As originally filed

FINE-GRAINED FILLER SUBSTANCES FOR PHOTOMETRIC REACTION FILMS

Field of the invention

The invention relates to a diagnostic test element for detecting an analyte in a sample of a body fluid and to a process for producing such a diagnostic test element. Such diagnostic test elements are used, for example, for detecting one or more analytes in body fluids such as whole blood, for example for detecting glucose, uric acid, ethanol, or lactate, or similar analytes. However, other applications are also possible.

Prior art

In the prior art, numerous diagnostic test elements are known which can be used for detecting at least one analyte in a sample of a body fluid. The at least one analyte can be, for example, a metabolite. Qualitative and also, or else, quantitative detection of the analyte can be carried out. Known analytes are, for example, glucose, more particularly blood glucose, uric acid, ethanol, and/or lactate. Other types of analytes are also alternatively or additionally detectable. The body fluid can be, for example, whole blood, blood plasma, interstitial fluid, saliva, urine, or other types of body fluids. The invention will now, without restricting further possible embodiments, be described essentially with reference to the detection of glucose in whole blood.

Test elements generally comprise at least one detection reagent for the qualitative and/or quantitative

detection of the analyte. A detection reagent is to be generally understood to mean a chemical substance or a mixture of chemical substances which, in the presence of the at least one analyte, changes at least one detectable property, more particularly a physically and/or chemically detectable property. Preferably, this property change occurs specifically only in the presence of the at least one analyte to be detected, but not in the presence of other substances. However, in practice, it is possible to tolerate an unspecific property change within certain limits, in the presence of other chemical substances whose presence in the sample of the body fluid is generally unlikely and/or which are present at only a very low concentration.

The at least one property change can be, for example, the change in an optically detectable property, more particularly a color change. Examples of diagnostic test elements having optical detection reagents are well known in the prior art. For example, EP 0 821 234 B1, to which reference can be made to a great extent in the context of the present invention, describes a diagnostic test support for determining an analyte from whole blood by means of a reagent system which is present in the support and which includes a color formation reagent. The diagnostic test support comprises a test field which a sample loading side, onto which the sample is added, and a detection side, on which an optically detectable change occurs as a result of the reaction of the analyte with the reagent system. The test field is configured such that the erythrocytes present in the sample do not reach the detection side. Furthermore, the test field has a transparent slide and a first film layer and also a second film layer applied to the first film layer. The first layer located on the transparent slide is in a moist state and thereby exhibits considerably less light scattering than the second layer lying over it.

The first film layer comprises a filler whose refractive index is close to the refractive index of water, whereas the second layer contains a pigment having a refractive index of preferably at least or even > 2.0, more particularly of at least 2.2, at a concentration of preferably at least 25% by weight or even more than 25% by weight, based on the dried second layer. For example, the first layer can comprise a sodium aluminum silicate as a filler.

US 4,312,834 discloses a diagnostic agent for the detection of a component material. A water-insoluble film is disclosed which is composed of a film former and a film opener in the form of fine, insoluble inorganic or organic particles. The film opener serves the purpose of rendering the film porous so that sufficient sample uptake by the film can occur. Accordingly, by way of example, it is proposed to use pigments, i.e., particles having a large particulate size, titanium dioxide pigments for example, as film openers.

WO 2006/065900 A1 describes a test strip or electrochemical sensor for measuring the amount of an analyte in a biological fluid. This comprises an enzyme system for the reaction with the analyte. The reactive system is blended into a water-soluble, swellable polymer matrix which comprises small, water-insoluble particles having a nominal size of about 0.05 to 20 micrometers. Thus, a reduced porosity using small particulates is described.

However, in practice, the test elements known from the prior art, more particularly test elements having at least one test field, have disadvantages and technical challenges. For instance, it has become apparent that customary test fields, as known in the prior art, may result in a granular, inhomogeneous color development.

However, such an inhomogeneous color development is generally irrelevant for conventional analytical test devices, since these have comparatively large measurement spots. For example, commercially available optical blood glucose measurement devices have measurement spots with a diameter of about 1.5 mm. With such diameters, a mean coefficient of variation, i.e., the ratio of a standard deviation to a mean value for the measurement, is, for example, typically about 1.5% over a measurement range of typically about 10 to 600 mg per dl blood glucose.

However, in terms of device technology, a trend toward analytical measurement devices having smaller measurement spots should be noted. However, with smaller measurement spots, inhomogeneities, especially in the color development of the detection reaction, become more strongly noticeable. For example, for measurement spots having a diameter of below 0.5 mm, the coefficient of variation distinctly increases, more particularly above the still clinically acceptable value of about 4%. Since the development of integrated blood glucose measurement systems is leading to increasingly smaller blood volumes, down to about 100 nanoliters, measurement spots of 10 micrometers x 10 micrometers in size, especially when using spatially resolved optics, have to be made possible here. However, the known test fields generally do not have the necessary precision for this purpose.

Object of the invention

It is therefore an object of the present invention to provide a diagnostic test element, a process for producing a diagnostic test element, and also a process for detecting an analyte in a sample of a body fluid, all of which avoid at least to a substantial extent the disadvantages of known diagnostic test elements and of

known processes. More particularly, high-precision quantitative detection of the at least one analyte shall also be made possible in very small amounts of fluid.

Disclosure of the invention

This object is achieved by the invention having the features of the independent claims. Advantageous further developments of the invention, which are realizable alone or in any combination, are presented in the dependent claims. There are proposed a diagnostic test element for detecting an analyte in a sample of a body fluid, a process for producing a diagnostic test element for detecting an analyte in a sample of a body fluid, and also a process for detecting an analyte in a sample of a body fluid. The diagnostic test element in one or more of the proposed embodiments may be obtainable as per a process according to the invention, and the production process may be used to produce a diagnostic test element in one or more of the described embodiments. Accordingly, for the description of possible embodiments of the diagnostic test element, reference can be made to the description of the production process, or vice versa. However, other embodiments are also possible in principle. The proposed process for detecting an analyte of a sample of a body fluid is carried out using a diagnostic test element in one or more of the embodiments described hereinafter.

In a first aspect of the present invention, there is accordingly proposed a diagnostic test element for detecting an analyte in a sample of a body fluid. For the possible embodiments of this test element, reference can be made in principle to the above description of the prior art. Thus, the at least one analyte can, for example, be detected quantitatively or

qualitatively. The at least one analyte can be in particular one or more of the analytes glucose, uric acid, ethanol, lactate, or a combination of these analytes and/or of other analytes. However, other analytes are also detectable in principle, for example one or more of the abovementioned analytes. The sample of the body fluid can be in particular whole blood. However, other embodiments are also possible in principle, and reference can be made to the above description.

The diagnostic test element comprises at least one test field having at least one detection reagent. A test field is to be understood to mean a continuous area of the detection reagent, more particularly a film having one or more layers, which, as will be more particularly elucidated below, can be applied to, for example, at least one support element. The detection reagent is set up to perform at least one detectable change in the presence of the analyte. More particularly, this detectable change can be a physically and/or chemically detectable change. Hereinafter, reference will be made in particular to physical changes in the form of an optically detectable change, more particularly a color change. In principle, however, other types of detectable changes are also alternatively or additionally conceivable, for example chemically and/or electrochemically detectable changes. The detection reagent can in particular comprise at least one enzyme, for example glucose dehydrogenase (e.g., FAD-, NAD^+-, or PQQ-dependent) and/or glucose oxidase. Thus, in general, there can be used, for example, glucose detection methods which comprise one or more of the following enzymatic detection methods or detection reagents: GOD, GlucDOR (PQQ-dependent GDH and mutants thereof), FAD-GDH, NAD-dependent GDH with mediator (e.g., diaphorase) for transferring the redox equivalents of NADH to a nitrosoaniline mediator.

Furthermore, the detection reagent can, alternatively or additionally, comprise one or more mediators, i.e., substances capable of transferring electrical charges from one substance to another. More particularly, mediators can be used which are suitable for electron transfer. For example, this substance can be nitrosoaniline. Furthermore, the detection reagent can, again alternatively or additionally, comprise at least one indicator. An indicator can be understood to be a substance which as such can change at least one property which can be detected, depending on in which form this substance is present. For example, substances can be used which, in an oxidized and a reduced form, can have different optical properties, for example different colors. Alternatively or additionally, the indicator can comprise a substance which, in different charge states, has different optical properties, for example different color properties. Thus, in general, a detection reagent can be understood to be a single substance or a mixture of substances, for example, as explained above, a mixture of at least one enzyme, at least one mediator, and at least one indicator. Such detection reagents are known in principle from the prior art, for example from the prior art described above.

The test field has at least one detection layer comprising the detection reagent. A system having a single detection layer can be used, or multiple detection layers can be used which can be applied on top of one another, directly or by interposing one or more further layers. However, particular preference is given to a system having only a single detection layer. A layer is to be understood in the context of the present invention to mean in general an element in which a layer material is applied flat to a support element or is formed as a freestanding film. The layer

can, but need not necessarily, be closed, but can have, for example, openings as well. However, particular preference is given to, as will be more particularly developed below, a substantially uniform, preferably hole-free, homogeneous embodiment of the detection layer having a homogeneous layer thickness. The layer thickness, i.e., the average thickness of the detection layer, is preferably 3 to 60 micrometers, more particularly 5 to 15 micrometers, for example 8 micrometers.

The detection layer comprises particles. Particles are to be understood in general in the context of the present invention to mean solid bodies in the micrometer range or nanometer range which are not directly connected to one another and which are thus able to form, for example, a free-flowing powder in the dry state and without other substances of the detection layer. Particles can, for example, form in general solid constituents of aerosols, suspensions, or powders.

According to the invention, it is proposed that the particles have a particle size distribution, more particularly in the dry state of the detection layer, in which at least 90% of all particles of the detection layer have an actual particle size of less than 10 micrometers, preferably of less than 3 micrometers, or even less than 1 micrometer.

The detection layer to which this condition shall apply is to be understood to mean the entire detection layer whose change is measureable. More particularly, it can be, when an optically detectable change such as a color change for example is measured, the entire optically recognizable detection layer, optionally right up to a reflection layer or removal layer which is applied to the detection layer on a sample loading side, as will

be more particularly elucidated below. For example, the detection layer can, as will be more particularly elucidated below, be overlaid by at least one further layer which can have, for example, reflective properties. The layers do not necessarily have to be clearly delimited from one another. Locally, the detection layer is, viewed from the detection side, to be understood to mean each layer which is measured, right up to, for example, the reflecting particles and/or another reflecting object of a directly or indirectly adjacent layer.

A particle size is to be understood to mean an equivalent diameter of a particle, i.e., a diameter of a bead which has a volume and/or a surface similar to that of the particles. Various processes for determining the particle size distribution can be used. There are different cases which have to be distinguished from one another. For raw materials, such as for example one or more fillers which may be constituents of the detection layer, the particle size can, for example, be determined by means of laser scattering and/or laser diffraction. In a layer assembly, for example of the detection layer and optionally of a removal layer applied thereto, optical processes can also be used, for example processes which are based on image recognition. In this way, a particle size distribution for example, within the detection layer for example, can be determined down to a range of 3 micrometers to 10 micrometers. On the other hand, other processes as well can be alternatively or additionally used, such as for example scanning electron microscopy of samples, for example microtome cross sections. By means of such processes, it is possible to determine, for example, particle sizes and particle size distributions in the detection layer and, optionally, also in one or more layers applied thereto, such as the optional removal layer for example. A clear

identification of the particles can also be carried out by, for example, additionally using energy dispersive X-ray spectroscopy (EDX). When using electron microscopy processes, the resolution is typically sufficient in the nanometer range, where it is possible to record, for example, all particles having a particle size > 1 nanometer. In general, apparatuses and processes for determining the particle size distribution are known to a person skilled in the art and commercially available. In the context of the present invention, optical determination of the particle size distribution can be used, since preferably the detectable change is an optically detectable change. For example, it can be automatic image analysis of an image of the detection layer, as will be more particularly developed below. This automatic image recognition can be carried out, for example, by capturing an image of at least one part of the detection layer by means of a camera or another spatially resolving image detector and then by recognizing individual particles by means of image recognition and assigning them to a size distribution. It is possible in general to use, for example, all recognized particles to determine the particle size distribution. However, in practice, since particles are generally only recognized as such only above a minimum size, only particles above a predefined minimum size as well, for example, can be considered in the determination of the particle size distribution, for example only above a minimum size of 10 nanometers to 200 nanometers, more particularly a particle size of 50 nanometers to 100 nanometers.

An actual particle size is, in the context of the present invention, to be understood to mean the particle size of the particles in the detection layer, in the form in which the particles are actually present in the detection layer. If the particles in the

detection layer are composed of multiple primary particles, for example in the form of agglomerates and/or aggregates which adhere together, the equivalent diameter of the aggregate or agglomerate should be used, and not the equivalent diameter of the primary particles. The present invention thus does not include cases in which the detection layer is produced such that production thereof makes use of powders which nominally have the mentioned properties but in which the particles of the powder, for example during the production of the detection layer, interact with one another such that agglomerates and/or aggregates are present in the final and preferably dry detection layer and so, altogether for all particles in the finished detection layer, the mentioned condition is no longer fulfilled.

More particularly, at least 80% of all particles of the detection layer can have an actual particle size of less than 5 micrometers, more particularly of less than 1 micrometer. It is particularly preferred for at least 70% of all particles of the detection layer to have an actual particle size of less than 900 nanometers, preferably of less than 800 nanometers.

The particles of the detection layer can have in particular an average particle size of 10 nanometers to 5 micrometers, preferably of less than 1 micrometer. The average particle size can be preferably from 20 nanometers to 1 micrometer and particularly preferably from 20 nanometers to 500 nanometers. Alternatively or additionally, the average particle size can be preferably from 70 nanometers to 5 micrometers, more particularly from 70 nanometers to 1 micrometer, and particularly preferably from 70 nanometers to 500 nanometers.

The particles of the detection layer can have in particular an average particle size of less than 1 micrometer, more particularly of less than 500 nanometers, and particularly preferably of up to 300 nanometers, or even less than 100 nanometers, for example 25 nanometers or less.

An average particle size can be understood to mean, for example, the median of all particle sizes of the particle size distribution, which is usually referred to as d_{50}. This median is selected such that about 50% of the particles have a particle size below the d_{50} value, and about 50% of the particles have a particle size above this median.

The particles can comprise in particular one or more of the following materials: SiO_2; diatomaceous earth; a silicate, more particularly a sodium aluminum silicate; a metal oxide, more particularly an aluminum oxide and/or a titanium oxide; a synthetic oxidic material, more particularly a nanoparticulate oxidic material, more particularly a nanoparticulate silicon oxide and/or aluminum oxide and/or titanium oxide; kaolin; powder glass; precipitated silica; calcium sulfate x 2 H_2O.

It is particularly preferred for all particles of the detection layer having a particle size of more than 10 nanometers, more particularly of more than 20 nanometers or of more than 100 nanometers, to be inorganic particles. As already defined above, the term "particle" shall not include an organic film former and an organic film formed therefrom, since films are generally not composed of loose particulates which are not connected with one another, but since films generally form a continuous layer. However, the particles of the detection layer can, as will be more

particularly developed below, be embedded in at least one such film former.

The detection layer can have in particular a refractive index of 1.1 to 1.8, preferably of 1.2 to 1.5. Thus, the detection layer can have in particular, whether in a dry state or in a moist state, a refractive index which is close to the refractive index of water (about 1.33).

The diagnostic test element, more particularly the at least one detection reagent and/or the at least one detection layer, can be set up in particular such that the detectable change is completed within a period which is less than 60 seconds, preferably less than 40 seconds, and particularly preferably 20 seconds or less. This period can also be referred to as the reaction time. If, for example, the detectable change includes an optical change in the form of a color change, reaction time can be defined by, for example, that timespan from the application of the sample to the test field within which a color reaction is completed to the extent that the relative reflectance subsequently changes by less than 1% per half a second. The relative reflectance can, for example, be the ratio of the reflectance to a reflectance of a test element with no sample and/or to a calibration standard. The reaction time can, for example, be set by appropriate selection of the test chemistry of the detection reagent and/or by the total composition of the test field and/or by the particle size distribution used in the context of the present invention.

The test field can have in particular a loading side for applying the sample of the body fluid and a detection side for detecting a change in the detection reagent, more particularly an optical change, for example a color change. In addition, the test field can

have at least one removal layer. This removal layer can have multiple functions. For example, this layer can be set up for partitioning coarse constituents of the sample, more particularly for partitioning erythrocytes. Alternatively or additionally, the removal layer can also be set up to cover the inherent color of the sample, for example the inherent color of blood. For this purpose, the removal layer can, as is yet to be explained in detail below, comprise, for example, at least one pigment, preferably at least one white pigment. On the other hand, alternatively or additionally, the removal layer can also be set up to fulfill a reflective function, for example to reflect a measurement light which is interspersed into the detection layer, and/or light emitted in the detection layer, such as fluorescent light for example.

The removal layer can be arranged in particular on a side of the detection layer facing the loading side. For example, the removal layer can be applied directly or indirectly to the detection layer. An indirect application can be understood to mean, for example, the interposition of one or more further layers. The removal layer can be set up in particular such that coarse constituents of the sample, more particularly in the case of whole blood erythrocytes, are not able to reach the detection side of the detection layer or are not able to reach the detection layer at all. Coarse constituents can be understood to mean in general constituents which have a size, for example a particle size and/or an equivalent diameter, of more than 1 micrometer, more particularly of more than 5 micrometers. Erythrocytes in particular, which have a characteristic and intensive inherent color, are able to interfere with or even prevent the customary color detection of blood glucose, for example by means of the detection reagents described above, on the detection side.

The removal layer can in particular be coarse-grained, i.e., can be likewise particulate, and the particles of this removal layer can be coarser than the particles of the detection layer. More particularly, the removal layer can have particles of more than one micrometer in size. More particularly, the removal layer can have at least one pigment, i.e., one particulate dye, preferably an inorganic dye, with particles having an average particle size which is above the light wavelength used for optical detection, for example above a wavelength of 660 nanometers. More particularly, the removal layer, as explained above, can have at least one pigment for optically covering any inherent color of blood. The removal layer can comprise in particular at least one white pigment. The removal layer can comprise, for example, one or more of the following pigments: titanium dioxide; zirconium dioxide; barium titanate; barium zirconate; zirconium silicate. A combination of the mentioned pigments and/or of other pigments is also possible. Particular preference is given to the use of zirconium dioxide and/or titanium dioxide. The pigment preferably has an average particle size of between 200 nanometers and 400 nanometers for optimal reflection of the light.

Alternatively or additionally, the removal layer can optionally have at least one filler, preferably a filler with a refractive index of < 2.0. This filler makes it possible to confer, for example, a sucking behavior and/or a transparency on the removal layer. The at least one filler can comprise, for example, silica and/or a silicate. For example, the filler can have an average particle size of < 5 micrometers.

More particularly, the removal layer can have a pigment with a refractive index of at least 2.0, preferably of at least 2.2 or even at least 2.5, at a concentration

of at least 25% by weight, based on a dried layer, i.e., a dried removal layer. This pigment can be in particular titanium dioxide particles and/or zirconium dioxide particles, or the pigment can comprise these types of particles. However, other embodiments are also possible. The titanium dioxide particles or zirconium dioxide particles can have in particular an average particle size of, for example, at least approximately 300 nanometers. However, deviations of preferably not more than 50%, particularly preferably of not more than 10%, may be tolerable. A particle size of 300 nanometers is generally optimal for white pigment reflecting visible light. Titanium dioxide particles have in particular light scattering properties so that the removal layer can also act at the same time as a reflection layer in order to reflect light radiated from the detection side. However, alternatively or additionally, the layer assembly of the test field can also comprise in addition at least one reflection layer which can have the mentioned properties.

The diagnostic test element can, as explained above, be formed as a layer assembly and/or can comprise a layer assembly. In addition to the at least one detection layer, it can comprise in addition the at least one removal layer and/or at least one reflection layer. The test field can be applied to at least one support element with its detection side. More particularly, the diagnostic test element can thus comprise at least one support element, with the support element preferably having at least one transparent region. The test field can be applied to the transparent region with its detection side. The support element can be, for example, a flat support element, more particularly a support element in the form of a strip. For example, the support element can comprise a plastics layer, a paper layer, a ceramic layer or a laminate assembly and/or a combination of the mentioned layers. For

example, the support element can be substantially opaque outside the transparent region so that the detection side of the test field is perceptible only through the transparent region. The loading side of the sample can then be arranged on a side of the test field facing away from the support element. The diagnostic test element can be formed such that the sample of the body fluid is directly applied to the loading side and so, for example, the loading side is directly accessible to a user of the diagnostic test element and the user can, for example, directly drip, dab, or apply in some other way a sample onto the area of the loading side which is at least partly accessible. Alternatively, a transport system may also be provided which is set up to transport the sample of the body fluid from a loading site arranged at another location to the loading side, but this is less preferred.

The detection layer can, as already mentioned repeatedly above, comprise not only the particles and the detection reagent but also further substances. The particles are preferably not identical to the detection reagent or at least not completely identical to the detection reagent, and, as described above, the detection reagent can also be a mixture of multiple detection reagents or multiple substances which together form the detection reagent. The detection layer can be, for example, analogous to the first film layer described in EP 0 821 234 B1 of the diagnostic test support, apart from the particle size distribution described above. Thus, the detection layer can comprise, for example, at least one organic film former. For example, this at least one film former can comprise a polyvinyl propionate dispersion. However, other film formers can also be alternatively or additionally used.

In a second aspect of the present invention, there is proposed a process for producing a diagnostic test element for detecting an analyte in a sample of a body fluid. As explained above, this diagnostic test element can be more particularly a diagnostic test element as per one or more of the embodiments described above or one or more of the exemplary embodiments yet to be described below. The diagnostic test element has at least one test field having at least one detection reagent. The detection reagent is set up to pass through at least one change in the presence of the analyte, more particularly an optical change. The test field has at least one detection layer comprising the detection reagent. In the process, the detection layer is generated such that these particles has, with at least 90% of all particles of the detection layer having an actual particle size of less than 10 micrometers, preferably of less than 3 micrometers or even of less than 1 micrometer. For particularly preferred particle size distributions, reference can be made to the above description.

The detection layer can be generated in particular by means of at least one wet chemical process, more particularly from one or more dispersions, preferably aqueous dispersions. Such layer-forming processes from one or more dispersions are known in principle to a person skilled in the art, and reference can be exemplarily made in turn to, for example, the abovementioned prior art, more particularly EP 0 821 234 B1.

To ensure that the mentioned conditions for the particle size distribution are present in the finished detection layer, different processes can be used. More particularly, at least one powder, for example a pigment powder, can be used to provide the particles in the detection layer. This powder may comprise

agglomerates of primary particulates, which may already be directly present in the starting powder or which may temporarily form as well only during the production process, for example in the dispersion. However, the pigment powder in the proposed production process is processed by means of at least one mechanical dispersion process in order to break up the agglomerates at least partly so that the abovementioned particle size distribution is present in the detection layer. A dispersion process is generally to be understood to mean a process in which the powder, for example the pigment powder, is distributed in at least one liquid medium, preferably an aqueous medium, without the powder dissolving in this medium, so that a dispersion forms. The dispersion can be admixed with further substances. A mechanical dispersion process is - in contrast to chemical dispersion processes, which may nevertheless be additionally used, though this is less preferred - to be understood to mean a dispersion process in which the distribution of the powder in the medium is maintained by means of a mechanical action on the dispersion. This mechanical action can be effected in particular such that, with this mechanical action, high shear forces have an effect on the dispersion and more particularly on the powder and the agglomerates present therein, whereof aggregates shall also be included, and so these are broken up at least partly to form smaller particles which fulfill the abovementioned particle size distribution condition.

More particularly, a dissolver can be used for carrying out the mechanical dispersion process. A dissolver is generally to be understood to mean an apparatus which can maintain the distribution of the powder in a medium, more particularly a substantially homogeneous distribution, and can exert at the same time high shear forces on the dispersion. For example, these shear forces can be exerted by means of two or more surfaces

running against one another and closely spaced to one another, between which the dispersion is received. For example, dissolvers in the form of disk stirrers are commercially available in which high shear forces are exerted on the dispersion by means of a stirring disk, which is brought into a rotary motion, and so the agglomerates are torn apart.

Alternatively or additionally, dissolvers in accordance with a rotor/stator principle can be used. By means of such dissolvers, it is thus possible to generate or process a dispersion from which the detection layer is generated, and so the abovementioned condition for the particle size distribution is fulfilled.

Alternatively or additionally, a three-roll mill (also called a three-roller mill) can be used for carrying out the mechanical dispersion process, more particularly for dispersing the fillers. With such a three-roll mill, use is made of at least three rolls or cylinders which run against one another at different speeds. The gap between the rolls or cylinders is generally set such that it is comparatively small, for example to 1 mm, down to the nanometer range.

To provide the particles, one possible option, as will be more particularly elucidated below, is to use commercially available particles which fulfill the mentioned condition for the particle size distribution. However, grinding processes can also be alternatively or additionally used. For instance, to provide the particles, use can be made of, for example, at least one powder, more particularly at least one pigment powder, the powder being subjected to at least one grinding step. A grinding step is to be understood to mean a process in which the powder in a dry or in a wet state is ground by the action of mechanical forces. Various grinding processes are known. For instance, the at least one grinding step can comprise, for example, a

wet grinding step, more particularly in a bead mill, and/or a dry grinding step, more particularly in an air jet mill. Other grinding processes are also known to a person skilled in the art and are commercially available, and so corresponding mills can be selected which can be adapted to the type of powder and/or the type of desired particle size distribution.

Use can be made in particular of a powder of a synthetic oxidic material when providing the particles. Such synthetic oxidic materials are already in part, as is yet to be exemplarily explained in detail below, commercially available in the mentioned particle sizes, for example from material manufacturers that have specialized in micromaterials and/or nanomaterials. More particularly, the at least one synthetic oxidic material can be a nanoparticulate oxidic material. A nanoparticulate material is to be understood in the context of the present invention to mean in general a material which has particles having an average particle size of below 100 nanometers. More particularly, the oxidic material can be silicon oxide and/or aluminum oxide and/or titanium oxide, for example Al_2O_3 and/or TiO_2 and/or SiO_2. More particularly, the mentioned oxides, which may also be present as mixed oxides, can be present in nanoparticulate form.

In a further aspect of the present invention, there is proposed a process for detecting an analyte in a sample of a body fluid, more particularly whole blood. Use is made here of a diagnostic test element in one or more of the embodiments described above and/or in one or more of the embodiments yet to be described in detail below. The sample has a volume of less than 2 microliters, more particularly of less than 0.5 microliters, and particularly preferably of less than 0.3 microliters, for example of 100 nanoliters. More particularly, the detectable change of the at least one

detection reagent of the test field of the diagnostic test element used, as explained above, can be an optically detectable change. In this case, it is particularly preferred for a spatially resolving optical detector to be used for detecting the detectable change. A spatially resolving optical detector is to be understood to mean an optical detector which has a multiplicity of optical sensors which are able to record regions of the detection side of the detection layer which are not completely congruent. More particularly, the spatially resolving optical detector can comprise at least one image sensor, i.e., an array of optical detectors which can be one-dimensional or else two-dimensional. More particularly, the optical detector can thus comprise a CCD chip and/or CMOS chip. In addition, the spatially resolving optical detector can comprise at least one optical element for imaging the detection side and/or the detection layer onto an image-sensitive surface of the spatially resolving optical detector. A spatially resolving optical detector and the mentioned small sample volumes make the advantages of the present invention, which are yet to be described in detail below, particularly apparent, since conventional detection layers lead to great uncertainty in the detection owing to the disadvantageous wetting effects and the coarseness of the detectable change, more particularly of the optically detectable change.

The proposed diagnostic test element, the proposed production process, and the proposed detection process have numerous advantages compared to known apparatuses and processes of the type mentioned. For instance, an important basis for the present invention is the recognition that the use of small particulates in the form of particles for producing a detection layer generally results in aggregate formation, and so the detection layer is no longer able to profit from the

low particulate size of the primary particles. A test strip which is produced as per known processes of the prior art from substances having the described particulate sizes will thus in general have accordingly only particulates in an aggregated state. The particulate sizes which are known from customary production processes and which are used as starting material in powders are thus only nominal values which are generally not found again in the actual particle size distribution in the detection layer. Generally, the starting material (which is referred to here in general as a filler or which may contain at least one filler) has to be finely dispersed in the production of the detection layer. In the case of some fillers, such as Aerosils and/or Aeroxides for example, this does not generally have to be considered in particular, since such substances have been optimized by the manufacturer in many cases for easy disk convenience.

In contrast, according to the invention, production of the diagnostic test element having a detection layer can be carried out which results in a considerably more favorable particle size distribution in the finished detection layer. For example, use can be made of starting powders having average particle sizes of, for example, up to 50 nanometers, preferably up to 30 nanometers, in which dispersing of the particles can be provided before the production of the detection layer, for example before the application of a dispersion for producing the detection layer to the support element, and so the particle sizes fulfill the mentioned condition. The particle sizes of the primary particles of the starting powder can, for example, remain substantially preserved, or agglomeration and/or aggregation of the primary particles may occur only to a slight extent during production.

Starting materials which can be used for producing the dispersion are commercially available materials, for example commercially available powders which already have the desired particulate size or particle size distribution. However, alternatively or additionally, it is also conceivable for at least parts of the starting substances, for example of the at least one powder, to be initially ground as described above before the generation of the detection layer in order to achieve a corresponding particle size distribution having preferred grain sizes.

Furthermore, it has become apparent that, as will be more particularly elucidated below, not all materials are suitable for such a process, since, depending on the selected material during dispersing, such materials may also act as thickeners, and this may lead to gel formation. More particularly, certain materials may possibly act as thickeners from, for example, a concentration of more than 3% by weight, more particularly more than 5% by weight, or even more than 20% by weight, based on the dispersion. However, the abovementioned materials have been found to be particularly advantageous, since such a thickening action does not occur or at least generally does not occur, at least at concentrations of up to 3% by weight in the dispersion, preferably of more, for example of up to 5% by weight or up to 20% by weight.

The present invention is further based on overcoming technical prejudices which are often advanced against fine-grained detection layers. For example, the effect of small particles on depth of shade and reaction time in detection layers was hitherto unknown. To achieve the necessary precision, it is necessary in the case of, for example, optical detection to achieve in general reflectance differences, also referred to as a reaction shift, of more than 40%, preferably of more

than 50% or even more than 60%, relative reflectance, based on the blank value of the dry detection layer, between the glucose concentrations of 10 mg/dl and 600 mg/dl, which typically form a measurement range. Reflectance is generally to be understood to mean the diffuse, undirected reflection of waves, more particularly of light, in contrast to a regular, directed reflection. Reflectance is often related to the surface of the detection side and also referred to as degree of reflectance. Degree of reflectance is to be understood to mean the ratio of the luminance remitted by a surface to the luminance of a surface in a reference white. Reflectance is a customary measured value in optical test elements, such as the test elements described in the prior art for example, and known in this field to a person skilled in the art.

Furthermore, reaction times of less than 10 seconds have to be achieved in general with customary diagnostic test elements. Reaction time is to be understood to mean the time within which a substantially steady state has occurred after application of the sample to the test field. However, particularly in the case of reaction times, it had, until now, been feared that more densely packed ingredients of the detection layer need a longer time for penetrating and dissolving through the sample fluid. The sample liquid can be, for example, blood or blood plasma recovered therefrom after removal of the erythrocytes.

It was all the more surprising that, as is yet to be explained in detail below, in the case of the preferred particle size distributions, the reflectance shift practically did not change compared to coarse-grained detection layers and also the reaction time remained at least approximately the same. However, at the same time, considerably more homogeneous wetting of the test

fields was achieved, and as a result, as is likewise yet to be explained in detail below, the coefficient of variation in particular was distinctly reduced. By overcoming the mentioned prejudices, it is thus possible to generate diagnostic test elements which have a considerably higher precision compared to conventional diagnostic test elements and which, at the same time, are also suitable for measuring very small volumes of samples, more particularly by using spatially resolving optical detectors.

Brief description of the figures

Further details and features of the invention are revealed in the following description of preferred exemplary embodiments, more particularly in conjunction with the subclaims. The exemplary embodiments are, at least in part, depicted schematically in the figures. The same reference symbols in the individual figures refer to elements which are the same or similar in function or to elements which correspond to each other in terms of their functions. The invention is not restricted to the exemplary embodiments.

In particular:

Figure 1	shows a schematic cross-sectional view of a diagnostic test element according to the present invention;
Figures 2A and 2B	show examples of wetting a test field surface of a test field of a conventional diagnostic test element (figure 2A) and of a diagnostic test element according to the invention (figure 2B);

Figure 3 shows reflectance curves of diagnostic test elements as per figures 2A and 2B;

Figure 4 shows reflectance curves of further exemplary embodiments of diagnostic test elements according to the present invention;

Figure 5 shows reflectance curves of samples with ingredients dispersed in different ways;

Figures 6A to 6D show microscope images of samples of varying granularity;

Figures 7A and 7B show standard deviations of the gray values in figures 6A to 6D; and

Figures 8A and 8B show autocorrelation functions of the gray values in figures 6A to 6D.

Exemplary embodiments

Figure 1 schematically shows a possible assembly of a diagnostic test element 110 in a cross-sectional view, which assembly can also be used in the context of the present invention. In this exemplary embodiment, the diagnostic test element 110 comprises a support element 112 which can be, for example, in the form of a strip. As a whole, the diagnostic test element 110 can thus be in the form of a test strip.

The support element 112 comprises at least one transparent region 114. In the region of the transparent region 114, there is applied to the support

element 112 a layer assembly which can completely or partly cover the transparent region 114. In the exemplary embodiment depicted, this comprises two layers and forms a test field 116. In the exemplary embodiment depicted, this test field 116 comprises by way of example a detection layer 118 having a detection side 120 facing the support element 112 and the transparent region 114. In addition, in the exemplary embodiment depicted, the test field 116 optionally comprises a removal layer 122 on the side of the detection layer 118 facing away from the support element 112. This removal layer 122 serves to remove coarse constituents of a sample 126 of a body fluid which, on a loading side 128, can be applied to a test field surface 124.

The transparent region 114 can, for example, be simply in the form of an opening, for example a hole, in the support element 112. In this case in particular, but also in other embodiments, there can be additionally applied to the support element 112 a support slide or another type of support, preferably a transparent support slide. This optional support slide is indicated in figure 1 by the reference number 119. This support slide 119 can, for example, be introduced between the support element 112 and the detection layer 118 in the layer assembly shown in figure 1. For example, the support slide 119 can be part of a reaction film, where the at least one detection layer 118 and, optionally, the at least one removal layer 122 are applied to the support slide 119, for example by means of a printing process and/or a blade-coating process. Subsequently, this reaction film is then applied to the actual support element 112 having the transparent region 114, and so the detection layer 118 is perceptible through the transparent region 114.

Alternatively, the transparent region 114 can, however, also be completely or partially filled with a

transparent material, for example a transparent plastics material, and/or the entire support element 112 can be in the form of a transparent support element. In this case in particular, but also in other cases, the layer assembly having the at least one detection layer 118 and, optionally, the at least one removal layer 122 can also be directly applied to the support element 112. Alternatively, it is also possible here to again use a reaction film according to the above embodiments which is applied to the support element 112.

It should be pointed out that the assembly depicted in figure 1 of the diagnostic test element is to be understood merely as an example and that other types of assemblies are also possible. For example, multiple detection layers 118 and/or multiple removal layers 122 or no removal layer 122 at all can be provided. In addition, the assembly shown in figure 1 can be supplemented by various other elements which are not depicted. For example, a spreading mesh can be provided on the test field surface 124. In addition, parts of the test field surface 124 can be covered, for example with a hydrophobic material, in order, for example, to make only part of the loading side 128 accessible for applying the sample 126. For possible embodiments of the diagnostic test element 110, reference can be made, for example, to the abovementioned EP 0 821 234 B1 or to other known test strip assemblies.

Example 1

The present invention relates essentially to configuring and producing the detection layer 118. To compare diagnostic test elements 110 having the above-described particle size distribution according to the invention with conventional diagnostic test elements, use may be made in principle of layer assemblies, as

described in EP 0 821 234 B1 for example. However, in the present example, use is made of layer assemblies of the test field 116, which are produced as follows:

Sample A:

The comparative sample (sample A) produced is a diagnostic test element 110 corresponding to the following assembly:

a) Detection layer:

To produce a dispersion for the detection layer 118, two part solutions (part solutions 1 and 2) are initially prepared, and these are then combined to form a part batch. In this context, the term "solution" is used irrespective of whether a real solution is actually present or only a dispersion for example. An enzyme solution is prepared, and the part batch 1 and the enzyme solution are mixed to give a coating composition. To this end, the following is carried out:

Part solution 1: 0.34 g of xanthan gum are preswollen for 24 h in 35.5 g of 0.02 M glycerol 3-phosphate buffer, pH 6.5 and mixed with 5.0 g of polyvinyl propionate dispersion.

Part solution 2: 5.2 g of Transpafill are dispersed for 10 min with an Ultraturrax in 21.5 g of water.

Part batch 1: Both part solutions are combined and, after addition of 0.15 g of tetraethylammonium chloride, 0.17 g of N-octanoyl-N-methylglucamide, 0.06 g of N-methyl-N-octadecenyl taurate ("Geropon T 77"), and 0.88 g of PVP (MW: 25 000), are stirred moderately for 1 h with a paddle stirrer. Then the following part solutions are added in the order shown:

- 0.10 g of bis-(2-hydroxyethyl)-(4-hydroximinocyclohexa-2,5-dienylidene)ammonium chloride in 1.5 g of water,
- 0.65 g of 2,18-phosphomolybdic acid hexasodium salt in 1.5 g of water,

whereupon the pH is adjusted to 6.7 with NaOH.

Enzyme solution: 5 mg of PQQ disodium salt and 0.28 g of GDH (mutant 31) and also 0.16 g of a 1 M $CaCl_2$ solution are added to 25.6 g of 0.1 M glycerol 3-phosphate buffer, pH 6.5 and stirred for > 3 h.

The part batch 1 and enzyme solution are mixed, admixed with a solution of 20 mg of $K_3[Fe(CN)_6]$ in 0.4 g of water and also 1.0 g of 2-methyl-2-butanol, and stirred for 30 min. This gives a coating composition for producing the detection layer 118.

The coating composition produced in this way is, with a grammage of 90 g/m^2, applied to a support slide 119 in the form of a polycarbonate slide having a thickness of 125 micrometers and dried.

Transpafill® is a commercially available sodium aluminum silicate powder from Evonik Industries AG. The precision-improving effect of N-methyl-N-octadecenyl taurate ("Geropon T 77") has been described in EP 0 995 994.

b) Removal layer:

In the present exemplary embodiment, the removal layer 122 is also produced by initially preparing two part solutions (part solution 1 and part solution 2) and then combining them. This is carried out as follows:

Part solution 1: A slurry of 1.37 g of Gantrez S 97 in 13.5 g of water is admixed with 2.2 g of 16% NaOH and

preswollen overnight. Then 0.40 g of tetraethylammonium chloride, 0.34 g of N-octanoyl-N-methylglucamide, 0.06 g of N-methyl-N-octadecenyl taurate ("Geropon T 77"), and 1.87 g of PVP (MW: 25 000) are added and stirred for 1 h.

Part "solution" 2: 14.3 g of titanium dioxide E 1171 from Kronos and 1.95 g of precipitated silica FK 320 from Degussa are dispersed for 10 min with an Ultraturrax in 36.4 g of water.

After combining the part solutions, there are added 5.7 g of polyvinyl propionate dispersion, 0.15 g of bis-(2-hydroxyethyl)-(4-hydroximinocyclohexa-2,5-dienylidene)ammonium chloride in 4.2 g of water, 1.85 g of 2,18-phosphomolybdic acid hexasodium salt in 4.2 g of water, 10 mg of $K_3[Fe(CN)_6]$ in 0.4 g of water, and the pH is adjusted to 6.8 with NaOH. After addition of 1.0 g of 2-methyl-2-butanol, stirring is carried out for 1 h.

The name Gantrez® is a product name from ISP International Speciality Products, Cologne, Germany. Chemically, it is a copolymer of maleic acid and methyl vinyl ether.

The coating composition produced in this way by combining part solutions 1 and 2 is then, with a grammage of 45 g/m^2, applied to the first coated polycarbonate support slide 119 described as above, i.e., to the detection layer 118, and dried.

Sample B:

To produce a diagnostic test element 110 according to the invention, a grinding process is carried out in the detection layer 118 on the raw material Transpafill® which is substantially responsible for the coarseness

of this detection layer 118. Optionally, the likewise coarse-grained raw material diatomaceous earth in the removal layer 122, which acts as precipitated silica, can also be subjected to a grinding process. However, in the removal layer 122, there should be no grinding of the titanium dioxide, which serves as a white pigment and should thus exhibit reflectivity for the radiated light, for example light having a wavelength of 660 nanometers. This light is, for example, radiated through the transparent region 114, radiated through the detection layer 118, and is reflected at the removal layer 122, and so the removal layer 122 in the exemplary embodiment depicted in figure 1 can simultaneously serve as a reflection layer.

To make the abovementioned coarse-grained fillers Transpafill® and, optionally, precipitated silica finer, they are subjected to a wet grinding step. This can be carried out with an agitator bead mill, for example for 20 minutes, which correspondingly leads to a measurement giving a particle size d_{50} of about 0.3 micrometers and a particle size d_{90} of about 0.5 micrometers. The value d_{90} refers to the particle size at which 90% of the particles are finer than the value d_{90}.

Samples A and samples B produced in this way enable various comparative experiments to be carried out. These comparative experiments can eliminate in particular the prejudice that more densely packed ingredients need a longer time for penetrating and thus need to dissolve through the sample fluid.

Figures 2A and 2B depict wetting experiments which were carried out on samples of type A (figure 2A) and samples of type B (figure 2B). These experiments firstly show the influence of the grinding step on wetting. The comparative experiments each show test

fields 116 having a test field surface 124 to which a drop 130 of the sample 126 is applied. Subimages 132 in figures 2A and 2B each show microscope images of the test field surface 124, whereas subimages 134 show the change in gray value in the microscope images 132 along an intersection line 136 through the drop 130 of the sample 126. The sample 126 used was a test fluid having a concentration of 50 mg/dl glucose.

In subimages 134, the pixel position, indicated by #, along the intersection line 136 is plotted on the vertical axis in arbitrary units. The horizontal axis specifies the time t in seconds after application of the sample 126. In subimage 134, the changes in gray value are depicted in each case. The right-hand side of this subimage depicts a scale which specifies the changes in gray value (ΔI) in arbitrary units. Figure 2A shows a test field surface 124 of sample A, i.e., a test field material as is currently used in commercially available test strips. Figure 2B shows by contrast a test field 116 having the test field material according to sample B as per the present invention.

Without going into the numerical details of the measurement, particularly subimages 134 of the change in gray value in figures 2A and 2B show in a direct comparison that grinding of the test field material results in a distinctly more homogeneous temporal change in reflectance characteristics along the intersection line 136. Accordingly, the initiation of the reaction, which is specific for the detection of the analyte to be detected, is effected virtually at the same time along the intersection line 136 in the exemplary embodiment as per figure 2B, whereas in the experiment with unground test field material as per figure 2A, a strong temporal offset of the initiation of the reaction can be found. For instance, a temporal offset between individual locations along the

intersection line 136 can occur which can be up to 3 seconds or more. Also, there can be observed locations along the intersection line 136 at which the reaction occurs instantaneously, and also locations at which the reaction does not appear to proceed at all.

Furthermore, in both figures 2A and 2B, a majority of particles 137 in the detection layer 118 is perceptible. In the microscope images in subimages 132, almost solely the detection layer 118 is visible in each case, since light rays which enter the detection layer 118 through the transparent region 114 are reflected no later than at the pigments of the removal layer 122, more particularly titanium dioxide pigments.

It can be clearly discerned that the particles 137 in conventional sample A as per figure 2A are considerably larger and have a broader particle size distribution than the particles 137 in sample B according to the invention as per figure 2B. By means of a microscope image of this kind as per subimage 132 in figures 2A und 2B, a particle size distribution can also be readily created by image recognition and automatic recognition of particles at an appropriate magnification. Alternatively or additionally, a change in gray value as per subimages 134 may also be used. Analytical processes of this kind with automatic image recognition are known in principle from the field of image processing to a person skilled in the art.

Overall, an evening-out of the course of the analyte-specific reaction can thus be observed as the first positive effect of using a ground test field material. Furthermore, as is clear from figures 2A and 2B, a homogenization of the reaction across the wetted test field surface 124 can be established overall.

Furthemore, for the experiments overall, the reaction time for ground and unground samples remains overall on average approximately the same. For instance, in all cases, a reaction time of about 6 to 7 seconds was established. However, as is clear from the above-described results, the spatially resolved, local reaction time for ground test field materials is strongly evened out, and so local variations in reaction times can be considerably improved by the ground test field material.

In a further experiment, experiments on the reflectance shift are carried out with samples A and B. According to the prejudice outlined above that ground test field materials result in incomplete penetration of the detection layer 118, a distinct reduction in the reflectance shift would have to be observed for samples of type B, since only a smaller region of the detection layer 118 should be penetrated by the sample 126 and is thus available for the detection reaction.

The results of these reflectance measurements are depicted in figure 3. The concentration c of glucose in the sample 126 is shown on the horizontal axis, whereas the relative reflectance R is plotted on the vertical axis. EDTA venous blood was used as the sample 126, and the concentrations of glucose were varied in this test fluid. The curve 138 in figure 3 shows the remissions which were measured on a conventional diagnostic test element, i.e., a sample of type A, whereas the curve 140 shows remissions of a sample according to the invention of type B. As can be recognized from these depicted data, there is practically no change in the reflectance shift, i.e., the change in reflectance across the entire measurement range, which is typically between 10 and 600 mg/dl. Wet grinding of the fillers therefore does not result in impairment of optical properties and/or detection properties.

Thus, the experiments depicted in figures 1 to 3 clearly show that the use of ground fillers is not accompanied by impairment of the properties of the diagnostic test elements 110 in the form of a lengthening of the reaction time or in the form of a worsening of the reflectance shift. At the same time however, as figures 2A and 2B clearly demonstrate, the homogeneity and the precision of the measurements can be distinctly improved by the use of ground test field chemistry. On an Accu-Chek® Active measuring device, it has already been established in several measurements that there is an improvement in the coefficient of variation (CV), which reports the ratio of the standard deviation to the mean value of the measurements, from 1.5 to 1.2% following the transition of samples of type A to samples of type B.

As an alternative to the wet grinding described above according to sample B, grinding can also be alternatively or additionally carried out with, for example, a dry grinding step. Accordingly, use can be made of, for example, an air jet mill, by means of which particulate sizes of up to, for example, 100 nanometers are achievable in principle.

Furthermore, experiments were carried out in which the complete coating compositions for the detection layer 118 and the removal layer 122 were ground. In the detection layer 118, there was no cogrinding of the detection reagent, more particularly the enzyme, because of the energy input in the grinding process. However, such processes brought overall no or only a slight improvement in the homogeneity, in the reflectance shift, and in the reaction time. Thus, grinding the complete coating compositions does not have any advantage compared to grinding the filler prebatches. However, the latter is considerably simpler

in terms of production technology, since a stock can be ground.

Example 2

Grinding raw materials for the detection layer 118 represents an additional process step and can increase the costs of the diagnostic test elements 110. Therefore, in a second phase, raw materials are tested which are commercially available and which entail from the start an average particulate size in the range of << 1 micrometer. Available for this purpose is, inter alia, the Aerosil product range from Evonik Industries AG. These are hydrophilic, nanoparticulate oxides, more particularly metal oxides.

For instance, the following substitute materials were identified as substitutes for the above-described Transpafill® in sample B:

Material:	Type:	Average particle size:
SiO_2:	Hardly thickens during dispersion: Aerosil EG 50, Aerosil 90	20 nm
	Strongly thickens during dispersion: Aerosil 200, Aerosil COK 84	
TiO_2:	Aeroxide TiO_2 P 25	21 nm
Al_2O_3:	Aeroxide Alu 65	17 nm

Table 1: Examples of further possible substitute materials for Transpafill®.

The experimentally determinable property of certain materials that these materials have a thickening effect on the dispersion during the dispersion procedure is indicated by "thickens during dispersion" or "does not thicken during dispersion".

For the use of these substitute materials, there is naturally initially the fear, owing to the abovementioned prejudice, that the pores may then ultimately be too small in size to enable penetration of the detection layer 118, and the reflectance shift and the reaction times therefore worsen compared to standard samples.

Accordingly, samples are produced in which, compared to sample A above, the Transpafill® in the detection layer 118 has been replaced 1:1 with the following materials:

Sample C:

SiO_2, Aerosil COK 84 (mixed oxide with 10% Al_2O_3), average particle size 20 nm

Sample D:

TiO_2, Aeroxide TiO_2 P 25, average particle size 21 nanometers

and

Sample E:

Al_2O_3, Aeroxide Alu 65, average particle size 17 nanometers

Sample F:

In a fourth sample in this second example, compared to sample A, a wet-ground mixture of precipitated silica and titanium dioxide having an overall average particle size of 0.3 micrometers is used instead of Transpafill®.

Reflectance measurements are again carried out in part on these samples, analogously to the experiment as per figure 3. The results of these measurements are plotted in figure 4, the data depicted analogously to those in figure 3. The curve 142 indicates the reflectance of sample A, the curve 144 indicates the reflectance of sample D, the curve 146 indicates the reflectance of sample E, and the curve 148 indicates the reflectance of sample F.

Initially, it was found that the reaction times for all the samples were 6 seconds and therefore unchanged compared to comparative sample A. Furthermore, the reflectance shifts, as can be clearly discerned in figure 4, also remain substantially the same across the measurement range. The curves 142 and 148 are even overlapping to a great extent in figure 4.

However, it is apparent in this experiment that the very finely divided fillers should be dispersed close to their primary particulate size in order to avoid agglomeration and/or aggregate formation. For this purpose, conventional dissolvers are used, and use can be made of, for example, Polytron® or Megatron® devices from Kinematica AG or Ultra-Turrax® devices for example from IKA Maschinenbau.

For SiO_2 Aerosil types, such as samples C as per the description above for example, it is apparent that dissolvers can be used only with difficulty. Dissolvers are therefore preferably used for samples of types D and E, i.e., for titanium oxides and aluminum oxides, having in particular nominal average particle sizes of the starting powders of 30 nanometers or less. For samples of type C, in contrast, the use of dissolvers results in a dispersion only up to concentrations of about 3% by weight in the wet starting mixture owing to the thickening effect of the SiO_2 Aerosil types and to

gel formation. However, stirrers with lower shearing rates can be used here, but which provided in the experiments virtually the same results as the curves in figure 4. This indicates that the Aerosils have been optimized for dispersibility. For Aeroxide TiO_2 P 25 and Aeroxide Alu 65, thickening occurred only negligibly in the experiments.

As an alternative or in addition to the abovementioned fillers, ground or already commercially available as nanoparticulates, a search was made for further substances which are usable as fillers in the detection layer 118, for example as a substitute for Transpafill® in the above-described sample A. In addition to the above-described Aerosil types, the following substances, among others, were investigated:

- Kaolin
- Powder glass (TROVOtech, Wolfen)
- Precipitated silica
- Calcium sulfate x 2 H_2O
- Sodium aluminum silicates, such as Sipernat 44 MS (Degussa/Evonik) for example.

These fillers are either already available in the desired particle size or particle size distribution or can be processed by grinding to the required particulate size or particle size distribution.

The above experiments essentially show that the manufacturers have adjusted the Aerosil/Aeroxide types for easy dispersability, and so incorporation is largely independent of the shearing force.

In order to study more closely the necessity of dispersing down to primary particulate size, further experiments are performed. For this purpose, the above sample D is studied again in different ways. Therefor,

Aeroxide TiO_2 P 25 is dispersed either with a dissolver (sample G) or with a propeller stirrer having a low speed. The addition of Aeroxide TiO_2 P 25 is carried out either after mixing beforehand with water to form a paste (sample H) or by solid introduction into the thickener solution (xanthan gum) (sample I). The comparative sample used is, as before, sample A as per the above description. Altogether, the samples for the experiment described below are thus as follows:

Sample A': as per sample A above

Sample G: as per sample D, but dispersing of Aeroxide TiO_2 P 25 with a dissolver,

Sample H: as per sample D, but dispersing of Aeroxide TiO_2 P 25 with a propeller stirrer having a low speed, addition after mixing beforehand with water to form a paste, and

Sample I: as per sample D, but dispersing of Aeroxide TiO_2 P 25 with a propeller stirrer having a low speed by solid introduction into the thickener solution (xanthan gum).

In this way, samples G to I are prepared in which Transpafill® is replaced 1:1 by weight with Aeroxide TiO_2 P 25. Otherwise, the diagnostic test elements 110 are produced as described above.

With these samples of diagnostic test elements 110 in the form of test strips, measurements are carried out with 15 different glucose concentrations in EDTA venous blood on the commercially available Accu-Chek Active blood glucose measurement system, where n = 10 individual measurements per concentration are analyzed.

Figure 5 depicts, analogously to the data depicted in figure 4, reflectance curves for samples A', G, H, and I. The curve 150 indicates reflectance measurements for sample A', the curve 152 indicates reflectance measurements for sample G, the curve 154 indicates reflectance measurements for sample H, and the curve 156 indicates reflectance measurements for sample I.

The measurement curves show that the depth of shade is virtually the same for the four different coatings. The reaction rate as well is in the range of 6 to 8 seconds for all the samples.

This shows that the Aeroxide TiO_2 P 25 in all three cases, i.e., in samples G, H, and I, is present finely dispersed, since aggregates of TiO_2 have a pigment character and would attenuate the reaction color. Color attenuation would be caused by, in the case of aggregate formation, a pigment being present in the detection layer 118. Finely dispersed TiO_2 is, however, not a pigment, since in this case the particulate size is smaller than the light wavelength. This property is, for example, taken advantage of in sunscreen agents.

The major advantage of the detection films having fine-grained fillers is the distinctly improved homogeneity of the reaction colors, which therefore allows the measurement of smaller areas and thus smaller blood volumes.

This can be shown again in a comparative experiment in which different samples are studied. For this purpose, diagnostic test elements 110 in the form of test strips are spotted with plasma containing 100 mg/dl glucose, and the reaction color is measured with a CCD camera. Since the individual pixels can be read separately, a number of pixels are analyzed statistically with regard

to their precision (i.e., with regard to their standard deviation). Here, 10 pixels (edge length: 10 micrometers) are analyzed at the highest resolution, i.e., an overall area of 1000 μm^2. For lower resolutions, i.e., larger areas, averaging is carried out over more pixels.

Again, different samples are studied here, analogously to the above samples:

Sample A'': as per sample A above, unground fillers, comparative sample

Sample J: as per sample A'', but Transpafill® and precipitated silica are ground,

Sample A''': as per sample A above, coarse ingredients, comparative sample, and

Sample K: replacement of Transpafill® with Aeroxide TiO_2 P 25.

Figures 6A to 6C show microscope images of the measurement spots which are the basis of the subsequent measurements. Figure 6A shows a microscope image of a region of sample A'' spotted with the solution, i.e., of a sample having unground fillers. Figure 6B shows an analogous image for sample J, i.e., of a sample having ground test chemistry. Figure 6C shows an image for sample A''', which essentially represents again a sample having coarse ingredients and corresponds to sample A'', and figure 6D shows an analogous image for sample K, in which Transpafill® is replaced with Aeroxide TiO_2 P 25.

The axes labels in figures 6A to 6D display in each case the pixel position on a CCD chip in arbitrary units. Furthermore, measurement fields in figures 6A to

6C are indicated by means of corresponding squares, the coordinates of which are displayed in the images.
Both figures 7A and 7B depict standard deviations for the samples of figures 6A to 6D. Plotted on the vertical axis in each case is the standard deviation s of the gray values in figures 6A to 6D. This standard deviation s is specified as a percentage, based on the average gray value shift between a blank measurement and a fully reacted sample. This standard deviation s is specified as a function of the area A plotted on the horizontal axis, via which averaging was carried out.

7A shows a comparison of samples A'' and J, i.e., a comparison of the standard sample with a sample having ground test chemistry. The curve 158 displays the course of the standard deviation for sample A'', whereas the reference number 160 indicates the curve of sample J having ground test chemistry. In figure 7B, standard sample A''' (reference number 162) is compared with sample K (reference number 164) in terms of its standard deviation.

The measured results show that the standard deviation s and thus any possible measurement errors strongly increase below about 30 x 30 μm^2, i.e., an area <0.01 mm^2. It can be further recognized that this rise in the standard deviation for sample J (curve 160) having ground chemistry is distinctly lower, and the ground chemistry is therefore advantageous for the miniaturization of blood volumes, i.e., for miniaturization of the measurement spot in figures 6A to 6D. The same can also be said for sample K (curve 164 in figure 7B).

Various processes were specified above to determine the particle size of samples. A further option which can be alternatively or additionally applied involves calculating autocorrelation functions via the gray

value distribution in microscope images, such as those in figures 6A to 6D for example. The autocorrelation function is a cross-correlation function of a signal with itself, which is a function of a shift τ.

Autocorrelation functions (indicated by ACF) for samples A'' to K are plotted in figures 8A and 8B. Figure 8A shows a comparison of comparative sample A'' (curve 166) with sample J having ground ingredients (curve 168), and figure 8B shows a comparison of comparative sample A''' having coarse ingredients (curve 170) with sample K having fine ingredients (curve 172). In each case, the autocorrelation function ACF is shown on the vertical axis, and the shift τ of the autocorrelation function in millimeters is shown on the horizontal axis. The autocorrelation functions were determined by analyzing figures 6A to 6D.

It can be discerned in a comparison of the autocorrelation functions that the coarse fillers (curves 166, 170) have distinctly broader autocorrelation functions than the fine-grained fillers (curves 168, 172). The data depicted show that the autocorrelation function ACF correlates with the particle size distribution. Simply from microimages, such as the images in figures 6A to 6D for example, it is thus possible to determine the granularity of a sample. For example, the half-height width of the autocorrelation functions 166 to 172 can be a measure of the granularity of the detection layer 118. Admittedly, direct reading of the particle size distribution from these curves 166 to 172 is not possible. By means of one or more calibration measurements on samples having known particle size distributions, the particle size or the particle size distribution can be directly deduced from the curves 166 to 172.

Reference symbol list

110	Diagnostic test element
112	Support element
114	Transparent region
116	Test field
118	Detection layer
119	Support slide
120	Detection side
122	Removal layer
124	Test field surface
126	Sample
128	Loading side
130	Drop
132	Subimage, microscope image
134	Subimage, change in gray value
136	Intersection line
137	Particle
138	Reflectance, sample A
140	Reflectance, sample B
142	Reflectance, sample A
144	Reflectance, sample D
146	Reflectance, sample E
148	Reflectance, sample F
150	Reflectance, sample A'
152	Reflectance, sample G
154	Reflectance, sample H
156	Reflectance, sample I
158	Standard deviation, sample A''
160	Standard deviation, sample J
162	Standard deviation, sample A'''
164	Standard deviation, sample K
166	Autocorrelation, sample A''
168	Autocorrelation, sample J
170	Autocorrelation, sample A'''
172	Autocorrelation, sample K

CLAIMS

1. A process for detecting an analyte in a sample (126) of a body fluid by using a diagnostic test element (110), wherein the diagnostic test element (110) comprises at least one test field (116) having at least one detection reagent, wherein the detection reagent is set up to pass through at least one detectable change in the presence of the analyte, wherein the test field (116) has at least one detection layer (118) comprising the detection reagent, wherein the detection layer (118) comprises particles (137), wherein at least 90% of all particles (137) of the detection layer (118) have an actual particle size of less than 10 micrometers, wherein the test field (116) has a loading side (128) for applying the sample (126) and a detection side (120) for detecting a change in the detection reagent, wherein the test field (116) further has at least one removal layer (122), wherein the removal layer (122) is arranged on a side of the detection layer (118) facing the loading side (128), wherein the removal layer (122) comprises at least one pigment, wherein the sample (126) has a volume of less than 2 microliters, wherein the detectable change is an optically detectable change, and wherein a spatially resolving optical detector is used for detecting the detectable change, wherein the diagnostic test element (110) further comprises at least one support element (112), wherein the support element (112) has at least one transparent region (114), wherein the test field (116) is applied at least in part to the transparent region (114) with its detection side (120).

2. The process as claimed in claim 1, wherein at least 80% of all particles (137) of the detection layer (118) have an actual particle size of less than 5 micrometers.

3. The process as claimed in claim 1 or 2, wherein at least 70% of all particles (137) of the detection layer (118) have an actual particle size of less than 900 nanometers.

4. The process as claimed in claim 1 or 2, wherein the particles (137) of the detection layer (118) have an average particle size of 10 nanometers to 5 micrometers.

5. The process as claimed in claim 1 or 2, wherein the particles (137) of the detection layer (118) have an average particle size of less than 1 micrometer.

6. The process as claimed in claim 1 or 2, wherein the particles (137) comprise one or

more of the following materials: SiO_2; diatomaceous earth; a silicate; a metal oxide; a synthetic oxidic material; kaolin; powder glass; precipitated silica; calcium sulfate x 2 H_2O.

7. The process as claimed in claim 1 or 2, wherein the particles having a particle size of more than 100 nm among the particles (137) of the detection layer (118) are inorganic particles.

8. The process as claimed in claim 1 or 2, wherein the detection layer (118) has a refractive index of between 1.0 and 1.5.

9. The process as claimed in claim 1 or 2, wherein the pigment comprises a white pigment.

10. A process for producing a diagnostic test element (110) for detecting an analyte in a sample (126) of a body fluid, wherein the diagnostic test element (110) comprises at least one test field (116) having at least one detection reagent, wherein the detection reagent is set up to pass through at least one change in the presence of the analyte, wherein the test field (116) has at least one detection layer (118) comprising the detection reagent, wherein the detection layer (118) is generated such that the detection layer (118) comprises particles (137), wherein at least 90% of all particles (137) of the detection layer (118) have an actual particle size of less than 10 micrometers, wherein the test field (116) has a loading side (128) for applying the sample (126) and a detection side (120) for detecting a change in the detection reagent, wherein the test field (116) further has at least one removal layer (122), wherein the removal layer (122) is arranged on a side of the detection layer (118) facing the loading side (128), wherein the removal layer (122) comprises at least one pigment, wherein the diagnostic test element (110) further comprises at least one support element (112), wherein the support element (112) has at least one transparent region (114), wherein the test field (116) is applied at least in part to the transparent region (114) with its detection side (120).

11. The process as claimed in claim 10, wherein at least one powder is used to provide the particles (137), wherein the powder comprises agglomerates of primary particulates, wherein the powder is processed by means of at least one mechanical dispersion process in order to break up the agglomerates at least partly.

12. The process as claimed in claim 11, wherein at least one dissolver is used for carrying out the mechanical dispersion process, wherein a dispersion for producing the detection layer (118) is generated.

13. The process as claimed in claim 10, wherein at least one powder is used to provide the particles (137), wherein the powder is subjected to at least one milling step.

14. The process as claimed in claim 10, wherein a powder of a synthetic oxidic material is used when providing the particles (137).

15. The process as claimed in claim 2, wherein at least 80% of all particles (137) of the detection layer (118) have an actual particle size of less than 1 micrometers.

16. The process as claimed in claim 5, wherein the particles (137) of the detection layer (118) have an average particle size of less than 500 nanometers.

17. The process as claimed in claim 5, wherein the particles (137) of the detection layer (118) have an average particle size of up to 300 nanometers.

18. The process as claimed in claim 6, wherein the silicate is a sodium aluminum silicate.

19. The process as claimed in claim 6, wherein the metal oxide is at least one selected from the group consisting of an aluminum oxide and a titanium oxide.

20. The process as claimed in claim 6, wherein the synthetic oxidic material is a nanoparticulate oxidic material.

21. The process as claimed in claim 20, wherein the nanoparticulate oxidic material is at least one selected from the group consisting of a nanoparticulate silicon oxide, a nanoparticulate aluminum oxide and a nanoparticulate titanium oxide.

22. The process as claimed in claim 1, wherein the sample (126) has a volume of less than 0.5 microliters.

23. The process as claimed in claim 10, wherein the change is an optical change.

24. A diagnostic test element (110) for detecting an analyte in a sample (126) of a body fluid, comprising at least one test field (116) having at least one detection reagent, wherein the detection reagent is set up to pass through at least one detectable change in the presence of the analyte, wherein the test field (116) has at least one detection layer (118) comprising the detection reagent, wherein the detection layer (118) comprises particles (137), wherein at least 90% of all particles (137) of the detection layer (118) have an actual particle size of less than 10 micrometers, wherein the test field (116) has a loading side (128) for applying the sample (126) and a detection side (120) for detecting a change in the detection reagent, wherein the test field (116) further has at least one removal layer (122), wherein the removal layer (122) is arranged on a side of the detection layer (118) facing the loading side (128), wherein the removal layer (122) comprises at least one pigment, wherein the diagnostic test element (110) further comprises at least one support element (112), wherein the support element (112) has at least one transparent region (114), wherein the test field (116) is applied at least in part to the transparent region (114) with its detection side (120).

25. The diagnostic test element (110) as claimed in claim 24, wherein the change is an optical change.