

**HUPO PLASMA PROTEOME
PROJECT (PPP) PILOT PHASE
WORKSHOP**

Bethesda, 16-17 July, 2003

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OVERALL SCIENTIFIC GOALS OF PPP

Comprehensive analysis of plasma protein constituents in normal humans, with large cohorts

Determination of the extent of variation in plasma proteins

Across populations (around the world)

Within populations (in various countries)

Identification of biological sources of variation within individuals over time, with validation of biomarkers

Age, sex/menstrual cycle, exercise

Common medications, selected diseases

AIMS FOR PILOT PHASE OF PPP

1. Compare a broad range of technology platforms for the characterization of proteins in human plasma and serum. Assess resolution, sensitivity, time, cost, volumes of sample required, practicality.
2. Clarify influence of various technical variables in specimen collection, handling, and storage; especially anti-coagulation and plasma/serum.
3. Determine whether (and, if so, how) the most abundant plasma proteins should be depleted, and whether anti-protease cocktails are necessary or desirable.

AIMS FOR PILOT PHASE (cont'd)

4. Develop a database structure and repository for HUPO PPP results
5. Establish international collaborations for later-phase characterization of the normal human plasma proteome in the major ethnic groups.
6. Lay groundwork through evaluation of technology platforms and specimen handling for future studies of circulating proteins (biomarkers) in health and disease.

AIMS OF THIS WORKSHOP

Provide assessment of progress to date

**Review the Technology Platforms being used
across the 46 participating laboratories**

**Demonstrate the Data Submission and Data
Analysis tools and formats**

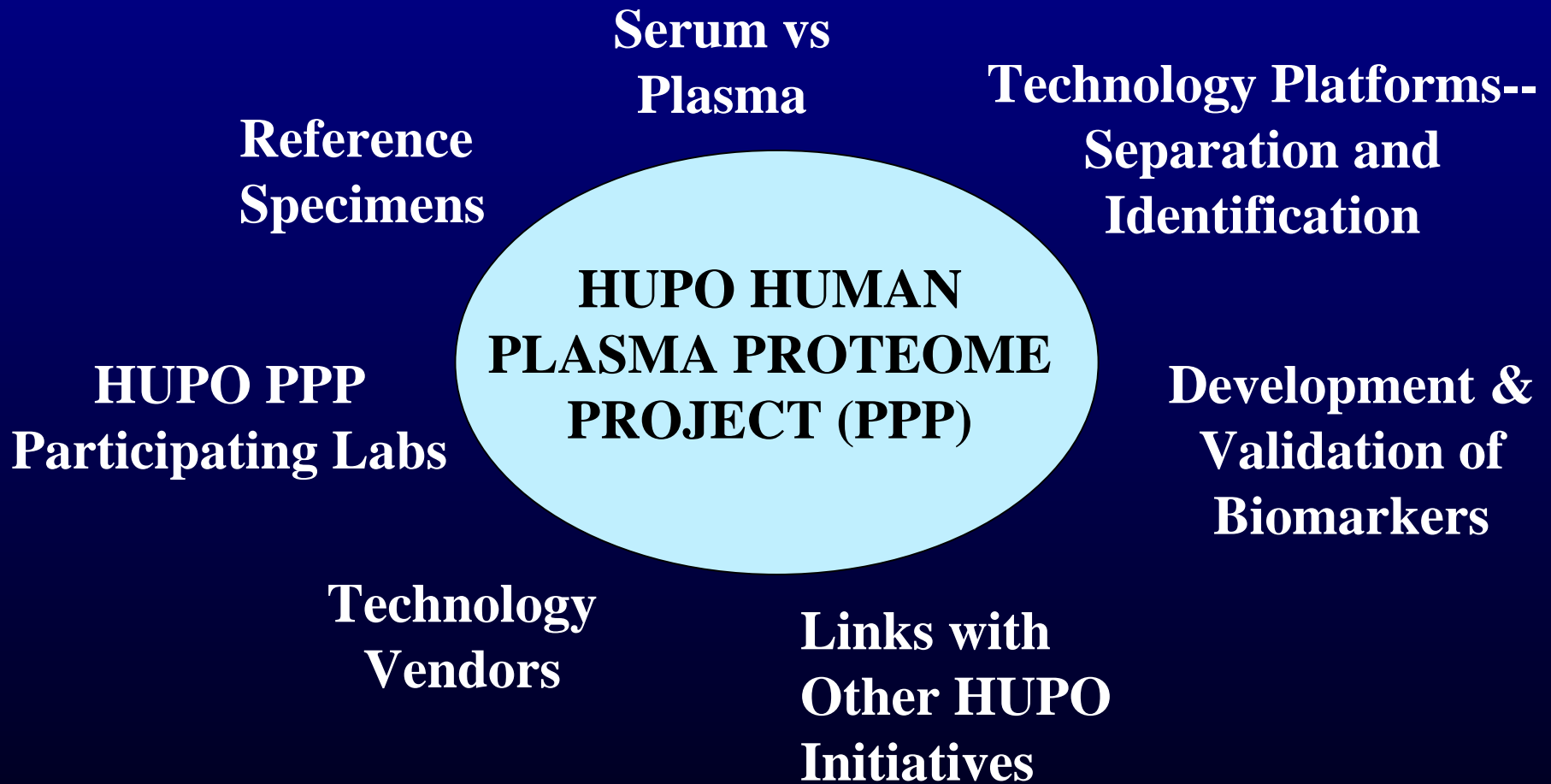
**Clarify and establish commitments for
parameters to be studied with Reference
Specimens, with specific protocols and with
additional specimens**

AIMS FOR WORKSHOP (cont'd)

**Outline activities for PPP at Montreal
HUPO Congress**

**Begin preparations for “Execution Phase”
with population cohorts and disease-
oriented studies of potential biomarkers
and mechanisms**

**Discuss involvements with other HUPO
initiatives**



CHALLENGES/ISSUES FOR PILOT PHASE

Initial Planning Meeting, Bethesda, April, 2002

- 1. Sensitivity of various techniques for dynamic range of proteins and of peptides**
- 2. Technical aspects of specimen collection, handling, storage: need to standardize; antiprotease needed?**
- 3. Methods of depleting or fractionating high abundance proteins**
- 4. Comparisons of serum and plasma**
- 5. Enumeration and categorization of proteins: post-translational modifications, tissue of origin**

CHALLENGES/ISSUES (cont'd)

- 6. Separation of protein digests vs proteins**
- 7. Liquid phase multi-dimensional methods vs gel-based methods**
- 8. Parameters for high-throughput link to MS**
- 9. MALDI and SELDI comparisons**
- 10. Various labeling methods for comparison of paired samples**

TECHNOLOGY PLATFORMS (illustrated by HUPO PPP Sept 2002 Ann Arbor presentations)

- Liquid phase separations--Sam Hanash (Michigan)
- Free Flow Electrophoresis--Askar Kuchumov (Tecan Inc)
- Microsol IEF Pre-Fractionation--David Speicher (Penn)
- 2-D LCMS/Automated - Steve Cohen (Waters)
- Systems Approach with Peptide Digest--Karin Rodland (Pacific Northwest National Laboratory, Dept of Energy)
- Swellgels - Walid Qoronfleh (PerBio, Pierce Chemical)
- AntiBio Mix - Moncef Jendoubi (Milagen Inc)
- Chicken IgY Anti-Human Antibodies—Zhang (GenWay)

MAJOR ACTIVITIES IN VERSAILLES WORLD CONGRESS (Nov 2002)

ELICITED INTEREST/PROVIDED INFO ABOUT ROLES

**HEARD PROPOSALS FOR REFERENCE SPECIMENS
AND DECIDED WHICH TO DEVELOP**

**INVITED QUESTIONS ABOUT TECHNOLOGY PLATFORMS
AND SENSITIVITY OF DETECTION OF DYNAMIC RANGE**

ELICITED INTEREST FOR COMMITTEES:

Specimens Committee

Technology and Protocol Review/Feedback Committee

Data Management and Bioinformatics Committee

Executive Committee (including corporate & govt partnerships)

GENERAL REFERENCE SPECIMEN

Proposal by American Red Cross

(Kenneth Ingham)

Purpose: broadly available reference specimen for comparisons of many different technologies for separation and identification of proteins.

Utilize Red Cross general pool of plasma (citrated) from several thousand donors of blood (or plasma) at various centers, frozen and thawed at plasma processing centers, tested for pathogens, aliquoted to 0.25-0.5-1.0 ml volumes in appropriate tubes, frozen at -80°C , and stored for distribution to HUPO labs. Conduct extensive clinical lab analyses for future use.

[Defer until after Pilot Phase]

SERUM AND PLASMA REFERENCE SPECIMENS

in cooperation with BD Biosciences

(Bruce Haywood, David Warunek, Catherine Skobe)

1. Recruit and consent equal numbers of healthy, fasting, ethnically diverse male and female donors (volume required to be determined, according to number of participating labs) under an IRB-approved donor Program. [Separate ethnic pools will be prepared.]
2. Draw blood into bags or tubes with appropriate concentrations of K-EDTA, lithium heparin, or sodium citrate for plasma and without clot activator for serum. [Leave anti-protease cocktail to side experiments in individual laboratories.]

3. Centrifuge donor specimens at 2-6C, pool, filter, aliquot, freeze and store at -70C within 60 min of processing.
4. Distribute to each participating HUPO PPP laboratory a set of specimens with 1 ml (in four aliquots) each from Serum Pool and from K₃EDTA, sodium citrate and lithium heparin-anti-coagulated Plasma Pools for use with PPP-approved protocols.
5. Distribute internationally through regional lead sites.

PROPOSAL FROM CHINESE ACADEMY OF MEDICAL SCIENCES (Xiaohang Zhao)

The Chinese Academy of Medical Sciences will collect a large pool from plasma donors at its hospitals, under procedures agreed upon with the HUPO PPP. Specimens can be prepared also from individuals.

Specimens will be characterized carefully for pathogens and for clinical analytes. Aliquots of the large pool will be made available for distribution to HUPO PPP participating laboratories especially in Asia-Oceania HUPO.

[Plan to prepare identically to BD specimens.]

GENERAL REFERENCE SPECIMEN FREEZE-DRIED PLASMA

**National Inst Biological Standards & Control U.K.
(Trevor Barrowcliffe & David Wood)
from FDA meeting 10 January, 2003**

Reference Specimen for Hemostasis and Thrombosis
(for Intl Soc Thrombosis & Hemostasis/Standards Committee).

Prepared from 25 donors as whole blood anticoagulated with citrate-phosphate-dextrose; double-spun, HEPES added to 0.05M; aliquot tested for HIV, HBV, HCV; 1 ml aliquots in 5000 ampoules frozen at -70°C . Thawed at room temperature to avoid cryoprecipitation, then freeze-dried at -35°C for 4 days and desiccated over P_2O_5 for 6 days, filled with dry N_2 . Tested for longterm stability. Will be assayed for clinical analytes by HUPO.

MOST ABUNDANT PLASMA PROTEINS (mg/ml)

From Chemical Rubber Handbook of Biochemistry 1970, pp C-36-39,
and current data from GenWay, Inc, at (Sept 2002 PPP Workshop)

albumin 35-45

haptoglobin 0.3-2

IgG, IgA, IgM 12-18

alpha-1 acid

fibrinogen 2-6

glycoprotein 1

alpha-1 antitrypsin 2-5

hemopexin 1

alpha-2 macroglobulin 2-4

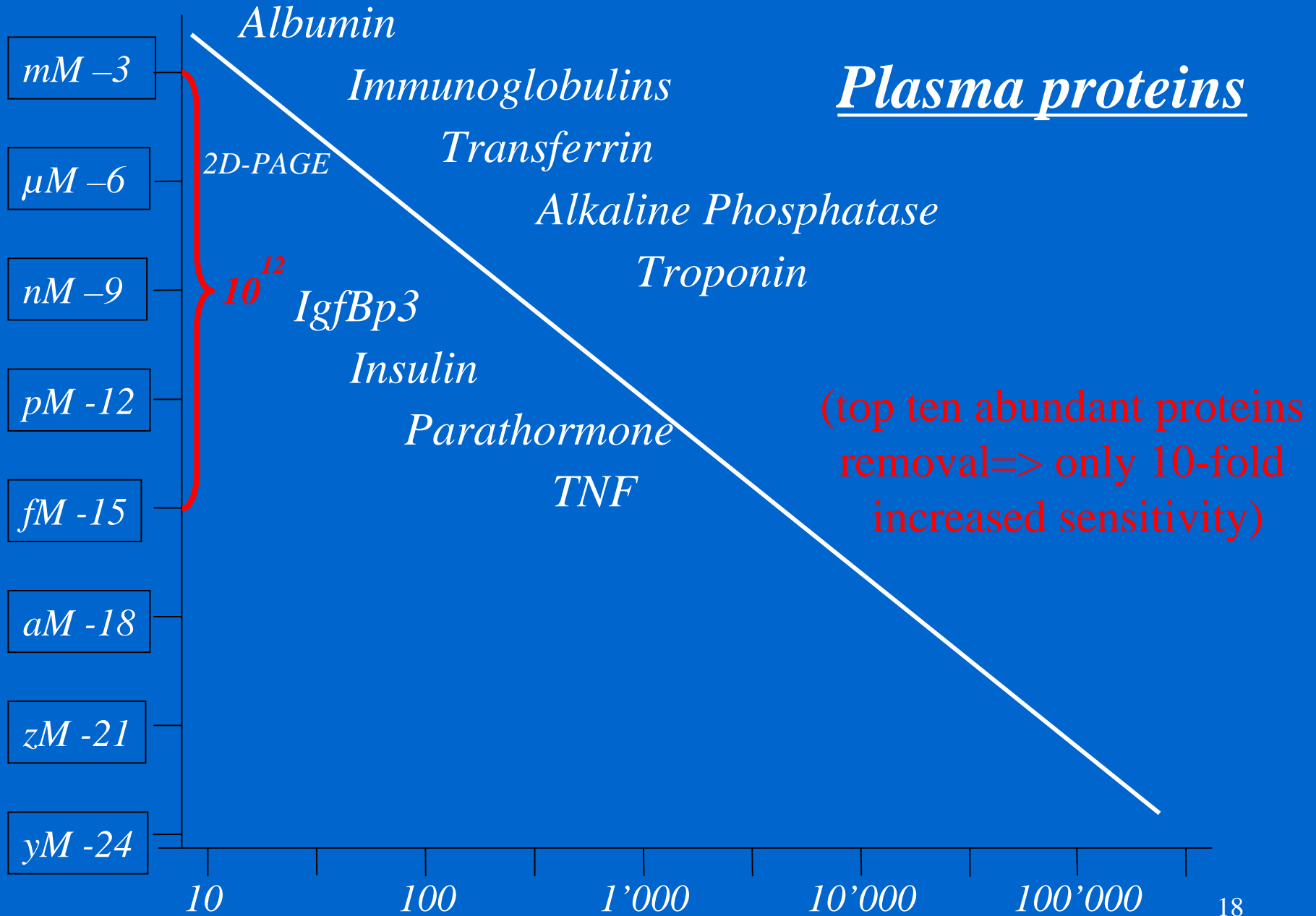
pre-albumin 0.3-0.4

transferrin 2-3

ceruloplasmin 0.3

alpha-2 + beta-lipoproteins (LDL) 4-7

alpha-lipoproteins (HDL) 0.6-1.5



SUMMARY OF LAB EFFORTS

(see tabulation provided in Workshop folder)

45 Total Participating Labs (26 US, 19-International):

16 – US Academic

6 – US Federal

4 – US Corporate

Other Countries: 7 – Europe,

1– Israel, 9 – Asia, 2 – Australia

Number requesting/running reference specimens:

40 – UK NIBSC

41 – BD b1

18 – BD b2

18 – CAMS

SUMMARY OF LAB EFFORTS (cont'd)

25 – Number volunteering for multi-parameter specimen handling protocol

30 – Number testing depletion or pre-fractionation

21 – Number requesting vendor input

Numbers running different kinds of technology platforms:

28 – 2D gels

26 – Liquid chromatography

23 – Peptide digest first

30 – MALDI or LC/MS, MS/MS

15 – SELDI

17 – Labeled proteins

9 – Other

PPP PILOT PHASE ACTIVITIES & MILESTONES

1. Prepared HUPO PPP reference specimens and distributed to participating labs (May 2003)
2. Defined initial roles for participating laboratories:
Run reference specimen set(s), as well as other specimens chosen by the Lab, using each lab's preferred technology platform(s), with guidance from PPP technical committee (sent 16 May, 2003).

PILOT PHASE (cont'd)

3. In subsets of labs (with assistance from this Workshop and from vendors):
 - a) Run the three alternative plasma preps (EDTA, heparin, citrate) & serum from other ethnic groups
 - b) Compare with vs without anti-protease cocktail and with vs without depletion of albumin and/or other highly abundant proteins
 - c) Test technical parameters of sample handling, e.g. duration at various temps before frozen, temp of frozen storage, duration of storage, freeze/thaw (see Chan/Moore, www.hupo.org).

4. Database development

- a) Develop methods for identification, enumeration, and comparison of proteins, accommodating a broad array of technology platforms for separation and then identification, including mass spectrometry and antibody arrays
- b) Assure specimen tracking and methods comparisons
- c) Link with databases of HUPO/PSI, Swissprot, EBI; DOE/PNNL; others
- d) Build inventory of plasma/serum proteins from HUPO PPP studies

Data Development (cont'd)

e) Consider system for identifying and classifying related proteins (in cooperation with EBI); an example:

1.0 Primary amino acid backbone sequence of gene-coded protein (including splice variants and SNPs)

1.1 Modification of backbone: cleavage (activation, inactivation)

1.2 Modification of side-chains

1.2.1 Phosphorylation (sites) and dephosphorylation

1.2.2 Glycosylation (sites)

1.2.3 Other classes of post-translation modifications

1.3 Aggregation (dimers, etc)

1.4 Interactions: protein-protein,-nucleic acid,-others

PILOT PHASE (cont'd)

5. Administration and Dissemination

- a) Develop administrative hub (responsibility assigned by HUPO to U. Michigan)
- b) Raise funds for budget: private & public
- c) Clarify intellectual property matters: committed to public domain, with ample opportunity for proprietary development of biomarkers and targets/agents
- d) Link with related HUPO initiatives: liver, protein standards, brain, antibodies

Administration & Dissemination (cont'd)

- e) Organize PPP Workshop in 2003 (this Workshop) to drive pilot studies and to determine further work plans for presentation of results at 2nd World Congress, Montreal, 11-12 October and at 2004 “jamboree”.
- f) Publish presentations with comparisons of samples, of technologies, from different populations around the world, from participating laboratories: already arranged for Special Issue of Proteomics.

CORPORATE SPONSORS (to date)

Johnson & Johnson

Pfizer

Invitrogen

Procter & Gamble

BD Clinical Preanalytical Solutions

Bristol Myers Squibb

Agilent

PPP TECHNICAL COMMITTEE STRUCTURE

- **Reference Specimens and Specimen Handling Issues**
(Dan Chan, chair)
- **Technology Platforms & Protocols (Richard Simpson)**
- **Database Development and Links with EBI (HUPO/PSI)**
(Henning Hermjakob)
- **Population Cohorts/Specimen Banks (Gerard Siest)**
- **Education & Training Committee (Peipei Ping)**
- **Executive Committee (including Partnerships) (Omenn)**

POTENTIAL POPULATION COHORTS AND SPECIMEN RESOURCES FOR PPP

Stanislas Cohort: Nancy, France (Siest):

4600 in 1006 families: cardiovascular/metabolic disease emphasis; 700 families returning at 10 yrs 2003-04; clot activator for serum; EDTA for plasma; 280 ul straws, 10 each

Women's Health Initiative (NHLBI/NIH, Fred Hutch, Seattle, CC): 67,000 women; CHD, breast/endometrial CA/osteoporosis emphases

CARET: Seattle, WA (Goodman et al):

18,314 current & former smokers

PLCO (Prostate/Lung/Colon/Ovary) Study, US: NCI/Detroit site

General population: Leeds, UK (Grant)

Alcoholism Cohort: Colorado, US (Tabakoff)

Children and families, The Children's Hospital, Boston, US (Castillo)

Early Detection Research Network, US (NCI)

Population Cohorts/Specimen Banks Committee

Gerard Siest (Nancy, Stanislas Cohort), chair

Sudhir Srivastava (NCI/EDRN)

**M. Tammemagi, Henry Ford Health System,
Detroit, PLCO (part of national NCI study)**

**Xiaohang Zhao (Chinese Academy of Medical
Sciences Hospitals, Beijing)**

**Mark Thornquist (Director, Coordinating
Center, CARET, or Gary Goodman, PI)**

JULY 17, 2003, LAB UPDATE FOR PPP

Name of Lab _____

Name of Individual _____

Timelines feasible?

7/21: XML-based data submission format__

7/31: Experimental protocol____(suitable for
cross-analyses and for publication

9/15: Initial data____; replication ____

June 2004: Jamboree

Dates_____

Location: Bethesda _____ **Ann Arbor** _____

INSTRUCTIONS (5.16.03)/FORMS: OK?

i: Technology & Resources Summary

ii: Identified Proteins: DB used___ Conf/prob'y___

a) Primary protein product/gene acc #

b) All spots/peaks identified

iii: Experimental Protocol(s): details important

iv: Method of ID/Validation

v: Estimates of abundances (method)

high/mod/low/very low: mg, ug, ng, pg/ml

Least abundant protein detected & identified

BIOINFORMATICS & DATABASES

Database Searched: IPI___; other _____

Software for Submission: Excel _____

XML _____ PEDRo (if rec'd)_____

PTM's _____

Statistical analyses_____

**Do you have your own bioinformatics
expert? _____**

**Do you want some technical assistance from
EBI? _____**

UPDATE ON SPECIMENS PROTOCOLS

Any changes from tabulated response?

Will do Chan Protocol (time, temp, F/T)

with PPP___/own___specimens (BD anti-coag details)

Depletion (method)

Pre-fractionation (method)

Anti-proteases: cocktail___; tube___

At start of collection___; at thaw___

Phosphatase inhibitor (method)

Clot activator