The Debate Rages On: Heparinized Plasma and Serum Collection in Chemistry Testing

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About the Speaker

- Bench tech experience in hematology, hemostasis, urinalysis, toxicology, and chemistry in a large multi-system medical center
- Program Director of a hospital based MT Program as well as Safety and Education Coordinator
- Lab Director of Immediate Response Lab and Critical Access Lab
- MLS instructor at University of Cincinnati for online and on campus students
Objectives

1. Identify the biological differences of plasma and serum.

2. Compare benefits for appropriate use of heparinized plasma and serum samples.

3. Review best practices for analyte testing with either heparinized plasma or serum.
The Collection Process

The quality of the analytical results begin with the quality of the collection process

- How has the collector been trained?
- Has the patient been identified correctly?
- Has the order of draw been followed in collection?
- Are samples treated appropriately following collection?
  - Mixing
  - Centrifugation
  - Storage
What are the differences between plasma and serum?

- **Serum** is the clear liquid supernatant remaining after centrifugation of *clotted* blood.
- It contains electrolytes, certain proteins, antigens, hormones, immunoglobulins, enzymes, and dissolved gas.
What are the differences between plasma and serum?

- **Plasma** is the clear liquid supernatant remaining after centrifugation of *anticoagulated* blood.

- It contains electrolytes, certain proteins including fibrinogen and clotting factors, antigens, hormones, immunoglobulins, enzymes, and dissolved gas.
Collection Tubes

1950’s Glass collection tubes

1980’s PET plastic tubes

- Clot activator
  - Surfactant for clot activation

- Serum separator gel
  - Separates based on specific gravity
    - Gel 1.04 g/cm³
    - Formed clot 1.09 g/cm³
    - Serum 1.02 g/cm³
  - Inert
Serum Collections

‘Red top’ – serum or Red top with Gold Ring serum separator
Contains clot activator and/or inert gel

‘Gold top’ – serum separator
Contains clot activator and inert gel

End over end inversion 5-10x following collection

Allow for clotting time up to 30 minutes

Centrifuge
Plasma Collections

Heparinized plasma

- Minimal chelating properties
- Minimal effects on water shifts in erythrocytes
- Low cation concentration
- Lithium, Sodium, or Ammonium Heparin
  Anticoagulant that prevents coagulation through thrombin inhibition
- Invert 5-10x after collection
- ‘Green top’
  - Tubes are spray-dried with heparin
  - Plasma separator of inert acrylic gel
Advantages of Gel Separator Tubes

- No need to pour off sample aliquot
- Single patient identification
  - Decreased errors
- Allows for storage in primary tubes
Advantages of Gel Separator Tubes

Minimizes serum or plasma contact with cellular elements

- Phosphates decrease as erythrocytes convert ATP to ADP
- Glucose decreases due to glycolysis by erythrocytes
- Enzymes AST and LDH increase due to red cell release
- Potassium increases through the action of the Na+/K+ ATPase pump
Recommendations when using Gel Separator Tubes

- Use a ‘swing out’ centrifuge
- Centrifuge at recommended speed and time
- Store and transport in an upright position
Plasma Versus Serum

World Health Organization:

Plasma is more applicable than serum in most clinical tests as plasma is a better reflection of the pathological condition of a patient.
Advantages of Heparinized Plasma

Heparinized plasma saves time:

- Decreases turn around time on pre-analytical testing
  - Benchmark 90% completion of tests within 60 minutes
  - Sample order to result verification

- Serum samples require a 10-30 minute clotting time prior to centrifugation

- Plasma samples can be centrifuged immediately after collection
Advantages of Heparinized Plasma

Heparinized plasma provides a higher yield in volume for testing

- 15-20% more volume from the same volume of blood collected

- ISO 15189
  - Periodic review of sample volume requirements to ensure that excessive blood sample volumes are not collected
Advantages of Heparinized Plasma

Heparinized plasma prevents coagulation induced fibrinogen formation

- Fibrin in the serum sample may plug pipettes or analyzer probes
  - Short sample
  - Plugged probe
  - Increased testing time

- In serum tubes, fibrin can continue to be formed during or after centrifugation, especially in the anticoagulated patient
Advantages of Heparinized Plasma

Heparinized plasma prevents other coagulation induced interferences

- Decreased likelihood of hemolysis
- Decreased in release of platelet factors
- More accurate total protein value
Disadvantages of Heparinized Plasma

Interference in testing

- Interference with other cations
  - Sodium can be falsely elevated with sodium heparin collection
  - Lithium levels can be falsely elevated with lithium heparin collection

- Less stable due to presence of platelets, white cells, and red cells

- Interference by fibrinogen in protein analysis
Disadvantages of Heparinized Plasma

Less stable upon storage

- Refrigeration promotes latent formation of fibrin
- If stored refrigerated in the primary tube, concentration changes may occur in commonly ordered tests
  - Calcium most affected within 24 hours
  - After 24 hours: K+, Gluc, LD
Issues with Heparinized Plasma Gel Tubes

PST tubes should not be inverted or re-centrifuged

- Inversion or re-centrifugation may release cells trapped in the gel back into the suspended liquid
- Elevated AST, LDH, K+ identified in one study
Issues with Heparinized Plasma Gel Tubes

PST gel tubes may separate

- Specific situations
  - Increased proteins present in Multiple myeloma
  - Patients with high specific gravity
  - Iodine contamination from contrast media

- Clogged analyzer probe

- Insufficient sample aspiration

- Delayed patient care
Advantages of Serum

‘Gold Standard’ for certain tests

- Cells trapped within clot
- No fibrinogen interference
  - Total protein
  - SPE
Advantages of Serum

Increased storage and preservation

- Longer preservation time
  - SST good for transporting from remote locations
  - If a serum separator is used, stable for up to 48 hours
  - Glucose stable with gel tubes
Disadvantages of Serum

Latent clot formation

- Fibrin formation post centrifugation

- Inadequate clotting time
  - Patient anticoagulant therapy
  - Patient with coagulopathy
Disadvantages of Serum

Platelet substances are released during clot formation

- Pseudo-hyperkalemia due to potassium release from platelets during the clotting process
- LD, Ca$^{2+}$, Mg$^{2+}$ can be higher in serum than plasma
Disadvantages of Serum

SST tubes may not be suitable for certain analytes

- Antidepressants
- Benzodiazepines
- Transferrin
- Testosterone
Variables in Sample Effectiveness

1. Collection technique
   a) Fill volume
   b) Mixing
   c) Lack of hemolysis
2. Centrifugation
3. Aliquot of samples
4. Assay compatibility
5. Population/setting
6. Temperature/storage
Summary

- Lithium heparin gel tubes and serum gel separator tubes are routinely used in chemistry and immunochemistry.
- Serum may show increased TAT and latent fibrin formation.
- Plasma can be centrifuged immediately after collection.
- Gel separator tubes provide increased stability by creating the barrier between cellular and liquid components.
Conclusion

- Emphasize collect collection protocol
  - Patient identification, phlebotomist training, collection technique
- Recommend assessing each specimen type with the analyzer/test for best outcomes
- Establish reference ranges based on specimen type
- Follow tube manufacturer guidelines and recommendations for centrifugation, fill volume, and analyte testing
References

Ahuja, Aparna Jah. MD, Plasma – the preferred sample type for clinical chemistry testing? MEDLAb. 2016;1

ARUP Labs. www.aruplabs.com/testing


Cleveland Clinic Labs. www.clevelandclinicslabs.com

References


Inversion of lithium heparin gel tubes after centrifugation is a significant source of bias in clinical chemistry testing


References


- Serum or Plasma? Preanalytical Pulse. Greiner Bio One

- Tube Chart. Greiner Bio-One


- UC Health Laboratory. http://laboratory.uchealth.com/

- Vacuette Preanalytics Manual. Greiner Bio-One

- WHO. Use of anticoagulants in diagnostic laboratory investigations. 2002
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