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The Effects of Hypertonic Saline (7.5%)/Dextran-70 (HSD) on Human Red Cell Typing, Lysis, and Metabolism in Vitro

Gerald L. Moore, James J. Summary
Michael A. Dubick, Mary E. Ledford
Barbara A. Ryan, Armando Gonzales
Charles E. Wade

DIVISION OF MILITARY TRAUMA
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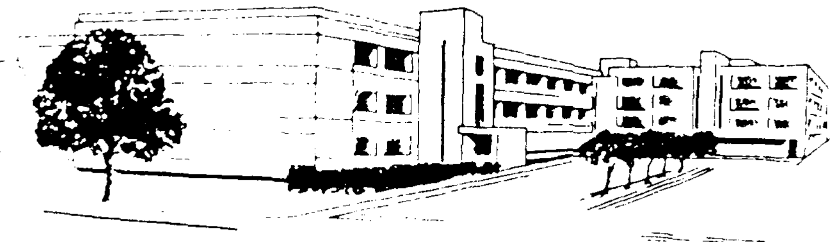

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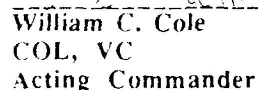

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ABSTRACT

The introduction of a 7.5% hypertonic saline/6% Dextran-70 (HSD) solution into clinical trials for the treatment of hypovolemic states, and the past concerns regarding possible interference of dextran with blood serology, prompted us to investigate the effects of HSD on human red cell typing and stability. HSD was evaluated with fresh and 35-day stored CPDA-1 red cells from 12 healthy donors. A 1:5 mixture of HSD to blood had no effect on ABO, Rh, and MN typing in both fresh and stored blood. HSD produced no significant lysis with fresh cells and a minimal level with stored blood. No evidence of metabolic or morphologic changes were seen after HSD treatment. The results of this study suggest that clinical use of HSD for treatment of hemorrhagic shock will not affect blood group determinations or red cell stability from stored blood which may be infused after the HSD treated patient is transported to a hospital.

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The Effects of Hypertonic Saline(7.5%)/Dextran-70 (HSD) on Human Red Cell Typing, Lysis, and Metabolism in vitro. -- Moore et al.

INTRODUCTION

In trauma scenarios initial treatment of hemorrhage and shock is frequently done with crystalloid solutions. Present medical doctrine calls for three volumes of saline or Ringer's solution per estimated volume of blood lost. More recently a 7.5% hypertonic saline / 6% Dextran-70 solution (HSD) has been shown effective in reducing volume requirements from 3 to 0.25 volumes for treatment of hypoxia (1,2). Preliminary studies in human trauma patients suggest that it may increase survival (3,4).

Previous experience with dextran has indicated that it may interfere with the ability to type and crossmatch red cells (5-7), presumably through its induction of rouleaux formation (7). Effects of dextran on typing or crossmatching are a direct function of the dextran molecular weight and the quality of the stored red cells (6). Many of the adverse effects are associated with dextran average molecular weights above 150,000 (9,10). This could be a problem for the HSD resuscitated patient who subsequently is transfused in the emergency room.

An additional problem has been the in vitro observation of increasing lysis when dog red cells were exposed to doses of hypertonic saline solutions above 7.5% (8). While it is well known that dog red cells are more fragile than human, and readily lyse under slight stress, stored human blood is also well known to increase its fragility during banked storage.

In this study we have examined the effects of HSD on both fresh and 35 day stored human red cells to determine if HSD altered the cell's metabolism, degree of lysis, or ability to be typed.

METHODS

Units of whole blood (450 ml each) were drawn with informed consent from twelve healthy adult volunteers. The blood was collected into Fenwal CPDA-1 bags according to American Association of Blood Banks Standards (11). Volunteers were chosen to insure that all ABO blood types were represented. Forty ml of blood was removed for day zero analysis, and the remainder of the blood was stored at 3°C for 35 days, then re-analysed.

On day zero and day 35 the 40 ml aliquot of blood from each donor was divided into two 20 ml volumes in centrifuge tubes. To

one tube was added 5 ml of isotonic saline containing 200 mg/dl of glucose (CTR). To the other paired tube was added 5 ml of the HSD test solution (HSD),which was prepared by Pharmacia AB, Uppsala, Sweden. Both tubes were capped, mixed, and incubated for 30 min. in a 37°C water bath with remixing at 10 min. intervals. The tubes were then centrifuged at 3400xg for 10 min. The supernatant was removed and saved for hemoglobin analysis. The packed cells were resuspended in 25 ml of isotonic saline plus glucose and centrifuged to wash out residual hypertonic saline and to return the cells to normal volume. These washed cells were then suspended in an equal volume of isotonic saline and evaluated. The mixing ratio of 1:5 was chosen as a worse-case situation. A typical 70 kg man has a blood volume of about 5000 ml. If half of this volume were lost, then resuscitation would be done with 250 ml of HSD, for a 1:10 ratio. Smaller people and larger blood loss might reduce the ratio further, but 1:5 is the maximum ratio conceived for surviving patients.

During evaluation the cells were typed (11) and assayed for osmotic fragility (12), morphology (13), ATP (14), and 2,3-DPG concentrations (14). The saline wash solution was saved for hemoglobin analysis (15). All bags were cultured at the end of the study to insure that they had remained sterile. Blood typing for ABO, D(Rho) and MN antigens were performed using commercial (American Hospital Supply, Miami, FL) antisera and controls. Agglutination results were scored on the basis of no agglutination (N) to (+4). Statistical comparisons were made using the paired t-test in BMDP 3D. A 0.05 level of probability was used.

RESULTS

Typical osmotic fragility curves for one donor are shown in Figure 1. HSD treatment of red cells causes a slight right shift in the curve which is statistically but not clinically significant. The osmotic fragility data was evaluated by linear regression of the linear, mid portion, of the curve (0.425 to 0.525 percent saline) and testing for differences in the 50% lysis salt concentration, the slope of the regression, and the correlation coefficient (16). These data are summarized in Table 1. Statistical differences were seen between CTR and HSD at both time periods and between time periods with both CTR and HSD 50% lysis means. Similar statistical differences were seen between the slopes of the regression lines, but no significant differences were seen among the correlation coefficients.

Red cell lysis was monitored by measuring both the supernatant hemoglobin levels following 37°C incubation of the cells, and the isotonic saline wash solution subsequent to

incubation. The data are summarized in Figure 2. There were no significant differences between the mean supernatant values at Day 0. Significant differences (control vs HSD) were seen between Day 0. washes, Day 35 supernatants, and Day 35 wash means.

Figure 3 shows the mean values for red cell morphology index, ATP and 2,3-DPG. There were no significant differences between CTR and HSD groups. The control results were typical of previous storage studies in CPDA-1 (17).

No pseudoagglutination or rouleaux formation occurred in the blood of the 12 donors typed in this study, resulting in no ABO, D, or MN discrepancies in the fresh or stored red cells following HSD treatment. The strength of the agglutinations (Table 2) were identical, except for one case.

DISCUSSION

In over 40 years of clinical use, primarily as a volume expander in the management of shock, dextran was occasionally observed to effect the behavior of red blood cells (7,9,18). Depending on the average molecular weight of the preparation, dextrans could decrease as well as increase red cell aggregation, even to the extent of promoting rouleaux formation (9,18,19). such concerns about possible pseudoagglutination induced by dextran prompted a number of investigators to evaluate the effects of dextran on typing and crossmatching of blood. Following an extensive review of the literature, Gruber (9) reported that interference with red cell serology was observed only with large molecular weight (>150,000 dalton) dextrans and not with dextrans of 40-70,000 daltons. The present study, which uses Dextran of 70,000 daltons in the HSD, shows no effect on red cell typing and thus confirms the earlier efforts. In addition we have previously observed that infusion of HSD at therapeutic doses to euvolemic or hemorrhaged rabbits and pigs (4) does not induce significant rouleaux formation (20), suggesting that HSD infusion would not effect red cell typing or crossmatching. The hypertonic saline component of HSD also does not effect typing which is consistent with the observation that red cell typing or crossmatching is not affected by intravenous infusion of electrolyte solutions (21). This is also shown in the deglycerolization of frozen-thawed red cells which are treated with 12% saline prior to washing with isotonic saline (22). The final washed cells show no loss in ability to be typed or crossmatched.

Red cells are good osmometers with great resistance to osmotic lysis. Treatment of thawed glycerolized cells with 12% saline lyses only a small fraction of 1% of the cells (22). This

resistance to lysis was also shown by Rocha and Silva for dog red cells which did not hemolyse until saline concentrations exceeded 15% (8). The Day-0 arm of our study confirmed this resistance to lysis with fresh CPD banked blood. These cells did shrink in the presence of HSD and were somewhat difficult to resuspend in isotonic saline, but did not show evidence of rouleaux formation. When resuspended in isotonic saline the cells returned to normal suspension behavior and had normal size and morphology. HSD treatment did not alter the levels of ATP or 2,3-DPG in the cells, implying that this treatment did not affect red cell metabolism by causing egress of metabolites, salt inactivation of glycolytic enzymes, or general leakyness of the membranes. Stored red cells are more prone to lysis than fresh cells, as observed in our study between fresh and 35 day controls. HSD did cause a significant increase in lysis of the 35 day stored red cells when compared to controls. However, this lysis, which could equal 2 or 3 percent of the cells, may not represent an increased decrement of therapeutic red cells because the 35 day cells would only have a 24 hour survivability of about 75 %. Thus the cells lysed by HSD may be the oldest cells which would lyse or be cleared anyway upon infusion. It is interesting that all of the HSD-induced lysis we saw occurred in the wash, not when HSD was added to the cells. This suggests that lytic damage is a function of excess salt leaving the red cells, but not entering them.

The MN antigen system was measured because it is attached to the membrane protein glycophorin A. This protein is involved with protein 4.1 and band 3 protein in a mechanism, not yet understood, which seems to help bind together the major structural proteins in the membrane skeleton (23). Change in ability to measure MN, which was not seen in our study, may be associated with loss of membrane integrity.

The effects of HSD on fresh or stored banked red cells seems to be limited to a slight amount of lysis after exposure to HSD and during return to isotonicity. This effect becomes somewhat more pronounced as the blood ages and becomes more fragile, but is not of sufficient magnitude cause concern. No changes in ability to measure red cell antigens was observed. Therefore, at the doses used to treat hemorrhagic shock, it does not appear that HSD poses a significant clinical problem with respect to cell typing or the stability of banked blood.

REFERENCES

1. Kramer GC, English TP, Gunther RA, Holcroft JW. Physiological mechanisms of fluid resuscitation with hyperosmotic/hyperoncotic solutions. Perspectives in Shock Research, Alan Liss, New York, 1989:311-320.

2. Kramer GC, Perron PR, Lindsey DC, Ho HS, Gunther RA, Boyle WA, Holcroft JW. Small-volume resuscitation with hypertonic saline dextran solution. Surgery 1986;100:239-46.

3. Holcroft JW, Vassar MJ, Turner JE, Derlet RW, Kramer GC. 3% NaCl and 7.5% NaCl/Dextran 70 in the resuscitation of severely injured patients. Ann Surg 1987;206:279-88.

4. Holcroft JW, Vassar MJ, Perry CA, Ganaway WL, Kramer GC. Perspectives on clinical studies for hypertonic saline/dextran solutions for the treatment of traumatic shock. Brazilian J Med Biol Res 1989;22:291-3.

5. Roche JP, Dodelin RA, Bloom WL. Effect of dextran on blood typing and crossmatching. Blood 1952;7:373-5.

6. Marston NA. Crossmatching of blood in the presence of dextran. Lancet 1954;2:688.

7. Wallace J. Crossmatching of blood in the presence of dextran. Lancet 1954;2:761.

8. Rocha E, Silva R, Velasco IT, Porfirio MF. Hypertonic saline resuscitation: saturated salt solutions are also effective but induce hemolysis in dogs. Circ. Shock 1988;24:246.

9. Gruber UF. Blood replacement. Berlin, Springer-Verlag, 1969.

10. Bartholomew JR, Bell WR, Kickter T, Williams GM. A prospective study of the effect of dextran administration on compatibility testing. Transfusion 1986;26:431-3.

11. American Association of Blood Banks Technical Manual, 9th Ed, AABB Press, Arlington, VA, 1985.

12. Parpart, AK et al. The osmotic resistance of human red cells. J Clin Invest 1947;26:636-40.

13. Hogman CF, deVerdier C, Ericson A, Hedlund K. Studies on the mechanism of human red cell loss of viability during storage at 4°C in vitro. Vox Sang 1985;48:257-68.

14. Moore GL, Ledford ME, Unruh KA, Brummell MR. Red cell storage in modified CPD-adenine. Transfusion 1981;21:699-701.

15. Moore GL, Ledford ME, Merydith A. A micromodification of the Drabkin hemoglobin assay for measuring plasma hemoglobin in the range of 5 to 2000 mg/dl. Biochem Med 1981;26:167-73.

16. Moore GL, Ledford ME, Gonzales A. Analysis of osmotic fragility curve data. MS in preparation.

17. Moore GL, Peck CC, Sohmer PR, Zuck TF. Properties of blood stored in anticoagulant CPDA-1 solution. Transfusion 1981;21:135-8.

18. Hint H. Relationships between chemical and physicochemical properties of dextran and its pharmacological effects. IN: Derrick JR, Guest MM Eds. Dextrans, Current Concepts of Basic Actions and Clinical Applications. Springfield, Charles C. Thomas, 1971;3-26.

19. Singh BA, Joseph KP. Erythrocyte sedimentation profiles under gravitational field as determined by He-Ne laser. VII Influence of dextrans, albumin, and saline on cellular aggregation and sedimentation rates. Biorheology 1987;24:53-61.

20. Ryan BA, Summary JJ, Dubick MA, Bowman PD, Wilson L, Wade CE. The hemostatic and hematogolic effects of hypertonic saline (7.5%) Dextran-70 (HSD) in swine and rabbits. FASEB J 1989;3:A1210.

21. DeViltorio AA. Plasma volume expanders and blood typing. J Am Med Assoc 1954;154:1398.

22. Valeri CR. Blood Banking and The Use of Frozen Blood Products. Boca Raton: CRC Press, 1976.

23. Bennett V. The spectrin-actin junction of erythrocyte membrane skeletons. Biochem Biophys Acta 1989;988:107-21.

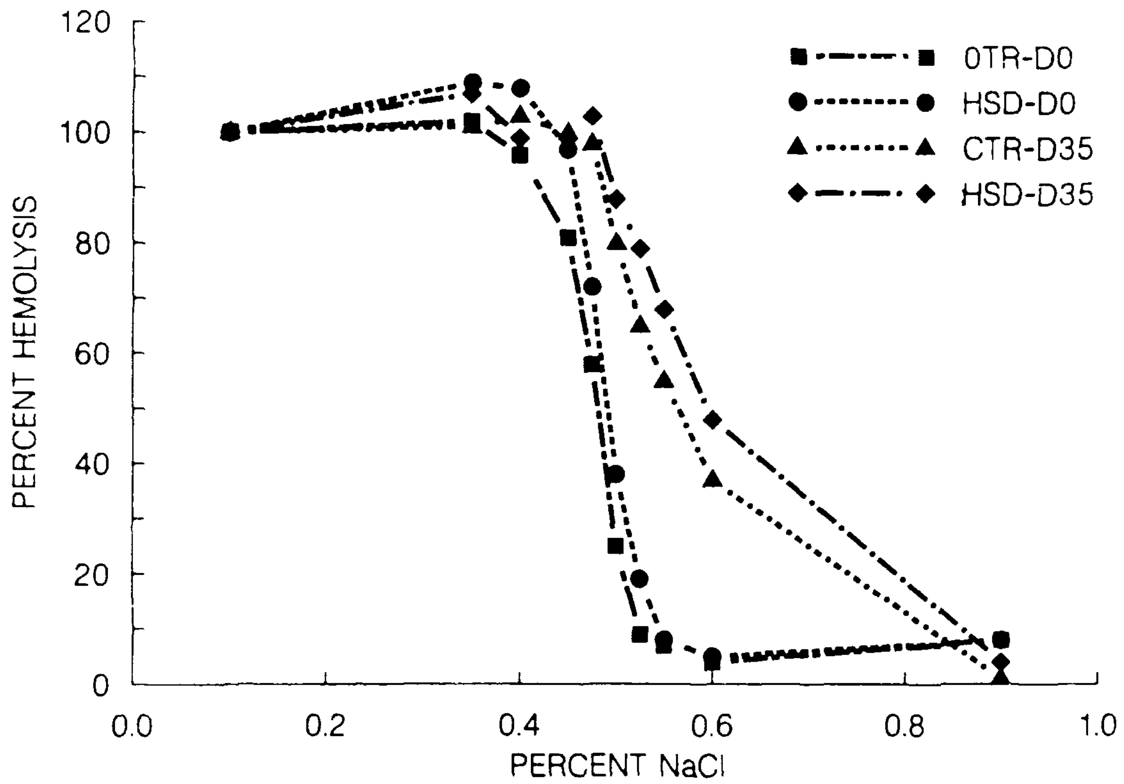

Figure 1: Typical osmotic fragility curve shown for one donor in the study. Statistical data calculated on the linear drop portion of the curve between salt % of 0.425 and 0.5, or 0.45 and 0.60.

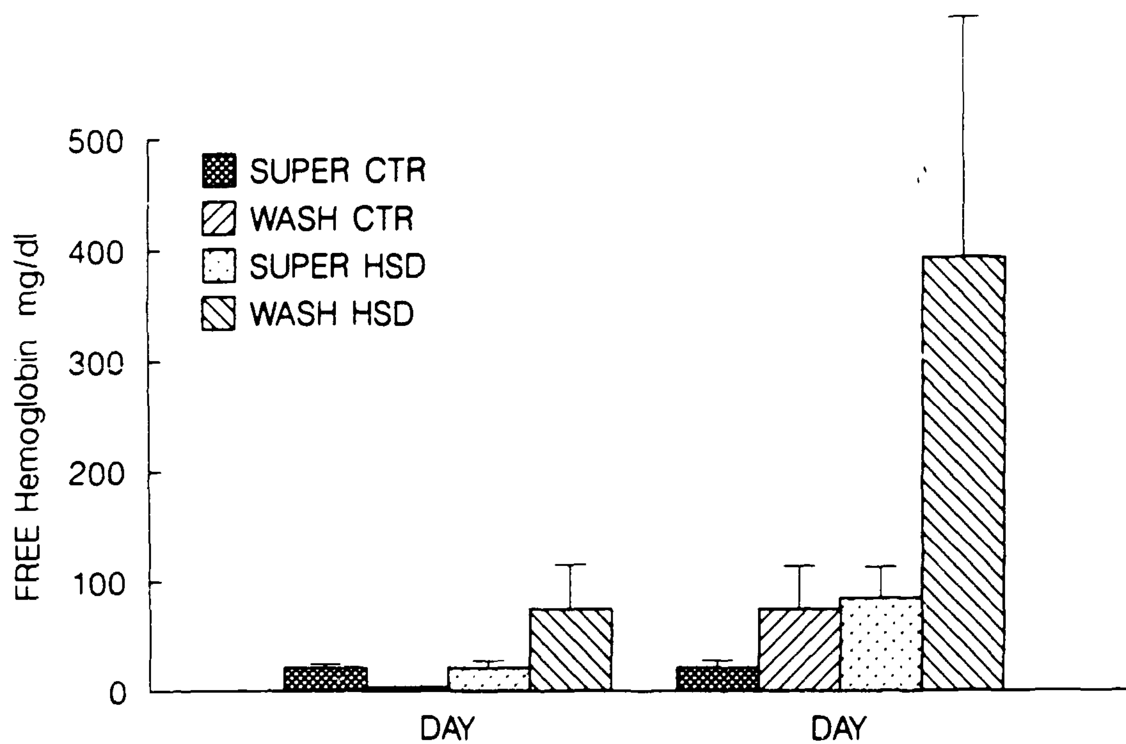

Figure 2: Supernatant hemoglobin from lysed red cells. A concentration of about 150 mg/dl would be equivalent to 1% lysis of the red cells. Error bars are SD.

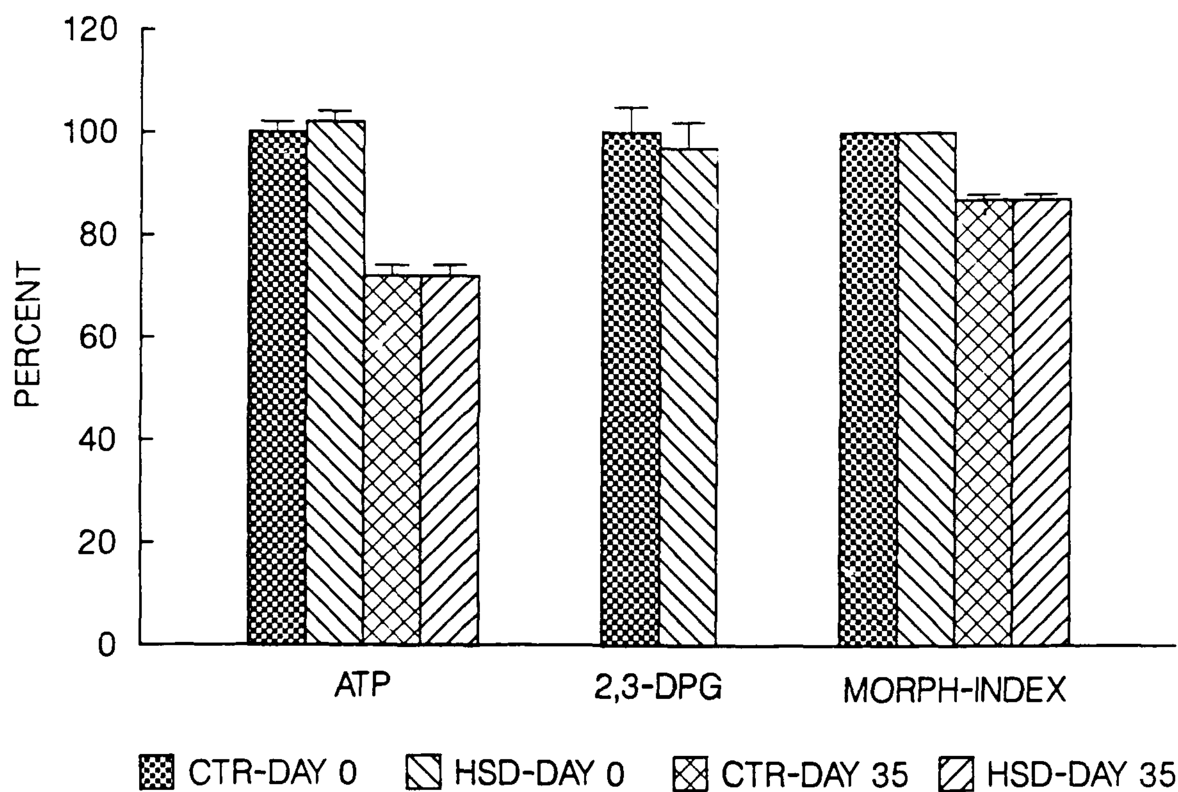

Figure 3: Red cell Morphology Index expressed as percent (100% is all biconcave disks) and red cell ATP and 2,3-DPG expressed as percent of initial values. Day 35 2,3-DPG values are near zero. Error bars are SEM.

Table 1

SUMMARY DATA OF OSMOTIC FRAGILITY CURVES
(MEAN ± SD)

	CTR-Day0	HSD-Day0	CTR-Day35	HSD-Day35
50% Lysis Point	0.4610±.02	0.4775±.01	0.5083±.02	0.5262±.04
Regres. Slope	881±215	1041±156	699±136	626±152
Regres. r-value	0.952±.03	0.961±.04	0.974±.02	0.960±.04

Table 2

The Effect of HSD[1] and/or Storage on Red Blood Cell Typing for ABO Groups and M/N Antigens*

Specimen #	Type	Anti-A	Anti-B	Anti-AB	Anti-M	Anti-N	Anti-D
100	AB+	4+	4+	4+	3+	3+	3+
102	B+	N	4+	4+	3+	3+	3+
103	AB+	2+(3+)	4+	4+	3+	3+	3+
104	B+	N	4+	4+	3+	N	3+
105	A+	4+	N	4+	3+	3+	3+
106	O+	N	N	N	3+	N	3+
107	A+	4+	N	4+	3+	N	3+
108	O+	N	N	N	3+	3+	3+
109	O+	N	N	N	3+	N	3+
110	A+	4+	N	4+	3+	3+	3+
111	B+	N	4+	4+	3+	3+	3+
112	O+	N	N	N	3+	3+	3+

N = No agglutination, Negative
[1]HSD = 7.5% Hypertonic Saline in 6% Dextran-70

* = Identical results were obtained following 30 min incubation of normal saline or HSD with fresh blood at a ratio of 1:5 (v/v). Further, repeating the incubations with whole blood stored for 35days produced identical results as with fresh blood.

Specimen # 103 (3+): Strength of agglutination increased after 35 days in both the control and HSD incubation.

For the above specimens, all Rh and saline controls were negative.

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