1R01 AG028321 & NO1-HC-25195, (PI Philip A Wolf)**
Lab	FHS Lab
Contact:	Patrice Sutherland: patrices@bu.edu

2. Method:

3. Technical Aspects

Commercial kit including all reagents	
Vendor	Dade Behring BN 100 – High Sensitivity CRP Agent http://www.dadebehring.com
Minimum detectable dose Units	0.15 mg/L
Measuring range	0.15 – 550 mg/L
Actual range measured	0.15 – 66.33

4. FHS Specimen Characteristics

a. Serum, run in single
b. Frozen samples (- 80C)

5. QC aspects

CV intra-assay:	2.8%
CV intra phantoms	6.7%
Number per cycle	116
CV inter-assay	5.5%
CV threshold for re-measuring:	n/a
Bar code reader:	n/a
Internal controls	Yes

6. FHS participant aspect

a. Markers run: 1/3/05 – 8/2/05
b. Measured in: mg/L
c. Count: Generation 3 Exam 1 = 4071, Omni Group 2 Exam 1 = 407

7.Descriptive Statistics – Gen3 Exam1

	Mean	SD	Minimum	Maximum	Median	Q1	Q3
Unadjusted:	2.59	4.84	0.15	66.33	1.05	0.45	2.72
Log-transformed	0.13	1.23	-1.90	4.19	0.05	-0.80	1.00

Descriptive Statistics – Omni Group 2 Exam1

	Mean	SD	Minimum	Maximum	Median	Q1	Q3
Unadjusted:	2.61	4.49	0.15	45.86	1.13	0.52	2.75
Log-transformed	0.17	1.23	-1.90	3.83	0.12	-0.65	1.01

8. Publication[1-12]{Jefferson, 2007 8407 /id}

Fibrinogen

1. Funding Source/Lab

Framingham specimens	
Grant #	**NO1-HC-25195, (PI Philip A Wolf)**
Lab	FHS Lab
Contact:	Patrice Sutherland: patrices@bu.edu

2. Method: Clauss method (clot time)

3. Technical Aspects

Commercial kit including all reagents	
Vendor	Diagnostico Stago Diagnostico Stago Start 4 http://www.diagnosticostago.com
Minimum détectable dose units	90 mg/dL
Measuring range	90 –1800 mgd/L
Actual range measured	87-787

4. FHS Specimen Characteristics

a. Serum, run in duplicate
b. Frozen samples (- 80C)

5. QC aspects

CV intra-assay:	1.1%
intra phantoms ICC	0.925
Number per cycle	178
CV inter-assay	2.5%
CV threshold for re-measuring:	n/a
Bar code reader:	n/a
Internal controls	Yes

6. FHS participant aspect

a. Markers run: 11/06/03 – 8/02/05
b. Measured in: mg/dL
c. Count: Generation 3 Exam 1 = 4050 & Omni 2(1) = 405

7. Descriptive Statistics – Gen 3

	Mean	SD	Minimum	Maximum	Median	Q1	Q3
Unadjusted:	341.02	71.13	87	787	331	293	378
Log-transformed	5.81	0.20	4.47	6.67	5.80	5.68	5.93

Descriptive Statistics – Omni gen 2

	Mean	SD	Minimum	Maximum	Median	Q1	Q3
Unadjusted:	371.57	80.43	149	693	362	315	418
Log-transformed	5.89	0.22	5.00	6.54	5.89	5.75	6.04

8. Publication

Intercellular adhesion molecule 1 (ICAM 1)

1. Funding Source/Lab

Framingham specimens	PI: Emelia J. Benjamin, MD, ScM Emelia@bu.edu
Grant #	**RO1 HL076784 & 1R01 AG028321**
Lab	JFK/IL
Contact:	Izabella Lipinska: lipinska@bu.edu;

2. Method: Quantitative ELISA

3. Technical Aspects

Molecular Devices VersaMax microplate reader	
Commercial kit including all reagents	
Vendor	R & D Systems (Cat. No. BBE 1B) **http://www.rndsystems.com/**
Minimum detectable dose	<0.35 ng/ml
Standard curve range	0 - 50 ng/mL

4. FHS Specimen Characteristics

a. Serum, run in duplicate
b. Frozen samples, run on 1st thaw

5. QC aspects

CV intra-assay:	
CV intra FHS IDs:	2.56
CV intra phantoms	
Number per cycle	
CV inter	No inter CVs since some phantoms and the original IDs are run in the same plate or on same day.
CV threshold for re-measuring:	
Bar code reader:	Yes
Internal controls	Yes

6. FHS participant aspect

a. Markers run: 07/2006
b. Measured in: ng/mL
c. Count Gen 3 n = 4069 & Omni Gen 2 n **= 372**

Descriptive Statistics – Gen 3

	Mean	**SD**	**Minimum**	**Maximum**	**Median**	**Q1**	**Q3**
Unadjusted:	256.21	76.01	96.47	1323.80	239.80	207.63	287.71
Log-transformed	5.51	0.26	4.57	7.19	5.48	5.34	5.66

Descriptive Statistics – Omni group 2

	Mean	**SD**	**Minimum**	**Maximum**	**Median**	**Q1**	**Q3**
Unadjusted:	214.87	69.65	57.71	594.67	204.14	172.65	250.87
Log-transformed	5.318	0.329	4.055	6.388	5.319	5.151	5.525

8. Publications[1;3-7;9;13]

Interleukin-6 (IL6) serum

1. Funding Source/Lab

Framingham specimens	PI: Emelia J. Benjamin, MD, ScM & PAW emelia@bu.edu; **Offspring Exam 8 Omni 1(3)**
Grant #	**RO1 HL076784, 1R01 AG028321 & NO1-HC-25195, (PI Philip A Wolf)**
Lab	Freedman Lab.
Contact:	Kahraman Tanriverdi, Kahraman.tanriverdi@umassmed.edu

2. Method:

3. Technical Aspects

Commercial kit including all reagents	
Vendor	R & D Systems (Cat. No. D6050) **http://www.rndsystems.com/**
Minimum detectable dose Units	0.039 pg/mL
Measuring range	0 - 10 pg/mL
Actual range measured	0.039 - 10 pg/mL

4. Specimen Characteristics

a. Serum, run in single
b. Frozen samples (- 80C)

5. QC aspects

CV intra-assay:	7.8%
CV intra phantoms	
Number per cycle	
CV inter-assay	
CV threshold for re-measuring:	
Bar code reader:	yes
Internal controls	yes

6. FHS participant aspect

a. Markers run: 5/08 – 7/08
b. Measured in: pg/mL
c. Count: Generation 3 Exam 1 = 4032, Omni Group 2 Exam 1 = 406

7.Descriptive Statistics – Gen3 Exam1

	Mean	SD	Minimum	Maximum	Median	Q1	Q3
Unadjusted:	1.88	2.75	0.15	101.48	1.31	0.88	2.03
Log-transformed	0.35	0.67	-1.90	4.62	0.27	-0.12	0.71

7.Descriptive Statistics – Omni Group 2 Exam1

	Mean	SD	Minimum	Maximum	Median	Q1	Q3
Unadjusted:	2.13	2.91	0.18	38.58	1.43	0.90	2.34
Log-transformed	0.42	0.73	-1.69	3.65	0.36	-0.11	0.85

8. Publication

Isoprostanes (8-epi-PGF2α/Urinary creatinine =Urinary isoprostanes)

1. Funding Source/Lab

Framingham specimens	PI: Emelia J. Benjamin, MD, ScM & PAW emelia@bu.edu; **Offspring Exam 8 Omni 1(3)**
Grant #	**RO1 HL076784, 1R01 AG028321 & NO1-HC-25195, (PI Philip A Wolf)**
Lab	Freedman Lab.
Contact:	Kahraman Tanriverdi, Kahraman.tanriverdi@umassmed.edu

2. Method:

3. Technical Aspects

Commercial kit including all reagents	
Vendor	Cayman Chemical (Cat. No. 516351) **http://www.caymanchem.com/app/template/Home.vm**
Minimum detectable dose Units	0.8 pg/mL
Measuring range	3.9 – 500 pg/mL
Actual range measured	3.9 – 500 pg/mL

4. Specimen Characteristics

a. Serum, run in duplicate
b. Frozen samples (- 80C)

5. QC aspects

CV intra-assay:	7.3%
CV intra phantoms	
Number per cycle	
CV inter-assay	
CV threshold for re-measuring:	
Bar code reader:	Yes
Internal controls	Yes

6. FHS participant aspect

a. Markers run: 2/09 – 3/09
b. Measured in: pg/mL
c. Count: Generation 3 Exam 1 = 4053, Omni Group 2 Exam 1 = 408

7.Descriptive Statistics – Gen3 Exam1

	Mean	SD	Minimum	Maximum	Median	Q1	Q3
Unadjusted:	1270.12	1425.93	6.93	19561.42	901.09	424.57	1641.73
Log-transformed	6.68	1.03	1.94	9.88	6.80	6.05	7.40

7.Descriptive Statistics – Omni Group 2 Exam1

	Mean	SD	Minimum	Maximum	Median	Q1	Q3
Unadjusted:	1082.51	2200.85	29.88	41688.80	757.91	422.49	1280.26
Log-transformed	6.56	0.90	3.40	10.64	6.63	6.05	7.15

8. Publications

Lipoprotein-Associated Phospholipase A2 (LP-PLA2) Activity Analysis by CAM

1.Funding Source/Lab

Framingham specimens	PI: Emelia J. Benjamin, MD, ScM Emelia@bu.edu
Grant #	**RO1 HL076784 & 1R01 AG028321** **Lp-PLA2 activity levels was measured by GSK at no cost to the FHS**
Lab	GlaxoSmithKline(GSK)
Contact:	Dr. Terry Walker or J.J. Nelson: jeanenne.j.nelson@GSK.com
Submitted By:	**Kim Hamlet** Research Associate – Biomarkers High Throughput Biology – RTP Discovery Research, GSK 919-483-8710

2. Method: Activity

3.Technical Aspects

Discovery Research, GSK	
CAM Kit (Lot # TN02)	
Vendor	diaDexus http://www.diadexus.com/
Units	nmol/mL/min
Measuring Range	2-300 nmols/mL/min
Mass	measured with 2-site ELISA (diaDexus Inc, South San Francisco, Calif)

4.FHS Specimen Characteristics

Gen 3 Exam 1 EDTA Plasma stored at -80

Framingham Heart Study Samples

Date received:
Samples received
Samples tested:
Date Reported

Study Design:

1. p-Nitrophenol calibrators were run in duplicate on a separate plate at the beginning of each week to verify accepted range for p-nitrophenol curve. However, all Lp-PLA2 activity values were calculated from a single slope of 0.02713 from an average of twenty-two p-Nitrophenol curves performed just prior to the start of the study.
2. Controls run at the beginning and end of each plate in duplicate (12 wells, 6 averages, 3 averages of Low QC and 3 averages of High QC)
3. All samples run in duplicate on consecutive microtiter plates (mean values reported)
4. Samples with duplicate CV greater than 12.4% were repeated. Repeat results reported. Samples with repeat CV exceeding 12.4% were not repeated and the mean of the duplicates with the lowest CV reported.

CAM Assay Acceptability Criteria:

1. At least four of six QC duplicate averages must fall within the acceptable ranges shown below. These ranges were established during the first week of the study.

   CAM Controls:

   Low QC range (C3, 10580213): 117.9 - 165.0 nmol/mL/min
   High QC range (C2, 10580220): 208.8 - 269.5 nmol/mL/min

2. Additionally, the mean plate CV of the six duplicate averages must be less than 8.5%.
3. Each calibrator on the p-nitrophenol curve must fall within predefined ranges in order to be accepted.

   Standard 1: 0.02 to 0.08 OD_{405nm}
   Standard 2: 0.15 to 0.21
   Standard 3: 0.26 to 0.38
   Standard 4: 0.57 to 0.88
   Standard 5: 1.15 to 1.74
   Standard 6: 1.53 to 2.65
   Standard 7: 2.23 to 3.26

   In addition, the slope of the weekly standard curve must fall within the pre-defined range of 0.023 - 0.031 ΔOD_{405}/nmol.

4. Samples which had activity values outside 2 to 300 nmols/mL/min exceed dynamic range of assay and reported as such.

Lipoprotein-Associated Phospholipase A2 (LP-PLA2) Mass Analysis by PLAC

1. Funding Source/Lab

Framingham specimens	PI: Emelia J. Benjamin, MD, ScM Emelia@bu.edu
Grant #	**RO1 HL076784 & 1R01 AG028321** **Lp-PLA2 mass was measured by diaDexus at no cost to the FHS**
Lab	GlaxoSmithKline(GSK)
Contact:	Dr. Terry Walker or J.J. Nelson: jeanenne.j.nelson@GSK.com
Submitted By:	Mary Jane Cerelli diaDexus Inc. 343 Oyster Point Blvd South San Francisco, CA 94080 650-246-6539

2. Method: Mass

3.Technical Aspects

Discovery Research, GSK		
Kit Lp-PLA2 P/N 90103 L/N 509003, PLAC® Test		
Vendor	diaDexus http://www.diadexus.com/	
Units	ng/mL	
Measuring Range		
PLAC Controls	C 1 90018-508069 C 2 90019-508070	mean 192.8ng/mL mean 424.3ng/mL

4.FHS Specimen Characteristics

Offspring Exam 7 EDTA Plasma stored at -80

Framingham Samples

Date Received:
Samples Supplied:
Samples tested:
Date Reported

Study Design

1. Calibrators and controls run in duplicate
2. Controls run throughout the plate
3. Samples run in single point, 20% of the samples run in duplicate, same plate (mean values reported)
4. Samples with duplicate CV's > than 15 % repeated, repeat results reported.

Assay Acceptability criteria:

1. Calibrators: calibrator 6 OD ≥ 1.6
2. Duplicates CV on calibrators and calibrators ≤ 15%
3. PLAC Controls:
   - C 1 range 153.9 - 231.7 ng/mL
   - C 2 range 302.8 - 545.9 ng/mL

Quality Control

Twenty-four percent of the total samples assayed by PLAC test were run in duplicate. Of the samples tested, all produced CVs < 15% between duplicates.

QC Control Summary

N.B. Lp-PLA2 should not be confused with secretory PLA2 group IIA; they have different functions.

- Secretory PLA2 is involved in arachidonic acid mobilization.
- Lp-PLA2 cleaves polar oxidized fatty acids; it does not affect arachidonic acid release.
- Lp-PLA2 is also referred to as Type VIIa PLA2.
-

References for secretory PLA2:

- Mallat Z, Steg PG, Benessiano J, Tanguy ML, Fox KA, Collet JP, Dabbous OH, Henry P, Carruthers KF, Dauphin A, Arguelles CS, Masliah J, Hugel B, Montalescot G, Freyssinet JM, Asselain B, Tedgui A. Circulating secretory phospholipase A2 activity predicts recurrent events in patients with severe acute coronary syndromes. *J Am Coll Cardiol*. 2005;46:1249-1257.
- Boekholdt SM, Keller TT, Wareham NJ, Luben R, Bingham SA, Day NE, Sandhu MS, Jukema JW, Kastelein JJ, Hack CE, Khaw KT. Serum levels of type II secretory phospholipase A2 and the risk of future coronary artery disease in apparently healthy men and women: the EPIC-Norfolk Prospective Population Study. *Arterioscler Thromb Vasc Biol*. 2005;25:839-846.

Monocyte chemoattractant protein-1 (MCP1)

1.Funding Source/Lab

Framingham specimens	PI: Emelia J. Benjamin, MD, ScM emelia@bu.edu
Grant #	**RO1 HL076784 & 1R01 AG028321**
Lab	JFK/IL
Contact:	Izabella Lipinska: lipinska@bu.edu

2. Method: Quantitative ELISA

3. Technical Aspects

Molecular Devices VersaMax microplate reader	
Commercial kit including all reagents	
Vendor	R & D Systems (Cat. No. DCP00) **http://www.rndsystems.com/**
Minimum detectable dose	<5.0 pg/mL
Standard curve range	0 – 2000 pg/mL

4. FHS Specimen Characteristics

a. Serum, run in duplicate
b. Frozen samples, run on 3rd thaw
c. Samples were subjected to 1-3 freeze-thaw cycles.

5. QC aspects

CV intra-assay:		CV intra-assay: (FHS IDs) 3.8±3.3		
CV intra FHS IDs:				
CV Intra-assay final data set. If sample re-measured because CV is higher than threshold then use the lowest CV.		3.83±3.32		
CV intra phantoms		CV phantoms: Yes		
Number per cycle				
CV inter		No inter CVs since some phantoms and the original IDs are run in the same plate or on same day		
CV threshold for re-measuring:		13.1		
Bar code reader:		Yes		
Internal controls	mean	std	min	max
	314	28	256	376

Mean and CV by month:

Month	Aug 2006	Sep 2006	Oct 2006
N	37	974	37
Mean±std	231.0 ± 89.4	333.9 ± 154.1	372.8 ± 126.0
CVmean±std	2.1 ± 1.6	1.9 ± 1.7	1.2 ± 1.8

6. FHS participant aspect

a. Markers run: 06/02 – 11/02
b. Measured in: pg/mL
c. Count Gen 3 n = 3995 & Omni Gen 2 n = **406**

Descriptive Statistics – Gen 3 Exam 1

	Mean	SD	Minimum	Maximum	Median	Q1	Q3
Unadjusted:	359.50	227.58	29.38	9432.10	333.70	270.08	416.70
Log-transformed	5.82	0.34	3.38	9.15	5.81	5.60	6.03

Descriptive Statistics – Omni Group 2 Exam 1

	Mean	SD	Minimum	Maximum	Median	Q1	Q3
Unadjusted:	368.68	459.99	134.39	7970.67	304.56	238.73	403.85
Log-transformed	5.76	0.44	4.90	8.98	5.72	5.48	6.00

MCP-1 Kits Shipped in 2002

Ship Date	Catalogue #	Description	Qty	Lot#
8/29/2006	SCPOO	MCP-1, R/D	126	240267

8. Publications[1;3-7;9;15]

Osteoprotegerin (OPG)
Generation 3 Exam 1 Omni Gen 2 Exam 1

1. Funding Source/Lab

Framingham specimens	PI: Emelia J. Benjamin, MD, ScM & PAW emelia@bu.edu; **Offspring Exam 8 Omni 1(3)**
Grant #	**RO1 HL076784, 1R01 AG028321 & NO1-HC-25195, (PI Philip A Wolf)**
Lab	Freedman Lab.
Contact:	Kahraman Tanriverdi, Kahraman.tanriverdi@umassmed.edu

2. Method:

3. Technical Aspects

Commercial kit including all reagents	
Vendor	Biomedica Gesellschaft mbH, Vienna, Austria American Vendor ALPCO (Cat. No. 04-B1-20402) **http://www.moleculardevices.com/pages/instruments/versamax.html**
Minimum detectable dose Units	0.14 pmol/L
Measuring range	0 – 30 pmol/L
Actual range measured	0 – 30 pmol/L

4. Specimen Characteristics

a. Plasma, run in single
b. Frozen samples (- 80C)

5. QC aspects

CV intra-assay:	10.1%
CV intra phantoms	
Number per cycle	
CV inter-assay	9.6%
CV threshold for re-measuring:	
Bar code reader:	yes
Internal controls	yes

6. FHS participant aspect

a. Markers run: 8/08 – 11/08
b. Measured in: pmol/L
c. Count: Generation 3 Exam 1 = 4087, Omni Group 2 Exam 1 = 410

7.Descriptive Statistics – Gen3 Exam1

	Mean	SD	Minimum	Maximum	Median	Q1	Q3
Unadjusted:	4.42	1.59	0.13	13.18	4.20	3.38	5.20
Log-transformed	1.42	0.36	-2.04	2.58	1.43	1.22	1.65

7.Descriptive Statistics – Omni Group 2 Exam1

	Mean	SD	Minimum	Maximum	Median	Q1	Q3
Unadjusted:	3.93	1.46	2.21	10.27	4.57	3.03	4.57
Log-transformed	1.31	0.35	-0.11	2.33	1.29	1.11	1.52

8. Publication

P-Selectin (plasma)- Generation 3 Exam 1 Omni Gen 2 Exam 1

1. Funding Source/Lab

Framingham specimens	PI: Emelia J. Benjamin, MD, ScM & PAW emelia@bu.edu; **Offspring Exam 8 Omni 1(3)**
Grant #	**RO1 HL076784, 1R01 AG028321 & NO1-HC-25195, (PI Philip A Wolf)**
Lab	Freedman Lab.
Contact:	Kahraman Tanriverdi, Kahraman.tanriverdi@umassmed.edu

2. Method:

3. Technical Aspects

Commercial kit including all reagents	
Vendor	R & D Systems (Cat. No. BBE 6) **http://www.rndsystems.com/**
Minimum detectable dose Units	<0.5 ng/mL
Measuring range	0 – 50 ng/mL
Actual range measured	0 – 50 ng/mL

4. Specimen Characteristics

a. Plasma, run in single
b. Frozen samples (- 80C)

5. QC aspects

CV intra-assay:	3.6%
CV intra phantoms	
Number per cycle	
CV inter-assay	7.5%
CV threshold for re-measuring:	
Bar code reader:	yes
Internal controls	yes

6. FHS participant aspect

a. Markers run: 8/08 – 11/08
b. Measured in: ng/mL
c. Count: Generation 3 Exam 1 = 4086, Omni Group 2 Exam 1 = 410

7.Descriptive Statistics – Gen3 Exam1

	Mean	SD	Minimum	Maximum	Median	Q1	Q3
Unadjusted:	49.35	18.93	0.85	250.39	47.45	36.56	58.96
Log-transformed	3.83	0.38	-0.16	5.52	3.86	3.60	4.08

7.Descriptive Statistics – Omni Group 2 Exam1

	Mean	SD	Minimum	Maximum	Median	Q1	Q3
Unadjusted:	49.45	18.99	11.23	160.60	47.45	35.41	60.94
Log-transformed	3.83	0.38	2.42	5.08	3.86	3.57	4.11

8. Publication

Tumor necrosis factor receptor II (TNFRII)
Generation 3 Exam 1 Omni Gen 2 Exam 1

1. Funding Source/Lab

Framingham specimens	PI: Emelia J. Benjamin, MD, ScM & PAW emelia@bu.edu; **Offspring Exam 8 Omni 1(3)**
Grant #	**RO1 HL076784, 1R01 AG028321 & NO1-HC-25195, (PI Philip A Wolf)**
Lab	Freedman Lab.
Contact:	Kahraman Tanriverdi, Kahraman.tanriverdi@umassmed.edu

2. Method:

3. Technical Aspects

Commercial kit including all reagents	
Vendor	R & D Systems (Cat. No. DR **http://www.rndsystems.com/**T 200)
Minimum detectable dose Units	0.6 pg/mL
Measuring range	0 – 500 pg/mL
Actual range measured	0 – 500 pg/mL

4. Specimen Characteristics

a. Plasma, run in single
b. Frozen samples (- 80C)

5. QC aspects

CV intra-assay:	
CV intra phantoms	
Number per cycle	
CV inter-assay	4.6%
CV threshold for re-measuring:	
Bar code reader:	yes
Internal controls	yes

6. FHS participant aspect

a. Markers run: 08/08 – 11/08
b. Measured in: pg/mL
c. Count: Generation 3 Exam 1 = 4087, Omni Group 2 Exam 1 = 410

7.Descriptive Statistics – Gen3 Exam1

	Mean	SD	Minimum	Maximum	Median	Q1	Q3
Unadjusted:	2642.02	17403.37	1038.90	948113.88	2159.41	1878.48	2506.67
Log-transformed	7.70	0.27	6.95	13.76	7.68	7.54	7.83

7.Descriptive Statistics – Omni Group 2 Exam1

	Mean	SD	Minimum	Maximum	Median	Q1	Q3
Unadjusted:	2275.26	1020.30	1478.12	17631.70	2110.89	1826.73	2478.30
Log-transformed	7.68	0.27	7.30	9.78	7.65	7.51	7.82

8. Publication

Acknowledgements

The Inflammatory Marker Measurements detailed in this manual were made possible due to funding supplied by the National Institute of Health/National Heart Lung & Blood Institute (NIH/NHLBI). A brief summary of the grant specific aims are detailed below:

Framingham: Inflammation, Genes & Cardiovascular Disease
Agency: NHLBI Type: RO1 HL076784
PI: Emelia J. Benjamin, MD, ScM

DESCRIPTION:

Recent experimental and clinical studies support the concept that vascular inflammation is central to the development of atherosclerosis, and that systemic inflammatory markers predict a wide array of CVD events. There is increasing interest in the role of genetic variation in inflammation contributing to the susceptibility to CVD. To date mostly small case-control studies have suggested that polymorphisms in inflammatory genes are associated with subclinical and clinical CVD, but the studies have differed with regard to which genes are central, with many only finding the association in specific subject subgroups.

We have previously measured systemic markers of vascular inflammation (*i.e.*, CRP, sICAM-1, MCP-1, IL-6) and oxidative stress (*i.e.*, isoprostanes), in a population-based sample of 3800 middle-aged and elderly men and women of the Framingham Heart Study offspring cohort. We propose to genotype inflammatory candidate genes in the Framingham offspring cohort have been phenotyped for CVD risk factors, subclinical CVD. We also propose to measure systemic inflammatory markers in the Framingham Study Generation III cohort, who are the children of the offspring cohort.

The central hypothesis of this proposal is that systemic vascular inflammation represents a complex phenotype that evolves over a lifetime and is influenced by both environmental and genetic factors. We further postulate that variations in the inflammatory phenotype (marker levels) and genotype predispose to the development of CVD. The purpose of this proposal is to determine the contribution of genetic and environmental factors to vascular inflammation, and to define the extent to which inflammatory phenotypes and genotypes predict subclinical and clinical CVD, and enhance risk prediction models. Our proposal's specific aims are as follows:

Aim 1. **To examine the environmental determinants of systemic inflammation in the community.** .

Aim 2. **To investigate the genetic determinants of systemic inflammation.**

Aim 3. **To identify the inflammatory phenotypic and genetic determinants of subclinical CVD.**

Aim 4. **To determine the contribution of inflammatory phenotype versus genotype to prevalent and incident CVD and to incident hypertension.**

The investigation will increase understanding as to whether inflammation is a core risk factor for CVD or is merely a marker of presence and burden of other CVD risk factors. These insights will fundamentally contribute to knowledge about the pathophysiology of CVD and may lead to improved prevention, risk stratification and management of CVD.

PERFORMANCE SITE(S) *(organization, city, state)*

NHLBI's **The Framingham Heart Study** 73 Mount Wayte Ave. Suite 2 Framingham, MA 01702-5827	**BUSM, Keaney Laboratory** Whitaker Cardiovascular Institute, 715 Albany St., Rm. W507 Boston, MA 02118-2393	**BUSM Genetics Laboratory** Whitaker Cardiovascular Institute, 715 Albany Street, W408 Boston, MA 02118-2393

KEY PERSONNEL. See instructions. *Use continuation pages as needed* to provide the required information in the format shown below. Start with Principal Investigator. List all other key personnel in alphabetical order, last name first.

Name	Organization	Role on Project
Benjamin, Emelia J.	Fram. Heart Study/Boston Univ.	Principal Investigator
Baldwin, Clint	Boston University	Geneticist
Keaney, John F.	Fram. Heart Study/Boston Univ	Co-investigator
Larson, Martin G.	Fram. Heart Study/Boston Univ	Senior Statistician
Levy, Daniel	NHLBI/Fram. Heart Study	Co-investigator
Massaro, Joseph	Fram. Heart Study/Boston Univ	Statistician
Mitchell, Gary	Cardiovascular Engineering Inc.	Consultant
O'Donnell, Christopher J.	NHLBI/Fram. Heart Study	Co-investigator
Ramachandran, Vasan S.	Fram. Heart Study/Boston Univ.	Co-Principal investigator
Vita, Joseph	Fram. Heart Study/Boston Univ	Co-investigator

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