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(71) Applicant and
(72) Inventor: CAMPBELL, Andrew, T. [NO/NO]; Grindastua, Slogumveien, N-2015 Leirsund (NO).

(74) Agent: OSLO PATENTKONTOR AS; P.O. Box 7007 M, N-0306 Oslo (NO).

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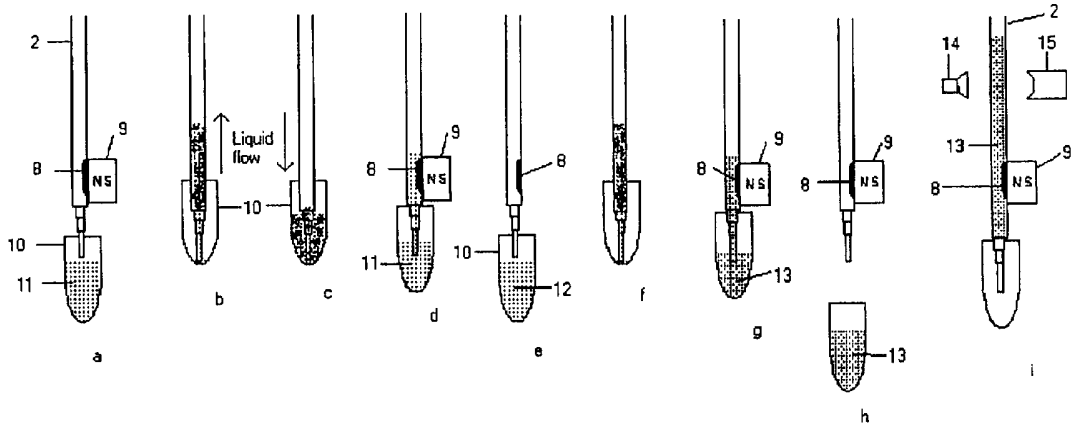


(57) Abstract: A method and device is provided for the efficient mixing, separation, collection and washing of magnetic particles from fluids. The fluid in a suitable container containing the target entities and magnetic particles having an affinity for the said target entities is placed in a device of the present invention in a removable manner and the efficient mixing is effected additionally to a magnetic field collection system. Magnetic particles in small volumes can be washed and concentrated. Procedures for the further manipulation and detection of the target entities bound to the magnetic particles or the residual fluid are described. Depending on the embodiment employed, the device can be applied to a range of volumes from less than 100 µl to in excess of 100 ml.

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# A DEVICE AND METHOD FOR MANIPULATING MAGNETIC PARTICLES

## TECHNICAL FIELD

A method and device is provided for the efficient mixing, separation, collection and washing of magnetic particles from fluids.

## BACKGROUND OF THE INVENTION

A commonly used procedure for the isolation of specific entities in biological systems is immuno-magnetic separation (IMS). This procedure, now common practice in the field, utilizes magnetic or paramagnetic species (hereinafter referred to as magnetic particles) as a solid phase to which target entities are bound. These magnetic particles are formulated or processed by a number of known techniques to have an affinity for the target entity, e.g. by having surface-immobilized ligands or receptors, or via ionic, hydrophobic, hydrophilic or other specific or non-specific interactions to the target entity. These processes and procedures are well documented and described by others and will not be discussed in further detail herein.

In brief, IMS includes a reaction phase to promote complex formation between the magnetic particles and the target entity, generally facilitated by mixing the magnetic particles in a tube or vessel containing the target entity within a test medium. The magnetic particles are subsequently isolated using a magnetic field gradient, for example by the application of a magnet to the side of the tube or vessel to effect localized concentration, magnetizing a magnetizable matrix within the tube or vessel to immobilize the magnetic particles on the magnetizable

matrix or move the magnetic particles to an area distinct from the test medium, thereby effecting isolation. The magnets used include both permanent and electromagnets. During this magnetic isolation step, unwanted material is substantially removed from the target entities, with almost complete removal being effected using sequential wash steps with an aqueous buffer of defined composition. The isolated and purified target entities can then be readily processed further, depending on the application. This has included the subsequent labelling, wash and detection steps described for a range of immunoassays (including enzyme linked, radioactive, fluorescent or luminescent), nucleic acid detection (e.g. PCR), as well as the isolation of cells, viruses, proteins, prions or other targets for further use, manipulation or detection. IMS using negative selection has also been described. In this procedure, the unwanted material is bound to the magnetic particles and removed from the desired target entities when magnetic separation is effected.

The magnetic particles that have been described for such applications include mono-, and poly-disperse or cluster-type particles or aggregates, with both monomodal and polymodal size distribution employing spheres, flakes or other solid phases as the base matrix. The sizes of the magnetic particles have been described from 10 nm to 10 mm, but are more commonly 50 nm to 5 μm.

IMS has many advantages over other separation or isolation procedures, including speed of reaction, efficiency, retention of viability or structure of the target and the theoretical application to automation. The speed of reaction and efficiency of the system is generally considered to be a function of the surface area available for interaction with the target and the advantageous mixing kinetics of the magnetic particles. For this reason, theoretically, smaller (increased surface area available for interaction) monodisperse, non-aggregated particles

with a density approaching unity are to be preferred, although, in practice, this has been demonstrated not to be case. The improved viability or structural retention of the target is considered to be a consequence of the gentler magnet based isolation over other techniques like centrifugation or column based procedures.

Others have described automation of IMS systems. These are generally complex instruments requiring multiple components including pumps, valves, robotic arms, high precision dispensers and complex tubing arrangements. Often the mixing device is separate from the magnetic capture device. They are also commonly described as requiring recirculation facilities to effect complete magnetic particle isolation or specialist high gradient magnetic separation (HGMS) media to reduce the non-specific entrapment of material and to maintain flow (i.e. speed of assay and blocking of matrix). When HGMS media is presented in a column format, it is generally recognized that these HGMS media columns block (i.e. fluid flow through the media ceases) when processing test matrices that contain a high quantity of particulate material, consequently limiting the application of HGMS columns. The automated IMS systems previously described often require the accurate placing of the magnet. The closer the magnet is to the sample, the faster the quantitative isolation of the magnetic particles. This can be technically complex. It is an attractive feature to continually move or agitate the magnetic particles throughout the procedure using agitators, rods, alternating electromagnets or moving magnets that often require highly specialised equipment. The IMS automates described to date are also generally designed to process a fixed, or relatively narrow range of volumes. This is especially the case when rods are used for mixing or the separation is conducted within a pipette tip. These systems have been, in general, designed with a single application in mind, e.g. to retrofit existing high-throughput robotic workstations or for processing a single, fixed volume.

Other important advantages of IMS automates are that they can be readily designed to prevent contamination of the target entity with other samples being co-processed, or from extraneous material and will be safer for the operators when processing pathogenic or infections material (e.g. specific microorganisms).

There therefore exists a requirement for an IMS system that can:

1) Mix, collect and wash using the same principle

2) Continually agitate the matrix

3) Operate for a range of volumes

4) Protect the sample and operator

5) Use relatively standard disposable or reusable materials as required

**SUMMARY OF THE INVENTION**

According to the present invention there is provided a method for the efficient mixing of magnetic particles in a fluid and the subsequent separation of the magnetic particles from the fluid by means of a magnetic field. The device comprises a pipette with a larger diameter flexible or movable walled "bulb" area, that preferably contains a high gradient magnetic separation (HGMS) matrix which can be compressed, pushed or squeezed to effect fluid movement within the system that is connected or integral to said pipette, a magnet, a device for compressing, pushing or squeezing the larger diameter flexible or movable walled bulb area, and a device for moving the magnet toward the pipette and away from it.

**FIGURES**

**FIG. 1a & 1b:** Device for mixing, separating and washing magnetic particles.

**FIG. 2a, 2b & 2c:** HGMS device for mixing, separating and washing magnetic particles.

**FIG. 3:** Device for concentrating and collecting magnetic particles.

**FIG. 4:** Method for labelling and detecting specific entities using the device.

**FIG. 5:** A description of an automated device of the invention.

**DETAILED DESCRIPTION OF THE METHOD**

An objective of the invention is to provide a device and a method for mixing, separating and washing magnetic particles which does not require pumps, valves, mixing rods or separate suction or pressure facilities to manipulate the fluid containing the suspension of magnetic particles.

This objective is accomplished by providing a device for the separation of magnetic particles from a suspension of magnetic particles in a fluid by means of a magnetic field. The device comprises a pipette with a larger diameter flexible-walled bulb area which can be compressed, pushed or squeezed, connected or integral to said pipette, a magnet, and a device for moving the pipette toward and away from said magnet, or a device for moving the magnet toward and away from said pipette to enable the magnetic particles to be deposited or immobilized within the larger diameter flexible-walled bulb area of said pipette.

A preferred embodiment is an apparatus in accordance with the invention comprising a pipette with the larger diameter flexible-walled bulb connected to said pipette as an integral part of the pipette, however pipette/bulb arrangements that are manufactured as separate units, with assembly post-manufacture, are also envisaged in the present invention. Pipettes with a cylindrical cross-section are preferred, with a single opening at the end of the pipette longitudinal extension tip. It is also possible to use pipettes that have areas with rectangular cross-sections. It is essential to the invention that at least one retaining wall of the larger diameter bulb area of the pipette is flexible or movable and the interior of the pipette can be penetrated by a magnetic field, with plastic usually being employed as the material.

In a preferred embodiment, the fluid held in the pipette is substantially contained within the larger volume flexible-

walled bulb area where separation of the magnetic particles is achieved. If possible, the interior of the pipette/bulb arrangement should not have any recesses or edges as this may interfere with the quantitative release of magnetic particles separated in the pipette. Pipettes that have a uniform inner diameter over their longitudinal extension are suitable; it can however be advantageous to have pipettes with tapered ends.

The inner wall of the pipette/bulb arrangement should be smooth. It is also possible to use pipettes whose inner walls are provided with a non-adherent coating, for example, a silane coating.

In a preferred embodiment the pipette/bulb arrangement is produced by standard blow moulding techniques, the whole unit being produced in plastic that can be discarded after use.

Magnetic particles are understood to be particles that are attracted by means of a magnetic field. Hence, the magnetic particles can themselves be magnetized. Preferred materials are paramagnetic materials, i.e., those that exhibit only a minor remanence (i.e., magnetism remaining after the magnetic field has been removed). The material of the particles can be a compound material, e.g. a matrix that contains magnetically attractable particles. Magnetically attractable materials are, for example, iron, iron oxide, nickel, cobalt, or chromium oxide. One or several particles of this material can be embedded in a matrix. The matrix can consist of a multitude of materials, e.g. organic or inorganic polymers.

In order to introduce suspensions and solutions into the pipette/bulb arrangement the larger diameter bulb is first compressed then inserted into the solution containing the magnetic particles. The compression is then controllably released to draw the fluid into the body of the pipette.

In one embodiment of the invention, the magnet is located at an external wall of the larger diameter bulb area of the pipette. This will allow collection of the magnetic particles at the inner wall of the larger diameter bulb area of the pipette. An advantage of this invention is that the magnet will automatically come into direct contact with the pipette bulb when the pipette bulb is compressed as the magnet operates as at least one containment wall of the device that retains the pipette bulb. Therefore in this invention, it is not necessary to have a fine adjustment of the distance between magnet and pipette bulb.

The surface of the magnet located in the vicinity of the pipette bulb should have a cross section that is essentially not smaller than the diameter of the pipette bulb. The magnet surface should be planar for the pipette bulb, however magnet geometry's that match the profile of the pipette bulb can be employed and more than one magnet can be employed.

Magnets for separating the magnetic particles can be electrical magnets as well as permanent magnets. Permanent magnets are preferred as the alloys commonly available today exhibit a higher magnetic force per magnet area and do not generate heat during operation, which could interfere with the system. Strong permanent magnets exhibit a magnetic flow that is sufficient to readily separate most magnetic particles from a suspension with a thickness of up to a few centimetres. The magnets used preferably have the form of a block magnet. It is, however, also possible to use magnets with various other shapes or geometry's.

The magnet can be fixed in its position inside the apparatus or attached to a device for moving it spatially. Further, a device in accordance with the invention comprises a device for moving the magnet toward the pipette. The invention is also intended to encompass an embodiment where the pipette is moved towards the magnet.

A preferred embodiment of the invention uses a fluid permeable magnetizable high gradient magnetic separation (HGMS) matrix located within the larger diameter flexible-walled bulb area of the pipette. This embodiment is particularly suitable for application to the smaller magnetic particles for example, ferrofluids. The magnetizable HGMS matrix can be secured in position within the pipette bulb area or can be discharged within pipette bulb area. The orientation of the HGMS matrix should permit the compression of the pipette bulb.

The magnetizable HGMS matrix can be made of materials such as steel wool, metal-coated fibres, and metal spheres, plates, bars, filings or wires. In a preferred manner the magnetizable HGMS matrix is constructed in such a fashion which creates substantially homogeneous fluid flow during the procedure and can either be a rigid production or malleable in organization. The construction of said magnetizable HGMS matrices are well known to one skilled in the art and will not be covered in more detail herein.

In one embodiment of the invention that employs a magnetizable HGMS matrix, the HGMS matrix is located within the base of the larger diameter flexible-walled bulb area of the pipette. This configuration will ensure that it is imperative that the fluid containing the magnetic particles passes through the magnetizable HGMS matrix. A known disadvantage of column based HGMS systems can occur when processing test media that contain a high level of particulate material, for example, water sample concentrates, food or feed washings or other environmental material and when manipulating matrices containing aggregates, large cell types or viscous polymers. In these cases, the magnetizable HGMS matrix has a propensity to become blocked thus obstructing fluid flow through the HGMS matrix or it can be difficult to recover the magnetic particles from the HGMS matrix. A solution to these disadvantages is an advantage conferred by the present

invention. The fluid flow is readily and controllably repeatedly reversed, i.e. back-flushed, during the operation, thus minimizing these negative effects experienced for the column based HGMS matrix systems.

Another preferred embodiment of this invention locates the magnetizable HGMS matrix in the middle of the larger diameter flexible-walled bulb area of the pipette. A significant advantage of this embodiment is that the fluid suspension containing the magnetic particles is not forced to pass through the HGMS matrix as occurs for column separation systems. This minimizes non-specific entrapment of extraneous material, retains flow, reduces shear forces and facilitates the collection of the magnetic particles at the end of the procedure. To magnetize the HGMS matrix the magnet is introduced at the outer wall of the pipette bulb in such a fashion that the magnet induces a magnetic flux within the HGMS matrix, the magnetic flux thereby magnetizing the HGMS matrix, to which the magnetic particles will be attracted and immobilized.

In a preferred manner, the magnet is attached at a place adjacent to the larger diameter flexible-walled bulb area pipette wall in a configuration that will induce maximum magnetic flux within the HGMS matrix contained within the pipette bulb.

The invention further addresses methods for mixing the magnetic particles to effect affinity capture of the target entity or for labelling a target entity previously bound to the magnetic particles comprising the following steps:

1. a) providing a suspension of magnetic particles in a vessel containing the target entity or labelling reagent within a test medium fluid,

b) transferring a portion of the magnetic particle suspension into a pipette/bulb arrangement, effected

by compressing the pipette bulb then inserting the pipette tip into the test medium fluid and releasing the compression on the pipette bulb,

c) compressing the pipette bulb to expel the fluid from the body of the pipette back into the vessel,

d) releasing the compression on the pipette bulb to draw a portion of the magnetic particle suspension back into the body of the pipette,

e) repeatedly and sequentially compressing and releasing the pipette bulb (points c & d above) to effect efficient mixing.

This method will effect the efficient mixing of magnetic particles in a fluid.

2. a) providing a suspension of magnetic particles in a container,

b) transferring a portion of the magnetic particle suspension into a pipette/bulb arrangement containing a magnetized HGMS matrix, effected by compressing the pipette bulb then inserting the pipette tip into the suspension containing the magnetic particles and releasing the compression on the pipette bulb,

c) compressing the pipette bulb to expel the fluid from the body of the pipette back into the vessel,

d) releasing the compression on the pipette bulb to draw a portion of the magnetic particle suspension back into the pipette bulb containing the magnetized HGMS matrix,

e) repeatedly and sequentially compressing and releasing the pipette bulb (points c & d above) to

effect complete immobilization of the magnetic particles onto the magnetized HGMS matrix contained within the pipette bulb,

f) compressing the pipette bulb to expel the fluid from the body of the pipette back into the vessel,

g) introducing a second vessel containing the target entity or labelling reagent within a test medium fluid,

h) releasing the compression on the pipette bulb to draw a portion of the test medium into the pipette bulb containing the immobilized magnetic particles on the magnetized HGMS matrix,

i) repeatedly and sequentially compressing and releasing the pipette bulb (points g & h above) to effect efficient mixing.

This method will effect the efficient mixing of target entities or labelling reagents in a fluid in the presence of, or through an HGMS matrix containing immobilized magnetic particles.

An advantage of the above methods is that the volume contained within the pipette body does not solely govern the volume that can be mixed. The volume that can be effectively contained in a vessel will be the maximum volume capacity of the system. In a preferred manner, the volume drawn into the pipette bulb would be a minimum of 5% to 10% of the total volume of the system, preferably at least 25% to effect rapid and efficient mixing.

The pipette should not draw air during the cycle. Therefore, the diameter and length of the longitudinal extension of the pipette will govern the minimum volume of the system.

The pipette bulb compression can be readily and accurately controlled for the appropriate volumes (i.e. little compression for small volumes, maximum for large). Devices that can process <100 µl to >1000 ml can therefore be readily designed using this invention. Diluting the sample in an appropriate buffer can process smaller volumes. Larger volumes can be processed with increasing time and by employing larger volume containment vessels and pipettes with bulbs that retain a larger volume of fluid.

The invention further addresses a method for washing magnetic particles comprising the following steps:

a) providing a suspension of magnetic particles in a first fluid,

b) compressing the pipette bulb and releasing the compression to transfer the suspension into the body of the pipette,

c) sequentially compressing and releasing the compression on the pipette bulb with a magnet located at the pipette bulb to effect magnetic capture,

d) removing the first fluid from the body of the pipette,

e) transferring a second fluid into the pipette and removing the magnet,

f) removing the second fluid together with the magnetic particles suspended therein from the pipette into a collecting container.

The washing of magnetic particles in the course of an analysis procedure is necessary to remove non-bound reaction partners.

In a preferred manner, the magnet is attached at a place adjacent to at least one wall of the larger volume flexible-walled bulb area of the pipette.

In a preferred manner, the pipetting procedures are carried out such that the total volume of fluid can be ejected from the pipette, if required. This is done by only using maximum compression on the pipette bulb during the eject cycle.

After the particles have been deposited at the inner wall of the pipette or immobilized on the magnetized HGMS matrix, the fluid from which the particles have been removed is ejected from the pipette. Following this, a second fluid is drawn into the pipette to wash the magnetic particles. This can be done while the magnet is still at the wall of the pipette bulb or, if this is not desired, the magnet can be moved away from the pipette bulb prior thereto. In the first procedure, the separated magnetic particles are washed in the flow entering the pipette bulb; in the second case the particles are at least partly mixed in the flow entering the pipette bulb. The fluid in which the magnetic particles are suspended, and the washing fluid may be water or an inert fluid. In a preferred manner, the fluids contain surface-active agents and/or blocking agents as this increases the washing effect.

In a simple washing procedure, the particles can be ejected directly with the fluid. If more extensive washing procedures are required, the fluid can be ejected from the body of the pipette while the magnet is still at the wall of the pipette bulb, and further washing fluids introduced according to the already described procedure. Alternatively, the particles can be suspended in the washing fluid according to the already described procedure and be again deposited at the wall of, or immobilized on the magnetizable HGMS matrix contained within the pipette bulb.

The efficiency of a washing procedure depends on how homogeneous the magnetic particles are suspended in the washing fluid. Hence it is preferred, particularly when not using an HGMS matrix, to resuspend the magnetic particles in the washing fluid. This can be achieved by removing the magnet from the pipette bulb area before the washing fluid is drawn into the pipette. Moving the fluid repeatedly between the pipette bulb and the wash fluid container can enhance the suspension resulting from swirling up. Magnetic particles that have been deposited on the inner wall of the pipette bulb or immobilized on the HGMS matrix can thus be efficiently resuspended in the washing fluid. With this method it is not necessary to install a mechanical agitator or an ultrasound device to resuspend the particles.

The described methods for resuspension can also be applied to eject the particles together with the fluid from the pipette.

The invention also encompasses a method to concentrate the magnetic particles into a small volume following capture of the magnetic particles in the larger diameter flexible-walled bulb area of the pipette. This comprises a magnet placed at the wall of the pipette towards the tip of the longitudinal end of the pipette, near the opening that the suspension is introduced into the pipette. After the particles have been deposited at the inner wall of the pipette bulb or immobilized on a magnetized HGMS matrix within the pipette bulb, the fluid from which the particles have been removed is ejected from the pipette. Next, a final system or storage fluid is drawn into the pipette with the magnet moved away from the pipette prior thereto, to resuspend the particles, with fluid oscillation to effect complete resuspension of the particles as required. Following this, a magnet is placed at the wall of the pipette towards the tip of the longitudinal end of the pipette before controllably ejecting the fluid from the pipette. In such a manner, the magnetic particles will be

concentrated at the inner wall or on a suitable magnetizable HGMS matrix, as described above, at the tip of the longitudinal extension of the pipette. The fluid can be repeatedly, controllably drawn into and expelled from the pipette to effect complete concentration of the magnetic particles. These concentrated particles can then be collected in a suitable system or storage fluid at the required volume using the procedures described above.

The invention also addresses a method for carrying out an analysis comprising the steps: Providing a suspension of magnetic particles in a first fluid and either incubating the suspension with an analyte prior to transferring the reaction mixture to a pipette/bulb assembly and moving a magnet to the pipette bulb, or first immobilizing the magnetic particles onto a magnetized HGMS matrix prior to the introduction of the analyte into the pipette/bulb assembly. Next, the fluid phase is ejected from the body of the pipette and a system fluid transferred into the pipette/bulb assembly, then moving the pipette away from the magnet, ejecting the system fluid together with the particles in a receiving container, and determining the analyte concentration based on the specific properties of the system fluid or the particles.

Detection methods can include the use of fluorescent, luminescent, enzyme, radio, phosphorescent, spin, dye, nucleic acid, nucleic acid analogue, chelating or heavy metal labels. These assays are known from prior art and their detailed description is therefore omitted here.

The subsequent steps of an analysis procedure occur as described for the washing procedure. However, in the analysis procedure, a system fluid instead of a washing fluid is drawn into the pipette. Said system fluid can either be water or an inert fluid containing detergents, reagents, or auxiliary substances. Once the particles have been ejected from the pipette together with the system

fluid, the analyte bound to the magnetic beads reacts with an active reagent, e.g. an antibody carrying a label (e.g. an enzyme, ruthenium label). In a preferred manner, the bound analyte is detected after it has been washed again with conventional washing solutions. The analyte can be detected via a colour reaction, for example. In a preferred manner, the particles are separated in a measurement cell where a measuring signal (e.g. colouration, fluorescence) is generated. Quantification of the label can also be conducted within the body of the pipette by incorporating suitable optics or other detection devices in the device.

The device shall be described further with the aid of the aforementioned figures.

**FIG. 1a** shows a device of the invention for mixing magnetic particles in a fluid. The top part (1) of the pipette is a cylindrical flexible-walled body with which the longitudinal extension part (2) of the pipette is an integral part. The complete unit is a disposable plastic Pasteur pipette supplied by Labtech International, UK. A compressing device with piston (3) and non-magnetic plate (4) is directly in contact with the top part (1) of the pipette. When the piston (3) is moved towards the non-magnetic plate (4), a fluid (5) containing magnetic particles can be expelled from the pipette through the longitudinal extension (2) into a sample container (6). When piston (3) is moved away from the top part (1) and compression of the top part (1) is released, fluid contained in the sample container (6) will be drawn into the pipette. The piston (3) is mechanically connected to a motor, therefore can be controllably oscillated to effect fluid movement between the pipette (1 & 2) and the sample container (6), thereby effect the efficient mixing of magnetic particles in a fluid. The maximum volume of the sample container (6) is the maximum volume of the system.

**FIG. 1b** further shows a device of the invention for separating and washing the magnetic particles. It is the same Pasteur pipette used described in figure 1a with a top part (1) and an integral longitudinal extension part (2). The compressing device with piston (3) and magnet (4) is directly in contact with the top part (1) of the pipette. Due to the magnetic field, magnetic particles (7) that were suspended in the fluid (5) contained within the top part (1) of the pipette have been deposited at the inner wall of the pipette adjacent to the magnet (4). When the piston (3) is moved towards the magnet (4), the fluid (5) can be expelled from the pipette through the longitudinal extension (2) into a sample container (6) containing the residual fluid (5). When piston (3) is moved away from the top part (1) and compression of the top part (1) is released, fluid contained in the sample container (6) will be drawn into the pipette. Magnetic particles (7) that were suspended in the fluid (5) that enters the top part (1) of the pipette will be deposited at the inner wall of the pipette adjacent to the magnet (4). The piston (3) is mechanically connected to a motor, therefore can be controllably oscillated to effect fluid movement between the pipette (1 & 2) and the sample container (6), thereby effect the efficient collection of the magnetic particles (7) from the fluid (5).

After a suitable number of oscillations sufficient to collect the magnetic particles (7) at the inner wall of the pipette adjacent to the magnet (4), the fluid (5) can be expelled from the pipette by moving piston (3) towards magnet (4). To wash the magnetic particles the sample container (6) containing the fluid (5) is removed from the device and replaced with a wash container (8) containing a wash solution (9). The magnet (4) is removed and the compression on the top part (1) of the pipette is released to draw the wash solution into the pipette and resuspend the magnetic particles (7) in the wash solution (9).

Depending on application, the magnet (4) can be immediately replaced and the magnetic particles (7) immobilized to the inner wall of the top part (1) adjacent to the magnet (4) according to the procedure described above. Alternatively, if more extensive washing is required, the procedure for mixing described in figure 1a should be followed, prior to magnetic capture as described above.

After a suitable number of wash cycles the magnetic particles (7) are immobilized at the inner wall of the pipette adjacent to the magnet (4) as previously described. The wash solution (9) is expelled from the pipette by moving piston (3) towards magnet (4). To collect the washed particles, the wash container (8) containing the wash solution (9) is removed from the device and replaced with a wash container (8) containing fresh wash solution (9). The magnet (4) is removed and a non-magnetic plate inserted (not shown) and the compression on the top part (1) of the pipette is released to draw the wash solution (9) into the pipette and resuspend the magnetic particles (7) in the fresh wash solution (9). The piston (3) is moved towards the non-magnetic plate, and the wash solution (9) containing magnetic particles can be expelled from the pipette through the longitudinal extension (2) into a suitable collection container (not shown).

**FIGS. 2a & 2b** show devices of the invention for separating and washing the magnetic particles using a magnetizable HGMS matrix. The HGMS matrix in figure 2b is malleable in organization. Both figures utilize a similar Pasteur pipette used described in figure 1a with a top part (1) and an integral longitudinal extension part (2), but includes a magnetizable HGMS matrix (7) within the top part (1) of the pipette. The compressing device with piston (3) and magnet (4) is directly in contact with the top part (1) of the pipette. The magnet is placed such that it will magnetize the magnetizable HGMS matrix (7). Due to the magnetic field induced within the magnetizable HGMS matrix (7) by the

magnet (4), magnetic particles that were suspended in the fluid (5) contained within the magnetized HGMS matrix (7) have been deposited on the magnetized HGMS matrix (7). When the piston (3) is moved towards the magnet (4), the fluid (5) is expelled from the top part (1) of the pipette, passing through the magnetized HGMS matrix (7) then via the longitudinal extension (2) into a sample container (6) containing the residual fluid (5). Magnetic particles that pass through the magnetized HGMS matrix (7) are deposited on the magnetized HGMS matrix (7). When piston (3) is moved away from the magnet (4) and compression of the top part (1) is released, fluid contained in the sample container (6) is drawn into the pipette. Magnetic particles that are suspended in the fluid (5) that enters the magnetized HGMS matrix (7) are deposited on the HGMS matrix (7). The piston (3) is mechanically connected to a motor, therefore can be controllably oscillated to effect fluid movement between the top part (1) of the pipette and the sample container (6), thereby repeatedly passage the fluid (5) containing magnetic particles through the magnetized HGMS matrix (7) and effect the efficient collection of the magnetic particles onto the magnetized HGMS matrix (7) from the fluid (5).

After a suitable number of oscillations sufficient to collect the magnetic particles on the magnetized HGMS matrix (7), the fluid (5) is expelled from the pipette by moving piston (3) towards magnet (4). To wash the magnetic particles the sample container (6) containing the fluid (5) is removed from the device and replaced with a wash container (8) containing a wash solution (9). The magnet (4) is retained in position and the compression on the top part (1) of the pipette is released to draw the wash solution (9) into the pipette and through the magnetized HGMS matrix (7) on which the magnetic particles are immobilized. The wash solution (9) can be repeatedly passed through the magnetized HGMS matrix (7) onto which the magnetic particles are immobilized by controllably moving

the piston (3) towards and away from the magnet (4) to effect the sequential compression on the top part (1) of the pipette, thus inducing the wash solution to flow through the magnetized HGMS matrix (7).

After a suitable number of wash cycles the wash solution (9) is expelled from the pipette by moving piston (3) towards magnet (4) and the wash container (8) containing the wash solution (9) is removed from the device and replaced with a wash container (8) containing fresh wash solution (9). The magnetic particles immobilized on the magnetized HGMS matrix (7) are collected from the HGMS matrix (7) by releasing the compression on the top part (1) of the pipette to draw the fresh wash solution (9) into the top part (1) of the pipette. The magnet (4) is removed and replaced with a non-magnetic plate (not shown) before moving the piston (3) towards the non-magnetic plate (not shown). The wash solution (9) entering the magnetizable HGMS matrix (7) from the top part (1) of the pipette resuspends the magnetic particles that were immobilized on the magnetizable HGMS matrix (7). The wash solution (9) containing magnetic particles can be expelled from the pipette through the longitudinal extension (2) into a suitable collection container (not shown). If required, partial piston compression and release (flow agitation) can be effected to facilitate the complete resuspension of the magnetic particles from the magnetizable HGMS matrix (7) before expelling the wash solution (9) from the pipette.

**FIG. 2c** shows a device of the invention for separating and washing the magnetic particles from fluids that have a high particulate content using a magnetizable high gradient magnetic separator. It is a similar pasteur pipette used described in figure 2a with a top part (1) and an integral longitudinal extension part (2) and a magnetizable HGMS matrix (7) contained within the top part (1) of the pipette. The compressing device with piston (3) and a non-magnetic plate (4) is directly in contact with the top part

(1) of the pipette. The magnet (8) is placed such that it will magnetize the magnetizable HGMS matrix (7). Due to the magnetic field induced within the magnetizable HGMS matrix (7) by the magnet (8), magnetic particles that were suspended in the fluid (5) contained within or adjacent to the magnetized HGMS matrix (7) have been deposited on the magnetized HGMS matrix (7). When the piston (3) is moved towards the non-magnetic plate (4), the fluid (5) is expelled from the top part (1) of the pipette via the longitudinal extension (2) into a sample container (6) containing the residual fluid (5). A proportion of the fluid (5) passes through the magnetized HGMS matrix (7), magnetic particles that pass through the magnetized HGMS matrix (7) are deposited on the magnetized HGMS matrix (7). When piston (3) is moved away from the non-magnetic plate (4) and compression of the top part (1) is released, fluid (5) contained in the sample container (6) is drawn into the pipette. Magnetic particles that are suspended in the fluid (5) that enters the magnetized HGMS matrix (7) are deposited on the HGMS matrix (7). The piston (3) is mechanically connected to a motor, therefore can be controllably oscillated to effect fluid movement between the top part (1) of the pipette and the sample container (6), thereby repeatedly passage the fluid (5) containing magnetic particles through the magnetized HGMS matrix (7) and effect the efficient collection of the magnetic particles onto the magnetized HGMS matrix (7) from the fluid (5). Larger particulate material will not enter the magnetizable HGMS matrix (7) and therefore does not have the potential to block the magnetized HGMS matrix (7).

After a suitable number of oscillations sufficient to collect all the magnetic particles on the magnetized HGMS matrix (7), the fluid (5) is expelled from the pipette by moving piston (3) towards the non-magnetic plate (4). To wash the magnetic particles the sample container (6) containing the fluid (5) is removed from the device and replaced with a wash container (9) containing a wash

solution (10). The magnet (8) is retained in position and the compression on the top part (1) of the pipette is released to draw the wash solution (10) into the pipette and into the magnetized HGMS matrix (7) on which the magnetic particles are immobilized. The wash solution (10) can be repeatedly passed through the magnetized HGMS matrix (7) onto which the magnetic particles are immobilized by controllably moving the piston (3) towards and away from the non-magnetic plate (4) to effect the sequential compression on the top part (1) of the pipette, thus inducing the wash solution (10) to flow through the magnetized HGMS matrix (7).

After a suitable number of wash cycles the wash solution (10) is expelled from the pipette by moving piston (3) towards the non-magnetic plate (4) and the wash container (9) containing the wash solution (10) is removed from the device and replaced with a wash container (9) containing fresh wash solution (10). The magnetic particles immobilized on the magnetized HGMS matrix (7) are collected from the HGMS matrix (7) by releasing the compression on the top part (1) of the pipette to draw the fresh wash solution (10) into the top part (1) of the pipette. The magnet (8) is removed from the device before moving the piston (3) towards the non-magnetic plate (4). The wash solution (10) entering the magnetizable HGMS matrix (7) from the top part (1) of the pipette resuspends the magnetic particles that were immobilized on the magnetizable HGMS matrix (7). The wash solution (10) containing magnetic particles can be expelled from the pipette through the longitudinal extension (2) into a suitable collection container (not shown). If required, partial piston compression and release (flow agitation) can be effected to facilitate the complete resuspension of the magnetic particles from the magnetizable HGMS matrix (7) before expelling the wash solution (10) from the pipette.

In figures 1 & 1b and figures 2a, 2b & 2c, a restricted volume of fluid can be processed by not completely releasing the compression on the top part (1) of the pipette.

**FIG. 3** shows a device of the invention for collecting and concentrating magnetic particles in a fluid after processing and separating magnetic particles with one of the devices described in figures 1 & 2. A compressing device with piston (3) and non-magnetic plate (4) is directly in contact with the top part (1) of the pipette. When the piston (3) is moved towards the non-magnetic plate (4), a fluid (5) containing magnetic particles can be expelled from the top part (1) of the pipette through the longitudinal extension (2) into a fluid container (6) containing the residual fluid (7). Due to the magnetic field, magnetic particles (8) that were suspended in the fluid (5) contained within the top part (1) of the pipette have been deposited at the inner wall of, or on a HGMS matrix within the longitudinal extension (2) of the pipette adjacent to the magnet (9). If required, the tip at the end of the longitudinal extension (2) of the pipette can be inserted into the residual fluid (7) contained within the fluid container (6) and partial piston compression and release can be effected to repeatedly pass the fluid (5) containing the magnetic particles by the magnet (9) to facilitate the complete collection of the magnetic particles. The fluid is expelled from the pipette by moving piston (3) towards the non-magnetic plate (4) and the fluid container (6) containing the residual fluid (7) is removed from the device and a collection or reaction container (not shown) containing a system or storage fluid is inserted into the device. The magnet (9) is removed from the wall of the longitudinal extension (2) of the pipette, before partially releasing the piston (3) compression on the top part (1) of the pipette to draw the system or storage fluid (7) into the longitudinal extension (2) of the pipette sufficient to resuspend the magnetic particles from the

wall or HGMS matrix of the longitudinal extension (2) of the pipette. The storage or system fluid can then be expelled from the pipette into the collection container by moving piston (3) towards the non-magnetic plate (4).

**FIG. 4** shows a possible method of processing the magnetic particles for the detection of a specific target entity bound to the magnetic particles using the device of the invention described in figure 3.

Referring in part to figure 3:

**FIG 4a:** fluid is expelled from the pipette by moving piston (3) towards the non-magnetic plate (4) and the fluid container (6) containing the residual fluid (7) is removed from the device and a fluid container (10) containing a system fluid (11), is inserted into the device.

**FIG 4b & 4c:** magnet (9) is removed from the wall of the longitudinal extension (2) of the pipette, before partially releasing the piston (3) compression on the top part (1) of the pipette to draw the system fluid (11) into the longitudinal extension (2) of the pipette sufficient to resuspend the magnetic particles (8) from the wall of the longitudinal extension (2) of the pipette or from an HGMS matrix (not shown). The piston (3) is controllably moved towards the non-magnetic plate (4) to expel the system fluid containing the magnetic particles into the fluid container. Mixing is effected by controllably repeatedly and sequentially partially moving the piston (3) away from and towards the non-magnetic plate (4).

**FIG. 4d:** after sufficient incubation time for specific labelling to occur, the magnet (9) is placed at the wall of the longitudinal extension (2) of the pipette to collect the processed magnetic particles. Complete collection of the magnetic particles (8) is effected by controllably and sequentially partially moving the piston (3) away from and

towards the non-magnetic plate (4) so that the system fluid (11) containing the magnetic particles repeatedly flows past the wall or HGMS matrix adjacent to the magnet (9). The system fluid (11) is expelled from the pipette by moving piston (3) towards the non-magnetic plate (4) and the fluid container (10) containing the residual system fluid (11) is removed from the device. The magnetic particles can be washed, as many times as required, by following the procedure detailed in FIG. 4a to d inc., substituting a wash solution for the system fluid.

If the system fluid used was a direct label, for example, a fluorescent-, luminescent- or radio-label, the magnetic particles can be processed directly as will be described for figures 4h and 4i

**FIG. 4e & 4f:** a detection fluid container (10) containing a detection fluid (12), for example, containing an enzyme substrate or similar, is inserted into the device. The magnet (9) is removed from the wall of the longitudinal extension (2) of the pipette, before partially releasing the piston (3) compression on the top part (1) of the pipette to draw the detection fluid (12) into the longitudinal extension (2) of the pipette sufficient to resuspend the magnetic particles (8) from the wall of the longitudinal extension (2) of the pipette, or via an HGMS matrix. Mixing, if required, is effected by controllably repeatedly and sequentially partially moving the piston (3) away from and towards the non-magnetic plate (4).

**FIG. 4g.** After sufficient incubation time the detection reaction to occur, the magnet (9) is placed at the wall of the longitudinal extension (2) of the pipette to collect the processed magnetic particles. Complete collection of the magnetic particles (8) is effected by controllably and sequentially partially moving the piston (3) away from and towards the non-magnetic plate (4) so that the reacted detection fluid (13) containing the magnetic particles

repeatedly flows past the wall or HGMS matrix adjacent to the magnet (9) and are collected in the pipette.

**FIG. 4h:** If the detection instrumentation is external to the device of the invention the reacted detection fluid (13) is expelled from the pipette by moving piston (3) towards the non-magnetic plate (4) and the reaction container (10) containing the reacted detection fluid (13) is removed from the device for further processing.

**FIG. 4i:** If the detection instrumentation is integral to the device of the invention the reacted detection fluid (13) is drawn further up the longitudinal extension (2) of the pipette, by controllably moving the piston (3) away from non-magnetic plate (4) until the reacted detection fluid (13) is at an area corresponding to an energy source (14) for example a light source, if required and a suitable detector (15).

**FIG. 5** shows an automated device of the invention. This figure displays the side elevation of an automated device that can process a plurality of samples (not shown) using multiple pipette/bulb arrangements (only one of which is shown) using the processes described in detail for figures 1, 2, 3 and 4. The top part (1) of the pipette is a cylindrical flexible-walled body with which the longitudinal extension part (2) of the pipette is an integral part. It is a similar plastic Pasteur type pipette described in the preferred embodiments detailed in FIGS. 1, 2 and 3. A compressing device with piston (3) and a series of magnets (4) that are housed in a magnet holder (5) are directly in contact with the top part (1) of the pipette. The piston (3) is connected to a motorized controller (6) to effect the required movement of the piston (3). When piston (3) is moved towards the magnets (4) housed in the magnet holder (5) the top part (1) of the pipette is compressed effecting fluid movement and magnetic capture of magnetic particles as previously described in detail for

figures 1, 2 and 3. The piston (3) has been designed to accommodate a removable non-magnetic plate (7) or a second magnet holder (as in 5) if a higher magnetic field is required in a given application. The pipette/bulb arrangement (1 & 2) is held in position in the device using a fixed housing (8). In this embodiment the magnet (4) can be displaced laterally relative to the top part (1) of the pipette by moving the magnet holder (5), which is connected, to a magnet holder motorized controller (not shown). When the magnet holder (5) is displaced laterally from the top part (1) of the pipette, it remains in contact with the top part (1) of the pipette and a non-magnetic face of the magnet holder (5) contacts the top part (1) of the pipette. When the magnet holder (5) is in the non-magnetic position and the piston (3) moves towards magnet holder (5) the top part (1) of the pipette is compressed, effecting fluid movement within the body of the pipette, without magnetic separation occurring (i.e. during mixing or particle concentration). An additional magnet holder (9) is shown in the device for particle concentration (see figure 3 for details), or for processing the captured magnetic particles in conjunction with detection procedures (see figure 4 for details). This magnet holder is a duplicate of magnet holder (5) and therefore contains both magnet positions and non-magnetic positions. The magnet holder is connected to the magnet holder motorized controller (not shown) and can be moved in the same plane as magnet holder (5) i.e. displaced laterally from the pipette longitudinal extension (2) to present a magnet or non-magnetic plate as required (refer to figure 3 and 4 for details). Furthermore, the device has a mobile platform (10) for retaining and moving the fluid containers (not shown) used in the assays (see figures 1, 2, 3 and 4 for details). This mobile platform (10) is connected to a motorized controller (not shown) to effect the vertical movement of the fluid containers with the result that the opening at the tip at the end of the longitudinal extension (2) of the pipette is inserted into the fluid of choice

retained within the fluid containers as and when required. An apparatus (not shown) is located on the mobile platform (10), which holds a plurality of fluid containers and can move the fluid containers to a position below or away from the longitudinal extension (2) of the pipette in preparation for the mobile platform to present the fluid container to the longitudinal extension (2) of the pipette. The various piston (3), magnet holder (5), mobile platform (10) and fluid container apparatus motorized controllers are linked using standard electronic components (not shown) that can effect the coordinated movement of the components automatically without user intervention.

Furthermore, the method according to the present invention can be applied to various types of apparatus, and in this case a mechanism required for controlling magnetic particles in a reaction can be substantially improved.

Although the invention has been described with respect to a series of specific embodiments for a complete and clear disclosure, the appended claims are not to be thus limited but are to be interpreted as embodying all modifications and alternative constructions that may occur to one skilled in the art which fairly fall within the basic teaching herein set forth.

**EXAMPLES**

The following experimental examples describe the invention in greater detail. However, the examples provided should not be considered as limiting the invention; it should be understood that they are provided by way of example only.

Three replicate matched water samples containing *Cryptosporidium parvum* oocysts (bovine source) were prepared. A 30 ml aliquot of the water sample was placed

into a 50 ml centrifuge tube and approximately 15000 *C. parvum* oocysts, were added and mixed thoroughly to create a homogeneous suspension. This 30 ml water sample was split into 3 equal 10 ml aliquots to generate the 3 matched water samples each containing approx. 5000 oocycts. Magnetic particles (Dynal Biotech, Norway) with an affinity for *C. parvum* oocysts and a blocking buffer system were added to each aliquot. Two of the aliquots were processed separately, as described below using 3 ml plastic pasteur pipettes (Labtech International, UK). The remaining aliquot was processed according to a standard validated IMS method (described in US EPA Method 1622) and served as a performance control.

Pipette trials

Mixing of the magnetic particles in the samples was effected by inserting the tip of the Pasteur pipette into the sample and manually compressing, then releasing the pipette bulb for 30 min, with one compression-release cycle every 3 sec. Following this, a 10 x 10 x 6 mm N38H grade block magnet (Magnet Sales & Service, UK) was placed at the base area of the pipette bulb and the magnetic particles were captured by repeating the compression-release cycles for 2 min (approx. 40 cycles). With the magnet still in place, all fluid was ejected from the pipette using a single complete compression. The magnet was removed and 1 ml of wash buffer was drawn into the pipette to resuspend the magnetic particles from the inner wall of the pipette bulb. The magnet was placed towards the end of the longitudinal extension of the pipette, approximately 20 mm from the opening at the end of the longitudinal extension of the pipette and the 1 ml wash fluid was slowly (taking approximately 10 seconds) ejected from the body of the pipette. The magnetic particles were concentrated at the inner wall of the longitudinal extension of the pipette

adjacent to the magnet. A 50 µl aliquot of 0.1 M HCl was prepared in a microtube (Eppendorf). The magnet was removed from the pipette and the 50 µl HCl solution was drawn into the pipette to resuspend the magnetic particles, before ejecting the acid solution containing the magnetic particles back into the microtube. Oocysts were dissociated from the magnetic particles and enumerated by microscopy using standard methods as described elsewhere (US EPA Method 1622).

The results of this experiment are summarized in table 1 below.

Table 1. Results of trial 1 comparing the pipette separation technique with US EPA Method 1622 technique.

| Percentage recovery | | |
|---|---|---|
| US EPA Method 1622 | Pipette (Replicate 1) | Pipette (Replicate 2) |
| 81.2 | 86.7 | 83.9 |

A second experimental trial was conducted as above, in which four replicate matched water samples containing *C. parvum* oocysts were prepared. A 40 ml aliquot of the water sample was placed into a 50 ml centrifuge tube and approximately 200 *C. parvum* oocysts, were added and mixed thoroughly to create a homogeneous suspension. This 40 ml water sample was split into four equal 10 ml aliquots to generate the four matched water samples each containing approximately 50 oocysts. Magnetic particles (Dynal Biotech, Norway) with an affinity for *C. parvum* oocysts and a blocking buffer system were added to each aliquot. Three of the aliquots were processed separately, as described above using 3 ml plastic pasteur pipettes. For this trial, magnetic capture was performed for 3 min (approx. 60 cycles) The remaining aliquot was processed according to a

standard validated IMS method (described in US EPA Method 1622) and served as a performance control.

The results of this experiment are summarized in table 2 below.

Table 2. Results of trial 2 comparing the pipette separation technique with US EPA Method 1622 technique.

| Percentage recovery | | | |
|---|---|---|---|
| US EPA Method 1622 | Pipette (Replicate 1) | Pipette (Replicate 2) | Pipette (Replicate 3) |
| 44.2 | 96.7 | 60.8 | 121.5 |

The above data in Tables 1 and 2 clearly indicate the sensitivity and performance competence of the pipette system.

**PATENT CLAIMS**

1. A device for mixing, separating, collecting and washing magnetic particles from a suspension in a fluid; the said device comprises:

   a) a pipette having a larger volume containment area of which at least one retaining wall is flexible or movable, in addition to a longitudinal extension with an opening;

   b) a movable piston that can compress said flexible or movable walled larger volume area to effect fluid movement within the body of the pipette and cyclically between the pipette and a suitable fluid containment vessel, said fluid containing a suspension of magnetic particles;

   c) a magnet exterior to the pipette and positioned to apply a magnetic field to at least part of the fluid contents such that the magnetic particles are deposited or immobilized at an area interior to the flexible or movable walled larger volume area of said pipette;

   d) a secondary magnet facility to concentrate previously deposited or immobilized magnetic particles at an area interior to the longitudinal end of said pipette;

   e) a mechanism for effecting relative movement of the pipette and the magnets, such that at least one of them moves toward the other.

2. A device for mixing, separating, collecting and washing magnetic particles from a suspension in a

fluid; the said device comprises:

a) a pipette having a larger volume containment area of which at least one retaining wall is flexible or movable, in addition to a longitudinal extension with an opening;

b) a high gradient magnetic separation (HGMS) matrix retained within the larger volume separation containment area;

c) a movable piston that can compress said flexible or movable walled larger volume area to effect fluid movement within the body of the pipette, through the HGMS matrix and cyclically between the pipette and a suitable fluid containment vessel, said fluid containing a suspension of magnetic particles;

d) a magnet exterior to the pipette and positioned to apply a magnetic field such that the HGMS matrix becomes saturated with the magnetic field so that the magnetic particles are deposited or immobilized within or on the HGMS matrix contained within said pipette;

e) a secondary magnet facility to concentrate previously deposited or immobilized magnetic particles at an area interior to the longitudinal end of said pipette;

f) a mechanism for effecting relative movement of the pipette and the magnets, such that at least one of them moves toward the other.

3. A device according to claim 2, characterised by that the HGMS matrix is fashioned in such a manner that all the fluid must pass through the HGMS matrix.

4. A device according to claim 2 characterised by that the HGMS matrix is fashioned in such a manner that a proportion of the fluid must pass through the HGMS matrix.

5. A device according to claim 2, characterised by that the HGMS matrix is rigid in construction.

6. A device according to claim 2, characterised by that the HGMS matrix is malleable in formation.

7. A device according to claim 2, characterised by that the magnetic particles are immobilised onto the HGMS matrix prior to the introduction of the fluid containing the target entities.

8. A device according to claim 2, characterised by that the magnetic particles are immobilized on the HGMS matrix throughout the washing and subsequent labelling procedures.

9. A device according to claim 1, characterised by that the longitudinal extension of the pipette contains a magnetizable HGMS matrix.

10. A device according to claim 2, characterised by that the longitudinal extension of the pipette contains a magnetizable HGMS matrix.

# AMENDED CLAIMS

[Received by the International Bureau on 04 January 2004 (04.01.04): original claims 1-10 replaced by amended claims 1-10, original claims 1, 9 cancelled, new claims 4, 6 added (2 pages)]

## PATENT CLAIMS (Ammended 29 December 2003)

1. A device for mixing, separating, collecting and washing magnetic particles from a suspension in a fluid; the said device comprises:

a) a pipette having a larger volume containment area of which at least one retaining wall is flexible or movable, in addition to a longitudinal extension with an opening;

b) a high gradient magnetic separation (HGMS) matrix contained within said pipette;

c) a movable piston that can compress said flexible or movable walled larger volume area to effect fluid movement within the body of the pipette, through or adjacent to the HGMS matrix and thereafter cyclically between the pipette, the HGMS matrix and a suitable fluid containment vessel, said fluid containing a suspension of magnetic particles;

d) a magnet exterior to the pipette and positioned to apply a magnetic field such that the HGMS matrix becomes saturated with the magnetic field so that the magnetic particles are deposited or immobilized within or on the HGMS matrix contained within said pipette;

e) a secondary magnet facility to concentrate previously deposited or immobilized magnetic particles at an area interior to the longitudinal end of said pipette;

f) a mechanism for effecting relative movement of the pipette and the magnets, such that at least one of them moves toward the other.

2. A device according to claim 1, characterised by that the HGMS matrix is fashioned in such a manner that all the fluid must pass through or adjacent to the HGMS matrix.

3. A device according to claim 1, characterised by that the HGMS matrix is fashioned in such a manner that only a proportion of the fluid must pass through or adjacent the HGMS matrix.

4. A device according to claim 1, characterised by that the HGMS matrix is retained within the larger volume containment area of said pipette.

5. A device according to claim 1, characterised by that the HGMS matrix is retained within the longitudinal extension of said pipette.

6. A device according to claim 1, characterised by that the HGMS matrix is not retained within the body of said pipette, but freely dispersed within the fluid phase of said device until the magnet is introduced.

7. A device according to claim 1, characterised by that the HGMS matrix is rigid in construction.

8. A device according to claim 1, characterised by that the HGMS matrix is malleable in formation.

9. A device according to claim 1, characterised by that the magnetic particles are immobilised onto the HGMS matrix prior to the introduction of the fluid containing the target entities.

10. A device according to claim 1, characterised by that the magnetic particles are immobilized on the HGMS matrix throughout the washing and subsequent labelling procedures.

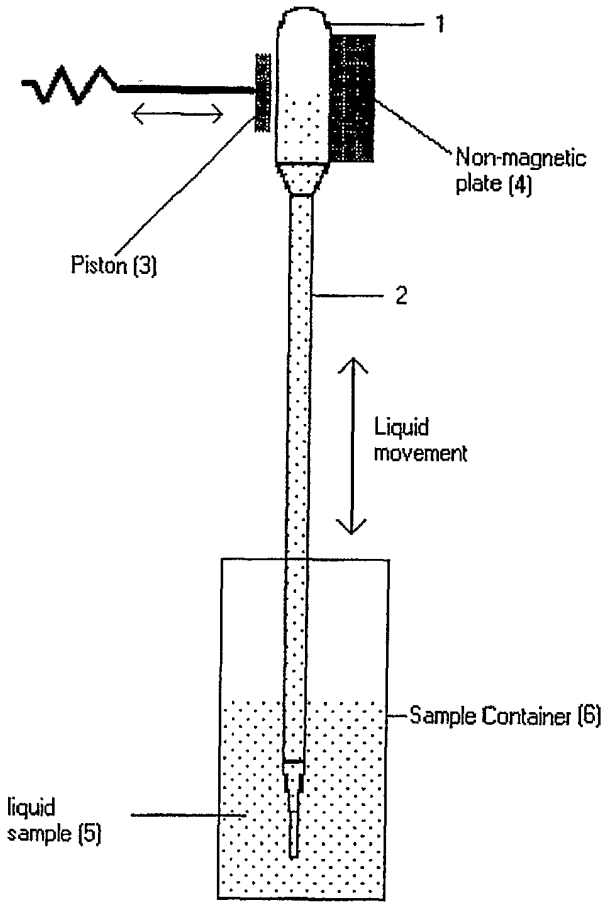
1
Non-magnetic plate (4)
Piston (3)
2
Liquid movement
Sample Container (6)
liquid sample (5)

FIG. 1a

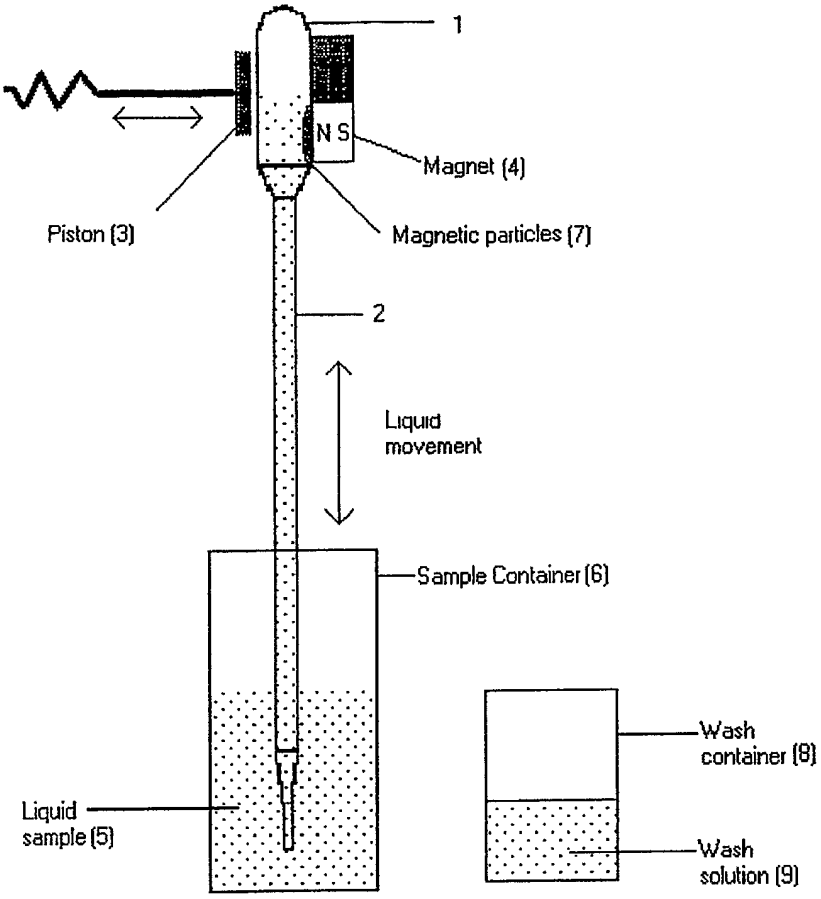
1
N S
Magnet (4)
Piston (3)
Magnetic particles (7)
2
Liquid
movement
Sample Container (6)
Wash
container (8)
Liquid
sample (5)
Wash
solution (9)

FIG. 1b

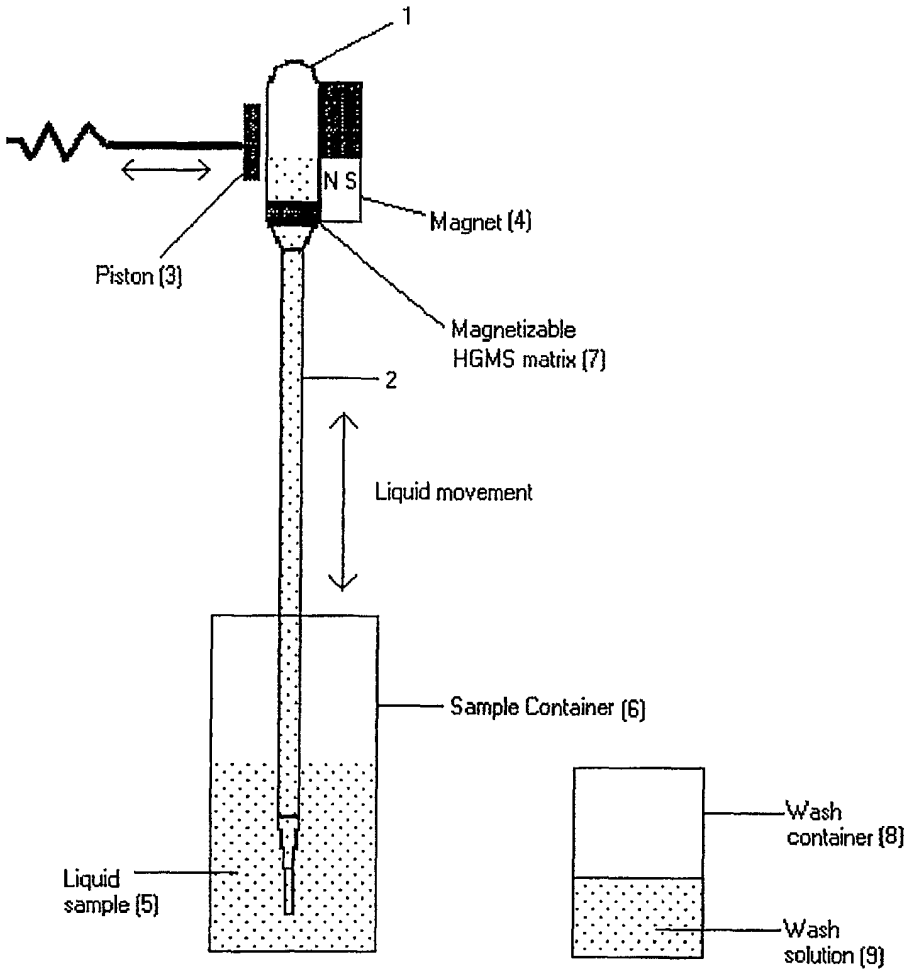
1
N S
Magnet (4)
Piston (3)
Magnetizable
HGMS matrix (7)
2
Liquid movement
Sample Container (6)
Wash
container (8)
Liquid
sample (5)
Wash
solution (9)

FIG. 2a

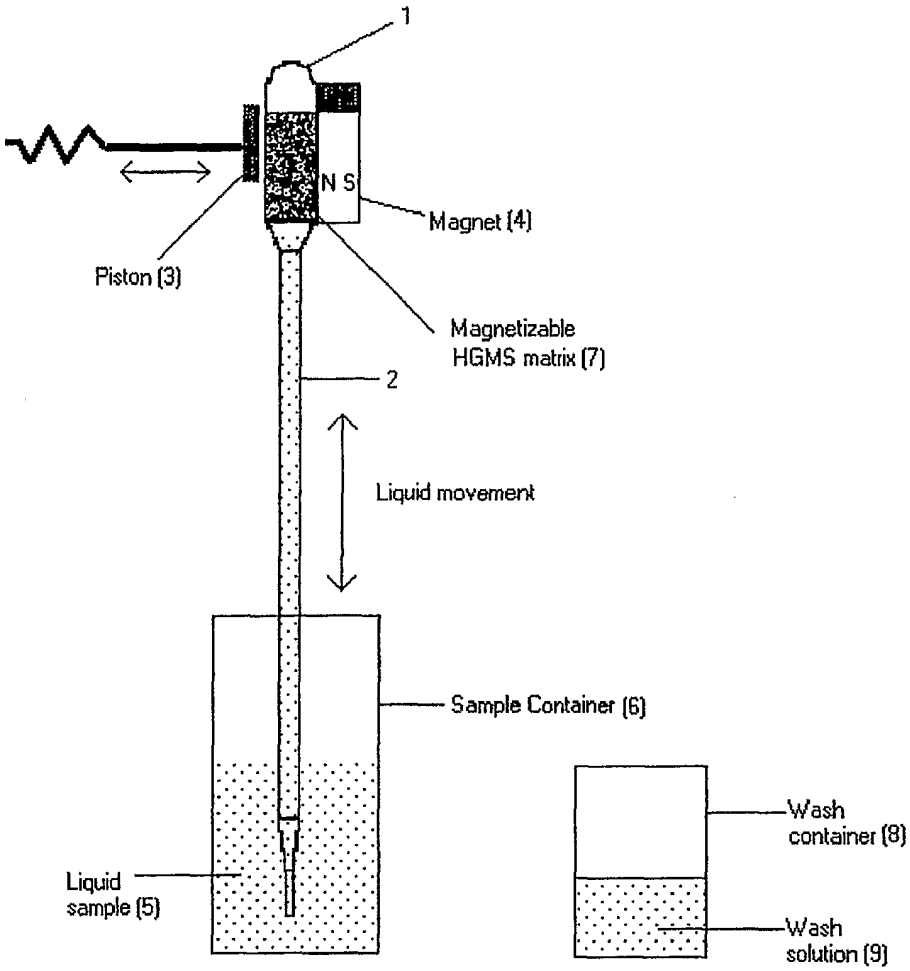
1
N S
Magnet (4)
Piston (3)
Magnetizable
HGMS matrix (7)
2
Liquid movement
Sample Container (6)
Wash
container (8)
Liquid
sample (5)
Wash
solution (9)

FIG. 2b

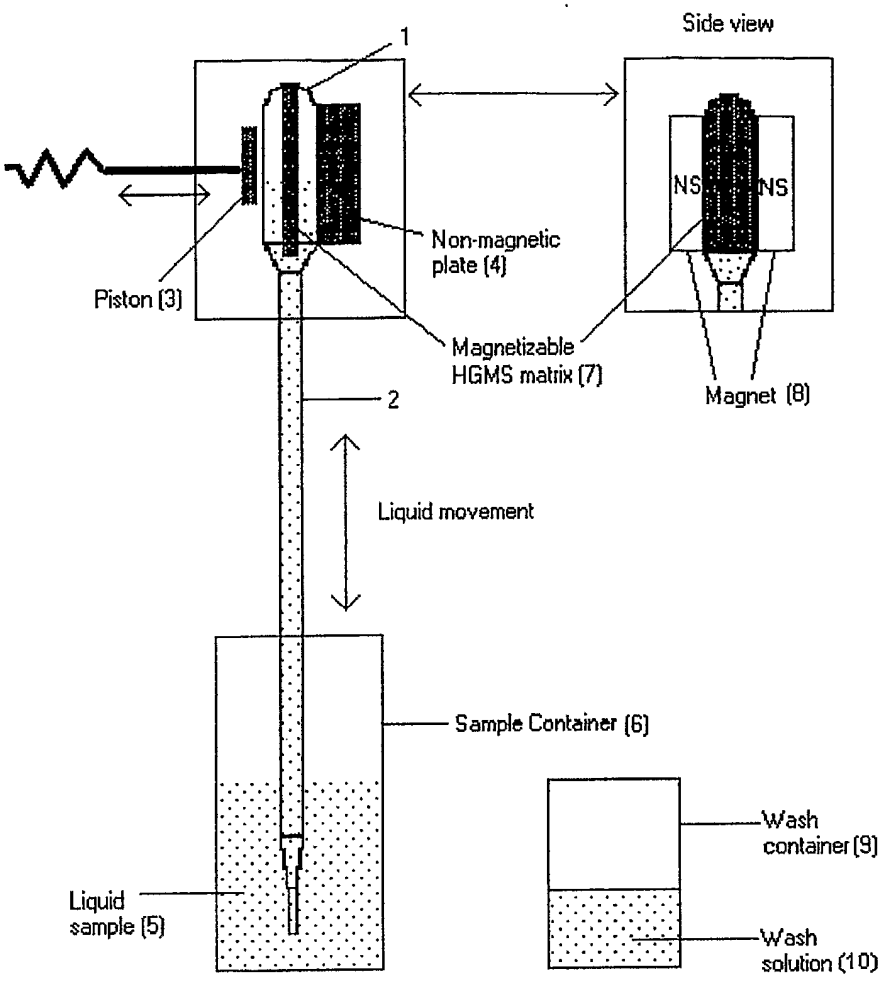
Side view
1
NS
NS
Non-magnetic plate (4)
Piston (3)
Magnetizable HGMS matrix (7)
Magnet (8)
2
Liquid movement
Sample Container (6)
Wash container (9)
Liquid sample (5)
Wash solution (10)

FIG. 2c

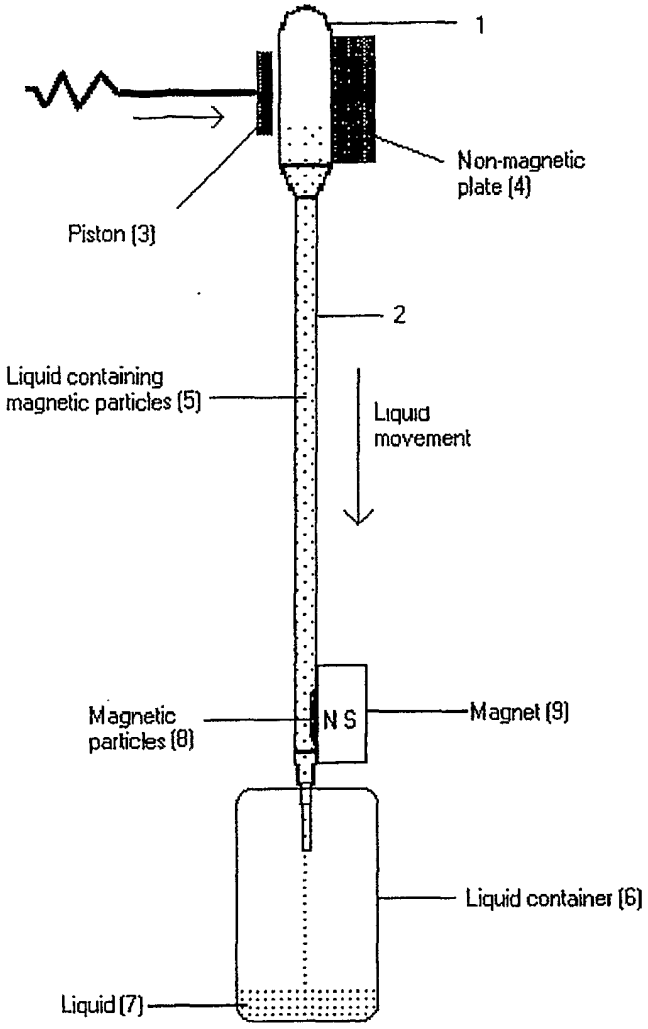
1
Non-magnetic plate (4)
Piston (3)
2
Liquid containing magnetic particles (5)
Liquid movement
Magnetic particles (8)
N S
Magnet (9)
Liquid container (6)
Liquid (7)

FIG. 3

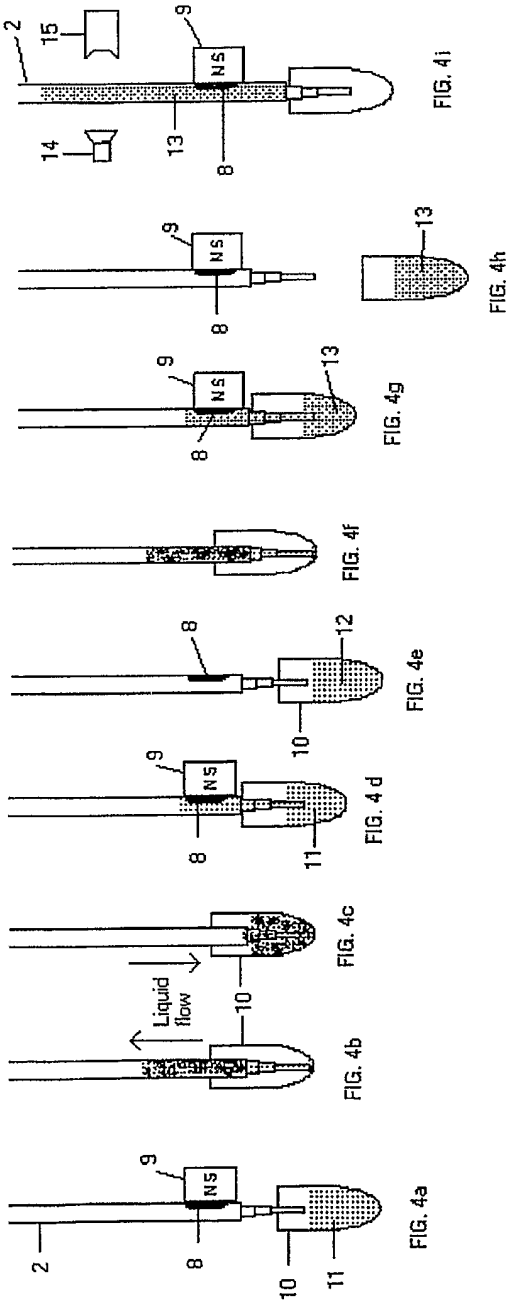


FIG. 4i
FIG. 4h
FIG. 4g
FIG. 4f
FIG. 4e
FIG. 4 d
FIG. 4c
FIG. 4b
FIG. 4a

FIG. 4

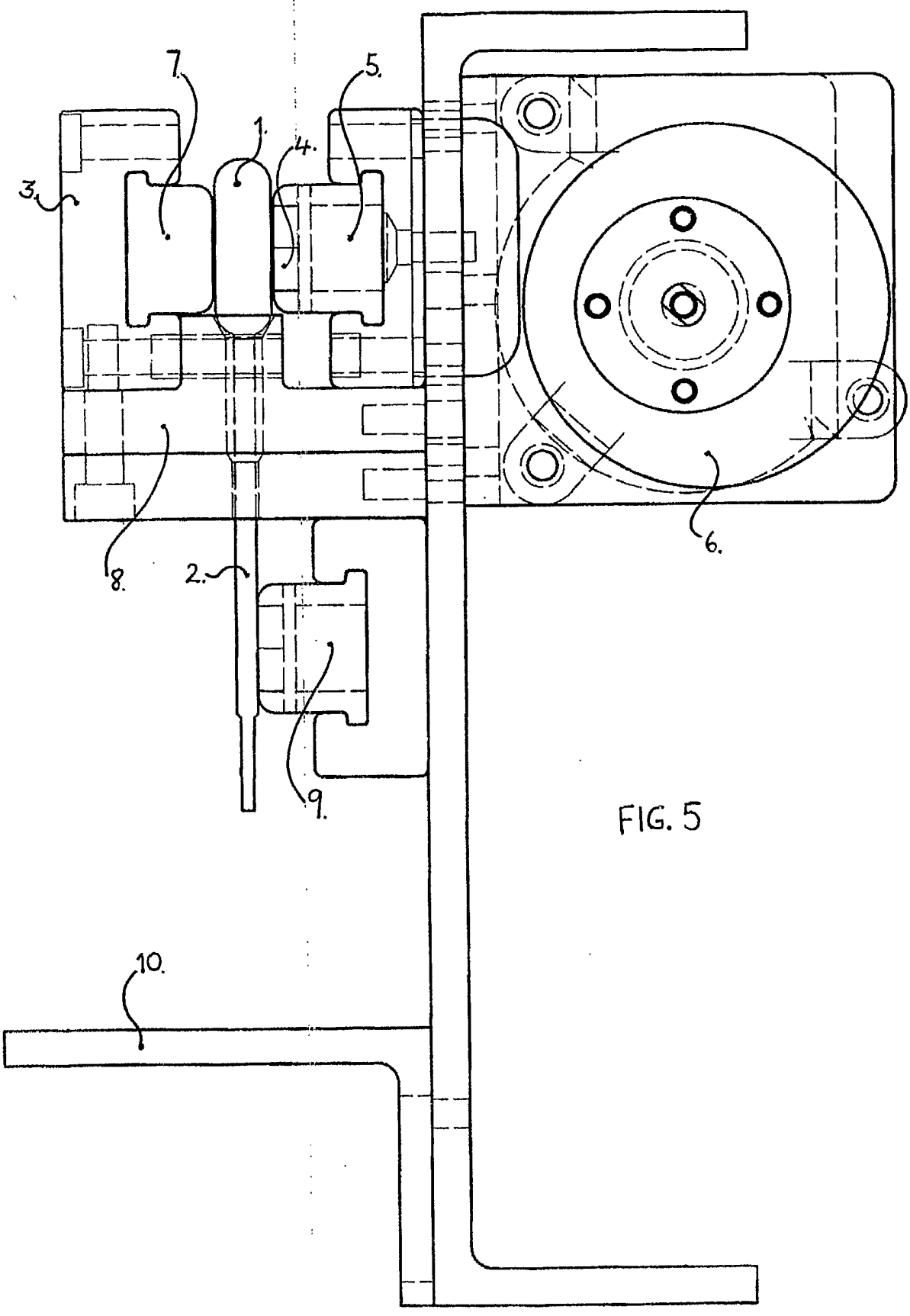


FIG. 5

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/NO 03/00261

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: B01L 3/02, B03C 1/00, G01N 33/543
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)

IPC7: G01N, B01L

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

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-INTERNAL

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[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

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| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 31 October 2003 | 04-11-2003 |
| Name and mailing address of the ISA/<br>Swedish Patent Office<br>Box 5055, S-102 42 STOCKHOLM<br>Facsimile No. +46 8 666 02 86 | Authorized officer<br>Mats Raidla /LR<br>Telephone No. +46 8 782 25 00 |

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International application No.
PCT/NO 03/00261

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