4. Mix the whole blood sample and Anti A1 lectin mixture well with an applicator stick.
5. Tilt the slide back and forth and observe for agglutination within 1 minute. Test that do not show agglutination within 1 minute are considered NEGATIVE. Do not interpret drying or fibrin strands as agglutination.

INTERPRETATION OF RESULTS
Agglutination of the red blood cells is a positive test result and indicates the presence of the A1 antigen and absence of agglutination indicates that the cells do not possess A1 Antigen and are A2 subgroup.

LIMITATIONS OF PROCEDURE
1. Test using antisera of the ABO blood group system should be performed at room temperature (25°C±5°C) and never incubated at high temperatures.
2. The A1 antigen is not fully expressed on the red blood cells of new born infants and false negative results may occur.
3. Red cells upto 28 days old may be tested with this reagent; however positive reactions may be weaker than those obtained with fresh red cells.
4. Contaminated blood specimens and/or supplementary materials used in the procedures described in this protocol may interfere with the results.

SPECIFIC PERFORMANCE CHARACTERISTICS
When properly stored and used according to the procedures described under Direction for Use, this reagent will agglutinate red cells which have the A1 antigens. The potency of this reagent is standardised by comparison to a previously approved lot. The reactivity of each lot is demonstrated in tests with the recommended procedure using a panel of cells. The specificity of each lot is shown by the recommended tube method using a panel of cells which lack the antigen against which the antiserum is directed but contain as many other antigens having a frequency of 1% or greater as possible.

BIBLIOGRAPHY
3. Ibid.

LIMITED EXPRESSED WARRANTY DISCLAIMER
The manufacturer limits the warranty to the test kit, in as much as that the test kit will function as an in vitro diagnostic assay within the limitations and specifications as described in the product instruction manual, when used strictly in accordance with the instructions contained therein. The manufacturer disclaims any warranty expressed or implied including such expressed or implied warranty with respect to merchantability, fitness for use or implied utility for any purpose. The manufacturer’s liability is limited to either replacement of the product or refund of the purchase price of the product and in no case liable to for claim of any kind for an amount greater than the purchase price of the goods in respect of which damages are likely to be claimed. The manufacturer shall not be liable to the purchaser or third parties for any injury, damage or economic loss, howsoever caused by the product in the use or in the application thereof.
DESCRIPTION OF SYMBOLS USED

The following are graphical symbols used in or found on J. Mitra diagnostic products and packing. These symbols are the most common ones appearing on medical devices and their packing. They are explained in more detail in the British and European Standard BS EN 15223-1:2012.

- In vitro diagnostic medical device
- See Instruction for use
- Temperature
- Limitation
- Caution
- See instruction for use

WARNING FOR USERS

WARNING: ALL THE SAMPLES TO BE TESTED SHOULD BE HANDLED AS THOUGH CAPABLE OF TRANSMITTING INFECTION. NO TEST METHOD CAN OFFER COMPLETE ASSURANCE THAT HUMAN BLOOD PRODUCTS WILL NOT TRANSMIT INFECTION.

1. The use of disposable gloves and proper biohazardous clothing is STRONGLY RECOMMENDED while running the test.
2. In case there is a cut or wound in hand, DO NOT PERFORM THE TEST.
3. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
4. Tests are for in vitro diagnostic use only and should be run by competent person only.
5. Do not pipette by mouth.
6. All materials used in the assay and samples should be decontaminated in 5% sodium hypochlorite solution for 30-60 min. before disposal or by autoclaving at 121°C at 15psi for 60 min. Do not autoclave materials or solution containing sodium hypochlorite. They should be disposed of in accordance with established safety procedures.
7. Wash hands thoroughly with soap or any suitable detergent, after the use of the kit. Consult a physician immediately in case of accident or contact with eyes, in the event that contaminated material is ingested or come in contact with skin puncture or wounds.
8. Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.
9. The reagent contains Sodium Azide as a preservative. If these material are to be disposed off through a sink or other common plumbing systems, flush with generous amounts of water to prevent accumulation of potentially explosive compounds. In addition, consult the manual guideline “Safety Management No. CDC-22”, Decontamination of Laboratory Sink Drains to remove Azide salts” (Center for Disease Control, Atlanta, Georgia, April 30, 1976.)
10. Extreme turbidity may indicate microbial contamination. Serologic testing is necessary to recognize reagent deterioration. It is recommended that the reagent should be tested with appropriate positive and negative controls according to the methods described in this protocol.

Positive Control: Red blood cells known to possess the antigen(s) towards which the antisera is directed.

Negative Control: Red blood cells known to lack the antigen(s) towards which the antisera is directed.

SPECIMEN COLLECTION AND PREPARATION

No special preparation of the patient is required prior to specimen collection. Blood collected with an anticoagulant (ACD, CPD, Sodium Citrate, Ammonium Oxalate, EDTA and Heparin) should be used in the test procedures. The blood sample should be tested as soon as possible following collection. If a delay in testing occurs, the sample should be stored at 2-8°C.

Red blood cells collected and stored in ACD or CPD have been shown to maintain reactivity for 28 days while RBCs collected and stored in sodium citrate or oxalate are reactive for atleast 14 days. If red blood cells are collected in EDTA or heparin they should be tested within 48 hours.

Blood obtained by finger puncture may be tested directly in the slide method. However to avoid clotting, blood collected in this manner should be mixed quickly with the test serum.

ADDITIONAL MATERIALS REQUIRED

Glass slides, Test tubes (10X75mm), Pasteur Pipettes, Normal saline, Beakers, Centrifuge, Timer & Mixing sticks.

Required Supplementary materials

DIRECTION FOR USE

Tube Method
1. Prepare a 3-4% suspension of red blood cells in Isotonic Saline.
2. To a test tube add 1 drop of Anti-A, lectin.
3. Using a Pasteur pipette add 1 drop of the 3-4% red blood cell suspension to the above test tube.
4. Mix well and Centrifuge for 1 minute at 1000 rpm (100-125 rcf). The centrifugal force applied to cell/serum mixture should be the minimum required to produce a “button” of red cells and a clear supernatent. Overcentrifugation i.e. the application of forces in excess of the minimum required to produce a button causes the cells to adhere to the bottom of the test tube. Vigorous agitation is then necessary before they can be resuspended. During such agitation weak agglutination may be dispersed causing a positive reaction to be missed under centrifugation i.e. the failure to apply forces necessary to cause the cells to form a button and a clear supernatent may result in weak or negative reaction. Exact speed and time of centrifugation cannot be recommended which will cover the wide variety of centrifuges available. Therefore each laboratory must calibrate its own equipment and determine the time required at a given speed to achieve the desired result.
5. Resuspend the cells by gentle agitation and examine macroscopically for agglutination.

Slide Method
1. Take whole blood sample to be tested.
2. Using a Pasteur pipette, place 1 drop of the whole blood sample on the slide.
3. Place 1 drop of Anti A, lectin next to the whole blood sample on the slide.

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