Potato starch extract as an alternative serum separator gel and its effects on glucose testing

Donna Dane G. Aldana[1], Sheena Lyn A. Tanagon[1], Ria Rose Ann G. Manantan[1], Nico A. Pudlao[1], Joha D. Valenciano[1], Rio Rose L. Fabro[1] & Allan Jay C. Espiritu[2]

[1] Student researcher; [2] Faculty researcher
Department of Medical Laboratory Science, College of Natural Sciences
Saint Louis University
Contact information: ajc_espiritu@yahoo.com

Abstract

This study examines the potential of potato starch extract as an alternative serum separator gel and its effects in glucose testing. It sets off by determining the ability of the devised potato starch serum separator gel to form a barrier between the formed elements of blood and the serum and then testing the serum collected if the starch gel had an effect on glucose determination. The results show that potato starch extract, upon the application of heat and in the presence of water, effectively yields a gel with thixotropic property that has the ability to separate the serum from the formed elements and would not affect glucose testing. This confirms, thus, the potential of potato starch extract as a natural serum separator gel. With same characteristics as the commercial serum separator gel, the potato starch serum separator gel proves to be an effective and economically feasible alternative to the latter.

Keywords: *natural serum separator gel, potato, starch, thixotropic, glucose*

Serum separator tubes (SST) were introduced in the USA in 1976 (Bush & Cohen, 2003), and since then these have become very useful in the clinical laboratory. SST are evacuated tubes containing special gels that separate serum from blood cells as well as particles that cause blood to clot quickly (Serum Separating Tube, 2003).

The special gels have a thixotropic property which is the ability to liquefy during centrifugation and impart a solid-like behavior during transient periods (Czerwinski, 2006; Arzoumanian, 2005; Thixotropic, 2007). The use of gel barriers has provided a large benefit in collecting, processing and storing the specimen used in the clinical laboratory (Bush & Cohen, 2003; Arzoumanian, 2005; Rosa-Fraile, et al., 2003).

The gel prevents contamination and ion-exchange between the serum and the clotted red blood cells after centrifugation (Ernst 2005; Bush & Cohen, 2003; Dojki & Haldopolouis, 1984). As an example, all cells in the body contain a high concentration of potassium. During the blood clotting and spinning processes, potassium may leak out from the formed elements, but with the help of the gel separator, the potassium from cells is isolated from the serum and prevents increased potassium in the serum (Baer, Ernst, Willeford, & Gambino, 2006). It has also been proven that the longer contact of the serum and the formed elements contribute to the inaccuracy of results. Thus, the barrier formation of the serum separator gel between the serum and the formed elements is very important (Ernst, 2004). In addition, serum separator gels allow longer storage of specimen (Rosa-Fraile, et al., 2003).

The serum separator gels available in the market today are made up of synthetic polymers such as silica polymers and other synthetic materials such as latex, polyurethane, and syran (Bush & Cohen, 2003; Cohen & DiCesare, 2006; Fiehler, 1990; Iskra, 2002; Spiritus, Zaman, & Desmet, 2003; White, 1983). However, there are natural polymers that can be used to prepare thixotropic gels and starch found in potatoes is one of them (Potato Solanum tuberosum, 2007).

Starch is a polysaccharide containing two types of molecules, amylose and amylopectin (Malhotra, Kumar, East, & Jaffe, 2007). Amylose and amylopectin are crystalline and amorphous areas of starch granules respectively (Starch, 2007; Tester, Karkalas, & Qi, 2004). A study by Douzals and company (1999) confirmed that when water-starch suspension is heated, a phase transition is observed for starch and granule swelling increases with temperature. When the starch granules are heated at temperatures 60˚C-90˚C, they will gradually adsorb water, swell in size, and leach amylase, losing their crystallinity and causing the water to thicken and gelatinize (Gelatinization, 2007; Hashem, Afifi, El-Alfy, & Hebeish, 2005).

This study aims to look at the potential of potato extract as an alternative to the artificially manufactured polymers as constituents for the commercial serum separator tubes. Potato (*Solanum tuberosum*) is known to have a high concentration of starch which has the ability to form a thixotropic gel with the addition of normal saline solution in the presence of heat (Gelatinization, 2007). With this given, the current study intends to (a) determine the ability of the potato starch serum separator gel to form a barrier between the formed elements of blood and the serum, and (b) test the serum collected from the potato starch serum separator tube if the starch gel had an effect on glucose determination.

Serum glucose determination is incorporated in this study to determine if amylase may act upon the starch present in the potato starch serum separator gel which may eventually cause false increase in the serum glucose concentrations. Digestive enzymes (e.g. amylase) in the body have normal occurrences in the blood stream that are released by the exocrine glands (Isenmana, Liebow, & Rothman, 1999). The amylase content in the blood is capable of digesting the amylose content of the starch serum separator gel. However, the enzyme activity only happens at a pH of 6.9 and a temperature of 37˚C (Bishop, Fody, & Shoeff, 2005). In addition, amylase is a metalloenzyme which needs the presence of calcium as an ion activator for it to digest starch present in the potato starch serum separator gel (Calcium (Ca) in Blood 2006). Furthermore, the normal blood pH is 7.35-7.45 and the procedure will be done in vitro at ambient room temperature. This way, amylase may not act upon the starch found in the starch serum separator gel (Bishop, Fody, & Shoeff, 2005). It is then hypothesized that no starch digestion will occur and that there will be no false increase in the serum glucose concentration because the optimum conditions for amylase to digest starch are not fulfilled.

Beyond suggesting a natural alternative to the commercial serum separator gels currently used in the clinical laboratory, the findings of this study likewise bear socio-economic significance. The introduction of an alternative serum separator gel made from locally available materials lessens the need to import SST and thus reduce overall cost. Moreover, the increased demand for potatoes because of the alternative SST will benefit the local farmers' livelihood especially those in the Cordillera region where potatoes are easily grown.

Materials and methods

Potato starch serum separator gel preparation. The Igorota variety of potato, having the most abundant concentration of starch, was used. Different concentrations of starch solutions and Normal Saline Solution (NSS) which served as the solvent were prepared and then heated at 60˚C – 90˚C until a gel was formed (Hashem, Afifi, El-Alfy, & Hebeish, 2005). Normal Saline Solution was used since it does not cause lysis of the red blood cells when the gels came in contact with the red blood cells during blood collection. The prepared gel concentrations (0.21 g/ml, 0.23 g/ml and 0.25 g/ml) were incorporated into the test tube and then used in blood collection. To serve as control, yellow-topped vacutainer tubes with serum separator gel were employed. This was used to compare the separation of the serum and the formed elements of blood as mediated by the serum separator gel and to determine if there is significant difference in the result of the glucose determination using the serum collected from the different concentrations of potato starch serum separator gel and this control.

Barrier formation between the serum and the formed elements. After centrifugation, the potential of the different concentrations of the potato starch extract to serve as a serum separator gel was determined by their ability to migrate between the serum and the formed elements of blood. The potato starch serum separator gel has specific gravity between that of the formed elements, namely erythrocytes and leukocytes (i.e. 1.10) (Blood, 2003) and platelets (i.e. 1.053-1.064) (Savage, McFadden, Hanson, & Harker, 1986), and that of the serum (i.e. 1.03) (Blood, 2003). The potato starch serum separator gel, with the help of the centrifugation, separated the formed elements and the serum based on their densities or specific gravities (Centrifugation, 2007).

Glucose concentration and determination. The serum was collected and subjected to glucose determination using the glucose oxidase method to test the digestion of starch by amylase in blood which may cause a false increase in the glucose concentration (Bishop, Fody, & Shoeff, 2005). Glucose oxidase is the most common method used in blood glucose testing. It is frequently employed in studies such as in the researches of Greenfield and Samaras (2006) and Anderson et al. (1999).

Results

The comparison of the separation of serum and the formed elements by the potato starch serum separator gels is presented in Table 1. The researchers observed that all concentrations of the potato starch serum separator gel and the control have all successfully formed a barrier between the two components of whole blood.

Table 1. Separation of the serum and the formed elements with the use of different serum separator gels.

Starch Concentration	Ability of Separation
0.21 g/ml	+
0.23 g/ml	+
0.25 g/ml	+
Control	+

Legend: (+) = positive for the formation of a barrier between the serum and formed elements.

The serum was subjected to glucose oxidase test and the results are presented in Table 2. The glucose values are observed to be different from each other. The values presented in Table 2 were subjected to ANOVA (Two-factor with Replication) statistical tool to determine if the differences in serum glucose results are significant.

Table 2. Glucose determination results

Patients	Concentrations			
	0.21 g/ml	**0.23 g/ml**	**0.25 g/ml**	**Control**
Patient 1				
Trial 1	4.4	4.0	3.0	4.3
Trial 2	3.6	5.1	3.9	4.6
Trial 3	3.9	4.0	3.5	4.8
Average	4.0	4.4	3.5	4.7
Patient 2				
Trial 1	3.0	3.6	3.8	3.6
Trial 2	4.0	2.7	5.3	3.6
Trial 3	4.7	3.4	4.1	3.5
Average	3.9	3.2	4.4	3.6
Patient 3				
Trial 1	4.0	3.1	4.4	3.3
Trial 2	2.1	4.4	3.9	4.6
Trial 3	5.8	4.8	4.9	5.0
Average	4.0	4.1	4.4	4.3
Patient 4				
Trial 1	5.8	4.8	4.5	3.4
Trial 2	4.5	3.5	5.8	5.3
Trial 3	3.9	4.0	3.1	5.6
Average	4.7	4.1	4.5	4.8
Patient 5				
Trial 1	5.2	3.0	2.4	3.6
Trial 2	3.1	2.9	2.9	4.5
Trial 3	2.8	4.5	3.0	4.9
Average	3.7	3.5	2.8	4.3

The results of the ANOVA: Two-Factor with Replication are shown in Table 3. It could be observed that the F value of the patients is 2.14 and is lower than the F critical value at α.05 of 2.61, which means that there is no significant difference among the glucose values obtained from the patients. Likewise, the F value of the different concentrations is 0.83 and is lower than the F critical value at α.05 which is 2.84, which means that the difference between the glucose values at the serum collected from the different concentrations of potato starch serum separator gel and the control is not significant.

Table 3. Summary of the critical values in ANOVA (two-factor with replication)

Source of Variations	SS	Df	MS	F	F crit α.05
Patients	6.53	4	1.63	2.14	2.61
Concentrations	1.91	3	0.64	0.83	2.84
Patient x					
Concentrations	7.43	12	0.62	0.81	2.00
Within	30.65	40	0.77	-	-
Total	46.52	59	-	-	-

Discussion

The ability of the different concentrations of starch serum separator gel to form a barrier between the serum and the formed elements was determined. The researchers found out that the serum from all the different concentrations have migrated above the gel and the formed elements under the gel. As compared to the control, they all successfully separated the serum and the formed elements. Since the application of centrifugal force would allow the different particles to migrate and settle at an area on the basis of specific gravity or density, it would be safe to say that the specific gravity of the potato starch serum separator gel is between the serum and the formed elements as the potato starch serum separator gel migrated between the serum and the formed elements. Thus, the potential of starch as an alternative serum separator gel is demonstrated by the abovementioned results. The thixotropic property imbibed by starch when formed into a gel was important in the gel's ability to travel in between the serum and the formed elements by allowing the different particles to flow through it during centrifugation (Thixotropic, 2007).

The different glucose values that could be observed in Table 2 may be caused by several factors, one of which is patient variability because the patients from whom the blood samples were collected were not controlled in terms of their sex, food intake, daily activities and metabolism (Magee 2005; Holtschlag, Gannon, & Nuttall 1998). Another factor could be the time of collection as the blood samples were not taken at the same time and the trials for each patient were performed with different intervals.

As discussed in the result section of this article, Table 3 shows that there is no significant difference in the glucose values of the serum collected from the different potato starch serum separator gels including that of the control as stated. Hence, even if there are varied results in glucose concentrations as seen in Table 2, these differences are still not significant and of no consequence in the glucose determination tests that were performed on the serum samples collected. It means that the differences in the glucose results obtained are still very minimal and not clinically significant. This is confirmed by the results of the ANOVA (Two factor with replication) statistical tool which shows that the F values are below the F critical values at α.05 (refer to Table 3).

Conclusion and recommendations

The study confirmed the potential of potato starch extract as a natural serum separator gel. The experiment demonstrated that the potato starch extract, upon the application of heat and in the presence of water, has effectively yielded a gel with thixotropic property that has the ability to separate the serum from the formed elements and would not affect glucose testing.

Having the same characteristics as the commercial serum separator gel, the potato starch serum separator gel devised by the researchers is an effective alternative to the expensive SST used in the laboratory today. Moreover, it is more economically feasible than the commercial SST because of the cheap and locally available materials used in its production.

The study points out to the examination of the potential of extracts from other starch-rich tubers in the preparation of alternative, natural serum separator gels. It also suggests the incorporation of a natural coagulant in the prepared gel to enhance clotting, as well as the testing of other blood analytes to determine the effectiveness of the prepared serum separator gels.

References

Anderson, J., Allgood, L., Turner J., Oeltgen P., & Daggy, B. (1999). Effects of psyllium on glucose and serum lipid responses in men with type 2 diabetes and hyperchloremia. *The American Journal of Clinical Nutrition*, 4, 466-473.

Arzoumanian, l. (2005). What is the importance of properly processing a BD vacutainer SST tube? *Tech Talk*,4, 1.

Baer, D. Ernst, D., Willeford, S., & Gambino, R. (2006). Investigating elevated potassium values. *Clinical Issues*, 24-31.

Bishop, M. L., Fody, E. P., Shoeff, L. E. (2005). Clinical Chemistry. : Principles, Procedures, Correlations. 5th Edition. Lippincott, Williams & Wilkins. P.257.

Blood. (2003). Bioengineering, Hofstra University. Retrieved on September 28, 2007 from http://people .hofstra.edu/sina_y_rabbany/engg81/blood.html

Bush, V., & Cohen, R. (2003). The evolution of evacuated blood collection tubes. *Laboratory Medicine*, 4.

Calcium (Ca) in Blood (2006). Retrieved on October 15, 2007 from http:/www.webmd.com/a-to-z-guides/calcium-ca-in-blood

Centrifugation. (2007). Retrieved on October 22, 2007 from http://www.answers.com/topic/centifugation-2?cat=technology

Cohen, R., DiCesare, P.C. (2006). Device and method for separating components of a fluid sample. Retrieved on June 28, 2007 from http://www.freepatentsonline/7153477.html

Czerwinski, F. (2006). Solidification and semi-solid processing. JOM, 58, 17-20.

Dojki, K., Haldopolouis, I. (1984). Device for separating serum from blood sample. Retrieved on October 17, 2007 from http://www.freepatentsonline.com/4464254.html

Douzals, J., Marechal, P., Coquille, J., & Gervails, P., (1996). Microscopic study of starch gelatinization under high hydrostatic pressure. J. Agric. *Food Chem*, 44, 1403-1408.

Ernst, D. (2004). Re-spinning gel tubes. *Medical Laboratory Observer*, 10, 36.

Ernst, D. (2005). Applied Plebotomy. Lippincott, Williams & Wilkins. p. 167.

Fiehler, W. (1990). Blood Serum Separator Tube. Retrieved on October 21, 2007 from http://www.google.com/patents?id=YgkAAAAABAJ&dq= Blood+serum+separator+William+R.+Fiehler

Gelatinization of starch. (2007) Food Resources. Oregon Satae University 2007. Retrieved on September 1, 2007, from http://food.oregonstate.edu/starch/gelatinzation.html

Greenfield J., Samaras K. (2006). Evaluation of pituitary function in the fatigued patient: a review of 59 cases. *European Journal of Endocrinology*, 154, 147-157.

Hashem, A., Afifi, M.A., El-Alfy, E.A., & Hebeish, A. (2005). Synthesis, characterization and saponification of poly (AN)-starch composites and properties of their hydrogels. *American Journal of Applied Sciences*, 2, 614-621.

Holtschag, D., Gannon, M., & Nuttall, F. (1998). Sate-space models of insulin and glucose responses to diets of varying nutrient content in men and women. *Journal of Applied Physiology*, 85, 935-945.

Isenmana, L., Liebow, C., & Rothman, S. (1999). The endocrine secretion of mammalian digestive enzymes by exocrine glands. *AJP Endocrinology and Metabolism*, 276, 223-232.

Iskra, M. (2002). Device and method for separating components of a fluid sample. Retrieved on October 21, 2007 from http://www.google.com/patents?id=bLMKAAAAEBAJ&dq=Device+and+method+for+separating+components+of+a+fluid +sample+Micahel+J.+Iskra

Magee, L. (2005). Preanalytical variables in the chemistry laboratory. *Labnotes*, 15, 1-5.

Malhotra, S., Kumar, V., East, A., & Jaffe, M., (2007). Applications of corn-based chemistry. *National Academy of Engineering*, 37.

Potato. *Solanum tuberosum* L (2007). Retrieved on June 28, 2007 from http://en.wikipedia.org/wiki/potato.

Rosa-Fraile, M., Sampedro, A., Rodriguez-Granger, J., Camacho, E., & Manrique, E. (2003). Suitability of frozen serum stored in gel separator primary sampling tubes for serological testing. *Clinical Diagnostic Laboratory Immunology*, 11, 119-121.

Savage, B., McFadden, P.R., Hanson, S.R. Harker, L.A (1986). Relation of Platelet Density to Platelet age. Retrieved on September 30, 2007 from http://www.osti.gov/energycitations/product.bibilio.jsp?osti_id+5013472

Serum Separating Tube. (2003). Retrieved on October 21, 2007 from http://en.wikipedia.org/wiki/Serum-separating_tube

Spiritus, T., Zaman, Z., & Desmet W. (2003) Iodinated contrast media interfere with gel barrier formation in plasma and serum separator tubes. *Clinical Chemistry*, 49, 1187-1189.

Starch. Retrieved on: June 28, 2007, from http://www.answers.com/topic/starch?cat=health

Tester, R,, Karkalas, J., Qi, X. (2004). Starch structure and digestibility enzyme-substrate relationship. World's Poultry Science Journal. 60:186-195.

Thixotropic. Retrieved on September 29, 2007, from http://dictionary.reference.com/browse/thixotropic

White, F. (1983). Serum separating method and apparatus. Retrieved on October 21, 2007 from http://www.google.com?patents?id=ULgPAAAAEBAJ&dq+Serum+separating+method+and+apparatus+Fred+K.White