Device for multiparametric assays

An assay device (C) compatible with multi-well format for performing a biological reaction between capture and target molecules, comprising:

- a cap (A) comprising side walls (7), and top surface (5) and a bottom surface (6), wherein the top surface (5) and bottom surface (6) are connected by at least one inlet channel (10) and at least one outlet channel (11) and wherein the top surface (5) of the cap (A) comprises a stopper indentation (15),
- a well (B) comprising side walls (3) and a bottom surface (4) upon which capture molecules are immobilized, wherein the cap (A) and the well (B) are made of different polymeric materials being preferably cycloolefin polymer for the cap (A) and polypropylene for the well (B),
- wherein the side walls (7) of the cap (A) and the side walls (3) of the well (B) comprises complementary screwing means (12,13) to seal the cap (A) inside the well (B) to form a chamber (1) between the bottom surface (6) of the cap (A) and the bottom surface (4) of the well (B) and wherein the two said surfaces are essentially parallel to each other with a variation of distance between the two surfaces lower than 100 \( \mu \text{m} \), better lower than 20 \( \mu \text{m} \) and,
- wherein the chamber (1) has a height of less than 5 mm, preferably less than 2 mm and which is vapour and fluid tight, when the cap (A) is sealed inside the well (B).
Description

Field of the invention

[0001] The present invention is related to an assay device compatible with multi-well format for performing biological reactions between target molecules and capture molecules, wherein these capture molecules present are preferably fixed upon a surface of a well in the form of a micro-array.

[0002] The present invention is also related to a device for performing multiple assays in compartmentalized chambers.

Background of the invention

[0003] Biological samples are difficult to analyze, because of the complexity of the molecules being present in biological organisms and the large number of molecules having similar chemical or physical characteristics. On the other side, there is a need to perform analyses on materials as small as possible given the fact that in many analyses, the amount of material available is very limited. The miniaturized assays for multiple components are now performed on a miniaturization scale using micro-arrays. Currently, micro-array assays are performed on an individual basis along with the different steps of the method from the incubation with the sample to the scanning of the array and the data analysis. These methods are not adapted for handling multiple samples simultaneously. The ability to do so would be advantageous for routine assays requiring automated procedure, (preferably upon multi-wells plate) such as in clinical diagnostic laboratories or in high throughput screening.

[0004] Assays in molecular biology required reaction temperature being high for controlling the specificity of the polynucleotides reaction. At these high temperatures, evaporation may occur, if devices used for performing these reactions are not perfectly sealed. Furthermore, a possible deformation of substrates comprising the reactive molecules by this heating will also hamper the specific detection of multiple targets at the end of the assay.

State of the art

[0005] US patent application 2003/0049862 discloses an assay device comprising a cap having inlet and outlet channels and a well having an array of capture molecules on its bottom surface wall. Two channels are present in this cap and seals have been seen to close the chamber liquid or gas tight.

[0006] However, a fixing of the cap upon the wall is not adequate for avoiding evaporation of liquid during assay performed in this well, to prevent drying out of air of element (such as array), that may be comprised in this well and to avoid possible cross contamination between wells.

[0007] The US patent application 2005/0023672 describes well cap combinations comprising integrated micro-arrays in a capillary gap. This US patent application discloses to seal the cap to this well by screwing means.

[0008] Mendoza et al. (1999, Biotechniques, 27, 778-80 782-6, 788) provides biochips in multi-well format for the detection of proteins wherein the solid support is made of an optically flat, glass plate containing 96 wells formed by an enclosing hydrophobic Teflon mask. Each of these wells contains at its bottom four identical 36-element arrays (144 elements per well) comprising 8 different antigens and a marker protein.

[0009] However, none of these documents addresses the particular problem of simultaneous detection of multiple targets in a biological sample in a device comprising immobilized capture molecules on a flat surface, said device and having an inlet and outlet to inject the sample remaining light without significant evaporation during the reaction between target and probe molecules even for long incubation times at elevated temperature, nor that flat surface comprising the capture molecule remains flat during the incubation at high temperature for purposes of imaging the surface there after.

Aims of the invention

[0010] A first aim of the present invention is to provide an assay device, a multi-well plate or an apparatus comprising this device, for an selective and efficient detection and/or quantification of multiple targets in a miniaturized format and that does not present the drawbacks of the state of the art.

[0011] A preferred aim is to propose such assay device, plate or (apparatus) that improves micro-fluidic handling of sample(s) in a 24 or 96-well plate format for a simultaneous detection of multiple targets present in one or multiple biological sample(s), to avoid evaporation during assay, to prevent drying out of arrays comprised in wells, to avoid cross contamination between wells or compartments present in the same wells, to avoid bubble formation inside the wells, to handle small volume(s) of sample(s) homogeneously distributed upon capture molecules present in the different wells and to allow an easy efficient and reproductive detection of targets bound on these capture molecules thereafter.

[0012] Another aim of the invention is to provide such device that is compatible with multi-well format and which allows modulation of the assay size (number of dilutions, ...) modulation of the incubation volume needed for a particular application, if required by the consumer need and by the number of samples to be tested.

Summary of the invention

[0013] The present invention is related to an assay device (C) compatible with multi-well plate format and adaptable upon the frame of a multi-well plate for performing a biological reaction between capture and target molecules, comprising:
- a cap A comprising side walls 7, a top surface 5 and a bottom surface 6, being connected by at least one inlet channel 10 and at least one outlet channel 11 and wherein the top surface 5 of the cap A comprises a stopper indentation 15,
- a well B comprising side walls 3 and a (flat) bottom surface 4 upon which capture molecules are immobilized,
- wherein the cap A and the well B are made of different polymeric materials, being preferably a cycloolefin polymer material for the cap A and a polypropylene material for the well B,
- wherein the side walls 7 of the cap A and the side walls 3 of the well B comprise complementary screwing means (or features 12, 13) to seal the cap A inside the well B, to form a chamber 1 having an height of less than 5 mm preferably less than 2 mm or 1 mm (the chamber being formed between the bottom surface 6 of the cap A and the (flat) bottom surface 4 of the well.

**[0014]** The device according to the invention is configured in order to obtain a chamber 1 that is efficiently vapor and fluid tight, when the cap A is sealed inside the well B, to reduce evaporation from this chamber 1 and to prevent or avoid drying out of capture probes comprising in this chamber. Therefore, the side wall(s) 7 of the cap A and the side wall(s) 3 of the well B comprise complementary screwing means (or features 12, 13) to seal the cap A inside the well B, so that the (flat) bottom surface 4 of the well B and the bottom surface 6 of the cap A are essentially parallel to each other and present a variation of distance (tolerance) between them (between the two surfaces) lower than 100 μm, preferably lower than 20 μm. Advantageously, the screwing means 13 (internal female, thread) of the well B located on side walls 3 is complementary to the screwing means 12 (external male, thread) of the cap A located on the side wall 7 as depicted in figure 1, figure 3 and figure 4.

**[0015]** This variation of distance (tolerance) is measured between a first distance value d1 and a second distance value d2 as provided in figure 1, wherein the distance value d1 is located in the center 4a of the (flat) bottom surface 4 of the well B comprising a micro-array and wherein the second distance value d2 being obtained from a location 4b at the periphery of the (flat) bottom surface 4 of the well B, preferably at a distance of at least 1 mm from the well walls 3, the distance value d1 differs by less than 100 μm, preferably less than 20 μm from the distance value d2.

**[0016]** Advantageously, as schematically provided in figure 1, the device C according to the invention has a cap A having a cavity 20 which present a (flat) bottom surface 6.

**[0017]** According to another embodiment of the present invention, as schematically provided in figure 9, the well B presents an additional cavity 22 with a bottom (flat) surface 4 and a shoulder 23 serving as contact with the cap A, when the cap A is sealed into the well B.

**[0018]** According to a preferred embodiment, the capture molecules are immobilized on the (flat) bottom surface 4 of the well B in discrete regions in the form of a micro-array having a density of at least 4, preferably 10, 100, 1000 or more discrete region per cm². Advantageously, the sealing of the cap A into the well B reduce, prevent or avoid drying out of this (micro)array.

**[0019]** Advantageously, the distance (tolerance) between this micro-array comprising capture molecules and the well B side walls 3 is higher than 1 mm, for allowing an efficient delivery of capture molecules upon this (flat) bottom surface 4 of the well B by a spotting arrayer. The distance of at least 1 mm also avoids border effects during incubation with the sample (capillary movements along the walls 3).

**[0020]** The device C of the invention allows many tests upon many arrays to be set up and processed together; because they allow much higher throughput of test samples and greatly improves the efficiency for performing assays on biological micro-arrays.

**[0021]** The device C allows proper formation of a tight chamber 1 resulting from the sealing of two complementary parts A, B by avoiding the use of glue that can interfere with biological reaction, which is difficult to remove from the support and which can induce a fluorescent background (disturbing the detection upon the micro-array).

**[0022]** Advantageously, the volume of liquid above capture molecules present on a (flat) bottom surface 4 of the well B is small and remains homogeneous on the overall bottom surface 4 comprising these immobilized capture molecules during the different processing steps, this requirement improves the reproducibility of the assay, particularly when these capture molecules are present in a micro-array format.

**[0023]** A sealing of a cap A on a well B, to form the device C of the invention is also configured to avoid contamination of a sample solution contained in a first well by another sample solution possibly contained in an adjacent well. An efficient sealing by screwing avoids also capillary effects bringing solution out of the well.

**[0024]** The screwed device C according to the invention allows easy access to the bottom surface 4 of fixed by capture molecules as required for the scanning of the array. Also the chamber 1 can be dissociated and re-associated according to the constraints of the fabrication or of the multiple steps of a detection method. These sealings and opening step have to be easily performed either manually or by using a robotic automate that can be also used for the formation of the array on this bottom surface 4 and for a scanning of this bottom (flat) surface 4 comprising the capture molecules.

**[0025]** The device can be subjected to a plurality of processing steps, including hybridization, washing, staining and scanning steps. The cap A can be removed after a sample incubation, thus making the well B easily accessible for successive working and labeling either man-
The device C according to the invention is compatible with a multi-well format. Therefore, another aspect of the present invention is related to a multi-well plate comprising a multi-well chassis D and at least one, preferably at least two (one or more) device(s) C according to the invention or at least one well B of the device C of the invention.

Preferably, this chassis D comprises 24 frames E (6 x 4) for inserting up to 24 device(s) C or well(s) B of the device(s), 96 frames E or 384 frames E for inserting respectively up to 96 or up to 384 device(s) C or well(s) B of the device(s) according to the invention.

Advantageously, the top surfaces of different devices C are complementary to the attachment feature 16 of the frame E. Advantageously, the contact is made only between the solid support surface and the chassis D of the multi-well plate at a predefined position 25 of a ring 24 on the top surface of the well B. As a consequence, the devices are aligned in the same orientation in the frames E of the multi-well chassis D. Advantageously, the stopper indentation 15 present on the top surface 5 of the cap A is blocked at a ridge site 25 presented on the well B, when the cap A is tightly screwed into the well B. This feature allows an alignment of the devices C in the frames E of the multi-well chassis D. A correct alignment of the devices C is important for an efficient positioning of automatic handling elements into the devices C and also for obtaining an efficient detection of target molecules fixed in their capture molecules. Examples of automatic handling elements are: pipette tips which are inserted into the inlet 10 and outlet 11 channels of the cap A or screwing pipes which are inserted into the screw channels 14 of the cap A.

Advantageously, the number of devices C inserted in the frames E of the chassis D is adapted according to the customer need and the number of samples to be processed in parallel.

Preferably, when inserted into the frame of the multi-well chassis D, the distance between the external bottom surface of the device C or the well B of the device C and a support surface (table surface) is of at least 1 mm. When the device C according to the invention is incorporated in the chassis D, the surface of the chassis D in contact with a support (table surface) is preferably aligned with the external bottom surface 26 of the well B, this surface 26 is opposite to the inner bottom surface 4 comprising the immobilized capture molecules.

To avoid any contact between the external bottom surface 26 of the well B and a solid support, the well B is inserted in the frame E of the multi-well plate at a position which avoid any contact between this external bottom surface 26 and the solid support preferably. In this position the external bottom surface is located at the distance of at least 1 mm from this solid support surface. Preferably, the contact is made only between the solid support surface and the chassis D of the multi-well plate. The external bottom surface 26 of the well B may present also an o-ring format 27 of polymer in a height of at least 1 mm.

This feature avoids scratches of the external bottom 26 of the well B due to contact with various tables surfaces. An o-ring 27 of polymer of at least 1 mm high surrounding the external bottom surface 26 of the well B is depicted in figure 4.

For an easy production, the cap A and the well B have preferably a cylindrical shape for an easy introduction into the frame E of the multi-well chassis D. However, other shapes or others sections of the wells could be selected by the person skilled in the art.

The device C according to the invention may further comprise means for sealing inlet and outlet channels (10,11) at the top surface 5 of the cap A so as to obtain a sealing of inlet port and an outlet port of this inlet a sealing of the cap A inside the well B at a fixed and predefined position 25 of a ring 24 on the top surface of the well B. As a consequence, the devices are aligned in the same orientation in the frames E of the multi-well chassis D. Advantageously, the stopper indentation 15 present on the top surface 5 of the cap A is blocked at a ridge site 25 presented on the well B, when the cap A is tightly screwed into the well B. This feature allows an alignment of the devices C in the frames E of the multi-well chassis D. A correct alignment of the devices C is important for an efficient positioning of automatic handling elements into the devices C and also for obtaining an efficient detection of target molecules fixed in their capture molecules. Examples of automatic handling elements are: pipette tips which are inserted into the inlet 10 and outlet 11 channels of the cap A or screwing pipes which are inserted into the screw channels 14 of the cap A.
channel and outlet channel.

[0037] In the device according to the invention, the capture molecules are selected from the group consisting of nucleotide sequences (polynucleotide sequences or oligonucleotide sequences), proteins, peptides (preferably antibodies or hypervariable portions thereof) polysaccharides or a mixture thereof. These capture molecules are capable of binding complementary target molecules which are preferably selected from the group consisting of nucleotide sequences, proteins, peptides, haptoxens or a mixture thereof.

[0038] Advantageously, the capacity volume of the chamber 1 formed in the device C according to the invention is comprised between about 10 μl and about 200 μl, preferably about 100 μl.

[0039] Preferably, the height specifications of the chamber 1 are the same across the surface comprising the immobilized capture molecules, but may be larger near the well walls 3 or above the 2 parts. This feature allows having a homogeneous distribution of sample solution across the overall surface comprising the capture molecules. As all the surface is constantly covered, this avoids drying effects.

[0040] In a preferred embodiment, the capacity volume of the well B, when not sealed by the cap is comprised between about 100 μl and about 1000 μl.

[0041] In a preferred embodiment, the cap A and the well B have a cylindrical shape. Round corners of the well walls are preferred to avoid capillary effects both in open well B or closed well B with screw cap A.

[0042] The device C according to the invention is made of two parts: the well B and the cap A which are advantageously made of different polymeric materials. Preferably, the well B is made of cycloolefine polymer while the cap A is made of polypropylene. Advantageously, the cap A is sealable and removable from the well B.

[0043] Preferably, the polymer of the cap A is softer than the polymer of the well B and/or has thermal expansion coefficient which is higher than the thermal expansion coefficient of the polymer of the well B.

[0044] Advantageously, the cap A is made of a flexible material such as polypropylene to better seal the wells. Preferably the (linear) thermal expansion coefficient of the cap A material is higher by at least 5 and better 10 and even better than 20 10^-6/K at 20°C in m/m.K x 10^-6 than the thermal expansion coefficient of the well material. The thermal expansion coefficient is a fractional change in length per degree of temperature change. It is usually defined as followed:

\[ \alpha = \frac{1}{L_0} \frac{\partial L}{\partial T} \]

where \( L_0 \) is the original length, \( L \) the new length, and \( T \) the temperature.

The linear thermal expansion is the one-dimensional length change with temperature.

\[ \frac{\Delta L}{L_0} = \alpha_L \Delta T \]

The linear thermal expansion coefficient is related to the volumetric thermal expansion. The change in volume with temperature can be written:

\[ \frac{\Delta V}{V_0} = \alpha_V \Delta T \]

For exactly isotropic materials, the volumetric thermal expansion coefficient is very closely approximated as three times the linear coefficient.

\[ \frac{\Delta V}{V_0} = 3 \alpha \Delta T \]

[0045] Furthermore, the cap A could be also formed by two different polymers wherein, a first polymer being in contact with the well B to form the chamber 1 is softer than the other polymer.

[0046] Similarly, the well B could be also made of two different polymers wherein, a first polymer being in contact with the cap to form the chamber 1 is harder than the other polymer (used in the composition of the well).

[0047] The well B is preferably made of a rigid low fluorescence material, such as cycloolefine polymer Zeonex™. Other polymers like polycarbonate, polyacrylate, polyethylene may also be used. The cap A is preferably made of a semi-rigid polymer, preferably polypropylene.

[0048] The bottom surface 4 onto which the capture molecules are immobilized is preferably transparent and distortion free for purposes of imaging the surface. This material should preferably also be non-fluorescent in order to minimize the background signal level and allow detection of low level signals from low intensity features on the surface.

[0049] The capture molecules are preferably covalently immobilized on Zeonex™ (330R) using the chemistry described in the EP Patent application EP 1847316 incorporated herein by reference. The well B bottom thickness is preferably of less than 1 mm with 50 μm tolerance.

[0050] Advantageously, in the device C according to the invention the bottom surface 4 of the well B is comprised between about 4 mm² and about 280 mm², preferably between about 20 and about 200 mm². A portion of this bottom surface 4 comprises capture molecules, preferably present upon a surface of about 4 mm² and
preferably more than 1 cm wide and about 1.5 mm wide and been separated from each other by about 6 mm. Preferably, the attachment features 17 are separated from the mounting foot 19 by a distance of at least 1 mm, creating a gutter 18 allowing the insertion of attachment feature 16 of about 1 mm wide being present on inner wall of the frame E. Preferably said attachment feature 16 is located on the inner wall of the frame E at a distance of 1 mm from the top of the frame and about 6 mm from the bottom of the frame. Preferably, two attachment features 17 are present at one side of the well external side wall 3 and two other attachment features 17 are present on the opposite side of the side wall 3 allowing the insertion of two attachment features 16 being present on facing inner walls of the same frame.

Preferably, the cap A has a cylindrical shape with a diameter of about 14 mm and height of about 10 mm. Preferably, the top surface 5 of the cap comprises a stopper indentation 15 of about 1 x 2 mm. The top surface 5 also comprises at least one inlet channel 10 and at least one outlet channel 11 which are separated from each other by a pitch of about 9 mm. The diameter of inlet channel 10 or outlet channel 11 is about 1 mm. The top surface 5 may also comprise screw channels 14 which are separated from each other by a pitch of about 10 mm. The diameter of the screw channel 14 is about 2 mm. The bottom surface 6 comprises preferably a cavity 20 of 10 mm in diameter and height of about 1.4 mm. When the cap A is sealed into the well B it prevents less than 10% evaporation of liquid present in the chamber after 24 hours incubation at 65°C, preferably less than 5% evaporation of liquid volume. Furthermore, the sealed device C (when the cap A is sealed into the well B) resists to agitation and may be incubated in adequate temperature in a mixing apparatus like the thermomixer from Eppendorf AG (Hamburg, Germany).

Advantageously, the device C according to the invention is also configured for a detection of different target compounds present upon different separated areas or compartment 2 of the flat surface of the well as schematically outlined in figures 5, 7, 8 and 10.

Therefore, the chamber 1 formed between the cap A and the well B comprises at least two compartments 2 which are separated from each other by physical separations 9 wherein each compartment 2 comprises at least one inlet port/channel 10 and at least one outlet port/channel 11 which are connected with the top surface 5 of the cap A. Advantageously, these physical separations 9 are obtained when the cap A is sealed into the well B. Therefore, according to a first embodiment of the present invention schematically outlined in figure 5, the cap A may comprise these physical separations 9 in the cavity 20 of its bottom surface 6. Figure 6 represents top 5 and bottom surfaces 6 of the cap of figure 5. According to a second embodiment, the compartments 2 are obtained by using a well B comprising physical separations 9 fixed on its bottom (flat) surface 4 (figure 7), preferably in its cavity 22 (figure 10). According to a last embodiment of the present invention schematically outlined in figure 8 the cap A comprises a first portion 9a of the physical separation 9 (possibly present in the cavity 20) of its bottom surface 6 and the well B comprises a second portion 9b of this physical separation 9 on its bottom surface 4 or on its cavity 22. These two portions 9a, 9b, are complementary and form the separation between the two compartments 2.

Advantageously, the two compartments 2 are fixed with different capture molecules or are fixed with the same capture molecules. The same capture molecules could be used to bind different target molecules to be detected in different compartments 2.

Advantageously, the device A according to the invention comprises at least two or more compartments (preferably four compartments) separated by one or more physical separations 9.

Advantageously, these physical separations 9 have a height comprised between about 0.3 and about 3 mm and the well inside wall 3 has a height comprise between about 4 and about 10 mm.

The volume of the compartment is preferably comprised between about 1 µl and about 20 µl, preferably about 10 µl.

Furthermore, each of these compartments 2 has preferably a distinctive sign different from the distinctive sign of another compartment which is detectable together with the detection of the target molecule present in this compartment and bound upon its corresponding capture molecule.

The device of the present invention comprising compartments 2 that are preferably used as described in the European patent application EP1852186.

The target molecules may be labeled to allow their detection. The labelled associated detections are numerous. A review of the different labelling molecules is given in WO 97/23717. The most frequently used and preferred labels are fluorochromes like Cy3, Cy5 and Cy7.

Radioactive labelling, cold labelling or indirect labelling with small molecules recognised thereafter by specific ligands (streptavidin or antibodies) are common methods. The resulting signal of target fixation on the array is either fluorescent, colorimetric, diffusion, electrolymuseline, bio- or chemiluminescent, magnetic,
A preferred label is the use of the gold labelling of the bound target in order to obtain resonance light scattering (RLS) detection or silver staining which is then easily detected and quantified by a scanner. Gold particles of 10-30 nm are required for silver amplification while particles of 40-80 nm are required for direct detection of gold particles by RLS or by Photothermal Heterodyne Imaging.

In a preferred method, gold particles of 10-30 nm are amplified by silver enhancement preferably using the silverquant analysis platform including the Silverquant kit for detection, the Silverquant Scanner for slide scanning and Silverquant Analysis software for image quantification and data analysis (Eppendorf, Germany). Due to the non linear detection of the presence of silver, the data analysis requires a linearization of data before data processing. The data are then processed according to the invention. An algorithm of curve fitting is applied to a positive detection curve spotted on the array. Then each spot signal is linearized in ‘concentration units’ using the fitting curve.

Assays on biological reactions generally include contacting immobilized capture molecules with target molecules contained in a sample under the selected reaction conditions, optionally washing the surface comprising the capture molecule to remove un-reacted target molecules, and analyzing the surface comprising the capture molecules for evidence of reaction between target and capture molecules. These steps involve handling fluids. The methods of this invention automate these steps so as to allow multiple assays to be performed concurrently. Accordingly, this invention employs automated fluid handling systems for concurrently performing the assay steps in each device. Fluid handling allows uniform treatment of samples in the devices. Microtiter robotic and fluid-handling devices are available commercially, for example, from Tecan AG or Eppendorf AG (Epmotion).

In a preferred embodiment, handling of the device C is performed by using a (multi) pipette and/or 96-wells plate format automate. Samples solutions are preferably introduced into the device C through inlet port of an inlet channel 10. The air present in the chamber 1 of the device C is moved out by the outlet channel 11. Preferably the inlet port of the inlet channel 10 and the air duct port of the outlet channel 11 are not at the same level, one being at a lower level than the other in the chamber 1. In case of injection of the sample solution, the inlet port is preferably lower than the air duct port in the chamber 1. The steps after the incubation step with the sample are preferably performed without removing the cap A by just removing sealing means applied on the inlet elements of the cap top surface 5. When carrying washing steps, the washing solution is preferably injected by an inlet port which is higher than the outlet port in the chamber 1. The outlet port is lower to ensure complete removing of washing solution from chamber 1.

Drying of the surface area comprising the capture molecules is also possible using the device of the invention. Air aspiration is performed by one of the port.

Just before analyzing the surface comprising the capture molecules for evidence of reaction between target and capture molecules, the cap A is preferably removed to allow a scanning of the well B bottom surface 4 from the top.

Scanning of multiple devices C inserted into the multi-well chassis D may be performed by several means.

Scanners adapted for multi-well plate reading have usually a limited focusing tolerance. As in the present invention, the devices C mounted on the chassis D are not linked to each other by a common external surface, they may not be all in the same focal plan of the scanner. One proposed solution to this problem is to use a flexible chassis D allowing a limited movement of the device inside the frame E. The chassis D comprising multiple devices C to scan is preferably positioned on a high precision glass plate allowing the alignment of the devices C in a same focal plan before introduction in a scanner.

The scanning can be performed by using a scanner having independent focusing system for each device.

The cap A may further comprise at least one screw channel 14 on its top surface 5 to allow the insertion of screwing pipes. The screwing pipes are preferably mounted on the head of an automate. There are preferably two pipes which are not exactly parallel to each other to allow a good retention of the screwing pipes into the cap A of the device C during transfer movements. The screw pipes can load and screw the cap A into the well B and then remove it and discard it.

The top surface 5 of the cap A may also further comprise the stopper indentation 15 allowing the cap A to stop at a fixed position 25 of the well B (once tightly screwed into the well B). Such alignment structure is depicted in figures 3, 4 and 6. These support structures may be used to mount or position the cap A into the well of the device at the same position for the different devices C. Each frame E of the multi-well chassis D matches the shape of a device C that is preferably asymmetric to avoid rotation of the device C once positioned into the frame E. Asymmetric and unidirectional positioning of the device C into the frame E allows unique horizontal orientation in the chassis D. The devices are also aligned vertically into the frames E due to complementary attachment feature 16 of the frame E with attachment feature 17 of the well B (figures 2 and 4). Thus, the devices which are inserted in the multi-well chassis D may be easily aligned by an automatic handling device or on detection or imaging system. This feature ensures proper orientation and alignment for liquid handling and scanning of the target molecules bound to complementary capture molecules.

The plate of the invention can be introduced
into a holder in the fluid-handling device. This robotic device is programmed to set appropriate reaction conditions, such as temperature, add samples to the well B of device C through inlet channel 10, incubate the test samples for an appropriate time, remove un-reacted samples, wash the wells B, add substrates as appropriate and perform detection assays. The particularity of the reaction conditions depends upon the purpose of the assay. For example, the assay may involve testing whether a sample contains target molecules that react to a probe under a specified set of reaction conditions. In this case, the reaction conditions are chosen accordingly.

Another aspect of the present invention is related to an apparatus (high trough put screening apparatus) which comprise the device C or the multi-well plate comprising the device C or the well(s) B of the device C according to the invention, and possibly multi pipettes and other element of a 96 well plate format or an automatic.

This apparatus further comprises head with screwing pipe(s) for an insertion of screwing pipe(s) into the screw channel 14 of the top surface 5 of the cap A for obtaining an efficient manipulation of the cap A and the sealing of the cap A inside the well B.

The present invention will be described in more details in the enclosed figures presented as nonlimiting illustrations of the various embodiments of the present invention.

Another aspect of the present invention is related to a kit of part comprising the cap A and the well B of the device C according to the invention and possibly the chassis D of the multi-well plate.

Short description of the figures and tables

Figure 1 represents a side view of a preferred device of the invention.

Figure 2 represents a 3D view of the device of figure 1 being inserted into a frame of a multi-well chassis by complementary attachment features.

Figure 3 represents top surface, bottom surface and side view of the cap of figure 1.

Figure 4 represents top surface and side views (external and internal) of the well of figure 1 comprising attachment features creating a gutter for insertion in the frame of figure 2 by clipping.

Figures 5, 7, 8 and 10 represent side views of preferred devices of the invention wherein the chamber formed between the cap and the well comprises at least two compartments.

Figure 6 represents top and bottom surfaces of the cap of figure 5.

Figure 9 presents a schematic view of a device similar to the one presented in figure 1 but with a cavity being present in the well and serving for the formation of a chamber once the cap is screwed on the well.

Figure 10 presents a schematic view of a device similar to the one presented in figure 9 but with physical separation being present in the well and serving for the formation of compartments once the cap is screwed on the well.

Example 1: Detection of GMOs on micro-array spotted at the bottom (flat) surface of the well B of the device C

1. Preparation of cycloolefin polyaldehyde wells B

2. The microarray

Aldehyde functions are introduced into a cycloolefin (Zeonex™ 330R) well using the chemistry described in the EP Patent application EP01847317A1 incorporated herein by reference. The dimensions of the well B are the following: diameter of the bottom (flat) surface 4 = about 14.19 mm, height of the side wall 3 = about 12 mm.

The microarray comprises common and event-specific genetic event nucleotide sequences. The microarray is commercially available as DualChip GMO V 2.0 (Eppendorf AG, Hamburg, Germany). The capture molecules (probes) are immobilized upon the bottom (flat) surface 4 of the well B to form an array of 16 x 18 spots having a pitch of 400 micron between the spots. The spotting protocol described by Schena et al. (1996 PNAS, USA 93:10614) is used for the grafting of aminated DNA to the aldehyde derivatized well B surfaces. Each capture polynucleotide sequence (capture probe) is present in triplicate on the micro-array. The capture nucleotide sequences are printed with a home made robotic device using 250 μm diameter plain pins. The spots have around 400 μm in diameter and the volume dispensed is about 0.5 nl. After washing, the wells are dried at room temperature and stored at 4 °C until used.

The capture probe sequences are described in the EP Patent application EP1724360 incorporated herein by reference. These capture probes are able to identify genetically modified event nucleotide sequences by screening simultaneously multiple genetic elements: P35S, T-Nos, Pnos-nptII, pat, Bar, Cry1AB, Cry3Bb1, EPSPS, Invertase (Maize), Lectin (Soybean), Cruciferin (Rapeseed) and gut or hox (PCR control) sequences. The micro-array also includes advantageously a control element CaMV.

The micro-array is also able to detect a specific genetic event nucleotide sequence of a given specified GMO.
[0087] The capture probe (polynucleotide sequence) Cry1Ab-1 is specific for BT176, the Cry1Ab-2 for Mon810 and the Cry1Ab-3/Cry1Ac for BT11, Mon531 and Mon15985. The capture probe (polynucleotide sequence) EPSPS-A is specific for GA1, the EPSPS-B for RRS and Mon603 and the EPSPS-C for GT73, Mon1445 and H7-1.

3. The PCR

[0088] In order to amplify the sequences of the genetic elements that could be detected on the DualChip GMO V 2.0, 3 PCR reactions are processed according to the kit manual (Eppendorf AG, Hamburg, Germany). The primers used and PCR conditions are also described in EP Patent application EP1724360.

PCR 1 (PCR S) allows the amplification of common genetic event nucleotide sequences of the GMO: 3SS Promotor, Nos terminator, Pnos-ntpl, CaMV, Pat, Bar, EPSPS, Cry1Ab, Cry3Bb1 and Gut (PCR S control) sequences.

PCR 2 (PCR P) allows the amplification of plant elements which are specific of the following plant species: Maize (Invertase gene), Soybean (Lecitin gene), Brassicaceae (Cruciferin gene), Cotton (Acp1 gene), Rice (Gos9 gene), Sugar beet (Glutamine synthetase (gs) gene), Potato (UGPase gene), gut (PCR P control).

PCR 3 (PCR E) allows the amplification of event-specific genetic element nucleotide sequences of 12 specified GMOs: GA21, Mon603, Bt11, RRS, Mon531, T45, GT73, Bt176, Mon810, MonN863, Mon15985, Mon1445 and hox (PCR E control).

[0089] The PCR amplification are performed in a final volume of 25 μl containing: 1X QIAGEN Multiplex PCR Master Mix containing HotStarTaq® DNA Polymerase, Multiplex PCR buffer and dNTP Mix, 0.15 μM of each biotinylated primer, 0.1 μM of each non-biotinylated primer (except for maize and sugar beet for which 0.3 μM of biotinylated primer and 0.2 μM of non-biotinylated primer are used) and containing 200 ng of Genomic DNA that is extracted from a sample of Maize seeds using a CTAB-based method (Rogers, S.O. and Bendich, A.J. 1985. Extraction of DNA from milligram amounts of fresh, herbarium and mumified plant tissues. Plant Mol. Biol. 5: 69-76) and quantified using the “Quant-it™ PicoGreen dsDNA assay kit” (Invitrogen, USA) as described in the kit manual.

[0090] The DNA of the samples are denatured at 95 °C for 15 min. Then 32 cycles of amplification are performed consisting of 30 sec at 95 °C, 90 sec at 56 °C and 30 sec at 72 °C and a final extension step of 10 min at 72 °C.

4. Hybridization and colorimetric detection

[0091] In this experiment, the 3 PCR products (amplified sequences) are hybridized in the well B having fixed the micro-array at its bottom surface 4. The cap A is screwed within the well B to form an assay device C which is inserted in a multi-well plate having a 96-well plate format.

[0092] To prepare the hybridization mix, 9 μl of each PCR product (27 μl in total) is mixed in a 1.5 ml microtube with 5 μl of SensiHyb solution, 4 μl of hybridization control and 4 μl of water. 5 μl of 0.5 N NaOH is added and incubated for 5 min at room temperature. Then 50 μl of hybridization solution (Genomic HybriBuffer, Eppendorf, AG) is added and 100 μl of the hybridization mix solution is injected inside the well B though the inlet channel 10 of the cap. The inlet and outlet channels are sealed and hybridization is performed for 1h at 60°C. The hybridization is immediately followed by the detection step. The colorimetric detection is performed using the Silverquant detection kit (Eppendorf, Hamburg, Germany). Pixels are captured and the signal is processed to generate a heat map.

[0093] The cap A is removed and the well B is washed 2 times with Post Hybridization Buffer for 1 min and 3 times with Washing Buffer for 1 min. The well is incubated for 10 min at temperature in Pre-Blocking Buffer and for 45 min at room temperature in the Diluted Gold-Conjugate. After incubation, the well is washed 4 times with Washing Buffer for 1 min, then once with Rinsing Buffer for 1 min. Then, an equal volume of Silverquant A and B solutions are incubated for 5 min at room temperature. After incubation, the well is washed 2 times with distilled water for 30 sec at room temperature and air dried before detection of the target molecule upon corresponding capture molecules. The detection is performed in the scanner ArtixScan 4500t (Mircrotek International Inc., Taiwan) which is adapted for reading a multi-well plate using a home made holder.

Claims

1. An assay device (C) compatible with multi-well format for performing a biological reaction between capture and target molecules, comprising:

- a cap (A) comprising side walls (7), and top surface (5) and a bottom surface (6), wherein the top surface (5) and bottom surface (6) are connected by at least one inlet channel (10) and at least one outlet channel (11) wherein the top surface (5) of the cap (A) comprises a stopper indentation (15),

- a well (B) comprising side walls (3) and a bottom surface (4) upon which capture molecules are immobilized,

- wherein the cap (A) and the well (B) are made of different polymeric materials, being preferably a cycloolefin polymer material for the cap (A) and a polypropylene material for the well (B),

- wherein the side walls (7) of the cap (A) and the side walls (3) of the well (B) comprises complementary screwing means (12, 13) to seal the cap (A) inside the well (B) to form a chamber (1)
between the bottom surface (6) of the cap (A) and the bottom surface (4) of the well (B) wherein the two said surfaces are essentially parallel to each other with a variation of distance between the two surfaces lower than 100 \( \mu \text{m} \), better lower than 20 \( \mu \text{m} \),

- wherein the chamber (1) has a height of less than 5 mm, preferably less than 2 mm and which is vapour and fluid tight, when the cap (A) is sealed inside the well (B).

2. The device of claim 1, wherein the cap (A) has a cavity (20) on its bottom surface (6), said cavity having a flat surface.

3. The device according to the claim 1 or 2, wherein the bottom surface (4) of well (B) has a cavity (22) and has a shoulder (23) serving as contact with the cap (A) when sealed into the well (B).

4. The device of claim 1, wherein the capture molecules (4) preferably selected from the group consisting of a nucleotide sequences, proteins, peptides, preferably antibodies or hypervariable portions thereof, polysaccharides or a mixture thereof are immobilized onto the bottom surface (4) of the well (B) in discrete regions in the form of an micro-array having a density of at least 4, preferably 10, 100 or 1000 discrete regions per \( \text{cm}^2 \).

5. The device according to any of the preceding claims, wherein the top surface (5) of the cap (A) comprises at least one screw channel (14) to allow insertion of screwing pipes.

6. The device according to any of the preceding claims, wherein the polymer of the cap (A) is softer than the polymer of the well (B).

7. The device according to any of the preceding claims, wherein the polymer of the cap (A) has a thermal expansion coefficient which is higher than the thermal expansion coefficient of the polymer of the well (B).

8. The device according to any of the preceding claims, wherein the cap (A) is formed by two polymers components and wherein the polymer of the cap (A) being in contact with the well (B) to form the chamber (1) is softer than the other polymer.

9. The device according to any of the preceding claims, wherein the chamber (1) comprises at least two compartments (2) with at least one physical separation (9) and wherein the physical separations are present in the cavity (22) of the bottom surface (4) of the well (B) and wherein each compartment (2) comprises at least one inlet channel (10) and at least one outlet channel (11).

10. The device according to the claim 3, wherein the chamber (1) comprises at least two compartments (2) with at least one physical separation (9) and wherein the physical separations are present in the cavity (22) of the bottom surface (4) of the well (B) and wherein each compartment (2) comprises at least one inlet channel (10) and at least one outlet channel (11).

11. A multi-well plate having a multi-well plate format and comprising inserted into a frame (E) of a multi-well chassis (D) at least one of the device (C) according to any of the preceding claims 1 to 10.

12. The plate according to the claim 11, wherein the chassis (D) comprises 24 frames (6 x 4) for inserting up to 24 devices (C) according to any of the preceding claims 1 to 10.

13. The plate according to the claims 11 to 12, wherein the frame (E) comprises attachment feature (16) complementary to attachment feature (17) of the well (B).

14. An apparatus, preferably a high through put screening apparatus of target molecules which comprise the device according to any of the preceding claims 1 to 10 or the multi-well plate according to the claims 11 to 13.

15. The apparatus according to the claim 14 which comprises a head with a screwing pipe for an insertion of the screw channel (14) of the device (C) according to the claim 5.

16. A kit of part comprising the cap (A) and the well (B) of the device (C) according to any of the preceding claims 1 to 10, or the multi-well plate according to the claims 11 to 13.
FIG. 2
<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document with indication, where appropriate, of relevant passages</th>
<th>Relevant to claim</th>
<th>CLASSIFICATION OF THE APPLICATION (IPC)</th>
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</thead>
<tbody>
<tr>
<td>A</td>
<td>EP 1 852 186 A (EPPENDORF ARRAY TECHNOLOGIES [BE]) 7 November 2007 (2007-11-07)</td>
<td>1,9,10</td>
<td>B01L3/00</td>
</tr>
<tr>
<td>A</td>
<td>WO 2007/131999 A (EPPENDORF ARRAY TECHNOLOGIES S [BE]; REMACLE JOSE [BE]; ALEXANDRE ISAB) 22 November 2007 (2007-11-22)</td>
<td>1-5,8-10</td>
<td>B01L3/00</td>
</tr>
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</table>

The present search report has been drawn up for all claims
This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on the 08-12-2008.

For more details about this annex: see Official Journal of the European Patent Office, No. 12/82.
REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- US 20030049862 A [0005]
- US 20050023672 A [0007]
- EP 1847316 A [0049]
- EP 1852186 A [0063]
- WO 9727317 A [0064]
- US 5312527 A [0065]
- EP 01847317 A1 [0083]
- EP 1724360 A [0085] [0088]

Non-patent literature cited in the description

- Schena et al. PNAS. USA, 1996, vol. 93, 10614 [0084]