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(71) Applicant
Fuji Photo Film Co., Ltd.,
No. 210, Nakanuma,
Minami Ashigara-shi,
Kanagawa, Japan
(72) Inventors
Masao Kitajima,
Fuminori Arai,
Asaji Kondo
(74) Agent
Gee & Co.,
Chancery House, Chancery
Lane, London WC2A 1QU

(54) Sheet Material for Blood Analysis

(57) An analysis sheet for determining the hematocrit value (volume % of erythrocytes) of blood comprises (a) a water-impermeable planar support having thereon (b) a porous, fibrous or non-fibrous spreading layer which has a hydrophilic surface and in which the surface of the internal voids or the interior is hydrophilic and water-insoluble, with, optionally, a binder layer (c) between support (a) and layer (b).

A non-fibrous spreading layer can have a mean pore diameter from 0.1 to 2.5 μm. A fibrous layer can be made of a two-fold fabric of a porosity of 20 to 80%. The spreading layer thickness is 30 μm to 3 mm.

For hematocrit determination, a drop of whole blood is applied to the porous surface and it spreads uniformly therein to form two concentric circles, the inner of which is more colored and contains the erythrocytes. The diameter of at least the outer circle is measured and is compared to a calibration graph to indicate the hematocrit value.

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FIG. 1

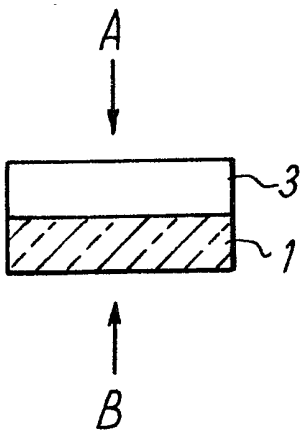


FIG. 2

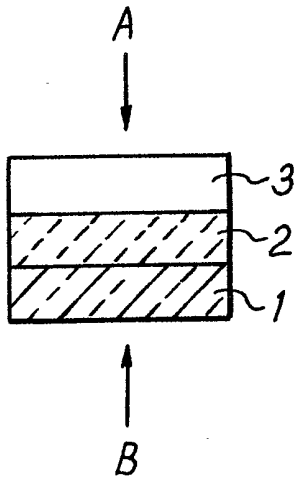


FIG. 3

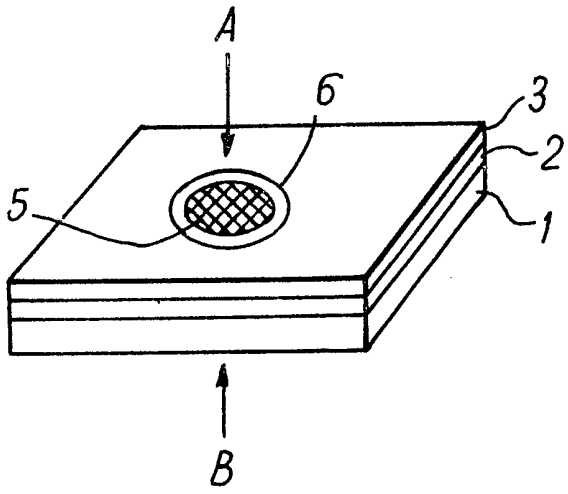


FIG. 4

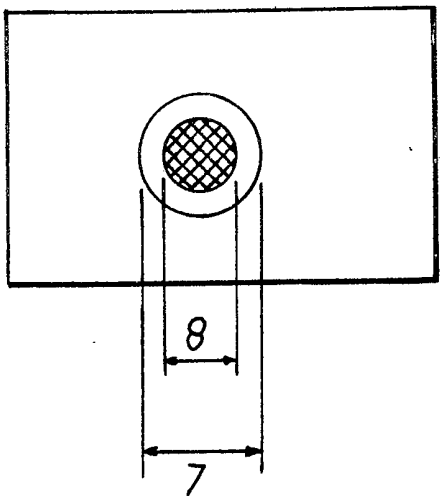
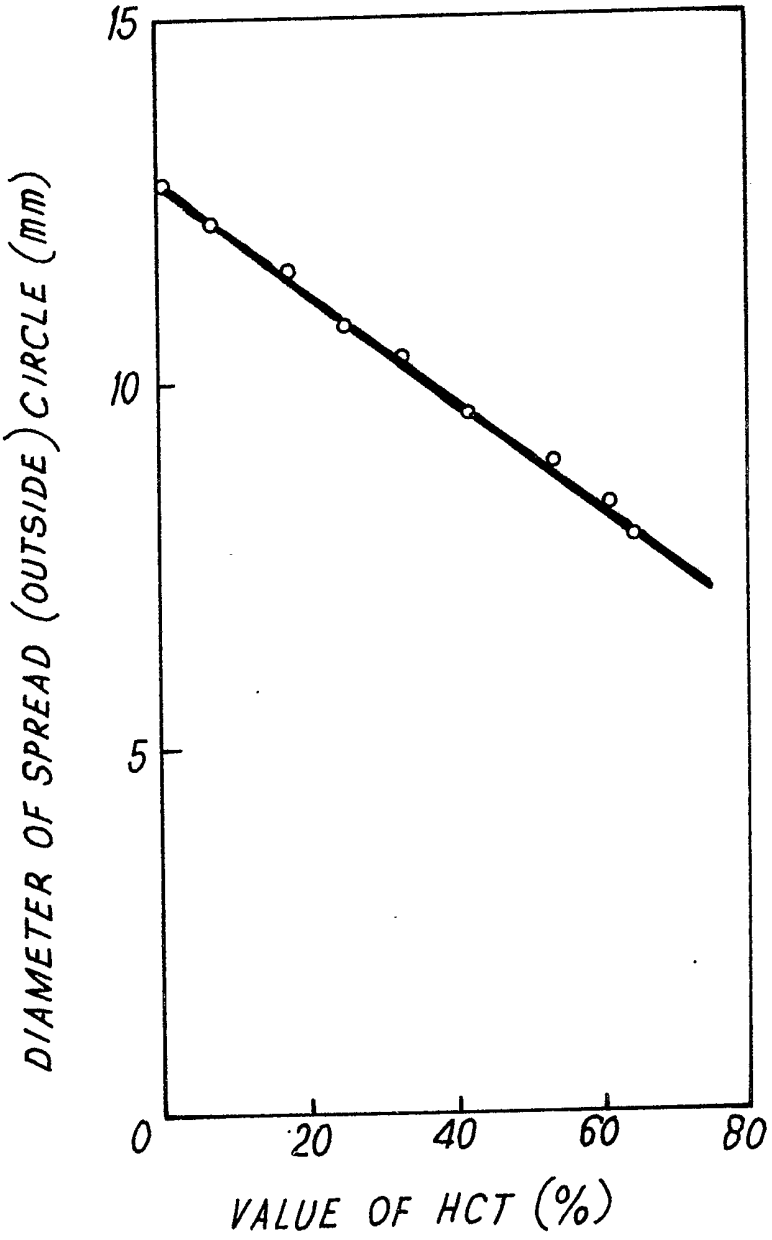


FIG. 5


15
10
5
0
20
40
60
80
DIAMETER OF SPREAD (OUTSIDE) CIRCLE (mm)
VALUE OF HCT (%)

SPECIFICATION

Sheet Material for Blood Analysis

This invention relates to a layered sheet material for determining the hematocrit value of blood, which is an important test item in the field of medical, clinical or physiological hematology,and to a method of use thereof.

As is well known, blood is composed of plasma and cell ingredients (i.e., erythrocytes, leucocytes and thrombocytes). The volume percentage of erythrocytes based on the volume of whole blood is called the hematocrit value, which is an important value to know in assessing the degree of anemia of a patient. Methods for determining the hematocrit value (hereinafter abbreviated as "HCT") that have so far been utilized include a centrifuging method, an electrical resistance method, and measurement of specific gravity. However, these methods have the defects that they require a long time and complicated wet procedures.

An object of the present invention is to provide a simple and dry HCT-determining material which enables HCT to be determined by only applying thereto one drop of an extremely small amount of whole blood.

The present invention provides a blood analysis sheet material comprising a water-impermeable planar support having provided thereon a porous spreading layer which has a hydrophilic surface and in which the surface of the internal voids of the interior is hydrophilic and water-insoluble (such being hereinafter simply referred to as "hydrophilic"), the porous spreading layer being such that blood can be spread therethrough depending upon the spreading nature, e.g. viscosity of the blood.

A binder layer can be provided between the support and the porous spreading layer.

The invention will be described with reference to the accompanying drawings, wherein

Figure 1 is a schematic sectional view showing one embodiment of an HCT-determining material comprising a water-impermable planar support having directly provided thereon a hydrophilic porous spreading layer;

Figure 2 is a schematic sectional view showing one embodiment of an HCT-determining material comprising a water-impermeable planar support having provided thereon, in sequence, a binder layer and a hydrophilic porous layer;

Figure 3 is a perspective view showing a spread blood sample applied to a porous spreading layer of the HCT-determining sheet shown in Figure 2;

Figure 4 is a surface view showing a blood sample spread through a porous spreading layer of a sheet as shown in Figure 1 or 2; and

Figure 5 is a graph showing the relationship between HCT as determined in Example 1(C), and the diameter of the spread (outside) circle.

Figure 1 shows the simplest form of the sheet of the present invention, wherein a porous spreading layer 3 is provided on a water-impermeable support 1 to form an integral sheet.

In Figure 2, a binder layer 2 and a porous spreading layer 3 are provided on a hydrophilic planar support 1 to form an integral sheet. The binder layer 2 is not an essential but, in a usual production process, It is preferably used in order to adhere the hydrophobic support 1 to the porous spreading layer 3 in a "fluid contact state", which state is defined in U.S. Patents 3,922,158 and 4,042,335 and U.S. Reissue Patent 30,267.

In determining HCT, a drop of whole blood is applied to the HCT-determining sheet of the present invention from side A of the porous spreading layer, as shown in perspective in Figure 3. The amount of whole blood applied is suitably between 1 and 100 μl, for example 10 μl. The thus applied whole blood immediately spreads, within several tens of seconds, into the hydrophilc porous spreading layer concentrically in a horizontal direction to form a colored concentric inside circle 5 containing erythrocytes and a less colored concentric outside circle 6; the spreading of a sample is thus completed.

The HCT can be determined, as shown in Figure 4, by measuring the diameter of the spread outside circle 7 or both the diameter of the spread outside circle and that of the spread inside circle 8, by viewing from the direction indicated by arrow A or, with a transparent support, arrow B, and referring to a previously prepared conversion table or calibration, e.g. as in Figure 5.

The spreading layer for the HCT-determining sheet of the present invention can be a fibrous or non-fibrous porous membrane having a hydrophilic and water-insoluble surface and having internal voids whose surface is hydrophilic and water-insoluble or having a hydrophilic and water-insoluble interior. The desired degree of porosity varies depending upon the degree of hydrophilicity, state of the pores and form and distribution of pores, and it is difficult to set forth specific ranges. By way of general guidance, however, where the spreading layer comprises a non-fibrous porous material, the mean pore diameter can range from 0.1 μm to 2.5 μm, preferably from 0.5 μm to 2 μm and, where the spreading layer comprises a fibrous porous material such as a fabric (broad cloth), fabrics formed using two folded yarns with a yarn number count of 100 to 200, preferably from 120 to 160, are used. A suitable degree of porosity is from 20 to 80%, preferably from 25 to 80%.

Such fabrics can be rendered hydrophilic as described for the absorbent layer in U.K. Specification No. 2,013,338. Fibrous fabrics which can be used may be knitted or woven, and can be

composed of natural or synthetic fibres or mixed yarns, natural fibres and synthetic polymers.

Hydrophilic porous spreading layers which can be used include non-fibrous porous membranes as disclosed in U.S. Patents 3,553,067, 3,594,263, 3,922,158 and 4,050,898, those prepared by rendering hydrophilic non-fibrous porous membranes, porous particulate membranes and sheets composed of glass fibers or papers. Of these, membrane filters having the characteristics described hereinafter are particularly preferred; such membrane filters can have a pore size of from 0.1 μm to 3 μm, preferably 0.4 μm to 2 μm.

The thickness of the porous spreading layer is in practice at least 10 μm and in view of the amount of liquid applied, measurement accuracy and handling properties, the thickness generally ranges from 30 μm to 3 mm, preferably from 50 μm to 1 mm, more preferably from 100 μm to 500 μm.

The porous spreading layer used in the present invention may have a uniform structure composed of a single material or may be formed by combining two or more materials having different physical properties so as to obtain improved properties.

Suitable processes for integrally providing a porous spreading layer on a water-impermeable planar support or on a binder layer include integrally laminating a previously prepared porous membrane or fabric on the support or the binder layer, and coating a solution or dispersion capable of forming a porous spreading layer on the support or the binder layer to thereby integrally form a porous spreading layer.

The porous spreading layer to be used for the hematocrit value determining sheet of the present invention must possess properties such that the surface to which the sample is to be applied and the surface of voids in the porous spreading layer or the interior thereof have high hydrophilicity and that, when applying a drop of blood thereto, the blood spreads almost isotropically within the plane to which the blood is applied. In order to obtain such properties, the porous materials must be subjected to a treatment to render such hydrophilic. Suitable treatments which can be used are a surface treatment using a hydrophilic compound such as a cationic, anionic, or nonionic, surfactant or a plasticizer, or a colloid (e.g., gelatin), a treatment to render such hydrophilic by, for example, hydrolysis with an acid or an alkali, a treatment of chemically rendering such hydrophilic by reaction with a hydrophilic compound, a corona treatment, a flame treatment, an ultraviolet light irradiation treatment, an electric discharge treatment, a vacuum deposition treatment or a spraying treatment.

Addition of agents for stabilizing the hue of hemoglobin, such as a pH buffer, an anticoagulant, an antihemolytic agent, an antioxidant or an oxidizing agent, and erythrocyte membrane-modifying agents such as a dye, a pigment, an inorganic salt, an aldehyde, an isocyanate or hydrogen peroxide to the porous spreading layer serves to improve accuracy or improve procedures like readout.

Suitable water-impermeable planar support materials which can be used to form the HCT-determining sheet of the present invention include plate-like materials of metal, wood, paper or glass, a synthetic resin film such as of polyester (e.g., polyethylene terephthalate, bisphenol A polycarbon), cellulose ester (e.g., cellulose diacetate, cellulose triacetate, cellulose acetate propionate) or polymethylmethacrylate. The thickness of the water-impermeable planar support ranges from 10 μm to 3 mm, preferably from 20 μm to 1 mm, most preferably from 50 to 500 μm. Of these, transparent planar supports are convenient because they permit the diameter of the spread circle to be measured from either side of the HCT-determining sheet.

When the sheet of the invention includes a binder layer, this functions to uniformly adhere the porous spreading layer to the support in a fluid contact state and to control the spreading of a blood sample through the porous spreading layer within a suitable range.

A hydrophobic binder may be used for the binder layer as long as it does not completely repel water, but, for determining HCT with high accuracy, it is desirable to primarily used a hydrophilic binder.

A blood sample applied to the porous spreading layer is uniformly spread within the spreading layer and, when a hydrophilic binder layer is employed, it simultaneously permeates into the binder layer. The binder layer has essentially semipermeable properties, and hence water and low molecular weight compounds diffuse and permeate into the binder layer while particles such as erythrocyates, leucocytes, thrombocytes, etc. do not permeate into the binder layer but are uniformly spread into the porous spreading layer and at the interface between the porous spreading layer and the binder layer. High molecular weight compounds such as albumin and globulin penetrate or do not penetrate into the binder layer depending upon the molecular weight and crystallinity of the binder polymer constituting the binder layer matrix.

Suitable hydrophobic binders which can be used for the binder layer include thermoplastic resins such as polyvinyl acetate, polystyrene, polymethyl methacrylate, vinyl chloride-vinyl acetate copolymers, cellulose esters, etc. and the thermosetting resins such as polyurethanes, melamine, etc.

Suitable hydrophilic binders which can be used for the binder layer include a wide variety of materials such as natural high molecular weight materials (e.g., gelatin, agarose, dextran) and hydrophilic synthetic high molecular weight materials (e.g., polyvinyl alcohol, polyacrylamide,

polyacrylic acid). Of these, gelatin for photographic use is most preferred due to its excellent swelling properties for water, gel-forming capability, adhesion properties, water-absorbing properties, etc. and ease of production.

Various additives may be added to the binder layer for the purpose of stabilizing the hue of hemoglobin and preventing penetration, diffusion, or precipitation of hemoglobin, as well as controlling spreading. Illustrative additives include low molecular weight or high molecular weight materials such as nonionic, cationic, or anionic surfactants, plasticizers, inorganic salts, organic acid salts, pH buffers, pigments, dyes, solid fine particulate fibers, oxidizing agents, reducing agents, acids, alkalis, etc. For the purpose of facilitating readout of the diameter of the spread circle, a pH indicator which becomes colored or discolored depending upon the pH of the blood serum, or a color reagent which reacts with a blood serum ingredient such as albumin to produce a color such as Bromocresol Green can be used.

The binder layer may in some cases be formed by two or more sub-layers for the purpose of improving adhesiveness and ease of readout and preventing curling. The thickness of the binder layer can range from 0.1 μm to 1 mm, preferably from 1 μm to 100 μm, particularly preferably from 5 μm to 50 μm.

The HCT-determining sheet of the present invention can also be used in a process of determining HCT by merely measuring the diameter of the spread circle of blood formed by applying a drop of blood onto a porous spreading layer of the sheet.

This process is based on the principle that the hydrophilic porous spreading layer is greatly dependent, in its blood-spreading properties, upon the viscosity of the sample, or the content of particulate ingredients in the blood. Thus, this porous spreading layer has different characteristics from that of the above described spreading layer used in multi-layered integral analytical elements for chemically analyzing blood and described in Japanese Patent Application (OPI) Nos. 53,888/74 (corresponding to U.S. Patent 3,922,158) and 131,786/77 (corresponding to U.S. Patent 4,050,898) and U.K. Specification No. 2,013,338. That is, the spreading layer used in the multi-layered integral analytical elements must possess the above-described characteristics such that, when a definite amount of liquid is applied onto the sheet, a definite liquid-spread area results so that quantitative analysis can be conducted. In this case, the liquid-spread area must be definite even when the viscosity of the sample to be analyzed somewhat varies, and various means are incorporated therein to attain such characteristics.

On the other hand, the hydrophilic porous spreading layer used in the HCT-determining sheet of the present invention is such that the ease with which blood spreads is directly dependent upon the viscosity of the blood. That is, the porous spreading layer has characteristics such that blood with a low viscosity, i.e., blood with low HCT, forms a spread circle with a larger diameter due to the greater ease with which the blood spreads, whereas blood with a high viscosity, i.e. blood with high HCT, forms a spread circle with a smaller diameter due to the lesser ease with which the blood spreads. This means that the diameter of the spread circle and the viscosity of blood, or hematocrit value, are directly related to a negative proportional constant. In determining HCT using the HCT-determining sheet of the present invention, the relation between the diameter of the spread circle (or the area of the spread circle) and HCT is previously obtained by using a definite amount of standard blood, thus preparing a conversion table or a calibration curve. The HCT of an unknown blood sample can be determined simply by applying the sample to the porous spreading layer in the same amount as used in preparing the conversion table or the calibration curve, and measuring the diamater or the area of the spread circle.

The HCT-determining sheet of the present invention has another important advantage in that HCT can be determined without accurately weighing a blood sample. Blood is composed of a hemocyte component and a blood serum component and, when applied onto the surface of the porous spreading layer of the HCT-determining sheet in accordance with the present invention, double circles are formed as a result of spreading, with the inside circles containing the hemocyte component and the outside circle comprising the blood serum component alone. It has now been found that the ratio of diameter or area of the inside circle to that of the outside circle is in a definite relation with the HCT of an applied blood sample. Thus, HCT can be determined without accurately weighing a blood sample, by previously measuring the ratio of diameter or area of the inside circle to that of the outside circle and preparing a calibration curve of HCT versus the diameter or area ratio similarly with the above-described method of spreading a definite amount of sample to measure the diameters of outside and inside spread circles.

The present invention is described in more detail by the following non-limiting examples of the present invention.

Example 1

A. Preparation of HCT-determining Sheet:

A solution containing 0.2% polyoxyethylene nonylphenoxy ether (nonionic surfactant, HS210, made by Nippon Oils & Fats Co., Ltd.) and 10% gelatin was coated on a 185-μm thick transparent polyethylene terephthalate (PET) film subbed with gelatin to thereby form a binder layer of a dry thickness of 15 μm composed of gelatin. This coated layer was swollen with water, and Microfilter FM-120 (a trademark for cellulose tracetate manufactured by Fuji Photo Film Co., Ltd.) of a mean pore size

of 1.2 μm containing 0.5% (by weight) of surfactant, HS210, was press-laminated thereon to prepare an HCT-determining sheet.

B. Preparation of Blood Samples:

A fresh ACD preserved blood (preserved blood containing as preserving agents sodium citrate, citric acid and dextrose) was centrifuged to separate such into a hemocyte component and a blood serum component. Then, they were mixed with each other in various ratios to prepare samples. The HCT of each of the thus prepared samples was measured using an AMCO centrifuge (E-24) and a capillary hematocrit tube. Thus, standard samples having an HCT of from 0 to 70% were obtained.

C. Measurement:

The HCT-determining sheet prepared in (A) above was cut into 30x30 mm squares. 10 μl of each blood sample of 0 to 70% in HCT prepared in (B) was taken up in a micropipette to applied to the square of HCT-determining sheet. 30 seconds after the application, double circles resulted, with the inside circle containing erythrocytes and the outside circle comprising blood serum. The outside diameter of each circle was measured from the side opposite the sample-applied side, i.e., from the side of the transparent PET film. The diameter of the outside circle and the HCT obtained in (C) were confirmed to be in such a relation that the diameter of the (outside) spread circle was in a direct relation with the HCT, with a negative proportional constant, as shown in Figure 5.

Example 2

A drop (about 20 μl) of each blood sample of 7 to 61% in HCT prepared in Example 1, (B), was applied to a 30x30 mm square of HCT-determining sheet prepared as described in Example 1, (A). 30 seconds after the application, the diameter of the inside circle containing erythrocytes and the diameter of the outside circle comprising blood serum were measured to determine the ratio. Thus, the results shown in Table 1 below were obtained. Table 1 shows that the ratio of inside circle/outside circle in diameter is linearly related with HCT.

Table 1

	HCT(%)					
	7	*16*	*27*	*38*	*44*	*61*
Spread Circle Inside Diameter/ Outside Diameter	0.33	0.41	0.48	0.56	0.58	0.67

Example 3

Onto a porous spreading layer of HCT-determining sheet produced as described in Example 1 was applied to 10 μl of fresh blood taken from a human vein using heparin as an anticoagulant. 30 seconds after the application, the diameter of the outside spread circle was measured to be 9.5 mm. The HCT was determined to be 43% from the calibration curve shown in Figure 5.

On the other hand, the HCT of the same blood sample was determined according to the centrifugation method using a hematocrit tube in the same manner as in Example 1, (B). Thus, the HCT was determined to be 43%.

This showed that the HCT determined by using the HCT-determining sheet of the present invention agreed well with the HCT determined according to the conventional centrifugation method using a hematocrit tube.

Example 4

Onto a 18-mm wide commercially available adhesive tape (made by Nitto Electric Industrial Co., Ltd.) comprising a 100 μm thick transparent PET film having coated thereon a pressure sensitive adhesive composition was press-laminated a micro-filter (FM-80, made by Fuji Photo Film Co., Ltd.) of 0.8 μm in mean pore size which had been rendered hydrophilic by impregnation with 0.2% alkylphenoxy polyethoxyethanol (nonionic surfactant, Triton X-405, made by Rohm and Haas) to thereby prepare an HCT-determining sheet.

A drop of each of the standard blood samples prepared in Example 1, (B), was applied to this sheet to measure the diameter of the spread outside circle. A linear relationship was observed between the HCT determined according to the conventional configuration method and the diameter of the spread circle.

Claims

1. A blood analysis sheet material for determining the hematocrit value of blood, comprising (a) a

water-impermeable planar support having thereon (b) a porous spreading layer which is fibrous or non-fibrous and has a hydrophilic surface and in which the surface of the internal voids or the interior is hydrophilic and water-insoluble, said porous spreading layer being such that blood spreads therethrough.

2. A sheet as claimed in Claim 1, wherein the porous spreading layer is non-fibrous and has a mean pore diameter ranging from 0.1 to 2.5 micrometres (μm).

3. A sheet as claimed in Claim 1, wherein the porous spreading layer is water-insoluble.

4. A sheet as claimed in Claim 1, wherein the porous material comprises a non-fibrous porous membrane.

5. A sheet as claimed in Claim 1 or 2, wherein said porous material is glass fiber, a fabric or paper.

6. A sheet as claimed in Claim 5, wherein the fabric is a broad cloth formed using two folded yarns with a yarn number count of 100 to 200.

7. A sheet as claimed in any preceding claim, wherein the porous layer has a thickness of 30 micrometres to 3 millimetres.

8. A sheet as claimed in any preceding claim, wherein the support is transparent.

9. A sheet as claimed in any preceding claim, wherein the support is made of metal, wood, paper, glass or a synthetic resin film.

10. A sheet as claimed in any preceding claim, wherein a binder layer is provided between the support and the porous spreading layer.

11. A sheet as claimed in Claim 10, wherein said binder is a hydrophilic material.

12. A sheet as claimed in Claim 11, wherein the binder is gelatin.

13. A sheet as claimed in any preceding claim, wherein the porous layer also contains an agent which stabilizes the hue of hemoglobin and/or an agent which modifies the membrane of erythrocytes.

14. A sheet for blood analysis, substantially as hereinbefore described with reference to part A of Example 1 or to Example 4.

15. A method of determining the hematocrit value of human or animal blood, which comprises applying to the surface of the porous spreading layer of a sheet as claimed in any preceding claim a drop of whole blood, measuring the diameter or area of at least the outer of the two circles formed on the surface, and calculating the corresponding hemocrit value therefrom.

16. A method as claimed in Claim 15, wherein the amount of blood applied is 1 to 100 microlitres.

17. A method as claimed in Claim 15, substantially as hereinbefore described in Part C of Example 1 or in Example 2, 3 or 4.

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