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Review Article

Serum & Plasma Sampling from Small Animal Species

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ABSTRACT

In preclinical research, the laboratory animals are used to study the pharmacokinetics, pharmacodynamics, and Toxicity of the drugs. These studies are generally assessed in blood, plasma or serum metrices. Whole blood with and without anticoagulant are collected from small animals like rat or mouse by withdrawal technique to study a drug PK profiling, biochemical parameters and hematology assay. Hence, the plasma and serum are separated from the anticoagulated blood and coagulated blood, respectively, by the centrifugation technique under refrigerated temperature. Further, the storage conditions, if analysis delayed, for plasma and serum samples must be specified. Thus, the objective of the review is to demonstrate the fundamental and pragmatic understanding about the collection of blood samples from the animal species, precise handling of blood samples, use of centrifugation machine for matrix separation, and sample storage conditions.

Keywords: *Serum sampling; Plasma sampling; Animal species.*

Introduction

An ethical committee approval or IAEC is required before collecting any blood sample from experimental species like mice, rats, rabbits, etc. In preclinical studies, plasma kinetic profile of drug and serum biochemistry are commonly investigated in Pharmacokinetics (PK) /Toxicokinetic (TK) study of NCE/drug and toxicity study of NCE/drug, respectively. Therefore, the blood samples with and without anticoagulant are collected by retro orbital bleeding or any other bleeding techniques in order to separate the plasma (drug kinetic profile) and serum (serum biochemistry) by centrifugation method under refrigeration temperature (4^0C). Further, hematologic parameters are determined in whole blood containing anticoagulant in toxicity study. An analysis of serum sample for biochemical parameters is usually performed within 2 hour of serum collection and recommended to store at -20^0C, if analysis delayed. The collected plasma (drug PK profile) samples are recommended to be stored at frozen temperature (-80^0C), if analysis delayed or repetition necessitated. Whole blood for haematology should be analysed same day within 12 h of blood collection [1].

Definition

Plasma: The liquid part of the blood and lymphatic fluid, which makes up about half of the volume of blood. Plasma is devoid of formed elements and, unlike serum, has not clotted. Anticoagulant is needed for plasma collection.

Serum: The clear liquid that can be separated from clotted blood. Serum differs from plasma, the liquid portion of blood without clotting factors and formed elements of the blood.

Whole Blood: Blood with all its components (as white and red blood cells, platelets, and plasma) intact that has been withdrawn from an animal or subject into a tube containing with and without anticoagulant. The separated liquid is called serum, if whole blood is collected without anticoagulant whereas the separated liquid is called plasma, if whole blood is collected with anticoagulant [2,3].

Icteric: of, relating to, or affected with jaundice.

Lipemic: Excessive amounts of fat and fatty substances in the blood; hyperlipemia.

K_2EDTA: Di Potassium Ethylene Diamine Tetra acetic acid is used as anticoagulant.

RCF (x g) or RPM: gravity or revolution per minute,

RCF to RPM conversion formula:

$G = (1.118 \times 10^{-5}) \times R \times S^2$; Where, R radius of the rotor in centimetres, S = Speed of the centrifuge in revolution per minute (RPM).

PPE: It is defined the personnel protective equipment, which includes gloves, face mask, head mask and apron.

Principle

Serum is the liquid fraction of the clotted blood. The serum is separated by centrifugation method after whole blood is allowed to clot for 20 min. The serum is meticulously removed and collected using a Finn pipette into an eppendorf tube. Plasma is produced when whole blood is collected into a tube containing an anticoagulant. The plasma is separated by the centrifugation method. The supernatant, which is obtained by centrifugation, is designated as plasma. Using a Finn pipette, the plasma is collected from the cell pellet into an eppendorf tube. The plasma and serum matrices are commonly recommended in pharmacokinetics and toxicity studies of NCE or Drug, respectively [4].

Material

Species: Albino Mouse/Albino Rat

Sex: Male and/or Female

Weight range: Mice- 19 to 28 g & Rats – 160 to 280g

Equipment- Syringe, needle, eppendorf tube, vacutainer.

Chemicals- anticoagulant (EDTA, Heparin etc.)

Procedure

Serum Separation

The whole blood can be collected in a covered test tube (1.5 ml eppendorf tube) without anticoagulant from experimental species by blood withdrawal technique. If commercially available tubes are to be used, then the researcher should use the red topped tubes. These are available from Becton Dickinson (BD). The BD's trade name for the blood handling tube is vacutainer. The whole blood is allowed to clot for 20 min by leaving it undisturbed at room temperature (15-25^{0}C). The whole blood samples can be centrifuged at 5000 RPM under refrigeration temperature (4^{0}C) for 10 min. An obtained supernatant is designated as serum. Usually, serum should be separated within 45 min of blood collection to prevent the blood analytes from deterioration. Following centrifugation, serum is immediately transfer into a clean polypropylene tube using a finnpipette. The serum is usually applied for determination of biochemical parameters in drug toxicity testing or diagnostic test. It is advisable that serum samples should be analyzed at room temperature (15-25^{0}C) within 2 h of serum separation. The serum samples are otherwise recommended to be stored at -20^{0}C (stability for 30 days) if analysis delayed/repeated. Serum samples should meet the aforementioned handling condition for precise and accurate analysis.

Note: - It is important to avoid freeze-thaw cycles because this is detrimental to many serum components. Samples which are hemolysis, icteric or lipemic can invalidate certain tests [5].

Plasma Separation

The whole blood can be collected into commercially available anticoagulant-treated tubes e.g., EDTA-treated (lavender tops) or citrate-treated (light blue tops) from experimental species by blood withdrawal technique. Heparinized tubes (green tops) are indicated for some applications; however, heparin can often be contaminated with endotoxin, which can stimulate white blood cells to release cytokines. Plasma is separated from cell pellet by centrifugation at 5000 RPM under refrigeration temperature (4^{0}C) for 10 min. An obtained supernatant is designated as plasma. Following centrifugation, plasma is immediately transfer into a clean polypropylene tube using a finnpipette. The plasma samples should be maintained at 2–8°C while handling. Usually, plasma should be apportioned into 0.5 ml aliquots, and transported and stored at -80°C, if no. of samples is more and analysis takes time. Plasma is mostly recommended to determine the drug Pharmacokinetics (EDTA tubes) or glucose (tubes containing Na fluoride/Potassium oxalate, which has antiglycolytic property). Plasma obtained from Na citrate treated blood, is usually used for Prothrombin time determination.

Note: - It is important to avoid freeze-thaw cycles. The samples, which are hemolyzed, icteric or lipemic can invalidate certain tests. Various commercially available tubes of specified size for blood collection can be used.

Hematology Assay (CBC & DLC)

Fresh whole blood is collected into an eppendorf (1.5 mL) tube containing EDTA or Vacutainer- EDTA tube

from experimental species by blood withdrawal technique. Typical sample size of ~150 µL is required for hematologic parameters determination by sophisticated bioanalyzer. Whole blood samples are recommended to be analyzed within 12 h of blood collection and should be maintained at refrigeration temperature (2-8^{0}C) till analysis. Hematology testing using hematology analyzer is performed on EDTA- (purple top tube) blood to determine red cells, white cells, platelets, hemoglobin, etc. The hematology studies are mostly performed in drug toxicity evaluation or in disease progression [6].

Note:- Heparin (green top tube) is not recommended as an anticoagulant for cell count, because the cells clump in heparin, invalidating count. Citrate (blue top tube) is not recommended due to the dilution of the blood by the liquid citrate.

Conclusion

In preclinical research, the laboratory animals commonly used are mouse and rat in pharmacokinetics or toxicity studies. These studies have mostly used the biological metrices to determine the drug levels in plasma or to investigate the drug toxicity effects on serum biochemistry and blood hematology. Plasma and serum are the essential matrices, which are separated from whole blood collected with and without anticoagulant, respectively, by centrifugation under cold temperature (2-8^{0}C). The storage and handling of these samples in specified conditions is obligatory to avert the drug/biological analytes from deterioration. Thus, it can be concluded that a careful collection of matrices, which include serum and plasma, and their effective handling result into better analytical outcomes.

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Conflict of Interest

None declared.

References

1. There AW, Chawale PA and Bais AG: A review on preclinical pharmaceutical research: principal and common routes of administration in small rodents. Int J Pharm Sci & Res 2023;14(3):1076-97.
2. Thavasu, PW et al. Measuring cytokine levels in blood. Importance of anticoagulants, processing, and storage conditions. J Immunol Methods, 1992; 153:115–124.
3. Kughapriya P, Elanchezhian JA. Stability of Common Biochemical Analytes in Serum when Subjected to Changes in Storage Conditions and Temperature. Indian J Med Biochem, 2019;23(1):178-181.
4. Dong-wen Wu, Yu-meng Li, Fen Wang. How Long can we Store Blood Samples: A Systematic Review and Meta-Analysis. eBioMedicine. 2017 Sep 23;24:277–285.
5. Ozmen Sevda Unalli, Yesim Ozarda. Stability of hematological analytes during 48 hours storage at three temperatures using Cell-Dyn hematology analyzer. J Med Biochem. 2021 Jun 5;40(3):252-260.
6. Kamal Kachhawa, Poonam Kachhawa, Meena Varma, et al. Study of the Stability of Various Biochemical Analytes in Samples Stored at Different Predefined Storage Conditions at an Accredited Laboratory of India. J Lab Physicians 2017 Jan-Mar;9(1):11–15.