Title

Lateral flow test strips, kits, and methods of detecting a target molecule.

Technical Field

The present invention relates to lateral flow test strips which can be used to detect the presence of a target molecule in a liquid sample.

The invention also relates to kits comprising said lateral flow test strips and an extraction solution for suspending a test sample.

The invention also relates to methods of detecting a target molecule using a lateral flow test strip of the present invention.

Background

Lateral flow tests are designed to look for a target molecule that may be present in a liquid sample. For instance, lateral flow tests are used to diagnose infections caused by bacteria and viruses, and are also used for home pregnancy tests. For instance, in the case of lateral flow tests for COVID-19, the target molecule being detected is the spike protein from the SARS-CoV-2 virus. A significant benefit of lateral flow tests is their simplicity of use, and the ability to produce a result, i.e. a coloured line, within minutes that can be read with the naked eye.

Lateral flow tests rely on the interaction between an antibody and its target. Antibodies are 'Y' shaped proteins with two arms that specifically recognise other molecules and bind to them. An antibody which is specific for a certain molecule will bind only to that molecule and not to any others. Therefore, the use of antibodies in these tests allows for highly specific detection of a target molecule.

Lateral flow tests work by drawing a liquid sample along a test strip using capillary flow to meet a detection agent that shows a visual positive or negative result. The liquid to be tested is
applied to a sample pad and flows downstream (i.e. in the direction of the capillary flow of the liquid) from the sample paid to a conjugate pad. The conjugate pad stores a detection agent comprising conjugates of an antibody and a coloured particle. The antibody specifically recognises and binds the target molecule in the sample and, once the antibody is bound to the target molecule, the target molecule-conjugate flows down the strip towards a reaction membrane. The reaction membrane has a test line comprising additional antibodies that are immobilised in a line across the reaction membrane and specifically recognise and bind to the target molecule. The immobilised antibodies capture the target molecule-conjugate, creating a visible line which indicates a positive result.

D1, "Pregnancy testing throughout the ages", describes the use of lateral flow tests in home pregnancy tests. In the lateral flow tests in D1, the detection agent is an antibody conjugated

to a blue-coloured latex particle.

D2, "Product website: Goldilocks^{TM} gold nanoparticles", describes gold nanoparticles. D2 states that the gold nanoparticles are suitable for conjugation to various types of biological molecules. However, D2 provides no discussion of how conjugates comprising the gold nanoparticles could be used.

There is a continuing need to develop new detection agents that can be used in lateral flow tests strips and provide improved sensitivity, so that a result can be shown on the test strip even when the concentration of the target molecule in a liquid sample is very low.

Summary of the Invention

It is the object of the present invention to provide lateral flow tests with improved sensitivivity.

According to a first aspect of the invention, there is provided a lateral flow test strip according to claim 1.

Advantageously, the gold nanoparticles used in the detection agent of the present invention perform extremely well, and provide significantly improved sensitivity.

In experiments conducted by the inventors, gold nanoparticles were conjugated to an antibody that is specific for the spike protein of the SARS-CoV-2 virus and were compared to the same antibody conjugate to the blue-coloured latex particles described in D1. Unexpectedly, the conjugates containing the gold nanoparticles exhibited a 10-fold sensitivity improvement compared to conjugates containing blue-coloured latex particles.

This improved sensitivity is beneficial because it means that a visible result can be detected even when the quantity of the target molecule is very low. This improved sensitivity was unexpected, as D1 and D2 do not teach that gold nanoparticles would provide such a significant improvement in sensitivity compared to blue-coloured latex particles

In a preferred embodiment, the gold nanoparticles have a diameter of 40 nm, as 40 nm diameter particles provide the best sensitivity.

In a preferred embodiment, the reaction membrane further comprises a control line, positioned such that the test line is between the conjugate pad and the control line, wherein the control line comprises antibodies that specifically recognise and bind to the conjugates in the detection agent. The control line shows that the test has been carried out correctly, because it confirms that the liquid sample containing the conjugates successfully reached the test line and had a chance to interact with it.

In a preferred embodiment, the reaction membrane is a nitrocellulose membrane. Nitrocellulose membranes are advantageous because nitrocellulose has a natural ability to bind to proteins. This means that antibodies can be applied directly and immobilised firmly without needing any additional treatment.

In a more preferred embodiment, the nitrocellulose membrane has a pore size of 8 - 12 microns. The pore size can be adjusted to control the sample flow rate, and it has been determined that the best results are obtained using a pore size of 8-12 microns.

In a preferred embodiment, the lateral flow test strips further comprise a wicking pad positioned such that the reaction membrane is between the conjugate pad and the wicking pad. The wicking pad works by soaking up the liquid and therefore increases the volume of sample that enters the test strip. The increased volume washes away excess conjugates and this improves the sensitivity of the test. Various types of material can be used for the wicking pad. However, in a more preferred embodiment, the wicking pad is made from a cellulose filter, as this material is highly absorbent.

In a preferred embodiment, the lateral flow test is housed in a plastic cassette, which protects the test strip and is labelled to clearly indicate the position of the test and control lines.

In one embodiment, the sample pad is made of cellulose fibre.

In one embodiment, the conjugate pad is made from non-woven glass fibre.

In one embodiment, the detection agent is stored on the conjugate pad in dried form.

In one embodiment, the antibody has a binding affinity of $Kd \leq 10^{-7}M$ for the target molecule.

In one embodiment, the antibodies in the detection agent and the test line are the same.

In one embodiment, the antibodies in the detection agent and the test line are different.

In one embodiment, the antibodies in the detection agent and the test line specifically recognise and bind to the spike protein from the SARS-CoV-2 virus.

According to a second aspect of the invention, there is provided a kit according to claim 12. In addition to the lateral flow test strip, the kit contains an extraction solution. A test sample (e.g. a nose or throat sample) can be suspended in the extraction solution to provide a liquid sample that is suitable for use with the lateral flow test kit. Therefore, the kit provides the user with the necessary components to carry out the test at home.

In one embodiment, the extraction solution comprises phosphate buffered saline.

According to a third aspect of the invention, there is provided a method for detecting the presence of a target molecule according to claim 14.

As discussed in the background section, the lateral flow tests of the present invention can be adapted to detect any suitable target molecule, by using antibodies in the detection agent and the test line that specifically recognise and bind to the target molecule.

In one embodiment, the target molecule is the spike protein from the SARS-CoV-2 virus.

Claims

1. A lateral flow test strip comprising a sample pad (1), a conjugate pad (2), and a reaction membrane (3), wherein the conjugate pad (2) is positioned between the sample pad (1) and the reaction membrane (3),

wherein the conjugate pad (2) stores a detection agent (11) comprising conjugates of an antibody (10) and a spherical gold nanoparticle (9) with a diameter of 20 - 100 nm, wherein the antibody (10) specifically recognises and binds to a target molecule (6),

wherein the reaction membrane (3) has a test line (7) comprising antibodies (12) that are immobilised in a line across the surface of the reaction membrane (3) and specifically recognise and bind to the target molecule (6).

2. The lateral flow test strip of claim 1, wherein the gold nanoparticles (9) have a diameter of 40 nm.

3. The lateral flow test strip of claim 1 or claim 2, wherein the reaction membrane (3) further comprises a control line (8), positioned such that the test line (7) is between the conjugate pad (2) and the control line (8), wherein the control line comprises antibodies (13) that specifically recognise and bind to the conjugates in the detection agent (11).

4. The lateral flow test strip of any one of claims 1 to 3, wherein the reaction membrane (3) is a nitrocellulose membrane.

5. The lateral flow test strip of claim 4, wherein the nitrocellulose membrane has a pore size of 8 - 12 microns.

6. The lateral flow test strip of any one of claims 1 to 5, further comprising a wicking pad (4) positioned such that the reaction membrane (3) is between the conjugate pad (2) and the wicking pad (4).

7. The lateral flow test strip of claim 6, wherein the wicking pad (4) is made from a cellulose filter.

8. The lateral flow test strip of any one of claims 1 to 7, wherein the antibodies (10, 12) specifically recognise and bind to the spike protein from the SARS-CoV-2 virus.

9. The lateral flow test strip of any one of claims 1 to 8, wherein the antibody (10) has a binding affinity of $K_d \leq 10^{-7}$M for the target molecule (6).

10. The lateral flow test strip of any one of claims 1 to 9, wherein the sample pad (1) is made of cellulose fibre.

11. The lateral flow test strip of any one of claims 1 to 10, wherein the conjugate pad (2) is made from non-woven glass fibre.

12. A kit comprising a lateral flow test strip of any one of claims 1 to 11 and an extraction solution for suspending a test sample.

13. The kit of claim 12, wherein the extraction solution comprises phosphate buffered saline.

14. A method for detecting a target molecule (6), the method comprising the steps of:

(i) applying a liquid sample (5) to the sample pad (1) of a lateral flow test strip of any one of claims 1-11,

(ii) checking for the presence of a coloured test line (7) that indicates the presence of the target molecule (6) in the liquid sample (5).

15. The method of claim 14, wherein the target molecule (6) is the spike protein from the SARS-CoV-2 virus.