(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 December 2012 (27.12.2012)

WIPO | PCT

(10) International Publication Number
WO 2012/176202 A1

(51) International Patent Classification:
G01N 33/493 (2006.01) *G01N 33/52* (2006.01)

(21) International Application Number:
PCT/IL2012/050209

(22) International Filing Date:
18 June 2012 (18.06.2012)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/498,648 20 June 2011 (20.06.2011) US
61/607,017 6 March 2012 (06.03.2012) US

(71) Applicant *(for all designated States except US)*: **COMMON SENSE LTD.** [IL/IL]; P.O. Box 3567, 38900 Caesarea (IL).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **PARDO-YISSAR, Vered** [IL/IL]; 10 Tarshish Street, 6019000 Yehud - Neve Monoson (IL). **HERSHCOVITZ-FUNDOIANO, Yifat** [IL/IL]; 6 Ha'yovel Street, 3752037 Karkur (IL). **KESSARY SHOHAM, Hadar** [IL/IL]; 32 Ha'Gidonim Street, 30900 Zichron Yaakov (IL).

(74) Agents: **WEBB & CO.** et al.; P.O. Box 2189, 76121 Rehovot (IL).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*

WO 2012/176202 A1

(54) Title: ARTICLES AND METHODS FOR MONITORING URINARY TRACT INFECTION

(57) Abstract: The present invention relates to the field of medical diagnostics and more specifically, to an improved identification of urinary tract infection (UTI), using a urine-testing article comprising nitrite-indicator polymer matrix to identify nitrites in urine. The present invention further describes methods for preparing and using said urine-testing article.

ARTICLES AND METHODS FOR MONITORING URINARY TRACT INFECTION

FIELD OF THE INVENTION

The present invention relates to the field of medical diagnostics and more specifically, to an improved identification of urinary tract infection (UTI), using a urine-testing article comprising nitrite-indicator polymer matrix to identify nitrites in urine. The present invention further describes methods for preparing and using said urine-testing article.

BACKGROUND OF THE INVENTION

Urinary tract infections are most common among females, with a high recurrence rate of about 25%-30%. UTI are considered the most frequent bacterial infections in women and it is estimated that about one third of all adult women have experienced an episode of symptomatic cystitis (bladder infection) at least once. UTI are common in infants and among young boys and young girls, albeit with considerably lower estimates.

Most episodes of UTI are caused by bacterial infection; however in many cases the etiology is not clear. Typically, diagnosis of UTI includes dipping a dipstick in a urine sample for identifying elements, such as, leukocyte esterase or nitrites, which are indicative of infection. Common diagnosis also includes examining proliferation of microorganisms in a urine culture.

The presence of nitrites in urine is a well-known marker for UTI, as the majority of bacteria that cause UTI induce reduction of nitrates to nitrites, thus causing a substantial elevation in nitrite level in the subject's urine.

One of the approaches to detecting nitrites in urine includes the use of a diazotizing agent and a coupling agent. For example, U.S. Patent No. 4,631,255 discloses a composition for assaying for nitrites comprising an acid component, a diazotizing agent and a coupling agent, wherein said composition changes color upon contacting a urine sample containing nitrites.

Most diagnostic tools for UTI require the involvement of health care professionals, and therefore are used only in medical facilities. There are, however, diagnostic tools for UTI that are suitable for personal use at home. For example, U.S. Patent No. 6,203,496 discloses a disposable article, such as, a diaper, a panty liner and a pad, for UTI preliminary diagnosis even by personnel having limited medical training. The article comprises one or more chemical reagents that change color upon contact with urine containing abnormal levels of substances, including nitrites. Suitable reagents include naphthyl ester, diazonium salt, arsenilic acid and tetrahydrobenzo (h) quinolin.

U.S. Patent No. 6,464,635 discloses a diaper comprising chemicals for visual examination of urine status, other than UTI, based on the level of various substances such as nitrates, bilirubin, ketones, blood, proteins, urobilinogen and glucose.

In products which are intended for home use, the stability of a color-based indication is of high importance. A secretion-monitoring article for identifying a secreted biological fluid, other than urine, providing a stable visual indication is disclosed in U.S. Patent No. 6,921,647. The article comprises a pH indicator agent, a polymer and an ion-balance reagent where the indication is stable for at least 48 hours.

There is currently a need for a urinary tract infection detection system, which can be easily used by both a health care giver and an untrained user and which would present a clear, long lasting and non-leaching indication.

SUMMARY OF THE INVENTION

The present invention provides indicator compositions, diagnostic articles and uses thereof for detecting the presence of nitrites in a subject's urine, thereby diagnosing urinary tract infections. The invention relies on the unexpected discovery that incorporating a chemically-based detection system for nitrites with a polymer provides a stable color indication. The resulting indication does not leach and remains indicative for at least 24 hours. Moreover, the color indication is maintained even after several cycles of drying and re-wetting of the indicator system. Advantageously, the indication provided by the article of the invention is not affected by interfering substances and conditions, such as glucose, ascorbic

acid and changes in urine pH. Such an indicator composition would enable a reliable early detection of urinary tract infection at the comfort of the user's home, would accelerate treatment at early stages of the inflammation and thereby may prevent unnecessary discomfort and pain for the subject.

According to one aspect, the present invention provides an indicator composition comprising a polymer, a plasticizer, a diazotizing reagent and a coupling agent, wherein said indicator composition provides a color indication in the presence of nitrites in urine.

According to another aspect, the present invention provides a diagnostic article for detection of urinary tract infection in a subject, comprising an absorbent substrate configured to absorb urine and an indicator composition comprising a polymer, a plasticizer, a diazotizing reagent and a coupling agent, wherein said indicator composition provides a color indication in the presence of nitrites in said absorbed urine.

According to another aspect, the present invention provides a diagnostic article for detection of urinary tract infection in a subject, comprising an absorbent substrate configured to absorb urine and the indicator composition of the invention, wherein said indicator composition provides a color indication in the presence of nitrites in said absorbed urine.

According to one embodiment, the indicator composition further comprises at least one component selected from the group consisting of: a pH indicator, a dye, an anti-oxidant, an acid, a trap, a photo-resistant agent and a wetting agent.

It is to be understood that during preparation of the indicator composition, a solvent is added in order to obtain a homogenous solution of all the components in the composition. The solvent may contain a buffer. In some embodiment, the buffer maintains an acidic pH. In other embodiments, the buffer maintains a transition pH range of 2.0-5.0. However, upon applying the indicator composition onto an absorbent substrate (thereby forming the article of the invention), the solvent is evaporated.

According to another embodiment, the amount of the polymer in the indicator composition is within the range of 30% to 90%, the amount of the plasticizer in the indicator composition is within the range of 5% to 50%, the amount of the diazotizing reagent in the indicator composition is within the range of 0.1% to 12%, the amount of the coupling agent

in the indicator composition is within the range of 0.01% to 5%, wherein the percentages presenting the amount of each component in the indicator composition refer to weight/weight percentages based on the total weight of the indicator composition and the total weight of the indicator composition equals 100%.

According to yet another embodiment, the indicator composition further comprises pH indicator wherein the amount of the pH indicator in the indicator composition is within the range of 0.0005% to 1%.

According to yet another embodiment, the indicator composition further comprises a dye wherein the amount of the dye in the indicator composition is within the range of 0.05% to 2.5%.

According to yet another embodiment, the indicator composition further comprises an anti-oxidant, wherein the amount of the anti-oxidant in the indicator composition is within the range of 1% to 20%.

According to yet another embodiment, the indicator composition further comprises an acid, wherein the amount of the acid in the indicator composition is within the range of 0.1%-10%.

According to yet another embodiment, the indicator composition further comprises a trap, wherein the amount of the trap in the indicator composition is within the range of 0.5% to 2.5%.

According to yet another embodiment, the indicator composition further comprises a wetting-agent, wherein the amount of the wetting agent in the indicator composition is within the range of 1% to 25%.

According to yet another embodiment, the indicator composition further comprises a photo-resistant agent, wherein the amount of the photo-resistant agent in said indicator composition is within the range of 0.1% to 1%.

According to yet another embodiment, the sensitivity of the indicator composition to nitrites is in the range of about 0.01 to 2 mg/dL.

According to yet another embodiment, said polymer comprises one or more of the polymers selected from the group consisting of: cellulose acetate, dextran, nitrocellulose, ethyl cellulose, carboxymethyl cellulose, cellulose acetate phthalate, cellulose acetate propionate, cellulose acetate butyrate, hydroxypropyl methylcellulose (HPMC), polyethylene glycol (PEG), polyvinyl alcohol, polyvinyl alcohol-polyethylene glycol graft copolymer, silicone dioxide and derivatives thereof.

According to yet another embodiment, said polymer is cellulose acetate.

According to yet another embodiment, said plasticizer is selected from the group consisting of: 1,2-cyclohexanedicarboxylic acid di-isononyl ester; bis(2-ethylhexyl) sebacate, acetyl tributyl citrate, triethylhexyl trimellitate, triethyl citrate, ethyl lactate, alkylsulfonic acid phenylester, dioctylphthalate, polylactide, ethyl hexyl phthalate, polyvinyl pyrrolidone (povidone) and combinations thereof.

According to yet another embodiment, said plasticizer is 1,2-cyclohexane dicarboxylic acid di-isononyl ester.

According to yet another embodiment, said diazotizing agent is selected from the group consisting of: p-arsanilic acid, methyl anthranilate, sulfanilamide, sulfanilic acid, 2-amino-6-methoxy benzothiazole, 2-amino- benzothiazole, 2-amino-6-nitro benzothiazole, 2-amino-6-ethoxybenzothiazole, 2-amino-6-methylbenzothiazole, 2-amino-3,5-dinitrothiophene, 4-[(4-methyl-1-piperazinyl)sulfonyl]aniline, 4-({4'-[(4-aminophenyl)sulfonyl][1,1'-biphenyl]-4-yl}sulfonyl)aniline, Sulfadimethoxine, sulfathiazole, Sodium sulfanilate hydrate or any aromatic primary amine with a sulfonyl group and a combination thereof.

According to yet another embodiment, said diazotizing agent is sulfanilamide.

According to yet another embodiment, said diazotizing agent is 4-[(4-methyl-1-piperazinyl)sulfonyl]aniline.

According to yet another embodiment, said diazotizing agent is 4-({4'-[(4-aminophenyl)sulfonyl][1,1'-biphenyl]-4-yl}sulfonyl)aniline.

According to yet another embodiment, said coupling agent is selected from the group consisting of: N-(1-Naphthyl)ethylenediamine dihydrochloride, 4-({4'-[(4-aminophenyl)sulfonyl][1,1'-biphenyl]-4-yl}sulfonyl)aniline, 4-[(4-methyl-1-piperazinyl)sulfonyl]aniline, 1-Naphthylamine and 2-(1-Naphthylamino) ethanol.

According to yet another embodiment, the pH indicator has a transition pH range between 0-4.0. According to yet another embodiment, the pH indicator with a transition range between 0-4.0 is selected from: leucomalachite green and gentian violet.

According to yet another embodiment, the pH indicator has a transition pH range between 8.0-14.0.

According to yet another embodiment, said dye is selected from the group consisting of: Indigo (2,2'-Bis(2,3-dihydro-3- oxoindolyliden)), Trypan Blue ((3Z,3'Z)-3,3'-[(3,3'-dimethylbiphenyl-4,4'-diyl)di(1Z)hydrazin-2-yl-1-ylidene]bis(5-amino-4-oxo-3,4-dihydronaphthalene-2,7-disulfonic acid), Food Blue No. 1 Aluminum Lake, Alizarin Blue Black B (disodium 4,4'-[(4-hydroxy-9,10-dioxo-9,10-dihydroanthracene-1,3-diyl)diimino]dibenzenesulfonate or other food or cosmetic grade color.

According to yet another embodiment, said dye is Indigo, also known as (2,2'-bis(2,3-dihydro-3- oxoindolyliden).According to yet another embodiment, said anti-oxidant is selected from the group consisting of titanium dioxide pigments, zinc oxide, polyvinyl alcohol, ascorbic acid, D-Isoascorbic acid, t-butyl hydroquinone, polystyrene and vitamin E.

According to yet another embodiment, said trap is selected from the group consisting of: beta-cyclodextrin and pentasodium pentetate.

According to yet another embodiment, said wetting agent is selected from the group consisting of: glycerol, 2-ethoxy ethanol and sodium carboxy methyl starch.

According to yet another embodiment, said wetting agent is glycerol.

According to yet another embodiment, said acid is selected from the group consisting of: citric acid, tartaric acid, adipic acid, picric acid, methacrylic acid, stearic acid and acetic acid.

According to yet another embodiment, said acid is a salt derivative of an acid selected from the group consisting of: citric acid, tartaric acid, adipic acid, picric acid and acetic acid.

According to yet another embodiment, said acid is citric acid.

According to yet another embodiment, the photo-resistant agent is 4-methacryloxy-2-hydroxy benzo- phenone.

According to yet another embodiment, said indicator composition provides an indication which is stable, does not leach through several cycles of drying and re-wetting and is indicative for at least 3 hours upon contact with nitrites in urine, preferably for at least 12 hours, preferably for at least 24 hours and preferably for at least 48 hours. According to another embodiment, the indication is stable for at least 3 hours. According to another embodiment, the indication is stable for at least 12 hours. According to another embodiment, the indication is stable for at least 24 hours. According to another embodiment, the indication is stable for at least 48 hours.

According to yet another embodiment, the absorbent substrate is selected from the group consisting of: gauze, panty shield, hygienic napkin, diaper,inter-labial absorbent structure, an absorbent sticker and a combination thereof.

According to yet another embodiment, the absorbent substrate comprises a fabric material. According to yet another embodiment, the fabric material is selected from the group consisting of: woven fabric, non-woven fabric, hydrophilic fabric material, hydrophobic fabric material and combinations thereof.

According to yet another embodiment, the diagnostic article further comprises a protective film separating the indication composition from the environment. According to yet another embodiment, the protective film is configured to dissolve upon contacting urine. According to yet another embodiment, the protective film comprises a polymer selected from the group consisting of: polyvinyl alcohol, polyethylene glycol, hydrous magnesium silicate, hydroxypropyl methyl cellulose, sodium starch glycollate, Polyethylene glycol (PEG), xanthan gum and a combination thereof. According to yet another embodiment, the protective film comprises talc.

According to another aspect, the present invention provides a method for detecting urinary tract infection in a subject, the method comprising

(a) providing a diagnostic article comprising an absorbent substrate configured to absorb urine and an indicator composition, wherein said indicator composition comprises a polymer, a plasticizer, a diazotizing reagent and a coupling agent and wherein said indicator composition provides a color indication in the presence of nitrites in said absorbed urine;

(b) mounting said diagnostic article so as to receive urine from the subject;

(c) contacting the indicator composition with urine; and

(d) detecting the color indication, thereby determining whether an abnormal level of a nitrites indicative of UTI, is present in the urine.

According to one embodiment detecting comprises observing the color indication of the indicator composition, wherein a color that is different from the color of said indicator composition prior to the step of mounting, is indicative of abnormal level of nitrites.

According to another embodiment, contacting the indicator composition with urine takes place upon receiving urine secreted from said subject.

According to yet another embodiment, the diagnostic article comprises a protective film wherein step (c) further comprises removing the protective film.

According to yet another aspect, the present invention provides a method for preparing the article of the invention, the method comprising preparing a solution comprising the indicator composition and a solvent. According to one embodiment, said solvent is selected from the group consisting of: water, isopropyl alcohol, ethanol, acetone and a combination thereof.

Further embodiments, features, advantages and the full scope of applicability of the present invention will become apparent from the detailed description and examples given hereinafter. However, it should be noted that the detailed description, while indicating preferred embodiments of the invention, is given by way of illustration only, since various

changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides an indicator composition for detecting nitrites, a diagnostic article comprising the indicator composition and a method of using same to detect urinary tract infections in a subject in need thereof. The article and methods of the invention are configured to be used by the user himself or a care taker, and do not require the involvement of personnel with medical training. Furthermore, the article and method of the invention may be applied outside medical facilities, providing theretofore outstanding patient compliance together with stable and reliable indication of UTI. A main advantage of the indicator composition of the invention is the stable indication that it provides. The indication is maintained through cycles of drying and wetting, and is not affected by interfering substances and conditions, such as, glucose, and/or ascorbic acid and/or changes in urine pH. Without being bound to any theory or mechanism, these outstanding qualities are conferred by the specific combination of components in the indicator composition, including the presence of a polymer and a trap among other components.

According to one aspect, the invention provides an indicator composition for detection of urinary tract infection in urine of a subject, the indicator composition comprising a polymer, a plasticizer, a diazotizing reagent and a coupling agent; wherein the indicator composition provides a color reaction in the presence of nitrites in the urine of said subject. According to another embodiment, the color reaction provided by the indicator composition results in s color indication. According to some embodiment, the terms “color reaction” and “color indication” are used interchangeably.

According to another aspect, the invention provides a diagnostic article for detection of urinary tract infection in a subject, wherein the diagnostic article comprises an absorbent substrate configured to absorb urine and the indicator composition of the invention. The indicator composition comprises a polymer, a plasticizer, a diazotizing reagent and a coupling agent, wherein the indicator composition provides a color reaction in the presence of nitrites in the absorbed urine.

The terms "urinary tract infection" and "UTI" are interchangeably used herein to define an infection of the urinary tract typically induced by bacteria. The bacteria may be *Escherichia coli* and *Staphylococcus saprophyticus* among others.

Without being bound to any theory or mechanism, the microorganisms that cause UTI induce reduction of nitrates to nitrites. As a result, the level of nitrites in the infected urine is elevated. Thus, nitrites give an indication of UTI.

According to yet another embodiment, the color indication provided by the indicator composition of the present invention is based on a chemical reaction between nitrites, in the urine, and the indicator composition. Without being bound to any theory or mechanism, the chemical reaction occurs between the nitrites and the diazotizing agent, followed by a coupling reaction, which produces an azo dye. The color of the azo dye is thereby indicative of the presence of nitrites. This type of chemical reaction is based on the Griess reaction which has been described in the art (Jay B. et al., Analytical Chemistry 1979, 51(9): 1493-1502).

Preferably, the color resulting from the reaction with the indicator-composition is stable. According to yet another embodiment, the stable color indication provided by the indicator composition of the invention is distinctive from the color of urine, excrement or blood. Preferably, the color indication is of colors other than brown, red, or yellow. According to yet another embodiment, the color indication is dependent on the combination of diazotizing reagent and coupling agent. According to yet another embodiment, the color of the color indication is selected from the group consisting of: blue, green, orange, pink, purple, violet and white.

Typically, the color of the indication is determined by the diazotizing agent and the coupling agent or their combination. The article of the invention may combine several indications corresponding to different physiological conditions. For example, the article may further include an indication for vaginosis. To this end, the indicator disclosed in U.S. Pat. No. 6,627,394 may be incorporated in the article of the invention. The variety of colors provided by the various diazotizing agents and coupling agents and their combination enable choosing distinct colors for each medical condition diagnosed by the same article.

The terms "stable color", "stable color indication" and "stable indication" as used herein, are interchangeable. These terms refer to a color indication which maintains its color, or essentially the same color, over time. According to another embodiment, the stable color indication is a color indication that does not leach through several cycles of drying and re-wetting. According to another embodiment, the stable color indication is a color indication that is indicative for at least 3 hours after contact with nitrites in urine. According to another embodiment, the stable color indication is a color indication that is indicative for at least 12 hours following contact with nitrites in urine. According to another embodiment, the stable color indication is a color indication that is indicative for at least 24 hours following contact with nitrites in urine. According to another embodiment, the stable color indication is a color indication that is indicative for at least 48 hours after contact with nitrites in urine. According to another embodiment, the stable color indication is an indication which remains of essentially the same color, and does not leach or fade through several cycles of drying and re-wetting, for at least 3 hours upon contact with nitrites in urine or a combination thereof. According to yet another embodiment, the polymer of the indicator-composition enables the stable color indication. According to yet another embodiment, the plasticizer changes the plasticity of the polymer.

According to yet another embodiment, the amount of the polymer in the indicator composition is within the range of 30% to 90%, the amount of the plasticizer in the indicator composition is within the range of 5% to 50%, the amount of the diazotizing reagent in the indicator composition is within the range of 0.1% to 12% and the amount of the coupling agent in the indicator composition is within the range of 0.01% to 5%. The percentages presenting the amount of each component in the indicator composition refer to weight/weight percentages based on the total weight of the indicator composition. The total weight of the indicator composition equals 100%. According to yet another embodiment, the amount of plasticizer in the indicator composition is within the range of 15% to 50%. According to yet another embodiment, the density of the plasticizer and wetting agent solutions is within the range of 0.6-1.26 gr/ml. According to yet another embodiment, the density of the plasticizer and wetting agent solutions is within the range of 0.3-1.5 gr/ml.

According to another embodiment, the amount of the polymer in the indicator composition is within the range of 30% to 90%, the amount of the plasticizer in the indicator composition is within the range of 5% to 50%, the amount of the diazotizing reagent in the

indicator composition is within the range of 0.1% to 28%, the amount of the coupling agent in the indicator composition is within the range of 0.01% to 5%, wherein the percentages presenting the amount of each component in the indicator composition refer to weight/weight percentages based on the total weight of the indicator composition and the total weight of the indicator composition equals 100%.

According to another embodiment, the amount of the polymer in the indicator composition is within the range of 30% to 90%, the amount of the plasticizer in the indicator composition is within the range of 15% to 50%, the amount of the diazotizing reagent in the indicator composition is within the range of 0.1% to 12%, the amount of the coupling agent in the indicator composition is within the range of 0.01% to 5%, wherein the percentages presenting the amount of each component in the indicator composition refer to weight/weight percentages based on the total weight of the indicator composition and the total weight of the indicator composition equals 100%.

According to yet another embodiment, the polymer comprises one or more polymers selected from the group consisting of: cellulose acetate, dextran, nitrocellulose, ethyl cellulose, carboxymethyl cellulose, cellulose acetate phthalate, cellulose acetate propionate, cellulose acetate butyrate, hydroxypropyl methylcellulose (HPMC), polyethylene glycol (PEG), polyvinyl alcohol, polyvinyl alcohol-polyethylene glycol graft copolymer, silicone dioxide and derivatives thereof. According to yet another embodiment, said polymer is cellulose acetate. According to yet another embodiment, said polymer is cellulose acetate phthalate. According to yet another embodiment, the polymer consists a single polymer. According to yet another embodiment, the polymer consists of a mixture of polymers.

According to yet another embodiment, the plasticizer is selected from the group consisting of: 1,2-cyclohexane dicarboxylic acid di-isononyl ester, bis(2-ethylhexyl) sebacate, acetyl tributyl citrate, triethylhexyl trimellitate, triethyl citrate, ethyl lactate, alkylsulfonic acid phenylester, dioctylphthalate, polylactide, ethyl hexyl phthalate, polyvinyl pyrrolidone (povidone) and combinations thereof. Each possibility represents a separate embodiment of the present invention. According to yet another embodiment, said plasticizer is 1,2-cyclohexane dicarboxylic acid di-isononyl ester.

The terms “plasticizer” and “binder”, as used herein, are interchangeable.

The term "diazotizing agent" as used herein, refers to an agent which forms a diazonium salt in the presence of nitrites. A diazotizing agent includes, but is not limited to, p-arsanilic acid, , methyl anthranilate, sulfanilamide, sulfanilic acid, 2-amino-6-methoxy benzothiazole, 2-amino- benzothiazole, 2-amino-6-nitro benzothiazole, 2-amino-6-ethoxybenzothiazole, 2-amino-6-methylbenzothiazole, 2-amino-3,5-dinitrothiophene, 4-[(4-methyl-1-piperazinyl)sulfonyl]aniline, 4-({4'-[(4-aminophenyl)sulfonyl][1,1'-biphenyl]-4-yl}sulfonyl)aniline, Sulfadimethoxine, sulfathiazole, Sodium sulfanilate hydrate or any aromatic primary amine with a sulfonyl group and a combination thereof. Each possibility represents a separate embodiment of the present invention. According to yet another embodiment, the diazotizing agent is sulfanilamide. According to yet another embodiment, the diazotizing agent is 4-[(4-methyl-1-piperazinyl)sulfonyl]aniline. According to yet another embodiment, the diazotizing agent is 4-({4'-[(4-aminophenyl)sulfonyl][1,1'-biphenyl]-4-yl}sulfonyl)aniline.

The term "coupling agent" as used herein refers to an agent which forms an azo dye in the presence of a diazonium salt. A coupling agent includes, but is not limited to, N-(1-Naphthyl)ethylenediamine dihydrochloride, 4-({4'-[(4-aminophenyl)sulfonyl][1,1'-biphenyl]-4-yl}sulfonyl)aniline, 4-[(4-methyl-1-piperazinyl)sulfonyl]aniline, 1-Naphthylamine, and 2-(1-Naphthylamino) ethanol. Each possibility represents a separate embodiment of the present invention. According to yet another embodiment, the coupling agent is N-(1-Naphthyl)ethylenediamine dihydrochloride.

According to yet another embodiment, the indicator composition further comprises at least one component selected from the group consisting of: a pH indicator, a dye, an anti-oxidant, an acid, a trap and a wetting agent.

According to yet another embodiment, the indicator composition further comprises a pH indicator. According to yet another embodiment, the pH indicator is selected from: leucomalachite green and gentian violet. According to yet another embodiment, the pH indicator is leucomalachite green. According to yet another embodiment, the pH indicator is gentian violet. According to yet another embodiment, said pH indicator has a transition pH range between 0-4.0. In yet another embodiment, said pH indicator has a transition pH range between 8.0-14.0. According to yet another embodiment, the amount of the pH indicator in the indicator composition is within the range of 0.0005% to 1%.

According to yet another embodiment, the indicator composition further comprises a dye. The dye may serve as a reactant which changes color upon exposure to nitrites. The dye may further serve to change the indicative color shade of the indicator composition. According to yet another embodiment, the dye is selected from the group consisting of: Indigo (2,2'-Bis(2,3-dihydro-3- oxoindolyliden)), Trypan Blue ((3Z,3'Z)-3,3'-[(3,3'-dimethylbiphenyl-4,4'-diyl)di(1Z)hydrazin-2-yl-1-ylidene]bis(5-amino-4-oxo-3,4-dihydronaphthalene-2,7-disulfonic acid), Food Blue No. 1 Aluminum Lake, Alizarin Blue Black B (disodium 4,4'-[(4-hydroxy-9,10-dioxo-9,10-dihydroanthracene-1,3-diyl)diimino]dibenzenesulfonate or other food or cosmetic grade color. Each possibility represents a separate embodiment of the present invention. According to yet another embodiment, the amount of the dye in the indicator composition is within the range of 0.05% to 2.5%. According to yet another embodiment, the amount of the dye in the indicator composition is within the range of 0.005% to 2.5%.

According to yet another embodiment, the indicator composition further comprises an anti-oxidant. According to yet another embodiment, the anti-oxidant is selected from the group consisting of titanium dioxide pigments, zinc oxide, polyvinyl alcohol, ascorbic acid, D-Isoascorbic acid, t-butyl hydroquinone, polystyrene and vitamin E. Each possibility represents a separate embodiment of the present invention. According to yet another embodiment, the amount of the anti-oxidant in the indicator composition is within the range of 10% to 20%. According to yet another embodiment, the amount of the anti-oxidant in the indicator composition is within the range of 1% to 20%. According to yet another embodiment, the amount of the anti-oxidant in the indicator composition is within the range of 0.1% to 20%.

According to yet another embodiment, the anti-oxidant is polystyrene. According to yet another embodiment, the anti-oxidant is t-butyl hydroquinone. According to yet another embodiment, the anti-oxidant is polyvinyl alcohol.

According to yet another embodiment, the indicator composition further comprises an acid. The acid provides an acidic environment, which is favorable for the Griess reaction. The acid may also prevent interference from ammonia in urine. Ammonia reacts with nitrites and

thus reduces the sensitivity of the indicator composition. According to yet another embodiment, the acid is selected from the group consisting of: citric acid, tartaric acid, adipic acid, picric acid, methacrylic acid, stearic acid and acetic acid. According to yet another embodiment, the acid is citric acid. Each possibility represents a separate embodiment of the present invention.

According to yet another embodiment, the acid is a salt derivative of an acid selected from the group consisting of: citric acid, tartaric acid, adipic acid, picric acid and acetic acid. Each possibility represents a separate embodiment of the present invention. According to yet another embodiment, the acid is a salt derivative of citric acid. According to yet another embodiment, the amount of the acid in the indicator composition is within the range of 0.1% to 10%.

According to yet another embodiment, the indicator composition further comprises a trap. According to yet another embodiment, the trap is a glucose trap or an ascorbic acid trap. According to yet another embodiment, the trap is selected from beta-cyclodextrin and pentasodium pentetate. Each possibility represents a separate embodiment of the present invention. According to yet another embodiment, the trap is beta-cyclodextrin. According to yet another embodiment, the amount of the trap in the indicator composition is within the range of 0.5% to 2.5%. According to yet another embodiment, the amount of the trap in the indicator composition is within the range of 0.05% to 2.5%. The terms "trap" and "scavenger", as used herein, are used interchangeably.

Without being bound by any theory or mechanism, the trap is intended to mask interference to the stable color indication. The interference may be caused by glucose and/or ascorbic acid. According to yet another embodiment, the trap bind to at least one of glucose and ascorbic acid, thereby masking interference.

According to yet another embodiment, the indicator composition further comprises a photo-resistant agent. According to yet another embodiment, the photo-resistant agent is 4-methacryloxy-2-hydroxy benzo- phenone. According to yet another embodiment, the amount of the photo-resistant agent in the indicator composition is within the range of 0.1% to 1%. Without wishing to be bound to any theory or mechanism, the photo-resistant agent protects

the indicator-composition from damages related to ultra-violet radiation, such as, but not limited to, formation of free radicals.

According to yet another embodiment, the indicator composition further comprises a wetting agent. The wetting agent may act to increase the hydrophilic characteristics of the absorbent substrate. The increase in hydrophilic characteristics improves the interaction between the hydrophilic urine and the absorbent substrate. According to yet another embodiment, the wetting agent is selected from the group consisting of: glycerol, 2-ethoxy ethanol and sodium carboxy methyl starch. Each possibility represents a separate embodiment of the present invention. According to yet another embodiment, the wetting agent is glycerol. According to yet another embodiment, the amount of the wetting agent in the indicator composition is within the range of 1% to 25%. According to yet another embodiment, the amount of the wetting agent in the indicator composition is within the range of 1% to 40%.

The terms "wetting agent" and "surfactant", as used herein, are interchangeable.

According to yet another embodiment, the sensitivity of the indicator composition to nitrites is high and an indication is obtained even for traces of nitrites in the urine. According to yet another embodiment, the sensitivity of said indicator composition to nitrites is in the range of 0.01 to 2 mg/dL.

According to yet another embodiment, the indicator composition is incorporated in an absorbent substrate. According to yet another embodiment, incorporating the indicator composition in an absorbent substrate forms the article of the invention. According to yet another embodiment, the indicator composition is applied onto the absorbent substrate by spraying, printing or coating. According to yet another embodiment, the absorbent substrate is incorporated in a device selected from the group consisting of: gauze, panty shield, hygienic napkin, diaper, inter-labial absorbent structure, an absorbent sticker and a combination thereof. Each possibility represents a separate embodiment of the present invention.

The term "absorbent sticker", as used herein, refers to an absorbent material comprising a sticky surface. According to yet another embodiment, the absorbent sticker is configured to stick to any other absorbent substrate known in the art. According to yet

another embodiment, the indicator composition is initially applied to an absorbent sticker and the sticker is then applied to another absorbent substrate.

According to yet another embodiment, the absorbent substrate may be of many suitable absorbent materials known in the art such as polyester membranes, polypropylene membranes, cellulose membranes, paper, cotton and linen.

According to some embodiments, the absorbent substrate comprises a fabric material. According to yet another embodiment, the fabric material consists of a woven fabric. According to yet another embodiment, the fabric material consists of a non-woven fabric. According to yet another embodiment, the fabric material consists of a hydrophilic fabric. According to yet another embodiment, the fabric material consists of a hydrophobic fabric. According to yet another embodiment, the fabric material is selected from the group consisting of: woven fabric, non-woven fabric, hydrophilic fabric material, hydrophobic fabric material and combinations thereof.

Details of manufacture of pads, panty shields, sanitary napkins and diapers, including the attachment of indicators to substrates, are well known to those skilled in the art and have been fully described in the prior art, for example, U.S. Pat. Nos. 5,217,444; 5,897,834; 6,149,590 and 8,044,257.

According to yet another embodiment, the diagnostic article further comprises a protective film. Without wishing to be bound to any theory or mechanism, the protective film may improve the stability of the indicator composition throughout the indicator composition's shelf-life. According to yet another embodiment, the protective film is configured to prevent the indicator composition from providing a color reaction until the protective film is dissolved, preferably, until the protective film dissolves upon contact with urine. According to yet another embodiment, the protective film is configured to prevent the indicator composition from reacting with air and/or nitrites until the protective film is dissolved, preferably, until the protective film dissolves upon contact with urine. According to yet another embodiment, the protective film is configured to dissolve upon contacting urine.

According to yet another embodiment, the protective film comprises a polymer selected from the group consisting of: polyvinyl alcohol, polyethylene glycol, hydrous

magnesium silicate, hydroxypropyl methyl cellulose, sodium starch glycollate, Polyethylene glycol (PEG), xanthan gum and a combination thereof. Each possibility represents a separate embodiment of the present invention. According to yet another embodiment, the protective film comprises talc. According to yet another embodiment, the protective film comprises talc and a polymer selected from the group consisting of: polyvinyl alcohol, polyethylene glycol, hydrous magnesium silicate, hydroxypropyl methyl cellulose, sodium starch glycollate, Polyethylene glycol (PEG), xanthan gum and a combination thereof. Each possibility represents a separate embodiment of the present invention. As used herein, the term "talc" refers to a hydrated magnesium silicate having a chemical formula selected from the group consisting of: $H_2Mg_3(SiO_3)_4$ and $Mg_3Si_4O_{10}(OH)_2$.

According to yet another embodiment, the protective film has one or more of the following properties: water soluble, biocompatible, partially or completely impermeable to gases or a combination thereof.

According to some embodiments, the protective film is applied onto the absorbent substrate, thereby covering and/or protecting the absorbent substrate. According to yet another embodiment, the protective film is applied onto the absorbent substrate by coating, spraying or any suitable method known in the art According to yet another embodiment, the protective film is applied onto the absorbent substrate wherein the absorbent substrate already includes the indicator composition. According to some embodiments, the article of the invention is prepared as follows: first, the indicator composition is applied onto the absorbent substrate, thereby forming an absorbent substrate containing the indicator composition. Second, the absorbent substrate containing the indicator composition is covered, or otherwise protected, with the protective film.

According to another aspect, the present invention discloses a method for detecting urinary tract infection in a subject. According to this aspect, a diagnostic article comprising an absorbent substrate, configured to absorb urine, and an indicator composition is used. The indicator composition comprises a polymer, a plasticizer, a diazotizing reagent and a coupling agent. The indicator composition provides a color reaction in the presence of nitrites in the absorbed urine. According to the method of the present invention, the article is mounted in a way allowing receiving urine from the subject and the indicator composition is contacted with urine. Upon receipt of urine, the color of the article, or parts thereof which incorporate

the diagnostic composition, is detected, thereby allowing to determine whether an abnormal level of nitrites, indicative of UTI, is present in the urine.

According to one embodiment, the article used in the method further comprises mounting means for placing the absorbent substrate in a position to receive urine secreted from the subject, such as adhesive strips.

According to another embodiment, the color reaction of the article is monitored by comparing color in the article to a color scale provided by a manufacturer.

The method of the present invention may be used by any subject in need thereof, men, women and infants, whether autonomously or with the help of a care provider. The care provider may have limited medical training. According to yet another embodiment, the subject may mount the article within undergarments (such as in the case of a panty shield) or using mounting means such as adhesive strips (in the case of a diaper).

According to yet another embodiment, the article of the present invention is prepared as follows: initially, a polymer solution is pre-formed, by mixing dry polymer and a plasticizer with a solvent. Typically, 1.5 g of polymer is dissolved in between 4 ml and 60 ml of solvent, preferably between 30 ml and 50 ml solvent. Next, the essential components of the indicator composition are added to the solution, namely a diazotizing agent and a coupling agent (the essential components may be separately dissolved in a solvent prior to being added to the polymer solution). Optionally, at least one of the additional components is added into the solution, namely, a pH indicator, a dye, an anti-oxidant, an acid, a trap and a wetting agent. Once the indicator solution is ready, it is applied by suitable means to an absorbent substrate. Application may be carried out by any suitable method known in the art, such as, spraying or spreading the mixture onto an absorbent substrate or by dipping the absorbent substrate in the mixture. Optionally, a protective film is further applied to the absorbent substrate by any of the abovementioned application methods.

The solvent may be selected from the group consisting of: water, isopropyl alcohol, ethanol, acetone and a combination thereof. According to yet another embodiment, the solvent further comprises a buffer. According to yet another embodiment, the buffer maintains a transition pH range of 2.0-5.0. According to yet another embodiment, the buffer

comprises one or more of the buffers selected from the group consisting of: citrate buffer, phosphate buffer, acetic buffer, tartaric buffer and adipic buffer.

Following incorporation of the indicator solution into the absorbent substrate, the solvent is allowed to evaporate. As a result the final article is obtained. Alternatively, the absorbent substrate may be further integrated into another article to form the final article. For example, the absorbent material which contains the indicator composition may be incorporated into a panty liner.

Additional objects, advantages, and novel features of the present invention will become apparent to one ordinarily skilled in the art upon examination of the following examples, which are not intended to be limiting. Additionally, each of the various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below finds experimental support in the following examples.

EXAMPLES

Example 1. Indicator compositiosn containing polyvinyil alcohol.

An indicator composition for detection of nitrites in urine containing polyvynil alcohol was prepared in a solution of water and acetone. The components of several indicator compositions, including the solvents, are described in Tables 1 and 2.

Table 1:

Material	Function	Amount	Units
Sulfanilamide	Diazotizing agent	0.2	g
N-1-napthylethylenediamine dihydrochloride	Coupling agent	0.02	g
Water	Solvent	2	ml
Acetone	Solvent	52	ml
Cellulose acetate	Polymer	1.5	g
Polyvinyl alcohol	Anti-oxidant	0.1	g
Hexamoll dinch	Plasticizer	1	ml

Table 2:

Material	Function	Amount	Units
Sulfanilamide	Diazotizing agent	0.2	g
N-1-napthylethylenediamine dihydrochloride	Coupling agent	0.02	g
Water	Solvent	2	ml
Acetone	Solvent	42	ml
Cellulose acetate phthalate	Polymer	1.2	g
Polyvinyl alcohol	Anti-oxidant	0.1	g
Hexamoll dinch	Plasticizer	0.8	ml

Example 2. Indicator compositions containing sulfanilamide as a diazotizing agent.

An indicator composition for detection of nitrites in urine containing Sulfanilamide as a diazotizing agent was prepared in a solution of water and acetone. The components of several indicator compositions, including the solvents, are described in Tables 3, 4 and 5.

Table 3:

Material	Function	Amount	Units
Sulfanilamide	Diazotizing agent	0.2	g
N-1-napthylethylenediamine dihydrochloride	Coupling agent	0.02	g
Water	Solvent	2	ml
Acetone	Solvent	52	ml
Cellulose acetate	Polymer	1.5	g
Hexamoll dinch	Plasticizer	1	ml

Table 4:

Material	Function	Amount	Units
Sulfanilamide	Diazotizing agent	0.1	g
N-1-napthylethylenediamine dihydrochloride	Coupling agent	0.01	g
Water	Solvent	1	ml
Acetone	Solvent	51	ml
Cellulose acetate	Polymer	1.5	g
Triethyl citrate	Plasticizer	0.5	ml

Table 5:

Material	Function	Amount	Units
Sulfanilamide	Diazotizing agent	0.2	g
N-1-napthylethylenediamine dihydrochloride	Coupling agent	0.02	g
Water	Solvent	2	ml
Acetone	Solvent	42	ml
Cellulose acetate phthalate	Polymer	1.2	g
Hexamoll dinch	Plasticizer	0.8	ml

Example 3. Indicator-compositions containing glycerol as a wetting agent.

An indicator composition for detection of nitrites in urine containing Glycerol as a wetting agent was prepared in a solution of water and acetone. The components of several indicator compositions, including the solvents, are described in Tables 6-15.

Table 6:

Material	Function	Amount	Units
4-({4'-[(4-aminophenyl)sulfonyl][1,1'-biphenyl]-4-yl}sulfonyl)aniline	Coupling agent	0.005	g
4-[(4-methyl-1-piperazinyl)sulfonyl]aniline	Diazotizing agent	0.005	g
Acetone	Solvent	50.5	ml
Cellulose acetate	Polymer	1.5	g
Hexamoll dinch	Plasticizer	1	ml
Glycerol	Wetting agent	0.6	ml

Table 7:

Material	Function	Amount	Units
Sulfathiazole	Diazotizing agent	0.2	g
N-1-napthylethylenediamine dihydrochloride	Coupling agent	0.05	g
Water	Solvent	5	ml
Acetone	Solvent	20	ml
Cellulose acetate phthalate	Polymer	0.75	g
Glycerol	Wetting agent	0.6	ml
Dioctylphthalate	Plasticizer	0.2	ml

Table 8:

Material	Function	Amount	Units
Sulfathiazole	Diazotizing agent	0.4	g
N-1-napthylethylenediamine dihydrochloride	Coupling agent	0.08	g
Water	Solvent	8	ml
Acetone	Solvent	40	ml
Cellulose acetate phthalate	Polymer	1.875	g
Glycerol	Wetting agent	0.6	ml
4-methacryloxy-2-hydroxy benzo- phenone	Photo-resistant agent	0.02	g
Polystyrene	Anti-oxidant	0.002	g
Dioctylphthalate	Plasticizer	50	µl

Table 9:

Material	Function	Amount	Units
Sulfathiazole	Diazotizing agent	0.4	g
N-1-napthylethylenediamine dihydrochloride	Coupling agent	0.08	g
Water	Solvent	8	ml
Acetone	Solvent	20	ml
Cellulose acetate phthalate	Polymer	1.875	g
Glycerol	Wetting agent	0.6	ml
Polystyrene	Anti-oxidant	0.01	g
Dioctylphthalate	Plasticizer	0.2	ml

Table 10:

Material	Function	Amount	Units
Sulfathiazole	Diazotizing agent	0.4	g
N-1-napthylethylenediamine dihydrochloride	Coupling agent	0.08	g
Water	Solvent	8	ml
Acetone	Solvent	40	ml
Cellulose acetate phthalate	polymer	1.875	g
Glycerol	Wetting agent	0.6	ml
Polystyrene	Anti-oxidant	0.01	g
Dioctylphthalate	Plasticizer	0.2	ml

Table 11:

Material	Function	Amount	Units
Sulfathiazole	Diazotizing agent	0.4	g
N-1-napthylethylenediamine dihydrochloride	Coupling agent	0.08	g
Water	Solvent	8	ml
Acetone	Solvent	20	ml
Cellulose acetate phthalate	Polymer	1.875	g
Glycerol	Wetting Agent	0.6	ml
T-butyl hydroquinone	Anti-oxidant	0.02	g
Dioctylphthalate	Plasticizer	0.2	ml
Ascorbic acid	Anti-oxidant	50	µl

Table 12:

Material	Function	Amount	Units
Sulfanilamide	Diazotizing agent	0.6	g
N-1-napthylethylenediamine dihydrochloride	Coupling agent	0.06	g
Water	Solvent	6	ml
Acetone	Solvent	26	ml
Cellulose acetate phthalate	Polymer	0.75	g
Glycerol	Wetting Agent	0.9	ml
Dioctylphthalate	Plasticizer	0.3	ml

Table 13:

Material	Function	Amount	Units
Sulfanilamide	Diazotizing agent	0.6	g
N-1-napthylethylenediamine dihydrochloride	Coupling agent	0.06	g
Water	Solvent	6	ml
Acetone	Solvent	26	ml
Cellulose acetate phthalate	Polymer	1.25	g
Glycerol	Wetting agent	0.9	ml
T-butyl hydroquinone	Anti-oxidant	0.01	g
Dioctylphthalate	Plasticizer	0.3	ml

Table 14:

Material	Function	Amount	Units
Sulfanilamide	Diazotizing agent	0.6	g
N-1-napthylethylenediamine dihydrochloride	Coupling agent	0.06	g
Water	Solvent	6	ml
Acetone	Solvent	28	ml
Cellulose acetate phthalate	Polymer	0.75	g
Glycerol	Wetting agent	0.9	ml
Polystyrene	Anti-oxidant	0.02	g
Dioctylphthalate	Plasticizer	0.3	ml

Table 15:

Material	Function	Amount	Units
Sulfanilamide	Diazotizing agent	0.8	g
N-1-napthylethylenediamine dihydrochloride	Coupling agent	0.08	g
Water	Solvent	8	ml
Acetone	Solvent	30	ml
Cellulose acetate phthalate	Polymer	1.25	g
Glycerol	Wetting agent	0.9	ml
Polystyrene	Anti-oxidant	0.02	g
Dioctylphthalate	Plasticizer	0.3	ml

Example 4. Indicator compositions containing 4-[(4-methyl-1-piperazinyl)sulfonyl]aniline as a diazotizing agent.

An indicator composition for detection of nitrites in urine containing 4-[(4-methyl-1-piperazinyl)sulfonyl]aniline as a diazotizing agent was prepared in a solution of water and acetone. The components of several indicator composition, including the solvents, are described in Tables 16-20.

Table 16:

Material	Function	Amount	Units
4-[(4-methyl-1-piperazinyl)sulfonyl]aniline	Coupling agent	0.005	g
2-amino-3,5-dinitrothiophene 1%	Diazotizing agent	0.005	g
Water	Solvent	0.5	ml
Acetone	Solvent	50.5	ml
Cellulose acetate	Polymer	1.5	g
Hexamoll dinch	Plasticizer	1	ml
Citric acid	Acid	0.025	g

Table 17:

Material	Function	Amount	Units
N-1-napthylethylenediamine dihydrochloride	Coupling agent	0.0025	g
4-[(4-methyl-1-piperazinyl)sulfonyl]aniline	Diazotizing agent	0.005	g
Water	Solvent	1	ml
Acetone	Solvent	50	ml
Cellulose acetate	Polymer	1.5	g
Hexamoll dinch	Plasticizer	1	ml

Table 18:

Material	Function	Amount	Units
N-1-napthylethylenediamine dihydrochloride	Coupling agent	0.0025	g
4-[(4-methyl-1-piperazinyl)sulfonyl]aniline	Diazotizing agent	0.005	g
Water	Solvent	1	ml
Acetone	Solvent	50	ml
Cellulose acetate	Polymer	1.5	g
Hexamoll dinch	Plasticizer	1	ml
Indigo	Dye	0.05	g

Table 19:

Material	Function	Amount	Units
N-1-napthylethylenediamine dihydrochloride	Coupling agent	0.005	g
4-[(4-methyl-1-piperazinyl)sulfonyl]aniline	Diazotizing agent	0.05	g
Water	Solvent	2	ml
Acetone	Solvent	50	ml
Cellulose acetate	Polymer	1.5	g
Hexamoll dinch	Plasticizer	1	ml

Table 20:

Material	Function	Amount	Units
N-1-napthylethylenediamine dihydrochloride	Coupling agent	0.005	g
4-[(4-methyl-1-piperazinyl)sulfonyl]aniline	Diazotizing agent	0.05	g
Water	Solvent	2	ml
Acetone	Solvent	50	ml
Cellulose acetate	Polymer	1.5	g
Triethyl citrate	Plasticizer	0.5	g

Example 5. Indicator compositions containing 4-({4'-[(4-aminophenyl)sulfonyl][1,1'-biphenyl]-4-yl}sulfonyl)aniline as a diazotizing agent.

An indicator composition for detection of nitrites in urine containing 4-({4'-[(4-aminophenyl)sulfonyl][1,1'-biphenyl]-4-yl}sulfonyl)aniline as a diazotizing agent was prepared in a solution of water and acetone. The components of several indicator compositions, including the solvents, are described in Tables 21-23.

Table 21:

Material	Function	Amount	Units
N-1-napthylethylenediamine dihydrochloride	Coupling agent	0.01	g
4-({4'-[(4-aminophenyl)sulfonyl][1,1'-biphenyl]-4-yl}sulfonyl)aniline	Diazotizing agent	0.02	g
Water	Solvent	2	ml
Acetone	Solvent	52	ml
Cellulose acetate	Polymer	1.5	g
Hexamoll dinch	Plasticizer	1	ml
Indigo	Dye	0.05	g

Table 22:

Material	Function	Amount	Units
N-1-napthylethylenediamine dihydrochloride	Coupling agent	0.005	g
4-({4'-[(4-aminophenyl)sulfonyl][1,1'-biphenyl]-4-yl}sulfonyl)aniline	Diazotizing agent	0.01	g
Tetrasodium 3,3'-[(3,3'-dimethyl[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[5-amino-4-hydroxynaphthalene-2,7-disulphonate]	Dye	0.0025	g
Water	Solvent	1.5	ml
Acetone	Solvent	51	ml
Cellulose acetate	Polymer	1.5	g
Hexamoll dinch	Plasticizer	1	ml
Indigo	Dye	0.05	g
Citric acid	Acid	0.025	g

Table 23:

Material	Function	Amount	Units
N-1-napthylethylenediamine dihydrochloride	Coupling agent	0.005	g
4-({4'-[(4-aminophenyl)sulfonyl][1,1'-biphenyl]-4-yl}sulfonyl)aniline	Diazotizing agent	0.01	g
Tetrasodium 3,3'-[(3,3'-dimethyl[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[5-amino-4-hydroxynaphthalene-2,7-disulphonate]	Dye	0.0025	g
Water	Solvent	1.5	ml
Acetone	Solvent	51	ml
Cellulose acetate	Polymer	1.5	g
Hexamoll dinch	Plasticizer	1	ml
Indigo	Dye	0.05	g
Citric acid	Acid	0.025	g

Example 6. Indicator compositions containing 2-amino 6-methoxybenzothiazole as a diazotizing agent.

An indicator composition for detection of nitrites in urine containing 2-amino 6-methoxybenzothiazole as a diazotizing agent was prepared in a solution of water and acetone. The components of the indicator composition, including the solvents, are described in Table 24.

Table 24:

Material	Function	Amount	Units
N-1-napthylethylenediamine dihydrochloride	Coupling agent	0.01	g
2-amino 6-methoxybenzothiazole	Diazotizing agent	0.1	g
Water	Solvent	4	ml
Acetone	Solvent	50	ml
Cellulose acetate	Polymer	1.5	g
Hexamoll dinch	Plasticizer	1	ml
Citric acid	Acid	0.2	g

Example 7. Indicator compositiosn containing sulfadimethoxine as a diazotizing agent.

An indicator composition for detection of nitrites in urine containing Sulgadimethoxine as a diazotizing agent was prepared in a solution of water and acetone. The components of the indicator composition, including the solvents, are described in Table 25.

Table 25:

Material	Function	Amount	Units
Sulfadimethoxine	Diazotizing agent	0.06	g
N-1-napthylethylenediamine dihydrochloride	Coupling agent	0.01	g
Water	Solvent	2	ml
Acetone	Solvent	42	ml
Cellulose acetate phthalate	Polymer	1.2	g
Hexamoll Dinch	Plasticizer	0.8	ml

Example 8. Indicator compositiosn containing Sodium sulfanilate hydrate as a diazotizing agent.

An indicator composition for detection of nitrites in urine containing Sodium sulfanilate hydrate as a diazotizing agent was prepared in a solution of water and acetone. The components of the indicator composition, including the solvents, are described in Table 26.

Table 26:

Material	Function	Amount	Units
Sodium sulfanilate hydrate	Diazotizing agent	0.1	g
N-1-napthylethylenediamine dihydrochloride	Coupling agent	0.01	g
Water	Solvent	1	ml
Acetone	Solvent	42	ml
Cellulose acetate phthalate	Polymer	1.2	g
Hexamoll Dinch	Plasticizer	0.8	ml

Example 9. Indicator compositions containing methyl anthranilate.

An indicator composition for detection of nitrites in urine containing Methyl anthranilate was prepared in a solution of water and acetone. The components of the indicator composition, including the solvents, are described in Table 27.

Table 27:

Material	Function	Amount	Units
Methyl anthranilate	Diazotizing agent	0.1	g
N-1-napthylethylenediamine dihydrochloride	Coupling agent	0.01	g
Water	Solvent	1	ml
Acetone	Solvent	42	ml
Cellulose acetate phthalate	Polymer	1.2	g
Hexamoll Dinch	Plasticizer	0.8	ml

Example 10. Formulation of indicator-composition containing Beta-cyclodextrin as a trap.

An indicator composition for detection of nitrites in urine containing Beta-cyclodextrin as a trap was prepared in a solution of water and acetone. The components of several indicator compositions, including the solvents, are described in Tables 28-30.

Table 28:

Material	Function	Amount	Units
Sulfanilamide	Diazotizing agent	0.2	g
N-1-napthylethylenediamine dihydrochloride	Coupling agent	0.02	g
Water	Solvent	2	ml
Acetone	Solvent	42	ml
Cellulose acetate phthalate	Polymer	1.2	g
Beta-cyclodextrin	Trap	0.04	g
Hexamoll dinch	Plasticizer	0.8	ml

Table 29:

Material	Function	Amount	Units
Sulfanilamide	Diazotizing agent	0.2	g
N-1-napthylethylenediamine dihydrochloride	Coupling agent	0.02	g
Water	Solvent	2	ml
Acetone	Solvent	42	ml
T-butyl hydroquinone	Anti-oxidant	0.4	g
Beta-cyclodextrine	Trap	0.04	g
Cellulose acetate phthalate	Polymer	1.2	g
Hexamoll dinch	Plasticizer	0.8	ml

Table 30:

Material	Function	Amount	Units
Phosphate citrate buffer (pH 3.0, 0.1 M)	Buffer	4	ml
Sulfanilate hydrate	Diazotizing agent	0.4	g
N-1-napthylethylenediamine dihydrochloride	Coupling agent	0.02	g
Acetone	Solvent	42	ml
T-butyl hydroquinone	Anti-oxidant	0.4	g
Beta-cyclodextrine	Trap	0.04	g
Cellulose acetate phthalate	Polymer	1.2	g
Hexamoll dinch	Plasticizer	0.8	ml

Example 11. Detection of nitrites in nitrite solutions using an indicator composition

Polyester non-woven fabric was coated with a solution containing an indicator composition of the formulation laid down in Table 31. The fabric was dipped into the indicator composition solution and dried using hot air, resulting in a white-colored fabric. The fabric was cut into pieces, and each piece was placed in a diaper. Each piece was treated with a water-based nitrite solution containing either 0.01 mg/dL, 0.025 mg/dL or 0.05 mg/dL

nitrite. A clear purple stain appeared on each piece and remained stable for 24 hours. Similar results were obtained when the fabric was coated with a commercial mixture of OPADRY II - Colorcon (5% in distilled water), as a protective-film, prior to treatment with the nitrite solution. Similar results were obtained when coated fabrics were treated with human urine samples to which nitrites were added (0.01 mg/dL, 0.025 mg/dL or 0.05 mg/dL).

Table 31:

Material	Function	Amount	Units
Sulfathiazole	Diazotizing agent	0.6	g
N-1-napthylethylenediamine dihydrochloride	Coupling agent	0.15	g
Water	Solvent	10	ml
Acetone	Solvent	20	ml
Cellulose acetate phthalate	Polymer	1.25	g
Glycerol	Wetting agent	0.6	ml
Polystyrene	Anti-oxidant	0.01	gr
Dioctylphthalate	Plasticizer	0.4	ml

The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying current knowledge, readily modify and/or adapt for various applications such specific embodiments without undue experimentation and without departing from the generic concept, and, therefore, such adaptations and modifications should and are intended to be comprehended within the meaning and range of equivalents of the disclosed embodiments. It is to be understood that the phraseology or terminology employed herein is for the purpose of description and not of limitation. The means, materials, and steps for carrying out various disclosed functions may take a variety of alternative forms without departing from the invention.

CLAIMS

1. An indicator composition comprising a polymer, a plasticizer, a diazotizing reagent and a coupling agent, wherein said indicator composition provides a color indication in the presence of nitrites in urine.

2. The indicator composition of claim 1, wherein the sensitivity of said indicator composition to nitrites is in the range of 0.01 to 2 mg/dL.

3. The indicator composition of claim 1, wherein said indication is stable, for at least 3 hours.

4. The indicator composition of claim 3, wherein said indication is stable for at least 12 hours.

5. The indicator composition of claim 3, wherein said indication is stable for at least 24 hours .

6. The indicator composition of claim 3, wherein said indication is stable for at least 48 hours.

7. The indicator composition of claim 1, further comprising at least one component selected from the group consisting of: a pH indicator, a dye, an anti-oxidant, an acid, a trap, a photo-resistant agent and a wetting agent.

8. The indicator composition of claim 1, wherein the amount of said polymer in the indicator composition is within the range of 30% to 90%, the amount of said plasticizer in the indicator composition is within the range of 5% to 50%, the amount of said diazotizing reagent in the indicator composition is within the range of 0.1% to 12%, the amount of said coupling agent in the indicator composition is within the range of 0.01% to 5%, wherein the percentages presenting the amount of each component in the indicator composition refer to weight/weight percentages based on the total weight of the indicator composition and the total weight of the indicator composition equals 100%.

9. The indicator composition of claim 7, wherein the amount of the pH indicator in said indicator composition is within the range of 0.0005% to 1%.

10. The indicator composition of claim 7, wherein the amount of the dye in said indicator composition is within the range of 0.05% to 2.5%.

11. The indicator composition of claim 7, wherein the amount of the anti-oxidant in said indicator composition is within the range of 1% to 20%.

12. The indicator composition of claim 7, wherein the amount of the acid in the indicator composition is within the range of 0.1% to 10%.

13. The indicator composition of claim 7, wherein the amount of the trap in said indicator composition is within the range of 0.5% to 2.5%.

14. The indicator composition of claim 7, wherein the amount of the wetting agent in said indicator composition is within the range of 1% to 25%.

15. The indicator composition of claim 7, wherein the amount of the photo-resistant agent in said indicator composition is within the range of 0.1% to 1%.

16. The indicator composition of claim 1, wherein said polymer comprises one or more polymers selected from the group consisting of: cellulose acetate, dextran, nitrocellulose, ethyl cellulose, carboxymethyl cellulose, cellulose acetate phthalate, cellulose acetate propionate, cellulose acetate butyrate, hydroxypropyl methylcellulose, polyethylene glycol, polyvinyl alcohol, polyvinyl alcohol-polyethylene glycol graft copolymer, silicone dioxide and derivatives thereof.

17. The indicator composition of claim 1, wherein said polymer is cellulose acetate.

18. The indicator composition of claim 1, wherein said diazotizing agent is selected from the group consisting of: p-arsanilic acid, methyl anthranilate, sulfanilamide, sulfanilic acid, 2-amino-6-methoxy benzothiazole, 2-amino- benzothiazole, 2-amino-6-nitro benzothiazole, 2-amino-6-ethoxybenzothiazole, 2-amino-6-methylbenzothiazole, 2-amino-3,5-dinitrothiophene, 4-[(4-methyl-1-piperazinyl)sulfonyl]aniline, 4-({4'-[(4-aminophenyl)sulfonyl][1,1'-biphenyl]-4-yl}sulfonyl)aniline, sulfadimethoxine,

sulfathiazole, sodium sulfanilate hydrate or any aromatic primary amine with a sulfonyl group and a combination thereof.

19. The indicator composition of claim 1, wherein said diazotizing agent is sulfanilamide.

20. The indicator composition of claim 1, wherein said diazotizing agent is 4-[(4-methyl-1-piperazinyl)sulfonyl]aniline.

21. The indicator composition of claim 1, wherein said diazotizing agent is 4-({4'-[(4-aminophenyl)sulfonyl][1,1'-biphenyl]-4-yl}sulfonyl)aniline.

22. The indicator composition of claim 1, wherein said coupling agent is selected from the group consisting of: N-(1-Naphthyl)ethylenediamine dihydrochloride, 4-({4'-[(4-aminophenyl)sulfonyl][1,1'-biphenyl]-4-yl}sulfonyl)aniline, 4-[(4-methyl-1-piperazinyl)sulfonyl]aniline, 1-Naphthylamine and 2-(1-Naphthylamino) ethanol.

23. The indicator composition of claim 1, wherein said plasticizer is selected from the group consisting of: 1,2-cyclohexane dicarboxylic acid di-isononyl ester, bis(2-ethylhexyl) sebacate, acetyl tributyl citrate, triethylhexyl trimellitate, triethyl citrate, ethyl lactate, alkylsulfonic acid phenylester, dioctylphthalate, polylactide, ethyl hexyl phthalate, polyvinyl pyrrolidone and combinations thereof.

24. The indicator composition of claim 1, wherein said plasticizer is 1,2-cyclohexane dicarboxylic acid di-isononyl ester.

25. The indicator composition of claim 7, wherein said pH indicator is selected from: leucomalachite green and gentian violet.

26. The indicator composition of claim 7, wherein said pH indicator has a transition pH range between 0-4.0.

27. The indicator composition of claim 7, wherein said pH indicator has a transition pH range between 8.0-14.0.

28. The indicator composition of claim 7, wherein said dye is selected from the group consisting of: 2,2'-Bis(2,3-dihydro-3- oxoindolyliden), (3Z,3'Z)-3,3'-[(3,3'-dimethylbiphenyl-4,4'-diyl)di(1Z)hydrazin-2-yl-1-ylidene]bis(5-amino-4-oxo-3,4

dihydronaphthalene -2,7-disulfonic acid) and disodium 4,4'-[(4-hydroxy-9,10-dioxo-9,10-dihydroanthracene-1,3-diyl)diimino]dibenzenesulfonate.

29. The indicator composition of claim 7, wherein said anti-oxidant is selected from the group consisting of: titanium dioxide pigments, zinc oxide, polyvinyl alcohol, ascorbic acid, D-Isoascorbic acid, t-butyl hydroquinone, polystyrene and vitamin E.

30. The indicator composition of claim 7, wherein said trap is selected from the group consisting of: beta-cyclodextrin and pentasodium pentetate.

31. The indicator composition of claim 7, wherein said wetting agent is selected from the group consisting of: glycerol, 2-ethoxy ethanol and sodium carboxy methyl starch.

32. The indicator composition of claim 7, wherein said wetting agent is glycerol.

33. The indicator composition of claim 7, wherein said acid is selected from the group consisting of: citric acid, tartaric acid, adipic acid, picric acid, methacrylic acid, stearic acid and acetic acid.

34. The indicator composition of claim 7, wherein said acid is a salt derivative of an acid selected from the group consisting of: citric acid, tartaric acid, adipic acid, picric acid and acetic acid.

35. The indicator composition of claim 7, wherein said acid is citric acid.

36. The indicator composition of claim 7, wherein said photo-resistant agent is 4-methacryloxy-2-hydroxy benzo- phenone.

37. A diagnostic article for detection of urinary tract infection in a subject, comprising an absorbent substrate configured to absorb urine; and the indicator composition of any one of claims 1-36, wherein said indicator composition provides a color indication in the presence of nitrites in said absorbed urine.

38. The article of claim 37, wherein said absorbent substrate is selected from the group consisting of: gauze, panty shield, hygienic napkin, diaper ,inter-labial absorbent structure, an absorbent sticker and a combination thereof.

39. The article of claim 37, wherein said absorbent substrate comprises a fabric material.

40. The article of claim 39, wherein said fabric material is selected from the group consisting of: woven fabric, non-woven fabric, hydrophilic fabric material, hydrophobic fabric material and combinations thereof.

41. The article of claim 37, wherein the article further comprises a protective film.

42. The article of claim 41, wherein said protective film is configured to dissolve upon contacting urine.

43. The article of claim 41, wherein said protective film comprises a polymer selected from the group consisting of: polyvinyl alcohol, polyethylene glycol, hydrous magnesium silicate, hydroxypropyl methyl cellulose, sodium starch glycollate, polyethylene glycol, xanthan gum and a combination thereof.

44. The article of claim 43, wherein said protective film further comprises talc.

45. A method for detecting urinary tract infection in a subject, the method comprising:

(a) providing a diagnostic article comprising an absorbent substrate configured to absorb urine and an indicator composition, wherein said indicator composition comprises a polymer, a plasticizer, a diazotizing reagent and a coupling agent and wherein said indicator composition provides a color indication in the presence of nitrites in said absorbed urine;

(b) mounting said diagnostic article so as to receive urine from the subject;

(c) contacting the indicator composition with urine; and

(d) detecting the color indication, thereby determining whether an abnormal level of a nitrites indicative of urinary tract infection, is present in the urine.

46. The method of claim 45, wherein detecting comprises observing the color indication of the indicator composition, wherein a color that is different from the color of said indicator composition prior to the step of mounting, is indicative of abnormal level of a nitrites.

47. The method of claim 45, wherein the sensitivity of said indicator composition to nitrites is in the range of 0.01 to 2 mg/dL.

48. The method of claim 45, wherein said indication is stable for at least 3 hours.

49. The method of claim 45, wherein said indication is stable for at least 12 hours.

50. The method of claim 45, wherein said indication is stable for at least 24 hours.

51. The method of claim 45, wherein said indication is stable for at least 48 hours.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL2012/050209

A. CLASSIFICATION OF SUBJECT MATTER

IPC (2010.01) G01N 33/493, A61F 13/49, G01N 33/52

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC (2010.01) G01N 33/493

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Databases consulted: PATENTSCOPE, THOMSON INNOVATION, Esp@cenet, WPI Data, EPODOC
Search terms used: color detecting, nitrite, urine, UTI, dizotizing agent, coupling agent, polymer, plasticizer, diagnostic, article

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0160240 B1 TERUMO KABUSHIKI KAISHA 06 Nov 1985 (1985/11/06) page 3, examples	1,2,7,13,16,18,19,22,23,33,34,37-41,45-47
Y		1-51
Y	WO 2007027198 A1 KIMBERLY CLARK CO 08 Mar 2007 (2007/03/08) p. 7, l. 3-14, p. 8, 2nd par. – p. 9, 1st par	1-51
Y	US 3718543 A LAGOMARSINO J. 27 Feb 1973 (1973/02/27) abstract, c. 2, l. 22-29, c. 3, l. 27-33	1-51
Y	WO 2010131127 A2 KIMBERLY CLARK CO 18 Nov 2010 (2010/11/18) p.9, l. 13-23; p.11, l. 22-30, p.7, l. 12 – p. 8, l. 3, p. 17, l. 9-21	1-51

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 Aug 2012	16 Aug 2012

Name and mailing address of the ISA:	Authorized officer
Israel Patent Office The Technology Park, Bldg.5, Malcha, Jerusalem, 96951, Israel Facsimile No. 972-2-5651616	SIN-MALIA Yoav YoavSo@justice.gov.il Telephone No. 972-2-5651738

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL2012/050209

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 7947467 A KRITZMAN AMNON et al 25 May 2011 (2011/05/25) abstract, c. 5, l. 39-55, c. 5, l. 2-4, 25-27; c. 6, l. 10-17, c. 7, l. 58-61; c. 8, l. 6-7; examples, c. 5, l. 15-20, claim 6	1-51

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/IL2012/050209

Patent document cited search report	Publication date	Patent family member(s)	Publication Date
EP 0160240 B1	06 Nov 1985	JP60233552 (A)	20 Nov 1985
US 7947467 A	25 May 2011	CA2632588 (A1)	21 Jun 2007
		CA2670403 (A1)	30 May 2007
		EP1419268	19 May 2004
		EP1565140 (A2)	24 Aug 2005
		EP1960762 (A2)	27 Aug 2008
		IL159918 (A)	03 Nov 2008
		JP2006504954 (A)	09 Feb 2006
		US2003017605 (A1)	23 Jan 2003
		US2003166293 (A1)	04 Sep 2003
		US2007003993 (A1)	04 Jan 2007
		US2008086060 (A1)	10 Apr 2008
		US201036707 (A1)	03 Jun 2010
		US2009275071 (A1)	05 Nov 2009
		US2007134740 (A1)	14 Jun 2007
		US2012003685 (A1)	05 Jan 2012
		WO03007997 (A2)	03 Jan 2003
		WO2004040253 (A2)	13 May 2004
		WO2007069240 (A2)	21 Jun 2007
		AU2002354890 (B2)	30 May 2007
		AU2003278582 (A1)	25 May 2004
		AU2003278582 (B2)	07 Feb 2008
		CA2454338 (A1)	03 Jan 2003
		CA2504323 (A1)	13 May 2004
WO 2007027198 A1	08 Mar 2007	EP1920251 (A1)	14 May 2008
		EP1920251 (B1)	29 Dec 2010
		US2007048182 (A1)	01 Mar 2007
		US8003399 (B2)	03 Aug 2011

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/IL2012/050209

Patent document cited search report	Publication date	Patent family member(s)	Publication Date
WO 2010131127 A2	18 Nov 2010	US2010290948 (A1)	18 Nov 2010
		WO2010131127 (A3)	24 Mar 2011