TO: PARTICIPATING LABORATORIES FOR HUPO PLASMA PROTEOME PROJECT (PPP)

FROM: Gil Omenn & HUPO PPP Executive Committee

DATE: 27-January-2003

We are pleased to confirm that we are *ready to launch the Pilot Phase* of this major collaborative project. The overall goal of the Project is a comprehensive analysis of plasma/serum proteins in human populations in health and diseases. The pilot phase aims to utilize HUPO reference specimens to compare a broad range of technology platforms for the characterization of proteins in human plasma and serum. Attached – for your information – is the most recent summary of the project, following the San Diego CHI-HUPO Conference 15 January 2003.

We are seeking your prompt response to the simplified questionnaire below. We will seek a brief protocol from you subsequently, not to exceed five pages, so that we can assure a full range of technology platforms and to enable constructive feedback to you from our Technology Platforms and Protocol Review Committee, chaired by Richard Simpson.

We have several reference specimens being specially prepared for this Project, including those discussed at our January 10 meeting with FDA, WHO and NIBSC (UK). **We need your response to plan the volumes and distribution of the reference specimens.** The Specimen Collection & Handling Committee led by Dan Chan will soon finalize the choices:

(a) National Institute for Biologics Standards & Control (NIBSC): freeze-dried citrated plasma

(b) BD: small donor pools by ethnic group in sets of four specimens, comprising EDTA-, heparin-, and citrate-anticoagulated plasma plus serum (b1 = Caucasian set; b2 = African-American and Asian-American sets); and

(c) Chinese Academy of Medical Sciences (probably similar to BD, perhaps best for distribution throughout Asia-Oceania HUPO).

We expect all labs to run specimen (a) and the Caucasian four-specimen set (b1); we encourage requests for sets (b2) and (c). We also ask for interested labs to run additional samples of your choosing that will permit comparing results from:

(1) protease inhibitor cocktail, or not;

(2) depletion of albumin or gamma globulins or both, using immunoaffinity or protein A/G columns or beads, versus not (vendors have offered HUPO special access to immunoaffinity products); and

(3) various practical variables in timing, temperature, freeze/thaw, and other handling of specimens (for those interested, more information will be provided; see Chan/Moore at www.hupo.org, Plasma Project Project).

We are working with the HUPO Protein Standards/European Bioinformatics Institute staff, led by Rolf Apweiler and Henning Hermjakob, to provide the data structure and data management for this initiative. All data will be public domain, with ample opportunities for intellectural property through diagnostic biomarker tests and target-based agents.

Human Plasma Proteome (HPP) Project Questionnaire

Date: _____

To: **Gil Omenn, HUPO PPP Leader** (email: gomenn@umich.edu; Ph: 734-763-7583)

Please tick boxes,use tab to go to next item

☐ We confirm our interest in participating actively in the HUPO Plasma Proteome Project as a Participating Laboratory.

☐ We are willing to submit a brief protocol.

Name of laboratory	
Contact person	
Detailed address for specimen distribution	
Phone	
Email	

Reference specimens to be studied, 1 ml in four 250 µl aliquots/specimen (choose as many as desired)

☐ (a) Single specimen NIBSC freeze-dried citrated-plasma sample

☐ (b1) Four-specimen set (from BD): EDTA-, heparin-, and citrated-plasmas plus serum (Caucasian), frozen

☐ (b2) Similar four-specimen sets from African-Americans and from Asian-Americans

☐ (c) Similar four-specimen set from Chinese Academy of Medical Sciences

Preference (choose one)

☐ frozen (on dry ice)

☐ freeze-dried/lyophilised (simply re-suspend in 1 ml distilled water)

☐ doesn't matter

Willingness to investigate additional variables:

use of ☐ protease cocktail – *indicate what cocktail:* …

use of ☐ immunoaffinity to deplete
☐ albumin ☐ immunoglobulins ☐ additional proteins

or ☐ other affinity chromatography methods to deplete – *specify:* …

☐ albumin ☐ immunoglobulins ☐ others, *specify:* …

☐ Are you willing to work with Dan Chan et al on technical variables of specimen handling (see above) and duplicate runs?

☐ Please connect me with potential vendors/suppliers.

Additional lab-specific specimens likely to be included in the studies: *(please specify)*

Technology platforms to be utilized (check all that apply)

☐ 2D PAGE	☐ Free-Flow Electrophoresis
☐ Multi-dimensional liquid separations	☐ Separation of intact proteins
☐ LC/MS or LC/MS-MS	☐ Digestion, then separation of peptides
☐ Chips/MS (SELDI)	☐ Analysis of sub-proteomes – *specify:*
☐ Protein Microarrays	☐ Other:

Volume

☐ regular volume = 1 ml equivalent for (a); 4 ml for (b1) or (c); 4 or 8 ml for (b2)

☐ additional volume – *please specify what you think you need, according to assays to be performed: ml*
Indicate number of duplicates you will run per assay: (0,1,….)

What data elements in what format do you expect to submit to the Project? *(please specify; we will use your responses to help prepare standard formats with XML)*

We welcome comments on means of comparing output/results from various studies across laboratories and technologies:

a) Sensitivity and Resolution	no. distinct proteins (criteria: via genome databases; via antibody arrays; other?)	
	no. protein spots/isoforms or fractions identified	
	specific analytes detected among a set of clinical analytes in various concentration ranges determined on the reference specimen	
b) Practical aspects	Time and effort for various components of the study	
	Equipment and training required	
	Costs	
	Variability/complications	

Assuming protocol submission and review and distribution of specimens is completed by the end of March, I will be able to complete the **initial** studies in time for data submission in advance of the October 8-11 2nd World Congress of Proteomics in Montreal. Yes ☐ No ☐

Would you be interested in a “World Jamboree” (together for 7-10 days, or possibly virtual gathering) for intensive analyses of findings, modeled after the Drosophila Genome Jamboree and/or the preparation of the Human Genome Special Issue of Nature (15-Feb-01) and Science (16-Feb-01)? Yes ☐ No ☐

Thank you!