

METHODS AND DEVICES FOR DETECTING ANALYTES

FIELD

5 The present invention concerns methods, systems and kits of parts suitable for detecting the presence of analytes within samples. When target analytes are present in the sample, sandwich complexes are formed comprising the analyte, a magnetically moveable particle and a catalyst. Complexes are separated from other components of the sample by magnetic separation and detected.

10 BACKGROUND

High sensitivity immunoassays are a crucial tool in the point of care diagnostics industry. The need for high sensitivity immunoassays is increasing as new and clinically important biomarkers are identified, many of which are present in very low concentrations. Lab based high sensitivity immunoassays exist but typically require
15 numerous assay steps which need to be carried out by trained laboratory technicians. Many immunoassays incorporate labelled reagents conjugated on to antibodies which bind specifically to target antigens.

Enzyme-linked immunosorbent assays (ELISA) are used to detect the presence of an analyte, such as an antigen, in a sample through the use of probes (such as
20 antibodies) that bind to the analyte. The analyte is immobilised on a solid surface to which the probe is applied such that the probe binds to the analyte. The probe is typically linked to a redox catalyst such as an enzyme. Thus, when analyte is present, a complex comprising analyte, probe and redox catalyst is formed. Any unbound probe is removed from the assay by washing. Then, a substrate of the redox catalyst is
25 added to the assay such that, when analyte is present, the redox catalyst of the complex formed is able to act on the substrate and catalyse its conversion to another product. This conversion reaction may be detectable, for example, by a change in colour, light emission or a change in electrical signal.

Sandwich ELISA is a type of ELISA in which the analyte is not itself directly
30 immobilised on a surface. Rather, an additional probe (further to that bonded to a redox catalyst), such as another antigen, is used to bind to a different position on the analyte and immobilise the analyte on a surface. The result is the formation of a sandwich complex comprising the immobilisable probe, the target analyte and the probe bonded to a redox catalyst. Again, any unbound probe is removed the assay by
35 washing before addition of the substrate of the redox catalyst and, when analyte is

present, the redox catalyst of the sandwich complex formed is able to act on the substrate and catalyse its conversion to another product. See D. Neupane and K. J. Stine, *Appl. Sci.*, 2021, 11, 7087 for a review of electrochemical sandwich assays for biomarkers incorporating aptamers, antibodies and nanomaterials for detection of specific protein biomarkers.

These assays require wash steps to remove any unbound labelled antibody so that after the wash step the only redox catalyst that remains is that bonded to a probe which is bonded to the captured analyte (i.e. that forming part of a sandwich complex with the target analyte). In this way, a signal can be generated which is proportional to the amount of analyte present in the sample. Many products are currently under development which attempt to automate the assay approach, often by carrying out an automated wash step. Such automated wash steps require complex and expensive equipment and often require special wash solutions. These existing automated products are expensive, complex and have multiple potential failure modes. Expensive instrumentation in turn means that such point of care solutions cannot be provided to users at low cost and this limits product adoption.

Immunosensing probes comprising horseradish peroxidase (HRP)-labelled anti-carcinoembryonic antigen antibody-functionalised magnetic beads are described by N. Gan, L. Jia and L. Zheng in *Int. J. Mol. Sci.*, 2011, 12(11), 7410-7423. As part of the synthesis of the immunosensing probes, it is described that unadsorbed antibodies can be separated from the probes by magnetic separation, followed by washing the final mixture. The immunosensing probes are used as part of sandwich assays in which sandwich complexes are immobilised on the surface of an electrode and analytes are detected electrochemically. In order to update the electrode surface, it is described that the magnetic nanoprobe can be freed from the electrode surface by mixing the solution comprising the electrode in the presence of a 0.3 mT magnetic field, wherein the field lines are oriented perpendicular to the direction of the surface of the electrode.

In WO 2010/004241 (The Secretary of State for Innovation Universities & Skills of her Majesty's Britannic Government), an assay is described in which an antigen becomes attached to a carrier device, a magnetic particle and to a silver sol particle. It is described that the antigens can be made to move, for example by application of a magnetic field, and can then be detected using the silver of the label. In WO 2020/032294 A1 (BBB Inc.), a further example of a biosensor using magnetic nanoparticles is described.

In WO 2007/010368 (Inverness Medical Switzerland GmbH), an assay device is described including a first reagent including a magnetic particle and a second reagent including a detectable component. The first and second reagent can each independently bind to an analyte in a sample and it is described that applying a magnetic field can selectively concentrate the detectable component in a detection zone.

In US 10,509,032 B (Alere Switzerland GmbH), an assay method and device are described. The device is configured to create a sample liquid-air interface with the sample liquid and magnetically susceptible particles can be located (via an applied magnetic field) at the liquid-air interface when a second liquid contacts the interface to form a liquid-liquid interface. The magnetically susceptible particles are configured to transport an analyte across the interface into the second liquid.

In US 2008/0160634 A1 (Intel Corp), a device for detecting an analyte in a sample is described. The device comprises a fluidic network and an integrated circuitry component. The fluidic network comprises multiple zones such as a sample zone, a cleaning zone and a detection zone. The fluidic network contains a magnetic particle and/or a signal particle. A sample containing an analyte is introduced, and the analyte interacts with the magnetic particle and/or the signal particle through affinity agents. A microcoil array or a mechanically movable permanent magnet is functionally coupled to the fluidic network, which are activatable to generate a magnetic field within a portion of the fluidic network, and move the magnetic particle from the sample zone to the detection zone. A detection element is present which detects optical or electrical signals from the signal particle, thus indicating the presence of the analyte.

Many of the assays and devices described above suffer from one or more drawbacks including low sensitivity (e.g. due to high background signal), multiple fluidic chambers, wash steps, complex and/or expensive test strip designs and/or the need for relatively large volumes of sample. There remains a need for low cost high sensitivity immunoassays that deliver the high level of target signal amplification made possible by enzyme amplification but that do not require a wash step. The immunoassays are preferably easy to use and reliably produced at low cost and high volume. The present invention provides alternative methods, systems and kits to address one or more of these needs and the above-noted problems and/or limitations.

SUMMARY

It is described herein that a target, such as an analyte, in a sample may be detected in a sandwich ELISA-type process that does not require any washing steps. When present, the target is detected as part of a sandwich complex comprising the target, a target binding moiety tethered to a magnetically moveable particle, and a target capture moiety conjugated to a catalyst. The resulting sandwich complex is movable to and immobilisable at a second position on or within a test strip by activating or generating (e.g. applying) a magnetic field. When magnetically moved to the second position, the complex is separated from unbound target capture moiety conjugated to a catalyst such that the only catalyst present at the second position is that which is conjugated to a target capture moiety that is bonded to a target.

The ability to selectively move the complex to the second position can facilitate the provision of a method and system for detecting a target in a sample without the need for wash steps. In particular, by positioning any additional reagents needed for spontaneous amplification of a reaction modulated by the catalyst (e.g. an excess of catalyst substrate and optionally also a mediator species) at only the second position can mean any signal generated by the catalyst is restricted to those catalysts which are bound as part of a complex. The signal generated by the catalysis process may be detected, for example by detecting the presence of product generated by the catalyst, or, in some cases, by detecting the presence of a converted mediator species.

In order to avoid the need for wash steps and/or complex strip designs with multiple fluid chambers, the present inventors have particularly identified that certain configurations of test strip are particularly advantageous in minimising the risk of cross-contamination from unbound/uncomplexed catalyst moving (e.g. diffusing) towards the catalyst substrate at the second position (e.g. in the detection zone) when the test strip is used in the various methods described herein. By positioning the sample inlet between the first and second positions, following the introduction of the sample fluid, the flow of sample across the first position means that any target capture moiety conjugated to a catalyst re-suspended in the sample fluid is less likely to move and/or diffuse towards the catalyst substrate positioned at the second position (as the flow of fluid is away from the second position). Thus, the risk of background signal associated with unbound/uncomplexed catalyst may be reduced. This may assist in improving the overall sensitivity of the method.

In some examples, the inventors have found that certain catalysts are able to convert catalyst substrate to another product that is detectable, for example by sensing an electrical response on application of an electrical stimulus, or optically. When

catalyst substrate is positioned on or within the test strip at the second position, the catalyst within the complex catalyses the conversion of the catalyst substrate to another product, which other product is detectable. Therefore, once the complex has been magnetically moved and immobilised at the second position, and when catalyst substrate is in excess relative to the concentration of the complex, detectable product may quickly accumulate. Detectable product is generated only when the complex is moved to the second position (optionally immobilised at the second position) and catalyst substrate is available to the complex. Detection of the product may be by sensing an electrical response on application of an electrical stimulus or optically, for example by detecting a colour change. The signal detected may be directly proportional to the concentration of the complex at the electrode, which, in turn, may be directly proportional to the concentration of target.

Where detection is by application of an electrical stimulus, the second position may be in proximity to a working electrode. The electrical stimulus applied at the working electrode may be selected such that electrons are transferred between the working electrode and detectable product, thus a response is generated only when detectable product is present. Accordingly, a response is generated only when the complex is moved to (and optionally immobilised at) the second position and catalyst substrate is available to the complex. In other words, and without being bound by theory, where the catalyst substrate present at or in proximity to the working electrode does not generate a signal (e.g. at a potential applied to the working electrode) then no signal (e.g. current) will be generated when the electrical stimulus (e.g. a measuring potential) is turned on in those cases where there is no catalyst (e.g. enzyme) present. However, where a catalyst (e.g. enzyme) is present or in proximity with the working electrode, as a result of it binding to the target to form a complex, then the catalyst substrate can be spontaneously converted to detectable product by the catalyst in proportion to the concentration of the target.

In yet further examples, when the catalyst is a redox catalyst, redox mediator and redox catalyst substrate are positioned on or within the test strip at the second position, and when the redox mediator is present in an "active" state (i.e. an oxidation state that allows for the transfer of electrons to or from the redox catalyst), the redox catalyst within the complex catalyses the conversion of the redox catalyst substrate to another product whilst simultaneously catalytically converting active redox mediator to converted redox mediator or inactive redox mediator (i.e. redox mediator in an oxidation state that does not allow for the transfer of electrons to or from the redox

catalyst). Therefore, once the complex has been magnetically moved to (and optionally immobilised at) the second position, and when active redox mediator is in excess relative to the concentration of the complex, converted (or inactive) redox mediator may quickly accumulate. Converted (or inactive) redox mediator is generated only when the complex is moved to (and optionally immobilised at) the second position and redox mediator and redox catalyst substrate are available to the complex. Converted (or inactive) redox mediator may then be detected. Detection of the converted or inactive redox mediator may be by sensing an electrical response on application of an electrical stimulus or optically, for example by detecting a colour change. The signal detected may be directly proportional to the concentration of the complex at the electrode, which, in turn, may be directly proportional to the concentration of target.

Where detection is by application of an electrical stimulus, the second position may be in proximity to a working electrode. The electrical stimulus applied at the working electrode may be selected such that electrons are transferred between the working electrode and converted or inactive redox mediator (but not unconverted or active redox mediator), thus a response is generated only when converted or inactive redox mediator is present. Accordingly, a response is generated only when the complex is moved to (and optionally immobilised at) the second position and redox mediator and redox catalyst substrate are available to the complex. In other words, and without being bound by theory, where the active or unconverted mediator present at or in proximity to the working electrode is initially in an oxidation state that does not generate a signal (e.g. at a potential applied to the working electrode) then no signal (e.g. current) will be generated when the electrical stimulus (e.g. a measuring potential) is turned on in those cases where there is no redox catalyst (e.g. enzyme) present. However, where a redox catalyst (e.g. enzyme) is present or in proximity with the working electrode, as a result of it binding to the target to form a complex, then the active or unconverted mediator can be spontaneously converted to another oxidation state (e.g. its converted or inactive state) by the catalyst in proportion to the concentration of the target.

Unexpectedly, where detection is by application of an electrical stimulus, after the complex has been moved to, and optionally immobilised, at the second position, it may be detected with amplified signal by sensing an electrical response immediately following the application of an electrical stimulus, i.e. there is no need to delay sensing the response for long periods of time (such as tens of minutes to hours) after first

applying the electrical stimulus, for example to allow the signal to build-up. This is because the electrical response is generated by transfer of electrons between the electrode and the redox mediator when the redox mediator is in its “converted” or “inactive” state, i.e. in the oxidation state that forms after the mediator has transferred
5 electrons to or from the redox catalyst, or the electrical response is generated by transfer of electrons between the electrode and the detectable product. As described above, once the complex has been magnetically moved to the second position (and optionally immobilised at the second position), and when active (or unconverted) redox mediator and/or catalyst substrate is in excess relative to the concentration of the
10 complex, converted or inactive redox mediator or detectable product may quickly accumulate, thereby providing an amplified signal. In other words, and without being bound by theory, the inventors believe that by positioning suitable amplification reagents at or in proximity with the working electrode (and, for example, not elsewhere on the test strip), conditions can be created which can allow spontaneous catalytic (e.g.
15 enzymatic) signal amplification to occur. In other words, once the redox catalyst reaches the working electrode area it can start to convert substrate and, in the case where the catalyst is a redox catalyst, can then be regenerated by the active or unconverted mediator so that the redox catalyst (e.g. enzyme) can continue to convert substrate. As this happens the active or unconverted mediator stock may be
20 continuously converted to the inactive or converted mediator. This stock of converted mediator may be detected (e.g. measured electrochemically) at the end of the assay. In such examples, the signal generated from this converted mediator will be proportional to the analyte concentration in the sample. In examples where the substrate and the active unconverted mediator are present in excess this reaction can continue, thus
25 amplifying the signal (and the longer it is left to amplify the greater the amplification). Accordingly, the described methods, kits and systems may provide exceptional signal amplification, which may further be spontaneous and continuous.

As stated above, the present inventors have particularly identified that certain configurations of test strip are particularly advantageous in minimising the risk of
30 background signal associated with unbound/uncomplexed catalyst moving towards the catalyst substrate at the second position.

Accordingly, viewed from a first aspect, the invention provides a method of detecting a target in a sample using a system comprising a test strip, the test strip comprising:

35 an assay chamber;

a sample inlet in fluid communication with the assay chamber;
a target capture moiety conjugated to a catalyst positioned on or within the assay chamber at a first position; and
a catalyst substrate positioned on or within the assay chamber at a second position;
5 wherein the sample inlet is positioned between the first and second positions;
and the system further comprising:

a target binding moiety tethered to a magnetically moveable particle;
wherein the method comprises:

- 10 (a) introducing a sample to the assay chamber via the sample inlet such that the sample travels or flows across the first position in a direction that is away from the second position;
- (b) at the first position, incubating a mixture of the sample, the target capture moiety conjugated to the catalyst, and a target binding moiety tethered to a magnetically moveable particle for a period of time such that, when the target is
15 present, a complex is formed, the complex comprising the target capture moiety conjugated to the catalyst, and a target binding moiety tethered to a magnetically moveable particle, each being independently bound to the target;
- (c) activating or generating a magnetic field to cause the complex to move to the second position such that the complex is separated from one or more other components of the mixture and such that the catalyst catalyses the conversion of the catalyst substrate to another product; and
20 (d) detecting the catalysis.

The detection may be carried out using any suitable method of detection. In some
25 embodiments the detecting may comprise electrochemically detecting or optically detecting.

Viewed from a second aspect, the invention provides a test strip comprising:

- an assay chamber;
a sample inlet in fluid communication with the assay chamber;
30 a target capture moiety conjugated to a catalyst positioned on or within the assay chamber at a first position; and
a catalyst substrate positioned on or within the assay chamber at a second position;
wherein the sample inlet is positioned between the first and second positions.

The test strip may be configured such that the introduction of a sample into the assay chamber via the sample inlet causes the sample to travel or flow across the first position in a direction that is away from the second position.

Viewed from a third aspect, the invention provides a system comprising:

- 5 (a) a test strip comprising:
an assay chamber;
a sample inlet in fluid communication with the assay chamber;
a target capture moiety conjugated to a catalyst positioned on or within the assay chamber at a first position; and
10 a catalyst substrate positioned on or within the assay chamber at a second position;

wherein the sample inlet is positioned between the first and second positions, such that the introduction of a sample into the assay chamber via the sample inlet causes the sample to travel or flow across the first position in a direction that is away
15 from the second position;

(b) a target binding moiety tethered to a magnetically moveable particle; and

(c) a magnetic field generator configured to activate or generate a magnetic field;

wherein the system is configured such that when the magnetic field is activated or generated, the magnetically moveable particles move to the second position.

20 Viewed from a fourth aspect, the invention provides a kit of parts comprising, as separate components:

(a) a test strip comprising:

an assay chamber;

a sample inlet in fluid communication with the assay chamber;

25 a target capture moiety conjugated to a catalyst positioned on or within the assay chamber at a first position; and

a catalyst substrate positioned on or within the assay chamber at a second position;

wherein the sample inlet is positioned between the first and second positions;

30 (b) a target binding moiety tethered to a magnetically moveable particle; and

(c) an electromagnet positionable such that activation of the electromagnet generates a magnetic field capable of moving the magnetically moveable particle to the second position or a permanent magnet positionable such that a magnetic field capable of moving the magnetically moveable particle to the second position is generated.

Viewed from a fifth aspect, the invention provides for the use of the test strip of the second aspect, the system of the third aspect, or the kit of parts of the fourth aspect to detect the presence or absence of a target in a sample.

5 In some embodiments of any of the aspects described above and herein, the first position may be a reagent zone. In some embodiments, the first position may be at a first end of the assay chamber. In some embodiments, a target binding moiety tethered to a magnetically moveable particle may be positioned at or in proximity to the first position.

10 In some embodiments, the second position may be a detection zone. In some embodiments, the second position may be at or in proximity to a second end of the assay chamber.

15 In some embodiments, the method of detecting a target in a sample may be conducted in a single liquid (e.g. a single liquid mixture comprising the sample and/or a single body of fluid or liquid). In some embodiments, the method of detecting a target in a sample does not comprise contacting the sample with one or more fluidic interfaces (e.g. on the test strip). In some embodiments, the method of detecting a target in a sample may not require the use of one or more wash steps (e.g. with a buffer or the like). In some embodiments, the method of detecting a target in a sample may not comprise the use of any wash steps. In some embodiments, the method of detecting a target in a sample may be conducted in a single fluid chamber (e.g. the assay chamber may be a single fluid chamber). In some embodiments, the method of detecting a target in a sample may be conducted on a test strip that does not comprise multiple fluid chambers. In some embodiments, the complex is only contacted with the catalyst substrate (and optionally also a mediator when present) upon application (e.g. 20 activation or generation) of the magnetic field and movement of the complex to the second position.

25 As stated above, in the test strips described herein, the test strip may be configured such that the introduction of a sample into the assay chamber via the sample inlet causes the sample to travel or flow across the first position in a direction that is away from the second position.

30 Without being bound by theory, this configuration of test strip is particularly advantageous in minimising the risk of cross-contamination and/or background signal from unbound/uncomplexed catalyst moving (e.g. diffusing) towards the catalyst substrate at the second position (e.g. in the detection zone) when the test strip is used in the various methods described herein. By positioning the sample inlet between the 35

first and second positions, following the introduction of the sample, the flow of sample across the first position means that any target capture moiety conjugated to a catalyst re-suspended in the sample fluid is less likely to move and/or diffuse towards the catalyst substrate positioned at the second position (as the flow of fluid is away from the second position). Thus, the risk of background signal associated with unbound/uncomplexed catalyst may be reduced.

The issue of background signal is a particular issue when using sandwich complexes formed from a target analyte, a magnetically moveable particle and a catalyst such as those described herein - as the signal associated with any unbound/uncomplexed catalyst is also subject to amplification. Thus, in these types of assays, it is critical to keep unbound/uncomplexed catalyst away from any catalyst substrate (and optionally mediator) that is positioned in a detection zone. In order to minimise background signal resulting from such unbound/uncomplexed catalysts, prior art methods typically employ multiple fluidic chambers, wash steps and/or complex strip designs. However, surprisingly, the present inventors have identified that positioning a sample inlet between a first and second position and causing the fluid flow of a sample to move across the first position and away from the second position can minimise background signal associated with unbound/uncomplexed catalyst and avoid the need for such complex and expensive test strips.

Furthermore, the present inventors have additionally identified that these configurations can allow the distance between the first and second positions to be reduced, minimising the overall size of the test strip and/or the volume of sample required. Typically strip designs with unidirectional fluid flow paths (e.g. a unidirectional flow of fluid following introduction of a sample, from a sample inlet through a reagent zone to a detection zone) require longer flow paths, multiple fluid chambers and/or wash steps, meaning that relatively larger volumes of sample are required.

Thus, as stated above, in the test strips described herein, the test strip may be configured such that the introduction of a sample into the assay chamber via the sample inlet causes the sample to travel or flow across the first position in a direction that is away from the second position.

In some embodiments, the test strip is configured such that the introduction of a sample into the assay chamber via the sample inlet causes the sample to travel or flow in multiple directions away from the sample inlet and/or towards a periphery and/or opposing ends of the assay chamber. For example, the positioning of the sample inlet

between the first and second positions facilitates a multi-directional fluid flow following introduction of the sample into the assay chamber via the sample inlet.

5 In some embodiments, the sample inlet is positioned between the first and second positions such that introduction of a sample causes a substantially bi-directional fluid flow, one portion of sample flowing away from the sample inlet and across the first position in a direction that is away from the second position, and a second portion of the sample flowing away from the sample inlet and across the second position in a direction that is away from the first position.

10 In some embodiments, the sample inlet is positioned such that it is substantially equidistant or substantially midway between the first position and the second position. In some embodiments, the sample inlet may be positioned asymmetrically (e.g. asymmetrically on or within the test strip with respect to the first and second positions). In some embodiments, the sample inlet may be positioned closer to the first position than the second position. In some embodiments, the sample inlet may be positioned
15 closer to the second position than the first position.

In some embodiments, the sample may move towards and across the first position comprising the target capture moiety conjugated to a catalyst. In some embodiments, the sample fluid may further move towards and across the second position comprising the catalyst substrate. In some embodiments, the flow of liquid
20 from the sample inlet furcates into two or more different directions (e.g. bifurcates). The result of this substantially bi-directional fluid flow (or a multi-directional fluid flow) may be to push the respective components at the first and second positions apart (the introduction of the sample fluid may be considered to wash the resuspended components in opposite directions and/or towards opposite ends of the assay
25 chamber). In some embodiments, the flow of liquid from the sample inlet bifurcates into 2 or more different directions such that diffusion between the first position and the second position is substantially reduced or eliminated. The test strip configurations as described herein assist in minimising the amount of unbound/uncomplexed catalyst that contacts the substrate at the second position.

30 Further examples of the disclosure are described below. It will be appreciated that the following methods, systems, kits of parts and uses may employ the test strip designs and systems of the second and thirds aspects as outlined above. Thus, any examples described below are equally applicable to the aspects described above.

The disclosure provides a method of detecting a target in a sample using a
35 system, the method comprising:

- (i) contacting a mixture on or within a test strip, the mixture comprising:
- (a) a target binding moiety tethered to a magnetically moveable particle;
 - (b) a target capture moiety conjugated to a catalyst; and
 - (c) a sample;

5 wherein component (b) is positioned on or within the test strip at a first position and the system further comprises:

- (d) a catalyst substrate;

 wherein component (d) is positioned on or within the test strip at a second position;

10 (ii) incubating (a), (b) and (c) for a period of time such that, when the target is present, a complex is formed, the complex comprising components (a) and (b) each being independently bound to the target;

 (iii) activating or generating a magnetic field to cause the complex to move to the second position such that the complex is separated from one or more other components of the mixture and such that the catalyst catalyses the conversion of the catalyst substrate to another product; and

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 (iv) detecting the catalysis. As described above, the test strip may be the test strip of the first aspect.

 Detecting the catalysis may be directly or indirectly. For example, it may comprise detecting a detectable product formed during and/or as a result of the catalysis step. In some examples, the detectable product may be formed by the conversion of the catalyst substrate to another product. Thus, the detectable product may be the another product. In some examples, the detectable product may be the inactive or converted mediator as described herein and above. Thus, as used herein

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25 and unless the context indicates otherwise, the detectable product may refer to the another product and/or the inactive or converted mediator.

 The catalyst may be a redox catalyst. In such cases, the system may further comprise a redox mediator positioned on or within the test strip at the second position. In those cases, activating or generating a magnetic field causes the complex to move to the second position such that the complex is separated from one or more other components of the mixture and such that the redox mediator is able to transfer electrons to or from the redox catalyst such that the redox catalyst catalyses the conversion of the redox catalyst substrate to another product and catalyses the conversion of the redox mediator from an active to an inactive state. Detecting the catalysis may comprise detecting the inactive redox mediator.

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The detecting of (iv) may comprise:

(i) positioning a working electrode at a third position, which third position is at least in proximity to the second position, and away from the first position; (ii) applying an electrical stimulus to the working electrode; and

5 (iii) sensing an electrical response.

The disclosure further provides a method of separating a complex from one or more other components of a mixture on or within a test strip using a system, wherein the complex comprises:

10 (a) a target binding moiety tethered to a magnetically moveable particle; and

(b) a target capture moiety conjugated to a catalyst;

wherein component (b) is positioned on or within the test strip at a first position and components (a) and (b) are each independently bound to a target to form the complex and wherein the system further comprises a catalyst substrate positioned at a second position; and

15 wherein the method comprises:

(i) activating or generating a magnetic field to cause the complex to move to the second position such that the complex is separated from one or more other components of the mixture and such that the catalyst catalyses the conversion of the catalyst substrate to another product; and

20 (ii) detecting the catalysis.

The catalyst may be a redox catalyst. In such cases, the system may further comprise a redox mediator positioned on or within the test strip at the second position.

25 Activating or generating a magnetic field at the second position on or within the test strip causes the complex to move to the second position such that the complex is separated from one or more other components of the mixture and such that the redox mediator is able to transfer electrons to or from the redox catalyst such that the redox catalyst catalyses the conversion of the redox catalyst substrate to another product and catalyses the conversion of the redox mediator from an active to an inactive state.

30 Detecting the catalysis may comprise detecting the inactive redox mediator.

The detecting of (ii) may comprise:

(i) applying an electrical stimulus to a working electrode positioned at a third position, which third position is at least in proximity to the second position, and away from the first position; and

35 (ii) sensing an electrical response.

The disclosure further provides a system comprising:

(a) a test strip;

(b) a target capture moiety conjugated to a catalyst positioned on or within the test strip at a first position;

5 (c) a magnetic field generator configured to activate or generate a magnetic field;

(d) a target binding moiety tethered to a magnetically moveable particle; and

(e) a catalyst substrate;

10 wherein component (e) is positioned at a second position and the system is configured such that when the magnetic field is activated or generated, the magnetically moveable particles move to the second position.

15 When a sample comprising a target is contacted with (b) and (d), a complex is formed, the complex comprising components (b) and (d) each being independently bound to the target. In such cases, when the magnetic field is activated or generated, the complex moves to the second position such that the complex is separated from one or more other components of the mixture and such that the catalyst catalyses the conversion of the catalyst substrate to another product.

20 The catalyst may be a redox catalyst and the system may further comprise a redox mediator positioned at the second position such that when the magnetic field is activated or generated, the redox mediator is able to transfer electrons to or from the redox catalyst such that the redox catalyst catalyses the conversion of the redox catalyst substrate to another product and catalyses the conversion of the redox mediator from an active to an inactive state.

25 The system may further comprise a detector, which may be a working electrode positioned at a third position, which third position is at least in proximity to the second position, and away from the first position, such that when an electrical stimulus is applied to the working electrode, electrons are transferred between the working electrode and either the catalyst substrate or the redox mediator in an inactive state.

30 The disclosure further provides a kit of parts comprising, as separate components:

(a) a test strip comprising:

(i) a target capture moiety conjugated to a catalyst; and

(ii) a catalyst substrate;

35 wherein component (i) is positioned at a first position and component (ii) is positioned at a second position;

(b) a target binding moiety tethered to a magnetically moveable particle; and

(c) an electromagnet positionable such that activation of the electromagnet generates a magnetic field capable of moving the magnetically moveable particle to the second position or a permanent magnet positionable such that a magnetic field capable of moving the magnetically moveable particle to the second position is generated.

The catalyst may be a redox catalyst and the kit of parts may further comprise a redox mediator positioned at the second position.

The kit may further comprise a detector, which may be a working electrode positioned at a third position at least in proximity to the second position, and away from the first position.

The disclosure further provides for the use of any of the systems, or the kit of parts as described above to detect the presence or absence of a target in a sample.

BRIEF DESCRIPTION OF FIGURES

Fig. 1. Formal drawings of a test strip that may be used for electrochemical detection of magnetic separation of a magnetically moveable particle tethered to a target binding moiety, which may itself be bonded to a target, and a target capture moiety conjugated to a catalyst. Lengths are given in mm. RE is a reference electrode, WE is a working electrode and CE is a counter electrode.

Fig. 2. a. Photographs showing the magnetic accumulation of magnetite beads coated with horseradish peroxidase (HRP) enzyme. Urea hydrogen peroxide (UHP) is used as an enzyme substrate and 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) is used as a redox mediator. The change in colour occurs as the HRP-labelled beads react with substrate and mediator to oxidise ABTS. This colour change is detectable using an electrode-potentiostat system concomitant with the colour change. The photograph on the right is a negative sample with no magnet present at the working electrode.

Fig. 2. b. Histogram showing the current detected by the electrode-potentiostat system using the system shown in the photographs of Fig. 2. a. where $n = 5$ and error bars denote the standard deviation. The electrochemical response of the magnetic separation system coupled to the enzyme-amplified redox signal was measured and a significant difference can be seen between the positive (magnet in place) and negative (no magnet present) samples.

Fig. 3. a. Schematic showing capture of an IL-6 antigen by a magnetic bead coated with an IL-6 antibody, and a further IL-6 antibody conjugated to biotin. The

streptavidin of a poly-horseradish peroxidase (HRP) conjugated to streptavidin is able to bind to the biotin of the antibody. A solution comprising the complex is applied to a reagent zone of a dumbbell strip, shown in the centre of the schematic. The complex may be transported along the strip to an electrode zone on the right of the strip, where substrates for the HRP (shown as reagent A + B) may interact with the HRP, producing an electrochemical signal that may be detected at the electrode zone.

Fig. 3. b. Photograph of a dumbbell strip in a magnetic rig.

Fig. 3. c. Graph of atomic unit charge (a. u. C) detected using the set-up shown in Fig. 3. a. and Fig. 3. b. as a function of concentration of IL-6 antigen.

Fig. 4. a. Schematic showing capture of one part of a nucleic acid target by a biotin-conjugated nucleic acid probe strand, which is in turn bonded to a streptavidin-conjugated magnetic bead. Another part of the nucleic acid target is captured by a detection strand, which is in turn bonded to an enzyme.

Fig. 4. b. Graph of current detected when targeting a nucleic acid target (*Oxa1* – oxacillin resistance gene) as a function of the concentration of the target in the presence of another nucleic acid (*tetA* – tetracycline resistance). Horseradish peroxidase (HRP) was used as the enzyme, hydrogen peroxide was used as the substrate and 3,3',5,5'-Tetramethylbenzidine (TMB) was used as a redox mediator. Electrochemical detection carried out via chronoamperometry.

Fig. 4. c. Graph of current detected when targeting a nucleic acid target (*Oxa1* – oxacillin resistance gene) as a function of the concentration of the target in the presence of another nucleic acid (*tetA* – tetracycline resistance). Alkaline phosphatase was used as the enzyme, and p-nitrophenyl phosphate (pNPP) was used as the substrate. Electrochemical detection carried out via linear sweep voltammetry (LSV).

Fig. 5. Labelled line drawing of a test strip suitable for use in the methods disclosed herein.

Fig. 6. Graph showing the charge density from: (i) a plasma sample comprising 1000 ng/mL of PCT antigen; and (ii) a plasma sample comprising 0 mg/mL of PCT antigen; as determined using a test strip as shown in Figure 5.

DETAILED DESCRIPTION

In the discussion that follows, reference is made to a number of terms, which have the meanings provided below, unless a context indicates to the contrary. The nomenclature used herein for defining compounds, in particular the compounds according to the invention, is in general based on the rules of the IUPAC organisation

for chemical compounds, specifically the "IUPAC Compendium of Chemical Terminology (Gold Book)".

5 The term "comprising" or variants thereof will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

10 The term "consisting" or variants thereof will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, and the exclusion of any other element, integer or step or group of elements, integers or steps.

The term "about" herein, when qualifying a number or value, is used to refer to values that lie within $\pm 5\%$ of the value specified. For example, where the third position is separated from the first position by at least about 1.0 cm, a range of 0.95 cm to 1.05 cm is included.

15 As described above, according to a first aspect there is provided a method of detecting a target in a sample using a system comprising a test strip, the test strip comprising:

an assay chamber;

a sample inlet in fluid communication with the assay chamber;

20 a target capture moiety conjugated to a catalyst positioned on or within the assay chamber at a first position; and

a catalyst substrate positioned on or within the assay chamber at a second position;

wherein the sample inlet is positioned between the first and second positions;

25 and the system further comprising:

a target binding moiety tethered to a magnetically moveable particle;

wherein the method comprises:

30 (a) introducing a sample to the assay chamber via the sample inlet such that the sample travels or flows across the first position in a direction that is away from the second position;

(b) at the first position, incubating a mixture of the sample, the target capture moiety conjugated to the catalyst, and a target binding moiety tethered to a magnetically moveable particle for a period of time such that, when the target is present, a complex is formed, the complex comprising the target capture moiety

conjugated to the catalyst, and a target binding moiety tethered to a magnetically moveable particle, each being independently bound to the target;

(c) activating or generating a magnetic field to cause the complex to move to the second position such that the complex is separated from one or more other components of the mixture and such that the catalyst catalyses the conversion of the catalyst substrate to another product; and

(d) detecting the catalysis.

The test strip may comprise a base and a lid. For the avoidance of doubt, the first position may be positioned anywhere on the base or the lid. In some embodiments the first position is within the test strip, for example the first position may be enclosed by the positioning of the base with respect to the lid. In some embodiments, the first position is on the base and within the test strip, e.g. on the base and enclosed by the lid of the strip. As used herein, the base (e.g. the base of the test strip) may be considered as a test substrate and the two terms may be used interchangeably within the context of the present disclosure.

The assay chamber may be positioned on or within the test strip (e.g. on or within the base of the test strip). The assay chamber may define a volume on or within the test strip and/or base into which a sample may be introduced and/or retained during the assays and methods as described herein. The assay chamber may comprise a single fluid chamber. The assay chamber may comprise a plurality of zones in fluid communication with one another (for example, at least two zones). The assay chamber may comprise a reagent zone at the first position. The assay chamber may comprise a detection zone at the second position. The first position and/or reagent zone may be at a first end of the assay chamber. The second position and/or detection zone may be at a second end of the assay chamber. The first end of the assay chamber and the second end of the assay chamber may be opposing ends of the assay chamber.

The assay chamber may be enclosed, optionally by a lid of the strip. The assay chamber may be partially enclosed (e.g. one portion of the assay chamber, such as the reagent zone and/or first position, may be enclosed, and another portion of the assay chamber, such as the detection zone and/or second position, may not be enclosed). In some embodiments, the lid may be transparent and/or at least a portion of the lid may be transparent. For example, the portion of the lid enclosing the detection zone and/or second position may be transparent.

The assay chamber may comprise any suitable dimensions and/or shape to facilitate the sample to travel or flow across the first position in a direction that is away

from the second position (and optionally also to facilitate the sample to travel or flow across the second position in a direction that is away from the first position). In some embodiments, the assay chamber may comprise a first enlarged end (comprising the first position and/or reagent zone) and/or a second enlarged end (comprising the second position and/or detection zone). The first and second enlarged ends may be connected by a connecting channel. The connecting channel may be narrower than the first and second enlarged ends. The first enlarged end of the assay chamber may taper towards the connecting channel. The second enlarged end of the assay chamber may taper towards the connecting channel. In some embodiments, the first and/or second ends (e.g. enlarged ends) taper towards the sample inlet. Such configurations may facilitate and/or promote the desired flow of sample following introduction of the sample into the assay chamber.

The test base and/or the assay chamber may be dumbbell-like in shape. The first position may be at one end of the dumbbell-like shape. The second position may be at a second end of the dumbbell-like shape. The base and/or assay chamber may comprise an hourglass-like shape. For example, an hourglass-like shape with an elongated mid-portion. The first position may be at one end of the hourglass-like shape. The second position may be at a second end of the hourglass-like shape.

As stated above, the sample inlet is in fluid communication with the assay chamber and is positioned between the first and second positions. The positioning of the sample inlet between the first and second positions is sometimes referred to herein as a "centre-fill" design or configuration. However, the sample inlet does not need to be positioned exactly halfway between the first and second positions. In some embodiments, the sample inlet may be positioned away from the first and/or second positions. In some embodiments, the sample inlet may be positioned approximately midway between the first and second positions. In some embodiments, the sample inlet is positioned or located such that the sample is introduced into the connecting channel of the assay chamber.

The sample inlet may be positioned on the base of the test strip, optionally at a position adjacent to the assay chamber. In some embodiments, the sample inlet may be located such that the inlet substantially lies in the same plane as the assay chamber. In such configurations, the sample is introduced into the assay chamber such that the sample is delivered substantially parallel to the surface of the base and/or travels or flows across the surface of the base in a direction that is substantially parallel to the surface of the base.

The sample inlet may be located on, positioned on, or defined by a lid. In some embodiments, the sample inlet may be located such that the inlet lies substantially above the assay chamber. In such configurations the sample is introduced into the assay chamber such that the sample is delivered from a first direction that is substantially perpendicular to the surface of the base (e.g. from above when the test strip is held in a horizontal configuration). In some embodiments, the sample may strike and/or impinge the surface of the base prior to travelling or flowing across the first position in a direction that is away from the second position (and optionally also to facilitate the sample to travel or flow across the second position in a direction that is away from the first position).

The sample may be deposited in the sample inlet. The inventors have found that it is advantageous to position the sample in the sample inlet in the described configurations to minimise the risk of potential diffusion of the target binding moiety tethered to a magnetically moveable particle and/or the target capture moiety conjugated to a catalyst to the second position on sample deposition.

In use, the sample may be deposited in a sample inlet, positioned in between the first and second positions. For example, the sample may be deposited in a sample inlet positioned in between the two ends of the base.

As stated above, the target capture moiety conjugated to the catalyst is positioned on or within the test strip (e.g. on or within the assay chamber) at a first position. The positioning of the target capture moiety may be by depositing the target capture moiety at the first position, for example by any suitable means of contacting. In some cases, the target capture moiety conjugated to a catalyst may be deposited onto the strip as a solution or suspension, for example by spraying or dripping, and the strip is allowed to dry. In some embodiments, the target capture moiety conjugated to a catalyst is printed onto the test strip at the first position. As stated above, the first position may be at one end of the test strip, for example at one end of a base.

In some embodiments, the target binding moiety tethered to a magnetically moveable particle is positioned on or within the test strip. As will be appreciated, this component may be positioned at any suitable position at the start of the method as described herein provided that it is present at the first position for the incubation stage. In some embodiments the target binding moiety tethered to a magnetically moveable particle is positioned on or within the test strip by any suitable means of contacting. In some embodiments, the target binding moiety tethered to a magnetically moveable particle is deposited onto or within the strip as a solution or suspension, for example by

spraying or dripping, and the strip is allowed to dry. In some embodiments, the target binding moiety tethered to a magnetically moveable particle is printed onto the test strip, e.g. onto the base.

5 In some embodiments, the target binding moiety tethered to a magnetically moveable particle is positioned on or within the test strip at the first position. In such embodiments, the target binding moiety tethered to a magnetically moveable particle is already present at the first position prior to the incubation stage. In some embodiments, the target binding moiety tethered to a magnetically moveable particle is positioned on or within the test strip at a different position to the first position. For example, the target
10 binding moiety tethered to a magnetically moveable particle may be positioned between the first and second positions, or at the second position. In such embodiments, the target binding moiety tethered to a magnetically moveable particle may be moved to the first position prior to the incubation stage. For example, the target binding moiety tethered to a magnetically moveable particle may be magnetically
15 moved to the first position. Additionally, or alternatively, the target binding moiety tethered to a magnetically moveable particle may be moved to the first position following introduction of the sample (e.g. may be moved with the sample as the sample travels or flows across the first position and away from the second position).

In some embodiments, the target binding moiety tethered to a magnetically moveable particle and the sample may be contacted on or within the test strip by any
20 suitable means. For example, each may be deposited separately on or within the test strip. Alternatively, both components may be added to the base simultaneously. In some cases, the target binding moiety tethered to a magnetically moveable particle and the sample may be independently deposited onto or within the strip as a solution or
25 suspension, for example by spraying or dripping.

The target binding moiety tethered to a magnetically moveable particle and the target capture moiety conjugated to a catalyst may each be separately or simultaneously deposited onto or within the strip as a solution or suspension, for example by spraying or dripping, and the strip is allowed to dry. The target binding
30 moiety tethered to a magnetically moveable particle and the target capture moiety conjugated to a catalyst may each be separately or simultaneously printed onto or within the test strip, e.g. at the first position.

The target binding moiety tethered to a magnetically moveable particle may be positioned at one end of the test strip, such as one end of a base, for example at the
35 same end as the target capture moiety conjugated to a catalyst. Where the test strip

comprises an assay chamber and/or base that is dumbbell-like in shape, both the target capture moiety conjugated to a catalyst and the target binding moiety tethered to a magnetically moveable particle may be positioned at the same one end of the dumbbell-like shape.

5 The inventors have found that combining the target binding moiety tethered to a magnetically moveable particle and the target capture moiety conjugated to a catalyst before deposition onto the test strip can result in aggregation of the two, increasing the chance of false positive results. Thus, the target binding moiety tethered to a magnetically moveable particle is typically deposited onto or within the strip separately
10 from the target capture moiety conjugated to a catalyst. In some embodiments, the target binding moiety tethered to a magnetically moveable particle is positioned on or within the test strip at a position, such as a section of the first position, that does not overlap with the target capture moiety conjugated to a catalyst. In some embodiments, the target binding moiety tethered to a magnetically moveable particle and target
15 capture moiety conjugated to a catalyst are each present (e.g. deposited) in different sections of the reagent zone (e.g. such that they do not overlap or do not substantially overlap). During the test assay these components may become re-suspended as the sample travels or flows across the first position.

 As stated above, the catalyst substrate is positioned on or within the test strip
20 (e.g. on or within the assay chamber) at a second position. As above, and for the avoidance of doubt, the second position may be positioned anywhere on the base or the lid. In some embodiments the second position is within the test strip, for example the second position may be enclosed by the positioning of the base with respect to the lid. In some embodiments, the second position is on the base and within the test strip,
25 e.g. on the base and enclosed by the lid of the strip.

 In some embodiments, the catalyst substrate (and optionally the redox mediator) is or are positioned on or within the test strip e.g. on or within the assay chamber) at the second position, for example by any suitable means of contacting. In some embodiments, either or both of these components is or are deposited onto or
30 within the strip as a solution or suspension, for example by spraying or dripping, and the strip is allowed to dry. In some embodiments, the catalyst substrate (and optionally also the redox mediator) is printed onto the test strip, e.g. onto the base. In some embodiments, a redox catalyst and redox mediator may be pre-mixed and simultaneously deposited or printed onto the test strip, e.g. onto the base.

The catalyst substrate is a chemical species which may be acted upon by the catalyst and catalytically converted by the catalyst to one or more different products. For example, the catalyst substrate may be cleaved at one or more positions by the catalyst to produce one or more different products. Any one type of catalyst may have
5 more than one suitable catalyst substrate, which it is capable of catalytically converting to one or more different products. Any suitable substrate may be used in combination with its corresponding catalyst.

The catalyst substrate is positioned on or within the test strip (e.g. on or within the assay chamber) at a second position. The second position is positioned away from
10 the first position, i.e. the first and second positions do not overlap. In some embodiments, the first and second positions are separated by at least about 0.1, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5, 2, 2.5 or 3 cm. For example, the first and second positions may be separated by at least about 1 cm. In some embodiments, the first and second positions are separated in the range of from about 0.5 cm to about 2 cm. In
15 other examples, the catalyst substrate is positioned on or within the test strip at a second position and elsewhere on or within the test strip, e.g. it may be positioned across or within a partial or an entire surface of a base.

In some embodiments, the catalyst is a redox catalyst. For the avoidance of doubt, in such embodiments, the catalyst substrate is a redox catalyst substrate. In
20 such embodiments, the test strip may further comprise a redox mediator positioned on or within the test strip (e.g. on or within the assay chamber) at the second position.

The redox mediator is a chemical species which acts as an electron shuttle between the redox catalyst's oxidising and reducing forms. As described above, it has been found by the inventors that, when the method of the invention is applied, the
25 redox mediator may be used in its "active" state, i.e. in an oxidation state that allows for the transfer of electrons to or from the redox catalyst such that the redox catalyst catalyses the conversion of the redox catalyst substrate to another product. Typically, the redox mediator is introduced to the system in its active state.

Both the redox mediator and the redox catalyst substrate may each be
30 positioned on or within the test strip (e.g. on or within the assay chamber) at the second position. The redox mediator may also be positioned elsewhere on the test strip, for example it may overlap with the second position or it may be positioned across or within a partial or an entire surface of a base including at the second position. Alternatively, the redox mediator may be positioned only at the second position, and
35 the redox catalyst substrate may be positioned at the second position and elsewhere

on the test strip. In this case, the redox catalyst substrate may overlap with the second position or it may be positioned across or within a partial or an entire surface of a base including at the second position.

5 The second position may be at one end of the test strip, such as at one end of a base. For example, where the base is dumbbell-like in shape, the first position may be at one end of the dumbbell-like shape and the second position may be at the other end.

10 In particular embodiments, both the target capture moiety conjugated to the catalyst and the target binding moiety tethered to the magnetically moveable particle are each positioned at the same one end of the test strip, such as at the same one end of a base, and the catalyst substrate (and optionally the redox mediator) is (or are) positioned on the other end. For example, where the base is dumbbell-like in shape, the target capture moiety conjugated to a catalyst and the target binding moiety tethered to a magnetically moveable particle may each be positioned at the same one end of the dumbbell-like shape and the catalyst substrate (and optionally the redox mediator) may be positioned at the other end of the dumbbell-like shape.

15 In use, following introduction of a sample to the test strip, a mixture is formed comprising the target binding moiety tethered to a magnetically moveable particle, the target capture moiety conjugated to a catalyst and the sample. The mixture on the test strip comprising the target binding moiety tethered to a magnetically moveable particle, the target capture moiety conjugated to a catalyst and the sample may be a liquid. For example, the sample comprising the target may be a liquid which, when added to or within the strip, forms the mixture, i.e. forms a liquid comprising the target binding moiety tethered to a magnetically moveable particle, the target capture moiety conjugated to a catalyst and the sample.

20 As described herein, the method of the first aspect comprises incubating the target binding moiety tethered to a magnetically moveable particle; the target capture moiety conjugated to a catalyst; and the sample for a period of time such that, when the target is present, a complex is formed, the complex comprising the target binding moiety tethered to a magnetically moveable particle and the target capture moiety conjugated to a catalyst, each being independently bound to the target. When the target is present, the complex often forms within minutes or seconds. The incubation time typically ranges from about 0.5 seconds to about 10 minutes, about 2 seconds to about 5 minutes, about 5 seconds to about 60 seconds, or about 10 to about 30 seconds. In some embodiments, the incubation time ranges from about 2 minutes to about 5 minutes.

The method of the first aspect further comprises activating or generating a magnetic field to cause the complex to move to the second position such that the complex is separated from one or more other components of the mixture and moved to or into proximity with the catalyst substrate and, in some cases, the redox mediator.

5 The magnetic field may be activated or generated by activating an electromagnet or mechanically activating, e.g. moving, a permanent magnet into proximity with the second position.

The magnetic field strength is at least strong enough to attract and cause the magnetically moveable particles of the complex to move towards the second position
10 on or within the strip, thereby separating the complex from one or more other components of the mixture. In some embodiments, the complex is separated from at least any unbound target capture moiety conjugated to a redox catalyst within the mixture. The magnetic field typically has a strength of about 0.05 mT to about 5 T, such as about 0.1 mT to about 2 T, about 1 mT to about 500 mT, or about 10 mT to
15 about 50 mT. In some embodiments, the magnetic field has a strength of approximately 1.8 Tesla.

The magnetically moveable particle may be a paramagnetic particle capable of movement by the magnetic field. The paramagnetic particle may be a paramagnetic bead, such as a "magnetic bead". Magnetic beads are well known in the art and may
20 comprise any suitable paramagnetic material, such as iron oxide, e.g. magnetite (Fe_3O_4).

The shape and the precise position of the magnetic field at the second position need not be limited, provided that the second position is physically separate from (e.g. not overlapping with) the first position. To reduce the probability of diffusion of any
25 unbound target capture moiety conjugated to a redox catalyst to the second (or third) position, it may be beneficial to position the first position as far away as practically possible from the second (or third) position on or within the test strip.

In some embodiments, activating or generating a magnetic field causes the complex to move in a lateral direction, e.g. along the test strip to the second position
30 such that the complex is separated from one or more other components of the mixture (e.g. any unbound target capture moiety conjugated to a catalyst within the mixture). By a lateral direction is meant that the complex moves across the horizontal rather than the vertical plane of the test strip. For example, where the strip comprises a substrate that is a thin sheet of material, the complex may move horizontally across the surface
35 with the largest surface area.

In particular embodiments, the test strip comprises a base comprising one or more capillary channels such that, on activating or generating a magnetic field, the complex is able to move through the one or more capillary channels from the first to the second position.

5 Where the catalyst is a redox catalyst and requires redox mediator in order to catalytically convert the redox catalyst substrate, both the redox mediator and redox catalyst substrate are positioned at the second position. In the absence of redox mediator, the redox catalyst may be capable of converting one redox catalyst substrate to another product. However, after conversion, the redox catalyst is not capable of
10 acting on any further redox catalyst substrate until it is oxidised or reduced back to its active state (the active state being the state in which the redox catalyst is able to convert redox catalyst substrate to another product). In order to catalytically convert the redox catalyst substrate to one or more other products, and amplify the electrochemical signal generated per target, redox mediator and redox catalyst
15 substrate should be available to the redox catalyst at the second position. In some embodiments, the redox mediator and redox catalyst substrate are present in excess (e.g. molar excess) with respect to the redox catalyst making up the complex.

 Where the catalyst does not require redox mediator in order to catalytically convert the catalyst substrate, catalyst substrate is positioned at the second position
20 but redox mediator need not be present. In order to catalytically convert the catalyst substrate to one or more other products, and amplify the electrochemical signal generated per target, catalyst substrate should be available to the redox catalyst at the second position. In some embodiments, the catalyst substrate is present in excess (e.g. molar excess) with respect to the redox catalyst making up the complex.

25 The target capture moiety conjugated to a catalyst is positioned on or within the test strip at the first position, which is positioned away from the second position. Activating or generating a magnetic field causes the target capture moiety conjugated to a catalyst that forms part of the complex (also comprising the target and the target binding moiety tethered to a magnetically moveable particle) to move to the second
30 position. In some embodiments, the second position is at least in proximity with the detector, e.g. the working electrode.

 In some embodiments, the second position at least partially overlaps with the detector, e.g. the working electrode. In such embodiments, at least some of the catalyst substrate and, in some cases, the redox mediator may be positioned at the
35 detector, e.g. working electrode, for example by adsorption or deposition at the surface

of the detector. Alternatively, one of the redox catalyst substrate and the redox mediator may be positioned at the detector, e.g. working electrode, and the other may be positioned in a non-overlapping part of the second position. In some embodiments, the catalyst substrate and, in some cases, the redox mediator are adsorbed on the surface of the detector, e.g. working electrode and the second position overlaps with the entirety of the third position. In some embodiments the detector is a working electrode.

In further embodiments, the second position partially overlaps with the detector and the catalyst substrate is positioned at the detector. On activating or generating the magnetic field, the magnetically moveable particle and thus (where target is present) the complex moves to the second position. The magnetically moveable particles may be moved to a part of the second position in proximity to but not overlapping with the detector, i.e. the magnetically moveable particles need not overlap with the detector in order to detect catalysis.

When both the redox mediator and the redox catalyst substrate are positioned at the second position they are unavailable to any unbound target capture moiety, thus any unbound target capture moiety conjugated to a redox catalyst cannot convert the redox catalyst substrate to another product or the redox mediator from an active to an inactive state. In contrast, the redox mediator and the redox catalyst substrate are available to the complex that, on activating or generating a magnetic field, moves to the second position. Consequently, the redox catalyst comprised within the complex at the second position is able to catalytically convert the redox catalyst substrate to one or more other products, and simultaneously convert the redox mediator from an active to an inactive state, thus amplifying the signal per target.

Provided both the redox mediator and the redox catalyst substrate are available to any complex that, on activating or generating a magnetic field, moves to the second position, the redox catalyst comprised within the complex at the second position will be able to catalytically convert the redox catalyst substrate to one or more other products and simultaneously convert the redox mediator from an active to an inactive state, and thus amplify the signal per target.

Where the catalyst does not require redox mediator in order to catalytically convert the catalyst substrate, when the catalyst substrate is positioned at the second position it is unavailable to any unbound target capture moiety, thus any unbound target capture moiety conjugated to a catalyst cannot convert the catalyst substrate to another product. In contrast, the catalyst substrate is available to the complex that, on

activating or generating a magnetic field, moves to the second position. Consequently, the catalyst comprised within the complex at the second position is able to catalytically convert the catalyst substrate to one or more other products, thus amplifying the signal per target.

5 Provided the catalyst substrate is available to any complex that, on activating or generating a magnetic field, moves to the second position, the catalyst comprised within the complex at the second position will be able to catalytically convert the catalyst substrate to one or more other products and simultaneously convert the redox mediator from an active to an inactive state, and thus amplify the signal per target.

10 In some embodiments, the method of the first aspect further comprises positioning a detector such as a working electrode at a third position, which third position is at least in proximity with the second position, and away from the first position. The third position may further be located in the detection zone of the assay chamber. The second position may at least partially overlap with the position of the
15 detector, e.g. working electrode, such that activating or generating a magnetic field causes at least some of the complex to move to the detector. The third position is away from the first position. In some embodiments, the third position is at a distance from the first position such that diffusion of any unbound target capture moiety conjugated to a catalyst to the third position is negligible, i.e. does not contribute to
20 measurable signal. In some embodiments, the third position is separated from the first position by at least about 0.1, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5, 2, 2.5 or 3 cm. For example, the third position may be separated from the first position by at least about 1 cm. In some embodiments, the first and third positions are separated in the range of from about 0.5 cm to about 2 cm.

25 The third position may be at one end of the test strip. In some examples, the first position may be at a first end of the test strip and the second and third positions may be at a second end of the test strip (e.g. an opposing end). For example, where the test strip is dumbbell-like in shape, the first position may be at one end of the dumbbell-like shape and the second and third positions may be at the other end.

30 In particular embodiments, both the target capture moiety conjugated to a catalyst and the target binding moiety tethered to a magnetically moveable particle are each positioned at the same one end of the test strip and the catalyst substrate and the working electrode are positioned on the other end. For example, where the test base is dumbbell-like in shape, the target capture moiety conjugated to a catalyst and the
35 target binding moiety tethered to a magnetically moveable particle may each be

positioned at the same one end of the dumbbell-like shape and the catalyst substrate and working electrode may be positioned at the other end of the dumbbell-like shape.

Where the detector is a working electrode, the method of the first aspect also comprises applying an electrical stimulus to the working electrode and sensing an electrical response. Where the catalyst is a redox catalyst and requires redox mediator in order to catalytically convert the redox catalyst substrate, the system may be configured such that at the time of applying the electrical stimulus to the working electrode, electrons are transferred between the working electrode and the redox mediator in an inactive state (i.e. inactive redox mediator). In such cases, the electrical response is indicative of the concentration of inactive redox mediator. Alternatively, the system may be configured such that at the time of applying the electrical stimulus to the working electrode, electrons are transferred between the working electrode and detectable products resulting from conversion of the redox catalyst substrate. In such cases, the electrical response is indicative of the concentration of detectable products produced on conversion of the redox catalyst substrate.

Where the catalyst does not require redox mediator in order to catalytically convert the catalyst substrate, the system may be configured such that at the time of applying the electrical stimulus to the working electrode, electrons are transferred between the working electrode and detectable products resulting from conversion of the catalyst substrate. In such cases, the electrical response is indicative of the concentration of detectable products produced on conversion of the catalyst substrate.

The electrical stimulus is applied after the activation or generation of a magnetic field. The time in between activating or generating the magnetic field and applying the electrical stimulus need not be limited, provided (where the target is present) at least some of the complex is able to move to the second position on or within the strip and generate either redox mediator in an inactive state or detectable product before the electrical stimulus is applied. The inventors have found that magnetic field-induced movement of the complex to the second position is surprisingly quick. Thus, in some embodiments, the electrical stimulus is applied about 0.1 s to 20 s after the activation or generation of a magnetic field. However, it may be beneficial to allow more time in between activation or generation of a magnetic field and application of an electrical stimulus, for example to allow for greater amounts of inactive redox mediator or detectable product to build up, thereby allowing for a greater response to be sensed. In some embodiments, the method comprises a first incubation step in which the complex is formed and a second incubation step, following movement of the complex

to the second position. In embodiments comprising a second incubation step, the method further comprises incubating the complex with the catalyst substrate (and optionally the redox mediator) at the second position and/or in the detection zone. This second incubation step may be for a period of time to allow an increased amount of inactive redox mediator or detectable product to build up. In some embodiments, the electrical stimulus is applied several minutes after the activation of a magnetic field. The electrical stimulus may be applied from about 0.5 seconds to about 15 minutes, about 1 seconds to about 10 minutes, about 2 seconds to about 5 minutes, about 5 seconds to about 60 seconds, or about 10 to about 30 seconds after the activation of a magnetic field.

Electrochemical signal generated when electrons are transferred between the working electrode and the redox mediator in an inactive state is amplified when the conversion of the redox mediator from an active to an inactive state is catalysed by the redox catalyst. Activating or generating a magnetic field causes the target capture moiety conjugated to a redox catalyst that forms part of the complex (also comprising the target and the target binding moiety tethered to a magnetically moveable particle) to move to the second position.

In cases where the detector is a working electrode, the second position is at least in close proximity with the working electrode, thus the magnetic field causes at least some of the complex to move near to the electrode, thereby separating the target capture moiety comprising the complex from any unbound target capture moiety. The proximity of the complex and the working electrode is such that, on application of an electrical stimulus to the working electrode, electrons are transferred between the working electrode and the redox mediator in an inactive state or detectable product, thereby generating a response that is sensed. Where the second position does not overlap with the working electrode, the redox mediator or detectable product may be capable of diffusing to the working electrode.

When target is present, the strength of the response detected by the detector is directly proportional to the amount of redox mediator and/or catalyst substrate that is converted by the catalyst, which (when the redox mediator and/or the catalyst substrate are in excess) is dependent on the concentration of catalyst at the second position, which is in turn dependent on the concentration of target present in the sample. Thus, in some embodiments, the strength of the response is proportional to the amount of target in the sample.

Typically, the redox mediator and/or the catalyst substrate are present in the system in molar excess relative to each of the target binding moiety tethered to a magnetically moveable particle and the target capture conjugated to a catalyst. Often, the target binding moiety tethered to a magnetically moveable particle and the target capture conjugated to a catalyst are present in substantially equal amounts (i.e. the mean molar concentration of each lie within $\pm 10\%$ of each other). Typically, the redox mediator and/or the catalyst substrate are present in molar amounts of 10^1 to 10^{20} times greater than each of the target binding moiety tethered to a magnetically moveable particle and the target capture conjugated to a catalyst, such as 10^3 to 10^{18} , 10^5 to 10^{15} . In some embodiments, the redox mediator and/or the catalyst substrate are present in molar amounts of at least 10^{10} times greater than each of the target binding moiety tethered to a magnetically moveable particle and the target capture conjugated to a catalyst.

The test strip may comprise a base. The base may comprise a film. The film may comprise a hydrophilic or hydrophobic polymer, or a hydrophilic or hydrophobic treatment of a polymer to either promote or hinder wetting or fluid movement or diffusion. Hydrophilic polymers, such as some polyesters, may be beneficial owing to improved wetting of the base by the sample, and improved wicking of the sample across the surface of the base.

The test strip, e.g. the base, may further be treated with a polymer suitable for hindering diffusion of large molecules (such as molecules of a molecular weight greater than about 100 Da, in some embodiments molecules of a molecular weight greater than about 4000 Da, in some embodiments molecules of a molecular weight in the range of 1 MDa) across the surface of the base. This way, the diffusion of any unbound target capture moiety conjugated to a catalyst to the second or third positions on the base may be hindered. For the avoidance of doubt, the polymer suitable for hindering diffusion of large molecules may also hinder, but to a much smaller extent, the movement of the complex to the second position on activation or generation of the magnetic field. In some embodiments, the polymer is positioned at least at the first position. The polymer may comprise or consist of one or more of the group consisting of carboxymethyl cellulose, polyethylene glycol, dextran, dextrin, and polystyrene.

The polymer suitable for hindering diffusion of large molecules may also hinder the diffusion of the redox mediator and/or the catalyst substrate so that diffusion of redox mediator and/or catalyst substrate to the first position may be hindered.

The first and second positions on or within the test strip may be at least partially or entirely covered by the mixture. In some embodiments, the first and third positions on or within the strip are at least partially or entirely covered by the mixture. In some embodiments, the first, second and third positions are at least partially or entirely covered by the mixture. In particular embodiments, first, second and third positions on or within the strip are at least partially or entirely covered by the mixture, which comprises a single body of fluid, i.e. the mixture is not broken up.

The target capture moiety may be conjugated to the catalyst via a linker, i.e. a molecule bonded to both the target capture moiety and the catalyst. The linker may be a particle, such as a latex particle. Alternatively, the linker may form by conjugation techniques known in the art, such as biotin-streptavidin or 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC)-N-hydroxysuccinimide (NHS). For example, the catalyst may be conjugated to biotin and the target capture moiety may be conjugated to streptavidin. The biotin and streptavidin may bind to one another, thereby linking the catalyst and the target capture moiety.

Similarly, the target binding moiety may be conjugated to the magnetically moveable particle via a linker, i.e. a molecule bonded to both the target binding moiety and the magnetically moveable particle. The linker may be a particle, such as a latex particle. Alternatively, the linker may form by conjugation techniques known in the art, such as biotin-streptavidin or 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC)-N-hydroxysuccinimide (NHS). For example, the magnetically moveable particle may be conjugated to streptavidin and the target binding moiety may be conjugated to biotin. The biotin and streptavidin may bind to one another, thereby linking the magnetically moveable particle and the target binding moiety.

In some embodiments, the catalyst is an enzyme. For the avoidance of doubt, in such embodiments the catalyst substrate is an enzyme substrate. Suitable catalyst (e.g. enzyme) substrates will be selected in accordance with the type catalyst or enzyme selected. Thus, the enzyme substrate may be any substrate of the enzymes described herein. In some examples, the enzyme substrate may be glucose or hydrogen peroxide (e.g. urea hydrogen peroxide).

In some embodiments, where the catalyst is an enzyme, the enzyme is any one selected from the group consisting of horseradish peroxidase, alkaline phosphatase, glucose dehydrogenase, glucose oxidase, glutathione reductase, xanthine oxidase, laccase, glutaredoxin, cytochrome c oxidase, alcohol dehydrogenase, pyruvate dehydrogenase and sorbitol dehydrogenase.

In some embodiments, the catalyst is a redox catalyst such as a redox enzyme. In some embodiments, the redox enzyme is a reducing enzyme. Where the enzyme is a reducing enzyme, redox mediator is typically added to the system as a reduced mediator. In this way, the redox mediator is able to reduce the oxidised enzyme that forms following reduction of the enzyme substrate, to re-form the reduced form of the enzyme. The reduced form of the enzyme is then able to convert another molecule of substrate, and the cycle is able to repeat.

In particular embodiments, the enzyme is a reducing enzyme and the redox mediator is a reducing mediator. The reducing enzyme may be glutathione reductase.

In alternative embodiments, the redox enzyme is an oxidising enzyme. In such embodiments, redox mediator is typically added to the system as an oxidised mediator. The oxidising enzyme may be any one selected from the group consisting of an oxidase, peroxidase and dehydrogenase, such as horseradish peroxidase, glucose dehydrogenase, glucose oxidase, xanthine oxidase, laccase, cytochrome c oxidase, alcohol dehydrogenase, pyruvate dehydrogenase and sorbitol dehydrogenase.

The redox mediator may be any one selected from the group consisting of ferri/ferrocyanide; ruthenium (II) and (III) complexes (e.g. ruthenium (III) hexamine chloride; ferrocene and ferrocenium derivatives; cobaltocene, rhodocene and other metallocenes; p-nitrophenyl phosphate (pNPP); quinones; nicotinamide adenine dinucleotide; nicotinamide adenine dinucleotide phosphate; flavin adenine dinucleotide; methylene blue and derivatives; 2,6-dichlorophenolindophenol; and phenylenediamine and derivatives.

In some embodiments, the redox enzyme is an oxidising enzyme and the redox mediator is added to the system as a reduced mediator. Alternatively, the redox enzyme may be a reducing enzyme and the redox mediator may be added to the system as an oxidised mediator.

The skilled person is able to assess which redox mediators are compatible with which redox catalysts. By way of example only, horseradish peroxidase is compatible with at least 3,3',5,5'-tetramethylbenzidine, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid, diaminobenzidine and phenylenediamine. Glucose dehydrogenase is compatible with at least ferricyanide, ruthenium (III) hexamine (and salts, such as chloride salts thereof), tris(bipyridine)ruthenium(III) chloride, ferrocene and ferrocene derivatives.

The target capture moiety and target binding moiety need not be limited, provided they are capable of binding to the target at separate binding sites. For

example, in certain embodiments the target binding moiety and the target capture moiety each independently comprise at least one of: a nucleic acid, such as a single stranded DNA, RNA, PNA or LNA molecule, an antibody, or an antigen binding fragment thereof, a cell surface receptor or its ligand, or a biologically active fragment of a cell surface receptor or its ligand, an aptamer, a molecular imprinted polymer, an enzyme, a lipid, a glycan, a glycoprotein, a glycolipid, or a proteoglycan. In some embodiments the target capture moiety and target binding moiety include a fusion of one or more of the above moiety types. Moreover, the target capture moieties and target binding moieties may be fused or otherwise bound to the magnetically moveable particle or redox catalyst using techniques well known in the art.

Tagging with nucleic acid sequences is well-known in the art and may be carried out using standard bio-conjugation chemistries (see, for example O. Koniev and A. Wagner, *Chem. Soc. Rev.*, 2015, 44, 5495; and C. Sornay et al., *R. Soc. Open Sci.*, 2022, 9:211563). For example, N-hydroxysuccinimidyl (NHS) ester coupling with amines, carbodiimide (such as 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), dicyclohexylcarbodiimide (DCC) or diisopropylcarbodiimide (DIC)) coupling with amines, biotin coupling with Streptavidin (see, for example, C. M. Dundas et al., *Appl. Microbiol. Biotechnol.*, 97, 9343–9353 (2013)); or maleimide coupling with thiol moieties (see, for example, S. S. Ghosh et al., *Bioconjug. Chem.*, 1990, 1:71-6) may be used.

In some embodiments, the target binding moiety and the target capture moiety each independently comprise one or more selected from the group consisting of protein, DNA and RNA. The target binding moiety and the target capture moiety may each independently comprise one or more selected from the group consisting of an antibody, an antigen-binding antibody fragment, an antibody mimetic and a nucleic acid strand (e.g. a DNA or RNA strand). Often, the target binding moiety and the target capture moiety each independently comprise one or more selected from the group consisting of an antibody, an antigen-binding antibody fragment and an antibody mimetic.

As used herein, the phrase “antigen-binding fragment thereof”, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen. The antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term “antigen-binding fragment thereof” include (i) a Fab fragment, a monovalent fragment consisting of the VH, VL, CL and CH1 domains; (ii) a F(ab')₂ fragment, a bivalent fragment

comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VH and VL domains of a single arm of an antibody, (v) a dAb fragment (Ward et al., (1989) Nature 341:544 546), which consists of a VH domain; and (vi) an isolated
5 complementarity determining region (CDR) or (vii) a combination of two or more isolated CDRs which may optionally be joined by a synthetic linker. Furthermore, although the two domains of the Fv fragment, VH and VL, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VH and VL regions pair
10 to form monovalent molecules (known as single chain Fv (scFv); see e.g., Bird et al. (1988) Science 242:423 426; and Huston et al. (1988) Proc. Natl. Acad. Sci. USA 85:5879 5883). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding fragment thereof". These antigen binding fragments are obtained using conventional techniques known to those with skill in the art, and the
15 fragments are screened for utility in the same manner as are intact antibodies.

The inventors have found that the method of the invention is capable of detecting the presence of target in a sample at concentrations less than 50 pg/mL, e.g. as low as 10 pg/mL or 1 pg/mL. In particular embodiments, the method is capable of detecting the presence of a target at a concentration of 1pM, 5 pM or 10 pM in a
20 sample, e.g. 1 pM or 10 pM or more of a target in a sample.

In cases where the detector is a working electrode, the working electrode is typically part of an electrode system comprising at least one further electrode, i.e. the method of the invention typically comprises an electrode system comprising two or more electrodes. In some embodiments, the electrode system is electronically
25 connected to a sensor such as a potentiostat. In particular embodiments, the electrical stimulus is a potential and the electrical response is a current. In such cases, the electrode system may be any system suitable for the application of a potential difference to the working electrode. To supply a potential difference, the electrode system comprises a minimum of two electrodes. For example, the working electrode
30 may apply the desired potential to the inactive redox mediator and transport charge to (thereby acting as an anode and reducing the inactive redox mediator) or from (thereby acting as a cathode and oxidising the inactive redox mediator) the inactive redox mediator. A second electrode is required with a known potential, with which to use as a reference, and to complete the circuit and balance the charge, for example if the
35 working electrode acts as an anode and reduces the redox mediator, then the second

electrode balances the charge by oxidising a component of the system, thereby acting as a cathode. The second electrode acts as both a reference and a counter electrode.

Typically, the electrode system comprises 2 to 4 electrodes, often 3 or 4. Commonly, the electrode system comprises 3 electrodes. When the electrode system comprises 3 electrodes, it comprises a working electrode, a reference electrode (of a known potential and with which to use as a reference) and a counter electrode (to complete the circuit and balance the charge). In such an electrode system, changes in the potential difference at the working electrode are measured independently to the changes in the potential difference at the counter electrode. This set-up is advantageous over a 2 electrode system because analysis of the electrical response is simpler.

When the electrode system comprises 4 electrodes, it comprises a working electrode, a reference electrode, a counter electrode and a working sense electrode. In this set-up, the potential difference is measured at the working sense electrode (relative to the reference electrode), and is independent to the electrochemical reaction occurring at the working electrode, i.e. the effect of an applied current on, the system itself is being measured. Such a set-up is useful for the measure of impedance across the system (discussed below).

The electrodes of the electrode system may be made of any material suitable for conducting electrons. It is preferable that the material is resistant to corrosion, and is able to conduct a suitable current load. Sometimes, the current load required is in the range of ± 1 nA to ± 1 mA; ± 10 nA to ± 0.1 mA; or ± 100 nA to ± 0.01 mA (with an error of $\pm 1\%$). Suitable materials include any one or a selection from the group consisting of gold, silver, platinum, palladium, titanium, graphite, carbon, brass, tungsten, ruthenium, iridium, titanium, nickel, aluminium, tin, or one or a selection of their oxides. Often, the electrodes of the system are made from any one or a selection from the group consisting of gold, silver, platinum, palladium, titanium, graphite and carbon. Typically, the electrodes are made from any one or a selection from the group consisting of gold, silver, platinum and palladium, preferably gold and silver. Commonly, the electrodes are made of one type of material, i.e. they are not made of a selection of materials.

The electrode system may be produced via additive printing processes, such as 3D-printing or screen-printing. These are suitable approaches for the production of cost-effective electrode systems and sensors (Tan, C., Nasir, M. Z. M., Ambrosi, A., Pumera, M., 2017. Anal. Chem. 89, 8995-9001). The electrode system may be

microfabricated and may be produced by depositing the desired material, patterning the material with the desired micro features (e.g. by UV photolithography), and if necessary, removing or etching material. Preferably, the electrode system is screen-printed. Screen printed electrodes (SPEs) feature many advantages over more traditional electrodes such as ease of fabrication and cleaning procedures, reliability, low-cost, repeatability and provide rapid time to result. SPEs are amenable to mass production, whereby a large volume of electrodes can be produced at relatively low-cost compared to traditional macro or microelectrodes (Hayat, A., Marty, J. L., 2014. Sensors. 14, 10432-10453). Due to these advantages, SPEs lend themselves nicely to prototyping and for the development of novel sensing technologies, as reported here.

The electrodes may be electronically connected to the potentiostat. The electronic connections may be any suitable connections to carry an electronic signal between the potentiostat and the electrode system. Typically, such electronic connections are electric wires. Each electrode of the electrode system is electronically connected to the potentiostat to allow the application and detection of electrical signals to and from each electrode.

In some embodiments, amperometry (such as chronoamperometry), electrochemical impedance spectroscopy (EIS), open circuit potentiometry, chronocoulometry, pulse voltammetry, cyclic voltammetry, and/or linear sweep voltammetry are used to apply an electrical stimulus and sense an electrical response.

In some embodiments, amperometry (such as chronoamperometry), electrochemical impedance spectroscopy (EIS), open circuit potentiometry, chronocoulometry, pulse voltammetry, and/or cyclic voltammetry are used to apply an electrical stimulus and sense an electrical response. The potentiostat of the system may be suitable for cyclic voltammetry, open circuit potentiometry measurements, chronoamperometry, EIS, square wave voltammetry (SWV), and differential pulse voltammetry (DPV).

DPV can be used to sensitively investigate electron transfer to and from an electrode surface. An electric potential is measured between the working electrode and the reference electrode, while the current is measured between the working electrode and the counter electrode. The electric potential is increased or decreased linearly with time to a set potential (a potential linear sweep), or is incrementally increased or decreased with time (a staircase waveform). A series of regular voltage pulses are superimposed upon the potential linear sweep or staircase waveform. The current is measured before (initial current) and after (final current) the voltage pulse,

and the difference between the final and initial current is plotted as a function of the applied electric potential. In this way, the effect of the non-Faradaic charging current is minimised, i.e. only the Faradaic current (the current generated by the reduction or oxidation of components of the system) is measured, thus electron transfer may be analysed more precisely.

The peak current (I_{pk}) measured in DPV plots corresponds to the current generated on oxidation of a component of the system. Changes in the peak current are reflective of changes in complex concentration at the working electrode: as the amount of complex increases, the peak current increases. Thus, measuring the peak current over time gives an indication of the rate of complex build up at the working electrode.

Sometimes the electrical signal applied to the electrode system is a series of regular voltage pulses superimposed upon the potential linear sweep or staircase waveform, with a potential applied in the range of about -1.0 V to 1.0 V. Other times, the potential applied is in the range of about -0.5 V to 0.7 V or about -0.3 V to 0.5 V.

In one embodiment, the electrical stimulus comprises a potential applied between the working electrode and the reference electrode and the electrical response consists of a current between the working electrode and the counter electrode. The type of electrical response detected from the electrode system depends on the measurement. If the measurement is DPV, then the electrical response is a direct current (DC). The current is measured before (initial current) and after (final current) the voltage pulse. Thus, when measuring DPV, the electrical signal applied is a series of regular voltage pulses superimposed upon the potential linear sweep or staircase waveform and the electrical response is a direct current measured before and after the voltage pulse.

EIS is capable of real-time data capture. The impedance of the working electrode-structure interface may be studied using an alternating potential difference across a range of frequencies to establish information regarding the interface, its electron transfer properties and surrounding diffusional behaviour. The term "impedance" used herein, is a measure of the frequency dependant resistance of the first substance to a current flow of a circuit, and is calculated according to the formula below, where E_ω is equal to the frequency-dependant potential and I_ω is equal to the frequency-dependent current.

$$Z_\omega = \frac{E_\omega}{I_\omega}$$

Changes in impedance are reflective of changes at the electrode surface as a function of time: as the number of complexes at the working electrode increases, the frequency dependent resistance at the surface of the working electrode to a current flow (i.e. the impedance) increases.

The skilled person is aware that an electrical stimulus may be applied to the working electrode and an electrical response may be sensed from the working electrode at multiple time points. For example, a response may be measured before activating or generating the magnetic field and after activating or generating the magnetic field and the two responses compared to assess whether or not the target is present in the sample.

For the avoidance of doubt, each of the embodiments described in relation to the method of the first aspect apply mutatis mutandis to any of the other methods, test strips, systems, kits and uses described above and herein.

The second aspect of the invention provides a test strip comprising:
an assay chamber;
a sample inlet in fluid communication with the assay chamber;
a target capture moiety conjugated to a catalyst positioned on or within the assay chamber at a first position; and
a catalyst substrate positioned on or within the assay chamber at a second position;

wherein the sample inlet is positioned between the first and second positions.
The test strip may be configured such that the introduction of a sample into the assay chamber via the sample inlet causes the sample to travel or flow across the first position in a direction that is away from the second position.

The third aspect of the invention provides a system comprising:
(a) a test strip comprising:
an assay chamber;
a sample inlet in fluid communication with the assay chamber;
a target capture moiety conjugated to a catalyst positioned on or within the assay chamber at a first position; and
a catalyst substrate positioned on or within the assay chamber at a second position;

wherein the sample inlet is positioned between the first and second positions, such that the introduction of a sample into the assay chamber via the sample inlet

causes the sample to travel or flow across the first position in a direction that is away from the second position;

(b) a target binding moiety tethered to a magnetically moveable particle; and

5 (c) a magnetic field generator configured to activate or generate a magnetic field;

wherein the system is configured such that when the magnetic field is activated or generated, the magnetically moveable particles move to the second position.

When a sample comprising a target is contacted with a target capture moiety conjugated to a catalyst and a target binding moiety tethered to a magnetically moveable particle, a complex is formed, the complex comprising the target capture moiety conjugated to a catalyst and the target binding moiety tethered to a magnetically moveable particle, each being independently bound to the target. In such cases, when the magnetic field is activated or generated, the complex moves to the second position such that the complex is separated from one or more other components of the mixture and such that the catalyst catalyses the conversion of the catalyst substrate to another product.

The catalyst may be a redox catalyst and the system may further comprise a redox mediator positioned at the second position such that when the magnetic field is activated or generated, the redox mediator is able to transfer electrons to or from the redox catalyst such that the redox catalyst catalyses the conversion of the redox catalyst substrate to another product and catalyses the conversion of the redox mediator from an active to an inactive state.

The system may further comprise a detector. This may be any detector suitable to sense a response that is characteristic of a result of the catalysis, such as the inactive redox mediator or an active product formed by conversion of the catalyst substrate. For example, the detector may be suitable for detecting the wavelength of absorption of the inactive redox mediator or an active product formed by conversion of the catalyst substrate, e.g. a UV-vis spectrometer or the like.

Alternatively, the detector may be a working electrode positioned at a third position, which third position is at least in proximity to the second position, and away from the first position, such that when an electrical stimulus is applied to the working electrode, electrons are transferred between the working electrode and the redox mediator in an inactive state or an active product formed by conversion of the catalyst substrate.

In some embodiments, the system of the third aspect further comprises a sample that may or may not comprise a target. The system of the third aspect may further comprise a sensor to sense an electrical response.

5 The fourth aspect of the invention provides a kit of parts, comprising, as separate components:

- (a) a test strip comprising:
 - an assay chamber;
 - a sample inlet in fluid communication with the assay chamber;
 - a target capture moiety conjugated to a catalyst positioned on or within the
 - 10 assay chamber at a first position; and
 - a catalyst substrate positioned on or within the assay chamber at a second position;
 - wherein the sample inlet is positioned between the first and second positions;
- (b) a target binding moiety tethered to a magnetically moveable particle; and
- 15 (c) an electromagnet positionable such that activation of the electromagnet generates a magnetic field capable of moving the magnetically moveable particle to the second position or a permanent magnet positionable such that a magnetic field capable of moving the magnetically moveable particle to the second position is generated.

20 The catalyst may be a redox catalyst and the kit of parts may further comprise a redox mediator positioned at the second position.

The kit may further comprise a detector, which may be any detector suitable to sense a response that is characteristic of a result of the catalysis, such as inactive redox mediator or an active product formed by conversion of the catalyst substrate. For example, the detector may be suitable for detecting the wavelength of absorption of

25 the inactive redox mediator or an active product formed by conversion of the catalyst substrate, e.g. a UV-vis spectrometer or the like.

Alternatively, the detector may be a working electrode positioned at a third position on the test strip, at least in proximity to the second position, and away from the first position.

30 In some embodiments, redox mediator and/or the catalyst substrate are each positioned at the working electrode.

The kit of parts may further comprise one or more selected from:

- (i) at least one further electrode;
- (ii) a sensor such as a potentiostat; and

(iii) one or more electronic connections capable of completing a circuit between the electrodes and sensor.

The fifth aspect of the invention provides use of the test strip of the second aspect, the system of the third aspect, or the kit of parts of the fourth aspect to detect the presence or absence of a target in a sample. For example, the test strip of the second, third and fourth aspects may further comprise a sample that may or may not comprise a target. The sample may be added to the test strip to form a mixture as defined in the first aspect. The first, second and third positions may be at least partially or entirely covered by the mixture.

For the avoidance of doubt, each of the embodiments described in relation to the method of the first aspect apply mutatis mutandis to the test strip of the second aspect, the system of the third aspect, the kit of parts of the fourth aspect, and the use of the fifth aspect. For example, both redox mediator and redox catalyst substrate may be positioned at a working electrode; the redox catalyst may be a redox enzyme; and/or the test strip may further comprise a polymer suitable for hindering diffusion of large molecules, for example across the surface of the test strip, wherein the polymer is positioned at least at the first position.

The methods, test strips, systems, kits and uses described herein are intended for use in detecting the presence of a target in a sample. The target may be an analyte. In particular, the target may be a component found (or potentially found) in a biological sample. In other examples, the target may be a component (such as a contaminant) found (or potentially found) in a sample taken from a manufacturing process.

In some examples, the target may be a protein, a peptide, a nucleic acid, a metabolite, a saccharide or polysaccharide, a lipid, a drug or drug metabolite, or the like.

The sample may be a biological fluid. By way of example only, the biological fluid may be blood, plasma, serum, urine, sweat, stool, cerebrospinal fluid, interstitial fluid, saliva, sputum, nasal fluid or any other bodily fluid.

The sample may be a liquid sample. The sample may be diluted and/or mixed with a liquid (e.g. a solution or buffer) prior to introduction to the sample inlet. Accordingly, in some examples, the sample may comprise a biological fluid diluted and/or mixed in a liquid (e.g. a solution or buffer).

As stated above, the test strips and systems described herein can allow the distance between the first and second positions to be reduced, minimising the overall size of the test strip and/or the volume of sample required. In particular, the volume of a

biological sample required may be minimised. In some embodiments, the methods may be suitable to detect the presence of a target in from about 1 μ L to 1 mL of a biological sample (e.g. from about 1 μ L to about 100 μ L, or from about 1 μ L to about 10 μ L, e.g. from about 5 μ L to about 10 μ L of a biological sample). Such biological samples may be diluted and/or mixed with a biologically compatible liquid or buffer prior to introduction to the sample inlet.

Each and every patent and non-patent reference referred to herein is hereby incorporated by reference in its entirety, as if the entire contents of each reference were set forth herein in their entirety.

The invention may be further understood with reference to the following examples.

EXAMPLES

Magnetic accumulation of magnetite beads coated with horseradish peroxidase (HRP) enzyme was demonstrated using a test strip of the invention.

Urea hydrogen peroxide (UHP) was used as the enzyme substrate and 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) was used as a redox mediator. These were deposited onto the surface of the working electrode using dextran and polyethylene glycol and air-dried.

A change in colour appears between $t=0$ (left) and $t=60s$ (middle) (see Fig. 2. a.) as a magnetic field causes the HRP-labelled beads to move to the second position at the top of the strip and react with substrate and mediator, causing an electrochemical change (i.e. oxidation of ABTS) detectable using an electrode-potentiostat system concomitant, with the colour change. A significant difference in the current detected can be seen between the positive (magnet in place) and negative (no magnet present) samples (see Fig 2. b).

A negative sample where no magnet is present at the working electrode is shown in the photograph on the right of Fig. 2. a., taken after 300s.

Interleukin 6 (IL-6) assay

A wash-free IL-6 chronoamperometry magnetic assay was carried out using a magnetic rig (shown in Fig.3.b.), dumbbell strips (shown in Fig.3.a.) and IL-6 reagents in a wet format.

Equipment and materials

- poly-HRP lot P366115 (cat 898387 from RD system): poly-horseradish peroxidase conjugated to Streptavidin
- IL-6 conjugate lot P355923 (cat. 898934 from RD System): polyclonal antibody specifically for human IL-6, and conjugated to biotin
- 5 - IL-6 antigen lot P318690 (cat. 840115 from RD System): Human IL-6 standard
- Reagents substrate A lot P361118 (cat 895000 from RD System): stabilized hydrogen peroxide
- Reagents substrate B lot P361121 (cat 895001 from RD System): stabilized tetramethylbenzidine
- 10 - Tween 20
- Phosphate buffered saline (PBS)
- Lidded dumbbell strip
- Palmsense connector
- Magnetic rig

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The dumbbell strip comprises a reagent zone (see Fig.3.a.), to which was added a solution comprising anti-IL-6 antibody-coated magnetic beads, IL-6 antigens, IL-6 detection antibodies conjugated to biotin, and HRP enzymes conjugated to biotin. Prior to addition to the dumbbell strip, the solution was allowed to incubate for 20 minutes so that a complex could form comprising: magnetic bead - IL-6 antibody – IL-6 antigen – IL-6 antibody conjugated to biotin – streptavidin conjugated polymer HRP.

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The dumbbell strip also comprises a detection zone (e.g. an electrode zone) (see Fig.3.a.), comprising a working electrode, on which substrates for the HRP enzyme (hydrogen peroxide and tetramethylbenzidine) were positioned.

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Tween20 and phosphate buffered saline (PBS) solution were added to the dumbbell strip at the position shown in Fig.3.a.. These act as a stacker solution, i.e. they are suitable for easing diffusion of molecules, particularly larger molecules such as the complex comprising magnetic bead - IL-6 antibody – IL-6 antigen – IL-6 antibody conjugated to biotin – streptavidin conjugated polymer HRP, or magnetic bead - IL-6 antibody.

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Once the reagents were added to the dumbbell strip, a magnet was moved along the magnetic rig to drag the magnetic beads (both complexed and uncomplexed) into the electrode zone, where they mixed with the HRP substrate.

Chronoamperometry was used to measure the electrochemical response generated by HRP acting on the substrates.

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Formal method steps

- 5 A) Formation of an immunocomplex between anti-IL-6 antibody coated magnetic beads, biotinylated detection antibody, streptavidin poly-HRP and the IL-6 antigen were performed as follows:
- 1) 1% coated magnetic beads were prediluted in RD System Reagents Diluent, 1:1.
 - 2) 5 uL of the coated beads, at 0.5%, was added to a protein low bind tube.
 - 3) 2.5 uL of biotin conjugated detection IL-6 antibody from HS IL-6 RD system kit was then added to the tube.
 - 10 4) 7.5 uL of streptavidin conjugated poly HRP from HS IL-6 RD system kit was then added to the tube.
 - 5) IL-6 antigen was dissolved in deionised water to a final concentration of 60 ng/mL and further dilution steps were made in RD System Reagents Diluent.
 - 6) 5 uL of one of various IL-6 antigen solutions was added to the tube or Reagents Diluent was added as a blank sample.
 - 15 IL-6 solutions concentration made were 4 ng/mL, 2 ng/mL, 400 pg/mL and 200 pg/mL.
 - 7) The tube was quickly vortexed to mix reagents, spun down to collect all the volume at the bottom of tube and incubated static for 20 minutes.
 - 8) After 20 minutes, the tube was placed on a magnet to separate the beads and 15 uL of the supernatant was removed.
 - 20 B) The magnetic assay was performed as follows:
 - 1) The lidded dumbbell strip was inserted into the Palmsense connector which was embedded onto the magnetic rig.
 - 2) The magnetic rig was connected to the laptop and the magnet was positioned at the top of the strip (reagents zone)
 - 25 3) 5 uL of a 1:1 mix solution of RD System Reagents Substrate A and B was added to the bottom of the strip (electrode zone)
 - 4) 8 uL of Tween20 0.5% PBS was added to the electrode zone, and also filled the area of the strip shown in Fig.3.a..
 - 30 5) 5 uL of the solution prepared in A) was added to the top of the strip (reagents zone).
 - 6) The magnetic rig button was pushed in order to activate the magnet movement; the magnet dragged the beads into the electrode zone.
 - 7) A chronoamperometry measurement was taken at -0.1V, 20 sec scan.
 - Two rigs and two Potentiostats were used. The following repeats were performed per
 - 35 IL-6 concentration levels: 5 reps for blank, 4 reps for 50 pg/mL, 3 reps for 100, 500 and

1000 pg/mL.

Results

5 A linear correlation was observed (see Fig.3.c.) between IL-6 antigen concentration and electric charge detected at the working electrode via chronoamperometry as in step B) 7), described above, in the range of 50 to 1000 pg/mL (R square = 0.995).

A limit of the blank (LoB) was calculated as equal to Mean Blank + (1.645 x SD Blank) resulting in 29.8 pg/mL.

10 A limit of detection was calculated as = LoB + (1.645 x SD) resulting in lowest detectable analyte concentrations of 83.7 pg/ml of IL-6.

The exemplary method described above was successfully applied to detect the IL-6 target using antibody coated magnetic beads and biotin conjugated antibodies, together with streptavidin conjugated polymer HRP, and substrates of HRP.

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Nucleic acid assay

An exemplary method for the detection of nucleic acid target strands is described.

Method

- 20 1) A nucleic acid probe strand was connected to magnetic beads (see Fig.4.a.) via common conjugation techniques, e.g. biotin-streptavidin or 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC)-N-hydroxysuccinimide (NHS).
- 2) The nucleic acid target bonded to the probe strand, exposing an overhanging region on the target complementary for a detection strand.
- 25 3) An enzymatically labelled (e.g. HRP, glucose dehydrogenase, Alkaline phosphatase etc.) detection strand bonded to the overhanging region to form a complex on the magnetic bead
- 4) The complex was added to one part of a test strip.
- 5) The substrate for the enzyme (e.g. HRP = horseradish peroxidase substrate, TMB and ABTS mediators; glucose dehydrogenase = glucose substrate; alkaline phosphatase = pNPP substrate etc.) was added on or in proximity to a
- 30 6) The complex was dragged using a magnetic to the working electrode, where the substrate was acted on by the enzyme.

- 7) The enzymatic signal was electrochemically measured via incubation of the full complex with a redox mediator (e.g. 3,3', 5,5"-tetramethylbenzidine (TMB), p-nitrophenyl phosphate (pNPP), ferricyanide, ruthenium hexamine chloride etc.)
- 8) Electrochemical detection was carried out via chronoamperometry, DPV, SWV, linear sweep voltammetry or cyclic voltammetry.

Data

A linear relationship was observed in both calibration curves obtained for (1) specific target (*Oxa1* – oxacillin resistance gene) versus a non-specific target (*tetA* – tetracycline resistance) with HRP, hydrogen peroxide and TMB as the enzyme, substrate and mediator combination and via chronoamperometry (see Fig.4.b.), and (2) specific target (*Oxa1* – oxacillin resistance gene) versus a non-specific target (*tetA* – tetracycline resistance) with alkaline phosphatase and pNPP as the enzyme and substrate combination and via linear sweep voltammetry (LSV) (see Fig.4.c.).

Conclusion

It was possible to produce dose response curves for a specific genetic sequence (*oxa1*) with minimal signal interference seen from another genetic sequence (*tetA*), which was used as an example negative control sequence.

Example Test Strip

A line drawing of an exemplary test strip for use in the methods disclosed herein is shown in Fig. 5. This test strip comprises a base that is a dumbbell-like shape, one of the ends being a reagent zone, where the target binding moiety tethered to a magnetically moveable particle (such as a capture antibody tethered to a magnetic bead, as in Fig. 5) and a target capture moiety conjugated to a catalyst (such as a detection antibody conjugate tethered to an enzyme, as in Fig. 5) are positioned.

The other end of the base is a detection zone, where the catalyst substrate (in Fig. 5, this is an enzyme substrate) is positioned and, when used, the redox mediator is positioned. In Fig. 5, the enzyme substrate and redox mediator are pre-mixed prior to deposition on the test strip. When detecting the catalysis comprises (i) applying an electrical stimulus to a working electrode positioned at a third position, which third position is at least in proximity to the second position, and away from the first position; and (ii) sensing an electrical response, the working electrode may be positioned at the detection zone (shown as a carbon electrode in Fig. 5). The base of Fig. 5 further

comprises a silver/silver chloride reference electrode and a carbon counter electrode, both positioned at the detection zone. A connection pad is used to electronically connect the electrodes. The electrodes may be connected to a potentiometer.

5 The base of Fig. 5 further comprises a sample inlet, which is positioned between the reagent and detection zones. The sample is positioned in the sample inlet. Whilst the sample can be positioned at the reagent zone, the inventors have found that it is advantageous to position and/or introduce the sample by way of the sample inlet. If the sample is applied as a liquid to the reagent zone, it could run to and overlap with the detection zone, allowing the target binding moiety tethered to a
10 magnetically moveable particle and/or the target capture moiety conjugated to a catalyst to move to the detection zone and potentially give a false positive result. Positioning the sample inlet between the first and second position and introducing the sample via this inlet, minimises the risk of diffusion of the target binding moiety tethered to a magnetically moveable particle and/or the target capture moiety conjugated to a
15 catalyst to the detection zone on sample application.

The reagents in the reagent zone and the sample in the sample inlet may be controllably re-suspended, allowing the components to mix and, where target is present, form a complex. A magnetic field may then be activated or generated to cause the complex to move to the detection zone such that the catalyst catalyses the
20 conversion of the catalyst substrate to another product. The catalysis can be detected by applying an electrical stimulus to a working electrode positioned in the detection zone; and (ii) sensing an electrical response. The base of Fig. 5 further comprises two air escapes at either end of the strip, to prevent the build-up of air, which could hinder the mixing of the components and the movement of the complex.

25 The reference numerals indicated on Fig. 5 designate the following features of the test strip: 5 – air escape; 10 – magnetic beads-capture antibody; 15 - enzyme-detection antibody conjugate; 20 – reagent zone; 25 - detection zone; 30 – Working Electrode (Carbon); 35 – Reference Electrode (Silver/Silver Chloride); 40 – Counter Electrode (Carbon); 45 – air escape; 50 – connection pad; 55 – catalytic substrate and
30 electrochemical redox mediator (pre-mixed prior to deposition); 60 – sample inlet (the centre-fill design allows for resuspension of reagents whilst preventing magnetic beads or enzyme conjugate moving to the detection zone upon sample application).

Example Assay Using “Centre-Fill” Test Strip

35 Materials Used:

Substrate/Mediator	Enzyme-Detection Antibody Conjugate	Magnetic beads-capture antibody
250mM Ruthenium (III) Hexamine Chloride 250mM D-Glucose 1% PEG 20kDa 0.05% Tween20	15ug/mL GDH Enzyme conjugated to antibody 1% PEG 20kDa 5% Dextran 10kDa 1% BSA 5mM Tris pH 8.0 0.05% Tween20	0.075% magnetic bead conjugated to antibody 1% PEG 20kDa 5% Dextran 10kDa 1% BSA 5mM Tris pH 8.0 0.05% Tween20

Method:

Solutions of the substrate/mediator (pre-mixed), enzyme-detection antibody conjugate and magnetic beads-capture antibody were deposited and dried on the test strip as per the general deposition pattern shown on Fig. 5.

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- An exemplary assay on a plasma sample was carried out using the test strip configuration as shown in Fig. 5. One sample was spiked with 1000 ng/mL of procalcitonin and the other left as the control (no procalcitonin was added). The stock of procalcitonin (PCT) antigen was made in plasma rather than buffer to ensure plasma concentration on-strip remained at 100%. The sample (25 μ L) was added to the test strip by way of sample inlet 60. The test strip was incubated for 2 minutes to form the immunocomplex and then the magnetic beads were magnetically moved using a 0.5T magnetic field to the working electrode, where the substrate was acted on by the enzyme, and incubated for 5 minutes. Electrochemical detection was carried out via chronoamperometry, using a potential of +0.25V for 20 seconds.
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- Fig. 6 shows the signal (charge density) generated from a positive plasma sample comprising 1000 ng/mL of procalcitonin (PCT) antigen and also from a control plasma sample (comprising 0 ng/mL of PCT antigen). This assay did not require the use of any wash steps following introduction of the sample to the test strip.
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CLAIMS:

1. A method of detecting a target in a sample using a system comprising a test strip, the test strip comprising:
- 5 an assay chamber;
a sample inlet in fluid communication with the assay chamber;
a target capture moiety conjugated to a catalyst positioned on or within the assay chamber at a first position; and
a catalyst substrate positioned on or within the assay chamber at a second
10 position;
wherein the sample inlet is positioned between the first and second positions;
and the system further comprising:
a target binding moiety tethered to a magnetically moveable particle;
wherein the method comprises:
- 15 (i) introducing a sample to the assay chamber via the sample inlet such that the sample travels or flows across the first position in a direction that is away from the second position;
- (ii) at the first position, incubating a mixture of the sample, the target capture moiety conjugated to the catalyst, and the target binding moiety tethered to a
20 magnetically moveable particle for a period of time such that, when the target is present, a complex is formed, the complex comprising the target capture moiety conjugated to the catalyst, and the target binding moiety tethered to a magnetically moveable particle, each being independently bound to the target;
- (iii) activating or generating a magnetic field to cause the complex to move to
25 the second position such that the complex is separated from one or more other components of the mixture and such that the catalyst catalyses the conversion of the catalyst substrate to another product; and
- (iv) detecting the catalysis.
- 30 2. The method of claim 1, wherein introducing the sample to the assay chamber via the sample inlet is also such that the sample travels or flows across the second position in a direction that is away from the first position.
3. The method of claim 1 or 2, wherein the catalyst is an enzyme.
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4. The method of any one of claims 1 to 3, wherein the catalyst is a redox catalyst and the system further comprise a redox mediator positioned on the test strip at the second position.
5. The method of any one of claims 1 to 4, wherein detecting the catalysis comprises:
- (a) detecting a detectable product formed by and/or as a result of the catalysis, optionally by sensing an electrical response on application of an electrical stimulus or by detecting a colour change.
6. The method of any one of claims 1 to 4, wherein the detecting comprises:
- (i) applying an electrical stimulus to a working electrode positioned at a third position, which third position is at least in proximity to the second position, and away from the first position; and
 - (ii) sensing an electrical response.
7. The method of claim 6, wherein the catalyst substrate is positioned at the working electrode, and optionally wherein both a redox mediator and a redox catalyst substrate are positioned at the working electrode.
8. The method of any one of claims 6 to 7, wherein the working electrode is part of an electrode system comprising at least one further electrode, optionally wherein the electrode system is electronically connected to a sensor such as a potentiostat.
9. The method of any one of claims 6 to 8, wherein the electrical stimulus is a potential and the electrical response is a current.
10. The method of any one of claims 6 to 9, wherein amperometry (such as chronoamperometry), pulse voltammetry, electrochemical impedance spectroscopy, and/or cyclic voltammetry are used to apply an electrical stimulus and sense an electrical response.
11. The method of any one of claims 6 to 10, wherein the strength of the electrical response is proportional to the amount of the catalyst substrate converted.

12. The method of any one preceding claim, wherein activating or generating a magnetic field causes the complex to move in a lateral direction to the second position such that the complex is separated from one or more other components of the mixture.
- 5 13. The method of any one preceding claim, wherein the magnetic field is generated by:
- (i) activating an electromagnet; or
 - (ii) mechanically activating a permanent magnet into proximity with the second position.
- 10 14. The method of any one preceding claim, wherein the target binding moiety tethered to the magnetically moveable particle is also positioned on or within the assay chamber at the first position.
- 15 15. The method of any one preceding claim, wherein at least one or more, or all, of the following:
- the method is conducted in a single liquid;
 - the method does not comprise the use of wash steps;
 - the method does not comprise contacting the sample with one or more fluidic interfaces; and
- 20 the assay chamber is a single fluid chamber.
- 25 16. The method of any one preceding claim, wherein the complex is only contacted with the catalyst substrate, and optionally also a mediator when present, upon activation or generation of the magnetic field and movement of the complex to the second position.
- 30 17. The method of any one preceding claim, wherein the first and second positions are at least partially or entirely covered by the sample following introduction of the sample to the assay chamber.
18. The method of any one preceding claim, wherein the target binding moiety and the target capture moiety each independently comprise amino acids and/or nucleic acids.

19. The method of any one preceding claim, wherein the target binding moiety and the target capture moiety each independently comprise one or more selected from the group consisting of protein, DNA and RNA.
- 5 20. The method of any one preceding claim, wherein the target binding moiety and the target capture moiety each independently comprise one or more selected from the group consisting of an antibody, an antigen-binding antibody fragment and an antibody mimetic.
- 10 21. A test strip comprising:
an assay chamber;
a sample inlet in fluid communication with the assay chamber;
a target capture moiety conjugated to a catalyst positioned on or within the assay chamber at a first position; and
15 a catalyst substrate positioned on or within the assay chamber at a second position;
wherein the sample inlet is positioned between the first and second positions, such that the introduction of a sample into the assay chamber via the sample inlet causes the sample to travel or flow across the first position in a direction that is away
20 from the second position.
22. A system comprising:
(a) a test strip comprising;
an assay chamber;
25 a sample inlet in fluid communication with the assay chamber;
a target capture moiety conjugated to a catalyst positioned on or within the assay chamber at a first position; and
a catalyst substrate positioned on or within the assay chamber at a second position;
30 wherein the sample inlet is positioned between the first and second positions, such that the introduction of a sample into the assay chamber via the sample inlet causes the sample to travel or flow across the first position in a direction that is away from the second position;
(b) a target binding moiety tethered to a magnetically moveable particle; and

(c) a magnetic field generator configured to activate or generate a magnetic field;

wherein the system is configured such that when the magnetic field is activated or generated, the magnetically moveable particles move to the second position.

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23. The test strip of claim 21 or the system of claim 22, wherein the first position:

- (i) is or comprises a reagent zone;
 - (ii) is positioned at or in proximity to a first end of the assay chamber; and/or
 - (iii) further comprises the target binding moiety tethered to the magnetically
- moveable particle.

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24. The test strip of claim 21 or 23, or the system of claim 22 or 23, wherein the second position:

- (i) is or comprises a detection zone; and/or
- (ii) is positioned at or in proximity to a second end of the assay chamber.

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25. The test strip of any one of claims 21 or 23 to 24, or the system of any one of claims 22 to 24, wherein the assay chamber is a single fluid chamber.

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26. The test strip of any one of claims 21 or 23 to 25, or the system of any one of claims 22 to 25, further comprising a detector, optionally wherein the detector comprises a working electrode positioned at a third position, which third position is at least in proximity to the second position, and away from the first position.

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27. The test strip of any one of claims 21 or 23 to 26, or the system of any one of claims 22 to 26, wherein the catalyst is a redox catalyst and the test strip further comprises a redox mediator positioned at the second position.

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28. The test strip or system of claim 27, wherein both the redox mediator and redox catalyst substrate are positioned at the working electrode.

29. The test strip of any one of claims 21 or 23 to 28, or the system of any one of claims 22 to 28, wherein the sample inlet is positioned between the first and second positions, such that the introduction of a sample into the assay chamber via the sample

inlet is also such that the sample travels or flows across the second position in a direction that is away from the first position.

30. A kit of parts, comprising, as separate components:
- 5 (a) a test strip comprising:
an assay chamber;
a sample inlet in fluid communication with the assay chamber;
a target capture moiety conjugated to a catalyst positioned on or within the assay chamber at a first position; and
- 10 a catalyst substrate positioned on or within the assay chamber at a second position;
wherein the sample inlet is positioned between the first and second positions;
- (b) a target binding moiety tethered to a magnetically moveable particle; and
- (c) an electromagnet positionable such that activation of the electromagnet
- 15 generates a magnetic field capable of moving the magnetically moveable particle to the second position or a permanent magnet positionable such that a magnetic field capable of moving the magnetically moveable particle to the second position is generated.
31. The kit of parts of claim 30, comprising the test strip as defined in any one of claims 21 or 23 to 29.
- 20 32. The kit of parts of claim 31, further comprising one or more selected from:
- (i) at least one further electrode;
- (ii) a sensor such as a potentiostat; and
- 25 (iii) one or more electronic connections capable of completing a circuit between the electrodes and sensor.
33. Use of the test strip of any one of claims 21 or 23 to 29, the system of any one of claims 22 to 29, or the kit of any one of claims 30 to 32 to detect the presence or
- 30 absence of a target in a sample, optionally in accordance with the method of any one of claims 1 to 20.