Serum vs Plasma

Which specimen should you use?

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Introduction

This presentation is intended to provide laboratorians with an in-depth discussion/review of serum and heparin plasma.

Heparin plasma is the specimen of choice in clinical chemistry for an increasing number of laboratories.

Heparin plasma represents a more complex specimen than serum.

We will cover:

- TAT
- Factors Influencing Specimen Quality
- Analyte Stability
- Instrument/Assay Considerations

Summary
Serum vs Plasma

Chemistry

Clot activator
30 min clot time

Centrifuge
1,100 - 1,300g
10 min

Serum

Lithium Heparin
Mix and spin

Centrifuge
1,100 - 1,300g
10 min

Plasma
Serum Tubes

- Non gel blood collection tube
  - Serum tube 30 – 60 min clot
  - Need to be aliquot to avoid cell contamination
- Gel blood collection tube
  - Serum tube with activator gel separates cells from serum
  - 30 minute clot for routine chemistry
- Thrombin blood collection tubes (RST)
  - 5 minutes for STAT serum determination in chemistry
- Inversion at blood collection: 8-10
- Centrifugation:
  - 1100-1300 g for 10 minutes at room temperature
Heparin plasma

- Heterogeneous mixture of straight-chain anionic mucopolysaccharides
- Molecular weight distribution of roughly 3000 to 30000 Da.

- Li-Heparin (used for routine clinical chemistry) or Na-Heparin (used if Li needs to be determined and for whole blood cell assays), 17 IU/mL, spray dried
- Characteristics of heparin plasma:
  - White particulate matter
  - Cold activation of clotting still possible
Polling Question

Which sample does your laboratory use?

A. Only serum
B. Only plasma
C. Mainly serum with some plasma
D. Mainly plasma with some serum
E. Almost even split
F. Don’t know
How Important is TAT?

- TAT represents top 3/5 categories listed by physicians as the most important

TAT = 36.5%

### Table 8. Aggregate Percentage of Most Important Laboratory Service Category

<table>
<thead>
<tr>
<th>Service Category*</th>
<th>Respondents, No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quality/reliability of results</td>
<td>1191 (31.7)</td>
</tr>
<tr>
<td>Routine test TAT</td>
<td>554 (14.8)</td>
</tr>
<tr>
<td>Inpatient stat test TAT</td>
<td>455 (12.1)</td>
</tr>
<tr>
<td>Test menu adequacy</td>
<td>409 (10.9)</td>
</tr>
<tr>
<td>Outpatient stat test TAT</td>
<td>361 (9.6)</td>
</tr>
<tr>
<td>Accessibility of pathologists</td>
<td>160 (4.3)</td>
</tr>
<tr>
<td>Critical value notification</td>
<td>152 (4.0)</td>
</tr>
<tr>
<td>Clinical report format</td>
<td>90 (2.4)</td>
</tr>
<tr>
<td>Accessibility of laboratory staff</td>
<td>90 (2.4)</td>
</tr>
<tr>
<td>Esoteric test TAT</td>
<td>81 (2.2)</td>
</tr>
<tr>
<td>Staff courtesy</td>
<td>71 (1.9)</td>
</tr>
<tr>
<td>Phlebotomy services</td>
<td>58 (1.5)</td>
</tr>
<tr>
<td>Laboratory management responsiveness</td>
<td>34 (0.9)</td>
</tr>
<tr>
<td>Accessibility of laboratory manager</td>
<td>26 (0.7)</td>
</tr>
<tr>
<td>Courier services</td>
<td>22 (0.6)</td>
</tr>
</tbody>
</table>

* TAT indicates turnaround time.

How Important is TAT?

- TAT is on the bottom for level of satisfaction

<table>
<thead>
<tr>
<th>Service Category†</th>
<th>No. of Institutions</th>
<th>10th</th>
<th>50th (Median)</th>
<th>90th</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quality/reliability of test results</td>
<td>138</td>
<td>75.0</td>
<td>89.9</td>
<td>100.0</td>
</tr>
<tr>
<td>Staff courtesy</td>
<td>138</td>
<td>71.4</td>
<td>89.9</td>
<td>100.0</td>
</tr>
<tr>
<td>Accessibility of laboratory staff</td>
<td>136</td>
<td>66.7</td>
<td>87.6</td>
<td>100.0</td>
</tr>
<tr>
<td>Accessibility of pathologist</td>
<td>137</td>
<td>69.6</td>
<td>87.5</td>
<td>100.0</td>
</tr>
<tr>
<td>Critical value notification</td>
<td>138</td>
<td>66.7</td>
<td>85.4</td>
<td>95.7</td>
</tr>
<tr>
<td>Accessibility of laboratory manager</td>
<td>136</td>
<td>65.6</td>
<td>84.6</td>
<td>100.0</td>
</tr>
<tr>
<td>Phlebotomy services</td>
<td>136</td>
<td>60.0</td>
<td>84.6</td>
<td>100.0</td>
</tr>
<tr>
<td>Test menu adequacy</td>
<td>138</td>
<td>65.5</td>
<td>84.6</td>
<td>97.2</td>
</tr>
<tr>
<td>Laboratory management responsiveness</td>
<td>136</td>
<td>59.6</td>
<td>82.6</td>
<td>100.0</td>
</tr>
<tr>
<td>Courier services</td>
<td>132</td>
<td>50.0</td>
<td>81.5</td>
<td>100.0</td>
</tr>
<tr>
<td>Inpatient stat test TAT</td>
<td>132</td>
<td>60.0</td>
<td>80.9</td>
<td>95.8</td>
</tr>
<tr>
<td>Clinical report format</td>
<td>138</td>
<td>62.5</td>
<td>80.6</td>
<td>93.8</td>
</tr>
<tr>
<td>Routine test TAT</td>
<td>138</td>
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<td>80.2</td>
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<td>138</td>
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</tr>
<tr>
<td>Esoteric test TAT</td>
<td>136</td>
<td>33.3</td>
<td>54.5</td>
<td>80.0</td>
</tr>
</tbody>
</table>

*Higher percentile ranks indicate better relative performance.
†TAT indicates turnaround time.

### How Important is TAT?

- Stat TAT represents most important category listed by nurses.

<table>
<thead>
<tr>
<th>Most Important Category</th>
<th>Respondents, No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stat test TAT</td>
<td>2432 (37.8)</td>
</tr>
<tr>
<td>Accuracy of test results</td>
<td>981 (15.2)</td>
</tr>
<tr>
<td>Abnormal results notification</td>
<td>523 (8.1)</td>
</tr>
<tr>
<td>Telephone courtesy</td>
<td>487 (7.6)</td>
</tr>
<tr>
<td>Phlebotomy responsiveness</td>
<td>455 (7.1)</td>
</tr>
<tr>
<td>Phlebotomy courtesy toward patients</td>
<td>404 (6.3)</td>
</tr>
<tr>
<td>Routine test TAT</td>
<td>364 (5.7)</td>
</tr>
<tr>
<td>Ability to answer telephone questions</td>
<td>292 (4.5)</td>
</tr>
<tr>
<td>Promptly answered phones</td>
<td>183 (2.8)</td>
</tr>
<tr>
<td>Phlebotomy courtesy toward nursing</td>
<td>94 (1.5)</td>
</tr>
<tr>
<td>Laboratory management responsiveness</td>
<td>94 (1.5)</td>
</tr>
<tr>
<td>Accessibility of laboratory management</td>
<td>71 (1.1)</td>
</tr>
<tr>
<td>Laboratory POC support</td>
<td>55 (0.9)</td>
</tr>
</tbody>
</table>

* TAT indicates turnaround time; POC, point of care.

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How Important is TAT?

- TAT is on the bottom for level of satisfaction

<table>
<thead>
<tr>
<th>Table 4. Distribution of Percentage of Very Satisfied/Usually Satisfied Ratings for Each Interaction Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category†</td>
</tr>
<tr>
<td>Accuracy of test results</td>
</tr>
<tr>
<td>Phlebotomy courtesy toward patients</td>
</tr>
<tr>
<td>Phlebotomy courtesy toward nursing</td>
</tr>
<tr>
<td>Abnormal results notification</td>
</tr>
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</tr>
<tr>
<td>Routine test TAT</td>
</tr>
<tr>
<td>Phlebotomy responsiveness</td>
</tr>
<tr>
<td>Accessibility of laboratory management</td>
</tr>
<tr>
<td>Laboratory management responsiveness</td>
</tr>
<tr>
<td>Stat test TAT</td>
</tr>
</tbody>
</table>

* Higher percentile ranks indicate better relative performance.
† POC indicates point of care; TAT, turnaround time.

How To Reduce TAT?

- Fast Analytical Phase
- Speed Up Sample Transport
- Reduce Preanalytical Handling & Processing
- Use Plasma
Recommended clotting times for serum blood collection tubes generally range from 30-60 minutes.

Use of plasma allows laboratories to process and test specimens upon receipt, while avoiding latent fibrin formation due to incomplete clotting.
Serum Specimen Quality

- Specimen quality has been another factor prompting some laboratories to switch to plasma.

- Serum specimens are subject to latent fibrin formation when clotting is inadequate.
  - insufficient clotting time
  - patients receiving anticoagulant or thrombolytic therapy

- Fibrin can range from thin strands to large cloud-like masses.
Fibrin / Micro clots

- Visible clot
- Fibrin mass
- Fibrin strands
- “Microclots”

**SERUM**
Incomplete clotting fibrin

**PLASMA**
Micro clots from filtered heparinized specimen
Fibrin – Tube Mixing

• Mixing immediately after collection facilitates dispersion of additive into the blood.

• Incomplete mixing may lead to incomplete or delayed clotting (serum) or incomplete anticoagulation (plasma)

• Typical manufacturer recommendations:
  Serum tubes: 5-6 inversions
  Plasma (heparin) tubes: 8-10 inversions

= 1 inversion
Fibrin

- Can cause significant disruption to instrument operation and process workflow.
Issues Due To Fibrin

- Physical obstruction of sampling probe
- Insufficient sampled volume
- Gradual deposition of fibrin in reaction chambers or pathways
- Interference with measurement systems or reagents

**Potential consequences**: instrument downtime, failure to provide test results, or erroneous test results.
## Fibrin – Instrument Operation

<table>
<thead>
<tr>
<th>Location</th>
<th>Event</th>
<th>Type of Interference</th>
<th>Potential Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sampling Probe</td>
<td>Aspiration of fibrin causing probe obstruction</td>
<td>Physical</td>
<td>- Sampling problem, insufficient quantity aspirated</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Erroneous results</td>
</tr>
<tr>
<td>Reaction Pathway or Reaction Chamber</td>
<td>Aspiration of &quot;micro clots&quot; not sufficiently large to obstruct probe</td>
<td>Physical, chemical or immunological</td>
<td>- Gradual deposition of fibrin in reaction pathway; &quot;plaque&quot;</td>
</tr>
<tr>
<td></td>
<td>Latent fibrin formation inside instrument</td>
<td></td>
<td>- Build up leads to obstruction</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Even with no obstruction, potential interference from light scattering or reagent interference</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Erroneous results</td>
</tr>
</tbody>
</table>
Addressing Fibrin Issues

- Approaches to minimize the impact of fibrin in serum specimens often require user intervention, increase TAT, and may not be recommended.

- To help reduce these issues, some laboratories have switched to plasma.

- However, plasma specimens also have unique characteristics concerning specimen quality and integrity.
Plasma Trends

- World wide generally increasing use of plasma
- Increasing use of plasma in some European countries
- US also increasing number of labs are moving to plasma due to TAT
Gel Movement

The presence of a solid clot in serum gel tubes also leads to a difference in the movement of gel during centrifugation.

**Serum:** Gel must move up and around the clot, against the tube wall.

**Plasma:** Gel moves up in pieces similar to a ‘lava lamp’.
Simulated Gel Movement

serum tube

plasma tube
What type of sample is this?

unmixed  mixed
Plasma Specimen Quality

- Separation of blood based on density gradient:
  platelets (least dense) > white blood cells > red blood cells (most dense)

- Platelets most abundant followed by WBC
- Fibrin – where present, generally exists in form of thin strands
- May lead to formation of 'microclots'
Plasma Specimen Quality

- As a result of the potential for variable amounts of cells, platelets, fibrin, and WPM, heparin plasma is generally a more complicated matrix to manage than serum.

- A proper understanding of the factors that influence plasma specimen quality is needed.
Ideal Plasma Specimen

- Ideal plasma specimen would be one which is cell/platelet free and in which the anticoagulant functions to inhibit clotting and fibrin formation for extended periods of time.

- Often not attained with heparin plasma specimens.
Problem: Supernatant Balance

TAT  Specimen
In general, most assays in clinical chemistry are compatible with both serum and heparin plasma, and test results are sufficiently equivalent that the same reference ranges can be used.

However for certain assays or test methods, plasma specimens may be unacceptable, or differences in results may be sufficient to warrant a change in reference range.
Changes due to clotting

- Clotting is proteolytic process
- During clotting, some cells will lyse

Serum Potassium around 0.7 mmol/L higher than plasma
Serum ref range: 3.5 – 5.2 mmol/L
Plasma ref range: 3.5 – 4.5 mmol/L
Potassium

- Potassium and phosphorus increased in serum due to release from cells/platelets during clotting.
- A linear correlation has been shown between platelet count and the increase in serum potassium.


Total Protein

- Slightly increased in plasma due to presence of fibrinogen.


Other Tests

- Differences in certain enzymes (e.g., LD, ALKP, AST) may be seen.
- Lithium/sodium increased with use of lithium or sodium heparin.
- Interference from fibrinogen may also make plasma an unsuitable specimen for certain protein analysis methods (e.g., SPEP - protein electrophoresis).
- Heparin may interfere with certain immunoassays.

Differences
Serum
vs
Plasma

Guder et al., 1996. Samples: From the Patient to the Laboratory.
Effects over Time

- Reduced stability in plasma of certain common analytes that are involved in cell/platelet-mediated metabolic processes and/or are present in higher concentrations in cells or platelets.

- Serum-plasma differences may be evident with these analytes depending on plasma cell/platelet content and time between centrifugation and testing.
Heparin plasma specimens with increased cell/platelet concentrations exhibit reduced stability of certain common analytes. Analytes affected are involved in cell/platelet-mediated metabolic processes and/or are present in higher concentration in cells or platelets.

**Table 6. Storage Stability of Selected Analytes in Lithium Heparin Plasma (mean ± sd)**

<table>
<thead>
<tr>
<th>ANALYTE</th>
<th>UNITS</th>
<th>TIME 0 Allquot from Non-Gel Tube</th>
<th>TIME 0 Gel Tube</th>
<th>TIME 24h Allquot from Non-Gel Tube</th>
<th>TIME 24h Gel Tube</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>mg/dL</td>
<td>100.8 ± 50.8</td>
<td>98.3 ± 50.8</td>
<td>101.2 ± 51.0</td>
<td>62.0 ± 52.4</td>
</tr>
<tr>
<td>Potassium</td>
<td>mmol/L</td>
<td>3.87 ± 0.26</td>
<td>3.96 ± 0.27</td>
<td>3.88 ± 0.25</td>
<td>4.25 ± 0.29</td>
</tr>
<tr>
<td>Carbon Dioxide</td>
<td>mmol/L</td>
<td>20.6 ± 2.2</td>
<td>19.6 ± 2.0</td>
<td>20.7 ± 2.1</td>
<td>16.0 ± 1.8</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>mg/dL</td>
<td>3.06 ± 0.56</td>
<td>3.11 ± 0.55</td>
<td>3.09 ± 0.55</td>
<td>3.46 ± 0.53</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>mg/dL</td>
<td>134.4 ± 91.1</td>
<td>131.8 ± 88.7</td>
<td>126.2 ± 86.8</td>
<td>125.9 ± 85.9</td>
</tr>
<tr>
<td>GGT</td>
<td>U/L</td>
<td>32.9 ± 51.2</td>
<td>33.1 ± 49.9</td>
<td>32.6 ± 51.4</td>
<td>34.7 ± 49.8</td>
</tr>
<tr>
<td>AST</td>
<td>U/L</td>
<td>21.6 ± 7.4</td>
<td>22.5 ± 7.1</td>
<td>21.0 ± 6.9</td>
<td>23.9 ± 6.8</td>
</tr>
<tr>
<td>LD</td>
<td>U/L</td>
<td>139.1 ± 16.6</td>
<td>154.8 ± 16.5</td>
<td>138.1 ± 16.9</td>
<td>184.9 ± 25.7</td>
</tr>
</tbody>
</table>
Dependence on Handling and Test Methodology

- The occurrence and magnitude of serum-plasma differences can depend on specimen handling and processing procedures and/or the specific instrument/assay methodology used.

- Plasma specimens may also exhibit an increased frequency of duplicate errors with certain instrument/test combinations, due to platelets, cell aggregates, or microclots.


Fibrin – Test Interference

• Erroneous FSH results caused by insufficient clotting of serum specimens and fibrin formation within analyzer reaction vessel.¹

• Falsely elevated Troponin-I due to fibrin in serum samples.²

• Duplicate errors in LD due to micro clots or cell aggregates in plasma samples.³

Do instruments support serum and plasma for all analytes?

<table>
<thead>
<tr>
<th>Instrument Company</th>
<th>Serum</th>
<th>Plasma</th>
<th>% Plasma</th>
<th>% Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beckman Coulter N=20</td>
<td>1160</td>
<td>1106</td>
<td>95%</td>
<td>92% - 97%</td>
</tr>
<tr>
<td>Ortho J &amp; J N=6</td>
<td>212</td>
<td>197</td>
<td>93%</td>
<td>89% - 100%</td>
</tr>
<tr>
<td>Siemens N=10</td>
<td>612</td>
<td>571</td>
<td>93%</td>
<td>90% - 97%</td>
</tr>
<tr>
<td>Abbott N=10</td>
<td>756</td>
<td>667</td>
<td>88%</td>
<td>78% - 95%</td>
</tr>
<tr>
<td>Roche N=11</td>
<td>613</td>
<td>520</td>
<td>85%</td>
<td>71% - 92%</td>
</tr>
</tbody>
</table>

*(BD non-published data)*
Specimen Yield

The formation of a physical clot in serum blood collection tubes also leads to other differences between serum and plasma specimens.

% Yield of serum is slightly lower due to serum trapped between the clot and the tube wall.
Serum vs. Plasma

- nearly cell-free
- good storage stability for most analytes
- wide range of assays available

- shorter TAT: can be centrifuged immediately
- faster gel movement in gel tubes
- more reproducible gel barrier formation
- increase supernatant yield 15-20% > serum
Serum vs. Plasma

- longer TAT
- instrument or test interference from fibrin, esp. with anticoagulation therapy
- may cause pseudohyperkalemia
- analytical variation due release from cells/platelets during clotting

- Higher cell counts
- reduced storage stability for certain analytes
- fibrin formation during storage
- interference from anticoagulant
- interference from fibrinogen
Summary

Plasma can provide significant benefits in reducing TAT

Plasma can provide significant benefits reducing analyte variability & increasing supernatant yield

However the benefits and implications of using plasma Need to be weighed against the long term stability

Specimen management protocols are of particular importance for plasma samples
Proper Sample Selection

- The selection of serum vs. heparin plasma may be dependent on the specific setting/population.

- *Example*: plasma for stat testing or patients on heparin therapy.

- *Example*: serum to preserve sample quality over extended periods of time or transportation.

- Standardizing on one sample type may be desirable but not always practical.
TOP 10 LIST
Preparing High Quality Specimens

1. Select appropriate sample type (serum vs. heparin plasma) based on pre-centrifugation time and patient population (and assay compatibility).

2. Ensure correct collection technique to minimize hemolysis.

3. Fill evacuated blood collection tubes to the stated draw volume. This will ensure the proper blood-to-additive ratio.

4. Ensure correct number of complete tube inversions immediately after collection to ensure blood and additive are mixed thoroughly.

5. Ensure correct (minimum or longer) clotting time for serum tubes.
6. Ensure centrifuge g-force (RCF) and spin time are sufficient to obtain adequate sedimentation of cells, platelets and other debris.

7. Carefully aliquot samples from non-gel tubes after centrifugation.

8. Avoid mixing/agitation of plasma gel tubes between centrifugation and testing.

9. To help reduce fibrin formation over time, keep heparin plasma at room temperature.

10. Recentrifugation of sample aliquots can help “clean up” samples but, do not re-spin gel tubes.
Thank you