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A NEW PERMANENT STANDARD FOR SAHLI'S HEMOGLOBINOMETER.

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Under optimum conditions the estimations of hemoglobin by Sahli's method are surprisingly accurate. If the diluting test-tube is accurately graduated and has the same inside diameter as the standard tube, and if the blood pipette has the proper capacity, very satisfactory determinations can be made provided the standard solution is correct.

The manufacturers can readily fulfil all the requirements except the last one. The unreliability of the standard has brought the method into disrepute. Acid hematin standards deteriorate and soon become worthless. I have examined permanent standards made by two leading manufacturers. Those of one firm have a color which does not resemble that of acid hematin. Estimations made with these standards showed that they did not have the hemoglobin equivalence that was claimed for them. The standards of the other firm were better, but they became darker after some months. They were discontinued by the manufacturer.

The writer has been able to prepare a permanent standard from inorganic material that has shown no change in 10 months. It is made by mixing 50 cc. of ferric sulfate solution (containing 53.3 gm. in 100 cc.), 15 cc. of cobalt sulfate solution (10 gm. $CoSO_4 \cdot 7H_2O$ in 100 cc.), and 10 cc. of water. Unfortunately, ferric sulfate (either in dry form or as a solution) of uniform color value seems to be unobtainable. Different samples secured from the same manufacturer varied widely in the character of the color. If one wishes to duplicate my standard, he must check it up against 1 per cent acid hematin that has been prepared with research accuracy. It is probable that different proportions of ferric sul-

fate and cobalt sulfate than those given above will have to be used.[1]

Technique.—Put (approximately) 0.2 N HCl in the graduated test-tube to about the 10 mark. Deliver 0.02 cc. of blood with the pipette into the solution and mix quickly. Draw the liquid into the pipette twice blowing it out, then rinse the tip with a drop of the acid solution. Keep the test-tube in a beaker of hot water (55–60°) for 7 minutes. In that length of time the full acid hematin color is developed—1 hour at room temperature did not prove to be as reliable as some investigators claim.

TABLE I.

Comparison of Hemoglobin Estimations by Van Slyke's Method and by Sahli's Method Using the New Permanent Standard.

Blood No.	Hemoglobin.	
	Van Slyke's method.	New method.
	per cent	*per cent*
1	96.7	97.0
2	99.7	99.0
3	104.6	104.0
4	114.5	115.0
5	117.1	117.0
6	119.3	120.0
7	122.6	123.0
8	122.0	123.0
9	132.0	132.0

Cool the tube and dilute the mixture gradually with careful mixing until it matches the standard. The standard must be at 19–20° to give strictly accurate results. Immerse the standard tube in water at that temperature for a few minutes before using it, if the room temperature is different.

At higher temperatures the standard becomes darker (increased hydrolytic dissociation) and underestimation results. The error is approximately 1 per cent for each degree.

[1] Because of the difficulty of reproducing the standard, we shall check up the solution before it is distributed. Hynson, Westcott and Dunning of Baltimore, will furnish the material.

The method was carefully standardized against Van Slyke's method.[2] A reading of 100 on the graduated tube indicates that the blood contains 13.8 gm. of hemoglobin in 100 cc. The readings will not, therefore, be in terms of percentage of the average normal. The work of Meyer and Butterfield[3] seems to show that the Sahli readings will average 116 for normal males and 109 for females provided the blood contains 5 million red cells per cubic millimeter.

When the method is carried out with great care the estimations are almost as accurate as those by Van Slyke's method, as will be seen by examining Table I. Routine estimations should give results within 2 per cent of the correct amount.

SUMMARY.

A permanent standard made of inorganic materials is proposed for the Sahli hemoglobinometer. The estimations are accurate if the pipette and tubes are correctly calibrated.

[2] Van Slyke, D. D., *J. Biol. Chem.*, 1918, xxxiii, 127. Van Slyke, D. D., and Stadie, W. C., *J. Biol. Chem.*, 1921, xlix, 1.

[3] Meyer, E., and Butterfield, E. E., *Arch. Int. Med.*, 1914, xiv, 94.