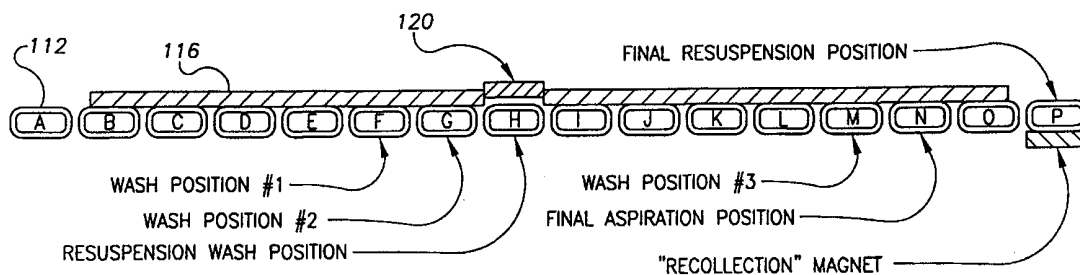




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(54) Title: METHOD AND APPARATUS FOR WASH, RESUSPENSION, RECOLLECTION AND LOCALIZATION OF MAGNETIZABLE PARTICLES IN ASSAYS USING MAGNETIC SEPARATION TECHNOLOGY



## (57) Abstract

Method and apparatus for enabling resuspension wash and magnetic localization of sample components bound to particles with magnetic properties in reaction vessels during separation and wash for enhanced chemiluminescent signal generation in biomedical assays. The assays involve moving reaction vessels past magnets that partially localize the particles prior to passing a reduced strength magnet where washing occurs, with or without resuspension, after separating out the unbound particles and liquid. The band of particles is subsequently resuspended in acid for chemiluminescent purposes. A variety of magnet configurations are employed to realize the reduced strength magnet. Reduced strength magnets adjacent the full width magnets prevent the band of magnetic particles from becoming split. The localized particles enable more efficient resuspension by reagent.

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Method and Apparatus for Wash, Resuspension, Recollection  
and Localization of Magnetizable Particles in Assays  
Using Magnetic Separation Technology

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RELATED APPLICATIONS

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This application is a continuation-in-part of U.S. Patent Application No. 08/644,909, filed May 10, 1996.

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FIELD OF THE INVENTION

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The invention generally relates to the field of biomedical assays employing magnetic separation techniques, and specifically to a method and apparatus for focusing or localizing magnetizable particles during separation and wash in such assays.

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BACKGROUND OF THE INVENTION

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Heterogeneous immunoassays typically require the separation of sought-for components bound to component-selective particles from unbound or free components of the assay. To increase the efficiency of this separation, many assays wash the solid phase (the bound component) of the assay after the initial separation (the removal or aspiration of the liquid phase). Some chemiluminescent immunoassays use magnetic separation to remove the unbound assay components from the reaction vessel prior to addition of a reagent used in producing chemiluminescence or the detectable signal indicative of the amount of bound component present. This is accomplished by using magnetizable particles including, but not restricted to, paramagnetic particles, superparamagnetic particles, ferromagnetic particles and ferrimagnetic

1 particles. Tested-for assay components are bound to  
2 component-specific sites on magnetizable particles during the  
3 course of the assay. The associated magnetizable particles  
4 are attracted to magnets for retention in the reaction vessel  
5 while the liquid phase, containing unbound components, is  
6 aspirated from the reaction vessel.

7 Washing of the solid phase after the initial separation  
8 is accomplished by dispensing and then aspirating a wash  
9 solution, such as de-ionized water or a wash buffer, while  
10 the magnetizable particles are attracted to the magnet.

11 Greater efficiency in washing is accomplished by moving  
12 the reaction vessels along a magnet array having a gap in the  
13 array structure proximate a wash position, allowing the  
14 magnetizable particles to resuspend during the dispense of  
15 the wash solution. This is known as resuspension wash.  
16 Subsequent positions in the array include magnets, allowing  
17 the magnetizable particles to recollect prior to aspiration  
18 of the wash solution and introduction of reagent beyond the  
19 end of the magnet array.

20 Prior art wash block configurations have employed iron-  
21 based or non-iron-based inserts in the gap of the magnet  
22 array at the wash position. Rather than simply removing a  
23 magnet from the resuspension position, the insert is intended  
24 to maintain the accumulation of magnetic particles in the  
25 absence of resuspension wash, and to orient these particles  
26 for thorough resuspension if resuspension wash is employed.  
27 In addition, the insert prevents a reaction vessel from  
28 becoming misaligned and jammed in the magnet array. While  
29 functioning adequately for assays which employ resuspension  
30 wash, it is evident that the provision of such inserts in  
31 place of a magnet at the wash position adversely affects  
32 assays which do not use the resuspension in washing but which  
33 proceed through the wash position without resuspension. With  
34 a non-iron-based insert such as of aluminum or ceramic, a  
35 single band of magnetizable particles which is normally  
36 formed along the interior of the reaction vessel as it passes  
37 the magnet array, during the initial separation, is split

1 into two smaller bands on either side of the reaction vessel  
2 due to attraction by the magnets on either side of the insert  
3 at the resuspension and wash position. This is due to the  
4 minimal effect by the insert on the magnetic flux patterns.  
5 Since reagent is introduced into the reaction vessel in a  
6 stream directed toward where the magnetizable particles  
7 collected before splitting, the split in the banding of the  
8 magnetizable particles results in the stream missing the main  
9 concentration of magnetizable particles. Poor resuspension  
10 of the magnetizable particles during resuspension wash and  
11 upon addition of an acid reagent used to condition the bound  
12 component reagent in the generation of a chemiluminescent  
13 signal results.

14 Similarly, the use of an iron-based insert such as of  
15 steel may result in split-banding of the magnetic particles,  
16 but may further introduce a band of particles in the middle  
17 of the cuvette wall adjacent the insert. This is due to the  
18 tendency of an iron-based insert to shunt magnetic fields.  
19 While the formation of a band or pellet of particles in the  
20 middle of the cuvette wall is desired, the reproducibility of  
21 this response across varying assays for a single iron-based  
22 insert configuration is unlikely. This is due to the varying  
23 characteristics of the assays, including concentration of  
24 surfactants and the particle masses.

25 Other prior art approaches for facilitating resuspension  
26 wash have employed inserts of reduced width with the  
27 intention that magnetic fields extending from the adjacent  
28 magnets will hold the magnetic particles in position.  
29 However, this approach has also resulted in the split-banding  
30 of the particles.

31 Therefore, the prior art fails to provide a wash region  
32 which enables the efficient washing of magnetizable particles  
33 during the wash phase of a magnetic separation assay without  
34 adversely effecting assays not employing resuspension wash.

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1 position. As with assays not employing resuspension wash,  
2 magnetizable particle focusing results in improved  
3 chemiluminescent reaction.

4 It is a further object of the present invention to  
5 provide a wash region which enables the accurate and  
6 predictable collection of magnetizable particles, whether or  
7 not resuspension wash is performed at that region.

8 This object is achieved, in an alternative embodiment,  
9 through the provision of a magnet of reduced strength,  
10 relative to the other magnets in the array, at the wash  
11 position. For instance, in a preferred embodiment, the  
12 reduced strength magnet provides a magnetic field at the  
13 respective reaction vessel position one-half that as provided  
14 by the other magnets. This reduced strength magnet acts as a  
15 replacement for the soft magnetic insert previously  
16 mentioned, which, on its own, provides no magnetic field.  
17 Additionally, the magnets of the array which are disposed at  
18 reaction vessel positions before and after the wash position  
19 are not trimmed, but instead extend across the full extent of  
20 the respective reaction vessel position. At the last  
21 reaction vessel position in the array, the respective magnet  
22 is disposed on a side of the reaction vessel opposite that of  
23 all previous magnets in order to avoid the dense packing in  
24 collected particles which sometimes results in incomplete  
25 resuspension in the stream of final reagent. A further  
26 feature of this alternative embodiment includes lowering the  
27 focal point of the magnetic field, generated by magnets of  
28 the array, at a reaction vessel position immediately  
29 following the reaction vessel position having the reduced  
30 strength magnet proximate thereto.

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#### BRIEF DESCRIPTION OF THE DRAWINGS

33 This invention is pointed out with particularity in the  
34 appended claims. The above and further advantages may be  
35 more fully understood by referring to the following  
36 description and accompanying drawings of which:

1 Fig. 1A is an elevation view of a magnet array and a  
2 sequence of reaction vessels passing therethrough according  
3 to the present invention;

4 Fig. 1B is an elevation view of the magnet array of Fig.  
5 1A in which resuspension wash is performed;

6 Fig. 2 is an elevation view of the magnet array of Fig.  
7 1A illustrating a reaction vessel transport mechanism;

8 Fig. 3 is a rear elevation view of the magnet array of  
9 Fig. 1A illustrating a magnet array support structure;

10 Fig. 4A is a side elevation view of a non-resuspension  
11 wash nozzle oriented proximate a reaction vessel for use in  
12 the magnet array of Fig. 1A;

13 Fig. 4B is a side elevation view of a resuspension wash  
14 nozzle oriented proximate a reaction vessel for use in the  
15 magnet array of Fig. 1B;

16 Fig. 5A is an elevation view of a magnet array and a  
17 sequence of reaction vessels passing therethrough according  
18 to a further embodiment of the present invention;

19 Fig. 5B is an elevation view of the magnet array of Fig.  
20 5A in which resuspension wash is performed; and

21 Fig. 6 is a top view of the magnet array of Fig. 5A.  
22

#### 23 DETAILED DESCRIPTION

24 To increase the efficiency of the separation of bound  
25 components from free components in immunoassays, many assays  
26 wash the solid phase (bound component) of the assay after the  
27 initial separation (removal of the liquid phase and unbound  
28 component). The present invention operates in the context of  
29 a chemiluminescent immunoassay of known type which uses  
30 magnetic separation to remove unbound assay components from a  
31 reaction vessel such as a cuvette.

32 The presently disclosed method and apparatus enables a  
33 resuspension wash of magnetizable particles with improved  
34 wash efficiency and focuses magnetizable particles from a  
35 band to a small region or dot, enabling a more efficient  
36 resuspension of magnetizable particles for a signal  
37 generation portion of the assay.



1           In all of the following discussions, it is assumed that  
2 the reaction vessels progress from the left-hand side of the  
3 illustrations to the right-hand side past a fixed magnet  
4 array at regularly timed intervals, although continuous  
5 motion is not excluded. Means for imparting lateral  
6 translation of the reaction vessels is described subsequently  
7 with regard to Fig. 2. In an exemplary embodiment, such  
8 interval is approximately 15 seconds. Additionally,  
9 throughout this description, aspiration and dispense  
10 functions are executed via means known in the art without  
11 full details being shown.

12           The magnet array of Figs. 1A and 1B includes a  
13 succession of reaction vessels such as cuvettes 12, each  
14 containing assay components and magnetizable particles 14  
15 which are initially in a freely distributed state within the  
16 respective cuvette 12. The concentration of solid phase  
17 (bound component) of the assay remaining in free suspension  
18 in the cuvette at position B is less than that of the first  
19 cuvette 12 in position A due to the initial collection of  
20 solid phase proximate magnets of the array 16 at position B.  
21 In the cuvette 12 of position C, this effect is more  
22 evident. By the time a cuvette has progressed to position D,  
23 the majority of the solid phase 14 has collected proximate  
24 respective magnets of the array 16.

25           References to "magnets" adjacent a respective position  
26 are understood to refer to a pair of adjacent magnets of  
27 oppositely oriented polarity, one above the other, proximate  
28 the respective cuvette position. A band of magnetizable  
29 particles 14 forms along the junction of these two magnets,  
30 where the magnetic gradient is at a maximum.

31           Non-resuspension washes are provided at positions F, G,  
32 and M in the illustrative embodiment of Fig. 1A, and at  
33 positions F and M in the embodiment of Fig. 1B. At these  
34 positions, liquid phase is aspirated from the cuvette 12 via  
35 tubes (15, 17, 19 in Fig. 1A and 15, 19 in Fig. 1B) and wash  
36 solution is reintroduced via nozzles (30, 32, 34 in Fig. 1A  
37 and 30, 34 in Fig. 1B). The nozzles are positioned in front

1 of respective tubes in the view of Figs. 1A and 1B. In  
2 particular, the nozzles are angled toward the front of the  
3 respective cuvette 12 (out of the page in Figs. 1A and 1B) to  
4 avoid disturbing the pellet of solid phase 14 collected at  
5 the respective magnets of the array 16.

6 The tube 21 at position N of Figs. 1A and 1B is employed  
7 to aspirate liquid phase from the respective cuvette 12 prior  
8 to the introduction, at position P, of reagent via nozzle 36,  
9 the reagent facilitating a subsequent chemiluminescent  
10 reaction within a luminometer. In contrast to the non-  
11 resuspension wash nozzles (30, 32, 34 in Fig. 1A and 30, 34  
12 in Fig. 1B), the reagent dispensing nozzles 36 are angled  
13 toward the pellet of solid phase 14 in order to thoroughly  
14 disperse it.

15 In prior art magnet arrays, a portion of the liquid  
16 phase may remain trapped within the solid phase 14 prior to  
17 introduction of the reagent at position P, even after  
18 repeated non-resuspension washes, such as at positions F, G,  
19 and M in Fig. 1A and positions F and M in Fig. 1B. This  
20 trapped liquid phase limits the accuracy of the assay.

21 At position K of Fig. 1A, the magnets of the array 16  
22 proximate the cuvettes 12 are disposed at a lower position.  
23 This provides the solid phase pellet 14 with time to  
24 recollect at the lower position prior to the introduction of  
25 assay reagent at position P. Thus, when reagent is directed  
26 at the pellet 14 in position P by the nozzle 36, the solid  
27 phase 14 will be centrally located in the reaction vessel 12  
28 when the acid is applied at position P. However, such  
29 repositioning of the pellet does not necessarily enhance the  
30 ability of the non-resuspension washes to rid the solid phase  
31 14 of trapped liquid phase.

32 In Fig. 1A, a resuspension wash is not employed, and as  
33 such the focused, or localized, solid phase remains proximate  
34 respective magnets 16 as the cuvette 12 progresses through  
35 the wash block.

36 In contrast, the magnet array of Fig. 1B does employ a  
37 resuspension wash. Resuspension washing of the solid phase

1 involves the aspiration of the liquid phase containing the  
2 unbound components of the assay from the cuvette 12 at  
3 position G via the tube 17 while the bound components are  
4 held in place by respective magnets in the array 16. This is  
5 followed by re-introduction of wash solution into the cuvette  
6 12 at position H by a dispense nozzle 32 angled at the solid  
7 phase pellet 14 collected at the back of the cuvette 12  
8 proximate the magnets 16.

9 At position H, magnets of the array 16 have been  
10 replaced by a soft magnetic insert 20. By dispensing wash  
11 solution onto the magnetizable particles via the nozzle 32 in  
12 the absence of magnets in the array 16, the magnetizable  
13 particles are resuspended, exposing more surface area, and  
14 freeing liquid phase trapped during initial magnetizable  
15 particle collection. After the solid phase has been  
16 resuspended, it is recollected by a subsequent series of  
17 magnets in the array 16 at positions I *et seq.* prior to  
18 aspiration of the wash solution and introduction of the acid  
19 reagent at position P. Other wash stages, in addition to  
20 those illustrated, are possible.

21 The wash block of Figs. 1A and 1B is provided with a  
22 large gap in the magnet array at position H, thus enhancing  
23 resuspension wash. Prior art magnet arrays employed narrower  
24 gaps, resulting in split bands of magnetizable particles due  
25 to the attractive forces of array magnets on either side of  
26 the narrow gap.

27 The present invention avoids the splitting of the solid  
28 phase material into bands at opposite sides of the cuvette  
29 12, in part, by providing a focusing of the solid phase 14  
30 into a smaller band or dot 24. The gap at the resuspension  
31 wash position is filled with an insert 20 made of a soft  
32 magnetic material such as low carbon steel. Further, the  
33 magnets of the array 16 at positions G and I on either side  
34 of the resuspension wash position, position H, are trimmed  
35 such that the gap in the array of magnets 16 and the insert  
36 20 extend proximate a region of the reaction vessels 12

1 previously occupied by the solid phase band 14 adjacent to  
2 the resuspension wash position.

3 As a result, magnetizable particles linearly banded by  
4 the magnets in the previous positions, but which are no  
5 longer directly aligned with magnets of the array 16, migrate  
6 along the reaction vessel 12 walls towards portions of the  
7 reaction vessel interior proximate the trimmed magnets 16.  
8 For instance, in position G, the magnets 16 are trimmed on  
9 the right-hand side. Magnetizable particles formerly aligned  
10 in the trimmed region now migrate to the center of the vessel  
11 12, over the trimmed magnets 16.

12 The magnetizable particle banding pattern in the  
13 reaction vessel at the resuspension wash position, position  
14 H, remains unchanged in the absence of resuspension wash  
15 (Fig. 1A). With resuspension wash (Fig. 1B), the large soft  
16 magnetic insert 20 enables the complete resuspension of the  
17 solid phase 14 free of influence of magnets at positions G  
18 and I. Also, the provision of magnets trimmed on a left-hand  
19 side at position I downstream of the resuspension wash  
20 position, position H, further serves to avoid influencing the  
21 magnetizable particles during the resuspension wash in Fig.  
22 1B.

23 The array 16 magnets at position I, downstream of the  
24 resuspension wash position, position H, and the soft magnetic  
25 insert 20, is also trimmed on its left-hand side in Fig. 1A.

26 This serves to focus the solid phase 14 downstream of the  
27 resuspension wash position, position H. The magnetizable  
28 particles on the left side of the reaction vessel 12 are no  
29 longer directly aligned with magnets 16 at position I.  
30 Rather, they migrate toward the right, into the center of the  
31 vessel 12. The net effect is a conversion of the  
32 magnetizable particles from a wide band 14 to a more compact,  
33 centrally located band 26.

34 For the embodiment of Fig. 1A, the single magnetizable  
35 particle band at position H does not split into two bands as  
36 in the prior art because the soft magnetic insert 20 acts to  
37 short out, or minimize, the magnitude of the field gradient

1 in the resuspension wash position, position H, and because  
2 trimming the magnets of the array 16 at positions G and I  
3 reduces the reach of the fields, from the same, into the  
4 resuspension position H.

5 At position M, trimmed magnets 27 are provided to  
6 further narrow the band of collected magnetizable particles.

7 In a further embodiment, even smaller magnets 28, focusing  
8 magnets, are employed at position N to focus the magnetizable  
9 particles into yet a smaller area, thus providing a smaller  
10 target of solid phase 24 at position P for more efficient  
11 resuspension upon dispense of reagent. Smaller, focusing  
12 magnets 28 are not used in a preferred embodiment for the  
13 initial collection of the solid phase because, amongst other  
14 things, the larger the magnet surface area, the faster the  
15 collection of the magnetizable particles.

16 In an alternative embodiment, all of the magnets in the  
17 array 16 along the length of the wash block are provided as  
18 focusing magnets 28, though the resuspension wash position,  
19 position H, would continue to be provided with a gap such as  
20 that provided by the soft magnetic block 20 of Figs. 1A and  
21 1B. However, such an embodiment would require more time for  
22 each reaction vessel 12 to be proximate the magnets 28 in the  
23 array to provide an equivalent degree of capture capability  
24 due to the smaller size of the magnets in such an embodiment.

25 In yet another embodiment of the present invention, it  
26 is possible to enable further focusing of the magnetizable  
27 particles by employing another gap in the magnet array 16  
28 prior to the focusing magnets 28 at position N. For  
29 instance, such a gap could be employed at position L. Here,  
30 the magnetizable particles 14 have already been gathered at  
31 an interior wall of the reaction vessel 12. A gap at  
32 position L would allow the magnetizable particles to become  
33 released from the interior wall, though they would generally  
34 remain localized. Thus, re-attraction by subsequent focusing  
35 magnets 38 would not take an excessive amount of time.

36 Illustratively, in a first embodiment illustrated in  
37 Fig. 2, the reaction vessels 12 containing the suspended

1 solid phase 14 are laterally translated along the magnet  
2 array 16 by a linked conveyor belt 40 comprised of a sequence  
3 of reaction vessel receptacles 42. A sequence of freely  
4 rotatable rollers 44 are employed to provide support for the  
5 conveyor belt 40. At least one such roller 46 is  
6 mechanically connected to a motor 48, wherein the motor 48  
7 rotates this roller 46, which in turn causes the conveyor  
8 belt 40 and the reaction vessels 12 disposed therein to  
9 translate relative to the magnet array 16.

10 The rear view of the magnet array in Fig. 3 illustrates  
11 a first embodiment of a magnet array 16 support structure 50.

12 The magnet array 16 of Fig. 3 is a reverse view of the  
13 magnet array 16 of Figs. 1A and 1B. The magnets of the array  
14 are backed by a conductive material such as high-iron, low-  
15 carbon steel to focus the magnetic field toward the reaction  
16 vessels 12. The support structure 50, which attaches to the  
17 magnet backing material, is preferably provided from a  
18 magnetically non-reactive material such as aluminum or one of  
19 its alloys to avoid unwanted disturbances in the magnetic  
20 field established within the reaction vessels. The magnets  
21 of the array 16 and the backing material are fastened to the  
22 support structure 50 in a variety of ways, including via the  
23 use of adhesive or mechanical fasteners. The support  
24 structure 50 is itself suspended by being mechanically  
25 attached to a wall of an enclosure (not illustrated), either  
26 by adhesive, mechanical fasteners, or some combination  
27 thereof.

28 In the illustrated embodiment of the support structure  
29 in Fig. 3, the element is segmented into three portions: an  
30 initial portion to the right of Fig. 3, a central portion,  
31 and a small final portion on the left. The latter provides  
32 support for the focusing magnets 24. In an alternative  
33 embodiment, the central portion and the final portion are  
34 combined, such that the support structure is formed of two  
35 portions.

36 Fig. 3 also illustrates a rear view of the soft magnetic  
37 insert 20. Disposed in a central location thereof is a

1 cross-section of a mechanical fastener 52 such as a screw  
2 employed in securing the insert 20 to a wall of the  
3 enclosure. In alternative embodiments, the soft magnetic  
4 insert is supported by a respective support element such as a  
5 stanchion or by an extension of the array magnet support  
6 element 50. In the latter alternative, the support element  
7 50 would then be one continuous element, if the final portion  
8 and the central portion are continuous, or two elements if  
9 the focusing magnets 24 is supported independently.

10 The orientation of wash solution nozzles as employed  
11 along the magnet array 16 of the foregoing is illustrated in  
12 Figs. 4A and 4B. In particular, a nozzle 30 such as that  
13 used for reintroduction of wash solution at position F in  
14 Figs. 1A or 1B is shown in cross-section in Fig. 4A. Solid  
15 phase 14 has collected proximate the magnet array 16  
16 (supported by the support element 50) at the rear of the  
17 reaction vessel 12. The nozzle 30 is oriented with respect  
18 to the reaction vessel 12 to provide a stream 60 of wash  
19 solution from a wash solution reservoir 62 via a pump 64 to a  
20 front, interior surface of the reaction vessel 12. This  
21 avoids disturbing the solid phase collected at the rear of  
22 the vessel 12.

23 In Fig. 4B, the orientation of a nozzle 32 such as that  
24 used for resuspension wash at position H in Fig. 1B is  
25 illustrated in Fig. 4B. A stream 66 of wash solution from  
26 the reservoir 62 via the pump 64 is directed at the solid  
27 phase previously collected proximate magnets in the array 16,  
28 but now adjacent to the soft magnetic insert 20. The solid  
29 phase is therefore not retained by magnets, and is easily  
30 washed back into suspension by the stream 66 of wash solution  
31 from the nozzle 32.

32 Having described preferred embodiments of the invention,  
33 it will be apparent to those skilled in the art that other  
34 embodiments incorporating the concepts may be used.

35 For instance, though the present invention has been  
36 described in the context of a chemiluminescent immunoassay,  
37 it can be applied to other assay environments in which the

1 separation of bound and unbound components by magnetic  
2 separation is required. Further, the exact number of  
3 positions in which magnetizable particles are exposed to  
4 magnets 16 depends upon the exact nature of the desired  
5 separation, the configuration of the magnets 16, the  
6 characteristics of the magnetizable particles and the  
7 associated bound component, etc.

8 Nozzle 32 has been shown in two locations in Figs. 1A  
9 and 1B, specifically position H in Fig. 1A and position I in  
10 Fig. 1B. While provided as one nozzle with a like reference  
11 identifier in both figures, each embodiment of Fig. 1A and 1B  
12 could be provided with a nozzle at position G for non-  
13 resuspension wash, and another nozzle at position H for use  
14 in an embodiment employing resuspension wash. Thus, the same  
15 array configuration could be used for assays both employing  
16 and not employing resuspension wash.

17 In addition to the illustrated embodiment of Fig. 2,  
18 other means for translating the conveyor belt are envisioned,  
19 such as a friction drive disposed on either side of the  
20 conveyor at one or more positions.

21 In yet another embodiment of the present invention, the  
22 reaction vessels 12 are translated along the magnet array 16  
23 by way of a sequence of respective reaction vessel yokes (not  
24 illustrated) connected to the respective reaction vessel near  
25 the top of the vessel.

26 The arrangement of elements in Figs. 4A and 4B is a  
27 generalized illustration of the relationship between the  
28 elements, and is not intended to represent a preferred  
29 layout. For instance, the nozzle 30, 32 in Figs. 4A and 4B  
30 can also be located at the same relative position above a  
31 respective reaction vessel 12, but angled in opposite  
32 directions to properly direct the respective stream 60, 66.  
33 Further, the pump and reservoir can be provided in a variety  
34 of ways, as known to one skilled in the art.

35 The embodiments described in the foregoing are best  
36 suited to particles and particle mixtures that relocate  
37 easily within and along the wall of a reaction vessel in



1 response to changes in magnetic fields in the vicinity of the  
2 reaction vessel. By "particle mixtures," it is meant  
3 mixtures including magnetic particles, the sample, primary  
4 reagents, ancillary reagents and wash solutions. Such easily  
5 relocatable mixtures form consistently shaped and accurately  
6 positioned pellets. Factors which contribute to the  
7 responsiveness of mixtures to applied magnetic fields include  
8 the size of the magnetic particles, the "stickiness" of  
9 substances in the reaction mixture, the inclusion of  
10 "slippery" surfactants in the reaction mixture, etc.

11 Assay mixtures which do not relocate easily or  
12 consistently in response to moving magnetic fields tend to  
13 form pellets of variable shapes in unpredictable locations,  
14 particularly at positions N, O and P of Figs. 1A and 1B.  
15 This inconsistency diminishes the benefit of shaping and  
16 positioning the particle mass, since it introduces  
17 variability in the resuspension step where the final reagent  
18 is introduced. In addition, some assay mixtures tend to pack  
19 the aggregate of particles more densely as the magnetic  
20 forces shape and position them, thus frustrating efforts  
21 taken to remove unbound label which might otherwise  
22 improperly effect the desired reaction.

23 In general, it is desirable to minimize the number of  
24 different wash steps in the separation and wash process;  
25 additional process steps have the potential for introducing  
26 variability. However, in certain assays, additional wash  
27 steps are necessary to localize the magnetic particles into a  
28 band on the side wall of a reaction vessel. Some assays  
29 benefit significantly from a resuspension wash step.  
30 Resuspension wash is used to wash out any unbound label that  
31 may be trapped in the particle aggregate which would  
32 otherwise contribute a non-specific signal to the true signal  
33 at the assay read step. Therefore, as provided in the  
34 previous embodiment, a further embodiment provides the  
35 ability to selectively employ a resuspension wash, but with  
36 an emphasis placed on minimizing unnecessary manipulation of  
37 the particle aggregate.

1           Fig. 5A is an illustration of a system having a  
2 continuous magnet array proximate a series of consecutive  
3 reaction vessel positions. The array is comprised of  
4 vertically oriented pairs of magnets 116, one above the  
5 other, arranged such that the magnetic gradient generated by  
6 the magnets at each reaction vessel position has a horizontal  
7 maximum proximate the intersection of the two magnets. As in  
8 the previously described embodiment, each reaction vessel, or  
9 cuvette, 112 contains assay components and magnetizable  
10 particles 114 which are initially in a freely distributed  
11 state within the respective cuvette 112 (for instance, at  
12 position A). The concentration of unbound solid phase  
13 decreases as each cuvette traverses the magnet array (from  
14 left to right in Figs. 5A and 5B).

15           For ease of reference, reaction vessel positions B  
16 through G are referred to as an initial separation stage, at  
17 which the solid phase is initially collected against the  
18 interior wall of the cuvette, proximate the respective  
19 magnets 116. One or more non-resuspension washes (discussed  
20 subsequently) may be provided within the initial separation  
21 stage. Position H is referred to as a resuspension wash  
22 position, at which a resuspension wash may occur according to  
23 the needs of each assay. Positions I through M are referred  
24 to as a subsequent separation stage, even though in the case  
25 of Fig. 5A, there is no resuspension wash and so the  
26 separation begun in the initial separation stage is merely  
27 continued. In the case of Fig. 5B, however, the subsequent  
28 separation stage is utilized for the purpose of separating  
29 the magnetic particles into a pellet against the cuvette  
30 interior wall most proximate the magnet array. One or more  
31 non-resuspension washes may occur within the subsequent  
32 separation stage (discussed subsequently). Position N is  
33 referred to as a final aspiration stage, where liquid phase  
34 is removed from the cuvette prior to the final reagent  
35 resuspension. Position O is an optional rest stage.  
36 Position P is referred to as a final resuspension stage,  
37 where a final reagent is introduced into the cuvette at such

1 an angle that the previously collected pellet 114 is washed  
2 off the cuvette wall and is freely suspended in the reagent  
3 solution.

4 As in Fig. 1A, optional non-resuspension wash positions  
5 are provided at positions F, G and M. Here, liquid phase is  
6 aspirated from the cuvette 112 via tubes 115, 117, 119, and  
7 wash solution is introduced via nozzles 130, 132, 134. While  
8 these nozzles are labeled "WASH BUFFER" in the figures, it is  
9 understood that any wash solution desired may be dispensed  
10 from the nozzles. Each of the washes at these positions is  
11 optional. As in the prior embodiment, the nozzles may be  
12 oriented in a variety of ways in order to avoid dislodging  
13 the pellet of solid phase 114 collected at the respective  
14 magnet 116; the goal is to remove liquid phase, then refill,  
15 without disturbing the collected particles.

16 In Fig. 5A, a separation and wash process is illustrated  
17 wherein resuspension wash is not used. Typically, the assay  
18 will pass the resuspension wash position (position H in the  
19 figures) with the reaction vessel being filled with wash  
20 solution that was added either at position F or G. By  
21 providing "full width" magnets, or magnets which extend  
22 across the entire extent of the reaction vessel position, at  
23 positions G and I, any tendency to split the particle band to  
24 the left or right is reduced. Depending upon the response of  
25 a particular reagent mixture to magnetic field manipulation,  
26 this tendency may be evident with the trimmed magnets of the  
27 foregoing embodiment.

28 For instance, certain "sticky" mixtures react to the  
29 trimmed magnet at position G of Fig. 1A by accumulating on  
30 the left side of the cuvette wall most proximate the magnets  
31 (as viewed in Fig. 1A). This accumulation is maintained  
32 through position H. Then, with the trimmed magnet at  
33 position I, part of the magnetic particles break free of the  
34 previous accumulation and gather on the right side, while  
35 another part of the accumulation remains on the left side of  
36 the cuvette wall. Since the nozzle 136 which dispenses the  
37 final reagent at position P is preferably aimed at the middle

1 of the cuvette wall most proximate the magnets, this split  
2 band of particles is not directly impinged by the final  
3 reagent stream, and incomplete resuspension is achieved.

4         However, in the embodiment of Figs. 5A and 5B, full  
5 width magnets at positions G and I avoid this split-banding,  
6 and consequently result in more complete resuspension in the  
7 final reaction mixture at position P.

8         Without resuspension wash at position H, the reduced  
9 strength magnet at position H is strong enough to hold the  
10 previously collected particle band 114 and prevent the  
11 particles from being attracted to the stronger magnetic  
12 forces at the neighboring positions, positions G and I, which  
13 would otherwise result in split-banding.

14         The reduced strength magnet may be provided as a magnet  
15 having dimensions similar to that of the remaining magnets of  
16 the array but which provides a weaker magnetic field.  
17 Alternatively, it may be preferred to provide a magnet  
18 identical to the remaining magnets of the array, only  
19 disposed further from the respective reaction vessel  
20 position, such as by recessing the magnet, thus resulting in  
21 a weaker effective magnetic field at the cuvette. See Fig. 6  
22 for an illustration of a recessed magnet at position H.  
23 Also, the material behind or around the magnet can be  
24 utilized to impact the effective magnetic field strength that  
25 acts on the particles to separate them from the solution and  
26 hold them to the cuvette wall during washing. Further, the  
27 reduced strength magnet may be implemented by recessing a  
28 single magnet of the same strength as all other magnets of  
29 the array, though all of the remaining reaction vessel  
30 positions employ two magnets of opposing polarity. Thus, in  
31 Figs. 5A and 5B, the magnet at position H appears to be half-  
32 height with respect to the magnet pairs of the remaining  
33 positions.

34         In Fig. 5B, the use of a resuspension wash in the  
35 instant configuration is illustrated. Here, wash fluid is  
36 aspirated out of the cuvette at position G but will not be  
37 replaced there. Rather, the cuvette is moved to the

1 resuspension wash position, position H, without any liquid  
2 phase in the cuvette. The resuspension wash nozzle 133 is  
3 aimed at the particle aggregate on the "back" wall of the  
4 cuvette, most proximate the magnets. Since the magnetic  
5 forces holding the particles against the wall at this  
6 position are of reduced strength, the force of the  
7 resuspension wash stream is capable of resuspending much of  
8 the particle aggregation. However, the particles will  
9 immediately begin recollecting and will continue to do so at  
10 the stepped down magnet positions which follow (i.e., at  
11 positions I, J, K, ...). Any non-specific label that was  
12 trapped in the aggregate will now be freed into the wash  
13 solution and will be aspirated out of the reaction vessel at  
14 either wash position M and/or N.

15 In either case, the magnet array of Figs. 5A and 5B  
16 typically provides a particle aggregation of consistent shape  
17 and position within the respective cuvette at the critical  
18 final resuspension position, position P, regardless of the  
19 characteristics of the contents of the assay mixture.

20 The tube 121 at position N of Figs. 5A and 5B is  
21 employed to aspirate liquid phase from the respective cuvette  
22 112 prior to the introduction, at position P, of the final  
23 reagent via the nozzle 136. This reagent facilitates the  
24 subsequent chemiluminescent reaction within a luminometer.  
25 The reagent dispensing nozzle 136 at position P is preferably  
26 angled towards the pellet 114 for maximum dispersion of the  
27 accumulated particles.

28 In order to facilitate the complete dispersion of the  
29 pellet 114 in the stream of final reagent at position P, the  
30 respective magnet for that reaction vessel position, which  
31 may be referred to as a "recollection" magnet, is preferably  
32 positioned on the opposite side of the cuvette with respect  
33 to all other reaction vessel positions, as shown in the top  
34 view of Fig. 6. This "recollection" magnet is not shown in  
35 Figs. 5A and 5B as it would otherwise obscure a portion of  
36 the cuvette 112 at position P. The purpose of positioning  
37 the magnet at this manner is to assist the stream of reagent

1 in releasing the pellet from the wall of the cuvette and to  
2 aid in fully resuspending the magnetic particles in the final  
3 reagent mixture. The cuvette does not remain at position P  
4 long enough for the magnetic particles to begin re-  
5 accumulating proximate this final, oppositely positioned  
6 magnet to any significant degree. This is especially so  
7 since reagent is being introduced into the cuvette for most  
8 of the dwell time at this position.

9 In contrast to the system illustrated in Figs. 1A and  
10 1B, the magnets at reaction vessel position I and subsequent  
11 positions are disposed lower than the magnets at positions A  
12 through G. The magnets at the start of the separation (the  
13 initial separation stage) are positioned with respect to the  
14 height of the cuvettes to accommodate a wide range of initial  
15 reaction volumes. For example, if a high volume of liquid  
16 phase is present in a cuvette, the magnetic forces as  
17 provided by the embodiments of Figs. 5A and 5B are sufficient  
18 to collect particles from the farthest distances in the  
19 reaction fluid both above and below the magnetic centerline.

20 Once so collected, however, it is desirable to shift the  
21 particle aggregation lower within the cuvette so that the  
22 aggregate is positioned below the fluid level of the final  
23 resuspension fluid volume. The lower magnets after this  
24 step-down achieve the desired aggregate repositioning more  
25 completely than the previous embodiment for certain reaction  
26 mixtures. Since the resuspension wash volume does not  
27 participate in the final reaction, being withdrawn at the  
28 latest at position N, it can be selected such that the lower  
29 set of magnets is capable of recollecting all particles,  
30 including those in the upper portion of the resuspension wash  
31 volume.

32 Assay mixtures which resist repositioning of the  
33 particle mass tend to get even less responsive the longer  
34 they are maintained in one location. Thus, delaying the  
35 step-down to a later reaction vessel position within the  
36 subsequent separation stage, such as at position K in Figs.  
37 1A and 1B, only tends to make the aggregate more difficult to

1 accurately position. Locating the step-down immediately  
2 following the resuspension wash position avoids this  
3 difficulty.

4       These and other examples of the invention illustrated  
5 above are intended by way of example and the actual scope of  
6 the invention is to be determined from the following claims.

CLAIMS

What is claimed is:

- 1 1. A system, for use in an assay apparatus, for enabling  
2 separation and wash of magnetic particles in a reaction  
3 vessel, said magnetic particles having sample components  
4 bound thereto, the system comprising:  
5 an array of plural, consecutive reaction vessel  
6 positions, each position having a respective magnet adjacent  
7 thereto, past which said reaction vessel transits in  
8 sequence, said array comprising  
9 an initial separation stage comprised of a first  
10 consecutive plurality of said reaction vessel positions,  
11 a resuspension wash position comprised of one of  
12 said reaction vessel positions, adjacent said initial  
13 separation stage,  
14 a subsequent separation stage comprised of a  
15 second consecutive plurality of said reaction vessel  
16 positions, adjacent said resuspension wash position,  
17 a final aspiration position comprised of one of  
18 said reaction vessel positions, adjacent said subsequent  
19 separation stage, and  
20 a final resuspension position comprised of one of  
21 said reaction vessel positions, adjacent said final  
22 aspiration position;  
23 an initial aspiration element, adjacent a first of said  
24 reaction vessel positions of said initial separation stage,  
25 adapted for selectively aspirating liquid phase from said  
26 reaction vessel at said initial separation stage first  
27 reaction vessel position;  
28 a resuspension wash dispense element, adjacent said  
29 resuspension wash position, adapted for selectively  
30 dispensing resuspension wash solution into said reaction  
31 vessel at said resuspension wash position;  
32 a final aspiration element, adjacent a first reaction  
33 vessel position of said subsequent separation stage, adapted  
34 for selectively aspirating liquid phase from said reaction



35 vessel at said subsequent separation stage first reaction  
36 vessel position; and

37 a reagent dispense element, adjacent said final  
38 resuspension position, adapted for selectively dispensing  
39 reagent into said reaction vessel at said final resuspension  
40 position to resuspend said magnetic particles in said  
41 reagent,

42 wherein said array is configured such that said  
43 reaction vessel transits, in sequential order, said initial  
44 separation stage, said resuspension wash position, said  
45 subsequent separation stage, said final aspiration position,  
46 and said final resuspension position,

47 wherein said magnet adjacent said resuspension wash  
48 position is of reduced magnetic strength relative to all  
49 other magnets adjacent said array of plural consecutive  
50 reaction vessel positions.

1 2. The system of claim 1, wherein each magnet of said  
2 initial separation stage, said subsequent separation stage,  
3 and said final aspiration position provides a magnetic field  
4 which tends to attract said magnetic particles into a  
5 substantially horizontal cluster on an interior wall of said  
6 reaction vessel most proximate said magnet.

1 3. The system of claim 2, wherein said magnets adjacent  
2 said subsequent separation stage, said final aspiration  
3 position, and said final resuspension position are disposed  
4 such that said substantially horizontal cluster develops at  
5 a point, on said interior wall of said reaction vessel most  
6 proximate said magnet, below a point where said  
7 substantially horizontal cluster develops at said initial  
8 separation stage.

1 4. The system of claim 1, further comprising at least one  
2 rest position intermediate said final aspiration position  
3 and said final resuspension position past which said  
4 reaction vessel transits in sequence.

1 5. A method of separating and selectively washing magnetic  
2 particles within a reaction vessel, said magnetic particles  
3 having sample components bound thereto, the method  
4 comprising the steps of:

5 passing said reaction vessel through an array of  
6 reaction vessel positions of an assay apparatus, comprising  
7 an initial separation stage, a resuspension wash position, a  
8 subsequent separation stage, a final aspiration position,  
9 and a final resuspension position, for separating said  
10 magnetic particles from a liquid phase in said reaction  
11 vessel and washing said magnetic particles free of unbound  
12 sample components, wherein:

13 said initial separation stage comprises a first  
14 consecutive plurality of said reaction vessel positions and  
15 an initial aspiration element, adjacent a first of said  
16 reaction vessel positions of said initial separation stage,  
17 adapted for selectively aspirating liquid phase from said  
18 reaction vessel at said initial separation stage first  
19 reaction vessel position;

20 said resuspension wash position comprised of one of  
21 said reaction vessel positions, adjacent said initial  
22 separation stage, and a resuspension wash dispense element,  
23 adjacent said resuspension wash position, adapted for  
24 selectively dispensing resuspension wash solution into said  
25 reaction vessel at said resuspension wash position;

26 said subsequent separation stage comprised of a second  
27 consecutive plurality of said reaction vessel positions,  
28 adjacent said resuspension wash position;

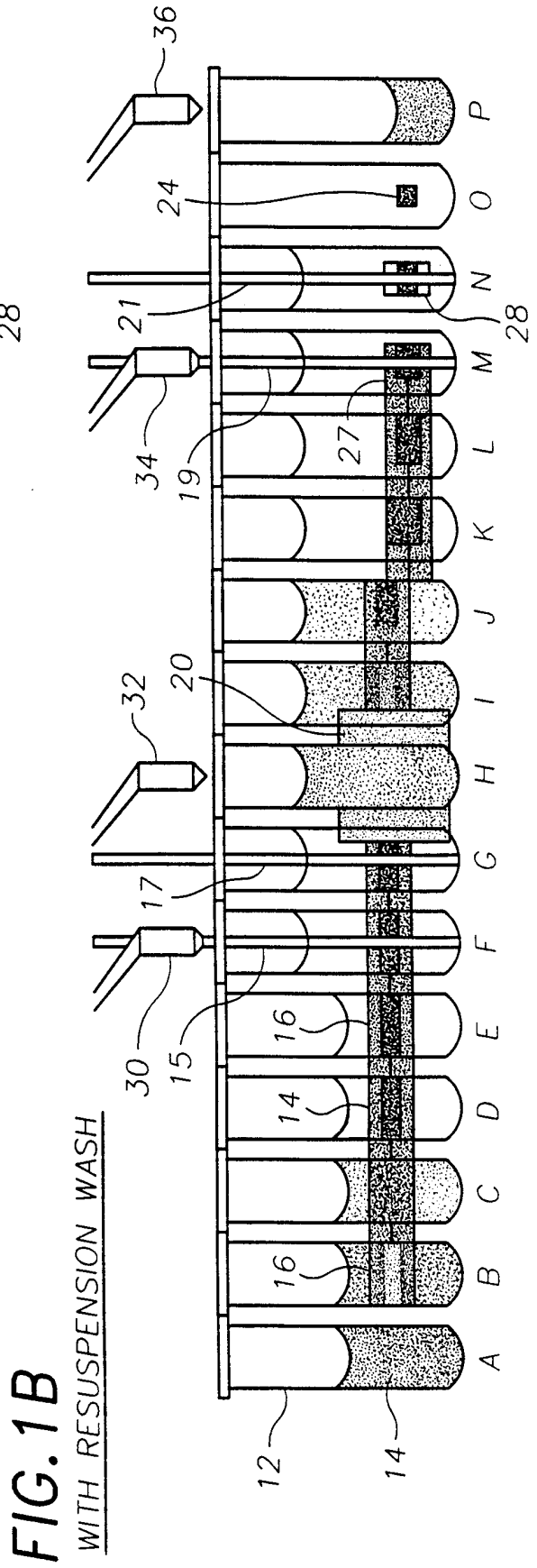
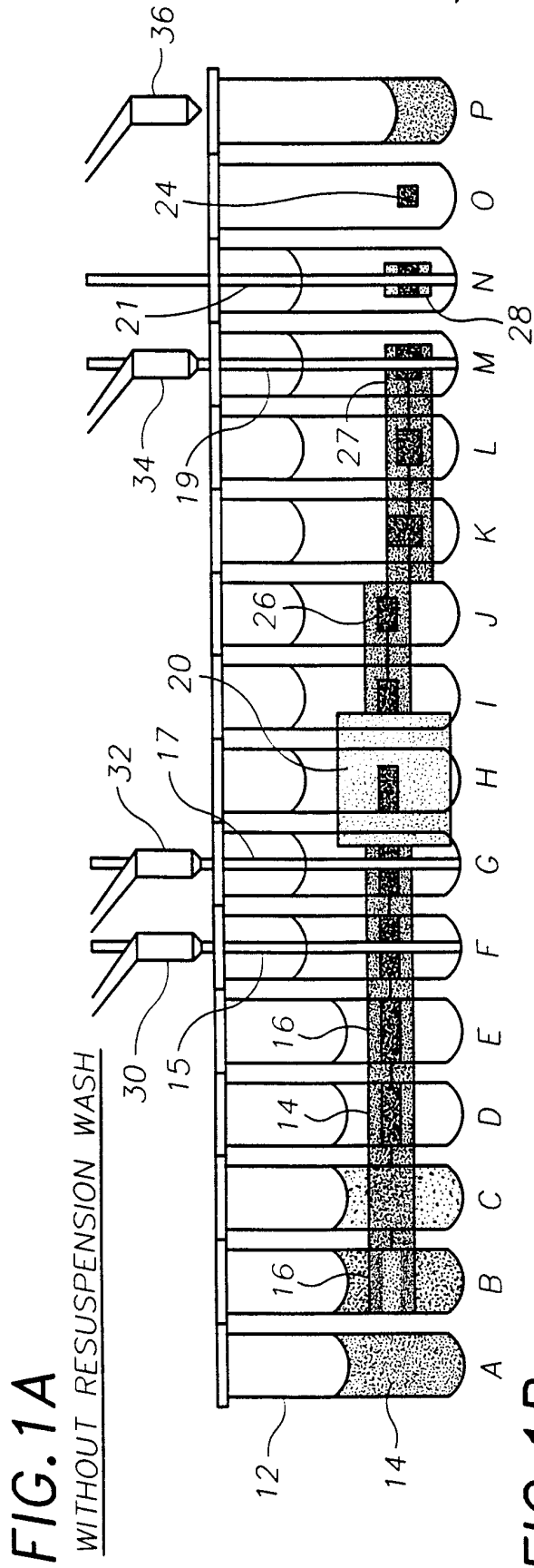
29 a final aspiration position comprised of one of said  
30 reaction vessel positions, adjacent said subsequent  
31 separation stage, and a final aspiration element, adjacent a  
32 first reaction vessel position of said subsequent separation  
33 stage, adapted for selectively aspirating liquid phase from  
34 said reaction vessel at said subsequent separation stage  
35 first reaction vessel position; and

36 a final resuspension position comprised of one of said  
37 reaction vessel positions, adjacent said final aspiration  
38 position, and a reagent dispense element, adjacent said  
39 final resuspension position, adapted for selectively  
40 dispensing reagent into said reaction vessel at said final  
41 resuspension position to resuspend said magnetic particles  
42 in said reagent,

43 wherein said array is configured such that said  
44 reaction vessel transits, in sequential order, said initial  
45 separation stage, said resuspension wash position, said  
46 subsequent separation stage, said final aspiration position,  
47 and said final resuspension position, and

48 wherein said magnet adjacent said resuspension wash  
49 position is of reduced magnetic strength relative to all  
50 other magnets adjacent said array of plural consecutive  
51 reaction vessel positions.

1/6



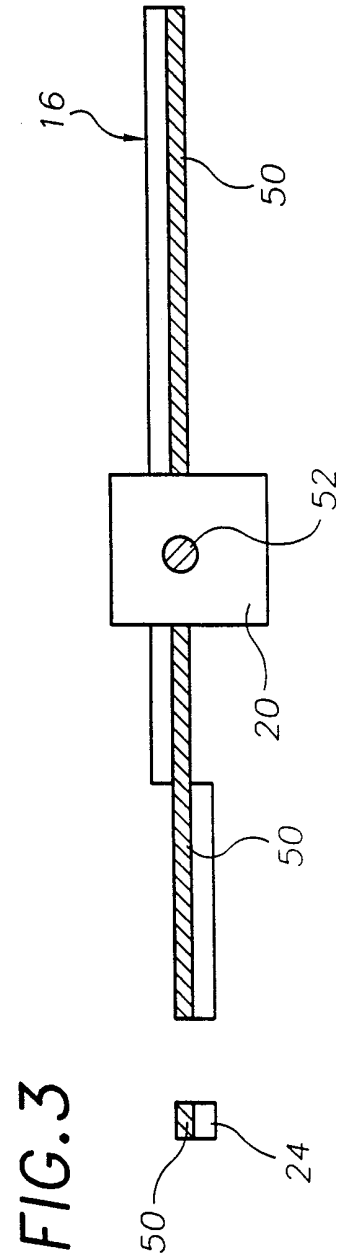
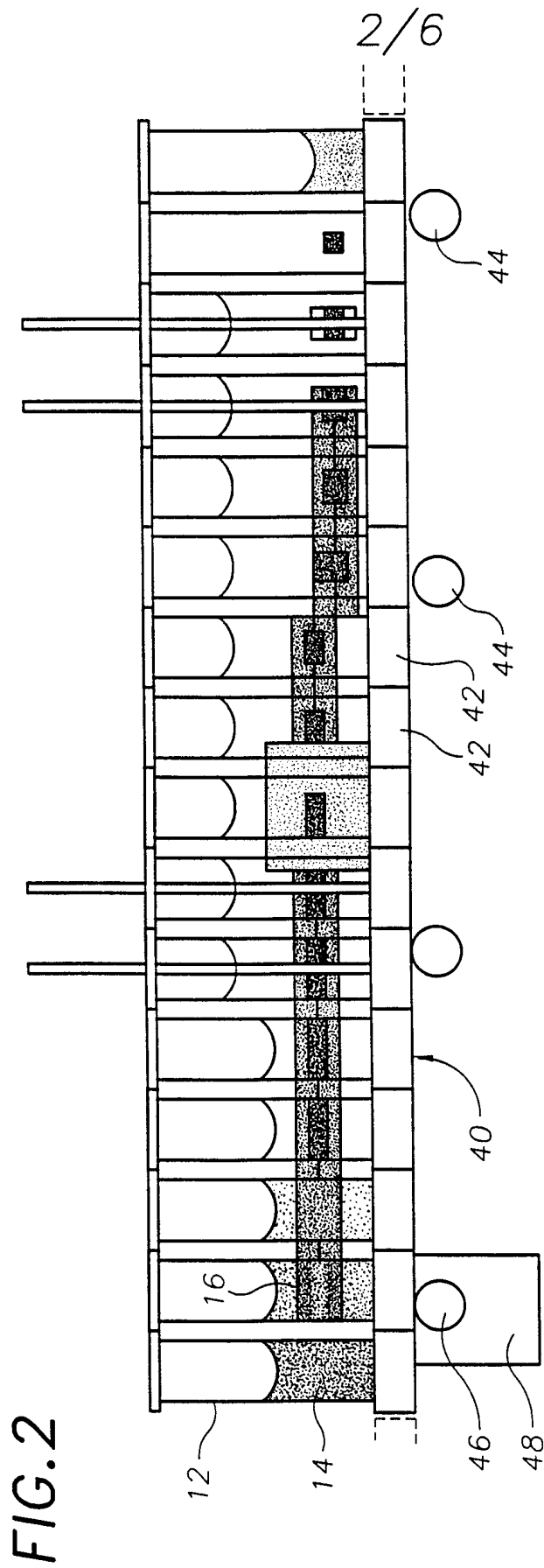


FIG. 4B

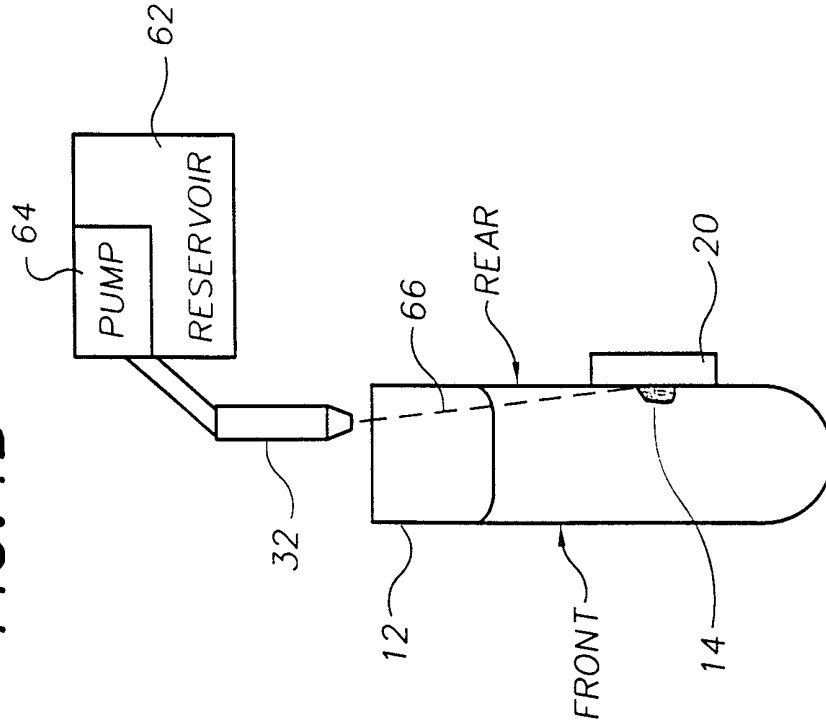
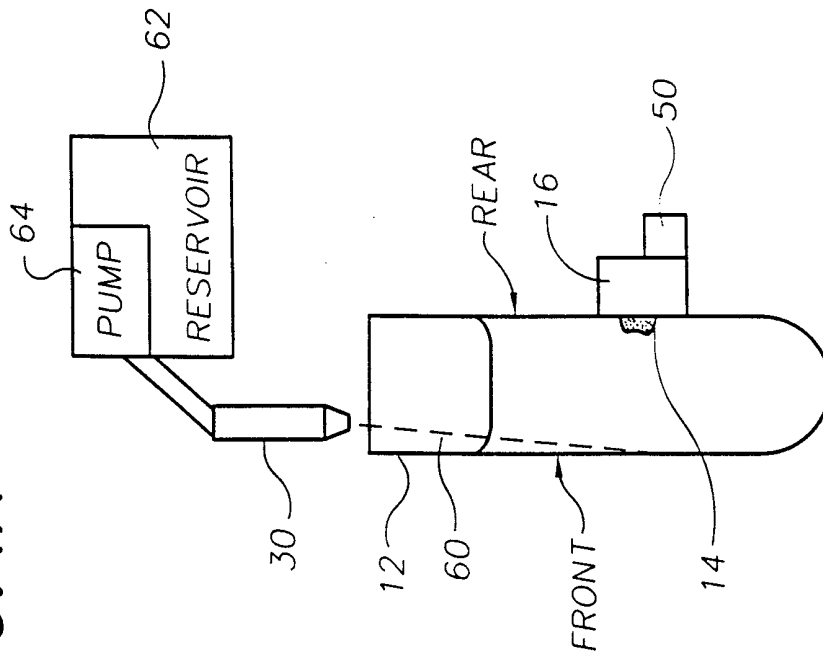
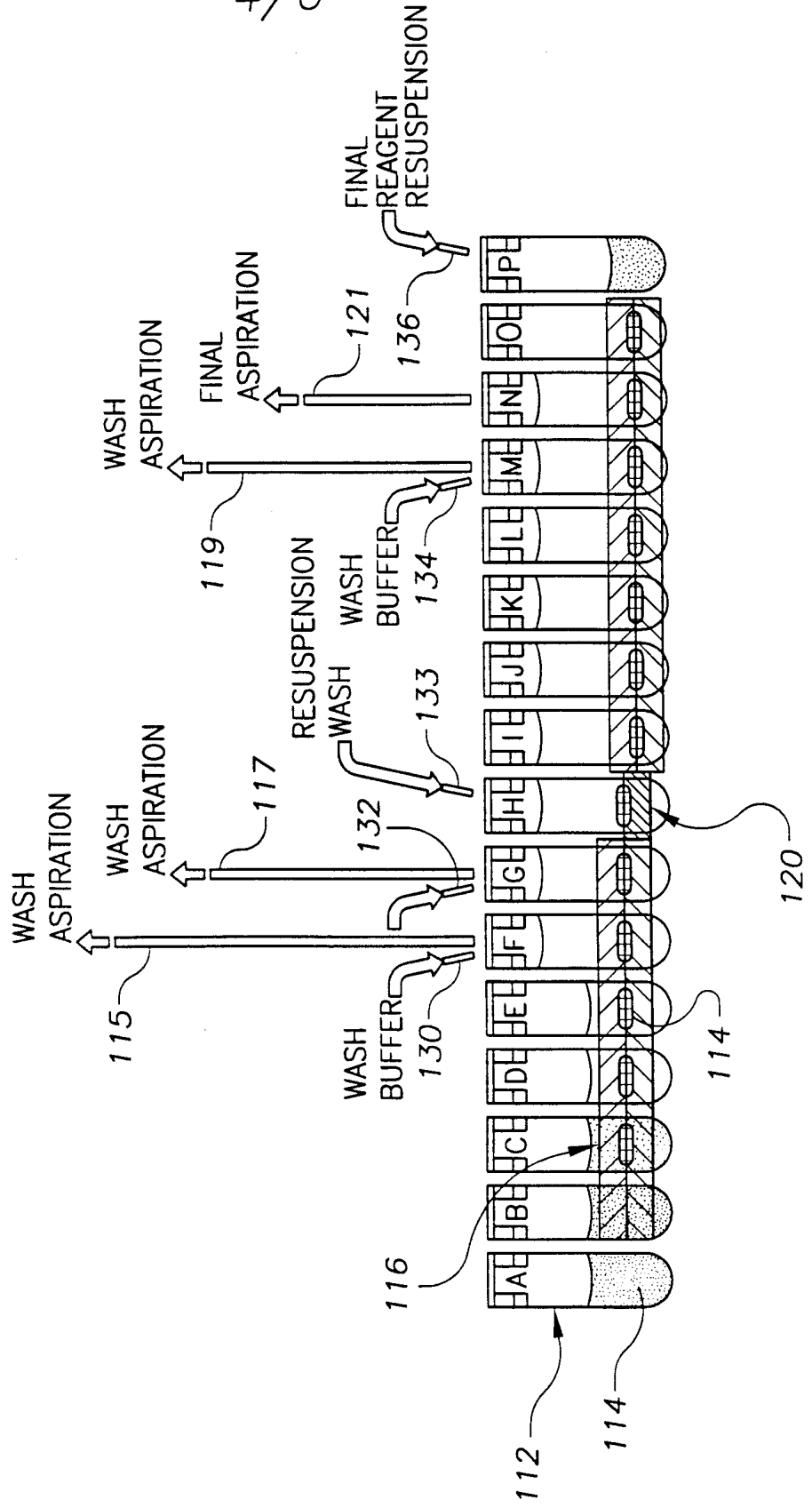


FIG. 4A



**FIG. 5A**  
**WITHOUT RESUSPENSION WASH**



**FIG. 5B**  
WITH RESUSPENSION WASH

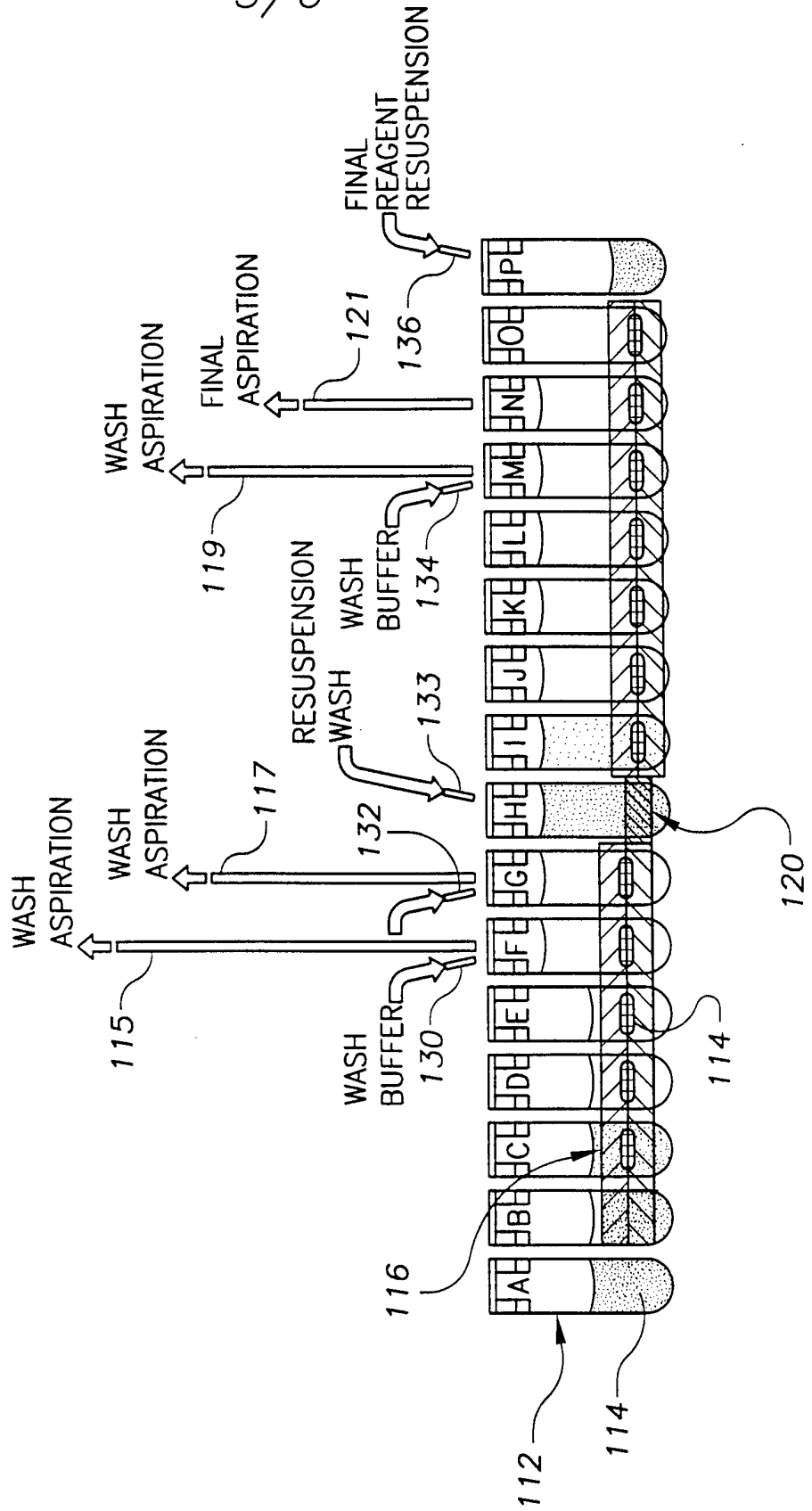
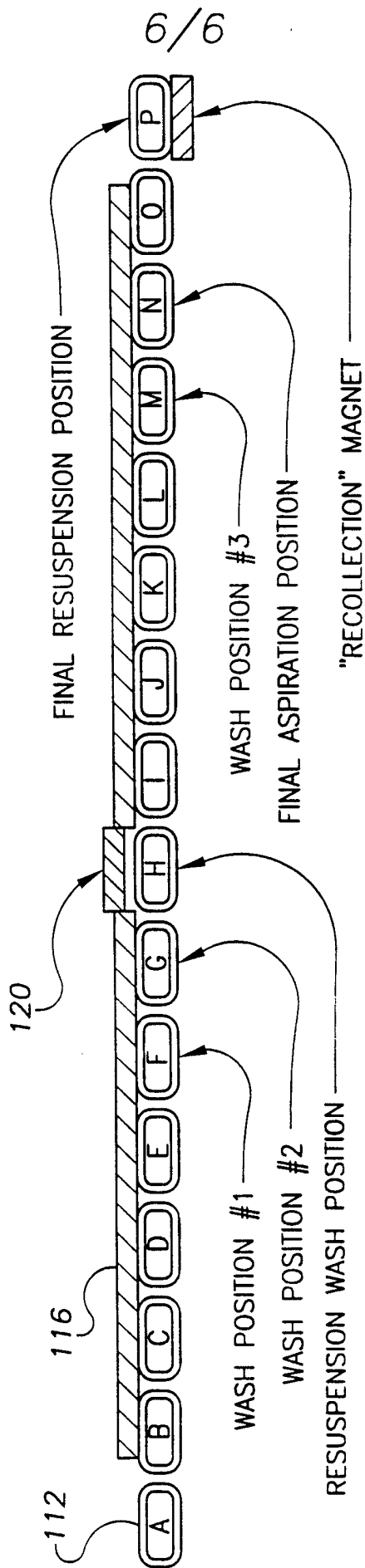




FIG. 6



# INTERNATIONAL SEARCH REPORT

|  |
|--|
| International Application No<br><b>PCT/IB 99/01646</b> |
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**A. CLASSIFICATION OF SUBJECT MATTER**  
 IPC 7 G01N35/00 B03C1/28 G01N33/543

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)  
 IPC 7 G01N B03C

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 practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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Further documents are listed in the continuation of box C.       Patent family members are listed in annex.

\* Special categories of cited documents :

|  |  |
|--|--|
| <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> | <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&amp;" document member of the same patent family</p> |
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| Date of the actual completion of the international search<br><br><b>13 December 1999</b> | Date of mailing of the international search report<br><br><b>20/12/1999</b> |
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|--|---|
| Name and mailing address of the ISA<br>European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Authorized officer<br><br><b>Hocquet, A</b> |
|--|---|

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/IB 99/01646

| C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT |  |                       |
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Information on patent family members

International Application No

PCT/IB 99/01646

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