DEPARTMENT OF THE NAVY
NAVAL MEDICAL RESEARCH CENTER
503 ROBERT GRANT AVENUE
SILVER SPRING, MARYLAND 20910-7500

IN REPLY REFER TO
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20 Sep 19

MEMORANDUM

From: Commander, Naval Medical Research Center
To: Defense Technical Information Center

Subj: FREEDOM OF INFORMATION ACT REQUEST CASE NUMBER DON-NAVY-019-009357, DTIC-R (FOIA 2019-131)

Ref: (a) Freedom of Information Act (FOIA) Request from Mr. Thomas Cuff (Attachment 1)

1. I have reviewed the file associated with Case Code Number DON-NAVY-019-009357, Defense Technical Information Center (DTIC) document number AD-5258 entitled, "The Reproducibility and Constancy of the Platelet Counts" (Attachment 2). I have determined that the Naval Medical Research Center (NMRC) is the correct recipient for this review request.

2. I have determined that the document is published and publicly available through PubMed at the following link: https://academic.oup.com/ajcp/article-abstract/23/1/15/1767409?redirectedFrom=fulltext. The DTIC document AD-5258, is very similar to that available online with similar dates. Due to public availability, this document is exempt from FOIA and releasable. No redaction is required to the information pertaining to DTIC document number AD-5258 and should be made delimited and made available to the public.

3. My point of contact for this matter is LT Kelly Nobles, NMRC Legal Officer who can be reached via e-mail: kelly.n.nobles.mil@mail.mil or via phone: (301) 619-1507.

F. R. JONES
Acting

AD-

5258

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NAVAL MEDICAL RESEARCH INSTITUTE

NMRI

THE REPRODUCIBILITY AND CONSTANCY OF THE PLATELET COUNTS

RESEARCH REPORT
Project NM 006 012.05.10

THE REPRODUCIBILITY AND CONSTANCY OF THE PLATELET COUNTS

GEORGE BRECHER, M.D.
The National Institute for Arthritis and Metabolic Diseases,
The National Institutes of Health, Bethesda, Md.

MARVIN SCHNEIDERMAN, B.S.
The National Cancer Institute,
The National Institutes of Health, Bethesda, Md.

EUGENE P. CRONKITE
Commander MC USN

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RESEARCH REPORT
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NAVAL MEDICAL RESEARCH INSTITUTE
NATIONAL NAVAL MEDICAL CENTER
BETHESDA, MARYLAND

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ABSTRACT

1. Direct platelet counts performed with the phase microscope on venous blood collected in siliconed test tubes accurately reflect the circulating platelet level. The error of the single count is 11 per cent, and can be reduced only by doing multiple counts.

2. Platelet counts on capillary blood from a finger puncture are subject to greater errors (24%) and the counts are on the average 2½ per cent lower. This is presumably due to a variable loss of platelets in the puncture wound and does not necessarily indicate a lower circulating capillary platelet level.

3. The main advantage of the platelet counts with the phase microscope is the easy and certain recognition of individual platelets, and it is believed that this accounts for its satisfactory reproducibility, even by inexperienced technicians.

4. Other methods of platelet counting are reviewed and the necessity for detailed statistical analysis of counts is illustrated.

5. In 13 healthy males, platelet levels varied greatly between individuals, but individual platelet counts showed only minor variations during a five months' period.

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INTRODUCTION

Ready agglutination and disintegration of platelets have been held responsible for the difficulties in counting platelets (1,2). Yet platelets in suitable anticoagulants have been observed over extended periods without evidence of disintegration (3). The adhesiveness of platelets has been nearly completely prevented by the use of siliconed surfaces, certain anticoagulants or rapid dilution (3,4,5). The difficulty of differentiating platelets from extraneous particles has been overcome by the use of the phase microscope (3,6). The theoretical considerations which appeared to preclude accurate platelet counts are therefore no longer valid, and practical experience on venous blood samples has shown that platelet counts are in fact reproducible subject only to those errors inherent in any hemocytometer count (3). The purpose of this paper is to report on the relative accuracy of venous and capillary platelet counts, and to review the merits of some of the counting methods in current use.

METHODS

The counting method consists essentially in the use of one per cent ammonium oxalate as diluting fluid, and the counting of the platelets in a hemocytometer with a phase microscope. The use of one per cent ammonium oxalate insures clearing of the background by hemolysis of red blood cells, and the use of the phase microscope assures easy recognition of the individual platelets. The principle of the method is identical with that of Feissly (6), but avoids the use of a cocaine solution as diluent.

1. Venous blood was collected by inserting a 20 gauge needle, without syringe, into a cubital vein, and by allowing one-two cc. of blood to flow directly into a siliconed test tube. The siliconed test tube was kept in a beaker with ice water both before and after the collection of blood. Immediately after collection, the blood was diluted in eight RBC pipettes selected from a pool of 16. An alternate method which has given equally satisfactory results in our experience is to collect the blood with 19 or 20 gauge needle and a clean, dry syringe with a minimum of suction to avoid air bubbles. After removing the needle, the blood is gently expelled into a siliconed test tube. If only single or duplicate counts are contemplated, the cooling of the test tube in ice water may be omitted. Regardless of the method of collection, the blood must be diluted in the RBC pipettes without delay.

Capillary blood was collected from the tip of a finger after cleaning it with acetone. The puncture was made with the lancets described by Walters, *et al* (7). These lancets are sharp, cause little pain, and can be sterilized in an autoclave. Pipettes were filled directly from the finger puncture. Again eight pipettes from a pool of 16 were used. Usually all eight pipettes could be filled from a single puncture, and only rarely was it necessary to do a second finger puncture. Undue pressure to obtain a sufficiently large drop of blood was avoided. In the experiments here reported, the venous and capillary counts were taken within a few minutes of each other. In half of the experiments the capillary count was done first; in the other half the venous blood was collected first.

2. The blood was drawn up to the 1 mark of a RBC pipette and one per cent ammonium oxalate to the 101 mark, giving a 1:100 dilution. This step must be completed without delay. Subsequently, the pipettes were kept rotating in one of the commercially available pipette rotors until the chambers could be conveniently filled and counted. Rotation of pipettes for as long as eight hours does not affect the counts (3). The one per cent ammonium oxalate solution is kept in the ice box to prevent growth of molds and bacteria.

3. A hemocytometer was filled in the usual fashion, except that a No. 1 or 1½ cover slip is used rather than the standard hemocytometer cover glass of 0.4 to 0.6 mm. thickness. The use of a thin cover slip is essential in phase microscopy, and it has been previously shown its use does not lead to an increase in the error of the count (3). The counting chamber must have a flat bottom, since a concavity in the bottom of the counting chamber vitiates the phase effect.

4. The chamber was set aside for 10 to 15 minutes, a wet cotton pledget placed next to it, and chamber and pledget covered with one-half of a Petri dish, to allow settling of platelets while avoiding drying of the preparation. In the present investigation, four counting chambers were used in rotation.

5. Platelets were counted in 10 blocks of small squares (as for RBC counts), 5 blocks being counted in each half of the chamber. The total number of platelets so counted x 2,500 gave the platelet count per cu. mm. A 43x phase objective with long working distance condensor and 10x eyepieces were used. In this procedure, the platelets stand out as individual round or oval bodies with pink or purple sheen. The presence of any platelet clumps indicates that the dilution of the RBC pipette has been delayed too long and a fresh sample must be taken. This occurs but rarely, and no platelet clumps were seen in any of the counts reported below. On focussing up and down, platelets can be seen to have one or more fine processes. Crystals, dirt, bacteria are readily distinguished by their refractility and absence of pink-purple sheen. With even slight experience, it is not necessary to visualize the processes of individual platelets and the count can be completed quickly, once the platelets have settled out, without more than occasional refocussing.

The procedure of recording counts was to have the technicians write down the count for each of the 10 blocks of small squares in each chamber. This allowed computation of the field error due to chance distribution of the cells in the chamber. The counts for the individual blocks of squares were not added until after completion of the day's work.

One technician filled all of the pipettes, and did all the counts presented in table 1, except for the fourth series of counts, which was done by two observers.

The necessary phase equipment consists of a *long working distance condenser* with 43x phase objective. Most firms manufacture only one type of 43x phase objective, and the performance of all makes now available in this country appears comparable. American Optical Company supplies several types of 43x phase objectives and a "medium dark contrast" phase objective should be used.

Unfortunately the instructions supplied by the manufacturers of phase microscopes are unnecessarily involved and difficult to follow, which has resulted in the erroneous impression that particular skill is required in the use of the phase equipment. This is by no means the case if a few essentials are kept in mind.

1. The microscope lamp must be centered on the mirror and focussed in the entrance plane of the condenser. A ribbon filament lamp is preferred, but coil filaments with ground glass give usable results. The lamp diaphragm must be kept open. Since the condenser setting is critical, the condenser cannot be used to change the light intensity of the image. This can only be done by neutral grey filters. Green filters, useful for other purposes in phase microscopy, should not be used for the platelet count.

2. The phase condenser produces a *hollow* cone of light, and the vertex of the cone must be in the plane of the microscope slide. Therefore, the position of the condenser is critical. Preliminary adjustment of the condenser setting is readily accomplished by placing a piece of lens paper on the counting chamber. As the condenser is moved up and down, the area illuminated by the condenser changes from a ring to a small dot and again to a ring. The smallest dot that can be produced represents the optimal condenser height.

3. The phase effect is produced when the image of the annular diaphragm of the condenser and the ring shaped phase plate of the objective overlay. To ensure this, the condenser must be centered while viewing the images of the annular diaphragm and the phase plate through a telescopic eyepiece supplied by the manufacturer. This procedure is difficult for the uninitiated because of confusing diffraction patterns that appear in the telescopic eyepiece.

The difficulty can be readily overcome if the following procedure is used: After proper focussing and centering of light source, an empty counting chamber is put on the stage and the condenser with a 43x annulus in place is adjusted as described above. Using an *ordinary* 43x objective, the mirror is adjusted to give optimal illumination and the microscope is focussed on the lines of the chamber. One eyepiece is then replaced by the special telescopic eyepiece. When the eyepiece is focussed, a white ring is seen which represents the image of the condenser annulus. The next step is to remove the 43x annulus from the condenser (or to turn the condenser to zero position, depending on the make of the equipment), and to change to the 43x phase objective. A black ring will now be seen, representing the phase plate. This black ring is approximately of the size, and in the position of the white ring seen before. Having visualized the images of the condenser annulus and the phase plate, it is now easy to make them overlay by centering the condenser with the 43x annulus again in place. Since the black ring is slightly larger, a fine black concentric ring (no crescent) remains at the inner aspect of the white ring when the condenser is properly centered. Moving the condenser down gives a red color to the white ring, moving it up, a blue color. In optimal position the ring is white. Moving the mirror makes the ring grey instead of white. The ring must be *uniformly* white.

RESULTS

Table 1 gives the range and means of capillary and venous platelet counts on 13 healthy males. Eight counts were performed on *one* sample of venous blood and eight counts on *successive* drops of capillary blood on each of four different dates. The four series were spaced four to six weeks apart over a five month period. There was no overall tendency of the platelet counts to increase or decrease during the five month period of observation. Some individuals (e.g.EA) showed only slight variation in the platelet count, while others (e.g.GB) showed substantial changes on successive examinations, but no definite trend. The greatest variation between the *mean* venous counts on different dates in any individual was 55,000, while the greatest variation between individuals was 268,000.

The statistical method of analysis of variance permitted consideration of the different sources of variation of counts separately. After excluding the components of variance due to differences between individuals and differences in time at which the samples were taken, the observed errors of the count could be compared with those expected from Berkson's formula (8) discussed below. This part of the analysis of variance is given in table 2. The two major components of the overall error of the count are given, namely, the variation from square to square *within* the chamber, referred to as field error and the variation *between* chambers. Since each chamber was filled from a different pipette, the variation between chambers in table 2 includes the pipette error and must be compared with the combined pipette and chamber error of Berkson. There was no significant difference between expected and observed field errors or expected and observed pipette-chamber errors in the venous counts. For the capillary counts, the observed field error also agreed well with expectations, but the variation between chambers was significantly higher, the coefficient of variation being 21.7 per cent compared with an expected 6.5 per cent. Since the same pipettes and chambers were used for all counts, the greater variation of the capillary as compared with venous counts must be ascribed to actual variation of the number of platelets in successive drops of blood obtained from the finger puncture.

The mean of all venous platelet counts was 248,000, that of all capillary counts 242,000/mm.^{3}. The difference of 2% per cent, though small, is highly significant ($P < .01$).

The errors of a single count and of the mean of multiple counts are given in table 3. The component errors of the venous count are the minimum errors inherent in any hemocytometer count. The figures given in table 3 represent a single standard deviation in percent of the mean. If the true platelet count is 250,000, about one-third of the counts made on a

single sample will, therefore, be lower than 223,000 or higher than 273,000, and one count in 20 will be below 195,000 or above 305,000. This wide spread of individual counts cannot be reduced, but the mean count can be determined with increasing accuracy by multiple counts. Reduction of the error much below four per cent for the venous count appears impractical because 32 counts would be necessary to bring it down to two per cent.

For 6 of the 13 individuals used in the present study, earlier counts on venous blood were available and are presented in table 4. This comparison of counts, taken over a period of two years, further emphasizes that the variation of the platelet level of healthy men is relatively minor, compared with differences between individuals.

Of the two technicians responsible for the counts here reported, one was highly skilled and has had three years' experience with the phase microscope technique. The other had been instructed in the use of the standard and phase microscopes only recently, and had performed fewer than 50 red cell and platelet counts before the present experiment was begun. Table 5 shows, for one of the four series counts, the field errors obtained by these two observers. As previously pointed out (3), the best estimates of the field error of the platelet count, like that of the red cell count, is 92 to 93 per cent of the theoretical Poisson error. Both technicians closely approached this expected error, notwithstanding the very brief experience of one of them.

DISCUSSION

The expected error of the platelet count in the hemocytometer is given by Berkson's formula for the red cell count (3,8)

$$V_c = \sqrt{\frac{(0.92)^2 \times (100)^2}{n} + \frac{(4.6)^2}{n_p} + \frac{(4.7)^2}{n_c}}$$

when V_c = standard error in per cent of mean,

n = number of cells counted,

n_c = number of chambers used,

n_p = number of pipettes used

The first term under the square root represents the *field* error, due to chance distribution of cells in the chamber. There is no known way of reducing this error except by counting more cells. This is best done by doing multiple counts, using different pipettes and chambers. This reduces simultaneously the *pipette* and *chamber* errors, represented by the second and third terms under the square root. Theoretically one might expect to reduce pipette and chamber errors also by more accurate equipment. However, these errors include technical errors of filling pipettes and chambers, as well as errors of calibration. Pipettes and chambers from reputable manufacturers are usually already within the tolerances allowed by the National Bureau of Standards. In our experience the actual errors obtained did not vary significantly from Berkson's figures of 4.6 and 4.7 (3), and neither the use of certified equipment nor prolonged experience in pipetting significantly reduced these errors. Other investigations obtained similar results (9).

The platelet counts on venous blood here reported show the predicted errors. This is in accord with our previous conclusion (3) that the counts are reproducible and represent the actual circulating platelet level. The counts on capillary blood show a greater variation. Most likely there is admixture of tissue fluid leading to a very slight dilution of the blood or loss of platelets in the puncture wound or both. Neither admixture of tissue fluid or loss of platelets could be expected to be constant, and this would explain the greater variation between successive drops of blood, reflected in the combined pipette-chamber error.

It would also explain the somewhat lower mean platelet count in capillary as compared with venous blood. It may be recalled at this point that our conclusion that the venous count accurately reflects the circulating platelet level (3) was based on the observation that counts from multiple venipunctures showed only the minimum predicted error (11%). It was postulated that any loss of platelets during venipuncture would have manifested itself in a greater variation of counts. This notion is fully borne out by the markedly greater variation between capillary counts (24%), although the average loss of platelets is only about 2½ per cent.

Several methods have been proposed to avoid platelet adhesion by letting blood from a finger puncture well up into a drop of anticoagulant solution placed over the puncture wound. Whatever the effectiveness of this procedure may be, it precludes a direct hemocytometer count and requires the determination of the platelet red cell ratio. In measuring a ratio of 1:20 (250,000 platelets to 5,000,000 RBC in healthy males), the minimum error is 14 per cent when platelets among 1,000 red cells are counted and 7 per cent when 4,000 are counted (10). In addition, a red cell count, which has an error of about 8 per cent, is required to obtain the platelet level per cu. mm. The combined errors are 16 per cent and 11 per cent, when platelets are counted among 1,000 or 4,000 red blood cells respectively. Thus, any increase in accuracy by possible avoidance of platelet adhesion is counterbalanced by the greater variability of the count, unless at least 4,000 red cells are counted. Moreover, the theoretically computed errors of the platelet red cell ratio presuppose a uniform distribution of platelets among red cells. Even from cursory examination of wet smears, which are generally preferred in the indirect methods, it can be readily seen that there is definite lack of uniformity. It has been suggested that only smears with a uniform distribution of platelets should be counted (11). Unfortunately, this basically sound advice is difficult to follow in practice, since there is no ready criterion by which one can judge when the variation in the number of platelets from field to field exceeds that due to mere chance distribution. In the very nature of things, the per cent variation in the number of platelets from field to field becomes less evident as their absolute number increases, and in the search for uniform smears, those with a disproportionately high number of platelets are likely to be chosen. In addition, even though the original method calls for counts of different areas in at least two smears, technicians are liable to pick areas where platelets can readily be seen, i.e., areas with a high number of platelets. Such selection of smears or areas rather than avoidance of platelet loss probably accounts for the high counts obtained by the indirect method.

Selection of smears or areas, moreover, sacrifices the randomness of sampling, which is essential to insure reproducibility of counts. Apparently workers in the same laboratory can agree on the details of the procedure and duplicate their own counts. However, mean counts for large groups of healthy adults reported from different laboratories using the indirect method have varied from 200,000 to 716,000, underscoring the hazards of selection of smears or areas. Direct methods have shown better agreement, reported values summarized by Tocantins (12) varying only from 250,000 to 350,000.

While these considerations suggest a definite superiority of the direct counting methods, it can be pointed out that both the indirect and direct method have been reported to be readily reproducible. Standard errors of only 1.8 per cent for one indirect method (13) and of 0.02 per cent for a direct method (14) have been claimed. As emphasized by Biggs (15), errors due to chance distribution of cells alone are considerably higher. These unexpectedly low errors must, therefore, be viewed in the same light as the consistent throwing of sevens and elevens with a pair of dice. No hesitation is generally felt to ascribe such "luck" to loaded dice, and some type of non-randomness is the only explanation for unexpectedly close agreement of counts. In contrast to the conscious use of loaded dice, the bias involved in very close agreement is largely due to unconscious equalization of counts. Satisfactory

duplication of counts on the same sample cannot rule out unconscious equalization and does not necessarily insure reproducibility. Biggs (16) has shown this experimentally by subdividing a sample of blood for multiple red cell counts by two technicians. The two sets of counts showed a significant difference, even though the multiple counts of each technician agreed very well between themselves. Rejection procedures also result in apparently closer agreement of counts than expected, again at the expense of accuracy (17). Thus, the reported excellent reproducibility does not dispose of the objections to the indirect method. Rather, it becomes apparent that a small standard deviation is not in itself an assurance of the reproducibility of either the direct or indirect method.

Statistical treatment of data, to be of greatest value, should include an analysis of the various components of the total error, using Berkson's or some similar procedure. The actual errors can then be compared with the theoretical minimum error due to chance distribution of cells in the chamber or smear and known errors of the equipment. Smaller errors imply some improprieties, however unintentional. Greater errors can be due to non-random sampling or unexpected technical difficulties. Using this general plan of analysis, Biggs performed multiple counts on capillary blood with both Lempert's direct and Dameshek's indirect method (15). The errors were greater than the computed minimum errors in both instances, but the direct method approached the expected values more closely. The counts done on one individual with the direct method showed an approximately normal distribution around the mean of 247,000 platelets per cu. mm. The mean of the counts done on the same individual with the indirect method was 365,000, but the distribution was irregular and showed two peaks. The total error was 41 per cent for Dameshek's, but only 23 per cent for Lempert's method, thus demonstrating the expected superiority for the direct method.

In Biggs' experiments there was a large observer error both in the direct and indirect counts. There can be little doubt that identification of individual platelets is readily made with the indirect method using a 97x objective. We therefore suspect that the large observer error obtained by Biggs for the indirect method is due to differences in the technician's opinion of what is a "representative" area to be counted. The two peaks in the distribution of counts that appeared in Biggs' data would be readily accounted for by different selection by different observers. As already noted, this selection of areas can be standardized for technicians working in the same laboratory, but in general this will not give an unbiased estimate of the true count and no standardization can be achieved from laboratory to laboratory. In contrast, the observer error for the direct method is probably due to the notorious difficulty of differentiating platelets from extraneous particles in the counting chamber when ordinary illumination is used. This difficulty can largely be overcome by trained observers, as shown by Sloan (18), using the Rees-Ecker method. It is therefore concluded that the primary and probably sole advantage of the use of phase microscopy in the method presented lies in the ease with which any observer can identify individual platelets with certainty. As a result, even inexperienced technicians can obtain the optimal accuracy that can be approached with other direct methods only by highly skilled observers.

In either case, the accuracy of the single platelet count is limited by the unavoidable field, pipette and chamber errors, which amount to 11 per cent, so that a single direct count on venous blood determines the true platelet count (with 95 per cent confidence limits) only within ± 22 per cent. The mean of eight simultaneous counts has an error of 4 per cent and gives a 95 per cent probability that the interval, the mean ± 8 per cent, encompasses the true count. For the capillary count these errors are approximately twice as large.

For clinical practice and some research problems, only moderate accuracy is required. Great strides in the knowledge of thrombocytopenic diseases have been made, in part, by the use of the rather inaccurate indirect methods. However, differences of opinion as to the platelet levels in splenic arterial as compared with splenic venous blood might be resolved by multiple counts with the method here presented. Both in clinical practice and clinical

research, it is desirable to be able to compare counts from different laboratories, which can best be done by using a direct method, preferably using the phase microscope and multiple counts. The case with which individual platelets can be recognized by the inexperienced technician is a worthwhile advantage of the method presented, even when greater accuracy is not a major consideration.

SUMMARY

1. Direct platelet counts performed with the phase microscope on venous blood collected in siliconed test tubes accurately reflect the circulating platelet level. The error of the single count is 11 per cent, and can be reduced only by doing multiple counts.

2. Platelet counts on capillary blood from a finger puncture are subject to greater errors (24%) and the counts are on the average 2½ per cent lower. This is presumably due to a variable loss of platelets in the puncture wound and does not necessarily indicate a lower circulating capillary platelet level.

3. The main advantage of the platelet counts with the phase microscope is the easy and certain recognition of individual platelets, and it is believed that this accounts for its satisfactory reproducibility, even by inexperienced technicians.

4. Other methods of platelet counting are reviewed and the necessity for detailed statistical analysis of counts is illustrated.

5. In 13 healthy males, platelet levels varied greatly between individuals, but individual platelet counts showed only minor variations during a five months' period.

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TABLE 1

Capillary and Venous Platelet Counts in 1000's per cu mm. for 13 Normal Males
Four Series of Eight Counts Each

Individual	Source		Series 1 (Jan 10-Feb 27)	2 (Feb 28-Mar 31)	3 (Apr 8-May 6)	4 (May 15-June 18)
LL	Venous	Range	320-400	342-415	305-412	300-372
		Mean	347	388	355	338
	Capillary	Range	305-388	333-398	315-408	292-380
		Mean	346	367	374	331
TT	Venous	Range	270-358	302-352	308-345	305-428
		Mean	315	325	328	351
	Capillary	Range	278-340	220-375	285-348	278-335
		Mean	315	309	321	307
GD	Venous	Range	255-335	252-320	282-345	245-292
		Mean	299	287	309	269
	Capillary	Range	285-395	250-328	262-338	245-298
		Mean	324	288	303	276
RW	Venous	Range	263-300	252-295	248-310	225-265
		Mean	276	272	273	241
	Capillary	Range	235-322	222-300	228-283	212-265
		Mean	266	263	249	234
GB	Venous	Range	212-295	282-338	210-285	250-335
		Mean	252	307	253	293
	Capillary	Range	212-270	278-335	228-285	220-308
		Mean	250	310	255	263
PW	Venous	Range	205-273	185-240	200-280	208-248
		Mean	243	214	234	233
	Capillary	Range	200-248	178-212	205-240	170-255
		Mean	221	191	224	212
DH	Venous	Range	192-260	190-280	188-310	208-268
		Mean	235	246	242	238
	Capillary	Range	192-250	192-250	190-252	200-282
		Mean	229	221	220	232
EC	Venous	Range	212-260	200-265	208-260	200-272
		Mean	230	232	232	244
	Capillary	Range	203-275	212-270	190-280	250-292
		Mean	239	241	231	266
EA	Venous	Range	182-252	178-258	222-245	188-250
		Mean	225	213	232	214
	Capillary	Range	230-260	175-232	180-250	195-252
		Mean	244	209	215	228
TB	Venous	Range	180-235	175-222	178-208	155-208
		Mean	209	202	194	183
	Capillary	Range	168-238	170-220	170-210	148-198
		Mean	192	189	191	169
HS	Venous	Range	160-218	170-240	158-278	182-250
		Mean	187	205	225	207
	Capillary	Range	148-230	165-237	190-248	175-225
		Mean	178	180	218	206
CS	Venous	Range	170-220	160-245	188-310	152-215
		Mean	186	214	212	188
	Capillary	Range	160-230	165-220	168-215	152-190
		Mean	192	198	199	174
RF	Venous	Range	138-185	160-248	140-202	142-195
		Mean	160	214	174	169
	Capillary	Range	122-232	185-230	160-195	158-188
		Mean	175	212	177	175

TABLE 2

Field and Chamber Variation in Capillary and Venous Counts
Comparison of Observed and Expected Mean Squares for a Single Count

Source of Variation	Expected*		Observed			
			Finger Puncture		Venepuncture	
	Mean Square	Coefficient of Variation	Mean Square	Coefficient of Variation	Mean Square	Coefficient of Variation
Chambers**	13.17***	6.57	52.9***	21.68	12.53***	5.97
Field error	9.03	9.29	8.75	9.17	9.02	9.12

*Computed from Berkson's formula

**Includes pipette error and any variation among drops

***The term usually given in the analysis of variance for "chamber variation" = field error + 10 x chamber error ($= \sigma_f^2 + 10\,\sigma_c^2$)

TABLE 3

Coefficient of Variation* of Single and Multiple Counts

Number of counts	Capillary	Venous
1	24	11
2	17	8
4	12	6
8	8	4

*Computed from data given in Table 2 from the formula $C.V = \sqrt{(\text{field error})^2 + (\text{chamber + pipette error})^2}$

e.g. $11.0 = \sqrt{(9.12)^2 + (5.97)^2}$

TABLE 4

Variations of Venous Counts in Seven Individuals Taken Over a Two-year Period

Individual	July 1950*	Dec 1950**	Feb 1951***	Jan-June 1952****
LL	310		360	357
GD	232	242	265	291
GB	288	378	340	276
PW	262	232	235	231
EC	235		245	235
CS		218		200
RF	186	162	182	179

*Mean of 40 counts on 5 blood samples, taken on successive days.
**Mean of 4 counts on 1 blood sample.
***Mean of 8 counts on 1 blood sample.
****Mean of 32 counts on 4 blood samples (details in Table 1).

TABLE 5

Field Errors Made by Two Different Technicians in One Series of Counts

Technician	Number of Individuals Counted	Finger Puncture		Venepuncture	
		Mean Square	Percent of Poisson Error	Mean Square	Percent of Poisson Error
D	5	9.06	95.3	8.77	91.2
H	8	8.04	89.4	8.31	94.7