IOWA STATE UNIVERSITY
Extension and Outreach

Sample Collection and Handling for Vitamin and Mineral Analyses

Blood Sample Collection For Nutrient Analysis

Collecting a blood sample is a minimally invasive procedure. However, a basic understanding of blood components and sampling equipment can help ensure accurate and appropriate sampling. Certain factors should be considered prior to submitting a blood sample for analysis.

Breakdown of blood

Blood consists of various components such as white blood cells (granulocytes, lymphocytes, and monocytes), red blood cells, platelets, proteins, and various nutrients[1]. These components are different in dimensional sizes and weights. When blood is spun at a high rotation, centrifugal forces (gravity) cause these different components to separate into various fractions based on the density of each component[1]. The fractions typically are denoted as serum/plasma, red blood cell layer, and buffy coat layer (Figure 1). Serum and plasma commonly are used inversely and the liquid fraction may not appear noticeably unique from one another; however, the two are inherently different. If whole blood is allowed to clot prior to centrifuging the sample, the clear portion is denoted as serum. If the whole blood does not clot prior to centrifuging, the clear fraction is denoted as plasma. The main differences between serum and plasma are that serum does not contain fibrinogen because it is used in the clotting process, and plasma typically is higher in protein concentration[1,2].

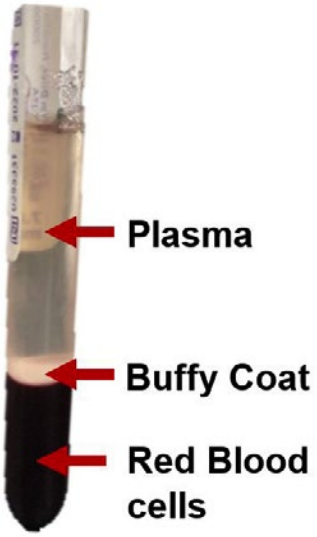

Figure 1.

Blood tube type

Blood samples can be used to test for a variety of parameters. The diagnostic tests may require either whole blood, serum, or plasma. If you are unsure which type of supernatant the diagnostic assay requires, contact the supporting diagnostic laboratory.

After determining whether an assay requires serum or plasma, the technician needs to select between different types of blood tubes. The tube types initially can be divided into plasma and serum tubes. Plasma tubes will contain an anticoagulant that prevents the blood from clotting. Anticoagulants for plasma tubes include EDTA, heparin, and citrate. Plasma tubes must be inverted several times after the blood is collected in the blood tube to distribute the anticoagulant to ensure the blood will not clot. If blood is collected in a plasma tube and clots, the sample is no longer usable and blood needs to be redrawn.

A serum tube will allow blood to clot due either to a silica particle in the blood tube that activates clotting, or because there is no additive so the blood clots naturally. Another type of blood tube contains a gel separator. When this tube is centrifuged, the gel within the tube separates the clotted red blood cells from the supernatant creating a serum fraction. Blood tube tops often are color coded based on which additive the tube contains. (See Table 1).

Table 1. Brief summary of blood tubes, their additives, and their functions[3]

Tube cap color	Additive function
Red (some red caps have a "tiger" pattern)	3.2% sodium citrate – prevents clotting
Gold	No additive – blood will clot (gel will separate serum from cells)
Green	Sodium (dark green) or lithium (light green) heparin – prevents clotting
Lavender/pink	Potassium EDTA – prevents clotting
Gray	Sodium fluoride, and sodium or potassium oxalate – prevents clotting

Timing

The timing of when the blood is drawn relative to when the animal last ate should be noted, particularly if the nutrient status of the animal is in question. When food is consumed, the body begins to digest and redistribute the nutrients to the body via the bloodstream[4]. This process takes time and results in a spike in circulating nutrients in the body when the nutrients enter the bloodstream for redistribution to the peripheral tissues.

Nutrient redistribution depends on the nutrient in question, as some are digested quicker than others. The best way to avoid potential nutrient spikes in circulation is to take the desired blood sample before the animal eats. Sick animals can sequester nutrients to help fight illnesses, and thus their overall nutrient status can be different.

Sampling to detect pathogens or antibody levels needs to be conducted on an appropriate number of animals within a population to ensure there are appropriate detection allowances. Therefore, when taking a blood sample for a particular analysis, it is important to understand how timing of feed consumption and health status could impact the findings.

Hemolysis

Hemolysis (Figure 2) is when red blood cells rupture and cause the contents of the red blood cell to intermingle with other components of the blood. Hemolysis typically occurs when a shear force is applied to the blood sample or when blood is drawn via a syringe and then transferred to a blood tube. Hemolysis also may occur when trauma or force is applied to the blood during the collection process and after the blood is collected. If blood is collected via a syringe and then delivered into a blood collection tube, forceful expulsion of the blood through the needle can cause the red blood cells to burst.

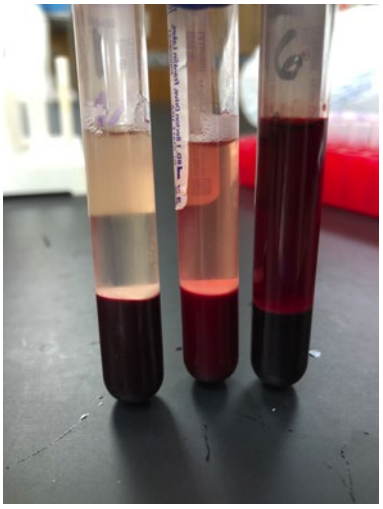

Figure 2. The sample on the left is an ideal non hemolyzed sample. The middle blood tube is a sample that has moderate hemolysis and can be found in the field after a blood draw. The right blood tube is an extreme case of hemolysis, and the sample should not be used.

Hemolysis could be problematic for nutrient sampling. When a red blood cell lyses, the components of the red blood cell can interact with the other nutrients in the plasma/serum. When some nutrients interact, they form complexes that alter the analyzed levels of some nutrients. For example, red blood cells contain zinc and iron, both of which increase the analyzed levels upon lysis and interfere with other nutrients such as vitamin A[5].

Summary

A blood sample is a non-invasive and simple procedure that can provide insight into an animal's health. Knowing what type of blood tube is needed for the desired laboratory analysis is essential. Your diagnostic laboratory can assist in choosing the correct type of blood tube. Timing of the blood sample collection also is essential to avoid any nutrient spikes. Avoid sampling a sick pig as it may have altered blood chemistry. Not sampling enough pigs to detect a pathogen also may affect results, as an illness can cause altered nutrient status.Take care when collecting a blood sample because when a shear force is applied to blood, hemolysis can occur and skew the results of the sample.

See more information on blood samples in Iowa State University Extension and Outreach publication IPIC 205A: Sample Collection and Handling for Vitamin and Mineral Analyses: Collecting Feed and Biological Samples for Vitamin and Mineral Testing. https://store.extension.iastate.edu/Product/16573.

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Resources

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[2]Issaq HJ, Xiao Z, Veenstra TD. Serum and plasma proteomics. Chem Rev. 2007;107(8):3601-3620. doi:10.1021/CR068287R/ASSET/IMAGES/LARGE/CR068287RF00012.JPEG

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[4]Goodman BE. Insights into digestion and absorption of major nutrients in humans. Am J Physiol - Adv Physiol Educ. 2010;34(2):44-53. doi:10.1152/ADVAN.00094.2009

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