FRAMINGHAM HEART STUDY

INFLAMMATORY MARKERS MANUAL

OFFSPRING EXAM 7 & OMNI 1(2)

The FHS Offspring Exam 7 & Omni 1(2) Inflammatory Marker Manual was prepared by:
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Product Information of Assay Kits for each marker

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.

RO1 HL064753 (PI: Emelia J. Benjamin, MD, ScM ***)*** 07/01/00 - 06/30/2004
NIH/NHLBI
Inflammation: Correlates and Prognosis in Framingham
Specific Aims: To determine the relation between inflammatory markers & CVD & its risk factors in the FHS Offspring examination 7.

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. Linking to the FHS data base, specimen bar code labels are generated that include FHS participant ID number and draw date. When the inflammation project was initiated the FHS had about 7 freezers, one of which was @-70C. As of 2007 the FHS has 22 freezers (1 @ ~ -70C, 21 @ -80C). The vast majority of samples were stored at -80 degrees Celsius. Specimens are aliquoted after centrifugation and stored at -80^{o}C without freeze thaw cycles until assay. The stability of specimens that have been stored at -80^{o}C for years has been verified for CRP and other antigens. Shipped specimens are identified by FHS ID number, blinding laboratory personnel, and maintaining confidentiality.

FHS timing of phlebotomy and urine collection. Some biomarkers potentially could be affected by time of day or food; consecutive 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's are measured on serum, using a nephelometric method on a Dade Behring BN100 with Dade Behring reagents. CRP assays are run in single. Reproducibility is assessed using phantom samples and random repeats.

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

Random urine samples were collected and frozen at -20C for subsequent analysis. Urine creatinines were measured using a modified Jaffe reaction on an Abbott Spectrum CCX with Abbott reagents. Samples are diluted 1:20 with normal saline and run in duplicate.
Testing is repeated if:
- for creatinine ≤50mg/100ml; if the difference is >4.0 mg/100ml
- for creatinine >50 mg/100ml; if the difference is >6.5%.

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 20. 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 $\mid y - y \mid$ • Range $\mid y - y \mid$ • CV%
	Daily	Phantom variability • 4% specimens assigned a dummy ID and rerun as phantoms	• $\mid y_1 - y_2 \mid$ 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			

CD40 Ligand (CD40L) Plasma

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:	Izabella Lipinska: lipinska@bu.edu

2. Method: Quantitative ELISA

3. Technical Aspects

Molecular Devices VersaMax microplate reader	
Commercial kit including all reagents	
Vendor	Bender MedSystems (Cat. No. BMS 293) http://www.bendermedsystems.com/
Minimum detectable dose	0.005 ng/mL
Standard curve range	0.08 – 5 ng/mL

4. FHS Specimen Characteristics

a. EDTA Plasma, 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:		4.59±3.63		
CV intra FHS IDs:		4.58±3.63		
CV Intra-assay final data set. If sample re-measured because CV is higher than threshold then use the lowest CV.		4.43±3.40		
CV intra phantoms		4.79±3.70		
Number per cycle		148		
CV inter		no inter CVs since some run in the same plate or same day.		
CV threshold for re-measuring:		12.9		
Bar code reader:		Yes		
Internal controls	mean	std	min	max
	0.40	0.06	0.27	0.56

Mean and CV by month:

Month	09/2004	10/2004	11/2004	12/2004	01/2005
N	78	1217	989	1028	604
Mean±std	3.25±4.90	3.10±4.59	3.36±4.80	3.26±4.78	3.30±4.73
CVmean±std	5.45±4.15	4.83±3.92	4.57±3.44	4.45±3.47	4.26±3.48

6. FHS participant aspect

a. Markers run: 10/04 – 2/05
b. Measured in: ng/mL
c. Count Offspring n = 3305 & Omni n = 400

Descriptive Statistics

	Mean	SD	Minimum	Maximum	Median	Q1	Q3
Unadjusted:	3.27	4.76	0.068	29.45	1.17	0.53	3.89
Log-transformed	0.39	1.24	2.69	3.38	0.15	0.64	1.36

7. Marker residuals (studentized)

Adjustment	Mean	SD	Minimum	Maximum	Media	Q1	Q3
Age & Sex	5.20E-6	1.000	-2.59	2.57	-0.20	-0.83	0.75
Multivariable	2.45E-5	1.000	-2.73	2.77	-0.18	-0.82	0.75
covariates: idtype, age, sex, bmi, smoke, cvd, glucose, tot_hdl, lipidrx, hrx, sbp, dbp, hrtnow, asa3week, waist, diab, trig							

Publication[1]

CD40 Ligand (CD40L, Serum)

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:	Izabella Lipinska: lipinska@bu.edu

2. Method: Quantitative ELISA

3. Technical Aspects

Molecular Devices VersaMax microplate reader	
Commercial kit including all reagents	
Vendor	Bender MedSystems (Cat. No. BMS 239) **http://www.bendermedsystems.com/**
Minimum detectable dose	0.062 ng/mL
Standard curve range	0.16 – 10 ng/mL

4. FHS Specimen Characteristics

a. Serum, 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:		5.19±6.40		
CV intra FHS IDs:		5.22±6.49		
CV Intra-assay final data set. If sample re-measured because CV is higher than threshold then use the lowest CV.		4.69±4.45		
CV intra phantoms		4.37±3.37		
Number per cycle		144		
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:		11.5		
Bar code reader:		Yes		
Internal controls	mean	std	min	max
	5.2	0.8	3.4	6.7

Mean and CV by month:

Month	06/2003	07/2003	08/2003	09/2003	10/2003
N	102	651	646	831	850
Mean±std	1.10±1.42	4.02±3.38	2.72±2.47	3.00±3.01	5.17±3.57
CVmean±std	11.8±23.4	5.18±6.67	4.50±7.13	4.88±3.63	5.00±3.38

Month	11/2003	12/2003	02/2004	03/2004	04/2004
N	416	267	252	21	26
Mean±std	4.89±4.00	5.56±3.90	2.21±2.18	1.84±2.12	1.86±2.50
CVmean±std	5.26±3.22	5.43±3.52	5.40±5.01	4.08±2.50	7.94±19.2

6. FHS participant aspect

a. Markers run: 09/03 – 04/04
b. Measured in: ng/mL
c. Count Offspring n = 3281 & Omni n = 397

Descriptive Statistics -

	Mean	SD	Minimum	Maximum	Median	Q1	Q3
Unadjusted:	3.91	3.44	0.005	33.18	2.98	1.13	5.87
Log-transformed	0.75	1.51	-5.30	3.50	1.09	0.13	1.77

7. Marker residuals (studentized)

Adjustment	Mean	SD	Minimum	Maximum	Median	Q1	Q3
Age & Sex	-2.05E-6	1.000	-4.21	1.92	0.23	-0.41	0.68
Multivariable	-4.49E-6	1.000	-4.26	1.89	0.23	-0.40	0.67
covariates: idtype, age, sex, bmi, smoke, cvd, glucose, tot_hdl, lipidrx, hrx, sbp, dbp, hrtnow, asa3week, waist, diab, trig							

C-reactive protein (CRP)

1. Funding Source/Lab

Framingham specimens	PI: Emelia J. Benjamin, MD, ScM emelia@bu.edu; **Offspring Exam 7** PI: Ramachandran S. Vasan, MD vasan@bu.edu; **Offspring Exam 6** PI: Peter W.F. Wilson, MD, **Offspring Exam 2**
Grant #	**RO1 HL 064753**, **RO1 HL076784 (Benjamin) & NO1-HC-25195 (Wolf)**
Lab	FHS Lab
Contact:	Patrice Sutherland: patrices@bu.edu

2. Method: Particle enhanced immunonephelometry

3. Technical Aspects

Commercial kit including all reagents	
Vendor	Dade Behring BN 100 – High Sensitivity CRP reagent http://www.dadebehring.com
Minimum detectable dose Units	0.16 mg/L
Measuring range	0.16 - 1100 mg/L
Actual range measured	0.15 – 250.50
	0.14mg/L – Cases measured below the lowest detectable assay limit of 0.15mg?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, run in single
b. Frozen samples (- 80C)

5. QC aspects

CV intra-assay:	3.20 %
CV intra phantoms	3.9 %
Number per cycle	116
CV inter-assay	5.3 %
CV threshold for re-measuring:	n/a
Bar code reader:	No
Internal controls	yes

6. FHS participant aspect

a. Markers run:
b. Measured in: mg/L
c. Count Offspring n = 3301 & Omni n = 398

Descriptive Statistics -

	Mean	SD	Minimum	Maximum	Median	Q1	Q3
Unadjusted:	4.32	7.71	0.16	250.5	2.14	0.99	5.12
Log-transformed	0.81	1.13	-1.83	5.52	0.76	-0.01	1.63

7. Marker residuals (studentized)

Adjustment	Mean	SD	Minimum	Maximum	Median	Q1	Q3
Age & Sex	-7.47E-6	1.000	-2.62	4.24	-0.0261	-0.72	0.72
Multivariable	-1.52E-5	1.000	-3.30	5.09	-0.0262	-0.71	0.62
covariates: idtype, age, sex, bmi, smoke, cvd, glucose, tot_hdl, lipidrx, hrx, sbp, dbp, hrtnow, asa3week, waist, diab, trig							

8. Publication[1-12]{Benjamin, 2007 10160 /id}

Fibrinogen

1.Funding Source/Lab

Framingham specimens	NO1 HC 25195 (Wolf)
Specimens examining technical aspects of Fibrinogen	
Lab	FHS Lab
Contact:	Patrice Sutherland: patrices@bu.edu

2. Method: Clauss method (clot time)

3. Technical Aspects

Commercial kit including all reagents	
Vendor	Diagnostica Stago Reagents – STart 4 http://www.stago.com/gb/asp/home_global.asp
Units	mg/100ml
Measuring range	90 – 1800 mg/100ml
Actual Measuring Range	181-1194 mg/100ml

4. FHS Specimen Characteristics

a. Run in duplicate
b. Citrate plasma not previously thawed

5. QC aspects

CV intra-assay:	1.1 %
CV intra phantoms	3.1%
Number per cycle	118
CV inter	4.4 %
CV threshold for re-measuring:	n/a
Bar code reader:	n/a
Internal controls	yes

6. FHS participant aspect

a. Markers run: 12/18/02 – 11/04/03
b. Measured in: mg/100ml
c. Count Offspring n = 3178 & Omni n = 397

Descriptive Statistics -

	Mean	SD	Minimum	Maximum	Median	Q1	Q3
Unadjusted:	379.8	74.7	181.0	1194	371	329	422
Log-transformed	5.92	0.19	5.20	7.09	5.92	5.80	6.05

7. Marker residuals (studentized)

Adjustment	Mean	SD	Minimum	Maximum	Median	Q1	Q3
Age & Sex	-1.05E-7	1.000	-3.77	6.21	-0.0259	-0.65	0.65
Multivariable	-1.26E-5	1.000	-4.36	6.95	-0.0081	-0.66	0.66
covariates: idtype, age, sex, bmi, smoke, cvd, glucose, tot_hdl, lipidrx, hrx, sbp, dbp, hrtnow, asa3week, waist, diab, trig							

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:	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
c. Samples were subjected to 1-3 freeze-thaw cycles.

5. QC aspects

CV intra-assay:		3.93±2.86		
CV intra FHS IDs:		3.94±2.97		
CV Intra-assay final data set. If sample re-measured because CV is higher than threshold then use the lowest CV.		3.70±2.40		
CV intra phantoms		3.71±2.67		
Number per cycle		147		
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:		8.8		
Bar code reader:		Yes		
	mean	std	min	max
R&D controls	269.67	23.19	161	335
Internal controls	218.14	15.50	180	245

Mean and CV by month:

Month	07/2001	08/2001	09/2001	10/2001	11/2001
N	485	614	593	1431	831
Mean±std	249±60	261±67	241±61	257±89	259±120
CVmean±std	3.56±2.68	3.74±2.67	4.63±2.68	3.98±2.65	3.74±3.48
Month	12/2001	02/2002	04/2002		
N	35	39	11		
Mean±std	280±86	371±150	256±84		
CVmean±std	4.16±2.61	3.14±2.15	2.75±1.93		

6. FHS participant aspect

a. Markers run: 07/01 – 04/02

b. Measured in: ng/mL

c. Count Offspring n = 3303 & Omni n = 398

Descriptive Statistics -

	Mean	SD	Minimum	Maximum	Median	Q1	Q3
Unadjusted:	255.98	86.82	2.47	1384	241.26	209.74	283.13
Log-transformed	5.5	0.30	0.90	7.23	5.49	5.35	5.65

7. Marker residuals (studentized)

Adjustment	Mean	SD	Minimum	Maximum	Median	Q1	Q3
Age & Sex	-2.0E-6	1.000	-14.90	6.27	-0.062	-0.55	0.49
Multivariable	-2.59E-5	1.001	-15.82	6.53	-0.032	-0.53	0.49
covariates: idtype, age, sex, bmi, smoke, cvd, glucose, tot_hdl, lipidrx, hrx, sbp, dbp, hrtnow, asa3week, waist, diab, trig							

ICAM-1 Kits Shipped in 2001

Ship Date	Catalogue #	Description	Quantity	Lot#
7/16/2001	BBE 1B	Human sICAM-1CD54 Parameter ELISA Kit	10	204247
7/26/2001	BBE 1B	Human sICAM-1CD54 Parameter ELISA Kit	10	204247
8/13/2001	BBE 1B	Human sICAM-1CD54 Parameter ELISA Kit	10	204546
9/17/2001	BBE 1B	Human sICAM-1CD54 Parameter ELISA Kit	15	205051
9/24/2001	BBE 1B	Human sICAM-1CD54 Parameter ELISA Kit	15	205051
10/1/2001	BBE 1B	Human sICAM-1CD54 Parameter ELISA Kit	15	205051
10/8/2001	BBE 1B	Human sICAM-1CD54 Parameter ELISA Kit	5	205169
11/27/2001	BBE 1B	Human sICAM-1CD54 Parameter ELISA Kit	1	205628
12/3/2001	BBE 1B	Human sICAM-1CD54 Parameter ELISA Kit	3	205628
10/23/2001	BBE 1B	Human sICAM-1CD54 Parameter ELISA Kit	15	205241
11/13/2001	BBE 1B	Human sICAM-1CD54 Parameter ELISA Kit	7	205628
11/13/2001	BBE 1B	Human sICAM-1CD54 Parameter ELISA Kit	5	205628
Generation 3 samples				
11/15/2005	BBE1B	sICAM-1, R/D	126	233487

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

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:	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 - (Human IL-6 Quantikine HS ELISA Kit) **http://www.rndsystems.com/**
Minimum detectable dose	<0.70 pg/mL
Standard curve range	0 – 300 pg/mL

4. FHS Specimen Characteristics

a. Serum, run in duplicate
b. Frozen samples, run on 2nd thaw
c. Samples were subjected to 1-3 freeze-thaw cycles.
d. Volume of sample: 200uL

5. QC aspects

CV intra-assay:		3.12±2.19		
CV intra FHS IDs:		3.11±2.18		
CV Intra-assay final data set. If sample re-measured because CV is higher than threshold then use the lowest CV.		3.05±2.12		
CV intra phantoms		3.42±2.42		
Number per cycle		145		
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:		7.9		
Bar code reader:		Yes		
Internal controls	mean	std	min	max
	1.3	0.2	1.0	1.7

Mean and CV by month:

Month	12/2001	01/2002	02/2002	03/2002	04/2002	05/2002
N	138	855	308	1040	775	775
Mean±std	3.23±3.31	3.89±3.38	4.00±4.58	4.11±6.31	4.26±4.91	3.73±5.22
CVmean±std	3.67±2.51	3.28±2.32	3.21±2.14	3.07±2.14	2.90±2.10	3.09±2.11

6. FHS participant aspect

a. Markers run: 01/02 – 05/02
b. Measured in: pg/mL
c. Count Offspring n = 3297 & Omni n = 398

Descriptive Statistics -

	Mean	SD	Minimum	Maximum	Median	Q1	Q3
Unadjusted:	3.99	5.11	0.37	104.37	2.68	1.80	4.27
Log-transformed	1.07	0.71	-0.99	4.65	0.99	0.59	1.45

7. Marker residuals (studentized)

Adjustment	Mean	SD	Minimum	Maximum	Median	Q1	Q3
Age & Sex	4.66E-6	1.000	-2.65	5.03	-0.12	-0.68	0.51
Multivariable	3.09E-5	1.000	-2.46	5.44	-0.13	-0.66	0.45
covariates: idtype, age, sex, bmi, smoke, cvd, glucose, tot_hdl, lipidrx, hrx, sbp, dbp, hrtnow, asa3week, waist, diab, trig							

HS Il6 Kits Shipped in 2002

Ship Date	Catalogue #	Description	Quantity	Lot#
3/4/2002	HS600	Human Il-6 Quantikine HS ELISA Kit	10	207027
4/30/2002	HS600	Human Il-6 Quantikine HS ELISA Kit	6	207925
3/20/2002	HS600	Human Il-6 Quantikine HS ELISA Kit	6	207305
5/15/2002	HS600	Human Il-6 Quantikine HS ELISA Kit	4	208038
4/29/2002	HS600	Human Il-6 Quantikine HS ELISA Kit	6	207925
4/2/2002	HS600	Human Il-6 Quantikine HS ELISA Kit	6	207541
4/22/2002	HS600	Human Il-6 Quantikine HS ELISA Kit	6	207925
3/12/2002	HS600	Human Il-6 Quantikine HS ELISA Kit	6	207305
3/25/2002	HS600	Human Il-6 Quantikine HS ELISA Kit	6	207305
4/1/2002	HS600	Human Il-6 Quantikine HS ELISA Kit	6	207541
5/13/2002	HS600	Human Il-6 Quantikine HS ELISA Kit	6	208038

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

Interleukin -18 Serum

1. Funding Source/Lab

Framingham specimens	PI: Emelia J. Benjamin, MD, ScM Emelia@bu.edu
Grant #	**RO1 HL 064753** & **RO1 HL076784**
Lab	University of Mainz, Mainz, Germany
Contact:	Tanja Zeller, PhD Tanja Zeller: zellert@uni-mainz.de Renate Schnabel, MD, MSc Renate Schnabel [schnabelr@gmx.de]

2. Method: Quantitative ELISA

3. Technical Aspects

Commercial kit including all reagents	
Vendor	**MBL(www.mblintl.com): Human IL-18**
Minimum detectable dose	128 pg/ml
Standard curve range	**128 – 5000 pg/ml**

4. FHS Specimen Characteristics

a. Serum, 10% duplicate
b. Frozen samples (- 80C)

5. QC aspects

CV intra-assay:		
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.		3.0% (combined Offspring and Omni)
CV intra phantoms		Offspring – 9.3% Omni 7.9%
Number per cycle		Offspring – 91, Omni - 17
CV inter		13.1%
CV threshold for re-measuring:		
Bar code reader:		
Internal controls		No data available

6. FHS participant aspect

a. Markers run: 7/08 – 8/08
b. Measured in: pg/mL
c. Count Offspring n = 3189 & Omni n = 396

Descriptive Statistics -

	Mean	SD	Minimum	Maximum	Median	Q1	Q3
Unadjusted:	255.67	127.40	128.00	1469.70	227.61	167.04	305.64
Log-transformed	5.45	0.42	4.85	7.29	5.43	5.12	5.72

7. Publications

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

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:	Izabella Lipinska: lipinska@bu.edu

2. Method: ACE Competitive EIA

3.Technical Aspects

Molecular Devices VersaMax microplate reader	
Commercial kit including all reagents	
Vendor	Cayman Chemical (Cat. No. 516351) **http://www.caymanchem.com/app/template/Home.vm**
Minimum detectable dose	n/a
Standard curve range	3.9 – 500 pg/mL

4.FHS Specimen Characteristics

a. Urine, run in duplicate

b. Frozen samples - S*amples were subjected to 1-3 freeze-thaw cycles.*

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:		9.6±6.8		
CV intra FHS IDs:		9.6±6.8		
CV Intra-assay final data set. If sample re-measured because CV is higher than threshold then use the lowest CV.		9.12±5.78		
CV intra phantoms		10.0±6.4		
Number per cycle		135		
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:		21.3		
Bar code reader:		Yes		
Internal controls	mean	std	min	max
	663.19	116.72	407	881

Mean and CV by month:

Month	01/2001	02/2001	03/2001	04/2001	05/2001
N	599	763	759	439	548
Mean±std	148±87	142±103	150±95	168±109	187±149
CVmean±std	9.7±8.9	10.1±6.5	9.9±6.3	9.1±5.6	9.3±6.6

Month	06/2001	08/2001			
N	278	58			
Mean±std	148±86	178±100			
CVmean±std	9.0±5.6	7.6±5.4			

6. FHS participant aspect

a. Markers run: 01/01 – 08/01
b. Measured in: pg/mL
c. Count Offspring n = 2828 & Omni n = 372

Descriptive Statistics -

	Mean	SD	Minimum	Maximum	Median	Q1	Q3
Unadjusted:	158.30	109.39	1.25	1844.85	132.80	89.32	194.93
Log-transformed	4.89	0.60	0.22	7.52	4.89	4.49	5.27

7. Marker residuals (studentized)

Adjustment	Mean	SD	Minimum	Maximum	Median	Q1	Q3
Age & Sex	-8.70E-7	1.000	-7.58	4.60	0.0020	-0.65	0.64
Multivariable	-1.46E-5	1.000	-7.97	5.15	0.0141	-0.65	0.66
covariates: idtype, age, sex, bmi, smoke, cvd, glucose, tot_hdl, lipidrx, hrx, sbp, dbp, hrtnow, asa3week, waist, diab, trig							

8. Algorithm to get indexed isoprostane values

Calculation process:
1. Isoprostane units are pg/mL and were changed to ng/mL by dividing by 1000.
2. Creatinine units are mg/100mL and were changed to mmol/mL by dividing by 100 and then by the formula weight of creatinine (131)
3. The final value was created by dividing the isoprostane ng/mL by creatinine in mm/mL to get a value in ng/mmol

Calculations**:**
isonew=iso/1000;
creatnew=(creat/100)/131;
isocreat=isonew/creatnew;

9. Publications[9;14]{Meigs, 2007 10202 /id}

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; Jaffe reaction

3. Technical Aspects

Commercial kit including all reagents	
Vendor	Abbott Spectrum CCX
Minimum detectable dose	
Measuring Range:	6-1000mg/100ml

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:	2.0%
CV intra phantoms	6.2%
Number per cycle	154
CV inter –assay	4.0%
CV threshold for re-measuring:	Creatinine </=50mg/100ml; repeated if delta >4.0 mg/100ml Creatinine > 50 mg/100ml; repeated if delta > 6.5% of mean
Bar code reader:	No
Internal controls	yes

6. FHS participant aspect

a. Markers run: 4/23/01 – 11/1/01
b. Measured in: mg/100mL
c. Count Offspring n = 2828 & Omni n = 390

Descriptive Statistics -

	Mean	SD	Minimum	Maximum	Median	Q1	Q3
Unadjusted:	121.70	75.41	3.10	608.80	112.40	63.95	162.15
Log-transformed	4.57	0.75	1.13	6.41	4.72	4.16	5.09

7. Publications[9;14]

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 HL 064753** & **RO1 HL076784** **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

CAM = a colormetric activity measurement; it is not an ELISA although the reaction to generate a colored product is performed in plates with microtiter wells, much as an ELISA setup

3.Technical Aspects

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

4.FHS Specimen Characteristics

Offspring Exam 7 EDTA Plasma stored at -80

Framingham Heart Study Samples

Date received:	9/8/05
Samples received	3416
Samples tested:	3416
Date Reported	01/16/06

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 nmol/min/ml exceed dynamic range of assay and reported as such.

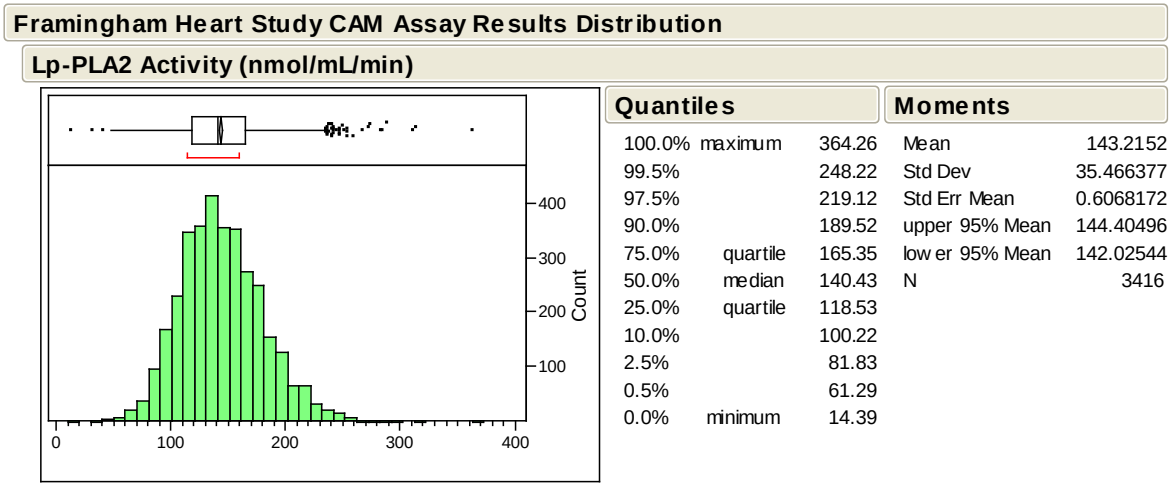
Results:

Total number of plates run including repeats: 106

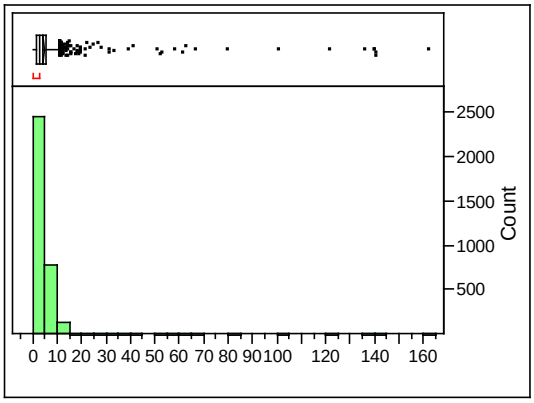
Sample results: File attached

Summary:

Results	Sample Number	Mean	Min	Max	5% Percentile	95% Percentile	Median
CAM nmol/min/ml	3416	143.2	14.4	364.3	90.3	204.4	140.4



Duplicate CV (%)



Quantiles		
100.0%	maximum	162.43
99.5%		41.75
97.5%		11.97
90.0%		8.15
75.0%	quartile	5.40
50.0%	median	3.05
25.0%	quartile	1.42
10.0%		0.59
2.5%		0.14
0.5%		0.01959
0.0%	minimum	0.00

Moments	
Mean	4.2786957
Std Dev	7.8013808
Std Err Mean	0.1334789
upper 95% Mean	4.5404023
low er 95% Mean	4.0169892
N	3416

Quality Control:

All samples assayed by CAM assay were run in duplicate. 291 samples which had duplicate CV's exceeding 12.4% were repeated in duplicate and the mean was reported. Of the repeated samples, some samples had CV's exceeding 12.4% for both runs of the assay, in which case, the mean of the duplicates with the lowest CV was reported.
All plates met the acceptance criteria set forth for the QC's; no entire plate required repeating.

QC Control Summary:

Control	Mean	% CV	n
CAM Low QC	144.2	7.0%	318
CAM High QC	246.1	5.9%	318

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 HL 064753** & **RO1 HL076784** **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 – measured with an ELISA

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 mean 192.8ng/mL C 2 90019-508070 mean 424.3ng/mL
Generation	▪ Offspring Exam 7 - diaDexus Generation 2 mass assay ▪ Omni 1 Exam 2 - diaDexus Generation 3 mass assay ▪ Generation 3 - diaDexus Generation 3 mass assay ▪ Omni 2 Exam 1- diaDexus Generation 3 mass assay

4.FHS Specimen Characteristics

Offspring Exam 7 EDTA Plasma stored at -80

Framingham Samples

Date Received:	10/5/05, 10/11/05 replaced, 10/25/05 box 21 returned
Samples Supplied:	3456 tubes, 40 empty
Samples tested:	3416
Date Reported	1/13/06

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

Results:

Total number of plates run: 55 kits

Sample Results: **File Attached**

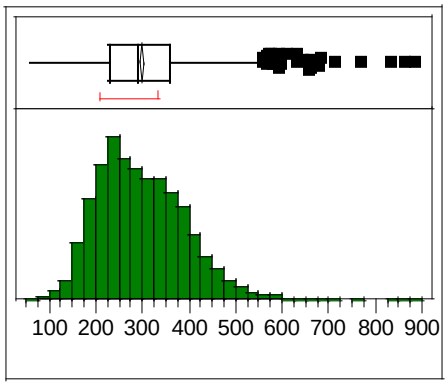
Summary:

Results

Results	Sample Number	Mean	Min	Max	5% Percentile	95% Percentile	Median
PLAC ng/mL	3416	299.1	63.7	886.3	167.4	460.7	288.3

Framingham PLAC Test Distribution

Lp-PLA2 ng/mL



Quantiles

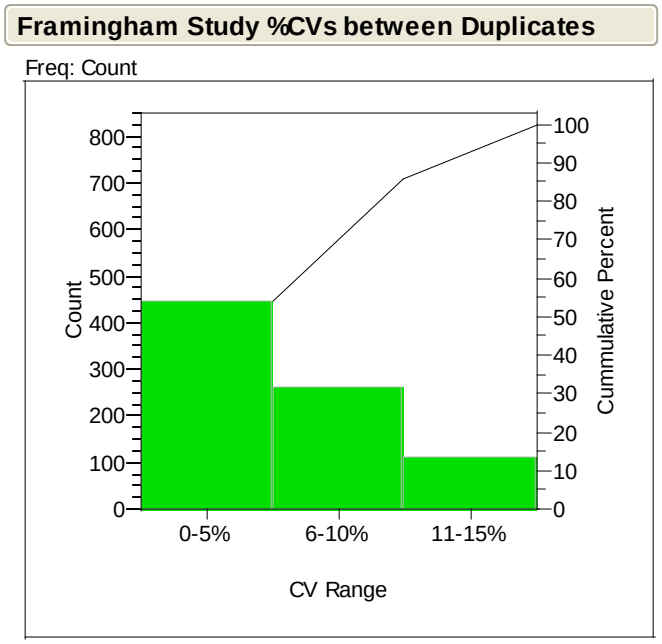
100.0%	maximum	886.27
99.5%		594.80
97.5%		500.27
90.0%		419.12
75.0%	quartile	360.37
50.0%	median	288.30
25.0%	quartile	229.13
10.0%		189.38
2.5%		151.84
0.5%		110.81
0.0%	minimum	63.70

Moments

Mean	299.05034
Std Dev	93.984264
Std Err Mean	1.6080376
upper 95% Mean	302.20315
low er 95% Mean	295.89753
N	3416

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

Control	Beginning controls	Middle controls	End controls	Mean All	%CV All
	ng/mL	ng/mL	ng/mL	ng/mL	%
PLAC Control 1 ng/mL 153.9 - 231.7	185.9	180.8	174.0	180.3	6%
PLAC Control 2 ng/mL 302.8 - 545.9	380.4	355.9	339.0	358.7	8%
N (total number of wells)	128	128	124	190	190

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 HL 064753** & **RO1 HL076784**
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	06/2002	7/2002	8/2002	9/2002	10/2002	11/2002
N	653	281	700	1195	990	24
Mean±std	329±145	314±121	330±142	327±102	309±120	322±199
CVmean±std	6.3±3.0	7.1±2.8	6.1±3.42	2.0±1.8	1.8±1.5	2.7±1.8

6. FHS participant aspect

a. Markers run: 06/02 – 9/02
b. Measured in: pg/mL
c. Count Offspring n = 3242 & Omni n = 393

Descriptive Statistics -

	Mean	SD	Minimum	Maximum	Median	Q1	Q3
Unadjusted:	321.34	124.89	2.00	2139.82	306.95	246.57	378.44
Log-transformed	5.71	0.36	0.69	7.67	5.73	5.51	5.94

7. Marker residuals (studentized)

Adjustment	Mean	SD	Minimum	Maximum	Median	Q1	Q3
Age & Sex	-3.28E-6	1.000	-13.58	5.43	0.0290	-0.58	0.61
Multivariable	-4.83E-5	1.000	-13.71	5.69	0.0324	-0.59	0.62
covariates: idtype, age, sex, bmi, smoke, cvd, glucose, tot_hdl, lipidrx, hrx, sbp, dbp, hrtnow, asa3week, waist, diab, trig							

MCP-1 Kits Shipped in 2002

Ship Date	Catalogue #	Description	Qty	Lot#
5/13/2002	PDCP00	Human CCL2MCP-1 QuantikinePhampak (50 Plates)	1	1506043
5/14/2002	PDCP00	Human CCL2MCP-1 QuantikinePhampak (50 Plates)	1	207984
10/24/2002	DCP00	Human CCL2MCP-1 Quantikine ELISA Kit	7	209990
Generation 3 samples				
8.29.2006	SCPOO	MCP-1, R/D	126	240267

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

Myeloperoxidase (MPO)

1.Funding Source/Lab

Framingham specimens	PI: Emelia J. Benjamin, MD, ScM Emelia@bu.edu PI: Ramachandran S. Vasan, MD vasan@bu.edu
Grant #	**RO1 HL 064753** & **RO1 HL076784**
Lab	JFK/IL/AB
Contact:	Izabella Lipinska: lipinska@bu.edu

2. Method: Quantitative ELISA

3.Technical Aspects

Molecular Devices VersaMax microplate reader	
Commercial kit including all reagents	
Vendor	Oxis (Cat. No. 21013) http://www.oxis.com/
Minimum detectable dose	0.17 ng/mL
Standard curve range	0 – 25 ng/mL

4.FHS Specimen Characteristics

a. Serum, 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:		3.15±2.69		
CV intra FHS IDs:		3.15±2.69		
CV Intra-assay final data set. If sample re-measured because CV is higher than threshold then use the lowest CV.		3.02±2.48		
CV intra phantoms		3.18±2.68		
Number per cycle		141		
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:		11.5		
Bar code reader:		Yes		
Internal controls	mean	std	min	max
	80.9	13.0	45.9	102.6

Mean and CV by month:

Month	6/2003	7/2003	8/2003	9/2003	10/2003
N	170	269	389	267	652
Mean±std	45±34	54±44	50±35	49±30	46±24
CVmean±std	2.4±2.1	2.4±2.5	2.6±2.5	3.1±2.6	3.3±2.8

Month	11/2003	12/2003	1/2004	2/2004	
N	268	698	788	305	
Mean±std	52±37	46±27	41±28	48±28	
CVmean±std	3.2±2.7	3.1±2.5	3.8±2.9	2.9±2.5	

6. FHS participant aspect

a. Markers run: 06/03 – 05/04

b. Measured in: ng/mL

c. Count Offspring n = 3190 & Omni n = 395

Descriptive Statistics -

	Mean	SD	Minimum	Maximum	Median	Q1	Q3
Unadjusted:	47.08	30.72	0.86	376.99	39.03	27.31	58.19
Log-transformed	3.68	0.58	-0.15	5.93	3.66	3.31	4.06

7. Marker residuals (studentized)

Adjustment	Mean	SD	Minimum	Maximum	Median	Q1	Q3
Age & Sex	-3.11E-7	1.000	-6.22	3.73	-0.0203	-0.64	0.66
Multivariable	-1.60E-7	1.000	-6.21	3.75	-0.0173	-0.65	0.65
covariates: idtype, age, sex, bmi, smoke, cvd, glucose, tot_hdl, lipidrx, hrx, sbp, dbp, hrtnow, asa3week, waist, diab, trig							

8. Publication[1]

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:	Izabella Lipinska: lipinska@bu.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 (Cat. No. 04-B1-20402) **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, run in duplicate
b. Frozen samples, run on 4th thaw
c. Samples were subjected to 1-3 freeze-thaw cycles.

5. QC aspects

CV intra-assay:		3.73±2.87		
CV intra FHS IDs:		3.72±2.87		
CV Intra-assay final data set. If sample re-measured because CV is higher than threshold then use the lowest CV.		3.72±2.87		
CV intra phantoms		3.94±2.79		
Number per cycle		147		
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:		12.7		
Bar code reader:		Yes		
Internal controls	mean	std	min	max
	8.1	0.4	7.4	9.3

Mean and CV by month:

Month	06/2005	07/2005	08/2005	09/2005	10/2005
N	29	685	1192	1360	581
Mean±std	5.0±1.3	5.6±1.7	5.7±1.9	5.7±2.0	5.1±1.7
CVmean±std	4.2±3.6	3.3±2.5	3.5±2.7	3.9±3.0	4.2±3.1

6. FHS participant aspect

a. Markers run: 06/05 – 10/05
b. Measured in: pmol/L
c. Count Offspring n = 3299 & Omni n = 400

Descriptive Statistics -

	Mean	SD	Minimum	Maximum	Median	Q1	Q3
Unadjusted:	5.59	1.91	0.59	27.80	5.32	4.38	6.44
Log-transformed	1.67	0.31	-0.53	3.32	1.67	1.48	1.86

7. Marker residuals (studentized)

Adjustment	Mean	SD	Minimum	Maximum	Median	Q1	Q3
Age & Sex	1.32E-5	1.000	-8.19	6.19	0.025	-0.57	0.59
Multivariable	4.26E-5	1.000	-8.36	6.06	0.0173	-0.57	0.60
covariates: idtype, age, sex, bmi, smoke, cvd, glucose, tot_hdl, lipidrx, hrx, sbp, dbp, hrtnow, asa3week, waist, diab, trig							

P-Selectin Kits Shipped in 2005

Ship Date	Catalogue #	Description	Qty	Lot#
10/5/2005	BI-20402	OPG, Alpco	100	353SKU

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.

8. Publication[1]

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:	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 6) **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, 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:		3.20±2.41		
CV intra FHS IDs:		3.21±2.40		
CV Intra-assay final data set. If sample re-measured because CV is higher than threshold then use the lowest CV.		3.04±2.16		
CV intra phantoms		2.99±2.46		
Number per cycle		148		
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:		8.5		
Bar code reader:		Yes		
	mean	std	min	max
R&D controls	576.4	68.6	422.4	750.6
Internal controls	38.6	5.59	27.0	52.4

Mean and CV by month:

Month	6/2004	7/2004	8/2004	9/2004	
N	1415	1092	587	868	
Mean±std	38±14	38±14	42±18	37±12	
CVmean±std	3.4±2.6	3.1±2.3	3.1±2.2	3.0±2.3	

6. FHS participant aspect

a. Markers run:06/04 – 10/04

b. Measured in: ng/mL

c. Count Offspring n = 3304 & Omni n = 400

Descriptive Statistics -

	Mean	SD	Minimum	Maximum	Median	Q1	Q3
Unadjusted:	38.23	14.32	1.79	194.91	36.32	28.69	45.66
Log-transformed	3.58	0.37	0.58	5.27	3.59	3.36	3.82

7. Marker residuals (studentized)

Adjustment	Mean	SD	Minimum	Maximum	Median	Q1	Q3
Age & Sex	-1.90E-6	1.000	-8.08	4.32	0.0391	-0.60	0.65
Multivariable	2.22E-5	1.000	-7.85	4.60	0.0554	-0.59	0.64
covariates: idtype, age, sex, bmi, smoke, cvd, glucose, tot_hdl, lipidrx, hrx, sbp, dbp, hrtnow, asa3week, waist, diab, trig							

P-Selectin Kits Shipped in 2004

Ship Date	Catalogue #	Description	Qty	Lot#
5/24/2004	SBBE6	Human sP-Selectin/CD62P Parameter SixPak(6 Plates)	9	221312
7/8/2004	SBBE6	Human sP-Selectin/CD62P Parameter SixPak(6 Plates)	9	221312

8. Publications[1;6]

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:	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. DRT 200) **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:		2.25±1.62 Low Range 7.57% High Range 5.63% (Meigs 060406))		
CV intra FHS IDs:		2.25±1.62		
CV Intra-assay final data set. If sample re-measured because CV is higher than threshold then use the lowest CV.		2.25±1.61		
CV intra phantoms		2.33±1.78		
Number per cycle		148		
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:		6.3		
Bar code reader:		Yes		
Internal controls	mean	std	min	max
	1835.3	190.2	1366.3	2197.0

Mean and CV by month:

Month	2/2005	3/2005	4/2005	5/2005	
N	303	1470	1235	842	
Mean±std	1976±815	2176±822	2137±756	2098±745	
CVmean±std	2.2±1.6	2.2±1.6	2.2±1.6	2.4±1.7	

6. FHS participant aspect

a. Markers run: 2/05 – 5/05

b. Measured in: pg/mL

c. Count Offspring n = 3227 & Omni n = 400

Descriptive Statistics -

	Mean	SD	Minimum	Maximum	Median	Q1	Q3
Unadjusted:	2126.70	788.06	671.22	8383.43	1957.85	1643.88	2400.13
Log-transformed	7.61	0.31	6.51	9.03	7.58	7.40	7.78

7. Marker residuals (studentized)

Adjustment	Mean	SD	Minimum	Maximum	Median	Q1	Q3
Age & Sex	1.58E-5	1.000	-3.90	4.81	-0.0830	-0.65	0.57
Multivariable	4.7E-5	1.000	-3.67	4.99	-0.0574	-0.65	0.58
covariates: idtype, age, sex, bmi, smoke, cvd, glucose, tot_hdl, lipidrx, hrx, sbp, dbp, hrtnow, asa3week, waist, diab, trig							

sTNF RII Kits Shipped in 2005

Ship Date	Cat #	Description	Qty	Lot#
2/22/2005	SRT200	Human sTNF RII/TNFRSF1B Quantikine SixPak(6 Plates)	9	221312
2/22/2005	SRT200	sTNF-r2 , R/D	108	227150

8. Publication[1]

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 is detailed below:

Inflammation: Correlates and Prognosis in Framingham
Agency: NHLBI Type: RO1 HL064753
PI: Emelia J. Benjamin, MD, ScM

DESCRIPTION:

Increasingly, researchers understand that inflammation is critical to the development of atherosclerosis and the progression to cardiovascular (CVD) events. We hypothesize that a pathophysiologic link between systemic inflammation and CVD events is through endothelial injury and dysfunction. Endothelial dysfunction with subsequent loss of the vasodilator, anti-thrombotic, and anti-inflammatory properties of the vascular endothelium plays a dynamic role in the development of atherosclerosis and the activation of plaques culminating in CVD events.

Most prior studies of inflammatory markers have been limited to small samples of highly selected patients. The relation between the markers and cardiovascular risk factors remains unclear and their relation with endothelial dysfunction and subclinical disease remains largely unexplored. Most importantly, prior studies have not demonstrated if inflammatory markers predict incident CVD in the community. Completion of such a study will require assessment of inflammatory markers in a large, well-characterized population. We propose to assess inflammatory markers in about 3,800 men and women of the Framingham Study. The markers will include inflammatory (C-reactive protein, fibrinogen, soluble intercellular adhesion molecule-1, endothelin-1, monocyte chemotactic protein-1, tumor necrosis factor-α) and oxidative stress markers (8-epi-$PGF_{2\alpha}$, thromboxane B_2). The specific aims of this proposal are to:

1. Determine the relation between CVD risk factors and systemic markers of vascular inflammation.

2. Analyze the relations between inflammatory markers, endothelial dysfunction, and subclinical disease.

3. Relate markers of inflammation to prevalent and incident CVD events adjusting for standard risk factors.

Our central hypothesis is that inflammatory markers are independent risk factors for CVD events with endothelial dysfunction operating in the causal pathway. The Framingham Study is uniquely suited for this proposal by virtue of the single site population-based design, the availability of extensive antecedent and contemporary risk factor data, and the availability of long-term, longitudinal follow-up. The proposed study provides a unique opportunity to assess the prognostic importance of inflammatory markers and is likely to yield new information that will directly improve the prevention and management of CVD.

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

The Framingham Heart Study, NHLBI
5 Thurber Street
Framingham, MA 01702-6334

Boston University School of Medicine
Whitaker Cardiovascular Institute
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KEY PERSONNEL.

Name	Organization	Role on Project
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Martin G. Larson	The Framingham Study, BUSM	Co-Investigator, Statistician
Joseph A. Vita	Boston University School of Medicine	Co-Investigator, Endothelial Function
Peter W. F. Wilson	The Framingham Study, BUSM	Co-Investigator, Lab Director, FHS
Christopher J. O'Donnell	The Framingham Study, NHLB	Co-Investigator, Subclinical Disease

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.

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Ramachandran, Vasan S.	Fram. Heart Study/Boston Univ.	Co-Principal investigator
Vita, Joseph	Fram. Heart Study/Boston Univ	Co-investigator

Aging and Inflammation: Longitudinal Markers and Genetics in the Framingham Study
Agency: NHLBI Type: 1R01AG028321
PI: Emelia J. Benjamin, MD, ScM

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
Framingham, MA 01702-5827

KEY PERSONNEL.

Principal Investigator/Program Director (Last, First, Middle): Benjamin, Emelia J.

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OTHER SIGNIFICANT CONTRIBUTORS			
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List of Covariate Labels
Defined at exam the marker was measured, unless otherwise specified

idtype	=	Omni (ethnic/minority cohort; 7) vs. Offspring (predominantly white, European descent; 1)
sex	=	male/female
Age	=	Age, years
sbp	=	Clinic physician's systolic blood pressure, mm Hg (average of 2 measures)
dbp	=	Clinic physician's diastolic blood pressure, mm Hg (average of 2 measures)
bmi	=	Body Mass Index (BMI) kg/m^2
smoke	=	Current smoking (regular within year prior to exam), %
hrx	=	Hypertensive medication, %
lipidrx	=	Lipid lowering medication, %
tot_hdl	=	Fasting total/HDL cholesterol, ratio
glucose	=	Fasting blood glucose, mg/dl
cvd	=	Prevalent cardiovascular disease diagnosis includes any one of the following events at or prior to the exam: Angina Pectoris (AP), Congestive Heart Failure (CHF), Coronary Insufficiency (CI), Cerebrovascular Accident (CVA), Intermittent Claudication (IC), Myocardial Infarction (MI)
diab	=	Diabetes mellitus (fasting blood sugar ≥126 mg/dl or on treatment)
trig	=	Fasting triglycerides mg/dl,
hrtnow	=	Hormone replacement therapy
asa3week	=	Aspirin (3 per week)
waist	=	Waist measurement, inches

Note: medications and smoking are by self-report