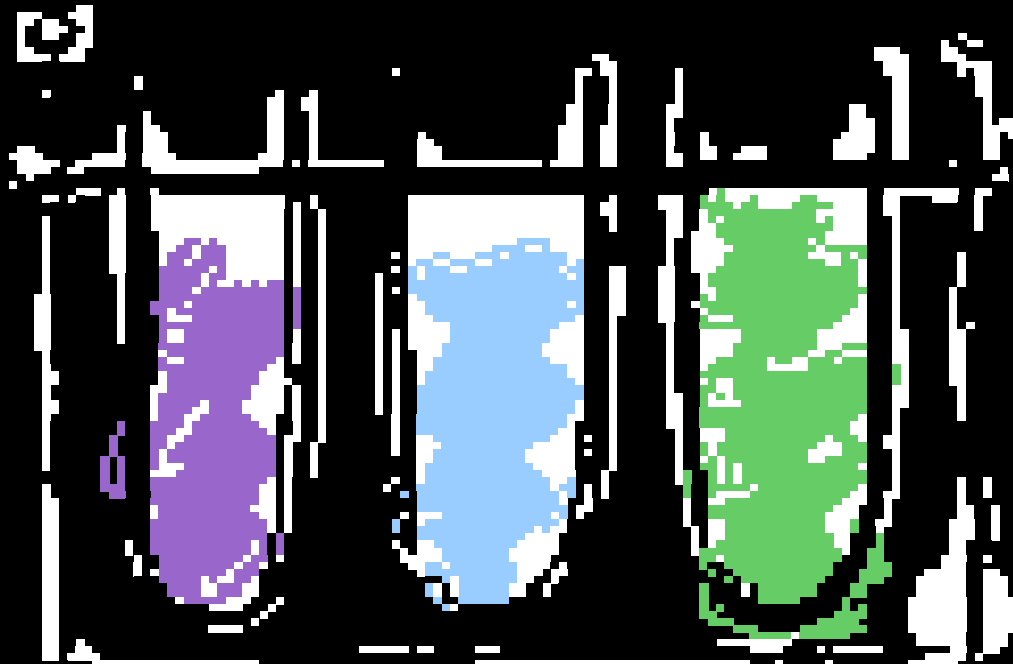


Evaluation of three different specimen types for analysis of certain analytes



Outline of Presentation

- **Organisation and Planning**
 - Introduction –Literature Review /Historical Perspective
 - Aims and Objective
 - Study Design: Ethical Approval, Health & Safety
- **Analysis**
 - Materials and Methods
- **Statistical Analysis**
- **Results**
- **Discussion, Summary and Conclusions**
- **Future**

Historical Perspective

Specimen Type

- Evacuated Tubes
- Plain/separating material
- Additives – anticoagulants
- Color-coded



Specimen Type

- Sources of error in biochemical tests are described in 3 categories
- Preanalytical- Time, Biological and Technical
- Analytical- Instrument and reagent dependent
- Post analytical- Result reporting and clinical interpretation

One of the most important pre-analytical factors that can introduce systematic bias is specimen type

Causes of Preanalytical Error

Collection Related Causes

- Storage of collection devices
- Filling of tube (blood:additive ratio)
- Order of draw
- Type of tube
- Type of additive
- Leakage of cellular constituents
- Incomplete separation

Serum Separator tubes SST's

- Customarily contain an inert polymer gel
- Functions to separate the supernatant from the cells
- Density is between RBC (1.094g/ml) and serum or plasma (1.026 g/ml)
- Gel does not move until sufficient force is applied by the clot pushing down on the gel
- Gel must flow around the clot along the wall of the tube

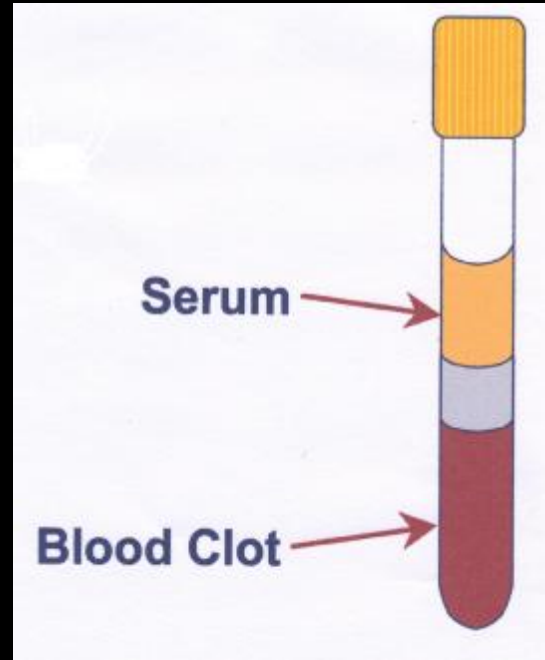
Centrifugation

Serum Separation



Function of Gel Tubes

- Provide an immovable barrier between the serum and blood clot
- Allows analytes to maintain their stability



Serum Separator Tubes SST

- A review of the literature indicates that the use of SST tubes for many analytes is controversial e.g. Phenytoin
- Although BD SST™ has been extensively investigated, but with conflicting results, the serum BD SST™ 11 has not been as widely researched.

Introduction

- There is a lack of consensus regarding the most appropriate specimen type for analysis:
- Serum vs. Plasma
 - Serum increased turnaround time
 - e.g. Patients on anticoagulant therapy
 - Risk of fibrin clots
- Certain analytes cannot be performed on Plasma

Introduction Cont...

- Published reports are conflicting whether or not biochemistry results are similar for serum and plasma samples taken from the same individual or population ¹⁻⁴
- Manufacturers state that either serum or plasma may be used for renal, lipid and Phenytoin analysis ⁵⁻⁸. NCEP ⁹ guidelines recommend serum for lipid analysis, and there is conflicting evidence regarding the suitability of plasma lithium heparin for lipid analysis ¹⁰⁻¹¹

Aims of the Study

- To Determine if the results for renal and lipid profiles and phenytoin are interchangeable in serum (plain and gel) and plasma lithium blood collection tubes.
- To investigate the stability of these analytes in serum and plasma; after
 - (1) prolonged contact with cells or gel at room temperature
 - (2) aliquoted and stored at 4⁰C

Overall Objective

- The overall objective was to assess if one sample type would be suitable for renal, and lipid profiles and phenytoin analysis.
- Identification of a single specimen type suitable for most analytes would result in significant savings and efficiencies.

Materials and Methods

Blood Specimen Types:

- S 6 ml serum plain BD Vacutainer
- P 6 ml plasma heparinized (LH 102 I.U.)
- G 5 ml serum gel separator BD SST™11

Evaluation

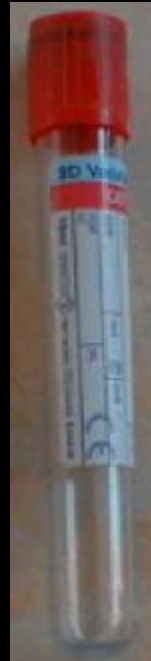
Consisted of clinical differences in results and efficiency in use.

Reference Samples

Specimen Types



**Plasma Lithium
Heparin**



Serum Plain



**Serum Separator Tube
(Gel Tube)**

Specimen Collection

- Triplicate blood samples (S, P & G) from a single venipuncture were obtained sequentially
- Serum samples were collected before plasma samples to prevent anticoagulant contamination¹²
- N = 56 study 1 Renal and Lipid analysis
- N = 21 study 2 Phenytoin analysis

Specimen Handling

- Primary specimens S,P and G were simultaneously centrifuged once ¹³ @ 3000g for 10 minutes and analysed in duplicate within 2 hours (T₀). Immediately following centrifugation two cell free aliquots from each of the primary tubes were separated and stored at 4°C.
- The primary specimens and their corresponding aliquots were subsequently analysed in duplicate at T₂₄ and T₄₈ respectively.

Instrumentation

TDx



LX20



Test	Methods
Renal Profile	
Na, K, Cl, CO ₂	ISE
Urea	Conductivity
Creatinine	Colorimetric
Lipid Profile	
Cholesterol	Enzymatic /colorimetric timed end-point
Triglycerides	
HDL	Bio-Stat (1997)
LDL	Friedewalde Equation
Phenytoin	FPIA

Computations



- Computations were performed on a Dell Inspiron PC using Microsoft Office 2000 Excel® and the software package Analyse-it.
- Microsoft Office Excel database were set-up for each parameter.

Statistical Analysis

- The Shapiro-Wilk W Test was employed to determine whether observations of a sample were normally distributed ¹⁴
- Values >0.1 indicated a normal distribution.
- Statistically significant changes ($p < 0.05$) from control samples were determined for each analyte by Friedman 1- way ANOVA by rank ¹⁵

Statistical Analysis Cont.

- When the Friedman analysis indicated significant differences, the **Critical Range Test**¹⁶ was used to establish which pairs of conditions differed significantly .
- A statistically significant difference was deemed clinically significant if the value fell outside the Total Allowable Error (TE)¹⁷ range (reference mean +/- TE%)
- TE was calculated by a combination of imprecision and bias

$$TE = 1.65(\text{Imprecision}) + \text{Inaccuracy (allowable bias)}$$

Statistical Analysis Cont.

- **Clinically significant changes** for Phenytoin were determined by the **Significant Change Limit (SCL)**¹⁸ defined as

$$\text{SCL} = \text{Initial value} \pm 2.8\text{USD.}$$

- The USD was obtained by averaging the quality control data for the period of analysis for Phenytoin.

Results

- There was no failure in barrier gel formation for the SST tubes used during the study
- Except for CO₂ , Phenytoin and Triglycerides the data exhibited non-parametric distribution.
(*Shapiro-Wilk Test, p<0.1*)

RESULTS OF THE SHAPIRO-WILK W TEST

Analyte	P-Value	Distribution
Sodium	0.0080	Non-Parametric
Potassium	<0.001	Non-parametric
Chloride	0.0595	Non-parametric
Urea	<0.001	Non-parametric
Creatinine	0.0506	Non-parametric
Choloesterol	<0.0010	Non-parametric
Triglycerides	0.2810	Parametric
HDL	0.0009	Non-parametric
LDL	0.0023	Non-parametric
Phenytoin	0.4465	Parametric
CO ₂	0.6049	Parametric

Results at T₀

- While most of the analytes displayed statistical significance (Friedman 1-way ANOVA by rank, $p < 0.05$) the only analyte that displayed both a statistical and a clinical significance at T₀ was Potassium;
serum and gel K were respectively 9.6% & 7.1% higher than plasma K.
- All other parameters demonstrated equivalence between plasma and serum (plain and gel) at T₀.

Results of Stability of Analytes standing on cells/gel at RT.

- All analytes except K, TCO_2 , Urea, Creatinine and Phenytoin were stable in all three tubes over T_{24} and T_{48}

K

Stable in gel tubes up T_{48}

Increased

-by 32% in serum plain at T_{48}

-and in plasma by 9.6% at T_{24} and 70.6% at T_{48}

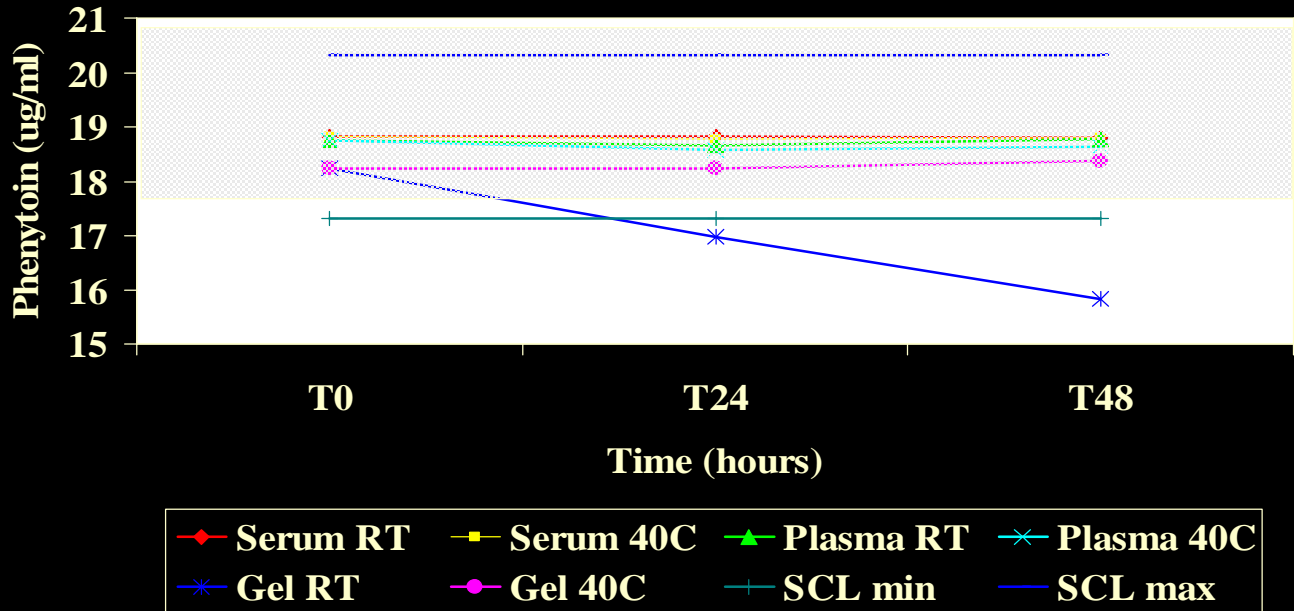
Results of Stability of Analytes Standing on Cells/Gel at RT

- Plasma urea was marginally above the clinically significant range at T₄₈
- Creatinine in plasma and serum (plain) increased to a clinically extent (14.5% and 28.3% respectively) at T₄₈
- CO₂ was unstable in all 3 tubes (% differences >20%)

Results – Stability of analytes standing on cells/gel over T_{24} and T_{48} at RT

- This study demonstrated a progressive clinical significant decrease in Phenytoin concentration over time in serum in contact with the gel.
- Phenytoin concentrations were stable in both serum (plain) and plasma tubes standing on cells at RT.

Phenytoin (mean, ug/ml) initially, and during storage at RT and 4°C in serum (plain & gel) and plasma tubes



Results of Stability of Analytes aliquoted and stored at 4°C

- CO₂ was the only parameter that displayed both a statistical and clinical significant change at 4°C in all tubes at T₂₄ and T₄₈.
- All other analytes were stable when aliquoted and stored at 4°C.

Discussion-

Performance of tubes at T_0

- The lower plasma K than in serum is consistent with those obtained by previous authors ¹⁹⁻²¹ who investigated serum (plain) vs. plasma.
Inclusion of serum gel in our 3-way study is new
- Anticoagulant prevents the rupture of platelets and the consequence release of K
Number and functional behavior determines magnitude of difference
- K requires specific reference ranges for Serum and Plasma

Discussion – Stability of analytes standing on cells/gel over T_{24} and T_{48} at RT

- The increase in K over time in plain serum and plasma is most likely attributable to Na-K ATPase pump failure with passive diffusion of K from erythrocytes.
- Stability of K in Gel tubes demonstrates the efficiency of the gel barrier at preventing metabolically active analytes from interacting with the cells and supernatant.

Discussion – Stability of analytes standing on cells/gel over T₂₄ and T₄₈ at RT

CO₂ :The decrease in TCO₂ demonstrated in the three tubes was probably due to exchange with ambient atmosphere ²².

Urea: The movement of water into cells, causing hemoconcentration could be the aetiology of the increase in plasma urea over T₄₈.

Hemoconcentration was less pronounced in Gel tubes than Plasma and serum plain

*Discussion – Stability of analytes standing on
cells/gel over T_{24} and T_{48} at RT*

Phenytoin

- The progressive reduction of Phenytoin in the Gel tube was possibly due to slow adsorption of the drug by the gel
- In contrast, a number of studies have found favorable results with the Gel Tubes ²³⁻²⁴

Discussion- Stability of analytes aliquoted and stored at 4°C

- Although the stability of analytes immediately separated from cells has previously been described by several authors , these studies are difficult to evaluate because study design varied considerably.

Summary

- At T_0 , except for K, all analytes in Serum, Plasma and Gel tubes were equivalent
- Plasma tubes may be used for lipid analysis
- Stability of CO_2 was independent of tube type and storage temperature
- Apart from Phenytoin most analytes were stable in Gel tubes and these tubes demonstrated enhanced stability for U/E analysis compared to serum (plain) and plasma.

Conclusions

- We concluded that Serum Gel is the specimen of choice for these analytes:
 - Increased stability
 - Convenient sample handling
 - Protection against possible contagion
(aliquoting only required for Phenytoin)
 - Increased efficiencies

Future

- Further investigation required to determine the overall performance of Gel tubes in a clinical setting including a broader range of analytes
- Reference ranges for Serum and Plasma K could be investigated as they are not interchangeable