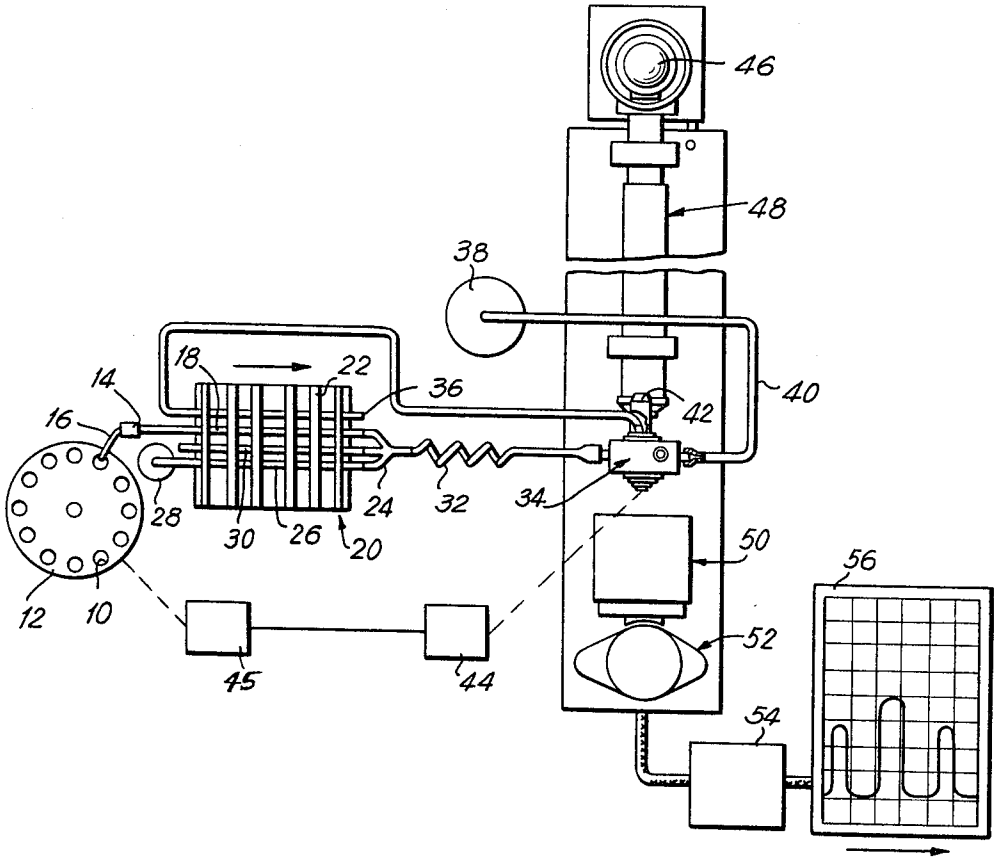
Nov. 19, 1968 N. GOCHMAN ETAL 3,412,037

METHOD AND MEANS FOR THE CALIBRATION OF
PARTICLE COUNTING APPARATUS
Filed Jan. 26, 1966



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United States Patent Office 3,412,037
Patented Nov. 19, 1968

3,412,037

METHOD AND MEANS FOR THE CALIBRATION OF PARTICLE COUNTING APPARATUS

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Filed Jan. 26, 1966, Ser. No. 523,136
2 Claims. (Cl. 252—408)

ABSTRACT OF THE DISCLOSURE

A standard for calibrating blood counting apparatus is provided in a suspension of a substantially uniform minute unicellular fungi, substantially disc shaped with a diameter of the same order of magnitude as the blood cells to be counted by the apparatus, such as saccharomyces cerevisiae; in a solution of sodium chloride in a concentration of 5 to 15% for the purpose of reducing the deterioration rate of the cell structure; and sodium fluoride in a concentration of one half of 1% or greater for the purpose of inhibiting sugar breakdown and thereby precluding growth of the yeast.

This invention relates to the calibration of apparatus for counting the number of particles suspended in a fluid medium; and more particularly to the calibration of apparatus for counting the number of red or white cells contained in a volume of diluted blood.

Such counting apparatus is disclosed in U.S. patent application, Ser. No. 427,593 of Jack Isreeli, filed Jan. 25, 1965. Briefly described, such apparatus has a support for a plurality of whole blood samples, which are sequentially diluted and treated, a flow cell of small cross-section through which the volume of diluted blood is passed, illuminated optical means coupled to said flow cell for detecting the passage of individual blood cells therethrough and for providing an output pulse signal in response thereto, electronic means coupled to said detecting means for receiving and totaling the number of pulses provided per unit of time and providing an output signal responsive thereto, and means for automatically and periodically cleansing said passageway.

The customary method of calibrating such apparatus has been to provide a whole blood standard by repeatedly counting such blood by manual hemacytometer techniques to establish its value. Such a standard is then diluted and counted by the apparatus to calibrate its output. Although satisfactory, such a whole blood standard has the disadvantages that the standard is usable for only one day, and that each time a fresh whole blood standard is prepared, the manual counts must be repeated.

Another method has been to provide a red blood standard which has been stabilized, as by tannic acid, so that it will be usable for a longer period of time, if kept under refrigeration. Such a standard, however, is not usable for white cell counting in a system which provides for the destruction of red cells prior to the counting of the white cells.

Yet another method is to provide a standard comprising a suspension of polystyrene latex or of ragweed pollen particles. Such standards, however, are not very satisfactory as a whole blood standard because it is difficult to pack enough particles into a suspension of liquid. This is due to these particles being substantially spherical while blood cells having the same diameter are disc shaped and thus have a smaller unit volume.

It is, therefore, an object of this invention to provide a standard, and a method of preparing such a standard, which is usable for both red cell counting, white cell counting involving the prior destruction of red cells, and

which is readily reproducable with a minimum of labor over an extended period of time.

A feature of this invention is the provision of a standard comprising yeast having an average particle size of the order of magnitude of seven microns diameter in a liquid suspension, said cells having been treated with an agent for inhibiting sugar breakdown.

Another feature of this invention is the method of providing a standard comprising the steps of providing active dry yeast having an average particle size of the order of magnitude of seven microns diameter; determining the particle count per unit of weight; adding a quantity of liquid containing an agent for inhibiting sugar breakdown to a quantity of the yeast to provide a volume of suspended yeast particles having a predetermined quantity of particles per unit of volume.

These and other objects, features and advantages of the invention will be appreciated from the following disclosure taken in conjunction with the accompanying drawing in which:

The figure is pictorial diagram of an exemplary apparatus with which the present invention may be utilized.

In the figure the whole blood samples are disposed in respective sample containers **10** supported by an indexible turntable **12** which sequentially presents each container **10** to an off-take mechanism **14**. The off-take mechanism has an off-take tube **16** which is linked to the turntable and its inlet is inserted into each container presented thereto. The outlet of the off-take tube is coupled to a pump tube **18** which is disposed in a proportioning peristaltic type pump **20** having a plurality of rollers **22** which each progressively occlude the length of the pump tube. The outlet of the pump tube **18** is coupled to one leg of a four legged fitting **24**. The inlet of a companion pump tube **26** is coupled to a source **28** of saline solution in the case of counting for red cells, or of red cell lysing agent, such as acetic acid, in the case of counting for white cells. The outlet of the pump tube **26** is coupled to another leg of the fitting **24**. The inlet of another companion pump tube **30** is coupled to a source of relatively inert gas, such as the atmosphere, and its outlet is coupled to another leg of the fitting **24**. The outlet leg of the fitting **24** is coupled to the inlet of a mixing coil **32** whose outlet is coupled to an inlet of a flow cell assembly **34**. The diluted, air segmented sample passes through a vertical passageway in a light permeable flow cell and is discharged to waste through a pump tube **36**. A source **38** of a wash liquid may be coupled by a conduit **40** to the flow cell passageway and a valve **42** controlled by a rotary solenoid **44** which is conterolled by a sampler-cam operated switch **45** may control the passage of sample or wash liquid, in the alternative, through the flow cell passageway.

A light source **46** passes a light beam through a lense system **48** to a focus within the flow cell passageway. Light which is intercepted by a particle is deflected and passes through a collector lens assembly **50**, having a central dark field disc, to a light detector **52**. The light detector provides signals responsive to the detection of light pulses from particles to a count/rate circuit **54**, whose output signal is recorded by a chart recorder **56**.

The diameter of red blood cells averages normally at seven microns while the diameter of white blood cells averages normally in the range of ten to fifteen microns.

We have found that an active dry form of saccharomyces cerevisiae, such as is marketed as "Fleischmann's Active Dry Yeast Type 1821" by Standard Brands Incorporated, has a particle shape which approximates a disc and a diameter which averages seven microns. The particle size and density are homogeneous, and a given weight provides a reproducible volume and particle concentration.

This yeast may be prepared as a standard for red or white blood cells as follows: A weighed volume of dried yeast is mixed and suspended in a diluent to provide a stock suspension which has an approximately known cell count. This suspension is then counted repetitively by conventional manual hemacytometer technique and this determined count is assigned to the stock suspension. Subsequent dilutions can be prepared from the stock suspension and a calibration curve of recorder signal versus particle concentration can be drawn for the calculation of the blood samples. Different stock suspensions are prepared for the red count standard of approximately seven million cells per cubic millimeter, and for the white count standard of approximately twenty-five thousand cells per cubic millimeter.

The use of a standard in this manner compensates for the dilution at the fitting **24**, which will affect the standard and the blood samples equally. The yeast cells produce approximately the same intensity of signals in the light detector as red and white blood cells. The yeast cell standard curve also automatically compensates for coincidence effects, that is, single counts of two or more particles, which occur at high cell concentration.

A diluting fluid for the yeast comprises one percent sodium fluoride and ten percent sodium chloride in water. The sodium fluoride serves as an enzyme poison and inhibits sugar breakdown, thereby precluding growth of the yeast. Concentrations of sodium fluoride of one half of one percent or greater have been found to be effective. The sodium chloride improves the stability of the yeast

cells, that is, reduces the deterioration rate of the cell structure. Concentrations of sodium chloride in the range of five to fifteen percent have been found to be effective.

While activated dry saccharonyces cerevisiae has been found to be effective as a standard, and conveniently available in commercial quantities, it will be apparent to one skilled in the art that other minute unicellular fungi having a substantially disc shape with a diameter of the same order of magnitude as red and white blood cells will also be effective.

What is claimed is:

1. A method for the preparation of a standard, for the calibration of a blood counting apparatus, comprising the step of:

suspending saccharomyces cerevisiae in a solution of sodium chloride in a concentration of five to fifteen percent and sodium fluoride in a concentration of one half to one percent.

2. A standard, for the calibration of a blood counting apparatus, consisting essentially of:

saccharomyces cerevisiae suspended in a solution of sodium chloride in a concentration of five to fifteen percent and sodium fluoride in a concentration of one half to one percent.

References Cited

FOREIGN PATENTS

690,849 7/1964 Canada.

MAYER WEINBLATT, *Primary Examiner.*