

## A METHOD OF PREPARING HEMOGLOBIN - FREE SERUM

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Existing methods for obtaining blood sera depend on the fact that whole blood coagulates in vitro. As a rule, these methods permit some admixture of hemoglobin in the serum. In cases where this slight admixture of hemoglobin has no practical significance, these methods are adequate.

However, in tests such as photometric analysis and setting up colloidal reactions, for example, the presence of even a small amount of hemoglobin in the serum can lead to serious errors. In animals such as dogs, the erythrocytes are fragile and can break up during manipulations needed to obtain the serum. To obtain hemoglobin-free serum from them is no easy matter.

We decided to look for a method for obtaining blood serum free of hemoglobin. We developed a method involving the clotting of plasma instead of whole blood, as done in other procedures.

Paraffined centrifuge tubes could be prepared on the day (or day before) the blood was collected. Carefully washed and dried tubes were taken and filled with melted paraffin which would be immediately poured out, the tubes being then cooled. The walls of the tubes would thus be covered with a thin paraffin coating. Some 20-30 minutes before drawing the blood, we placed tubes in an ice pack, thus assuring their refrigeration.

In addition to the paraffined tubes, an equal number of well-dried centrifuge tubes is needed.

To draw off the plasma, there should be on hand several pipettes having rubber pyriform attachments.

The blood must be drawn from the dogs by means of short needles having large openings, but thin walls. One may employ needles used for spinal punctures, but these should be shortened to a length of 3-3.5 cm.

The anterior external metatarsal vein of the dog is pierced without application of a tourniquet, or by only applying one at the very moment of entering the vein with the needle. The blood flowing freely through the needle lumen is collected into the refrigerated tube. The effort should be made to have the blood drop directly to the bottom without flowing down the sides.

The blood is immediately centrifuged at 2500 rpm for 3.5-4 minutes. In this time interval, the formed elements of the blood are fully precipitated, while the plasma has had no opportunity to clot.

The blood plasma is carefully taken up by the pipette having the rubber bulb enlargement on it and transferred to the dry centrifuge tube.

To hasten coagulation of the plasma in the tube, it is placed in a thermostat at a temperature of 37-38° for 30-40 minutes.

The coagulated plasma is then placed in a refrigerator at 3-5°. Here clot retraction occurs.

After 20-24 hours, the tube is taken out and a fine glass stick is used to free the clot from the centrifuge tube walls after which it is centrifuged at 2500 rpm for 2-3 minutes. As a result, the plasma clot, consisting

and can be found at the bottom of the tube under the layer of the serum. This hemoglobin is poured into a clean test tube.

there is obtained 3-4 cc of serum.

Technical factor in the entire procedure of obtaining hemoglobin-free serum lies in the formed elements precipitated before the blood is permitted to clot. For the adequate result, the procedure as just outlined has to be adhered to rigorously.

#### SUMMARY

whereby hemoglobin-free serum may be drawn from dogs. It depends on the pre-treatment before clotting has taken place.