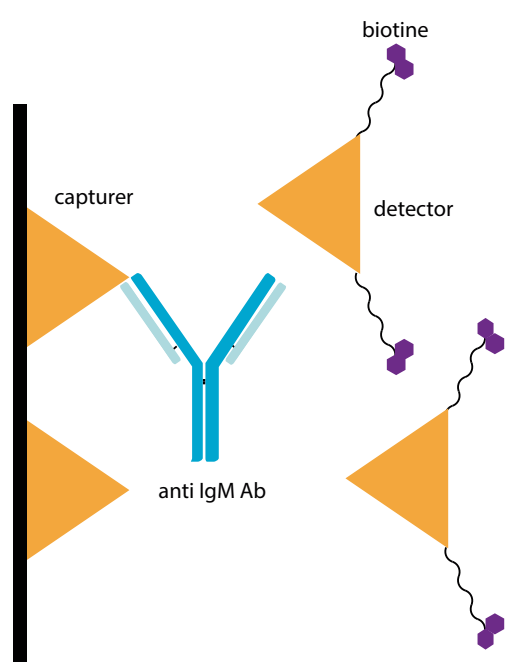


Raw Material for Double Antigen Sandwich ELISA

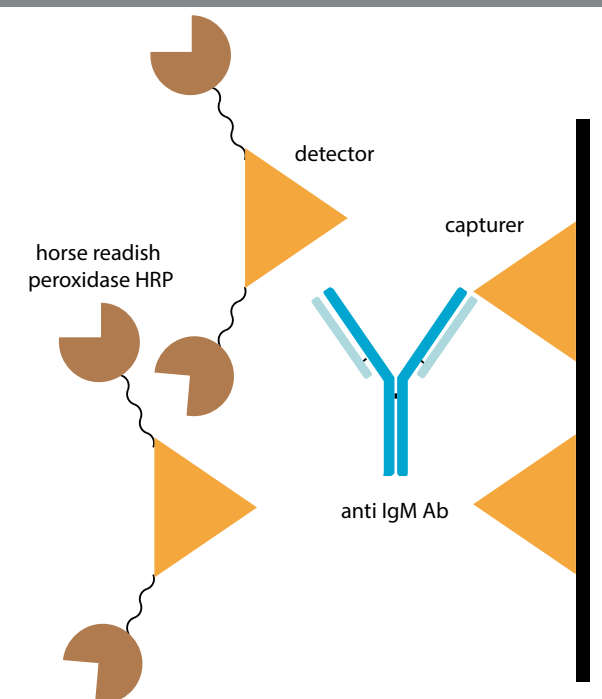
The double-antigen sandwich based enzyme-linked immunosorbent assay (DAS-ELISA) is a new ELISA format that provides an advantage. It detects total rather than class-specific antibodies and has been used with success in third-generation ELISAs to improve their sensitivity for the detection of human immunodeficiency virus infection. This allows the system to be applied to samples from outbreak investigations, in which the detection of both IgM and IgG antibodies is needed, or to the testing of specimens of animal origin. Employing a DAS format instead of the original indirect format can substantially improve the assay's specificity.

In double antigen sandwich ELISA kit, an antigen is pre-coated on the plate, which acts as the **capturer**. A second antigen conjugated to tracer enzyme -horseradish peroxidase (HRP) or to biotin as indirect label (which will react with streptavidine-HRP), is used as the **detector**. The pre-coated and conjugated antigens will be bound to the **two variable domains of the antibody**. Then the specific antigen-antibody-antigen-HRP immuno complex (RIGHT SIDE) or antigen-antibody-antigen-biotin-estreptavidin-HRP immuno complex (LEFT SIDE) will be developed.

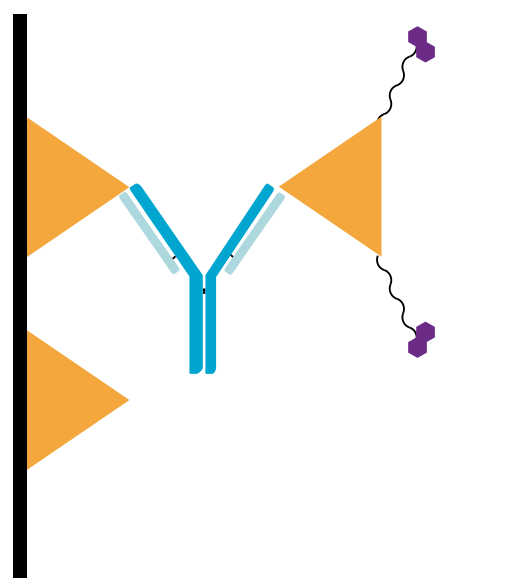
1) CAPTURE PHASE



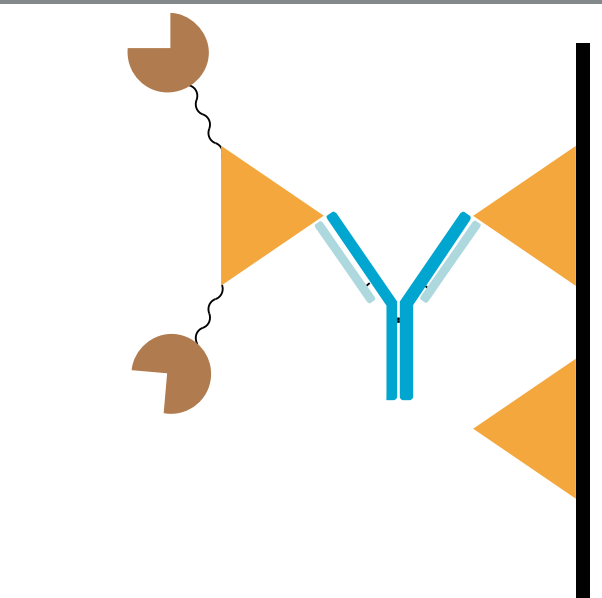
THE ANTIBODIES IN THE SAMPLE ARE FIRST ALLOWED TO REACT WITH THE PRE-COATED ANTIGENS DURING THE FIRST INCUBATION. DURING THIS PROCESS, THE TARGET ANTIBODIES, IF PRESENT, WILL BE CAPTURED ON THE POLYSTYRENE MICROWELL STRIPS.



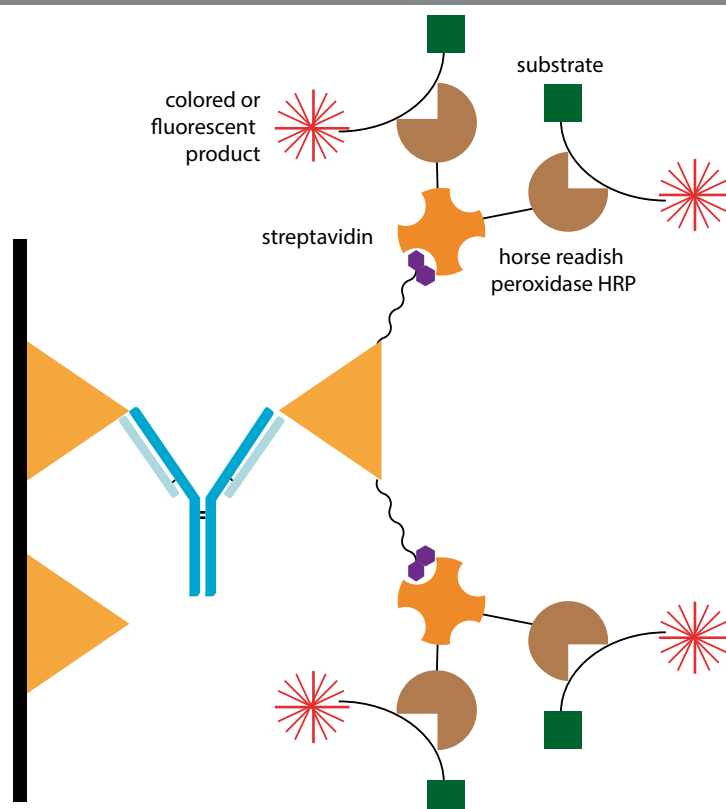
2) DETECTION PHASE



THEN, THEY ARE DETECTED BY THE ADDITION OF THE HRP-CONJUGATED ANTIGEN (RIGHT SIDE) OR THE BIOTIN-CONJUGATED ANTIGEN (LEFT SIDE). IN THE SECOND CASE, A STREPTAVIDINE-HRP CONJUGATED WILL BE NECESSARY FOR THE ASSAY DEVELOPMENT.



3) DEVELOPING PHASE



FINALLY, CHROMOGEN SOLUTIONS CONTAINING TETRAMETHYL BENZIDINE (TMB) AND UREA PEROXIDE WILL BE ADDED TO THE WELLS AND, IN PRESENCE OF THE "SANDWICH" IMMUNO COMPLEX, THE COLORLESS CHROMOGENS WILL BE HYDROLYZED BY THE BOUND HRP CONJUGATE TO THE BLUE COLORED PRODUCT, WHICH WILL TURN YELLOW AFTER STOPPING THE REACTION WITH SULFURIC ACID. THE AMOUNT OF COLOR CAN BE MEASURED AND IS PROPORTIONAL TO THE AMOUNT OF ANTIBODY IN THE SAMPLE. WELLS CONTAINING SAMPLES NEGATIVE FOR TARGET ANTIBODY REMAIN COLORLESS.

