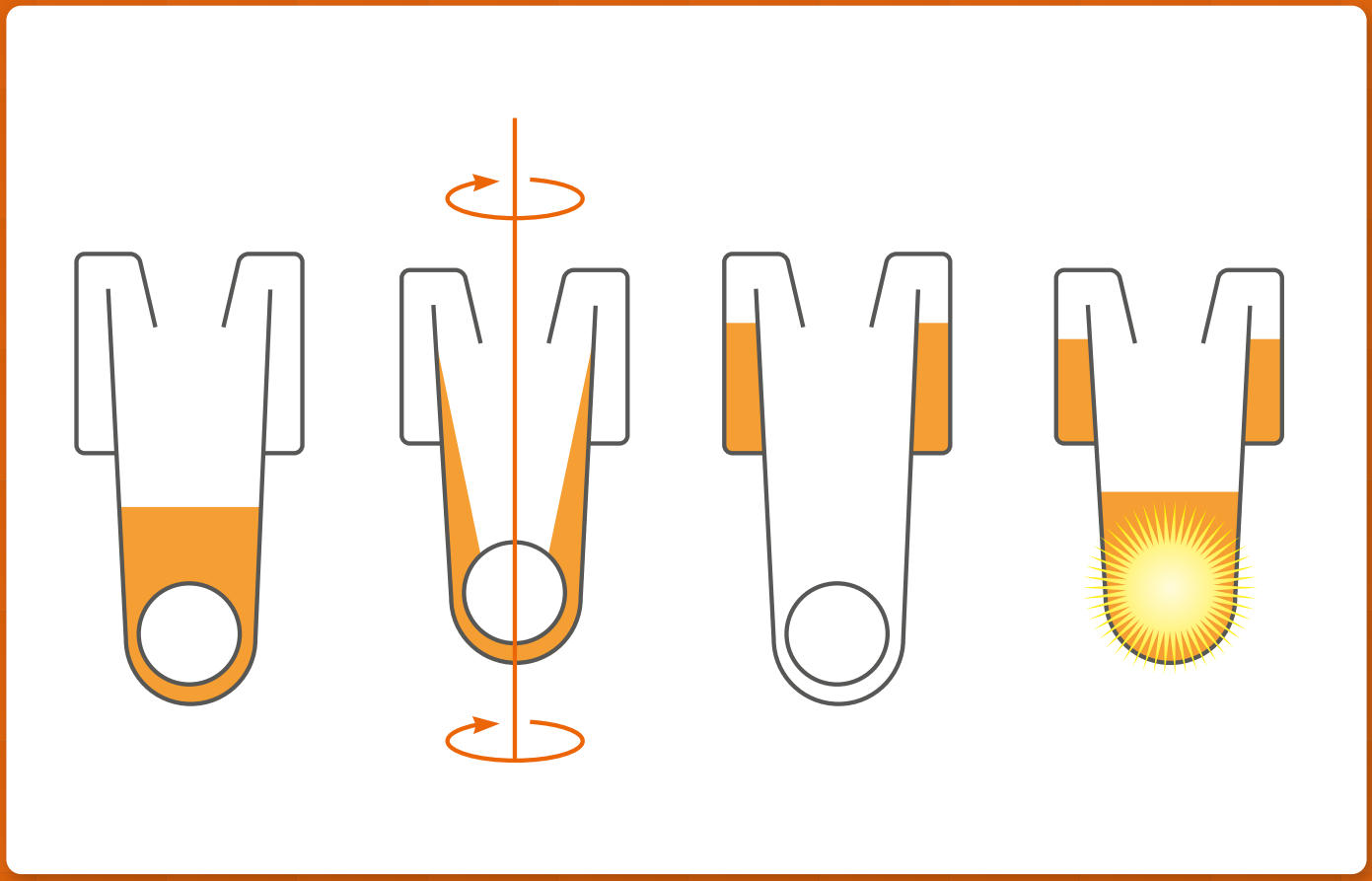
The basic guide for the use of magnetic bead in ChemiLuminiscent ImmunoAssays (CLIA)

Choose the right CLIA reagents for your assay and determine how your choice will affect your results.

by Dr. Fabrice Sultan



Sepmag®

SUMARY

About the author: Dr. Fabrice Sultan, OEM Diagnostics- Estapor, Merck Chimie SAS (France)

Fabrice Sultan obtained his PhD in Life Sciences from the Biomedical Institute in Paris in 1990. He joined the B.Braun Medical as product manager in charge of the parenteral nutrition product line of the company. After 8 years he started as sales and marketing manager with Prolabo, a french company of the Merck group. In charge of the Estapor Microspheres product line as sales & marketing manager,

Fabrice Sultan combines his background in biology, biochemistry and immunology, together with his experience in sales & marketing and his knowledge in international business. Fabrice Sultan has gained a solid reputation in Microspheres Technologies and provide useful technical trainings in bead-based immunoassays.

Introduction. Chemiluminescent ImmunoAssays

Well-established classical detection methods that are packed in kit form include Radioimmunoassays (RIAs) or Enzyme Linked Immunoassays (ELISAs). These two detection methods were highly sensitive and used for a variety of biological and chemical testing. Even though RIAs are very sensitive assays, by their very nature, RIAs are comprised of highly hazardous, radioactive reagents. The handling and disposal of RIA refuse can be difficult to regulate in the lab environment as well as expensive.

ELISAs are much cleaner assays. However, ELISAs do not have the dynamic range of RIAs and are not sensitive when an analyte exists in very low concentrations. In addition, ELISA steps are often long and drawn out with the final reactive step requiring a stop reagent so that the color of each test well does not continue to develop. One must be very precise when timing the addition of substrate and stop reagent in an ELISA in order to have an accurate result.

More recently, a newer, faster technique has revolutionized biological and chemical detection. Chemiluminescent Immunoassays (CLIAs) are based on the detection of a protein that glows or emits light in a chemiluminescent reaction. No stop reaction is required and incubations are short in these assays. CLIAs have a much wider detection range than either RIAs or ELISAs and are also extremely sensitive under low analyte concentration conditions. Enhanced CLIAs use horseradish peroxidase (HRP)-linked substrates to allow detection of femtomoles of protein. The materials used in CLIA reagents are, in general, non-hazardous and much easier to dispose of than both RIA and ELISA reagents. CLIAs can also be automated with a luminometer.

One of the easiest ways to use a CLIA assay to isolate a particular analyte is to attach the substrate to magnetic beads. Magnetic bead CLIAs perform both detection and isolation tasks concurrently. In addition, your analyte (along with your luminescent reagent) can be quickly separated from any contaminants or from free substrate and at the same time, concentrated. For your particular assay, choosing the correct magnetic bead can determine how successful your analyses and separations will be. This e-book aims to help you determine how to choose your CLIA reagents and how your choice will affect your results.

No stop reaction is required and incubations are short in these assays. CLIAs have a much wider detection range than either RIAs or ELISAs and are also extremely sensitive under low analyte concentration conditions.

Chapter 1. The Two Key Reasons for Selecting Chemiluminescence for Immunoassays

Retrieving analytes from their original sources (especially when working on protein) can be tricky and difficult. In addition, various methods of analyte recovery are highly concentration dependent. For example, analytes can be extracted from very concentrated samples, e.g. greater than or equal to 1 mg/ml, with methods such as nephelometry or turbidmetry. Lower concentrations such as 1 ug/ml require the use of an ELISA or Immunofluorescence assays to isolate the desired analyte.

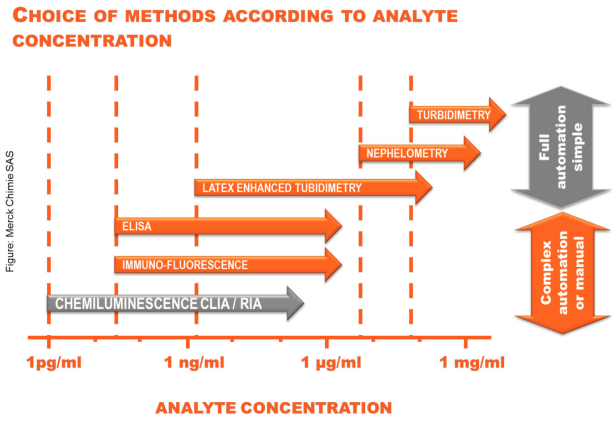
If you have analyte concentrations that are very low, however, ELISA and Immunofluorescence techniques become unreliable and inefficient. More sensitive assays are needed to efficiently isolate analytes at concentrations lower than 500 pg/ml. Radioimmunoassays (RIAs) or Chemiluminescence Immunoassays (CLIAs) are the assays of choice for mixtures with low analyte concentrations.

High throughput is greatly desired for companies that are trying to determine a large number of analytes in a short amount of time. Chemiluminescence is the preferred technique because you can identify hundreds of analytes per hour with an automated Chemiluminescent Immunoassay. Previously, ELISAs were used for this purpose. Although ELISAs are excellent assays, you cannot automate an ELISA, or analyze such a large number of substances in a short amount of time. Therefore, ELISAs are now considered ‘old’ technology and are no longer deemed efficient enough for high throughput analyte identification.

Using a high throughput method such as Chemiluminescence is very advantageous for settings such as hospital labs that need to analyze, for example, thousands of patient samples a day. If you want to look at 5-10 analytes per patient, you will be performing tens of thousands of tests daily. This becomes an impossible task using ELISAs or other older techniques, but with high throughput techniques such as automated Chemiluminescent Immunoassays (CLIAs), this task becomes quite doable.

Therefore, if your lab needs to isolate an analyte present at a very low concentration you can choose between an RIA and the CLIA. However, since RIAs use radioactive chemicals and generate radioactive waste, the best choice is to use CLIAs which are much safer than RIAs and more environmentally friendly. Larger labs more concerned with high throughput analysis should also use CLIAs since they can analyze large numbers of analytes in a small period of time in an automated fashion.

High throughput is greatly desired for companies that are trying to determine a large number of analytes in a short amount of time. Chemiluminescence is the preferred technique because you can identify hundreds of analytes per hour with an automated Chemiluminescent Immunoassay.



Chapter 2. How Do Chemiluminescent Assays Work?

There are two ways in which CLIAs are used in the lab: the one step sandwich technique and the two step immunocapture technique. In both techniques the first step is to attach a highly specific protein to magnetic beads. Almost all assays using this technique utilize a very sensitive antibody as the attached protein. The antibodies used are very specific for the analytes to be recognized and analyzed. The coated magnetic beads are considered 'the reagent'.

The reagent is mixed and incubated with a fraction of blood, serum, urine or other biosample (depending on your application) and is designed to recognize one specific biomarker or protein. If the biomarker exists in the sample, there will be a reaction with the antibody on the coated beads and the analyte.

The reagent is mixed and incubated with a fraction of blood, serum, urine or other biosample (depending on your application) and is designed to recognize one specific biomarker or protein.

After incubation, there are critical steps to wash the reagent in order to eliminate contaminants and decrease background or noise in the sample.

In the sandwich technique, the next step involves a secondary antibody which recognizes the same analyte as the primary antibody coated on the magnetic beads. The analyte is, therefore, 'sandwiched' between two antibodies. The secondary antibody is also conjugated with a stable enzyme that catalyzes the final reaction with the substrate, resulting in chemiluminescence. The amount of chemiluminescence is proportional to the concentration of analyte captured by the coated magnetic beads.

Immunocapture is similar to the above technique, but is specific to analytes that are also antibodies. In this case, the antibody-coated magnetic beads recognize and react with the desired antibody in serum, urine, or another bodily fluid; is washed several times; reacted with an enzyme-conjugated anti-analyte antibody; washed again; and combined with substrate.

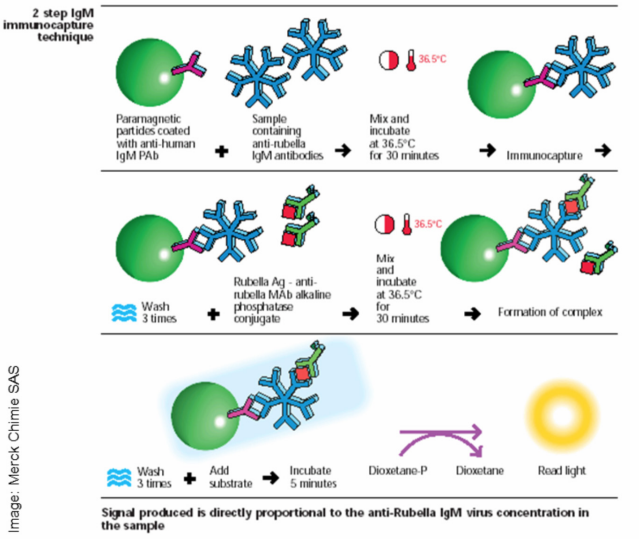


Image: Merck Chimie SAS

Chapter 3. Developing a Chemiluminescent Immunoassay in Eight Basic Steps

When developing and using a CLIA, it is important to follow eight basic steps in order to ensure that your assay will be the most efficient and accurate that it can be.

1. Define the expected dynamic range.

It is important to figure out the approximate concentration range of the analyte in your sample. In addition, you need to figure out what the optimal concentration range is recommended in order to detect your desired analyte. If analyte concentrations are high, you may not need to use such a sensitive technique as CLIA.

2. Define the assayed specimen (e.g. blood, serum, urine, etc.).

The type of specimen will help you understand the expected dynamic range of analyte contained within the specimen. Therefore, it is important to know what type of specimen you will be working with when analyzing an analyte.

3. Select the antibodies to be used in the assay and whether you will need polyclonal or monoclonal antibodies.

You will want to select antibodies that are highly specific for your unique analyte. Specificity is key to the success of your assay. Polyclonal vs. monoclonal is important to determine as well and will depend on the character of the antibody itself and how specific each are for your analyte.

4. Choose your best magnetic beads.

You will need to determine what the optimal bead size, bead surface area and iron oxide content needs to be for your specific analyte assay.

5. Optimize the coating procedure.

When coating your beads, you will want to take into account the adsorption properties of the beads, any covalent binding you will need to create and the biological effects of linking proteins to your beads.

6. Select your homogenization method.

Your choice of homogenization will depend on the equipment you have, the personnel who work on the equipment, the size of your samples and the ease of use within the assay. Your choices are roller homogenization, overhead homogenization, vortexing or sonication.

7. Select the correct biomagnetic separation technology for removing free antibodies and other contaminants.

In other words, this step reminds you that you need to use systems that have been tested with

other beads. Systems that can help you easily validate your process and can help you scale up your process are preferred.

8. Enhance your immunoassays when required.

If you can make your assay better, it is important to do so. Making your assay more efficient, more accurate and easier to perform is important not only to the final results of your analysis, but also to the bottom line of your company.

It is vital to remember that all of these steps are important. You should not skip even one step in this process of developing your CLIA.

Chapter 4. Three Antibody Choices for Chemiluminescent Immunoassays

When choosing antibody types for your CLIA, you need to consider a number of factors. One of the most important considerations is the concentration of your analyte. If your analyte is very low in concentration, you will need an antibody that will combine high specificity and high affinity.

Polyclonal Antibodies (pAb)

- These antibodies tend to be 'hyperimmune' and have higher affinity. Use these antibodies if the epitopes are not well defined. In other words, polyclonal antibodies are not highly specific to one particular epitope. In fact, if polyclonal antibodies are not pre-adsorbed with the secondary antibody, there can be problems with non-specific interactions.
- Polyclonal antibodies are typically affinity purified – more so than global IgGs, so specificity is assured. The level of specificity can range and must be determined before you can relate your antibody reaction to the concentration of your analyte.
- Make sure you use fully specific antibodies and take all precautions to validate specificity and any non-specific interactions.

Monoclonal Antibodies (mAb)

- If you happen to have a polymeric antigen and decide to use monoclonal antibodies, you should use only one mAb. Using more than one mAb can confuse the final analysis of analyte concentration.
- However, if you have separate epitopes and well-defined mAbs, you can react your specimen with a mixture of mAbs and analyze each epitope separately.
- Monoclonal antibodies can have very high affinity (i.e. < 10-10 M-1) and very high specificity (higher specificity than polyclonal antibodies) for a defined epitope

Indirect Method

Be aware that sometimes if you coat your beads with your antibody of choice, it may be difficult for the antibody to react with the antigen. In this case, you may need to use indirect means to reach your desired results. You can use a spacer (a chemical or biological arm) between the bead and the analyte. Spacers are especially helpful when working with very small peptides or epitopes.

.

Chapter 5. Five Points to Consider when Selecting the Proper Magnetic Microsphere for Chemiluminescent Immunoassays

When determining which magnetic microsphere to use for your CLIA, it is important to take into consideration a variety of different variables:

1. Size of the beads.

Magnetic microspheres come in a wide range of sizes, from less than 100 nm to more than 3.0 microns in diameter for CLIAs. Working with smaller beads gives the analyst a bit more advantage over the larger beads, however the magnetic separation is slower. Trying different sizes is advantageous and will help you determine what size is easiest to work with and what size bead will give you the best results. The gold standard seems to be a 1.0 µm bead size, so starting at this size may help speed your size determination efforts.

2. Bead material.

Beads are made of various types of polymers including: polystyrene (PS) and polystyrene-divinylbenzene (PS/DVB). Understanding the properties of each of these materials and determining how each affects your assay is important in the final resulting analysis of your analyte.

3. Density of the beads greater than 1.2 g/cc.

Your beads have a magnetic coating. The magnetic coating on your bead has a higher density than the bead polymer. As a result, the higher the percentage of magnetic pigment, the faster the magnetic beads will separate. Higher density also implies faster sedimentation rates.

4. Surface properties to consider:

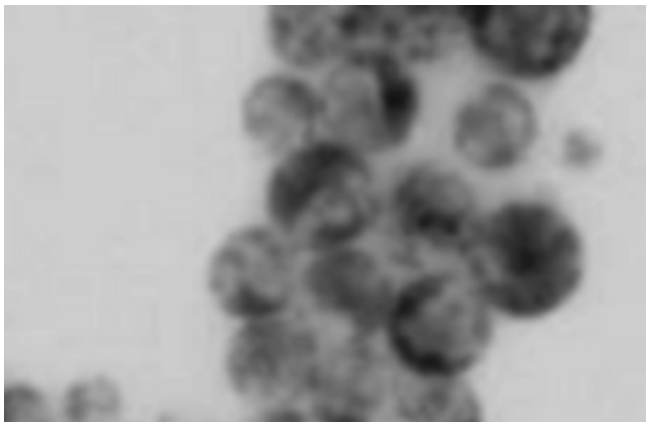
- Plain surface. This type of surface allows for passive coupling to your antibody.
- Modified surface. Typically the modifications on magnetic beads are -COOH groups, -NH2 groups, -OH groups or -SH groups. These modifications allow you to couple your antibody onto the bead covalently, thus giving you a more stable coating.
- Pre-activated surface. Pre-activated groups on the surface of the beads include tosyl, chloromethyl, or epoxy groups. Once again these modifications allow for stable covalent coupling of your antibody/antigen.
- Bio-activated surface. Bead surfaces can also be activated with streptavidin, anti-IgG, or protein A or G. This type of surface allows you to attach your antibody via bio-coupling techniques. The advantage of this type of surface is that you only need to use biomagnetic separation in order to separate the free antibody or antigen from those that have reacted with one another. There is no centrifugation required.

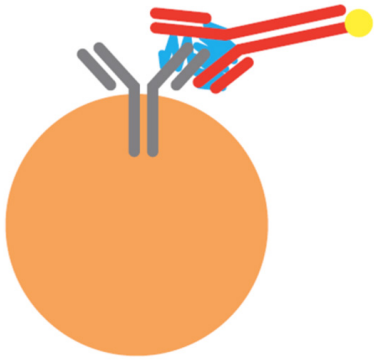
5.Batch size.

Will you need 100 g of beads, 10 kg of beads or even more? This is an important question to answer especially if you are planning to mass-market the production of your assays. Small batches must be validated separately while a large batch can be validated quickly.

As always, it is vitally important to consider all of

your variables before you dive headlong into production. Small scale testing to determine what type of bead to use will save you a lot of trouble in the future when you decide to scale up your assay production.





Chapter 6. How to Select the Best Surface for Your Chemiluminescent Immunoassays

Depending on your antibody and your assay, you have a number of ways to couple your protein or antibody to the surface of your magnetic beads. Once again, forethought is important in choosing which bead surface to use.

Plain surface.

This is the cheapest alternative and allows you to coat your beads passively. If, however, you are experiencing a great deal of non-specificity and background problems, you may need to consider the modified alternatives below.

Modified surface.

This is the simplest of the surface modifications that allow you to covalently and stably couple your antibody to the magnetic beads. Carboxyl, amino, hydroxyl and sulfates are typical groups that are added to the surface. Some problems can occur such as aggregation and nonspecific binding. If these problems are an issue, you may need to use pre-activated surfaces.

Pre-activated surface.

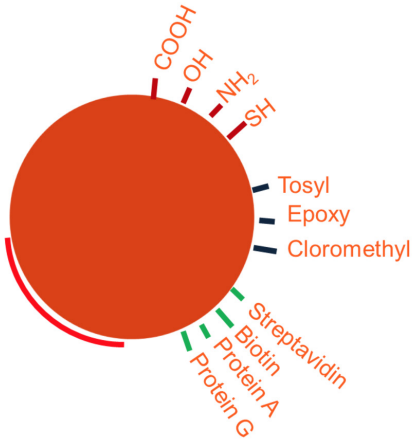
These surfaces are very 'sticky' and also allows for very stable covalent coupling of your antibody to the beads. Activated groups used are tosyl, epoxy, and chloromethyl groups. These are also very easy to couple by incubating the pre-activated beads in the correct buffer, correct pH or other conditions and coupling is automatic.

Bio-activated surface.

This is a very expensive, but highly effective option. A coat such as streptavidin is placed on the magnetic beads and the bead bound to the analyte can easily be removed from any background noise or free antigen merely by using biomagnetic separation. No additional washes are necessary.

Encapsulated surface.

The beads are encapsulated in a 'shell' structure. These beads give a very low non-specific background reading, improved sensitivity and improved colloidal stability.
By choosing the correct surface for your beads you can determine the sensitivity and accuracy level of your CLIA. For very high resolution/sensitive assays, the better the surface, the better the results.



Some problems can occur such as aggregation and nonspecific binding. If these problems are an issue, you may need to use pre-activated surfaces.

Chapter 7. The Five Most Popular Magnetic Microsphere Currently for Chemiluminescent Immunoassays

Although there are many choices you can make when determining bead surfaces, there are five predominant types of magnetic beads currently used most often by Chemiluminescent Immunoassay companies. These magnetic bead types are:

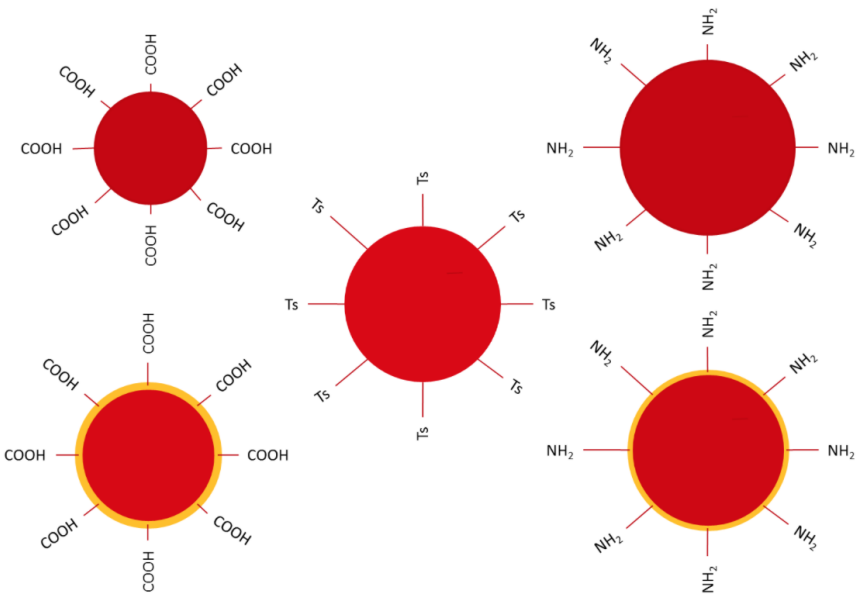
1. Surface modified beads with carboxyl (COOH) groups. The beads used are typically 700 nm – 1.2 microns in diameter and are comprised of 35-65% magnetic pigment (by weight).

2. Surface modified encapsulated beads with carboxyl (COOH) groups. These beads are typically 0.9 to 1.4 µm in diameter and, depending on your application, are comprised of 15-50% magnetic pigment (by weight).

3. Surface modified beads with amino (NH2) groups. These beads are 1-2 microns in diameter and typically contain 35-65% magnetic pigment (by weight).

4. Surface modified encapsulated beads with amino (NH2) groups. These beads can be a bit smaller and come in a size range of between 900 nm and 1.8 microns in diameter. The magnetic pigment content range is 35-50% (by weight).

5. Surface pre-activated with tosyl groups. These beads are either 1.1 – 1.4 µm in diameter with a magnetic pigment content of greater than 30% (by weight).



Once again, all options remain viable and available, however 80-90% of all companies that develop CLIAs tend to use beads from the above five categories. These beads seem to have the widest applicability and reliability combined with reasonable cost for the customer.

Chapter 8. Five Things One Should Consider when Launching Magnetic Bead IVD Kit Stability Studies

Stability over time is important and should reflect the environment that is typically encountered by the reagents. For example, if the reagents will be frozen and thawed, stability should be addressed in these conditions. Likewise, length of storage of the reagent should also be addressed while looking at stability. The following common reagent conditions should be considered when looking at stability of IVD kits:

1. Accelerated instability.

- Sometimes the kit can lose significant activity at 37oC (approximately 20% activity loss after three days). This usually happens because the ligand is unstable.
- This accelerated instability is not acceptable in a CLIA IVD kit since many of the enzymes used in catalyzing the final reaction need to work at physiological temperatures.

2. Long-term stability issues

In order to test for long term stability, reference lots should always be stored at -20oC and tested periodically. Long term stability, especially while stored frozen, is highly desirable since customers can purchase in quantity ahead of their demand and store the material until it is needed. Long term stability is also important when considering cost issues – both cost of production and cost for the customer. The longer you can store your product, the larger your batches can be and the less your production cost will be.

3. Real-time stability issues.

Ultimately, you would like to aim for your IVD kit to remain stable in the refrigerator (at 4oC) for at least a year. This speaks once again to cost issues and consumer demand.

4. Bench top storage and delivery issues.

It is reasonable to aim for room temperature stability of up to three months. This allows the end user to store reagents on their bench during a prolonged period of assays and also alleviates worries about instability during delayed delivery times.

5. Freeze/thaw and Heating/cooling cycles.

You should aim for a loss of activity after freeze/thaw or heating/cooling cycles of less than 10% over time. Labs may overestimate what they need at any given time and need to re-freeze their inventory. In addition, you also want your product to be refractory to ambient environmental temperature variances.

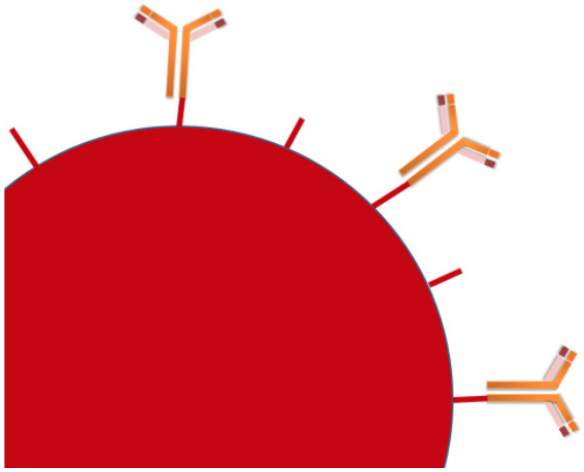
When end users look at products to use in their assays, they look at ease of use, reliability, precision and of course, stability. If a product isn't stable, the accuracy from one assay to another is in question and the product becomes useless to the end user. When proper steps are taken to guarantee stability in a variety of conditions, there will be great demand for your product.

When end users look at products to use in their assays, they look at ease of use, reliability, precision and of course, stability. If a product isn't stable, the accuracy from one assay to another is in question and the product becomes useless to the end user.

Chapter 9. How to Select the Best Blocking Reagent for Chemiluminescent Immunoassays

Non-specific background and auto-aggregation are often caused by the presence of exposed hydrophobic surfaces on the magnetic beads. The use of a blocking reagent combined with gentle homogenous bio-magnetic separation will help reduce background and auto-aggregation of your coated beads.

A blocking step is standard in all immunoassays. For CLIA beads, blocking reagents are often coated onto the microspheres using adsorption. This step occurs after the coupling reaction to the antibody. Blocking reagent concentrations as high as 0.1% are recommended in order to saturate all of the exposed hydrophobic surfaces. After the beads are processed, it is important to store the beads in a standard blocking buffer concentration of 0.05% so that blocking will be maintained throughout the storage life of the reagents.



The most commonly used blockers are:

- Bovine Serum Albumin (BSA). BSA is used either alone or in combination with a surfactant.
- Tween-20 and Triton X-100. These two are non-ionic surfactants that are used at a concentration of 0.05% in combination with 1% BSA.
- Casein/Pepticase. These are cheap proteins that contain free biotin. Therefore, these blocking reagents are not suitable to use in a system that involves biotin.
- 'Irrelevant' IgG. This blocking reagent is used when coupling a very specific IgG to the microspheres. It is important to always test that this type of blocking reagent is truly non-reactive with the analyte.
- Fish Skin Gelatin (FSG). This is a type of pure gelatin/hydrolysate. Other types of gelatins are also used as blocking reagents, depending on your preferences.
- Polyethylene glycol (PEG). This is a very versatile blocking reagent because it comes in a wide variety of molecular weights and charges.
- Sera. Sera from horse or fish are often used as a blocking reagent since they are typically inert when testing for cross-reactivity with many types of antibodies.
- Commercial blockers. Since most commercial blocking reagents are proprietary and, therefore, have an unknown composition, they are not recommended for use in IVD reagent production.

Without the proper blocking reagent, it is unlikely that the end user will be able to garner the sensitivity they require for their assays, especially if their analyte exists in very low concentrations. Blocking reagent choice, therefore, is extremely important to the quality of the final product.

Chapter 10. The Nine Key Questions to Ask Before Selecting your Magnetic Bead Supplier

Magnetic bead suppliers vary in how they produce their product, how they deliver their product and how they guarantee their product. Before deciding on a supplier, here are nine questions to ask your potential sources of magnetic beads:

1. Does the supplier conduct periodic audits of the product for quality control purposes? All suppliers should periodically check their product and validate that it meets their standard specifications.

2. Does the supplier offer bulk pricing for large orders? Do they offer specials if you want to evaluate and validate their products? Good suppliers will not shy away from allowing you to 'test' their products before you buy. Often you can get free samples to test and validate before you decide to buy in quantity.

3. Does the supplier guarantee delivery of the product in good condition? Is delivery reliable and can the supplier act quickly if you need material urgently? Does the supplier have a 'safety stock' supply in case of emergencies?

4. Does the supplier respond promptly when they receive a purchase order? Does the supplier respond promptly to customer service technical issues?

5. Does the supplier offer custom products? Does the supplier have a proactive attitude toward offering new products that will help improve your product and business?

6. Can the supplier help with scaling up issues? Have they tested their beads on biomagnetic separation systems that can cope with different production volumes and validation requirements?

7. Can your potential supplier help you achieve a better market position? Can they verbalize how they would do this?

8. Does the supplier try to anticipate changes in the technology and respond to those changes?

9. Does the supplier happily offer regular and easy to use technical support? Will the supplier meet with you face to face?

It is always important to have a supplier that is not only responsive to your needs, but who anticipates what your needs may be in the future. In addition to quality of the material and guarantee of the product, a responsive supplier is the best way to make a customer happy.

Summary. CLIAs and Magnetic Beads

CLIAs are excellent assays for high throughput, low analyte concentration, time sensitive testing and isolation. Using coated magnetic beads as the reagent in a CLIA is an easy and established technique favored among many clinical scientists. Making sure the CLIA you develop is accurate and consistent will be key for creating a high demand detection product. In order to develop the best product possible, it is important that you consider all of the steps discussed in this ebook.

1. Make sure you know the expected concentration range of your analyte and make sure you understand the assay specimen type.

2. Choose polyclonal antibodies if your epitopes are not well defined and choose monoclonal antibodies if you have a well defined epitope and need very high specificity. Polyclonal antibodies are not specific for one epitope but tend to recognize the range of epitopes 'seen' by the immune system.

3. Choose the proper magnetic bead for your particular assay. Be sure to consider all of the following variables:

a. Bead size (try to work with medium-sized beads, around 1.0 µm if possible)
b. Bead material (PS or PS/DVB, other types of materials or encapsulated if possible)
c. Bead with a higher magnetic pigment content (between 30% and 50% if possible)
d. Bead surface properties (plain, modified, pre-activated or bio-activated)
e. Bead batch size
f. Bead 'à la carte'. If what you need is not available 'à la carte', ask your supplier to develop a custom product to fit your specific needs.

4. Think carefully about the bead surface you need for your assay. While plain surfaces are the cheapest and are the easiest to work with, they also can have specificity problems and high background readings. Modified surfaces will stably couple, but require chemical activation that can cause aggregation. Pre-activated surfaces are easy to couple and bio-activated surfaces are usually free from background noise (although a very expensive modification). Encapsulated beads improve your assay's sensitivity and stability.

5. Some magnetic bead types are used more than others. You may want to consider the well-used, well-established beads or decide that you need unique modifications.

6. Make sure you test the stability of your reagent and aim to address short term, long term and real-time stability issues. In addition, pay attention to storage conditions, hot/cold cycles and delivery when considering stability of your beads.

7. Test a variety of different blocking reagents and choose the blocking reagent that gives the least background noise and stability.

8. Make sure you have vetted your magnetic bead supplier thoroughly before beginning your development.

By following all of these steps, your CLIA assays using magnetic bead technology will be high quality and will have a much better chance of becoming high demand products.

About the Author: Dr. Fabrice Sultan, OEM Diagnostics- Estapor, Merck Chimie SAS (France)

Fabrice Sultan obtained his PhD in Life Sciences from the Biomedical Institute in Paris in 1990. He joined the B.Braun Medical as product manager in charge of the parenteral nutrition product line of the company. After 8 years he started as sales and marketing manager with Prolabo, a french company of the Merck group. In charge of the Estapor Microspheres product line as sales & marketing manager, Fabrice Sultan combines his background in biology, biochemistry and immunology, together with his experience in sales & marketing and his knowledge in international business. Fabrice Sultan has gained a solid reputation in Microspheres Technologies and provide useful technical trainings in bead-based immunoassays.

www.sepmag.eu

sepmag®

Follow us on:

SEPMAG SYSTEMS
contact@sepmag.eu
+34 935 820 161

Parc Tecnològic del Vallès
E-08290 Cerdanyola del Valles. Barcelona