Analysis of Analytes in Human Serum Under Conditions of Routine and Delayed Centrifugation

University of New Hampshire
College of Life Sciences and Agriculture

Grace DiPersio

Faculty Advisor: Stephanie Clarke MS, MT (ASCP)

CORE PHYSICIANS

Study Objectives

To determine:

- **Diagnostic significance** of 10 commonly measured and diagnostically valued analytes when human serum is separated from blood cells after **routine** and **extended clotting times**
- How erroneous test results are **interpreted by physicians** and subsequently referenced for diagnoses and treatment plans
- How **improvements** can be made logistically for optimal specimen handling by the phlebotomist

Background

VACUETTE® Serum Separation Tube

- Silica coating; clot activator
- Polymer gel
- Chemistry and serology tests

Chemistry Tests

Glucose	Magnesium	Thyroid Stimulating Hormone
Calcium	ALT/AST	
Potassium	Creatinine	
Sodium	HDL Cholesterol	

Observations of delayed centrifugation as a phlebotomist with Core Physicians, LLC.

- Courier pick-ups moments after blood draw → tube unspun for questionable amount of time
- The challenge of cost efficiency faced by laboratory companies- the need for more centrifuges vs the high cost of the product, maintenance, and repair
- Contradicting clot times recommended by manufacturers and laboratories ranging from 15 minutes to two hours
- Gel barrier breakdown (see figure 2)

Literature

Conclusions:

- Most analytes remain stable up to 24 hours prior to centrifugation, however, glucose was among those to degrade in several studies

Lacking:

- Acceptable delay times for the unstable analytes
- If and how invalid test results lead to improper diagnosis
- How the issue of delayed centrifugation can be resolved

Methodology

- Volunteer patients of Core Physicians donated three plastic VACUETTE® SST type (serum separation tube) tubes of blood in addition to the tests that brought them to the laboratory
- Each tube then sat for a specified amount of time prior to centrifugation as follows:
  - Tube 1: 15 minutes – Control
  - Tube 2: One hour – Experimental
  - Tube 3: Four hours – Experimental

- Specimens sent to Karen Crago, Core Physicians Medical Laboratory Technologist, for processing
  - Ran in Beckmann Coulter AU680

Figure 1. Hettich Rotofix 32A Centrifuge used for this study

Results

- Statistically significant changes in response variables via ANOVA **were not** observed
- Clinically significant changes in response variables over time prior to centrifugation **were** observed

Figure 2. Gel barrier breakdown after 15, 60, and 240 minutes of clotting from left to right

Observed Gel Barrier Breakdown

- **SSTs, if handled properly, should:**
  - Be easy to use (one centrifugation step)
  - Yield high serum levels for testing
- **Improper specimen handling lead to:**
  - Potential impact on specific gravity (the basis of gel separation of clot and serum)
  - Breakdown of gel barrier leading to RBC leakage into serum (as seen in figure 2)
  - Potential release of gel material into serum

Glucose

- **Result:**
  - Blood glucose levels steadily decreased over time
  - The most significant differences seen across all analytes tested
- **Probable cause:**
  - Cells utilizing glucose as a source of energy
- **Potential impact on patient:**
  - Glucose levels from a tube that sat for over 1 hour could be falsely negative for pre-diabetes, a reversible condition if caught, but could lead to type II diabetes if not

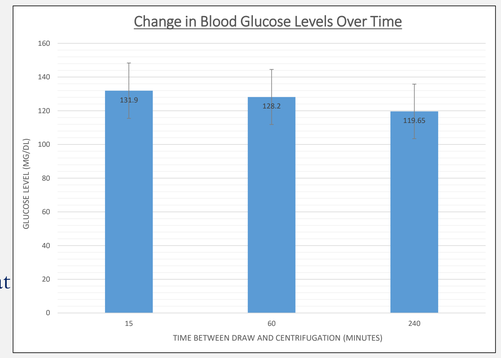
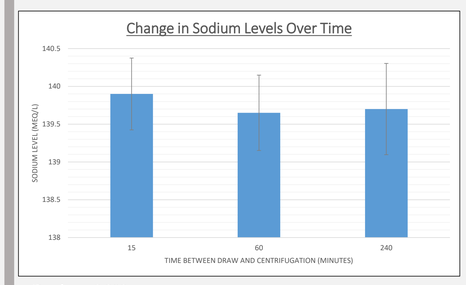
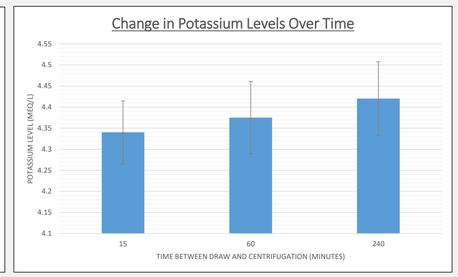


Figure 3. Changes in blood glucose levels of control vs experimental tubes
P-value= 0.863

Sodium & Potassium



P-value= 0.939



P-value= 0.792

Figures 4&5. Changes in sodium and potassium levels of control vs experimental tubes

Result: Sodium decreased over time while potassium increased

Probable cause: Potassium is mostly present *within* the cells, so RBC leakage due to gel barrier breakdown → falsely elevated results

Potential impact on patient: These electrolytes work together to maintain acid-base balance & regulate fluid in the body. Minor decreases/increases in potassium could have significant consequences (respiratory failure, heart disturbances). Results could lead to immediate & unnecessary action

Thyroid Stimulating Hormone

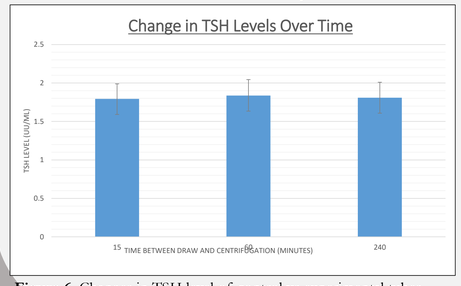


Figure 6. Changes in TSH level of control vs experimental tubes
P- value= 0.988

- **Result:**
  - Increase in levels in 60 minute tube and then decrease in 240 minute tube
- **Probable cause:**
  - Inconclusive, but likely related to age and storage conditions
- **Potential impact on patient:**
  - False hypothyroid/ hyperthyroid diagnosis
  - Poor monitoring of disease

Conclusions

- Phlebotomists, laboratory personnel, physicians and patients can be assured that most analytes will remain stable if centrifugation is delayed up to 4 hours
- Falsely decreased glucose levels will result if serum is not separated from blood clot within one hour
  - Leading to missed diagnosis of pre-diabetes (see figure 7)
- Despite the extended stability of most analytes, since glucose is included in a comprehensive metabolic panel, tubes should not be left un-centrifuged for greater than one hour to ensure accurate test results and subsequent diagnosis and treatment plan, or lack thereof

Glucose Findings: Pre-diabetes Missed Diagnosis

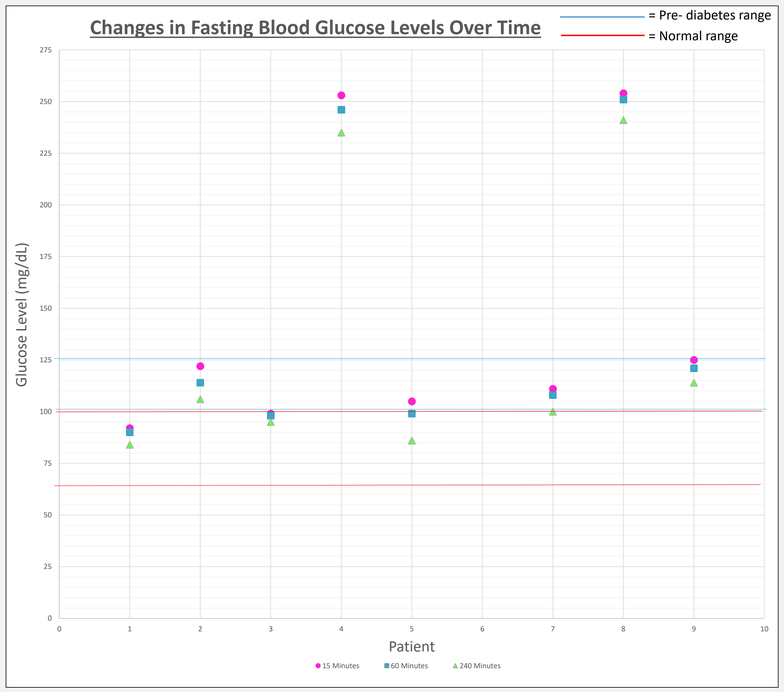


Figure 7. Changes in fasting glucose levels over time to show how a pre-diabetic patient could be missed diagnosed

Proposed Solution & Further Investigation

- Tube racks with imbedded timers
  - Add tube; start timer to count up, taking note when it gets to approximately 30 minutes
  - Final tube; set timer to 15 minute countdown
  - Improves communication amongst phlebotomists
  - Low cost solution to improve test results

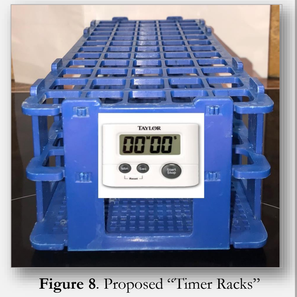


Figure 8. Proposed "Timer Racks"

- Ongoing Research
  - Interviewing physicians to conclude how erroneous test results are interpreted and used for diagnosis and treatment plans
    - How often are tests reordered due to discrepancies between results and patient presentation
  - Interviewing phlebotomists
    - How do they feel logistics could be improved to ensure proper handling of specimen
  - Continued data collection

References

1. Boyanton, BL Blick, KE . Stability studies of twenty-four analytes in human plasma and serum. Clin Chem 2002;48:2242–7 Google Scholar, Medline, ISI.
2. Oddoze, CE Lombard, EE Portugal, HI. Stability study of 81 analytes in human whole blood, in serum and in plasma. Clinical Biochemistry 2012; 45: 464-469 Google Scholar, Elsevier, Sceince Direct.
3. Tanner, MA Kent, NL Smith, BN Fletcher, SN Lewer, ME. Stability of common biochemical analytes in serum gel tubes subjected to various storage temperatures and times pre-centrifugation. Annals of Clinical Biochemistry: International Journal of Laboratory Medicine 2008; 45 SAGE Journals.