APPENDICES

Appendix-1 : Test of Kell :

1.1 Diamed – ID Micro type system :

K1 and K2 detected by using ID – Microtyping system .

1.2. Introduction :

The Kell (Kell) system (ISBT number 006) is currently known to contain 22 antigens, numbered K1 to K 25 (K8, K9 and K15 are now obsolete) .

According to Mollison, the phenotype frequencies of the antithetical antigens K (K1) and k (K2) in the Caucasian population are as follows:

KK 0.2%
Kk 8.8%
kk 91.0%

The antibodies of the Kell system have been associated with transfusion reaction and HDN.

The clinical importance of anti-K has resulted in the systematic determination of the Kell and Cellano antigens in both donors and patients . The ID-Cards" anti-K and anti-k can be used to determine the antigen status of donor blood prior to cross

matching, for confirmation of the antigen after antibody identification, or for paternal/ fetal antigen typing in allo-immunized pregnancies [81].

The ID-Card (anti-K and anti-k) allow testing of six samples simultaneously.

1.3. Principle :

The gel is a suspension of porous micro sphere whose size and distribution were selected to produce settling of non-agglutinated red cells at the buttom of the microtube and retention of agglutinated in the gel at a variable levels according to their size. The retention of some red cells at the top this indicate the positive result . If all red cells are settled at the bottom these indicate the negative result [82] .

The ability of gel to separate red cells from serum protein obviates the need washing , thus simplifying the technical procedure in most application .

The other advantage of gel technology is to provide stable end point reading with semi quantitative standardize inter pretation that minimizes the risk errors .

1.4. Limitation :

- Bacterial or other contaminations of materials used can cause false positive or false negative results .
- Fibrin residues in the red ecll suspension may trap non-agglutinated cells presenting a fine pink line on top of

the gel while most of the cells are on the bottom of the microtube after centerifugation .

- Strict adherence to the procedures and recommended equipments should be checked regularly according to GLP procedures.
- Use of suspension solution for red cells other than ID-Diluent 2 may modify the reaction .
- Too haevy or too weak red cell suspensions can cause aberrant reaction [81].
- Cells that have become poly agglutinable , due to crypto antigen exposure e.g. T antigen, either in vivo or in vitro, may react with all human antisera . Further investigation of such reactions is required .
- 1.5. Reagent :
    1. I.D-Card anti-K with 6 micro tubes containing anti-K of human origin within the gel matrix . Preservative: <0.1% Na N^3 .
    2. ID-Card anti-k with 6 micro tubes containing anti-k of human origin within the gel matrix . Preservative: < 0.1% Na N^3 [81].

1.6. Further material required :

1. ID-dispenser .
2. ID-pipetor .
3. ID-tips (pipetor lips) .

4. Suspension tubes .
5. ID-working table .
6. ID-centrifuge-6, 12 or 24 .

1.7. Sample material :

For optimal result, the determination should be µwith local laboratory procedures for sample acceptance criteria, blood sample should be drawn into criteria, EDTA or CPD-A anticoagulant . Sample drawn into plain tube (no-anticoagulant) may also be used.

1.8. Preparation of blood sample :

5% red cell suspension in ID-Diluent 2 was prepared as follow :

The diluents were allowed to reach room temperature before used .

1- 0.5 ml of ID-Diluent 2 was dispensed into a cleaned glass tube .
2- 50 µl of whole blood or 25µl of packed cells was added and mixed gently .
3- Was incubated for 10 minutes at room temperature (18-25ºC) .
4- Was used with in 15 minutes after incubation .

1.8. Controls :

Known positive and negative samples should be included in accordance with the relevant guidelines of quality assurance .