

# INACCURACIES IN THE SIZE OF HEMACYTOMETER CHAMBERS AND PIPETS

M. W. LYON, JR., M.D., PH.D., WASHINGTON, D. C.  
Professor of Bacteriology and Pathology, George Washington University  
Medical School

The accidental discovery of a hundred per cent. error in the depth of a recently purchased chamber of the Helber type led to an examination of the accuracy of others and to inquiry at the Bureau of Standards as to the accuracy of hemacytometers in general.

The gross error referred to was discovered by an enthusiastic student who used the Helber chamber in making a count of red blood corpuscles. In calculating the number of corpuscles from the data obtained he found twice the number of corpuscles that one of his companions found in the same blood. He repeated the work with a similar result. The conclusion was obvious that the Helber chamber was about 40 microns deep instead of the labeled 20. Probably other counting chambers are on the market or in use as carelessly labeled as is this one.

Before it was learned that the Bureau of Standards was prepared to test hemacytometers, measuring the depth of chambers was attempted by allowing blood or India ink to dry on the ruled disk and on the surrounding plateau and calculating their difference in focus when viewed with a  $\frac{1}{12}$  objective, a microscope with the fine adjustment graduated to indicate depressions or elevations of a micron being used. The results of this crude method are given in Table 1. Measuring the thickness of the ruled disk and slide on which it is mounted, and then the thickness of the plateau and the slide, and taking their difference with an ordinary micrometer caliper was found to be an equally crude method, owing to the fact that the glass slide on which the ruled disk and plateau are mounted does not have its upper and lower surfaces parallel, and probably these surfaces are not perfect planes.

The method employed by the Bureau of Standards in measuring the depth of chambers is to apply a heavy glass disk of known thickness to the counting chamber and to take the combined thickness of that, the plateau surrounding the ruled disk, and the glass slide on which the plateau and ruled disk are mounted, by means of a micrometer measuring machine, and to use the mean of four readings. The mean of

man makes, comes up to these requirements. An error of 10 per cent. in the case of the Helber chamber could not be considered important if it is used in counting the number of organisms in bacterins. If used for cytologic work, it ought to be more accurate. One and 2 per cent. errors in ordinary blood counting are negligible.

Another possible source of error in the counting chamber is that the square millimeters on it and the  $\frac{1}{400}$  square millimeters on it are not what they are labeled. I have measured

TABLE 2.—CAPACITY OF DILUTING AND MIXING PIPETS  
EXPRESSED IN CUBIC CENTIMETERS, AND SHOWING  
LABELED AND ACTUAL CAPACITY RATIOS AS  
TESTED BY U. S. BUREAU OF  
STANDARDS

Manu- fac- turer	Capacity of Interval, C.c.				Nominal Capacity Ratio		
	0 to 0.5	0 to 1.0	0 to 11	0 to 101	1:2*	1:10†	1:100‡
					Actual Capacity Ratio		
A	0.0176	0.0348	0.2376	.....	1:1.98	1: 5.8	
A	0.0151	0.0313	0.2043	.....	1:2.07	1: 7.4	
A	0.0041	0.0090	.....	0.6004	1:2.20	.....	1: 73
A	0.0025	0.0052	.....	0.5428	1:2.08	.....	1:103
B	0.0145	0.0279	0.3019	.....	1:1.92	1: 9.8	
B	0.0224	0.0430	0.2690	.....	1:1.95	1: 5.2	
B	0.0021	0.0044	.....	0.4585	1:2.10	.....	1:103
C	0.0125	0.0255	0.2039	.....	1:2.04	1:10.5	
C	0.0020	0.0042	.....	0.4519	1:2.10	.....	1:106
D	0.0124	0.0248	0.2738	.....	1:2.00	1:10.0	
D	0.0103	0.0216	0.2518	.....	1:2.04	1:10.6	
D	0.0096	0.0196	0.2222	.....	1:2.04	1:10.3	
D	0.0086	0.0071	.....	0.8003	1:1.97	.....	1:112
D	0.0042	0.0084	.....	0.8917	1:2.00	.....	1:105
D	0.0040	0.0082	.....	0.8353	1:2.05	.....	1:101

\* Actual capacity ratio calculated from intervals 0.5 and 1.0.

† From 0 to 1, and 1 to 11 [i. e., (0 to 11) minus (0 to 1)].

‡ From 0 to 1, and 1 to 101 [i. e., (0 to 101) minus (0 to 1)].

some of them by means of a stage micrometer and micrometer eyepiece, and they were found to be as near their alleged size as that method is capable of measuring. It is practically possible to rule the square millimeters to within 1 micron of accuracy, yielding thus an error of 0.1 per cent. Slight variations in the small squares may be tolerated, provided that, in making counts, diagonal blocks are used, for example, by using the twenty-five small squares in the upper left hand corner, then the block immediately and diagonally to the right and below, then the next similarly placed block of twenty-five small squares finally ending up with the block of twenty-five squares in the lower right hand corner. Any slight variations in the spacing of the lines that make up the small squares is thus compensated for, as squares formed by all the lines are involved.

Attention has been previously called to variations in the calibration of the mixing and diluting pipets.<sup>1</sup> I have never had the accuracy of the pipets under my observations tested. The figures in Table 2, supplied by Mr. H. W. Bearce of the Bureau of Standards, are very significant in this connection. The errors range from 50 per cent. down to none. Only six out of the fifteen pipets listed have an error of less than 5 per cent., and two of less than 2 per cent.; one pipet for making counts of white corpuscles is perfect. The most perfect pipet for making red counts is 1 per cent. wrong. Probably the general run of pipets in use is not more accurate. Mr. Bearce is of the opinion that it is practically possible to calibrate pipets to have an error not greater than 2 per cent.

An error of tolerance of 2 per cent. in the pipets and of 2 per cent. in the counting chambers, with a practically negligible error of 0.1 per cent. in the size of the square millimeter of the ruled disk gives an instrument with an error of 4 per cent. As shown by Emerson,<sup>2</sup> 4 per cent. represents a reasonable error in blood counting. An instrument designed to give counts within 4 per cent. is almost as near perfection as is the user of the instrument, and ought to be satisfactory, at least for clinical purposes. For the highest degree of accuracy as might be demanded in some scientific investiga-

1. Wood, F. C.: Chemical and Microscopical Diagnosis, Ed. 2, 1909, p. 48.

2. Emerson, C. P.: Clinical Diagnosis, Ed. 4, 1913, p. 469.

TABLE 1.—LABELED AND ACTUAL DEPTHS OF COUNTING  
CHAMBERS \*

	Helber Count- ing Chamber Maker M.	Fuchs-Rosenthal Counting Cham- ber, Maker M.	Thoma Count- ing Chamber Maker N.	Thoma Count- ing Chamber Maker O.	Thoma Count- ing Chamber Maker M.
Labeled depth of chamber.....	20	200	100	100	100
Depth of chamber as measured by the Bureau of Standards.....	46	203.4	100.2	104.2	104.4
Estimation of depth by noting change in focus of particles dried on the ruled disk and on the plateau.....	42	186	101	99	97

\* The figures are thousands of a millimeter, or microns.

four similar measurements for the thickness of the ruled disk and the mounting slide is then ascertained. The difference between these two means, less that of the glass disk of known thickness, gives the depth of the chamber. This method of measuring them is accurate to less than 1 micron. It is the opinion of Mr. D. R. Miller, of the Bureau of Standards, that makers of hemacytometers can with care make chambers accurate to within 2 microns of the labeled depth. This corresponds to an error of 2 per cent. on the ordinary blood counting chamber, of 1 per cent. on a Fuchs-Rosenthal chamber, and of 10 per cent. on a Helber chamber. From Table 1 it will be seen that only one of the five counting chambers measured for me, representing three standard Ger-

tions, cell counts could be calculated on the basis of the actual size of the instrument. Manufacturers, however, if possible ought at the same time to keep the error within 1 per cent. in the case of the counting chamber and the pipets, as Emerson has shown that experts in blood counting are able to keep their errors within 2 per cent.

The suggestion is offered that the counting chamber of hemacytometers should be made of one solid piece of glass instead of the usual three cemented pieces. Wood<sup>1</sup> has called attention to variations in the depth of chambers due to changes in temperature affecting the cement. In addition to the name of the maker and other information placed on them, the chambers should be given a serial number, as is frequently done with the pipets. Each chamber could then be furnished with a certificate of accuracy. It is possible to number the chambers as made in the usual manner with the ruled disk and the plateau cemented to a heavy plate. This is actually done with those chambers measured by the Bureau of Standards, but there is always the possibility of the cemented pieces of glass coming off, and there would be nothing to interfere with irresponsible parties cementing them on again or even cementing them on other numbered glass plates.

#### SUMMARY

Several hemacytometers measured by the Bureau of Standards show gross inaccuracies as to the depth of counting chamber and calibration of pipets.

An error of tolerance not exceeding 2 per cent. in the case of the depth of the counting chamber, and in the calibration of the pipets should be adhered to by the manufacturers. If possible this error should be kept to within 1 per cent. in each case. An error of tolerance not exceeding 0.1 per cent. should be allowed for the size of the square millimeters of the chambers.

It would be desirable to have the counting chambers made of a solid piece of glass.

Each hemacytometer should be furnished with a certificate as to its accuracy or as to its errors.

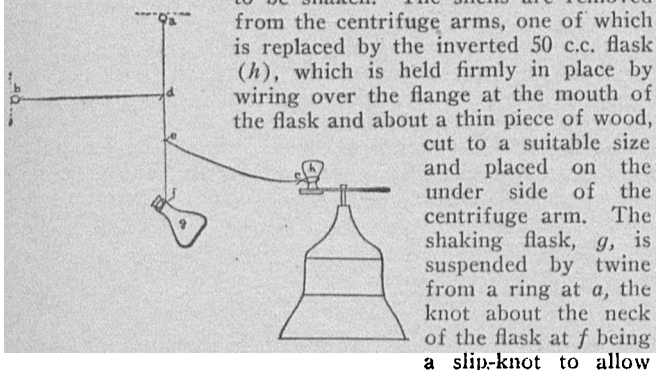
The Bureau of Standards, Washington, D. C., is prepared to test the accuracy of hemacytometers for a nominal fee.

#### AN IMPROVIZED SHAKING MACHINE

R. M. LeComte, M.D., WASHINGTON, D. C.

Pathologist, Washington Asylum Hospital

The essential parts of this contrivance are an electric centrifuge, some heavy twine and two flasks, one of about 50 c.c. capacity and the other large enough to carry the material to be shaken. The shells are removed from the centrifuge arms, one of which is replaced by the inverted 50 c.c. flask (h), which is held firmly in place by wiring over the flange at the mouth of the flask and about a thin piece of wood,



cut to a suitable size and placed on the under side of the centrifuge arm. The shaking flask, g, is suspended by twine from a ring at a, the knot about the neck of the flask at f being a slip-knot to allow changing the flask without disturbing the whole apparatus. A cord is tied firmly to the cord af at e, and fastened loosely about the neck of the flask h at c so that the flask can revolve in the grasp of the knot. This cord, ec, is of such length as to reach from the point e to the shaft of the centrifuge. The centrifuge is then run at moderate speed. A third cord, bd, may be tied at the points indicated in the diagram to control the extent of the motion of the shaking flask, g. If a spiral spring is inserted between the points a and f, an additional vertical motion is obtained.

1424 K Street N.W.

#### IMPROVED ASPIRATING APPARATUS

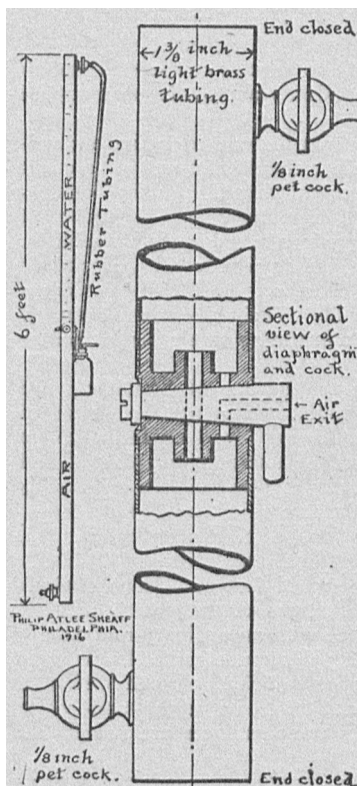
PHILIP ATLEE SHEAFF, M.D., PHILADELPHIA

In THE JOURNAL, Sept. 16, 1916, I described an aspirating apparatus for office and hospital use in obtaining gastric contents, and, Dec. 2, 1916, Dr. Harvey G. Beck of Baltimore described an apparatus designed for the use of partial vacuum

in diagnosis and treatment.

Both of these devices supply a needed want, but are restricted in their usefulness by the fact that Beck's requires a faucet and running water, and mine is a permanent fixture in proximity to the wash-stand.

I have so modified my apparatus that it is portable, self-contained, always ready for use, and lends itself to various uses in diagnosis and treatment in which a partial vacuum is desirable, and in principle might be spoken of as a "hydrostatic hour-glass." It is brass throughout and consists of a piece of 1 3/8 inch light brass tubing closed at each end, near which is a 3/8 inch pet-cock. A diaphragm containing a cock divides the cylinder into two compartments of equal capacity. The upper half is filled with water, the lower half with air. In operation the tube is placed in the vertical position, a rubber tube connects the upper pet-cock with the aspirating bottle (which for convenience is held to the tube by a clip) as shown in the sketch, a cupping glass, or other container for blood or fluid, the pet-cock is opened, and the diaphragm cock turned with its handle down.



Improved apparatus for aspirating gastric contents.

This allows the water in the upper chamber to pass into the lower chamber, thus displacing its contained air, as shown in the sketch, and with a tube of the length shown, generating a negative pressure of 65 mm. of mercury in the aspirating bottle. After the operation is over, any remaining water in the upper chamber is allowed to flow into the lower chamber and all cocks are closed, when the apparatus is instantly ready for use again by turning it upside down and repeating the manipulation after connecting the tubing of the aspirating bottle to the upper pet-cock.

No water is lost, the device is simple, and while somewhat long, can be carried easily; it does not take up much space when not in use, as it can stand in a corner; is easily maintained in the upright position by throwing a bandage around it and the back of a chair; has no valves to get out of order; requires no wash-stand and running water, and the three cocks which it contains need no more attention than an ordinary gas fixture.

4006 Baring Street.

**Judgment in Health Expenditures.**—To spend the resources of the department on other peoples' pet projects, on things which, even though desirable, are not in given circumstances essential, is to tithe mint, anise and cummin and neglect the weightier matters of the law. These weightier matters are the activities which experience has shown to be fruitful of real results. They follow paths clearly marked, toward a definite goal—the health of the state.—Alice Hamilton, *Survey*, Jan. 20, 1917.