can be further amplified by fluorescence methods. Each PCR amplicon that is labeled by fluorescent tags(s) can produce 1000 or 10,000 more protons in a single excitation/measurement period when a fluorescence beacon or probe is used to detect the amplicon. Another example is a Campylobacter-like organism test, in which H. pylori cells multiplies in the culture and is thus amplified, and where urease secreted from the amplified cells can be detected after the number of cells passes the detection threshold. Yet another example is the screening of MRS A. This detection method differentiates the colonies of the multiplied and amplified cells on a culture medium.

[0005] However, current methods which rely on removal of various combinations of background interference and amplification detection have not filled the need for a robust, cost effective, rapid, easy to use detection method for targets, which is compatible in resource limited environments. The requirement for an electrical power source to perform the current tests by powering the instrument or providing the temperature incubation has restricted access to this technology in resource-poor regions or conditions. The time required to practice the current methods is long. Many hours or days are required to produce enough biological targets to generate sufficient detection signals. The amplification step used for pathogen detection, metabolite determination, or nucleic acid sequence detection usually requires expensive laboratory instruments and trained specialists to run the instruments. In the case of PCR, care is required to handle labile reagents, and special diligence is essential to avoid contamination between samples. Moreover, the instruments required to perform PCR are expensive and complex. The above considerations are severe limitations that prevent POC (point of care) use or provide rapid sample-to-result in less than 30 minutes. ~~The~~Embodiments of the present invention provides solutions to these and other needs.

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 ~~for~~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, wherein the detection zone comprises a capture component for the analyte and an indicator; ii) Contacting the sample application 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, 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 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)

[0016] In a further embodiment of this aspect, the detection of the product of the proficient enzyme is by colorimetric change, where the colorimetric change is due to fluctuations as a result of proficient enzyme assisted reaction, for example, protonation or deprotonation of the dye material or silver ion reduction.

[0017] In yet further embodiments of this aspect, the detection of the product of the proficient enzyme is by precipitation as the result of the effect of the proficient enzyme-assisted reaction, for example, precipitation of a soluble component, which can be a protein including BSA or pH sensitive polymer, which forms aggregates with changes in pH. The change of pH results in the shift of the surface charge of the polymer. As a consequence of the surface charge variation, the 3-D structure of the polymer molecules changes and the hydrophobic part of the polymer is exposed. The exposure of the hydrophobic region of the polymer increases the entropy. These hydrophobic parts of the polymer would form noncovalent interactions thus forming aggregates. Examples of pH sensitive polymers include: methyl acrylic acid, methyl methacrylate, methacrylic acid 2-(dimethylamino) ethyl ester, and N-hydroxymethyl acrylamide.

[0018] ~~In another aspect, the present invention provides~~Disclosed herein is a molecular diagnostic device that has (a) a sample loading zone, (b) a reporting carrier zone, where the reporting carrier zone contains a reporting carrier and a proficient enzyme, (c) a source of substrate for the proficient enzyme; and (d) a detection zone, where the detection zone contains a capture component.

[0019] In some embodiments of this aspect, the device also has a positive control zone.

[0020] In some embodiments of this aspect, the sample loading zone is a sample loading pad.

[0021] In some embodiments of this aspect, the reporting carrier zone is a conjugate pad.

[0022] In some embodiments of this aspect, the detection area comprises a porous membrane.

0030] In yet further embodiments of this aspect, the detection of the product of the proficient enzyme is by precipitation as the result of the fluctuation of the proficient enzyme-assisted reaction, for example, precipitation of a soluble component, which can be a protein including BSA or pH sensitive polymer. Examples of pH sensitive polymers include: methyl acrylic acid, methyl methacrylate, methacrylic acid 2-(dimethylamino) ethyl ester, and N-hydroxymethyl acrylamide.

[0031] ~~In another further aspect, the present invention provides~~Disclosed herein is a molecular diagnostic device that has (a) a sample loading zone, (b) a pre-reporting carrier zone, if there is one, where the pre-reporting carrier zone contains a reporting carrier and a proficient enzyme, (c) a source of substrate for the proficient enzyme and a reporting carrier for the pre-reporting carrier; and (d) a detection zone, where the detection zone contains a capture component.

[0032] ~~In another further aspect, the present invention provides~~Disclosed herein is a molecular diagnostic device that has a detection zone, where the detection zone contains a capture component. The pre-reporting carrier, reporting carrier, or substrate could be added to the detection sequentially.

[0033] ~~In another further aspect, the present invention provides~~Disclosed herein is a molecular diagnostic device that has a detection zone, where the detection zone contains a capture component. The detection zone could be placed and moved amongst multiple reagent containers that contain one or more of the following: pre-reporting carrier, reporting carrier, and substrate.

[0034] ~~In yet another further aspect, the present invention provides~~Disclosed herein is a molecular diagnostic device that utilizes combinations of the process highlighted in paragraphs [0031], [0032], and [0033].

BRIEF DESCRIPTION OF THE DRAWINGS

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 analyte is a small molecule.~~

~~6. The method of claim 1, wherein the analyte is a nucleic acid~~.

~~7~~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.

~~8. The method of claim 1, wherein the proficient enzyme conjugate comprises a nucleic acid.~~

~~9. The method of claim 1, 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.~~

~~10~~ 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.

~~11~~ 7. The method of claim ~~7~~ 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.

~~12~~ 8. The method of claim ~~8~~ 5, wherein the nucleic acid is covalently attached to a proficient enzyme to form the proficient enzyme conjugate.

~~13. The method of claim 7, wherein the antibody is covalently attached to a proficient enzyme to form the proficient enzyme conjugate.~~

~~14~~ 9. The method of claim 1, further providing one or more inactive pro-enzymes in the detection zone.

~~15~~ 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.

~~16. The method of claim 15, wherein the effect of the proficient enzyme assisted reaction is proton release.~~

~~17~~ 11. The method of claim ~~16~~ 10, wherein the proton release produces a pH change; wherein the pH change is determined using a pH sensitive potassium I-hydroxy-4-[1-(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.

~~18. The method of claim 17, wherein the pH change is determined using a pH sensitive potassium I-hydroxy-4-[1-(2-hydroxyethylsulphonyl) phenylazo]-naphthalene-2-sulphonate indicator polymer that is immobilized on the membrane.~~

~~19. The method of claim 17, wherein the pH change is determined using a pH sensitive cellulose acetate coupled dye.~~

~~20~~ 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.

~~21. The method of claim 1, wherein the activity of the proficient enzyme conjugate is detected by fluorescence emission.~~

~~22. The method of claim 1, wherein the activity of the proficient enzyme conjugate is detected by electrochemical methods.~~

~~23~~ 13. The method of claim ~~18~~ 12, wherein the colorimetric change is due to silver ion reduction.

~~24~~ 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.

~~25. The method of claim 24, wherein the soluble component is a protein or pH sensitive polymer.~~

~~26. The method of claim 25, wherein the protein is BSA.~~

~~27. The method of claim 25, 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.~~

~~28~~ 15. The method of claim 1 further comprising adding a pre-reporter carrier to the test sample prior to contacting the sample application zone with the test sample.

~~29~~ 16. The method of claim 1 further comprising an absorptive pad at the distal end of the chromatographic medium.

~~30~~ 17. The method of claim 1, wherein the substrate is allyl hexanoate and proficient enzyme is carboxylesterase.

~~31~~ 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, 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.

~~32~~ 19. The lateral flow assay device of claim ~~31~~ 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.

~~33~~ 20. The lateral flow assay device of claim ~~31~~ 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.

~~34. The lateral flow assay device of claim 31 wherein the proficient enzyme conjugate comprises a nucleic acid.~~

~~35. The lateral flow assay device of claim 31 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.~~

~~36~~ 21. The lateral flow assay device of claim ~~31~~ 18 wherein the antibody interacts non-covalently with a proficient enzyme to form the proficient enzyme conjugate.

~~37~~ 22. The lateral flow assay device of claim ~~31~~ 18 wherein the nucleic acid is covalently attached to a proficient enzyme to form the proficient enzyme conjugate.

~~38~~ 23. The lateral flow assay device of claim ~~31~~ 18 further providing one or more inactive pro-enzymes in the detection zone.

~~39~~ 24. The lateral flow assay device of claim ~~31~~ 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 1-hydroxy-4-[1-(2-hydroxyethylsulphonyl) phenylazo]-naphthalene-2-sulphonate; and/or wherein the indicator is a pH sensitive indicator is a pH sensitive cellulose acetate coupled dye.

~~40. The lateral flow assay device of claim 31, wherein the indicator is a pH sensitive indicator.~~

~~41. The lateral flow assay device of claim 31, wherein the indicator is a pH sensitive indicator is Potassium 1-hydroxy-4-[1-(2-hydroxyethylsulphonyl) phenylazo]-naphthalene-2-sulphonate.~~

~~42. The lateral flow assay device of claim 31, wherein the indicator is a pH sensitive indicator is a pH sensitive cellulose acetate coupled dye.~~

~~43~~ 25. The lateral flow assay device of claim ~~31~~ 18, wherein the reporting carrier zone comprises a conjugate pad.

~~44~~ 26. The lateral flow assay device of claim ~~31~~ 18, wherein the sample loading zone comprises a sample loading pad.

~~45~~ 27. The lateral flow assay device of claim ~~31~~ 18, further comprising a rigid or flexible backing material.

~~46~~ 28. The lateral flow assay device of claim ~~31~~ 18, wherein the antibody is associated with the proficient enzyme by noncovalent interactions.

~~47~~ 29. The lateral flow assay device of claim ~~31~~ 18, wherein the antibody or nucleic acid is covalently attached to the proficient enzyme.

~~48~~ 30. The lateral flow assay device of claim ~~31~~ 18, further comprising a source of one or more inactive pro-enzymes.

~~49~~ 31. The lateral flow assay device of claim ~~31~~ 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.

~~50~~ 32. The lateral flow assay device of claim ~~31~~ 18, wherein the pH change is determined using a pH sensitive hydrogel.

~~51. The lateral flow assay device of claim 31, wherein the detection zone detects the product of the proficient enzyme by colorimetric change.~~

~~52. The lateral flow assay device of claim 31, wherein the detection zone detects the product of the proficient enzyme by fluorescence emission.~~

~~53. The lateral flow assay device of claim 31, wherein the detection zone detects the product of the proficient enzyme by electrochemical methods.~~

~~54~~ 33. The lateral flow assay device of claim ~~51~~ 31, wherein the colorimetric change is due to silver ion reduction.

~~55. The lateral flow assay device of claim 31, the detection zone detects the product of the proficient enzyme by precipitation of a soluble component.~~

~~56~~ 34. The lateral flow assay device of claim ~~31~~ 18, 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 protein is BSA.~~

~~58~~ 35. The lateral flow assay device of claim ~~56~~ 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.

~~59~~ 36. The lateral flow assay device of claim ~~31~~ 18 further comprising an absorptive pad at the distal end of the chromatographic medium.

~~60~~ 37. The lateral flow assay device of claim ~~31~~ 18, wherein the substrate is allyl hexanoate and proficient enzyme is carboxylesterase.

~~61~~ 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 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.

~~62~~ 39. The lateral flow assay kit of claim ~~61~~ 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.

~~63~~ 40. The lateral flow assay kit of claim ~~61~~ 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.

~~64. The lateral flow assay kit of claim 61 wherein the proficient enzyme conjugate comprises a nucleic acid.~~

~~65. The lateral flow assay kit of claim 61 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.~~

~~66~~ 41. The lateral flow assay kit of claim ~~61~~ 38 wherein the antibody interacts non-covalently with a proficient enzyme to form the proficient enzyme conjugate.

~~67~~ 42. The lateral flow assay kit of claim ~~61~~ 38 wherein the nucleic acid is covalently attached to a proficient enzyme to form the proficient enzyme conjugate.

~~68~~ 43. The lateral flow assay kit of claim ~~61~~ 38 further providing one or more inactive pro-enzymes in the detection zone.

~~69~~ 44. The lateral flow assay kit of claim ~~61~~ 38, wherein the activity of the proficient enzyme conjugate is detected by monitoring the effect of the proficient enzyme assisted reaction.

~~70~~ 45. The lateral flow assay kit of claim ~~61~~ 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.

~~71. The lateral flow assay kit of claim 70, wherein the indicator is a pH sensitive indicator is Potassium I-hydroxy-4-[l-(2-hydroxyethylsulphonyl) phenylazo]-naphthalene-2-sulphonate.~~

~~72. The lateral flow assay kit of claim 70, wherein the indicator is a pH sensitive cellulose acetate coupled dye.~~

~~73~~ 46. The lateral flow assay kit of claim ~~61~~ 38, wherein the reporting carrier zone comprises a conjugate pad.

~~74~~ 47. The lateral flow assay kit of claim ~~61~~ 38, wherein the sample loading zone comprises a sample loading pad.

~~75~~ 48. The lateral flow assay kit of claim ~~61~~ 38, further comprising a rigid or flexible backing material.

~~76~~ 49. The lateral flow assay kit of claim ~~61~~ 38, wherein the antibody is associated with the proficient enzyme by noncovalent interactions.

~~77~~ 50. The lateral flow assay kit of claim ~~61~~ 38, wherein the antibody or nucleic acid is covalently attached to the proficient enzyme.

~~78~~ 51. The lateral flow assay kit of claim ~~61~~ 38, further comprising a source of one or more inactive pro-enzymes.

~~79~~ 52. The lateral flow assay kit of claim ~~61~~ 38, wherein the detection zone detects a product of the proficient enzyme by detection of a pH change.

~~80~~ 53. The lateral flow assay kit of claim ~~79~~ 38, wherein the pH change is determined using a pH sensitive hydrogel.

~~81~~ 54. The lateral flow assay kit of claim ~~61~~ 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.

~~82. The lateral flow assay kit of claim 61, wherein the detection zone detects the product of the proficient enzyme by fluorescence emission.~~

~~83. The lateral flow assay kit of claim 61, wherein the detection zone detects the product of the proficient enzyme by electrochemical methods.~~

~~84~~ 55. The lateral flow assay kit of claim ~~81~~ 54, wherein the colorimetric change is due to silver ion reduction.

~~83. The lateral flow assay device of claim 61, the detection zone detects the product of the proficient enzyme by precipitation of a soluble component.~~

~~84~~ 56. The lateral flow assay device of claim ~~83~~ 54, wherein the soluble component is a protein or pH sensitive polymer; or wherein the protein is BSA.

~~85. The lateral flow assay device of claim 84, wherein the protein is BSA.~~

~~86~~ 57. The lateral flow assay device of claim ~~84~~ 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.

~~87~~ 58. The lateral flow assay device of claim ~~60~~ 37, further comprising an absorptive pad at the distal end of the chromatographic medium.

~~88~~ 59. The lateral flow assay device of claim ~~60~~ 37, wherein the substrate is allyl hexanoate and proficient enzyme is carboxylesterase.