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 \textsuperscript{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 microsphere whose size and distribution were selected to produce settling of non-agglutinated red cells at the bottom 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 \textsuperscript{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 endpoint reading with semi quantitative standardize interpretation 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 cell 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 heavy or too weak red cell suspensions can cause aberrant reaction.
- Cells that have become polyagglutinable, 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. I.D-Card anti-k with 6 micro tubes containing anti-k of human origin within the gel matrix. Preservative: < 0.1% Na N\(^3\).

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 with 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 (18-25°C).
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.