MagRetriever[™] - a new instrument for large-volume immunomagnetic separation Filtaflex Ltd, Almonte, ON, Canada (contact: *tsharpe@filtaflex.ca*)

Background

The fundamental step of extracting bacteria, viruses, allergens or other analytes from samples before detection can be time-consuming. In bacteriological analysis, for example, traditional enrichment may require days to bring analytes to detectable concentrations. Immunomagnetic separation (IMS) applied to samples prior to selective enrichment can often concentrate target analytes faster than older procedures. IMS reagents are usually called immunomagnetic *beads* or IMBs. The rapid decrease of magnetic field strength with distance from a magnet tends to restrict IMS to volumes of ≤ 10 mL, but for some applications ability to handle suspension volumes up to 250 mL is needed. Only one commercial system (PathatrixTM, Matrix MicroScience) currently is able to retrieve IMBs from 250 mL suspension volumes - Pathatrix pumps suspensions through a set of tubes, sponge filter and conventional magnet, during 30 minutes or more.

Filtaflex Ltd has developed a new instrument for large-volume IMS work. This document shows how *MagRetriever*TM is able to retrieve IMBs from 250 mL food suspensions in ≤ 6 minutes, and provides related information. Ability to retrieve IMBs from suspensions does not conclusively prove ability to extract a particular target analyte – Filtaflex Ltd expects initial interest will be by research groups.

MagRetriever™ is the subject of patent applications Canada 2690453, USA 12968283, and is trademarked in Canada



How does MagRetrieverTM retrieve IMBs ?

MagRetriever's proprietary *Tractor Magnet* focuses a powerful magnetic field through a *Retrieval Vessel* containing IMB suspension. Though its field strength decreases with distance far less rapidly than from a regular magnet, the field near the top of a suspension is still weaker than at the base of the Retrieval Vessel. In an initial *Streaming* phase MagRetriever circulates the IMBs closer to the most intense parts of the magnetic field, enhancing their sedimentation to the base of the Retrieval Vessel. Then in a *Compacting* phase the Tractor Magnet coerces sedimented IMBs to move towards, fall into, and become trapped in the Retrieval Vessel's central *Well*. At this point the user removes the Vessel, pipets out the IMBs and discards the Vessel. Run duration is typically 3-8 min.



During *Streaming* the user is unlikely to see much happening within the Retrieval Vessel, but MagRetriever's action becomes visible during the *Compacting* phase, as shown in these photos.



Compacting under way



IMBs falling into Well



IMBs trapped in Well

How to use MagRetrieverTM

Fill a Retrieval Vessel to the 250 mL mark with suspension, slide it into MagRetriever and press a button to begin retrieval. MagRetriever also has buttons to select different retrieval protocols and run durations if desired. For clear suspensions a Pasteur pipet may be all you need to transfer retrieved IMBs to the next stage of the analysis; for obscure suspensions a "magnetic pipet" such as PickPenTM may be preferable.

A unique advantage of MagRetriever is operational *flexibility*. MagRetriever does not commit users to a particular IMB or target-trapping protocol - it is just a tool to retrieve any IMB reagent that has been deployed in a sample suspension. Of five commercial IMB reagents tested in 250 mL of water, Tween 80, celery, carrot and lean ground beef suspensions, using a standard 6 min retrieval program, MagRetriever retrieved essentially 100% of:

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Dynabeads TM	 "uniform superparamagnetic polyystyrene, 2.8 μm dia";
MagnaBind [™]	- "silanized iron oxide, superparamagnetic,1-4 μm dia";
Pathatrix TM	- no corporate description found - irregular particles of varied size;
MagaBeads TM	- " paramagnetic iron oxide in polymer supports, 8.8 or 15.6 µm dia";
-	(Note: MagRetriever retrieved MagaBeads TM in as little as 2 min).

Interestingly, in 6 min runs with water or Tween 80 solution MagRetriever retrieved only a small fraction of:

Bio-AdembeadsTM - "Uniform superparamagnetic nanoparticles, 300 nm dia" most probably because in free suspension these tiny IMBs experience an exaggerated viscous drag and sediment slowly. But MagRetriever retrieved a large proportion of them from ground beef suspension, probably because when attached to the (more massive) beef particles remaining after prefiltration (see below), viscosity had less effect.

Images of suspensions and IMB retrieval

These photos illustrate how MagRetriever retrieved IMBs from different food suspensions. All suspensions were 25g samples in 250 mL 0.5% Tween 80, pulsified 15s and prefiltered through a paint strainer or S-90 foam-stuffed funnel. Figures at the sides of Vessels refer to MagRetriever settings - it is not necessary to know what they mean, however, for the sake of inter-sample and IMB comparison, a standard runtime of 360s was used. Many analytical situations would allow shorter runtimes.



Pathatrix™ E.coli O157 in celery



Pathatrix™ E.coli O157 below ground beef



MagnaBind[™] in carrot



viewed from underneath



and in ground beef, suspension poured off



and from above after suspension poured off

Prefiltration prior to addition of IMBs

For some samples the only pre-retrieval step needed will be to simply fill a Retrieval Vessel with suspension. With foods or similar samples one other step may be needed, as tissue debris may stop IMBs falling completely into the Vessel's Well, even with MagRetriever's powerful magnet system. Large particles may also trap and reduce the availablity of IMBs to the target. Thus it is desirable to exclude detritus from suspensions before adding the IMB reagent. On the other hand (see **Bio-Adembeads** above) if particles are not too large they may actually enhance retrieval. Food suspensions prepared by paddle-type blender usually tend to have too much detritus to be immediately used in IMS, and with some foods even a PulsifierTM may generate large particles. Routine coarse prefiltration of suspensions (below) was found to be very beneficial.







250 mL pulsified ground beef

As MagRetriever does not lock the IMB binding step to retrieval, binding the analytical target is conveniently done in a 500mL conical flask. Simply prefilter suspension into the flask, add IMBs and agitate for whatever time and conditions yield optimum binding. For prefiltration, "medium grade" paint strainers obtained from hardware stores (inexpensive and sterilizable) were found to be excellent - they filtered 250 mL of even ground beef suspension in seconds and gave excellent retrieval results. A polyester foam (S-90), which was used in the Filtaflex's "FiltaTips" pipet-tip prefilters and which does not affect viable counts from food samples, also is useful - plugs of this foam pushed into funnels rapidly filtered 250 mL celery and carrot suspensions, though with lean ground beef suspension they quickly clogged.



paint strainers make excellent prefilters



S-90 foam plugs in funnels are quite good

MagRetrieverTM specifications

MagRetrieverTM

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Size:	width 203 mm (8"); height 254 mm (10");
	depth 380 mm (15")
Weight:	9.7 kg (21.4 lb)
Power:	130/250 VAC (12VDC at instrument)
Other:	serial interface for users who wish to
	develop their own retrieval programs

Retrieval Vessel Size:

dia 110 mm (4.2"); height 61 mm (2.4") Other: Sterile, DNA-free, etched at 250 mL level. In cartons of 60, individually packed. Loose-fit lids lift off or replace easily. Etched area for writing sample IDs.

Trademark ownerships

Dynabeads	Dynal Biotech (Ullernchausseen 52, Oslo, N-0309, Norway) Dynal Biotech
MagnaBind	Pierce Biotechnology (3747 N. Meridian Rd, P.O. Box 117, Rockford, IL 61105, USA)
Pathatrix	Matrix MicroScience Limited (Lynxx Business Park, Fordham Rd, Newmarket, UK)
MagaBeads	Cortex Biochem, Inc. (1933 Davis St. Suite 321, San Leandro, California 94577, USA)
Bio-Adembeads	Ademtech. (Parc Scientifique Unitec 1, 4, allée du doyen Georges Brus, F-33600 Pessac, France)
PickPen	Bio-Nobile Oy (Tykistokatu 4B, Turku 20521, Finland).
Pulsifier	Microgen Bioproducts Ltd (1 Admiralty Way, Camberley, Surrey GU15 3DT, UK)
S-90 Foam	Engineered Foam Products Ltd (111-B Snidercroft Road, Concord, ON L4K 2J8, Canada)