ABSTRACT

The present study has been taken for comparing two blood collection and sera separation methods i.e. test tubes in parallel with vacutainers. Study has been carried out in four months (April to July, 2012) on one hundred and eighty five human volunteers from across Niger State, Nigeria. About 4ml of blood sample were collected aseptically from cephalic vein and divided into two equal parts, first one placed into pre-labeled test tube and the second into vacutainer (plain) separately for each volunteer. Sera from both test tube and vacutainer for each volunteer were extracted and volume measured separately and in combination. The results revealed that a range of sera of between 0.4 - 0.7 µl for test tubes and 0.7 - 1µl for vacutainers were recovered, respectively. Findings of the present study clearly indicate that vacutainers are of significantly superior over test tubes in separating sera from whole blood particularly, if larger volume of serum is required.

KEYWORDS
Human Blood Sera
Test tubes
Vacutainers
Collection Technique

* Corresponding author

E-mail: agarba_nvri@yahoo.co.uk (Garba A)

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1 Introduction

Circulating blood in both humans and animals consists of already formed elements including red blood cells (RBC), white blood cells (WBC), and platelets; suspended in plasma. Usually blood when collected out of the circulatory system will clot due to the polymerization of fibrinogen to fibrin unless if an anticoagulant such as EDTA, heparin, and sodium oxalate etc is added to prevent the clotting (Versieck, 1985). This clot of blood usually retracts, expressing serum which differs from plasma chiefly in that it contains no fibrinogen. Further centrifugation of clotted blood or anti-coagulated whole blood will separate serum or plasma respectively from the blood.

Over the years test tubes were used to clot blood and extract serum or with anti-coagulants to centrifuge whole blood to express plasma. However, in 1947 tubes containing different color stoppers known as vacutainers were developed by Joseph Kleiner (Russell-Delucas, 2011). The vacutainer is currently being manufactured by Becton, Dickinson and Company (BD), a global company providing medical supplies to the healthcare and pharmaceutical industry. Red top tube (vacutainer) which also has a clot activator is used to collect serum to test for infectious diseases, or routine blood donor screening (Russell-Delucas, 2011). Generally, blood is collected from human or animal subjects for various laboratory analyses which could be performed on whole blood or blood fractions, which include serum, plasma, and lymphocytes (Vaught, 2006).

For good quality laboratory test result, a number of variables are required to be optimal including a skilled phlebotomists, blood collecting device, well prepared patient, adequate volume of blood (for sera or plasma retraction). In most of the diagnostic laboratories it was suggested to collect 2 to 2.5 times volume of blood compare to the volume of serum required (Quest Diagnostics - http://www.nicholsinstitute.com/SpecimenCollection/SerumPlasma.aspx). In this study, we employed the use of test tubes parallel with vacutainers (as an improved technology) to compare which of the two will yield more serum from human blood in field condition.

2 Materials and Methods

2.1 Collection of sample

A total of three hundred were target but only 185 blood samples were collected from volunteers between the age of 18 and 67 groups across Niger State of Nigeria. Four ml of blood was collected aseptically from the cephalic vein of each volunteer using 5ml syringe and 18 Guage needle.

2.2 Comparative study

The collected blood sample was divided into two equal parts (2ml each), one part into a test tube and the other in vacutainer. The test tubes/vacutainers were allowed to stand at an angle of 90°C on the surface of a cold man box for a period not less than one hour (30 minutes on the surface and 30 minutes inside the ice packed cold man box) (Plate 1).

Plate 1. Test tubes and vacutainers containing 2ml of blood each standing at right angles to enable clotting and sera retraction.
Serum sample separation was done according to TDSHS (2010) with minor modification. A Pasteur pipette was used to collect the generated serum from the clotted blood in both the test tube and vacutainer respectively. Volumes of sera which were earlier centrifuged at 3000 rpm for 10 minutes were measured separately and combined using a 5 ml syringe. Paired students t-test was used to test for significance difference in volumes generated by test tubes and vacutainers using Microsoft office 2010 excel tool data analysis. p value less than 0.05 (p<0.05) was considered a significant difference. The results were summarized using descriptive statistics into tables and charts.

3 Results and Discussion

Results showed that after upright standing for one hour of clotted blood; flake of clotted blood covers the surface of the test tube and the serum accumulated beneath (at the bottom) as compared with the vacutainers whose serum appeared on the surface and surrounding the clotted blood (Plate 2). Larger volumes of sera samples were generated in vacutainers than in the test tubes for all the volunteers (Plate 3, Figure 1). Between 0.4 to 0.7 µl (in test tubes) and 0.7 to 1µl (in vacutainers) of serum was regenerated from 2ml whole blood (Figure 1). The mean volume of serum in all the two observations was 0.56±0.075 µl (in test tubes) and 0.8±0.06µl (in vacutainers) (Table 1). A statistical significant difference (p < 0.001) exists between volumes of sera generated in test tubes versus vacutainers (Table 1). It was further observed that between 1.2 to 1.6 ml of serum can be generated from 4 ml of blood when divided into two places (test tube and vacutainer) at the same time (Plate 3).

In most of the developing countries for some reasons, very few medical outlets are constantly engaged with the use of vacutainers in blood collection and particularly, sera and plasma separation despite the modernization of medical practices globally. Vacutainers being a modern technology may have some promises than the traditional (test tubes) abundantly in use in most developing countries. In this study however, it revealed more sera generation with vacutainers than test tubes and the formation of flakes of clotted blood on the surface with more serum at the bottom of the test tube (Plate 2). This flakes formation could probably be responsible for low generation of serum by vacutainer. Additionally, the test tube is wider in width than the vacutainer, suggesting more surface area coverage of blood in the vacutainer and indeed sera extract than in the test tube as shown in Plate 2. Thirdly, due to flakes and clot formation on the surface of the test tube; serum being at the bottom, it makes penetration of the pipette tips more difficult to reach the bottom to aspirate serum in test tube than vacutainer as observed in this study. Furthermore, there is the risk of rupture of clotted blood in such manipulations in the test tube than the vacutainer where the serum is on the top and to some extent surrounding the clotted blood. It should also be noted that to generated a particular volume of serum, you required two times or two and half times the volume of blood says Quest Diagnostics (http://www.nicholsinstitute.com/SpecimenCollection/SerumPlasma.aspx).

However, our findings contradict this claim and suggest that this claim may not be tenable when test tubes are used but probably if vacutainers are used. As can be seen in this study the mean volume of serum generated by test tube from a 2 ml whole blood was 0.56 ml (Table 1). It is interesting to note that all the experimental conditions ranging from field blood sample collection by the same phlebotomist, using the same syringe per volunteer, clotting of blood on flat top and inside ice packed of a cold man box for an hour etc were the same. Yet, more sera were generated in vacutainer significantly (p < 0.001) than in the test tube; suggesting an inherent property of the vacutainer beyond chance than test tube, probably clot activator as asserted by some researchers (Russell-DeLucas, 2011). Another important thing to note in this study, is that even within the two methods variation of serum generation from as low as 0.4 ml to as high as 0.7 ml (in test tubes) while 0.7 to 1ml serum generation in vacutainers were observed (Figure 1).

Table 1. Average volume of serum realized from paired 2ml of blood each in test tubes and vacutainers from 185 human volunteers.

<table>
<thead>
<tr>
<th>t-Test: Paired Two Sample for Means</th>
<th>Test tubes</th>
<th>Vacutainers</th>
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<tbody>
<tr>
<td>Mean</td>
<td>0.56ml</td>
<td>0.80ml</td>
</tr>
<tr>
<td>Stderror</td>
<td>±0.075</td>
<td>±0.06</td>
</tr>
<tr>
<td>Observations</td>
<td>185</td>
<td>185</td>
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P(T< =t) two tail = 2.7298E-114  (p<0.001) = statistical significant difference exists
Figure 1. Frequency of serum recovered from 2ml of human blood in both test tubes and vacutainers each from 185 human volunteers.

Plate 2. Quantity of serum generated by test tube (left) and vacutainer (right) before pipetting after standing for 1 hour. Note flake of clotted blood (white arrow for test tube) and serum output (green arrows) in both, from same volunteer.

This observation may be due to physiologic conditions of volunteers as there were no prior preparations (such as, fasting the volunteers, controlled climatic conditions etc) being field collection at spot not under controlled laboratory conditions which could affect the outcome. This assertion has also been observed by some researchers (Frederick et al., 2011) though will not invalidate the fact that vacutainers yield more serum than test tube. Because in the same volunteer test tube may yield 0.4 or 0.5ml while the vacutainer will yield 0.7 or 0.8 ml as observed and statistically proven beyond chance (Table 1, Figure 1). This study appeared to be the first to compare such difference of sera generation from test tube and vacutainer. It therefore highly recommends the use of vacutainer particularly in serum or plasma generation than test tube. Assessment of the quality of serum generated by test tube versus vacutainer is further recommended.
Figure 3. Combined volume of (test tube and vacutainer) sera generated per volunteer ranging from 1.2 ml (blue arrow), to 1.4ml (green arrow) and highest 1.7ml (white arrow) where seen in this study.

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References


