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METHOD OF SAMPLE PREPARATION ON A SPECTROMETRIC SAMPLE SUPPORT

Field of the invention

[0001] The invention relates to a method for sample preparation on a spectrometric sample support, in which (a) a sample support is provided which comprises an arrangement of individual droplets of liquid, each of which encloses microorganism sediments, (b) a plate of an absorbent material is provided, (c) the plate and sample support are brought together in such a way that droplet liquid is absorbed into the absorbent material, (d) the plate locally enriched with liquid is lifted off the sample support (vertically), whereby the microorganism sediments that are depleted of liquid are exposed, and (e) the exposed microorganism sediments are prepared for a spectrometric measurement.

Background of the invention

[0002] The prior art is explained below with reference to a specific aspect. This should not, however, be understood as limiting. Useful developments and changes from the known prior art can also be applicable beyond the relatively narrow context of this introduction and will be available to experienced practitioners in this field after reading the following disclosure.

[0003] Previous experiments have shown that microorganisms suspended in a droplet of nutrient solution accumulate in a microorganism deposit on a flat surface after a relatively short standing time (or “resting time”) of up to one hour. The microorganisms that have sedimented there in a kind of “biofilm” can be carefully freed from residual liquid and remaining suspended particles, for example by bringing an absorbent cloth into contact with the droplets. After this “dehumidification”, the type of microorganisms can be reliably determined with a subsequent mass spectrometric measurement; see international application WO 2018/099500 A1. This finding was astonishing since it was found, contrary to expectations, that the microorganism cell sediment of interest was not removed at the same time as the extracted liquid. This finding allows microorganism cultivation (or incubation) for growth promotion and preparation for an analytical measurement on the same substrate, such as a sample support plate for insertion into the ion source of a mass spectrometer or a slide for insertion into the measurement well of an infrared spectrometer.

[0004] There is no fully formed scientific explanation yet for this microbial behaviour

in a droplet on a flat substrate. However, it is assumed that physical interactions between the sample support surface and the microorganism cells as well as adhesion processes due to the biochemical and biophysical properties of the microorganism cell surface are responsible for the preferred accumulation or sedimentation on the substrate.

[0005] In a further development of the technology known from WO 2018/099500 A1, the application EP 3 376 202 A1 proposes a mask with indentations or holes, the inner surfaces of which are brought into contact with the edge regions of the droplets and suck off the droplet liquid laterally by means of capillary forces. The centre of the droplets, under which most of the microorganism sediment is located, should remain untouched during this process, since it has been feared that direct contact with the sediment would necessarily lead to the depletion of the microorganisms ("stamp effect") and thus to a loss of sensitivity in the subsequent analytical measurement.

[0006] However, the local non-contact removal of the liquid, either via the profiled mask or the placement of a dimensionally stable plate on a vertical spacer surrounding the droplets, as described in EP 3 376 202 A1, has the disadvantage that such a quantity of droplet residual liquid can still remain on the substrate of the sediment that subsequent examination of the microorganism sediment is impaired and further purification measures may be necessary.

[0006a] European application EP 1 053 784 A2 discloses the processing of very small sample amounts, for example in chemical or enzymatic reactions, purifications or analytical studies, in very small amounts of liquid of the order of one microlitre and less, in particular for very large numbers of samples in simultaneous processes. The disclosure is concerned with reducing disproportionately growing container wall contact surfaces with their mostly harmful effects in the case of very small amounts of liquid by using standing (or hanging) droplets as microreactors for processing, wherein the droplets sit on wetting-friendly (lyophilic) anchors in otherwise wetting-hostile (lyophobic) surfaces and therefore have a well-defined wall contact area. The anchors can be passivated as desired or made surface-active, the latter to support certain processing steps. Further configurations include the integration of electrical, optical, fluidic or other microstructured elements into the support to assist processing.

[0007] There is therefore a need to separate individual microorganism sediments sufficiently completely from covering droplets of liquid and to remove as little material as possible from the microorganism sediments with the liquid during this separation process. Further problems addressed by the invention will become readily apparent to a

person skilled in the art upon reading the following disclosure.

Summary of the invention

[0008] The invention relates to a method for sample preparation on a spectrometric sample support, comprising the steps: (i) providing the sample support, which contains an arrangement of individual droplets of liquid, each of which has microorganism sediments enclosed in it, (ii) providing a plate of absorbent material, (iii) lowering the plate vertically onto the sample support in such a way that the microorganism sediments and plate come into contact, wherein the droplets of liquid are absorbed into the absorbent material, (iv) lifting the plate, which is locally enriched with droplet liquid, off the sample support, whereby the microorganism sediments that are depleted of liquid are exposed, and, if necessary, after any remaining residual liquid film left on the microorganism cell sediments has been allowed to dry, (v) preparing the exposed microorganism sediments for spectrometric measurement, for example a subsequent infrared or mass spectrometric measurement.

[0009] In various embodiments, the plate can contain a swelling absorbent material and can be lowered until there is only a small gap between it and the sample support so that a supernatant of the droplets comes into contact with the plate and is absorbed by it, wherein the absorbent material undergoes localised swelling at the sites where the droplet liquid is absorbed and thus makes contact with the microorganism sediments. A plate of blotting paper, the material of which has a corresponding swelling behaviour, has proven to be particularly suitable for this variant. Blotting paper is known in particular from the restoration and archiving of moisture-sensitive works of art and is substantially a neutral, acid-free, absorbent and low-lint bibulous paperboard with a high proportion of cotton fibres, which can be more than 50%, in particular 60%.

[0010] The selection of the initially still contact-free gap can be tested very simply by lowering a plate provided with a staining paint against the sample support. If traces of colour can be found on the sample support after lifting the plate again, the gap was not large enough to avoid direct contact and must be readjusted. In this way, gaps of fractions of a millimetre, which can be closed by the swelling behaviour of an absorbent material such as blotting paper when it absorbs liquid locally until it comes into contact with the microorganism sediment, can be easily adjusted experimentally.

[0011] In various alternative embodiments, the plate can be placed directly onto the sample support and gently pressed against it so that the arrangement and plate make all-over contact.

[0012] The invention is based on the unexpected and thus surprising finding that it is possible to lower a flat and low-lint plate of an absorbent material against a flat sample support and bring it into contact with same, sometimes even placing it on the same and pressing it gently, in such a way that capillary forces absorb almost all of the liquid supernatant from a droplet into the material without the microorganism sediment concealed underneath being depleted to such an extent that a subsequent spectrometric investigation of the sample support would not provide any meaningful results due to insufficient biomaterial. Although a film of liquid remains after the plate is lifted (vertically), it is so thin that it dries up within seconds.

[0013] The sample support and/or plate are preferably spring-mounted with relative respect to each other so that any excessive pressure applied, when the plate is pressed down, is taken up by the springs. The elastic deformation of the springs ensures that a uniform contact pressure of the plate on the sample support is maintained. By suitably selecting the spring constant, this pressure can be adapted to requirements and adjusted in such a way that the microorganism sediment is not impaired by the pressure applied.

[0014] In various embodiments, a plate surface facing toward the sample support can be moistened slightly before being lowered, for example with deionised water or methanol/ethanol, to accelerate the absorption of the droplet liquid.

The slight moistening and superficial wetting of the fibres lying on the outer plate surface facilitates in particular the formation of a liquid connection or liquid bridge between the droplets deposited on the sample support and the fibre mesh of the plate so that the surface of the fibre fabric on the outside is already wet from the start and does not first have to be wetted or saturated with the droplets on the sample support with liquid contact in a time-consuming manner.

[0015] The plate preferably has absorbent fibres made from cellulose and/or plant fibres, in particular cotton, nitrocellulose, nylon, glass fibre, polyvinylidene difluoride or combinations thereof. Blotting paper, a particularly suitable material, can contain, for example, a mixture of 60% cotton fibres and 40% bleached (pH-neutral and acid-free) cellulose.

[0016] Usual droplet volumes can correspond to about one to twelve microlitres. These volumes in the form of uniform droplets correspond approximately to a diameter of two or three millimetres over the surface of the sample support.

[0017] In particular in infrared spectrometry or mass spectrometry analysis, for example MALDI time-of-flight analysis, 48, 96, 384 or 1536 individual droplets can be

arranged in a predefined (and regular) pattern on the sample support. Of course, it is also possible to mark the sample support with preferred droplet spots, for example lyophilic circular areas in a lyophobic environment. The diameter of the droplets will then adjust according to the dimension of the lyophilic circular area, as with standardised anchor plates of the AnchorChip™ type.

[0018] In particular, the droplets can mainly contain liquid that is used in sample pretreatment methods and sample preparation methods for infrared spectrometry or mass spectrometry. For example, the droplets may comprise nutrient solution and the sedimented material may comprise microorganisms cultivated and then deposited in these nutrient solution droplets. Separation of the nutrient solution from sedimented microorganisms can be used in particular as a preparation step of a sample for infrared spectrometric or mass spectrometric identification (e.g. using IR transmission spectroscopy or MALDI time-of-flight mass spectrometry) according to type/subspecies or another characterisation of the microorganisms, such as rapid determination of resistance/sensitivity of microorganisms to antimicrobial substances such as antibiotics and antimycotics.

[0019] If necessary, an antimicrobial substance can be added to the nutrient solution, for example in order to determine by incubation the minimum inhibitory concentration of microorganisms in the droplet of nutrient solution enriched with antibiotics or antimycotics.

[0020] Additionally or alternatively, the droplets can contain washing liquid (such as an aqueous solution or pure deionised water) or other liquid working media to be used for a sample preparation method. For example, the washing liquid from sample spots covered with previously dried microorganism sediment or with dried matrix substance and microorganism sediment can be absorbed with the plate. The washing liquid is preferably selected in such a way that the matrix substance or the matrix crystal lattice with built-in sample crystals is not dissolved, for example in the case of an α -cyano-4-hydroxycinnamic acid affinity preparation for in-situ desalting (see Gobom et al., Anal. Chem. 73, 2001, 434-438).

[0021] In various embodiments, a metal, glass or ceramic plate can be used as the spectrometric sample support. In particular, polished stainless steel plates or variations thereof, such as so-called anchor plates (AnchorChip™; Bruker Daltonik GmbH), which contain alternately mutually delimited lyophilic and lyophobic surfaces on a stainless steel substrate, come into consideration. A glass plate is also possible as an infrared spectrometric slide for a transmission measurement. The sample support can be

reusable or disposable.

[0022] A common, standardised sample support for matrix-assisted laser desorption/ionisation (MALDI) may comprise a regular arrangement of 48, 96, 384 or 1536 sample spots. The dimensions of a sample support and a plate of absorbent material can each be 127.76 mm (length) x 85.48 mm (width), corresponding to a microtitre plate with a thickness of about two to five millimetres, provided they are congruent. However, the sample spot arrangement and thus the arrangement of the droplets on the sample support takes up a smaller area than the sample support itself.

[0023] A frame for the plate or a well for the sample support into which the plate is inserted may be provided to assist in guiding and aligning the plate during lowering onto the droplet arrangement as described in EP 3 376 202 A1, the contents of which are hereby incorporated by reference in their entirety. The frame can, for example, be firmly connected to the plate, in particular by folding a protruding cut edge of the absorbent material and then impregnating it with a plastic which then hardens. The frame can also be injection-moulded onto the outer perimeter of the plate using an injection-moulded plastic.

[0024] The plate is preferably made of a stiff and low-lint fabric. Plant fibres and/or cellulose are suitable for this. For example, it can have fibre materials such as cotton fibre, nitrocellulose, nylon, glass fibre, polyvinylidene difluoride or combinations thereof, for example blotting paper with about 60% cotton fibres and 40% bleached (pH-neutral and acid-free) cellulose. Dimensionally stable materials are particularly useful in automated methods for absorbing liquid. The dimensionally stable plates can be easily removed from a storage facility by specially adapted handling devices such as robotic arms, clamped in an adapted holder, fed to the liquid absorption step and then deposited at a storage location, and possibly also disposed of.

[0025] With regard to the manufacture of the plate, it is possible to provide a sheet of the absorbent material and then cut the pieces of plate to length according to the desired outer contour. For use in a microbiological laboratory, it is advisable to select the outer dimensions of the plate in such a way that the absorbed liquid with any suspended microorganisms remaining in it cannot penetrate to the narrow side of the plate or to the top of the plate so that the locally liquid-enriched plate can be gripped there without the risk of contamination and can be moved.

Brief description of the figures

[0026] Reference is made to the following figures in order to better understand the

invention. The elements in the figures are not necessarily drawn to scale, but are primarily intended to illustrate the principles of the invention (largely schematic). In the figures, corresponding elements in the different views are identified by the same reference signs.

[0027] Figures 1A to 1E schematically show an exemplary embodiment of a method in which a plate of absorbent material is placed on a droplet-carrying sample support.

[0028] Figures 2A to 2D schematically illustrate a modification of the method in which springs absorb excess pressure applied during pressing of the plate on the sample support and thus ensure that the pressure is applied evenly.

[0029] Figure 3 schematically shows an exemplary embodiment of a method in which a plate of swelling material is lowered to a small gap from the sample support.

[0030] Figure 4 shows MALDI time-of-flight mass spectrometry spectra after removal of culture supernatants from an enterobacterial sediment, once using a non-contact method, top diagram A, as exemplified by E. A. Idelevich et al., Clin. Microbiol. Infect. 2018 (7): 738-743, in which Whatman® cellulose filter paper was used as absorbent material, and once using a contacting method in accordance with principles of the invention, bottom diagram B; vertical axis: intensity in arbitrary units; horizontal axis: charge-related mass m/z .

Detailed description

[0031] Although the invention has been illustrated and explained with reference to a number of embodiments, a person skilled in the art will recognise that various changes can be made to the form and detail thereof, without departing from the scope of the technical teaching defined in the attached claims.

[0032] Figure 1A shows an arrangement of droplets (2) along a row of eight on a sample support (4), which can correspond to a MALDI sample support plate or a slide suitable for infrared spectrometry. A plate (6) of an absorbent material is positioned over the sample support (4). The plate (6) is slowly moved vertically against the sample support (4), the droplets (2) coming into contact with the absorbent material of the plate (6) from a certain lowering point so that the droplet liquid can be absorbed by the material of the plate (6) via capillary forces (figure 1B). In contrast to earlier disclosures, this lowering movement can end with the plate (6) being placed on the sample support (4), as shown in figure 1C. In this way, all-over contact between the plate (6) and the droplet arrangement (2) can be produced, which leaves less residual liquid from the droplets (2) on the microorganism sediments and thus facilitates subsequent

spectrometric measurements of the sediments.

[0033] Surprisingly, it has been found that a previously suspected stamp effect is significantly less pronounced than feared, according to which effect many microorganism cells, when they come into direct contact with the absorbent material of the plate (6), are removed from the sediment by transfer to said plate, thereby making subsequent measurement more difficult due to the reduction in available biomaterial, which is why contactless removal of the liquid has been advocated in earlier disclosures. Although in-house experiments of the applicant, in which the plates used to absorb liquid were incubated, have shown that colonies of microorganisms can form on the plate surface at the points where droplet liquid has been absorbed, the amount of “stamped” biomaterial, contrary to expectation, turned out to be so low that subsequent spectrometric investigations of the material remaining on the sample support (4) were not impaired.

[0034] No fully formed scientific explanation has been found for these beneficial properties. However, it is believed that the interaction between fibres of the plate material and microorganisms is very small, so the two do not adhere well to each other, which counteracts the stamp effect. Furthermore, it is believed that restricting the plate movement to a purely vertical lowering onto the sample support while avoiding lateral shearing movements, in contrast to the earlier disclosure, where lateral movements were recommended, also reduces and possibly eliminates the risk of smearing the microorganism biofilm, which could lead to rearrangement of cells from the sample support to the plate material.

[0035] After contact, the droplet liquid is absorbed in a very short period of time (usually fractions of a second), possibly facilitated by previous slight moistening of the plate material. Thereafter, the locally liquid-enriched plate (6) can be lifted up again and removed (figure 1D). The absorbed liquid is held securely in the capillary mesh of the plate (6) so that there is no risk of it dripping out again when it is lifted (vertically) and contaminating the sample support (4). Rather, it is a very safe and reliable way of removing liquid. The partially soaked plate (6) is typically disposed of as a consumable, which is advantageous in microbiological applications. However, it could optionally also be washable and then reusable.

[0036] As already explained above, the microorganism sediments enclosed by the droplets (2) are not removed by the gentle absorption of the liquid by capillary forces, but mostly remain in the middle of the surface of the sample support (4) where the droplets (2) were deposited. The sedimented material, which has now been freed from

liquid, is thus available for further processing, such as sample preparation for infrared spectrometry, matrix-assisted laser desorption/ionisation or similar processing steps, indicated by the pipetting of a working liquid, e.g. matrix substance for MALDI, with (8) in figure 1E.

[0037] The gap from the edge of the plate to the nearest droplet preferably corresponds to at least the droplet radius on the surface of the sample support, and more assuredly is the droplet diameter in order to avoid, when liquid is absorbed into the absorbent material, biohazardous components being sucked too close to the edge, where the plate can be gripped by laboratory personnel if necessary. A similar safety margin should be maintained for the minimum thickness of the plate to prevent penetration of the absorbed liquid to the plate surface, facing away from the liquid absorption.

[0038] In a variant of the method explained above, the plate (16) can also be pressed slightly after it has been lowered against and placed on the sample support (14) in order to ensure that the plate (16) and the arrangement (12) or sample support surface are in all-over contact to ensure the most complete liquid absorption possible. In order to avoid excessive pressure being exerted (e.g. by inexperienced laboratory personnel), which can impair the microorganism sediment, particularly when pressing by hand, the plate (16) and sample support (14) can be spring-mounted relative to one another, as schematically indicated by the helical springs (18) in figures 2A to 2D. It goes without saying that the sample support (14), the plate (16) or both elements can be spring-mounted in order to achieve the desired effect, although only the springs of the sample support (14) are illustrated in the sequence from figure 2.

[0039] A plate (16) of absorbent material is lowered against the sample support (14) carrying the droplets of liquid (12), figure 2A. In this state, the springs (18) are relaxed. As soon as the plate (16) comes into contact with the droplet supernatant, which occurs before contact with the sample support surface, the droplet liquid is absorbed into the absorbent material of the plate (16); figure 2B. Even in this state, the springs (18) are still relaxed. As soon as the plate (16) touches down on the sample support (14), resistance can be felt during the lowering movement. Pressure can now be applied in order to ensure that the plate (16) and the sample support surface or arrangement (12) come into contact evenly and, in particular, over the entire surface and absorb as much residual liquid as possible; figure 2C. The springs (18) are now compressed according to the applied pressure N and the spring constant, thus preventing excessive pressure on the microorganism sediments. After a period of fractions of a second, when the liquid

has been absorbed into the plate material, the plate (16) can be lifted vertically again and thus exposes the microorganism sediments that are depleted of liquid, which are then accessible for further processing on the sample support (14); figure 2D. The springs (18) relax again.

[0040] It goes without saying that the helical springs (18) from the above example are only used to illustrate the spring principle. Other spring mechanisms may also be used in the methods presented herein, as deemed appropriate by a practitioner.

[0041] Embodiments have been described above in which the absorbent plate (26) is placed directly on the sample support (24). However, it is also possible to space the plate (26) slightly above the sample support (24), for example by resting it on a lateral ridge (28) if it is made of a swelling absorbent material; figure 3. When the plate surface comes into contact with the droplet supernatants, liquid is locally absorbed into the plate (26) and leads to an increase in volume ("swelling") there so that the swollen material comes into gentle contact with the microorganism sediments; see the enlarged detail in figure 3. It has been found that this gentle contact does not further impair the sediment, in particular only a few microorganism cells are transferred to the plate surface ("stamping effect"), but it allows the droplet liquid to be absorbed more completely than is possible with the previously published strictly gap-based techniques using non-swelling materials. The amount of residual liquid that remains on the sample support (24) after the (vertical) lifting of the locally soaked plate (26) is thereby reduced, which facilitates the subsequent spectrometric measurements.

[0042] In order to simplify the handling of the plate, it can be inserted or clamped in a frame. For example, the frame can be dimensioned in such a way that it encloses a sample support in a flush manner on which a droplet array is disposed, as described in EP 3 376 202 A1. It can be designed as a disposable item that is disposed of with the soaked plate, or it can be washable and reusable. Possible configurations include a frame with a stepped inner contour on which the plate can be frictionally placed. When the frame slides down around the outer contour of the sample support, liquid contact is established at a certain point. The frame also has the advantage of providing reliable alignment and guidance of the plate relative to the droplet array.

[0043] In an alternative embodiment, also described in principle in EP 3 376 202 A1, the frame can be firmly connected to the plate. For example, a protruding cut edge of the absorbent material can be folded and then impregnated with a plastic, which then hardens to ensure strength and dimensional stability (one-piece variant). If necessary, the frame can also be injection-moulded onto the outer perimeter of the plate using an

injection-moulded plastic.

[0044] EP 3 376 202 A1 also discloses inserting a sample support, which carries the array of droplets, into a well that encloses it on all sides. The plate can then be dimensioned similarly to the sample support and slowly slide downwards onto the sample support from the upper well opening. Recessed grips on the well walls can make it easier to insert and remove the sample support and plate.

[0045] Figure 4 shows measurement data that illustrate the advantages of the method described here. The (almost) complete removal of a droplet supernatant, here culture or nutrient solution supernatant after bacterial incubation directly on a MALDI sample support, according to the principles of the present disclosure (bottom diagram B), and thus also the removal of contamination, e.g. components of the nutrient medium or salts that could disrupt subsequent measurement, lead to improved quality of measurement data in the form of higher intensities of the ion signals and an improved signal-to-noise ratio.

[0046] Regarding determination of the antibiotic susceptibility (cephalosporin antibiotic: ceftazidime) of examined gram-negative bacteria, the contacting method according to the invention and the non-contact method from the prior art after 4h/4.5h/5h incubation gave consistent results in relation to a conventional nutrient solution microdilution method (20h incubation) at 95%/100%/100% or 93%/93%/92%, which underlines the great benefit of these processes.

[0047] In addition to the exemplary embodiments explained, further embodiments of the invention are also conceivable. With knowledge of this disclosure, it is possible for the person skilled in the art to design further advantageous preparation methods for samples for infrared spectrometric measurement or mass spectrometric measurement using a desorbing ionisation method, which should be covered by the scope of protection of the patent claims including any equivalents.

FREMANGSMÅDE TIL PRØVEFORBEREDELSE PÅ EN SPEKTROMETRISK PRØVEHOLDER

Patentkrav

1. Fremgangsmåde til prøveforberedelse på en spektrometrisk prøveholder, som omfatter trinnene:
 - Tilvejebringelse af prøveholderen, der omfatter et arrangement af individuelle dråber af væske, som hver omgiver mikroorganismesedimenter,
 - Tilvejebringelse af en plade af et absorberende materiale,
 - lodret nedsenkning af pladen på prøveholderen, så mikroorganismesedimenter og pladen berører hinanden, hvorved væskedråberne optages i det absorberende materiale,
 - løft af pladen, der er lokalt beriget med dråbevæske, af prøveholderen, hvorved de væskereducerede mikroorganismesedimenter blotlægges, og
 - præparering af de blotlagte mikroorganismesedimenter til en spektrometrisk måling.
2. Fremgangsmåden ifølge krav 1, hvor pladen rummer et svulmende absorberende materiale og sænkes til kort over prøveholderen, så en supernatant af dråberne kommer i kontakt med pladen og absorberes af den, hvor det absorberende materiale lokalt svulmer på de steder, hvor dråbevæsken er absorberet, og kommer i kontakt med mikroorganismesedimenterne.
3. Fremgangsmåden ifølge krav 1, hvor pladen sættes direkte på prøveholderen og forsigtigt presses mod den, så arrangementet og pladen er i berøring over hele fladen.
4. Fremgangsmåden ifølge krav 3, hvor prøveholderen og/eller pladen er fjederlagret i forhold til hinanden, så for stort tryk ved nedtrykning af pladen optages af fjedringen.
5. Fremgangsmåden ifølge krav 1 til 4, hvor en pladeflade, der vender mod prøveholderen, fugtes let før nedsenkning for at fremskynde absorberingen af dråbevæsken.

6. Fremgangsmåden ifølge et af kravene 1 til 5, hvor pladen har absorberende fibre af cellulose og/eller plantefibre.
7. Fremgangsmåden ifølge et af kravene 1 til 6, hvor et dråbevolumen svarer til omkring én til tolv milliliter.
8. Fremgangsmåden ifølge kravene 1 til 7, hvor mikroorganismesedimenterne i væskedråberne tilvejebringes fra næringsopløsning eller vaskevæske.
9. Fremgangsmåden ifølge krav 8, hvor næringsopløsningen er iblandet en antimikrobiel substans.
10. Fremgangsmåden ifølge et af kravene 1 til 9, hvor præpareringen af de eksponerede mikroorganismesedimenter efterfølges af en infrarød eller massespektroskopisk måling fra prøveholderen.
11. Fremgangsmåden ifølge et af kravene 1 til 10, hvor der mellem løft af pladen og præparering er en kort ventetid, hvor eventuelt resterende væskefilm på mikroorganismecellesedimenterne lades tørre.

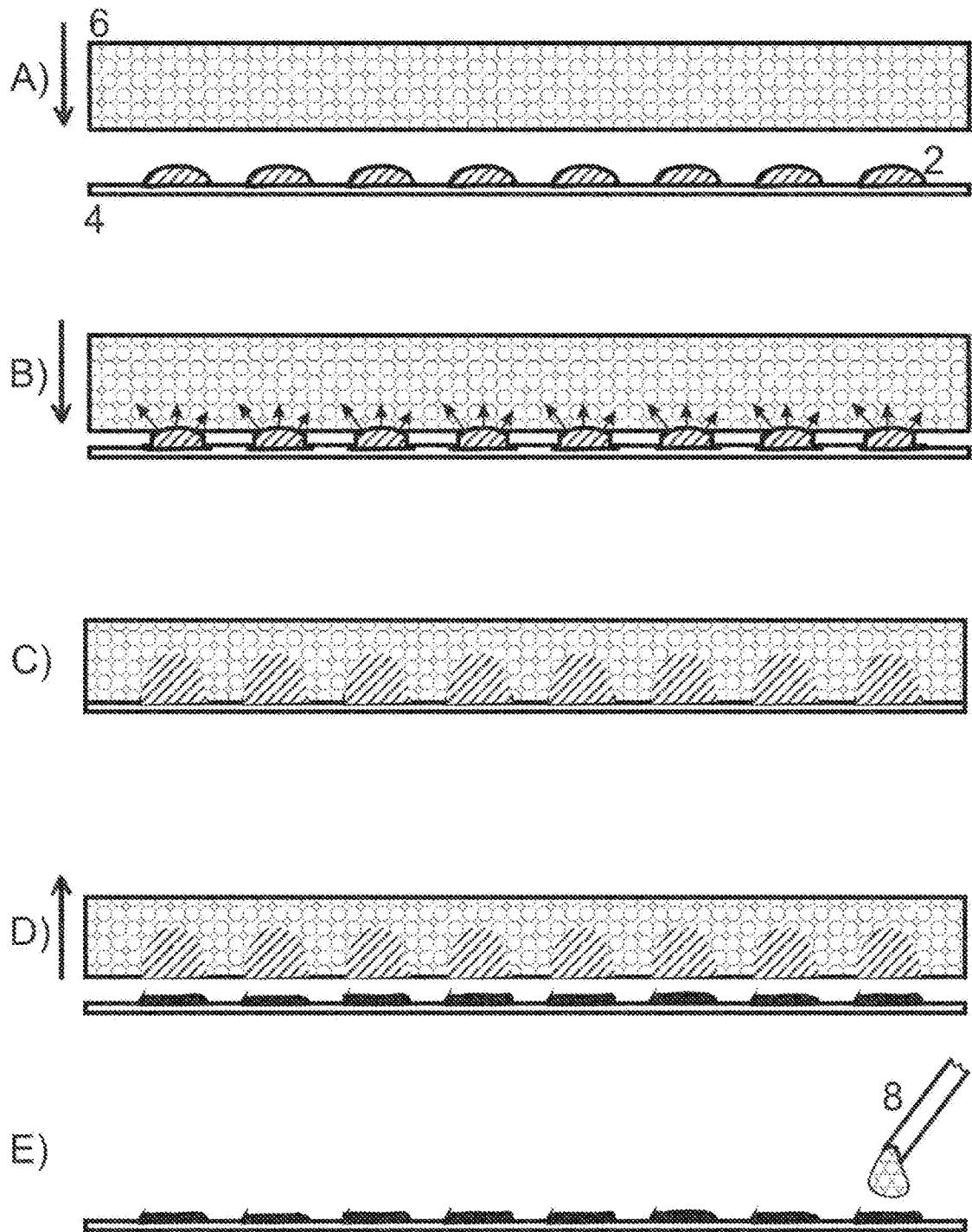


Figure 1

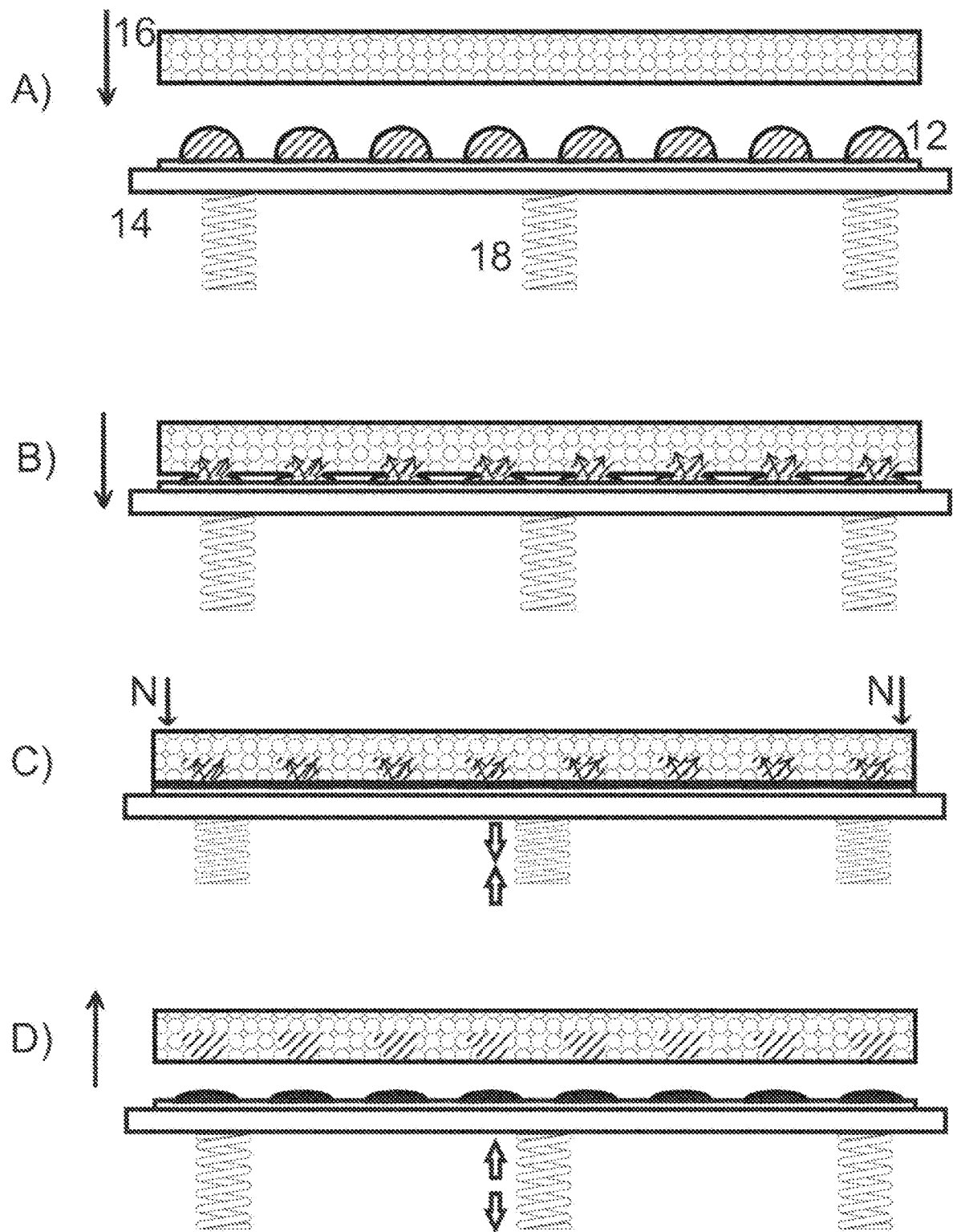


Figure 2

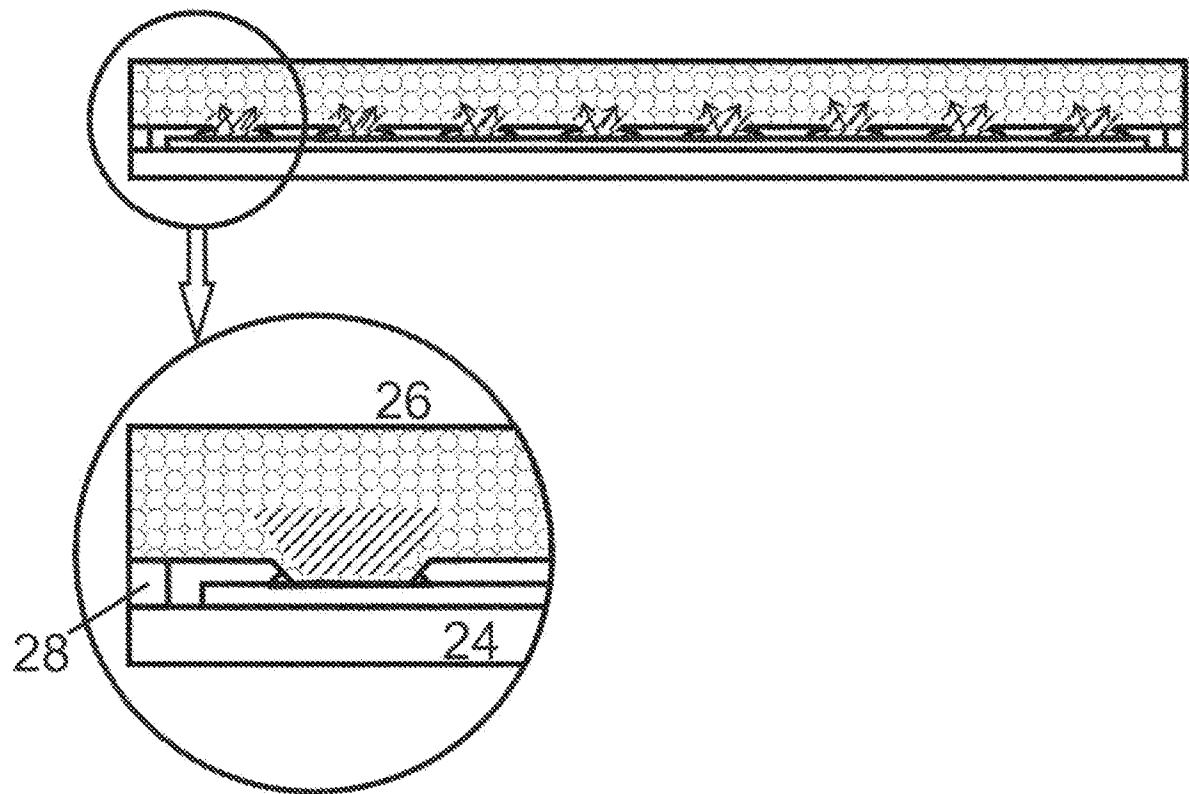


Figure 3

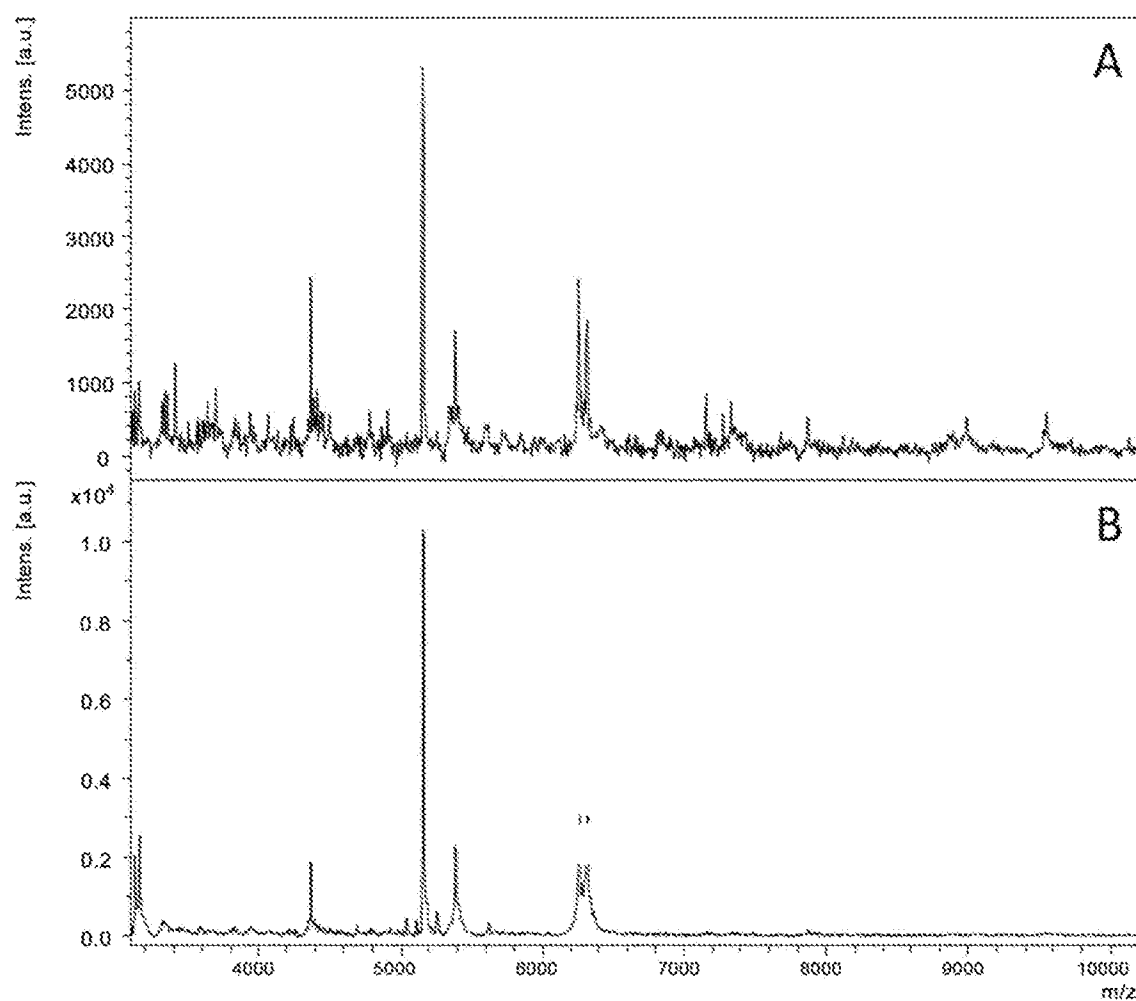


Figure 4