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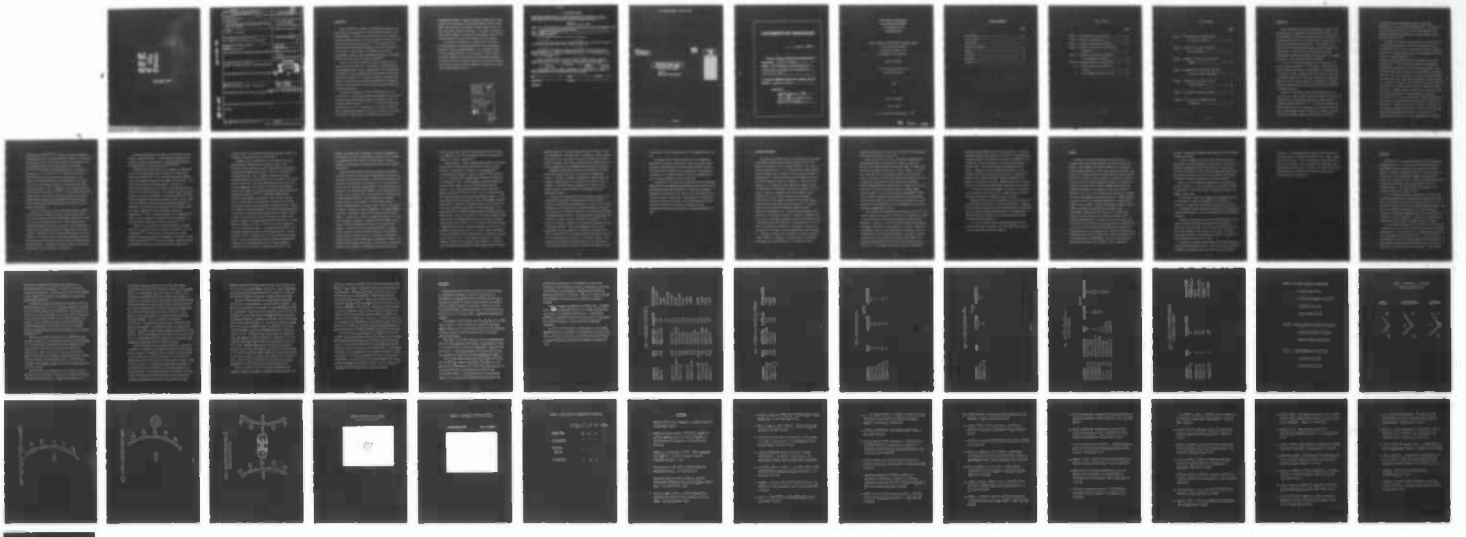

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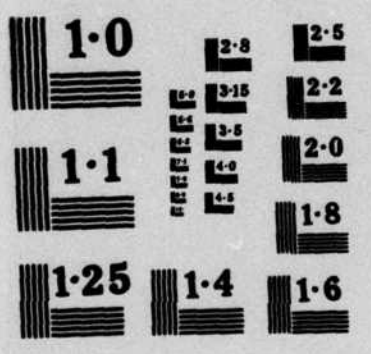

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Introduction

Severe anaphylactic transfusion reactions most frequently occur in patients who are IgA-deficient and have formed antibodies to the IgA immunoglobulin.[1] Selective IgA deficiency is the most commonly recognized immunodeficiency and is characterized by the absence of IgA from the serum and external secretions.[2] Other components of the immune system including antibody response and cellular immunity are usually normal. IgA deficiency is associated with recurrent sinopulmonary infections, gastrointestinal disturbances or autoimmune diseases but also may occur in healthy individuals.

The cause of selective deficiency of IgA is unknown but two theories have been proposed.[2, 3] The majority of individuals with this deficiency appear to have a normal number of IgA synthesizing B-lymphocytes but these lymphocytes lack the ability to mature into IgA secreting plasma cells. The second category of IgA deficiency appears to result from the presence of class-specific T-suppressor cells which prevent B-cells from maturing into IgA synthesizing and secreting plasma cells.

The mode of inheritance of selective IgA deficiency is not entirely clear. Several family studies have suggested a multifactoral inheritance mode demonstrated by either an autosomal recessive or autosomal dominant pattern.[4, 5, 6, 7, 8] One report[8] suggests that IgA deficiency is due to the inheritance of an aberrant regulatory gene of immunological synthesis rather than a structural gene defect. There exists in family members of IgA-deficient individuals an

sorbent-sorbate system. These K_d values were regressed with various properties of the sorbates, which included molecular weight, dipole moment, polarizability, molar volume, parachor, aqueous solubility, and octanol-water partition coefficients. The results of these correlation studies showed that polarizability and aqueous solubility are the best predictors of K_d values investigated in this research.

The sorption free energy (ΔG^o), enthalpy (ΔH^o), and entropy (ΔS^o) were measured by conducting isotherms at three temperatures. The mean ΔH^o values were approximately -1 to -2 kcal/mol, and the ΔG^o values ranged from about -1 to -4 kcal/mol. These relatively low values, together with the results of the correlation studies, implicated the London (dispersion) forces and the ion-induced dipole interaction as the most plausible mechanisms to account for the sorption of these compounds by soils.

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AUTHOR: Bobby C. Springer

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Kay R. Zelenski, Ph.D.

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Susan L. Wilkinson

THE EVALUATION OF QUALITATIVE AND QUANTITATIVE PROCEDURES IN THE DETECTION OF IgA -DEFICIENT DONORS

A thesis submitted to the Division of Graduate Studies
of the University of Cincinnati
in partial fulfillment of the
requirements for the degree of

MASTER OF SCIENCE

of the College of Medicine at the
Hoxworth Blood Center

1984

by

Bobby C. Springer

Captain, USAF

B.S., University of North Alabama, 1970

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Introduction

Severe anaphylactic transfusion reactions most frequently occur in patients who are IgA-deficient and have formed antibodies to the IgA immunoglobulin.[1] Selective IgA deficiency is the most commonly recognized immunodeficiency and is characterized by the absence of IgA from the serum and external secretions.[2] Other components of the immune system including antibody response and cellular immunity are usually normal. IgA deficiency is associated with recurrent sinopulmonary infections, gastrointestinal disturbances or autoimmune diseases but also may occur in healthy individuals.

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increased incidence of hypogammaglobulinemia and selective deficiencies or increases of other immunoglobulins. The incidence of IgA deficiency varies between 1/300 and 1/3000 depending on the population studied and the sensitivity of the techniques used for detection (Table 1).[12-25]

The $IgA_2m(1)$ and $IgA_2m(2)$ allotype genes are alternative alleles in a biallelic system closely linked to the Gm genes of the IgG immunoglobulin system. The gene frequencies of $IgA_2m(1)$ and $IgA_2m(2)$ are 0.98 and 0.02 respectively in the Caucasian population, and 0.36 and 0.64 in American Negroes.[9,10,11]

Individuals with IgA deficiency may form antibodies to the IgA immunoglobulin.[14] Most often these antibodies are classified as class-specific as they may react with determinants common to both subclasses of IgA, IgA_1 and IgA_2. Anti-IgA antibodies of a limited specificity have also been reported. Anti-IgA of limited specificity is specific for either a particular IgA subclass or allotype, $IgA_2m(1)$ or $IgA_2m(2)$. The class-specific antibodies are usually formed in individuals with a complete absence of IgA who presumably recognize human IgA as a foreign antigen. They often have titers greater than 1000 and are associated with serious anaphylactic transfusion reactions.[26, 27] The anti-IgA antibodies of limited specificities are most often found in individuals with normal levels of IgA who have a deficiency of a specific IgA subclass or allotype. Some have also been reported in IgA-deficient persons. These subclass or allotype-specific antibodies usually have titers less than 250 and are associated with relatively less severe anaphylactoid or urticarial

transfusion reactions.[28,29,30,31] However, one fatality associated with anti-IgA of limited specificity has been reported. The incidence of class-specific anti-IgA has been reported to vary between 0% and 24% in otherwise normal, healthy IgA deficient donors (Table 2).[14,16,19] In IgA-deficient patient populations, the frequency is much higher, 44 to 57% (Table 3),[31,32] and may be related to the higher incidence of blood transfusion in these patients. The frequency of limited specificity anti-IgA antibodies is much lower than that of class-specific antibodies in normal donor and patient populations (Tables 2 and 3). Additional varied patient populations have also been studied (Table 4).[28,33,34] These studies indicate high incidence rates of anti-IgA in IgA-deficient patients with ataxia telangiectasia, patients who have experienced anaphylactoid transfusion reactions, postpartum women and patients who have received multiple transfusions.

There are four possible causes of anti-IgA antibody formation. These include the transfusion of blood or blood products, fetal-maternal hemorrhage, maternal-fetal hemorrhage or absorption of human or animal IgA via the intestinal wall.[31] The concepts of fetal-maternal hemorrhage and intestinal absorption of IgA immunoglobulins stimulating the formation of anti-IgA antibodies have been proposed primarily to explain the lack of a known immunizing event in certain patients. It is unlikely that fetal-maternal hemorrhage would cause the production of anti-IgA since the fetus does not normally produce IgA while in utero. Stimulation of anti-IgA formed as the result of dietary items is, at this point, only theoretical.

Anti-IgA antibodies have been shown to be of the IgG class and capable of binding complement. A kinin-mediated mechanism has been proposed to explain the pathogenesis of IgA associated anaphylactic and anaphylactoid transfusion reactions.[28,35,36,37,38]

Precipitating IgG antibodies to the Fc fragment of ruminant IgM have also been reported with varied frequencies in IgA-deficient individuals (Table 5).[38-42] It is postulated that these antibodies result from antigenic stimulation from ingested dairy products. It is believed that absorption of these antigens is increased in IgA-deficient individuals due to the lack of secretory IgA which may normally limit mucosal absorption. Anti-ruminant antibodies become of practical importance when detecting or quantitating human IgA by agar diffusion techniques using antisera produced in goats, cows, or sheep. If the IgA deficient serum being tested contains anti-ruminant IgM antibodies, the reactions of this antibody with the ruminant IgM will give results suggesting that the human serum contains IgA immunoglobulin. The use of rabbit anti-human IgA in immunodiffusion tests or close examination of precipitin lines for nonidentity in double diffusion techniques will prevent such an error.[41,42]

Estimates on the incidence of severe anaphylactic transfusion reactions in IgA-deficient patients range from 1/20,000 to 1/47,000 transfusions.[35,43] This low rate of occurrence does not seem plausible considering the reported frequencies of anti-IgA in various patient populations.[28,33,34] One author, however, suggests that many minor anaphylactoid reactions are not reported and many serious

reactions may not be investigated properly due to adequate methods of testing not being available to all blood banks.[35]

Management of the transfusion needs of patients with anti-IgA presents special problems for the transfusion service. Once identified, these patients must receive blood products lacking IgA to avoid potential anaphylactic reactions. There are three methods to accomplish this task. First, storage of autologous blood when transfusion needs can be anticipated should be considered. The use of washed red blood cells and frozen-thawed-washed red blood cells have also been suggested as safe methods of transfusing these patients. However, both products as routinely prepared have been associated with anaphylactic reactions.[44-46] Yap et al[47] have shown that one additional wash, beyond the four washes with two liters of normal saline routinely used in the preparation of washed red blood cells, significantly reduced the IgA supernatant concentration from .2 mg/dl to .08 mg/dl. Frozen-thawed-washed red blood cells washed eight times in two liters of hypertonic saline and two liters of isotonic saline resulted in the reduction of IgA concentrations to less than .0003 mg/dl as compared to .01 mg/dl IgA in routinely washed units. A third method of providing red cells, as well as other blood components, is the use of IgA-deficient donors. As previously noted (Table 1), an appreciable number of otherwise normal blood donors have a selective deficiency of IgA. Techniques used to identify IgA-deficient individuals in large donor populations include automated hemagglutination-inhibition (HI), radioimmunoassay (RIA), and immunoprecipitin procedures as well as manual gel immunodiffusion and HI.[19] The

automated techniques have the advantage of efficient processing for large numbers of tests but the equipment required is costly. Manual gel immunodiffusion and HI methods, however, require no special equipment and are easily and economically performed in most clinical laboratories.

Manual gel immunodiffusion techniques described for screening large donor populations include radial immunodiffusion (RID) and the Ouchterlony double diffusion methods. These methods are based on the principle of antigen-antibody interaction as one or both substances diffuse through a semisolid support medium. At and near their zone of equivalence, antigen and antibody complex to form visible precipitin patterns. RID is primarily used as a quantitative technique but also has qualitative applications. The RID support matrix contains antisera specific for the antigen. Wells are punched into the antibody containing medium and receive antigen samples. This system is allowed to incubate at constant temperature. At antigen-antibody equivalence, a precipitin ring forms if antigen is present in the samples. If quantitative results are required, the size of the ring may be measured and compared to standards since the diameter of the precipitin ring is mathematically related to the concentration of antigen. In the Ouchterlony double diffusion method, an antigen sample is allowed to passively diffuse through a neutral support medium toward an antibody solution. At the point of equivalence, a precipitin line will form demonstrating the presence of antigen. The double diffusion technique also permits interpretation of immunologic identity, partial identity or nonidentity of specific proteins by

virtue of the patterns formed by adjoining precipitin lines (Figure 1). Sensitivity of the RID and Oucherlony methods are approximately 2 mg/dl and 3.5 mg/dl, respectively.[48]

A more sensitive, .1 mg/dl, hemagglutination-inhibition procedure has been used to further quantify potential low levels of IgA not detected by immunodiffusion techniques. Gold and Fudenberg,[49] utilizing methods developed by Jandl and Simmons,[50] first described the use of an IgA-anti-IgA hemagglutination-inhibition procedure for the quantitation of IgA. Jandl and Simmons' research described the use of metallic cations to coat red blood cells with specific proteins. Their research demonstrated that certain multivalent metallic cations (eg, Cr^{+++}, Fe^{+++}, Be^{+++}, etc.) in solution combine with carboxyl groups on the red blood cell membrane (Figure 2). This interaction between the multivalent cations and the membrane carboxyl groups caused a reduction of the negative charge on the red cell and resulted in spontaneous agglutination in saline suspension. The agglutination was not visibly distinguishable from antibody mediated agglutination. When the cations and red cells were suspended in human serum, cations were more efficiently bound to the red cell membrane before agglutination occurred. It was postulated that this inhibition of agglutination was due to competitive binding of the cations by the negatively charged serum proteins. By virtue of their multivalency, the cations may share bonds simultaneously with the protein molecules and the red blood cells. This shared bonding results in covalent binding of protein to the membrane surface (Figure 3). Red blood cells thus coated with protein were rendered agglutinable by specific

antisera (Figure 4). Gold and Fudenberg used this principle to bind IgA immunoglobulin to red blood cells. A solution of chromic chloride ($CrCl_3$) was used to coat group O red cells with purified IgA myeloma protein as antigen. These cells were then used as indicator cells for passive hemagglutination and hemagglutination-inhibition procedures.

Hemagglutination-inhibition involves the incubation of specific antibody with samples suspected of containing the corresponding antigen. If antigen is present, the antibody is complexed with the antigen and agglutination of the antigen coated indicator cells is inhibited. On the other hand, if antigen is not present in the sample, antibody is free to bind with the indicator cells and agglutination results.

A review of the literature indicates no apparent consensus on a concentration of IgA which constitutes IgA deficiency (Table 1). Criteria for defining IgA deficiency appear to be more a factor of the sensitivity of the available test, rather than results of controlled clinical trials to determine how much IgA can be tolerated by patients possessing anti-IgA antibodies. Reported acceptable values range from <5 mg/dl at one major center to $\leq$0.05 mg/dl at another.[51,52] One study of the amounts of IgA that can be tolerated in patients at risk is presently ongoing.[52] This report suggests, however, that no generalization may be possible due to individual variations in the affinity and specificity of anti-IgA antibodies which may play a major role in determining the likelihood of a transfusion reaction. This possibility seems feasible considering the fact that some patients with anti-IgA experience transfusion reactions and others do not and,

at this time, no test is available which will distinguish between the two.

An additional fact concerning levels of IgA in IgA-deficient individuals has recently been reported. Using a very sensitive RIA procedure, $\leq$0.002 mg/dl IgA, this study found fluctuations ranging from 0.0002 mg/dl to 0.38 mg/dl IgA in some donors when testing was performed at 3 to 6 month intervals.[52] This report emphasizes the need to verify IgA deficiency with each donation.

The purpose of this study was to evaluate the RID and Ouchterlony immunodiffusion and hemagglutination-inhibition procedures for use in identifying IgA-deficient individuals in a large donor population. IgA-deficient was defined as <0.1 mg/dl as this was the limit of the HI test sensitivity in this study. Identification of such donors is necessary to create a rare donor file of IgA-deficient donors which should provide safe blood products for patients at risk of anaphylactic transfusion reactions due to a selective deficiency of IgA.

Materials and Methods

RID plates were prepared using locally available goat anti-human IgA (Children's Hospital Medical Center, Cincinnati, OH). This antibody was diluted 1:20 in a 1.5% agar solution. Ten ml of this media was pipetted into 6.5 x 8.5 cm immunodiffusion plates (Madison Company, Columbia, MD) to a depth of 3 mm and allowed to harden at room temperature. A total of 36 wells, 3 mm in diameter, were then individually punched in each plate (Figure 5). These plates were stored at 4 C. On each day of use, plates were allowed to warm at room temperature for 30 minutes. Following the addition of 10 µℓ of sample or controls to appropriate wells and incubation at room temperature for 18 to 24 hours, these plates were examined qualitatively for the presence or absence of a precipitin ring to identify potential IgA-deficient samples (Figure 5). Ouchterlony double diffusion plates were obtained commercially (Meloy Laboratories, Springfield, VA). Storage and daily preparation was identical to that used for RID plates. Following the addition of 20 µℓ of sample or anti-human IgA (Meloy Laboratories, Springfield, VA) to appropriate wells and incubation at room temperature for 18 to 24 hours, these plates were examined for the presence or absence of a precipitin line to identify potential IgA-deficient samples (Figure 6). Precipitin lines were also evaluated for evidence of identity, partial identity or nonidentity. The sensitivity of the RID and Ouchterlony plates was determined to be 10 mg/dl and 4 mg/dl

respectively using serial dilutions of a commercial human IgA standard (Sigma Chemical Company, St. Louis, MO).

Indicator cells for use in the HI and passive hemagglutination procedures were group O red blood cells coated with IgA by the metallic cation method described by Jandl and Simmons.[50] These cells were stored in Alsever's solution for at least 3 days at 4 C prior to use. Stored red cells were initially washed x 10 in 0.9% normal saline. One tenth ml of these washed red cells was added to 0.1 ml of purified IgA_1 human myeloma protein (American Red Cross Blood Services, Bethesda, MD) at a concentration of 1 mg/ml in a 12 x 75 mm test tube. One tenth ml of freshly prepared 0.1% $CrCl_3$ (Sigma Chemical Company, St. Louis, MO) was added and immediately vortexed. This mixture was incubated at room temperature for 10 minutes with frequent gentle agitation. These cells were then washed x 10 in 0.9% normal saline and resuspended to a 2% concentration for use in the HI and passive hemagglutination tests.

The HI procedure was performed as described by Vyas et al.[36] One volume of sample, either serum or plasma at a 1:10 dilution, was added to an equal volume of diluted goat anti-human IgA (Sigma Chemical Company, St. Louis, MO) in V-bottom microtiter plates (Cooke Engineering Company, Alexandria, VA) and incubated for 1 minute. Dilutions of anti-IgA were chosen that would agglutinate the indicator cells when incubated with a serum specimen containing <0.1 mg/dl IgA. Optimal dilutions in 3% bovine albumin (Miles Laboratories, Elkhart, IN) giving the required sensitivity were most often within the range of 10^{-3} to 10^{-5}. One volume of a previously prepared 2% suspension of

indicator cells was added to the serum-antibody mixture. The plates were gently agitated and incubated for 30 minutes at room temperature. Following incubation, the microtiter plates were centrifuged for 12 to 15 seconds at 1300 x g and placed on a microtiter plate holder at a 60-degree angle. The red cell patterns were examined after 30 minutes. Streaming of red cells indicated the presence of IgA in sufficient amounts to neutralize the goat anti-IgA. Agglutination, demonstrated as tight solid buttons, indicated insufficient IgA present to neutralize the anti-IgA. Samples negative for IgA at this initial 1:10 dilution were then tested undiluted by the HI procedure.

A microtiter well containing sample and indicator cells only was run concurrently with the HI procedure. Agglutination in this well indicated the presence of antibody to IgA in the sample.

IgA-deficient and low level IgA samples for use in evaluation of test methods were submitted by the following institutions - American Red Cross (ARC) Blood Services, Buffalo Region, Buffalo, NY; ARC Blood Services, Southeast Michigan Region, Detroit, MI; Minneapolis War Memorial Blood Bank, Minneapolis, MN; ARC Blood Services, Central California Region, San Jose, CA.

A total of 1,995 donor serum samples were obtained from the Hoxworth Blood Center, Cincinnati, OH. All samples were stored at 4 C and tested within 96 hours of donation.

Results

The RID, HI and passive hemagglutination procedures were evaluated with 47 serum samples submitted by several regional blood centers. These samples were initially screened for IgA using the locally prepared RID plates. Those samples giving no precipitin ring by RID were considered potentially IgA-deficient at the limit of test sensitivity, 10 mg/dl. The more sensitive, 0.1 mg/dl, HI procedure was then used to test these RID negative samples for the presence of potential low levels of IgA. Passive hemagglutination tests were performed simultaneously to determine the presence or absence of antibody to IgA. Four red cell patterns were noted with the combined HI and passive hemagglutination methods (Figure 7). Samples containing normal levels of IgA completely inhibited the anti-IgA and the indicator cells streamed. IgA-deficient specimens, however, did not inhibit the dilutions of anti-IgA and the indicator cells agglutinated, forming tight solid buttons in all antibody wells. Samples that were IgA-deficient and contained antibody to IgA additionally agglutinated the indicator cells in the control wells. The presence of sufficient anti-IgA to agglutinate the indicator cells in the control well invalidated the IgA-deficient interpretation of agglutinated cells in the antibody wells. However, the presence of class-specific anti-IgA in a sample can be taken as an indication that the individual must be IgA-deficient. The fourth cell pattern was demonstrated by samples with low levels of IgA, <10 mg/dl and

0.1 mg/dl. These samples inhibited agglutination only with the weaker dilutions of anti-IgA.

A total of 47 samples were received from other blood centers. Thirty-six of these were submitted as IgA-deficient. Twenty-one were confirmed as IgA-deficient without anti-IgA but 11 tested as IgA-deficient with anti-IgA. Three of these 36 samples were negative for IgA by RID but found to contain low levels of IgA by the HI method. One sample was positive for IgA by RID, >10 mg/dl (Table 6a).

Seven of the 47 specimens were submitted as IgA deficient with anti-IgA (Table 6b). In six, the presence of anti-IgA was confirmed by agglutination in the control wells. One specimen, however, was negative for anti-IgA.

The remaining 4 samples were submitted as containing low levels of IgA (Table 6c). Three were confirmed by the HI method as containing >0.1 mg/dl IgA. One sample was positive for IgA by RID.

All samples that gave unexpected results by RID were evaluated on double diffusion plates to rule out the possibility of anti-ruminant antibodies.

Following the evaluation tests on the submitted samples, serum samples from 995 blood donors were screened for potential IgA-deficiency by the RID method. Serum from one donor was negative for IgA. This specimen was then tested by the HI assay. HI results indicated the sample was IgA-deficient, <0.1 mg/dl. No antibody to IgA was demonstrated in the control well.

An additional 1,000 donor serum samples were screened using the Ouchterlony double diffusion plates. Sera from 3 donors gave negative reactions, no precipitin lines, at the limit of test sensitivity,

4 mg/dl IgA. All sample precipitin lines were closely examined to evaluate partial or nonidentity and none were noted. These 3 samples were then tested by HI. Two of the specimens were IgA-deficient, <.1 mg/dl, without antibody. One of the samples was IgA-deficient and demonstrated anti-IgA in the control well. All samples identified as IgA-deficient were confirmed by the American Red Cross Special Services Laboratory, Bethesda, MD.

Discussion

Evaluation of the 47 submitted samples by RID and HI gave some discrepant results. In the first group of 36 samples submitted as IgA deficient without anti-IgA, 21 were confirmed in this study. However, 11 tested as containing anti-IgA and 4 others were found to contain IgA. All of the discrepant samples in this group were submitted by the same blood center. Personal communication with this center proved inconclusive in clarifying these results due to incomplete records. This center, however, did suggest that the original testing was most likely in error.

Of the 7 specimens submitted as IgA deficient and positive for anti-IgA, one sample failed to agglutinate the IgA-coated indicator cells. This sample was reported as having an antibody titer of 40 which was the lowest of any of the anti-IgA antibodies submitted. The amount of IgA coating these indicator cells, which affects the sensitivity of the passive hemagglutination test, might provide an explanation for this discrepancy. In addition, the anti-IgA in this sample may have been affected by repeated freezing or thawing.

Of the remaining 4 samples that were submitted as containing low concentrations of IgA, 3 were confirmed by the HI method as containing approximately 0.1 to 10.0 mg/dl IgA. One specimen tested positive by the less sensitive, 10 mg/dl, RID procedure. The possibility that this specimen and any of the others giving unexpected reactions in RID might contain an anti-ruminant antibody that reacted with the goat anti-IgA used in the RID plates was investigated. These specimens

were tested in conjunction with normal donor specimens by the Ouchterlony double diffusion method. Precipitin lines indicating identity were demonstrated. Therefore, the precipitin rings generated by these samples by RID was due to the presence of IgA and not by anti-ruminant antibodies.

Although the evaluation of the RID and HI procedures with the 47 submitted samples was not in total agreement, no specimen containing IgA was incorrectly identified as IgA-deficient. The possibility of missing the presence of anti-IgA, as occurred in this evaluation with the low titered anti-IgA, would be of no clinical consequence to a patient. This is supported by reports that blood with anti-IgA antibodies administered to normal patients lead to no untoward effects, apparently because of dilution of antibody in the recipient's circulation.[16,17]

Based on this preliminary evaluation, the combined RID and HI procedures appeared to be an acceptably accurate screening methodology for the identification of IgA-deficient donors. Therefore, the evaluation was continued by screening 995 donor samples for IgA-deficiency. Serum from 1 donor was found to be potentially negative for IgA by RID and confirmed as IgA-deficient by HI. These results indicated an apparent incidence rate of 1/1000 which was consistent with previously reported rates in normal donor populations of 1/300 to 1/3000.

The possibility was considered that this incidence rate was inaccurate due to the reported presence of anti-ruminant antibodies in some IgA-deficient individuals. Although the reported rates of

anti-ruminant antibodies range from 24% to 75%, these studies consisted for the most part of patients with varied clinical problems (Table 5). To investigate this factor further in a normal donor population, an additional 1,000 donor specimens were screened for potential IgA deficiency using the Ouchterlony double diffusion method with goat anti-human IgA. Using this procedure, IgA-deficient serum would give no precipitin lines. However, IgA-deficient samples with anti-ruminant antibody would give lines of nonidentity. Three potential IgA-deficient donors were identified using this double diffusion technique. All of the samples were confirmed as IgA-deficient. One specimen contained anti-IgA. Precipitin lines were closely examined for all samples tested and all demonstrated identity. Therefore, no anti-ruminant antibodies demonstrated in this sampling. Based on this sampling the incidence rate was 1/333, which was not a statistically different proportion ($p>.20$) from the 1/1000 incidence rate found when the RID technique was used.

A larger sample size comparing the two procedures or more data concerning the frequency of anti-ruminant antibodies in IgA-deficient donors is required to determine the more efficient screening method. If the incidence of anti-ruminant antibodies in this population is significant, then the double diffusion method would be superior to RID as used in this study in identifying IgA-deficient individuals. If the incidence rate is not significant, then either method would be equally efficient in identifying IgA-deficient individuals. The use of rabbit anti-human IgA would dissuade this concern, since anti-ruminant antibodies do not react with antisera from this source.

However, commercially available rabbit antisera is more expensive and the cost per test would be higher. This cost is a major consideration since many thousands of donors would need to be tested to create and maintain an extensive rare donor file of IgA-deficient individuals.

The use of the HI procedure as a sensitive technique for the detection of low concentrations of IgA has been well documented. The procedure performed well in this study as it was consistently reliable and reproducible. The critical factor affecting the use of HI was the coating of red blood cells with the IgA immunoglobulin as the extent of binding influences the sensitivity of the test. The red cells used in this study required storage at 4 C for 3 days in Alsever's solution before they could be optimally coated. Coating prior to this time produced indicator cells that gave agglutination with anti-IgA at a dilution of 10^{-4}. Coating at 3 days or soon thereafter resulted in cells that agglutinated with the antisera at a dilution of 10^{-5} and, therefore, were more sensitive indicators of the presence of IgA in donor serum. Personal communication with other centers using HI indicate that red cells from different individuals are highly variable in their ability to bind with IgA, as some red cells coat optimally on the day they are drawn and others require storage for as long as 10 days. Indicator cells in this study could be stored and used for approximately one week before their performance deteriorated.

The use of indicator cells in the passive-hemagglutination procedure to detect the presence of antibody to IgA is limited. The

IgA used to prepare the indicator cells in this study was IgA_1 immunoglobulin. IgA_2, $IgA_2m(1)$ and $IgA_2m(2)$ proteins were unavailable due to their rarity. Indicator cells coated with IgA_1 will react only with anti-IgA that is class-specific. Therefore other anti-IgA antibodies would go undetected. However, as previously noted, the presence of antibody to IgA in an IgA-deficient donor should be of no clinical consequence to the recipient. The purpose of these cells as a control is, therefore, required only to indicate that there is no excess of anti-IgA contributed by the donor serum to influence the reactions in the antibody wells. This use of a control well may be of no consequence in the identification of IgA-deficient donors since the presence of anti-IgA that would give false positive agglutination in the antibody wells is generally considered to indicate IgA-deficiency.

The RID as used in this study was an extremely cost effective method of screening a large donor population. Using locally available goat anti-human IgA the material cost amounted to only $0.02/sample tested. The Ouchterlony double diffusion method using commercially purchased plates and antisera was $0.56/sample tested. If Ouchterlony plates were locally prepared and locally available antisera were used this material cost would be approximately the same as the RID plates. In addition to this low cost/test, the immunodiffusion methods require minimal technologist time to plate and read. The cost of the HI is negligible since many samples may be batched and tested.

Conclusion

IgA-deficient blood products are required to avoid potentially fatal anaphylactic transfusion reactions in patients who are IgA-deficient and have formed anti-IgA antibodies. Red blood cells for these patients may be provided through autologous transfusion or extensive washing of banked or frozen units. However, when other blood components such as fresh frozen plasma or cryoprecipitates are needed, the use of a rare donor file of IgA-deficient individuals is required.

The frequency of IgA deficiency has been reported to vary between 1/300 to 1/3000 in normal, healthy donor populations. The large number of samples to be tested to create a donor file of IgA-deficient individuals requires a sensitive, accurate and cost effective screening methodology.

This study was performed to evaluate qualitative immunodiffusion and quantitative hemagglutination-inhibition (HI) procedures for use in the identification of IgA-deficient individuals. The sensitivity and accuracy of radial immunodiffusion and double diffusion and HI were evaluated with previously identified IgA-deficient samples.

The RID method using locally prepared plates was both accurate and cost-effective. However, there remains some doubt as to the possibility of some false positive reactions with these plates due to the reported incidence of anti-ruminant antibodies in some IgA-deficient patients. The Ouchterlony double diffusion method appears to be the more accurate immunodiffusion technique in this

instance since precipitation can be evaluated for identity and nonidentity. The HI procedure was found to be sensitive, accurate and cost-effective. The preparation of indicator cells sufficiently coated with IgA proved to be variable. However, once ideal conditions are determined for each cell source, the results are simple to reproduce.

Initial screening of approximately 2,000 donors gave an incidence rate of 1/500 IgA-deficient individuals in this small sampling which is consistent with previously reported rates. As this combined methodology is implemented and more donors are tested, the sample size will increase and result in a more statistically significant frequency.

As a result of this evaluation, implementation of the combined immunodiffusion and HI procedures is anticipated at the Hoxworth Blood Center, Cincinnati, OH, to create a rare donor file of IgA-deficient individuals who should provide safe blood products for patients at risk.

Table 1 - Incidence Rates of IgA Deficiency

Investigators	Number	Population Type	Incidence (%)	Deficiency Criteria
Bachmann (12)	6,995	district survey	0.14	<0.2% of normal
Hobbs (13)	11,000	hospital oriented	0.06	<2 mg/dl
			0.17	<5 mg/dl
			0.22	<10% of normal
Holt et al (14)	29,745	blood donors	0.11	<0.05 mg/dl
Huntley/Stephenson (15)	5,764	hospital oriented	0.21	<6 mg/dl
Vyas et al (16)	73,569	blood donors	0.11	Not reported
	5,990	id & relative	0.40	<6 mg/dl
Clark et al (17)	6,240	blood donors	0.30	<.1 mg/dl
Johannson et al (18)	3,957	blood donors	0.23	<.1 mg/dl
	1,401	pregnant women	0.21	
Ropars et al (19)	108,000	blood donors	0.04	<2 mg/dl
Buckley/Dees (20)	2,486	hospital oriented	0.97	<10 mg/dl
Cassidy et al (21)	3,024	community survey	0.03	<2 mg/dl
Koistinen (22)	64,588	blood donors	0.25	<1 mg/dl

Table 1 - Incidence Rates of IgA Deficiency (Continued)

Investigators	Number	Population Type	Incidence (%)	Deficiency Criteria
Hayashi et al (23)	9,000	hospital oriented	0.31	<1 mg/dl
Natvig et al (24)	5,020	blood donors	0.08	<2 mg/dl
Frommel et al (25)	15,200	blood donors	0.03	<1 mg/dl

Table 2 - Anti-IgA in Normal Donors

Investigators	Number	Anti-IgA	
		Class-specific	Limited-specifity
Holt et al (14)	34	18%	12%
Vyas et al (16)	83	24%	0
Ropars et al (19)	77	5%	0
Vyas/Fudenberg (28)	255	0%	2%
Laschinger et al (32)	429	17%	1%

Table 3 - Anti-IgA in IgA Deficient Patients

Investigators	Number	Anti-IgA	
		Class-specific	Limited-specifity
Nadorp et al (31)	16	44%	6%
Laschinger et al (32)	21	57%	4%

Table 4 - Incidence of Anti-IgA in Various Clinical Conditions

Investigator	Clinical Condition	Number	Anti-IgA	
			Class-specific	Limited-specificity
Vyas/Fudenberg (28)	kidney transplants	18	0	5%
Vyas/Fudenberg (28)	open-heart surgery	55	0	16%
Vyas/Fudenberg (28)	anaphylactoid trans rx	29	10%	76%
Vyas/Fudenberg (28)	ataxia telangiectasia	32	44%	0%
Pai et al (33)	postpartum women	192	0	24%
Vyas et al (34)	postpartum women	not reported	0	15%
Vyas et al (34)	multiply trans pts	not reported	0	21%

Table 5 - Incidence of Anti-ruminant Antibodies

in IgA-Deficient Individuals

Investigators	Number	% ruminant antibodies	population studied
Buckley/Dees (38)	24	75%	histories of recurrent infection
Amman/Hong (20)	30	40%	patients
Amman/Hong (40)	15	60%	autoimmune disease
Tomasi/Katz (41)	17	24%	various clinical disorders
Leikola/Uyas (42)	45	29%	referred samples

Table 6a - 36 Samples Submitted as IgA-Deficient

- 21 tested as IgA deficient

- 11 tested as IgA deficient with anti-IgA

- 3 tested as low level IgA

- 1 tested as high level IgA

Table 6b - 7 Samples Submitted as Positive for Anti-IgA

- 6 samples were positive for anti-IgA

- 1 sample was negative for anti-IgA

Table 6c - 4 Samples Submitted as Low Level IgA

- 3 tested as low level IgA

- 1 tested as high level IgA

Figure 1 - Interpretation of Precipitin Lines on Ouchterlony Plates

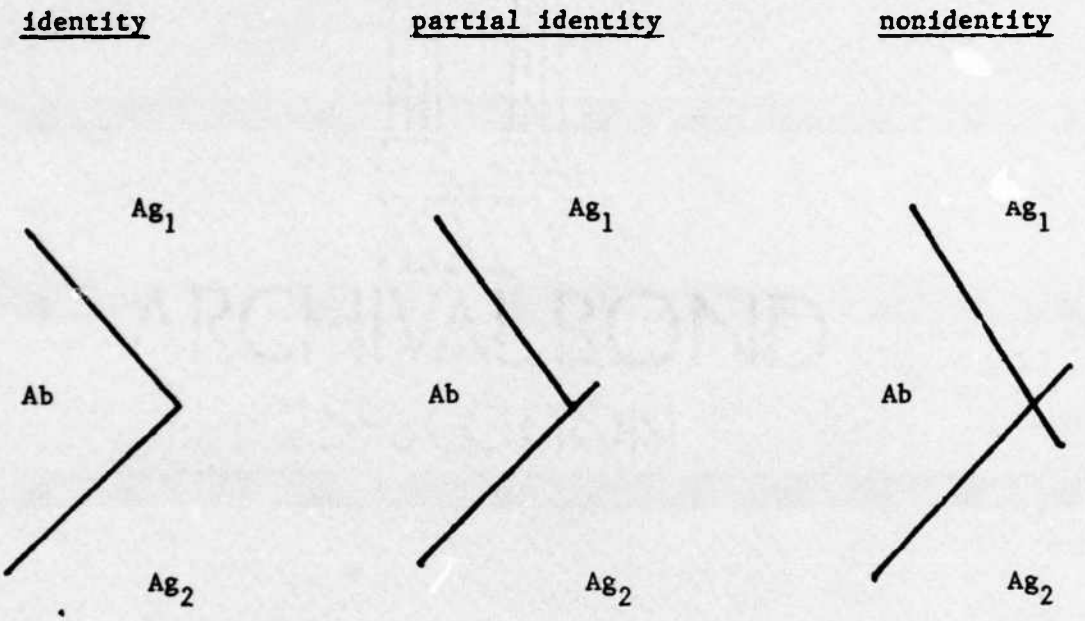


Figure 2 - Metallic Cation (Cr^{+++}) Binding to Red Cell Membrane

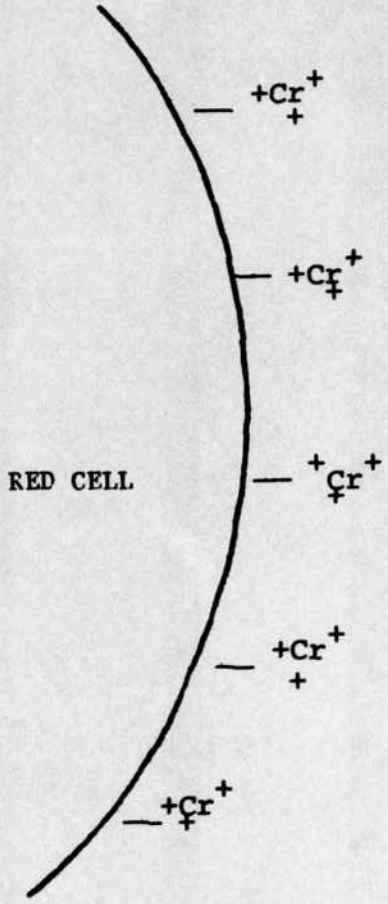


Figure 3 - Binding of Protein (IgA) to Red Cell Membrane

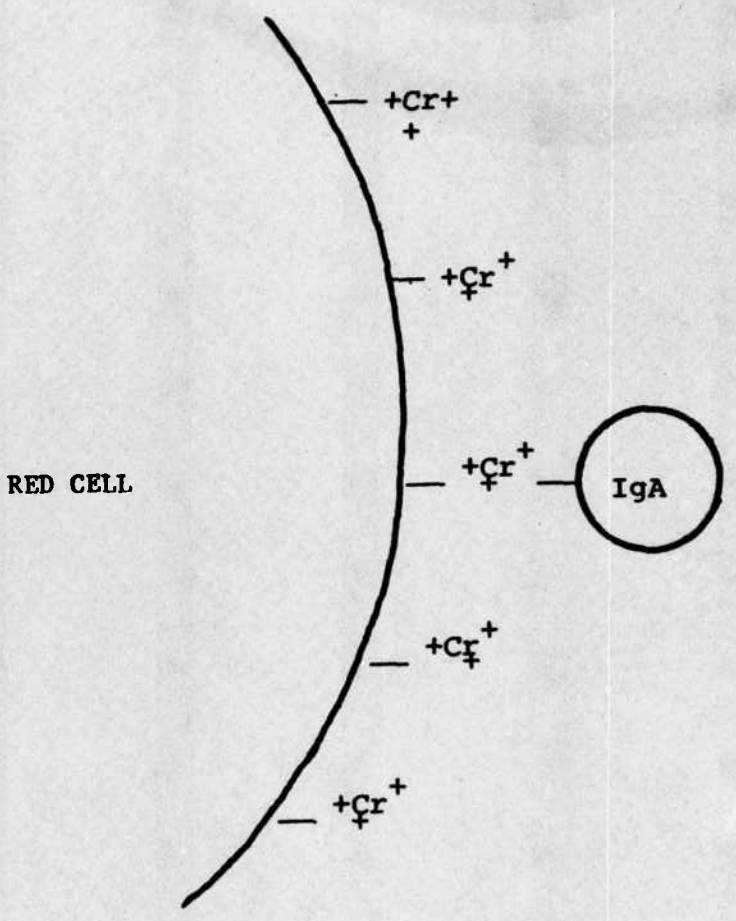


Figure 4 - Agglutination of Coated (IgA) Red Cells by Specific Antisera (Anti-IgA)

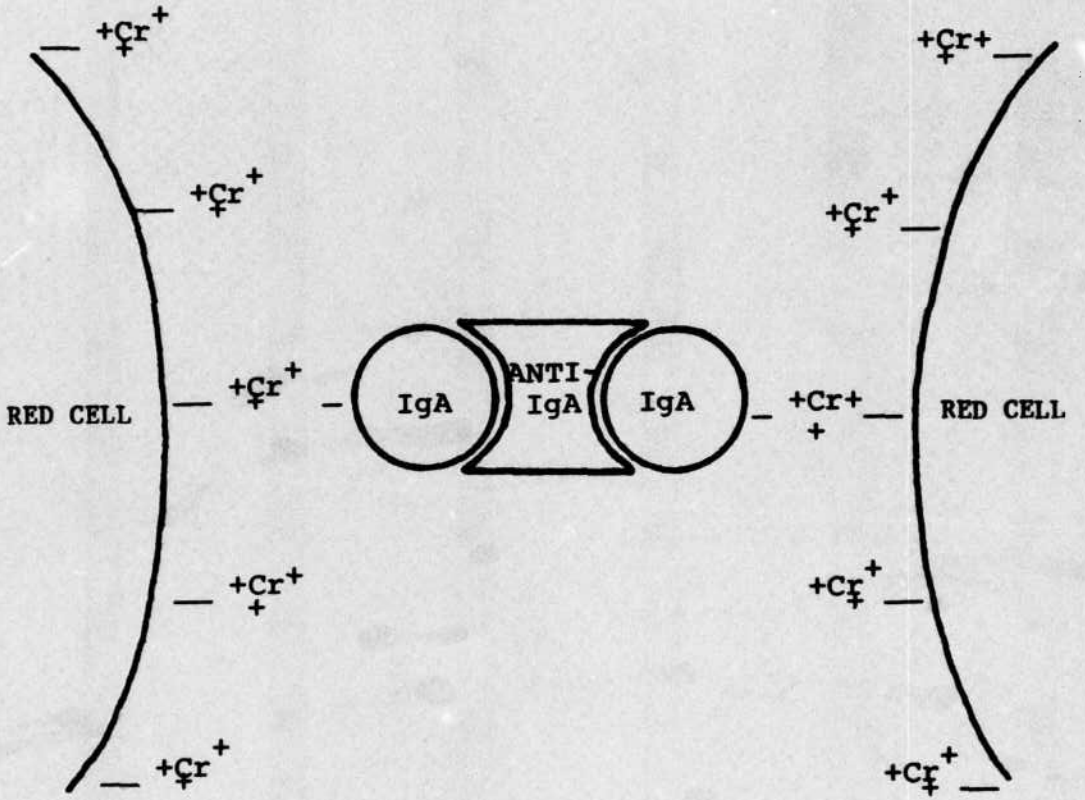


Figure 5 - Evaluation of RID Plates

for IgA-Deficient Samples

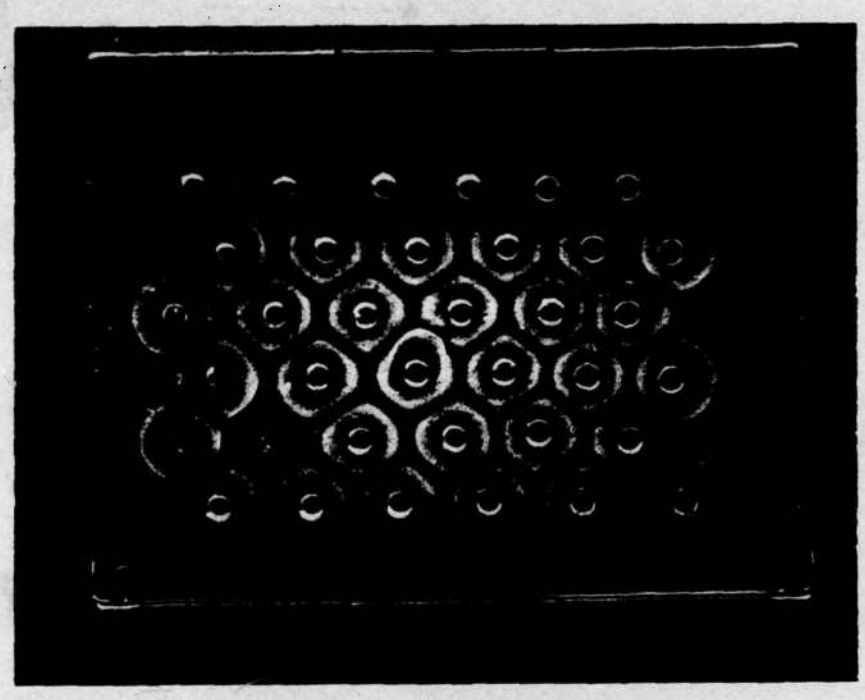


Figure 6 - Evaluation of Ouchterlony Plates

IgA-deficient sample lines of identity

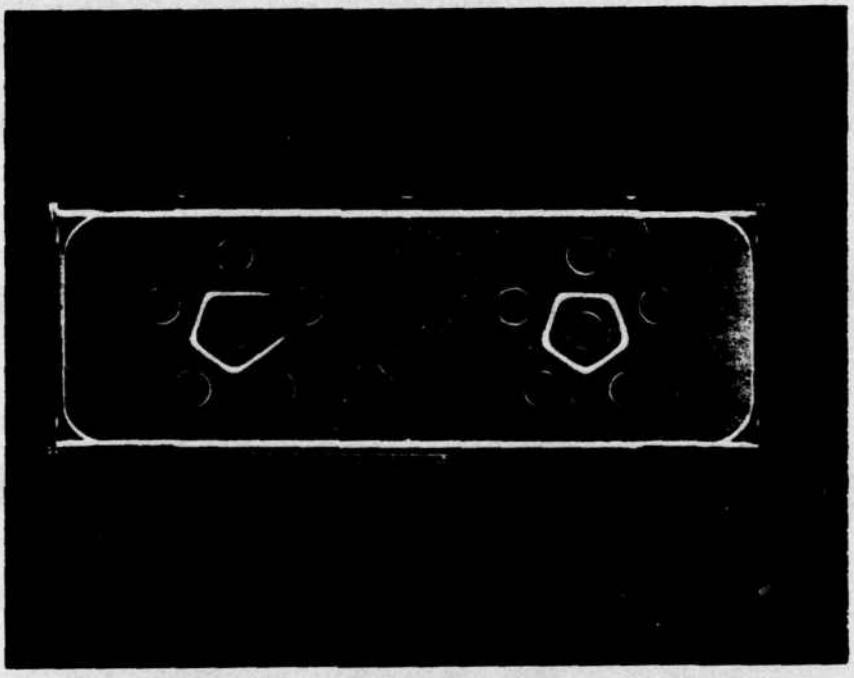
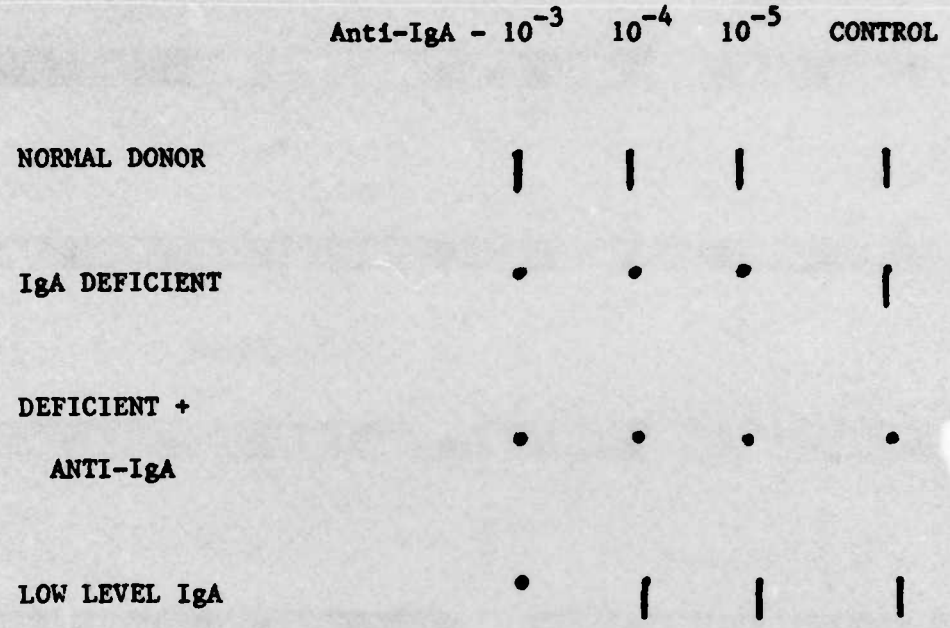


Figure 7 - Patterns Seen in Hemagglutination-Inhibition



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