The Advanced Guide to Scaling up Biomagnetic Separation Processes

Discover what you need to know to face the challenges of scaling up the manufacture of magnetic beads kits

Edited by Dr. Lluis M. Martínez

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SUMARY

About the author

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Chapter 1. How to scale-up biomagnetic separation process may increase profitability?

The main consumers of magnetic beads are In Vitro Diagnostic (IVD) companies who utilize these materials for their kits. Since some of these companies are highly successful, they obviously need to cope with higher demand for their kits by increasing production.

One way of increasing production of biomagnetic separation processes is to maintain the working volume, but increase the number of lots. Doing this would necessitate increasing the number of highly skilled workers needed to perform the separations. In addition, strict quality control would need to be enacted to make sure that each lot falls within an acceptable range of functionality and yield.

A better way of increasing production of biomagnetic separation processes is to increase the lot volume. Increasing lot volumes allows companies to maintain labor and QC costs, while simultaneously decreasing the cost per unit of materials, thus maintaining or decreasing the overall cost of producing the IVD kits.

The main limitation to increasing lot volume is the difficulty in keeping the working conditions and variables constant. In classic magnetic separators, the magnetic force changes as the volume changes. This means that conditions validated at small volumes cannot be automatically translated or reproduced at larger volumes. Thus, the advantages to scaling up (i.e. decreased cost of materials and workforce) are essentially lost because of the extra cost involved in validating the conditions at the new production volumes.

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Chapter 2. Why the force over different volume bottles is constant in advanced Biomagnetic Separation Systems?

The most common mistake when attempting to scale up production of magnetic beads using classical magnetic separation devices is to use the same magnetic field that was used at smaller volumes. But keeping the magnetic field constant at different volumes will not give you the same results because the separation conditions are completely different.

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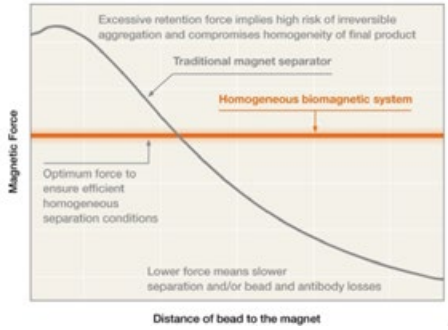


In this case the force is proportional to the gradient of the square of the magnetic field, the force that the beads experience in classical magnetic separation racks will change with the distance from the magnet. This is a major problem when scaling up production because beads experience very different conditions relative to what they experience during the production in smaller volumes.

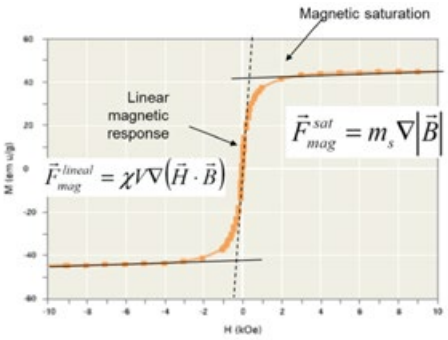
Advanced biomagnetic separation systems such as SEPMAG, however, generate homogeneous forces that do not vary with bead distance from the magnets. The force is maintained at a constant level by keeping the field high enough to magnetically saturate the beads. This allows the field to change linearly with distance. In this case, the distance of the beads from the magnet would have no effect on the results of the production and, therefore, the volume of the lot would not affect the separation behavior of the production process. The magnetic force over the beads will be the same in all the cases, then also the separation speed.

Therefore, using more advanced homogeneous biomagnetic separation systems allows your company to scale up production without the need for lengthy and costly validation experiments since the separation conditions do not change with volume size.

Chapter 3. Two conditions to have constant magnetic force regardless the lot volume

It is vitally important to understand the process and all of the variables of the process when scaling up a biomagnetic separation. If you do not understand the details of your process, you will throw away your initial investment you made in validating your initial process and jeopardize the product's time to market.

In biomagnetic separation processes, magnetic beads move because the magnetic force is greater than the drag force on the beads due to the buffer viscosity. Therefore, the key parameter to understand is the magnetic force. The magnetic force depends both on how the magnetic field changes spatially (i.e. the magnetic field gradient) and on the strength of the magnetization of the magnetic beads.



The two conditions that must be met in order for a constant magnetic force to be maintained in biomagnetic separation processes are:

1. The magnetic field needs to vary linearly with the distance of the beads from the magnet.
2. The beads should be magnetically saturated so that the field is high enough (e.g. B < 0.1 T for magnetite).

The newer, more advanced homogeneous biomagnetic separation systems such as SEPMAG fulfill these two conditions in virtually any volume desired. This is accomplished because the homogeneous systems are designed with a cylindrical geometry comprised of a constant radial magnetic field in the core. In these systems, the gradient is adjusted so that the magnetic field is over 0.1 T everywhere except in a small area around the axis.

Because of this constant force in the advanced systems, SEPMAG guarantees that no less than 93% of the beads are saturated when the process starts. After a few seconds, all of the beads have moved out of the central region and are in optimal conditions. The initial 7% of beads not in optimal conditions are considered the practical limit of the device.

Advanced homogeneous separation systems such as SEPMAG are therefore, easy to scale up because these conditions are determined in smaller volumes and remain the same when scaled up to larger volumes. When using standard magnetic separation racks, volume matters because the beads feel different forces relative to their distance from the magnets. Scaling up a process with standard devices forces the company to conduct validation and quality control experiments in order to find the new correct parameters in the larger volumes.

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Chapter 4. How to deal with the 4 typical magnetic separation beads scale up problems?

In standard magnetic separation racks, scaling up to a larger lot volume usually creates four common problems:

1. Larger batches do not have the same characteristics as smaller lots.
2. The percentage loss of magnetic beads and biomolecules increases.
3. Irreversible aggregation becomes a serious issue.
4. Different aliquots (i.e. single IVD kits) of the same batch in large volumes have very different characteristics.

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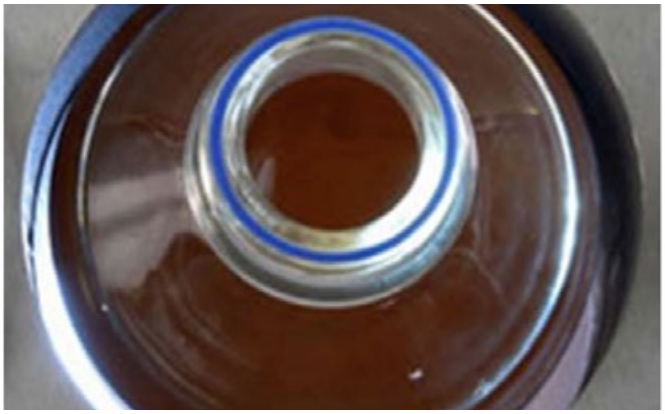
These problems are common in classical magnetic separation racks because these devices do not generate homogeneous magnetic forces. In classical systems the force on the bead varies with the distance from the magnet. Beads far from the magnet experience very low forces and beads in the retention area experience high, sometimes excessive, forces. Because the forces the beads experience are not constant, the characteristics of beads in larger batches will vary in depending on where in the container they were at the start of the separation process.. In addition, lot-to-lot variation is common because forces between lots of different volumes are not consistent.

Irreversible aggregation increases in classical magnetic separation racks It takes either longer separation times or higher forces to move distant beads to the retention area. Beads that are exposed to higher forces over longer periods of time are highly susceptible to irreversible aggregation. If you choose not to increase force in order to avoid problems with the beads near the magnet, you will suffer a decrease in magnetic bead yield.

Homogeneous systems such as SEPMAG maintain constant force on the beads no matter where the beads are in the container. Since the force is constant, the above limitations disappear. If the value of the magnetic force is chosen wisely, you can capture the beads lying far from the magnets while maintaining a gentle force in the retention area, thereby eliminating material losses and irreversible aggregation.

Chapter 5. How to avoid In-lot inconsistency when scaling up biomagnetic separation processes

Small volume classic magnetic separation racks are relatively cheap and do a fairly good job at separating magnetic beads. However, if the process involves multiple instances of capture and elution steps, irreversible aggregation becomes a real problem. In small volume separations (i.e. on the order of milliliters), using the appropriate techniques can give you excellent re-suspension results.



Large volume classic magnetic separation racks, however, typically have serious problems with irreversible aggregation. The way to avoid losses in magnetic bead yield is to increase the magnetic force. Since magnetic force in classic separation devices varies with distance, increasing the force causes beads close to the magnet and in the retention zone to experience extremely high forces over a longer period of time. Consequently, the magnetic beads have a higher probability of being irreversibly aggregated, forming clumps by overcoming the electrostatic barriers that normally keep the beads apart from one another. When the electrostatic barriers are breached, the beads become cross-linked. This irreversible aggregation and clumping effect in-lot consistency adversely because aliquots for individual kits from the same large volume lot are highly variable. Some beads were exposed only to a low magnetic force and some were exposed to very high forces.

There are two possible ways to deal with these inconsistencies:

1. You can devote a large amount of resources (money, materials, time, and personnel) toward recovering beads from the aggregates (i.e. using sonication and long sonication/homogenization cycles).
2. You can use a homogeneous biomagnetic separation system like SEPMAG.

Choosing to change production to an advanced

homogeneous magnetic separation system like SEPMAG is easier and cheaper in the long run than choosing to recover already aggregated beads. Beads produced with a homogeneous system and a wisely chosen magnetic force value will produce lots that are consistent throughout the entire volume since the same force is felt by all of the beads. In addition, irreversible aggregation is very rare in these systems. Therefore, SEPMAG systems will both increase in-lot consistency and will treat all beads gently and equally during the separation process.

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Chapter 6. Keeping lot to lot consistency when using Biomagnetic Separation at different volumes (from 1ml to 20L)

Classic magnetic separation racks are designed such that both the magnetic field gradient and the magnetic state of the beads vary with the position of the beads. This means that once a process has been validated at a specific volume (i.e. your process has low material losses and no irreversible aggregation), it is difficult to change batch size without needing to re-validate the process. In these devices, changing the volume means that the separation conditions (i.e. magnetic force vs. the distance of the beads from the magnet) are completely different from the original validated conditions.

One way to deal with differences of magnetic force with different production volumes in magnetic separation devices is to redesign the geometry of the device. But this also introduces a very different magnetic force map that will force the separation process to be re-validated with the new conditions. Neither increasing the volume with the original geometry nor redesigning the geometry of the classic magnetic separation racks will allow for lot-to-lot consistency when scaling up production.

The best option is to use advanced homogeneous biomagnetic separation devices such as the SEPMAG. Once you have correctly defined the optimal magnetic force in a homogeneous magnetic separation device, it is very simple to scale up production using the same validated conditions in a larger volume, with a different homogeneous magnetic separation system. It is important to note that the optimal magnetic force must be low enough to avoid clump formation (i.e. irreversible aggregation) and high enough to retain the beads while separating out the supernatant.

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Typically when using the SEPMAG devices, you would start with the SEPMAG A system which allows production of small volumes of beads (from 1 – 250 milliliters); validate the proper conditions for separating your beads; then scale up using the SEPMAG Q system for lot volumes of 1 Liter or greater. The SEPMAG Q system would utilize the same magnetic force you deemed optimal in the SEPMAG A system for your particular process.

Chapter 7. The secret to avoid magnetic bead (and biomolecule) separation losses when scaling up

When using standard magnetic separation racks, often you will experience a decrease in bead and biomolecule yield when scaling up your production process. This causes the scaled up process to be less economically efficient than it could be with yields commensurate to your original production.

Decreased yield during scaling up in inhomogeneous systems is largely due to the fact that the magnetic force varies with the distance of the bead to the magnet. The force that more distant beads feel is very low and increases quickly as you reach the retention area, close to the magnet. Lower forces cause beads to move slower. A combination of slower bead movement and longer distances for the beads to travel when using scaled-up volumes, results in very long separation times. If you choose to pump the supernatant out before these long separations are completed, you will subsequently have large losses of magnetic beads along with losses of the expensive biomolecules attached to them.

One option is to increase the size and weight of the magnetic separation device. This helps you gain increased magnetic force over the farthest beads, but doing so will also have a deleterious effect on the beads already in the retention area. When these beads are exposed to high forces over long periods of time, irreversible aggregation becomes a very serious issue. Aggregation causes the beads to form clumps that are tightly associated with each other. In a best case scenario, the clumps can be disaggregated without damaging the functional biomolecule, but at the cost of time, resources and personnel. You would need to subject the beads to added sonication steps along with several extra quality control checks in order to monitor the resuspension process.

By far, the best way to avoid all of these potential difficulties during magnetic separation is to generate a high force at distance from the magnet without increasing the force in the retention area. Homogeneous magnetic separation systems such as SEPMAG can accomplish this task. In systems such as SEPMAG, we choose the optimal magnetic force and you will not generate irreversibly aggregated clumps. In addition, since the magnetic force is constant across the entire volume of the vessel, separation times are shorter and material losses are minimal.

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Chapter 8. Scaling Biomagnetic Separation Process avoiding irreversible aggregation problems

A very common problem that occurs when scaling up inhomogeneous magnetic separation processes is irreversible aggregation of the magnetic beads. When the process is scaled up, the magnetic force experienced by the beads closest to the magnet is very high. Since the separation time typically must be increased in larger volumes in order to increase bead yield, the risk of irreversible aggregation and clump formation greatly increases. This can decrease yield and functionality of the beads by damaging the beads or by damaging the biomolecules attached to the beads. In addition, not all of the clumped beads will be recovered.

The main way to rid your production run of irreversible aggregated clumps is to use repeated cycles of sonication and homogenization. However, this implies the addition of several steps to the process, invest in new equipment, and additional QC points to ensure the re-suspension is correctly done.

The ideal way to deal with aggregation is to avoid it completely. You can avoid irreversible aggregation by decreasing the magnetic force in the retention area. In you are using an inhomogeneous magnetic separator rack, reducing the magnetic force near the magnet decreases the force felt by distant beads in a non-linear fashion. Unfortunately, this can lead to greater material losses and much longer separation times.

Optimally, you can use a homogeneous biomagnetic separation device where the magnetic force remains constant throughout the entire volume of the vessel. This makes it possible for you to reduce the magnetic force in the retention area without decreasing the force significantly over the beads that are more distant from the magnets. If your constant force is gentle enough, it will be below the irreversible aggregation force threshold. Even though this force is considered ‘gentle’, the force experienced by distant beads is still much higher than that experienced by distant beads in standard inhomogeneous magnetic separation rack. Therefore, homogeneous biomagnetic separation devices can speed separation while at the same time decreasing or eliminating clumping.

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Please note that while we are talking about gentle retention forces in homogeneous systems, the force should still be high enough to avoid excessive loss of beads that may remain in the supernatant. SEPMAG systems are designed to allow you to easily eliminate clumping, reduce separation times and at the same time, avoid undue loss of material.

Chapter 9. Permanent magnets vs. Electromagnets: Cost, maintenance, weight, reliability and other considerations for scaling up magnetic beads separation processes

Electromagnets are the classical way to generate intense magnetic fields. If you apply the electrical current across a coil, the magnetic field is quite small. But if you wrap the coils around an iron yoke, you can generate much stronger magnetic fields.

Unfortunately, if you need to scale up a magnetic separation process, you also need to increase the electrical power to the device and the amount of iron and copper used for the coil. The heat generated by the resistance of the larger coils will be significantly greater as you scale up and will require a substantial electromagnet cooling system. Theoretically, you can adjust the values of the magnetic fields or change the field profiles that generate the magnetic force in biomagnetic separation devices.
However, once you produce large lots you will need to accurately control the electromagnet adjustable parameters (electrical current, pole pieces, system temperature). In production facilities you will need to ensure you are always using exactly the magnetic separation conditions you have already validated.

Therefore, when you scale up a magnetic separation system that uses an electromagnet, you must consider the additional cost of the following:

- Much larger electric bill
- Power supply maintenance (for a power supply compliant with the Electromagnetic compatibility of your lab)
- Larger floor space
- A refrigeration infrastructure able to flow enough water to control the temperature of your device
- Maintenance of used coils, isolations, electronics and yoke.

MRIs typically use superconducting coils to avoid many of the above problems. However, for a system similar to an MRI, you will need to cryogenically cool the coils, making this technology far too expensive for a biomagnetic separation device.
A better alternative to electromagnets is the use of Rare Earth Permanent Magnets to generate the required magnetic field profile. Homogeneous biomagnetic separation systems can utilize these Rare Earth Magnets because the parameters of the separation process in these devices can be well defined (e.g. the optimal magnetic force and the field profile necessary to magnetically saturate the beads). With Rare Earth Permanent Magnets, conditions comparable to those using Electromagnets can be achieved with less weight, no need for cooling systems, no electrical power, no power supply and no maintenance costs. If the device is used at temperatures less than 80oC, conditions will remain constant for decades.

Therefore, Rare Earth Permanent Magnets provide a solution that gives you long term stability, a small footprint, and a one-time upfront cost for the system itself with no maintenance fees. Compared with Electromagnets, Rare Earth Permanent Magnets are a much more cost effective and reliable way to power biomagnetic separation devices.

Chapter 10. How to Avoid Safety issues when scaling up Biomagnetic Separation Process

Small magnetic separation racks (i.e. the types used to develop a prototype product before scaling up) generate magnetic fields that decay rapidly with distance. However, scaling up the process can be problematic because the size of the classical magnetic separation rack itself grows rapidly with desired batch volume. Because the magnetic field profile and the magnetic force are not the same in a larger device, the safety of users and the safety of ancillary equipment can become a serious issue.

Risk can be divided into two major zones:

1. Caution Zone: For fields > 0.5 mT (5 Gauss), ancillary equipment such as pacemakers, computers, magnetic recording media, credit cards and other such devices are at risk of being destroyed or altered. For this reason, MRI areas in hospitals will always warn you not to bring any electronics into a specified region surrounding the MRI system. A similar low level equipment risk area needs to be cordoned off for large classical magnetic separation racks.

2. Danger Zone: Fields > 3 mT (30 Gauss) pose a serious risk of mechanical injury for people working near the biomagnetic separation device. Ferromagnetic objects are strongly attracted by a field this strong and will move toward the magnet at increasing force and speed that can seriously injure a person in the path of the object. Since classical biomagnetic separation racks increase magnetic force quickly as you approach the magnet, there is very little time to react when you hold an ferromagnetic object (as scissors, screwdrivers or other magnet) and this is attached to the magnet. You have a highly chance that your hand, fingers or other part of the body keep trapped between the object and the magnetic separation rack, become seriously injured due the high forces involved.

The most obvious way to decrease these potentially dangerous stray magnetic fields is to use a closed system so that the risk is confined to a very small area immediately surrounding the magnetic device. Unlike classical biomagnetic separation racks advanced biomagnetic separation systems such as SEPMAG have been designed to reduce the caution and danger zones as much as possible, thus decreasing the lab space necessary for operating your system and increasing safety for workers.

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Chapter 11. Why separation time is not the critical parameter when scaling up magnetic beads separation

Although separation time may be one of the most obvious parameters to validate in your biomagnetic separation production, it is certainly not the most critical. Classical magnetic separation rack use inhomogeneous magnetic separation. When scaling up production on these devices, larger volumes can lead to longer and longer separation times. The way to keep separation times constant in classical systems is by increasing the magnetic force on the beads farthest from the magnets. But increasing the force on these distant beads will necessitate increasing the force by a great deal in the retention zone. Magnetic beads in the retention zone exposed to such great forces over time will irreversibly aggregate, causing a great deal of difficulty in the final separation. It is very difficult to balance time of separation and strength of magnetic force so that you have a yield that is comparable to smaller production volumes.

The way to keep separation times constant in classical systems is by increasing the magnetic force on the beads farthest from the magnets. But increasing the force on these distant beads will necessitate increasing the force by a great deal in the retention zone.

In order to easily move from one volume to another in biomagnetic separation processes, it is imperative to have well-defined conditions for the separation. Homogeneous biomagnetic separation systems such as SEPMAG allow the magnetic force to remain constant throughout the entire volume of the device. If the magnetic force is well defined, it is easy to scale up production because the parameters do not need to be changed.

A well-defined magnetic force is a force that is high enough for the beads to be magnetically saturated, but low enough that irreversibly aggregated beads do not form. The force must also be high enough to retain the beads during supernatant removal. With the optimal magnetic forces known for various volumes, the only parameter that needs to be determined in the new volume is the correct time of separation. The separation time for larger systems can be estimated as:

$$t_{large\ volume} = t_{small\ volume} * (R_{large\ volume} * F_{large\ volume}) / (R_{small\ volume} * F_{small\ volume})$$

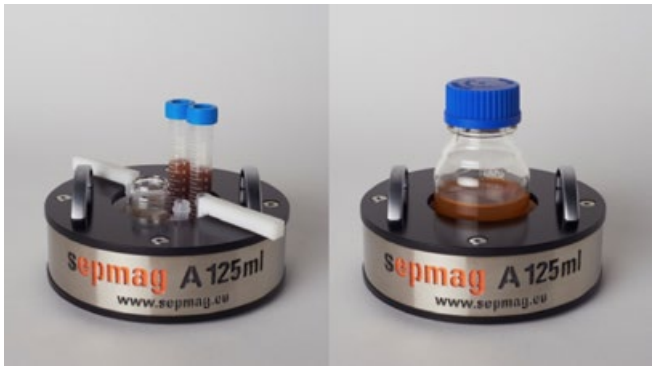
Where R is the distance travelled by the farthest beads and F the magnetic force of the device. The magnetic bead separation speed is constant and is proportional to the magnetic force. Therefore, the separation time is only dependent on the distance travelled by the beads to the retention area (usually the vessel radius).

Unlike classical systems, SEPMAG's homogeneous magnetic separation systems can easily monitor homogeneous magnetic bead separation conditions by using built-in optical sensors, further simplifying the validation and quality control process. By making sure that all parameters are well-defined, scaling up using homogeneous systems becomes a much easier task performed at a fraction of the cost.

Chapter 12. How to correctly use small containers on large biomagnetic separation systems

Sometimes during biomagnetic separation, different steps in the process require using different volumes. For example, if you have produced a 'mother batch' of magnetic beads and this large batch needs to be aliquoted so that each aliquot of beads can be uniquely coated, you will need the ability to move easily between volumes.

With inhomogeneous biomagnetic separation rack, this flexibility is not practical because magnetic forces change quickly with distance from the magnet. Therefore, beads in each volume used will be subjected to varying magnetic forces, even if the same separation device is used.



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Since the force is the same the beads in a large bottle will experience the same force as the beads in a small bottle. What is crucial, however, is using the correct adaptors in order to guarantee consistency between the different volumes.

Another reason to utilize various volumes during a process is to validate the process at various steps using small aliquots, but the same conditions in homogeneous devices. For example, if there are concerns about irreversible aggregation, smaller volumes can be run using the same conditions as larger volumes, in order to determine how to deal with the situation. The only difference between the two volumes would be the distance the farthest beads must travel to the retention area. To be sure clumps are not formed in the retention area, you can experiment with separation times, testing beads in the retention area to see what the maximum separation time can be for your particular production process without irreversible aggregation occurring. If clumps do not form in the retention area during the small scale production, there is no reason to believe that no clumps will form in the larger volumes under homogeneous conditions.

Therefore, making sure the adaptors are properly positioned and utilized, understanding the difference in separation times and optimizing the magnetic force will allow you to use small vessels in larger biomagnetic separation systems effectively.

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