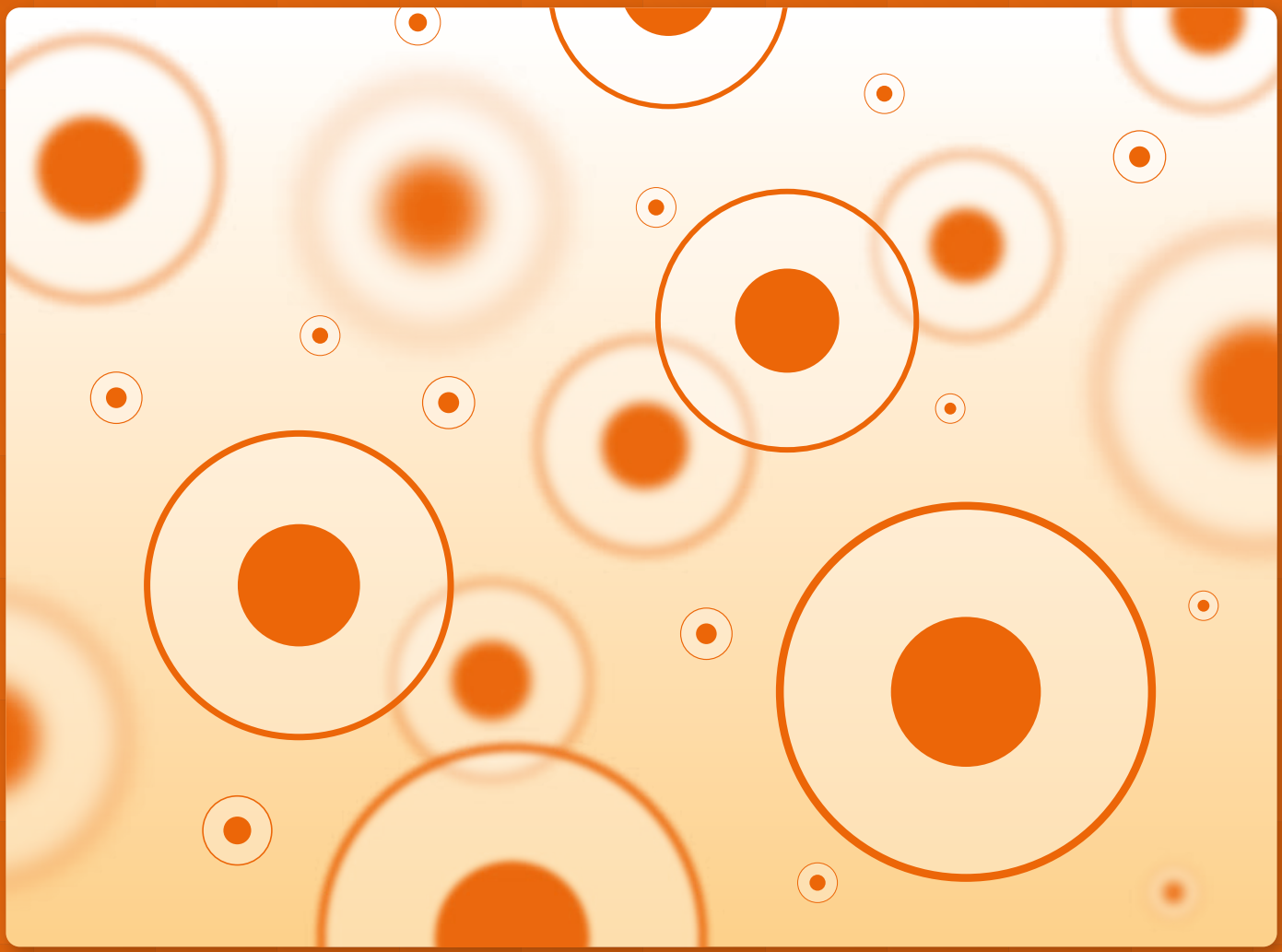
The Basic Guide to Use Biomagnetic Separation in Production Processes



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SUMARY

Introduction. Set up of Biomagnetic Separation Production Processes

Scientists in academic research labs and pharmaceutical labs, use magnetic bead kits for immunoassays and separation science. Doctors, lab technicians and scientists use magnetic beads in IVD kits as molecular diagnostics devices. Even though scientists typically use these beads at any given time in relatively small quantities, once the kit hits the market and is a product that has proven useful, the production volume for the kit must necessarily increase to meet demand. One needs to provide kits to many hospitals and labs globally and therefore one needs a cost-effective way to produce magnetic beads.

But since scientists only need a very small quantity of beads for any given use, why is production a problem? Normal lab and development techniques, while effective in the initial development and testing stage, are not effective in coping with the increased costs and attention to quality necessary in ramping up production volumes. Often there are in-lot inconsistencies, a great deal of material loss (from both beads and biomolecules), and aggregation of beads. In addition, when one has to use larger magnets, there are greater safety and health risks that must be considered.

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This guide was developed to help one review the key aspects of planning and creating a biomagnetic separation production facility for magnetic bead-based products. This manual further guides the facility toward separating the magnetic bead-based products as efficiently, safely and consistently as possible without incurring the typical problems of aggregation, material loss and in-lot inconsistencies. When a production facility is efficient, it will not waste necessary time, effort and resources.

1. Production of magnetic bead kits: To be or not to be magnetic.

What are the problems of classic separation techniques? Typically the classic ways to produce magnetic bead reagents and kits are slow, very high maintenance and costly to run. The three classic techniques, centrifugation, filtration and tangential filtration, are not straightforward techniques.

A company that produces magnetic beads-based products starts with the raw materials, namely, magnetic beads and antibodies (or other biological material). They incubate the two together and create a bead with an attached functional biological molecule. This is called 'functionalizing the beads'. Once the beads are functionalized, they are then separated from the supernatant, wash the beads and then aliquot the functional beads into doses. These doses will be placed in individual IVD kits and sold to researchers, hospitals and other biotech institutions.

The key aim of any production process is consistency. One does not want variability within a kit or between kits from a particular batch or even variability between batches if that is achievable. Normally most processing methods in the past used the classic techniques of centrifugation, filtration and/or tangential filtration. But these techniques are quite slow. For example, centrifugation takes time and material is lost in the filtrate; filters can clog; filters can change their performance over time and these pieces of equipment need a lot of care and maintenance to keep them running in good condition. Filtration methods require the operator to set the flow, the temperature, the pressure, the warm up time, and clean the filters regularly.

While the above mentioned classical techniques are the only ways to separate beads from a solution when the beads are not magnetic, biomagnetic separation wisely takes advantage of the properties of magnetic beads to more quickly and cleanly achieve separation. In addition, biomagnetic separation operates with minimal maintenance and very low costs. Even so, this technique is not guaranteed to give one consistent results unless one diligently validates the process and ensures that the parameters for the process are well established. This will be further explained, but in brief, homogeneous conditions that are well-controlled will provide consistent results from lot to lot and within a particular lot.

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2. Common mistakes that lead to inconsistency

Often when a lab produces a product that becomes popular, the impetus is to move forward and scale up production of that product. The problem is that moving from the production of small lots to full scale production usually produces surprising results. Scaling up is not trivial. When one scales up production, results become very inconsistent. Why would this be? Why can't researchers and technicians take the parameters from small production processes and translate them directly into large production processes?

The answer is quite simple. In small lab lots separation conditions are often defined on a very specific device for a very specific separation time. The process is dependent on empirical experience and on the condition of the machine at any given time. Usually this device used for lab lot production for testing and development of a product is a simple device with single magnets. Furthermore, the final use of IVD kits is typically in an automated diagnostic machine and so these lab lots are developed with that usage in mind.

But for all of these aforementioned cases, the linear and saturated magnetic state of the beads during the actual process is neither well-known nor well-quantified. If that is the case, then the magnetic force is also not well-defined. If one does not know these parameters well, then one cannot by extension, transfer well-defined biomagnetic separation conditions to a large scale production. When this happens, the consistency of the batches is greatly compromised.

There are two alternative solutions to these problems. The expensive solution is to investigate and define, in detail, what conditions are used in small scale production. Then, moving to larger scale production, try to redefine the conditions as best as possible. The better and less expensive solution is to use homogenous biomagnetic separation conditions from the start, since scaling up homogenous processes is straightforward.

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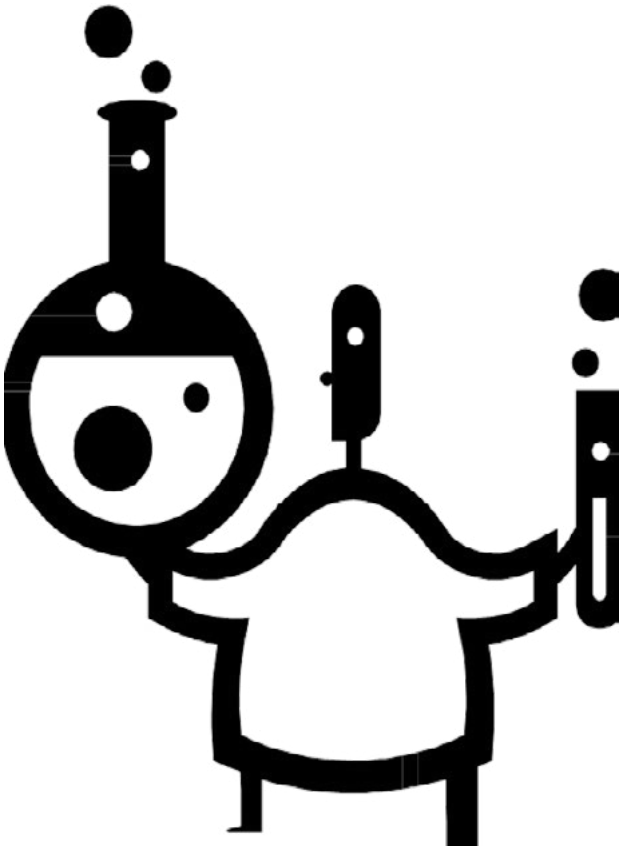

3. How do non-homogeneous magnetic separators jeopardize lot consistency?

It is understandably important to end users that every kit within a particular lot have the same properties. In other words, when one is producing lots of material to be used in an IVD kit, one necessarily strives for maximum reproducibility and minimal variability. With standard magnetic separators, it is very difficult to achieve this goal.

Standard, or non-homogeneous, magnetic separators generate magnetic field profiles whereby the forces felt by the magnetic beads vary with distance from the magnet. Magnetic beads sitting far from the magnet experience weak forces and therefore take a long time to reach the retention position, a position with higher forces. Therefore, these beads experience weak forces throughout most of the total processing time. Magnetic beads sitting near the magnets and near the retention position, however, experience very large forces throughout most of the total processing time. In other words, the magnetic forces experienced by different fractions of the beads are very different during the total processing time.

To keep losses at economical reasonable level, it is necessary use long separation times for capturing the beads experiencing low force. The longer the time, the greater the probability that the beads experiencing strong forces from the beginning of the process will undergo aggregation. Aggregation of magnetic beads is detrimental on many different levels because it is difficult to recover material and to gently separate the aggregates after they form. Aggregation costs time, money and effort that could be used in other ways.

Not only is aggregation wasteful, but if beads are disaggregated, the formerly aggregated beads seem to have different functional responses than beads that have never been aggregated. Therefore, kits will have aliquots of mixed material that have beads that have never aggregated, beads that are aggregated, and beads that were aggregated but are now disaggregated. Beads with different history would have different functionality. Because of this variable mix of functionality, different aliquots or different kits will likely have variable responses in the hands of the end user. Unfortunately, the high kit-to-kit variability necessitates costly quality assurance measures and controlled conjugation steps. This added cost can jeopardize the commercial success of the final product. The only way to reduce such risks (i.e. cost override and quality control issues) is to have as much control as possible over the biomagnetic separation conditions.

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4. Why do bead losses increase during production?

One of the biggest problems of producing magnetic beads when scaling up the production is that compared with smaller lot production, larger lot production seems to result in a much larger disproportionate loss of beads. This seems to happen even when the beads are produced in conditions that are similar to the small lot production process. The assumption is that when you scale up a process, you will have greater efficiency, but this does not happen when scaling up production of magnetic beads using classical separators.

What is going on? When one uses standard (non-homogeneous) separations devices, the magnetic field decreases with distance from the magnet. The magnetic forces are related to the field variation and so also decrease with distance from the magnet. When one scales up magnetic bead processing, the distance from the magnet increases, but the weight of the magnet cannot be increased proportionally to offset this distance. Worse yet, both the magnetic force and magnetic field usually scale up differently with an increase in magnet volume.

The consequence of the above is that in larger lot volumes, magnetic beads situated farther from the magnets experience lower forces. Lower forces increase the loss of beads during the processing time and also (because they are attached to the beads), a loss of biomolecules. If you want to overcome this, you need to wait more time to allow the farther beads to travel to the retention zone. But if one does this, the beads that are close to the magnet will experience high magnetic forces for a longer period of time and increase the probability that beads will aggregate. Therefore, one has to balance material loss due to distance from the magnet with material and functional loss due to aggregation.

The only way to overcome losses resulting from the above problems is to change from using a standard biomagnetic separation device to using one of the new devices that generates homogeneous forces regardless of the bead distance from the magnets.

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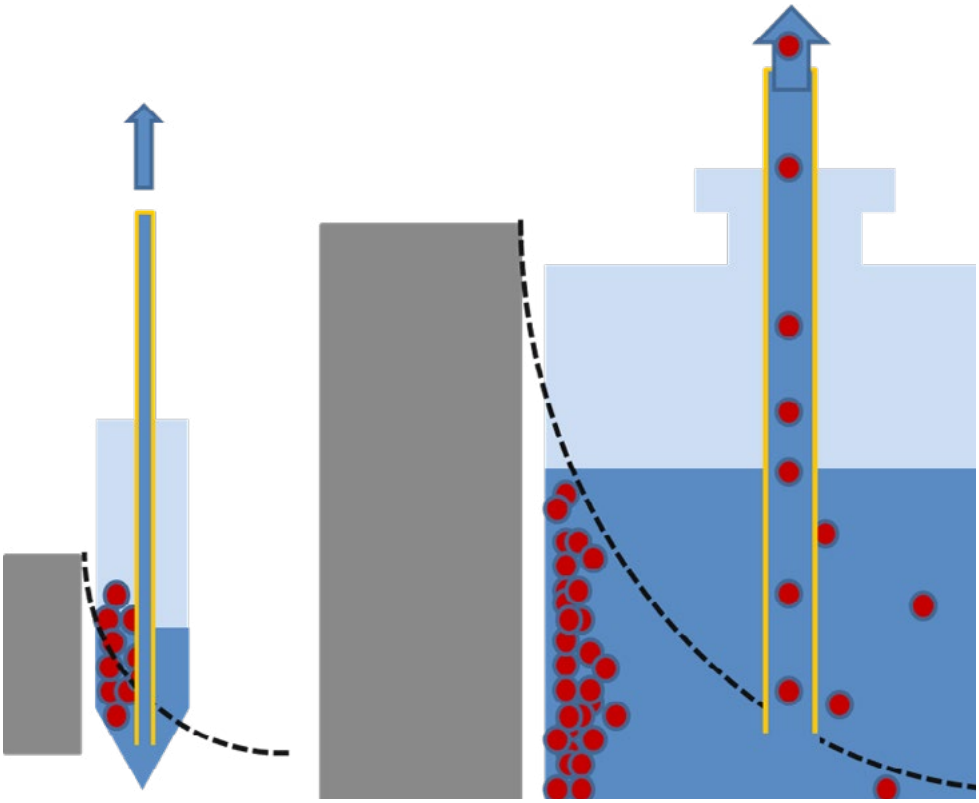

5. Why does separation time increase more quickly than the production volume?

A recognized problem in the biomagnetic separation industry is that when one increases the batch size to scale up production of magnetic beads, the separation time increases unproportionally to the increase in volume if one is working with standard magnetic separation devices. The fact is that classic magnetic separation systems generate, by their very nature, non-homogeneous magnetic fields which in turn generate non-homogeneous forces on the magnetic beads. Standard magnetic separation devices are not optimized to allow the beads to experience the same magnetic states (either linear or saturated).

Instead, under standard magnetic separation conditions, some beads are saturated and some beads are in the super paramagnetic range. In addition, these devices do not generate a controlled magnetic field pattern, thus preventing the technician from developing a clear understanding of the parameters necessary for efficient and consistent production.

If the magnetic beads are not saturated, thus are farther from the magnet, they will have a small magnetic moment and it will be difficult for them to interact with neighboring beads. When beads are saturated, the beads interact with each other and form long chains, allowing them to move more quickly to the retention zone. The result of non-saturation is that every bead will move as an insulated bead and will not be able to take advantage of the movement properties of the long chained-beads.

Individual bead movement can actually be several orders of magnitude slower than the movement of collective chains of beads. On a small scale, this is not a problem because separation takes only seconds to minutes. But if one moves to a larger volume, this difference is pronounced and full separation takes many hours or days instead of seconds or minutes.

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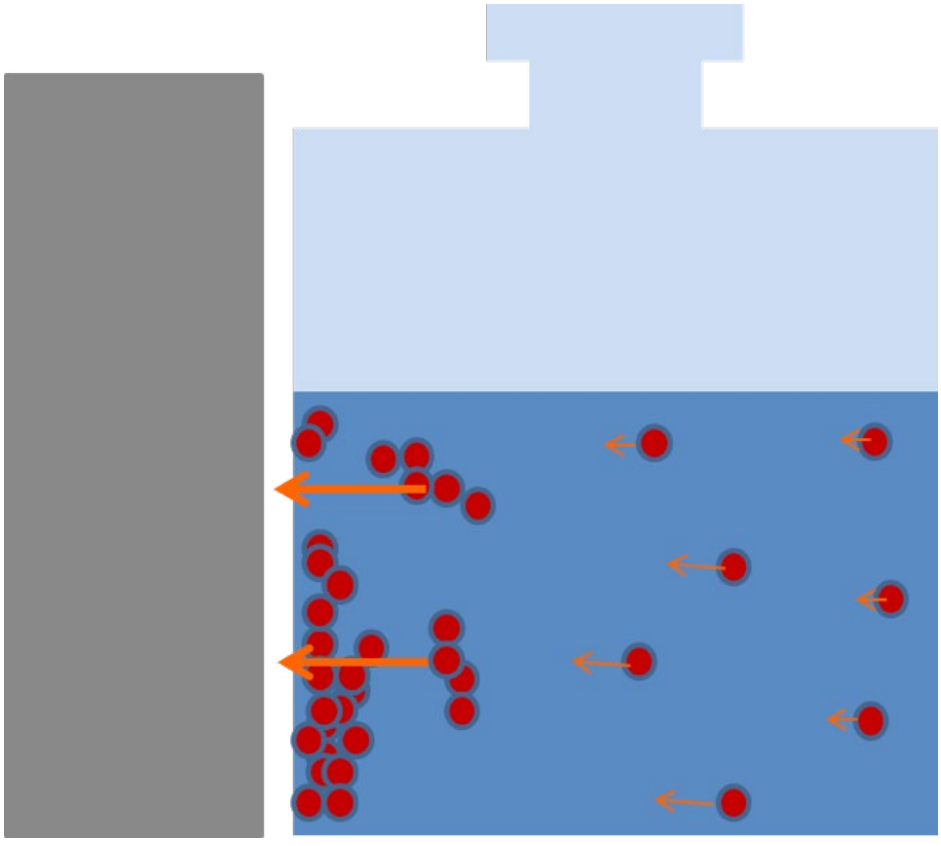

6. Safety Risks using Magnetic Systems in Production

When using biomagnetic separation systems, customers are always curious about how to comply with the various health and safety regulations that are in effect. When customers use small systems for their small scale development work, there is very little risk from the magnets. The only risk would be if the technician has a pacemaker and in that case, they would be extremely careful around even the smallest system. There is also a small risk of pinching one's fingers between two magnets. In terms of risk other than health, even a small scale magnet can erase your computer's magnetic hard drive, so computers need to be shielded and/or kept far from the device.

When a customer uses biomagnetic separation systems for production, it implies that one is using a large system that can generate very strong magnetic fields. In this case, the system can cause real harm and so workers and technicians should be on much higher alert than with smaller systems. Typically two areas are defined for magnetic systems: the caution area and the danger area.

The danger area is defined as the area where the magnetic field is higher than 3mT (30 Gauss). In the danger area, there is considerable risk of injuries due to mechanical problems. Forces from the magnets are so large that they can easily attract steel objects or tools and magnets can attract each other strongly. It is not a rare occurrence for workers to suffer from broken bones or a loss of fingers when precautions are not followed strictly.

The caution area is where the magnetic field is less than 3 mT, but more than 0.5 mT (5 Gauss). In this area, people with pacemakers are at risk and magnetic storage devices or electronic devices are at risk. Standard biomagnetic separation systems are open systems that allow large stray fields measuring as caution or danger zones as defined above. Therefore, there is a need to designate a large area surrounding standard devices so that safety areas can be delineated, even if the area is a clean room.

Alternatively, newer technologies apply closed systems with a very low level of stray fields, therefore with much smaller danger and caution areas. These devices should be used in places where space and/or safety is an issue.

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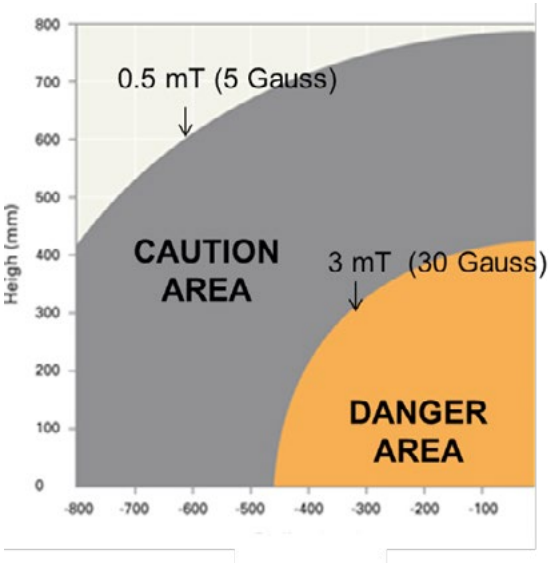

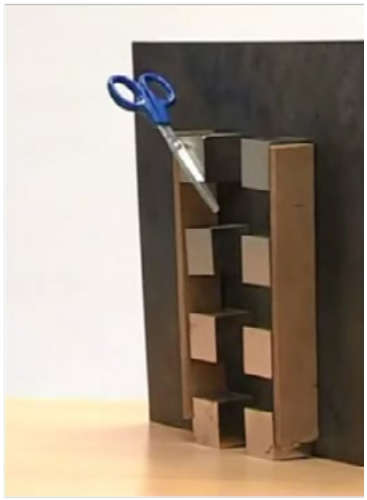

7. How to save space in the clean room

Classic magnetic separation equipment requires a large amount of space in order to comply with health and safety regulations. While using biomagnetic separation has numerous advantages, the magnetic fields surrounding the devices may be so large that they fall within the ‘danger’ and/or ‘caution’ areas. If that is the case, these areas must be designated in compliance with the laws and extra precautions need to be taken by all workers around the equipment. Danger areas are areas where the magnetic fields measure 3 mT (30 Gauss) or more. Caution areas are areas where the magnetic fields measure less than 3 mT or greater than 0.5 mT (5 Gauss).

Classic biomagnetic separation devices, by design, are open systems and therefore allow very large stray fields to escape. It is unfortunate that these stray fields can interfere, even at a great distance, with devices or systems containing magnetic components or recording media (as computers, magnetic cards, pacemakers). In addition, these stray fields can attract objects made of iron or steel (ferromagnetic objects), which can result in grave human injury.

Very large areas within the laboratory should be completely cleared of instruments and other objects to avoid potential injury by flying objects. It is not rare that people incur broken bones or other serious injuries from not taking the proper precautions and making sure that ferromagnetic objects are not in the same room as the biomagnetic separation device.

People with pacemakers should completely avoid being near these devices, but at the very least should stay outside of the ‘Caution’ zone.

Closed and/or well-shielded biomagnetic separation systems (where the majority of the magnetic field is contained) should be chosen if lab space is limited and costly. This newer type of system should be chosen especially if one needs to work in a controlled environment.

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8. Problems that stem from linking your production process to a specific type of equipment

When a lab has finally optimized their production process, they often link their process to a very specific piece of equipment and, by extension, have locked themselves into a constant volume. Often a lab develops its biomagnetic separation production process with a specific magnetic separation device – this is normal. Usually the only parameter that needs to be adjusted during production is the separation time.

If the biomagnetic separation device that is being used is non-homogeneous or if the conditions (parameters) are unknown, this process will not be reproducible when moving to a different device. The result of this is that there is a bottleneck in production because the lab has to determine how to optimize the production process on a different machine. This also often means that the lab has to run two duplicate lines for verification and quality control purposes until the new parameters are determined. All of this costs extra time, effort and money, essentially negating the cost reductions realized when the production is scaled up. Therefore, it is very important that when parameters are being defined, that the parameters are defined independent of the equipment. It is important to define parameters based on the properties of the material and the magnetic force, regardless of the vessel volume. By using a homogeneous biomagnetic separator like Sepmag, this becomes much easier.

If the parameters are equipment-independent and based on homogeneous conditions, changing the production process by scaling up or down does not need to include a bottleneck in production, but rather involves merely recalculating the separation time based on the speed of bead movement, since the magnetic force experienced by the beads is constant. Any new equipment should generate the same conditions. If this is true, separation time is merely a consequence of the distance the beads must travel. Standardizing parameters in this manner guarantees reproducibility of the process. It is much easier to link a production process to a technology that guarantees the same conditions no matter what size the batch or what volume of the vessel.

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9. Issues with lot-to-lot inconsistencies in magnetic bead processing

When magnetic bead reagents are produced in quantity, often you cannot know if you have the correct properties of the beads until the final quality control step. But if these properties are wrong, finding out the properties at the end of the production process does not allow you to salvage the lot. Knowing magnetic bead properties such as size, magnetic charge, and surface charge, is critical in order to have excellent reproducibility in the final product (e.g. IVD kits).

Not only is the final product at issue, but if you have beads with inappropriate properties, the biomagnetic protocols you have are useless. The force exerted on beads in a biomagnetic separation device depends both on the separator and on the bead properties. If the beads have variable properties, they will move at different speed and therefore a protocol would not work because you have no set parameters on which to base the protocol.

If the production process is not monitored all the way through, you have no way of detecting that there is a problem until the very end. In this scenario, Quality Control is tested hours or days after the separation steps are complete.

But, if you have a well-parameterized process such as a homogeneous biomagnetic separation, and you monitor the process as it proceeds, monitoring of the process will alert you to problems early in the protocol. This type of monitoring can allow the technician or scientist to modify the protocol by stopping the process and taking corrective measures. In this way you can save hours or even days as well as money by saving lots that may be lost completely with no monitoring.

Sometimes it is cheaper to start a new production cycle over instead of rectifying a lot that went wrong because the major cost is in running the process and not in the raw materials. Regardless, you will always save time when you use a monitoring system.

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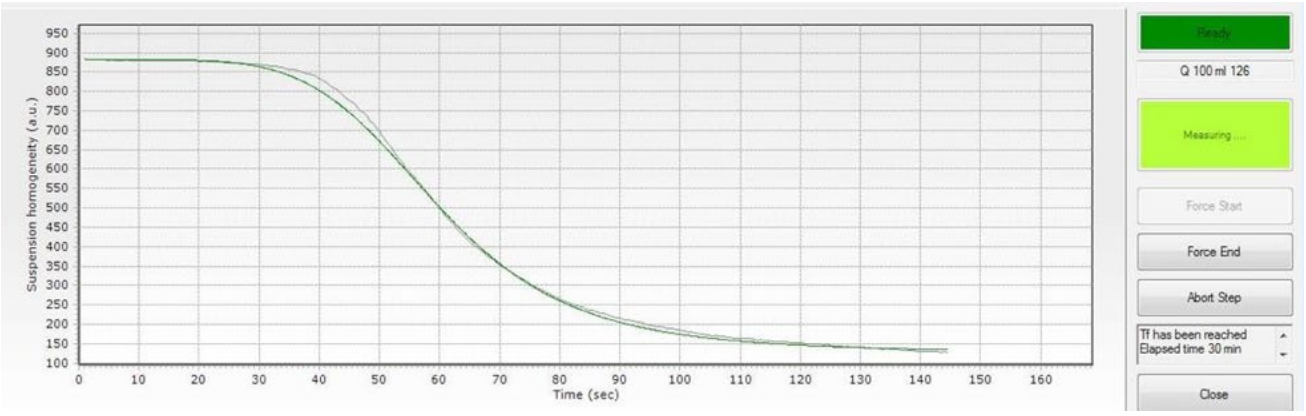
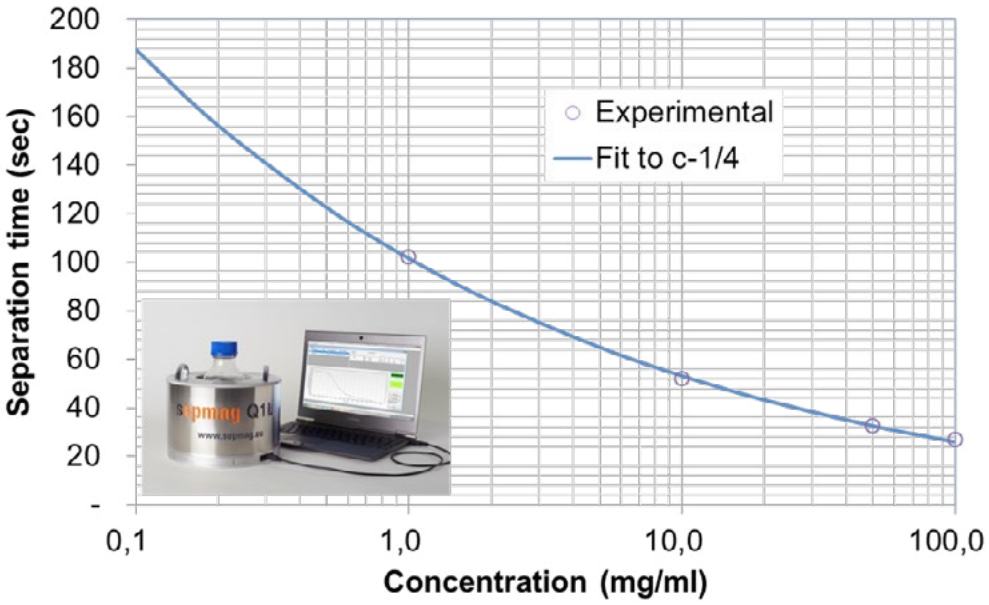


10. How do concentration changes affect Biomagnetic Separation?

In the Life Sciences, one of the most critical parameters for final IVD kit performance is magnetic bead concentration. The beads are functionalized with antibodies or other biological molecules, so the concentration of magnetic beads also delivers a specific concentration of biologically active reagent. If you do not have the correct amount of beads/biological molecules in your preparation, the sensitivity of the kit changes significantly. Therefore volume control of the suspension is quite important. The typical precision of volume control in laboratory processes in general, is approximately 2%. As the production process is scaled up, volume control becomes even more critical.

Efficient biomagnetic separation processes take advantage of bead to bead interactions. Beads interact with their neighbors, forming chains which then decrease the time of separation. Bead to bead interactions rely strongly on magnetic bead concentration. The more beads you have, the closer any given bead is to its neighbor and the faster they will interact and form these beneficial chain structures during the separation process. Once chains are formed, the chained structures move faster during separation than individual beads, thus affecting the time of separation. Therefore, not only does a higher concentration cause bead chains to be formed more quickly, it also facilitates a faster speed of separation.

In homogeneous biomagnetic separation, bead speed is proportional to the fourth root of the concentration. Under these conditions, if one monitors the production process, one can detect problems with the bead concentration if the separation time is off from the expected time. In other words, a 10% concentration change in the material can modify the separation time by 2.6%. Therefore, if you make a mistake somehow in creating your suspensions, the behavior of the beads separated with magnetic separation in homogeneous conditions will be very clearly different from the behavior of a suspension with the wrong concentration of beads.



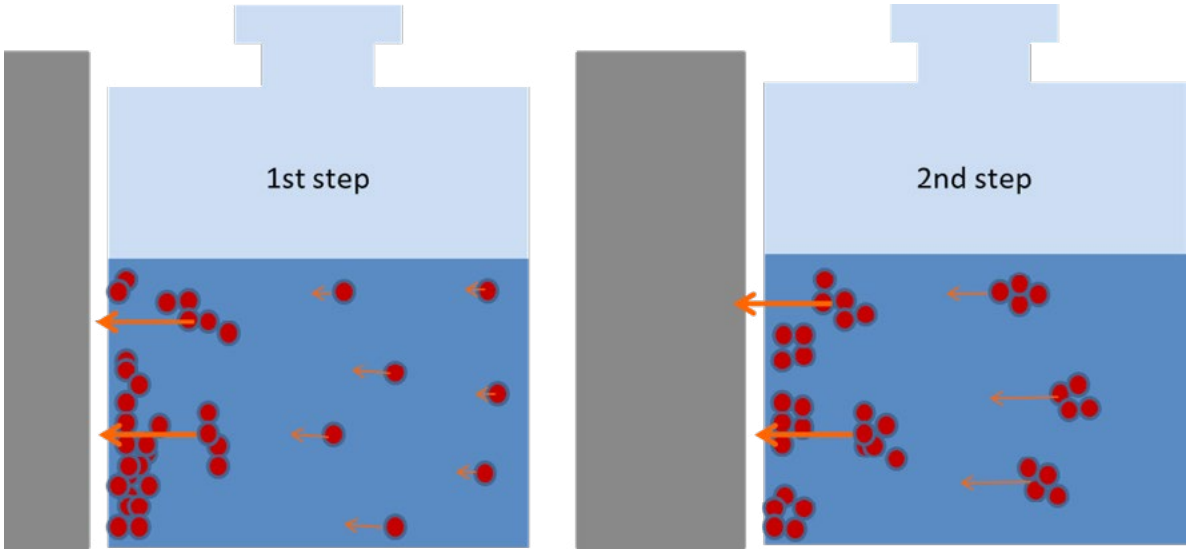
11. Resuspension problems during the biomagnetic separation process

End product variability is caused in large part by resuspension problems in your process. During biomagnetic separation, the retention forces in the system need to be high enough that losses of the beads will be avoided when the buffer is pumped out. But, the forces should not be too great because if they are excessive, beads will aggregate. Aggregated beads are tightly associated with each other, precluding the functionalization of part of their surface area with biomolecules such as antibodies, antigens or genetic material. Optimizing the retention forces (e.g. in homogeneous biomagnetic separation) and performing resuspension steps can help reduce the risk of aggregation.

However, waiting until the very end to check the performance of the process implies that one will not be able to detect any problems until days or hours after the start of the batch. If this happens, you cannot take corrective action or start a new batch if the problems are too severe.

With homogeneous biomagnetic separation techniques, one can monitor the process, allowing one to detect aggregation problems during the process. Clusters of aggregated beads behave in suspension as beads with a greater diameter and would move much quicker than expected. If you are including several washes, aggregation will increase with each step. Therefore, the monitor can detect that the aggregates move more quickly with each successive step in the process.

If you can detect aggregation early in the process, you would be able to take immediate corrective action by stopping the process and adjusting the settings or by discarding the lot in progress and starting a brand new lot. This decision depends on the policy of your company and how far along the beads are in the production process. Monitoring is an important part of saving the company time, effort and money in this way.



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