

Pathology

KEYWORDS: Hemoglobin, Estimation, Stock, Method, Copper sulphate

HEMOGLOBIN ESTIMATION BY USING COPPER SULPHATE METHOD



Volume - 7, Issue - 12, December- 2022

ISSN (O): 2618-0774 | ISSN (P): 2618-0766

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INTERNATIONAL JOURNAL
OF PURE MEDICAL RESEARCH

**Abstract**

The use of the copper sulfate method is based on the fact that when a drop of whole blood is allowed to fall into the copper sulfate solution, there is formed an insoluble copper proteinate, and the behavior of this single drop of blood in the solution will determine the rejection or the acceptance of the blood donor. This procedure of Hemoglobin Estimation holds a great relevance in blood bank as it is cheap and gives fast result. **PURPOSE** Hemoglobin estimation is done for every donor who presents himself / herself for blood donation to select healthy blood donor **SCOPE** Selection of healthy blood donors provides better post transfusion hemoglobin increment resulting in better oxygen carrying capacity as well as prevents donor to be anemic after donation. **RESPONSIBILITY**

Responsibilities

- i. Check the quantity of copper sulfate stock solution every day at 5:00 pm, if stock is insufficient; prepare stock solution for next day. Preparation of fresh copper sulfate working solution every day in the morning.
- ii. Standardization of copper sulfate solution
- iii. Take 50 ml of solution and change after performing 20–25 tests.
- iv. Dispose used solution as biohazardous material because of blood in the container.
- v. In case of doubtful result confirm by automated sysmex-1000 prior to accepting or deferring the donor.

Responsible person

Designated technician and / or Nursing staff

INTRODUCTION**Principle of method**

This method estimates the hemoglobin content of blood from its specific gravity. A drop of blood in contact with copper sulfate solution of specific gravity 1.053 becomes encased in a sac of copper proteinate, which prevents dispersion of fluid or any change in specific gravity for about 15 seconds. If the specific gravity of the donor's blood is higher than that of solution, the drop will sink within 15 seconds; if not, the drop will hesitate, remain suspended, or rise to the top of solution. A specific gravity of 1.053 corresponds to the hemoglobin concentration of 12.5 gm / dl.

Preparation**Stock solution**

- Labeled the preparation flask to be used appropriately (i.e. copper sulfate stock solution, date of preparation)
- Weigh 159.6 gm of anhydrous copper sulfate crystals using the balance
- Tip the copper sulfate into the 1000 ml volumetric flask and to it 1000 ml of distilled water
- Mix well and ensure that copper sulfate has dissolved completely.

Working solution

- i. Take 100 ml volumetric flask.

- ii. Put 52 ml stock solution of copper sulfate
- iii. Then add 48 ml of distilled water and mix properly
- iv. Check specific gravity of the solution, using the hydrometer It should be with in ± 0.0003 of the required value. (required value – 1.053)
- v. If necessary, adjust specific gravity using distilled water.

Specimen

One drop of capillary or venous blood

Material

CuSO₄ solution.
Wide mouth test tube or glass beaker of 50 ml capacity
Disposable sterile lancets
Sterile gauze, spirit
Container for sharp disposal
Graduated micropipette (50 μ l)

Procedure

- Take wide mouth test tube (50 ml) / glass beaker
- Dispense 50 ml of copper sulfate solution with proper labeling (Sp. gravity 1.053) to allow the drop to sink approximately three inches
- Clean the site of skin puncture (fingertip) of the donor with spirit solution and wipe dry with sterile gauze.
- Puncture the finger firmly, near the tip but slightly to the side, with a sterile disposable lancet. There should be free flow of blood. Do not squeeze the puncture site repeatedly as this may dilute the drop of blood with tissue fluid and lower the specific gravity. Take 50 μ l of blood in disposable tip fitted to fin pipette.
- Dispense one drop of copper gently at a height about one cm above the surface of copper sulfate solution.
- Observe for 15 second.

Interpretation

- If the drop of blood sinks to the bottom within 15 seconds, the hemoglobin is >12.5 gm / dl
- If the drop remains at the surface or rises from the bottom of the solution, the hemoglobin is below the acceptable value (<12.5 gm / dl). If time and equipment permit, it is desirable to perform a quantitative measurement of hemoglobin or hematocrit

Precaution

- a. The beaker / tube should be kept covered to avoid evaporation.
- b. Change the copper sulfate solution after 20 to 25 tests.
- c. Be sure the solution is adequately mixed before beginning each day's determination
- d. Dispose used solution as biohazardous material because of blood in the container.
- e. Dispose the lancets, fine tips, and blood contaminated gauze in appropriate biohazard container.
- f. Care to prevent blood from contaminating blood surfaces, the donor clothing or other persons or equipment's.

Limitations

- a. If the plasma protein level of the donor is on lower limit of normal,

the donor is liable to be deferred though he may have normal hemoglobin.

b. Plasma protein of donor higher side the donor with low hemoglobin (<12.5 gm/dl) can be bleed.

c. Donor with low Hb (<12.5gm/dl) but very high WBC count may be bleed.

Conclusion

The solution should be clear and free from precipitate. Routine quality control is performed in each batch of copper sulfate solution prepared Enter the hemoglobin values in donor selection and registration form Enter the hemoglobin values in donor record register. Record of copper sulfate solution and its functional validation in format sheet

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