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(54) Title: METHOD FOR ADJUSTING THE DISPENSING HEIGHT OF PIPETTES

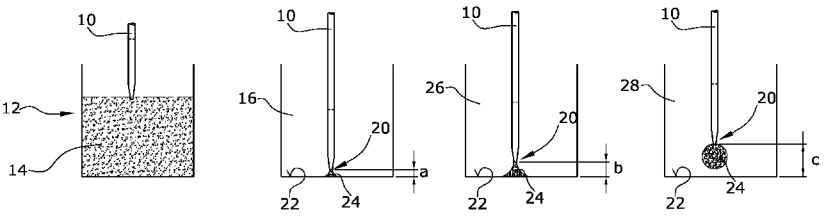

Fig.1

(57) Abstract: A method for adjusting the dispensing height of pipettes relative to an receiving element, comprising the following steps: causing an adjustment liquid (14) to be received in the at least one pipette (10), arranging a pipette tip (20) at a first distance (a) to the receiving element (18), passive dispensing of the adjustment liquid (14) into a first deepened portion (16) of the receiving element (18), arranging the pipette tip (20) at a further distance, passive dispensing of the adjustment liquid (14) into a further deepened portion (26), repeating steps d) and e) until no dispensing of the adjustment liquid (14) takes place anymore, whereby this is detected by visual means, and using a distance that had been set in one of the steps preceding the step in which no dispensing of the adjustment liquid (14) took place to define a dispensing height.

Method for adjusting the dispensing height of pipettes

The invention relates to a method for adjusting the dispensing height of pipettes relative to a receiving element.

Pipettes, being arranged e.g. in pipetting devices, are used for automatic dispensation of liquids into receiving elements. The receiving elements typically are microtitration plates comprising a plurality of deepened portions (wells) arranged in rows and columns. With the aid of such a pipetting device, liquid will each time be dispensed, e.g. from a plurality of pipettes arranged in a row, simultaneously into a plurality of deepened portions of a titration plate, said deepened portions being arranged in a row or column. The dispensing herein is preferably performed by means of passive pipettes. In passive pipettes, in contrast to active pipettes, the liquid will not be pressed out into the receiving element with the aid of a piezo element or the like; instead, a drop of the liquid will drip out of the tip of the pipette, due to a contact with a surface, e. g. the bottom of the receiving element or a liquid comprised within such receiving element. In the process, a droplet of the liquid issuing from the tip of the pipette can be placed in the receiving element if the liquid droplet is made to touch preferably the bottom of the deepened portion of the receiving element and, under the effect of the contact with the bottom, is pulled off the tip of the pipette. The droplet can be created by a liquid delivering system, for example a plancher pump. In view of this precondition, it must be safeguarded that the pipettes will be arranged close enough to the bottom of the deepened portion to allow for the liquid to dispensed. In case that the deepened portion does already contain a liquid, the dispensing of the liquid should then be performed in such a manner that the tip of the pipette will be positioned sufficiently close to the surface of the liquid which is contained in the deepened portion.

On the other hand, the problem exists that the tip of the pipette must not be immersed too deeply into the deepened portion, such as e.g. a well, of the titration plate because, otherwise, the liquid dispensation would cause the outer side of the pipette to be wetted with liquid. As a consequence, a part of the liquid would be removed again from the deepened portion, thus reducing the quantity of liquid remaining in the deepened portion. Further, a considerable problem is caused in that, during the next dispensation of liquid, the liquid adhering to the outer side of the pipette will be carried over in the next deepened portion, with a resultant risk of contamination. Thus, with respect to the dispensing height of the pipette tip, the demand exists that the pipette tip should have the maximum possible distance to the bottom of the deepened portion and respectively to the surface of the liquid already contained in the deepened portion.

Said two demands, i.e. the demand for reliable dispensation of liquid by contact of the droplet and the demand for said maximum possible distance, particularly in order to avoid contamination, are contrary to each other.

From US 5,143,849 a pipette system is known, comprising a pipette being connected to a pressure device to deliver liquid to the pipette. Furthermore, a pressure sensor is arranged to detect the pressure of the liquid within the pipette. To adjust the distance of the tip of the pipette from a surface, the liquid shall be delivered to, the tip of the pipette has a relatively large distance to that surface. By use of the pressure means, a meniscus is formed on the tip of the pipette. Thereafter, the distance between the tip of the pipette and the surface is decreased until the meniscus contact the surface. This contact is detected by a pressure decrease within the pipette. This measured distance is used to actively dispense liquid on the surface. The main drawback of this device is that a very sensitive pressure sensor has to be used. Another drawback is that the distance between the tip of the pipette and the surface may vary, for example due to unevenesses of the surface or tolerances. Since the exact height is used, even a small increase in

the distance will cause the problem that the meniscus or droplet does not contact the surface anymore.

It is an object of the invention to provide a method for adjusting the dispensing height of particularly passive pipettes relative to an receiving element, wherein, on the one hand, a safe dispensing of droplets is guaranteed and, on the other hand, the danger of contamination and respectively of undesired transfer of liquid via the outer side of the pipette is reduced.

According to the invention, the above object is achieved by a method as defined in claim 1.

In a first method of the invention, it is provided that, in a first step, an adjustment liquid is initially caused to be received in a pipette, e.g. by taking the adjustment liquid from a supply container by means of the at least one pipette and sucking it into the pipette. In the next step, a pipette tip of the filled pipette will be arranged at a first distance to the receiving element. According to the preferred embodiment of the invention the first distance is a small distance. This is followed by particularly passive dispensing of the adjustment liquid into a first deepened portion of the receiving element, said dispensing being performed by drop-wise dispensation. Due to the presently still small distance between the pipette tip and the first deepened portion, a safe dispensing of droplets is guaranteed.

In a further step, the pipette tip will again be arranged above a further empty deepened portion of the receiving element. In this step, the distance is enlarged so that the pipette tip will now be arranged at a second, larger distance to the receiving element. This is again followed by a passive dispensing of one or more droplets of the adjustment liquid into the further and respectively second deepened portion.

After also the dispensing of liquid into said further and respectively second deepened portion has been performed, this step will be repeated, wherein

the distance of the pipette tip to the receiving element is enlarged again. The dispensing is now performed into a still further and respectively third deepened portion. These dispensing steps, wherein dispensation takes place into different deepened portions with respective enlargement of the distance, will be repeated as often until no droplets are dispensed anymore. When the dispensing of droplets has ceased, the distance between the pipette tip and the receiving element has been made so large that the droplet will not touch anymore the bottom of the deepened portion or the surface of a liquid already existing in the deepened portion.

According to the invention, visual means are used to detect that no droplets are dispensed anymore. Since the distance between the tip of the pipette and the surface of the depended portion is increased each time before dispensing into a further deepened portion (e. g. a second, a third, etc. deepened portion) shall take place, it is very easy to visually recognize the first-time a deepened portion remains empty. This visualization can be easily performed by the person handling the device. Furthermore, by use of an optical sensor, such as a camera and an image processing means, it is also possible to detect automatically the first-time a deepened portion remains empty.

The specific optimum dispensing height will be set in the next step of the method. The optimum dispensing height is defined or for example calculated by using a distance that had been set in one of the steps preceding the step in which no dispensing of the adjustment liquid took place anymore. For example, the optimum dispensing height can be defined as the distance of the pipette tip in the second previous step. Additionally or alternatively it is possible to add or to deduct a certain safety margin to the used distance of the pipette tip to define the optimum dispensing height. The optimum dispensing height is preferably the distance of the pipette tip to the receiving element that the pipette tip had in the step immediately preceding the step in which no dispensing of adjustment liquid took place anymore.

Thus, in the method of the invention, as soon as it is detected that - due to the relative large distance - no dispensing has taken place anymore, the process is returned preferably by one step, and the dispensing height detected in this step will be stored and respectively fixed as the optimum dispensing height. The respective device can then be adjusted to this optimum dispensing height.

According to a second alternative method of the invention the pipette tip is in a first step arranged at a first large distance to the receiving element whereby this distance is reduced step by step. As soon as at least one of the receiving elements is filled with adjustment liquid which is detected by visual means, it is possible to define the optimum dispensing height. The optimum height can e.g. be the height of the pipette tip at the step liquid is dispensed. Also in this alternative method, it is possible to consider a certain safety margin to be added or deducted to the used distance of the pipette tip to define the optimum dispensing height.

This method for adjusting the dispensing height of particularly passive pipettes relative to an receiving element, comprises the following steps:

a) causing an adjustment liquid to be received in the at least one pipette,
b) arranging a pipette tip at a first particularly large distance (a) to the receiving element,
c) trying to passive dispense adjustment liquid into a first deepened portion of the receiving element,
d) arranging the pipette tip at a further distance (b), whereby this distance is particularly smaller than the first distance,
e) trying to passive dispense adjustment liquid into a further deepened portion,
f) repeating steps d) and e) until dispensing of the adjustment liquid takes place, whereby this is detected by visual means,
g) optionally repeating step d) at least once, and

h) using a distance that had been set in one of the step following the step in which a first dispensing of the adjustment liquid took place to define a dispensing height or using the distance that had been set in the step in which a first dispensing took place.

The method of the invention will on the one hand guarantee a reliable delivery of the liquid to be dispensed and, on the other hand, it will reduce the danger that larger quantities of liquid could be taken up by adherence to the outer side of the pipette and possibly cause contamination of further testing liquids.

Preferably, the enlarging or reduction of the distance between two successive dispensing steps will be performed always by the same amount. The amount of the enlargement or decrease of the distance is preferably in a range from 0.05 to 0.2 mm and with particular preference is 0.1 mm.

The small first distance is preferably selected to the effect that a dispensing of liquid is definitely guaranteed to occur. Since the approximate size of the droplets is known, the first distance is preferably smaller than the diameter of the droplets. According to a first variant of the method, it can thus be provided, in case that no droplets are dispensed already in the first step, to first reduce the distance by performing a corresponding intermediate step. Preferably, the small first distance is in a range from 0.05 to 0.1 mm.

Alternatively at the beginning of performing the adjustment method the enlarging or reduction of the distance between two successive dispensive steps may be by the same amount and is then followed by enlarging/reduction of the distance getting smaller from one step to the other.

According to a particularly preferred variant of the invention, a plurality of pipettes are filled with adjustment liquid, e.g. by taking up adjustment liquid simultaneously through the plurality of pipettes. Then, as described above, the adjustment liquid will be dispensed simultaneously into a plurali-

ty of deepened portions and respectively a group of deepened portions. In this process, the plurality of pipettes are preferably arranged in a row. It is particularly preferred herein that, for performing the method, use is made of a device in which a plurality of pipettes has been wound onto a coil. The pipettes are thus arranged parallel to each other in the configuration of a band. From this band, there will each time be severed or cut off a predetermined number of pipettes and then be used for filling the deepened portion with adjustment liquid, test liquid etc.

Then, in a first step of the filling process performed according to the first alternative of the method of the invention, a first group of deepened portions, preferably being arranged in a column of the receiving element, will be filled by use of a row of pipettes comprising a plurality of pipettes. In the next step, there will then be filled a second column, with the pipettes now having an enlarged second distance to the deepened portions. The corresponding filling steps are carried out in a column-wise manner, while the whole row of pipettes will each time be displaced by the preferably fixed amount of the distance. The enlarging of the distance is performed, as described above with respect to one pipette, until at least one deepened portion or a predetermined number of deepened portion in a column are not filled with adjustment liquid anymore. Thus, the distance that had been set in one of the steps preceding the step in which no dispensing of the adjustment liquid into at least one deepened portion or a predetermined number of deepened portions took place anymore is used to define the dispensing height.

A plurality of pipettes may likewise be used in the second alternative of the method of the invention, as described above.

As an adjustment liquid, preference is given to a colored liquid so that the adjustment method can be carried out in a simple manner by means of optical systems or through visual inspection. Preferably, use is made of an adjustment liquid having liquid properties similar to those of the test liquid

employed for testing. Particularly, the adjustment liquid has a similar - and preferably the same - viscosity as the test liquid. It is also possible, for adjusting, to use the test liquid itself as the adjustment liquid.

Preferably, the adjustment method is performed each time a new batch of receiving elements, e.g. titration plates, is used. Tests have shown that the manufacturing tolerances occurring within a batch are negligible. Further, it is preferred that the adjustment method of the invention is performed each time when a new band and respectively coil of pipettes is started to be used. Also in this regard, tests have revealed that the tolerances within a batch of pipettes, in particular within a coil of pipettes, are negligible.

According to a particularly preferred variant of the method of the invention, it is provided to carry out a test run after the adjusting process so as to verify whether the executed adjustment has been correct. By way of analyses performed with the aid of mass spectrometers, for instance, it can be detected whether an inacceptable contamination between liquids of different deepened portions has occurred or whether the required amount of liquid has possibly not been achieved.

Since the adjustment method of the invention allows for an adjustment to be achieved with high precision, a correspondingly adjusted device can be used for performing dilution series. Such dilution series, whose end volumes will be about 1 µl, have different dilution ratios of e.g. 1:2, 1:3 and 1:5. The pipettes will thus have to deliver quantities of liquid in the range of merely several nanoliters. Thus, already a possible transfer of even minimum amounts of liquid adhering to the outer side of the pipette would have correspondingly critical consequences.

In another preferred embodiment of the method according to the invention both methods starting at a small and large distance respectfully can be combined. In this combination both methods are performed one after the other so that two dispensing heights will be defined. The dispensing height

being finally defined can for example be the smaller defined dispensing height or the arithmetic average of both defined dispensing heights.

The invention will be explained in greater detail hereunder by way of a preferred embodiment.

In the drawing, the following is shown:

Fig. 1 a schematic diagram in lateral view for explanation of the method, and

Fig. 2 a schematic plan view of a titration plate for performing the adjustment method.

To begin with, the important steps for performing said method will be explained in greater detail hereunder by way of a single pipette 10 with reference to Figure 1. Within the discussed embodiment the pipette tip is located in a small first distance and the distance is increased step by step.

In the initial method step, the pipette 10 will be caused to take up an adjustment liquid 14 from a supply vessel 12.

In the next step, the pipette 10 will be introduced into a deepened portion 16 of a receiving element 18 (Fig. 2) until the pipette tip 20 is arranged at a first small distance *a* from the receiving element and respectively from a bottom 22 of the deepened portion 16. Then, dispensing of liquid takes place in a passive manner since the droplet 24 of the liquid will touch the bottom 22 and thus be pulled off from the pipette.

In the next step, the pipette 10 will be introduced into a further and respectively second deepened portion 26 wherein the pipette tip 20 now has a larger distance b from the bottom 22 of the deepened portion 26. Since,

now, a further droplet 24 will touch the bottom 22, also this droplet will be pulled off from the pipette.

In a further step - in the illustrated embodiment, the third step - of the adjustment method of the invention, the pipette 10 will be arranged in a further deepened portion 28 wherein the pipette tip 20 now has a still larger distance c from the bottom 22 of the deepened portion 28. The distance c is presently so large that the droplet 24 will not touch the bottom 22 anymore and thus will not be dispensed into the deepened portion 28 anymore. This can easily be detected by visual means.

In the illustrated embodiment, it is thus the dispensing height b which will be defined as the optimum dispensing height because this is the largest dispensing height at which a droplet 24 had still been dispensed.

The number of adjustment steps to be performed can also be distinctly higher than three. This will depend on various influential factors, such as e.g. the viscosity of the liquid, the change of the distance in successive adjustment steps, etc. Further, for performing the method of the invention, it is not essential to actually know said distance a. Moreover, it is important that the pipette tip 20 has a sufficiently small distance from bottom 22 to guarantee a reliable dispensing of a droplet. Also the optimum dispensing height detected at the end of the adjustment method does not necessarily have to be known as an absolute value.

The method described with reference to Fig. 1 is preferably carried out not only in a sole row of deepened portions 16,26,28 but, particularly when use is made of a titration plate 18 as a receiving element, in a plurality of rows. Thus, when using the titration plate 18 shown in Fig. 2, the first dispensing step is carried out in 16 deepened portions (column 1). The second step described with reference to Fig. 1 will then again be performed in 16 deepened portions corresponding to column 2, and the third step will be per-

formed in a like manner in column 3. As can be seen in the schematic representation, two deepened portions 28 in column 3 are empty.

Claims

1. A method for adjusting the dispensing height of preferably passive pipettes relative to an receiving element, comprising the following steps:

   a) causing an adjustment liquid (14) to be received in the at least one pipette (10),

   b) arranging a pipette tip (20) at a first distance (a) to the receiving element (18),

   c) passive dispensing of the adjustment liquid (14) into a first deepened portion (16) of the receiving element (18),

   d) arranging the pipette tip (20) at a further distance (b),

   e) passive dispensing of the adjustment liquid (14) into a further deepened portion (26),

   f) repeating steps d) and e) until no dispensing of the adjustment liquid (14) takes place anymore, whereby this is detected by visual means, and

   g) using a distance that had been set in one of the steps preceding the step in which no dispensing of the adjustment liquid (14) took place to define a dispensing height.

2. The method according to claim 1, wherein the distance to the receiving element (18) applied in two successive dispensing steps is each time increased.

3. The method according to claim 2, wherein the distance is increased each time by the same amount.

4. The method according to claim 2 or 3, wherein said amount is in the range from 0.05 mm to 0.2 mm.

5. The method according to any one of claims 1 to 4, wherein said further distance (b) being larger than said first distance (a) and larger than the previous distance.

6. The method according to any one of claims 1 to 5, wherein the dispensing height is calculated on the basis of a distance that had been set in one of the steps preceding the step in which no dispensing of the adjustment liquid took place anymore.

7. The method according to any one of claims 1 to 6, wherein the distance of the step immediately preceding the step in which no dispensing of the adjustment liquid took place anymore is used to define a dispensing height.

8. The method according to any one of claims 1 to 7, wherein said first distance (a) is smaller than the droplet diameter.

9. The method according to any one of claims 1 to 8, wherein a plurality of pipettes (10) simultaneously receive adjustment liquid (14) and simultaneously dispense said liquid into a group of deepened portions (16,26,28).

10. The method according to claim 9, wherein the pipettes (10) are arranged in a row.

11. The method according to claim 9 or 10, wherein groups of deepened portions (16,26,28) are arranged in columns or rows of the receiving element (18).

12. The method according to any one of claims 9 to 11, wherein the dispensing height is defined according to step (g) if no dispensing takes place anymore in at least one deepened portion (28) of a group of deepened portions.

13. The method according to any one of claims 1 to 12, wherein the receiving element is a microtitration plate (18).

14. The method according to any one of claims 1 to 13, wherein the adjustment liquid (14) has liquid properties similar to, particularly identical to, those of a test liquid used for examination.

15. The method according to claim 14, wherein a test liquid is used as the adjustment liquid (14).

16. The method according to any one of claims 1 to 15, wherein, after completion of the adjustment, a test series is performed for examination.

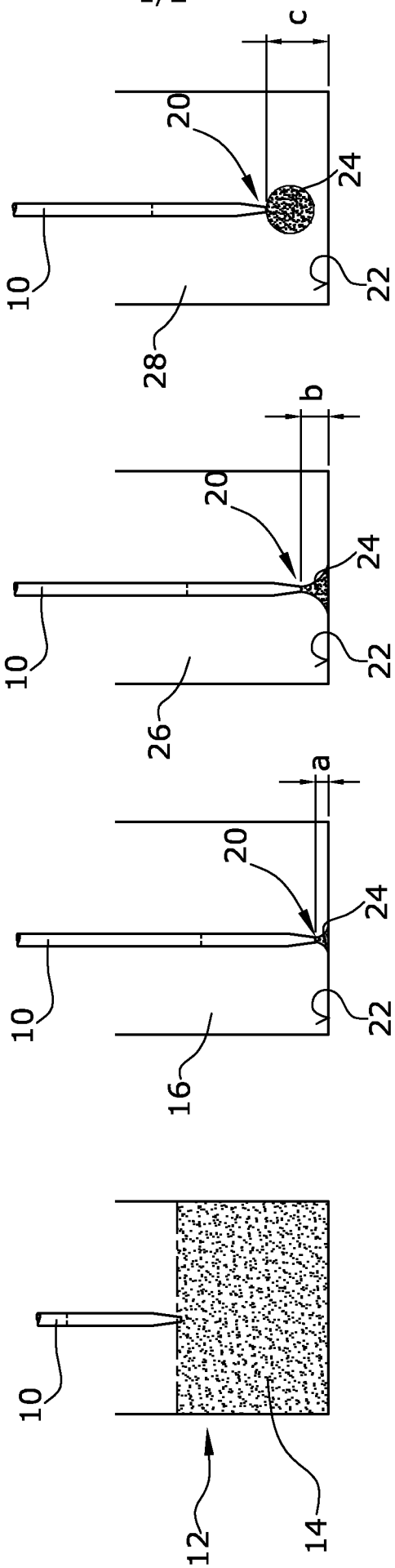

Fig.1

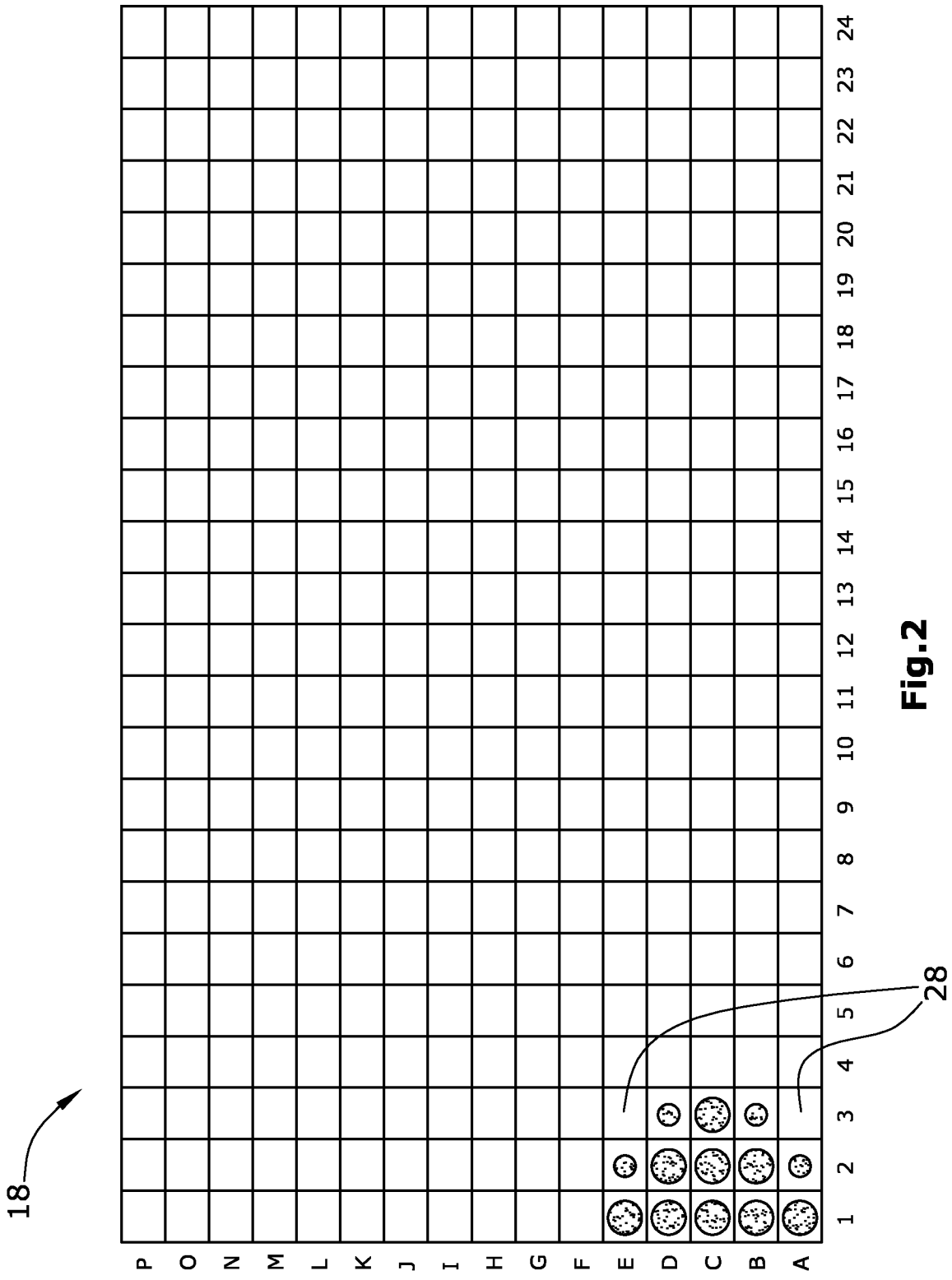
18
28
P O N M L K J I H G F E D C B A
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24

Fig.2

INTERNATIONAL SEARCH REPORT

International application No
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A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N35/10
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 143 849 A (BARRY JAMES V [US] ET AL) 1 September 1992 (1992-09-01) abstract figures 1A-1F column 3, lines 20-40 column 7, line 23 - column 8, line 17	1-16
A	WO 2005/121746 A1 (MARZIALI, ANDREA [CA] ET AL) 22 December 2005 (2005-12-22) abstract figures 8-10 paragraphs [0008] - [0013], [0028] - [0069]	1-16

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 August 2013	02/09/2013

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Seifter, Achim

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2013/064153

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