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(54) **METHOD FOR DETECTION OF MALARIA AND OTHER PARASITE INFECTIONS**

VERFAHREN ZUM NACHWEIS VON MALARIA UND ANDEREN INFEKTIONEN MIT PARASITEN

PROCEDE DE DETECTION DU PALUDISME ET AUTRES INFECTIONS PARASITAIRES

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(56) References cited:
US-A1- 2004 067 575 US-B1- 6 632 676

- **SCOTT C S ET AL: "Automated detection of malaria-associated intraleucocytic haemozoin by Cell-Dyn CD4000 depolarization analysis." CLINICAL AND LABORATORY HAEMATOLOGY APR 2003, vol. 25, no. 2, April 2003 (2003-04), pages 77-86, XP002522402 ISSN: 0141-9854**

- **FAWZI Z O ET AL: "Differences in automated depolarization patterns of Plasmodium falciparum and P. vivax malaria infections defined by the Cell-Dyn(R) CD4000 haematology analyser" TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE, ELSEVIER, GB, vol. 97, no. 1, 1 January 2003 (2003-01-01), pages 71-79, XP004545495 ISSN: 0035-9203**
- **PATTERSON K G ET AL: "Automated differential counting" BLOOD REVIEWS, CHURCHILL LIVINGSTONE, vol. 5, no. 2, 1 June 1991 (1991-06-01), pages 78-83, XP022954339 ISSN: 0268-960X [retrieved on 1991-06-01]**
- **FOURCADE C ET AL: "Automated detection of malaria by means of the haematology analyser Coulter GEN.S." CLINICAL AND LABORATORY HAEMATOLOGY DEC 2004, vol. 26, no. 6, December 2004 (2004-12), pages 367-372, XP002522403 ISSN: 0141-9854**
- **FOURCADE ET AL: 'Automated Detection of Malaria by Means of the Haematology Analyzer Coulter GEN.STM' CLINICAL LABORATORY HAEMATOLOGY vol. 26, 2004, pages 367 - 372, XP002522403**
- **LATHIA: 'Can hematological parameters discriminate malaria from nonmalarious acute febrile illness in the tropics?' INDIAN JOURNAL OF MEDICAL SCIENCES vol. 58, no. 6, June 2004, pages 239 - 244, XP008112824**
- **SCOTT ET AL: 'Automated detection of malaria-associated intraleucocytic haemozoin by Cell-Dyn CD4000 depolarization analysis' CLINICAL LABORATORY HAEMATOLOGY vol. 25, 2003, pages 77 - 86, XP002522402**

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DescriptionFIELD OF THE INVENTION

5 **[0001]** The present invention relates to a method for detection of malaria infections by measuring alteration of white blood cells of a blood sample. More specifically, it is related to a method of detection of a malaria infection based on specific alterations of cell volume properties of white blood cell subpopulations.

BACKGROUND OF THE INVENTION

10 **[0002]** Malaria remains one of the most common diseases in the world, with about 300 to 500 million cases of malaria infection estimated to occur annually. Approximately 1.5 to 2.7 million patients die annually from malaria, among them one million children are less than 5 year old. This disease, the incidence of which has been increasing for last 5 years, is today a major impediment to social and economical development in many countries. In France, 3400 cases of malaria are detected yearly, with 80% of them due to *Plasmodium falciparum*.

15 **[0003]** The pathogen agent of malaria is a protozoic parasite (*Plasmodium*) transmitted by the anopheles female to man, who is the intermediary host of the parasite. There are four types of plasmodium specific pathogen for human; *Plasmodium falciparum*, most frequently encountered in Africa, is occasionally fatal; *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale* are not fatal, but may result in relapses.

20 **[0004]** The laboratory diagnosis of malaria is usually established by standard procedures that include thin and thick film examinations, based on the observation of intraerythrocytic parasites in the blood smear, and possible investigation for soluble specific antigens. However, these investigations are only undertaken if there is a clinical suspicion that a patient may actually have malaria. Although a high level of suspicion may exist in endemic areas for malaria, this is not always the case in nonendemic regions, particularly if there is a failure to take an adequate patient history. Even when there is a clinical suspicion, it is not uncommon for laboratories in nonendemic areas to have relatively poor expertise for detecting and identifying malaria. It has been reported that although a blood count is almost always requested as part of the routine investigation of febrile patients, changes in leukocyte and platelet parameters are rarely sufficiently distinctive to suggest directly a diagnosis of malaria (Kueh et al, 1982, Scandinavian Journal of Haematology 29, 147-152 and Eriksson et al, 1989, Journal of Infectious Diseases 21, 434-441). The clinical implications of these limitations are illustrated by a Canadian study (Kain et al., 1998, Clinics in Infectious Diseases 27, 142-149) that reported an initially missed diagnosis in 59% of returning travelers with malaria. The consequent average delay before correct diagnosis and initiation of appropriate therapy was 7.6 days for *Plasmodium falciparum* and 5.1 days for *P. vivax*. Such delays can be an important contributory factor to the development of severe malarial complications and mortality rates (Humar et al., 1997, Canadian Medical association Journal 156, 1165-1167).

35 **[0005]** Malaria was shown to result in leukocyte alterations, reflected mainly by the presence of hemazoin-containing pigments, produced by the various plasmodium species and eventually phagocytosed by neutrophils (Amodu, et al., 1997, East African Medical Journal 74, 714-716) and monocytes (Amodu, et al., 1997, East African Medical Journal 74, 714-716; Amodu, et al. 1998, Transactions of the Royal Society of Tropical Medicine and Hygiene 92, 54-56; Arese et al, 1997, Annals of Tropical and Medical Parasitology 91, 510-516). These changes were recently analyzed for providing an automated diagnosis of malaria, either by means of a flow cytometer (Krämer, et al., 2001, Cytometry 45, 133-140), or of a haematology analyzer measuring light depolarization at two wavelengths (Hänscheid, et al., 1999, Emerging Infectious Diseases 5, 836-838; Mendelow, et al., 1999, British Journal of Haematology 104, 499-503; Hänscheid, et al, 2000, Parasitology Today 16, 549-551; Hänscheid, et al., 2001, American Journal of Tropical Medicine 64, 190-192; Grobusch, et al., 2003, Cytometry part B (clinical cytometry) 55B, 46-51; Scott, et al., 2003, Clinical and Laboratory Haematology 25, 77-86).

Fawzi et al., Transactions of the Royal Society of Tropical Medicine and Hygiene (2003) 97, 71-79 used automated depolarization analysis in order to detect malaria-associated haemozoin.

50 **[0006]** It is desirable to be able to detect malaria on an existing hematology analyzer during a blood analysis regularly performed on the instrument, which can assist an earlier detection of the disease without additional cost Any improved diagnosis and earlier detection can lead to a significant reduction in patient morbidity and mortality.

SUMMARY OF THE INVENTION

55 **[0007]** In one embodiment, the present invention is directed to a method of detecting a malaria infection using a blood sample. The method comprises the steps of: mixing the blood sample with a lytic reagent system to lyse red blood cells, and to form a sample mixture; performing a differential analysis of white blood cells of the sample mixture on a blood analyzer, and obtaining a cell volume distribution of a white blood cell subpopulation from a cell volume measurement used in the differential analysis; obtaining a cell volume parameter from the cell volume distribution of the white blood

cell subpopulation; evaluating the cell volume parameter against a predetermined criterion, and reporting an indication of the malaria infection if the cell volume parameter meets the predetermined criterion.

[0008] The malaria infection can be *Plasmodium* infection, The cell volume parameter is the mean cell volume, or standard deviation of variation of the cell volume distribution. The cell volume measurement can be an impedance measurement, a low angle light scatter measurement, or axial light loss measurement The white blood cell subpopulation is monocytes, lymphocytes, or neutrophils.

[0009] In a further embodiment, the method comprises the steps of: mixing the blood sample with a lytic reagent system to lyse red blood cells, and to form a sample mixture; performing a differential analysis of white blood cells of the sample mixture on a blood analyzer, and obtaining a first cell volume distribution of a first white blood cell subpopulation and a second cell volume distribution of a second white blood cell subpopulation from a cell volume measurement used in the differential analysis; obtaining a first cell volume parameter from the first cell volume distribution of the first white blood cell subpopulation and obtaining a second cell volume parameter from the second cell volume distribution of the second white blood cell subpopulation; obtaining a discriminant as a function of the first and second cell volume parameters; and evaluating the discriminant against a predetermined criterion, and reporting an indication of the malaria infection if the discriminant meets the predetermined criterion. In a preferred embodiment, the first and second cell volume parameters are standard deviations of the first and second cell volume distributions, respectively, and the discriminant is a function of the standard deviations of the first and second cell volume distributions.

[0010] Furthermore, the methods described above can further include analyzing cell distribution patterns obtained from the differential analysis or from an additional differential analysis of the blood sample.

BRIEF DESCRIPTION OF DRAWINGS

[0011] Fig. 1 shows the VCS scattergrams and the DC white blood cell distribution histograms of malaria negative (left) and malaria positive (right) samples.

[0012] Fig. 2 shows a table of DC (V), Opacity (C) and RLS (S) parameters provided by the Coulter Gen*S and LH 750 hematology analyzers..

[0013] Fig. 3 shows a ROC curve of the discriminant ($V_{sd}(\text{lymphocytes}) \times V_{sd}(\text{monocytes})$). The cut off and the corresponding sensitivity and specificity, as well as the Area Under the Curve (AUC) are provided.

[0014] Fig. 4 shows the variation of $V_{sd}(\text{monocytes})$ and $V_{sd}(\text{lymphocytes})$ of several groups of samples from blood donors collected over one month (each group includes 10 donors).

[0015] Fig. 5 shows the time dependent variation of $V_{sd}(\text{monocytes})$ and $V_{sd}(\text{lymphocytes})$ of a group of 15 blood donors.

DETAILED DESCRIPTION OF THE INVENTION

[0016] In one embodiment, the present invention provides a method for detecting a malaria infection by measuring alterations of white blood cells in a blood sample due to the disease using an automated hematology analyzer. The method comprises the steps of mixing a blood sample with a lytic reagent system to lyse red blood cells, and to form a sample mixture; performing a differential analysis of white blood cells of the sample mixture on a blood analyzer, and obtaining a cell volume distribution of a white blood cell subpopulation from a cell volume measurement used in the differential analysis; obtaining a cell volume parameter from the cell volume distribution of the white blood cell subpopulation; evaluating the cell volume parameter against a predetermined criterion, and reporting an indication of malaria if the cell volume parameter meets the predetermined criterion. As described in detail hereinafter, the cell volume can be measured using a direct current impedance measurement, low angle light scatter measurement or axial light loss measurement.

[0017] The term of cell volume parameter used herein refers to a parameter related to the cell volume distribution, such as the mean of cell volume or standard deviation from the cell volume distribution of a white blood cell subpopulation. The term of cell distribution parameter used herein refers to a parameter related to the cell distribution obtained from an impedance measurement, such as the mean, or standard deviation of the cell distribution of a white blood cell subpopulation as measured by either a direct current impedance measurement, or a radio frequency impedance measurement. It is noted that the cell distributions from these two impedance measurements represent different cell properties. The term of predetermined criterion used herein refers to a criterion made based on one or more of cell volume parameters, a function of cell volume parameters, such as a threshold or cut-off value of a parameter, or a function. The predetermined criterion further includes a characteristic cell distribution pattern. In general, the criterion is determined based on differences between a plurality of normal blood samples and a specific type of abnormal blood samples, such as malaria patient samples, which are illustrated in detail hereinafter.

[0018] In a preferred embodiment, the lytic reagent system comprises a lytic reagent and a stabilizing reagent The lytic reagent is mixed with a blood sample to lyse red blood cells, and the stabilizing reagent is added subsequently to

inhibit further lytic action on white blood cells in the sample mixture. Suitable examples of lytic reagents and the stabilizing reagents and the method of use have been described in U.S. Patent Nos. 5,155,044, 5,731,206, 5,786,224, 5,686,308 and 5,843,608. Using these lytic reagents and the stabilizing reagents, and the method of preparing a blood sample, the white blood cells are preserved in a near native state during the measurement, and their cellular membranes are not lysed. As described in the above-referenced patents, the lysing reaction, calculated from the time that the blood sample is in contact with the lytic reagent to the time starting measurement of the sample mixture, is in a range from about 8 to about 20 seconds. The reaction and measurement of the sample mixture are at a regular room temperature ranging from about 18°C to about 28°C.

[0019] It should be understood that other lytic reagents suitable to preserve white blood cells during the measurement of the sample mixtures can also be used for the purpose of the present invention.

[0020] In one embodiment, the differential analysis of white blood cells in the sample mixture is performed using the VCS measurement, which is utilized on various commercial hematology analyzers manufactured by Beckman Coulter, Inc. Fullerton, California. The VCS measurement refers to a multidimensional measurement of an impedance signal of direct current, referred to as DC, and an impedance signal of radio frequency, referred to as RF, and a medium angle light scatter signal, referred to as LS, generated by a cell in a conductive medium when the cell passes through a flow cell. Among these three measurements, both DC and RF measurements are impedance measurements, which detect the increase of impedance as a cell carried in a conductive medium passes through the flow cell. This technology has been fully described in U.S. Patent No. 5,125,737.

[0021] On the commercial hematology analyzer using the VCS measurement, a three-dimensional scattergram is produced. The three dimensions are DC as the z-axis, which is also referred to as "V" because a DC impedance signal directly correlates to the volume or size of a cell; Opacity (OP) as the y-axis, which is a function of RF and DC, and also referred to as "C" or conductivity because the RF signals further reflect cell contents of a cell; and Rotated Light Scatter (RLS) as the x-axis, which is a function of light scatter and DC, and also abbreviated as "S". The three-dimensional scattergram is usually displayed as a DC vs. RLS two dimensional scattergram, or a DC vs. OP two dimensional scattergram. In the DC vs. RLS scattergram, four major white blood cell subpopulations, i.e., lymphocytes, monocytes, neutrophils, and eosinophils, are differentiated from one another. In the DC vs. OP scattergram, three major groups of white blood cell subpopulations are differentiated from one another, i.e., lymphocytes, monocytes and a sum of neutrophils and eosinophils. The basophils can be differentiated using one or more gated scattergram. It is noted that to differentiate lymphocytes, monocytes, or neutrophils from other cell types, one can use all three measurements, i.e., DC, RF and RLS, or only two measurements, either DC and LS, or DC and RF. Furthermore, various other methods such as multi-angle light scatter measurement using forward scatter and side scatter, or low angle and medium angle light scatter can be used for differentiating white blood cell subpopulations, particularly lymphocytes, monocytes, or neutrophils from other cell types.

[0022] It has been found that the properties of white blood cells of a malaria patient are altered, particularly the volumes and the homogeneities of cell volume of specific white blood cell subpopulations are altered. More specifically, it has been found that when measured by the VCS measurement, the DC means of monocytes and neutrophils, and the standard deviations of DC and Opacity of the lymphocytes and monocytes of the malaria samples increase to the levels that the differences of these parameters from those of normal blood samples are statistically significant. Among these parameters, DC means directly relate to cell volume, and the standard deviation of a parameter reflects the homogeneity or heterogeneity of one or more cell properties represented by that parameter. The changes of these parameters of white blood cell subpopulations can be utilized as an indicator or discriminant for detection of malaria.

[0023] It should be understood that the increases of DC mean, standard deviation of DC, and Opacity of the monocytes are caused by the presence of large monocytic cells which mainly include activated monocytes, macrophages, and pigment-carrying monocytes. Similarly, the increases of the standard deviations of DC and Opacity of the lymphocytes are caused by the presence of large lymphocytes which mainly include reactive and activated lymphocytes; and the increase of the DC mean of the neutrophils is caused by the presence of large neutrophils which mainly include immature and activated neutrophils, and neutrophils phagocytosing parasites. Therefore, the term of "monocytes" used herein include monocytes, activated monocytes, macrophages, pigment-carrying monocytes, and other monocytic cells such as monoblasts, promonocytes, and myeloid related dendritic cells. The term of "lymphocytes," used herein include lymphocytes, reactive and activated lymphocytes. The term of "neutrophils" used herein include neutrophils, immature and activated neutrophils, and neutrophils phagocytosing parasites. The pigment-carrying white blood cells refer to the white blood cells that contain intracellular pigments produced by parasite digestion, such as hemozoin-carrying monocytes and neutrophils in a malaria patient's blood sample. The hemozoin is produced by *Plasmodium* species as an end product of hemoglobin digestion.

[0024] In one embodiment, as illustrated in the Example described hereinafter, the cell volume and its distribution of an individual white blood subpopulation are determined by the DC impedance measurement. It should be understood, however, the cell volume can also be determined by other suitable means, such as forward or low angle light scatter, or axial light loss measurement. In general, the low angle light scatter is defined as the light scatter signals measured

in less than 10° from the incident light. Axial light loss (ALL, also known as forward extinction) is generally the decrease in light energy due to a particle passing through a beam of incident light and being detected by a photo-detector. Generally ALL signals are detected at an angle from about 0° to about 1° from the incident light. Both low angle light scatter and ALL signals are strongly influenced by the size of the cell. The apparatus and method of measuring low angle light scatter and ALL signals known in the art can be used for the purpose of the present invention for measurement of the cell volume.

[0025] In a further embodiment, the detection of malaria can be accomplished by combining the information of cell property change from two or more different white blood cell subpopulations, for example, combining the cell volume parameters from two different white blood cell subpopulations, or combining a cell volume parameter of one white blood cell subpopulation with a standard deviation of Opacity of another white blood cell subpopulation. It has been found that using a discriminant as a function of more than one detectable parameters can further improve sensitivity and specificity than using one parameter from only one cell type.

[0026] In one embodiment, the discriminant defined by the following equation is used as a sensitive and specific indicator of malaria:

$$D = Vsd (\text{lymphocytes}) \times Vsd (\text{monocytes})/100$$

wherein D stands for the discriminant; V stands for the cell volume determined by DC measurement; sd stands for standard deviation; Vsd stands for the standard deviation of cell volume; and Vsd (lymphocytes) stands for standard deviation of cell volume of lymphocytes obtained from the DC measurement.

[0027] As illustrated in the Example, the method of the present invention using the discriminant described above has demonstrated high sensitivity and specificity for detecting malaria, which are comparable to known methods which utilize depolarized light scatter measurement.

[0028] The method of detection of malaria using a blood sample described above can also be used for detection of other parasite infections, such as *Toxoplasma gondii* infection, *Leishmania* infection, and *Babesia* infection. Similar to *Plasmodium* infection which is the cause of malaria, there are activated monocytes and macrophages in the peripheral blood of the patients when the patient has *Toxoplasma gondii*, *Leishmania*, or *Babesia* infections. The criterion for indication of a specific parasite infection, such as the cut-off value of a cell distribution parameter, can be determined for each specific infection.

[0029] Additionally, the method can further include pattern recognitions of cell distribution characteristics which relate to the disease. For example, as shown in Fig. 1, an additional population below the lymphocytes in DC is typically observed in the malaria samples. The specific cell distribution characteristics can be utilized to further enhance the sensitivity and specificity of the detection method.

[0030] In a further embodiment, the method of the present invention utilizes two separate white blood cell measurements for detecting malaria. More specifically, the method comprises the steps of exposing a first aliquot of a blood sample to a first lytic reagent system to lyse red blood cells and to form a first sample mixture; exposing a second aliquot of the blood sample to a second lytic reagent system to lyse red blood cells and to form a second sample mixture; performing a first differential analysis of white blood cells of the first sample mixture on a blood analyzer, and obtaining a cell volume distribution of a white blood cell subpopulation; performing a second differential analysis of white blood cells of the second sample mixture by a DC impedance measurement on the blood analyzer, and obtaining a cell distribution of white blood cells using the DC impedance measurement; obtaining a cell volume parameter from the obtained cell volume distribution of the first sample mixture; analyzing patterns of the obtained cell distribution of the second sample mixture; performing a combined analysis on a profile obtained by combining results of the two analyses, the combined analysis evaluating the profile against a predetermined malaria criterion; and reporting indication of malaria of the blood sample if the profile meets the predetermined malaria criterion.

[0031] The first lytic reagent system, the first differential analysis of white blood cells of the first sample mixture and the cell volume parameters from the obtained cell volume distribution of the first sample mixture are the same as those described above using the VCS measurement.

[0032] The second lytic reagent system suitable for lysing blood sample for the purpose of the present invention can comprise an isotonic blood diluent and a lysing reagents, such as those described in U.S. Patent Nos. 4,346,018, 4,521,518, 4,528,274, 4,962,038, 5,763,280, 5,834,315, 5,882,934, 5,935,857 and 6,573,102. One example of the second lytic reagent system is the Lyse S[®] III Diff lytic reagent and Isoton[®] III diluent manufactured by Beckman Coulter, Inc., California. Another suitable example is Lyse S[®] 4 lytic reagent and Isoton[®] 4 diluent manufactured by the same manufacturer. Alternatively, the reagent system can also be an isotonic lysing reagent as described in U.S. Patent No. 5,882,934. This reagent dilutes the blood sample and lyses the red blood cells at the same time for subsequent analysis.

[0033] The method of performing a second differential analysis of white blood cells of the second sample mixture by a DC impedance measurement on the blood analyzer, and obtaining a cell distribution of white blood cells using the DC

impedance measurement have been described fully in U.S. Patent Nos. 4,286,963, 4,485,175 and 6,673,618 B1.

[0034] On the hematology analyzer used herein, the second aliquot of the blood sample is diluted by a blood diluent and then mixed with a predetermined amount of a second lytic reagent to lyse the red blood cells, which forms the second sample mixture. Using the lytic reagents described above, the white blood cells are partially lysed. The obtained cell distribution depends on, in a certain degree, the nuclear volumes of white blood cell subpopulations, which is different from the cell volume distribution from the measurement of the first sample mixture. Typically, from this cell distribution the white blood cells can be differentiated into two or three subpopulations, commonly referred to as a two-part differential or a three-part differential. The three-part differential differentiates the white blood cells into lymphocytes, monocytes and granulocytes. It has been found that in the malaria samples there is an additional population present on the left side of lymphocytes, as shown in Fig. 1, which may be attributed to the parasites in the red blood cells or the ingested malaria pigments in the white blood cells. Such a cell distribution characteristics can be utilized to further improve sensitivity and specificity of detection of malaria using the method described above which is based on the analysis of the first sample mixture.

[0035] Therefore, in one embodiment, the analysis of cell volume parameters of the obtained cell volume distribution of the first sample mixture as described above can be combined with the analysis of patterns of the obtained cell distribution of the second sample mixture to produce a profile. A combined analysis evaluates the profile against a predetermined malaria criterion, and reports indication of malaria of the blood sample if the profile meets the predetermined malaria criterion.

[0036] The following examples are illustrative of the invention and are in no way to be interpreted as limiting the scope of the invention, as defined in the claims. It will be understood that other variations of the methods may be employed, in accordance with the proceeding disclosure.

EXAMPLE

(a) Patients

[0037] 89 clinical whole blood samples for investigations for malaria were analyzed. These samples were required for hematology analysis by physicians, on the basis of unexplained fever in patients either originating from or having traveled in endemic areas. 32 of them were recognized positive for malaria, due either to *Plasmodium falciparum* (28 cases) or *Plasmodium vivax* (4 cases). 57 were found negative by the reference method described hereinafter. The age of the patients ranged from 8 month to 72 year old.

(b) Hematology analyzer

[0038] All samples, either malaria positive or malaria negative, were run on a Coulter GEN*S™ hematology analyzer (manufactured by Beckman Coulter, Inc., California). The hematology analyzer is herein also referred to as instrument. The hematology analyzer provides two separate white blood cell differential analyses. The first differential analysis is performed using the VCS measurement of the first sample mixture prepared by mixing a first aliquot blood sample with an amount of Erythrolyse™ II to lyse red blood cells and subsequently mixing with an amount of Stabilyse™ to stabilize the white blood cells. The VCS measurement differentiates the white blood cells into five subpopulations, i.e., lymphocytes, monocytes, neutrophils, basophils and eosinophils. The second differential analysis is performed by using a DC impedance measurement of the second sample mixture prepared by diluting a second aliquot blood sample with Isoton® III diluent and mixing with an amount of Lyse S® III Diff reagent. The DC impedance measurement provides a total count of white blood cells and also differentiates the white blood cells into three subpopulations, i.e., lymphocytes, monocytes and granulocytes using an one dimensional DC histogram, which is commonly referred to as WBC histogram. For the purpose of reflecting the detection method, it is herein referred to as WBC DC histogram. All reagents described above are manufactured by Beckman Coulter, Inc., California.

[0039] Fig. 1 shows two DC vs. RLS scattergrams, one from a malaria negative patient and one from a malaria positive patient, and two WBC DC histograms. Several distribution features were observed in the malaria positive sample, including increased volume heterogeneity of the monocyte and lymphocyte populations, the presence of an additional population below the lymphocyte population, and the presence of an additional population on the left side of lymphocytes of the WBC DC histogram. These features have been frequently observed in malaria patients.

[0040] The parameters shown in Fig. 2 are accessible on an additional screen of the hematology analyzer, which include the mean values of DC or volume (shown as V), Opacity shown as (C) and Rotated Light Scatter (shown as S) for each of the four major populations, and the standard deviations (SD) of volume, Opacity and Rotated Light Scatter.

[0041] Furthermore, the hematology analyzer allows the user to create decision rules according to criteria chosen by the operator. These rules were recently shown to provide an accurate alarm system (Amouroux, et al., 2003, règles de décision. Annales de Biologie Clinique 61, 576-584) for detecting the blood abnormalities of the whole blood samples.

(c) Reference method

[0042] All samples were analyzed as "malaria positive" or "malaria negative" patients by a blood smear investigation, which showed the presence or the absence of plasmodium invasion of the red blood cells (RBC). In the 32 samples of the positive group, the percentage of invaded RBC ranged from 0.1 % to 4.65%.

(d) Statistical methods

[0043] The mean values and standard deviations (SD) of DC, Opacity and Rotated Light Scatter of lymphocytes, monocytes and neutrophils were taken from the table given by the hematology analyzer (Fig. 2) for each sample. DC, Opacity and Rotated Light Scatter are referred to in the table of the instrument display as V, C, and S, respectively.

[0044] The patients were divided into two groups, malaria negative or malaria positive. The parameters were first submitted to a non-parametric test (Wilcoxon rank test), in order to compare to each other the results of the negative and of the positive groups. The parameters which were found significantly different by this test were then submitted to a Receiver Operator Characteristic Curve (ROC) analysis, to determine whether the changes observed between the two groups were malaria specific and to determine the optimal cut off criteria for detection of the malaria. A ROC analysis was also performed on discriminants derived from the DC or volume parameters of the monocytes and lymphocytes.

(e) Evaluation of the selected parameters

[0045] Since the DC (volume) parameters of monocytes and lymphocytes were shown by the statistical analysis more efficient for distinguishing the malaria positive from the malaria negative patients, they were further evaluated in terms of repeatability, stability of the hematology analyzer over one month, variability from one instrument to another, time dependent variation of the parameters of the samples at different sample ages.

[0046] The repeatability was investigated with ten blood samples from blood donors. Each sample was run ten times on the hematology analyzer and the coefficient of variation (CV) of the volume parameters of monocytes and lymphocytes were calculated for each sample. The repeatability for each parameter was then expressed as the average CV of the ten samples. The repeatability of Vsd (lymphocyte) and Vsd (monocyte) was calculated according to the procedure described above. The mean coefficients of variation of these values were 4.4% and 5.5% respectively.

[0047] The sample age dependent variation was evaluated as the variation of a parameter over 77 hours after the blood collection. A group of 15 samples from blood donors were collected and run on the same instrument at 5, 8, 11, 17, 29, 53 and 77 hours after the blood collection. The averages of the volumes and standard deviations of the volume of lymphocytes and monocytes were calculated, respectively, to evaluate the variations of these parameters during a period of 77 hours. As shown in Fig. 5, the Vsd (lymphocyte) and Vsd (monocyte) are stable within 11 hours after the collection of the blood samples. The mean values of Vsd (lymphocyte) and Vsd (monocyte) at 5, 8 and 11 hours after the blood collection were 14.8, 15, 15.9 and 18.3, 17.5, 18.4 respectively.

[0048] The stability of the hematology analyzer was investigated separately on a LH750 hematology analyzer which had the same VCS detection system as that on the Gen*S, and was manufactured by the same manufacturer. The stability was investigated over one month by running periodically groups of blood samples from the donors, each group including ten samples. The average of the volumes and standard deviations of the volumes of lymphocytes and monocytes were calculated for each group, which allowed monitoring the variability of normal values in one month. The stability of the instrument is shown in Figure 4. Over a time period of one month, the average values of Vsd (lymphocyte) and Vsd (monocyte) of the blood samples from the groups of blood donors varied in the range 18.6-20.6 (mean: 19.6) and 15.3-16.9 (mean: 15.9) respectively, with a CV equal to 4% for both values.

[0049] The comparison between instruments was performed by using two LH 750 hematology analyzers, and both were set according to the manufacturer's requirements. 85 whole blood samples from blood donors were run on the first instrument and 81 on the second instrument. The means of Vsd (lymphocyte) and Vsd (monocyte) were compared by calculating the percentage variation between the two instruments. The results of comparison between instruments are given in Table 1. As shown, the Vsd (lymphocyte) and Vsd (monocyte) reported from two different instruments are consistent. The percentage variation of Vsd (lymphocyte) and Vsd (monocyte) between these two instruments were 3.2% and 1.5%, respectively.

Table 1. Variation between instruments of the Vsd of lymphocytes and the Vsd of monocytes

	Vsd lymphocytes	Vsd monocytes
Instrument 1 (85 donors)		
Mean	15.5	19.5

(continued)

5

10

	Vsd lymphocytes	Vsd monocytes
Instrument 1 (85 donors)		
95% confidence interval	15.2-15.9	19.3-19.8
Instrument 2 (81 donors)		
Mean	16	19.2
95% confidence interval	15.7-16.3	18.9-19.6
Variation (%)	3.2%	1.5%

(f) Results

15

[0050] A first analysis of the instrument reported DC, Opacity and RLS parameters was performed for lymphocytes, monocytes and neutrophils of the positive and negative groups. This analysis was performed using the non-parametric test (Wilcoxon rank test). Table 2 shows the statistical results of the two groups (mean and standard deviation for each parameter), as well as the significance of the differences.

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[0051] From Table 2, it can be seen that significant differences were observed in one neutrophil parameter, i.e., mean volume, two lymphocyte parameters, i.e., standard deviation of volume and standard deviation of Opacity, and three monocyte parameters, i.e., mean volume, standard deviation of volume and standard deviation of Opacity. These six parameters that exhibited significant differences between the two groups were submitted to a ROC analysis. Table 3 shows the results of the ROC analysis for each of the parameters investigated, in terms of sensitivity, specificity and cut off values. The area under the ROC curve (AUC) is also given.

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Table 2. Parameters exhibiting a significant difference between the malaria positive and the malaria negative groups.

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	Vmean	Cmean	Smean	Vsd	Csd	Ssd
Neutrophils						
Positive	151.8 ± 11.9	132.4 ± 5.3	142.9 ± 6.6	22.6 ± 3.5	8.0 ± 2.1	12.0 ± 2.3
Negative	145.7 ± 12.4	134.6 ± 4.7	140.9 ± 11.6	21.4 ± 4.3	7.3 ± 1.4	11.9 ± 2.5
Significance	p = 0.0059	NS	NS	NS	NS	NS
Lymphocytes						
Positive	85.3 ± 10.6	103.0 ± 3.1	67.1 ± 8.4	27.6 ± 4.2	16.1 ± 2.8	17.6 ± 2.3
Negative	82.6 ± 6.9	102.9 ± 4.6	66.5 ± 6.1	20.3 ± 4.4	13.0 ± 2.4	18.3 ± 1.7
Significance	NS	NS	NS	p = 0.0000	p = 0.0000	NS
Monocytes						
Positive	186.6 ± 12.1	111.3 ± 4.6	88.6 ± 3.9	24.7 ± 3.6	5.0 ± 0.9	9.3 ± 1.1
Negative	170.4 ± 11.9	111.7 ± 3.2	87.7 ± 4.6	20.1 ± 4.1	4.8 ± 0.4	9.6 ± 1.4
Significance	p = 0.0000	NS	NS	p = 0.0000	p = 0.0152	NS

45

[0052] As shown, the cut-off values obtained from the ROC analysis are used as the criterion for indication of malaria. The results described in Table 3 showed that two parameters (Vsd (lymphocytes) and Vsd (monocytes)) exhibited sensitivity higher than 90% and specificity higher than 60%, with AUC of 0.876 and 0.822, respectively. Thus, these two parameters are particularly favorable for distinguishing the malaria positive from the malaria negative samples.

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Table 3. ROC analysis of the six parameters significantly different in the malaria positive and the malaria negative groups.

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	Vmean neutrophils	Vmean monocytes	Vsd lymphocytes	Vsd monocytes	Csd lymphocytes	Csd monocytes
Cut off	141.5	181.6	22.9	19.9	14.4	4.9
Sensitivity (%)	84.4	68.7	90.6	96.9	81.3	68.7

(continued)

	Vmean neutrophils	Vmean monocytes	Vsd lymphocytes	Vsd monocytes	Csd lymphocytes	Csd monocytes
Specificity (%)	50.0	85.3	75.4	63.2	77.2	71.4
AUC	0.677	0.844	0.876	0.822	0.791	0.656

[0053] Furthermore, a discriminant (D) defined by the formula shown below was derived from these two parameters to further improve the specificity of the detection:

$$D = \text{Vsd (lymphocytes)} \times \text{Vsd (monocytes)} / 100$$

[0054] As shown in Fig. 3, the ROC analysis performed on this discriminant showed that a discriminant value higher than 5.06 indicated the presence of malaria with a sensitivity of 96.9% and specificity of 82.5%. Moreover, the cut off value corresponding to 100% sensitivity was 4.81, with a specificity of 77.2%.

[0055] Therefore, this discriminant significantly improved the specificity to 82.5% with a high sensitivity of 96.8%. As shown in Table 4, the performance of using this discriminant for detecting malaria is comparable with those known detection methods reported in the literature.

Table 4. Comparison of the performance of the D discriminant as a test for detecting the malaria positive patients with references.

Reference*	Detection Method/Instrument	Sensitivity	Specificity
Scott, <i>et al.</i> (2003)	Depolarization analysis (Abbott CD4000)	80.2%	87.3%
Grobusch, <i>et al.</i> (2003)	Depolarization analysis (Abbott CD3000)	48.6%	96.2%
Krämer, <i>et al.</i> (2001)	Depolarized side scatter -one wavelength (Cytomation Mo Flow)	92%	100%
Krämer, <i>et al.</i> (2001)	Correlated side scatter-two wavelengths (Cytomation MoFlow)	95%	94%
Häncheid, <i>et al.</i> (2001)	Depolarization analysis (Abbott CD3500)	95%	88%
Mendelow, <i>et al.</i> (1999)	Depolarization analysis (Abbott CD3500)	72%	96%
The method of the instant invention	Analysis of volume parameters (Beckman Coulter GEN*S)	96.8%	82.5%

* Scott, et al., 2003, Clinical and Laboratory Haematology 25, 77-86 Grobusch, et al., 2003, Cytometry part B (clinical cytometry) 55B, 46-51 Krämer, et al., 2001, Cytometry 45, 133-140 Hänscheid, et al., 2001, American Journal of Tropical Medicine 64, 190-192 Mendelow, et al., 1999, British Journal of Haematology 104, 499-503

[0056] While the present invention has been described in detail and pictorially shown in the accompanying drawings, these should not be construed as limitations on the scope of the present invention, but rather as an exemplification of preferred embodiments thereof.

Claims

1. A method of detecting a malaria infection using a blood sample comprising the steps of:

- 5 (a) mixing said blood sample with a lytic reagent system to lyse red blood cells, and to form a sample mixture;
(b) performing a differential analysis of white blood cells of said sample mixture on a blood analyzer, and obtaining a cell volume distribution of a white blood cell subpopulation from a cell volume measurement used in said differential analysis;
10 (c) obtaining a cell volume parameter from said cell volume distribution of said white blood cell subpopulation;
(d) evaluating said cell volume parameter against a predetermined criterion, and reporting an indication of said malaria infection if said cell volume parameter meets said predetermined criterion.

2. A method of detecting a malaria infection using a blood sample comprising the steps of:

- 15 (a) mixing said blood sample with a lytic reagent system to lyse red blood cells, and to form a sample mixture;
(b) performing a differential analysis of white blood cells of said sample mixture on a blood analyzer; and obtaining a first cell volume distribution of a first white blood cell subpopulation and a second cell volume distribution of a second white blood cell subpopulation from a cell volume measurement used in said differential analysis;
20 (c) obtaining a first cell volume parameter from said first cell volume distribution of said first white blood cell subpopulation and obtaining a second cell volume parameter from said second cell volume distribution of said second white blood cell subpopulation;
(d) obtaining a discriminant as a function of said first and second cell volume parameters; and
25 (e) evaluating said discriminant against a predetermined criterion, and reporting an indication of said malaria infection if said discriminant meets said predetermined criterion.

3. The method of claim 1 or 2, wherein said white blood cell subpopulation is monocytes.

4. The method of claim 1 or 2, wherein said white blood cell subpopulation is lymphocytes.

30 5. The method of claim 1 or 2, wherein said white blood cell subpopulation is neutrophils.

6. The method of claim 1 or claim 2, wherein said cell volume parameter is a standard deviation of said cell volume distribution.

35 7. The method of claim 1, wherein said cell volume parameter is a mean of said cell volume distribution.

8. The method of claim 1 or claim 2, wherein said cell volume measurement is an impedance measurement.

40 9. The method of claim 1 or claim 2, wherein said cell volume measurement is a low angle light scatter measurement, or an axial light loss measurement.

10. The method of claim 1 or 2, wherein said malaria infection is caused by Plasmodium infection.

45 11. The method of claim 2, wherein said first and second cell volume parameters are standard deviations of said first and second cell volume distributions, respectively; and said discriminant is a function of said standard deviations of said first and second cell volume distributions.

50 **Patentansprüche**

1. Verfahren zur Detektierung einer Malariainfektion durch Verwenden einer Blutprobe umfassend die Schritte:

- 55 (a) Mischen der Blutprobe mit einem lytischen Reagenzsystem, um rote Blutzellen zu lysieren, und um eine Probenmischung zu bilden;
(b) Durchführen einer Differentialanalyse von weißen Blutzellen der Probenmischung in einem Blutanalysator, und Erhalten einer Zellvolumenverteilung von einer weißen Blutzellensubpopulation von einer Zellvolumenmessung, welche in der Differentialanalyse verwendet wurde;

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- (c) Erhalten eines Zellvolumenparameters von der Zellvolumenverteilung von der weißen Blutzellsubpopulation;
- (d) Evaluierung des Zellvolumenparameters gegen ein vorbestimmtes Kriterium, und Berichten eines Hinweises auf die Malariainfektion, wenn der Zellvolumenparameter das vorbestimmte Kriterium erfüllt.

- 5 2. Verfahren zur Detektierung einer Malariainfektion unter Verwendung einer Blutprobe umfassend die Schritte:
- (a) Mischen der Blutprobe mit einem lytischen Reagenssystem, um rote Blutzellen zu lysieren, und um eine Probenmischung zu bilden;
 - 10 (b) Durchführen einer Differentialanalyse von weißen Blutzellen der Probenmischung in einem Blutanalysator; und Erhalten einer ersten Zellvolumenverteilung von einer ersten weißen Blutzellsubpopulation und einer zweiten Zellvolumenverteilung von einer zweiten weißen Blutzellsubpopulation von einer Zellvolumenmessung, welche in der Differentialanalyse verwendet wurde;
 - (c) Erhalten eines ersten Zellvolumenparameters von der ersten Zellvolumenverteilung von der ersten weißen Blutzellsubpopulation und Erhalten eines zweiten Zellvolumenparameters von der zweiten Zellvolumenverteilung von der zweiten weißen Blutzellsubpopulation;
 - 15 (d) Erhalten einer Diskriminante als eine Funktion von den ersten und zweiten Zellvolumenparametern; und
 - (e) Evaluierung der Diskriminante gegen ein vorbestimmtes Kriterium, und Berichten eines Hinweises auf die Malariainfektion, wenn die Diskriminante das vorbestimmte Kriterium erfüllt.
- 20 3. Verfahren nach Anspruch 1 oder 2, wobei die weiße Blutzellsubpopulation Monozyten ist.
4. Das Verfahren nach Anspruch 1 oder 2, wobei die weiße Blutzellsubpopulation Lymphozyten ist.
5. Verfahren nach Anspruch 1 oder 2, wobei die weiße Blutzellsubpopulation Neutrophile ist.
- 25 6. Verfahren nach Anspruch 1 oder Anspruch 2, wobei der Zellvolumenparameter eine Standardabweichung von der Zellvolumenverteilung ist.
7. Verfahren nach Anspruch 1, wobei der Zellvolumenparameter ein Mittelwert der Zellvolumenverteilung ist.
- 30 8. Verfahren nach Anspruch 1 oder Anspruch 2, wobei die Zellvolumenmessung eine Impedanzmessung ist.
9. Verfahren nach Anspruch 1 oder Anspruch 2, wobei die Zellvolumenmessung eine low angle light scatter-Messung oder eine axial light loss-Messung ist.
- 35 10. Verfahren nach Anspruch 1 oder 2, wobei die Malariainfektion durch Plasmodiuminfektion verursacht wird.
11. Verfahren nach Anspruch 2, wobei die ersten und zweiten Zellvolumenparameter Standardabweichungen von den ersten bzw. zweiten Zellvolumenverteilungen sind; und die Diskriminante eine Funktion von den Standardabweichungen von den ersten und zweiten Zellvolumenverteilungen ist.
- 40

Revendications

- 45 1. Procédé destiné à détecter une infection par la malaria en utilisant un échantillon de sang, comprenant les étapes consistant à :
- (a) mélanger ledit échantillon de sang avec un système de réactif lytique afin de lyser les globules rouges, et de former un mélange d'échantillon ;
 - 50 (b) réaliser une analyse différentielle des globules blancs dudit mélange d'échantillon sur un analyseur de sang, et obtenir une distribution du volume cellulaire d'une sous-population de globules blancs à partir d'une mesure du volume cellulaire utilisée dans ladite analyse différentielle ;
 - (c) obtenir un paramètre du volume cellulaire à partir de ladite distribution du volume cellulaire de ladite sous-population de globules blancs ;
 - 55 (d) évaluer ledit paramètre du volume cellulaire contre un critère prédéterminé, et rapporter une indication de ladite infection par la malaria si ledit paramètre du volume cellulaire répond audit critère prédéterminé.
2. Procédé destiné à détecter une infection par la malaria en utilisant un échantillon de sang, comprenant les étapes

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consistant à :

(a) mélanger ledit échantillon de sang avec un système de réactif lytique afin de lyser les globules rouges, et de former un mélange d'échantillon ;

(b) réaliser une analyse différentielle des globules blancs dudit mélange d'échantillon sur un analyseur de sang ; et obtenir une première distribution du volume cellulaire d'une première sous-population de globules blancs et une seconde distribution du volume cellulaire d'une seconde sous-population de globules blancs à partir d'une mesure du volume cellulaire utilisée dans ladite analyse différentielle ;

(c) obtenir un premier paramètre du volume cellulaire à partir de ladite première distribution du volume cellulaire de ladite première sous-population de globules blancs et obtenir un second paramètre du volume cellulaire à partir de ladite seconde distribution du volume cellulaire de ladite seconde sous-population de globules blancs ;

(d) obtenir un discriminant en tant que fonction desdits premier et second paramètres du volume cellulaire ; et (e) évaluer ledit discriminant contre un critère prédéterminé, et rapporter une indication de ladite infection par la malaria si ledit discriminant répond audit critère prédéterminé.

3. Procédé selon la revendication 1 ou 2, dans lequel ladite sous-population de globules blancs est des monocytes.
4. Procédé selon la revendication 1 ou 2, dans lequel ladite sous-population de globules blancs est des lymphocytes.
5. Procédé selon la revendication 1 ou 2, dans lequel ladite sous-population de globules blancs est des neutrophiles.
6. Procédé selon la revendication 1 ou 2, dans lequel ledit paramètre du volume cellulaire est un écart-type de ladite distribution du volume cellulaire.
7. Procédé selon la revendication 1, dans lequel ledit paramètre du volume cellulaire est une moyenne de ladite distribution du volume cellulaire.
8. Procédé selon la revendication 1 ou 2, dans lequel ladite mesure du volume cellulaire est une mesure de l'impédance.
9. Procédé selon la revendication 1 ou 2, dans lequel ladite mesure du volume cellulaire est une mesure de la diffusion de la lumière aux petits angles, ou une mesure de la perte de lumière axiale.
10. Procédé selon la revendication 1 ou 2, dans lequel ladite infection par la malaria est provoquée par une infection à Plasmodium.
11. Procédé selon la revendication 2, dans lequel lesdits premier et second paramètres du volume cellulaire sont des écarts-types desdites première et seconde distributions du volume cellulaire, respectivement ; et ledit discriminant est une fonction desdits écarts-types desdites première et seconde distributions du volume cellulaire.

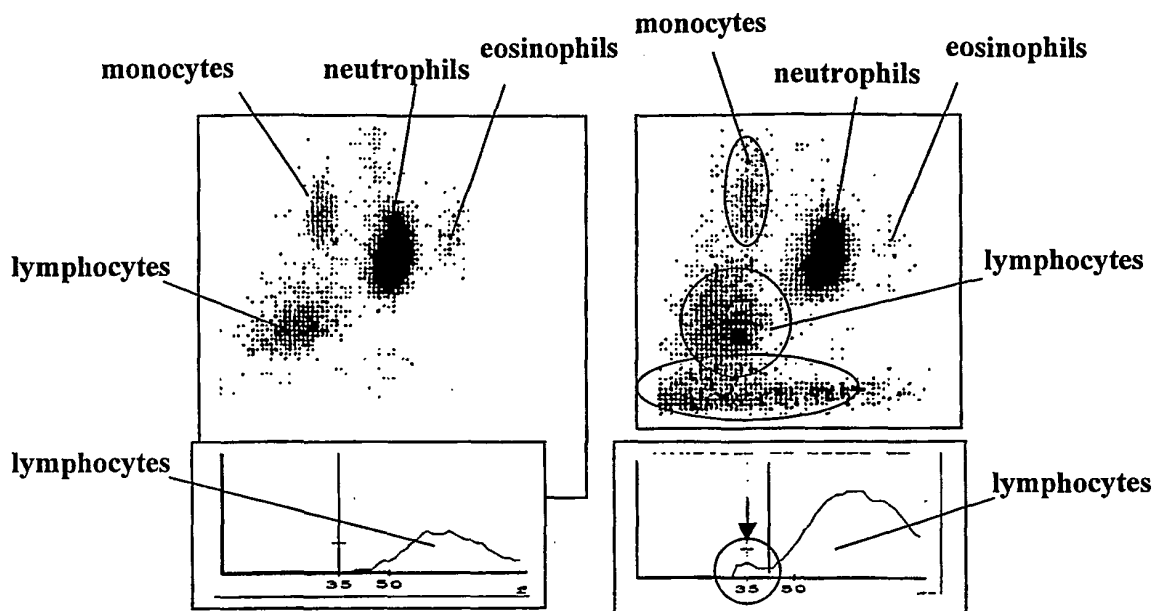


Fig. 1

	NE		LY		MO		EO	
	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD
V	169	49.92	82	25.42	184	26.63	161	28.86
C	147	17.86	130	31.82	130	9.74	143	9.48
S	128	17.81	69	22.53	92	11.40	193	7.88

Fig. 2

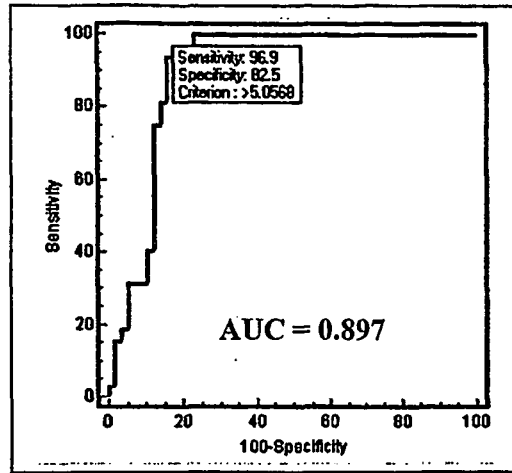


Fig. 3

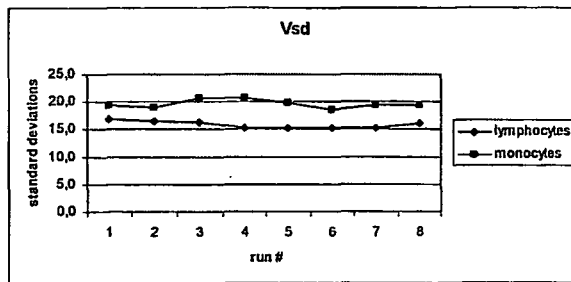


Fig. 4

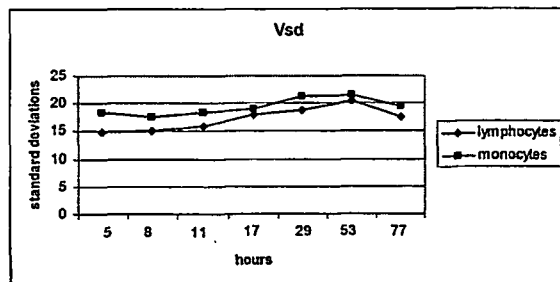


Fig. 5

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- US 5155044 A [0018]
- US 5731206 A [0018]
- US 5786224 A [0018]
- US 5686308 A [0018]
- US 5843608 A [0018]
- US 5125737 A [0020]
- US 4346018 A [0032]
- US 4521518 A [0032]
- US 4528274 A [0032]
- US 4962038 A [0032]
- US 5763280 A [0032]
- US 5834315 A [0032]
- US 5882934 A [0032]
- US 5935857 A [0032]
- US 6573102 B [0032]
- US 4286963 A [0033]
- US 4485175 A [0033]
- US 6673618 B1 [0033]

Non-patent literature cited in the description

- **KUEH et al.** *Scandinavian Journal of Haematology*, 1982, vol. 29, 147-152 [0004]
- **ERIKSSON et al.** *Journal of Infectious Diseases*, 1989, vol. 21, 434-441 [0004]
- **KAIN et al.** *Clinics in Infectious Diseases*, 1998, vol. 27, 142-149 [0004]
- **HUMAR et al.** *Canadian Medical Association Journal*, 1997, vol. 156, 1165-1167 [0004]
- **AMODU et al.** *East African Medical Journal*, 1997, vol. 74, 714-716 [0005]
- **AMODU et al.** *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1998, vol. 92, 54-56 [0005]
- **ARESE et al.** *Annals of Tropical and Medical Parasitology*. 1997, vol. 91, 510-516 [0005]
- **KRÄMER et al.** *Cytometry*, 2001, vol. 45, 133-140 [0005] [0055]
- **HÄNSCHEID et al.** *Emerging Infectious Diseases*, 1999, vol. 5, 836-838 [0005]
- **MENDELOW et al.** *British Journal of Haematology*, 1999, vol. 104, 499-503 [0005] [0055]
- **HÄNSCHEID et al.** *Parasitology Today*, 2000, vol. 16, 549-551 [0005]
- **HÄNSCHEID et al.** *American Journal of Tropical Medicine*, vol. 64, 190-192 [0005]
- **GROBUSCH et al.** *Cytometry part B (clinical cytometry)*, 2003, vol. 55B, 46-51 [0005] [0055]
- **SCOTT et al.** *Clinical and Laboratory Haematology*, 2003, vol. 25, 77-86 [0005]
- **FAWZI et al.** *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2003, vol. 97, 71-79 [0005]
- règles de décision. **AMOUREUX et al.** *Annales de Biologie Clinique*. 2003, vol. 61, 576-584 [0041]
- **SCOTT et al.** *Clinical and Laboratory Haematology*, vol. 25, 77-86 [0055]
- **HÄNSCHEID et al.** *American Journal of Tropical Medicine*, 2001, vol. 64, 190-192 [0055]

专利名称(译)	检测疟疾和其他寄生虫感染的方法		
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IPC分类号	G01N33/48 C12Q1/02 C12Q1/04 G01N33/50 G01N33/53 G01N33/569 G01N33/68		
CPC分类号	G01N33/56905 G01N2333/45 Y02A50/55 Y02A50/58 Y10T436/10 Y10T436/101666 Y10T436/107497		
优先权	60/548525 2004-02-27 US		
其他公开文献	EP1718966B1 EP1718966A1		
外部链接	Espacenet		

摘要(译)

一种检测寄生虫感染，特别是疟疾的方法，包括将血液样本与溶解试剂系统混合，以溶解红细胞，并形成样本混合物。在血液分析仪上对样品混合物中的白细胞进行差异分析，并从差异分析中使用的细胞体积测量中获得白细胞亚群的细胞体积分布；从白细胞亚群的细胞体积分布中获得细胞体积参数；根据预定标准评估细胞体积参数，并且如果细胞体积参数满足预定标准，则报告寄生虫感染的指示。该方法还使用根据预定标准从两个不同白细胞亚群的细胞体积参数获得的判别式。该方法还使用使用RF阻抗测量获得的小区分布的小区分布参数。

Table 2. Parameters exhibiting a significant difference between the malaria positive and the malaria negative groups.

	Vmean	Cmean	Smean	Vsd	Csd	Ssd
Neutrophils						
Positive	151.8 ± 11.9	132.4 ± 5.3	142.9 ± 6.6	22.6 ± 3.5	8.0 ± 2.1	12.0 ± 2.3
Negative	145.7 ± 12.4	134.6 ± 4.7	140.9 ± 11.6	21.4 ± 4.3	7.3 ± 1.4	11.9 ± 2.5
Significance	p = 0.0059	NS	NS	NS	NS	NS
Lymphocytes						
Positive	85.3 ± 10.6	103.0 ± 3.1	67.1 ± 8.4	27.6 ± 4.2	16.1 ± 2.8	17.6 ± 2.3
Negative	82.6 ± 6.9	102.9 ± 4.6	66.5 ± 6.1	20.3 ± 4.4	13.0 ± 2.4	18.3 ± 1.7
Significance	NS	NS	NS	p = 0.0000	p = 0.0000	NS
Monocytes						
Positive	186.6 ± 12.1	111.3 ± 4.6	88.6 ± 3.9	24.7 ± 3.6	5.0 ± 0.9	9.3 ± 1.1
Negative	170.4 ± 11.9	111.7 ± 3.2	87.7 ± 4.6	20.1 ± 4.1	4.8 ± 0.4	9.6 ± 1.4
Significance	p = 0.0000	NS	NS	p = 0.0000	p = 0.0152	NS