Appendix-1: Leaflets.

ID-Cards "Anti – Kp^a" /"Anti –Kp^b"
Human
Determination of the Kpa (KEL3) and (KEL4) Antigens.

Introduction:

The antithetical antigens Kp^a (KEL3), Kp^b (KEL4) and Kp^c (KEL21) are part of the Kell system (ISBT, number 006). Kp^a is present in approximately 2% of the Caucasian population while Kp^b is a high frequency (public) antigen. The Kp^c antigen has an occurrence of less than 0.01% but up to 0.32% in Japanese.

According to Mollison[(1)], the phenotype frequency in the Caucasian population of the two most important antigens is as follows:

Phenotype	**Frequency**
Kp (a+b-)	rare
Kp (a+b+)	$\pm$ 2.3%
Kp (a-b+)	$\pm$ 97.7%

Anti- Kp^a and anti-Kp^b are less common than anti-K but have similar serological characteristics and are considered to be clinically significant. They may occur after transfusion or by feto- maternal immunization.

The ID-Cards "Anti-Kp^a" and "Anti-Kp^b" allow testing of 6 samples simultaneously.

Reagents:

ID – Card "Anti-Kp^a" with 6 microtubes containing anti-Kp^a of human origin within the gel matrix. (Preservative: < 0.1% NaN_3).

ID-Card "Anti-Kp^b" with 6 microtubes containing anti-Kp^b of human origin within the gel matrix. (Preservative: <0.1% NaN_3).

Caution: The source materials, from which these products were manufactured, were found non-reactive for HBsAg, HCV and HIV (1+2) when tested with licensed reagents. However, no known test method can assure that infectious agents are absent. Products from human blood should be considered potentially infectious.

Additional Reagents required:

ID – Diluent 1: modified bromelin solution for red cell suspensions.

Further materials required:

ID – Dispenser	ID – Pipetor
ID – Tips (pipetor tips)	ID – Working table
Suspension tubes	ID- Centrifuge 6, 12, or 24

Sample material:

For optimal results, the determination should de performed using a freshly drawn sample, or in accordance with local laboratory procedures for sample acceptance criteria. Preferably, blood samples should be drawn into citrate, EDTA or CPD-A anticoagulant. Samples drawn into plain tubes (no anticoagulant) may also be used.

Preparation of blood sample:

Prepare a 5% red cell suspension in ID-Diluent 1 as follows:

Allow the diluent to reach room temperature before use.

1. Dispense 0.5 mL of ID-Diluent 1 into a clean tube
2. Add 50 µL of whole blood or 25 µL of packed cells, mix gently.
3. Incubate the red cell suspension 10 minutes at room temperature (18-25^{0}C)

Use within 15 minutes after incubation.

Controls:

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

Test procedure:

Do not use ID-Cards which show signs of drying, have bubbles, damaged seals or drops in the upper part of the microtubes and the seal.

Allow ID-Cards to reach room temperature before use.

1. Identify the appropriate microtube of the ID-Card "Anti-Kp^a and/or "Anti-Kp^b" with patient's name or number.
2. Take off the aluminum foil from as many microtubes as needed by holding the ID card in the upright position.
3. Add 10 or 12.5 uL of the red cell suspension to the appropriate microtubes of the ID-Card
4. Centrifuge the ID-Card for 10 minutes in the ID-Centrifuge
5. Read and record the results.

Interpretation of results:

A) **Principle**[(2)]

Positive: Agglutinated cells forming a red line on the surface of the gel or agglutinates dispersed in the gel.

Negative: Compact button of cells on the bottom of the microtube.

B) **Reactions for Kp^a and Kp^b**

* Positive reactions of + to ++++ indicate presence of the corresponding antigen.
* Positive reactions often appear as a double population. However, a double population may also indicate the presence of cells positive and negative for the corresponding antigen (e.g. Post-transfusion, if the antigen configuration of the transfused blood was different from the patient's).
* Negative reactions indicate absence of the corresponding antigen.

Remarks:

1. Prior to testing for the presence of antigens, it must be assured that the red cells are free of enzyme reactive autoantibodies.
2. Repeated centrifugation of the ID-Card does not affect the performance of the product.

Limitations:

a) ID cards which show air bubbles in the gel or drops in the upper part of the microtubes and/or the seal, must be centrifuged before use.
b) Cell that have become polyaglutinable, due to cryptantigen exposure e.g.. T antigen, either *in vivo* or *in vitro*, may react with all human antisera. Further investigation of such reactions is required.
c) Bacterial or other contamination of materials used can cause false positive or false negative results.
d) 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 centrifugation.
e) Strict adherence to the procedures and recommended equipment is essential. The equipment should be checked regularly according to GLP procedures.
f) Use of suspension solutions for red cells other than ID-Diluent 1 may modify the reactions, too heavy or too weak red cell suspensions can cause aberrant reactions.

ID-Diluent 1

Modified Bromelin solution for blood grouping and enzyme tests:

Introduction:

Proteolytic enzymes modify red blood cell antigens and thus enhance the reactivity of some antigen/antibody systems and suppress others such as M, N, S, Fy^a and Fy^b.The most commonly used enzymes in blood group serology are papain and bromelin. Papain is generally used for enzyme treatment of red blood cells prior to their use, whereas bromelin in addition is often used as an additive reagent.

"ID-Diluent 1" is a modified bromelin solution in which the enzyme activity is stabilized for a long period, specially prepared for the DiaMed-ID Micro Typing System. "ID-Diluent 1" is used fro preparing suspensions of red cells for blood grouping and as an additive for enzyme tests with untreated red cells for antibody detection and crossmatching.

Reagents:

ID-Diluent 1: modified bromelin solution for red cell suspension and reagent forenzyme tests, in 100 and 500 mL vials. Preservatives: the antibiotics trimetoprim and sulfamethoxazole

Further materials required:

ID – Dispenser	Pipette 25 & 50 uL
ID – Tips (pipetor tips)	Suspension tubes
ID-working table	

Sample material:

For optimal results, the determination should be performed using a freshly drawn sample, or in accordance with local laboratory procedures for sample acceptance criteria. Preferably, blood samples should be drawn into citrate, EDTA or CPD-A anticoagulant. Samples drawn into plain tubes(no anticoagulant) may also be used.

When the use of serum instead of plasma is required, the serum must be well cleared, by centrifugation at 1500g for 10 minutes, before use avoid fibrin residues, which may interfere with the reaction pattern.

Preparation of blood sample:

A) For blood group determinations

Prepare a 5% red cell suspension of the patient's red cells in "ID-Diluent 1" as follows:

Allow the diluent to reach room temperature before use.

1. Dispense 0.5 mL of "ID-Diluent 1" into a clean tube.

2. Add 50uL of whole blod or 25 uL of packed cells, mix gently.
3. Incubate the red cell suspension for 10 minutes at room temperature.

Use within 15 minutes after incubation.

B) For use as enzyme reagent additive (antibody detection, crossmatch)

Allow the diluent to reach room temperature before use.

After pipetting of the 0.8% red cell suspension and the serum or plasma sample to the appropriate microtubes of an ID-Card corresponding to the required test procedure, add 25 uL of "ID-Diluent 1" to each microtube.

Important: Consult the related instruction sheet of the ID-Cards for precise working procedures.

Limitations:

a) Bacterial or other contamination of materials used can cause false positive or false negative results.
b) Strict adherence to the procedures and recommended equipment is essential. The equipment should be checked regularly according to GLP procedures.

(1) Mollison, P.L., Engelfriet, C.P., Contreras, M.: Blood Transfusion in Clinical Medicine, 10th ed. 1997; Blackwell Scientific Publications, Oxford.
(2) Lapierre, Y., Rigal, D., Adam, J. The gel test: A new way to detect red cell antigen-antibody reactions. Transfusion 1990: 109-113.

Appendix-2: Consent form.

بسم الله الرحمن الرحيم

دراسة لنيل درجة الماجستير
جامعة السودان للعلوم والتكنولوجيا – كلية الدراسات العليا
برنامج ماجستير علوم المختبرات الطبية – تخصص أمرا ض الدم

الإسم: .....

سوف يتم أخذ عينة من الدم بحجم 2,5 مل من الوريد بواسطة حقنة طعن ، وذلك بعد مسح منطقة أخذ العينة بواسطة المطهر . كل الأدوات المستخدمة لأخذ العينة ، أدوات معقمة ومتبع فيها كل وسائل السلامة المعملية . وليس هنالك آثار جانبية للعملية.
ربما يحصل تورم بسيط في منطقة أخذ العينة وسوف

- **أوافق أنا المذكور أعلاه على أخذ عينة دم لإجراء الدراسة.**

الإمضاء: .....
التاريخ: .....

Appendix-3: <u>Questionnaire.</u>

بسم الله الرحمن الرحيم
دراسة لنيل درجة الماجستير
جامعة السودان للعلوم والتكنولوجيا – كلية الدراسات العليا
برنامج ماجستير علوم المختبرات الطبية – تخصص أمرا ض الدم
دراسة لتحديد وجود الانتجينات – ABO, Rh(D) and Kell3(Kp^a) (Kell4(Kp^b)

في قبيلة الشكرية

1- الإسم: .....
2- القبيلة: .....
3- السكن: .....
4- الموطن الأصلي: .....
5- رقم المنزل: .....
6- رقم التلفون: .....
7- العمر: .....
8- الجنس: .....
9- وجود مرض وراثي: .....
10- وجود مرض مزمن:

1/ انيمـياء ☐ **2/سك** ☐ **3/ـــــل** ☐ **4/لوكيميا** ☐

النتيجة: **Kell antigens:**

النتيجة:	**$Kell_4(Kp^b)$**	**$Kell_3(Kp^a)$**

ABO and D antigens:

النتيجة:	**D**	**AB**	**B**	**A**

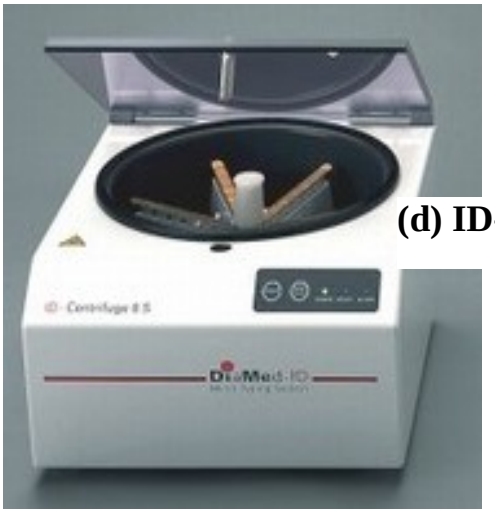
الإمضاء: ..... التاريخ: .....

Appendix-4: **Requirements of Gel Immune Diffusion Technique**

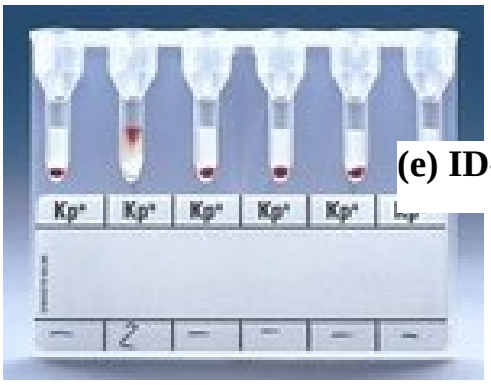
(a) ID- Diluent 1.

(b) ID- pipette.

(c) ID- working table.



(d) ID- centrifuge.



(e) ID- card KP^{a}.

(f) ID- card KP^{b}.