Part one **Sera & Vaccines** **Dr.Ikbal AL-Joofy**

…….INTRODUCTION……

Many unrecorded observes have noted that a contraction of and recovery from certain disease resulted in a permanent resistance to recurrence of a same disease…

Jenner discovered that cowpox or vaccinia induced protection against human small pox (fatal disease). Jenner called his procedure vaccination; this term is still used to describe the inoculation of healthy individuals with weakened or attenuated strains of disease-causing agents to provide protection from disease.

2 Centuries for smallpox vaccination to become universal, 1979 world health organization (WHO) was able to announce that small pox had been eradicated..

Jenner know nothing about an infectious agents that cause disease, until a late of the 19th century when Robert Koch proved that infectious diseases were caused by microorganisms ,each one responsible for a particular disease..

We now recognize four broad categories of disease-causing M.O.S ,or pathogens, these are viruses, bacteria , pathogenic

fungi ,and other relatively large & complex eukaryotic organisms collectively termed parasites .

The discoveries of Koch& microbiologists made a development of immunology possible and extended the example of Jenner s vaccination to other diseases.

Louis Pasteur in 1880s, devised a vaccine against Cholera in chickens, and developed a rabies vaccine proved success upon its first trial use in a boy bitten by a rabid dog . These practical led to a search for the mechanism of protection.

In 1890 , Emil Von Behring and Shibasaburo Kitasato discovered that a serum of vaccinated individuals contained substances-which they called antibodies-that specifically to relevant pathogen ..

Immunization and Vaccination

The history of immunization begins in 19th century, as we said before, observation had been made that individuals who recovered from certain disease were protected from recurrences, thus led to introduction of process known as "Variolation" (variola major is a small pox virus) , in W fluid extracts from the pustules caused by a small pox virus were

obtained from individuals who appeared to have recovered from infection & injected under the skin to uninfected individuals .The procedure was occasionally successful ,

So the term "immunization" can be used to denote an artificial process where by an individual is rendered immune. There are two broad categories of immunization: Active & Passive Active immunization is largely synonymous with vaccination.

Immunization is the means of providing specific protection against particular pathogens. The mechanism of immunity depends on the site of the pathogen and also the mechanism of its pathogenesis. Thus, if the mechanism of pathogenesis involves exotoxins, the only immune mechanism effective against it would be neutralizing antibodies that would prevent its binding to the appropriate receptor and promote clearance and degradation by phagocytes. Alternatively, if the pathogen produces disease by other means, the antibody will have to react with the organism and eliminate it by complement-mediated lysis or phagocytosis and intracellular killing. However, if the organism is localized intracellularly, it will not be accessible to antibodies while it remains inside. Thus, the cell harboring it will have to be

destroyed and only then can antibody have an effect. Most viral infections, intracellular bacteria, and protozoa are examples of such pathogens. In this case, the harboring cells can be destroyed by elements of cell mediated immunity. Alternatively, if they cause the infected cell to express unique antigens recognizable

by antibody, antibody-dependent, and complement mediated killing can expose the organism to elements of humoral immunity. As another possibility, cells harboring intracellular pathogens can be activated to kill the organism. Such is the case with pathogens that have the capability of surviving within phagocytic cells.

Specific immunity can result from either passive or active immunization and both modes of immunization can occur by natural or artificial processes (Figure 1).

Passive Immunity: Immunity can be gained, without immune system challenge, by transfer of serum or gamma globulins from an immune donor to a non-immune

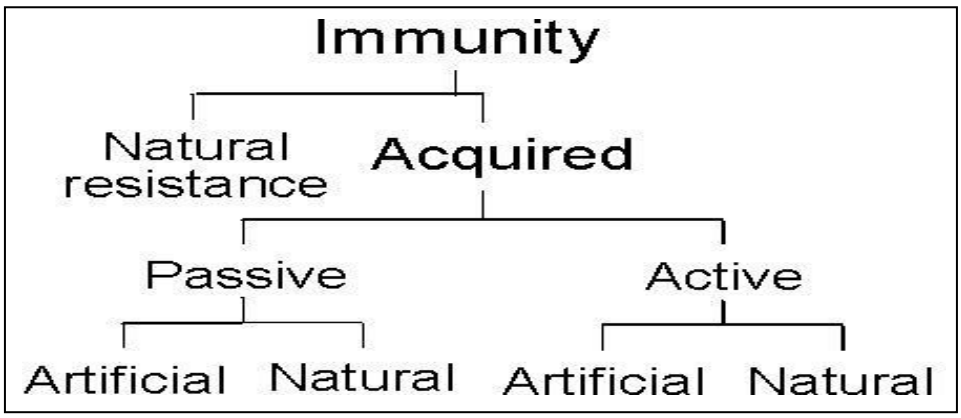


Figure 1. Different modes of acquiring

individual. Alternatively, immune cells from an immunized individual may be used to transfer immunity. Passive immunity may be acquired naturally or artificially.

Naturally acquired passive immunity:- Immunity is transferred from mother to fetus through placental transfer of IgG or colostral transfer of IgA.

Artificially acquired passive immunity:- Immunity is often artificially transferred by injection with gamma globulin from other individuals or from an immune animal. Passive transfer of immunity with immune globulin or gamma globulin is practiced in numerous acute infections (diphtheria, tetanus, measles, rabies, etc.), poisoning (insect-, reptile-bites, botulism), and as a prophylactic measure (hypogammaglobulinemia). In these situations, gamma globulin of human origin is preferable although specific antibodies raised in other species (usually horse) are effective and used in some cases (*e*.g., poisoning, diphtheria, tetanus, gas gangrene, botulism, *etc*.). While this form of immunization has the advantage of providing immediate protection, it is effective for only a short duration and often results in pathological

complications, such as serum sickness characterized by rash, fever, arthralgia, vasculitis, nephritis, *etc*., and anaphylaxis.

Passive transfer of cell-mediated immunity can also be accomplished in certain diseases (cancer, immunodeficiency). However, it is difficult to find histocompatible (matched) donors and there is severe risk of graft versus host disease.

Active Immunity:

This refers to immunity produced by the body following exposure to antigens.

Naturally acquired active immunity: Exposure to different pathogens leads to sub-clinical or clinical infections, which normally result in a protective immune response against these pathogens.

Artificially acquired active immunity: Immunization may be achieved by administering live or dead pathogens or their components. Vaccines used for active immunization consist of live attenuated organisms, killed whole organisms, microbial components, or detoxified toxins (toxoid).

***features of effective vaccines

1) Safe:-

Vaccine must not itself cause illness or death, because vaccine must be given to huge number of people, relatively few of whom aredying by a disease that vaccine is designed to prevent, this mean that ever a low level of toxicity is unacceptable.

2) Protective:-

Vaccine must protect against illness resulting from exposure to live pathogen, and able to produce protective immunity in a very high proportion of the people to whom it is given. Since it is unpractical to give large or dispersed rural populations regular booster vaccination. A successful vaccine must generate long-lived immunological memory; this means that both B&T lymphocytes must be primed by the vaccine

3) Give sustained protection:-

Protection against exposure must last few several years.

4) Induces neutralizing antibody:-

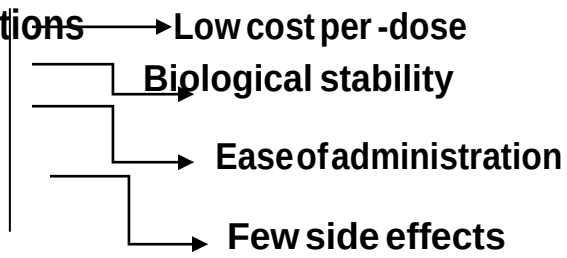
Some pathogens like polio virus W infect critical host cell within a short period after entering the body , this virus require pre- existing Ab for protection this is necessary in case of intracellular pathogens, in order to neutralize the pathogen before it enter the cells.

Some of infectious agents have a prolonged incubation period before they invade critical host cells, and for these pre-existing Ab is not necessary for protection.

Immune response to infections agents usually involve antibodies directed at multiple epitopes, only some of these Abs confer protection, thus an effective vaccine must be lead to the generation of Abs directed at the correct epitopes of the infectious agents .As we said before some pathogens like polio virus infect cells that cannot be replaced (e.g. neurons).Neutralizing antibody is essential to prevent infection of such cells.

5) **Induces protection T-cell:-some pathogens particularly intracellular are more effectively death with or by cell-mediated responses**

6) **Practical considerations**



******FACTORS AFFECTING THE IMMUNE RESPONSE TO VACCINES**

1) **Maternal antibodies:-**

Presence or absence of these Abs which disappear at the age of 6 months, and many persist until the age of 9 months or more, many interfere with vaccination.

2) **Nature of the vaccine, antigenic constituent & the form in which it is presented.**

3) **Intervals between successive doses of vaccine:**

In order to obtain typical response , it must be pointed out that active immunity of efficiency requires 5 to 14 days to develop after primary immunization, this is the time it takes for protective quantities of Abs to appear in serum . In passive immunization protection is provided immediately .

4) **Route of administration of the vaccines:-**

Either orally , so the vaccine will pass through GIT &undergo alteration by a action of enzyme &other substances as polio vaccine, while parenteral administration e.g. I.V. the substances or vaccine will enter the blood without change , which will give better immune reaction , other e.g. like BCG vaccine is given intradermal , and some of a vaccines are given S.C.

5) Presence of adjuvants, usually injected with an Ag improves the immune response. Although this often is thought of merely in terms of immunoglobulin response, it is clear that many adjuvants also simulate the activities of T-lymphocyte [cell-mediated immunity (CMT)] and activate macrophages.

6) Immune status:-

Live attenuated vaccines should not be given to patients with immunodeficiency disease. Furthermore, children suffering from malnutrition many have impaired immune response to vaccines.

Sera and Antisera

Serum or Sera

its solid and liquid components after it has been allowed to clot. It is clear and yellow in color. **That remains after blood has clotted and cells have been removed.**

Antisera is blood serum containing antibodies against a specific antigen, used to treat or provide immunity to a disease. It is extracted from an animal that has immunity to a particular disease **(Obtained from injecting an animal (horse, rabbit, goat) with antigen (snake venom, botulism or diphtheria toxin).**

SERUM It does not contain white or red blood cells or a clotting factor. It is the blood plasma without the fibrinogens. **Serum** includes all proteins not used in blood clotting (coagulation) and all the electrolytes, antibodies, antigens, hormones, and any extra substances such as drugs and microorganisms

Several lab techniques can be used to determine serum protein. Two proteins in the blood, globulin and albumin, are of particular interest. They typically make up the bulk of the protein in the blood and **the ratio between the two should remain relatively consistent.** Changes in the ratio can be caused many health conditions. Some conditions linked with changes in serum protein include: dehydration, diabetes, heart failure, kidney disease, tuberculosis, liver disease, autoimmune disease, and blood diseases like leukemia.

How it works Anti-Sera??

- Ab in the antiserum bind the infectious agent or antigen. The immune system then recognizes foreign agents bound to antibodies and triggers a more robust immune response. The use of antiserum is particularly effective against pathogens which are capable of evading the immune system in the unstimulated state but which are not robust enough to evade the stimulated immune system. The existence of antibodies to the agent therefore depends on an initial whose immune system by chance discovered a counteragent to the pathogen, or a "host species" which carries the pathogen, but does not suffer from its effects. Further stocks of antiserum can then be produced from the initial donor or from a donor organism that is inoculated with the pathogen and cured by some stock of preexisting antiserum. Diluted snake venom is often used as an antiserum to give a passive immunity to the snake bite itself

What is Epitopes?

An epitope refers to the specific target against which an individual antibody binds.
When an antibody binds to a protein, it isn't binding to the entire full-length protein. Instead, it is binding to a to a segment of that protein known as an epitope. In general, an epitope is approximately five or six amino acids in length. So, a typical full-length protein sequence actually contains many different epitopes against which antibodies can bind.

And, for any given protein sequence, one will typically find that multiple unique antibodies will recognize the

protein. Each of these antibodies binds to a specific epitope located on that protein.

Binding between the antibody and the epitope occurs at the Antigen Binding Site, which is called a paratope and is located at the tip of the variable region on the antibody. This paratope is only capable of binding with one unique epitope.

Within a protein sequence, one can find:

1. **Continuous epitopes, which are linear sequences of amino acids**
2. **Discontinuous epitopes, which exist only when the protein is folded into a particular conformation.**

In the context of developing a custom antibody, it is important to differentiate between targeting a specific epitope on a protein and in simply having antibodies (which may be against a number of epitopes) that recognize a particular protein.

How are Antibodies Produced?

Although detailed mechanics of the immune response are beyond the scope of this site, it is useful, in the context of developing a custom antibody, to have an overview of how antibodies are produced by the immune system.

When an organism's immune system encounters a foreign molecule (typically a protein) for the first time, specialized cells such as macrophages and dendritic cells capture the molecule and begin breaking it down

so that it can present these antigens to B cell lymphocytes.

Once Antigen Presentation to the B cell lymphocytes has occurred, a process known as Somatic Hypermutation allows the B cell to begin coding for a new antibody that will contain a unique Antigen Binding Site in the variable region that is capable of binding specifically to an epitope from the antigen. Each B cell lymphocyte produces one unique antibody against one unique epitope.

Once antibodies with sufficient specificity to the epitope can be encoded, the B cell begins to release antibodies into the bloodstream. These antibodies then bind specifically with the foreign molecule and allow the immune system to eliminate the molecule from the system.

In some cases, these antibodies can disable pathogens such as viruses directly due to the binding action. In other cases, such as with bacterial pathogens, these antibodies bind to surface proteins on the bacterium's surface, thereby signaling to the rest of the immune system that the pathogen should be destroyed.

After the foreign molecule has been eliminated, B cells remain in the bloodstream ready to produce antibodies if the antigen is encountered again.

From the perspective of developing a custom antibody against a protein antigen, the immune

system captures the protein, breaks it down into individual epitopes and presents these epitopes to the B cells so that development of antibodies specific to those epitopes can begin. These antibodies can then be collected directly in the serum or by isolating the individual B cells that produce antibody against the epitope of interest. With a full-length protein antigen, there will typically be multiple B cells generating antibodies against multiple epitopes from different regions of the protein.

***Polyclonal Antibodies

Polyclonal antibodies consist of many(poly) different antibodies That bind to many different epitopes on the target antigen.
Polyclonal Abs produced from different clones of B cells, each Of them bind to different epitopes on antigen.
Antigen is injected once into a rabbit, and wait about three weeks later to produce polyclonal Abs.

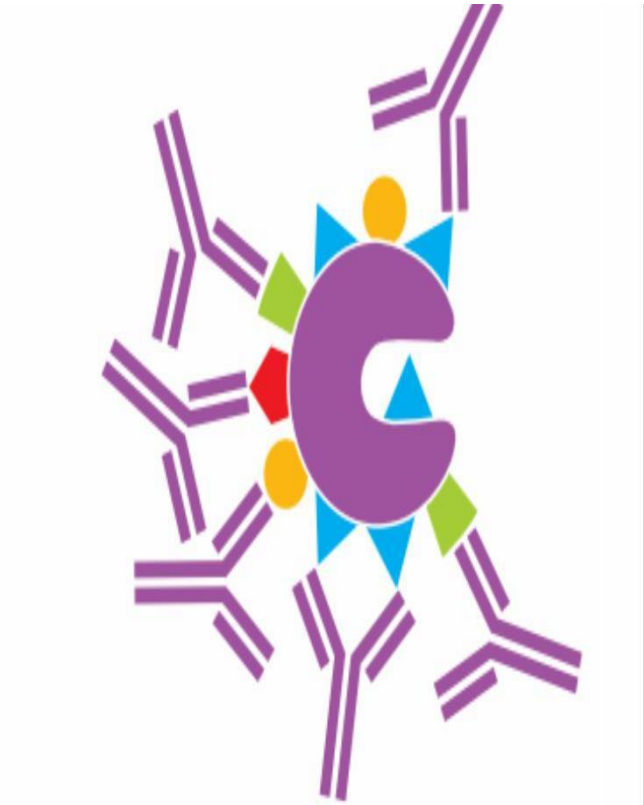


Figure 4: Schematic diagram of polyclonal antibodies binding to various epitopes on an antigen.

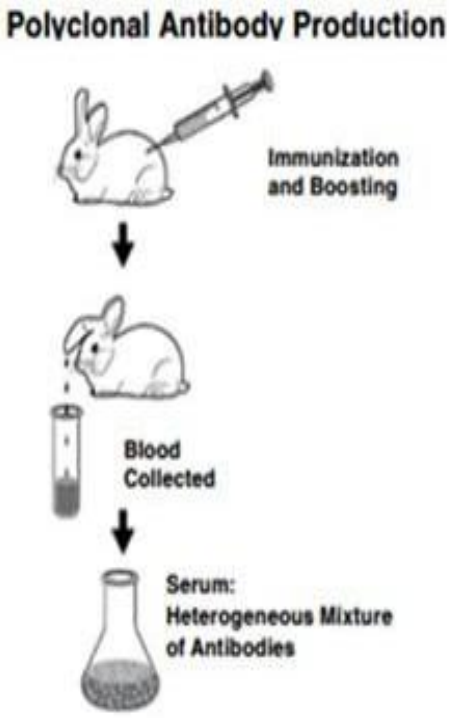


Figure 5. The process of polyclonal antibody production.

****Uses of Polyclonal Antibodies

1. . To detect a known or unknown isoforms of antige ns with high antigen homology .

2. . To detect low levels of a particular antigen

3. . To capture as much antigen as possible .

4. . To detect denatured proteins .

5. To detect a target in solutions with varying PH and salt

***Disadvantages of Polyclonal Antibody

1) **Shared epitopes on different proteins can label multiple proteins that are not the antigen protein.**
2) **Obtaining the antibody depends on a living animal and the ultimate death of the rabbit means no more antibody.**
3) **When a new rabbit is immunized with the same antigen, the exact epitopes generating antibodies will be different, and a different number of clones are generated.**

***Monoclonal Antibodies

Antibodies that are identical because they were produced by one type of cells, and always bind to single epitope.

Procedure of Monoclonal Antibodies Production

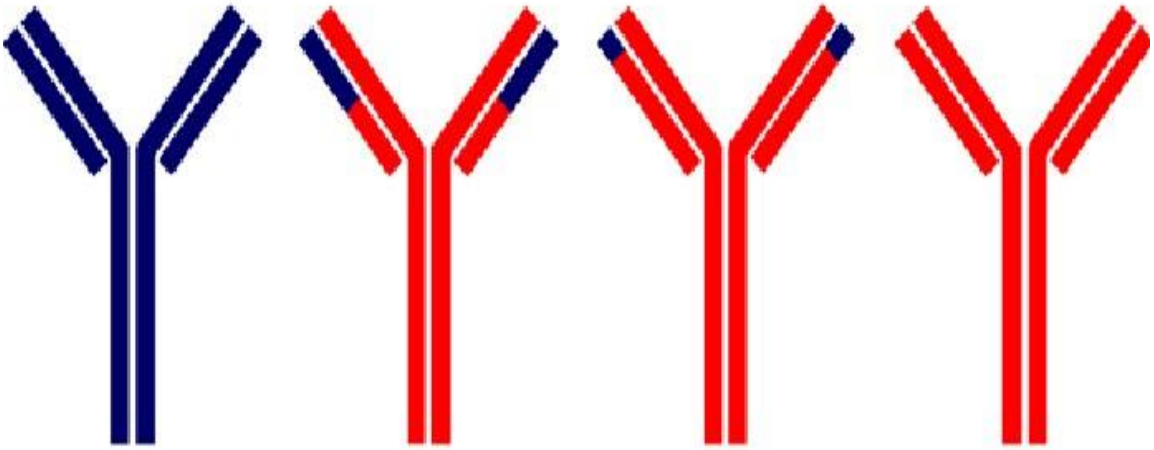
1) **Immunize animal**
2) **Isolate spleen cells**
3) **Fuse plasma cells with myeloma cells (e.g. using PEG)**
4) **Add HAT**
5) **Clone remaining cells**
6) **The hybridoma cells are screened to identify individuals that secrete desired Ab**
7) **Grow the chosen clone of cells in tissue culture indefinitely.**
8) **Harvest antibody from the culture**

Figure 6. A given clone of monoclonal antibodies reacts with a specific epitope on an antigen.

***Uses of Monoclonal Antibody

- **detecting a specific antigen**
- **detecting a single member of a protein family**
- **quantifying protein expression**
- **detecting changes in molecular conformation**
- **Immunotherapy**
-

The types of MAB Designed



Murine
100% mouse

Chimeric
33% mouse

Humanised
5-10% mouse

Human Protein
100% human

Problem with Using Mouse Monoclonal Antibodies

7) **Severe allergic response.**

8) **The fused human lymphocytes- mouse myeloma cells are very unstable.**

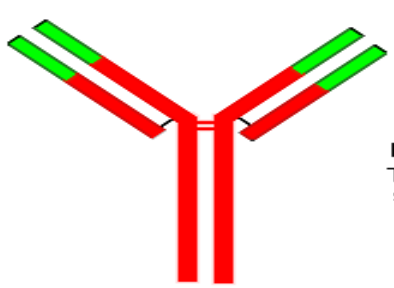
9) **There are no suitable myeloma cells in human that can replace mouse myeloma cells.**

***Antibodies Acte as Immunogens

- Antigenic Determinants on Abs Fall in 3 Categories
  - Isotypic
  - Allotypic
  - Idiotypic
- Isotypic
  - Constant Region Of Ab
  - If you inject Ab in a different species Anti-Isotype is generated

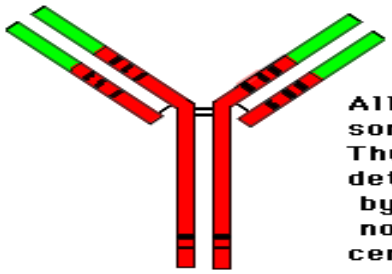
—If within same species, No Anti-isotype

Idiotype Vaccine contain antigenic determinant Ag-specific Ab which behave like epitopes similar to epitopes present on primary Ag (recognized by receptor on lymphocyte).The importance is regulation of immune responses.

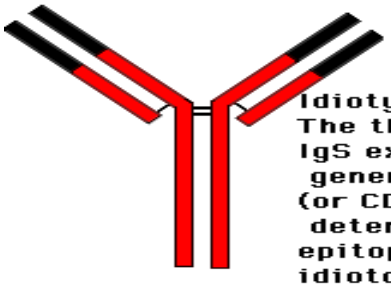


Antigenic Properties of antibodies

Isotypic determinant of C regions. They are found in all animals of the same species.



Allotypic determinant of C region. Found in some but not all members of the species. Thus, individuals may possess a given determinant. Antibodies can be obtrained by injection of the same species which does not have the determinant. Related to ceratin diseases.



Idiotypic determinants of V regions. The third group of antigenic determinants of IgS exists as a result of unique structures generated by the hypervariable subregions (or CDRs) on the L and H chains. These antigenic determinants are called idiotopes, analogous to epitopes antigens. There are three types of idiotopes:

(A) Idiotype (Id) of an immunoglobulin (Ig)-expressing lymphoma cell as vaccine to induce anti- lymphoma. immune response

(B) Schematic representation of the antigen-specific antibody (Ab1), the antigen (Ag)-mimicking anti-Ab1 anti-idiotypicantibody (Ab2), the anti-Ab2 anti-idiotypic antibody (Ab3) and their interactions. (C) Id of an Ab2 as vaccine to induce antigen-specific immune response to solid tumors Anti–idiotype:- an antibody that binds to the antigen-combining site of another antibody either suppressing or enhancing the immune response

Isotype refers to something that is (generally, genetic mutations are the exceptions) shared by all members of a species. All gentically normal humans make IgG, IgA, IgM, IgE and IgD, so these are antibody isotypes.

Allotypes refer to a property shared by some members of a species-in a nonimmunologic sense eye color is allotyic-some people have brown eyes, some blue, etc. In an immunologic sense it refers to allelic variants on normal genes and the proteins of the immune systemthey encode-for example, IgG antibodies made by some humans have a particular amino acid at a position and others have a different amino acid at that position. These substitutions are considered normal and do not affect the function of the protein.

Idiotypes refer to antigenic regions of antibody moelcules (and to T cell receptors as well) that are the part of these sturctures involved in antigen recognition and therefopre tend to be quite different among antibodies and T cell receptors that bind to different antigens.

Antibody Storage

Overview:

In comparison to sensitive proteins such as enzymes, antibodies are highly stable proteins that retain activity in a wide range of biological conditions. As a result, storage is reasonably straightforward and should focus on two key considerations: 1) antibodies are sensitive to repeated freeze/thaw cycles and 2) excessive protein concentration dilution, particularly of purified antibody, can result in material losses and reductions in antibody stability.

Short-term Storage:

Unpurified serum that contains sodium azide can be stored at 4°C for 2-3 months at a time. Unpurified serum without azide, however, should be kept at -20°C in order to avoid bacterial growth. Purified antibodies, which contain azide by default, can also be stored at

4°C for 2-3 months at a time to allow for use without repeated freeze/thaw cycles.

Long-term Storage:

For long-term storage, we recommend storing serum vials and aliquots of purified antibody at -20°C. Storage at -80°C isn't necessary.

Aliquoting Purified Antibody:

The purified antibody (which contains sodium azide) can be safely stored at 4°C for 2-3+ months at a time in order to avoid freeze/thaw cycles. For long term storage, however, we recommend aliquoting the purified antibody into several vials and storing these at -20°C. Each vial, which should contain enough purified antibody to cover use over a typical working period (2-3 months for example), can then be thawed a single time and stored at 4°C until depleted.

Antibody Concentration:

At low protein concentrations, antibody stability suffers and can lead to protein losses. Mechanically increasing antibody concentrations should also be avoided, since this will also typically cause protein losses. BSA can be added to purified antibody aliquots to increase concentration and improve stability (assuming that this doesn't interfere with the assay system).

Freeze/Thaw Cycles:

Repeated freeze/thaw cycles represent a significant risk to antibody stability, especially with purified antibody (which is at a lower protein concentration in

comparison to serum). In addition to following the guidelines above, we recommend the use of manual defrost freezers rather than frostless freezers.

Sodium Azide:

Sodium Azide is an antimicrobial agent that prevents the growth of bacteria in the serum or purified antibody. Azide is not added to the serum by default, but can be added upon request at no additional charge. Azide is added to all purified antibodies and columns by default in order to avoid contamination and to allow for storage at 4°C. It is important to note that sodium azide is not recommended for some applications. In particular, sodium azide blocks the cytochrome electron transport system, making it toxic to most organisms. Sodium azide can be dialyzed out of the serum if necessary.

Affinity Columns:

Affinity columns should be stored at 4°C and should not be frozen. Sodium Azide is added to these by default to avoid contamination and to allow for longer term storage at 4°C.

Glycerol:

The addition of glycerol can help avoid freeze/thaw cycles while keeping antibodies or serum at -20°C. However, it is not necessary if the above guidelines and aliquot strategies are followed.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