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 sample loading zone located upstream of a detection zone;

a pre-reporting carrier added to sample solution containing the test sample prior to loading the sample solution to the lateral flow assay device, wherein the pre-reporting carrier binds with the analyte to form a pre-reporting carrier-analyte complex;

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 containing at least one proficient enzyme conjugate,

wherein when the pre-reporting carrier-analyte complex comes into contact with the reporting carrier zone, the reporting carrier forms an association with the pre-reporting carrier-analyte complex; and

a detection zone adjacent to the reporting carrier zone, wherein the detection zone comprises a capture component for the analyte, and an indicator for visual indication,

wherein the association of the reporting carrier with the pre-reporting carrier-analyte complex immobilizes the reporting carrier via the interaction of the analyte and the capture component;

ii) contacting the sample 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 the analyte and the reporting carrier comprising a proficient enzyme; 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 molecule; or wherein the analyte is a nucleic acid.~~

~~5~~4. 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~~5. The method of claim 3, where the reaction temperature is maintained ~~to about~~ between 4°C to 95°C~~, to about 95°C, or higher~~ while still maintaining enzyme activity.

~~7~~6. The method of claim ~~5~~4, 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~~7. The method of claim ~~5~~4, wherein the nucleic acid is covalently attached to a proficient enzyme to form the proficient enzyme conjugate.

~~9~~8. The method of claim 1, further providing one or more inactive pro-enzymes in the detection zone.

~~10~~9. 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~~10. The method of claim ~~10~~9, 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~~11. 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~~12. The method of claim ~~11~~10, wherein the colorimetric change is due to silver ion reduction.

~~14~~13. The method of claim 1, wherein the activity of the proficient enzyme conjugate is detected by precipitation of a soluble component; and wherein the soluble component is a protein or pH sensitive polymer; and wherein the protein is BSA; ~~and/or~~ and 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~~14. The method of claim 1 further comprising an absorptive pad at the distal end of the chromatographic medium.

~~16~~15. The method of claim 1, wherein the substrate is allyl hexanoate and proficient enzyme is carboxylesterase.

~~17~~16. 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 pre-reporting carrier added to sample solution containing the test sample prior to loading the sample solution to the lateral flow assay device, wherein the pre-reporting carrier binds with the analyte to form a pre-reporting carrier-analyte complex;

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 containing at least one proficient enzyme conjugate,

wherein when the pre-reporting carrier-analyte complex comes into contact with the reporting carrier zone, the reporting carrier forms an association with the pre-reporting carrier-analyte complex; and

a detection zone adjacent to the reporting carrier zone, wherein the detection zone comprises a capture component for the analyte, and an indicator for visual indication,

wherein the association of the reporting carrier with the pre-reporting carrier-analyte complex immobilizes the reporting carrier via the interaction of the analyte and the capture component,

wherein the indicator detects a reaction of a substrate in the presence of a proficient enzyme thereby detecting the presence of the analyte.

~~18~~17. The lateral flow assay device of claim ~~17~~16 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.

~~19~~18. The lateral flow assay device of claim ~~17~~16 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.

~~20~~19. The lateral flow assay device of claim ~~19~~18 wherein the antibody interacts non-covalently with a proficient enzyme to form the proficient enzyme conjugate.

~~21~~20. The lateral flow assay device of claim ~~19~~18 wherein the nucleic acid is covalently attached to a proficient enzyme to form the proficient enzyme conjugate.

~~22~~21. The lateral flow assay device of claim ~~17~~16 further providing one or more inactive pro-enzymes in the detection zone.

~~23~~22. The lateral flow assay device of claim ~~17~~16, wherein the activity of the proficient enzyme conjugate is detected by monitoring the effect of the proficient enzyme assisted reaction; and wherein the indicator is a pH sensitive indicator; and wherein the pH sensitive indicator is Potassium I-hydroxy-4-[l-(2-hydroxyethylsulphonyl) phenylazo]-naphthalene-2-sulphonate; ~~and/or~~ and wherein the indicator is a pH sensitive indicator which is a pH sensitive cellulose acetate coupled dye.

~~24~~23. The lateral flow assay device of claim ~~17~~16, wherein the reporting carrier zone comprises a conjugate pad.

~~25~~24. The lateral flow assay device of claim ~~17~~16, wherein the sample loading zone comprises a sample loading pad.

~~26~~25. The lateral flow assay device of claim ~~17~~16, further comprising a rigid or flexible backing material.

~~27~~26. The lateral flow assay device of claim ~~19~~18, wherein the antibody is associated with the proficient enzyme by noncovalent interactions.

~~28~~27. The lateral flow assay device of claim ~~19~~18, wherein the antibody or nucleic acid is covalently attached to the proficient enzyme.

~~29~~28. The lateral flow assay device of claim ~~17~~16, further comprising a source of one or more inactive pro-enzymes.

~~30~~29. The lateral flow assay device of claim ~~17~~16, 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~~ or precipitation of a soluble component.

~~31~~30. The lateral flow assay device of claim ~~17~~16, wherein the pH change is determined using a pH sensitive hydrogel.

~~32~~31. The lateral flow assay device of claim ~~31~~30, wherein the colorimetric change is due to silver ion reduction.

~~33~~32. The lateral flow assay device of claim ~~30~~29, wherein the soluble component is a protein or pH sensitive polymer; or wherein the protein is BSA.

~~34~~33. The lateral flow assay device of claim ~~33~~32, 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.

~~35~~34. The lateral flow assay device of claim ~~17~~16 further comprising an absorptive pad at the distal end of the chromatographic medium.

~~36~~35. The lateral flow assay device of claim ~~17~~16, wherein the substrate is allyl hexanoate and proficient enzyme is carboxylesterase.

~~37~~36. 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 pre-reporting carrier added to sample solution containing the test sample prior to loading the sample solution to the lateral flow assay device, wherein the pre-reporting carrier binds with the analyte to form a pre-reporting carrier-analyte complex;

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, said reporting carrier comprising a carrier and one or more proficient enzyme cassettes containing at least one proficient enzyme conjugate,

wherein when the pre-reporting carrier-analyte complex comes into contact with the reporting carrier zone, the reporting carrier forms an association with the pre-reporting carrier-analyte complex;

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 for the analyte, and an indicator for visual indication,

wherein the association of the reporting carrier with the pre-reporting carrier-analyte complex immobilizes the reporting carrier via the interaction of the analyte and the capture component; 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.

~~38~~37. The lateral flow assay kit of claim ~~37~~36 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.

~~39~~38. The lateral flow assay kit of claim ~~37~~36 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.

~~40~~39. The lateral flow assay kit of claim ~~39~~38 wherein the antibody interacts non-covalently with a proficient enzyme to form the proficient enzyme conjugate.

~~41~~40. The lateral flow assay kit of claim ~~39~~38 wherein the nucleic acid is covalently attached to a proficient enzyme to form the proficient enzyme conjugate.

~~42~~41. The lateral flow assay kit of claim ~~37~~36 further providing one or more inactive pro-enzymes in the detection zone.

~~43~~42. The lateral flow assay kit of claim ~~37~~36, wherein the activity of the proficient enzyme conjugate is detected by monitoring the effect of the proficient enzyme assisted reaction.

~~44~~43. The lateral flow assay kit of claim ~~37~~36, wherein the indicator is a pH sensitive cellulose acetate coupled dye or a pH sensitive indicator~~;~~, and 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~~.

~~45~~44. The lateral flow assay kit of claim ~~37~~36, wherein the reporting carrier zone comprises a conjugate pad.

~~46~~45. The lateral flow assay kit of claim ~~37~~36, wherein the sample loading zone comprises a sample loading pad.

~~47~~46. The lateral flow assay kit of claim ~~37~~36, further comprising a rigid or flexible backing material.

~~48~~47. The lateral flow assay kit of claim ~~39~~38, wherein the antibody is associated with the proficient enzyme by noncovalent interactions.

~~49~~48. The lateral flow assay kit of claim ~~39~~38, wherein the antibody ~~or~~ and nucleic acid ~~is~~ are covalently attached to the proficient enzyme.

~~50~~49. The lateral flow assay kit of claim ~~37~~36, further comprising a source of one or more inactive pro-enzymes.

~~51~~50. The lateral flow assay kit of claim ~~37~~36, wherein the detection zone detects a product of the proficient enzyme by detection of a pH change.

~~52~~51. The lateral flow assay kit of claim ~~37~~36, wherein the pH change is determined using a pH sensitive hydrogel.

~~53~~52. The lateral flow assay kit of claim ~~37~~36, 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~~ or precipitation of a soluble component.

~~54~~53. The lateral flow assay kit of claim ~~53~~52, wherein the colorimetric change is due to silver ion reduction.

~~55~~54. The lateral flow assay device of claim ~~53~~52, wherein the soluble component is a protein or pH sensitive polymer; or wherein the protein is BSA.

~~56~~55. The lateral flow assay device of claim ~~55~~54, 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.

~~57~~56. The lateral flow assay device of claim ~~37~~36, further comprising an absorptive pad at the distal end of the chromatographic medium.

~~58~~57. The lateral flow assay device of claim ~~37~~36, wherein the substrate is allyl hexanoate and proficient enzyme is carboxylesterase.

~~59. 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.~~

~~60. The lateral flow assay device of claim 17, 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 kit of claim 37, 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.~~