United States Patent [19]

Katsuda

[54] CAPILLARY TUBE FOR BLOOD EXAMINATION

- [75] Inventor: Itsurou Katsuda, Tokyo, Japan
- [73] Assignee: Jintan Terumo Co., Ltd., Tokyo, Japan
- [22] Filed: Sept. 27, 1973
- [21] Appl. No.: 401,175

[30] Foreign Application Priority Data

- Sept. 29, 1972 Japan..... 47-113033
- [52] U.S. Cl. 128/2 F; 23/253 R; 23/259; 73/425.4 P; 128/2 G; 128/DIG. 5
- [51]
 Int. Cl.²
 A61B 5/14

 [58]
 Field of Search
 128/2 F, 2 G, DIG. 5;
 - 73/425.4 P; 23/253 TP, 253 R, 292, 259

[56] **References Cited** UNITED STATES PATENTS

2,487,077 2,940,448	11/1949 6/1960	Shepherd 23/253 TP X Furlong, Jr 23/259 UX
3,146,163	8/1964	Brewer 128/2 F X
3,518,804	7/1970	Gerarde 23/292 X
3,579,303	5/1971	Pickering 23/292
3,620,676	11/1971	Davis 23/253 TP

[11] **3,898,982**

[45] Aug. 12, 1975

3,732,079	5/1973	Davis 23/253 TP	
3,768,978	10/1973	Grubb et al 23/259	

OTHER PUBLICATIONS

Propper Manuf. Co. Brochure, Rec'd. in Gr. 330, Oct. 10, 1972, p. 1.

Hyland Co. Brochure, Rec'd. in Gr. 330, Oct. 10, 1972, pp. 1–4.

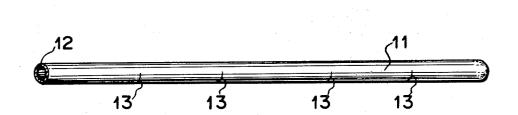
Primary Examiner—Kyle L. Howell Attorney, Agent, or Firm—Kemon, Palmer and Estabrook

ABSTRACT

[57]

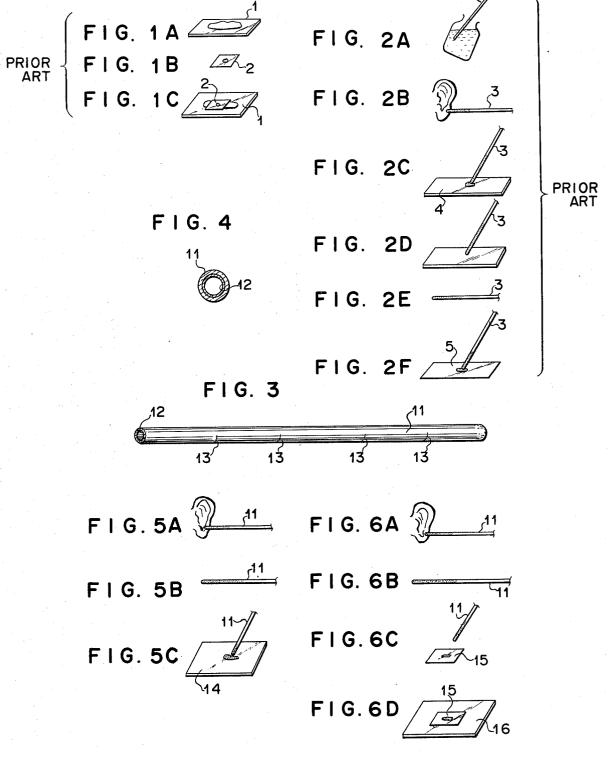
Capillary tubes of the double open-ended type used for blood collection and examination are provided over a portion of their internal surfaces with a dry coating of a reagent, e.g., a colorant for staining blood cells, a phagocytic substance for examining phagocytosis of leucocyte, etc. The blood introduction end of the tube is left uncoated to promote capillary action in blood sampling. The coating may include an anticoagulant and several different reagents may be mixed in the coating or coated at intervals longitudinally of the tube.

9 Claims, 18 Drawing Figures



3,898,982

PATENTED AUG 1 2 1975



5

CAPILLARY TUBE FOR BLOOD EXAMINATION

This invention relates to a capillary tube for use in staining cells and bacteria for observation and in a phagocytosis test of blood corpuscles or leucocyte.

The staining of a blood smear preparation is effected using generally a May-Giemsa staining method. This method is important in the diagnosis of blood disease by observing in detail the size, properties and color tone of cytoplasm, the state of granule, the presence or 10 absence of Auer bodies, inclusion body, and/or the size and shape of nuclear bodies. With the recent advance of medical science, in addition to the May-Giemsa staining method, it is conventional to also stain reticulocytes, mitochondria and intracellar granula. 15

The following are staining methods now used or to be used.

a. general staining

- b. reticulocyte staining
- c. Heinz bodies staining
- d. nuclear bodies staining
- e. mitchondria staining
- f. Golgi body staining
- g. oxidase
- h. peroxidase
- i. PAS staining

A staining capillary tube is designed to simplify these methods and one or all of these methods are intended to be effected within the capillary tube. The general staining includes a May-Giemsa staining; Giemsa staining; Wright staining; and a method using a fluorescent dye. In the last-mentioned method, the fluorescent dye is subject to an ultraviolet ray to produce a varying color tone dependent upon the kind of cells. Staining dyes used are as follows: 35

a. general staining

- Wright solution, Giemsa solution, May-Gruenwald solution, acridine orange (fluorescent dye)
- b. reticulocyte staining
- new methylene blue, brilliant cresyl blue
- c. Heinz bodies staining
- methyl violet d. nuclear bodies staining
- brilliant cresyl blue

e. mitochondria staining

- janus green + neutral red
- pinacyanol + neutral red

Various methods for staining cells such as blood corpuscles etc. have been known. For example, in a pappenheim method as shown in FIG. 1, 0.5 to 1 percent of a brilliant cresyl blue pure ethanol solution (dye solution) is dropped onto a glass plate 1 which is carefully cleaned and somewhat warmed, and is spread thin, if possible, for drying. Then, a droplet of blood is dropped at the center of a cover glass and the cover glass is placed on the glass plate. The resultant sample is sealed by petroleum jelly or fluid paraffin and is allowed to stand for 5 to 10 minutes to obtain a complete sample as shown in FIG. 1C. 60

In a Brecker method as shown in FIG. 2, a dye solution is sucked into a capillary tube to an extent that it reaches about one third of a length of the tube (FIG. 2A). Then, about the same amount of blood is sucked into the tube (FIG. 2B). The resultant solutuion is blow spattered on a slide 4 and intimately mixed (FIG. 2C). Then it is sucked back into the capillary tube (FIG. 2D) and allowed to stand for 10 minutes (FIG. 2E). The so-

lution is again blow spattered on a slide 5 and intimately mixed (FIG. 2F). A part of the solution is taken out and smeared thin on a spreader slider and air dryed. Thus a larger number of steps than those of the pappenheim method are required.

There is an examination method for testing the phagocytosis of leucocytes outside the living body. This method is broadly classified into a slide technique and a test tube method. The former performs a treatment similar to the pappenheim method while the latter performs a treatment similar to the Brecker method. Thus a number of steps are required as in the case of the general staining method.

Accordingly the object of this invention is to provide 15 a capillary tube for staining treatment or phagocytosis test which is capable of effecting a staining treatment or performing phagocytosis test with ease and with rapidity. For this purpose there is provided a capillary tube whose inner wall surface is coated with a dry sub-

20 stance consisting primarily of a dye or a phagocytic foreign matter.

This invention can be more fully understood from the following detailed description when taken in conjunction with the accompanying drawing, in which:

²⁵ FIG. 1 is an explanatory view of a pappenheim method, one of staining methods conventionally practised;

FIG. 2 is an explanatory view of a Brecker staining method;

FIG. 3 is a perspective view of a capillary tube according to this invention;

FIG. 4 is a cross-sectional view of the capillary tube; and

FIGS. 5 and 6 are explanatory views of the capillary ³⁵ tube.

There will be explained, by reference to FIGS. 3 to 6, the case where a staining treatment is effected. In FIG. 3 a reference numeral 11 is a transparent glass capillary tube. On the inner wall surface of the tube a dry material including a dye as a primary component is coated to provide a coating layer 12. The dry material differs in its components dependent upon the object to be stained. For example, where a reticular body of reticulocyte is stained, brilliant cresyl blue and new meth-45 ylene blue are used; where a blood corpuscle dye including an intracellar mitchondria is stained, janus green is used; where the nuclear bodies of blood corpuscle are stained; brilliant cresyl blue is used as a primary component; and where a substance such as leucocyte is stained, a fluorescent substance may be used. When the fluorescent substance is used, an observation can be made by exciting the substance by subjection to an ultraviolet ray etc. and when use is made of a blood from a vacuum fluid container containing an anticoagulant, no particular addition of an anti-coagulant is necessary, i.e., only a dye is required. However, when blood is collected directly from the human flesh, it is necessary to add an anti-coagulant. A plurality of dyes may be contained simultaneously. 60

Deposition of the dry material on the inner wall of the capillary tube 11 is effected, for example, in the following way.

A solution is prepared by dissolving into a solvent such as water, or suspending in a solvent, a dye, phagocytizing foreign matter and anti-coagulant. Then, one end of the capillary tube is inserted into the solution to permit a proper amount of the solution to be intro-

2

5

10

duced, under a capillary action, into the interior of the capillary tube. A hot air is blown into that end of the capillary tube from which the solution is introduced and the solution so introduced into the capillary tube is blown out towards the other end of the tube, i.e., an excessive amount of the solution is blown out towards the other end of the tube. The solution deposited on the inner wall of the tube 11 is immediately evaporated to form a coating layer 12. Graduations 13 are marked on the peripheral wall of the capillary tube.

Explanation is now made of the staining treatment effected using the above-mentioned capillary tube. In this case, a small amount of blood is collected from a human earlobe or a finger-tip. That is, these parts of the human body are sterilized by alcohol. After completely ¹⁵ dried, these parts are injured by a scalpel (in the case of the earlobe) or by a Schnepper or Franchesher (in the case of the fingertip). When blood that has naturally oozed out from the wound is accumulated to a 20 certain extent, one end of the capillary tube 11 is put on the blood pool as shown in FIG. 5A to permit the blood to be naturally sucked under a capillary action into the interior of the capillary tube. When a desired amount of blood is collected, a capillary tube 11 is 25 withdrawn from the wound. Then, the capillary tube is, for example, shaked to cause the dye etc. deposited on the inner wall of the capillary tube 11 to be dissolved into the blood to permit them to be sufficiently mixed as shown in FIG. 5B. A smear preparation is obtained 30 by blow spattering the blood within the capillary tube 11 onto glass plate as shown in FIG. 5C. The smear preparation can be permanently preserved. When a vital staining sample is prepared, the same process as shown in FIGS. 5A and 5B is effected. Then, the blood 35 within the capillary tube 11 is dropped onto a cover glass 15 as shown in FIG. 6C and the cover glass is inverted and put on a glass plate 16 as shown in FIG. 6D. The vital staining sample so obtained can not be preserved. 40

According to this invention, there is provided a capillary tube whose inner surface is coated with a reagent coating layer, for example, a dry material including as a primary component a colorant or dye or a phagocytizing foreign substance, for example, carbon, starch or ⁴⁵ polystyrene.

For example, when a blood corpuscle is stain-treated, one end of the capillary tube is contacted with the human blood to cause the latter to be naturally sucked into the interior of the capillary tube under capillary ⁵⁰ action to permit the dye coated on the inner wall of the tube to be dissolved into the blood. That is, the collection of blood and the staining of blood corpuscle or cells can be simultaneously effected. Therefore it is not necessary to adjust a staining fluid, as encountered in the prior art, in an attempt to stain cells. Furthermore, there is saved the trouble of forming a dye film on the slide as in the case of the pappenheim method.

If a transparent capillary tube is used, it is possible to observe the amount of blood sucked into the capillary tube. The amount of blood can be freely selected if the capillary tube is withdrawn when a predetermined amount of blood is reached. The operation is further facilitated when a graduation is marked on the outer wall of the capillary tube. Since the amount of blood can be freely selected, the mixing ratio between the dye and the blood can be easily adjusted.

As a powdered dye is used in this invention, the dye is highly stable as compared with a fluid dye as is used in the prior art and it is less likely that blood corpuscles etc. in the sample are changed due to a denaturation of the dye.

The same result can also be obtained in staining bacteria or performing the phagocytosis test of blood corpuscles etc.

EXAMPLE

On the inner wall of a capillary tube having a length of 75mm and an inner diameter of **1.2**mm the following reagents were coated:

EDTA - 2k (ethylene diamine tetraacetic acid)	0.12mg
Brilliant cresyl blue	0.14mg

Then, 50 micro-litre $(10^{-3}cc)$ of blood was introduced into the capillary tube and after mixing it was allowed to stand for five minutes. The capillary tube was again shaked for mixing and a smear preparation was microscopically observed.

For comparison purpose, the conventional Brecker method was also effected. The results are as follows:

Table	
-------	--

Sample No.	this invention	Brecker method
1	10 0/00	
2	12 0/00	12 0/00
3	9 0/00	8 0/00
4	8 0/00	9 0/00
5	12 0/00	12 0/00

Note: 0/00 denotes the number of reticulocytes stained in 1000 erythrocytes.

As will be evident from the Table, no substantial difference is observed between the number of reticulocytes stained using the capillary tube according to this invention and the number of reticulocytes counted according to the Brecker method. According to this invention, therefore, there is saved the trouble of preparing a staining solution or forming a dye film.

With the above embodiment there is explained the case where a dye or phagocytic foreign matter is coated on the whole inner wall surface of the capillary tube. However, a part of the inner wall of the capillary tube, for example one third of a length of the tube, may be free from any reagent coating layer. In this case, blood etc. can be quickly sucked into the capillary tube without being obstructed by a coated layer. Two or more reagents may be coated at intervals in a lengthwise direction of the tube in a manner that the layer is divided into sections of individual reagents. For example, the inner wall of one end portion of the tube is coated with a culture medium and the inner wall of the other end portion of the tube is coated with a dye with an intermediate portion of the tube left uncoated. A lymphocyte suspension is sucked from the culture medium side and cultured for a predetermined time period in a humid room and then mixed with the dye. Thus, the abnormality of chromosome can be easily and efficiently examined. The capillary tube according to this invention can of course be applied to not only a lymphocyte suspension, or whole blood, but also other blood cells. What is claimed is:

1. A tube for blood examination which comprises a double open-ended capillary tube having an inner diameter which allows the introduction of blood by capillary action through the inlet end of the tube, at least one reagent coating layer coated on a portion of the inner wall surface thereof and spaced apart from said inlet end, and an uncoated inner wall area for the introduction of blood, which runs from said inlet end to an 5 edge of the reagent coating layer.

2. The tube of claim 1 wherein the length of said uncoated area is about 1/3 the length of the tube.

3. The tube for blood examination according to claim 1 in which said reagent consists essentially of colorant 10 for staining cells in blood.

4. The tube for blood examination according to claim 1 in which said reagent consists essentially of phagocytic foreign substance for examining the phagocytosis of leucocyte.

5. The tube for blood examination according to claim 3 in which said reagent coating includes an anticoagulant.

6. The tube for blood examination according to claim 1 in which said reagent coating layer comprises at least 20 two kinds of reagent and each reagent is coated at intervals longitudinally of the tube.

7. The tube for blood examination according to claim

5 in which one kind of said reagent is culture medium and the other staining material.

8. A blood examining method comprising the steps of drawing by capillary action a prescribed amount of 5 fluid containing blood cells, from one end portion of a double open ended capillary tube where no reagent is coated to an extent corresponding to the prescribed amount of the fluid to be introduced, into said capillary tube having a portion where at least one blood examination reagent-containing layer is coated on the inner wall surface thereof, shaking the capillary tube to permit the reagent to be dissolved into the fluid, and blowing the fluid from the tube onto a substrate whereby an examination sample is prepared.
15 O The entropy of the state of the fluid fluid from the tube onto a substrate whereby an examination sample is prepared.

9. The method of claim 8 wherein said layer coating portion of said inner wall surface consists of two separate parts, one part being a culture medium and the other part being a staining material, and said shaking step comprises first mixing the fluid with the culture medium and then mixing the fluid-culture medium mixture with the staining material.

* * * * *

30

25

35

- 40
- 45

50

55

60

65