(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) **EP 1 982 183 B9**

(12) **CORRECTED EUROPEAN PATENT SPECIFICATION**

(15) Correction information:
Corrected version no 1 (W1 B1)
Corrections, see
Description Paragraph(s) 59

(48) Corrigendum issued on:
12.12.2012 Bulletin 2012/50

(45) Date of publication and mention of the grant of the patent:
27.06.2012 Bulletin 2012/26

(21) Application number: **07702519.5**

(22) Date of filing: **19.01.2007**

(51) Int Cl.:
G01N 33/62 *(2006.01)* ***G01N 33/543*** *(2006.01)*
G01N 33/50 *(2006.01)* ***G01N 33/52*** *(2006.01)*

(86) International application number:
PCT/DK2007/050005

(87) International publication number:
WO 2007/082545 (26.07.2007 Gazette 2007/30)

(54) **DRY STICK DEVICE AND METHOD FOR DETERMINING AN ANALYTE IN A SAMPLE**

TROCKENTESTSTREIFENVORRICHTUNG UND VERFAHREN ZUR BESTIMMUNG EINES ANALYTEN IN EINER PROBE

BANDELETTE D'ANALYSE ET PROCEDE POUR DETERMINER UNE SUBSTANCE A ANALYSER DANS UN ECHANTILLON

(84) Designated Contracting States:
AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IS IT LI LT LU LV MC NL PL PT RO SE SI SK TR

(30) Priority: **19.01.2006 DK 200600083**
19.01.2006 US 759952 P

(43) Date of publication of application:
22.10.2008 Bulletin 2008/43

(73) Proprietor: **Lattec I/S**
3400 Hillerød (DK)

(72) Inventor: **NYGAARD, Lars**
DK-3230 Græsted (DK)

(74) Representative: **Plougmann & Vingtoft A/S**
Rued Langgaards Vej 8
2300 Copenhagen S (DK)

(56) References cited:
EP-A- 0 339 331
EP-A- 1 321 478
EP-A1- 1 160 571
WO-A-2005/057216
WO-A1-2004/025301
WO-A2-03/023051
WO-A2-03/093788
AU-B2- 634 814
BE-A3- 1 011 487
GB-A- 2 023 815
US-A- 3 901 657
US-A- 4 215 995
US-A- 4 223 089
US-A- 4 245 096
US-A- 4 357 144
US-A- 4 732 736
US-A- 5 411 858
US-A- 5 776 779
US-A1- 2003 059 951
US-A1- 2003 073 073

- **ERBERSDOBLER H ET AL: "RAPID UREA DETERMINATION IN MILK BY A UREA STRIP METHOD AND A REFLECTION PHOTOMETRIC EVALUATION" MILCHWISSENSCHAFT, vol. 41, no. 5, 1986, pages 289-291, XP008068017 ISSN: 0026-3788**
- **GODDEN S ET AL: "Evaluation of the Azotest(R) strip as recommended for the estimation of milk urea nitrogen concentrations in individual cow, milk line and bulk tank samples." BOVINE PRACTITIONER, vol. 37, no. 1, February 2003 (2003-02), pages 36-42, XP008068042 ISSN: 0524-1685**
- **MELINDA M: "RAPID DETERMINATION OF UREA CONTENT IN MILK AS AN INFORMATORY METHOD FOR ESTIMATION OF PROTEIN-ENERGY RATIO OF CONSUMED FEEDSTUFF PRELIMINARY REPORT" MAGYAR ALLATORVOSOK LAPJA, vol. 40, no. 6, 1985, pages 359-359, XP008068041 ISSN: 0025-004X**

Printed by Jouve, 75001 PARIS (FR)

(Cont. next page)

- BUTLER W R ET AL: "Plasma and milk urea nitrogen in relation to pregnancy rate in lactating dairy cattle" JOURNAL OF ANIMAL SCIENCE, vol. 74, no. 4, 1996, pages 858-865, XP002395748 ISSN: 0021-8812
- RODRIGUEZ L A ET AL: "Effect of degradability of dietary protein and fat on ruminal, blood, and milk components of Jersey and Holstein cows" JOURNAL OF DAIRY SCIENCE, vol. 80, no. 2, 1997, pages 353-363, XP002395749 ISSN: 0022-0302
- HWANG SEN-YUAN ET AL: "Diurnal variations in milk and blood urea nitrogen and whole blood ammonia nitrogen in dairy cows" ASIAN-AUSTRALASIAN JOURNAL OF ANIMAL SCIENCES, vol. 14, no. 12, December 2001 (2001-12), pages 1683-1689, XP008068016 ISSN: 1011-2367

Description

FIELD OF THE INVENTION

[0001] The present invention relates to the field of analysing an analyte in a milk sample. In particular the present invention relates to an improved construction of a dry stick device for the determination of an analyte in a milk sample by the use of a chemical assay, where care is particularly taken to avoid precipitation of milk sample components on the top-face of the device causing limitation of the detectable signal.

PRIOR ART

[0002] Dairy Herd Improvement (DHI) has always been based on milk recording of individual cows in a herd. By increasing this knowledge of each individual cow, it is possible to both increase the quantity and to improve the quality of the milk. Furthermore, the general health situation is improved and the income for the farmers will thereby increase. One way for the farmers to increase their income may be to optimise feeding by monitoring the content of urea.

[0003] In dairy cow farming, it is highly important that the animals (e.g. cows) utilize the protein content in the feed optimally, as protein is one of the most expensive feed components. The utilization depends, inter alia, on the amount of energy and protein simultaneously present in the animal.

[0004] When a feed contains more protein than necessary, not all the ammonia formed therefrom can be processed in the rumen. The excess ammonia is taken up in the blood and discharged to the liver where the ammonia is converted to urea ($CO(NH_2)_2$). This urea is taken up in the blood and largely excreted via the urine. From the blood, a small part of the urea also finds its way to the milk. In a cow producing 25 kg milk, the urea content is generally about 5 to 8 g urea pr. 25 kg milk or (0.2 to 0.3 g/l). The more ammonia in the rumen, the more urea in the blood, which again means more urea in the milk.

[0005] Excess of protein (which also provides excess of nitrogen, because protein includes nitrogen) in the feed leads to higher urea content in the milk. Accordingly, from the viewpoint of nitrogen utilization, a low urea content is desirable. However, there is also a lower limit. Unduly low urea content indicates an improper energy ratio in the feed or an unduly low protein content of the feed. If the animals are feed less it will produce less milk than they are capable of, and/or the protein content in the milk decreases.

[0006] Another important point may be that it requires energy to process urea. In other words, a high nitrogen balance is also energetically unfavourable.

[0007] There is a direct relationship between the amount of protein fed and the concentration of urea in the blood and milk. High MUN (Milk Urea Nitrogen) results indicate an opportunity to reduce protein content of diets, without reducing the milk yield. This will reduce feeding costs and furthermore reduce nitrogen release to the environment. Overfeeding with protein, resulting in high urea levels in the milk, will have the following consequences:

- Energy is needed for cows to synthesize urea for excretion
- Reduction in the amount of energy available for milk production
- Less available energy may put early lactation at increased risk of ketosis
- High level of urea is toxic to sperm and embryos and can result in reduced fertility
- High urea levels contribute to environmental contamination

[0008] Today many farmers overfeed protein by 10-20%. Overfeeding protein by 20 %, will lead to increased cost of about USD 50/cow/year. The information given to the farmers by watching the Milk Urea Nitrogen will provide the basis for decisions on how to change the feeding, especially concerning the energy/protein ratio (see the table below). Because MUN levels are affected by a large number of cow-related factors, including age, stage of lactation, health status, water consumption and dry matter intake, cows fed the same ration often have very different MUN values. Therefore it is generally recommended using MUN results from a minimum of 10 cows for diagnosing feeding problems.

[0009] All the information from milk samples provided on urea content, plus many other data on individual cows, may be stored in or on several databases. A number of databases could be linked together and used by consultants, veterinarians, advisors and others without the need for on-farm visits. The information can be used to compare the situation in different countries, to improve the breeding values, to optimise the feeding and to reduce the problem with insufficient feeding.

[0010] Thus, the determination of urea in a sample has become increasingly popular and new technologies allowing measurement on a large number of samples and Milk Urea Nitrogen (MUN) is often used as an indicator of ammonia levels in the rumen. The MUN content is a quick, accurate reflection of the amount of nitrogen absorbed by the cow but not used for growth or milk protein synthesis. Determination of the urea content in a sample employing enzyme based test devices like Reflotest and Azotest®Strip/Azostix are well known to a person skilled in the art. Both the Reflotest and

Azotest®Strip/Azostix employ urease and azo dyes for the determination of urea in a sample.

[0011] The urea content in a sample may also be determined using a chemical assay utilising colouring agents such as o-phtalaldehyde, such chemical assays are as well known for the person skilled in the art. Often chemical assays involve compounds/controlling compounds (such as acids) which may cause predpitation of sample components.

[0012] US 4,215,995 discloses test means for determining the content of urea in a sample. The test means involves a single filter paper which is impregnated in a three stage application of reagents (including o-phtalaldehyde) separated from each other on an acidic modified carrier matrix. In this way a higher stability of the reagents are achieved and precipitation of the serum proteins may be avoided by providing a suitable acid matrix by using a strong cation exchange loaded paper. The problem with the device provided by US 4,215,995 is that the acidity component is not isolated from the reagents and will be contacted directly with pH sensitive liquid samples which may cause precipitation of sample components on the top-face of the device and thus reduce the readable signal.

[0013] Patent application JP 10-229023 discloses a test device comprising a solid support and a reagent pad and a developing pad for the determination of urea in a sample, such as blood, serum or plasma. The reagent pad is impregnated with o-phthalaldehyde glycerine acetal, polyvinyl pyrolidone and distilled water. The developing pad is impregnated with N-1-naphtyl-N'-diethylethylenediamine oxalate, 4-sulphophthalic acid in an aqueous solution, a surfactant and distilled water. The reagent pad is then coated onto the solid support, the developing pad is coated onto this reagent pad. The problem of using this or a similar test device for the determination of urea in a sample such as milk is that the acid impregnated in the developing pad (the top layer) causes the milk proteins to precipitate on the top of the device. This precipitation interferes with the colour developed when urea is determined and may increase the numbers of false results.

[0014] Thus, there is a need In the industry for a simple dry stick construction where the interference from precipitated sample components is limited or avoided.

SUMMARY OF THE PRESENT INVENTION

[0015] Accordingly, in a first aspect, the aim of the present invention is to provide a dry stick test device for the determination of an analyte in a milk sample by means of a chemical assay wherein said dry stick device is constructed in such a manner so as to limit or avoid precipitation of milk sample component(s). The dry stick test device comprises:

(i) optionally a solid support,

(ii) at least one reagent pad comprising a reagent capable of reacting with the analyte, a derivative of said analyte or an indicator compound for said analyte to provide a detectable signal when in moistened state,

(iii) a development pad which is located in contact with the at least one reagent pad, optionally between the solid support and the at least one reagent pad, said development pad comprises at least one controlling compound capable of providing a condition required for the reagent to react with the analyte to provide a detectable signal,

wherein the at least one reagent pad and the development pad are arranged to avoid precipitation of milk sample component(s) on the top-face of the device and wherein the sample is applied on the top-face of the device and a detectable signal is obtained from the top-face of the device and wherein the controlling compound is an acidic compound capable of providing a pH value of the milk sample below 6 and wherein the top-face of the device relates to the surface where the sampe initially gets into contact with the at least one reagent pad.

[0016] In another aspect of the present invention dry stick test device for the determination of an analyte in a milk sample by means of a chemical assay is provided. The dry stick test device comprises:

(i) optionally a solid support,

(ii) at least one reagent pad comprising a reagent capable of reacting with the analyte, a derivative of said analyte or an indicator compound for said analyte to provide a detectable signal when in moisten state,

(iii) a development pad which is located in contact with the at least one reagent pad, optionally between the solid support and the reagent pad, said development pad comprises at least one controlling compound capable of providing a condition required for the reagent to react with the analyte to provide a detectable signal,

wherein the at least one reagent pad is capable of providing a pH-value of the milk sample of 6 or above 6 in order to avoid precipitation of sample component(s), and the controlling compound present in the development pad is capable of providing a pH-value of the milk sample below 6, wherein the at least one reagent pad and the development pad are arranged to avoid precipitation of milk sample component(s) on the top-face of the device, wherein the sample is applied

on the top-face of the device and the detectable signal is obtained from the top-face of the device relates to the surface where the sample initially gets into contact with the at least one reagent pad.
Furthermore, it is an aspect of the present invention to provide a dry stick test device for the determination of urea in a milk sample, said dry stick test device comprises, optionally a solid support and at least 2 pads, said at least 2 pads comprise (i) at least one reagent pad comprising o-phthalaldehyde or a derivative thereof, a colouring compound and a detergent, and (ii) a development pad comprising at least one acid, wherein the development pad is located down-stream from the at least one reagent pad.

[0017] It is also the aim of the present invention to provide a method for the preparation of the dry stick device according to the present invention. The method comprises the steps of:

(i) providing at least one reagent pad by impregnating a first porous material with an aqueous solution comprising a reagent capable of reacting with the analyte, a derivative of said analyte or an indicator compound for said analyte to provide a detectable signal when in a moistened state,

(ii) thereafter drying the at least one reagent pad,

(iii) providing a development pad by impregnating a second porous material with an aqueous solution comprising at least one controlling compound which, when in a moistened state, is capable of providing a condition required for the reagent to react with the analyte to provide a detectable signal,

(iv) thereafter drying the impregnated second porous material, and

(v) immobilising the first porous material with the second porous material, optionally on a solid support, to obtain the dry stick device.

[0018] In a further aim of the present invention, a method for the determination of an analyte in a milk sample is provided. The method comprises the steps of:

(a) applying the sample suspected of containing the analyte to drystick test device, said drystick test device comprises:

(i) at least one reagent pad comprising a reagent capable of reacting with the analyte, a derivative of said analyte or an indicator compound for said analyte to provide a detectable signal when in a moistened state, and

(ii) in contact with said at least one reagent pad a development pad is located, said development pad comprises at least one controlling compound capable of providing a condition required for the reagent to react with the analyte to provide a detectable signal,
wherein the at least one reagent pad and the development pad are arranged to avoid precipitation of milk sample component(s) on the top-face of the device.

(b) permitting the sample to migrate into the at least one reagent pad and the developing pad and mobilising the at least one reagent and the at least one controlling compound, and

(c) permitting the at least one reagent and the analyte, the derivative of said analyte or the indicator compound for said analyte to react and provide a detectable signal.

[0019] The present invention will now be described in more detail in the following.

DETAILED DISCLOSURE OF THE PRESENT INVENTION

[0020] The inventors of the present invention surprisingly found and developed a new construction of a dry stick test device wherein the interference from precipitated components from the milk sample is limited or avoided.

[0021] The new construction of the dry stick test device for the determination of an analyte in a milk sample by means of a chemical assay comprises: (i) optionally a solid support, (ii) at least one reagent pad comprising a reagent or a combination of reagents capable of reacting with the analyte, a derivative of said analyte or an indicator compound for said analyte to provide a detectable signal when in moistened state, (iii) a development pad which is located in contact with the at least one reagent pad, optionally between the solid support and the at least one reagent pad, said development pad comprises at least one controlling compound capable of providing a condition required for the reagent to react with the analyte to provide a detectable signal, wherein the development pad is located down-stream from the at least one

reagent pad, wherein the at least one reagent pad and the development pad are arranged to avoid precipitation of milk sample component(s) on the top-face of the device and wherein the sample is applied on the top-face of the device and the detectable signal is obtained from the top-face of the device and wherein the controlling compound is an acidic compound capable of providing a pH value of the milk sample below 6 and wherein the top-face of the device relates to the surface where the sample initially gets into contact with the at least one reagent pad. In an embodiment of the present invention the at least one reagent pad comprises a reagent capable of reacting with the analyte, a derivative of said analyte or an indicator compound for said analyte and/or a reagent capable of participating in the determination of the analyte. The reagent capable of participating in the determination of the analyte may be a reagent taking part in the assay for providing a detectable signal, but which does not bind, react or interact directly with the analyte.

[0022] In the present context, the term "chemical assay" relates to the determination of the relative amount(s) of one or more components of the milk sample by means of a chemical and/or biochemical reaction. In an embodiment of the present invention the chemical assay involves the determination of the analyte which is not based on an enzyme-based determination.

[0023] An enzyme-based assay is an assay that depends on or uses enzymatic activity in order to produce a detectable signal.

[0024] As mentioned above the dry stick test device comprises at least one reagent pad and a development pad wherein the at least one reagent pad comprises the reagent or the combination of reagents and the development pad comprises at least one controlling compound capable of providing a condition required for the reagent or the combination of reagents to react with the analyte to provide a detectable signal. In an embodiment of the present invention the at least one reagent and the development pad are located relative to each other in such a manner that precipitation of milk sample components may be avoided, in particular, to avoid precipitation of milk sample components on the surface, where the reading/determination (of the colour) is performed.

[0025] In the present context the term "arranged to avoid precipitation" relates to the placing of the at least one reagent pad and the developing pad in such a manner that precipitation of milk sample components on the top-face of the dry stick test device is avoided when applying the sample. By precipitation of milk sample components is meant milk sample components of the fluid milk sample or part of the fluid milk sample that change into a solid or semisolid mass, often caused by the action of e.g. heat or chemical substances. It is preferred that the precipitation is provided by the action of a chemical substance (in the present context a controlling compound).

[0026] In the present context the term "top face" relates to the surface of the dry stick device according to the present invention where the milk sample is applied or where the milk sample initially gets into contact with the at least one reagent pad.

[0027] In an alternative embodiment of the present invention the term "top face" relates to the surface of the dry stick test device of the present invention from where the detectable signal is obtained. This surface is the same as the surface where the sample is applied.

[0028] In an embodiment of the present invention, the dry stick test device may comprise at least 2 reagent pads, such as at least 3 reagent pads, e.g. at least 4 reagent pads, such as at least 5 reagent pads, e.g. at least 6 reagent pads.

[0029] In the present context, the term "sample components" relates to all the substances present in the milk sample at the time of performing the assay, the "sample components" may in an embodiment of the present invention be one of the reagents of the assay. In an embodiment of the present invention the milk sample components that may tend to precipitate may be milk proteins, such as casein molecules.

[0030] The inventors of the present invention have also provided a new method for the determination of an analyte in a milk sample. The method comprises the steps of:

(a) applying the sample suspected of containing the analyte to drystick test device, said drystick test device comprises:

(i) at least one reagent pad comprising a reagent capable of reacting with the analyte, a derivative of said analyte or an indicator compound for said analyte to provide a detectable signal when in a moistened state, and

(ii) in contact with said at least one reagent pad a development pad is located, said development pad comprises at least one controlling compound capable of providing a condition required for the reagent to react with the analyte to provide a detectable signal,
wherein the at least one reagent pad and the development pad are arranged to avoid precipitation of milk sample component(s) on the top-face of the device and wherein the sample is applied on the top-face of the device and the detectable signal is obtained from the top-face of the device and wherein the controlling compound is an acidic compound capable of providing a pH value of the milk sample below 6 and wherein the top-face of the device relates to the surface where the sample initially gets into contact with the at least one reagent pad

(b) permitting the sample to migrate into the at least one reagent pad and the developing pad and mobilising the at

least one reagent and the at least one controlling compound, and

(c) permitting the at least one reagent and the analyte, the derivative of said analyte or the indicator compound for said analyte to react and provide a detectable signal.

[0031] The detectable signal may be any substance which directly or indirectly is capable of being observed by any kind of visual or instrumental means. The instrumental means may be e.g. a magno(magne)tometer, spectrophotometer, ELISA-reader. Various suitable compounds may be suitable as the colour producing compound. In the present invention the colour producing compound may be selected from the group consisting of chromogens, catalysts, fluorescent compounds, chemiluminescent compounds, radioactive labels, metals, magnetic particles, dye particles, organic polymer latex particles, liposomes or other vesicles containing signal producing substances and the like.

[0032] In the present context the term "in a moistened state" relates to the contact between the reagents in the reagent (s) pad and/or the controlling compound in the development pad and the milk sample whereby the reagent(s) pad and/or the development pad becomes wet or slightly wet. The effect of the moistened state is that the dried reagents, the dried controlling compound(s) are liberated and dissolved (mobilised) and the reaction in the dry stick device commences and a detectable signal is produced, which is dependent on the amount of analyte present in the milk sample.

The porous material

[0033] The materials selected to be used in the at least one reagent pad and/or the development pad may be selected from a porous material. In the present context the term "porous material" relates to a material which adsorbs the milk sample and thereby permits it to migrate.

[0034] The porous material selected may comprise a pore-size and capacity that make it possible to provide a high flow-rate which quickly dissolves the reagent or the combination of reagents and which provides a good and substantially even distribution of the samples. Preferably, the porous material may be selected for providing substantially no retention of triglyceride rich samples. In an embodiment of the present invention the retention of triglycerides is 0%, such as at the most 1%, e.g. at the most 2.5%, such as at the most 5%, e.g. at the most 10%, such as at the most 15%, e.g. at the most 25%, such as at the most 50%, or the retention of most or all of the triglycerides, e.g. at the most 75%, such as at the most 100%.

[0035] The porous material is selected from the group consisting of a nitrocellulose membrane, cellulose, a polymer (such as nylon, polyvinylidene fluoride or latex), glass fibre, woven fibres, non-woven fibres, a chromatographic gel membrane, diatomaceous earth, silica gel, silicium oxide and kieselguhr.

[0036] In an embodiment of the present invention, the porous material in the at least one reagent pad and/or in the development pad may be selected from a group of materials comprising a pore size preferably in the range of 1-1000 μm, such as in the range of 1-500 μm, such as in the range of 1-100 pm, for instance in the range of 1-75 μm, such as in the range of 5-500 μm, such as in the range of 5-100 μm, for instance in the range of 5-75 μm, such as in the range of 10-500 μm, such as in the range of 10-100 μm, for instance in the range of 10-75 μm, such as in the range of 10-50 μm, for instance in the range of 50-200 μm, such as in the range of 50-100 μm, for instance in the range of 100-500 μm, such as in the range of 50-300 μm, for instance in the range of 75-300 μm, such as in the range of 75-200 μm, for instance in the range of 75-150 μm, such as in the range of 75-120 μm.

[0037] In yet another embodiment of the present invention, the porous material in at least one reagent pad and/or in the development pad may be selected from a group of materials comprising a suitable pore size such as at most 500 μm, for instance at most 200 μm, such as at most 150 μm, for instance at most 100 μm, such as at most 75 μm.
Preferably, the porous materials used in the at least one reagent pad and/or the development pad may be the same in at least 2 pads, such as at least 3 of the pads, for instance 4 of the pads, such as at least 5 of the pads.

[0038] In accordance with the above porous material, it may be desirable to provide a device for detecting an analyte in a fast assay. In an embodiment of the present invention the assay time at approximately 20°C may be less than 20 minutes, such as less than 18 minutes, e.g. less than 15 minutes, such as less than 12 minutes, e.g. less than 10 minutes, such as less than 8 minutes, e.g. less than 5 minutes, such as less than 3 minutes, e.g. less than 2 minutes, such as in the range of 1 to 25 minute, e.g. in the range of 2-25 minutes, such as In the range of 5 to 20 minute, e.g. in the range of 8-18 minutes, such as in the range of 10 to 15 minute, e.g. in the range of 11-14 minutes, such as in the range of 12-13 minutes.

The solid support

[0039] The device according to the present invention may be supported by a solid support. In the present context, the term "solid support" refers to a material, which has no influence on the migration or on the reaction of the liquid milk sample or on reagent(s) or the agents capable of increasing the rate of the reaction. The solid support provides a

stabilising basis for the assay device and provides sufficient strength to maintain the desired physical shape and has substantially no interference with the production of a detectable signal.

[0040] In an embodiment of the present invention, the material for the solid support is selected from the group consisting of tubes, polymeric beads, nitrocellulose strips, membranes, filters, plastic sheets and the like.

[0041] Naturally, synthetic and natural occurring materials that are synthetically modified can be used as the material of the solid phase. Such materials include polysaccharides, for instance cellulosic materials such as paper and cellulosic derivatives, such as cellulose acetate and nitrocellulose, silica- orinorganic materials, such as, for example, deactivated alumina, diatomaceous earth, $MgSO_4$ or other inorganic finely divided material uniformly dispersed in a porous polymeric matrix, wherein the matrix may comprise one or more polymers such as homopolymers and copolymers of vinyl chloride, for instance, polyvinyl chloride, vinyl chloride-propylene copolymer, and vinyl chloride-vinyl acetate copolymer, cloth, both naturally occurring (for instance, cotton) and synthetic (for instance, nylon), porous gels, such as silica gel, agarose, dextran, and gelatin, polymeric films, such as polyacrylamide, and the like.

[0042] In an embodiment of the present invention, the solid support may be omitted from the dry stick test device. In this case the dry stick test device comprises at least one reagent pad and a development pad. When performing a determination of an analyte using a dry stick test device without a solid support, the sample is applied to the dry stick test device on one surface and the detectable signal may be detected on the same surface, thus any possible precipitation of milk sample components on the surface where the detectable signal is to be detected is limited or avoided.

The reagent pad

[0043] In the present context the term "reagent pad" relates to one or more pads comprising a reagent or a combination of reagents. The reagent or the combination of reagents may be impregnated into the reagent pad in such a manner that the reagent or the combination of reagents is/are immobilised when in dry state and mobile when in moistened state.

[0044] In the present context of the present invention the term "reagent" relates to the chemical substance that reacts with or participate in or is necessary for the determination of an analyte, a derivative of said analyte or an indicator compound for said analyte to provide a detectable signal. A similar definition of the combination of reagents may be provided which relates more specifically to 2 or more reagents, such as 3 or more reagents, e.g. 4 or more reagents, such as 5 or more reagents, e.g. 6 or more reagents.

[0045] In an embodiment of the present invention the dry stick test device comprises at least 2 reagent pads, such as at least 3 reagent pads, e.g. at least 4 reagent pads, such as at least 5 reagent pads, e.g. at least 6 reagent pads. In this embodiment the reagents that reacts with or participate in or is necessary for the determination of an analyte, a derivative of said analyte or an indicator compound for said analyte to provide a detectable signal may be introduced into different reagent pads. This may improve stability, storage properties and applicability of the dry stick device because non-compatible compounds can be included in different reagent pads of the dry stick device.

The development pad

[0046] In the present context, the term "development pad" relates to a pad capable of regulating the environment and the conditions for the milk sample comprising the analyte to an environment that facilitates the determination of the analyte, a derivative of said analyte or an indicator compound for said analyte.

[0047] In an embodiment of the present invention, the development pad comprise one or more controlling compounds capable of increasing the rate of the reaction between the analyte, a derivative of said analyte or an indicator compound for said analyte present in the milk sample and the reagent(s). In an embodiment of the present invention the controlling agent is an acid.

[0048] In yet another embodiment of the present invention, the development pad is in contact with at least one reagent pad by substantially fully overlapping, by partial overlap or by laying adjacent to at least one reagent pad. In an embodiment of the present invention the development pad is overlapping the at least one reagent pad by at least 5%, such as at least 10%, e.g. at least 25%, such as at least 50%, e.g. at least 75%, such as at least 80%, e.g. at least 90%, such as at least 95%. In the present context the term "substantially fully overlapping" relates to two separate pads (the developpement pad and the at least one reagent pad) being placed on top of one another. In the present context the term "partial overlap" relates to two separate pads (the developpement pad and the at least one reagent pad) overlapping with only part of the pad(s). A partial overlap of 100% relates to a full overlap and a deviation of 5% from the 100% full overlap relates to a substantially full overlap.

[0049] In an embodiment of the present invention the development pad and the at least one reagent pad(s) are laying adjacent to one another. This means that the pads are placed in contact with each other (touching each other). An overlap of 0% (but in contact) relates to the term "laying adjacent", furthermore, an overlap of less than 5% is considered being within the term of "laying adjacent", such as an overlap of at the most 4%, e.g. an overlap of the most 3%, such as an overlap of the most 2% or e.g. an overlap of the most 1%.

Controlling compound

[0050] In the development pad a controlling compound is immobilised. In the present context the term "controlling compound" relates to a substance that has the function as a propellant or a fuel in the specific assay for the determination of the analyte, a derivative of said analyte or an indicator compound for said analyte. The controlling compound may also be the chemical substance responsibly for the precipitation of sample components or the chemical compound causing the milk sample components not to precipitate. In an embodiment of the present invention the controlling compound may be separated from at least one of the reagents in order to improve the stability of the dry stick test device.

[0051] In yet another embodiment of the present invention the controlling compound is an acidic compound. The controlling compound is an acidic compound capable of providing a pH-value of the sample in the dry stick test device, when in a moistened state, below pH 6, such as below pH 5, e.g. below pH 4, such as below pH 3, e.g. below pH 2, such as below pH 1, e.g. below pH 0, such as in the range of pH 0-6, e.g. in the range of pH 0-5, such as in the range of pH 0-4, e.g. in the range of pH 0-3, such as in the range of pH 0-2, e.g. in the range of pH 0-1, such as in the range of pH 1-6, e.g. in the range of pH 2-6, such as in the range of pH 3-6, e.g. in the range of pH 4-6, such as in the range of pH 5-6.

The analytes to be determined

[0052] A device or a method based on the above principles can be used to determine a wide range of analytes by choice of appropriate colouring compounds known to the person skilled in the art, and the invention need not be limited to examples mentioned herein.

[0053] In an embodiment of the present invention the analytes to be assayed can be selected from the group consisting of a protein, a fat, a carbohydrate, an antibiotic, a steroid, such as hormones, a vitamin, a chemical compound, a hapten, a cell, such as a bacteria or such as leukocytes, an antibody, a drug of abuse and blood.

[0054] In an embodiment of the present invention, the analyte is a chemical compound and the chemical compound may be selected from the group consisting of urea, triglyceride and ketone bodies, such as acetoacetate, beta-hydroxybutyrate (BHB), acetone, ascorbic acid, nitrates, urobilinogen, cholesterol, and steroids such as pregnenolone, progesterone, testosterone, dihydrotestosterone, estrone, estradiol, cortisol, cortisone, aldosterone, corticosterone, androstenedione, 17α-OH- pregnenolone, 17α-OH- progesterone, 11-desoxy-corticosterone, 11-desoxycortisol and dehydroepiandrosterone, luteinising hormone or human chorionic gonadotropin.

[0055] The device and the method according to the present invention may also be suitable when the analyte is a carbohydrate and the carbohydrate may be selected from the group consisting of a monosaccharide, such as glucose or galactose, and a disaccharide, such as lactose.

[0056] In an embodiment of the present invention the dry stick test device is used for the determination of urea in a milk sample. The dry stick test device may comprise, optionally a solid support and at least 2 pads, said at least 2 pads comprise (i) at least one reagent pad comprising o-phthalaldehyde or a derivative thereof, a colouring compound and a detergent, and (ii) a development pad comprising at least one acid, wherein a development pad may be located downstream from the at least one reagent pad. In this embodiment the acidic compound is capable of providing a pH-value of the sample in the dry stick test device, when in a moistened state below pH 6, such as below pH 5, e.g. below pH 4, such as below pH 3, e.g. below pH 2, such as below pH 1, e.g. below pH 0, such as in the range of pH 0-6, e.g. in the range of pH 1-5, such as in the range of pH 1-4, e.g. in the range of pH 1-3, such as in the range of pH 1-2, e.g. in the range of pH 2-6, such as in the range of pH 3-6, e.g. in the range of pH 4-6, such as in the range of pH 5-6. Preferably, the at least one reagent pad is in contact with the development pad by substantially fully overlapping, by partial overlap or by laying adjacent. It is also preferred that the development pad may be located between the solid support and the at least one reagent pad.

The milk sample to be analysed

[0057] In order to wet the porous material used in the development pad and/or in the at least one reagent pad to permit migration, liquid milk sample is liquid milk applied. Furthermore, it is preferred that a minimum number of handling steps of the liquid milk sample is necessary before applying it to the dry stick test device. In the present context, the term "handling steps" relates to any kind of pre-treatment of the liquid milk sample before or after it has been applied to the assay device. This pre-treatment comprises separation, filtration, dilution, distillation, concentration, inactivation of interfering compounds, centrifugation, heating, fixation, addition of reagents, or chemical treatment.

[0058] In an embodiment of the present invention the milk sample may be collected from a mammal, preferably the mammal is selected from the group consisting of herd animals, cows, camels, buffaloes, pigs, horses, deer, sheep, goats, pets, dogs, cats and humans.

[0059] In an embodiment of the present invention, the sample is milk.

[0060] In a preferred embodiment of the present Invention a dry stick test device for the determination of an analyte in a milk sample by means of a chemical assay is provided. The device comprises:

(i) optionally a solid support,

(ii) at least one reagent pad comprising a reagent capable of reacting with the analyte, a derivative of said analyte or an indicator compound for said analyte to provide a detectable signal when in moisten state,

(iii) a development pad which is located in contact with the at least one reagent pad, optionally between the solid support and the at least one reagent pad, said development pad comprises at least one controlling compound capable of providing a condition required for the reagent to react with the analyte to provide a detectable signal,

wherein the at least one reagent pad is capable of providing a pH-value of the milk sample of 6 or above 6, and the controlling compound present in the development pad is capable of providing a pH-value of the milk sample below 6, wherein the at least one reagent pad and the development pad are arranged to avoid precipitation of milk sampe component(s) on the the top-face of the device, wherein the sample is applied on the top-face of the device and the detectable signal is obtained from the top-face of the device and wherein the top-face of the device relates to the surface where the sample initially gets into contact with the at least one reagent pad. In this embodiment the acidic compound is capable of providing a pH-value of the sample in the dry stick test device, when in a moistened state in the range of pH 1-5, such as in the range of pH 1-4, e.g. in the range of pH 1-3, such as in the range of pH 1-2, e.g. in the range of pH 2-5, such as in the range of pH 3-5, e.g. in the range of pH 4-5, such as in the range of pH 5-6. Preferably, the at least one reagent pad is in contact with the development pad by substantially fully overlapping, by partial overlap or by laying adjacent. It is also preferred that the development pad may be located between the solid support and the at least one reagent pad.

The ancillary compound

[0061] Because of the complexity of the liquid milk samples-to be assayed in the present invention it may occasionally be an advantage to use an ancillary compound in order to improve the flow and adsorption of the liquid milk sample in the regulation pad and/or in the one or more reagent pad(s) and to provide a fast, consistent and even release of the reagent(s) and the agents capable of increasing the rate of reaction. The ancillary compound may be supplied to the device either by a) adding it to the at least one reagent pad(s) and/or regulation pad alone or together with the liquid milk sample, b) incorporating the ancillary compound into at least one of the reagent pad(s) and/or the regulation pad, or c) a combination thereof.

[0062] In an embodiment of the present invention, the ancillary compound is added to the dry stick device before the liquid milk sample is added. Preferably the ancillary compound is a liquid.

[0063] In another preferred embodiment of the present invention, the ancillary compound and the liquid milk sample are added to the dry stick device in layers. In the present context, the term "layers" reefers to the splitting up of the volume of the ancillary compound and the volume of the liquid milk sample, and then the ancillary compound and the liquid milk sample are added to the first zone one after another. In this case, the ancillary compound may be added as a liquid as well as a solid compound. In an embodiment of the present invention, the ancillary compound and the liquid milk sample are split into at least 2 volumes each providing 4 alternating layers of ancillary compound and liquid milk sample, e.g. the ancillary compound and the liquid milk sample are split into at least 3 volumes each providing 6 alternating layers of ancillary compound and liquid sample, such as the ancillary compound and the liquid milk sample being split into at least 4 volumes each providing 8 alternating layers of ancillary compound and liquid milk sample, e.g. the ancillary compound and the liquid milk sample are split into at least 6 volumes each providing 12 alternating layers of ancillary compound and liquid milk sample, such as the ancillary compound and the liquid milk sample split into at least 8 volumes each providing 16 alternating layers of ancillary compound and liquid milk sample, e.g. the ancillary compound and the liquid milk sample are split into at least 10 volumes each providing 20 alternating layers of ancillary compound and liquid milk sample, such as the ancillary compound and the liquid milk sample being split into at least 20 volumes each providing 40 alternating layers of ancillary compound and liquid milk sample.

[0064] In yet an embodiment of the present invention the ancillary compound may be impregnated into at least one reagent pad(s) and/or into the regulation pad.

[0065] In another embodiment of the present invention at least one reagent pad and/or the development pad incorporating at least one ancillary compound capable of improving the flow of the liquid milk sample.

[0066] In yet another embodiment of the present invention the ancillary compound provides a fast, consistent and homogenous release of the reagent(s) in the at least one reagent pad and/or the agent capable of increasing the rate of reaction in the development pad. Additionally, the ancillary compound provides low affinity for protein binding.

[0067] Furthermore, the ancillary compound may provide low retention of triglyceride rich samples and/or decreases the viscosity of the sample.

[0068] In an embodiment of the present invention the ancillary compound contains chemical constituents selected from the group consisting of water, a surfactant, a salt, a metal, a sugar, a protein, a solvent and a lipid.

Preparation of the dry stick

[0069] The dry stick device according to the present invention may be prepared by any conventional methods provided for the preparation of dry stick devices. In a preferred embodiment the method for providing a dry stick device according to the present invention comprises the steps of:

(i) providing at least one reagent pad by impregnating a first porous material with an aqueous solution comprising a reagent capable of reacting with the analyte, a derivative of said analyte or an indicator compound for said analyte to provide a detectable signal when in a moistened state,

(ii) thereafter drying the at least one reagent pad,

(iii) providing a development pad by impregnating a second porous material with an aqueous solution comprising at least one controlling compound which, when in a moistened state, is capable of providing a condition required for the reagent to react with the analyte to provide a detectable signal,

(iv) thereafter drying the Impregnated second porous material, and

(v) immobilising the first porous material with the second porous material, optionally on a solid support, to obtain the dry stick device.

[0070] In an embodiment of the present invention the at least one reagent pad is located relative to the development pad to avoid precipitation of the milk sample component on the top-face of the device. Preferably, the development pad is located between the solid support and the at least one reagent pad. The milk sample component may as described earlier be selected from the group consisting of proteins, carbohydrate, fat, cells, or other components present in the sample.

[0071] In yet an embodiment of the present invention the first porous material may be impregnated with o-phthalaldehyde or a derivative thereof and a colouring compound. Furthermore, the first porous material may be further impregnated with a detergent.

[0072] In another embodiment of the present invention the first porous material may comprise 1, 2 or 3 different porous materials having 3, 2 or 1 reagents Impregnated, respectively. The reagents in this embodiment are selected from o-phthalaldehyde or a derivative thereof, a colouring compound and a detergent.

[0073] In an embodiment of the present invention the second porous material may be impregnated with an acid. Said acid may be capable of providing a pH-value of the sample in the dry stick test device, when in a moistened state below pH 6, such as below pH 5, e.g. below pH 4, such as below pH 3, e.g. below pH 2, such as below pH 1, e.g. below pH 0, such as in the range of pH 0-6, e.g. in the range of pH 0-5, such as in the range of pH 0-4, e.g. in the range of pH 0-3, such as in the range of pH 0-2, e.g. in the range of pH 0-1, such as in the range of pH 1-6, e.g. in the range of pH 2-6, such as in the range of pH 3-6, e.g. in the range of pH 4-6, such as in the range of pH 5-6

In an embodiment of the present invention the second porous material may be impregnated with one or more reagent (s) capable of reacting with the analyte, a derivative of said analyte or an indicator compound for said analyte to provide a detectable signal when in a moistened state, preferably together with the controlling compound.

Additionally embodiments

[0074] In an embodiment the dry stick test device according the present invention is used for the determination of an analyte in a milk sample. Preferably, the analyte is selected from the group consisting of a protein, a fat, a carbohydrate, an antibiotic, a steroid, a vitamin and a chemical compound. Preferably, the chemical compound is selected from the group consisting of urea, triglyceride and ketone bodies, such as acetoacetate, beta-hydroxybutyrate (BOHB), ascorbic acid (citric acid) and acetone. If the analyte is a carbohydrate the carbohydrate may be selected from the group consisting of glucose and lactose.

Determination of urea

[0075] As mentioned above the determination of protein utilisation may be an important parameter. As it is the case in cattle farming, it is highly important that the animals (e.g. cows) optimally utilize the protein contained in the feed, because protein is one of the most expensive feed components. The utilization depends, inter alia, on the amount of energy and protein simultaneously present in the animal.

[0076] The new construction of the dry stick test device according to the present invention may be very useful for the qualitative and quantitative determination of urea in a milk sample. The one or more reagent pad(s) of the dry stick test device may be impregnated with o-phthalaldehyde or a derivative thereof and a colouring compound and optionally a detergent, such as a polyoxyethylene alkyl phenyl ether or a polyoxyethylene alkyl ether.

[0077] The derivative of o-phthalaldehyde of the present invention means a compound which is converted to o-phthalaldehyde after the derivative is dissolved in the milk sample. The derivative includes o-phthalaldehyde glycerol acetal.

[0078] The colouring compound includes N-1-naphthyl ethylenediamine, N-1-naphthyl-N'-diethylethylenediamine and a salt thereof, such as salts of hydrochloric acid or oxalic acid are included.

[0079] The detergent may be a polyoxyethylene alkyl phenyl ether or a polyoxyethylene alkyl ether such as polyoxyethylene(9.5)p-t-octylphenylether (HLB: 13.5; Tradename: TRITON X-100), polyoxyethylene(40)p-t-octylphenylether (HLB: 17.9; Tradename: TRITON X-405), polyoxyethylene(20)octylphenylether (HLB: 16.2; Tradename: NISSAN NONION HS-220), polyoxyethylene(23)laurylether (HLB: 15.3; Tradename: Brij35), polyoxyethylene(20)palmitylether (HLB: 15.7; Tradename: Brij58), polyoxyethylene(20)laurylether (HLB: 16.2; Tradename: NISSAN NONION K-220), polyoxyethylene(20)stearylether (HLB: 15.3; Tradename: NISSAN NONION S-220), and so on.

[0080] The polyoxyethylene alkyl phenyl ether or polyoxyethylene alkyl ether of the present invention may have an HLB of 8 or more. In the present context, the term "HLB" relates to the Hydrophilic-Lipophilic Balance of the surfactant. This provides an indication of the hydrophilic portion of the molecule. More hydrophilic groups enable more solubility in water as more hydrogen bonds exist herein.

[0081] In an embodiment of the present invention, the development pad is impregnated with an acid necessary for the determination of urea. The acid used may be any kind of acid capable of providing and keeping a pH-value at 2.0 or lower when dissolved by the liquid sample. In particular, such acids Includes 4-sulfophthalic acid or an acid polymer.

[0082] The respective amount of o-phthalaldehyde or its derivative, the colouring compound (in the at least one reagent pad) and the acid (in the development pad) may be the amount which is necessary for the determination of urea in the specific assay. This means that the amounts are similar to those conventionally used for o-phthalaldehyde or its derivative, the colouring compound and the acid in the quantitative determination of urea. Normally, but not limited hereto, the amount of o-phthalaldehyde or its derivative is in the range sufficient to provide an amount of 0.05M - 2M when the o-phthalaldehyde or its derivative are dissolved in the milk sample. The amount of add provided in the development pad may be sufficient to provide and keep the pH-value of the milk sample at 2.0 or lower, when the milk sample is dissolved in the sample.

[0083] The amount of detergent may by sufficient to provide an amount of 0.1-20% (w/v) when the detergent becomes dissolved in the milk sample, such as in an amount of 0.1 - 10% (w/v), e.g. in an amount of 4-10% (w/v). In an embodiment of the present invention, the amount of detergent, when dissolved in the milk sample, can be calculated based upon the capacity of the sample kept in the dry stick test device.

[0084] Other known additives may be added to the dry stick test device which includes stabilisers, such as a surfactant etc. The surfactant may be used to improve extendibility and solubility of the milk sample and to enhance the coating properties of the liquid solution(s) upon production. In an embodiment of the present invention the amount of a specified surfactant may be the same as the amount conventionally used.

[0085] The structure of the dry stick test device of the present invention is not limited except that it should contain o-phthalaldehyde or its derivative, a colouring compound and an acid separated into at least one reagent pad and a development pad wherein the at least one reagent pad and the development pad are arranged to avoid precipitation of milk sample component(s) on the top-face of the device. The dry stick test device of the present invention may be produced in a manner similar to the production of conventional test pieces for measuring urea or as described in this document.

[0086] In an embodiment of the present invention the dry stick test device may be provided with a plurality of reagent pads where different reagent(s) is/are comprised in each reagent pad. Furthermore, an intermediate pad may be provided between the pluralities of reagent pads. In yet an embodiment of the present invention the reagents may not all be comprised in the at least one reagent pad, but one or more of the reagent(s) may be comprised in the development pad.

[0087] In figure 1, an example of the dry stick test piece is provided. The dry stick test piece comprises a solid support (1) having in one end a reagent section (2). The reagent section (2) comprises a development pad (3) located on the solid support (1) and on top of the development pad (3) is a reagent pad (4). In this example the milk sample is applied to the reagent pad (4) and the reagents (comprised herein are dissolved and migrates throughout the reagent pad and to the development pad (4) thereby dissolving the acid comprised herein and the reaction for determining urea progresses.

[0088] When a predetermined amount of a milk sample is dropped on the reagent pad (4) of the dry stick test piece (1), the sample is at first uniformly spread over the entire surface and throughout the entire reagent pad (4). The spread sample reaches the development pad (4) whereby the acid comprised in the development pad (4) is dissolved and the reaction proceeds. Urea determination comprised in the milk sample reacts with o-phthalaldehyde or its derivative present in the reagent pad (4) to produce 1,3.dihydroxyisoindrin (DHI). The carbonium ion of the resulted DHI reacts with a colouring compound from the reagent pad (4) under acidic conditions, provided by the compound present in the development pad (3), to produce a colour - a detectable signal. With the progress of the reaction the reagents comprised in the reagent pad (4) and the acid comprised are substantially completely dissolved and dispersed into the development pad (3) thereby dissolving the acid comprised therein, and the reagent pad and the development pad form in combination a detection pad.

[0089] It is obvious for the person skilled In the art that the controlling compound and the construction of the dry stick test device may be changed based on the concept of the present invention, if a different assay is to be provided or if another analyte is to be assayed.

[0090] Furthermore, it is also obvious for the skilled person how to choose the controlling compound to optimise the construction of the dry stick test device based on the knowledge provided by the concept of the present invention, namely, separating the controlling compound from the reagents (at least some of them) in different pads and subsequently arranging these pads in order to avoid precipitation of milk sample components, whereby the influence on the signal detected may be limited or avoided.

[0091] The concept of the present invention will be further illustrated in the following non-limiting figures and examples.

Description of the figures

[0092]

Fig. 1 shows one possible construction of the dry stick test device according to the present invention where (1) relates to the dry stick test device, (2) relates to the reagent section, (3) relates to the development pad and (4) relates to the reagent pad.

Fig. 2 shows dry stick test devices wherein the reagent pad is placed on top of the development pad and dry stick test devices wherein the development pad is placed upon the reagent pad. The pictures show different degrees of milk precipitation (white spots) on the reagent pad and development pad respectively.

Fig. 3 shows a magnification of the upper left reagent pad of figure 2.

Examples

Example 1: Influence of Precipitation on light diffusion on urea dry sticks

Experimental set up:

[0093] Pads comprising a strongly acidic bottom layer (development pad) and a dyed top layer reagent pad) are mounted either with development pad up or with the reagent pad up. Milk samples are applied and the distribution of milk in the pads is described. At the end of incubation / reaction the pads are scanned and green colour is measured in order to calculate influence of precipitation on light diffusion on pads.

Preparation of urea dry sticks:

[0094] Urea dry sticks (pads in plastic frames, lot number 20060928-1, produced according to "Production of UREA sticks ver. 4.0.2") comprising pads of 5x5 mm^2 are employed. Said pads consisting of two layers glued together, comprising:

1. a top layer comprising filterpaper impregnated with Triton X-405, N,N-diethyl-N'-1-Naphtyl-ethylenediamine Oxalate, the acetal of Glycerol and ortho-Phthaldialdehyde (development pad) and,
2. a bottom layer consisting of woven textile (nylon/PET) impregnated with 4-Sulfophthalic Acid (reagent pad).

[0095] The glued layers are cut into square pads, 5 by 5 mm^2 and placed in plastic frames either with the development pad on top of the reagent pad (invert mounted) or with the reagent pad on top of the development pad (correctly mounted).

Preparation of milk samples:

[0096] Two milk samples (in the following numbered #11 and #14) were enriched with 500 mg/ml urea.

Performance test of correctly and inverted mounted urea dry sticks:

[0097] Four correctly mounted sticks and four inverted sticks were fastened by tape.

[0098] 8 μl milk #11 and 8 μl milk #14 was applied on two inverted urea dry sticks separately and on two correctly mounted urea dry sticks separately. Following incubation at 25°C in 12 minutes and 30 seconds each urea dry stick was scanned inverted by Canon scanner ConoScan "2400U

[0099] On urea dry sticks comprising correctly mounted pads, milk is easily spread and enters freely into the pad. Following incubation colour is uniformly spread over the entire surface. On urea dry sticks comprising inverted mounted pads, the 8 μl milk remains as a small drop, slowly releasing liquid into the pad. Next to incubation coagulated milk constituents (protein ("cheese") with fat globules) remain on the pad surface, forming a white spot covering approx. $2/3^{rds}$ of the area (See figure 2 and 3).

[0100] Each of the eight scanned pads (with 300 dots per inch) was measured using Matlab software, a routine programme using red, green and blue colour. The Field measured on each urea dry stick constitutes approx. 4x4 mm^2 as the pads edges was left out.

[0101] In the table below green colour values are shown.

Pad orientation		Average green	Standard deviation of pixels (green color)
Development pad up "inverted"	first sample #14	213,2	25,6
	second sample #14	209,7	30,1
	first sample #11	205,3	35,4
	second sample #11	194,5	36,6
Reagent pad up "correct"	first sample #14	204,3	5,1
	second sample #14	213,7	8,7
	first sample #11	206,1	5,8
	second sample #11	199,4	5,1

Conclusion:

[0102] The above results clearly show, that the standard deviation of pixels (green color) is significantly higher (5-6 times) when pads is oriented with development pad up (inverted mounted pads) compared to pads with reagent pad up (correctly mounted pads). The results show that the color produced is not evenly distributed which causing false readings of the stick when the pads are mounted inverted and confirms that milk coagulates/precipitates significantly influence the assay and significantly influence dry stick performance.

REFERENCES

[0103]

US 4,125,995
JP 10-229023

Claims

1. A dry stick test device for the determination of an analyte in a milk sample by means of a chemical assay, said device comprises:

(i) optionally a solid support,
(ii) at least one reagent pad comprising a reagent capable of reacting with the analyte, a derivative of said

analyte or an indicator compound for said analyte to provide a detectable signal when in moistened state,
(iii) a development pad which is located in contact with the at least one reagent pad, optionally between the solid support and the at least one reagent pad, said development pad comprises at least one controlling compound capable of providing a condition required for the reagent to react with the analyte to provide a detectable signal, wherein the development pad is located down-stream from the at least one reagent pad

wherein the at least one reagent pad and the development pad are arranged to avoid precipitation of milk sample component(s) on the top-face of the device and wherein the sample is applied on the top-face of the device and the detectable signal is obtained from the top-face of the device and wherein the controlling compound is an acidic compound capable of providing a pH value of the milk sample below 6 and wherein the top-face of the device relates to the surface where the sample initially gets into contact with the at least one reagent pad.

2. A device according to any one of the preceding claims, wherein the at least one reagent pad comprises o-phthaladehyde or a derivative thereof, a colouring compound and a detergent.

3. A device according to any one of the preceding claims, wherein the analyte is selected from the group consisting of a protein, a fat, a carbohydrate such as monosaccharides, such as glucose or galactose and disaccharides, such as lactose, an antibiotic, a steroid, a vitamin,and a chemical compound such as urea, triglyceride and ketone bodies, such as acetoacetate, beta-hydroxybutyrate (BOHB), citric acid, lactic acid and acetone.

4. A dry stick test device for the determination of an analyte in a milk sample by means of a chemical assay, said device comprises:

(i) optionally a solid support,
(ii) at least one reagent pad comprising a reagent capable of reacting with the analyte, a derivative of said analyte or an indicator compound for said analyte to provide a detectable signal when in moisten state,
(iii) a development pad which is located in contact with the at least one reagent pad, optionally between the solid support and the at least one reagent pad, said development pad comprises at least one controlling compound capable of providing a condition required for the reagent to react with the analyte to provide a detectable signal, wherein the development pad is located down-stream from the at least one reagent pad

wherein the at least one reagent pad is capable of providing a pH-value of the milk sample of 6 or above 6, and the controlling compound present in the development pad is capable of providing a pH-value of the milk sample below 6, wherein the at least one reagent pad and the development pad are arranged to avoid precipitation of milk sample component(s) on the top-face of the device, wherein the sample is applied on the top-face of the device and the detectable signal is obtained from the top-face of the device and wherein the top-face of the device relates to the surface where the sample initially gets into contact with the at least one reagent pad.

5. A dry stick test device for the determination of urea in a milk sample, said dry stick test device comprises optionally a solid support and at least 2 pads, said at least 2 pads comprise (i) at least one reagent pad comprising o-phthalaldehyde or a derivative thereof, a colouring compound and a detergent, and (ii) a development pad comprising at least one acidic compound capable of providing a pH value of the milk sample below 6 , wherein the at least one reagent pad and the development pad are arranged to avoid precipitation of milk sample component(s) on the top-face of the device, wherein the sample is applied on the top-face of the device and the detectable signal is obtained from the top-face of the device and wherein the top-face of the device relates to the surface where the sample initially gets into contact with the at least one reagent pad.

6. A method for the preparation of the dry stick device according to any one of claims 1-5, said method comprises the steps of:

(i) providing at least one reagent pad by impregnating a first porous material with an aqueous solution comprising a reagent capable of reacting with the analyte, a derivative of said analyte or an indicator compound for said analyte to provide a detectable signal when in a moistened state,
(ii) thereafter drying the at least one reagent pad,
(iii) providing a development pad by impregnating a second porous material with an aqueous solution comprising at least one controlling compound which, when in a moistened state, is capable of providing a condition required for the reagent to react with the analyte to provide a detectable signal,
(iv) thereafter drying the impregnated second porous material, and

(v) immobilising the first porous material with the second porous material, optionally on a solid support, to obtain the dry stick device.

7. A method for the determination of an analyte in a milk sample, said method comprises the steps of:

(a) applying the sample suspected of containing the analyte to a dry stick test device according to any one of claims 1-6,
(b) permitting the sample to migrate into the at least one reagent pad and the developing pad and mobilising the at least one reagent and the at least one controlling compound, and
(c) permitting the at least one reagent and the analyte, the derivative of said analyte or the indicator compound for said analyte to react and provide a detectable signal

8. A method according to claim 7, wherein the analyte is selected from the group consisting of a protein, a fat, a carbohydrate such as glucose and lactose, an antibiotic, a steroid, a vitamin, a chemical compound such as urea, triglyceride and ketone bodies, such as acetoacetate, beta-hydroxybutyrate (BOHB) and acetone.

9. Use of a device according to any one of claims 1-5 for the determination of an analyte in a milk sample.

10. Use according to claim 9, wherein the analyte is selected from the group consisting of a protein, a fat, a carbohydrate, an antibiotic, a steroid, a vitamin and a chemical compound.

11. Use according to claim 10, wherein the chemical compound is selected from the group consisting of urea, triglyceride and ketone bodies, such as acetoacetate, beta-hydroxybutyrate (BOHB) and acetone.

Patentansprüche

1. Trockenindikatorprüfvorrichtung zur Bestimmung eines Analyten in einer Milchprobe mithilfe eines chemischen Assays, wobei die Vorrichtung umfasst:

(i) fakultativ einen festen Träger,
(ii) mindestens ein Reagenzkissen, umfassend ein Reagenz, das mit dem Analyten, einem Derivat des Analyten oder einer Indikatorverbindung für den Analyten zur Bereitstellung eines nachweisbaren Signals in feuchtem Zustand reagieren kann,
(iii) ein Entwicklungskissen, das in Berührung mit dem mindestens einen Reagenzkissen, fakultativ zwischen dem festen Träger und dem mindestens einen Reagenzkissen, angeordnet ist, wobei das Entwicklungskissen mindestens eine Steuerverbindungen umfasst, die eine Bedingung bereitstellen kann, die für die Reaktion des Reagenz mit dem Analyten zur Bereitstellung eines nachweisbaren Signals erforderlich ist, wobei das Entwicklungskissen dem mindestens einen Reagenzkissen nachgeordnet ist

wobei das mindestens eine Reagenzkissen und das Entwicklungskissen derart angeordnet sind, dass ein Ausfällen von einem oder mehreren Bestandteilen der Milchprobe auf der oberen Fläche der Vorrichtung vermieden wird, und wobei die Probe auf die obere Fläche der Vorrichtung aufgetragen wird und das nachweisbare Signal von der oberen Fläche der Vorrichtung erhalten wird und wobei die Steuerverbindung eine saure Verbindung ist, die einen pH-Wert der Milchprobe von unter 6 bereitstellen kann,
und wobei die obere Fläche der Vorrichtung in Bezug zu der Fläche steht, wo die Probe zu Beginn mit dem mindestens einen Reagenzkissen in Berührung kommt.

2. Vorrichtung nach einem der vorhergehenden Ansprüche, wobei das mindestens eine Reagenzkissen o-Phthaldehyd oder ein Derivat davon, eine Färbeverbindung und ein Detergens umfasst.

3. Vorrichtung nach einem der vorhergehenden Ansprüche, wobei der Analyt ausgewählt ist aus der Gruppe, bestehend aus einem Protein, einem Fett, einem Kohlehydrat, wie Monosacchariden, wie Glucose oder Galactose, und Disacchariden, wie Lactose, einem Antibiotikum, einem Steroid, einem Vitamin, einer chemischen Verbindung, wie Harnstoff, Triglycerid und Ketonkörpern, wie Acetacetat, beta-Hydroxybutyrat (BOHB), Zitronensäure, Milchsäure und Aceton.

4. Trockenindikatorprüfvorrichtung zur Bestimmung eines Analyten in einer Milchprobe mithilfe eines chemischen

Assays, wobei die Vorrichtung umfasst:

(i) fakultativ einen festen Träger,
(ii) mindestens ein Reagenzkissen, umfassend ein Reagenz, das mit dem Analyten, einem Derivat des Analyten oder einer Indikatorverbindung für den Analyten zur Bereitstellung eines nachweisbaren Signals in feuchtem Zustand reagieren kann,
(iii) ein Entwicklungskissen, das in Berührung mit dem mindestens einen Reagenzkissen, fakultativ zwischen dem festen Träger und dem mindestens einen Reagenzkissen, angeordnet ist, wobei das Entwicklungskissen mindestens eine Steuerverbindungen umfasst, die eine Bedingung bereitstellen kann, die für die Reaktion des Reagenz mit dem Analyten zur Bereitstellung eines nachweisbaren Signals erforderlich ist, wobei das Entwicklungskissen dem mindestens einen Reagenzkissen nachgeordnet ist

wobei das mindestens eine Reagenzkissen einen pH-Wert der Milchprobe von 6 oder über 6 bereitstellen kann und wobei die Steuerverbindung in dem Entwicklungskissen einen pH-Wert der Milchprobe von unter 6 bereitstellen kann, wobei das mindestens eine Reagenzkissen und das Entwicklungskissen derart angeordnet sind, dass ein Ausfällen von einem oder mehreren Bestandteilen der Milchprobe auf der oberen Fläche der Vorrichtung vermieden wird, wobei die Probe auf die obere Fläche der Vorrichtung aufgetragen wird und das nachweisbare Signal von der oberen Fläche der Vorrichtung erhalten wird und wobei die obere Fläche der Vorrichtung in Bezug zu der Fläche steht, wo die Probe zu Beginn mit dem mindestens einen Reagenzkissen in Berührung kommt.

5. Trockenindikatorprüfvorrichtung zur Bestimmung von Harnstoff in einer Milchprobe, wobei die Trockenindikatorprüfvorrichtung fakultativ einen festen Träger und mindestens 2 Kissen umfasst, wobei die mindestens 2 Kissen umfassen (i) mindestens ein Reagenzkissen, umfassend o-Phthalaldehyd oder ein Derivat davon, eine Färbeverbindung und ein Detergens, und (ii) ein Entwicklungskissen, umfassend eine saure Verbindung, die einen pH-Wert der Milchprobe von unter 6 bereitstellen kann, wobei das mindestens eine Reagenzkissen und das Entwicklungskissen derart angeordnet sind, dass ein Ausfällen von einem oder mehreren Bestandteilen der Milchprobe auf der oberen Fläche der Vorrichtung vermieden wird, wobei die Probe auf die obere Fläche der Vorrichtung aufgetragen wird und das nachweisbare Signal von der oberen Fläche der Vorrichtung erhalten wird und wobei die obere Fläche der Vorrichtung in Bezug zu der Fläche steht, wo die Probe zu Beginn mit dem mindestens einen Reagenzkissen in Berührung kommt.

6. Verfahren zur Herstellung der Trockenindikatorvorrichtung nach einem der Ansprüche 1-5, wobei das Verfahren folgende Schritte umfasst:

(i) Bereitstellen mindestens eines Reagenzkissens durch Tränken eines ersten porösen Materials mit einer wässrigen Flüssigkeit, umfassend ein Reagenz, das mit dem Analyten, einem Derivat des Analyten oder einer Indikatorverbindung für den Analyten zur Bereitstellung eines nachweisbaren Signals in feuchtem Zustand reagieren kann,
(ii) danach Trocknen des mindestens einen Reagenzkissens,
(iii) Bereitstellen eines Entwicklungskissens durch Tränken eines zweiten porösen Materials mit einer wässrigen Flüssigkeit, umfassend eine Steuerverbindung, die in feuchtem Zustand eine Bedingung bereitstellen kann, die für die Reaktion des Reagenz mit dem Analyten zur Bereitstellung eines nachweisbaren Signals erforderlich ist,
(iv) danach Trocknen des getränkten zweiten porösen Materials und
(v) Immobilisieren des ersten porösen Materials mit dem zweiten porösen Material, fakultativ auf einem festen Träger, zum Erhalt der Trockenindikatorvorrichtung.

7. Verfahren zur Bestimmung eines Analyten in einer Milchprobe, wobei das Verfahren folgende Schritte umfasst:

(a) Aufbringen einer vermutlich den Analyten enthaltenden Milchprobe auf eine Trockenindikatorprüfvorrichtung nach einem der Ansprüche 1-6,
(b) Ermöglichen der Migration der Probe in das mindestens eine Reagenzkissen und das Entwicklungskissen and Mobilisieren der mindestens einen Reagenz und der mindestens einen Steuerverbindung und
(c) Ermöglichen der Bereitstellung eines nachweisbaren Signals durch Reagieren des mindestens eine Reagenz und den Analyten, das Derivat des Analyten oder der Indikatorverbindung für den Analyten.

8. Verfahren nach Anspruch 7, wobei der Analyt ausgewählt ist aus der Gruppe, bestehend aus einem Protein, einem Fett, einem Kohlehydrat, wie Glucose und Lactose, einem Antibiotikum, einem Steroid, einem Vitamin, einer chemischen Verbindung, wie Harnstoff, Triglycerid und Ketonkörpern, wie Acetacetat, beta-Hydroxybutyrat (BOHB)

und Aceton.

9. Verwendung einer Vorrichtung nach einem der Ansprüche 1-5 zur Bestimmung eines Analyten in einer Milchprobe.

10. Verwendung nach Anspruch 9, wobei der Analyt ausgewählt ist aus der Gruppe, bestehend aus einem Protein, einem Fett, einem Kohlehydrat, einem Antibiotikum, einem Steroid, einem Vitamin und einer chemischen Verbindung.

11. Verwendung nach Anspruch 9, wobei die chemische Verbindung ausgewählt ist aus der Gruppe, bestehend aus Acetacetat, beta-Hydroxybutyrat (BOHB) und Aceton.

Revendications

1. Dispositif d'essai à bâtonnet sec pour le dosage d'un analyte dans un échantillon de lait au moyen d'un essai chimique, ledit dispositif comprenant :

(i) éventuellement un support solide,
(ii) au moins un tampon réactif comprenant un réactif capable de réagir avec l'analyte, un dérivé dudit analyte ou un composé indicateur pour ledit analyte afin de produire un signal détectable lorsqu'il se trouve à l'état humidifié,
(iii) un tampon de développement placé en contact avec l'au moins un tampon réactif, éventuellement entre le support solide et l'au moins un tampon réactif, ledit tampon de développement comprenant au moins un composé de contrôle capable d'apporter une condition requise pour que le réactif réagisse avec l'analyte afin de produire un signal détectable, où le tampon de développement est situé en aval de l'au moins un tampon réactif,

dans lequel l'au moins un tampon réactif et le tampon de développement sont disposés pour éviter la précipitation d'un ou de plusieurs composants de l'échantillon de lait sur la face supérieure du dispositif, et dans lequel l'échantillon est appliqué sur la face supérieure du dispositif et le signal détectable est obtenu à partir de la face supérieure du dispositif, et dans lequel le composé de contrôle est un composé acide capable de produire une valeur de pH de l'échantillon de lait inférieure à 6,
et dans lequel la face supérieure du dispositif correspond à la surface au niveau de laquelle l'échantillon entre initialement en contact avec l'au moins un tampon réactif.

2. Dispositif selon l'une quelconque des revendications précédentes, dans lequel l'au moins un tampon réactif comprend de l'o-phthalaldéhyde ou un dérivé de celui-ci, un composé colorant et un détergent.

3. Dispositif selon l'une quelconque des revendications précédentes, dans lequel l'analyte est choisi dans le groupe consistant en une protéine, une graisse, un hydrate de carbone tel que des monosaccharides, tels que du glucose ou du galactose, et des disaccharides, tels que du lactose, un antibiotique, un stéroïde, une vitamine et un composé chimique tel que de l'urée, un triglycéride et des corps cétoniques, tels que de l'acétoacétate, du bêta-hydroxybutyrate (BOHB), de l'acide citrique, de l'acide lactique et de l'acétone.

4. Dispositif d'essai à bâtonnet sec pour le dosage d'un analyte dans un échantillon de lait au moyen d'un essai chimique, ledit dispositif comprenant :

(i) éventuellement un support solide,
(ii) au moins un tampon réactif comprenant un réactif capable de réagir avec l'analyte, un dérivé dudit analyte ou un composé indicateur pour ledit analyte afin de produire un signal détectable lorsqu'il se trouve à l'état humidifié,
(iii) un tampon de développement placé en contact avec l'au moins un tampon réactif, éventuellement entre le support solide et l'au moins un tampon réactif, ledit tampon de développement comprenant au moins un composé de contrôle capable d'apporter une condition requise pour que le réactif réagisse avec l'analyte afin de produire un signal détectable, où le tampon de développement est situé en aval de l'au moins un tampon réactif,

dans lequel l'au moins un tampon réactif est capable de produire une valeur de pH de l'échantillon de lait supérieure ou égale à 6, et le composé de contrôle présent dans le tampon de développement est capable de produire une valeur de pH de l'échantillon de lait inférieure à 6, dans lequel l'au moins un tampon réactif et le tampon de déve-

loppement sont disposés pour éviter la précipitation d'un ou de plusieurs composants de l'échantillon de lait sur la face supérieure du dispositif, dans lequel l'échantillon est appliqué sur la face supérieure du dispositif et le signal détectable est obtenu depuis la face supérieure du dispositif, et dans lequel la face supérieure du dispositif correspond à la surface au niveau de laquelle l'échantillon entre initialement en contact avec l'au moins un tampon réactif

5. Dispositif d'essai à bâtonnet sec pour le dosage de l'urée dans un échantillon de lait, ledit dispositif d'essai à bâtonnet sec comprenant éventuellement un support solide et au moins 2 tampons, lesdits au moins 2 tampons comprenant (i) au moins un tampon réactif comprenant de l'o-phthalaldéhyde ou un dérivé de celui-ci, un composé colorant et un détergent, et (ii) un tampon de développement comprenant au moins un composé acide capable de produire une valeur de pH de l'échantillon de lait inférieure à 6, dans lequel l'au moins un tampon réactif et le tampon de développement sont disposés pour éviter la précipitation d'un ou de plusieurs composants de l'échantillon de lait sur la face supérieure du dispositif, dans lequel l'échantillon est appliqué sur la face supérieure du dispositif et le signal détectable est obtenu depuis la face supérieure du dispositif, et dans lequel la face supérieure du dispositif correspond à la surface au niveau de laquelle l'échantillon entre initialement en contact avec l'au moins un tampon réactif.

6. Méthode de préparation du dispositif à bâtonnet sec selon l'une quelconque des revendications 1 à 5, ladite méthode comprenant les étapes consistant à :

(ii) obtenir au moins un tampon réactif en imprégnant une première matière poreuse avec une solution aqueuse comprenant un réactif capable de réagir avec l'analyte, un dérivé dudit analyte ou un composé indicateur pour ledit analyte afin de produire un signal détectable lorsqu'il se trouve à l'état humidifié,
(ii) sécher ensuite l'au moins un tampon réactif,
(iii) obtenir un tampon de développement en imprégnant une deuxième matière poreuse avec une solution aqueuse comprenant au moins un composé de contrôle qui, lorsqu'il se trouve à l'état humidifié, est capable d'apporter une condition requise pour que le réactif réagisse avec l'analyte afin de produire un signal détectable,
(iv) sécher ensuite la deuxième matière poreuse imprégnée, et
(v) immobiliser la première matière poreuse avec la deuxième matière poreuse, éventuellement sur un support solide, pour obtenir le dispositif à bâtonnet sec.

7. Méthode de dosage d'un analyte dans un échantillon de lait, ladite méthode comprenant les étapes consistant à :

(a) appliquer l'échantillon suspecté de contenir l'analyte sur un dispositif d'essai à bâtonnet sec selon l'une quelconque des revendications 1 à 6,
(b) permettre à l'échantillon de migrer dans l'au moins un tampon réactif et le tampon de développement et mobiliser l'au moins un réactif et l'au moins un composé de contrôle, et
(c) permettre à l'au moins un réactif et à l'analyte, au dérivé dudit analyte ou au composé indicateur pour ledit analyte de réagir et de produire un signal détectable.

8. Méthode selon la revendication 7, dans laquelle l'analyte est choisi dans le groupe consistant en une protéine, une graisse, un hydrate de carbone tel que du glucose et du lactose, un antibiotique, un stéroïde, une vitamine, un composé chimique tel que de l'urée, un triglycéride et des corps cétoniques, tels que de l'acétoacétate, du bêta-hydroxybutyrate (BOHB) et de l'acétone.

9. Utilisation d'un dispositif selon l'une quelconque des revendications 1 à 5 pour le dosage d'un analyte dans un échantillon de lait.

10. Utilisation selon la revendication 9, dans laquelle l'analyte est choisi dans le groupe consistant en une protéine, une graisse, un hydrate de carbone, un antibiotique, un stéroïde, une vitamine et un composé chimique.

11. Utilisation selon la revendication 10, dans laquelle le composé chimique est choisi dans le groupe consistant en de l'urée, un triglycéride et des corps cétoniques, tels que de l'acétoacétate, du bêta-hydroxybutyrate (BOHB) et de l'acétone.

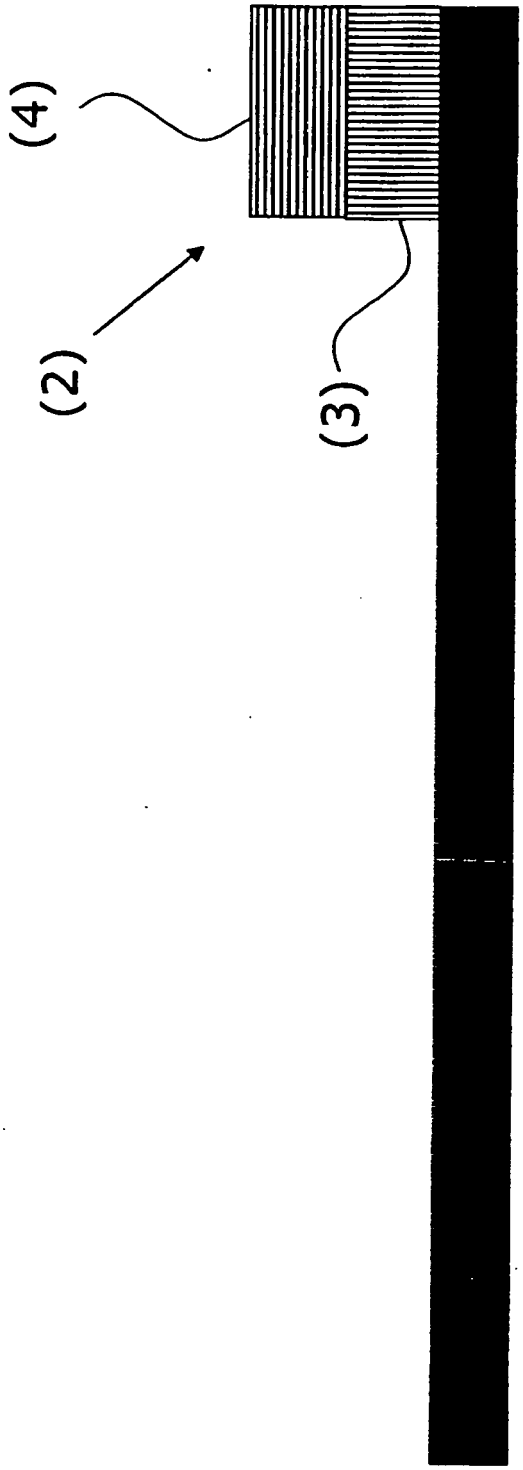

Fig. 1

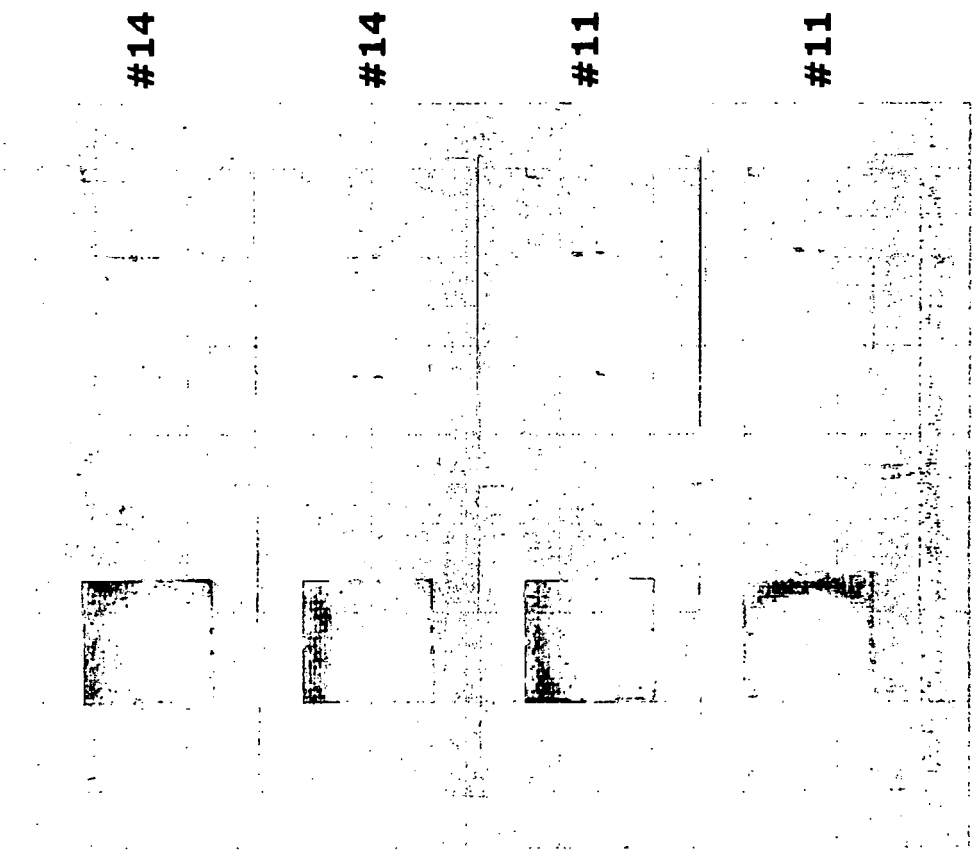
#14
#14
#11
#11
Development pad up – "Invert Mounted" pads
Reagent pad up – "Correctly mounted" pads

Fig. 2

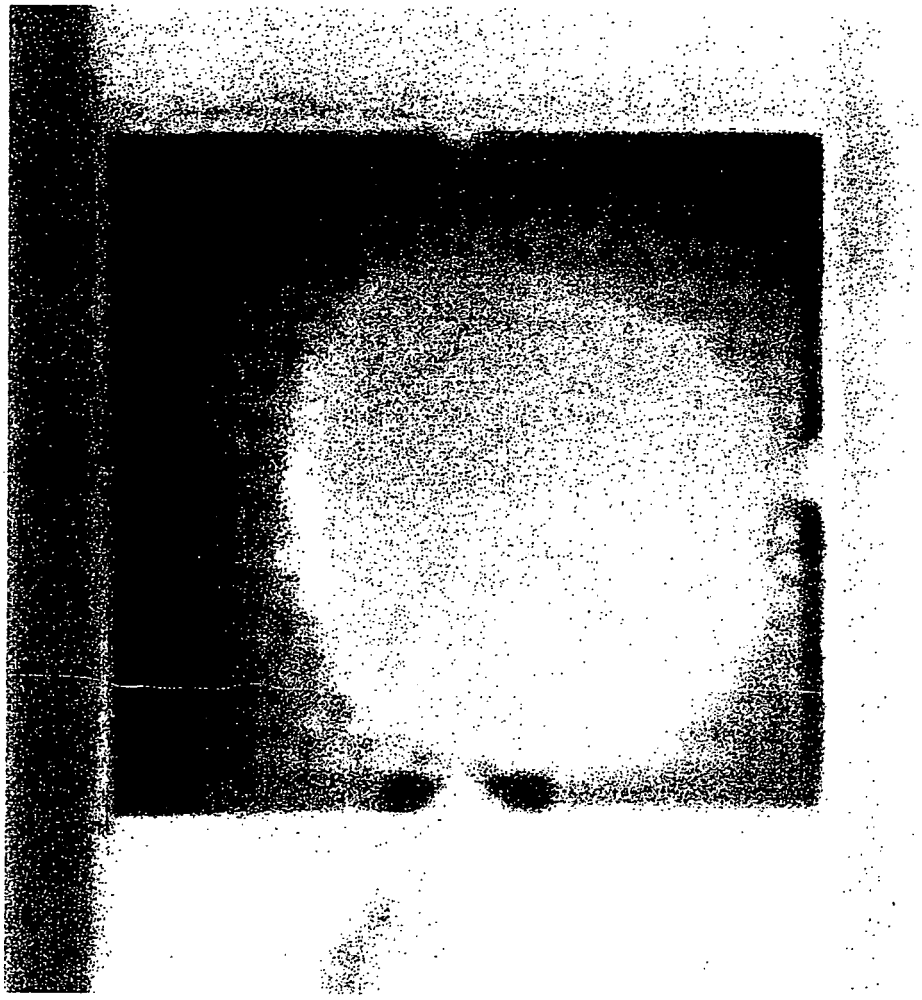

Fig. 3

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- US 4215995 A **[0012]**
- JP 10229023 A **[0013] [0103]**
- US 4125995 A **[0103]**