SUMMARY

[0006] Methods and devices for molecular detection or diagnostic assays are provided by the embodiments of the present invention. The methods disclosed herein are suitable for detection or monitoring of one or more targets that exist in very low concentrations in samples including biological, chemical and material but not exclusively limited to these, and generally allow detection in the absence of the replication of the target or amplification of a fragment or part of the target. The methods and devices of the present application are amenable to point of care detection without the requirement for access to electrical power.

[0007] In one aspect, the present invention provides a method of detecting an analyte in a test sample by performing the steps of i) providing a lateral flow assay device that comprises a chromatographic medium that includes: (a) a sample loading zone located upstream of a detection zone; (b) a reporting carrier zone located between the sample loading zone and a detection zone, wherein said reporting carrier zone comprises a reporting carrier capable of forming a complex with the analyte said reporting carrier comprising a carrier and one or more proficient enzyme cassettes; and (c) a detection zone adjacent to the reporting carrier zone, wherein the detection zone comprises a capture component for the analyte and an indicator; ii) ~~C~~contacting the sample ~~application~~loading zone with the test sample, wherein the test sample travels through the reporting carrier zone along the chromatographic medium from the sample loading zone to the detection zone and beyond the detection zone; iii) adding a substrate to the detection zone wherein the substrate undergoes a reaction in the presence of proficient enzyme analyte containing reporting carrier; and iv) generating a response of the indicator within the detection zone that corresponds to the presence or absence of the analyte in the test sample.

[0008] In another aspect, the present invention provides a lateral flow assay device for detecting the presence of an analyte within a test sample by comprising: a chromatographic medium that includes: a sample loading zone located upstream of a detection zone; a reporting carrier zone located between the sample loading zone and a detection zone, wherein said reporting carrier zone comprises a reporting carrier capable of forming a complex with the analyte said reporting carrier comprising a carrier and one or more proficient enzyme cassettes; and a detection zone adjacent to the reporting carrier zone, wherein the detection zone comprises a capture component for the analyte and an indicator wherein the indicator detects a reaction of a substrate in the presence of a proficient enzyme thereby detecting the product of the analyte enzyme reporting carrier complex and a substrate is detected, thereby detecting the presence of the analyte.;

[0009] In one aspect, the present invention provides a lateral flow assay kit for detecting the presence of an analyte within a test sample comprising a lateral flow assay device comprising: a porous membrane comprising:a sample loading zone; a reporting carrier zone down stream of the loading zone, wherein said reporting carrier zone comprises a reporting carrier capable of forming a complex with the analyte; a detection zone down stream of the reporting carrier zone, wherein the detection zone is adjacent to the reporting carrier zone and comprises a capture component and an indicator; and a substrate for the proficient enzyme; wherein the substrate is applied to the detection zone after the test sample has been allowed to flow through the lateral flow device and the product of the enzyme and substrate is detected.

[0010] In some embodiments, it is also possible to implement the method comprising these steps, but not in the order listed above. For example, it is possible to (a) immobilize the analyte from a sample suspected of containing the analyte (b) contacting the immobilized analyte to a reporting carrier to form a reporting carrier-analyte complex, where the reporting complex comprises a proficient enzyme, (c) providing a substrate for the proficient enzyme, and (d)

~~We Claim:~~CLAIMS

1. A method of detecting an analyte in a test sample, the method comprising;

i) providing a lateral flow assay device that comprises a chromatographic medium that includes:

(a) a sample loading zone located upstream of a detection zone;

(b) a reporting carrier zone located between the sample loading zone and a detection zone, wherein said reporting carrier zone comprises a reporting carrier capable of forming a complex with the analyte said reporting carrier comprising a carrier and one or more proficient enzyme cassettes; and

(c) a detection zone adjacent to the reporting carrier zone, wherein the detection zone comprises a capture component for the analyte and an indicator;

ii) ~~C~~contacting the sample ~~application~~loading zone with the test sample, wherein the test sample travels through the reporting carrier zone along the chromatographic medium from the sample loading zone to the detection zone and beyond the detection zone;

iii) adding a substrate to the detection zone wherein the substrate undergoes a reaction in the presence of proficient enzyme analyte containing reporting carrier; and

iv) generating a response of the indicator within the detection zone that corresponds to the presence or absence of the analyte in the test sample.

2. The method of claim 1, wherein the lateral flow device further comprises a control zone down stream of the detection zone and the control zone comprises a capture component for the reporting carrier and an indicator;

adding a substrate to the control zone wherein the substrate undergoes a reaction in the presence of proficient enzyme containing reporting carrier; and

generating a response of the indicator within the control zone that corresponds to the presence or absence of the reporting carrier.

3. The method of claim 1 further comprising maintaining a reaction temperature.

4. The method of claim 1, wherein the analyte is a protein; wherein the analyte is a small molecule; or wherein the analyte is a nucleic acid.

5. The method of claim 1, wherein the proficient enzyme conjugate comprises an antibody; wherein the proficient enzyme conjugate comprises a nucleic acid; or wherein the proficient enzyme conjugate comprises a proficient enzyme selected from the group consisting of: urease, phosphocholine phosphatase, betagalactosidase, xylose reductase, shikimate dehydrogenase, malate dehydrogenase, carboxylesterase, neopullulanase, subtilisin, 4-phytase, acetylcholinesterase, laccase, bacterial leucyl aminopeptidase, tripeptidyl-peptidase I, coagulation factor VIIa, trypsin, beta-fructofuranosidase.

6. The method of claim 3, where the reaction temperature is maintained to about 4°C, to about 95°C, or higher while still maintaining enzyme activity.

7. The method of claim 5, wherein the antibody interacts non-covalently with a proficient enzyme to form the proficient enzyme conjugate; wherein the antibody is covalently attached to a proficient enzyme to form the proficient enzyme conjugate.

8. The method of claim 5, wherein the nucleic acid is covalently attached to a proficient enzyme to form the proficient enzyme conjugate.

9. The method of claim 1, further providing one or more inactive pro-enzymes in the detection zone.

10. The method of claim 1, wherein the activity of the proficient enzyme conjugate is detected by monitoring the effect of the proficient enzyme assisted reaction; wherein the effect of the proficient enzyme assisted reaction is proton release.

11. The method of claim 10, wherein the proton release produces a pH change; wherein the pH change is determined using a pH sensitive potassium I-hydroxy-4-[l-(2-hydroxyethylsulphonyl) phenylazo]-naphthalene-2-sulphonate indicator polymer that is immobilized on the membrane; or wherein the pH change is determined using a pH sensitive cellulose acetate coupled dye.

12. The method of claim 1, wherein the activity of the proficient enzyme conjugate is detected by colorimetric change; wherein the activity of the proficient enzyme conjugate is detected by fluorescence emission; or wherein the activity of the proficient enzyme conjugate is detected by electrochemical methods.

13. The method of claim 11, wherein the colorimetric change is due to silver ion reduction.

14. The method of claim 1, wherein the activity of the proficient enzyme conjugate is detected by precipitation of a soluble component; wherein the soluble component is a protein or pH sensitive polymer; wherein the protein is BSA; and/or wherein the pH sensitive polymer is selected from the group consisting of methyl acrylic acid, methyl methacrylate, methacrylic acid 2-(dimethylamino) ethyl ester, and N-hydroxymethyl acrylamide.

15. The method of claim 1 further comprising adding a pre-reporter carrier to the test sample prior to contacting the sample ~~application~~loading zone with the test sample.

16. The method of claim 1 further comprising an absorptive pad at the distal end of the chromatographic medium.

17. The method of claim 1, wherein the substrate is allyl hexanoate and proficient enzyme is carboxylesterase.

18. A lateral flow assay device for detecting the presence of an analyte within a test sample, the lateral flow assay device comprising:

a chromatographic medium that includes:

a sample loading zone located upstream of a detection zone;

a reporting carrier zone located between the sample loading zone and a detection zone, wherein said reporting carrier zone comprises a reporting carrier capable of forming a complex with the analyte said reporting carrier comprising a carrier and one or more proficient enzyme cassettes; and

a detection zone adjacent to the reporting carrier zone, wherein the detection zone comprises a capture component for the analyte and an indicator, wherein the indicator detects a reaction of a substrate in the presence of a proficient enzyme thereby detecting the presence of the analyte.

19. The lateral flow assay device of claim 18 further comprising a control zone, wherein the control zone comprises a capture component for the reporting carrier and an indicator for detecting a reaction of a substrate in the presence of a proficient enzyme; wherein the product of the enzyme reporting carrier complex and substrate is detected thereby detecting the presence of the reporting carrier.

20. The lateral flow assay device of claim 18 wherein the proficient enzyme conjugate comprises an antibody; wherein the proficient enzyme conjugate comprises a nucleic acid; or wherein the proficient enzyme conjugate comprises a proficient enzyme selected from the group consisting of: urease, phosphocholine phosphatase, betagalactosidase, xylose reductase, shikimate dehydrogenase, malate dehydrogenase, carboxylesterase, neopullulanase, subtilisin, 4-phytase, acetylcholinesterase, laccase, bacterial leucyl aminopeptidase, tripeptidyl-peptidase I, coagulation factor VIIa, trypsin, beta-fructofuranosidase.

21. The lateral flow assay device of claim 18 wherein the antibody interacts non-covalently with a proficient enzyme to form the proficient enzyme conjugate.

22. The lateral flow assay device of claim 18 wherein the nucleic acid is covalently attached to a proficient enzyme to form the proficient enzyme conjugate.

23. The lateral flow assay device of claim 18 further providing one or more inactive pro-enzymes in the detection zone.

24. The lateral flow assay device of claim 18, wherein the activity of the proficient enzyme conjugate is detected by monitoring the effect of the proficient enzyme assisted reaction; wherein the indicator is a pH sensitive indicator; wherein the pH sensitive indicator is Potassium I-hydroxy-4-[l-(2-hydroxyethylsulphonyl) phenylazo]-naphthalene-2-sulphonate; and/or wherein the indicator is a pH sensitive indicator is a pH sensitive cellulose acetate coupled dye.

25. The lateral flow assay device of claim 18, wherein the reporting carrier zone comprises a conjugate pad.

26. The lateral flow assay device of claim 18, wherein the sample loading zone comprises a sample loading pad.

27. The lateral flow assay device of claim 18, further comprising a rigid or flexible backing material.

28. The lateral flow assay device of claim 18, wherein the antibody is associated with the proficient enzyme by noncovalent interactions.

29. The lateral flow assay device of claim 18, wherein the antibody or nucleic acid is covalently attached to the proficient enzyme.

30. The lateral flow assay device of claim 18, further comprising a source of one or more inactive pro-enzymes.

31. The lateral flow assay device of claim 18, wherein the detection zone detects a product of the proficient enzyme by detection of a pH change; wherein the detection zone detects the product of the proficient enzyme by colorimetric change; wherein the detection zone detects the product of the proficient enzyme by fluorescence emission; wherein the detection zone detects the product of the proficient enzyme by electrochemical methods; the detection zone detects the product of the proficient enzyme by precipitation of a soluble component.

32. The lateral flow assay device of claim 18, wherein the pH change is determined using a pH sensitive hydrogel.

33. The lateral flow assay device of claim 32, wherein the colorimetric change is due to silver ion reduction.

34. The lateral flow assay device of claim 18, wherein the soluble component is a protein or pH sensitive polymer; or wherein the protein is BSA.

35. The lateral flow assay device of claim 34, wherein the pH sensitive polymer is selected from the group consisting of methyl acrylic acid, methyl methacrylate, methacrylic acid 2-(dimethylamino) ethyl ester, and N-hydroxymethyl acrylamide.

36. The lateral flow assay device of claim 18 further comprising an absorptive pad at the distal end of the chromatographic medium.

37. The lateral flow assay device of claim 18, wherein the substrate is allyl hexanoate and proficient enzyme is carboxylesterase.

38. A lateral flow assay kit for detecting the presence of an analyte within a test sample, the lateral flow assay device comprising:

a porous membrane comprising:

a sample loading zone;

a reporting carrier zone down stream of the loading zone, wherein said reporting carrier zone comprises a reporting carrier capable of forming a complex with the analyte;

a detection zone down stream of the reporting carrier zone, wherein the detection zone is adjacent to the reporting carrier zone and comprises a capture component and an indicator; and

a substrate for the proficient enzyme; wherein the substrate is applied to the detection zone after the test sample has been allowed to flow through the lateral flow device and the product of the enzyme and substrate is detected.

39. The lateral flow assay kit of claim 38 further comprising a control zone, wherein the control zone comprises a capture component for the reporting carrier and an indicator for detecting a reaction of a substrate in the presence of a proficient enzyme; wherein the product of the enzyme reporting carrier complex and substrate is detected thereby detecting the presence of the reporting carrier.

40. The lateral flow assay kit of claim 38 wherein the proficient enzyme conjugate comprises an antibody; wherein the proficient enzyme conjugate comprises a nucleic acid; or wherein the proficient enzyme conjugate comprises a proficient enzyme selected from the group consisting of: urease, phosphocholine phosphatase, betagalactosidase, xylose reductase, shikimate dehydrogenase, malate dehydrogenase, carboxylesterase, neopullulanase, subtilisin, 4-phytase, acetylcholinesterase, laccase, bacterial leucyl aminopeptidase, tripeptidyl-peptidase I, coagulation factor VIIa, trypsin, beta-fructofuranosidase.

41. The lateral flow assay kit of claim 38 wherein the antibody interacts non-covalently with a proficient enzyme to form the proficient enzyme conjugate.

42. The lateral flow assay kit of claim 38 wherein the nucleic acid is covalently attached to a proficient enzyme to form the proficient enzyme conjugate.

43. The lateral flow assay kit of claim 38 further providing one or more inactive pro-enzymes in the detection zone.

44. The lateral flow assay kit of claim 38, wherein the activity of the proficient enzyme conjugate is detected by monitoring the effect of the proficient enzyme assisted reaction.

45. The lateral flow assay kit of claim 38, wherein the indicator is a pH sensitive indicator; wherein the pH sensitive indicator is Potassium I-hydroxy-4-[l-(2-hydroxyethylsulphonyl) phenylazo]-naphthalene-2-sulphonate; wherein the indicator is a pH sensitive cellulose acetate coupled dye.

46. The lateral flow assay kit of claim 38, wherein the reporting carrier zone comprises a conjugate pad.

47. The lateral flow assay kit of claim 38, wherein the sample loading zone comprises a sample loading pad.

48. The lateral flow assay kit of claim 38, further comprising a rigid or flexible backing material.

49. The lateral flow assay kit of claim 38, wherein the antibody is associated with the proficient enzyme by noncovalent interactions.

50. The lateral flow assay kit of claim 38, wherein the antibody or nucleic acid is covalently attached to the proficient enzyme.

51. The lateral flow assay kit of claim 38, further comprising a source of one or more inactive pro-enzymes.

52. The lateral flow assay kit of claim 38, wherein the detection zone detects a product of the proficient enzyme by detection of a pH change.

53. The lateral flow assay kit of claim 38, wherein the pH change is determined using a pH sensitive hydrogel.

54. The lateral flow assay kit of claim 38, wherein the detection zone detects the product of the proficient enzyme by colorimetric change; wherein the detection zone detects the product of the proficient enzyme by fluorescence emission; wherein the detection zone detects the product of the proficient enzyme by electrochemical methods; wherein the detection zone detects the product of the proficient enzyme by precipitation of a soluble component.

55. The lateral flow assay kit of claim 54, wherein the colorimetric change is due to silver ion reduction.

56. The lateral flow assay device of claim 54, wherein the soluble component is a protein or pH sensitive polymer; or wherein the protein is BSA.

57. The lateral flow assay device of claim 56, wherein the pH sensitive polymer is selected from the group consisting of methyl acrylic acid, methyl methacrylate, methacrylic acid 2-(dimethylamino) ethyl ester, and N-hydroxymethyl acrylamide.

58. The lateral flow assay device of claim 37, further comprising an absorptive pad at the distal end of the chromatographic medium.

59. The lateral flow assay device of claim 37, wherein the substrate is allyl hexanoate and proficient enzyme is carboxylesterase.

60. The method of claim 1, wherein the test sample is taken from the group consisting of blood, serum, plasma, urine, saliva, cerebrospinal fluid, lymph fluids, tissue and tissue and cell extracts, cell culture supernatants, biopsy specimens, paraffin embedded tissue, soil, fruit, juice, oil, milk, food and water.

61. The lateral flow assay device of claim 18, wherein the test sample is taken from the group consisting of blood, serum, plasma, urine, saliva, cerebrospinal fluid, lymph fluids, tissue and tissue and cell extracts, cell culture supernatants, biopsy specimens, paraffin embedded tissue, soil, fruit, juice, oil, milk, food and water.

62. The lateral flow assay kit of claim 38, wherein the test sample is taken from the group consisting of blood, serum, plasma, urine, saliva, cerebrospinal fluid, lymph fluids, tissue and tissue and cell extracts, cell culture supernatants, biopsy specimens, paraffin embedded tissue, soil, fruit, juice, oil, milk, food and water.