CDC-CROSSMATCH

**INTRODUCTION**

**Complement Dependent Cytotoxicity [CDC] test is a universally employed antibody against HLA antigen on cell membrane. It is an essential service of all histocompatibility testing laboratories .Its primary purpose is to assess the preformed antibodies in recipients which are capable of causing a hyperacute rejection.It is designed to include both positive and negative control.**

**During the cell lysis after addition of complement ,cells become permeable to dye.Therefore,cell death [positive test]indicates the presence of antigen against antibody ,while the cell viability [negative test]indicates absence of specific antigen.**

**SPECIMEN;**

**1. T and B lymphocytes suspension prepared by RosetteSep Technique.**

**2. Recipient serum.**

**INDICATIONS**

**1.As a part of histocompatibility work up for pre-transplant patients.**

**2.It is like a mimi in vitro Transparent .A positive T cell CDC crossmatch is an absolute contra-indication . A positive B cell crossmatch is a relative contraindication.**

**PRINCIPLE OF MODIFIED AHG-CDC CROSSMATCH**

**Anti human globulin [AHG].It is serum in a heterologous species and contains antibodies to human immunoglobulin.The AHG is raised in goats and is specific for the kappa light chain of all immunoglobulins.**

**Addition of AHG to mixture of target lymphocytes and human antiserum make these antibodies combine with human immunoglobulins which are bound to target cells.A few cell-bound molecules of antibody [the first immune complex] will bind many anti-human immunoglobulin molecules (the second complex)resulting is more and larger complexes which are capable of crosslinking and thus activating complement leading to cell lysis.The initial immune reaction is enhanced or magnified thus increasing the sensitivity of test .**

**Antibodies.although specific ,may sometimes fail to trigger complement in the lymphocytotoxic assay,giving false negative results.Possible reasons for this are that the antibodies are;**

**1)Of a class which does not fix a complement.**

**2)give cytotoxic negative absorption positive (CYNAP)reactions.**

**3)have a specificity for widely spaced determinants on the cell surface .**

**4)are present in concentration below the limits of detection in standard lymphocytotoxic assays.**

**5)are in excess and display prozone.**

**6)Are not at optimal ratios with antigen to trigger complement.**

**AHG will resolve these problems.**

**Depending upon the anti Ig selected one could identify the class of antibody binding to target cells if an antibody to heavy chain is used. All classes of immunoglobulins will be detected if the AHG is specific for the light chain.**

**READING**

**Reading is done with an inverted phase contrast microscope.Scoring is done by an objective scale estimating as closely as positive the percent dead cells in each well.**

**0-10%- cell death - Negative**

**11-20%cell death- Negative**

**21-50% cell death - Doubtful positive / weakly positive**

**51-80% cell death- Positive**

**RESULTS AND INTERPRETATIONS;**

**The negative control is used to assess viability of test cells and is used to ensure that the complement does not cause non-specific killing. The positive control validates complement activity. A ‘no complement’ control is added which is used to detect the presence of non complement dependent cytotoxicity .The failure of any of the controls indicates a requirement to repeat the crossmatch.**

**LIMITATIONS**

**Caution must be used with preparations containing significant numbers of B cells because their surface immunoglobulins are a natural target for the AHG and will result in false positive results.**

**If cells appear viable when prepared but cells die non specially on crossmatch tray ,the patient/donor may have been administered cytotoxic drugs affecting the viability of cells[eg; OKT3,ALG].**

**Cells may also die due to excessive incubation time.**

**QUALITY CONTROL**

**Negative control should contain minimal cell lysis and will indicate background for an individual tray.**

**Positive control must demonstrate lysis of the cells.If not,crossmatch must be repeated using fresh complement .**

**The serum of patients may contain anti-complimentary activity.To avoid this,serum is tested at increasing dilution or serum treated cells are washed before adding complement.**