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METHOD AND APPARATUS FOR COUNTING
BLOOD CORPUSCLES
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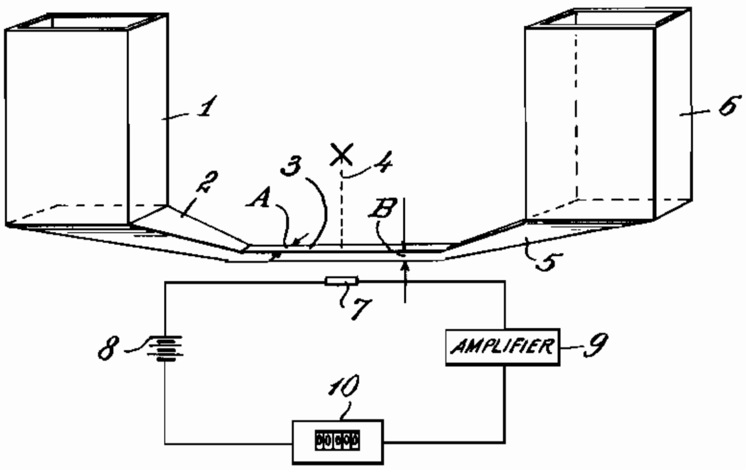
2 Sheets-Sheet 1

Fig. 1,



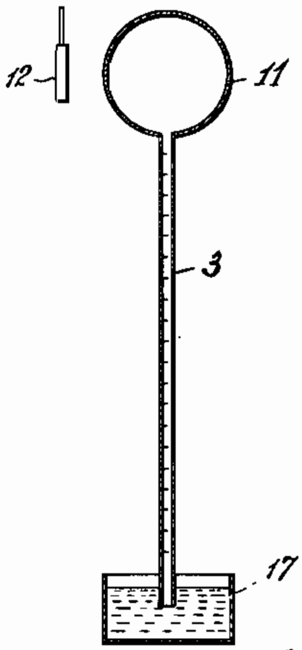


Fig. 2,

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Fig. 3,

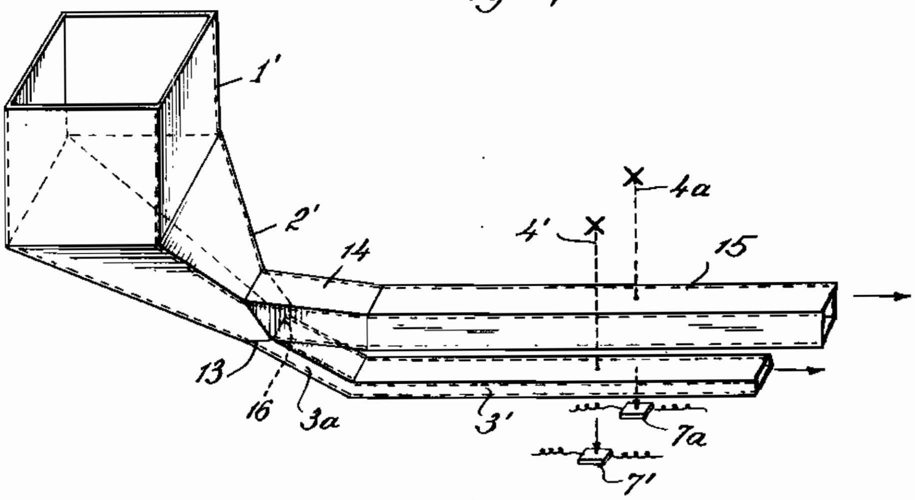
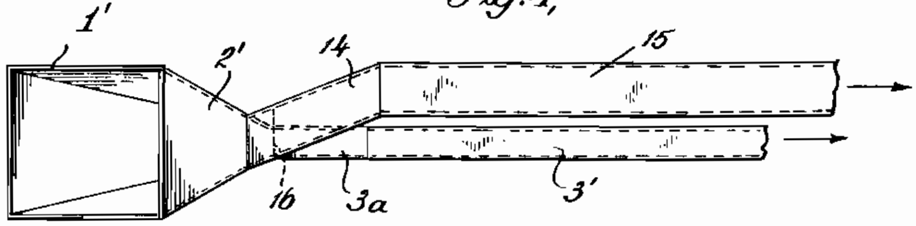


Fig. 4,



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ALIEN PROPERTY CUSTODIAN

METHOD AND APPARATUS FOR COUNTING BLOOD CORPUSCLES

Jan Kielland, Bergen, Norway; vested in the Alien Property Custodian

Application filed May 12, 1941

This invention relates to the counting of blood corpuscles and comprehends within its scope an improved process and apparatus for counting either red or white blood corpuscles present in a sample of blood. More particularly the invention comprises a method and apparatus for causing blood corpuscles to pass successively one after another past a given point at which they may be counted.

The standard practice of the art for counting blood corpuscles has involved the dilution of blood with a suitable diluting liquid in an appropriate proportion, and thereafter placing a drop of the diluted blood in a counting chamber of known height having engraved in its bottom surface a network of unit areas of known size. The number of blood corpuscles on one or more of the unit areas is then counted by an operator with the aid of a microscope. From the known dimensions of the apparatus the number of corpuscles per cubic mm. is calculated. Variations have been made in this procedure, but in general all methods, so far as I am aware, involve the counting by an operator of the number of corpuscles in a unit area. Such methods involve an error of ±5%.

In accordance with my invention a sample of blood is first diluted in a known proportion with a suitable dilution liquid, and thereafter the blood corpuscles while suspended in the dilution liquid are caused to pass successively one after another through a confined space and are counted while passing through this confined space. The blood corpuscles may be caused to move through the confined space and past the counting point by applying pressure to the liquid prior to its passing therethrough, or by the application of a reduced pressure at the outlet end of the confined space. The just recited method may be advantageously carried out by the use of the apparatus of the invention, which includes a vessel for diluted blood and connected therewith a channel member so dimensioned in cross section as to cause the blood corpuscles which are to be counted to pass therethrough and to pass the counting point in the channel individually and successively. At least a part of the oppositely disposed walls of the channel member must be of transparent material in order to permit the counting of the corpuscles passing successively through the channel. In practice it has been found advantageous to make the entire apparatus of a transparent material such as glass. The counting is with particular advantage accomplished by a counting device dependent for operation on the photoelectric principle either with or without a microscope.

The process of the invention provides a simpler, more rapid and more precise method of counting blood corpuscles than those heretofore employed. The apparatus is not complicated, and, if a counting device embodying a photoelectric cell in conjunction with a recording and counting arrangement is employed, the operation of the apparatus becomes automatic with an attendant increase in precision. In fact if the proper manipulative precautions are taken and the apparatus is properly proportioned, a precision approximating 100% may be attained either by the use of automatic means or by counting manually with the aid of a microscope. In actual practice a precision approximating 100% is seldom required, so that the dimensions of the apparatus and the manipulation thereof may be less carefully controlled, and the result will still be more exact than that obtained by previously known methods and apparatus.

In an apparatus designed to secure the greatest possible precision, the channel member is dimensioned on the basis of the size and shape of the blood corpuscles to be counted. Red blood corpuscles, which are plastic, are when stationary biconcave discs with a normal average diameter of 7.5 μ and a thickness of 2 to 3 μ. In blood diseases the diameter may vary from 4 to 13 μ and the thickness may vary up to about 5 μ. The white blood corpuscles are approximately spherical and have a diameter ranging from about 6 to 22 μ. All red blood corpuscles, even the largest, may because of their plasticity pass through a channel having a height of about 5 μ and a width of about 10 μ. The smallest of the red blood corpuscles may be caused to pass through such a channel individually by increasing the dilution of the blood sample. It has been shown by tests, however, that the red corpuscles in a suitably diluted blood sample will pass successively one after another through a channel considerably larger than the one just described. For example, a tube having a diameter of 20 μ may be successfully employed as the channel member. Standard calibrated tubes of this dimension are readily available at low cost, thus making possible in practice the use of a new tube for each test. Ordinarily in a typical sample of blood there is only about one white corpuscle to every thousand red ones, and even in extreme cases the proportion is less than ten white blood corpuscles for each thousand reds. Because of this fact a count of the usually desired precision will not be harmed by one or two white blood corpuscles passing through the channel, when a channel of sufficiently large dimensions is being used, and being erroneously counted together with the red corpuscles. In general the counting of approximately five hundred red corpuscles will provide a result of sufficient accuracy and the number of white corpuscles involved is thus negligible.

Best results are obtained by preventing the inlet of the counting passage from being clogged by the larger white corpuscles. This may be accomplished in several ways, among which may be mentioned the following. The diluted blood is first passed through a channel having both a width and height of at least 24 μ, and thereafter through a channel of the same width but having a height of about 5 μ. By this expedient the one or more white corpuscles occurring in an average sample being counted will be stopped at the entrance to the shallow channel, while a sufficient number of red corpuscles to give a precise count will continue through the shallow channel. The apparatus may also be made in such a way that the white corpuscles may actually be separated from the red, and if desired be counted separately. To accomplish this an inclined wall is placed at the point at which a large dimensioned channel narrows to connect with a smaller dimensioned channel. The large white corpuscles are impelled by the inclined wall laterally into a pocket or into a channel of appropriate size to permit their successive passage therethrough, it thus being possible to count the white corpuscles passing through the said channel. The red corpuscles will tend to be oriented with their flat sides facing the bottom of the channel and will continue forwardly through the narrow channel, which is positioned below the entrance to the referred to pocket or larger channel.

Appropriate means may be provided at either the inlet or outlet end of the channel for causing the diluted blood sample to pass through the counting channel at a desired rate. For example, means may be provided for increasing the pressure in a vessel connected with the inlet end of the channel. Such means may operate by increasing the air pressure over the diluted blood sample in the vessel. If the vessel is closed, the increased pressure may be produced by means of the application of heat. Alternatively a reduced pressure may be supplied in the outlet end of the channel or in a vessel connected thereto. In a particularly effective and simple modification of the apparatus of the invention, the channel member is closed at one end by being sealed into a bulb member. If the opposite end of the channel is immersed in a diluted sample of blood, the blood will rise in the channel member due to capillary action, but not longer than to the entrance of the bulb. This action may be expediated by preheating the bulb and letting it cool while the channel is being filled. The length of the channel will then contain a predetermined definite quantity of blood. If now heat is applied to the bulb, the blood sample will be forced outwards through the counting channel, and the blood corpuscles may be counted during their outward passage, or the counting operation may take place while the blood sample is filling the channel.

In order to explain in greater detail appropriate apparatus for carrying out certain embodiments of my invention, reference will be had to the accompanying drawings in which:

Fig. 1 includes a diagrammatic prespective view of an apparatus having a counting channel connected at each end to vessels for the diluted blood, and a diagrammatic plan view of a photoelectric counting means associated with the counting channel.

Fig. 2 is an axial section of an alternative structure in which the counting channel member is sealed to a bulb member.

Fig. 3 is a diagrammatic perspective view of an apparatus capable of separating red and white blood corpuscles and counting them separately.

Fig. 4 is a top plan view of the device shown in Fig. 3.

In accordance with one embodiment of my invention illustrated in Fig. 1, the lower end of a vessel 1 is connected to a channel 2 which is a narrowing passage, either progressively as illustrated or in a stepwise decreasing cross section. Channel 2 is integrally connected with a counting channel member 3 having a width A and a height B, the said dimensions in at least a portion of the length of channel 3 being such that the cross section of the interior passage will permit the blood corpuscles which are to be counted to pass individually and successively through the passage. As here illustrated the dimensions of the channel member 3 are substantially uniform throughout its length. As previously indicated the dimensions A and B may with advantage be made to equal approximately 10 μ and 5 μ respectively in an apparatus in which the highest precision in counting the red corpuscles is desired, but the member 3 may also be of larger dimension, such as for example a tube having a diameter of 20 μ. The counting channel 3 opens into and is integrally connected with a channel member 5, which increases in cross sectional dimension and provides a connection to a second vessel 6. In the application of the process in such an apparatus, a diluting liquid is with advantage added to container 1 in sufficient quantity so that it passes through and fills the three channel members and vessel 6 to a height equivalent to that in vessel 1. A measured quantity of blood is then introduced into the liquid in container 1 and is caused to pass through the channel together with a dilution liquid, for example, by the application of air pressure in container 1 or by the application of reduced pressure in container 6. The channel member 3 is made of a transparent material, at least in its central section. Opposed to this transparent section one may employ a microscope and count the blood corpuscles passing beneath the field. The microscope may be replaced by a source of light 4 which transmits light through the counting channel 3 against a light sensitive surface 7, for example, a selenium plate. The light sensitive member 7 is connected in an electrical circuit comprising a source of electrical current 8, an amplifier 9 and a counting apparatus 10. Such an arrangement counts the number of corpuscles passing through the channel by virtue of the interruption of current flow through the circuit, and automatically records the number of interruptions in the counting mechanism. A lens may with advantage be placed between the counting channel and the light sensitive surface, in which case and also in the case where a microscope is employed, it is advisable to surround the counting channel with an immersion oil in order to reduce the refraction of the light rays.

In an apparatus such as that diagrammatically represented in Fig. 2, the channel member **3** is closed at one end by being sealed into a bulb **11**, which may be warmed by a source of heat **12** such as the bank of electric bulbs as illustrated. As previously described a device of this sort is operated by immersing the end of the channel **3** in a sample of blood **17**, whereby the blood will rise in the channel **3** due to capillary action and possibly also by the aid of the cooling of the bulb, if this is preheated. The corpuscles may be counted with the aid of a microscope as they pass through into the bulb, or by means of a photoelectric cell associated with a counting mechanism as illustrated in Fig. 1. Alternatively the diluted blood sample may be permitted to pass into the channel and then be expelled therefrom by means of heating the bulb, the counting being done as the liquid passes outwardly through channel **3**.

In the case of special examinations it may be desirable to separate the white corpuscles from the red in order to secure a very precise count or in order to count both types of corpuscle. A device such as is illustrated in Figs. 3 and 4 provides a satisfactory means for accomplishing this in accordance with my invention. As here illustrated a container **1'** is connected to an inclined progressively narrowing passage **2'** which is integrally connected about its perimeter at **13** with a narrowed channel member ***3a*** which is also inclined but connects with a counting channel member **3'**. From the inclined upper wall of passage **2'** adjacent the point at which the passage connects with the shallow inclined extension ***3a***, there emerges a passage **14**, the axis of which is disposed at an oblique angle to the axis of the counting channel **3'**. This second passage may be extended to comprise an additional counting channel **15** of larger dimension and suitable for the free passage of white corpuscles, and may for convenience in counting be bent to run in a direction parallel to channel **3'** as illustrated. A counting arrangement similar to that illustrated in Fig. 1 may be disposed below the transparent walls of the channel and a source of light **4'** for directing light rays through the red corpuscle channel to impinge on a photosensitive surface **7'**. This photosensitive surface is connected in a counter-circuit of a type shown in Fig. 1. An additional source of light ***4a***, directed through the channel **15** to a photosensitive surface ***7a***, which in turn is in circuit with a counting device, is incorporated for the purpose of counting the white corpuscles passing through channel **15**. In an apparatus of this type the white corpuscles will be diverted by the inclined wall and the edge **16** of the two openings into the channel **14**, which in most cases should have a cross sectional dimension of not less than about 24μ. The narrower channel **3'** may have the same width as channel **14**, but should be substantially less in height, for example about 5μ.

In accordance with a further embodiment of my invention, blood corpuscles may be precisely counted by means of apparatus such as is disclosed in Figs. 1 and 2 under conditions such that the counting mechanism is sensitive to only the form of corpuscle to be counted. In such a method of operation a channel member having a diameter at least as large as the largest white corpuscle is employed. The red corpuscles are made colorless, for example by means of acetic acid, while the visibility of the white corpuscles is increased by the application of an appropriate dye. Under such conditions only the colored white corpuscles will register in the counting mechanism.

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