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Potency of Anti Human Globulin using cryopreserved C3d sensitized Cells over period of 10 days

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KEYWORDS

Anti Human Globulin, Cryopreserved C3d sensitized red blood cells

A B S T R A C T

The purpose of this study is to check the viability and recovery of Cryopreserved C3d sensitized red blood cells which is stored at different temperatures (- 70°C&-20°C) over a period of 10 days, and also checked the potency of polyspecific Anti Human Globulin (AHG) containing anti C3d using these cryopreserved C3d sensitized cells. To over come the problem of collection /transportation /and potency testing on the same day the performed study showed that freshly collected "O" positive red blood cells can be C3d sensitized and cryopreserved on the same day and stored at different temperatures (-20°C and -70°C) for a period of 10 days and can be used for the Quality control evaluation of Anti-C3d present in Polyspecific Anti-Human globulin (AHG) instead of freshly collected and sensitized C3d cells.

Introduction

Blood: Blood is fluid connective tissue and forms 7-8% of the body weight and comprises different components: Red blood cell (RBCs), White blood cell (WBCs), platelets and plasma. The RBCs make up 40-50% of total blood volume, whereas the WBCs make up only 1% of the blood volume. Blood plasma forms 55% of the blood's volume. (Greenwalt et al). The surface of the red cell carries a negative charge and thus repels from other red cells.

An important component of the red cell membrane is the blood group antigen which are capable of stimulating the production of specific antibody when introduced into a foreign circulation therefore they were known as Blood group antigen .These antigens are either proteins or sugars and adhere themselves to various components in the RBC membrane. There are different types of RBCs antigens however the most important types are the ABO and Rhesus types.

The term complement refers to a complex set of a distinct serum protein component that react with one another sequentially to form products with potent biological effect, including immune adherence, phagocytosis, and cell lysis some of these protein have specific enzymatic activity where as other are inhibitors that regulate the action of certain complement components, complement is important to the immunohematologist in that it can cause haemolysis of sensitized cell in vivo or in vitro when certain" complement binding" antibodies are involved(Bryant et al) . Three biochemical pathways activate the complement system: The Classical complement pathway, The Alternative complement pathway, and The Mannose-binding Lectin pathway. In all three pathways, a C3-convertase cleaves and activates component C3, creating C3a and C3b and causing a cascade of further cleavage and activation events. C3b binds to the surface of pathogens leading to greater internalization by phagocytic cells by opsonization.Where as C5a is an important chemo tactic protein helping recruit inflammatory cell. Both C3a and C5a have anaphylatoxin activity directly triggering deregulation of mast cells as well as increasing vascular permeability and smooth muscle contraction. C5b initiates the membrane attack pathway, which results in the membrane attack complex (MAC), consisting of C5b, C6, C7, C8, and polymeric C9. MAC is the cytolytic end product of the complement cascade and it forms a transmembrane channel, which causes osmotic lysis of the target cell.These all three pathwaysculminate in the formation of the convertases, which in turn generate the major effectors of the complement system and give rise to C3d complement. (Travers p, walport.M et al.).

C3d complement: It is 302-amino acid fragment in the alpha chain(672-1663)of C3b.It is generated when C3b is inactivated(iC3b)and its alpha chain is cleaved by complement factor into C3c and C3dg(955-1303)in the presence of complement factor H.Serum proteases further degrade the C3dg into C3d(1002-1303)and C3g(Mollison et al.).This complement component after sensitization is checked with Anti-human globulin test. (Bryant et al.)

Anti human globulin test: In 1945 Coomb's, Mourant, and Race described procedures for detecting attachment of antibodies that did not produce agglutination. This was first used to demonstrate antibody in serum, but later the same principle was used to demonstrate in-vivo coating of red blood cells with antibody or complement components. This test uses antibody to human globulins and is known as the Anti-Human globulin test. As used in immunohematology, AHG testing generates visible agglutination of sensitized red cells. (AABB technical manual 12th ed.).

Cryopreservation: Cryopreservation is the method that permits low temperature maintenance of diversity of cell. Cryos means cold and derives from the Greek language. Cryopreservation is cold storage for the purpose of preservation.

One of the most important early workers on the theory of cryopreservation was James Lovelock of Gaia theory fame. Dr. Lovelock's work suggested that damage to red blood cells during freezing was due to osmotic stresses. Lovelock in early 1950s had also suggested that increasing salt concentrations in a cell as it dehydrates to lose water to the external ice might cause damages to the cell. A controlled rate cooling process, allowing biological samples to equilibrate to optimal physical parameters osmotically in a cryoprotectant (a form of anti-freeze) before cooling in a

predetermined, controlled way proved necessary. The ability of cryoprotectants, in the early cases glycerol, to protect cells from freezing injury was discovered accidentally.(Lenny brioxy) .Therefore Glycerol is widely used as a cryoprotectant in case of red blood cell preservation due to its less toxicity, less penetrating power into RBCs membrane, requires less reagents for thawing of RBCs. And has less freezing power (Lenny brioxy).

Sensitization is the process in which there is specific binding of antibody to its antigenic receptor on Red blood cells without agglutination or lysis. During Previous studies it has been established that potency testing of anti-C3d Component of Polyspecific AHG requires freshly complement coated cells (sensitized) but the collection, sensitization and potency testing takes 5-6 hours which is not possible on same day. To overcome this problem the study was designed in which freshly sensitized cells were cryopreserved and stored at different temperature -70°C and -20°C over a period of 10 days and checked their viability and potency of Polyspecific AHG containing Anti-C3d using these cryopreserved cells.

Materials and Methods

Washed Red Blood Cells (O+ve) were Sensitized with C3d using solution of buffered sucrose solution(pH5.1), isotonic saline Magnesium chloride solution (0.4M),0.1%trypsin in sequence manner and then incubate at 37°C for 30 minutes . The C3d sensitized cells were cryopreservedusing glycerol,at different temperature(-70°C&-20°C) .Before cryopreservation we check their sensitivity with polyspecific AHG containing anti C3d.

Deglycerolization:Washed cryopreserved C3d sensitized cells with 12%NaCl, 1.6 % NaCl, 0.9% NaCl and isotonic saline till clear the supernatant is obtained. Viability and Potency of C3d sensitized cells were tested using polyspecific Anti human globulin (AHG) reagent

Results and Discussion

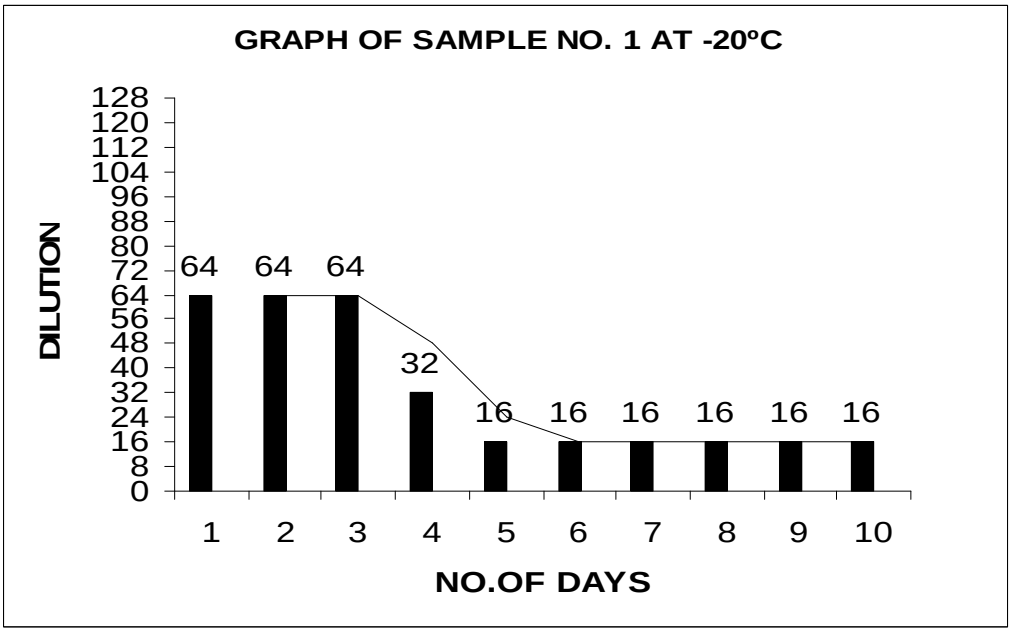
O+ve blood group collected from IRCS(Indian red cross society)for c3d sensitization to check their viability/potency of polyspecific AHG containing anti C3d at -20º &-70ºc temperature and record the results of each day over a period of 10 days.

Table.1 Shows the details of blood samples

No. of samples.	Date of collection	Date of sensitization	Date of cryopreservation	Percentage of recovery after Deglycerolization At(-70°C&-20°C)
1	27 April10	27 April 10	27 April 10	83%
2	4 May 10	4 May 10	4 May 10	83%
3	6 May 10	6 May 10	6 May 10	83%
4	14 May 10	14 May 10	14 May 10	83%
5	2 June 10	2 June 10	2 June 10	83%

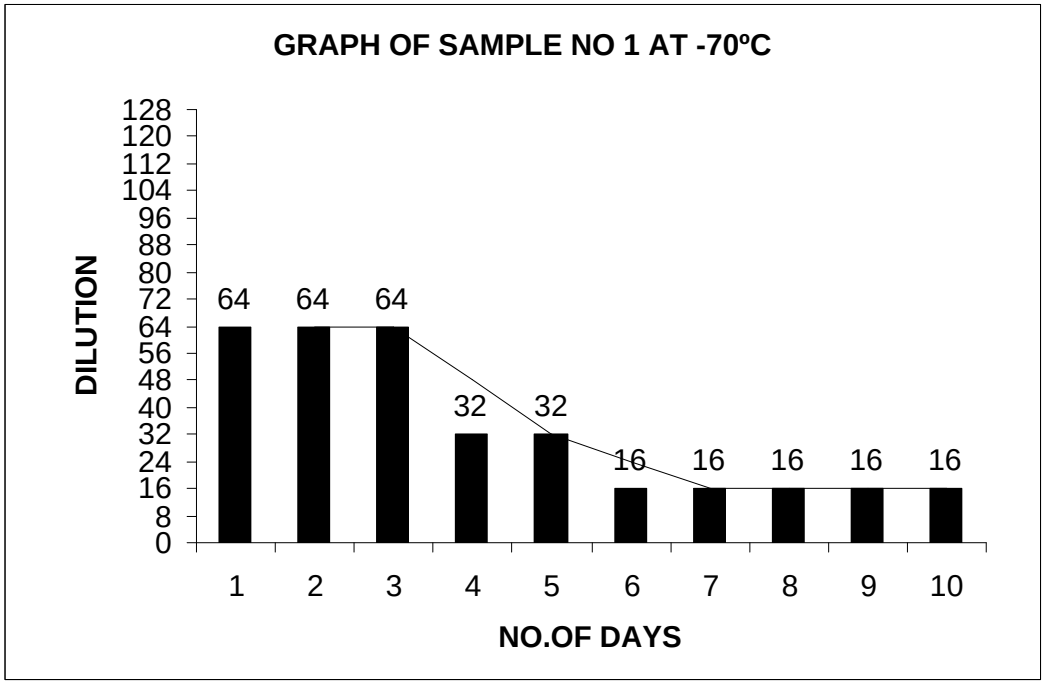
Sample no.1at -20ºC :

Graph 1A shows the potency of polyspecific AHG containing anti-C3d using cryopreserved C3d sensitized cells which were kept over period of 10 days.



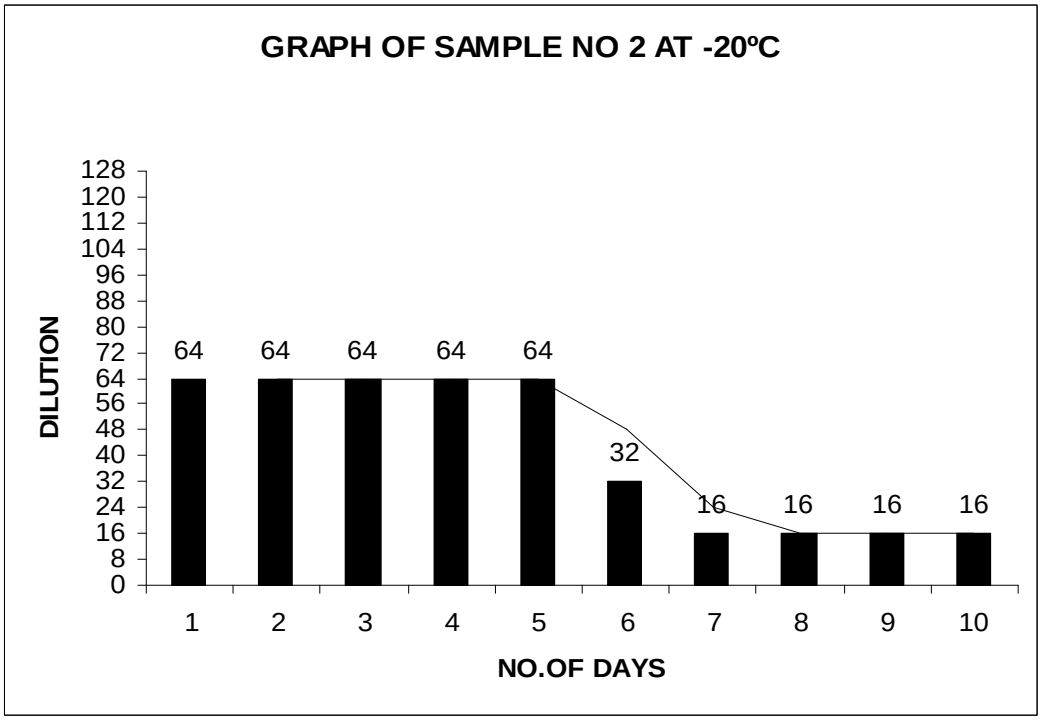
Sample no.1 at -70ºC

Graph 1Bshows the potency of Polyspecific AHG containing anti-C3d using cryopreserved cells which were kept at -70ºc over period of 10 days.



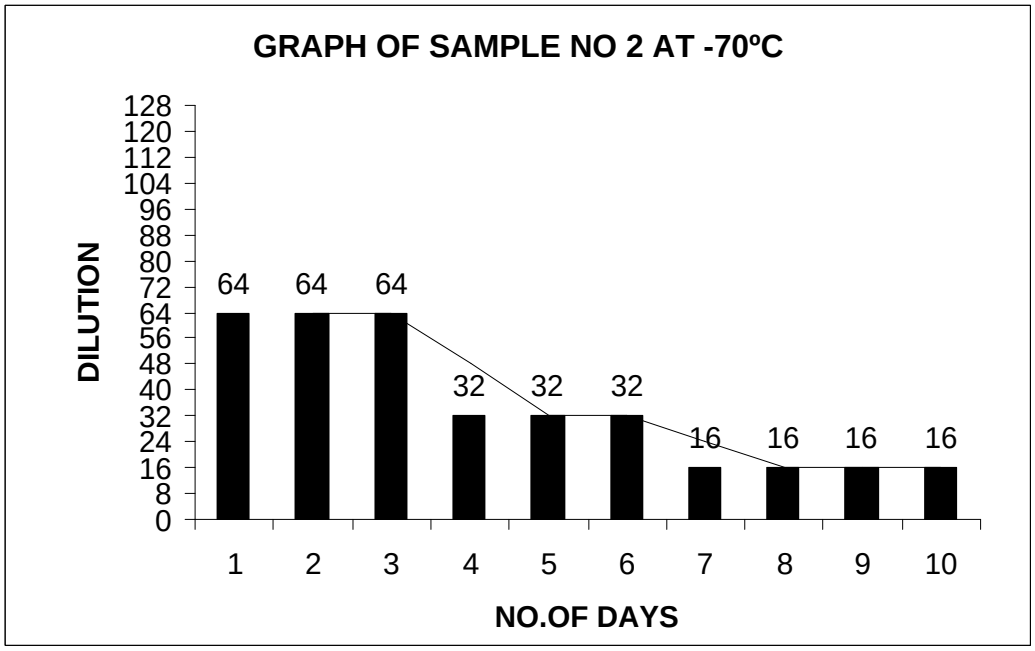
Sampleno.2 at-20^{o}C

Graph 2Ashows the potency of polyspecific AHG containing Anti-C3d using cryopreserved C3d sensitized Cells which were kept at -20^{o}C over period of 10 days.



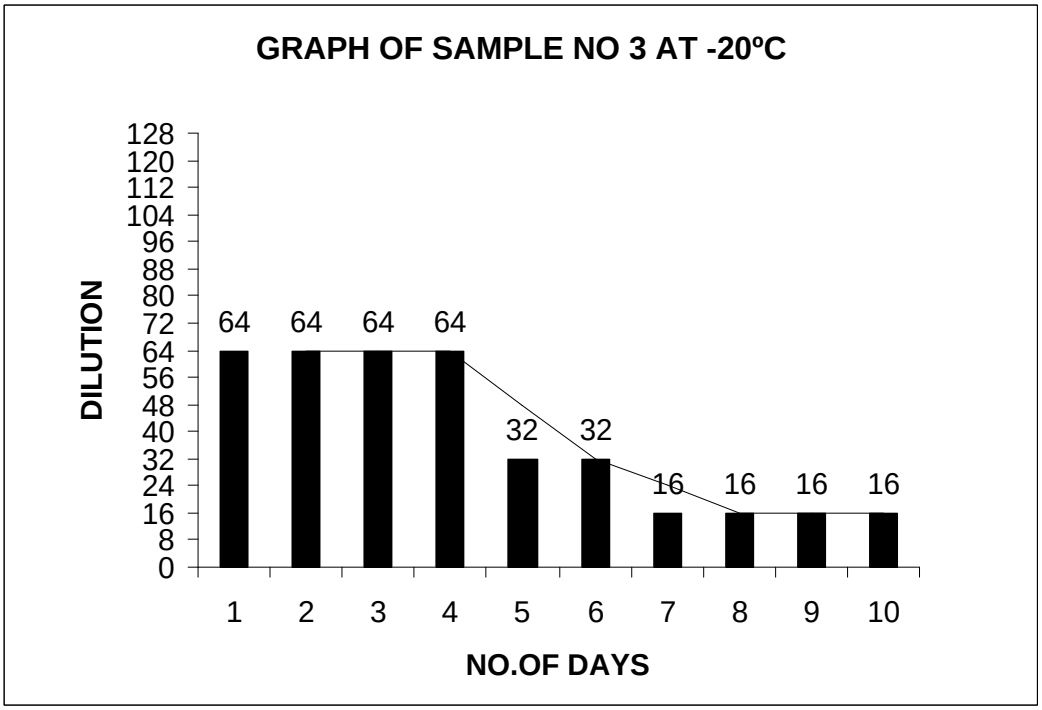
Sample no.2 at -70^{o}C

Graph 2B shows the potency of polyspecific AHG containing anti-C3d using cryopreserved C3d sensitized Cellswhich were kept at -70^{o}C over period of 10 days.



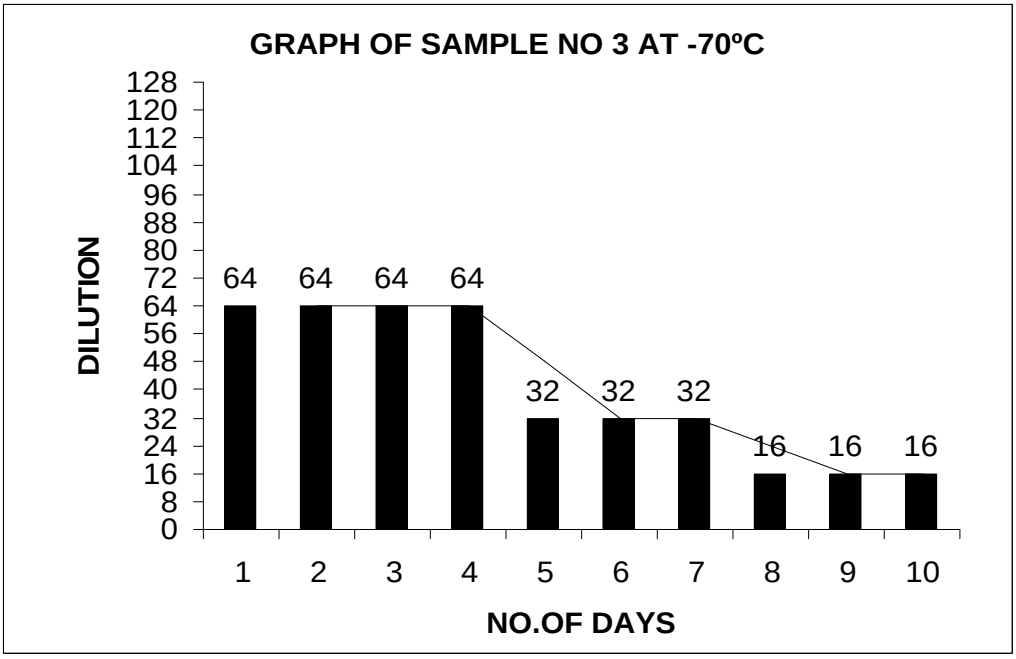
Sample no.3 at -20°C

Graph 3A shows the potency of polyspecific AHG containing anti-C3d using cryopreserved C3d sensitized Cells which were kept at -20ºC over period of 10 days.



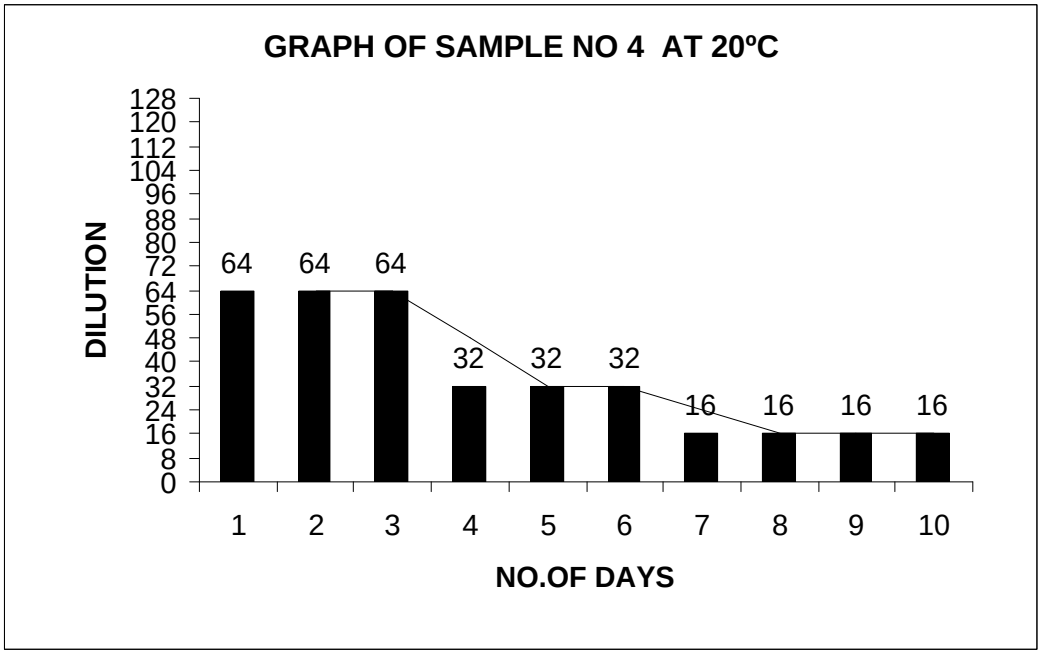
Sample no.3 at -70°C

Graph 3B shows the potency of Polyspecific AHG containing anti-C3d using cryopreserved C3d sensitized Cells which were kept at -70ºC over period of 10 days.



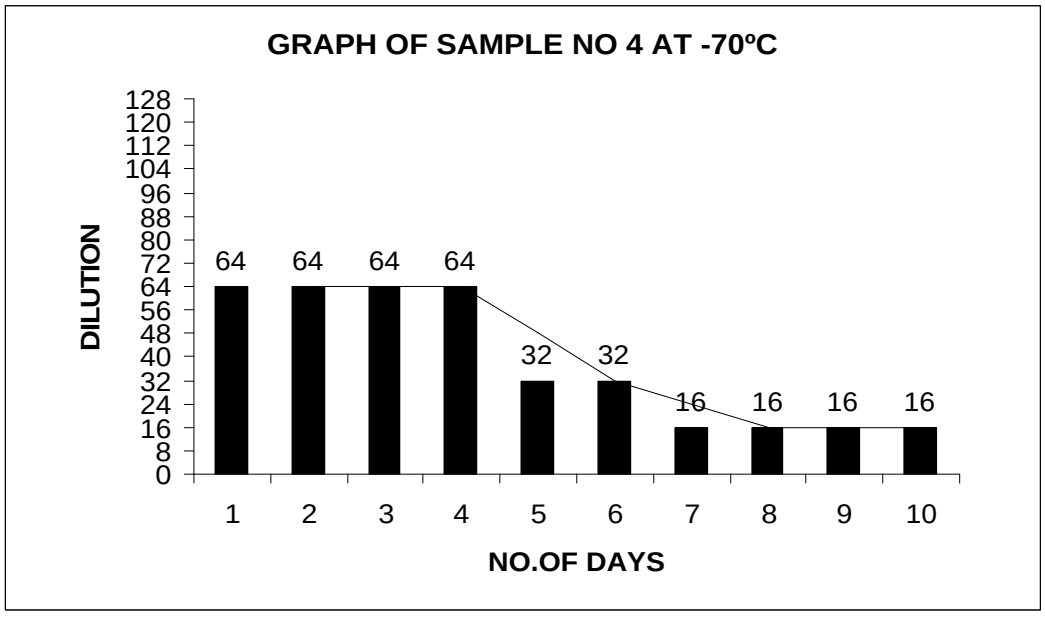
Sample no.4 at -20°C

Graph 4A shows the potency of polyspecific AHG containing anti-C3d using cryopreserved C3d sensitized Cells which were kept at -20ºC over period of 10 days.



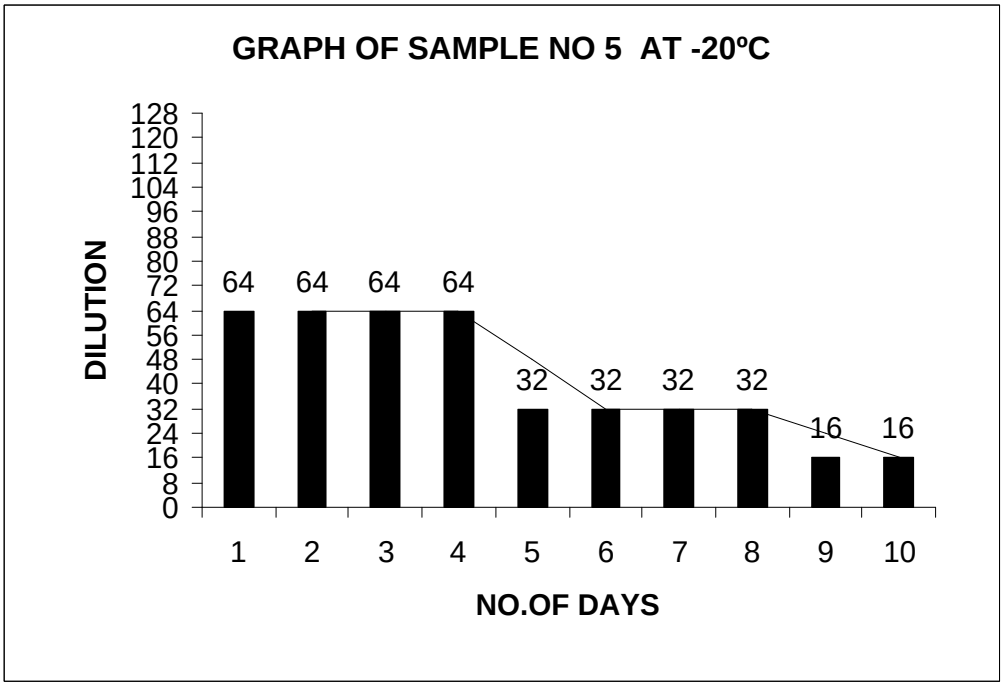
Sample no.4 at -70°C

Graph 4B shows the potency of Polyspecific AHG containing Anti-C3d using C3d sensitized Cells which were kept at -70ºC over period of 10 days.



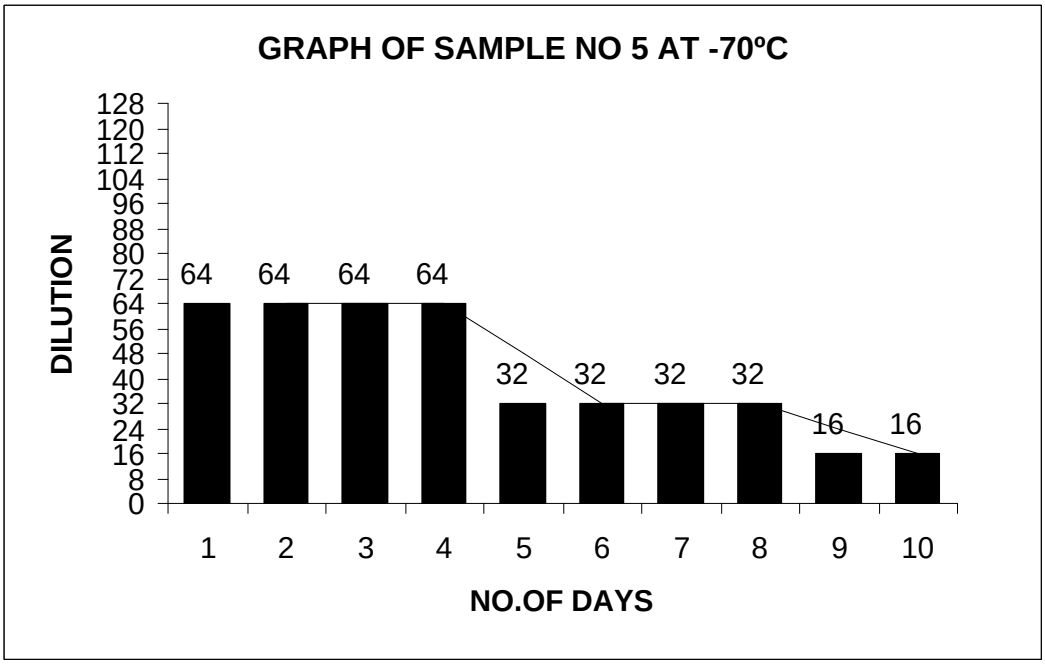
Sample no. 5 at -20ºC

Graph 5Ashows the potency of polyspecific AHG containing Anti-C3d using cryopreserved C3d sensitized Cells which were kept at -20ºC over period of 10 days.

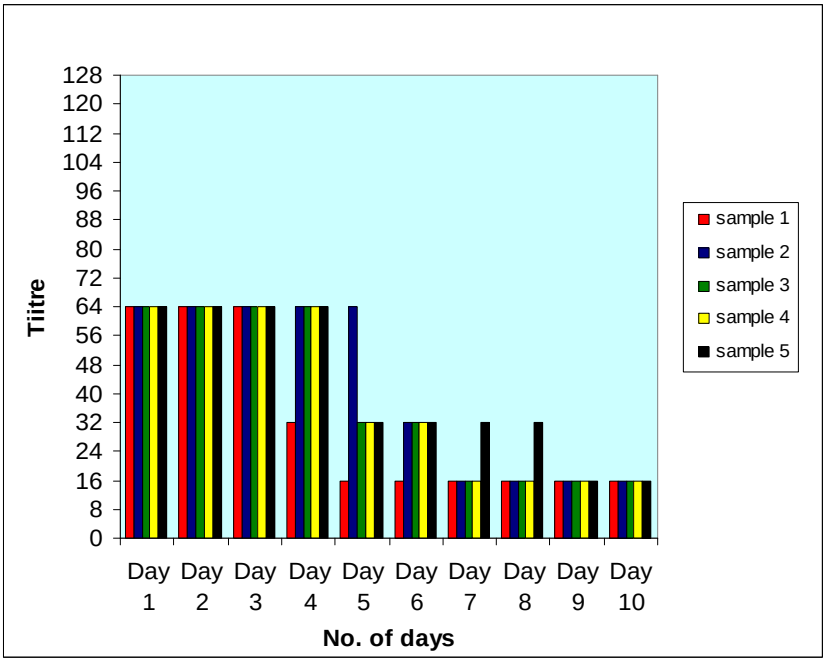


Sample no.5 at -70ºC

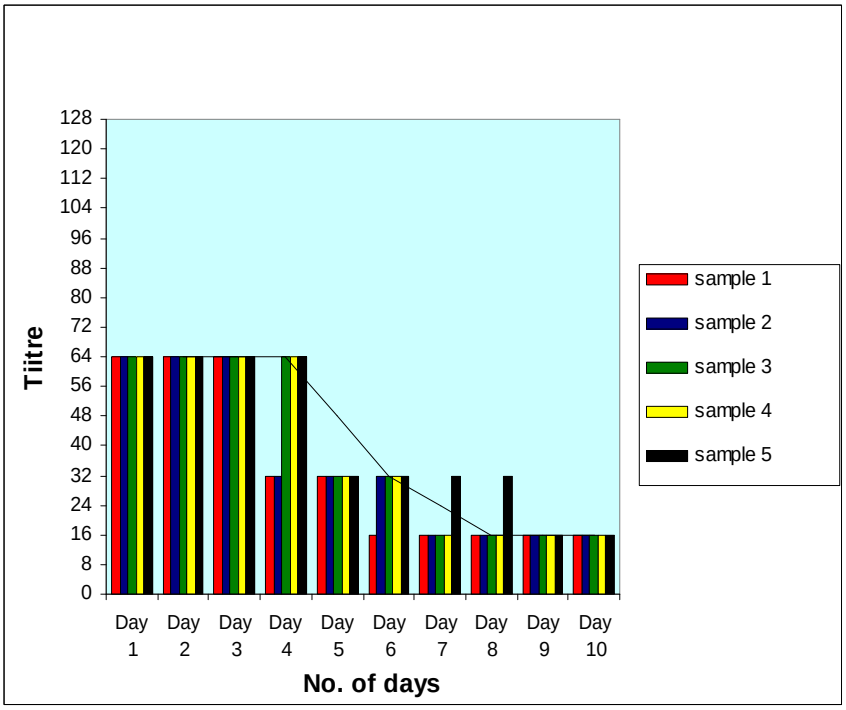
Graph5B shows the potency of polyspecific AHG containing Anti C3d using cryopreserved C3d sensitized Cells which were kept at -70ºC over period of 10 days.



Compiled graph of 5 samples stored at -70ºc to check the viability and potency of Polyspecific AHG containing anti-C3d over a period of 10 days.



Compiled graph of 5 samples stored at -20ºc to check the viability and potency of poyspecific AHG containing anti-C3d over a period of 10 days.



In this study we checked the viability of cryopreserved C3d sensitized cells stored at different temperature (-20°C and -70°C) over a period of 10 days and used the same cells to check the potency of anti-C3d present in polyspecific AHG. As Per the established standard procedure, It is recommended to use freshly collected and sensitized C3d cells to check the potency of anti C3d. For this purpose freshly collected O positive cells are sensitized and are used for potency testing of anti C3d on the same day which takes about 6-7 hours .To over come this problem of Collection/sensitization /and potency testing on the same day A study was designed in which the freshly collected O positive cells were C3d sensitized and were cryopreserved at different temperature (-20°C and -70°C) on the same day. These cell were then deglycerolized and checked for their viability and recovery. Under this study 5 different O positive Red blood samples were collected on different days from I.R.C.S (Indian Red Cross Society) New Delhi. All the five samples were sensitized and cryopreserved on the day of collection and 20 aliquots of each sample were prepared out of which 10 were preserved at -70°C and other at -20°C for a period of 10 days. On each day fresh aliquot were taken out from (-20°C and -70°C) and cells were thawed and deglycerolized to check the recovery of the sample. It was observed that all the 5 samples stored at (-20°C and -70°C) gave a recovery of 83% for a period of 10 days. Further it was found that 5 samples stored at -70°C gave a titer of 1:64 for first 4 days which is equal to the titer observed on the day of collection whereas the titer decreased by 1 dilution 1:32 on 5^{th},6^{th} and 7^{th} day and further titer reduce to 1:16 on 8^{th},9^{th},10 day and the sample stored at-20°C gave a titer of 1:64 first 3 days which is equal to the titer observed on day of collection where as the titer decreased by 1 dilution i.e,1:32 on 4^{th},5^{th},6^{th},7^{th} day and further titer reduce to 1:16 on 8^{th},9^{th} 10^{th} day. After deglycerolization the recovery of cell for the samples stored at (-20°C and-70°C) was constant 83%.There was a gradual decrease in the titer of anti C3d present in polyspecific AHG which may be attributed to loss of activity of C3d sensitized cell upon storage at both temperature (-20°C and -70°C).As per international acceptance criteria the potency of anti C3d component of Polyspecific AHG should be 1:4.Hence this study shows that in case of non availability of fresh cells the user can cryopreserved freshly sensitized cells at (-20°C and -70°C) for a period of 10 days.

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