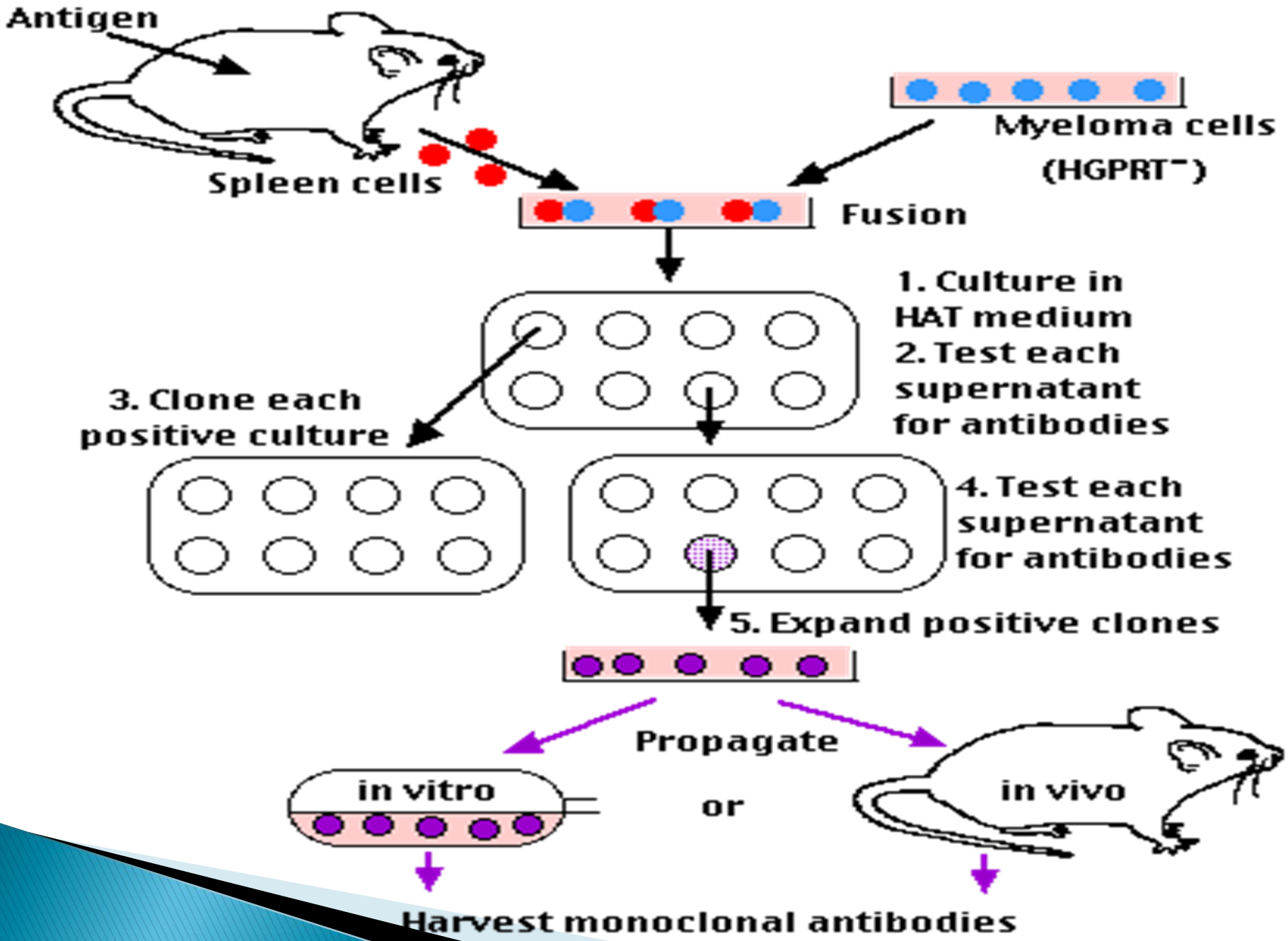
Monoclonal Antibody

Lab 8

Monoclonal Antibody Production

The importance of antibodies is well documented, but any antibody that is prepared or isolated by conventional methods is a group of different antibodies that target various agents, known as polyclonal antibodies. In 1975, Köhler and Milstein were able to devise a method to isolate **monoclonal antibodies** that would target only one antigen. It is based on injecting an animal with the antigen, collecting the B-lymphocytes from the serum or spleen (would give rise to polyclonal antibodies). Fusing the isolated B-lymphocytes with a cancerous Melanoma cell then creates a hybridoma. This hybrid cell can be cultured and cloned. Each of these daughter clones will secrete a single specific antibody (Monoclonal Antibodies). One limitation for Mab is the risk of an immune reaction to mouse proteins that may result in destroying the antibodies. This can be addressed by using **Chimeric** [using the human constant region] or **humanized** [using the constant plus some of the variable region] antibodies. This is achieved via splicing the mouse genes for the highly specific antigen-recognizing portion of the antibody and combining it with the human genes that encode the rest of the antibody molecule.


Antigen
Spleen cells
Myeloma cells
(HGPRT⁻)
Fusion
1. Culture in HAT medium
2. Test each supernatant for antibodies
3. Clone each positive culture
4. Test each supernatant for antibodies
5. Expand positive clones
Propagate
in vitro
or
in vivo
Harvest monoclonal antibodies

Application of the monoclonal antibodies

Those highly specific antibodies are used in treatment of some diseases as **Crohn's disease, coronary artery diseases, lower respiratory tract diseases caused by the RSV virus, moderate to severe persistent asthma,** cancer, in the immunosuppressive therapy, and widely used in diagnosis especially in serological kits.

Quality control for antisera vaccines and related products

Antisera are native sera containing substances that have a specific prophylactic or therapeutic action when injected in to animals exposed to or suffering from a disease due to specific microorganism or toxin (antitoxin sera, antibacterial sera and anti-viral sear).

General standards for antisera:

1- Acidity and alkalinity: native antisera have a pH of 7-8, solution of globulin or their derivatives have a pH of 6- 7.
2- Solids: native antisera contain not more than 10% of solid matter. Solution of globulin or their derivatives contain not much more than 20%
3- Sterility

4- Toxicity : antisera should be tested by

a- two healthy mice are injected subcutaneously with 0.5ml of antisera and observed for 5 days ,no abnormal reaction should develop

b- two healthy guinea pigs are each injected intraperitonially with at least 2 ml of antisera and observed for 10 days , no abnormal reactions should develop

5- Potency: the potency of antisera should be assessed by comparison with an established reference preparation and determined the potency by biological assay. it may be expressed in units . The unit is the specific biological activity contained in a specific amount of the international biological standard or other standard antisera.

6- Labeling : that information should be written on the product , including : name of product ,minimal total number of units in the container, volume, minimal no of units in 1 ml or 1gm,date of production, distinctive bath no., name of species in which it was prepared, name, address of the manufacture,

7- Storage: liquid preparations should be protected from light and stored at 5-3 C.

Quality control for the vaccines

1- Purity: that preparation should be tested for any type of contamination.

2- Freedom of abnormal toxicity : tested in same way of the antisera mentioned before

3- Safety;

4- Sterility

5- Potency should be assessed by comparison with an established reference preparation.