FRAMINGHAM HEART STUDY

INFLAMMATORY MARKERS MANUAL

OFFSPRING EXAM 8 OMNI 1(3)

The FHS Offspring Exam 8 Omni 1(3) Inflammatory Marker Manual was 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.

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.

FHS Inflammatory Marker
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 aliquotted. 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) – Offspring Exam 8 Omni 1

1. Funding Source/Lab

Framingham specimens	PI: Emelia J. Benjamin, MD, ScM & PAW emelia@bu.edu; **Offspring Exam 8**
Grant #	**RO1 HL076784 ,1R01 AG028321 & NO1-HC-25195, (PI Philip A Wolf)**
Lab	FHS Lab
Contact:	Patrice Sutherland: patrices@bu.edu

2. Method: Immuno Turbidometric

3. Technical Aspects

Commercial kit including all reagents	
Vendor	Roche Diagnostics Latex High Sensitivity Assay http://www.roche-diagnostics.us
Minimum detectable dose units	0.15 mg/L
Measuring range	0.165- 262,5 mg/L
Actual range measured	0.14 - 162.89
CODING MANUAL	0.14 mg/L -" Cases which measured below the lowest detectable assay limit of 0.15 mg/L were set to 0.14 mg/L (n = 21). The user should determine how to handle these cases.

4. FHS Specimen Characteristics

a. Serum, 8.4% run in duplicate
b. Frozen samples (- 80C)

5. QC aspects

CV intra-assay:	2.5%
intra phantoms inter class correlation (ICC)	0.997
Number per cycle	127
CV inter-assay	4.5%
CV threshold for re-measuring:	n/a
Bar code reader:	n/a
Internal controls	Yes

6. FHS participant aspect

a. Markers run: 4/8/09 – 7/22/09
b. Measured in: mg/L
c. Offspring Exam 8 n = 2885 & Omni Gen 1 Exam 3 n = 294

Descriptive Statistics -

	Mean	SD	Minimum	Maximum	Median	Q1	Q3
Unadjusted:	3.35	7.33	0.14	162.89	1.52	0.74	3.26
Log-transformed	0.48	1.11	-1.97	5.09	0.42	-0.30	1.81

Intercellular adhesion molecule 1 (ICAM 1)

1. Funding Source/Lab

Framingham specimens	PI: Emelia J. Benjamin, MD, ScM Emelia@bu.edu
Grant #	**RO1 HL 064753** & **RO1 HL076784**
Lab	JFK/IL
Contact:	Cheryl Ingram; Cheryl.Ingram@umassmed.edu

2. Method: Quantitative ELISA

3. Technical Aspects

Molecular Devices VersaMax microplate reader	
Commercial kit including all reagents	
Vendor	R&D Systems
Minimum detectable dose	0.35 ng/mL
Standard curve range	0-50 ng/mL

4. FHS Specimen Characteristics

a. Serum, 10% run in duplicate
b. Frozen samples, run on 2nd thaw
c. Samples were subjected to 1-3 freeze-thaw cycles.

5. QC aspects

CV intra-assay:	2.31
CV intra phantoms	11..6
Number per cycle	132
CV inter	--
CV threshold for re-measuring:	n/a
Bar code reader:	n/a

6. FHS participant aspect

a. Markers run: 5/29/08-7/3/08
b. Measured in: Ng/mL
c. Count Offspring n = 2907 & Omni n = 292 & Spouses n=100

Descriptive Statistics -

	Mean	**SD**	**Minimum**	**Maximum**	**Median**	**Q1**	**Q3**
Unadjusted:	296.02	106.4	1.86	1034.32	271.96	230.4	337.2
Log-transformed	5.63	0.36	0.62	6.94	5.60	5.44	5.82

ICAM-1 Kits Shipped in 2008

Ship Date	Catalogue #	Description	Quantity	Lot#
	DCD540	**Quantikine Human sICAM-1/CD54**		25687

Interleukin-6 (IL6) (High Sensitivity)

1.Funding Source/Lab

Framingham specimens	PI: Emelia J. Benjamin, MD, ScM Emelia@bu.edu
Grant #	**RO1 HL 064753** & **RO1 HL076784**
Lab	JFK/IL
Contact:	Cheryl Ingram; Cheryl.Ingram@umassmed.edu

2. Method: Quantitative ELISA

3. Technical Aspects

Molecular Devices VersaMax microplate reader	
Commercial kit including all reagents	
Vendor	R & D Systems **http://www.rndsystems.com/**
Minimum detectable dose	0.039 pg/mL
Standard curve range	0 – 10 pg/mL

4. FHS Specimen Characteristics

a. Serum, 10% run in duplicate
b. Frozen samples, run on 1st thaw
c. Samples were subjected to 1-3 freeze-thaw cycles.
d. Volume of sample: 100uL

5. QC aspects

CV intra-assay:	4.01
CV intra phantoms	12.29
Number per cycle	132
CV inter	3.73
CV threshold for re-measuring:	n/a
Bar code reader:	no
Internal controls	yes

6. FHS participant aspect

a. Markers run: 4/15/08 – 5/23/08
b. Measured in: pg/mL
c. Count Offspring n = 2907 & Omni n = 289 & Spouses n=100

Descriptive Statistics -

	Mean	SD	Minimum	Maximum	Median	Q1	Q3
Unadjusted:	2.64	2.95	0.15	27.30	1.77	1.19	2.87
Log-transformed	0.65	0.74	-1.90	3.31	0.57	0.17	1.05

HS Il6 Kits Shipped in 2008

Ship Date	Catalogue #	Description	Quantity	Lot#
	HS600B	QUANTIKINE HS HUMAN IL-6		254935

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 HL0767841, 1R01 AG028321 & NO1-HC-25195, (PI Philip A Wolf)**
Lab	FHS Lab
Contact:	Patrice Sutherland: patrices@bu.edu

2. Method: Colorimetric

3. Technical Aspects

Commercial kit including all reagents	
Vendor	Roche Diagnostics Roche Hitachi 911 http://www..roche-diagnostics.us
Minimum detectable dose Units	0.2 mg/L
Measuring range	0.2 – 650 mg/L
Actual range measured	4.4 – 477.2

4. FHS Specimen Characteristics

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

5. QC aspects

CV intra-assay:	1.2%
CV intra phantoms Offspring	5.3%
CV intra phantoms Omni	1.7%
Number per cycle	115
CV inter-assay	2.2%
CV threshold for re-measuring:	n/a
Bar code reader:	n/a
Internal controls	Yes

6. FHS participant aspect

a. Markers run: 2/8/07 – 1/28/08
b. Measured in: mg/L
c. Count: Offspring exam 8 = 2788 & Omni 1(3) = 288

Descriptive Statistics -

	Mean	**SD**	**Minimum**	**Maximum**	**Median**	**Q1**	**Q3**
Unadjusted:	1029.84	592.15	10.17	5048.95	958.4	599.24	1363.72
Log-transformed	6.74	0.70	2.32	8.53	6.87	6.40	7.22

Urinary Creatinine (run for Isoprostanes)

1. Funding Source/Lab

Framingham specimens	PI: Emelia J. Benjamin, MD, ScM emelia@bu.edu
Grant #	**RO1 HL 064753** & **RO1 HL076784**
Lab	FHS Lab
Contact:	Patrice Sutherland: patrices@bu.edu

2. Method: colorimetric

3. Technical Aspects

Commercial kit including all reagents

Vendor	Roche Diagnostics (Roche Hitachi 911) Creatinine/Rate-Blanked
Minimum detectable dose	n/a
Measuring Range:	0.2 - 650.0 mg/L (higher on dil'n)

4. FHS Specimen Characteristics

a. Urine, run in duplicate
b. Frozen samples (-20C)

Please note: The total N run for urinary isoprostanes is smaller than that for urinary creatinine. Two different sample sets were used to run these analytes. Collection of the isoprostane sample set was initiated approximately three months after the beginning of the exam cycle.

5. QC aspects

CV intra-assay:	1.2%
CV intra phantoms	Offspring N=115, CV=5.3% Omni N=12, CV= 1.7%
Number per cycle	Offspring N=115 Omni N=12
CV inter –assay	2.2%
CV threshold for re-measuring:	n/a
Bar code reader:	no
Internal controls	yes

6. FHS participant aspect

a. Markers run: 2/8/07 - 1/28/08
b. Measured in: mg/100mL
c. Count Offspring n = 3021 & Omni n = 298

Descriptive Statistics -

	Mean	SD	Minimum	Maximum	Median	Q1	Q3
Unadjusted:	103.54	61.92	4.4	477.2	95.9	56.7	138.2
Log-transformed	4.44	0.68	1.48	6.17	4.56	4.04	4.93

Lipoprotein-associated phospholipase 2 Activity

1. Funding Source/Lab

Framingham specimens	PI: Emelia J. Benjamin, MD, ScM Emelia@bu.edu
Grant #	**RO1 HL 064753** & **RO1 HL076784**
Lab	
Contact:	

2. Method: Quantitative ELISA

3. Technical Aspects

SpectraMax Plate reader: SN N-02741,	
Commercial kit including all reagents	
Vendor	DiaDexus Inc.
Minimum detectable dose	
Standard curve range	

4. FHS Specimen Characteristics

a. EDTA Plasma, run in duplicate
b. Frozen samples, run on 3rd thaw
c. Samples were subjected to 1-3 freeze-thaw cycles.
Samples were run in single point and 20% of total samples in duplicate points

5. QC aspects

CV intra-assay:	24.87
CV intra phantoms	10.98
Number per cycle	128
CV inter	6.8
CV threshold for re-measuring:	n/a
Bar code reader:	No
Internal controls	yes

6. FHS participant aspect

a. Markers run: March 30, 2009
b. Measured in: nmol/min/mL.
c. Count Offspring n = 2,972 Omni n=301

Descriptive Statistics -

	Mean	**SD**	**Minimum**	**Maximum**	**Median**	**Q1**	**Q3**
Unadjusted:	139.72	34.75	39.02	315.20	137.28	115.28	160.91
Log-transformed							

LpPLA2 mass Kits Shipped in 2008

Ship Date	Cat #	Description	Qty	Lot#
		Plate Washer (PLAC analysis):		DDX #36

Lipoprotein-associated phospholipase 2 Mass

1. Funding Source/Lab

Framingham specimens	PI: Emelia J. Benjamin, MD, ScM Emelia@bu.edu
Grant #	**RO1 HL 064753** & **RO1 HL076784**
Lab	
Contact:	

2. Method: Quantitative ELISA

3. Technical Aspects

Molecular Devices VersaMax microplate reader	
Commercial kit including all reagents	
Vendor	DiaDexus Inc.
Minimum detectable dose	
Standard curve range	

4. FHS Specimen Characteristics

a. EDTA Plasma, 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:	24.77
CV intra phantoms	8.12
Number per cycle	128
CV inter	3.1
CV threshold for re-measuring:	n/a
Bar code reader:	No
Internal controls	yes

6. FHS participant aspect

a. Markers run: March 30, 2009
b. Measured in: ng/mL
c. Count Offspring n = 2,972, Omni n= 301

Descriptive Statistics -

	Mean	**SD**	**Minimum**	**Maximum**	**Median**	**Q1**	**Q3**
Unadjusted:	202.90	50.29	38.20	556.30	202.20	171.80	230.63
Log-transformed							

LpPLA2 mass Kits Shipped in 2008

Ship Date	Cat #	Description	Qty	Lot#
		Plate Washer (PLAC analysis):		DDX #36

Monocyte chemoattractant protein-1 (MCP1)

1.Funding Source/Lab

Framingham specimens	PI: Emelia J. Benjamin, MD, ScM emelia@bu.edu
Grant #	**RO1 HL 064753** & **RO1 HL076784**
Lab	JFK/IL
Contact:	Cheryl Ingram; Cheryl.Ingram@umassmed.edu

2. Method: Quantitative ELISA

3.Technical Aspects

Molecular Devices VersaMax microplate reader	
Commercial kit including all reagents	
Vendor	R & D Systems **http://www.rndsystems.com/**
Minimum detectable dose	<5.0 pg/mL
Standard curve range	0 – 2000 pg/mL

4.FHS Specimen Characteristics

a. Serum, 10% 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:	1.39
CV intra phantoms	8.4
Number per cycle	131
CV inter	2.43
CV threshold for re-measuring:	n/a
Bar code reader:	n/a
Internal controls	yes

6. FHS participant aspect

a. Markers run: 7/14/08 – 9/4/08
b. Measured in: pg/mL
c. Count Offspring n = 2771 & Omni n = 278 & Spouses n=96

Descriptive Statistics -

	Mean	**SD**	**Minimum**	**Maximum**	**Median**	**Q1**	**Q3**
Unadjusted:	379.94	122.10	117.39	1655.63	363.28	296.93	440.14
Log-transformed	5.89	0.31	4.77	7.41	5.89	5.69	6.09

MCP-1 Kits Shipped in 2008

Ship Date	Catalogue #	Description	Qty	Lot#
	DCP00	QUANTIKINE HUMAN CCL2/MCP-1		240267

Osteoprotegerin (OPG)

1. Funding Source/Lab

Framingham specimens	PI: Emelia J. Benjamin, MD, ScM Emelia@bu.edu
Grant #	**RO1 HL 064753** & **RO1 HL076784**
Lab	JFK/IL/AB
Contact:	Cheryl Ingram;Cheryl.Ingram@umassmed.edu

2. Method: Quantitative ELISA

3. Technical Aspects

Molecular Devices VersaMax microplate reader	
Commercial kit including all reagents	
Vendor	Biomedica Gesellschaft mbH, Vienna, Austria American Vendor ALPCO () **http://www.moleculardevices.com/pages/instruments/versamax.html**
Minimum detectable dose	0.14 pmol/L
Standard curve range	0 – 30 pmol/L

4. FHS Specimen Characteristics

a. EDTA Plasma, 10% run in duplicate
b. Frozen samples, run on 1st thaw
c. Samples were subjected to 1-3 freeze-thaw cycles.

5. QC aspects

CV intra-assay:	4.68
CV intra phantoms	9.61
Number per cycle	128
CV inter	6.4
CV threshold for re-measuring:	n/a
Bar code reader:	n/a
Internal controls	yes

6. FHS participant aspect

a. Markers run: 9/8/08 – 12/4/08
b. Measured in: pmol/L
c. Count Offspring n = 2,885 & Omni n = 308

Descriptive Statistics -

	Mean	**SD**	**Minimum**	**Maximum**	**Median**	**Q1**	**Q3**
Unadjusted:	5.00	1.71	0.84	26.90	4.74	3.91	5.72
Log-transformed	1.56	0.31	-0.17	3.29	1.56	1.36	1.74

OPG Kits Shipped in 2008

Ship Date	Catalogue #	Description	Qty	Lot#
	04-B1-20402	OSTEOPROTEGERIN		383a

N.B.

Biomedica Gesellschaft mbH.via Alpco offered the Framingham Heart study a substantial discount on the purchase price of this kit. In accepting this offer all collaborators from this study agree to reference the kit source in any and all publications that result from these data sets. Both the manufacturer and US distribution source will be cited. Biomedica of Vienna, Austria, supplied by Alpco Diagnostics.

P-Selectin

1. Funding Source/Lab

Framingham specimens	PI: Emelia J. Benjamin, MD, ScM Emelia@bu.edu
Grant #	**RO1 HL 064753** & **RO1 HL076784**
Lab	JFK/IL/AB
Contact:	Cheryl Ingram; Cheryl.Ingram@umassmed.edu

2. Method: Quantitative ELISA

3. Technical Aspects

Molecular Devices VersaMax microplate reader	
Commercial kit including all reagents	
Vendor	R & D Systems **http://www.rndsystems.com/**
Minimum detectable dose	<0.5 ng/mL
Standard curve range	0 – 50 ng/mL

4. FHS Specimen Characteristics

a. EDTA Plasma, 10% run in duplicate
b. Frozen samples, run on 2nd thaw
c. Samples were subjected to 1-3 freeze-thaw cycles.

5. QC aspects

CV intra-assay:	0.68
CV intra phantoms	9.02
Number per cycle	128
CV inter	0.81
CV threshold for re-measuring:	n/a
Bar code reader:	no
Internal controls	yes

6. FHS participant aspect

a. Markers run: 12/22/08-2/21/09
b. Measured in: ng/mL
c. Count Offspring n =3,013 & Omni n = 308

Descriptive Statistics -

	Mean	**SD**	**Minimum**	**Maximum**	**Median**	**Q1**	**Q3**
Unadjusted:	41.16	13.65	9.94	223.10	39.82	32.68	48.02
Log-transformed	3.67	0.32	2.30	5.41	3.68	3.49	3.87

P-Selectin Kits Shipped in 2008

Ship Date	Catalogue #	Description	Qty	Lot#
	BBE6	HUMAN Sp-selectin		261632

Tumor necrosis factor receptor II (TNFRII)

1. Funding Source/Lab

Framingham specimens	PI: Emelia J. Benjamin, MD, ScM Emelia@bu.edu
Grant #	**RO1 HL 064753** & **RO1 HL076784**
Lab	JFK/IL/AB
Contact:	Cheryl Ingram; Cheryl.Ingram@umassmed.edu

2. Method: Quantitative ELISA

3. Technical Aspects

Molecular Devices VersaMax microplate reader	
Commercial kit including all reagents	
Vendor	R & D Systems **http://www.rndsystems.com**
Minimum detectable dose	0.2 pg/mL
Standard curve range	0 – 500 pg/mL

4. FHS Specimen Characteristics

a. EDTA Plasma, 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:	2.26
CV intra phantoms	7.64
Number per cycle	128
CV inter	1.63
CV threshold for re-measuring:	n/a
Bar code reader:	no
Internal controls	yes

6. FHS participant aspect

a. Markers run: 7/8/09-8/21/09
b. Measured in: pg/mL
c. Count Offspring n = 2892 & Omni n = 294

Descriptive Statistics -

	Mean	**SD**	**Minimum**	**Maximum**	**Median**	**Q1**	**Q3**
Unadjusted:	2625.48	1090.82	768.15	9528.70	2358.07	1910.71	3028.68
Log-transformed	7.80	0.36	6.64	9.16	7.77	7.56	8.02

sTNF RII Kits Shipped in 2008

Ship Date	Cat #	Description	Qty	Lot #
	DRT 200	QUANTIKINE HUMAN sTNF RII/TNFRSF1B		262177

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.

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
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Aging and Inflammation: Longitudinal Markers and Genetics in the Framingham Study

Agency: NHLBI Type:1R01AG028321
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DESCRIPTION:

Recent experimental and clinical studies have established that vascular inflammation is central to the nonvascular and vascular aging. To date studies with mostly single occasion assessments of markers or single nucleotide polymorphisms (SNPs) have suggested that variation in inflammatory pathway markers and SNPs are associated with the aging process and subclinical CVD. However, the studies have differed with regard to which markers and genes are central, and have left questions as to whether inflammation begets aging and subclinical CVD, whether aging and subclinical CVD lead to inflammation. We previously measured 11 systemic biomarkers and 3000 SNPs in over 200 candidate genes in inflammatory pathways in the community-based Framingham Offspring sample. The 3500 middle-aged and elderly men and women receive serial phenotyping for age-related phenotypes including physical function, CVD risk factors, and subclinical and clinical CVD. The extent to which inflammatory biomarkers increase with advancing age, independent of age-related CVD and its risk factors is uncertain. The relation of variation in inflammatory genes to aging-related phenotypes, including frailty, physical function and subclinical CVD is largely unknown. **The central hypothesis of this proposal is that the acceleration of systemic inflammation in midlife and advanced age is influenced by both risk factors and genetic variation. We postulate that variation in inflammatory pathway genes modulates longitudinal changes in inflammatory markers, and vascular aging (as assessed by increasing blood pressure and subclinical CVD), and the progression of frailty and declining physical function.** To address these hypotheses we propose to repeat at the measurements of 7 key inflammatory biomarkers, originally assessed 7 years. Specific Aims:

Aim 1. To examine the risk factors related to 7 year progression of systemic inflammation (between Offspring examinations 7 and 8, and Omni exams 2 & 3) in the community.

Aim 2. To investigate the genetic factors associated with systemic inflammation and to examine the relation between inflammatory SNPs/haplotypes and frailty and declining physical function.

Aim 3. 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.

Aim 4. To identify the relations between changes in inflammatory biomarkers to frailty and progression of declining physical function over 7 years of follow-up.

The proposed study will fundamentally contribute insight about the relations of inflammation and aging.

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

The Framingham Heart Study
73 Mount Wayte Ave. Suite 2
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KEY PERSONNEL.

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Framingham Heart Study (Offspring cohort exam 8)
Analysis by Lp-PLA2 Mass (PLAC) and Lp-PLA2 Activity (CAM) Assay
Quality Control Report

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Date Reported: 9/2/09

MATERIALS

1. Reagents

	Catalog No.	Lot No.
Lp-PLA2 Mass ELISA Kit	90123	906054
CAM Substrate	10080	903050
CAM Buffer	21072	903017

2. Controls

	Catalog No.	Lot No.
Lp-PLA2 Serum Control 1	65003	703028
Lp-PLA2 Serum Control 2	65004	703029
CAM Co03	70045	904013
CAM Co05	70047	904014

3. Instruments
   - SpectraMax Plate reader: SN N-02741
   - Plate Washer (PLAC analysis): DDX #365

4. Date Received: March 30, 2009
   Samples Received: Plasma samples for PLAC and CAM analysis
   Samples Tested: 2972 PLAC and CAM.

STUDY DESIGN

1. PLAC analysis
   1.1. Calibrators and controls were run in duplicate. Controls run throughout the plate.
   1.2. Results reported in units of ng/mL.
   1.3. Samples run in single points and 20% of total samples in duplicate points.
2. CAM analysis
   1.1. Controls were run in duplicate and tested on each plate.
   1.2. Results reported in units of nmol/min/mL.
   1.3. Samples were run in single point and 20% of total samples in duplicate points.

ACCEPTABILITY CRITERIA

1. PLAC
   1.1. CVs calculated on duplicate calibrators and controls are to be less than or equal to 15%.
   1.2. PLAC Controls:
   Serum C1 Range 165.2 – 240.4 (Mean 202.8) ng/mL
   Serum C2 Range 351.6 – 497.4 (Mean 424.5) ng/mL
2. CAM
   1.1. CVs calculated on duplicate controls are to be less than or equal to 15%.
   1.2. CAM Controls:
   Co03 Range 140.9 - 179.3 (Mean 160.1) nmol/min/mL
   Co05 Range 64.0 - 96.0 (Mean 80.0) nmol/min/mL

RESULTS

Sample Results

Excel File Attached

Sample Integrity and Processing

Sample volume generally observed at about 200 µL per samples. Samples were free of particulates, hemolysis, and lipemia except where noted in the comments section of the results.

PLAC Analysis

- 51 plates, including repeats were run by PLAC analysis.
- The reported control and sample results all passed acceptance criteria.
- A full distribution of results is shown in Figure 1.
- One sample is out of range > 1000ng/ml and is not included in figure 1.

CAM Analysis

- 51 plates, including repeat plates, were run by CAM analysis.
- The reported control and sample results all passed acceptance criteria.
- A full distribution of results is shown in Figure 2.

Results Summary

	Moments		Quantiles						
Test	Count	Mean	Min	5th	25th	Median	75th	95th	Max
PLAC (ng/mL)	2971*	202.90	38.2	122.15	171.85	202.20	230.58	288.59	556.30
CAM (nmol/min/mL)	2971	139.72	39.02	86.36	115.34	137.28	160.89	199.37	315.20

*****One sample out of range >1000ng/ml.

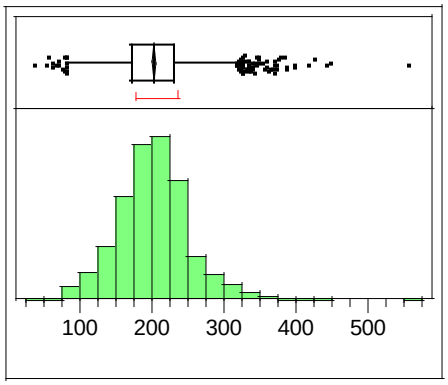
QC Control Summary

	N	Mean	% CV
PLAC Serum Control 1 (ng/mL)	150	201.5	5.6
PLAC Serum Control 2 (ng/mL)	150	405.2	6.6
CAM Co03 (nmol/min/mL)	51	161.6	4.7
CAM Co05 (nmol/min/mL)	51	83.2	3.6

Figure 1

Framingham Heart Study Offspring exam 8: Lp-PLA2 (PLAC) Distribution

PLAC ng/ml

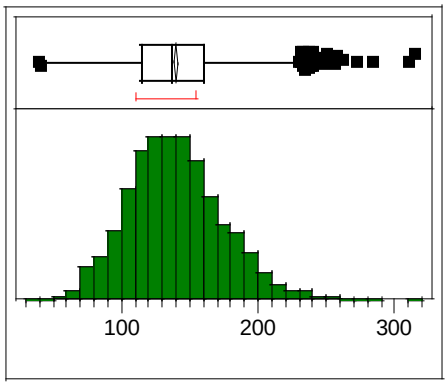


Quantiles			Moments	
100.0%	maximum	556.30	Mean	202.90182
99.5%		371.96	Std Dev	50.291453
97.5%		315.30	Std Err Mean	0.9228178
90.0%		262.60	upper 95% Mean	204.71125
75.0%	quartile	230.63	low er 95% Mean	201.09239
50.0%	median	202.20	N	2970
25.0%	quartile	171.80	Sum Wgt	2970
10.0%		141.53	Sum	602618.4
2.5%		105.08	Variance	2529.2302
0.5%		82.29	Skew ness	0.5016262
0.0%	minimum	38.20	Kurtosis	1.9249465
			CV	24.786103
			N Missing	0

Figure 2

Framingham Heart Study Offspring exam 8: Lp-PLA2 Activity(CAM) Distribution

CAM nmol/min/ml



Quantiles			Moments	
100.0%	maximum	315.20	Mean	139.71573
99.5%		244.32	Std Dev	34.75333
97.5%		213.23	Std Err Mean	0.6375953
90.0%		186.02	upper 95% Mean	140.9659
75.0%	quartile	160.91	low er 95% Mean	138.46555
50.0%	median	137.28	N	2971
25.0%	quartile	115.28	Sum Wgt	2971
10.0%		98.14	Sum	415095.43
2.5%		77.23	Variance	1207.794
0.5%		61.08	Skew ness	0.4373371
0.0%	minimum	39.02	Kurtosis	0.5384919
			CV	24.874315
			N Missing	0