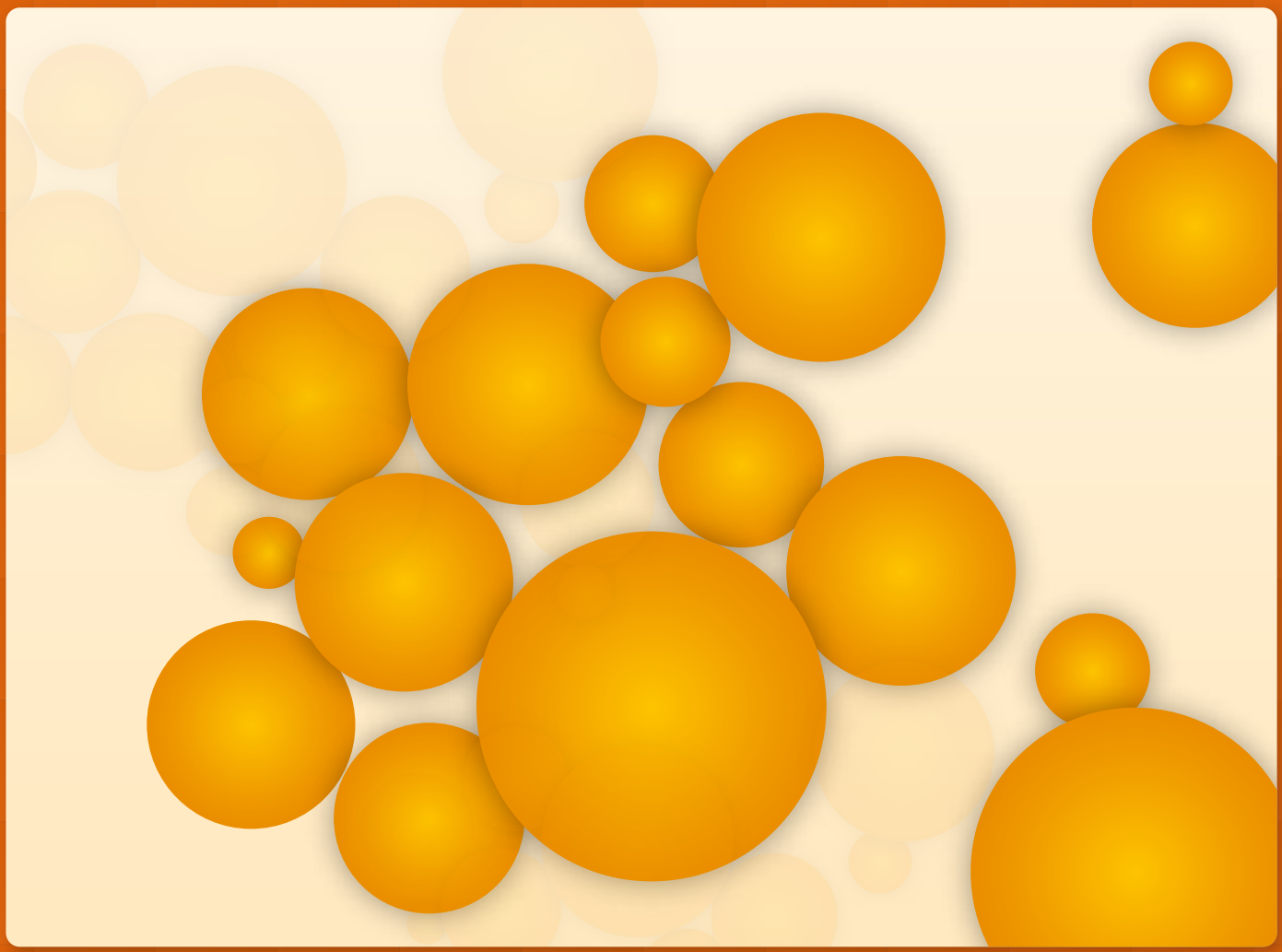
The Starting Guide to Validate Biomagnetic Separation Processes



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SUMARY

1. The weakest link in IVD production

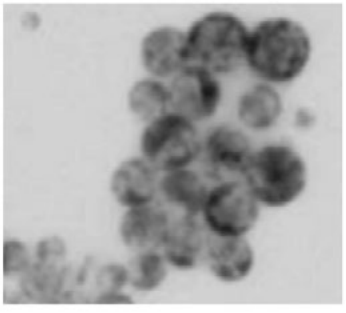
InVitro Diagnostic Immunoassays are some of the most successful life science magnetic bead and particle research applications that have come to market. The demand for such technology in hospitals and laboratories in particular, has grown dramatically. Because of this increased demand, the production of diagnostic kits needs to be scaled up. However, scaling up the production of kits is not always straightforward and can be limited by the need for strict reproducibility of every single piece in the kit. Variations must be minimized since doctors rely on repeatability and consistency when using diagnostic kits.

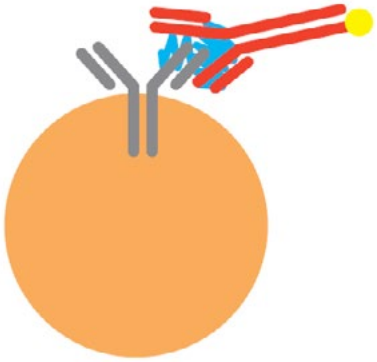
Companies that produce InVitro Diagnostic magnetic bead kits rarely pay much attention to the consistency from batch to batch of the magnetic separation steps, the measurements of the process or the control steps in the process. This becomes the weakest link in the production chain when attempting to scale up the production of diagnostic kits.

How does one ensure the reproducibility of these magnetic bead assays and thus strengthen the weakest link in the production process?

1. Assure that the assay's raw materials have very little, if any, batch-to-batch variation. This includes all buffers, magnetic beads, antibodies, etc.
2. Devote a large amount of effort and investment at each step of the assay:
   a. Production of stable and predictable polyclonal or monoclonal antibodies
   b. Making sure the coating of the beads is performed in a way that is highly consistent. For example, make sure the temperature, pH, and method of suspension are the same from batch to batch.
   c. Understand how to maintain a constant concentration of beads and chemicals from the first to the last aliquot in the lot.
3. Carefully validate magnetic separation
4. Understand how heterogeneities during separation can lead to undesirable and irreversible aggregation and uncontrolled losses in product.

The importance of controlling the consistency and reproducibility of IVD lots cannot be stressed enough. Without strict validation of every step, the substantial backing and investment that is put into each IVD lotcan be in serious jeopardy.





2. 6 Key factors affecting the behavior of magnetic beads

Biomagnetic separation technology is being widely adopted in many biotech and other life science industries. The managers of these industries spend a lot of energy, time and resources choosing the correct beads for their applications, however they often overlook very important variables that need to be controlled pertaining to the magnetic separation process. Problems with field and force, magnetic strength and bead aggregation tend to make the separation process difficult to reproduce and, ultimately, difficult to scale-up.

There are six key factors affecting the behavior of magnetic beads that are necessary to observe and control:

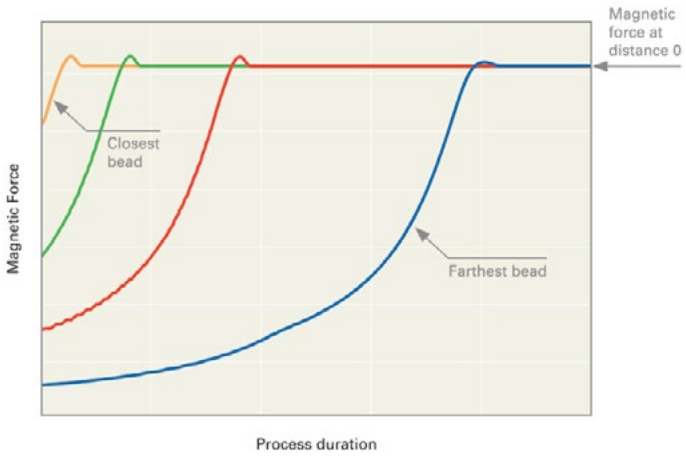
1. The viscosity of the buffer. Viscosity has a direct effect on the speed of the bead because the magnetic force acts against the drag force.
2. Ionic force and pH of the buffer. These will affect the surface charge and the stability of the suspension.
3. Temperature. This parameter can affect all of the other factors.
4. Magnetic content. The magnetic force will depend on how magnetized the beads are.
5. Bead size. Drag force depends on the size of the beads, so the size distribution (bead diameter) will have an impact on the drag force.
6. Homogeneity of magnetic force.

Problems with field and force, magnetic strength and bead aggregation tend to make the separation process difficult to reproduce and, ultimately, difficult to scale-up.

Likely, managers are already controlling factors 1 – 5, but the magnetic force itself is often overlooked. Is optimal magnetic force applied to your beads? Are you applying a homogeneous force to all of your beads?

If traditional magnetic separators are used, the magnetic forceis dependent on the distance to the magnet. Therefore, beads at various distances from the magnet will experience non-homogeneous fields and forces. Some beads will reside in the magnetic saturation regime while other beads will travel back and forth between weaker and stronger magnetization fields.

Furthermore, beads farther from the magnet are harder to attract because of the low magnetic force surrounding these beads. On the other hand, beads closer to the magnet experience excessive forces for longer periods of time, resulting in bead aggregation, loss of bead activity and resuspension problems. When beads aggregate, there is less viable bead surface area, the biomaterial to bead interaction is reduced, the biomaterial to biomaterial interactions become stronger and end product variability becomes a real problem. More time-consuming sonication and washing steps are needed when this happens. It is clear that not only understanding the material, but the process as well, is important to a company's bottom line and the consistent good quality of the end product.



3. The 2 basic points for understanding how magnetic separation works

Separation techniques using magnetic carriers (either beads or particles) are often used in the life sciences to 'capture' specific biomolecules. These techniques utilize immunocapture, DNA fragments, or electrical charge in order to specifically target the biomolecule of choice. After the capture of the biomolecule, magnetic forces can separate it from the rest of the milieu. Because of the seeming ease of separation, biomagnetic techniques are used by some as the 'gold standard' of separation technology.

While magnetic separation does have clear advantages over other techniques (e.g. centrifugation, filtration, affinity chromatography, gel purification, etc.) for purifying biomaterials, one needs to have a very good understanding of the physical principles behind magnetic separation in order to implement the technique correctly.

Two basic points are necessary for understanding magnetic separation:

1. Magnetic forces are generated by non-homogeneous magnetic fields.
   - **a.** A homogeneous magnetic field torques, but does not generate a force over a magnetic moment.
   - **b.** A magnetic force, however, is created by varying the magnetic field over a defined area (spatial variation).
   - **c.** A single permanent magnet separates because it generates a large spatial variation in its magnetic field, not because it generates a high magnetic field.
2. The value of the magnetic force depends on the magnetization of the beads.
   - **a.** The force of the magnetic field can be expressed as:

$$\vec{F}_{mag} = \nabla(\vec{m} \cdot \vec{B})$$

   - **b.** If the magnetic bead has a magnetic moment that varies (i.e. a linear response with a constant susceptibility), the magnetic moment will be χVH and the value of the force will depend on how it changes the square of the magnetic field: B2. (H=B/μ0):

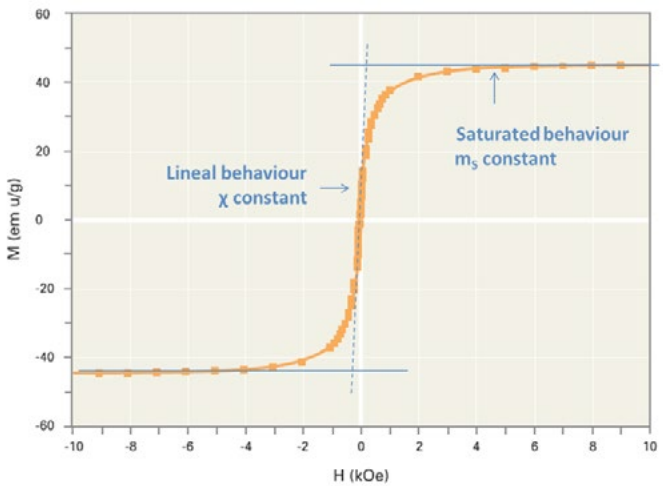
$$\vec{F}_{mag}^{linear} = \frac{\chi V}{\mu_0}\nabla B^2$$

   - **c.** If the magnetic bead has a constant magnetic moment (ms), the force depends on the magnetic field gradient:

$$\vec{F}_{mag}^{sat} = m_s \nabla|\vec{B}|$$

   - **d.** The magnetization of the beads will be linear at low magnetic fields and saturated at high magnetic fields.

The ability to understand how magnetic fields and beads interact, how magnetic forces are generated and how magnetic forces drive the beads against the viscosity of the suspension are key facets of knowledge to having reproducible, efficient and problem-free biomagnetic separation. It is important for scientists to understand both the spatial profiles of the magnetic field generated by the separation device and the magnetization curve of the magnetic beads. Once these variables and basic principles are mastered, one can create a much more robust purification process.



4. The two critical points necessary to achieve homogeneous biomagnetic separation conditions

During the development of a separation process that involves magnetic beads, scientists put great effort into reproducing the size of the beads, the magnetic charge on the beads, buffer composition, pH and temperature. What is often overlooked, however, is the importance of homogeneous biomagnetic separation conditions.

$$\vec{F_{mag}} = \nabla(\vec{m} \cdot \vec{B})$$

Typically, biomagnetic separation is performed on biomaterial that will be a part of the In Vitro Diagnostic (IVD) kit. The kits provide investigators with the necessary buffers, beads and specified biomolecules. These kits are produced in large batches. It is important during the production process, that each small aliquot from the batch has exactly the same properties as any other aliquot. In order for this to occur, all of the beads must experience the same conditions during processing. The generation of homogeneous biomagnetic separation requires that the value of the magnetic force be the same in the entire volume of the batch. The magnetic force is dependent on both the magnetization of the beads and the magnetic field profile.

There are two critical points that must be followed in order to achieve homogeneous biomagnetic separation conditions:

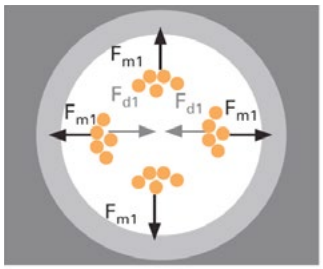
The magnetic field magnitude must be higher than 0.1 Tesla.

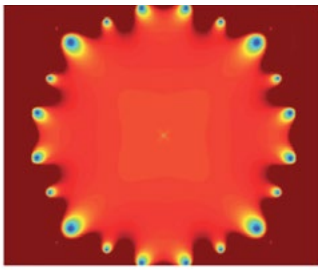
1. If the magnetic field is low, the magnetization of the beads will change linearly (constant susceptibility).
2. Constant force is obtained by the magnetic field having a variable spatial profile over the batch volume (the gradient of the square of the field modulus constant: B ~ r1/2).
3. Constant magnetic moment of the beads is obtained by making the intensity of the magnetic field high enough that they will be saturated.
4. Magnetite, the most common magnetic material used for magnetic beads, saturates at fields of approximately 0.1 Tesla (~ 80 kA/m).

Achieving a constant magnetic field gradient

1. If the magnetic beads are saturated, their magnetization will be constant.
2. Under constant magnetization, a constant magnetic force can be achieved by having a magnetic field profile with a constant gradient B ~ r.
3. This constant magnetic force can be achieved in smaller volumes by using a simple four pole permanent magnet arrangement.
4. In larger volumes, the magnet configuration is more sophisticated, but can be built by companies that specialize in this field. Cylindrical arrangements will fulfill this configuration, although 1 – 5% of the volume around the axis will not be perfectly homogenous.

Is your biomagnetic separation process homogeneous? Are you controlling the two critical points for homogeneity? New developments in biomagnetic separators provide you with the tools for biomagnetic homogeneity.





5. How to avoid resuspension problems during biomagnetic separation processes

There are no easy ways to bypass steps or simplify the production process of biomagnetic separation. Steps in the production process that seem easy or easily bypassed, turn the production into a nightmare if one attempts to take short cuts. One of these seemingly easy steps is the resuspension step.

Resuspension is relatively straightforward, but only if the magnetic beads have been treated properly throughout the process. If the beads are not treated gently, they will aggregate more easily and one will have difficulties during the separation steps.

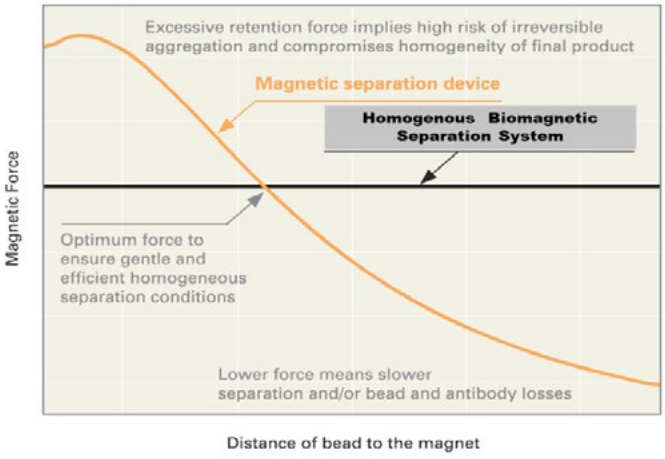
What is the explanation behind magnetic bead aggregation? If one uses traditional magnetic separators, a magnetic force dependent on distance from the magnet is created. Beads farther from the magnet will be harder to attract because of the low magnetic force in which they exist. This results in significant material loss and/or excessive separation time. On the other hand, beads close to the magnet will experience excessive magnetic force for long periods of time. Bead exposure to high magnetic forces results in bead aggregation (often irreversible), in a loss of activity and in resuspension problems. Laborious extra sonication steps are often required to rectify the aggregation.

Luckily, there are new developments in biomagnetic separation that allow scientists and engineers to apply optimum magnetic force to all beads equally. This ensures gentle, efficient and homogenous separation

Often, scientists try to increase the force reaching beads that normally experience low magnetic forces by adding permanent magnets. Unfortunately, this increases aggregation problems because it increases the force on the beads that exist closer to the magnet much more than it increases force on beads farther from the magnet.

Luckily, there are new developments in biomagnetic separation that allow scientists and engineers to apply optimum magnetic force to all beads equally. This ensures gentle, efficient and homogenous separation. It also avoids aggregation and resuspension problems. With a precision magnetophoresis system, all beads are exposed to a homogenous magnetic force.

It is important to take advantage of the new biomagnetic separator technology in order to achieve the best production process possible.



6. The 4 wrong ways to improve magnetic separation time (and 1 way to do it correctly)

It is vitally important that life science products be consistent from lot to lot. Two batches of the same product produced in the same way should have little variability. In order to achieve a high level of quality control, one must define a strict standard operating procedure (SOP). In the case of a permanent magnet biomagnetic separation device, conditions are usually very stable and so the main parameter to control is the time the vessel is exposed to the magnetic field during production.

However, using a standard biomagnetic separation device the resulting magnetic separation conditions will always be non-homogeneous. This happens because the magnetic field and magnetic force decrease quickly with distance from the permanent magnets in the separation device. The beads close to the permanent magnets experience much stronger magnetic fields and magnetic forces, causing irreversible aggregation problems if the forces are high enough and/or the exposure time in the magnetic field is long enough. The beads far from the magnets, on the other hand, experience weaker magnetic forces and magnetic fields and need much longer times to reach the retention positions. Usually this results in a loss of magnetic beads.

Investigators try to find the correct separation time, attempting to minimize both bead aggregation and bead losses. There are four wrong ways in which to try to improve separation time if one analyzes a batch and determines that the yield is not where it should be:

1. Use a shorter separation time. This method will reduce aggregation significantly, but because beads will have less time to reach the retention position, material loss (including both magnetic beads and biomaterial) will increase. If there are multiple steps, this loss will be multiplied.
2. Increase the magnetic force. Magnetic bead and biomaterial yield will increase (beads from far away will reach the retention position more quickly), but the beads near the magnets will experience much more force, increasing aggregation problems.
3. Reduce the magnetic force. Aggregation will decrease, but magnetic bead losses will increase.
4. Use a longer separation time. More magnetic beads will be collected, but beads near the magnets will be exposed to high forces longer and increase aggregation.

The upshot is one can never really find the correct separation time. There will always be compromises and losses using this method.

However, if a homogeneous biomagnetic separator is used, one can actually optimize the separation. The forces used are just enough to retain the beads during buffer extraction. The magnetic force experienced by the entire bead population is the same, so beads farther away will move to the retention position more quickly. All of the beads move at the same speed under homogeneous magnetic forces, balanced by the viscosity of the buffer. Because of this, one can determine the separation time and fully recover the magnetic beads. The separation time for full recovery of the beads is shorter, so the beads closer to the retention position are not exposed to high magnetic forces over a long period of time. The risk of irreversible aggregation is drastically lower using this new technology.

In addition, the beads are treated much more gently than when using standard magnets. The choice is clear. If you want optimal recovery with minimal bead damage, a homogeneous biomagnetic separator is the device to use.

7. Ensure 100% consistency from lot to lot

Biotech companies such as InVitro Diagnostic aim for 100% reproducibility in every single batch they produce and in every kit test in each batch. In fact, customers expect that they will receive a product that will perform exactly the same as the last time they purchased it. It does not matter what batch the product is or when it was produced. Customers expect that variation from batch to batch will be nonexistent. Therefore, it is incumbent on the company to make sure that there is consistency not only between lots over time but also within the same lots.

Traditional magnetic separation does not always deliver this level of consistency because if traditional magnetic separators are utilized, not all beads experience the same conditions. Beads closer to the magnet experience strong magnetic forces over a long period of time and are fully magnetized. Beads far from the magnet experience weak magnetic forces and variable magnetization. Weak forces cause magnetic beads to be lost during the process because they are not separated out with the other material. In addition, variable magnetization can affect the property of the beads , i.e. the magnetic force of the beads themselves. Strong forces over too long a period of time can lead to aggregation of the beads, loss of bead activity and resuspension problems. All of these things can be translated into lot to lot variation and inconsistencies.

As long as one performs separation using defined and homogeneous conditions that have been shown to be reproducible over time and scalable to any volume, modern biomagnetic separation techniques will be functionally consistent.

The latest developments in biomagnetic separation technology consist of separators that create a homogeneous and well defined magnetic force no matter how far away the bead is from the magnetic source. Therefore, all beads experience the same magnetic force and are essentially equal. With this new technology, conditions and results are reproducible over time, with any volume and from lot to lot. Therefore, if a company wants reproducibility, using the new biomagnetic separation technology is vitally important.

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8. The 2 ways you can demagnetize your biomagnetic separation device

Since reproducibility over time is a highly desired trait when using biomagnetic separation, especially when used in the life sciences, it is important to consider all possible disruptions of consistency. Biomagnetic separation devices use permanent magnets which maintain their properties over long periods of time.

Modern devices use a rare earth magnet: neodymium iron boron (NdFeB), because of its outstanding properties. While NdFeB magnets are considered permanent, (i.e. have constant and consistent properties), even these magnets have potential limitations over time and can very slowly lose their magnetic properties. It is important to consider these potential limitations and learn how to minimize them. Therefore, we have listed the two major ways below that human interaction can demagnetize a biomagnetic separator so that these conditions can be avoided.

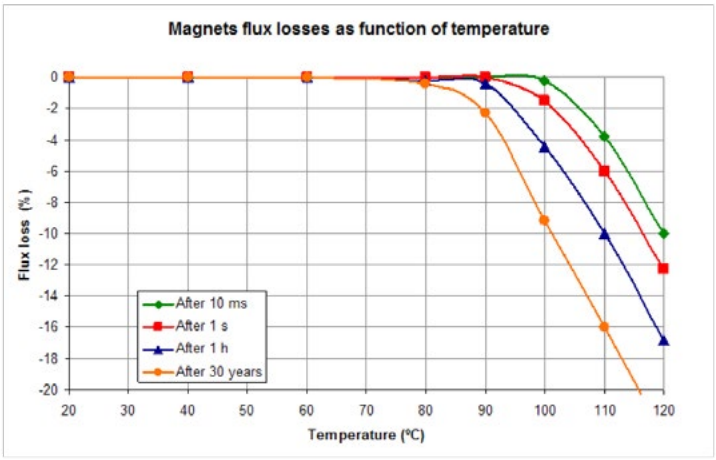
High magnetic fields

If your magnet is exposed to a magnetic field of greater than 1 Tesla (which is greater than 20,000 times the Earth's magnetic field), this can demagnetize a neodymium iron boron magnet.

The only device in a life science setting that can do this sort of damage is a hospital grade MRI.

High temperatures

If one heats a magnet high enough, a point can be reached which causes thermal agitation to overcome magnetic interactions, thus demagnetizing the material.
An NdFeB magnet heated at 80ºC or more, over a minimal period of 30 years will cause the magnet to lose 0.5% of its magnetic force.
Therefore, if you work with your samples at 20 – 40ºC, you are fine. You will not be doing any damage to your biomagnetic separator. However, do not put your device into an autoclave!
If you DO need to autoclave your biomagnetic separator, there are other permanent magnet material you can use in your device that will survive higher temperatures.
Therefore an NdFeB magnet is a highly stable magnet and should have highly desired consistent properties when reproducibility issues are at stake.



Therefore, if you work with your samples at 20 – 40ºC, you are fine. You will not be doing any damage to your biomagnetic separator. However, do not put your device into an autoclave!

9. Do you have full control of all your steps in your production process?

The cornerstone of any good production process is the ability to have robustness and reproducibility, especially in the biotech industry. Robustness means that process is efficient and easy to follow in order to obtain an end product. Reproducibility means that the process is repeatable over time and with different volumes of material. The biotech industry often uses live biomaterial that is complex, delicate and sometimes problematic to handle. Therefore, it is advantageous for a biotech company to spend resources and time to develop and define its production process and to train all of the technicians to follow procedures precisely.

In the biomagnetic separation industry, production managers tend to expend much of their efforts determining the parameters of the process to the nth degree, often leaving out one of the most important parameters: the magnetic separation parameter. They often use traditional magnetic separators to perform the task at hand without understanding all of the critical aspects and implications of using these devices in the resulting quality of their end product.

Unfortunately, with traditional magnetic separators, not all of the beads experience the same conditions. Beads that are close to the magnet are fully magnetized and are exposed to a high magnetic force over long periods of time, many times causing irreversible aggregation, loss of magnetic activity and problems with resuspension. Beads that are far from the magnet experience variable magnetization while they are traveling to their final point. Variable magnetization can affect the properties of the bead by reducing the magnetic force overall and slowing down the separation process. When doing this sort of work in high volumes, the distance problems are exacerbated.

Because of this, the magnetic separation process fails to achieve standard quality control. Often, beads are not consistent with each other within a lot and do not work consistently with varying batch volume of material.

The more modern biomagnetic separation techniques utilize separators that create a homogeneous, well-defined magnetic force no matter how far the bead is from the magnet. Therefore, all beads experience the same magnetic force. Because of this, results are reproducible over time and with all volumes of processed material; a crucial point to consider when deciding which separator to use.

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10. Why do magnetic beads move faster than expected?

The success of biomagnetic separation technology comes from its simplicity. Magnetic beads capture biomolecules and then they are extracted from the suspension. No filters are needed, there are no disposables, and there are no moving parts. The speed of separation, usually on the order of seconds for small volumes and minutes for large volumes, is fast compared to other separation techniques.

Since the speed of separation is fast when using traditional magnetic separation techniques, not much attention is typically paid to the details of bead movement. Even though separation conditions are not homogeneous and not well parameterized, the results are generally satisfactory for what is needed. However, as soon as batch volume increases using traditional methods, reproducibility problems become a serious issue. Apparently, volume matters for non-homogenous separators. On the other hand, newer biomagnetic separation devices should allow one to predict results with a great deal of accuracy. These devices have homogeneous and well parameterized conditions with constant magnetic force applied to each and every bead. For any bead, its velocity can be calculated by using the following formula:

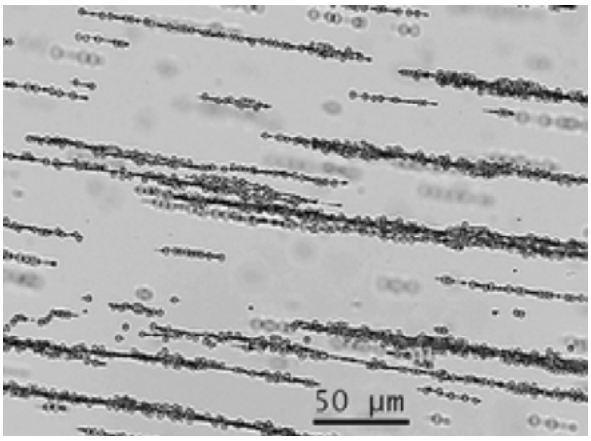
$$v = \frac{m_s \nabla |\vec{B}|}{3\pi\eta D} = \frac{M_s D^2 \nabla |\vec{B}|}{18\eta}$$

This formula considers the competition between the magnetic force and viscosity for each bead. Even so, experimental data consistently shows that bead movement is much faster than this formula predicts. What typically occurs is that the separation speed increases as the concentration of the magnetic beads increases, resulting in a shorter separation time than estimated.

What can explain this discrepancy?

In the presence of a magnetic field each bead is magnetized and acts as a tiny magnet. Each tiny bead magnet interacts with its neighbor. These interactions cause large chains of beads to form. These chains are similar in behavior to larger beads which move much faster along the magnetic field gradient than small, single beads. Therefore, when the magnetic interaction between beads is larger than thermal agitation, the beads always move faster than predicted by the above formula, a formula that is meant to calculate the speed of isolated particles and not long chains of beads.

Because of the complex interaction of all of the above variables, it is important to understand the scientific basis of the biomagnetic separation process and use the latest magnetic separation technology available.



11. How to monitor your biomagnetic separation process

Biomagnetic separation techniques are being used in more and more applications (e.g. immunoassays, collection of genetic material, protein purification). Most of the applications are industrialized and therefore require quality control protocols, validation audits and standard operating procedures. Because of the economic implications, production mistakes are not acceptable. Products must demonstrate reproducibility in order to be viable.

During processing, industry enacts many control measures in order to ensure compliance with manufacturing protocols. For example, for biomagnetic separation, much effort is devoted to controlling the correct temperature, pH, size distribution of the beads, and the exact volume of the lot. Amazingly, the control enacted on the magnetic separation step of the process is merely the measurement of how long the vessel is exposed to a magnetic field, capped with a technician's signature. If a lot does not conform to specifications, there is no way to check if there were any anomalies in the separation process itself.

The following are suggestions for monitoring the separation process more effectively in order to ensure full compliance with quality control protocols:

Amazingly, the control enacted on the magnetic separation step of the process is merely the measurement of how long the vessel is exposed to a magnetic field, capped with a technician's signature. If a lot does not conform to specifications, there is no way to check if there were any anomalies in the separation process itself.

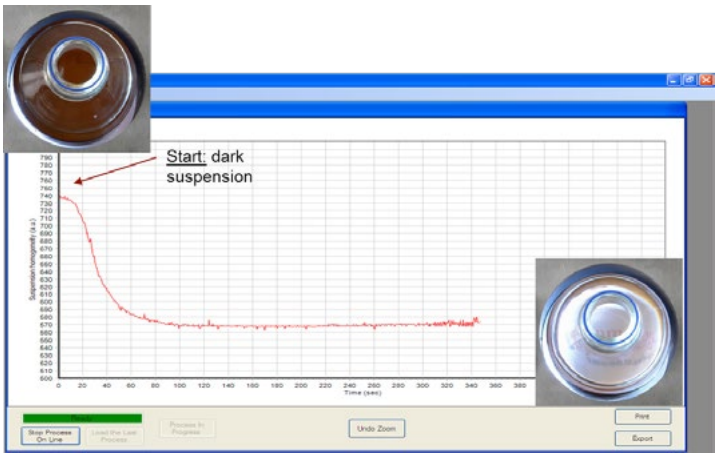
1. Use optical sensors

Initially, a biomagnetic separation suspension is dark and murky throughout. At the end of the process, the liquid is transparent and the beads are gathered at one end of the vessel. While direct observation is important, it is not a measurement.

Optical sensors will allow you to have measurements of the change of transparency or color of the liquid throughout the entire process. The sensors make recording these changes possible, data can be graphed vs. time, and the LED lights used in the process generate light without heat (thus not interfering in the separation process).

2. Use the optical sensor records to generate standard curves and parameters

If magnetic separation conditions are homogenous, the dynamics of separation will only depend on the characteristics of the suspension (i.e. viscosity, concentration, bead size, magnetization of the beads, etc.). Using an optical sensor we can generate standard curves for these various parameters, giving a baseline for production. Defining these standard curves will allow technicians to compare processes in progress against the standard curves. Any significant deviations of the parameters will show a clear modification of the curve. This allows quick visualization of errors early in production.



12. 4 ways to validate the homogeneity of your biomagnetic separation process

Biomagnetic separation needs validation in order to ensure reproducibility. The skills necessary to identify the key parameters affecting separation performance, measure those parameters, and enact the appropriate controls are specific and require an excellent background in physics.

Most biomagnetic separation industries take the easy road in order to validate their processes. They determine, by trial and error, the correct separation time. While this strategy is fine for one volume and one type of product, this does not work when scaling up production. Production after scaling up is then delayed until new conditions are defined, again by trial and error. Ideally, validation of a biomagnetic process should proceed by identifying and measuring key parameters such as the state of the magnetic beads and the magnetic field profile.

The following are suggestions for correctly validating biomagnetic separation production:

1. Check magnetic bead saturation levels

Fields over 0.1 Tesla will be enough for standard magnetite beads to acquire a magnetization near ms. Different materials will need different magnetic field saturation levels.

2. Make sure the magnetic field is strong enough.

Multiply the gradient by the vessel radius. The value should be much higher than the field necessary in order to saturate the beads.

3. Make sure the magnetic field gradient is large enough to retain the magnetic beads.

The magnetic force depends on both the field gradient and the moment of the magnetic beads. This value should be large enough to retain the beads at the wall of the vessel while removing the liquid completely. If you need a greater magnetic force, you will need to increase the magnetic field gradient.

4. Evaluate the 'non perfect' region and make sure it is small relative to the rest of the volume.

Magnetic field gradients will vary. A large magnetic gradient implies that in some areas, your magnetic field can be lower than that necessary to saturate the magnetic beads. Make sure this region is small enough that the beads can easily leave this region. Cylindrical vessels are the best shaped vessels to use. If the magnetic field gradient is radial, the volume of the sample in the 'non perfect' region is in the symmetry axis and any small force or agitation will push them out of the region. The volume also depends on r2, so, for example if 10% of the diameter of the vessel dips below 0.1 Tesla, it implies that only 1% of the sample volume is exposed to these conditions.

If you do not know how to put in place these 4 ways to validate the homogeneity of your biomagnetic separation process, you can look for new separators that do it for you.

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