RICCA CHEMICAL COMPANY
Arlington, TX 76103
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Blood Hemoglobin Screening (Specific Gravity Method)
Modified – Lower Specific Gravity Limits

Reagents Needed:

Copper Sulfate Solution, Specific Gravity 1.052 — RICCA CHEMICAL COMPANY Cat. No. R2329000
or Copper Sulfate Solution, Specific Gravity 1.054 — RICCA CHEMICAL COMPANY Cat. No. R2331000

Recommended Method:

1. Before opening each new bottle of Copper Sulfate solution, invert the container several times to remix contents.
2. Add a drop of blood to the Copper Sulfate solution from a height of about 1 cm.
3. Observe the behavior of the drop.
4. Change the Copper Sulfate solution after every 25 tests, daily, and if the solution turns cloudy during testing. Keep the solution covered to prevent evaporation between tests.

Test Results:

Samples with an acceptable Hemoglobin level will sink.

Samples with an unacceptably low Hemoglobin level will remain suspended or rise to the top.

Quality Assurance Testing (Recommended Method):

1. Obtain several specimens of anticoagulated samples of blood from persons with known Hemoglobin or Hematocrit values. The values should be previously determined by more accurate methods such as Hemoglobinometry or Microhematocrit. Use several specimens of borderline acceptable donors (see table) and several specimens of borderline unacceptable donors.

RICCA Cat. No.	Specific Gravity	Borderline Acceptable Donor Range	Borderline Unacceptable Donor Range
R2329000	1.052	12.5 – 13.0 g/dL Hemoglobin	10.5 – 11.5 g/dL Hemoglobin
R2331000	1.054	13.5 – 14.0 g/dL Hemoglobin	11.5 – 12.5 g/dL Hemoglobin

2. Add one drop of blood from the borderline acceptable donor samples to the Copper Sulfate Solution from a height of about 1 cm. If the specific gravity of the Copper Sulfate Solution is satisfactory, the drop will sink within 15 seconds. If the Copper Sulfate Solution specific gravity is not satisfactory, the sinking drop will hesitate, remain suspended, or rise to the top of the solution.
3. If the drop does not sink, repeat the test on a fresh aliquot of Copper Sulfate Solution.
4. Repeat step 2 for the borderline unacceptable donor samples. All of these drops should remain suspended or rise to the top.
5. If there is a clear and correct distinction between acceptable and unacceptable donor samples in this functional screening test, the lot of Copper Sulfate Solution tested is acceptable for use in blood donor screening. This functional test is really what counts in the final analysis. If the Copper Sulfate Solution screens these samples properly, then the Copper Sulfate Solution lot is acceptable for use.

Troubleshooting:

1. Temperature Variations:

Customers have mentioned that some inspectors have previously told them that the temperature of this Copper Sulfate solution must be well controlled near 25°C (77°F) during the actual use of this solution in Hemoglobin Screening of potential blood donors. This screening method was originally developed and validated by the United States Navy Research Unit at the Hospital of The Rockefeller Institute for Medical Research, New York, in 1949. Quoting from the published work, “No temperature controls or corrections are needed, because the temperature coefficients of expansion of blood and plasma are so near those of the corresponding Copper Sulfate Solutions that temperature changes between 10°C (50°F) and 40°C (104°F) do not significantly affect the gravity relations.” While

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this reference validates that the test solution temperatures can vary widely without significant error, the reference does also say that the temperature of the Copper Sulfate screening solution and the temperature of the blood samples should be within 5°C (9°F) of each other at the start of the testing. Details for this validation can be found in the original published work: "Measurement of Specific Gravities of Whole Blood and Plasma by Standard Copper Sulfate Solutions," Phillips, R. A., Van Slyke, D. D., Hamilton, P. B., Dole, V. P., Emerson, K., Jr., and Archibald, R. M., *Journal of Biological Chemistry*, **183**, March 1950, pp. 305-330.

2. Quality Assurance Testing (Hydrometer Methods):

The following are often overlooked sources of error in the hydrometer specific gravity method:

(1) Most hydrometer scales (60/60) are accurate only for a temperature of 60°F (15.6°C) for the test solution, and a temperature of 60°F (15.6°C) for the reference high purity water. These 60/60 hydrometer scales will not give an accurate reading at 25°C as required. If these 60/60 hydrometers are used for blood bank quality assurance testing of a representative Copper Sulfate test sample at 25°C (77°F), the result will be an incorrect, slightly lower specific gravity reading on the 60/60 scale. However, a correction to the reading at 25°C (77°F) can be determined by the customer. For the initial hydrometer validation, two or three samples of this product can be tested and approved by the functional monitoring method as described below. These approved Copper Sulfate samples can then be used as standards to determine the corrected reading at 25°C (77°F) for each hydrometer intended for routine Quality Assurance testing of future Copper Sulfate lots. These corrected target readings for each Hydrometer can then be used to approve or reject future Copper Sulfate batch samples when measured at 25°C (77°F).

(2) The specific gravity measurement temperature for this Copper Sulfate solution is expected to be 25°C (77°F). Specific gravity is temperature dependent. For this Copper Sulfate solution, the measured specific gravity will be lower if the measurement temperature is greater than 25°C (77°F) and the measured specific gravity will be higher if the measurement temperature is less than 25°C (77°F). For example, a Copper Sulfate Solution with a specific gravity of 1.0531 at 25°C (77°F) has a specific gravity of approximately 1.0536 at 60°F (15.6°C).

(3) The AABB Technical Manual says the hydrometer should be calibrated (checked for accuracy) before use. Some (such as typical urine hydrometers) are not accurate enough for precision measurement of the small specific gravity variations encountered.

(4) The hydrometer may have too wide a specific gravity range on its scale, making accurate estimates from the scale difficult. Use a precision or certified specific gravity hydrometer with an expanded scale covering as small a specific gravity range as possible. Since the reading must be visually estimated, an expanded scale covering a narrow specific gravity range improves the reading accuracy.

(5) Do not drop the hydrometer into the solution to be tested. Gently lower the hydrometer into the solution until it floats on its own. Drops of solution on the hydrometer stem above the liquid level will cause incorrect results. Do not allow the hydrometer to touch the sides of the solution container during measurement readings.

3. Quality Assurance Testing (Refractometer Methods):

Refractometer methods also suffer from some of the same sources of error as hydrometer methods. This includes incorrect solution measurement temperatures, unless the refractometer is self-temperature-compensating (typically to 20°C), and special refractometer scales for other solutions that are not suitable for Copper Sulfate testing. Suitable lots of these products have a refractive index of about 1.3425 at 20°C, or about 6.5° Brix (%) at 20°C on a Brix refractometer. A urine specific gravity refractometer refractive index scale can be used to determine the refractive index (ND) directly on this Copper Sulfate solution at 20°C. However a urine specific gravity refractometer cannot be used to directly read the specific gravity of this Copper Sulfate solution, since the scale is only accurate and suitable for urine specific gravity testing.

4. Quality Assurance Testing (Density Methods):

If density (g/mL) of the Copper Sulfate solution is determined at 25°C (77°F), divide the density value by 0.9970 g/mL (the density of water at 25°C) to convert to the specific gravity value.

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