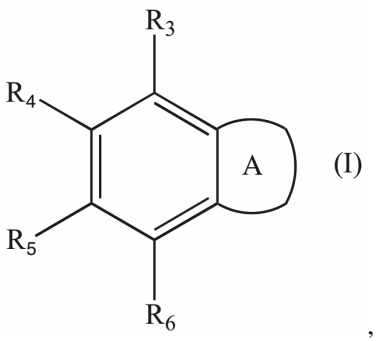
CLAIMS

What is claimed is:

1. A method for determining the presence of a microorganism in a sample using an electrochemically active reporter, wherein the method comprises

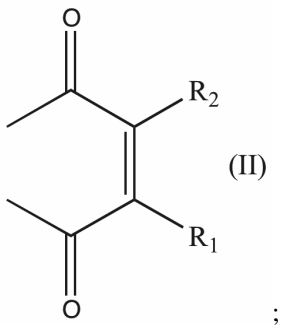
(a) contacting the sample with an electrochemically active reporter,

wherein the electrochemically active reporter is a conjugate comprising a sugar moiety and a redox active reporter moiety that are covalently linked such that the covalent bond can be enzymatically cleaved in the presence of the microorganism by an enzyme expressed by the microorganism, wherein the redox active reporter moiety is selected from the group consisting of compounds of formula (I) and resorufin

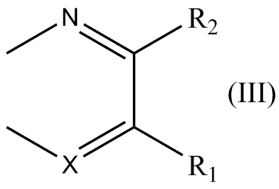


(I),

wherein A is an aryl or heteroaryl ring of formula (II) or (III):



(II);



(III),

each of R_1-R_6 is independently selected from the group consisting of H, NR_7R, C_{1-10} alkyl, OR_7, or R_1 and R_2 combine to form together with the carbon atoms to which they are attached a 5-6 membered alicyclic or aryl ring, wherein the alicyclic or aryl ring is substituted or unsubstituted, and if substituted the substituents are one or more groups selected from NR_7R_8, C_{1-10} alkyl, and OR_7;
X is N or S^+;
R_7 and R_8 are independently selected from the group consisting of H, C(O)-R_9, C_{1-10} Alkyl;
R_9 is C_{1-10} Alkyl,
under conditions that allow enzymatic cleavage of the covalent bond between the sugar moiety and the redox active reporter moiety and reduction of the redox active report moiety in the presence of the microorganism;

(b) electrochemically determining the released redox active reporter moiety; and

(c) determining the presence of the microorganism and, optionally, number of the microorganisms in the sample based on the determined released redox active reporter moiety.

2. The method of claim 1, wherein the method is carried out by a 3-electrode electrochemical system or a 2-electrode self-powering bioelectrochemical system comprising a working electrode and an electrolyte.

3. The method of claim 2, wherein step (a) comprises adding the sample and an effective amount of the electrochemically active reporter to the electrolyte.

4. The method of claim 3, wherein step (b) comprises measuring the electrical current or voltage resulting from the released redox active reporter moiety by chronoamperometry, potentiometry or voltammetry or other standard electrochemical techniques using the electrochemical system or by determining the potential difference between the anode and cathode in the self-powering bioelectrochemical system.

5. The method of claim 4, wherein the released redox active reporter is oxidized by interaction with the working electrode, and wherein the oxidized released redox active reporter is reduced by the microorganism and recycled for the generation of electrical current or voltage.

6. The method of claim 1, wherein step (c) comprises determining the number of the microorganisms in the sample based on the electrical current or voltage measured by comparing it to a predetermined standard curve.

7. The method of claim 1, wherein the microorganism is allowed to recover from dormancy or stress prior to being subjected to the detection.

8. The method of claim 1, wherein the microorganism is filtered or concentrated prior to being subjected to the detection.

9. The method of claim 1, wherein an agent is added to the sample to suppress microorganisms other than the target microorganism to be determined.

10. The method of claim 1, wherein the sugar moiety is a pyranose moiety, selected from the group consisting of β-D-glucopyranoside, β-D-galactopyranoside and β-D-glucuronide, and methyl ester, peracetylated and peracetylated methyl ester derivatives thereof.

11. The method of claim 10, wherein the electrochemically active reporter is selected from the group consisting of resorufin-β-D-glucopyranoside, methyl ester resorufin-β-D-glucopyranoside, peracetylated resorufin-β-D-glucopyranoside, peracetylated methyl ester resorufin-β-D-glucopyranoside, resorufin-β-D-galactopyranoside, methyl ester resorufin-β-D-galactopyranoside, peracetylated resorufin-β-D-galactopyranoside, peracetylated methyl ester resorufin-β-D-galactopyranoside, resorufin-β-D-glucuronide, methyl ester resorufin-β-D-glucuronide, peracetylated resorufin-β-D-glucuronide, peracetylated methyl ester resorufin-β-D-glucuronide, 2-hydroxy-1,4-naphthoquinone-β-D-glucopyranoside, methyl ester 2-hydroxy-1,4-naphthoquinone-β-D-glucopyranoside, peracetylated 2-hydroxy-1,4-naphthoquinone-β-D-glucopyranoside, peracetylated methyl ester 2-hydroxy-1,4-naphthoquinone-β-D-glucopyranoside, 2-hydroxy-1,4-naphthoquinone-β-D-galactopyranoside, methyl ester 2-hydroxy-1,4-naphthoquinone-β-D-galactopyranoside, peracetylated 2-hydroxy-1,4-naphthoquinone-β-D-galactopyranoside, peracetylated methyl ester 2-hydroxy-1,4-naphthoquinone-β-D-galactopyranoside, 2-hydroxy-1,4-naphthoquinone-β-D-glucuronide, methyl ester 2-hydroxy-1,4-naphthoquinone-β-D-glucuronide, methyl ester 2-hydroxy-1,4-naphthoquinone-β-D-glucuronide, peracetylated 2-hydroxy-1,4-naphthoquinone-β-D-glucuronide, peracetylated methyl ester 2-hydroxy-1,4-naphthoquinone-β-D-glucuronide, 5-hydroxy-1,4-naphthoquinone-β-D-glucopyranoside, methyl ester 5-hydroxy-1,4-naphthoquinone-β-D-glucopyranoside, peracetylated 5-hydroxy-1,4-naphthoquinone-β-D-glucopyranoside, peracetylated methyl ester 5-hydroxy-1,4-naphthoquinone-β-D-glucopyranoside, 5-hydroxy-1,4-naphthoquinone-β-D-galactopyranoside, methyl ester 5-hydroxy-1,4-naphthoquinone-β-D-galactopyranoside, peracetylated 5-hydroxy-1,4-naphthoquinone-β-D-galactopyranoside, peracetylated methyl ester 5-hydroxy-1,4-naphthoquinone-β-D-galactopyranoside, 5-hydroxy-1,4-naphthoquinone-β-D-glucuronide, methyl ester 5-hydroxy-1,4-naphthoquinone-β-D-glucuronide, peracetylated 5-hydroxy-

1,4-naphthoquinone-β-D-glucuronide, peracetylated methyl ester 5-hydroxy-1,4-naphthoquinone-β-D-glucuronide, 5,8-dihydroxy-1,4-naphthoquinone-β-D-glucopyranoside, methyl ester 5,8-dihydroxy-1,4-naphthoquinone-β-D-glucopyranoside, peracetylated 5,8-dihydroxy-1,4-naphthoquinone-β-D-glucopyranoside, peracetylated methyl ester 5,8-dihydroxy-1,4-naphthoquinone-β-D-glucopyranoside, 5,8-dihydroxy-1,4-naphthoquinone-β-D-galactopyranoside, methyl ester 5,8-dihydroxy-1,4-naphthoquinone-β-D-galactopyranoside, peracetylated 5,8-dihydroxy-1,4-naphthoquinone-β-D-galactopyranoside, peracetylated methyl ester 5,8-dihydroxy-1,4-naphthoquinone-β-D-galactopyranoside, 5,8-dihydroxy-1,4-naphthoquinone-β-D-glucuronide, methyl ester 5,8-dihydroxy-1,4-naphthoquinone-β-D-glucuronide, peracetylated 5,8-dihydroxy-1,4-naphthoquinone-β-D-glucuronide, and peracetylated methyl ester 5,8-dihydroxy-1,4-naphthoquinone-β-D-glucuronide.

12. The method of claim 1, wherein the covalent bond between the sugar moiety and the redox active reporter moiety is cleaved by an enzyme expressed by the microorganism to be determined.

13. The method of claim 12, wherein the expression of the enzyme is induced.

14. The method of claim 1, wherein the method further comprises a step of lysing the cell membrane or enhancing membrane permeability of the microorganism to facilitate the release of the redox active reporter moiety or the enzyme cleaving the electrochemically active reporter.

15. The method of claim 1, wherein the method is a multiplex method that allows simultaneous determination of a number of different organisms.

16. The method of claim 15, wherein for each microorganism to be detected a different electrochemically active reporter is used.

17. The method of claim 1, wherein the microorganism is a coliform bacterium.

18. The method of claim 17, wherein the microorganism is *Escherichia coli*, the enzyme expressed by the microorganism is β-glucuronidase, and the sugar moiety is β-D-glucuronide or a methyl ester, peracetylated or peracetylated methyl ester derivative thereof.

19. The method of claim 17, wherein the microorganism is *Escherichia coli*, the enzyme expressed by the microorganism is β-D-galactosidase, and the sugar moiety is β-D-galactopyranoside or a methyl ester, peracetylated or peracetylated methyl ester derivative thereof.

20. The method of claim 17, wherein the microorganism is *Escherichia coli* or *Enterococcus faecalis*, the enzyme expressed by the microorganism is β-glucosidase, and the sugar moiety is β-D-glucopyranoside or a methyl ester, peracetylated or peracetylated methyl ester derivative thereof.