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EUROPEAN QUALIFYING EXAMINATION 2025

Paper A

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Client’s letter

Dear Ms Florence,

[001] Our company was founded in 2020 to produce lateral flow tests for the SARS-CoV- 2 virus. These tests were in great demand during the COVID-19 pandemic. However, as the number of COVID-19 cases has declined, the demand for these tests has decreased and so we are looking into other markets for our products.

[002] Lateral flow tests can be used to diagnose infections caused by bacteria and viruses other than SARS-CoV-2, and are also used for home pregnancy tests. We would like to protect the latest developments of our technology with a new patent application that covers testing for COVID-19 along with other possible uses.

[003] We are about to leave for a meeting tomorrow with a potential investor based on the west coast of the US, so will be travelling for the rest of the day and will not be available to answer any questions. I have therefore enclosed all the information you will need to draft and file the application today, including documents D1 and D2 to give you further background. Please note that, in accordance with our company policy, we will not pay any additional claims fees and we wish to file only one application.

[004] 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 being tested in a COVID-19 test is usually a nose or throat swab sample that has been suspended in an extraction solution. The test is designed to look for part of the virus that may be in the sample. In our test, the target molecule being detected is the spike protein from the SARS-CoV-2 virus.

[005] Figure 1A shows a diagram of our lateral flow test. The liquid to be tested (5) is applied to the sample pad (1). This is typically an absorbent pad that acts as a sponge to hold the liquid sample (5) and is preferably made of cellulose fibre. The main function of the sample pad is to provide even and controlled distribution of the sample and so is an important part of the test.

[006] Downstream (i.e. in the direction of the capillary flow of the liquid) from the sample pad is the conjugate pad (2). The conjugate pad stores the detection agent (11) for the test, normally in a dried form. When the liquid sample reaches the conjugate pad, it can rehydrate and mobilise the detection agent. The conjugate pad is preferably made from non-woven glass fibre.

[007] The detection agent is a conjugate (11) of an antibody (10) and a coloured particle (9). The antibody must be able to specifically recognise and bind to the target molecule (6) in the liquid sample, which in our test is the spike protein produced by the virus. There are many different types of antibody commercially available and any of them can be used in the test. Ideally the antibody will have a high binding affinity for the target molecule. Binding affinity can be defined by the equilibrium dissociation constant (K_D). The lower the K_D value, the higher the affinity of the antibody for its target. Antibodies with a high binding affinity typically have a $K_D \leq 10^{-7}$M.

[008] The antibodies are attached to small, coloured particles which will provide the visible test result. These particles typically have a fairly uniform size and must have a spherical shape to ensure they move at a consistent rate. If the viral spike protein is present in the sample, it will bind to the antibody part of the coloured conjugate as shown in Figure 1B. The conjugate with bound target molecule will then flow downstream to the reaction membrane (3).

[009] The reaction membrane (3) has a test line (7) comprising additional antibodies (12) that are specific for the spike protein (the target molecule) and are immobilised in a line across the surface of the membrane. These antibodies can be either the same as the antibodies used in the detection agent conjugate or different to them, but for reasons of cost and ease of manufacturing we prefer to use the same type of antibody. When the liquid sample reaches the test line as shown in Figure 1C, any coloured conjugates with the target molecule attached will be caught and trapped. As more conjugates are trapped there, a coloured line will develop. This coloured line indicates that the sample contains the target molecule, which in the case of a COVID-19 test is the viral spike protein, and so shows that the subject who provided the sample is infected with the virus.

[010] Various materials can be used for the reaction membrane, such as cellulose acetate or nylon, but we prefer to use a nitrocellulose membrane. Nitrocellulose has an advantage over other conventional strip materials because it has a natural ability to bind to proteins. This means that antibodies (which are a type of protein) can be applied directly and immobilised firmly without needing any additional treatment. Furthermore, nitrocellulose membranes are readily available in a range of pore sizes (0.05 to 12 microns) and the pore size can be selected to control the sample flow rate. We find that a pore size of at least 5 microns works well, with the best results obtained using a pore size of 8-12 microns.

[011] Advantageously, the reaction membrane also comprises a control line (8) to show that the test has been carried out correctly. The control line contains a different type of antibody (13), which is specific for the conjugate, immobilised in a line across the membrane. Some of the remaining conjugates not trapped by the test line will instead be caught at the control line, causing a change in colour. This confirms that the liquid sample containing the conjugates successfully reached the test line and had a chance to interact with it. For this reason, the control line needs to be downstream of the test line.

[012] We find it helpful also to include a wicking pad (4) located at the downstream end of the test. 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, although we find the most absorbent wicking pads are made from a cellulose filter.

[013] For ease of use, our lateral flow tests are preferably 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 our commercial COVID-19 product, we typically sell the test together with an extraction solution that can be used to suspend a test sample (e.g. a nose or throat swab sample) before it is applied to the test. The extraction solution is usually a buffer solution, such as phosphate buffered saline, although it may contain additional components.

[014] We have previously used coloured latex particles in our conjugates. However, one of our scientists suggested using gold nanoparticles instead because of their intense ruby-red colour. We ordered some 40 nm colloidal gold nanoparticles from GoldiLocks™ to test, as described in document D2.

[015] We found that they gave a great result, and in fact improved the sensitivity of our lateral flow test 10-fold compared to the coloured latex particles. This means that it is possible to show results even when the concentration of spike protein (target molecule) is very low in the sample. We have experimented with gold nanoparticles from other manufacturers and found that any type which has a diameter of 100 nm or less is suitable for our test, since larger particles do not have the necessary red colour. However, 40 nm diameter particles provided the best sensitivity. We also found that gold nanoparticles with a diameter of less than 20 nm cannot carry sufficient antibodies to give an accurate result.

Client’s drawings

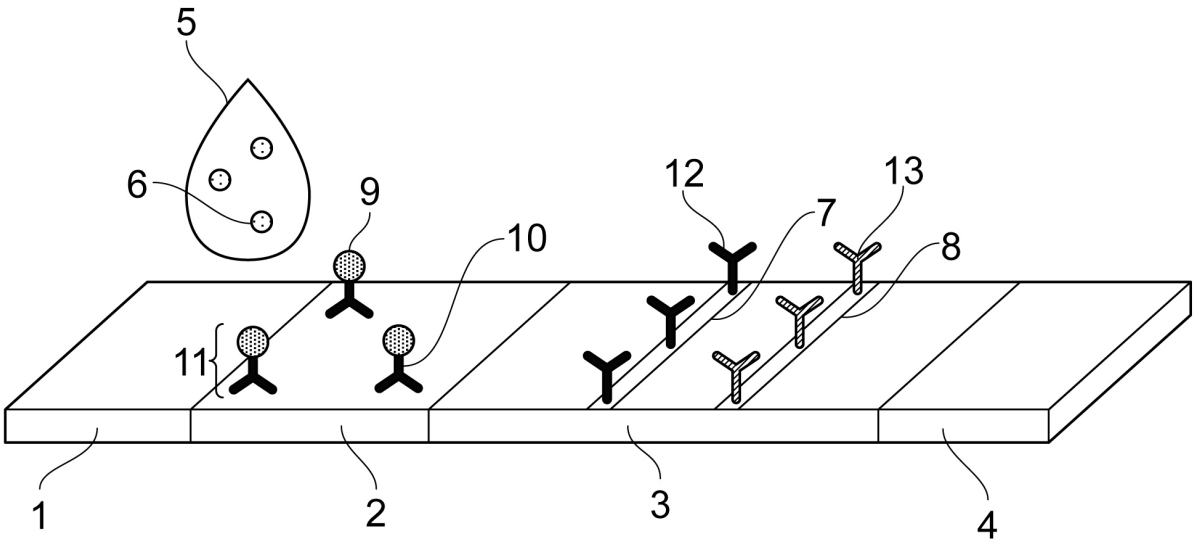

FIG. 1A

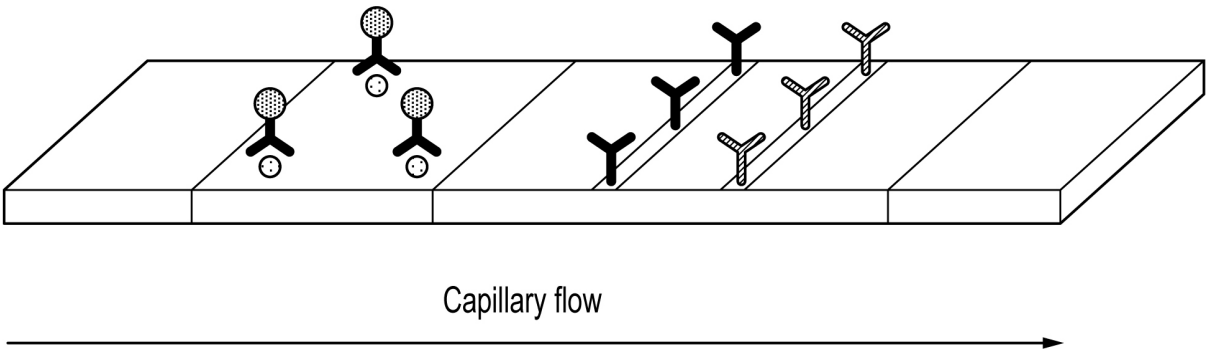

FIG. 1B

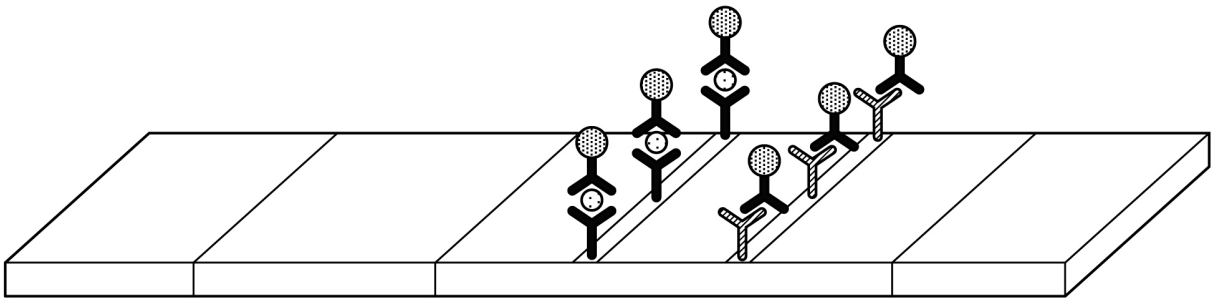

FIG. 1C

Excerpt from article: “Pregnancy Testing Through the Ages”

[001] For thousands of years, cultures have recognised that clues to whether a woman is pregnant could be discovered by inspecting her urine. By the early twentieth century, researchers realised that a woman’s body produces a hormone called human chorionic gonadotropin (hCG) after an egg is fertilised, and that this hormone is present in her urine.

[002] By the late 1970s the first pregnancy kits for home use were approved by the U.S. Food & Drug Administration. These kits required users to mix urine with solutions in test tubes and wait two hours for a result. In addition to being difficult to use, there was a high percentage of false negatives and so the results were not always reliable.

[003] The major breakthrough came in the 1990s with the development of lateral flow tests. A significant advantage is their simplicity of use, and the ability to produce a result within minutes that can be read with the naked eye. As many will know, the result is displayed as a coloured line, but how is this coloured line formed?

[004] As shown in Figure A, a lateral flow pregnancy test works by collecting a sample of urine on a sample pad (1). The urine runs along the test strip to the conjugate zone (2), which contains a detection agent (9) that reacts to a target molecule in the urine. This reaction then leads to a visible change that indicates a positive test.

[005] 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.

[006] The conjugate zone contains antibodies (8) that are specific to the hCG hormone. The antibodies are attached to small, blue-coloured latex particles (7). The antibody-latex conjugates are held in the conjugate zone in a dry state. If hCG is present in the urine sample, it binds to the conjugates and flows down the strip towards the test line (5) in the reaction zone (3).

[007] At the test line (5) there are more hCG-specific antibodies (10), but these ones are fixed to the strip. These fixed antibodies capture the hCG attached to the blue latex particles. This creates the blue line seen on these pregnancy tests.

[008] The reaction zone also contains a second line of fixed antibodies (11). These antibodies capture surplus conjugate not bound to hCG. This acts as a control line (6), showing that the test has been completed properly, and so is at the end of the test furthest away from the sample addition pad. Although the control line is a common feature of most commercially available pregnancy tests, there are some tests available that do not include one and rely on other means to show that the test has been performed successfully.

[009] The final part of the test is the wick (4) that absorbs the excess liquid sample. The wick (4) is made from an absorbent material such as cotton, cellulose filter or glass fibre. The pregnancy test is contained in a plastic cassette with labels to show where the test and control lines are located to help the user to read the test result.

[010] An important feature of the test is the material used for the strip containing the reaction zone (3). The best results are obtained with a nitrocellulose membrane that has a pore size of 9-10 microns. Other materials commonly used in these pregnancy tests include cellulose fibre for the sample pad (1) and non-woven glass fibre for the conjugate zone (2).

[011] To maximise sensitivity of the pregnancy test it is important to use antibodies that will bind strongly to the hCG hormone, preferably with a binding affinity (K_D) $\leq 10^{-7}$M.

[012] Since the 1990s, lateral flow tests have been developed for many different applications, such as detection of viral and bacterial molecules to diagnose infection, and can test a variety of samples including blood, saliva, and nasal and throat swabs. When testing nose and throat swab samples, these are usually first suspended in an extraction solution of phosphate buffered saline.

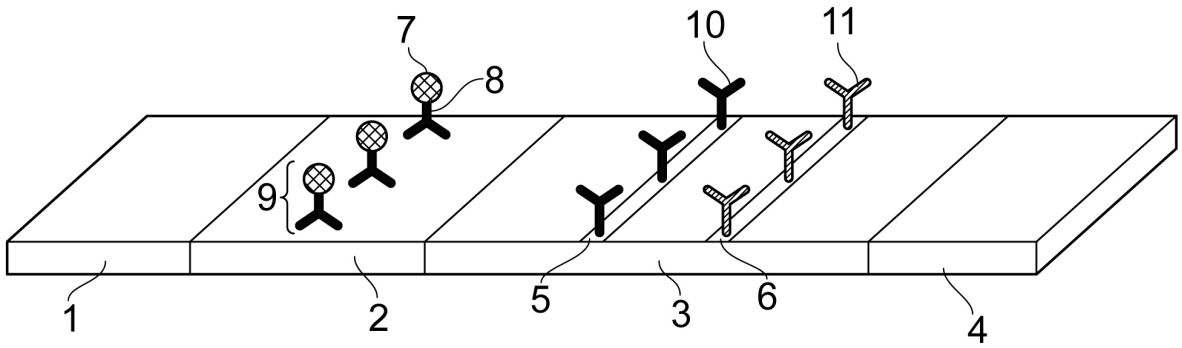

FIG. A

Product website: GoldiLocks™ gold nanoparticles

[001] GoldiLocks™ is recognised as both an innovator and a quality supplier of gold nanoparticle products. Our gold nanoparticles have been developed using specialised techniques that enable the production of extremely uniform spherical particles with a narrow size-distribution range. We are proud to offer a broad portfolio of gold nanoparticles for high-technology applications. Our spherical gold nanoparticles are available in sizes ranging from 5 nm to 400 nm in diameter, and our most popular product sizes are shown in the table below.

Product name	BabyBear™ gold nanoparticles	MummyBear™ gold nanoparticles	DaddyBear™ gold nanoparticles
Size (diameter, nm)	20	40	100
Sphericity (%)	99+	99+	99+
Odd shapes per 100 particles	<1	<1	<1

[002] Colloidal gold (a suspension of gold nanoparticles in a solvent) has been used by artists for centuries because of the nanoparticles' interactions with visible light. Gold nanoparticles absorb and scatter light resulting in colours ranging from vibrant reds (spherical particles that have a diameter of 100 nm or less) to blues to black and finally to clear and colourless. These colours occur because of localised surface plasmon resonance (LSPR), a phenomenon in which electrons on the surface of the nanoparticle oscillate in resonance with light.

[003] GoldiLocks™ gold nanoparticles are well suited for use in a wide variety of contexts, such as in solar cells, liquid crystals, catalysis and electronics. Our nanoparticles can also be conjugated to various types of biological molecules, for example peptides, proteins (including antigens and antibodies), DNA and RNA.