THE DETECTION OF IgG MATERNAL ALLOANTIBODIES FOR SAFETY TRANSFUSION IN NEWBORN AND INFANT LESS THAN 4-MONTH OLD IN SIRIRAJ HOSPITAL

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ABSTRACT

Hemolytic disease of the newborn (HDN) is a disease of the fetus and newborns caused by IgG maternal antibodies resulting from previous transfusions and pregnancies. These antibodies directed against fetal red cells can cross the placenta and cause red cell destruction. The disease has a wide range of severity due to the level of maternal antibodies and the characteristics of antigens with respect to fetal red cells. The serologic tests play an important role in diagnosis, predicting the severity, giving appropriate treatment, and prevention of the disease.

In this study, blood samples from 346 newborns with hyperbilirubinemia and 89 infants less than 4 months old were tested for ABO, Rh (D), direct antiglobulin test (DAT) and indirect antiglobulin test (IAT). Lui freeze-thaw and acid elution tests were used for the detection of IgG ABO antibodies and maternal alloantibodies attached to newborn red cells, respectively. ABO HDN was found in 63.9% of the cases which positive results for DAT, IAT and elution test were 30.8%, 63.3%, and 85.1%, respectively. It was found that Lui freeze- thaw elution test gave positive results in all positive DAT and in 78.4% of negative DAT cases. The test also gave positive results in 95.8% and 61.7% of positive and negative IAT cases, respectively. In addition, maternal clinically significant alloantibodies (anti-E, anti-E+Mi^{a} and anti-Jk^{a}) were found in 3 cases which the same antibodies were found in the eluates, but none required exchange transfusion. In the infant group, all tests were negative. No Rh negative cases were found in the study.

In conclusion, elution tests should be included as routine tests performed in the investigation of HDN. The causative antibodies could be demonstrated, as a result, definite diagnosis, appropriate management, especially selection of blood for exchange transfusion, and prevention for future pregnancies could be achieved.

KEY WORDS: HDN / IgG MATERNAL ANTIBODIES / ELUTION TESTS

57 pages.

การตรวจหาแอนติบอดีชนิด IgG ของมารดาเพื่อการให้เลือดที่ปลอดภัยในเด็กแรกเกิดและทารกที่อายุน้อยกว่า 4 เดือน ในโรงพยาบาลศิริราช

THE DETECTION OF IgG MATERNAL ALLOANTIBODIES FOR SAFETY TRANSFUSION IN NEWBORN AND INFANT LESS THAN FOUR - MONTH OLD IN SIRIRAJ HOSPITAL

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บทคัดย่อ

Hemolytic disease of the newborn (HDN) เป็นโรคที่เกิดในทารกซึ่งอยู่ในครรภ์จนถึงแรกเกิด เนื่องจากเม็ดเลือดแดงของทารกถูกทำลายโดยแอนติบอดีชนิด IgG จากแม่ ซึ่งเกิดขึ้นภายหลังการได้รับเลือดหรือเคยตั้งครรภ์มาก่อน ความรุนแรงของโรคแตกต่างกัน ขึ้นกับความแรงของแอนติบอดีจากแม่ และชนิดของแอนติเจนบนเม็ดเลือดแดงเด็ก การตรวจทางซีโรโลยีจะช่วยในการวินิจฉัยและการทำนายความรุนแรงของโรคซึ่งเป็นสิ่งสำคัญต่อการรักษาและการป้องกัน รวมทั้งการเตรียมเลือดเพื่อเปลี่ยนถ่ายเลือดที่เหมาะสม ในการศึกษานี้ได้ทำการตรวจเลือดทารกแรกเกิดที่มีอาการเหลือง จำนวน 346 ราย และเด็กที่มีอายุระหว่าง 8 วัน ถึง 4 เดือน จำนวน 89 ราย โดยทำการตรวจ ABO grouping, Rh (D) typing, Direct antiglobulin test (DAT), Indirect antiglobulin test (IAT) และเพิ่มการทำ elution test 2 วิธี คือ Lui freeze-thaw และ acid elution test สำหรับตรวจหา IgG anti-A หรือ anti-B และ แอนติบอดีชนิดอื่นจากแม่ที่จับบนเม็ดเลือดแดงเด็ก ตามลำดับ

ในการศึกษาพบ ABO HDN ร้อยละ 63.9 ซึ่งการตรวจ DAT, IAT และ elution test ให้ผลบวกร้อยละ 30.8, 63.3 และ 85.1 ตามลำดับ และพบว่ารายที่ DAT ให้ผลบวก การทำ elution test ให้ผลบวกด้วยทุกราย แต่รายที่ DAT ให้ผลลบ พบว่า elution test ให้ผลบวกร้อยละ 78.4 ในเด็กที่ IAT ให้ผลบวก พบว่ามี ร้อยละ 95.8 ที่ elution test ให้ผลบวกเช่นกัน แต่ในรายที่ IAT ให้ผลลบ พบว่ามีร้อยละ 61.7 ที่ elution test ให้ผลบวก นอกจากนี้ยังพบแอนติบอดีในเลือดแม่ 3 ราย ได้แก่ anti-E, anti-E+Mi^{a} และ anti-Jk^{a} ทุกรายไม่ได้ทำการเปลี่ยนถ่ายเลือด แม้ว่า 2 ใน 3 รายมี DAT บวก และใน eluate ทุกรายตรวจพบแอนติบอดีชนิดเดียวกับเลือดแม่ ส่วนในเด็กที่มีอายุต่ำกว่า 4 เดือน พบว่า DAT, IAT, elution test และการตรวจกรองหาแอนติบอดีของหมู่เลือดระบบต่าง ๆ ให้ผลลบทุกราย ในการศึกษานี้ไม่พบ Rh ลบ ทั้งในเลือดแม่และเลือดเด็ก

สรุป จากการศึกษาพบว่าในการตรวจเลือดเด็กที่มีภาวะเหลืองเพื่อวินิจฉัยโรค HDN และจัดเตรียมเลือดให้นั้น นอกจากตรวจหาหมู่เลือด ABO, Rh (D), DAT และ IAT แล้ว ควรทำ elution test ร่วมด้วยทุกราย เพื่อบอกชนิดของแอนติบอดีที่เป็นสาเหตุ ซึ่งจะเป็นประโยชน์ต่อการวินิจฉัย การรักษาโดยเฉพาะอย่างยิ่ง การเตรียมเลือดเพื่อเปลี่ยนถ่ายเลือดให้เด็ก และการให้การป้องกันสำหรับการตั้งครรภ์ครั้งต่อไป

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LIST OF ABBREVIATIONS

Abbreviations	Terms
-	Negative
+	Positive
%	Percent
<	Less than
=	Equal to; equals
>	Greater than
µl	Microliter
µg	Microgram
Ab	Antibody
AHG	Anti human globulin
°C	Degree Celsius
CCC	Coombs' Control Cells
CMV	Cytomegalovirus
DAT	Direct antiglobulin test
Exp.	Expiry date
g	Gram
HDN	Hemolytic Disease of the Newborn
IAT	Indirect antiglobulin test
Ig	Immunoglobulin
IS	Immediate spin
Min	Minute
Ml	Milliliter
PCR	Polymerase Chain Reaction
PUBS	Percutaneous Umbilical Blood Sampling
RBC	Red Blood Cell

LIST OF ABBREVIATIONS (cont.)

Abbreviations	Terms
RT	Room temperature
SC	Screening
Temp	Temperature
w	Weak

CHAPTER I
INTRODUCTION

Hemolytic disease of the newborn (HDN) is caused by the action of transplacentally transmitted maternal immunoglobulin (IgG) on paternally inherited antigens present on fetal red cells but lacking in the mother. The placenta is designed to promote the exchange of plasma constituents between mother and fetus. In this way, the fetus receives maternal nutrients and immunity by the transfer of maternal IgG antibodies (1). In HDN, maternal IgG antibodies produced following previous transfusions or pregnancies bind to fetal red cells, causing hemolysis. In severe cases, hydrops fetalis, kernicterus or death may develop.

Fetal red cells that are sensitized by maternal IgG antibody cannot continue to circulate and function normally, they are removed by the reticuloendothelial system for destruction (1), causing anemia in the fetus. Hemoglobin liberated from the damaged red cells is metabolized to indirect bilirubin. The indirect billirubin, the waste product of hemoglobin, conjugated by the maternal liver enzyme and excreted. As red cell destruction continues, the fetus becomes more anemic. Increasingly, severe fetal anemia causes enlargement of the fetal liver and spleen, which are the organs for both erythropoiesis and red cells destruction. Then immature red cells were released into the fetal circulation to compensate for anemia, and this referred to “erythroblastosis fetalis” (1). In untreated cases, cardiac failure accompanied by hydrops fetalis, a condition with edema and fluid accumulation in fetal cardiac, pleural and peritoneal cavities may occur and result in intrauterine death or stillborn.

Following delivery, the immediate neonatal problem is anemia without jaundice. But after birth, jaundice is detected within 24 hours because the maternal system is no longer available to conjugate and excrete indirect bilirubin. The newborn liver, which is deficient in enzyme glucuronyl transferase (2), is unable to conjugate the direct bilirubin into an excretable form, resulting in increased levels of indirect

bilirubin in the bloodstream a condition know as hyperbilirubinemia. Without adequate treatment, indirect bilirubin will bind to tissues which results in jaundice. In particular, it may bind with tissues of central nervous system especially in the brain cells of basal ganglia and cerebellum, causing a condition known as “kernicterus” a permanent brain damage, resulting in deafness, mental retardation or death (2).

HDN is classified into three categories, on the basis of their specificity of the causative IgG antibodies, as follow (1, 2, 3, 4):

1. ABO HDN, caused by IgG anti-A or anti-B in group O mother who has A or B newborn.

2. Rh HDN, the most severe form is caused by anti-D in Rh negative mother who has Rh positive newborn.

3. Less common causes of HDN include antibodies against antigens of many blood group systems such as Kell system (e.g., anti-K and anti-k), Kidd system (e.g., anti-Jk^a and anti-Jk^b) and Duffy system (e.g., anti-Fy^a and anti-Fy^b).

Laboratory diagnosis of HDN could be done both prenatal and postnatal. Prenatal study of the maternal history of pregnancies and transfusions and testing for ABO, Rh (D) and alloantibodies could predict the risk management of the fetus for future pregnancies. Prevention of Rh HDN due to anti-D, and appropriate treatment due to multiple antibodies or rare blood type of the mothers could be well planned. Postnatal study on both newborn and maternal samples could give definite diagnosis of HDN particularly the causative antibodies.

List of the tests routinely performed on newborns and maternal samples at the Department of Transfusion Medicine, Faculty of Medicine, Siriraj Hospital are:

1. Newborn less than-7 day old

1.1 Newborn’s sample

- ABO (cell grouping)
- Rh (D) typing and weak D test
- Direct antiglobulin test
- Indirect antiglobulin test for free anti-A, anti- B
- Antigen typing (if maternal alloantibody detected)

1.2 Maternal sample

- ABO (cell and serum grouping)
- Rh (D) typing and weak D test
- Antibody screening
- Antibody identification
- Antibody titration
- Antigen typing (if maternal alloantibody detected)

Blood prepared for exchange transfusion in ABO HDN should be red cells group " O ", same Rh (D) as newborn and resuspended with fresh frozen plasma group " AB " or same ABO group as newborn to obtain 500-600 ml whole blood of 50 - 60 % hematocrit as required. If alloantibody presents in maternal serum, red cells transfused should be lack of the antigen corresponding to the maternal antibody and using maternal serum for crossmatching.

2. Infant less than four-month old

- ABO (cell grouping)
- Rh (D) typing
- Directed antiglobulin test

Blood for transfusion should be the same ABO and Rh (D) groups, as an infant, using infant's serum for crossmatching.

It is abvious that our routine tests do not support the definite diagnosis of HDN due to ABO, Rh or other blood group systems. When mixture of alloantibodies are found in maternal serum, the tests performed do not give definite diagnosis of fetal red cell destruction.

In this study, the elution tests are used to detect IgG maternal antibodies that destroy fetal red cells. For ABO-HDN, Lui freeze-thaw elution test is used to detect IgG anti-A, anti-B, whereas, acid elution test is used for the detection of other alloantibodies (4, 5).

Research Question

Do the newborns and infants less than 4-month old have maternal IgG antibodies coated on their red cells?

Expected Results

The introduction of elution tests for the investigation of HDN could increase the detection of IgG antibodies in ABO HDN and IgG alloantibodies in other types of HDN. The definite diagnosis of HDN could be demonstrated. Finally, the management and the selection of appropriate blood for exchange transfusion could be applied.

CHAPTER II

OBJECTIVES

The objective of this study is to detect maternal IgG antibodies in newborns and infants less than 4-month old by including the elution tests in the investigation of HDN.

CHAPTER III
LITERATURE REVIEW

Hemolytic Disease of the Newborn

Hemolytic disease of the newborn (HDN) is caused by transplacentally transmitted maternal antibody on paternally inherited antigens present on fetal red cells (RBCs), but absent on the maternal RBCs. Maternal IgG antibody binds to fetal RBCs, and inturn these cells are destroyed by macrophages in the fetal liver and spleen, causing hemolysis (6). As a consequence of the hemolytic process, anemia, increased erythropoiesis, neonatal hyperbilirubinemia and sometimes result in fetal loss, death or disability may occur.

The first description of HDN is thought to be in 1609 by a French midwife who delivered twin babies, one baby was swollen and died soon after birth, and one developed jaundice and died several days later. For the next 300 years, many similar cases were described in which newborns failed to survive (7, 8, 9).

In 1932, Diamond and colleagues described the relationship among fetal hydrops, jaundice, anemia, and erythroblastosis in the circulation, a condition later called “erythroblastosis fetalis” (7, 8, 9).

In 1938, Darrow postulated that the hemolysis was due to transplacental passage of a maternal antibody into the fetal circulation (7). One year later (1939), an antibody that caused HDN was discovered by Philip Levine and Rufus E. Stetson who published their findings about a family whose a stillborn baby died of hemolytic disease of the newborn (7, 10, 11, 12). The mother suffered of blood loss at delivery. Her husband’s blood was transfused to her, with severe transfusion reactions. Since the mother and the father were both blood group O, they concluded that there must be another previously undiscovered antigen that was present on the husband’s RBCs but was not present on the mother’s RBCs and that the mother had formed an antibody

against this new antigen. This suggested for the first time that a mother could make antibodies because of immune sensitization to her fetus's RBCs (7, 10).

After Landsteiner and Weiner discovered the Rh factor on RBCs in 1940 (9, 10, 12, 13). One year later, Levine, Katzin and Burnham proposed that maternal sensitization to the Rh factor was due to an antigen that crossed the placental barrier into the maternal circulation (10, 13).

In 1966, two groups from the United States and the United Kingdom demonstrated, in a combined study, that giving anti-D immunoglobulin G (IgG) prophylaxis soon after delivery could prevent sensitization in Rh-negative women (7, 9). The World Health Organization (WHO) technical report in 1971 recommended that a dose of 125 IU of anti-D immunoglobulin G (IgG) should be given intramuscularly for every 1 ml of fetomaternal hemorrhage of Rh-positive packed RBCs or 2 ml of whole blood (7, 9).

In 1970s, routine antenatal care included screening of all unexpected maternal antibody to find which pregnancy may be at risk of HDN, and giving preventative treatment accordingly. This had led to a dramatic decrease in the incidence of HDN, particularly severe cases that were responsible for stillbirth and neonatal death (8).

In 1980's, ultrasonography was used to monitor Rh alloimmunized pregnancies, and in experienced hands, early hydrops can be reliably detected (7).

In 1998, the recommendation was reinforced by the American Association of Blood Banks and the American College of Obstetrics and Gynecologists with the inclusion of giving RhIG prophylaxis at 28 weeks'of gestation and within three days postpartum (9). Routine use of RhIG dose of 125 IU should be given intramuscularly for every 1 ml of fetomaternal hemorrhage of Rh-positive packed red blood cells or 2 ml of whole blood. At present, a dose of 300 µg of RhIG should be given intramuscularly at 28 weeks' of gestation and again within 72 hours after delivery of Rh-positive baby for every 15 ml of fetal packed red cells or 30 ml of whole blood (2, 7).

Disease Mechanism

HDN is caused by the destruction of the RBCs of the fetus by antibodies produced by the mother. Only antibodies of the immunoglobulin G (IgG) class are transported across the placenta, most IgG antibodies are directed against paternal antigens on the fetal red blood cells. (Figure 1)

Before birth, fetal red cells that are sensitized by maternal IgG antibody are removed by the reticulo-endothelial system for destruction, causing anemia in the fetus. Immature red cells were released into the fetal circulation to compensate for the anemia, and this referred to “erythroblastosis”. Indirect bilirubin is transported across the placenta for conjugation and excretion by the mother (10).

At birth, the immediate neonatal problem is anemia without jaundice but in severe cases, cardiovascular failure, hepatosplenomegaly, generalized edema, ascites, pericardial or pleural effusion and hydrops fetalis which may result in intrauterine death and stillborn could be found depending on the severity of the disease (14).

After birth, jaundice is detected within 24 hours because the maternal system is no longer available to conjugate and excrete indirect billirubin (7). The newborn liver which is deficient in enzyme “glucuronyl transferase”, is unable to conjugate indirect bilirubin into an excreable form, resulting in increased levels of indirect bilirubin in the blood stream.Without adequate treatment, the indirect bilirubin can reach the levels which is toxic to the newborn’s brain, causing “kernicterus” or permanent brain damage (8).

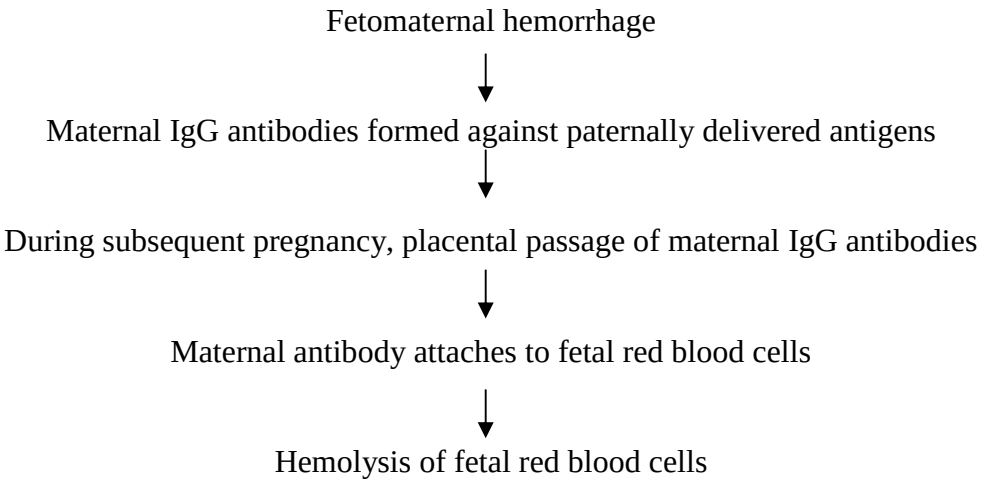

Figure 1 Pathogenesis of hemolytic disease of the newborn (10)

Classification of HDN (1, 2, 3, 4)

HDN is classified into three categories, on the basis of the specificity of the causative IgG antibodies.

1. ABO HDN, caused by IgG anti-A or anti-B in group O mother who has A or B newborn.

2. Rh HDN, the most severe form is caused by anti-D in Rh negative mother who has Rh positive newborn.

3. Less common causes of HDN include antibodies against antigens of many blood group systems such as Kell system (e.g., anti-K and anti-k), Kidd system (e.g., anti-Jk^a and anti-Jk^b) and Duffy system (e.g., anti-Fy^a and anti-Fy^b).

1. ABO Hemolytic disease of the newborn

ABO incompatibility is a common condition occurring in 20-25 % of pregnancies (15, 16, 17, 18), but produces HDN in 10 % of the cases. ABO HDN may affect the first pregnancy because IgG anti-A and anti-B are present normally in group “O”adults.

ABO incompatibility between the mother and newborn can cause HDN. Maternal IgG ABO antibodies can cross the placenta and attach to the ABO

incompatible antigens of the fetal RBCs leading to negative or weakly positive direct antiglobulin test (DAT). More commonly, the disease is manifested by the onset of hyperbilirubinemia and jaundice within 12 to 48 hours after birth. The increasing levels of bilirubin can be treated successfully with phototherapy (10). Severe cases requiring exchange transfusion are extremely rare. The severity of ABO HDN is generally mild and has negative or weakly positive DAT. This is because of poor development of ABO antigens and reduced number of A and B antigen sites on fetal or infant RBCs.

ABO antibodies are present in the sera of all individuals whose red cells lack the coresponding antigens. The high-titered IgG antibodies are commonly found in group O individuals than in group A or B individuals. Hence, ABO HDN is nearly always limited to A or B infants of group O mother with potent anti-A, B (10). The incidence of ABO HDN is several times greater than in other groups. However, hemolysis due to anti-A is more common than anti-B, but hemolysis due to IgG anti-B can be more severe and can lead to exchange transfusion. Because A and B antigens are widely expressed in various tissues besides RBCs such as epithelial cells, therefore only a small portion of antibodies crossing the placenta are available to bind to fetal RBCs due to neutralization (9). This is another reason why DAT in ABO HDN is usually negative or weakly positive.

2. Rh Hemolytic disease of the newborn

The Rh positive first born infant of Rh negative mother is unaffected because the mother has not yet been immunized. During gestation, and particularly at delivery when the placenta separates from the uterus, variable numbers of fetal RBCs enter the maternal circulation. These fetal RBCs, carrying Rh (D) antigen inherited from the father, immunize the mother and stimulate the production of anti-D. Once the mother is immunized to Rh (D) antigen, all subsequent offspring inheriting the D antigen will be affected. The maternal anti-D will cross the placenta and bind to the fetal Rh positive cells. The sensitized RBCs are destroyed by the fetal reticulo - endothelial system, resulting in anemia.

Anti-D is responsible for the most severe case of Rh HDN. In most cases, Rh negative mother will produce anti-D after the first Rh positive pregnancy. But in rare cases, alloimmunization can occur during the first pregnancy. The severity of Rh HDN can vary from mild to severe cases which need exchange transfusion to reduce bilirubin levels and prevent kernicterus. Phototherapy has no benifit in these cases.

The introduction of Rh immune globulin (RhIG) has reduced the incidence of Rh HDN. Routinely, to protect the mother against 30 ml of fetal Rh (D) positive blood (15 ml RBCs), Rh (D) negative mother will receive a 300 µg dose of RhIG intramuscularly at about 28 weeks of gestation (7, 9), which is about the time when fetal RBCs start to express the D antigen, and receive another dose within 72 hours after delivery of Rh positive baby. After delivery, Kleihauer-Betke test is used to estimate the feto-maternal hemorrhage and the correct dose of RhIG could be given to the mother (2).

To prevent anti-D production, RhIG should also be given to Rh negative mother after amniocentesis, abortions, termination of ectopic pregnancy, abdominal trauma, percutaneous umbilical blood sampling (PUBS) and intrauterine transfusions (2). Comparison of ABO HDN and Rh HDN is shown in Table 1.

3. Hemolytic disease of the newborn caused by other antibodies

Less common causes of HDN include antibodies directed against antigens of many blood group systems such as Kell system (e.g., anti-K and anti-k), Kidd system (e.g., anti-Jk^a and anti-Jk^b), Duffy system (e.g., anti-Fy^a and anti-Fy^b) and MNS system. To date, antibodies directed against the P and Lewis system have not been associated with HDN, except they are IgG antibodies (1, 2, 3, 4).

Table 1 Comparison of clinical data and laboratory findings in ABO HDN and Rh HDN

	ABO HDN	Rh HDN
Clinical findings		
Anemia	None to mild	Mild to severe
Jaundice	None to mild	Mild to severe
Hepatosplenomegaly	No	Mild to severe
Edema	No	Mild to severe
Serologic results		
ABO group	Mother group O Newborn group A or B	Any ABO group
D typing	D-positive or D-negative	Mother D-negative Newborn D-positive
Direct antiglobulin test	Negative or weakly positive	Positive
Antibody (IgG) in infant's serum	Anti-A, anti-B, anti-A,B	Anti-D
Hematological results		
Reticulocyte count	Mildly increased	Greatly increased
Blood smear		
Morphology	Spherocytes	Macrocytes Hypochromia
Nucleated red cells	Mildly increased	Greatly increased
Chemistry results		
Bilirubin	Mildly increased	Often > 20 g/dl
Value of prenatal testing	Not useful	Useful
Occurrence in first pregnancy	Often	Rare
Protection for next pregnancy	None	Yes, RhIG

From Basic and Applied Concepts of Immunohematology: *Hemolytic Disease of the Fetus and Newborn*, 2000 (2).

The severity of hemolytic disease of the newborn

The severity of HDN and infant determines whether the nature of HDN is mild, moderate, or severe (8). In mild hemolytic diseases, most of these newborns are not anemic and have minimal hemolysis. Apart from early phototherapy, they require no exchange transfusion. However, these newborns are at risk of developing more severe late anemia by 3-6 weeks of life (9).

In moderate hemolytic disease, these infants are not clinically jaundice at birth but rapidly develop increment of unconjugate bilirubinemia in the first 24 hours of life. These newborns often have hepatosplenomegaly and without adequate treatment, they are at risk of developing bilirubin encephalopathy. Early exchange transfusion is usually required. These newborns are also at risk of developing late anemia of infancy at 4-6 weeks of life (9).

In severe hemolytic disease, the fetal hydrops is predominantly caused by a capillary leak syndrome due to tissue hypoxia, and hypoalbuminemia secondary to hepatic dysfunction, and high-output cardiac failure from anemia. About half of these fetuses become hydropic before 34 weeks of gestation and need intensive monitoring and management (9).

Diagnosis and Management

Serologic and clinical tests performed at appropriate time during the pregnancy can accurately determine the level of antibody causing HDN also and determine the severity of RBCs destruction. If clinical and serologic data indicate that the fetus is becoming severely anemia, interventions such as intrauterine transfusion can be performed to treat the anemia and prevent the development of disease severity.

A. Prenatal Diagnosis

When a pregnant woman first visits her clinician, her obstetric history should be documented. Laboratory screening should be performed according to the

local guidelines to determine health status and to identify any health problems that may affect the pregnancy or the fetus. Lists of the tests are as follows:

1. ABO grouping: It is not essential during the prenatal period because ABO is unlikely to cause fetal morbidity or mortality (1).

2. Rh (D) typing: as unimmunized Rh negative women are potential candidates for anti-D immunoglobulin if Rh-positive fetus is being carried.

3. Antibody screening: the tests must be able to detect IgG alloantibodies that are reactive at 37 °C and in the antiglobulin phase. If the antibody screening is negative, repeat testing is recommended at 20 to 24 weeks of gestation and again at delivery (1, 10).

4. Antibody identification: if the antibody screening is positive, antibody identification should be able to determine the antibody specificity and whether antibodies are clinically significance (1). In Rh negative pregnant women who have weakly reaction anti-D, particularly during the third trimester, most of them may have received RhIG prenatally. This passively administered anti-D will be found with weakly reaction in the test and will remain demonstrable for 2 to 3 months or longer.

5. Antibody titration: when clinically significant antibody is identified, maternal serum/plasma samples should be regularly titrated (2, 19). The first serum specimen should be run in parallel with later specimens, only difference of greater than two dilutions or a score change of more than 10 should be considered as a significant change in titer.

6. Paternal phenotype: paternal specimen should be obtained and tested for the presence and zygosity of the corresponding antigens. The information is helpful in planning for further testing of the mother and counseling.

7. Amniocentesis: amniotic fluid analysis of fetal bilirubin level measure the clinical status of HDN directly (2, 19). In addition, amniocentesis is useful in determining fetal RBCs antigen typing using polymerase chain reaction (PCR) to amplify DNA from amniotic fluid.

B. Postnatal Diagnosis (1, 2, 19)

When a newborn develops anemia at birth and jaundice detected within 24 hours after birth, variety of causes should be identified. In case of suspected HDN, the following should be performed on newborn and maternal samples.

1. Newborn sample should be tested for :
   1.1 ABO grouping (cell grouping) and Rh (D) typing,
   1.2 DAT for diagnosis of HDN, the positive result indicates antibody coating on the infant's RBCs.
   1.3 Indirect antiglobulin test (IAT) in newborn serum, detectd for free IgG anti-A or anti-B from the mother.
   1.4 Elution tests may be helpful in determining IgG antibodies coated on infant's RBCs.
   1.5 Antigen typing if the mother has alloantibody.
2. Maternal samples should be tested for ABO grouping, Rh (D) typing, antibody screening, antibody identification and titration.

Management of HDN

A. Prenatal Management

An increase in maternal antibody titration (anti-D) suggests that amniocentesis should be performed. In Rh HDN due to anti-D, Liley graph (20) and Queenan curve (21) are used for evaluating data from spectrophotometric analysis of amniotic fluid with give three alternative management of the fetus (Figures 2 and 3). They allow the pregnancy to continue to term, perform intrauterine transfusion or induce early labour with exchange transfusion afterwards. Since the prediction of anemia from amniotic fluid analysis using Queenan curve gives high accuracy, could be performed at 14 weeks of gestation and early appropriate management could be done, it is considered to be superior to the Liley graph.

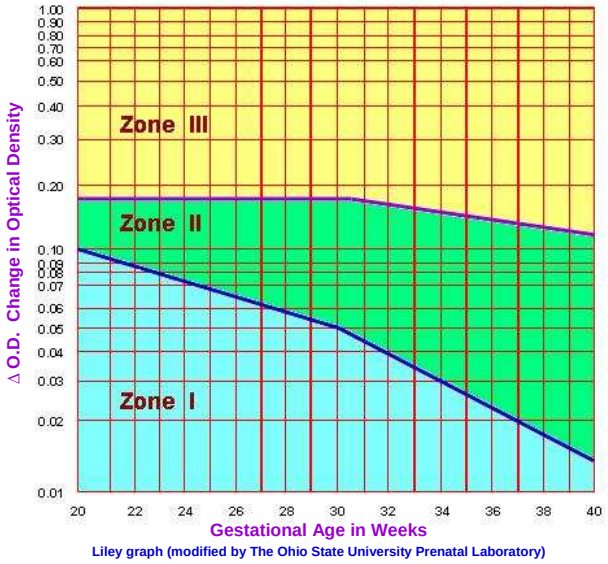

Figure 2 Liley graph. Liley graph for evaluating data from spectrophotometric analysis of amniotic fluid. The optical density at 450 nm and weeks of gestation are plotted to estimate the severity of HDN (Zone I, II, III) since 20 weeks of gestation. A reading of 0.206 at 35 weeks (Zone III) of gestation correlates with severe HDN and fetal death, which may necessitate immediate delivery. Zone I and II indicate unaffected or mildly affected and moderate disease, respectively (2, 10, 14, 20).

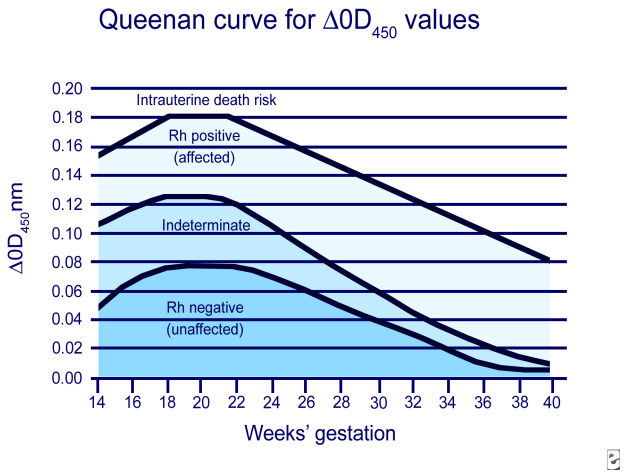

Figure 3 Queenan curve. Queenan curve is used for evaluating data from spectrophotometric analysis of amniotic fluid. The optical density at 450 nm and weeks of gestation are plotted to estimate the severity of HDN (unaffected, indeterminate and affected) since weeks of gestation. A reading of 0.18 since 14 weeks of gestation correlates with severe HDN and fetal risk of intrauterine death (9, 21).

Intrauterine transfusion (2, 10, 14, 22)

Intrauterine transfusions are given to correct anemia in utero and prevent potential heart failure. Blood provided should be packed red cells with a hematocrit of 75 to 80 % group O, Rh (D) negative, less than 7 days old, irradiated to prevent graft versus host disease and leukocyte reduced by filtration to prevent cytomegaloviral (CMV) infection. Donor RBCs are crossmatched with maternal serum. Percutaneous Umbilical Blood Sampling (PUBS) is a technique used to provide direct intravascular transfusion into the umbilical vein.

B. Postnatal Management

1. Phototherapy

Phototherapy is performed as an initial treatment for hyperbilirubinemia. Exposure of newborns to fluorescent blue light in the 420-475 nm range can successfully treat physiologic jaundice and mild cases of HDN, particularly ABO

HDN. Bilirubin, when exposed to light, undergoes photoisomerization to form photobilirubin, which can be excreted in the bile without conjugation (2, 10, 14, 24, 25). However when newborn with hyperbilirubinemia does not respond to phototherapy, exchange transfusions should be considered.

2. Exchange Transfusion

Newborn suffering from HDN is at risk from anemia and hyperbilirubinemia which can result in the permanent damage of the brain, exchange transfusion is indicated in severe HDN to overcome the problems. Exchange transfusions can correct anemia without increasing blood volume, remove sensitized newborn's red cells and replace with normal fresh donor's cells, reduce bilirubin level to prevent kernicterus and reduce maternal antibody level (2, 4, 10, 14). Blood selected for newborn ABO HDN is shown in Table 2.

Table 2 Selection of blood for exchange transfusion in ABO HDN

ABO group		Blood for exchange transfusion	
Mother	**Newborn**	**Red cells**	**FFP/Plasma**
O	A	O O	A or AB
O	B	O O	B or AB
A	B	O B,WB	B or AB
A	AB	A AB,WB	AB
B	A	O A,WB	A or AB
B	AB	B AB,WB	AB

WB = Whole blood FFP = Fresh frozen plasma

From *Work instruction. The Department of Transfusion Medicine, Faculty of Medicine Siriraj Hospital 2000.* (26)

A double-volume exchange will remove 85 % of red cells and lower bilirubin by 25% to 45% (30). Reconstituted whole blood such as, packed cells reconstituted with group AB fresh frozen plasma (FFP), with a final hematocrit of 50% to 60% is often used for exchange transfusion. Red cells should be ABO compatible with the newborn blood group and compatible with maternal serum. Blood collected in CPDA-1 and less than 7 days old is usually used to ensure maximal red cell viability and to avoid decreased 2,3-DPG and high potassium levels. FFP is used to provide plasma proteins, coagulation factors and albumin (4).

Compatibility testing in HDN (4)

First exchange

1. Use maternal serum to crossmatch with donor's red cells.
2. Use plasma / FFP to crossmatch with newborn's red cells.
3. Use plasma / FFP to crossmatch with donor's red cells.
4. Use eluate from newborn to crossmatch with donor's red cells (if maternal serum could not be obtained).

Read the results at RT, 37°C and IAT

Next exchange

If second exchange is required, crossmatching between newborn's serum and donor's red cells should be performed.

Blood transfusion for infant less than 4-month old

AABB standard requires only limited pretransfusion serologic testing for infants less than 4-month old, during any one hospitalization to prevent iatrogenic blood loss resulting in anemia, which may require more transfusions and to protect them from multiple donor exposures (4). The tests performed are:

1. ABO (cell grouping)
2. Rh (D) typing
3. Antibody screening in maternal or infant's serum / plasma

4. Crossmatching not required when using ABO, Rh (D) identical blood and negative antibody screening.

Packed red cells are usually given in small volume prepared from multiple-pack systems that allow preparation of several aliquots from a single donor unit. Infants usually do not form red cell antibodies during the first 4 months of life due to immature immune response, therefore, crossmatching is not considered unnecessary. But if antibody exists in the maternal or infant's serum, antigen-negative blood must be provided. ABO-identical or ABO-compatible blood, which has the same Rh (D) antigen as the infant can be transfused during the first 4 months.

In addition, using a single donor unit has many advantages, such as reduce the wastage of blood and reduce risks of transfusion-transmitted diseases.

CHAPTER IV
MATERIALS AND METHODS

Study design

Cross Sectional Description Study

Sample size

Sample size calculation is based on 95% confidence interval (CI) of true proportion of disparity of Hemolytic Disease of the Newborn (HDN).

$$n = \frac{Z_{\alpha/2}^{\ 2}\, pq}{d^2}$$

where p = estimate disparity of HDN = 0.25^{*}

q = $1 - p$ = 0.75

d = allowable error in estimating p = 0.05

α = probability of type I error = 0.05(2-sided)

$Z_{0.025}$ = 1.96

$$n = \frac{(1.96)^{2}\ (0.25)(0.75)}{(0.05)^2}$$

$$n = 288.12$$

$$\approx 290 \approx 300 \text{ cases}$$

The incidence of new born with hyperbilirubinemia at Siriraj Hospital is 20-25 %* (18) and it was considered to be the acceptable error. Using this formula, the sample size should be about 300 cases, however, 346 cases were recruited for this study.

Materials and Methods

Materials

1. Blood samples

1.1 Newborn's sample

Three hundred and forty six newborn's EDTA blood samples (0.5 ml) were sent from the Department of Pediatrics, Faculty of Medicine Siriraj Hospital, Mahidol University. They were diagnosed as hyperbilirubinemia and requested for blood grouping (ABO, Rh), DAT and IAT with or without blood for exchange transfusion.

1.2 Maternal samples

Three hundred and forty six maternal EDTA blood samples (8 ml), requesting for blood grouping (ABO, Rh) and screening for IgG alloantibodies were also obtained with newborn's samples.

1.3 Infant less than 4 month old (8 days to less than four- month old) samples

Eighty nine infant's EDTA blood samples (0.5 ml) were obtained from the Department of Pediatrics, requesting for blood transfusion.

2. Reagents

2.1 Anti-A, anti-B (National Blood Centre, Thai Red Cross Society)

2.2 Anti-D (DiaMed AG, Switzerland)

2.3 Antihuman globulin reagent (AHG) (DiaMed AG, Switzerland)

2.4 In house standard A, B and O cells

2.5 In house screen cells for detection of alloantibodies

- P+, P- to detect antibodies of Lewis system
- P_1, Mi^a to detect antibodies of P_1 and MNS system
- Screen cells to detect antibodies of Rh, Kidd, Kell and Duffy systems and other IgG alloantibodies.

2.6 In house panel cells for identification of maternal alloantibodies.

2.7 In house Coombs' control cells (CCC)

2.8 DiaCidel elution Kit (DiaMed AG, Switzerland)

2.9 0.9 % NSS

Methods

Conventional tube test (CTT) was used for all the tests of this study.

ABO cell grouping (4)

Procedure

1. Place 2 drops of anti-A and anti-B in each of 12x75 mm clean and labeled test tube.
2. Add to each tube 1 drop of 2-5% suspension in 0.9% NSS of red cells to be tested.
3. Mix and centrifuge at 1000 x *g*, 15 seconds.
4. Read, interpret and record the results.

Interpretation

a. Agglutination of tested red cells and either hemolysis are positive results.
b. A smooth cell suspension of the button is negative result.

ABO serum grouping (4)

Procedure

1. Label 12x75 mm, 3 clean test tubes as A, B and O
2. Add 2 drops of plasma in each tube.
3. Add 1 drop of 2-5% standard A, B and O cells suspension to each tube.
4. Mix and centrifuge at 1000 x *g*, 15 seconds.
5. Read, interpret and record the results.

Interpretation

a. Agglutination of tested red cells and either hemolysis are positive results.
b. A smooth cell suspension of the button is negative results.

Rh (D) typing (4)

Procedure

1. Place 2 drops of anti-D in 12x75 mm clean and labeled test tube.
2. Add 1 drop of 2-5% suspension in 0.9% NSS of red cells to be tested.
3. Mix and centrifuge at 1000 x *g*, 15 seconds.

4. Read, interpret and record the results.

   Interpretation

   a. Agglutination of tested red cells is positive result (Rh positive).
   b. A smooth cell suspension of the button is negative result.

If negative result obtained, weak D test should be performed.

5. Incubate for 15 minutes in 37 °C incubator.
6. Centrifuge at 1000 x *g*, 15 seconds.
7. Read for agglutination.
8. Wash 3 times with 0.9% NSS. Completely decant the final wash.
9. Immediately add 2 drops of AHG reagent, mix and centrifuge at 1000 x *g*, 15 seconds.
10. Read for agglutination and record the results.

    Interpretation

    a. Agglutination of tested red cells is weak D phenotype.
    b. A smooth cell suspension of the button is Rh negative.

Direct antiglobulin test (4, 28)

Procedure

1. Dispense 1 drop of 2-5% suspension in 0.9% NSS of red cells to be tested into 12x75 mm labeled tube.
2. Wash 3 times with 0.9% NSS. Completely decant the final wash.
3. Immediately add 2 drops of AHG reagent, mix and centrifuge them at 1000 x *g*, 15 seconds.
4. Read, interpret and record the result and check for microscopic agglutination.
5. Confirm the validity of negative result by adding Coombs' control cells.
6. Mix and centrifuge at 1000 x *g*, 15 seconds.
7. Examine for agglutination and record the results.

Indirect antiglobulin test (4, 28)

Procedure

1. Label 12x75 mm test tube, add 2 drops of test plasma and 1 drop of 2-5% cell suspension in 0.9% NSS.
2. Mix and incubate for 30 minutes in 37 °C incubator.
3. Centrifuge at 1000 x *g*, 15 seconds.
4. Read for agglutination.
5. Wash 3 times with 0.9% NSS. Completely decant the final wash.
6. Immediately add 2 drops of AHG reagent, mix and centrifuge at 1000 x *g*, 15 seconds.
7. Read for agglutination and check for microscopic agglutination. Record results.
8. Confirm the validity of negative result by adding Coombs' control cells.
9. Mix and centrifuge at 1000 x *g*, 15 seconds.
10. Examine for agglutination and record the results.

Lui freeze- thaw elution test (4)

Procedure for eluate preparation

1. Mix 1 volume of the RBCs to be tested with three volume of 0.9% NSS in a test tube.
2. Cap the tube, and then rotate the tube in order to coat the tube's wall with red cells.
3. Place the tube in a horizontal position in a freezer at -20 °C for 10 minutes.
4. Remove the tube from the freezer and thaw it quickly with warm, running tap water or 37 °C water bath.
5. Centrifuge for 2 minutes at 1000 x *g*.
6. Transfer the supernatant to a clean test tube and test it in parallel with the supernatant saline from the final wash.
7. Eluate is now ready for testing.

DiaCidel (Acid Elution) (29)

Procedure for eluate preparation

1. Wash the RBCs that have a positive DAT once with 0.9% NSS. At least 1 ml of packed cells is required.
2. Wash the packed cells 4 times with DiaCidel working wash solution.
3. Decant completely after last wash and keep part of supernatant to test for the presence of irregular antibodies.
4. Add to the washed packed cells with 1 ml DiaCidel elution solution. Mix well.
5. Centrifuge immediately for 1 minute at 900 x *g* (3000 rpm).
6. Transfer eluate into a clean test tube.
7. Add 5 drops (250 µL) of DiaCidel buffer solution to the eluate and mix well. Observe the forming of a blue color, indicating a neutral pH 6.5-7.5 is reached. If the blue color is not obtained, add more buffer (1 drop at a time) while mixing.
8. Centrifuge the eluate for 1 minute at 900 x *g* to completely remove any residual red cells.
9. Eluate is now ready for testing.

Testing the eluate

Procedure for detection of IgG anti-A/anti-B or other alloantibodies

1. Place 1 drop of eluate in 12x75 mm clean and labeled test tube.
2. Add 1 drop of 2-5% suspension (A/B or screen cells to alloantibodies detected, including negative control cells).
3. Perform indirect antiglobulin test.
4. Read the results macroscopically and microscopically.

Interpretation

a. Agglutination of the test cells with the eluate and no agglutination in the control tube shows that antibodies have been eluted from the RBCs surface.
b. No agglutination of the test cells with the eluate shows that no antibodies were eluted or that the corresponding antigen is not present on the test cells.

Antibody Screening test (25)

Routine antibody screening tests performed at the Department of Transfusion Medicine consist of 3 tests using four screening cells that were prepared from group O and had phenotyped for the most commonly encountered and clinically important RBC antigens.

1. Screening test for antibody of Lewis system, using papain treated cells
   P (+) = Le (a+b-) and Le (a-b+)
   P (-) = Le (a-b-) ll
2. Screening test for anti-P_1 and anti-Mi^a using P_1, Mi (a+) screen cells
3. Screening test for antibodies of Rh, Kidd, Kell and Duffy systems using SC screening cells (C, c, D, E, e, Jk^a, Jk^b, K, k, Fy^a, Fy^b).
4. Screening test for antibodies of Rh, Kidd and Kell systems using papainized SC screening cells.

Procedure

A. Method for screening antibody of Lewis system.

1. Appropriately label 2 tubes as P+ and P- .
2. Add 2 drops of serum to each tube.
3. Add 1 drop of P+ and P- screening cells to P+ tube and P- tube, respectively.
4. Incubate at 37 °C, 30 minutes.
5. Centrifuge and read for hemolysis.

Interpretation

Hemolysis at any stage of the test is positive result, indicating the need for antibody identification.

B. Method for screening antibody of P (anti-P_1) and MNS (anti-Mi^a) systems.

1. Appropriately label one tube for P_1, Mi (a+) cells.
2. Add 2 drops of serum to the labeled tube.
3. Add 1 drop of P_1, Mi (a+) screening cells to the tube.

4. Incubate at room temperature (RT) for 5 minutes, centrifuge, read for agglutination and incubation for another 30 minutes, then centrifuge and read for agglutination.

Interpretation

Agglutination at any stage of the test is positive result, indicating the need for antibody identification.

C. Method for screening antibody of Rh, Kidd, Kell and Duffy systems, using SC screening cells.

1. Appropriately label one tube as SC cell.
2. Add 2 drops of serum to SC tube.
3. Add 1 drop of the SC screening cells to SC tube.
4. Incubate at RT for 5 minutes, centrifuge, read for agglutination and incubation at 37 °C for 30 minutes.
5. Centrifuge and read for agglutination.
6. Perform indirect antiglobulin test.

D. Method for screening antibody of Rh, Kidd and Kell systems, using papainized SC screening cells (PS cells).

1. Appropriately label one tube as PS cell.
2. Add 2 drops of serum to PS tube.
3. Add 1 drop of the PS screening cells to PS tube.
4. Incubate at 37 °C for 30 minutes.
5. Centrifuge and read for hemolysis and agglutination.

Interpretation

Hemolysis and/or agglutination is positive test result, indicating the need for antibody identification.

Antibodies Titration (4)

Procedure

1. Label 10 test tubes according to the serum dilution (e.g., 1 in 1, 1 in 2 etc.) a 1 in 1 dilution means one volume of serum undiluted; a 1 in 2 dilution means one volume of serum in a final volume of two, or a 50 % solution of serum in the diluent.
2. Deliver one volume of 0.9 % NSS to all test tubes except the first (1 in 1) tube.
3. Add an equal volume of serum to each of the first 2 tubes (undiluted and 1 in 2 tube).
4. Using a clean pipette, mixes the content of the 1 in 2 dilution several times, and transfers one volume into the next tube (1 in 4 dilution).
5. Continue the same process for all dilutions, using a clean pipette to mix and transfer each dilution. Remove one volume of diluted serum from the final tube and save it for use if future dilutions are required.
6. Label 10 tubes (12x75 mm) for appropriate dilutions.
7. Using separate pipettes for each dilution, transfer 2 drops of each diluted serum into the appropriately labeled tubes and add 1 drop of 2-5% red cell suspension.
8. Mix well, and test by a serologic technique appropriate to the antibody.
9. Incubate at 37 °C 30 minutes.
10. Wash the cells three times with 0.9 % NSS and completely decant the final wash.
11. Add anti human globulin reagent to the dry cells button. Mix well.
12. Centrifuge and observe for agglutination. Grade and record the results.

CHAPTER V
RESULTS

1. Newborn less than 7-day old

We study 346 newborn samples who were diagnosed as hyperbilirubinemia or jaundice, and maternal samples which were sent from the Department of Pediatrics, Faculty of Medicine Siriraj Hospital to the Department of Transfusion Medicine for blood typing, DAT, IAT with or without request of blood for exchange transfusion, we found 221 (63.9%) cases of ABO HDN (mother O, newborn A or B), 47 (21.3%) cases of mother B/newborn AB, 30 (13.6%) cases of mother A/newborn AB, 26 (11.8%) cases of mother B/newborn A and 20 (9%) cases of mother A/newborn B as shown in Table 3.

In this study, only ABO incompatibilities between mothers and newborns were selected in order to detect maternal IgG antibodies attached to newborn's RBCs.

Table 3 Results of laboratory findings in 346 newborn and maternal samples

Sample No.	Newborn							Mother		
	ABO	Rh (D)	DAT	IAT		Elution		ABO	Rh (D)	Ab. screening, Identification
				anti - A	anti - B	anti - A	anti - B			
1	A	+	+	+		+		O	+	-
2	A	+	+	+		+		O	+	-
3	A	+	+	+		+		O	+	-
4	A	+	-	+		+		O	+	-
5	A	+	-	-		-		O	+	-
6	A	+	-	-		-		O	+	-
7	A	+	-	+		+		O	+	-
8	A	+	-	+		+		O	+	-
9	A	+	-	+		+		O	+	-
10	A	+	-	-		+		O	+	-
11	A	+	-	+		+		O	+	-
12	A	+	-	+		+		O	+	-
13	A	+	+	+		+		O	+	-
14	A	+	+	+		+		O	+	-
15	A	+	-	+		+		O	+	-
16	A	+	-	-		+		O	+	-
17	A	+	-	+		+		O	+	-
18	A	+	-	+		+		O	+	-
19	A	+	+	-		+		O	+	-
20	A	+	+	+		+		O	+	-

Table 3 Results of laboratory findings in 346 newborn and maternal samples (continued)

Sample No.	Newborn							Mother		
	ABO	Rh (D)	DAT	IAT		Elution		ABO	Rh (D)	Ab. screening, Identification
				anti - A	anti - B	anti - A	anti - B			
21	A	+	-	-		+		O	+	-
22	A	+	+	+		+		O	+	-
23	A	+	-	-		-		O	+	-
24	A	+	+	+		+		O	+	-
25	A	+	+	+		+		O	+	-
26	A	+	+	+		+		O	+	-
27	A	+	+	+		+		O	+	-
28	A	+	+	+		+		O	+	-
29	A	+	+	+		+		O	+	-
30	A	+	-	-		-		O	+	-
31	A	+	+	+		+		O	+	-
32	A	+	+	+		+		O	+	-
33	A	+	-	-		-		O	+	-
34	A	+	-	-		-		O	+	-
35	A	+	-	-		-		O	+	-
36	A	+	+	+		+		O	+	-
37	A	+	-	-		-		O	+	-
38	A	+	-	-		-		O	+	-
39	A	+	+	+		+		O	+	-
40	A	+	-	+		+		O	+	-
41	A	+	-	+		+		O	+	-
42	A	+	-	-		+		O	+	-
43	A	+	-	-		+		O	+	-
44	A	+	-	-		-		O	+	-
45	A	+	-	-		+		O	+	-
46	A	+	-	-		+		O	+	-
47	A	+	+	+		+		O	+	-
48	A	+	-	-		+		O	+	-
49	A	+	+	-		+		O	+	-
50	A	+	+	+		+		O	+	-
51	A	+	-	+		+		O	+	-
52	A	+	-	+		+		O	+	-
53	A	+	+	+		+		O	+	-
54	A	+	-	-		+		O	+	-
55	A	+	+	+		+		O	+	-
56	A	+	-	-		+		O	+	-
57	A	+	-	-		+		O	+	-
58	A	+	-	-		+		O	+	-
59	A	+	+	+		+		O	+	-
60	A	+	-	+		+		O	+	-
61	A	+	-	+		+		O	+	-
62	A	+	+	+		+		O	+	-
63	A	+	-	+		+		O	+	-
64	A	+	-	-		+		O	+	-
65	A	+	-	+		+		O	+	-
66	A	+	-	+		+		O	+	-
67	A	+	-	-		-		O	+	-
68	A	+	-	-		-		O	+	-
69	A	+	+	-		-		O	+	+
70	B	+	+		+		+	O	+	-
71	B	+	+		+		+	O	+	-
72	B	+	-		+		+	O	+	-
73	B	+	-		+		+	O	+	-
74	B	+	-		-		-	O	+	-
75	B	+	-		+		+	O	+	-
76	B	+	+		+		+	O	+	-
77	B	+	+		+		+	O	+	-
78	B	+	-		-		-	O	+	-
79	B	+	-		+		+	O	+	-
80	B	+	-		-		+	O	+	-

Table 3 Results of laboratory findings in 346 newborn and maternal samples (continued)

Sample No.	Newborn							Mother		
	ABO	Rh (D)	DAT	IAT		Elution		ABO	Rh (D)	Ab. screening, Identification
				anti - A	anti - B	anti - A	anti - B			
81	B	+	+		+		+	O	+	-
82	B	+	-		+		+	O	+	-
83	B	+	+		+		+	O	+	-
84	B	+	-		-		+	O	+	-
85	B	+	-		-		-	O	+	-
86	B	+	-		+		+	O	+	-
87	B	+	-		+		+	O	+	-
88	B	+	-		+		+	O	+	-
89	B	+	+		+		+	O	+	-
90	B	+	-		+		+	O	+	-
91	B	+	-		-		-	O	+	-
92	B	+	-		+		+	O	+	-
93	B	+	-		-		+	O	+	-
94	B	+	-		+		+	O	+	-
95	B	+	-		-		-	O	+	-
96	B	+	-		-		+	O	+	-
97	B	+	-		+		-	O	+	-
98	B	+	-		-		+	O	+	-
99	B	+	+		+		+	O	+	-
100	B	+	-		-		+	O	+	-
101	B	+	-		-		+	O	+	-
102	B	+	+		+		+	O	+	-
103	B	+	-		-		+	O	+	-
104	B	+	-		+		+	O	+	-
105	B	+	-		-		-	O	+	-
106	B	+	-		-		+	O	+	-
107	B	+	-		-		+	O	+	-
108	B	+	+		+		+	O	+	-
109	B	+	-		+		+	O	+	-
110	B	+	-		+		+	O	+	-
111	B	+	-		+		+	O	+	-
112	B	+	-		-		+	O	+	-
113	B	+	-		+		+	O	+	-
114	B	+	-		+		+	O	+	-
115	B	+	-		+		+	O	+	-
116	B	+	-		-		+	O	+	-
117	B	+	-		+		+	O	+	-
118	B	+	-		-		+	O	+	-
119	B	+	+		+		+	O	+	-
120	B	+	+		+		+	O	+	-
121	B	+	+		+		+	O	+	-
122	B	+	+		+		+	O	+	-
123	B	+	-		-		+	O	+	-
124	B	+	-		+		+	O	+	-
125	B	+	+		+		+	O	+	-
126	B	+	+		+		+	O	+	-
127	B	+	-		-		-	O	+	-
128	B	+	-		+		+	O	+	-
129	B	+	+		+		+	O	+	-
130	B	+	+		+		+	O	+	-
131	B	+	+		+		+	O	+	-
132	B	+	+		+		+	O	+	-
133	B	+	-		+		+	O	+	-
134	B	+	-		+		+	O	+	-
135	B	+	-		-		-	O	+	-
136	B	+	-		+		+	O	+	-
137	B	+	-		-		+	O	+	-
138	B	+	-		+		+	O	+	-
139	B	+	-		-		-	O	+	-
140	B	+	-		-		-	O	+	-

Table 3 Results of laboratory findings in 346 newborn and maternal samples (continued)

Sample No.	Newborn							Mother		
	ABO	Rh (D)	DAT	IAT		Elution		ABO	Rh (D)	Ab. screening, Identification
				anti - A	anti - B	anti - A	anti - B			
141	B	+	-		+		+	O	+	-
142	B	+	-		-		+	O	+	-
143	B	+	-		+		+	O	+	-
144	B	+	-		-		-	O	+	-
145	B	+	-		-		+	O	+	-
146	B	+	+		+		+	O	+	-
147	B	+	+		+		+	O	+	-
148	B	+	-		+		+	O	+	-
149	B	+	-		+		+	O	+	-
150	B	+	+		+		+	O	+	-
151	B	+	+		+		+	O	+	-
152	B	+	-		+		+	O	+	-
153	B	+	+		+		+	O	+	-
154	B	+	-		-		+	O	+	-
155	B	+	+		+		+	O	+	-
156	B	+	-		+		+	O	+	-
157	B	+	+		+		+	O	+	-
158	B	+	-		+		+	O	+	-
159	B	+	+		+		+	O	+	-
160	B	+	-		+		+	O	+	-
161	B	+	+		+		+	O	+	-
162	B	+	-		-		+	O	+	-
163	B	+	-		-		+	O	+	-
164	B	+	+		+		+	O	+	-
165	B	+	-		+		+	O	+	-
166	B	+	-		+		+	O	+	-
167	B	+	+		+		+	O	+	-
168	B	+	-		+		+	O	+	-
169	B	+	+		+		+	O	+	-
170	B	+	-		+		+	O	+	-
171	B	+	-		-		+	O	+	-
172	B	+	+		+		+	O	+	-
173	B	+	+		+		+	O	+	-
174	B	+	-		-		-	O	+	-
175	B	+	-		+		+	O	+	-
176	B	+	+		+		+	O	+	-
177	B	+	-		+		-	O	+	-
178	B	+	-		+		+	O	+	-
179	B	+	-		-		-	O	+	-
180	B	+	+		+		+	O	+	-
181	B	+	-		-		+	O	+	-
182	B	+	-		-		+	O	+	-
183	B	+	-		-		-	O	+	-
184	B	+	-		-		-	O	+	-
185	B	+	-		+		+	O	+	-
186	B	+	-		-		+	O	+	-
187	B	+	-		+		+	O	+	-
188	B	+	-		-		+	O	+	-
189	B	+	+		+		+	O	+	-
190	B	+	-		+		+	O	+	-
191	B	+	-		-		+	O	+	-
192	B	+	-		-		+	O	+	-
193	B	+	-		+		+	O	+	-
194	B	+	-		-		+	O	+	-
195	B	+	-		-		+	O	+	-
196	B	+	-		+		+	O	+	-
197	B	+	-		-		+	O	+	-
198	B	+	-		+		+	O	+	-
199	B	+	+		+		+	O	+	-
200	B	+	-		+		+	O	+	-

Table 3 Results of laboratory findings in 346 newborn and maternal samples (continued)

Sample No.	Newborn							Mother		
	ABO	Rh (D)	DAT	IAT		Elution		ABO	Rh (D)	Ab. screening, Identification
				anti - A	anti - B	anti - A	anti - B			
201	B	+	+		+		+	O	+	-
202	B	+	+		+		+	O	+	-
203	B	+	-		-		+	O	+	-
204	B	+	-		-		+	O	+	-
205	B	+	-		-		+	O	+	-
206	B	+	-		+		+	O	+	-
207	B	+	-		+		+	O	+	-
208	B	+	-		-		-	O	+	-
209	B	+	-		+		+	O	+	-
210	B	+	+		-		+	O	+	-
211	B	+	-		+		+	O	+	-
212	B	+	-		+		+	O	+	-
213	B	+	+		+		+	O	+	-
214	B	+	-		+		+	O	+	-
215	B	+	-		-		+	O	+	-
216	B	+	-		+		+	O	+	-
217	B	+	-		+		+	O	+	-
218	B	+	-		-		-	O	+	-
219	B	+	-		-		-	O	+	-
220	B	+	-		+		+	O	+	-
221	B	+	+		+		+	O	+	-
222	B	+	-		-		-	A	+	-
223	B	+	-		-		-	A	+	-
224	B	+	-		-		-	A	+	-
225	B	+	-		-		-	A	+	-
226	B	+	-		-		-	A	+	-
227	B	+	-		-		-	A	+	-
228	B	+	-		-		+	A	+	-
229	B	+	-		-		+	A	+	-
230	B	+	-		-		-	A	+	-
231	B	+	-		+		+	A	+	-
232	B	+	-		-		+	A	+	-
233	B	+	-		-		-	A	+	-
234	B	+	-		-		-	A	+	-
235	B	+	-		-		-	A	+	-
236	B	+	-		-		-	A	+	-
237	B	+	-		-		-	A	+	-
238	B	+	-		+		-	A	+	-
239	B	+	-		-		-	A	+	-
240	B	+	-		-		-	A	+	-
241	B	+	-		-		-	A	+	-
242	AB	+	-		-		-	A	+	-
243	AB	+	-		-		-	A	+	-
244	AB	+	-		-		-	A	+	-
245	AB	+	-		-		-	A	+	-
246	AB	+	-		-		-	A	+	-
247	AB	+	-		+		-	A	+	-
248	AB	+	-		-		-	A	+	-
249	AB	+	-		-		-	A	+	-
250	AB	+	-		-		-	A	+	-
251	AB	+	+		+		+	A	+	-
252	AB	+	-		-		+	A	+	-
253	AB	+	-		-		-	A	+	-
254	AB	+	-		-		-	A	+	-
255	AB	+	-		-		+	A	+	-

Table 3 Results of laboratory findings in 346 newborn and maternal samples (continued)

Sample No.	Newborn							Mother		
	ABO	Rh (D)	DAT	IAT		Elution		ABO	Rh (D)	Ab. screening, Identification
				anti - A	anti - B	anti - A	anti - B			
256	AB	+	-		-		-	A	+	-
257	AB	+	-		-		-	A	+	-
258	AB	+	-		-		-	A	+	-
259	AB	+	-		+		-	A	+	-
260	AB	+	-		-		-	A	+	-
261	AB	+	-		-		-	A	+	-
262	AB	+	-		-		-	A	+	-
263	AB	+	-		-		-	A	+	-
264	AB	+	-		-		-	A	+	-
265	AB	+	-		-		-	A	+	-
266	AB	+	-		-		-	A	+	-
267	AB	+	-		-		-	A	+	-
268	AB	+	-		-		-	A	+	-
269	AB	+	-		-		-	A	+	-
270	AB	+	-		-		-	A	+	-
271	AB	+	-		-		-	A	+	-
272	B	+	+					B	+	+
273	O	+	+					B	+	+
274	A	+	-	-		-		B	+	-
275	A	+	-	-		-		B	+	-
276	A	+	-	-		-		B	+	-
277	A	+	-	-		-		B	+	-
278	A	+	-	-		-		B	+	-
279	A	+	-	-		-		B	+	-
280	A	+	-	-		-		B	+	-
281	A	+	-	-		-		B	+	-
282	A	+	-	-		+		B	+	-
283	A	+	-	-		+		B	+	-
284	A	+	-	-		-		B	+	-
285	A	+	-	-		+		B	+	-
286	A	+	-	-		-		B	+	-
287	A	+	-	-		-		B	+	-
288	A	+	-	-		-		B	+	-
289	A	+	-	-		-		B	+	-
290	A	+	-	-		-		B	+	-
291	A	+	-	-		-		B	+	-
292	A	+	-	-		-		B	+	-
293	A	+	-	-		-		B	+	-
294	A	+	-	-		-		B	+	-
295	A	+	-	-		-		B	+	-
296	A	+	-	-		-		B	+	-
297	A	+	-	-		-		B	+	-
298	A	+	-	-		-		B	+	-
299	A	+	-	-		-		B	+	-
300	AB	+	-	-		-		B	+	-
301	AB	+	-	-		-		B	+	-
302	AB	+	-	-		-		B	+	-
303	AB	+	-	-		-		B	+	-
304	AB	+	-	-		+		B	+	-
305	AB	+	-	-		+		B	+	-
306	AB	+	-	-		-		B	+	-
307	AB	+	-	-		-		B	+	-
308	AB	+	-	-		-		B	+	-
309	AB	+	-	-		+		B	+	-
310	AB	+	-	+		+		B	+	-

Table 3 Results of laboratory findings in 346 newborn and maternal samples (continued)

Sample No.	Newborn							Mother		
	ABO	Rh (D)	DAT	IAT		Elution		ABO	Rh (D)	Ab. screening, Identification
				anti - A	anti - B	anti - A	anti - B			
311	AB	+	-	-		+		B	+	-
312	AB	+	-	-		-		B	+	-
313	AB	+	-	-		-		B	+	-
314	AB	+	-	-		-		B	+	-
315	AB	+	-	-		-		B	+	-
316	AB	+	-	-		+		B	+	-
317	AB	+	-	-		-		B	+	-
318	AB	+	-	-		-		B	+	-
319	AB	+	-	-		-		B	+	-
320	AB	+	-	-		-		B	+	-
321	AB	+	-	-		-		B	+	-
322	AB	+	-	-		+		B	+	-
323	AB	+	-	-		+		B	+	-
324	AB	+	-	+		+		B	+	-
325	AB	+	-	-		-		B	+	-
326	AB	+	-	-		-		B	+	-
327	AB	+	-	-		-		B	+	-
328	AB	+	-	-		-		B	+	-
329	AB	+	-	-		-		B	+	-
330	AB	+	-	-		-		B	+	-
331	AB	+	-	-		-		B	+	-
332	AB	+	-	-		+		B	+	-
333	AB	+	-	-		-		B	+	-
334	AB	+	-	-		-		B	+	-
335	AB	+	-	-		-		B	+	-
336	AB	+	-	-		-		B	+	-
337	AB	+	-	-		-		B	+	-
338	AB	+	-	-		+		B	+	-
339	AB	+	-	-		+		B	+	-
340	AB	+	-	-		-		B	+	-
341	AB	+	-	-		-		B	+	-
342	AB	+	-	-		-		B	+	-
343	AB	+	-	-		-		B	+	-
344	AB	+	-	-		-		B	+	-
345	AB	+	-	-		-		B	+	-
346	AB	+	-	-		-		B	+	-

DAT = Direct antiglobulun test + = Positive result

IAT = Indirect antiglobulun test - = Negativ result

Elution test = Lui freeze – thaw elution test

Ab. Screening = Antibody screening test

In the study of 346 newborns, 72 cases (20.8%) had positive DAT which 69 cases (95.8%) had positive Lui freeze-thaw elution test and 66 cases (91.7%) had positive for IgG ABO antibodies. Among 274 cases (79.2%) of negative DAT, positive Lui freeze-thaw elution test were found in 141 cases (51.5%) and positive IAT were found in 81 cases (29.6%).

Table 4 Summary of laboratory findings in 221 ABO HDN

	Mother / Newborn		
	O / A cases (%) N = 69	**O / B cases (%) N = 152**	**Total cases (%) N = 221**
Test for newborn red cells			
Rh (D) positive	69	152	221
DAT			
positive	25 (36.2)	43 (28.3)	68 (30.8)
negative	44 (62.8)	109 (71.7)	153 (69.2)
Elution test			
positive	56 (81.2)	132 (86.8)	188 (85.1)
negative	13 (18.8)	20 (13.2)	33 (14.9)
DAT and Elution test			
+ / +	25 (36.2)	43 (28.3)	68 (30.8)
- / +	31 (44.9)	89 (58.6)	120 (54.3)
- / -	13 (18.8)	20 (13.2)	33 (14.9)
Test for newborn serum			
IAT for anti-A/anti-B			
positive	41 (59.4)	99 (65.1)	140 (63.3)
negative	28 (40.6)	53 (34.9)	81 (36.7)
Test for maternal serum			
IAT for alloantibodies			
positive	1 (1.4)	0	1 (0.5)
negative	68 (98.6)	152 (100)	220 (99.5)

Elution test = Lui freeze – thaw elution test + = Positive result
Ab. Sc = Antibody screening test - = Negative result

Table 4, it was found that in 221 cases of ABO HDN, 68 cases (30.8%) and 153 cases (69.2%) had positive and negative DAT, respectively. Lui freeze-thaw elution test also gave positive results in 188 cases (85.1%). Furthermore, positive IAT for IgG ABO antibodies were found in 140 cases (63.3%).

In addition, alloantibody was found in 1 case (0.5%) of mother O with newborn A which acid elution test gave positive result (anti-E) that will be shown in Table 8.

Table 5 Summary of DAT, Elution test and IAT for anti-A /anti -B in 221 ABO HDN

DAT	Elution test		Total
	+	-	
+	**68***	**0**	**68**
-	**120****	**33*****	**153**
Total	**188**	**33**	**221**

+ = Positive result
- = Negative result
Elution test = Lui freeze – thaw elution test
* = Positive DAT, positive elution test and positive IAT (65 in 68 cases, 95.6%)
** = Negative DAT, positive elution test and positive IAT (73 in 120 cases, 60.8%)
*** = Negative DAT, negative elution test and positive IAT (2 in 33 cases, 6.1%)

Table 5 showed the summary of DAT, elution test and IAT for anti-A or anti-B. We found 68 cases that were positive for both DAT and elution test which 65 cases (95.6%) were also positive for IgG ABO antibodies. In 153 cases of negative DAT, 120 cases (78.4%) had positive elution test, which 73 cases (60.8%) also had positive for IgG ABO antibodies. Interestingly in 33 cases of negative DAT and elution test, 2 cases (6.1%) which had positive IgG ABO antibodies were detected. Negative DAT, elution test and IAT were found in 31 cases of 221 ABO HDN (14%).

Table 6 Summary of IAT and Elution test in 221 ABO HDN

IAT	Elution test		Total
	+	-	
+	138	2	140
-	50	31	81
Total	188	33	221

+ = Positive result
- = Negative result
Elution test = Lui freeze – thaw elution test

Table 6 showed the summary of IAT and elution test. One hundred and thirty eight cases in 140 cases of positive IAT also had positive elution test (98.6%). Fifty cases in 81 cases of negative IAT had positive elution test (61.7%). Negative IAT and elution test were found in 31 cases of ABO HDN (14%).

Table 7 Summary of laboratory findings in 123 other ABO incompatible newborns

	Mother / Newborn				
	A / B cases (%) N = 20	**B / A cases (%) N = 26**	**A / AB cases (%) N = 30**	**B / AB cases (%) N = 47**	**Total cases (%) N = 123**
Test for newborn red cells					
Rh (D) positive	20	26	30	47	123
DAT					
positive	0	0	1 (3.3)	0	1 (0.8)
negative	20 (100)	26 (100)	29 (96.7)	47 (100)	122 (99.2)
Elution					
positive	4 (20)	3 (11.5)	3 (10)	12 (25.5)	22 (17.9)
negative	16 (80)	23 (88.5)	27 (90)	35 (74.5)	101 (82.1)
DAT and Elution					
+ / +	0	0	1 (3.3)	0	1 (0.8)
- / +	4 (20)	3 (11.5)	2 (6.7)	12 (25.5)	21(17.1)
- / -	16 (80)	23 (88.5)	27 (90)	35 (74.5)	101(82.1)
Test for newborn serum					
IAT for anti-A/anti-B					
positive	2 (10)	0	3 (10)	2 (4.3)	7 (5.7)
negative	18 (90)	26 (100)	27 (90)	45 (95.7)	116 (94.3)
Test for maternal serum					
IAT for alloantibodies					
positive	0	0	0	0	0
negative	20 (100)	26 (100)	30 (100)	47 (100)	123 (100)

Elution test = Lui freeze – thaw elution test + = Positive result
Ab. Sc = Antibody screening test - = Negative result

Table 7 showed the summary of laboratory findings in 123 other ABO incompatible newborns. DAT positive was found in 1 case (0.8%) which also had positive elution test. Positive Lui freeze-thaw elution test and positive IAT were found in 22 (17.9%) and 7 (5.7%), respectively. In this group, no alloantibody was detected in maternal serum.

Table 8 Summary of DAT, Elution test and IAT for anti-A/anti-B in 123 other ABO incompatible newborns

DAT	Elution test		Total
	+	-	
+	1*	0	1
-	21**	101***	122
Total	22	101	123

+ = Positive result
- = Negative result
Elution test = Lui freeze-thaw elution test
* = Positive DAT, positive elution test and positive IAT 1 case (100%)
** = Negative DAT, positive elution test and positive IAT (3 in 21 cases, 14.3%)
*** = Negative DAT, negative elution test and positive IAT (3 in 101 cases, 3 %)

Table 8 showed the summary of DAT, elution test and IAT for anti-A or anti-B in ABO incompatible newborns. There was one case had positive DAT, elution test and also had positive IAT for ABO antibodies (100%). In 21 cases of negative DAT but positive elution test, 3 cases had positive IAT for ABO antibodies (14.3%). In 101 cases of both negative DAT and elution test, 3 cases had positive IAT for ABO antibodies (3%).

Table 9 showed the clinically significant maternal alloantibodies which were detected in 3 cases. They were anti-E (titer 64), anti-E+Mi^{a} (anti- E titer 8) and anti- Jk^{a} (titer 4). In the first case, the mother was O, D + with anti-E and the newborn was group A, D + and E -. DAT was 4+ but IAT for IgG anti-A was negative. Lui freeze-thaw was negative but DiaCidel was positive, anti-E was found in the eluate.

In the second case, the mother was group O, D+, anti-E and anti-Mi^{a} were found in her serum. The newborn was group B, D+, E+, Mi (a-), DAT 2+, only anti-E was detected in the eluate.

In the third case, the mother was group B, D+, anti-Jk^{a} was found in her serum. The newborn was group O, D+, Jk (a+b-), DAT was negative but weak anti-Jk^{a} was detected in the eluate.

Table 9 Maternal alloantibodies in 3 cases of 346 newborns with hyperbilirubinemia

Case No.	ABO, Rh (D)		Newborn				Mother			Exchange transfusion
	Mother	Newborn	DAT	Antigen typing	Elution test	Anti – A (IAT)	Antibody identification	Antibody titer	Antigen typing	
1	O, D +	A, D +	pos (4+)	E (+)	Lui = neg DiaCidel = anti – E	neg	anti – E	64	E (-)	No
2	B, D +	B, D +	pos (2+)	E (+) Mi (a-)	DiaCidel = anti - E	NT	anti - E and anti - Mi^a	8 (anti–E)	E (-) Mi (a-)	No
3	B, D +	O, D +	neg	Jk (a+b-)	DiaCidel = anti - Jk^a	NT	anti - Jk^a	4	Jk (a-b +)	No

pos /+ = Positive result
neg /- = Negative result
Lui = Lui freeze – thaw elution test
NT = Not tested
DiaCidel = Acid elution test

2. Infant less than 4-month old

Altogether 89 infant samples, who requested for blood transfusion from the Department of Pediatrics to the Department of Transfusion Medicine, Faculty of Medicine Siriraj Hospital, were studied.

Table 10 Results of laboratory findings in 89 infants less than 4-month old

Sample No.	ABO	Rh (D)	DAT	IAT		Eluate		Ab. screening
				Anti - A	Anti - B	Anti - A	Anti - B	
1	O	+	-	-	-	-	-	-
2	O	+	-	-	-	-	-	-
3	O	+	-	-	-	-	-	-
4	O	+	-	-	-	-	-	-
5	O	+	-	-	-	-	-	-
6	O	+	-	-	-	-	-	-
7	O	+	-	-	-	-	-	-
8	O	+	-	-	-	-	-	-
9	O	+	-	-	-	-	-	-
10	O	+	-	-	-	-	-	-
11	O	+	-	-	-	-	-	-
12	O	+	-	-	-	-	-	-
13	O	+	-	-	-	-	-	-
14	O	+	-	-	-	-	-	-
15	O	+	-	-	-	-	-	-
16	O	+	-	-	-	-	-	-
17	O	+	-	-	-	-	-	-
18	O	+	-	-	-	-	-	-
19	O	+	-	-	-	-	-	-
20	O	+	-	-	-	-	-	-
21	O	+	-	-	-	-	-	-
22	O	+	-	-	-	-	-	-
23	O	+	-	-	-	-	-	-
24	O	+	-	-	-	-	-	-
25	O	+	-	-	-	-	-	-
26	O	+	-	-	-	-	-	-
27	O	+	-	-	-	-	-	-
28	O	+	-	-	-	-	-	-
29	O	+	-	-	-	-	-	-
30	O	+	-	-	-	-	-	-

Table 10 Results of laboratory findings in 89 infants less than 4-month old (continued)

Sample No.	ABO	Rh (D)	DAT	IAT		Eluate		Ab. screening
				Anti - A	Anti - B	Anti - A	Anti - B	
31	A	+	-	-	-	-	-	-
32	A	+	-	-	-	-	-	-
33	A	+	-	-	-	-	-	-
34	A	+	-	-	-	-	-	-
35	A	+	-	-	-	-	-	-
36	A	+	-	-	-	-	-	-
37	A	+	-	-	-	-	-	-
38	A	+	-	-	-	-	-	-
39	A	+	-	-	-	-	-	-
40	A	+	-	-	-	-	-	-
41	A	+	-	-	-	-	-	-
42	A	+	-	-	-	-	-	-
43	A	+	-	-	-	-	-	-
44	A	+	-	-	-	-	-	-
45	A	+	-	-	-	-	-	-
46	A	+	-	-	-	-	-	-
47	B	+	-	-	-	-	-	-
48	B	+	-	-	-	-	-	-
49	B	+	-	-	-	-	-	-
50	B	+	-	-	-	-	-	-
51	B	+	-	-	-	-	-	-
52	B	+	-	-	-	-	-	-
53	B	+	-	-	-	-	-	-
54	B	+	-	-	-	-	-	-
55	B	+	-	-	-	-	-	-
56	B	+	-	-	-	-	-	-
57	B	+	-	-	-	-	-	-
58	B	+	-	-	-	-	-	-
59	B	+	-	-	-	-	-	-
60	B	+	-	-	-	-	-	-
61	B	+	-	-	-	-	-	-
62	B	+	-	-	-	-	-	-
63	B	+	-	-	-	-	-	-
64	B	+	-	-	-	-	-	-
65	B	+	-	-	-	-	-	-
66	B	+	-	-	-	-	-	-
67	B	+	-	-	-	-	-	-
68	B	+	-	-	-	-	-	-
69	B	+	-	-	-	-	-	-
70	B	+	-	-	-	-	-	-

Table 10 Results of laboratory findings in 89 infants less than 4-month old (continued)

Sample No.	ABO	Rh (D)	DAT	IAT		Eluate		Ab. screening
				Anti - A	Anti - B	Anti - A	Anti - B	
71	B	+	-	-	-	-	-	-
72	B	+	-	-	-	-	-	-
73	B	+	-	-	-	-	-	-
74	B	+	-	-	-	-	-	-
75	B	+	-	-	-	-	-	-
76	B	+	-	-	-	-	-	-
77	B	+	-	-	-	-	-	-
78	B	+	-	-	-	-	-	-
79	B	+	-	-	-	-	-	-
80	B	+	-	-	-	-	-	-
81	B	+	-	-	-	-	-	-
82	B	+	-	-	-	-	-	-
83	B	+	-	-	-	-	-	-
84	B	+	-	-	-	-	-	-
85	B	+	-	-	-	-	-	-
86	B	+	-	-	-	-	-	-
87	AB	+	-	-	-	-	-	-
88	AB	+	-	-	-	-	-	-
89	AB	+	-	-	-	-	-	-

\+ = Positive result

\- = Negative result

Table 10 showed the laboratory findings in 89 infants less than 4-month old from the Department of Pediatrics, Siriraj Hospital who requested for blood transfusion for ABO grouping, Rh (D) typing, DAT, antibody screening to detect alloantibody and Lui freeze-thaw elution testing. Antibody screening and elution testing were negative in all cases. IAT for anti-A/anti-B in infant's serum were also negative.

Table 11 Results of laboratory findings of ABO and Rh (D) blood groups in 89 infants less than 4-month old

ABO	Rh (D)	Number	%
O	positive	30	33.7
A	positive	16	18.0
B	positive	40	44.9
AB	positive	3	3.4
Total		89	100

Table 11 showed the laboratory finding of ABO and Rh (D) in 89 infants less than 4-month old. Among 89 infants, group O, A, B and AB were found in 30 (33.7%), 16 (18.0%), 40 (44.9%) and 3 (3.4%) cases, respectively. Rh negative was not found in this group.

CHAPTER VI
DISCUSSION

HDN is one of the diseases that Blood Banks play an important role in many aspects, such as, screening for high risk groups during pregnancy, investigation for the causative antibodies responsible for the disease, providing appropriate treatment and giving suitable prevention for the future pregnancies. HDN has various type of severity, mild, moderate and severe cases, due to the level of the maternal antibodies and characteristic of the antigens on fetal RBCs. Many blood groups system are responsible for HDN (1, 4, 7, 15 30). The most common type is ABO HDN which occurs mostly in group O mothers who have A or B newborns. The most severe type of HDN is Rh HDN, occurs in Rh negative mothers with Rh positive newborns. The maternal anti-D is produced as a result of previous transfusions of Rh positive blood or pregnancies. The destruction of fetal RBCs may result in severe anemia, hyperbilirubinemia, hydrops fetalis, kernicterus or even death. Therefore, all pregnant women should attend antenatal clinic for the benefit of herself and the babies. Additionally, serologic studies performed by the Blood Banks could suggest the appropriate management of the disease. The serological tests that should be done in the newborn's samples are ABO (cell) grouping, Rh (D) typing, DAT, elution test, screening for IgG ABO antibodies and for free maternal alloantibodies. The tests for the maternal samples are ABO grouping, Rh (D) typing, antibody screening and antibody identification. Then, HDN is diagnosed by the results of maternal and newborn ABO grouping and Rh (D) typing results which elution tests could help confirm the IgG ABO or alloantibodies bound to newborn's RBCs.

In this study of 346 newborns with diagnosis of hyperbilirubinemia, ABO HDN (mother O, newborn A or B) was the most common type found (63.9%) as shown in Table 3. In addition, clinically significant alloantibodies in maternal serum were found in 3 cases. Rh negative was not found in both mothers and newborns.

In Thailand, several studies on HDN and tests performed were reported. In 1997, Ratanasirivanich P, Noparat K and Chiewsilp P, studied clotted cord blood of 2100 Thai newborns at Ramathibodi Hospital and reported the prevalence of positive DAT was 1.57% which 96.96% were born to group O mothers. Antibodies in ABO system, confirmed by positive elution test were the most common cause of positive DAT but none of them required exchange transfusion. Anti-E was detected in the eluate of one case (mother O, newborn O) (31).

In 1997, Chuansumrit A, Siripoonya P, Nathalang O and Sriphaisal T. investigated infants with hyperbilirubinemia in the first week of life and found the positive rate of DAT in ABO incompatible group was similar by both conventional and the gel techniques, 54.5% amd 50%, respectively. They suggested that DAT using the gel technique is beneficial to the diagnosis of HDN (32).

In 2000, Cheepsattayakorn R, Fongsatitkul L and Pisaipong P, studied 60 clotted blood specimens of infants with hyperbilirubinemia in the first week of life at Chiang Mai which 30 cases were ABO incompatibility (mother O, newborn A or B). They reported the prevalence of positive DAT, positive IAT for ABO IgG antibodies and positive elution test were 36.67%, 66.67% and 50%, respectively. They concluded that the presence of specific ABO IgG antibodies by IAT in the serum and eluate correlated with clinical diagnosis of ABO HDN. Therefore, if positive IAT was found, the elution should be performed despite negative DAT (33).

Furthermore, in 2000, Chanachaiwan P, Sakuldamrongpanich T and Vutikornsombatkul V studied cord blood of 456 newborns at Police General Hospital and found the prevalence of ABO HDN (mother O, newborn A or B) and positive DAT were 9.2% and 1.74%, respectively. Positive DAT in ABO HDN was 19% which heat elution test was also positive. In addition, heat elution test positive was found in 67.6% of negative DAT cases. They concluded that elution test is more useful in diagnosis of ABO HDN (34).

In our study, positive DAT, positive IAT and positive elution test were 30.8%, 63.3% and 85.1%, respectively. We found high rate of negative DAT as previously described. The DAT of ABO HDN usually gives negative or weakly positive results, due to poor development of A and B antigens, reduced number of A and B antigenic sites, and the neutralization of maternal IgG ABO antibodies by A or

B substances in the fetal tissues which reduces the amount of ABO antibodies available to destroy fetal red cells. Therefore, DAT alone is not considered the test to diagnose HDN.

In the detection of IgG anti-A and anti-B by using IAT, we found positive results in 63.3% of the cases. The positive rate of IgG anti-B and anti-A were 65.1% and 59.4%, respectively. Similarly, Cheepsattayakorn R, Fongsatitkul L and Pisaipong P reported that IgG ABO antibodies was detected in 66.7% of their study. Although the test was helpful in a suspected case of HDN but it was not the only test to diagnose HDN as well (33).

When Lui freeze-thaw elution test was included in our study, the interesting results were found. Elution test gave positive results in 100% and 95.8% of positive DAT and positive IAT cases, respectively. Moreover, the positive elution tests were also found in 78.4% and 61.7% of negative DAT and IAT, respectively. It is obvious that, Lui freeze-thaw elution test is useful in the detection of IgG ABO antibodies. From our experience, the test is easy to handle and interpret the result, required small volume of RBCs and is inexpensive. Therefore, this elution test is useful in the investigation of ABO HDN. The benefit of freeze-thaw elution test is not only to confirm the diagnosis but eluate obtained could be used for compatibility test when maternal sample is unavailable. From this study, we recommend to perform Lui freeze-thaw elution test in the diagnosis of ABO HDN inspite of negative DAT or IAT.

Several studies of HDN from many countries in Asia and Australia were published. In 1987, a study in Singapore reported the incidence of HDN due to ABO incompatibility was 3.7% of all group O mothers. (35) In 1992, a review of infants with jaundice in Australia demonstrated 5.5% of ABO incompatibility and 1.8% of Rh incompatibility which 43.6% and 23.4% of Rh and ABO incompatibilities, respectively, required exchange transfusion (36). In 1996, 101 newborns with jaundice due to ABO incompatibility were studied in Sri Lanka, 84.2% were mother O with newborn A or B, 8.9% were mother A with newborn B or AB and 6.9% were mother B with newborn A or AB. They found high titer IgG anti-A and anti-B in maternal serum, resulting in 39% of newborns required exchange transfusion and 9% required multiple exchanges. ABO incompatibility in Sri Lanka appeared to be more severe

disease than elsewhere (37). In 1998, a study to determine the antibody specificity causing HDN in 79 cases in Korea demonstrated the prevalence of ABO HDN and Rh HDN (anti-D) were 25.3% and 8.9%, respectively. Anti-E + c and anti-E were the common alloantibodies detected in non-ABO incompatibility (38).

In the present study, maternal clinically significant alloantibodies were detected in 3 cases, they were anti-E, anti-E plus anti-Mi^a and anti-Jk^a as shown in Table 8. None of them required exchange transfusion due to the weak reactivity of anti-E and anti-Jk^a despite positive DAT and acid elution test. Anti –E is commonly found in the patients due to high incidence of R_1R_1 phenotype in Thai populations. Anti-Mi^a is another antibody that is commonly found in Thai patients and donors but it is an IgM type, therefore cannot cross the placenta and cause HDN (39). To demonstrate maternal IgG alloantibody attached on newborn's RBCs, we found acid elution test (DiaCidel) is suitable because it is easy to perform, require small volume of RBCs and gives clear-cut results even though the price is more expensive.

In the study of 89 infants less than 4-month old, the distribution of ABO group were O 33.7%, A 18%, B 44.9% and AB 3.4%. The incidence of B was higher than previously described (40). This is because our samples were the selected infants with anemia who required blood transfusions. All cases were Rh (D) positive. The results of DAT, IAT for IgG anti-A/anti-B, alloantibodies screening and Lui freeze-thaw elution test were all negative. These results indicated that, at the age of 8 days to 4 months, maternal IgG anti-A or anti-B were not found in our study. Unfortunately, we did not include the detection of IgM anti-A and anti-B in this study. When performed the tests for unexpected alloantibodies in infant's serum, the negative results obtained even though some of them may have received blood transfusions prior to referring to our hospital.

According to AABB Standards, repeat blood grouping (ABO, D) and crossmatching could be omitted if antibody screening is negative in the initial testing during the same hospitalization (41). This is because alloimmunization following red cell transfusions in neonate is unlikely to occur due to immature immune system (42, 43). Additionally, small aliquots from a single donor unit could be used to prevent blood wastage, multiple donor exposures and in particular, the risk of transfusion transmitted diseases (4, 27). The study results confirmed the recommendations of the

AABB Standard and the Standards for Blood Banks and Transfusion Services, National Blood Centre, Thai Red Cross Society (4, 27) that there is no need to take blood samples from these infants every time they need blood transfusion. Thus, iatrogenic anemia could be prevented.

CHAPTER VII
CONCLUSION

The purpose of this study was to detect maternal IgG antibodies in newborns and infants less than 4-month old in order to provide definite diagnosis of HDN and safe blood transfusion. In the study, Lui freeze-thaw elution test was used to detect IgG anti-A or anti-B and acid elution test (DiaCidel) was used in the detection of unexpected IgG alloantibodies bound to newborn red cells.

The study showed that Lui freeze-thaw elution test not only gave positive results in all cases of positive DAT but also gave positive results in 78.4% of negative DAT cases. In addition, the test gave positive results in 95.8% and 61.7% of positive and negative IAT cases, respectively. For alloantibodies detection, the use of acid elution test could detect the clinically significant alloantibodies in the maternal serum. For infants less than 4-month old, all the tests were negative, none of all IgG ABO antibodies or alloantibodies were found.

In conclusion, we recommend the use of elution tests as routine test performed in the investigation of HDN in order to improve the definite diagnosis and management of the disease.

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