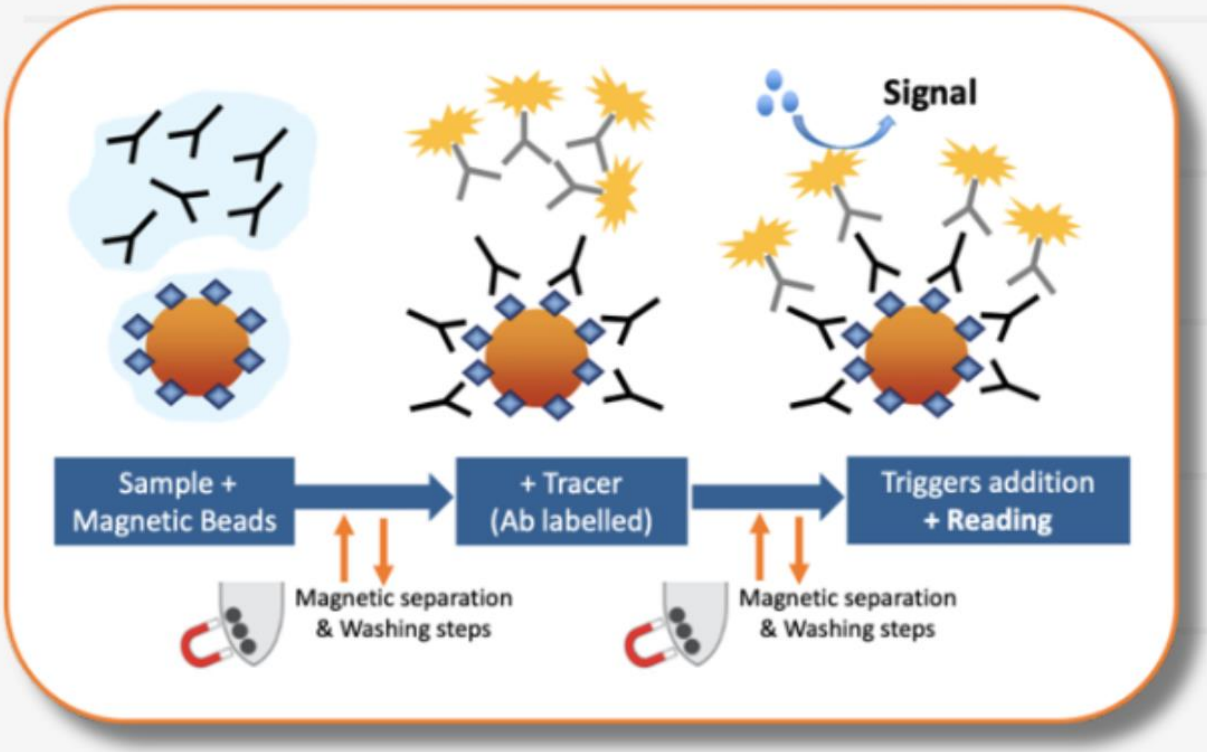
Biofuctionalization of magnetic beats in chemiluminescent (CLIA)



by Dr. Anabel Lermo

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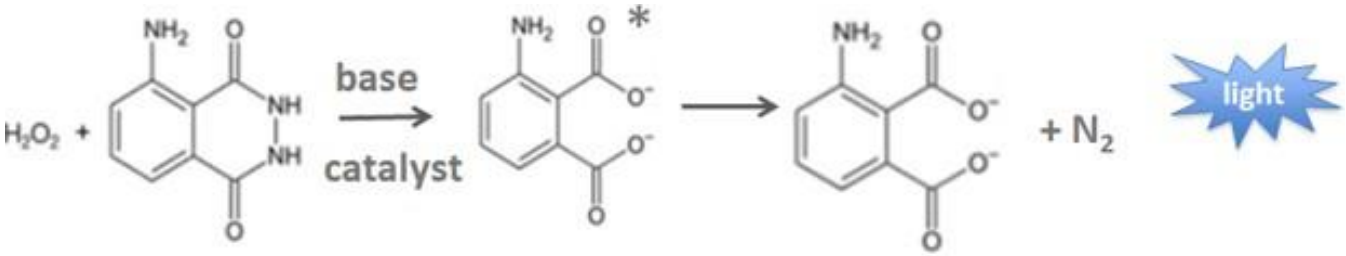
Overview on chemiluminescent immunoassay (CLIA)

General introduction

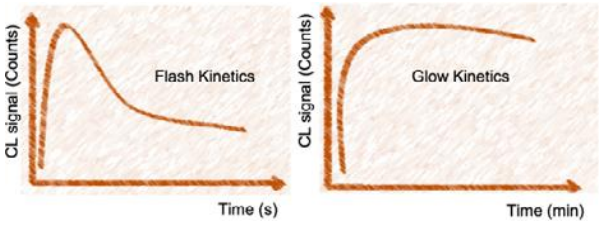
Luminescence is the emission of light, and it can occur in many ways. In research and biomedical industry fluorescence and chemiluminescence are often used. Fluorescence is when light is absorbed then emitted by a substance. A photon of a higher energy state is absorbed, then a lower energy photon is emitted in another range of the electromagnetic spectrum.
On the other hand, chemiluminescence (CL) is the conversion of the chemical energy into the emission of visible light as the result of an oxidation or hydrolysis reaction. The CL related reaction is shown as follows:

Chemiluminescence emits light without the need of excitation from a light source or a detector for a wide range of wavelengths not in the visible spectrum.

The luminol reaction is used as an example of CLIA reaction. In the presence of hydrogen peroxide, in basic conditions and with a catalyst, the luminol goes to an excited state and then to a ground state realizing light. The reaction is shown as follows:



In an immunoassay assay, CL is used as a label to display the information resulting from an immunobinding reaction. The CL applied to immunoassay is abbreviated as CLIA, and the emission of the lightcan be induced either in a glow-kinetics or in a flash- kinetics reaction. While the glow-kinetics reaction refers to an indirect method using enzyme labels such as HRP and AP+substrates, the Flash-kinetics reaction is a direct method which makes use of chemiluminescent labels such as luminol derivatives and acridinium. The glow-kinetics is characterized by a slow emission (minutes) of light that remains stable for minutes, while in the flash-kinetics thereis a rapid emission of light (tenths of second) that decays in a few seconds being a more convenient method for IVD automated platforms.



Current applications

Chemiluminescence is used in a wide range of applications such as environment monitoring, food analysis, forensic science, western blotting, DNA hybridization and CLIA, among others. For example, researchers monitor the presence of antibiotics in cows or the presence of pesticides in honey bees. Food and water can be tested for lead, antibiotics, or harmful bacteria such as *E.coli* using CLIA. Otherapplications in the biomedical field used CLIA to detect autoantibodies. Chemiluminescent immunoassays are also used for haemostasis markers, tumour markers, in clinical applications, Hepatits and infectious diseases detection. In 2020, CLIA was used for the detection of anti-SARS-CoV-2 IgG and IgM inpatient samples.

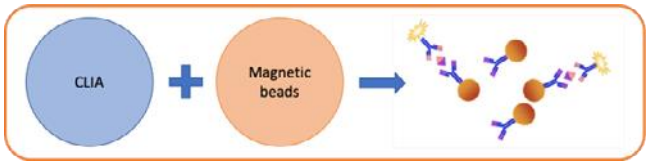
Advantages over other techniques

A major advantage of chemiluminescent immunoassays is the simplicity of the detector needed to re- cord data. Chemiluminescence emits light without the need of excitation from a light source or a detector for a wide range of wavelengths not in the visiblespectrum. This is also advantageous because therewill be no background from the substance being observed since the only light emitted will be from the chemiluminescent reaction. Unlike in fluorescent as- says where autofluorescence is an issue to monitor. Chemiluminescence also doesn't require separation of absorption and emission wavelengths to understand the readout, it is more direct.

Chapter 1. Magnetic beads in CLIA

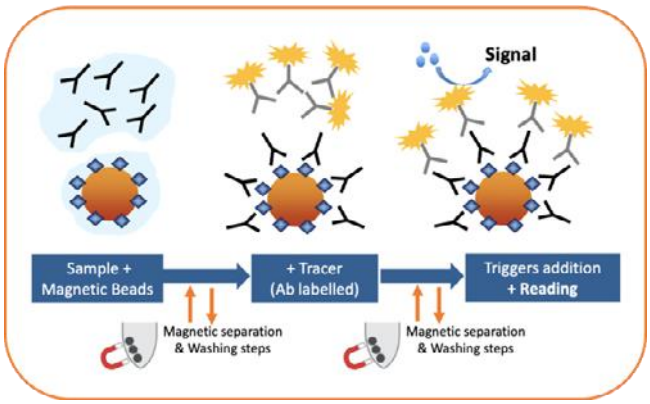
How can magnetic beads improve CLIA tests?

The combination of CLIA and magnetic beads brings together all the advantages of both parts. CLIA is known for its high sensitivity which allows the detection of analytes at very low concentrations, and thusproviding an excellent limit of detection in a wide dynamic range. Magnetic beads, on the other hand, are known for their simple protocols which help minimizing and even eliminating potential sources of error that occur in complex systems. Magnetic beads reduce the incidence of non-specific interactions during the capture and isolation processes. In addition, due to their large spherical surface area, magnetic beads allow for a more efficient binding tothe target substance.



Magnetic beads separation and washing steps

Separation is a crucial part of the CLIA protocol. Between each binding step, unbound material must be removed as efficiently as possible, using the optimum magnetic separation setup. Magnetic beads can be functionalized according to the assay that is required to the target analyte. For example, using CLIA to detect an antibody such as SARS-CoV-2 IgG, the first step will be to incubate the sample thatcan contain SARS-CoV-2 IgG antibodies to the magnetic beads, which are previously surface-functionalized with specific antigen/s that allow the antibodies to bind to the surface of the magnetic bead. Magnetic beads will capture only the target antibody of interest, which will increase the specificity of the assay at this crucial step. Next, a new incubation will be performed where a “tracer” (an anti-IgG labelled with a chemiluminescent tag in this example)is added and is bounded to the antibodies on the magnetic bead. Then there is another magnetic separation and washing step. This time it is crucial forremoving unbound tracer molecules, to ensure that all chemiluminescence response comes from specific bound antibodies. Finally, triggers are added, and signal is obtained, which is directly proportional to the material labelled with the chemiluminescent tag.



Magnetic beads washing

Modern magnetic separators ensure that washing steps occur carefully but still efficiently regarding time required for the separation. The constant magnetic force keeps all magnetic beads bound securelyto the walls of the container. Both modern magnetic separators and magnetic beads make it easy to per- form CLIA tests in plate format or at large scale. In fact, the separation of batches up to 20 L of magnetic beads suspensions is a well established practice for IVD-kits manufacturing. Using bottles and standard advanced Magnetic Bead Separation systems, theseparation processes are smoothly scaled up to re- ach 50L for customized systems.

Chapter 2. Preliminary considerations for CLIA design

Designing CLIA assays requires the consideration of different aspects, encompassing the raw materials for the reagent development & methods selection, together with the choice of the assay format. Material suppliers are a key factor for a successful design and development of an assay. An ideal supplier should be able to provide required raw materials not only at a reliable cost but also available to provide the required bulk quantities for scaling up the reagent. Moreover, suppliers should provide different lots to assess the lot-to-lot variability to check the impact in the assay to be developed.

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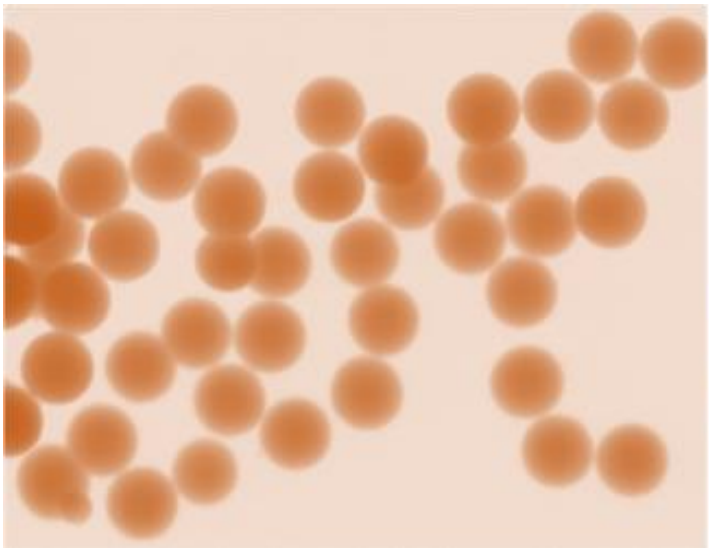
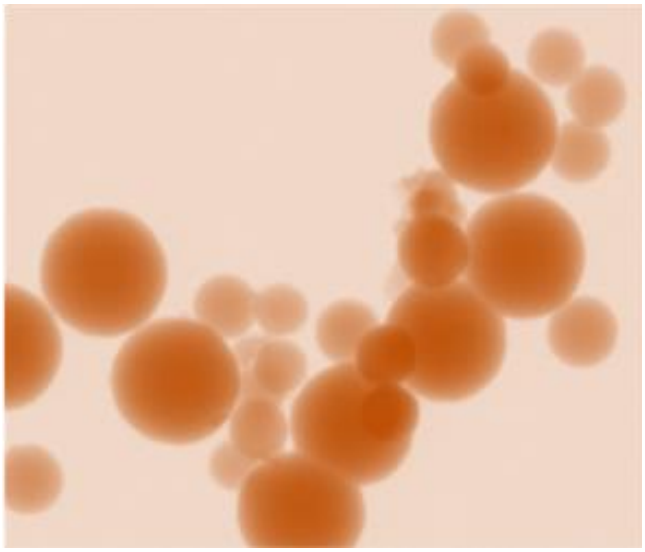
Strategies for choosing magnetic beads

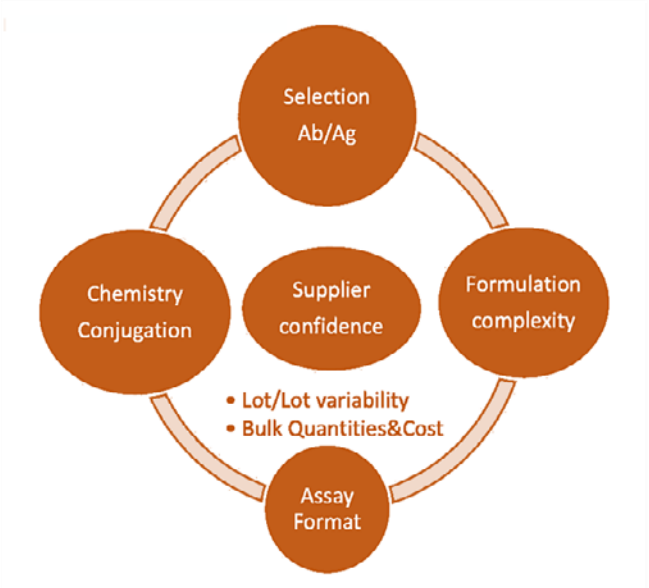
Magnetic beads are available in different sizes, ranging from nanometers to a few micrometers. Smaller beads will likely require more time to separate using a magnetic separator. The separation time of the magnetic beads depends strongly on the amount of the magnetic pigment. Higher pigment percentage will result in faster magnetic bead separation. A balance between the magnetic pigment and the size of the magnetic beads should be reached to maintain the correct density of the magnetic beads.





Chemical modified magnetic beads for CLIA

A simple but less stable strategy for conjugating a magnetic bead surface can be achieved through passive adsorption of the antigen or antibody directly on the surface of a “plain” magnetic bead. Alternatively, the modification of the surface chemistry of the magnetic beads is a great option if covalent attachment of the antigen or antibody is important for the assay. Active groups take advantage of the large sur-face area of the magnetic beads and provide abundant anchoring points for your antibodies or antigens to become covalently conjugated to the bead. Some examples of pre-activated chemicals that can beused are the popular Tosyl group or epoxy. There are other groups that are used which require activation,
such as carboxyl (-COOH) groups, amines$_{(-NH2)}$, and hydroxyls (-OH). This practice is also known as the chemical functionalization of magnetic beads.



Biological modified magnetic beads for CLIA

Biological modified magnetic beads (also known as surface biofunctionalization) are also a common strategy for CLIA development.

There are several types of biological modified magnetic beads. The antibody or antigen of choice can be modified with a biotin linker which binds to a magnetic bead modified with streptavidin. Protein A binds the Fc region of most immunoglobulins ma-king it an attractive choice for the immobilization of antibodies to the magnetic beads. Similarly, Protein G binds either the Fc or Fab region of a spectrum of immunoglobulins and can be used to immobilize antibodies. Sometimes Protein A and G are used together to increase the spectrum of potential antibody binding. It is also possible to conjugate specific proteins, but this requires optimization.

When biological modified magnetic beads are selected for a CLIA assay it is important to check the compatibility of these beads with the sample type, to prevent non-specific interactions with com-ponents of the sample with the biological surface of the magnetic beads.

Chapter 3. Most common assay formats

There are several types of CLIA formats that can be used depending on the target analyte of your assay. The choice of assay format will impact four major aspects of development. The first will be the choice of magnetic bead coated with antigen/s or antibody/ies for binding the target analyte. The tracer will then be required to match the target analyte using a conjugated antibody/ies or antigen/s conjugated with a CLIA label. The assay buffer will need to be optimized to improve the specificity and sensitivity for each step. Lastly there will be components such as blockers, other linking molecules or stabilizing molecules. These aspects can be optimized once an assay format is chosen.

The following examples represent typical assay formats depending on the target to be detected.

Assay formats type 1, simple CLIA assay formats

Antigen (Ag) detection-

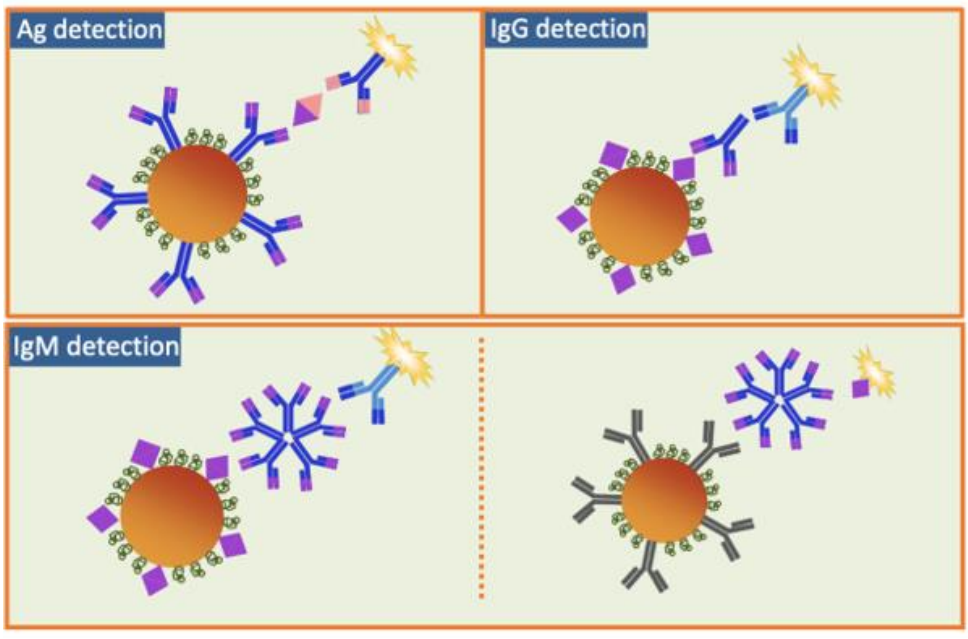
A sandwich assay can be used for Ag detection. In this case, magnetic beads are conjugated with antibodies specific to the target antigen. Then, a sample that contains the specific antigen is then binded to the antibody. Lastly, the tracer is a CLIA-labeled antibody that can also bind to the antigen that needs to be detected.

IgG detection-

For this type of assay format magnetic beads can be conjugated with an antigen specific to the IgG. Then, a sample that contains the specific IgG is then binded to the antigen. Lastly, the tracer is a CLIA-labeled anti-IgG antibody that binds to the IgG antibody that needs to be detected.

IgM detection-

Similarly to IgG format, IgM can be detected using magnetic beads conjugated with an antigen specific to the IgM. Then a CLIA-labeled IgM is used as a tracer. Alternatively, there is another format for the detection of IgM, where magnetic beads are conjugated with an anti-IgM and then the tracer in this case is an antigen specific to the target IgM. This alternative format implies the CLIA labelling of an antigen instead of an antibody and this can be more challenging for keeping the specificity of the antigen labelled.



Assay formats type 2, complex CLIA assay formats

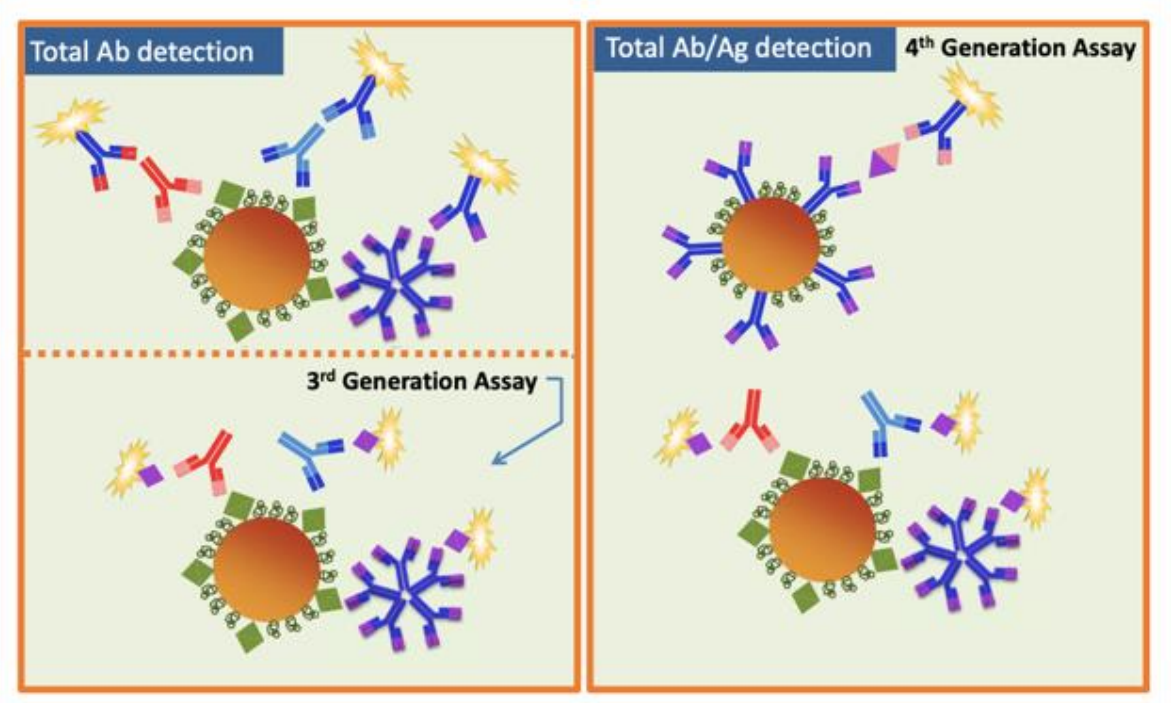
Total antibody (Ab) detection

More complex assay is required to detect multiple types of immunoglobulins. To detect IgG, IgA and IgM; magnetic beads can be conjugated with several types of antigens specific to various immunoglobulins. Then, the tracer consists of anti-IgG, anti-IgA and anti-IgM CLIA-labelled antibodies that bind to the antibodies.

The total antibody detection can be performed with a more sophisticated assay called “third generation assay”, in this case antigens for multiple types of antibody are conjugated to the surface of the magnetic beads. IgG, IgA, and IgM are binded specifically to the bead by the antigen. The tracer molecule in this case is a CLIA-labeled antigen. This assay format is more specific as the tracer molecules correspond directly to the antibody-antigen interaction of interest, rather than using a secondary antibody.

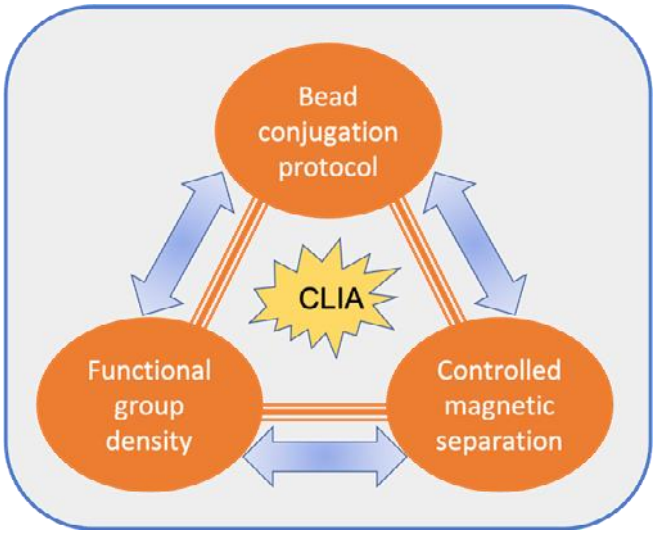
Total antibody (Ab) and antigen (Ag) detection

Sometimes, detecting in a sample both antibodies and antigens can be highly beneficial, as for example the case of HIV. For this assay format called “fourth generation assay”, a combination of magnetic beads conjugated to either antigen/s or antibody/ies are used. The beads conjugated with several antigens are specific for IgG, IgA and IgM antibodies and the tracer is a CLIA-labeled antigen. While the antibody conjugated beads will bind the antigen of interest and then a secondary CLIA-labeled antibodywill act as a tracer.



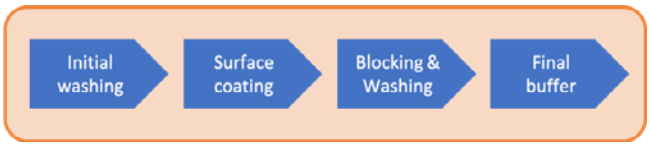
Chapter 4. Guide for successful magnetic bead conjugation

For a successful procedure for the magnetic bead conjugation, there are three important aspects to take in consideration when designing the assay: i) the planning of the conjugation protocol, ii) the density of the functional groups on the surface of the magnetic beads, and iii) the controlled Magnetic Separation of the Beads.



Typical bead conjugation protocol

1. First washing step - During this step the buffer in which the beads came is removed because it can contain components that can potentially interfere with the conjugation. Magnetic beads are attracted by a magnetic separator and the initial buffer is removed. When the bead buffer is removed, the appropriate buffer for antibody or antigen coating is added to the beads.
2. Coating the magnetic beads - The magnetic beads are then coated with either antibody or antigen depending on the assay format. The time for this step varies depending on the choice of the chemistry reaction of the bead and the protein to be attached. The optimal length for this step can last one, a few hours or overnight/s. It is important to consider the pH of the buffer used for the coating to make sure it is optimized for the specific isoelectric point of the chosen coating protein. Often coating is done at room temperature or 37 degrees if the protein of choice is stable at higher temperatures.
3. Blocking and Washing Steps - Blocking allows that free space or free chemical groups on the bead surface are blocked to prevent non-specific binding . Classic blocking agents are BSA, casein, PEG and glycine, which will bind to the free groups on the bead. Sometimes small amounts of detergent are also used to further prevent or reduce non-specific binding. This step is usuallydone at room temperature.
4. Final buffer - The final buffer for bead conjugation is added to keep the stability of the beads and compatible with the immunoassay re- action. It will prevent bead aggregation and help keep the beads stable. This buffer might includeblockers and should be optimized for pH and ionic strength. To prevent contamination antimicrobial agents are added, usually in the range of0.05 to 0.10%.



Influence of bead surface density

The amount of functional groups on the surface of the magnetic beads is an important parameter to take in consideration. This parameter is commonly quantified through the so-called “Parking Area (PA)”. In a coating process, the PA defines the space occupied with the functional groups on the surface of a magnetic bead. From a mathematical point of view, the calculated PA is the reciprocal of the surface charge density. PA is directly related to the beadsize (and this to its surface), the density of the so- lid bead and the amount of the surface functional groups. One of the benefits from knowing the PA, is the ability to select beads of different sizes with similar binding properties.

Controlled magnetic separation during bead conjugation

Modern magnetic separators for Magnetic Bead Separation can monitor the separations in real time. This is extremely valuable for the conjugation procedure. Separation of magnetic beads during conjugation should be efficient, avoiding residual stuck of the beads and easy to monitor. Efficiency is achieved when the separation is done quickly, steadily and repeatedly, all of which is a staple of modern magnetic separators. These specifications also reduce beads aggregation. Modern magnetic separators can also allow to scale up small lots to production size lots of conjugated beads as they are available in 10 liter sizes. Finally, modern separators come with the technology to follow the efficiency of the separation in real time on a computer. This gives more control over the conjugation procedure as you can monitor which buffer and conditions are best for your separation and getting consistent quality results. This also provides information between lots prepared of conjugated beads to check the robustness of the conjugation procedure.

Modern magnetic separators for Magnetic Bead Separationcan monitor the separations in real time. This is extremely valuable for the conjugation procedure.

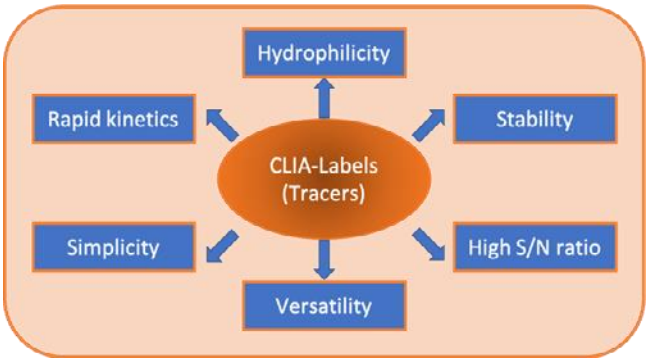
Chapter 5. Tracer optimization

The tracer, the antigen or antibody labelled with a chemiluminescent tag for CLIA, is the next vital optimization step of a chemiluminescent immunoassay. As mentioned earlier, chemiluminescent labels generate light from a chemical reaction. Widely used CLIA labels are based on luminol derivatives or acridinium esters.

CLIA label desired properties

CLIA labels should have the following characteristics for optimizing the Tracer design:

Stability- The CLIA label should be stable toprevent the decay of response over the time, providing extended reagent shelf life and extended rea- gent on-board stability. This is especially important during the reagent shipment to assure the stability of the CLIA label under extreme conditions of temperature.



High signal to noise ratio - For a CLIA assay it is important to have as much more signal than noise as possible. This helps ensure that the limit of detection can be low because the signal can be distinguishedfrom noise at small concentrations.

Versatility - The versatility of a tracer can expand your ability to design a CLIA. The same CLIA label should be used to bind small haptens to large antigens or antibodies, the label should be able to bindefficiently to any molecule of interest.

Simplicity - Ideally, the tracer mechanism should not be complicated so that the emission of light is easily triggered. A simpler tracer reaction will decrease the likelihood of error when using the assay.

Rapid Kinetics - For a CLIA assay it is important to provide a result the sooner the better so it is preferable that the light of emission will be completed in seconds for having high throughput.

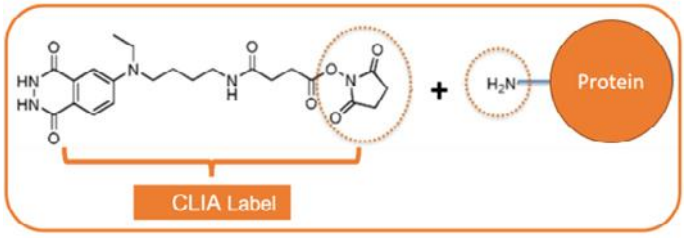
Hydrophilicity - The CLIA label should be hydrophilic to improve the tracer solubility, this can be made by modifying the CLIA label with groups that can improve solubility such as PEG structures. This will reduce the non-specific adsorption of the tracer being able to detect lower concentration of the analyte.

Chapter 6. CLIA-label protein conjugation

Once an optimal CLIA label (also called CLIA tag) is chosen, it must be conjugated to the protein which will bind to the analyte desired to detect. Isoluminol or Acridinium ester derivatives are often used as CLIA tags.

The chemistry of the CLIA label-protein conjugation

Conjugation of a protein with CLIA-label commonly involves labeling a protein with an acridinium ester derivative via an amine group on the protein. Primary amine groups are present on proteins either at the N-terminus of the polypeptide chain or at the end of a lysine amino acid side chain. These primary amines are often the target of protein labels due to their nucleophilic nature, their propensity to be charged and at exposed regions of a protein conformation. Acridinium ester has the N hydroxysuccinimide (NHS) ester group at the end, a known amine reactive group. When the reaction occurs, at pH between 7 and 9, the NHS is released and the rest of the ester is turned into an amide bond by binding the protein amine group.



Purification of the CLIA label-protein conjugation

After conjugation, the next step is to remove the free remaining labels and unbound protein. Chromatography is a classic method for separating remaining labels and unbound molecules. With chromatography the separation process can be monitored over time by absorbance and size information is Obtained. Dialysis can also be used for separation but provides less information than chromatography andis time consuming.

Considerations for CLIA label-protein conjugation

1. Avoiding amine buffers and additives in the protein matrix can minimize interference of the conjugation reaction, which occurs at amine groupson the target protein.
2. CLIA labels are highly unstable in aqueous solutions and to maintain its stability storing in organic solvents is best. DMSO or DMF are good examples of organic solvents for longer term storage of the CLIA label.
3. Be consistent with concentrations of protein and organic solvents throughout assay development. This will help maintain consistent results even as the proteins change (lot differences) or in scaling up the conjugation process.
4. The CLIA-label:protein ratio needs to be optimized to obtain an efficient conjugate. A higher molar or mass ratio, providing higher labelling can lead to insolubility problems and potentially decrease the activity of the protein. This becomes especially important for smaller proteins orhaptens.

Optimized CLIA label-protein conjugation

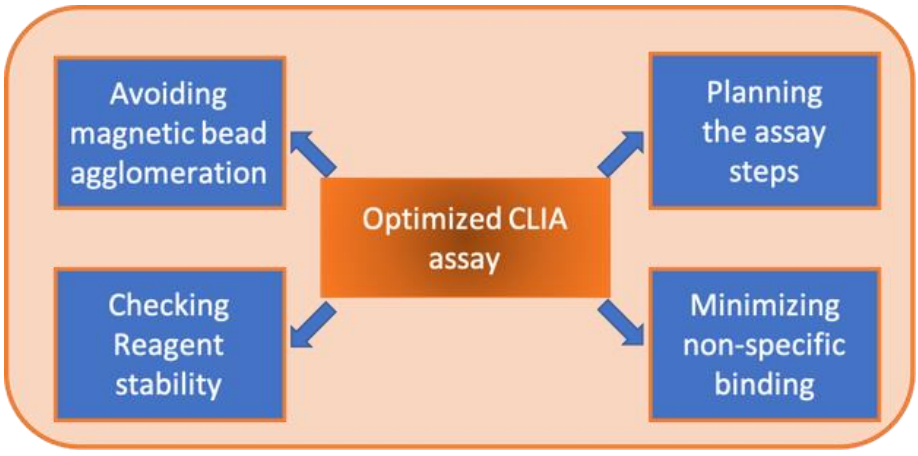
An optimized conjugation of the CLIA label to the target protein can be visualized during chromatography. During fractionation a large peak will ap- pear when your conjugated molecule goes through a filtration column, often based on size exclusion. Finally, when the CLIA performance is tested, the chemiluminescence from the immunoassay will be measured and the success of the conjugation will be assessed.

Chapter 7. Guide for optimizing CLIA performance and scaling-up

There are a few more considerations for optimizing the CLIA assay that in this chapter will be discussed. The following considerations are related to the performance of your assay.

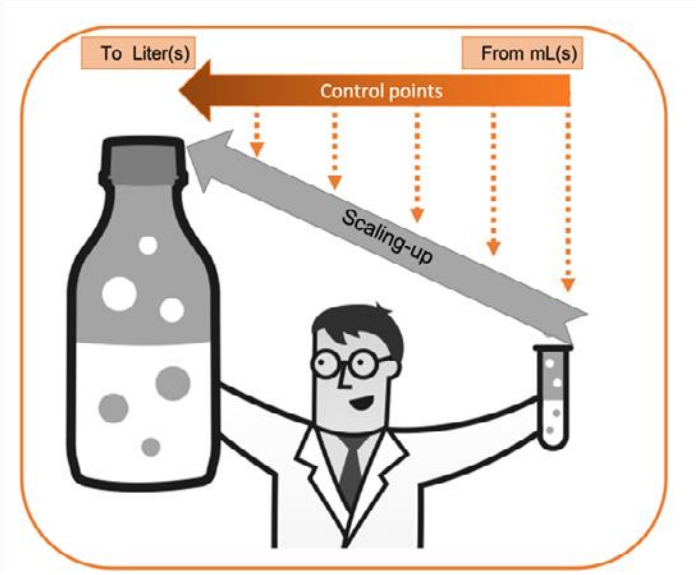
CLIA Performance considerations

1. Proper suspension of the magnetic beads- When magnetic beads are on board the analyzer and are not properly homogenized by the analyzer mixer, wrong results in the assay can be obtained. This can be called the “first sample effect”,where reactivity is too high or too low on the first replicate. If magnetic beads are not properly homogenized, the quantity of particles in the assaywill be different than the required. This problem needs to be troubleshooted as early as possible in the preliminary phase of the project. Many factors can modify this effect, such as particle size of the bead, the conjugation procedure or final resuspension buffer of the beads.
2. Using one step or two step assay- Depending on the analyte interested to be detected, a one or two step assay may be the preferred option.A two-step assay is a good option when there is concern about non-specific binding because thewashing steps can help to reduce it.
3. Other non-specific binding reduction methods- It is possible to include other antibodies in the assay to minimize unwanted binding events. The non-specific binding of heterophilic and human anti-mouse antibodies (HAMA) can be reduced using a mixture of polyclonal and mouse monoclonal antibodies. This mixture is usually included in the assay buffer formulation.
4. Check reagent stability- As is mentioned earlier in this eBook, the stability of your assay is vitally important. It is crucial to check how the reagents can handle transport and time spent unused. To have an idea of the reagent stability, accelerated stability is performed usually exposing the reagent at high temperatures such as 37 or 46 degrees for a period. The decrease of reactivity under these conditions can be related to low real stability. Of course, real time stabilityand transport simulation need to be assessed and cannot be replaced by the accelerated stability data.
5. In some cases, it can be really challenging to obtain acceptable real time stability for the reagent and some companies try to stabilize their rea- gent using lyophilization.



CLIA Scaling-up considerations

1. Control Points- Throughout the scaling up process it is useful to have control points at which you assess the variabilities in the process and in your materials. This will aid in troubleshooting when increasing the scale of each part of the process.
2. Instrumentation Consistency- It is better to use similar types of instrumentation to ensure that the process will still work the same way when going from small to large batches.
3. Key reagent scaling- The key reagents of the assay, such as the CLIA-labelled protein will be needed in larger quantities for scaling-up. Before scaling-up the entire procedure, it is important to be sure that the key reagents like the CLIA- labeled proteins can be successfully scaled up.
4. Other potential troubleshooting- For scaling-up, everything will need to increase. Storage capacities, temperature-controlled areas, magnetic separators, and other crucial materials will need to have the capacity to be scaled-up for the whole process to move in the larger direction.



Chapter 8. Aspects of available CLIA kits in the market

When developing a CLIA it is helpful to understand what is available commercially. CLIA kits are available from many different companies that formulate reagents or components of the assay specifics for the analyte to detect and tailor made for the company analyzer (platform). The solid phase can be based on superparamagnetic beads or polystyrene beads usually kept in liquid formulation. In both cases the-se beads require a CLIA-label reagent, discussedin more detail earlier in this eBook. The CLIA label can be based on either a flash chemiluminescent kinetics (such as using an acridinium ester label or an isoluminol derivate) or a glow chemiluminescent kinetics (such as using alkaline phosphatase or horseradish peroxidase). There are many chemiluminescent platforms from companies such as Siemens or Abbott. Companies aim to make their platforms with high throughput, panels with a broadrange of assays available and easy to use for multiple types of assays.

When developing a CLIA it is helpful to understand what is available commercially. CLIA kits are available from many different companies that formulate reagents or components of the assay specifics for the analyte to detect and tailor made for the company analyzer (platform).

CLIA-kit components

CLIA kit can come with reagents (beads, tracer, assay buffer…) in a unique cartridge or individual vials, depending on the company and analyzer. The kit will contain at least magnetic particles and tracer reagents. There is a usual characteristic for all platforms, magnetic beads are required to be homogenized on-board the analyzer, to prevent bead sedimentation. On the other hand, tracer is kept in opaque vials to be carefully protected from light exposure, maintaining its chemiluminescent properties. Calibrators and controls are also not typically included in the reagent kit but there are some companies that include in the reagent cartridge also the controls. Sometimes calibrators can be reagentlot dependent but is more versatile if calibrators can be used for any reagent lot. When the stability of calibrators and controls is compromised in liquid formulations, lyophilization of these materials can be an option to enlarge their stability although is worst from a point of view of easy to use for end users.

Instrumentation modules in a CLIA analyzer

There are three common modules in main analyzers needed to perform CLIA assays with magneticbeads.

It is required to have a washer station for the several washing steps included in the CLIA assay protocol. Together with a magnetic separation module integrated with the washer for the retention of the beads during the washing steps. Magnetic separation module require to be carefully designed to be strong enough to capture the beads, retaining the beads during the washing and preventing the stuck of the beads for an easy homogenization of the beads once washing steps have been performed.

The detector mode is also a common module to detect the light emitted from your chemiluminescent reaction.

All these three modules are integrated for common automated platforms for either benchtop analyzers or high-throughput routine analyzers.

Limitations and perspectives

Advantages of the CLIA

There are many types of assays that can be performed for detection of a molecule of interest, all with their own advantages and disadvantages. Many scientists choose to perform chemiluminescent immunoassays over the enzyme-linked immunosorbent assays (ELISA), fluorescence or radioimmunoassays. This is because the CLIA has been shown to have an improve detection at lower concentration and a wide dynamic range. This means that the CLIA can correctly detect a wide range of concentrations of the molecule of interest without the need for concentrating or diluting samples. The design of several types of CLIA-labels allows scientists to develop a CLIA with best precision at the low-end concentration of analyte and low background signal. Being able to correctly detect positive samples for a certain analyte, with fewer false negatives making sure that those with the presence of the analyte of interest are properly diagnosed.

The use of magnetic beads improves the separation steps in the CLIA assay, minimizing sample typical interferences and making easier the removal of the tracer that is not bound to the analyte of interest. This also makes development easier by ensuring consistent and easy washing and separating steps. Magnetic beads can be easily handled by automatic analyzers allowing streamlined assays. On the other hand, the detection method based on the CLIA label provides enhanced detection.

The main parts of the CLIA that determine the feasibility of your assay are the magnetic beads and the tracer. Time and care must be taken to design the optimum procedure to conjugate the magnetic beads with the Ab or Ag required. Then the conjugation of the CLIA-label to the tracer molecule must be efficient and effective without decreasing the specificity and sensitivity of the immunoreagent to be labelled and keeping required response from thedetector. Another limitation to consider is the lot-to-lot variability, an issue for all assays. Lot-to-lot differences in the reagents can be due to variability of raw materials used in the formulation of the reagents. This can impact the quality of the assay and must be carefully analyzed. On the other hand, It is important to check lot-to-lot differences in the calibrations and controls because response of certain reagents may change.

Although the development of a chemiluminescent immunoassay requires the optimization of several key parameters, with the help of solid expertise of researchers in the field, improved chemilumescentimmunoassays can be developed.

About the author
BIOGRAPHY: Dr. Anabel Lermo

Dr. Anabel Lermo is a Research Scientist within the field of In-Vitro Diagnostic (IVD). She holds a PhD in Chemistry from the UAB of Barcelo- na. After her postdoctoral work in the field of immunoassay development applied to biosensor design at the Analytical Chemistry Division UAB, she joined Biokit (a company of the Werfen group) for 7 years, developing chemiluminescent immunoassays for automated platforms in the field of infectious diseases. Later she worked for Pragmatic Diagnostics developing new assays where immunometric technologies are integrated with appropriate analytical platforms for IVD industry.

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

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