

TITLE OF INVENTION: FULL ENCLOSED ANALYTE DETECTION DEVICE

RELATED APPLICATIONS

[0001] This application claims priority to China Application Serial Number 202410677441.9, filed on May 29, 2024, which is incorporated herein by reference.

BACKGROUND

Field of Invention

[0002] The present invention relates to an analyte detection device, in particular to a fully enclosed analyte detection device including a paraffin wax tank.

Description of Related Art

[0003] A severe infectious pneumonia (coronavirus disease 2019, COVID-19) epidemic broke out at the end of 2019. During the epidemic, medical institutions experienced a shortage of clinical testing manpower and insufficient testing capacity. Decentralized testing technology and point-of-care (POCT) detection application value is gradually emerging. The international standard ISO 22870 defines POCT as “Testing that is performed near or at the site of a patient with the result leading to possible change in the care of the patient”. That is to say, POCT is different from the traditional laboratory testing model in medicine. POCT needs to conduct testing at a location close to the patient and can provide test results to clinicians more quickly to assist clinicians in disease diagnosis, monitoring, and treatment evaluation.

[0004] POCT testing products have the characteristics of simple sampling, intuitive operation interface, and easy to use by ordinary non-medical professionals. Therefore, the design of POCT testing products tends to follow new trends such as miniaturization,

multiplex tests, single-button operation, easy judgment of screening results, remote transmission of testing data, and integration with smart wearable devices.

[0005] Common POCT testing products currently on the market include disposable testing cassettes, test swabs, freeze-dried reagents, and handheld reading instruments. POCT testing products can conduct qualitative and semi-quantitative analysis of different target molecules such as coronavirus (SARS-CoV-2), influenza A virus, influenza B virus, respiratory syncytial virus (RSV), human papillomavirus (HPV), etc. to assist in determining the timing of medication.

[0006] The disposable testing cassettes of commercially available products LUCIRA[®] and PLUSLIFE use long and winding flow channels to transport human specimens to the reaction chambers for redissolution of freeze-dried reagents. The exhaust design of the flow channels and reaction chambers use breathable but waterproof PTFE porous breathable membranes to exhaust the remaining gas in the flow channels and reaction chambers. However, the above-mentioned commercially available products have the following shortcomings: (1) The design technology of the long and winding flow channels may cause cross contamination between the reaction chambers; and (2) The pressure relief technology of the porous breathable membranes may cause aerosol leakage due to contamination of the surrounding environment.

[0007] Therefore, developing an analyte detection device that avoids cross contamination between reaction chambers and contamination of the surrounding environment, resulting in detection abnormalities or failures, is an urgent problem in the art that needs to be solved.

SUMMARY

[0008] In order to solve the above-mentioned problems of the prior art, the object of the present invention is to provide a fully enclosed analyte detection device. (1) By discharging the gas existed in the flow channel and reaction tank of the lower cassette into the chamber of the upper cassette, where the body of the detection device is still a fully enclosed structure, the object of avoiding detection abnormalities or failures caused by contamination of the surrounding environment may be achieved. (2) By disposing the paraffin wax tank and the valve with the polygonal chamber, the object of avoiding detection abnormalities or failures caused by mutual contamination of tested nucleic acids between reaction tanks may be achieved.

[0009] In order to achieve the above-mentioned objects, the present invention provides a fully enclosed analyte detection device. The fully enclosed analyte detection device includes:

an upper cassette including:

a body including a top, a bottom, and a first chamber, wherein the bottom corresponds to the top, and the first chamber is located between the top and the bottom;

a tank being located adjacent to the bottom of the body and surrounding the body, wherein the tank has a second chamber; and

two connecting channels, wherein each of the connecting channels is arranged oppositely, and one end of each of the connecting channels is connected to the first chamber of the body, and the other end of each of the connecting channels is connected to the second chamber of the tank;

a lower cassette being engaged with the bottom of the body of the upper cassette, wherein the lower cassette includes:

a central hole corresponding to the bottom of the body;

at least one flow channel connected to the central hole; and

at least one reaction tank connected to the at least one channel, wherein the at least one reaction tank includes a breathable hole and a cavity, the breathable hole is arranged on a top of the reaction tank, and the cavity is located inside the reaction tank, and wherein the reaction tank is in gas connection to the second chamber through the breathable hole;

an aluminum film disposed between the bottom of the body of the upper cassette and the central hole of the lower cassette; and

a cap puncture needle removably covering the upper cassette and sealing the first chamber.

[0010] According to one embodiment of the present invention, in one case that the cap puncture needle is covered with the upper cassette, the first chamber is sealed and not connected to an external environment. In another case that the cap puncture needle is not covered with the upper cassette, the first chamber is not sealed and connected to the external environment, thereby injecting a lysis buffer.

[0011] According to one embodiment of the present invention, the fully enclosed analyte detection device further includes at least one paraffin wax tank. The central hole, the at least one paraffin wax tank, and the at least one reaction tank are connected to each other through the at least one flow channel. The at least one paraffin wax tank is disposed between the central hole and the at least one reaction tank, and the at least one paraffin wax tank is filled with paraffin. Before the paraffin is heated, the paraffin does not block a connection between the central hole and the at least one reaction tank. After the paraffin

is heated and solidified, the paraffin blocks a connection between the central hole and the at least one reaction tank.

[0012] According to one embodiment of the present invention, the fully enclosed analyte detection device further includes at least one valve. The central hole, the paraffin wax tank, the valve, and the reaction tank are connected to each other through the flow channel, and the at least one valve is disposed between the at least one paraffin wax tank and the at least one reaction tank. The at least one valve is configured to block a heated paraffin from flowing into the reaction tank, but does not block a lysis buffer from flowing into the reaction tank.

[0013] According to one embodiment of the present invention, the valve is a polygonal chamber, and the valve has a base and two legs. The base of the valve is connected to a lateral surface of the flow channel, and an angle θ between the bottom of the valve and the lateral surface of the flow channel is less than or equal to 90° . The two legs are respectively tapered from both ends of the base toward a direction of the flow channel and are connected to the flow channel.

[0014] According to one embodiment of the present invention, the cap puncture needle includes:

a sealing part being accommodated inside the body of the upper cassette and close to the top of the body, wherein the sealing part includes an outer wall, and an annular groove is provided on the outer wall of the sealing part; and

a puncture needle including at least one groove and a sharp bevel, wherein the at least one groove extends from one end of the puncture needle to the other end of the puncture needle and the one end of the puncture needle is connected to a center point of

a bottom of the sealing part, and wherein the sharp bevel is provided at the other end of the puncture needle relative to the sealing part.

[0015] According to one embodiment of the present invention, a side wall of the body adjacent to the bottom is tapered toward an inner direction of the body.

[0016] According to one embodiment of the present invention, each of the connecting channels forms an L-shape.

[0017] According to one embodiment of the present invention, the upper cassette further includes a barrier member. The barrier member includes a central hole. The barrier member is disposed inside the body and is located at a junction where the body connects to each of the connecting channels. An outer wall of the barrier member abuts against an inner wall of the body and the puncture needle passes through the central hole of the barrier member.

[0018] According to one embodiment of the present invention, the barrier member includes a soft cork or a rotary valve. When the barrier member is the soft cork, the barrier member blocks the first chamber from connecting to the second chamber of the tank through each of the connecting channels.

[0019] According to one embodiment of the present invention, when the barrier member is the rotary valve, the rotary valve is configured to be rotatable by the cap puncture needle to a first rotary configuration and a second rotary configuration. Under the first rotary configuration, the rotary valve blocks the first chamber from connecting to the second chamber of the tank through each of the connecting channels. Under the second rotary configuration, the rotary valve connects the first chamber, each of the connecting channels, and the second chamber of the tank through the at least one groove.

[0020] According to one embodiment of the present invention, the first chamber of the body is filled with a lysis buffer, and a total air volume of the first chamber of the body and the second chamber of the tank is greater than a volume of the lysis buffer.

[0021] According to one embodiment of the present invention, the lower cassette further includes an annular groove, and the annular groove is disposed on an outer wall adjacent to a top of the lower cassette.

[0022] According to one embodiment of the present invention, a shape of the at least one paraffin wax tank includes a circle, a triangle, a square, or a polygon.

[0023] According to one embodiment of the present invention, according to a total volume of the at least one paraffin wax tank, a volume of the paraffin ranges from 10% to 80% of the total volume of the at least one paraffin wax tank.

[0024] According to one embodiment of the present invention, an amount of the at least one flow channel, the at least one paraffin wax tank, the at least one valve, and the at least one reaction tank of the lower cassette is five, respectively.

[0025] According to one embodiment of the present invention, a shape of the central hole of the lower cassette is a polygon, such as a pentagon. Each side of the polygon of the central hole of the lower cassette is perpendicular to the connected at least one flow channel.

[0026] According to one embodiment of the present invention, a top of the at least one reaction tank is tapered from a periphery of the at least one reaction tank toward an axial direction of the at least one reaction tank.

[0027] According to one embodiment of the present invention, a top of the at least one reaction tank tapers from a periphery of the at least one reaction tank toward an axial

direction of the at least one reaction tank to the breathable hole. The breathable hole has a diameter of at least 1 mm.

[0028] According to one embodiment of the present invention, the breathable hole is breathable but not waterproof.

[0029] According to one embodiment of the present invention, the at least one reaction tank contains a solution with a volume between 5 μL and 50 μL .

[0030] According to one embodiment of the present invention, an amount of the at least one groove of the puncture needle is three.

[0031] According to one embodiment of the present invention, the fully enclosed analyte detection device further includes a first sealing ring. The first sealing ring is disposed in an annular groove on an outer wall of the sealing part of the cap puncture needle, and an outer edge of the first sealing ring abuts an inner wall of the body of the upper cassette, allowing the cap puncture needle to be slid on the body of the upper cassette and allowing the first chamber of the body to achieve the air-tight effect to prevent the lysis buffer from overflowing the body of the upper cassette and causing contamination.

[0032] According to one embodiment of the present invention, the cap puncture needle includes a handheld part connected to the sealing part. There is a stroke range between the first sealing ring and the handheld part, and the stroke range is between 0.1 mm and 10 mm. The stroke range may provide a fluid driving force for the lysis buffer in the first chamber of the body of the upper cassette. Therefore, the arrangement of the first sealing ring may provide the fluid driving force.

[0033] According to one embodiment of the present invention, the fully enclosed analyte detection device further includes a second sealing ring. The second sealing ring is disposed in an annular space, and the annular space is located between the aluminum film

and a side wall of the body adjacent to the bottom. The arrangement of the second sealing ring may achieve an air-tight effect between the upper cassette body and the lower cassette.

[0034] According to one embodiment of the present invention, the fully enclosed analyte detection device further includes at least one breathable membrane. The at least one breathable membrane is disposed between the breathable hole of the at least one reaction tank of the lower cassette and the second chamber of the upper cassette. When the lysis buffer sequentially passes the at least one paraffin wax tank and the at least one valve through the at least one flow channel, and enters the at least one reaction tank, at least one breathable membrane may discharge an air existed in the at least one flow channel into the second chamber of the upper cassette.

[0035] According to one embodiment of the present invention, an amount of the at least one breathable membrane is five.

[0036] According to one embodiment of the present invention, the fully enclosed analyte detection device further includes a third sealing ring. The third sealing ring is disposed in the annular groove of the lower cassette.

[0037] According to one embodiment of the present invention, the first sealing ring, the second sealing ring, and the third sealing ring are made of soft O-ring material.

[0038] According to one embodiment of the present invention, the fully enclosed analyte detection device further includes a sealing member. The sealing member adheres to and seals a bottom of the lower cassette.

[0039] According to one embodiment of the present invention, the sealing member is made of high-transparency thin pressure-sensitive adhesive material.

[0040] When the fully enclosed analyte detection device of the present invention is used, the first chamber of the body of the upper cassette is filled with the lysis buffer, and a user uses a nasopharyngeal swab to collect nucleic acid sample from the nasopharynx, swirls the nasopharyngeal swab in the lysis buffer 5 to 10 times, and then covers the cap puncture needle. The cap puncture needle is pressed toward the bottom of the body of the upper cassette, so that the cap puncture needle pierces the aluminum film, and then the lysis buffer containing the nucleic acid sample flows into the central hole of the lower cassette through the at least one groove and the sharp bevel of the puncture needle. Then, the nucleic acid sample sequentially passes the at least one paraffin wax tank and the at least one valve through the at least one flow channel, and enters the at least one reaction tank to dissolve a dry reagent placed in the at least one reaction tank. Finally, the fully enclosed analyte detection device is put into a fully automatic nucleic acid analyzer to perform amplification reaction of the nucleic acid, and the signal is read by an optical system to obtain the detection results.

[0041] The fully enclosed analyte detection device of the present invention may achieve the following effects. (1) The barrier member of the upper cassette may reduce the risk of the lysis buffer filled in the first chamber of the body of the upper cassette entering the second chamber of the tank through the two connecting channels during the transportation process, thereby moistening the breathable membrane. (2) The arrange of the first sealing ring may achieve an air-tight effect within the first chamber of the body and prevent the lysis buffer from overflowing the body of the upper cassette and causing contamination. The arrange of the second sealing ring may achieve an air-tight effect between the body of the upper cassette and the lower cassette. The arrange of the third sealing ring may achieve an air-tight effect between the body of the upper cassette and the lower cassette. (3) The reaction tank is connected to the second chamber through the breathable hole, but

the second chamber is not connected to the external environment. Thus, the fully enclosed analyte detection device is provided. When the user presses down the cap puncture needle, the lysis buffer enters the reaction tank through the central hole and the flow channel, and the gas in the flow channel and reaction tank is discharged into the second chamber through the breathable hole, thereby preventing the air in the flow channel and reaction tank from blocking the flow of the lysis buffer into the reaction tank. However, since the second chamber is not connected to the external environment, when the gas in the flow channel and reaction tank is discharged into the second chamber through the breathable hole, it is possible that the internal gas pressure will rise slightly, causing slight resistance for the lysis buffer to enter the reaction tank. However, since the volume of the second chamber is much greater than the volume of the gas discharged from the reaction tank and the flow channel into the second chamber, theoretically, the resulting pressure rise is very small and will not prevent the lysis buffer from entering the reaction tank, thereby achieving the effect of removing gas from the flow channel and the reaction tank in a sealed structure. In addition, rotating the rotary valve by rotating the cap puncture needle may allow the first chamber and the second chamber to be gas connected. The volume of the first chamber plus the volume of the second chamber is much greater than the volume of gas discharged from the reaction tank and the flow channel into the second chamber, and the resulting pressure rise may even be ignored and will not hinder the flow of lysis buffer from entering the reaction tank at all, satisfying the function of pressure balance during high-temperature reactions. (4) The paraffin filled inside the paraffin wax tank and located above the flow channel will be redissolved during the heating process of the reaction and then seal the flow channels connecting both sides of the paraffin wax tank. It may avoid the backflow of the lysis buffer and the well-well crosstalk of the amplicon in each reaction tank, causing mutual contamination of samples to be tested in different

reaction tanks. (5) When the paraffin is filled inside the paraffin wax tank and above the flow channel, in order to prevent the paraffin from flowing into the reaction tank through the flow channel during the filling process, an angle between the base of the valve of the polygonal chamber and the lateral surface of the flow channel is set less than or equal to 90° . The user presses down the cap puncture needle to provide a fluid driving force so that the lysis buffer may adhere to the base of the valve of the polygonal chamber, and enter the chamber of the valve, and then flow into the reaction tank. On the contrary, a molten paraffin without the fluid driving force may not adhere to the base of the valve of the polygonal chamber and enter the valve chamber, which may prevent the paraffin from flowing to the reaction tank through the flow channel during the filling process. (6) By bonding a high-transparency thin pressure-sensitive adhesive material to the bottom of the lower cassette, the fully enclosed analyte detection device is completely sealed, reducing the risk of detection abnormalities or failures caused by environmental contamination.

BRIEF DESCRIPTION OF THE DRAWINGS

[0042] The invention can be more fully understood by reading the following detailed description of the embodiment, with reference made to the accompanying drawings as follows:

[0043] FIG. 1A is a cross-sectional view of a first fully enclosed analyte detection device of the present invention.

[0044] FIG. 1B is a perspective view of the first fully enclosed analyte detection device of the present invention.

[0045] FIG. 2A is a top view of a lower cassette of the first fully enclosed analyte detection device of the present invention.

[0046] FIG. 2B is a partial perspective view of the lower cassette of the first fully enclosed analyte detection device of the present invention.

[0047] FIG. 3 is a perspective view of a combination of a soft cork of the upper cassette and a cap puncture needle of the first fully enclosed analyte detection device of the present invention.

[0048] FIG. 4A, FIG. 4B, and FIG. 4C respectively show the lysis buffer containing nucleic acid of the first fully enclosed analyte device of the present invention sequentially passing through the paraffin wax tank, the valve, and the reaction tank from the central hole of the lower cassette. Photos are taken during the outward filling process (FIG. 4A), the inward filling process after arriving at the reaction tank (FIG. 4B), and after the completion of filling (FIG. 4C).

[0049] FIG. 5A, FIG. 5B, and FIG. 5C respectively show the first fully enclosed analyte detection device of the present invention put in the nucleic acid analyzer for heating reaction at 65°C for 20 minutes. Photos are taken during heating at 1 minute (FIG. 5A), 10 minutes (FIG. 5B), and 20 minutes (FIG. 5C) to observe whether the lysis buffer containing nucleic acid in the reaction tank flows back and whether the paraffin inside the paraffin wax tank flows.

[0050] FIG. 6A shows a photo of dry reagents containing a primer pair of influenza A and reaction enzyme placed in the reaction tank of the lower cassette of the first fully enclosed analyte detection device of the present invention.

[0051] FIG. 6B show a photo of 1 μL of quality control sample of influenza A virus H1N1 subtype at 10^6 copies/ μL and 500 μL of nucleic acid lysis buffer flowing through the flow

channels of the lower cassette of the first fully enclosed analyte device into the reaction tank and reconstituting the dried reagents.

[0052] FIG. 6C show a photo of the lower cassette of the the first fully enclosed analyte detection device after the first fully enclosed analyte detection device is put into the nucleic acid analyzer for nucleic acid amplification reaction.

[0053] FIG. 7 shows that sampling is conducted with a swap by swabbing at an inner side of an outer cover of the first fully enclosed analyte detection device (P1), at a first sealing ring located in a first chamber of the upper cassette (P2), at a third sealing ring located in an annular groove of the lower cassette (P3), at an upper breathable membrane of handheld part of the cap puncture needle (P4), and at a breathable membrane outside the upper cassette (P5) for aerosol pollution testing.

[0054] FIG. 8A and FIG. 8B shows that the first fully enclosed analyte detection device of the present invention is used to sample from the areas of the inner side of the outer cover of the first fully enclosed analyte detection device (P1), the first sealing ring located in the first chamber of the upper cassette (P2), the third sealing ring located in the annular groove of the lower cassette (P3), the upper breathable membrane (P4) of handheld part of the cap puncture needle, and the breathable membrane outside the upper cassette (P5) with a swab. Photos of the reaction solution with 1x original solution and 100x dilution obtained by eluting the swab in 500 μ L eluent before (FIG. 8A) and after (FIG. 8B) the amplification reaction of the nucleic acid are taken.

[0055] FIG. 9A shows a photo of 1 μ L of quality control sample of influenza A virus H1N1 subtype at 10^6 copies/ μ L and 500 μ L of nucleic acid lysis buffer flowing through the flow channels of the lower cassette of the first fully enclosed analyte device into the reaction tank and reconstituting the dried reagents. The reaction tanks No. 1, No. 3, and

No. 4 includes dry reagents which contain RNA primer pairs of influenza A virus H1N1 subtype and reaction enzymes, while reaction tanks No. 2 and No. 5 contain RNA primer pairs of severe acute respiratory syndrome coronavirus type 2.

[0056] FIG. 9B shows a photo of the lower cassette of the first fully enclosed analyte detection device after the first fully enclosed analyte detection device is put into the nucleic acid analyzer for the amplification reaction.

[0057] FIG. 10A is a cross-sectional view of a second fully enclosed analyte detection device of the present invention.

[0058] FIG. 10B is a perspective view of the second fully enclosed analyte detection device of the present invention.

[0059] FIG. 10C is a perspective view of a combination of a rotary valve of the upper cassette and a cap puncture needle of the second fully enclosed analyte detection device of the present invention.

[0060] FIG. 11A is a perspective view of grooves of the rotary valve of the upper cassette connected to the connecting channels of the second fully enclosed analyte detection device of the present invention.

[0061] FIG. 11B is a perspective view of the second fully enclosed analyte detection device of the present invention in which the grooves of the rotary valve of the upper cassette are not connected to the connecting channels.

DETAILED DESCRIPTION

[0062] The following describes the embodiment of the present disclosure through specific examples. Those skilled in the field can understand other advantages and effects

of the present disclosure from the content disclosed in the present specification. However, the exemplary embodiments disclosed in the present disclosure are merely for illustrative purposes and should not be construed as a limiting the scope of the present disclosure. In other words, the present disclosure can also be implemented or applied by other different specific embodiments, and various details in the present specification can also be modified and changed based on different viewpoints and applications without departing from the concept of the present disclosure.

[0063] Moreover, the directional terms used in the present invention, for example, up, down, top, bottom, inside, outside, side, central, perpendicular, axial direction, etc. are only the directions shown in the attached drawings. Therefore, the directional terms are only used to illustrate and express the present invention, but not to limit the present invention.

[0064] Unless otherwise described herein, the singular forms “a” and “the” used in the specification and the appended claims of the present disclosure include plural entities.

[0065] Unless otherwise described herein, the term “or” used in the specification and the appended claims of the present disclosure includes the meaning of “and/or”.

[0066] Example 1: Provide a first fully enclosed analyte detection device

[0067] Referring to FIG. 1A, FIG. 1B, FIG. 2A, FIG. 2B, and FIG. 3, the fully enclosed analyte detection device includes an upper cassette 10, a lower cassette 20, an aluminum film 30, a cap puncture needle 40, a first sealing ring 50, a second sealing ring 60, five breathable membranes, a third sealing ring 70, and a high-transparency thin pressure-sensitive adhesive material 80.

[0068] The upper cassette 10 includes a body 11, a tank 12, two connecting channels 13, and a barrier member 14. The body 11 is cylindrical and has a top 111, a bottom 112

corresponding to the top 111, and a first chamber 113. The first chamber 113 is located between the top 111 and the bottom 112. A side wall of the body 11 adjacent to the bottom 112 is tapered toward an inner direction of the body 11. The tank 12 is located adjacent to the bottom 112 of the body 11 and surrounds the body 11. The tank body 12 has a second chamber 121. The two connecting channels 13 are arranged oppositely. Each connecting channel 13 is L-shaped, and one end of each connecting channel 13 is connected to the first chamber 113 of the body 11, and the other end of each connecting channel 13 is connected to the second chamber 121 of the tank 12. The barrier member 14 is in an annular shape and has a central hole. The barrier member 14 is disposed inside the body 11 and is located at a junction where the body 11 connects to each connecting channel 13. An outer wall of the barrier member 14 abuts against an inner wall of the body 11, so that the first chamber 113 is not connected to the second chamber 121 (in this example, the barrier 14 is a soft cork).

[0069] The lower cassette 20 is engaged with the bottom of the tank 12 of the upper cassette 10. The lower cassette 20 includes a central hole 21, five flow channels 22, five paraffin wax tanks 23, five valves 24, five reaction tanks 25, and an annular groove. The central hole 21 corresponds to the bottom 112 of the body 11 and the central hole 21 is connected to five flow channels 22. Each flow channel 22 is sequentially provided with the paraffin wax tanks 23, the valves 24, and the reaction tanks 25 in a direction away from the central hole 21, and the central hole 21 is connected to five flow channels 22, five paraffin wax tanks 23, five valves 24, and five reactions tanks 25. Each paraffin wax tank 23 is circular, and is filled with paraffin inside the paraffin wax tank 23 and above the flow channel 22. Each valve 24 is a triangular chamber and has a base 241 and two legs 242. The base 241 of the valve 24 is connected to the flow channel 22 connecting the paraffin wax tank 23. The angle θ between the base 241 and the legs of the flow channel

22 is 90°. The two legs 242 are respectively tapered from both ends of the base 241 toward a direction of the reaction tank 25 and are connected to the flow channel 22 located between the valve 24 and the reaction tank 25. The reaction tank 25 includes a breathable hole 251 and a cavity. The breathable hole 251 is arranged on a top of the reaction tank 25, and the cavity is located inside the reaction tank 25. The annular groove of the lower cassette 20 is disposed on an outer wall adjacent to the top of the lower cassette 20.

[0070] The aluminum film 30 is disposed between the bottom 112 of the body 11 of the upper cassette 10 and the central hole 21 of the lower cassette 20. An annular space is located between the aluminum film 30 and a side wall of the body 11 adjacent to the bottom 112.

[0071] The cap puncture needle 40 includes a handheld part 41, a sealing part 42, and a puncture needle 43. The sealing part 42 is connected to the handheld part 41 and the sealing part 42 is accommodated inside the body 11 of the upper cassette 10 and close to the top 111 of the body 11. The sealing part 42 includes an outer wall, and an annular groove is provided on the outer wall of the sealing part 42. The puncture needle 43 includes three grooves 431 and a sharp bevel 432. Each groove 431 extends from one end of the puncture needle 43 to the other end of the puncture needle 43 and the one end of the puncture needle 43 is connected to a center point of a bottom of the sealing part 42. The sharp bevel 432 is provided at the other end of the puncture needle 43 relative to the sealing part 42. The puncture needle 43 passes through the central hole of the barrier member 14.

[0072] When the cap puncture needle 40 is closed on the upper cassette 10, the first chamber 113 is sealed and not connected to the external environment. When the cap puncture needle 40 is not closed on the upper cassette 10, the first chamber 113 is not

sealed and is connected to the external environment. The user can fill the nucleic acid lysis solution into the first chamber 113.

[0073] The first sealing ring 50 is disposed in the annular groove on the outer wall of the sealing part 42 of the cap puncture needle 40, and an outer edge of the first sealing ring 50 abuts against an inner wall of the body 11 of the upper cassette 10, allowing the first chamber 113 of the body 11 to achieve the air-tight effect.

[0074] The second sealing ring 60 is disposed in an annular space, and the annular space is located between the aluminum film 30 and a side wall of the body 11 adjacent to the bottom 112. The arrangement of the second sealing ring 60 may achieve an air-tight effect between the body 11 of the upper cassette body 10 and the lower cassette 20.

[0075] Each breathable membrane is disposed between the breathable hole 251 of each reaction tank 25 of the lower cassette 20 and the second chamber 121 of the upper cassette 10.

[0076] The third sealing ring 70 is disposed in the annular groove of the lower cassette 20. The arrangement of the third sealing ring 70 may achieve an air-tight effect between the tank 12 of the upper cassette 10 and the lower cassette 20.

[0077] The high-transparency thin pressure-sensitive adhesive material 80 adheres to the bottom of the lower cassette 20 to form sealed flow channels.

[0078] In one embodiment, the first sealing ring 50, the second sealing ring 60, and the third sealing ring 70 are O-rings.

[0079] Example 2: Buffer filling test of the first fully enclosed analyte detection device

[0080] Referring to FIG. 1A and FIG. 1B, in order to detect the filling effect of the nucleic acid lysis buffer of the first fully enclosed analyte detection device, 500 μ L of nucleic

acid lysis buffer is filled into the first chamber 113 of the body 11 of the upper cassette 10, and 2 uL to 5 uL dyes are respectively placed in the five reaction tanks 25 of the lower cassette 20.

[0081] As shown in Table 1, from Test 1 to Test 3, the cap puncture needle 40 is pressed from the top 111 of the body 11 of the upper cassette 10 at a slow speed (press to the bottom in 5 seconds) or at a quick speed (press to the bottom in 1 second) to the bottom 112 and the aluminum film 30 is pierced, allowing the nucleic acid lysis buffer to be injected into the central hole 21 and then divided into five flow channels 22. After the nucleic acid lysis buffer sequentially passes through the paraffin wax tank 23 and valve 24 along each flow channel 22, the nucleic acid lysis buffer arrives at the reaction tank 25. The nucleic acid lysis buffer fills the reaction tank 25 in 12 seconds, 10 seconds, and 11 seconds, respectively and dissolves 2 uL to 5 uL dye. As shown in FIG. 4A to FIG. 4C, the results show that during the process of filling the reaction tank 25 with the nucleic acid lysis buffer, no bubbles are found in the reaction tank 25, which reduces the concern that the bubbles will interfere with the subsequent heating reaction.

[0082] Table 1: Results of buffer filling test of the first fully enclosed analyte detection device

	Test 1	Test 2	Test 3
Pressure speed of cap puncture needle (second)	5	1	1
The time for the nucleic acid lysis	12	10	11

buffer to fill the reaction tank (second)			
Amount of bubbles (unit)	0	0	0

[0083] Then, the first fully enclosed analyte detection device is put into the nucleic acid analyzer to perform a heating reaction. The reaction condition is performed by heating at 65°C for 20 minutes. During the heating process, the paraffin disposed inside the paraffin wax tank 23 and located above the flow channel 22 is melt, followed by sealing the flow channel 22 connected to the paraffin wax tank 23. During the heating period of 1 minute, 10 minutes, and 20 minutes, observe whether the nucleic acid lysis buffer in the reaction tank 25 flows back to the flow channel 22, the paraffin wax tank 23, or the valve 24, and whether the paraffin inside the paraffin wax tank 23 flows. As shown in FIG. 5A to FIG. 5C, the results show that the flow field of the first fully enclosed analyte detection device is stable during the heating process, and the nucleic acid lysis buffer in the reaction tank 25 does not flow back to the flow channel 22, the paraffin wax tank 23, and the valve 24. In addition, the paraffin inside the paraffin wax tank 23 do not flow.

[0084] Example 3: Aerosol pollution test of the first fully enclosed analyte detection device

[0085] In order to detect whether environmental pollution occurs after the first fully enclosed analyte detection device is placed in the nucleic acid analyzer for heating reaction, an aerosol pollution test with a limit of detection (LOD) of 1000 times is performed.

[0086] Control group: Refer to FIG. 2A, FIG. 6A, and FIG. 6B, the quality control sample of influenza A virus H1N1 subtype RNA is used as a control group. FIG. 6A shows that the reaction tank 25 of the lower cassette 20 of the first fully enclosed analyte detection device is provided with a dry reagent containing primer pairs of influenza A virus H1N1 subtype RNA and a reaction enzyme. FIG. 6B shows that 1 μL of quality control sample of influenza A virus H1N1 subtype at 10^6 copies/ μL and 500 μL of nucleic acid lysis buffer flows into the reaction tank 25 through the flow channel 22 of the lower cassette 20, and the dry reagents are redissolved. Finally, since the dry reagent contains phenol red, the solution in the reaction tank 25 of the lower cassette 20 is alkaline and red. FIG. 6C shows that the first fully enclosed analyte detection device is placed into the nucleic acid analyzer to perform the amplification reaction of nucleic acid. The results showed that after heating at 65°C for 20 minutes, the solution was acidic and yellow, indicating that the nucleic acid had been successfully amplified.

[0087] Experimental group: Referring to FIG. 2A and FIG. 7, sampling is conducted with a swap by swabbing at an inner side of an outer cover of the first fully enclosed analyte detection device (P1), at a first sealing ring 50 located in a first chamber 113 of the upper cassette 10 (P2), at a third sealing ring 70 located in an annular groove of the lower cassette 20 (P3), at an upper breathable membrane of handheld part of the cap puncture needle 40 (P4), and at a breathable membrane outside the upper cassette 10 (P5). The swap is eluted in 500 μL eluent to obtain an original solution. Dilute the original solution 100 times to obtain a 100-fold diluted solution.

[0088] 10 μL of the original solution or 10 μL of the diluted solution is added into the 500 μL nucleic acid lysis buffer. The nucleic acid lysis buffer is then added into the first chamber 113 of the upper cassette 10. The nucleic acid lysis buffer flows into the reaction

tank 25 through the flow channel 22 of the lower cassette 20, and the dry reagent is redissolved. The first fully enclosed analyte detection device is placed in the nucleic acid analyzer to perform amplification reaction of nucleic acid. The reaction condition is performed by heating at 65°C for 20 minutes. Referring to Table 2, FIG. 8A, and FIG. 8B, the results show that after qPCR reaction, no aerosol contamination is detected from P1 to P5.

[0089] Table 2

Number	Sampling area	Test results	
		Original solution	Diluted solution
#1	inner side of the outer cover (P1)	Not detected	Not detected
#2	first sealing ring (P2)	Not detected	Not detected
#3	third sealing ring (P3)	Not detected	Not detected
#4	upper breathable membrane of handheld part of the cap puncture needle 40 (P4)	Not detected	Not detected
#5	breathable membrane outside the upper cassette 10 (P5)	Not detected	Not detected

[0090] Example 4: Cross-contamination test of the first fully enclosed analyte detection device

[0091] In order to detect whether cross-contamination occurs in each reaction tank of the lower cassette after the detection device of the first fully enclosed analyte is placed in the

nucleic acid analyzer for heating reaction, a cross-contamination test of 1000 times the detection limit is performed.

[0092] Referring to FIG. 9A, dry reagents containing the primer pair of influenza A virus H1N1 subtype RNA and a reaction enzyme are provided in the reaction tanks numbered #1, #3, and #4 of the lower cassette of the first fully enclosed analyte detection device. In addition, dry reagents containing the primer pair of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA and a reaction enzyme are provided in the reaction tanks numbered #2 and #5 of the lower cassette of the first fully enclosed analyte detection device. Thus, the dry reagents containing influenza A virus H1N1 subtype and the dry reagents containing severe acute respiratory syndrome coronavirus 2 are placed separately in the reaction tank of the lower cassette middle. 1 μ L of quality control sample of influenza A virus H1N1 subtype at 10^6 copies/ μ L and 500 μ L of nucleic acid lysis buffer flows into the reaction tank through the flow channel of the lower cassette, and the dry reagents are redissolved. Since the dry reagent contains phenol Red, the solution in the reaction tanks numbered #1 to #5 of the lower cassette is alkaline and red.

[0093] Referring to FIG. 9B, the first fully enclosed analyte detection device is placed in the nucleic acid analyzer to perform the amplification reaction. The reaction condition is performed by heating at 65°C for 20 minutes. The results show that after the reaction, the solution in the reaction tanks numbered #1, #3, and #4 of the lower cassette is acidic and yellow, which indicates that the nucleic acid has been amplified successfully, while the solution in the reaction tanks numbered # 2 and # 5 of the lower cassette is still alkaline and red, which indicates that the nucleic acid amplification is not successful. The results showed that after the first fully enclosed analyte detection device is placed in the nucleic

acid analyzer for heating reaction, no cross-contamination occurred in each reaction tank of the lower cassette.

[0094] Example 5: Provide a second fully enclosed analyte detection device

[0095] Referring to FIG. 10A and FIG. 10B, this example provides a second fully enclosed analyte detection device. The second fully enclosed analyte detection device is similar to the first fully enclosed analyte detection device. The difference between the two devices is that in the second fully enclosed analyte detection device, the soft cork of the cassette in the first fully enclosed analyte detection device is replaced with a rotary valve 90. A bottom of the rotary valve 90 has a groove 91 extending from one end of the rotary valve 90 to the other end of the rotary valve 90.

[0096] Referring to FIG. 11A, the puncture needle 43A of the cap puncture needle 40A passes through a central hole of the rotary valve 90. The user may rotate the cap puncture needle 40A, so that the groove 91 of the rotary valve 90 is connected to the connecting channel 13A, and then the first chamber 113A is connected to the second chamber 121A. Then, the user may put the second fully enclosed analyte detection device into the nucleic acid analyzer to perform heating and nucleic acid amplification reactions. In addition, during the heating process, since the first chamber 113A and the second chamber 121A are connected, this air volume ratio may promote the paraffin to appear in a stable state when melted.

[0097] Referring to FIG. 11B, the user may also rotate the cap puncture needle 40A, so that the groove 91 of the rotary valve 90 is not connected to the connecting channel 13A and the first chamber 113A and the second chamber 121A are independent. As such, the user may fill the lysis buffer into the first chamber 113A to prevent the lysis buffer from flowing from the first chamber 113A to the second chamber 121A. The user may put the

second fully enclosed analyte detection device into the nucleic acid analyzer to perform heating and nucleic acid amplification reactions.

[0098] Although the present invention has been described in considerable detail with reference to certain embodiments thereof, other embodiments are possible. Therefore, the spirit and scope of the appended claims should not be limited to the description of the embodiments contained herein.

[0099] It will be apparent to those skilled in the art that various modifications and variations can be made to the structure of the present invention without departing from the scope or spirit of the invention. In view of the foregoing, it is intended that the present invention cover modifications and variations of this invention provided they fall within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A fully enclosed analyte detection device, comprising:

an upper cassette comprising:

a body comprising a top, a bottom, and a first chamber, wherein the bottom corresponds to the top, and the first chamber is located between the top and the bottom;

a tank being located adjacent to the bottom of the body and surrounding the body, wherein the tank has a second chamber; and

two connecting channels, wherein each of the connecting channels is arranged oppositely, and one end of each of the connecting channels is connected to the first chamber of the body, and the other end of each of the connecting channels is connected to the second chamber of the tank;

a lower cassette being engaged with the bottom of the body of the upper cassette, wherein the lower cassette comprises:

a central hole corresponding to the bottom of the body;

at least one flow channel connected to the central hole; and

at least one reaction tank connected to the at least one channel, wherein the at least one reaction tank comprises a breathable hole and a cavity, the breathable hole is arranged on a top of the reaction tank, and the cavity is located inside the reaction tank, and wherein the reaction tank is in gas connection to the second chamber through the breathable hole; and

an aluminum film disposed between the bottom of the body of the upper cassette and the central hole of the lower cassette; and

a cap puncture needle removably covering the upper cassette and sealing the first chamber.

2. The fully enclosed analyte detection device of claim 1, wherein in one case that the cap puncture needle is covered with the upper cassette, the first chamber is sealed and not connected to an external environment, and in another case that the cap puncture needle is not covered with the upper cassette, the first chamber is not sealed and connected to the external environment, thereby injecting a lysis buffer.

3. The fully enclosed analyte detection device of claim 1, wherein the fully enclosed analyte detection device further comprises at least one paraffin wax tank, and the central hole, the at least one paraffin wax tank, and the at least one reaction tank are connected to each other through the at least one flow channel, wherein the at least one paraffin wax tank is disposed between the central hole and the at least one reaction tank, and wherein the at least one paraffin wax tank is filled with paraffin, and before the paraffin is heated, the paraffin does not block a connection between the central hole and the at least one reaction tank, and after the paraffin is heated and solidified, the paraffin blocks a connection between the central hole and the at least one reaction tank.

4. The fully enclosed analyte detection device of claim 3, wherein the fully enclosed analyte detection device further comprises at least one valve, wherein the central hole, the at least one paraffin wax tank, the at least one valve, and the at least one reaction tank are connected to each other through the at least one flow channel, and the at least one valve is disposed between the at least one paraffin wax tank and the at least one reaction tank; and wherein the at least one valve is configured to block a heated paraffin from flowing into the at least one reaction tank, but does not block a lysis liquid from flowing into the at least one reaction tank.

5. The fully enclosed analyte detection device of claim 4, wherein the at least one valve is a polygonal chamber, the at least one valve has a base and two legs, the base of the at least one valve is connected to a lateral surface of the at least one flow channel, and

an angle θ between the base of the at least one valve and the lateral surface of the at least one flow channel is less than or equal to 90° , and wherein the two legs are respectively tapered from both ends of the base toward a direction of the at least one flow channel and are connected to the at least one flow channel.

6. The fully enclosed analyte detection device of claim 1, wherein the cap puncture needle comprises:

a sealing part being accommodated inside the body of the upper cassette and close to the top of the body, wherein the sealing part comprises an outer wall, and an annular groove is provided on the outer wall of the sealing part; and

a puncture needle comprising at least one groove and a sharp bevel, wherein the at least one groove extends from one end of the puncture needle to the other end of the puncture needle and the one end of the puncture needle is connected to a center point of a bottom of the sealing part, and wherein the sharp bevel is provided at the other end of the puncture needle relative to the sealing part.

7. The fully enclosed analyte detection device of claim 1, wherein a side wall of the body adjacent to the bottom is tapered toward an inner direction of the body.

8. The fully enclosed analyte detection device of claim 1, wherein each of the connecting channels forms an L-shape.

9. The fully enclosed analyte detection device of claim 1, wherein the upper cassette further comprises a barrier member; the barrier member comprises a central hole and the barrier member is disposed inside the body and is located at a junction where the body connects to each of the connecting channels, and wherein an outer wall of the barrier member abuts against an inner wall of the body and the puncture needle passes through the central hole of the barrier member.

10. The fully enclosed analyte detection device of claim 1, wherein the barrier member comprises a soft cork.

11. The fully enclosed analyte detection device of claim 9, wherein the barrier member blocks the first chamber from connecting to the second chamber of the tank through each of the connecting channels.

12. The fully enclosed analyte detection device of claim 9, wherein the barrier member is a rotary valve, and the rotary valve is configured to be rotatable by the cap puncture needle to a first rotary configuration and a second rotary configuration, and wherein under the first rotary configuration, the rotary valve blocks the first chamber from connecting to the second chamber of the tank through each of the connecting channels, and under the second rotary configuration, the rotary valve connects the first chamber, each of the connecting channels, and the second chamber of the tank through the at least one groove.

13. The fully enclosed analyte detection device of claim 1, wherein the first chamber of the body is filled with a lysis buffer, and a total air volume of the first chamber of the body and the second chamber of the tank is greater than a volume of the lysis buffer.

14. The fully enclosed analyte detection device of claim 1, wherein the lower cassette further comprises an annular groove, and the annular groove is disposed on an outer wall adjacent to a top of the lower cassette.

15. The fully enclosed analyte detection device of claim 3, wherein a shape of the at least one paraffin wax tank comprises a circle, a triangle, a square, or a polygon.

16. The fully enclosed analyte detection device of claim 3, wherein according to a total volume of the at least one paraffin wax tank, a volume of the paraffin ranges from 10% to 80% of the total volume of the at least one paraffin wax tank.

17. The fully enclosed analyte detection device of claim 4, wherein an amount of the at least one flow channel, the at least one paraffin wax tank, the at least one valve, and the at least one reaction tank of the lower cassette is five, respectively.

18. The fully enclosed analyte detection device of claim 17, wherein a shape of the central hole of the lower cassette is a polygon, and each side of the polygon of the central hole of the lower cassette is perpendicular to the connected at least one flow channel.

19. The fully enclosed analyte detection device of claim 1, wherein a top of the at least one reaction tank is tapered from a periphery of the at least one reaction tank toward an axial direction of the at least one reaction tank.

20. The fully enclosed analyte detection device of claim 1, wherein a top of the at least one reaction tank tapers from a periphery of the at least one reaction tank toward an axial direction of the at least one reaction tank to the breathable hole, and the breathable hole has a diameter of at least 1 mm.

21. The fully enclosed analyte detection device of claim 20, wherein the breathable hole is breathable but not waterproof.

22. The fully enclosed analyte detection device of claim 1, wherein the at least one reaction tank contains a solution with a volume between 5 μ L and 50 μ L.

23. The fully enclosed analyte detection device of claim 6, wherein an amount of the at least one groove of the puncture needle is three.

24. The fully enclosed analyte detection device of claim 1, wherein the fully enclosed analyte detection device further comprises a first sealing ring, and the first sealing ring is disposed in an annular groove on an outer wall of the sealing part of the cap puncture needle, and an outer edge of the first sealing ring abuts an inner wall of the body of the upper cassette.

25. The fully enclosed analyte detection device of claim 24, wherein the cap puncture needle comprises a handheld part connected to the sealing part, and there is a stroke range between the first sealing ring and the handheld part, and wherein the stroke range is between 0.1 mm and 10 mm.

26. The fully enclosed analyte detection device of claim 1, wherein the fully enclosed analyte detection device further comprises a second sealing ring, and the second sealing ring is disposed in an annular space, and wherein the annular space is located between the aluminum film and a side wall of the body adjacent to the bottom.

27. The fully enclosed analyte detection device of claim 1, wherein the fully enclosed analyte detection device further comprises at least one breathable membrane, and the at least one breathable membrane is disposed between the breathable hole of the at least one reaction tank of the lower cassette and the second chamber of the upper cassette.

28. The fully enclosed analyte detection device of claim 27, wherein an amount of the at least one breathable membrane is five.

29. The fully enclosed analyte detection device of claim 14, wherein the fully enclosed analyte detection device further comprises a third sealing ring, and the third sealing ring is disposed in the annular groove of the lower cassette.

30. The fully enclosed analyte detection device of claim 1, wherein the fully enclosed analyte detection device further comprises a sealing member, and the sealing member adheres to and seals a bottom of the lower cassette.

31. The fully enclosed analyte detection device of claim 30, wherein the sealing member is made of high-transparency thin pressure-sensitive adhesive material.