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16/MHS01/175
BCH 412 (ASSIGNMENT 2)

1. Highlight the principle of immunochemical techniques

Immunochemical techniques are based on a reaction of antigen with antibody, or more exactly, on a reaction of an antigenic determinants with the ~~bibding~~ binding site of the antibody. The exquisite specificity and the high affinity of antigens to their antibodies to cross link antigens, allow the identification and quantification of specific substances by a variety of methods including immunoassay, Radioimmunoassay, Enzyme Linked Immunosorbent assay (ELISA), Immunoprecipitation, Immunoelectrophoresis, Immunofluorescence and Immunohistochemistry

2. Describes the steps involved in Radioimmunoassay and state 3 of its disadvantages.

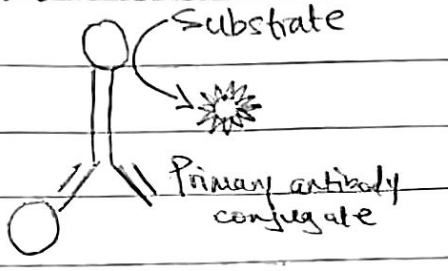
Classically, to perform a radioimmunoassay, a known quantity of antigen is made radioactive, frequently by labeling it with gamma-radioactive isotopes of iodine, such as I25-I, attached to tyrosine. This radiolabelled antigen is then mixed with a known amount of antibody for that antigen, and as a result, the two specifically bind to one another. Then, a sample of serum from a patient containing an unknown quantity of that same antigen is added. This causes unlabelled (or "cold") antigen from the serum to compete with the radiolabelled ("hot") antigen for antibody binding sites. As the concentration of "cold" antigen is increased, more of it binds to the antibody, displacing the radiolabelled variant and reducing the ratio of antibody-bound radiolabelled antigen to free

radiolabelled antigen. The bound antigens are then separated from the unbound ones, and the radioactivity of the free (unbound) antigen remaining in the supernatant is measured using a gamma counter.

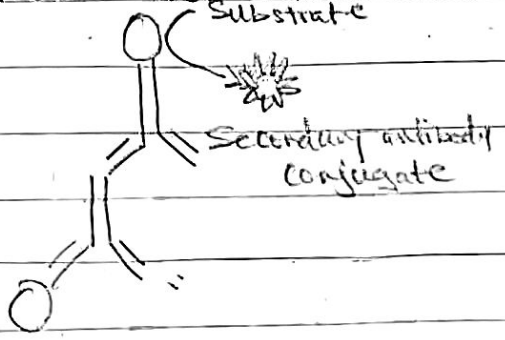
DISADVANTAGES OF RADIOIMMUNOSORBENT ASSAY

i High cost of equipment and reagents
ii Short shelf-life of radiolabelled compounds
iii Problems associated with the disposal of radioactive wastes

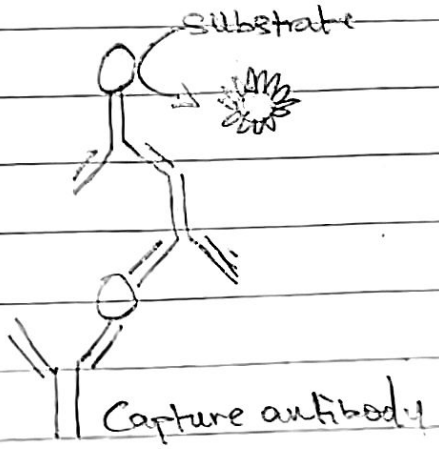
3. With the aid of schematic diagrams, differentiate between direct, indirect, competitive and sandwich enzyme-linked immunosorbent assay



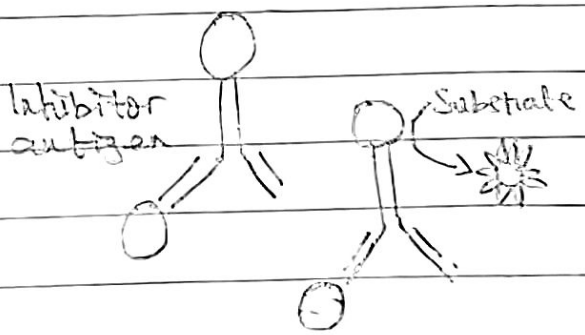
DIRECT ELISA



INDIRECT ELISA



SANDWICH ELISA



COMPETETIVE ELISA

4. State 5 differences between radio immunoassay and Enzyme linked Immunosorbed Assay

CHARACTER	ELISA	RIA
i Measurement	Relies largely on development of colour	Relies largely on production of radiation
ii Key molecule	Enzyme linked to antigen or antibody	The radioactive isotope
iii The types of reacting substance to produce detectable change	Many enzymes like horse raddish peroxidase	Mostly a single iodine isotope
iv Duration of experimental procedure	Very short and can be measured quickly	Very time consuming
v Skill requirement	Minimal skill and knowledge required	An efficient and highly skilled handler is needed to minimize exposure to radiation