Dec. 5, 1972 J. J. J. STAUNTON 3,705,048

CLINICAL SPINNER

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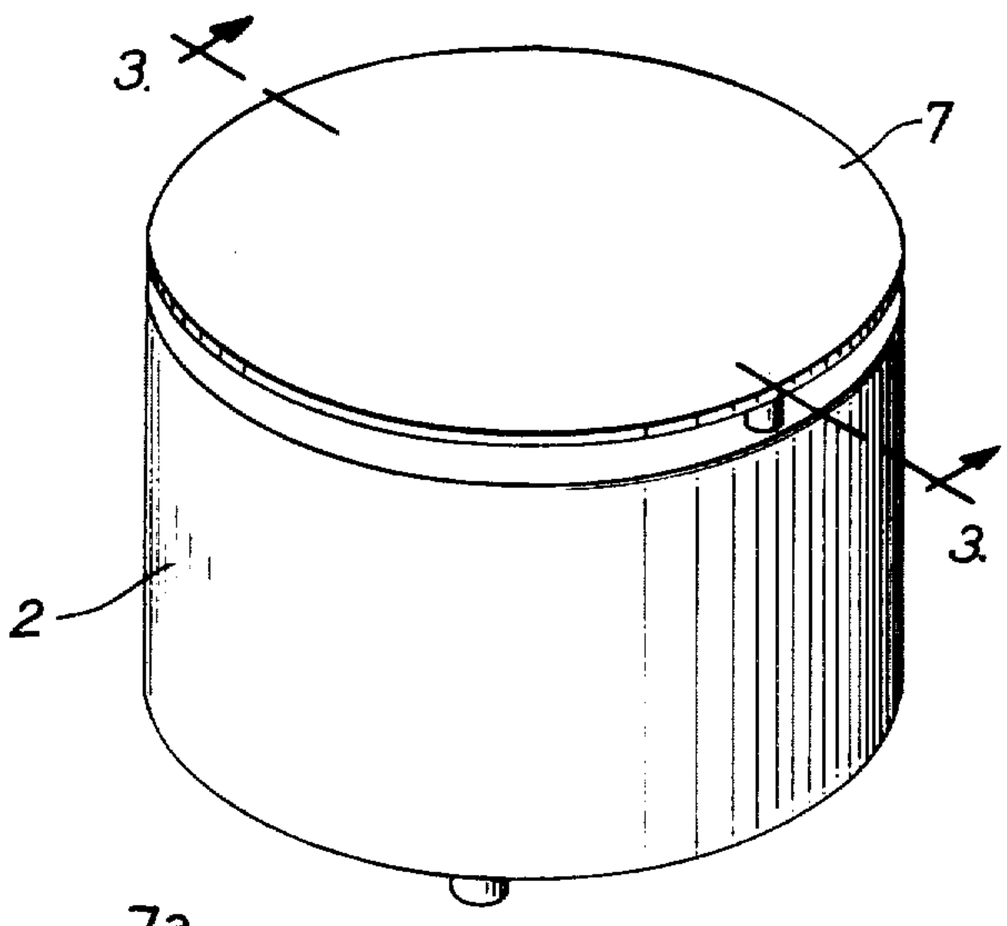
Fig. 1



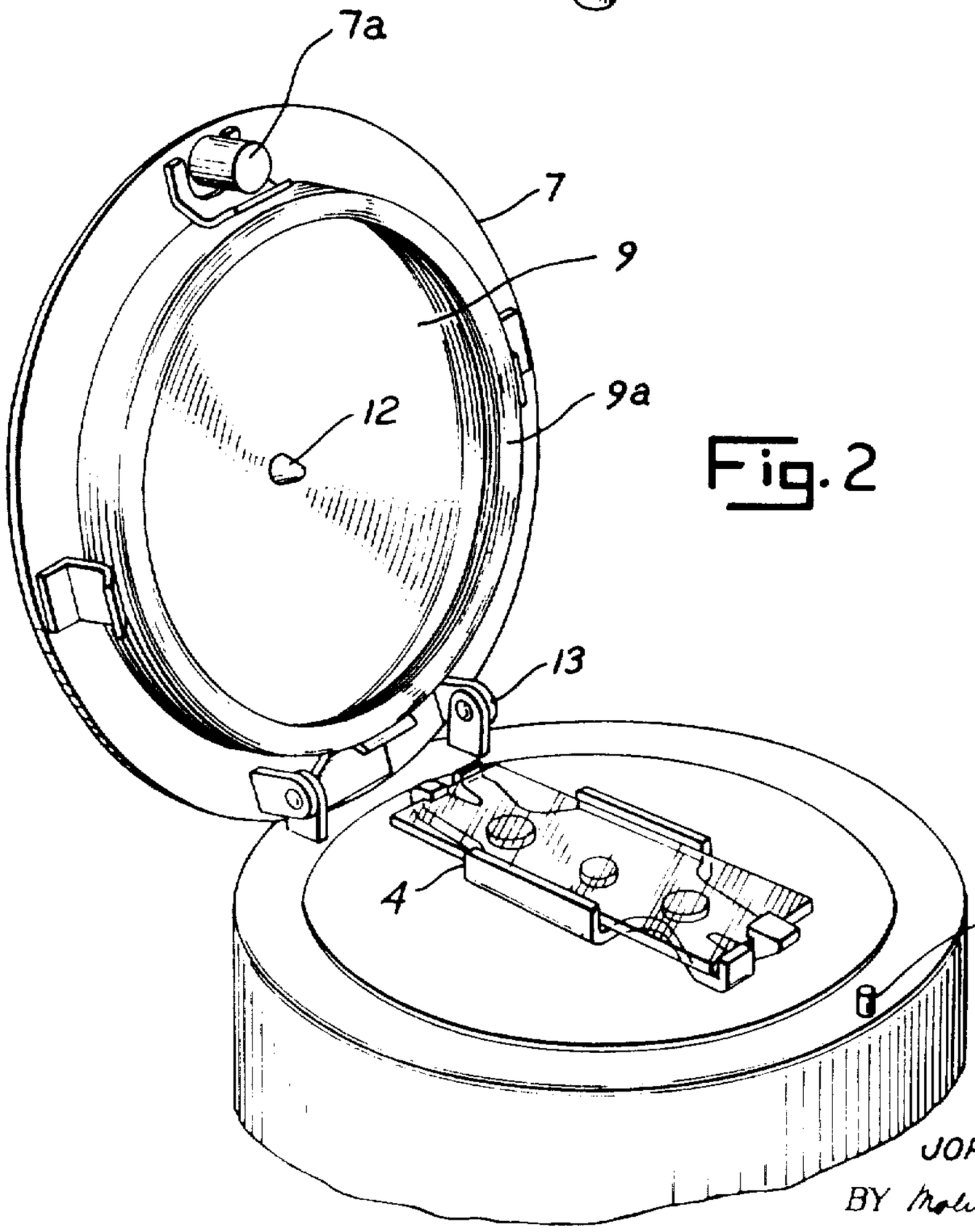


Fig. 2

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CLINICAL SPINNER

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Fig. 3

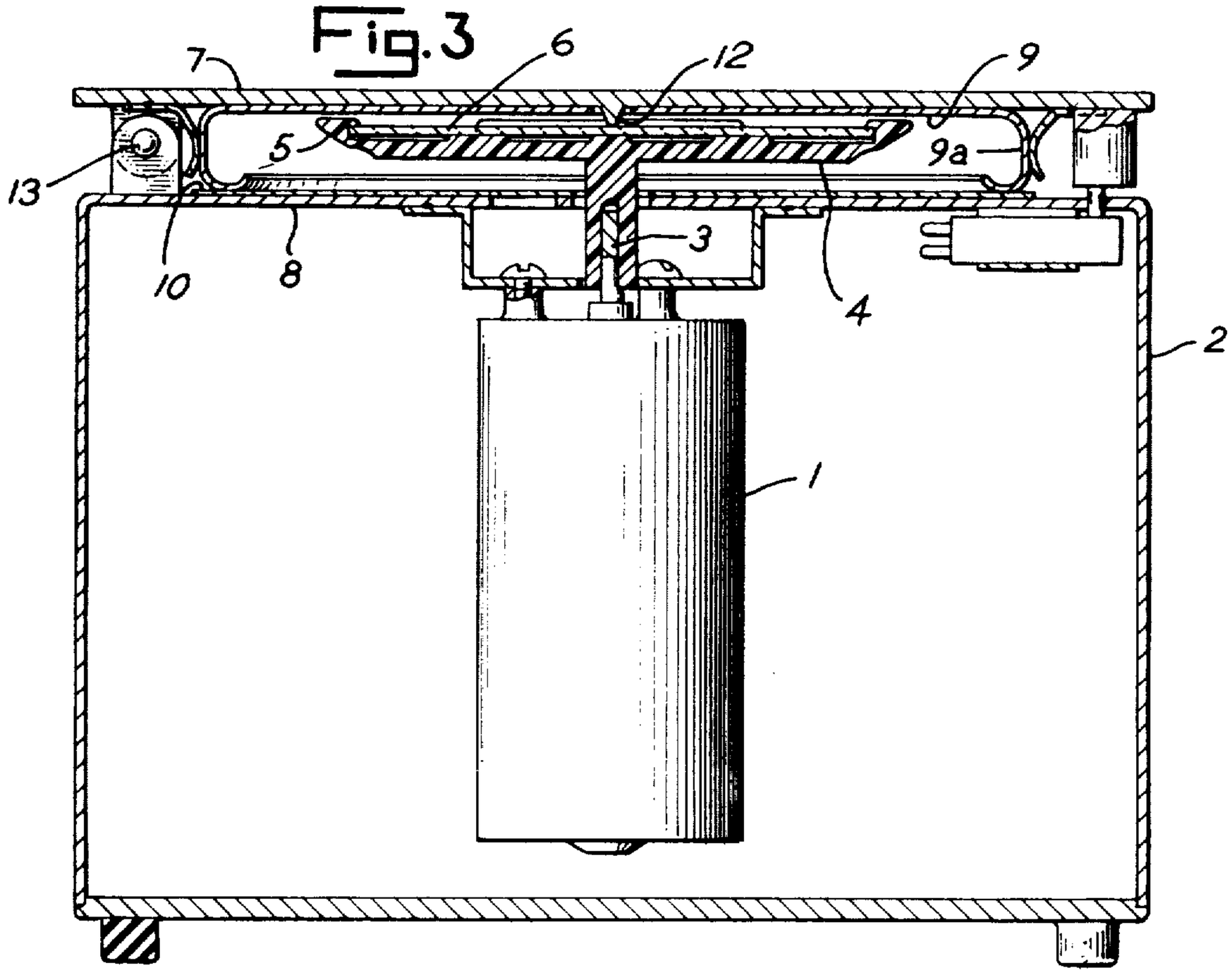
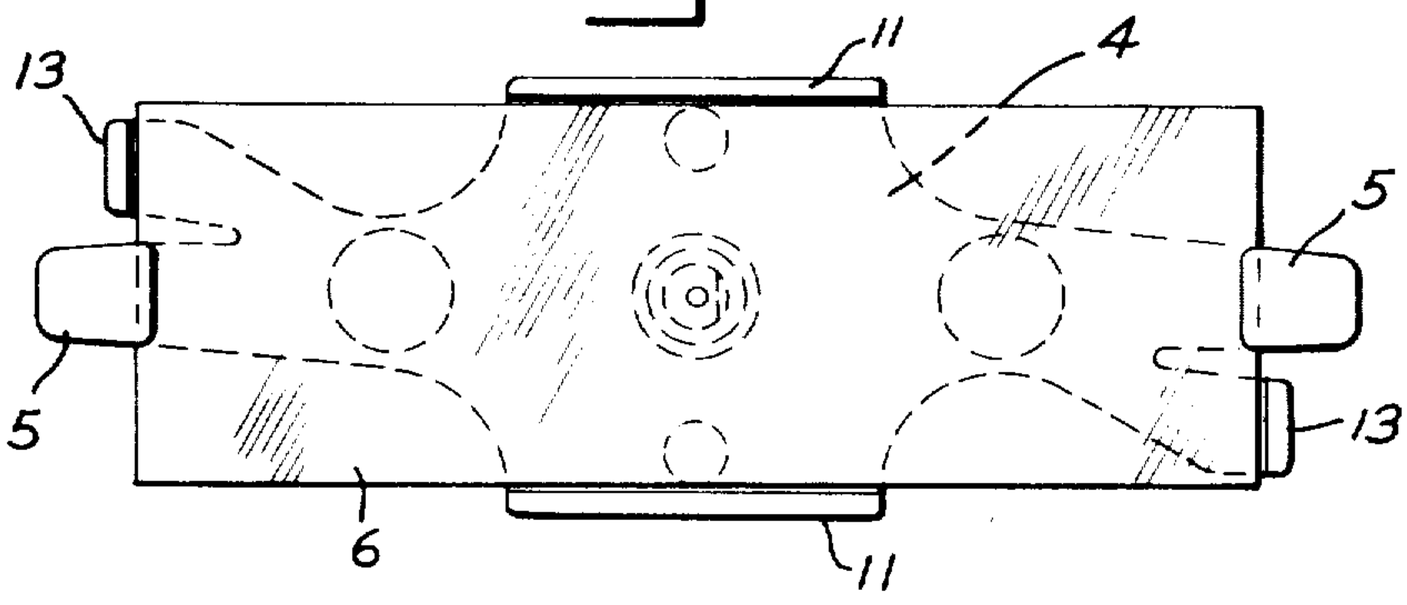


Fig. 4



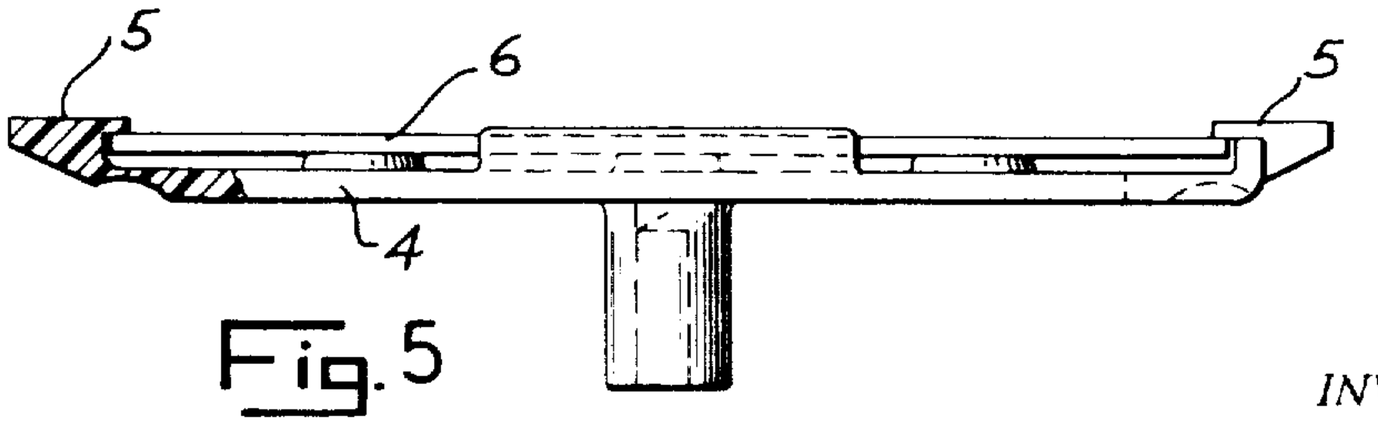


Fig. 5

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CLINICAL SPINNER

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Int. Cl. B44d 1/02
U.S. Cl. 117—3 7 Claims

ABSTRACT OF THE DISCLOSURE

An improved method and apparatus for preparing blood and similar samples for microscopic examination in which the sample is deposited on a slide and the slide is spun at high speed to throw off the excess liquid and leave a thin smear or film. The sample is shielded from air currents by means of a cover which fits over the rotating holder to which the sample slide is clamped. By shielding the sample it is protected from air buffeting which prevents distortion of the blood cells and obviates diagnostic errors due to change in cell shape. After the cells spread in a uniform layer, it is important to arrest rotation of the slide to prevent excess drying of the smear, which also results in distortion of the cell shape.

BACKGROUND OF THE INVENTION

This invention relates to a method and apparatus for preparing monolayer smears of blood and similar biological fluids for diagnostic evaluation. More particularly, the invention relates to an improved spinning device and method in which the liquid sample is protected from the cell-distorting influence of air moving rapidly past the sample.

PRIOR ART—THE PROBLEM

It has been proposed heretofore to produce a monocellular layer of blood for counting the cells therein by placing a couple of drops of blood on a glass slide, spinning the slide on a rapidly rotating holder to throw off the upper excess layers and to dry the very thin blood smear remaining on the slide. The dried sample can then be microscopically examined to count the erythrocytes and/or leucocytes. I have found that this prior procedure causes the erythrocytes to become distorted. These concave lenticular cells lose their characteristic central pallor (thin central region) and become rounded in appearance. Thus distorted, they resemble abnormal cells called spherocytes. This causes unacceptable diagnostic errors.

This undesirable distortion appears to result (1) from excessive drying as the sample continues to spin freely in air after the excess layers have been thrown off, and (2) from the buffeting and tumbling of the air currents set up by the rotating holder and the slide clamped thereto. The distortion of the cells does not result primarily from centrifugal force as might be expected. It appears that the residual liquid or serum, upon drying, tends by surface tension to pull down the relatively compliant cells flattening them out as discs. If the disc-like cell is on edge before flattening, the central pallor will be lost when flattened and the cell will incorrectly simulate a spherocyte.

SUMMARY OF THE INVENTION

The undesirable distortion of the erythrocytes in blood smears can be obviated by shielding the sample from surrounding air with a suitable enclosure during spinning and by accurately controlling the length of time the sample continues to spin after the excess liquid has been thrown off. The enclosure greatly reduces the radial pumping of air since there can be no central influx of air to the space between the enclosure cover and the slide. The air film confined between the holder and the enclosure cover is sheared into many small vortices which are effective in a gentle way in separating and toppling any erythrocytes that are standing on edge. Some spin time must be allowed to move these cells a bit so that those which are initially superimposed, and therefore, project above the liquid layer, are slid about to cause the cells in the film to spread out in a uniform monocellular layer. However, the spinning must be stopped while the serum is still wet so that the cells will not be caught and immobilized by the drying, increasingly-viscous serum while on edge and then be flattened out to simulate spherocytes. The displacement process of the moving air is a speed function, the rate of drying is an inverse speed function; hence there is a speed-time tradeoff that must be observed. The product of the velocity and the time equals a constant.

To protect the sample from the undesirable buffeting and drying, I provide a spinning apparatus having a cover completely enclosing the sample and spaced from 0.1 to 0.3 inch from the surface of the slide on which the sample is mounted. Additionally, the cover protects from the hazard of flying glass should the slide become disengaged from its holder and also serves to catch the slung-off sample for subsequent disposal. Preferably the cover is made from a disposable material so that it can be thrown away after use, thus avoiding the handling of possibly infectious samples.

DETAILED DESCRIPTION

Apparatus illustrating the invention is shown in the accompanying drawings in which:

FIG. 1 is a perspective view of the spinning apparatus;

FIG. 2 is an enlarged perspective view showing the cover and spinning portions of said apparatus;

FIG. 3 is a view taken along the line 3—3 of FIG. 1;

FIG. 4 is a plan view of the sample holder; and

FIG. 5 is a longitudinal sectional view through said holder.

A 12 volt permanent magnet DC motor **1** which has a starting torque of about 15 in. lbs. is vertically suspended from the top panel **8** of a suitable housing **2**. The motor shaft **3** has a flat or is splined for torque transferral and projects upwardly through panel **8** to receive an elongated holder **4**, preferably made of disposable plastic and having a hub that mates with the motor shaft. A microscope slide **6**, 25 x 75 x 1 mm. is retained by spring clips **5** mounted on either end of the holder. The clip may be integrally molded with the holder **4** from resilient plastic material. Side tabs **11** and end stops **13** are provided to locate the silde on the holder. A cover or cover frame **7** is hinged at **13** to the top panel **8** and a stiff paper or flexible plastic liner **9** having a downwardly-turned flange **9***a* is fitted into the cover. The liner **9** serves to catch the excess sample that is slung-off when the slide is spun. It also acts as a safety device should the slide become disengaged from the holder **4** during spinning. Primarily, however, the cover in cooperation with top panel **8** serves to isolate the sample on the slide from all air except that which is enclosed within the cover.

A flat paper disc **10** may be provided under the holder and supported by panel **8** as a second liner. Both liners and the holder **4** may be disposable to minimize clean up problems and also as a safety feature when handling infectious samples. It will be apparent that other configurations may be used for the liners.

The spacing between the cover **7** and the slide **6** must be accurately defined. Accordingly, I have provided spacing gauge or tip **12** integrally molded with the cover **7** and projecting coaxially with the spin axis from the underside of the cover. When the cover is closed over the slide, this tip contacts the center of the slide causing the cover to be spaced a predetermined distance therefrom.

The cover may be formed slightly convex toward the slide so that it will flatten out as the tip contacts the slide to provide substantially uniform spacing. By locating the gauge tip coaxially with the motor shaft **3**, it does not interfere with the movement of the slide during rotation. It also provides a safety hold-down for the slide should the clips **5** on the holder perform ineffectively. In the form of the invention shown in the drawing, a stud **7***a* on the cover **7** depresses the motor switch **11** mounted beneath panel **8** to initiate the spin cycle. The spin cycle is preferably electronically controlled by circuitry well known in the art. Such controls regulate accurately the period of time the motor accelerates, power cut off and braking of the motor shaft.

In operating the apparatus, a clean dry microscope slide is clipped into the holder. If desired, a number of holders can be preloaded preparatory to being placed in the spinner, one at a time, for spinning. A blood sample, typically 100μl. (2 drops) is deposited on the slide to cover the area where the final smear is desired. Usually the deposit will be near one end of the slide. Although reference is made to blood in illustrating the invention, it will be understood that other biological fluids and suspensions, such as tissue and bone marrow, may be treated in like manner. Any area of the slide not initially wet will be free of blood after spinning. When the slide is in place, the cover **7** is closed to actuate the motor and initiate the spin cycle. The motor accelerates typically for 0.05 second and reaches a predetermined speed of say 4000 r.p.m. during this period. The excess sample will be spun off at about three-quarters of this time. Upon reaching the desired pre-selected speed, the power to the motor is cut off and the spinning slide coasts at the pre-selected essentially constant speed for a predetermined time period, say 0.75 second. At this point, rotation is arrested rapidly for instance in 0.5 second or less. This may be accomplished by reverse excitation or a short applied to the motor. Thus the total time for the complete spinning cycle at 4000 r.p.m. is 1.3 seconds. The cover is then opened and the slide removed. The resulting monocellular smear will be dry in 10 to 15 seconds and ready for staining and examination.

I have found that a trade-off relationship exists between the lineal speed of the sample and the spin time. For example, instead of spinning at 4000 r.p.m. for 0.75 second, equivalent results may be achieved at 6000 r.p.m. and 0.5 second, when all other variables are held constant. The product of the speed and the time equals a constant which when observed will assure that the device will produce spin smears showing acceptably small distortion of the erythrocytes and leucocytes. Although the speed is conveniently specified in r.p.m. it will be understood that the significant speed is lineal. Hence the greater the distance between the sample and the shaft center, the higher will be the lineal speed of the sample.

After the motor is accelerated, the excess blood flies off during the initial 50 millisecond acceleration period. After this period, no further liquid is removed. The residual undried layer is about 2 or 3 microns thick. The erythrocytes in the blood have a concave lenticular shape about 1.5 to 3 microns thick and 7 microns in diameter. The leucocytes have a diameter of about 7 microns on the average but are more nearly spherical. These variously sized cells project up through the residual film surface like rocks on the water's edge.

I have found that excessive drying of the film while spinning results in distortion of the cells. If the rotation is discontinued immediately upon reaching full speed, the cells are still wet with serum and left in strings with some overlapping others rather than separated and easy to count. The length of time during which spinning continues after the excess liquid has been thrown off is critical. It must be sufficiently long to cause the cells to be spread out in a single layer. However, it must not be so long that the smear dries. The cover which encloses an air barrier protects the erythrocytes from becoming distorted due to the violent motion of the air, and slows the drying rate.

Examination of the blood cells in the central part of the spun smear adjacent to the axis of rotation, as compared with those in parts of the smear remote from the axis, indicates that the average separation and the displacement of the central pallor is largely random in direction and the amount of displacement is independent statistically of the length of the radius vector to the cell.

Consequently, the separation and distortion effects are not due primarily to centrifugal action but are largely caused by the air flow. In the absence of the cover, the flow of air is very strong and is radially directed away from the center at the slide surface due to centrifugal pumping, which results in distortion of the cells. If the distance from the sample to the cover increases beyond 0.3 inch, the air flow is increasingly strong and must be avoided to minimize distortion.

It is also important that the spinning be discontinued immediately after the cells have been separated and formed in a monolayer. This separation is affected in less than 0.5 second total time at 6000 r.p.m., whereupon the brake is applied. Continued high-speed spinning, even with the cover in place, causes the residual liquid or serum to dry and by surface tension to pull down the relatively compliant erythrocytes, flattening them out so that they assume the appearance of spherocytes.

Heretofore, the normal location for the blood smear is centrally disposed over the middle third of the slide, an area about 25 x 25 mm. It was previously considered to be necessary to locate the smear in this place in order to achieve uniform thickness and uniform statistical distribution of the cells. Since I have found that centrifugal force is not the decisive factor in these matters, I prefer to locate the smear on an area of about 25 x 25 mm. adjacent to an end of the slide. This makes the specimen easier to handle, allows more room for an identification label and avoids a hole in the smear where the gauge tip contacts the slide. It should be pointed out that the farther the smear is removed from the center, the lower the speed-time product because linear speed is the controlling factor not the number of revolutions at which the motor shaft rotates. Of course, in operating the present apparatus, the smear may be centrally located if desired.

It is to be understood that the embodiment of the invention which has been described is merely illustrative of one application of the principles of the invention. Numerous modifications may be made to the disclosed embodiment without departing from the true spirit and scope of the invention.

What is claimed is:

1. In a spinner device for the generation of monocellular layers of biological media,

a first, smooth surface for the reception of said media,

means to spin said first surface about an axis normal to said first surface at a rate sufficient to detach the part of said media not contiguous to said first surface by centrifugal action,

an enclosure surrounding said first surface, the improvement comprising

a second, continuous, stationary surface parallel to and spaced a predetermined distance above said first surface within said enclosure.

2. The improvement of claim **1** wherein said second surface is integrally associated with means to intercept the excess spin-off of said media.

3. The improvement of claim **1** wherein said predetermined spacing is approximately 0.1–0.3 inch.

4. A spinner device for generating blood smears comprising:

a horizontally disposed panel,

a motor suspended beneath said panel and having a shaft extending upwardly through said panel,

a sample holder removably secured to said shaft, said holder carrying means for securing sample slides thereto,

a cover enclosing said holder in a confined space above said panel,

said cover being spaced from said sample slide when the latter is mounted in the holder a distance of from 0.1–0.3 inch.

5. The spinner device of claim **4** in which said cover carries a disposable liner to catch blood thrown from the sample during spinning.

6. The spinner device of claim **5** in which said disposable liner is provided at the periphery of said confined space.

7. A method of producing a monocellular smear of a blood sample containing cells for microscopic examination which comprises:

depositing a minute quantity of said blood on a slide,

spinning the slide while shielding it from air currents for a period of time sufficient to spin-off the excesss

liquid and leave wet cells a few microns in thickness,

continuing to spin the slide to cause the cells in the wet film to spread out in a uniform monocellular layer and to partially dry the film, and

immediately arresting rotation of the slide to prevent further drying and distortion of the cells.

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U.S. Cl. X.R.

23—230 B, 258.5; 117—101; 118—52; 233—1 R; 424—3