

TABLE 1

| Reagent for a Glucose Test Pad  |             |
|---|-------------|
| Components  | Quantity    |
| Water   | 100 ml      |
| (2-[Morpholino]ethanesulfonic acid) sodium salt MES (MW 217.2, Sigma, St. Louis, MO, USA) Adjust pH to 5-7 by adding 6 M HCl) | 2.2 gm      |
| Tetonic 1307 (BASF Corporation, Mount Olive, New Jersey, USA)   | 1-3 gm      |
| PSSA, Polystyrenesulfonic acid, sodium salt (MW 70,000, Polysciences, Inc., Warrington, PA, USA)                              | 2-4 gm      |
| Croton (Croda Inc., Parsippany, NJ, USA)  | 2-4 gm      |
| Mannitol (MW 182, Sigma, St. Louis, MO, USA)  | 1-10 gm     |
| Phenazine Methosulfate (PMS, MW 306.34, Sigma, St. Louis, MO, USA)  | 30-300 mg   |
| WST-5 (MW 1331.37, Dojindo Laboratory, Japan)   | 0.8-4 gm    |
| Glucose Oxidase (GO, TOYOBO)  | 100-1000 KU |

TABLE 2

| Nitrite Reagent  |          |
|--|----------|
| Components   | Quantity |
| 10 mM Phosphate Buffer Saline, pH 7.4, (P-3813, Sigma, St. Louis, MO, USA) | 70 ml    |
| Ethanol  | 30 ml    |
| Sodium Nitrite (MW69, Aldrich Chemicals, Milwaukee, WI, USA)               | 5 gm     |
| Polyvinylpyrrolidone (MW 40,000, Sigma, St. Louis, MO, USA)                | 200 mg   |

TABLE 3

| Reagent for a Glucose Test Pad  |             |
|---|-------------|
| Components  | Quantity    |
| Water   | 100 ml      |
| (2-[Morpholino]ethanesulfonic acid) sodium salt MES (MW 217.2, Sigma, St. Louis, MO, USA) | 2.2 gm      |
| Poly(methyl vinyl ether-alt-maleic anhydride)* 6%   | 20 mL       |
| Adjust pH to 5.5-7 by adding 50% NaOH   |             |
| Triton X-305 (BASF Corporation, Moun Olive, New Jersey, USA)                              | 0.5-2 gm    |
| Mannitol (MW 182, Sigma, St. Louis, MO, USA)  | 1-10 gm     |
| Sodium Nitrite (MW69, Aldrich Chemicals, Milwaukee, WI, USA)                              | 1-5 gm      |
| WST-5 (MW 1331.37, Dojindo Laboratory, Japan)   | 0.8-4 gm    |
| Magnesium Chloride (MW 203, Sigma, St. Louis, MO, USA)                                    | 3-5 gm      |
| Phenazine Ethosulfate (PES, MW 334.4, Sigma, St. Louis, MO, USA)                          | 100-1000 mg |
| Glucose Oxidase (GO, TOYOBO)  | 100-1000 KU |

\*Poly(methylvinylether-alt-maleic anhydride), MW 1,080,000, Cat# 41632-0, Aldrich Chemicals, Milwaukee, WI, USA Weigh out Poly(methylvinylether-alt-maleic anhydride) 6% in water (w/v), and heat the suspension to 95 C. for 45 min. The resulting solution is ready to use upon cooling to room temperature.

Various glucose standards were tested on the non-charged and positively charged membranes. The signals were linear from 50 to 450 mg/dl glucose levels in blood. FIG. 1 shows the same dip was coated on different membrane. One is positive charged nylon membrane, one is no positive charged polysulfone membrane. The coated membrane was tested by 400 mg/dl glucose.

Using the following protocol, 10  $\mu$ L of aqueous samples comprising 400 mg/dL glucose were tested on strips as described above, where the membrane of the strips varied in

terms of the positively charged nylon membrane and (no positive charged)(non-charged)polysulfone membrane on the strip. A 10  $\mu$ l aqueous sample was applied onto a freshly prepared test strip. The strip was inserted into a reflectometer and data acquisition was commenced. The reflectance of the reading strip was monitored at 615 nm at one-second intervals for forty five seconds. Next, the data were uploaded from the reflectometer's memory buffer to a personal computer via a modified serial cable. The reaction profile was plotted by K/S versus seconds. (K/S is a measure of reflectance, discussed and defined in U.S. Pat. No. 4,935, 346, col. 14, the disclosure of which is herein incorporated by reference.)

It is evident from the above results and discussion that the subject invention provides for improvement over previous reagent test strip formats. By using a water soluble tetrazolium salt in combination with a positively charged substrate, the subject invention is the beneficiary of all of the positive attributes of tetrazolium compounds and is able to produce a non-washable reporter signal from the resultant water soluble formazan product. As such, the subject invention represents a significant contribution to the art.

All publications and patents cited in this specification are herein incorporated by reference as if each individual publication or patent were specifically and individually indicated to be incorporated by reference. The citation of any publication is for its disclosure prior to the filing date and should not be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it is readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

What is claimed is:

1. A composition of matter comprising:

a non-bibulous positively charged substrate; and at least one water soluble tetrazolium salt on at least one surface of said positively charged substrate.

2. The composition according to claim 1, wherein said water soluble tetrazolium salt is part of an analyte oxidizing signal producing system.

3. The composition according to claim 2, wherein said analyte oxidizing signal producing system comprises an analyte oxidase.

4. The composition according to claim 2, wherein said analyte oxidizing signal producing system comprises an analyte dehydrogenase.

5. The composition according to claim 2, wherein said analyte oxidizing signal producing system further comprises an electron transfer agent.

6. The composition according to claim 2, wherein said analyte oxidizing signal producing system further comprises an enzyme cofactor.

7. The composition according to claim 2, wherein said analyte oxidizing signal producing system is present as a reagent composition.

8. A reagent test strip comprising:

a positively charged substrate; and

an analyte oxidizing signal producing system present on said positively charged substrate, wherein said analyte oxidizing signal producing system includes a water soluble tetrazolium salt.