

Pilot studies with reference specimens

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Current Status - Specimens

- BD Biosciences
- Chinese Academy of Medical Sciences

Goals

- Develop protocols for blood specimen collection, handling, storage, freeze/thaw and high throughput issues.
- The protocols should be comprehensive, applicable to most methods/techniques, but also practical for routine or clinical uses.
- Collect and distribute reference specimens for proteomics analysis.

Sample Collection & Storage

SAMPLE SOURCE

Pooled sample, e.g. NIBSC.

Reference sample. from individual

SAMPLE TYPE (MATRIX)

Whole blood

Serum

Plasma

Other Body
Fluids

PRESERVATIVES and ANTI-COAGULANTS

Clot activated
(SST)

EDTA

heparin

NaF

Pot.oxalate

Citrate

STORAGE

Room temp (20C)

Refrigerated (4C)

Frozen
(-20)

Frozen
(-80)

SAMPLE SOURCE



Pooled
sample,
e.g.
NIBSC.

Reference sample from
healthy individual: Sex,
age, ethnicity,
physiological status, etc.
BD & CAMS

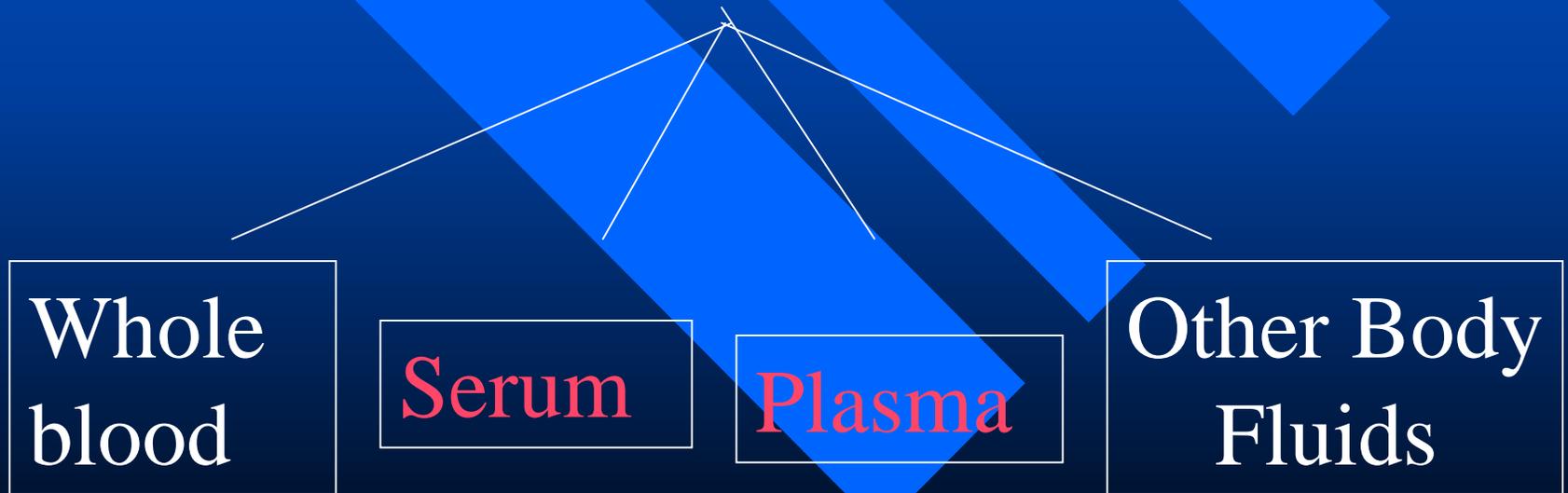
Sample Source

- What is a healthy (normal) individual? Serum with chemistry and hematological analytes within “normal” reference intervals?
- Age? Neonates, infants, children, adolescents, adults (young, middle age, geriatric).
- Physiological conditions, e.g. pregnancy.
- Pre- and post-menopausal status.
- Familial genetic variability – diabetes, cardiac disease, cystic fibrosis and other polymorphisms, esp. clinically silent variants.
- Lifestyle influences – Nutrition, smokers, alcohol consumption, exercise, medications, circadian variations.

Pool vs individual sample

- Pooling samples provide larger volume and more consistent, however, it could dilute unique low abundance proteins.
- An individual sample provides the detection of polymorphism, however, it limits the sample volume. Multiple samples are needed to account for the variation.

SAMPLE TYPE (MATRIX)



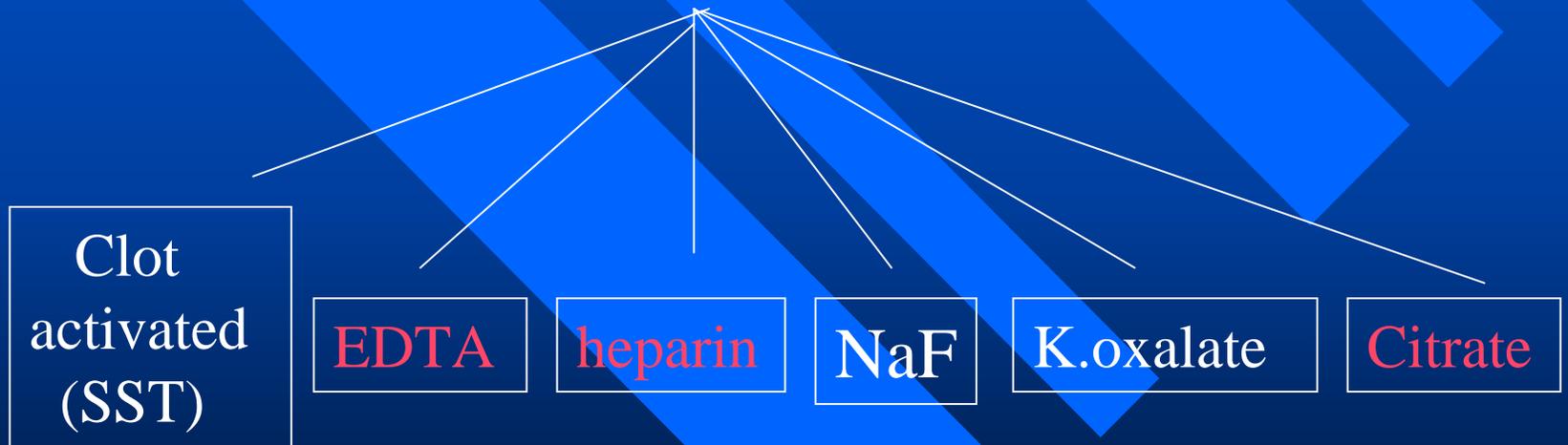
Sample Type

- Blood collection –venous recommended.
- Which sample type should be used – *serum or plasma?*
- For what purpose?

Serum vs plasma

- Serum has no additive and therefore it provides a cleaner specimen, except the use of clot activator.
- During the clotting process, some endogenous substances could be released.
- Loss of coagulation proteins, other proteins?
- Studies needed to be performed in selected labs.

PRESERVATIVES and ANTI-COAGULANTS



Preservatives or anticoagulants

- Should any of these substances be used? Need to study other additives, e.g. protease and phosphatase inhibitors.
- What are the effects on proteomic profiles or other methods? Samples in the pilot phase needs to be consistent with mass collection technique.

Sample Processing

- How soon after sample collection should the sample be processed? Immediately, 30 minutes, 1 hour, longer? Practical in clinic?
- Centrifugation – How long? At what temperature?
- Transportation issues.
- Fractionation? Extraction? Depletion of high abundant proteins?

STORAGE



Sample Stability Protocol

Storage Time

20C	4C	-20C	-80C
0	0	0	0
2 hrs	4 hrs	24 hrs	1 week
4 hrs	8 hrs	1 week	1 month
24 hrs	24 hrs	1 month	1 year
1 week	1 week		

Sample Storage

- How long was the sample stored? How long could the sample be stored?
- How was the sample stored? Refrigerated or frozen? Prefer flash freeze samples and store at -80°C .
- How many time could the sample be freeze and thaw? < 3 freeze-thaws (preferable only once).
- How should the sample be stored? Aliquot serum/plasma into 1 mL samples. Influence of container material – glass vs. polypropylene vs. polyethylene vs. PVC. Assign unique sample ID that does not identify the subject.

Other Issues

- Other in-house samples and studies could be performed.
- Duplicate analysis.
- Quantification.

Report from the Specimens Group (1)

- Protease inhibitors cocktail presentation:
BD blood collection tube for EDTA plasma
is available (convenience and consistency).
Phosphatase inhibitor: lower priority
- Depletion of high abundant proteins:
Agilent technologies (Ab column)
presentation.
- Stability studies: collection vs specimen
thawing, issues related to time zero.

Report from the Specimens Group (2)

- Recommend standard preparations for 2-D gel, MS etc. (NIST?).
- Study the effects of blood collection on plasma proteome (biological) and on protein interactions due to the ABO blood groups.
- Regulatory requirements for blood collection or use stored specimens: IRB, informed consent, HUPO lawyer?
- Next phase of blood collection, HUPO plasma/serum bank?
- Develop SOP (standard or selection) for analytical technology.











