

Blood Transfusion and Blood Components



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Blood Transfusion and Blood Components

by Dr C. Th. Smit Sibinga



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Foreword

The development of blood transfusion services and the availability and safety of blood, blood components and blood products in all countries of the Eastern Mediterranean Region has always been an important objective of the WHO Eastern Mediterranean Regional Office.

The transfusion of blood, its components and its products is a widely used form of supportive therapy, which has expanded greatly due to the development of more sophisticated medical procedures requiring the transfusion of blood. However, blood transfusion is not without hazards, including those related to the transmission of infection. Indeed, the safest blood unit is the one which is not transfused.

Unfortunately, the inappropriate use of blood is common, and adds to the problems and difficulties which impede the development of blood transfusion in the Region. Appropriate use of blood requires the use of critical clinical judgement. The medical indications for blood transfusion should be evaluated, and blood and blood products should not be transfused when alternative therapy is indicated.

Blood transfusion practice links blood donors to patients through the collection of the blood gift by clinical practitioners. Medical practitioners should be committed to the appropriate use of blood donations. In this connection, further education of physicians in the correct practice of transfusion medicine becomes essential.

To contribute to the bridging process between blood collection and its appropriate clinical use in countries of our Region, Dr. C. Th. Smit Sibinga, Director of the Red Cross Blood Bank, Groningen-Drenthe, The Netherlands, has kindly accepted to write the English version of his original valuable Dutch publication. Dr. Sibinga is a very well known authority in the area of transfusion medicine and within international scientific societies. Through the organization of annual symposia on blood transfusion, he has been able to establish Groningen-Drenthe as a well known centre for progress in transfusion medicine.

M. M. El-Nageh, M.D., Ph.D.
Regional Adviser
Health Laboratory Services
Regional Office for the
Eastern Mediterranean

‘A blood transfusion should never be ordered or given, unless it is worth the risk’

Karl Landsteiner

1. Introduction

The most successful and most widely used transplant in the history of medicine is blood. Despite the enormous knowledge gathered over many decades about what is possible and impossible in blood transfusion, each transfusion of blood or of a blood component remains a therapeutic procedure involving risk. The risks of transfusion are not only defined by compatibility, the degree of appropriateness of the donor for the recipient, but by the ever-present risk of transmitting infections such as hepatitis, syphilis and AIDS, and by the unjustified and often ignorant use of blood and blood components. The donation preceding every transfusion should be surrounded by such care that the donor suffers no harm and the blood and its respective components do not harm the recipient.

The World Health Organization (WHO) has recommended a procedure of focused recruitment and retention of voluntary nonremunerated blood donors, medical selection and affiliated donation. The blood of any donation should be subjected to effective control for HBsAg, syphilis, anti-HIV, anti-HCV, ABO blood group, Rhesus D factor and blood group alloantibodies.

2. Blood group systems

2.1 Red blood cells

Red blood cells or erythrocytes are produced in the bone marrow. Under normal circumstances they are present in blood flowing as mature cells without nuclei. Their function consists of the uptake, transport and release of oxygen and the transport of carbon dioxide from the tissues back to the lungs.

The red blood cell membrane consists of a large number of protein and sugar structures and antigens, which together form the blood groups. Each individual has his own specific blood group system, of which over 100 are currently known. Of some clinical importance are:

- ABO system
- Rhesus system
- Lewis system
- Kell, Duffy and Kidd systems
- MNSs system
- P system
- Lutheran system
- I system.

The majority of the blood group systems are cell-bound. Therefore, the antigens are not present in plasma or serum. However, the ABO and Lewis system are both cell-bound and soluble, which means that the corresponding antigens can be present in plasma and serum as well as in other body fluids.

2.1.1 ABO blood group system

The oldest known blood group system is the ABO or ABH system, discovered and described by Landsteiner and others in 1900.

TABLE 2.1

Blood group	Antigen on red blood cell	Antibody in serum
A	A + H	anti-B
B	B + H	anti-A
AB	A and B + H	none
O	H	anti-A and anti-B

The ABO system consists of the basic antigen H and 2 major groups of antigens:

- the A-antigen
- the B-antigen

Additionally the antibodies anti-A and anti-B are ‘naturally’ occurring in the plasma and serum. These antibodies are powerful haemolyzing antibodies and currently cause the most serious haemolytic transfusion reactions.

Within the ABO system four major blood groups can be recognized, depending on the presence or absence of one or both antigens:

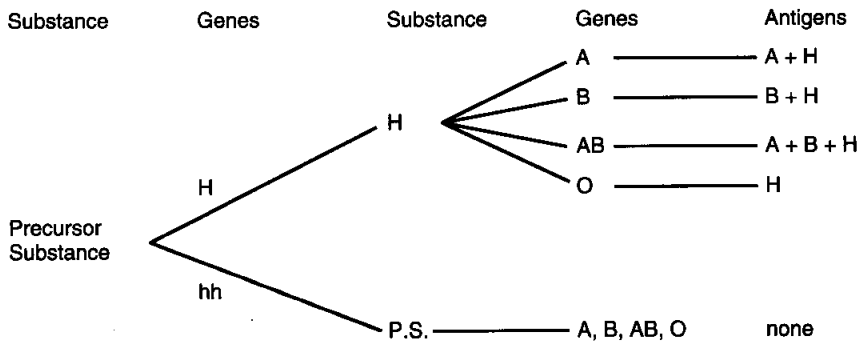
- blood group A
- blood group B
- blood group AB
- blood group O

The relationship within the ABO system between the blood group antigens on the red blood cells and the antibodies present in the plasma or serum is presented in **Table 2.1**.

The antigens of the ABO system develop early in the fetus. The antigens are not only present in the red blood cells, but are found in practically all tissues and body fluids in 78% of people.

The presence of ABO blood group antigens in body fluids is regulated by a dominant gene *Se*. The presence of the gene *Se* determines the so-called ‘secretor’ status. ‘Non-secretors’ are of the genotype *sese*. The production of A and B antigen is regulated by the *H* gene, determining the conversion of ‘precursor’ substance (P.S.) into H substance. In the presence of H substance the A gene regulates the production of A and H antigen, and the B gene regulates the production of B and H antigen. In the absence of both the A and B genes only the H antigen will be responsible for blood group O. In the absence of the H gene, a homozygous *hh* situation, there will be no conversion of ‘precursor’ substance into H substance. This means that even in the presence of A and/or B genes there will be no production of A and/or B antigen (**Figure 2.1**).

Figure 2.1



Such a situation is very rare and is known as type Oh or 'Bombay' group.

Certain microorganisms such as *E. coli* present in gastrointestinal carcinoma can cause the production of B antigen in individuals with blood group A. Also, in certain types of acute leukemia and refractory anaemia in blood group A individuals, there is a tendency to agglutinate erythrocytes with anti-A antibodies. These situations may cause confusion both in typing and in crossmatching.

Antibodies formed against the H substance may present as cold agglutinins and are also known in combination with antibodies against I.

Blood group A can be divided into a number of subgroups, of which A₁ and A₂ are the most relevant clinically.

The frequency of occurrence of the blood groups in the ABH system in Caucasians is presented in **Table 2.2**.

TABLE 2.2

Blood group	Percentage of the population
A	42%
B	8%
AB	3%
O	47%

2.1.2 Rhesus system

The presence of Rhesus antigen was discovered in 1939 by Levine following the injection of human erythrocytes in Rhesus monkeys. The monkeys produced specific antibodies against the human erythrocytes. *In vitro* the serum of these monkeys could haemolyze the type of red cells injected. The Rhesus system is much more complex than the ABO system and has become known specifically for its role in haemolytic anaemia of newborns.

The Rhesus system is cell-bound and not present in the serum. Antibodies against the various Rhesus antigens do not occur 'naturally'; they only develop following blood transfusion or pregnancy. The most known Rhesus antigen is the D factor. There are 4 other Rhesus antigens, namely C, c, E and e. The d antigen is a fictitious antigen which means that it has never been demonstrated and so far it has not been possible to generate antibodies against it. It is assumed that the production of Rhesus antigens is controlled by three pairs of closely related allelomorphous genes: Cc, Dd and Ee. Thus a Rhesus gene complex consists of 3 alleles that determine the production of the combination of 3 antigens. There are 8 Rhesus gene complexes that for reasons of simplicity are coded. The particulars and the frequency in a normal Caucasian population are given in **Table 2.3**.

TABLE 2.3 Prevalence of Rhesus gene complexes

Gene complex	Code	Percentage of the population
CDe	R ¹	42%
cDE	R ²	14%
cDe	R ⁰	2%
CDE	R ²	1%
cdE	r ¹	1%
Cde	r ²	1%
CdE	r ⁰	Very rare
cde	r	38%

For further simplification and ease of operation in practice, Smit Sibinga designed a system based on the general classification: Rhesus D positive = P, negative = N and D^u positive = U. The possible phenotypes and most probable genotypes in Rhesus D positive individuals are numbered in order of prevalence. In the absence of D the symbol N or U is used. **Tables 2.4 and 2.5** give an overview of the notice system as it can be applied in practice for a rapid and efficient identification of donors and selection of units of blood.

TABLE 2.4 Coding of combinations of antigens in the Rhesus system

DD or Dd = P	Cc ee = 1	cc ee = 6
dd = N	CC ee = 2	CC Ee = 7
D ^u or D ^u d = U	Cc Ee = 3	Cc EE = 8
	cc Ee = 4	CC EE = 9
	cc EE = 5	

TABLE 2.5 Classification of Rhesus phenotypes based on the coding of antigen combinations

Rhesus D positive	Rhesus D negative	Rhesus D ^u positive
CcDee (R ¹ r) = P1	Ccdee (r ¹ r) = N1	CcD ^u ee (R ^{1u} r) = U1
CCDee (R ¹ R ¹) = P2	CCdee (r ¹ r) = N2	ccD ^u Ee (R ^{2u} r) = U4
CcDEe (R ¹ R ²) = P3	ccdEe (r ¹ r) = N4	ccD ^u ee (R ^{ou} r) = U6
ccDEe (R ² r) = P4	ccdEE (r ¹ r ²) = N5	
ccDEE (R ² R ²) = P5	ccdee (rr) = N6	
ccDee (R ⁰ R ⁰) = P6		
CCDEe (R ¹ R ²) = P7		
CcDEE (R ² R ²) = P8		
CCDEE (R ² R ²) = P9		

Thus P1 represents the phenotype CcDee, and N1 the phenotype Ccdee.

Rhesus positive or negative is exclusively determined by the presence or absence of the D antigen or the variant D^u. The D^u antigen is often found together with the C antigen. The relationship C/D and C/D^u is determined by the so-called G substance. The presence of C and/or E antigen in the absence of D or D^u antigen in a normal Caucasian population is presented in **Table 2.6**.

TABLE 2.6 Prevalence of Rhesus phenotypes in the absence of D or D^u in a normal Caucasian population

Phenotype/genotype	Percentage of the population
ccdee (rr)	15%
Ccdee (r'r)	0.8%
ccdEe (r''r)	0.5%

The antibody most commonly found as a result of blood transfusion in the Rhesus system is anti-E. The incidence of anti-E and anti-C in Rhesus D-positive transfused patients seems to be higher than in Rhesus-negative transfused patients. Based on extensive research on the prevalence of Rhesus antigens in a large cohort of population, a number of gene complex pairs or genotypes could be identified. The most frequent genotypes are presented in **Table 2.7**.

TABLE 2.7 Prevalence of the frequent Rhesus phenotypes in a normal Caucasian population

Genotype	Percentage of the population
CDe/CDe R ¹ R ¹	17%
CDe/cDE R ¹ R ²	11.8%
CDe/cde R ¹ r	33.0%
cDE/cde R ² r	11.0%
cde/cde rr	15%

Besides the currently described Rhesus antigens there exist a number of variants, of the C antigen and the D antigen in particular. Clinically the most relevant variant is the D^u. Red blood

cells of the D^u type react in the laboratory with some but not all anti-D sera. The testing of red blood cells for D^u subtype is important because:

1. D^u antigen can provide immunization against D;
2. D^u red blood cells can be haemolyzed more strongly when transfused in patients with circulating anti-D antibodies.

The importance of the Rhesus system is the induction of Rhesus antibodies by transfusion with red cells loaded with antigens other than those present on the red cells of the recipient.

Clinically, the D antigen is of particular importance. A Rhesus D-negative recipient (dd) should always be transfused with D negative and thus also with D^u-negative red blood cells. However, D-negative donor cells can be transfused safely to a Rhesus D-positive recipient.

2.1.3 The other blood group systems

A. The Lewis system

The Lewis antigens Le^a and Le^b are present on the red blood cell and in saliva and plasma. In the newborn the red blood cells are not yet loaded with Lewis antigens. It is known that the red blood cell acquires the Lewis antigen by adsorption from the plasma. The system is controlled by the genes Le and le.

There is a relationship between the Lewis system, the ABH system and the 'secretor' genes. The Le gene produces Le^a antigen from the above-mentioned 'precursor' substance. The presence of the combination of Se and H gene leads to the conversion of Le^a antigen to Le^b antigen. In the absence of H or Se gene, there will be no production of Le^b antigen. In the absence of the Le gene, a homozygous lele situation, no 'precursor' substance can be converted into Le^a antigen, which means no production of Le^b antigen. The Lewis phenotypes as determined by the reaction of red blood cells with anti-Le^a and anti-Le^b, and their relation to the H and Sese genes are presented in **Table 2.8**.

TABLE 2.8

Phenotype	Reaction with anti-Le ^a anti Le ^b		Genes present	Frequency
1. Le(a-b+)	-	+	H, Se, Le	72%
2. Le(a+b-)	+	-	H, se, Le	22%
3. a. Le(a-b-)	-	-	H, Se, le	6%
b. Le(a-b-)	-	-	H, se, le	

Anti-Le^a antibodies are 'naturally' occurring and are quite often present in the serum of individuals of type lele (type 3 in Table 2.8) because there is no Lewis antigen present on their red blood cells. Anti-Le^b antibodies are acquired antibodies and are usually found in combination with anti-Le^a and anti-H antibodies. Lewis antibodies usually do not cause haemolytic reactions in the newborn. However, they may cause serious haemolytic transfusion reactions.

B. The Kell, Duffy and Kidd systems

Clinically, the Kell, Duffy and Kidd systems have much in common. The antibodies formed against the antigens of these systems are usually IgG with an optimal activity at 37°C. They are best demonstrated in the indirect Coombs test. All antibodies against Kell, Duffy and Kidd antigens can cause haemolytic disease of the newborn and haemolytic transfusion reactions.

1. The Kell system: the genotypes and their frequency are presented in Table 2.9.

TABLE 2.9

Reaction to anti-K	Genotype	Frequency
Kell positive	KK	0.2%
	Kk	10%
Kell negative	kk	90%

Besides the K or Kell and the k or Cellano antigen, there is another series of antigens known to this system. Anti-K antibodies are frequently found together with the immune-antibodies against the ABH and Rhesus system. Anti-k antibodies are rare because the k antigen is absent in only one in 500 Caucasians.

2. The Duffy system: the genotypes and their frequency are presented in Table 2.10.

TABLE 2.10

Reaction to anti-Fy ^a	Genotype	Frequency
Fy ^a positive	Fy ^a Fy ^a	17%
	Fy ^a Fy ^b	49%
Fy ^a negative	Fy ^b Fy ^b	34%

Fy^a antigen is a weak antigen. Antibodies against Fy^a and Fy^b are rarely found. However, their presence may cause haemolytic disease of the newborn and haemolytic transfusion reactions.

3. The Kidd system: the genotypes and frequencies are presented in Table 2.11.

Anti-Jk^a antibodies are more frequently found than anti-Jk^b antibodies. Both antibodies are complement-binding and their presence may cause serious haemolytic transfusion reactions as well as haemolytic disease of the newborn.

TABLE 2.11

Reaction to anti-Jk ^a	Genotype	Frequency
Jk ^a positive	Jk ^a Jk ^a	25%
	Jk ^a Jk ^b	50%
Jk ^a negative	Jk ^b Jk ^b	25%

The Duffy, as well as the Kidd system, shows so-called minus-minus phenotypes, Fy(a-b-) and Jk(a-b-), not found in Caucasians. Fy(a-b-) is found in 70% of negroes, while Jk(a-b-) is specifically found in Mongolians.

C. The MNSs system:

The MN blood groups were identified by Landsteiner and Levine during experiments in which rabbits were injected with human red blood cells. The population could be divided into 3 groups: M (28%), N (22%) and MN (50%) based on two antibodies, anti-M and anti-N. Through the discovery of a third antibody, the antigen S was established in relation to the MN groups. In a normal Caucasian population the S antigen is prevalent in about 55% of persons. Linked to M, N, and MN, S is present in 73%, 32% and 54%, respectively. Besides S, there is an s antigen that is controlled by an allelomorphic gene of S.

Both anti-S and anti-s can cause haemolytic disease of the newborn and haemolytic transfusion reactions. Both are incomplete IgG antibodies that are most active at 37°C.

Anti-M and anti-N antibodies occur rarely. When found in the serum they behave like cold agglutinins of the 'naturally' occurring type. Besides the major antigens of the MNSs system a number of rare antigens have been found in relation to the system. Of importance is the Mg antigen, controlled by the very rare Mg allele and almost exclusively found in Switzerland. Strangely enough antibodies against the Mg antigen occur fairly frequently. The production of the U antigen, in relation to the MNSs system, has been demonstrated. Antibodies against N are often found in dialysis patients.

D. The P system

The P system dates from the same period as the MN system and is composed of 2 alleles, P_1 and P_2 with the rare amorphous P and the even rarer P^k . There is a striking similarity in the relationship between the antigens P_1 and P_2 and the antigen p, and that between the antigens A_1 and A_2 and group O in the ABH system. This is presented in **Table 2.12**.

Anti- P_1 antibodies are cold agglutinins and are present as 'naturally' occurring antibodies in the majority of P_1 negative individuals. Anti- P_1 antibodies, however, are rarely of clinical importance. Truly P negative individuals, lacking both the P_1 and the P_2 antigen, are very rare. In the serum of these individuals usually a strong antibody anti- $P+P_1+P^k$ can be found, which may induce strong haemolysis at room temperature as well as at 37°C.

TABLE 2.12

Phenotype	Antigen	Serum antibody	Phenotype	Antigen	Serum antibody
A_1	A + A_1	None	P_1	P + P_1	None
A_2	A	Sometimes anti- A_1	P_2	P	Sometimes anti- P_1
O	None (H)	Anti-A + A_1	P	None	Anti-P + P_1

E. The Lutheran system

The Lutheran system is controlled by 2 allelic genes: Lu^a and Lu^b and the rare amorphous allele $Lu(a-b-)$. The Lutheran genes are localized on the same chromosome as the 'secretor' genes. There is a relationship between the Lutheran and the 'secretor' system. The Lutheran antigens are barely developed in the newborn. The most frequent genotype is $Lu(a+b+)$, which is present in 92% of the Caucasian population. Anti- Lu^a antibodies occur as 'natural' antibodies and are of the IgG saline agglutinin type, usually reacting below 37°C. Anti- Lu^b antibodies are usually incomplete antibodies that show up best in a Coombs technique at 37°C. The presence of anti- Lu^b antibodies may lead to slight transfusion reactions and haemolytic disease of the newborn.

F. The I system

The I system is characterized by 2 antigens, I and i. The I antigen is present on the red blood cells of basically every normal adult, while absent on the red blood cells of cord blood. In contrast the i antigen is present on cord red blood cells, while only exceptionally found on red blood cells of normal adults. The I antigen appears on the red blood cells only after the first 18 months of life. In patients with chronic haemolytic anaemia, thalassaemia major, certain hypoplastic anaemias and various forms of acute leukemia, the i antigen is found more

frequently on the red blood cells. The amount of I antigen on peripheral red blood cells is especially diminished in types of acute leukaemia.

Anti-I antibodies can occur in two ways:

- as an auto-antibody present in small amounts in the serum of most normal individuals and in strongly increased amounts in the serum of patients with acute pneumonias. In these latter cases they may cause a serious acquired autoimmune haemolytic anaemia of the cold type;
- more or less ‘naturally’ occurring in the serum of adults with red blood cells loaded with i antigen.

Anti-I antibodies are cold agglutinins forming agglutinates at 4°C. The agglutinates disappear when warmed up to 37°C and reappear when cooled down again to 4°C. They are IgM complement-fixing antibodies.

Anti-i antibodies are found as cold agglutinins in the serum of patients with mononucleosis infectiosa and other diseases of the reticulo-endothelial system. Anti-i antibodies are often incomplete IgG antibodies.

There is a relation between the ABH, P and I blood group systems, where the reactivity of anti-I antiserum varies with the ABH group of red blood cells. Some anti-I sera react more strongly with O than with A cells and vice versa. There are also antibody complexes such as anti-IH, anti-IB and anti-IP1. The clinical relevance of antibodies against the Ii antigens is determined by:

- the activity *in vitro* at room temperature and above 15°C;
- the height of the titre in which they occur.

Additionally these antibodies may mask the presence of other important antibodies. They must be clearly discriminated from antibodies directed against high-frequency blood group antigens, the so-called ‘high-incidence’ antigens.

2.2 White blood cells and platelets

Not only are the red blood cells loaded with blood group antigens, but the white blood cells and blood platelets appear to be loaded with a complex and not yet fully unraveled system of antigens, the ‘human leukocyte antigen’ or HLA system and the platelet antigen system. The significance of both antigen systems more or less parallels the developments in organ transplantation and the science of organ specificity. Also, with the transfusion of blood containing white blood cells and platelets one should take care against the transmission of infections and the sensitization against HLA and platelet antigens. The majority of the so-called non-haemolytic febrile transfusion reactions is based on the presence of HLA- and platelet-antibodies.

2.2.1 The HLA system

The MHC or Major Histocompatibility Complex is localized on the sixth chromosome pair. It comprises the HLA loci. There can be distinguished the LD (Lymphocyte Defined) or Major Locus, and three SD (Serum Defined) loci, namely LA or 1st, Four or 2nd, and AJ or 3rd locus. The HLA antigens are currently distinguished in four series: the first or A-series and the second or B-series, which contain most of the known HLA antigens together with a number of not yet completely identified antigens. These antigens are marked with a "w" (workshop).

There is the third or C-series and the fourth or D-series. The HLA-D locus comprises the various subtypes of D antigens: DR, DQ and DP-series.

The HLA-A, B and C loci comprise the genes responsible for the class I antigens, cell membrane antigens present on all nucleated cells and on platelets. These antigens are glycoproteins consisting of a heavy chain (mw 43 000) containing the antigen specificity and a light chain (mw 12,000) or β 2-microglobulin.

The genes of the HLA-D locus are responsible for the production of the class II antigens present on B lymphocytes, activated T lymphocytes, macrophages, vascular endothelium and dendritic cells. They are cell membrane glycoproteins which play an important role in the regulation of the immune process. Examples of HLA antigens in the various series are presented in **Table 2.13**.

Each of the chromosomes number 6 determines a haplotype of HLA antigens and these are therefore carried over *en bloc*. Each individual inherits one haplotype (one set of 3 HLA antigens) from the father and one from the mother. Evidently the greatest chance of complete HLA identity could be found amongst brothers and sisters. The optimal chance for identity is the identical twin. Thus, each individual has at his/her disposal a maximum of 6 HLA antigens. Systematic family investigations have revealed a number of HLA antigen combinations per haplotype. There are also a number of frequently occurring combinations known in the A- and B-series. Examples are: HLA-A1 and HLA-B8, HLA-A3 and HLA-B7, HLA-A2 and HLA-B12.

There is a relation between the presence of HLA antigens and certain diseases. HLA-A2 has an increased frequency in chronic glomerulonephritis. In multiple sclerosis HLA-A2 seems to be present less frequently while HLA-B7 is found more often. The presence of HLA-B27 is associated with ankylosing spondylitis. Not all HLA antigens have the same antigenicity. A number of HLA antigens are only slightly antigenic while others cause a rapid sensitization reaction.

HLA typing is important in the preparation for organ transplantation, but also in platelet and leukocyte transfusions. Here the class II antigens are of particular importance.

TABLE 2.13

HLA-A	HLA-B	HLA-B	HLA-C	HLA-D	HLA-DR	HLA-DQ	HLA-DP
A1	B5	Bw48	Cw1	Dw1	DR1	DQw1	DPw1
A2	B7	B49	Cw2	Dw2	DR2	DQw2	DPw2
A3	B8	Bw50	Cw3	Dw3	DR3	DQw3	DPw3
A9	B12	B51	Cw4	Dw4	DR4		DPw4
A10	B13	Bw52	Cw5	Dw5	DR5		DPw5
A11	B14	Bw53	Cw6	Dw6	DRw6		DPw6
Aw19	B15	Bw54	Cw7	Dw7	DR7		
A23	B16	Bw55	Cw8	Dw8	DRw8		
A24	B17	Bw56		Dw9	DRw9		
A25	B18	Bw57		Dw10	DRw10		
A26	B21	Bw58		Dw11	DRw11		
A28	Bw22	Bw59		Dw12	DRw12		
A29	B27	Bw60		Dw13	DRw13		
A30	B35	Bw61		Dw14	DRw52		
A31	B37	Bw62		Dw15			
A32	B38	Bw63		Dw16			
Aw33	B39	Bw64		Dw17			
Aw34	B40	Bw65		Dw18			
Aw36	Bw41	Bw67		Dw19			
Aw43	Bw42	Bw70					
Aw66	B44	Bw71					
Aw68	B45	Bw72					
Aw69	Bw46	Bw73					
	Bw47						
	Bw4 broad						
	Bw6 specif.						

2.2.2 The platelet antigen systems

Although most of the febrile non-haemolytic transfusion reactions to platelet transfusions and refractoriness are caused by HLA-antibodies, there are also specific antibodies engaged against platelet antigens.

Antibodies against platelet antigens usually play a role in neonatal alloimmune thrombocytopenia and post-transfusion purpura.

The most common platelet antigen (HPA) systems are the PL^A or Zw^a system and the systems Bak, Ko, PL^e and Duro.

The antigens PL^{A1} and PL^{A2} of the PL^A or Zw^a system relate to the platelet membrane glycoproteins. PL^{A1} occurs in 98% and PL^{A2} in 26% of the population. The Bak^a antigen is present in over 90% of the population.

PL^{A1}, PL^{A2} and Bak^a antigens are absent in patients with Glanzmann's disease (thrombasthenia). These patients are deficient in platelet membrane glycoproteins IIIa and IIb.

2.2.3 Granulocyte antigens

The neutrophilic granulocytes also have specific antigens on their cell surface. In the neutrophilic antigen system (NA) the antigens NA1 and NA2, NB1, ND1 and NE1 can be distinguished. The antigens HGA-1, -2 and -3 are present on monocytes.

Antibodies against these antigens are usually caused by pregnancies or transfusion.

3. Determination of blood groups

Testing for blood groups is usually restricted to the ABO and the Rhesus system. When testing for ABO blood group one has to look for both the antigens on the red cells (forward grouping) and the presence of antibodies in the serum (reverse grouping). Testing for the Rhesus type does not need 'reverse grouping', because there are no 'naturally occurring' antibodies.

3.1 Blood group antigens

Testing for blood group antigens is done with known antisera directed against one specific antigen. The bloodgroup antigen can be determined by adding one or more test sera to the red cells and observing the agglutination pattern.

The agglutination patterns of the ABO system are shown in **Table 3.1**.

TABLE 3.1

Red blood cells	Test sera		
	anti-A	anti-B	anti-(A+B)
A	+	-	+
B	-	+	+
AB	+	+	+
O	-	-	-

The agglutination patterns of the Rhesus system for the most common antigen combinations (genotypes) is shown in **Table 3.2**.

TABLE 3.2

Red blood cells	Test sera				
	anti-C	anti-D	anti-E	anti-c	anti-e
CDe/CD ϵ R ¹ R ¹	+	+	-	-	+
CDe/cDE R ¹ R ²	+	+	+	+	+
CDe/cde R ¹ r	+	+	-	+	+
cDE/cde R ² r	-	+	+	+	+
cde/cde rr	-	-	-	+	+

3.2 Blood group antibodies

Testing for the presence of blood group antibodies is divided into two groups:

- (a) testing for 'naturally occurring' antibodies, particularly of the ABO system,
 - (b) testing for so-called 'irregular' antibodies, developed through sensitization caused by transfusion, pregnancy or organ transplantation.
- (a) The 'naturally occurring' antibodies of the ABO system are determined by adding the serum of the individual to red cells of known antigenic type and reading the agglutination pattern. The agglutination patterns of the 'naturally occurring' antibodies of the ABO system are shown in **Table 3.3**.

TABLE 3.3

Serum sample	Test red blood cells	
	A	B
anti-A	+	-
anti-B	-	+
anti-(A+B)	+	+
none (group AB)	-	-

(b) Testing for 'irregular' antibodies consists of:

- determination of the presence or absence of such antibodies
- identification of any 'irregular' antibodies.

The search for 'irregular' antibodies is usually done as a type of crossmatch between fresh serum of the individual involved and group O red blood cells, specially selected for as broad and complete a range of antigens on the red cell membrane as possible. To demonstrate 'irregular' antibodies of the various known blood group systems with such a testcell panel, a variety of techniques must be applied at different temperatures. As a rule, the use of a saline at room temperature or lower is recommended for the detection of "irregular" antibodies. However, a saline-albumin test, a Coombs or an antiglobulin test and an enzyme test at 37°C may also be recommended. With these tests it is possible to detect haemolyzing, agglutinating and coating antibodies. Detection of 'irregular' antibodies is important in the prevention of transfusion reactions. Donor blood with 'irregular' antibodies can only be transfused as red cell concentrate, with or without washing.

3.3 Selection of donor blood for the patient: type and screen

Routine blood-grouping (ABO/Rhesus) of patients eligible for transfusion and testing their serum for the presence of 'irregular' antibodies enable a rapid selection with an abbreviated crossmatch procedure in case of a transfusion. Such an approach is called 'type and screen' (T&S).

When donor blood is tested routinely for ABO and Rhesus as well as for 'irregular' antibodies, the transfusion practice will become considerably safer and easier.

Irregular antibodies can be expected in women with multiple pregnancies (complete or incomplete) and in patients who had been transfused before or who have had transplants.

3.4 HLA antigens and antibodies

Testing for HLA antigens and antibodies is usually done in the same way as testing for blood group antigens and antibodies. In testing for HLA antigens, white blood cell suspensions are mixed with antisera against specific HLA antigens and the agglutination of the leukocytes is observed.

The major problem with leukocyte typing is the availability of mono-specific antisera (monoclonal antibodies). The detection of HLA antibodies in investigating the cause of non-haemolytic febrile transfusion reactions can be done with a leucoagglutination test, or in case of platelet antibodies, by means of a complement fixation or an immunofluorescence test.

4. The crossmatch

When donor blood has been selected for a patient on the basis of ABO and Rhesus blood group, a crossmatch must be carried out before transfusion of red cells. The purpose of the crossmatch is:

- detection of errors in the determination of donor and recipient ABO blood group;
- detection and identification of ‘irregular’ antibodies in the serum of the patient against antigens present on the red blood cells of the donor.

Donor selection on the basis of known blood group and Rhesus factor primarily determines the compatibility. Crossmatch then contributes to the specificity of that crossmatch.

4.1 Red cell crossmatch

There are two types of crossmatch:

1. The normal or ‘major’ crossmatch, where the serum of the patient is tested against the red cells of the donor. In fact, this procedure does not differ from the principle of the detection of ‘irregular’ antibodies. The difference is in the use of the red cells where, in case of a crossmatch these are exclusively the donor’s red cells and where in testing for ‘irregular’ antibodies a panel of group O cells is produced.
2. The reverse or ‘minor’ crossmatch in which the serum of the donor is tested against the red cells of the recipient. The purpose of this approach is to investigate whether the donor serum contains antibodies against antigens on the recipient’s red cells. However, this reverse crossmatch will not be needed when the donor blood is tested for ABO blood group and related ‘naturally occurring’ blood group antibodies and additionally is tested for ‘irregular’ antibodies. Only in case of an exchange transfusion for haemolytic disease of the newborn does the reverse crossmatch make sense.

The crossmatch must be done before the transfusion of red cells (and whole blood), although the need is less obvious when ‘type and screen’ has been done for both donor and recipient. This means a potential restriction of the crossmatch to those patients who are suspected of having developed clinically relevant ‘irregular’ antibodies. This is particularly so in multiparous women and those patients who have been transfused before. The results of the crossmatch or the ‘type and screen’ procedure should be affixed to the unit before a unit of red cells or processed whole blood is issued for transfusion. At the ward, all precautions should be taken to identify the correct recipient of the unit of blood to avoid and eliminate any potential clerical error and/or mix-up which could lead to fatal haemolytic transfusion reaction.

These administrative control procedures should be documented in the patient's file. When platelets are to be transfused, one can restrict the precautions to ABO and Rhesus compatibility. In other words, there should be no discrepancy between the ABO and Rhesus bloodgroup of donor and recipient.

4.2 White blood cell and platelet crossmatch

When a patient has developed HLA-antibodies, further transfusions should be restricted to leukocyte-depleted red cells and platelet concentrates. When needed, donors can be selected using HLA-typing and lymphocytotoxicity testing.

Platelet transfusions to sensitized or refractory patients can be done with leukocyte-depleted platelet concentrates, donor selection and/or a specific platelet crossmatch.

The techniques usually applied are immunofluorescence or ELISA in a microtitre system. However the predictive value of these methods is still not ideal.

In recent developments standard red cell concentrates stored at 4°C for over 12 days have shown a reduced capacity for generating sensitization and eliciting HLA-antibody formation.

5. Blood transfusion

5.1 Indications

The transfusion of blood or a blood component is no more or no less than a type of substitution therapy, a supportive treatment. Blood transfusion remains one of the most dangerous therapies. The injudicious use of blood and the use of whole blood instead of specific components not only represents a spillage of the voluntarily donated transplant, but could also lead to unnecessary risk to the patient. The optimal use of human blood therefore means that:

- blood transfusion should never be given unnecessarily;
- whole blood should only be used in exchange transfusion;
- only the missing component should be given in all other indications.

The above mentioned statements demand the following of the user of blood:

- the correct diagnosis of the condition for which any type of transfusion could be necessary or indicated;
- an up-to-date knowledge of the various blood components and which product is most appropriate in a given situation;
- ability to handle an adequate quantitative and qualitative transfusion policy to support the patient's needs.

TABLE 5.1 *In vivo* characteristics of the clotting factors

Factor	Plasma concentration for haemostasis	Half-life factor	% recovery	Stability at 4°C
I	100 mg/dl	4-6 days	50%	stable
II	40 IU/dl	2-3 days	40-80%	stable
V	10-15 IU/dl	12 hrs	80%	labile*
VII	5-10 IU/dl	2-6 hrs	70-80%	stable
VIII	10-40 IU/dl	8-12 hr	60-80%	labile**
IX	10-40 IU/dl	18-24 hrs	40-50%	stable
X	10-15 IU/dl	2 days	50%	stable
XI	30 %	3 days	90-100%	stable
XII	—	—	—	stable
XIII	1-5 IU/dl	6-10 days	5-100%	stable

* 50% after 14 days

** 25% after 24 hours

Everyday clinical practice, however, teaches us that usually through insufficient knowledge of the field of blood transfusion a series of problems can occur such as:

- irrational demand for 'fresh' and whole blood;
- unjustified use of red cells, platelets and granulocytes;
- 'elective' transfusion;
- incorrect use of albumin and plasma; and
- rational, but sometimes excessive use of clotting factor concentrates.

An overview of the *in vivo* characteristics of the various clotting factors is presented in Table 5.1.

5.2 Blood components

In clinical practice blood transfusion should be based on a justified transfusion policy, accomplished by optimal cooperation between the clinic and the blood bank. A useful and justified blood transfusion policy therefore should (1) focus on optimal use of the voluntary blood donation; and (2) promote an adequate and justified use of blood and blood components for the patient.

TABLE 5.2 Comparison of characteristics of red blood cell concentrate and whole blood

	Red blood cell concentrate	Whole blood
volume (ml)	300	450
haematocrit (%)	70	39
tot. protein (gm)	36	49
albumin (gm)	5	12
citrate (ml)	25	75
plasma sodium (mmol)	15	45
plasma potassium (mmol)	4	15
plasma ammonia (ug)	650	2000
plasma antigens	minimal	maximal
plasma alloantibodies	minimal	maximal
direct therapeutic effect	2.9% increase	1.1% increase
increase in Hct		
donor-patient ratio	1 donor unit - 6 patients	1 patient
	red blood cell concentrate	
	cryoprecipitate	
	platelets	
	plasma	
	albumin	
	gammaglobulins	

Such a policy is called a 'component policy' and comprises the primary use of red cells (RBC) instead of whole blood (WB). This implies that an increasing amount of blood must be processed into essential components such as cryoprecipitate, platelet concentrate, fresh plasma (frozen, cryosupernatant) albumin, prothrombin complex and plasmaprotein solution. A component policy in principle could be arranged as follows:

1. patients with non-acute (chronic) blood loss—it is of immediate importance and benefit for the individual patient that red blood cell concentrate always be given (see also **Table 5.2**);
2. patients with acute blood loss during surgery or following trauma—the volume reduction should be treated with plasma substitutes. Additionally red blood cell concentrates should be administered for the oxygen supply;
3. shock not caused by blood loss but as a result of sepsis, anaphylaxis, etc. is no indication for transfusion of red blood cell concentrate.

A comparable policy has already been in place for years in various parts of the world and can be found in the recommendations and guidelines for blood transfusion of the Council of Europe¹ and the WHO².

5.2.1 Whole blood

By definition whole blood should be: modified whole blood.

The use of whole blood is basically defined by two fundamental characteristics:

- the volume effect
- the oxygen-carrying capacity.

The indications that are valid for the use of (modified) whole blood are:

- acute massive blood loss with hypovolemic shock (see Chapter 8);
- exchange transfusion (see Chapter 9).

The demand for whole blood is regularly superseded by the demand for 'fresh' whole blood. There is still much confusion about the term 'fresh', as it is commonly used in the most literal sense of the word: donor out, patient in. When blood is transfused according to that principle, the patient will be at unnecessary and, above all, unacceptably high risk for blood-transmissible infections such as:

- hepatitis B virus (HBV);
- hepatitis C virus (HCV);
- human immunodeficiency virus (HIV 1&2);
- cytomegalo virus (CMV);
- Epstein-Barr virus (EBV);

¹ *Guide to preparation, use and quality assurance of blood components*. Council of Europe Press (Strasbourg: 1992).

² *Guidelines for the appropriate use of blood*. World Health Organization, WHO/GPA/INF/89.18 (Geneva: 1989).

- human T-cell leukemia virus (HTLV I&II);
- other so far unknown virus infections, and the risk of transmitting other infectious agents such as:
 - syphilis from seronegative asymptomatic donors when blood is used at less than 3 days shelf life;
 - malaria, babesiosis, Chagas' disease and others.

There is no question about the obligatory need for testing for HBsAg, anti-HIV and anti-HCV of any unit of blood or blood component before transfusion. The conventional idea that 'fresh' blood would be haemostatically the ideal product, has been abandoned a long time ago.

'Fresh' is a functional definition and implies that the blood component should fulfill criteria of functional usefulness.

5.2.2 Red blood cell concentrate

Red blood cell concentrate is usually prepared by removing about two-thirds of the plasma/anticoagulant-preservative part following centrifugation of a unit of citrated whole blood. The remaining red blood concentrate will then have a haematocrit of $\pm 70\%$. When a so-called 'additive solution' (SAG-M) is used, almost all plasma is removed and the remaining red blood concentrate is preserved with 100 ml of the 'additive solution', generating a haematocrit of $\pm 60\%$. When prepared in a closed multiple blood bag system, with no seals broken, a red blood concentrate will have a shelf life of at least 28 days, depending on the preservative used. The use of red blood concentrate has many distinct advantages over whole blood:

- smaller volume for the same amount of red cells;
- larger oxygen-carrying capacity per volume transfused;
- smaller amount of Na⁺, K⁺, ammonia and hydrogen ions, which is not only an advantage in cardiovascular insufficiency but also in patients with renal and liver insufficiency;
- less exposure to alloantibodies, which specifically plays a role in the transfusion of universal donor blood (O Rhesus D negative);
- less risk of transmission of virus infections;
- increase in availability of platelets, plasma, cryoprecipitate and albumin.

A comparison of the composition of whole blood and red blood concentrate is presented in **Table 5.2**.

It is important to note that when using red blood concentrate the transfusion represents no more than a substitution therapy for which not the laboratory but the patient with the symptomatology is defining the indication. Anaemia *per se* is never a reason for blood transfusion. Anaemia caused by iron deficiency, vitamin B12 or folic acid deficiency must be treated with iron medication, vitamin B12 injections or folic acid respectively. When treatment with such medication is insufficient, there may be an indication to support the transfusion of red blood concentrate.

Pre-operative transfusion of red blood concentrate is only justified in patients with severe anaemia. Blood loss during surgery usually requires substitution, first of volume and only in the second place for red blood concentrate. Plasma substitutes of non-human origin are preferred (crystalloids as well as colloids) primarily for reasons of safety and immediate availability. There is no reason for routine use of blood during surgical or obstetric procedures. Transfusion therapy anaemia with hypoxia can be indicated for various conditions, such as:

- *heart failure due to or accompanied by anaemia.* In such situations it is useful to transfuse red blood concentrate because of the volume restrictions and the lesser content of sodium and potassium of red blood concentrate (Table 5.2);
- *liver cirrhosis and liver insufficiency* with anaemia is a good indication for treatment with red blood concentrate. Here also the smaller volume as well as the reduced amount of citrate and ammonia are of importance;
- patients with *renal insufficiency* and uremia usually have chronic anaemia that normally requires transfusion only occasionally. Here too red blood concentrate is indicated because of the volume and the lesser contamination with crystalloids and hydrogen ions. Most patients with chronic anaemia and on dialysis are eligible for transplantation, which implies the processing of red blood concentrate to remove contaminating leukocytes and thrombocytes;
- in any type of *anaemia caused by bone marrow insufficiency* leukocyte-depleted red blood concentrate is indicated.

Red blood cell concentrates can be processed in various ways:

- *washing with saline* to remove contaminating plasma proteins.

Indications: IgA deficiency and paroxysmal nocturnal haemoglobinuria (PNH);

- *leukocyte depletion* by filtration. The purpose is the removal of HLA-antigen carrying cells, specifically the lymphocytes. This reduces and sometimes even prevents the occurrence of febrile non-haemolytic transfusion reactions.

Indications: patients who need frequent and long-term RBC support; prospective organ transplant recipients (renal, bone marrow) and patients who have become refractory to platelet transfusions. There is new information which relates the state of energy of the antigen-presenting white cell to its immunogenic effect—the older the cell the less anergic it is and the greater the chance of losing its antigen-presenting capacity. Red blood concentrate stored for over 12 days at 4°C has become anergic and seems to have lost almost all immunogenic capacity.

N.B. When leukocyte-depleted red blood concentrate is transfused, there should be no more than 10×10^6 remaining white cells (percentage removal of >99%). The removal of just the buffer coat is insufficient as over 40% of the white cells remain in the red blood concentrate.

- *freezing* red blood concentrate with a cryoprotectant such as glycerol at very low temperatures in liquid nitrogen.

Indications: autologous for those patients who have multiple clinically relevant ‘irregular’ blood group antibodies, transplant patients and in open-heart surgery.

After thawing, the cryoprotectant should be thoroughly washed away. The result is almost HLA-antigen free.

In summary, the following are important for adequate, optimal and justified use of red blood concentrate:

- transfusion of a single donor unit in an adult patient is never indicated (elective transfusion);
- anaemia not caused by acute blood loss should only be treated with red blood concentrate when adequate medication fails and the patient suffers from clear symptoms of anaemia;
- in patients with incurable malignancies, red blood concentrate should serve to support symptomatic treatment, leading to only temporary improvement of comfort;
- pre-operative transfusion is only indicated in severe anaemia and is not defined by the operation *per se*;
- massive blood loss must be treated with immediate and adequate substitution of volume followed by red blood concentrate in a slower transfusion rate;
- restricted acute blood loss is only an indication for transfusion when it has caused or tends to cause obvious and symptomatic anaemia.
Post-operative blood loss is generally determined by the dilution effect of massive transfusions or an open vessel;
- ‘fresh’ in blood transfusion has a functional definition.

5.2.3 Platelet concentrate

The prime indication for platelet transfusion still remains the bleeding tendency caused by thrombocytopenia. However, before any platelets are transfused, a search for the cause of the thrombocytopenia must be initiated. Platelet transfusions are indicated when a bleeding tendency exists, although under defined conditions platelets could also be transfused prophylactically:

- thrombocytopenia accompanied by decreased production as in leukemia, bone marrow insufficiencies such as aplastic anaemia, myelofibrosis and other forms of bone marrow suppression (medication-induced, irradiation aplasia);
- platelet function disorders such as Glanzmann’s disease and the acquired thrombocytopathies as in uremia;
- acute thrombocytopenia through massive transfusion in trauma and during major operations (vascular surgery), following extracorporeal circulation (ECC) in open-heart surgery.

When platelet transfusion is needed incidentally, a platelet pool of several random donations (‘random pool’) can be used. however, it is recommended rather to use leukocyte-depleted

concentrates ($<10 \times 10^6$ leukocytes) collected from a single individual donor ('single donor') in cases of frequent transfusions such as supportive therapy in patients with thrombocytopenia due to leukemia, bone marrow insufficiency or in the course of cytostatic therapy and all other forms of chronic congenital and/or acquired thrombocytopenia. When there are signs of sensitization or threatening refractoriness or when a bone marrow transplantation is scheduled, it is important to search for HLA compatible donors. The patient's relatives are usually an obvious source. When specially selected donors are used, the concentrate is produced by cytappheresis.

With automated haemapheresis techniques, one can collect from one single selected donor on average $3-4 \times 10^{11}$ platelets in about 2 hours with a minimum leukocyte contamination (elutriation or surge technique). Additional filtration of the concentrate over a special leukocyte-depletion filter could further reduce the contamination with leukocytes to $<10 \times 10^6$.

Platelet concentrates cannot be stored for long. At room temperature ($20-24^\circ\text{C}$) and with continuous gentle agitation, platelets can be stored for 3 to a maximum of 7 days, depending on the method of production (closed system, type of blood bag, preservative solution, concentration-to-surface-area ratio). At 4°C platelets cannot be stored for more than 24 hours, as they rapidly lose their haemostatic capacity due to swelling.

A standard platelet concentrate prepared from a standard whole blood donation of 450 ml contains $>0.5 \times 10^{11}$ thrombocytes in 50 ml plasma.

For platelet transfusion a crossmatch is not needed. However, ABO blood group and Rhesus compatibility should be assured.

Only in previously sensitized or refractory patients could a platelet crossmatch have a certain predictive value. Platelets should not be transfused to correct platelet numbers for a bleeding tendency due to thrombocytopenia or thrombocytopathy.

The transfusion of platelets should always be done through a clean and exclusively saline flushed giving set.

5.2.4 Leukocyte suspension

The need for supportive therapy with functional granulocytes has increased with the ability to treat patients with malignancies more intensively with chemotherapy. This development was paralleled by a more adequate policy to combat and prevent intercurrent infections by broad spectrum antibiotics and a selective gut decontamination (SGD) regime. So the real need for granulocytes has been diminished to situations where additional phagocytic capacity could be of benefit in the beginning of treatment of a severe and life-threatening septicæmia.

With the development of equipment to separate various cell types from the donor, it is now possible to selectively harvest granulocytes. The methods of separation are all based on centrifugation following continuous (CFC) or discontinuous flow (DFC) principles. The donor is connected to the centrifuge through a closed circuit. The outflowing blood is immediately centrifuged and separated into components. The cell type necessary for the

treatment of the patient can be harvested selectively. The donor is consequently given back the remaining blood and plasma. An optimal number of granulocytes can be harvested from a single donor by repeating such a 'run' several times in succession.

The transfusion of granulocytes or leukocytes should be ABO- and Rhesus-compatible and preferably also HLA-compatible.

The indication for granulocyte transfusion is defined by:

- granulocyte count $<0.5 \times 10^9/l$, and
- serious infection with or without septicaemia, and
- fever $>39^\circ\text{C}$ without reaction to adequate antibiotic regimen within 36 hours.

TABLE 5.3 Average recoveries

	C.F.C.	D.F.C.
leukapheresis	0.8×10^{10}	0.9×10^{10}

5.2.5 Cryoprecipitate

In 1959 Emile Rémigy described the production of a Factor VIII rich plasma concentrate. Freezing plasma rapidly in alcohol and dry ice, for example, at temperatures of at least -50°C , and subsequently slow thawing of the frozen plasma mass at 4°C , allows the plasma to go back into the fluid phase while the 'cryoprecipitate' remains in the gel phase. This fraction is rich in fibronectin, fibrinogen, anti-haemophilic globulin (Factor VIII), von Willebrand Factor (vWF) and Factor XIII.

Cryoprecipitate represents the cryoglobulin fraction of the plasma and is primarily used for further purification of Factor VIII for the treatment of haemophilia A patients.

The thawed plasma can be separated from the cryoprecipitate gel, recovering the Factor VIII as a crude concentrate from the originally processed unit of blood. The percentages of clotting proteins in cryoprecipitate relative to the original plasma are presented in **Table 5.4**.

For clinical use cryoprecipitate is produced from multiple donations and serves as a source of Factor VIII and von Willebrand Factor, but also as a source of fibrinogen, fibronectin and Factor XIII.

To increase safety and applicability, cryoprecipitate can be lyophilized and the virus inactivated by heat.

In general, the volume of cryoprecipitate is less than 6% of the volume of the starting plasma. Unless freeze-dried and virus-inactivated, the risk of transmission of hepatitis or AIDS is identical to that for fresh-frozen plasma and cellular components.

TABLE 5.4

Factor	Synonym	Percentage*
VIII	anti-haemophilic globulin	46.0
I	fibrinogen	33.0
II	prothrombin	1.5
V	proaccelerin	1.0
VII	proconvertin	1.8
IX	Christmas factor	5.5
X	Stuart-Prower factor	1.4
XI	plasma thromboplastin antecedent	1.1
XII	Hageman or contact factor	2.0
XIII	fibrin stabilizing factor	30.0
vWF	von Willebrand factor	50.0

* relative to the original plasma

Various fractionation methods are available for the production of highly purified Factor VIII concentrates, the preferred treatment modalities for haemophilia A patients, both prophylactically and therapeutically. These techniques aim for high purity in the production of Factor VIII preparation to allow a minimal amount of protein to be administered. For routine administration to patients with haemophilia A and von Willebrand disease, these preparations should preferably be lyophilized and treated to inactivate retroviruses, in particular HIV and HCV.

Currently, a recombinant DNA method for the production of virus-free human Factor VIII without contaminants has been developed.

For incidental use in surgery and obstetrics, for instance, cryoprecipitate can be stored deep-frozen (-30°C). It should be thawed in a water-bath at 37°C immediately before application. Once thawed, cryoprecipitate should be transfused without delay and never be refrozen. Cryoprecipitate contains at least 15 g fibrinogen in a total of 65 g protein per litre.

5.2.6 Plasma

The use of plasma is defined by:

- protein shortage;
- acute bleeding disorders due to shortage of clotting proteins; and
- hypovolaemia.

Plasma can be preserved in various ways. Here “fresh” also means functional.

Due to the relative risks of transmitting hepatitis and AIDS, plasma should be transfused preferably non-pooled ABO compatible, and adequately tested for HBsAg, anti-HIV and anti-HCV, donation by donation.

Fresh-frozen plasma (FFP): Non-cryoprecipitated plasma is frozen at -50°C within 6 hours of donation. Other than using it for fractionation, there is hardly any indication. A possible indication for FFP is in the treatment of haemostasis disorders due to deficiencies in clotting Factors II, V and VIII caused by massive blood loss or dilution due to massive transfusion. In paediatrics FFP can be used in the treatment of Gram-negative septicaemia because of its relatively high content of complement and opsonic factors.

However, the most important and universal indication is in fractionation into the various protein derivatives such as Factor VIII, Factor IX, prothrombin complex (PPSB), albumin, immunoglobulins, anti-thrombin III (AT III), etc.

Fresh-frozen plasma is stored at -30°C and thawed in a water-bath at 37°C before use. Once thawed it should be transfused within 4 hours and not refrozen.

Cryoglobulin-poor or cryosupernatant plasma (CPP): The remaining fraction after cryodepletion of fresh plasma. Cryoglobulin-poor plasma is as fresh as FFP. By thawing FFP at 4°C the cryoglobulin fraction is precipitated and the supernatant harvested as CCP. This plasma still contains 20-25% of the cryoglobulins (fibronectin, fibrinogen, etc.) and all other proteins in optimal concentration. It is the preferred preparation for treatment of haemostatic disorders due to deficiency in clotting factors with or without hypovolaemia due to blood loss. Cryoglobulin-poor plasma is stored at -20°C and thawed at 37°C before use, or stored at 4°C .

5.2.7 Prothrombin complex

Following the production of Factor VIII concentrate, the supernatant plasma can be further processed to recover the vitamin K-dependent prothrombin complex. This derivative contains prothrombin (II), proconvertin (VII), Christmas or haemophilia B Factor (IX) and Stuart-Prower Factor (X). This preparation is also lyophilized and virus-inactivated to reduce the risk of transmitting infections.

Prothrombin complex is used for the treatment of haemophilia B (Factor IX deficiency) patients. It is also used for the treatment of coagulation disorders due to deficiency of one or more factors of the prothrombin complex. Therefore, it is applied in serious coumarin overdosage (together with vitamin K1) and coagulation defects due to fulminant hepatitis or liver insufficiency of various origins.

Some prothrombin complex preparations such as FEIBA and Autoplex are activated and used for the treatment of haemophilia A inhibitor patients (Factor VIII antibodies).

These preparations are not without clinical risk, as in some cases thrombosis and disseminated intravascular coagulation have been observed.

Currently there are purified Factor IX and Factor VII concentrates available for treatment of haemophilia B (Factor IX deficiency) and Factor VII deficient patients.

5.2.8 Albumin and plasma protein solutions

Further fractionation of the remaining plasma recovers:

1. *Albumin* in a more or less purified form.
Albumin is marketed as a 4 or 5% solution, or a 20 or 25% sodium-poor solution.
2. *Plasma protein fraction (PPF)* is the fraction remaining following the extraction of Factor VIII, prothrombin complex, albumin and immunoglobulins in the Cohn cold-ethanol fractionation technique.

This preparation contains approximately 3% protein and is pasteurized. However, the fractionation procedure could lead to activation of the kallikrein system with the appearance of quantities of prekallikrein activator or PKA, causing febrile reactions and hypotension upon transfusion.

Plasma protein solutions and albumin are indicated in:

- hypoalbuminaemia or hypoproteinaemia (non-nutritional);
- hypovolaemic shock, whether haemorrhagic, septic and anaphylactic;
- severe burn wound injury;
- cerebral oedema.

Even for these indications albumin should be used selectively. The availability of albumin as a fractionation product is directly dependent on the optimal use of all other blood components such as RBC cryoprecipitate, platelet concentrate and above all, plasma.

5.2.9 Other blood components

In addition to the derivatives mentioned, plasma can be further fractionated into immunoglobulin fraction (polyvalent or specific), anti-thrombin III and C1-esterase inhibitor.

Polyvalent immunoglobulin fraction can be of value in the treatment of patients with hypogammaglobulinaemias or dys-gammaglobulinaemias, infections and idiopathic thrombocytopenic purpura (ITP). Clinically intravenous preparations are preferred, usually available as a 6% lyophilized protein fraction ($\geq 95\%$ IgG).

Infusion should be done slowly because of the risk of allergic reactions.

Apart from the standard polyvalent immunoglobulin preparations containing a great variety of antibodies, there are also specific immunoglobulin preparations available such as anti-D, anti-varicella Zoster, anti-CMV, and anti-HBV.

Anti-thrombin III concentrate is primarily used in inherited AT III deficiency and in cases of acquired deficiency, as in liver transplantation indicated for end-stage liver cirrhosis, and in serious cases of disseminated intravascular coagulation with massive consumption.

C1-esterase inhibitor concentrate is a protein fraction usually containing 50-60 I.U. of C1-esterase inhibitor, some albumin and ceruloplasmin. It is indicated in patients with threatening or acute episodes of angioneurotic oedema. It is administered intravenously.

An overview of the various blood components, their indications and hepatitis risk relative to whole blood is presented in **Table 5.5**.

TABLE 5.5

Product	Contents	Indications	Volume	Hepatitis risk and shelf life
1. rbc	red blood cells, ± 75 ml plasma, leuko's and platelets of their rest-fractions	anaemia, chronic and subacute blood loss	± 300 ml	= 28 ds: + 4°C
2. leukocyte-poor rbc	red blood cells, little plasma and saline	prevention of HLA sensitization	± 300 ml	= 24 hs: +4°C+
3. fresh-frozen plasma	plasma, all clotting proteins and other stable and labile proteins	fractionation	± 240 ml	= 2-4 hs: thawed 2 mns: -20°C
4. cryoglobulin-poor plasma	plasma, little labile clotting proteins and other labile proteins	consumption coagulopathie	± 210 ml	= 6 wk: +4°C 5 yr: -20°C
5a platelet concentrate (pooled)	platelets, little plasma, red blood cells and leuko's	bleeding due to thrombocytopenia and thrombocytopathie	± 50 ml/E	= 3-7 ds: 20-24°C
b platelet concentrate (cytapheresis)	idem	idem	± 200 ml	= 24 hs: 20-24°C+
6. leukocyte concentrate (cytapheresis)	leukocytes, platelets, little red blood cells to antibiotics	granulocytopenia bact. infect. (temp. 39°C) no reaction	± 300 ml	= 4 hs: 20-24°C
7. cryo-precipitate*	fibrinogen, fibronectin AHG (Factor VIII)	deficiencies in surgery/obstetrics	20-50 ml	= 4 hs post-sol. 24 mns: 2-8°C
8. Factor VIII concentrate*	AHG (Factor VIII)	haemophilia A	10-20 ml	> expiration date
9. prothrombin complex*	Factor II, VII, IX, X	haemophilia B, liver insufficiency, coumarin overdosage	10-30 ml/ampoule	> expiration date
10. albumin**	albumin (4-25%)	ac.neph. syndrome ac. liver cell necrosis burn wounds, shock	50-250 ml	<3-5 years
11. P.P.F.**	albumin ($\pm 3\%$)	idem	250 ml	<idem

* Freeze-dried and virus-inactivated

** Pasteurization inactivates hepatitis virus, chance for pka presence

= Hepatitis risk: smaller than, equal to, greater than in whole blood

+ When using a sterile connection system the shelf life can be extended.

6. Transfusion reactions

As mentioned in the introduction, blood transfusion is not without risk; firstly immunological as determined by the degree of compatibility of the donor with the recipient, secondly the inevitable risk of transmitting infections, mostly viral.

The reactions of the patient to transfused blood or blood components, the transfusion reaction, can be distinguished in:

- direct complications of transfusion;
 - a. febrile non-haemolytic transfusion reaction
 - b. allergic reactions
 - c. haemolytic reactions
 - d. overload of the circulation
 - e. bacteremia and sepsis
 - f. potassium intoxication, rarer types of electrolyte intoxication, hypothermia and acidosis (ACD blood)
 - g. air embolism (bottles)
- later complications of transfusion:
 - a. antigen-antibody reactions revealed in the immediate post-transfusion period
 - b. alloimmunization
 - c. transmission of infections such as hepatitis B and hepatitis C, cytomegalovirus, AIDS and other virus infections, syphilis, Chagas' disease and malaria.

6.1 Causes

The causes of transfusion reactions are generally:

1. *Insufficient attention given to compatibility and specificity.* With each type of transfusion where cellular elements are given to the patient, the ABO and Rhesus compatibility should be ensured. When RBC or whole blood are transfused, a test should also be done for specificity of the match between donor and patient: crossmatch.

When plasma is infused, ABO compatibility is preferred, based on the presence of ABO antibodies in the donor plasma.

2. *Insufficient attention given to the choice of therapy, the mode of administration and the mode of preserving blood and blood components.* This may lead to direct harm to the patient due to overload of the circulation with cardiac decompensation, air embolism due to too much pressure applied to bottles and the occurrence of bacteremia and in the worst

case septicæmia due to processing in open systems. Water-baths for thawing cryoprecipitate and fresh-frozen plasma are also major sources of contamination with, among others, *Pseudomonas* species and *E. coli*.

3. *Transmission of more or less serious infectious diseases due to contaminating viruses and bacteria of the donor blood.* The use of 'fresh' whole blood and plasma in particular may lead to the above-mentioned infections.

6.2 Symptomatology

The description of the symptomatology of transfusion reactions will be restricted to clinical aspects of serological transfusion reactions:

1. *Febrile non-haemolytic transfusion reactions* are the most frequently occurring reactions and are commonly due to antibodies against HLA and platelet antigens of the patient, directed against leukocytes and platelets of the donor. In a small percentage the febrile non-haemolytic transfusion reaction is caused by anti-serum protein antibodies. The clinical picture consists of the development of fever with or without chills but without pain and haemolysis.
2. *Allergic transfusion reactions* occur in the presence of allergens in the transfused blood. The patient's reaction may be itching, urticaria and sometimes generalized oedema. In more serious cases the reaction may lead to anaphylactoid shock. Such allergic reactions occur in $\pm 3\%$ of transfusions, especially following the transfusion of plasma, cryoprecipitate and plasma protein fraction.
3. *haemolytic transfusion reaction* is commonly due to a clerical error and usually manifests immediately following the start of the transfusion with fever, chills, headache, backache and lumbar pain, chest pain and nausea. The patient may go into shock and develop a haemorrhagic diathesis due to disseminated intravascular coagulation. The haemolysis and the shock may lead to oliguria and anuria with tubular obstruction due to haemoglobin cylinders. The urine produced is generally dark.

Haemolytic transfusion reactions are not always explicit but may occur in milder forms. Most haemolytic transfusion reactions are due to ABO incompatibility, Rhesus incompatibility and incompatibilities to the Lewis and the Kell, Duffy and Kidd blood group systems.

6.3 Prevention of transfusion reactions

This should always be pursued. First a thorough pre-transfusion specificity and compatibility testing is needed: 'type and screen' and crossmatch. Secondly, before each transfusion one should be convinced of the correct transfusion material, which implies the correct component and the correct donor with the correct ABO and Rhesus type. The crossmatch

should be done correctly without room for any type of incompatibility. It is self-evident that the conditions for preserving the blood and blood components should be stringently controlled to prevent reactions due to bad blood. Circulatory overload and electrolyte intoxications can be prevented by an adequate and optimal transfusion policy.

6.4 Treatment of transfusion reactions

First, stop the transfusion. The treatment should then be focused on prevention of anuria, the continuation of diuresis and the treatment of possible hyperpotassaemia. Allergic transfusion reaction commonly reacts well to parenteral administration of antihistamines.

Febrile non-haemolytic transfusion reactions can be treated symptomatically with antipyretics.

Once a transfusion reaction has occurred, reoccurrence of similar reactions should be seriously considered. This implies a more intense testing for specific compatibility and, in case of febrile non-haemolytic reactions, it is advisable to further transfuse leukocyte-depleted red blood concentrate.

A meticulous report and adequate administration in the patient's record should be done for each transfusion.

7. Transfusion-transmissible diseases

As stated before, blood transfusion may transmit diseases in principle defined by the presence of viral material in the blood to be transfused.

The majority of these viral infections cause post-transfusion hepatitis (PTH).

When the blood is collected from a stable voluntary non-remunerated repeat-donor population and adequately screened for HBV, HCV and HIV, the prevalence of transmission will be limited as shown in **Table 7.1**.

TABLE 7.1

Virus infection	Prevalence (%)
Hepatitis B	10
Hepatitis C	90
Hepatitis A	rare
CMV infection	rare
EBV infection	rare
HIV infection	very rare

7.1 Hepatitis B

HBsAg positive hepatitis is caused by the presence of hepatitis B virus in the donor. The hepatitis B virus can be detected by immunological techniques that test for the antigenic nature of the viral material.

Most techniques are based on the microtitre ELISA principle and have a very high degree of sensitivity.

The incubation period for HBsAg positive hepatitis is 6 months. The donor can be infected with HBV by contact with an HBsAg positive patient, by tattoos, ear-piercing or acupuncture. HBV can also be transmitted by sexual contact, injections or transfusion.

7.2 Hepatitis C

Hepatitis C is caused by one or more retroviruses and is the most common cause of PTH since the introduction of screening for HBsAg.

A retrovirus HCV has been identified as a direct causative agent. Currently there is a known series of specific 'markers', defined by both the structural and non-structural domains of the HCV genome. The available test for the detection of carriers amongst donors is focused on the presence of HCV antibodies against the various structural and non-structural markers. The test is an ELISA microtitre test that can be confirmed by a recombinant immunoblot assay (RIBA) and a polymerase chain reaction (PCR) test. There is a statistical relation between a positive anti-HBc test, elevated ALT levels and type C PTH. Because of this, testing for anti-HBc and ALT as surrogate markers has been introduced in several countries.

The real incidence of hepatitis C PTH is still not very well known. The incubation period is 2 weeks to 4 months, the disease is usually mild and subclinical, with only 20% of patients developing jaundice. The majority of patients seem to recover completely, but a small number develop a chronic persistent type of hepatitis with cirrhosis of the liver, which ultimately may lead to liver carcinoma.

The C retroviruses are lipid enveloped and are sensitive to virus-inactivation treatment with solvent-detergent or intensive heat-treatment at temperatures of $\geq 68^{\circ}\text{C}$.

7.3 Cytomegalovirus infection (CMV)

Cytomegalovirus infection is caused by the cytomegalovirus (CMV) and often bears a striking resemblance to Pfeiffer's disease. It could also cause hepatitis. CMV infection is a well known complication following the transfusion of fresh blood in open heart surgery and other surgical procedures. CMV is transmitted primarily by leukocytes. However, screening for anti-CMV antibodies and donor exclusion does not eliminate transmission as there are other casual and nosocomial routes for transmission of CMV.

CMV negative patients with seriously reduced immune-resistance (immune-compromise) and, in particular, low-birth-weight premature neonates born from CMV negative mothers are highly susceptible to CMV infection. The disease manifests generally in the form of the post-transfusion or post-perfusion syndrome characterized by the occurrence of fever, the development of hepato- and splenomegaly, the presence of atypical lymphocytosis and the development of more or less serious pathology of the lungs. Cytomegalovirus is highly susceptible to storage at 4°C . Therefore, the risk for transmitting CMV infection is greatly reduced when using blood that has been stored for at least 48 hours at 4°C .

7.4 AIDS

The 'acquired immunodeficiency syndrome' or AIDS is as yet a lethal disturbance of the cellular immunity, caused by the retrovirus 'human immunodeficiency virus' or HIV.

The general route of transmission of the infection is sexual contact or the sharing of needles in intravenous drug abuse. Transfusion of HIV-contaminated blood or blood components also leads to the development of AIDS in the recipient.

Since early 1985 a test for screening blood donations for anti-HIV antibodies is available and has been introduced as a mandatory routine quality control of all blood collected in many countries. However, the major preventative measure is in the selection of the donor. A reliable repeat-donor panel based on the principles of voluntaryism and nonremuneration and a careful and responsive medical selection, where the individual donor is well informed and made aware of the personal responsibility in answering the questions on risk factors, provides an important guarantee for a safe blood supply.

In the first period of testing, the incidence of seropositivity obviously will be greater than in the subsequent testing period. A stringent programme focused on risk-factor information and self-exclusion of the donor may limit the incidence as well as the risk for "window phase" donations to almost zero. Most transfusion-related AIDS cases have occurred amongst haemophilia patients who have been treated with cryoprecipitate or concentrates from large pools of plasma donations mostly originating from paid donations, that have been inadequately screened or not screened at all and have not been virus-inactivated.

The introduction of virus inactivation methods like solvent-detergent or heat treatment have reduced the chances for HIV transmission through these preparations to the negligible level.

7.5 Other virus infections

Besides CMV, hepatitis B, hepatitis C and AIDS, other viruses, in particular retroviruses, can be transmitted such as HIV-2 causing AIDS, HTLV-I and II which may cause a spastic paraplegia, or T-cell leukaemia following an extremely long incubation period.

Of the others, Epstein-Barr virus infection is the most common, causing Pfeiffer disease with a positive Paul-Bunnell reaction (in contrast to CMV mononucleosis). Adenoviruses causing influenza as well as slow viruses such as parvovirus can also be transmitted.

7.6 Syphilis and other infections

Syphilis is caused by a spirochaete. The infection is primarily sexually transmissible and causes a contagious disease that may lead to serious disability when neglected or not recognized. The infection can be treated successfully with antibiotics.

Blood stored for at least 4 days at 4°C is no longer a risk for transmitting syphilis. The incidence of seropositive as well as seronegative carriers of the spirochaete is usually significantly lower than hepatitis B virus-contaminated donors.

Apart from syphilis, other infections can be transmitted by blood transfusion such as brucellosis, toxoplasmosis, Chagas' disease, babesiosis, leishmaniasis and malaria.

Depending on the endemicity and epidemiology of these diseases, donor selection should include selective screening.

Vaccination of potential donors with living (attenuated) viral or bacterial material could also cause transmission within a defined period of time following the vaccination. *Rubella*-vaccinated donors are a source of transmissible infection within 3 months of the last injection. Donors vaccinated against measles, mumps, yellow fever, tuberculosis (BCG) and smallpox are a risk within 2 weeks of the vaccination. Donors vaccinated against tetanus, typhoid fever, paratyphus, cholera, diphtheria, influenza and poliomyelitis are a risk within the first 24 hours following vaccination.

8. Acute massive blood loss

Acute massive blood loss occurring in trauma, complicated surgical procedures, or in patients with a haemorrhagic diathesis generally leads to a life-threatening situation.

The problem in acute massive blood loss is defined by:

- hypovolaemia;
- anaemia and hypoxia;
- deficiency in clotting factors and other plasma proteins;
- deficiency in platelets.

Therefore, the transfusion policy in acute massive blood loss should be focused on:

- an optimal and adequate maintenance of circulating volume and perfusion of the tissues (combating shock);
- maintenance of an adequate haemoglobin level by transfusion of RBC;
- adequate policy for haemostasis; clotting factors and platelets.

Additionally, the policy should aim to:

- avoid transfusion of non-crossmatched blood; whenever necessary O Rhesus D negative RBC should be administered;
- minimize risks for infectious and immunological complications caused by the transfused blood or components.

An adequate transfusion policy in acute massive blood loss as instituted in the Trauma Centre of the University Hospital in Groningen could serve as a guideline:

- immediate insertion of large bore intravenous catheters in the upper as well as the lower limbs;
- immediate infusion of plasma expanders to a total volume of 1250 ml;
- depending on the clinical situation (blood pressure), continuation of the policy with infusion of colloids or RBC. When there is insufficient time for typing and crossmatching, O Rhesus D negative red cells should be used. However, a typing and crossmatching should always be initiated even in case the transfusion is given before the results are known;
- when volume is transfused under pressure it is advisable to adapt the temperature during the infusion. This can be achieved by using warming equipment;
- maintenance of adequate haemostatic capacity by transfusion of 2 donor units of (cryosupernatant) plasma following each 10 units of RBC. Following each 20 units of RBC 6 donor units of pooled ABO and Rhesus compatible platelets should be given. When necessary and available, cryoprecipitate could be used as a source of fibrinogen, fibronectin and Factor XIII for the stabilizing of the formed fibrin clots.

- calcium depletion due to plasma and whole blood infusions should be treated with intravenous calcium injection; follow each 4 donor units with one gram calcium gluconate intravenously.

The most important problems related to massive infusion of blood in these circumstances are:

- maintaining the circulation with adequate perfusion of the tissues;
- failure to control the cause of bleeding;
- blood chemical and biophysical problems such as hypothermia, changes in haemoglobin-oxygen affinity, disturbance of the acid-base equilibrium, citrate toxicity, hyperpotassaemia, microembolism, the infusion of large amounts of ammonia, the release of vasoactive amines, etc.

It may be clear that in a situation of acute massive blood loss, prompt and continuous consultation with the blood bank is an indispensable part of the treatment.

8.1 Plasma expanders

When there is acute massive blood loss, the circulating volume and tissue perfusion must be kept at an adequate level; the most readily available, fast, safe and cost-effective preparations are plasma expanders of non-human origin. Either crystalloid or colloid fluids may be used.

The clinically most important colloids are the dextrans, hydroxyethyl starch (HES) and the gelatin solutions.

The most essential advantages and disadvantages of the crystalloid and colloid solutions are listed in **Table 8.1**

TABLE 8.1

Solution	Advantages	Disadvantages
Crystalloids	<ul style="list-style-type: none"> • immediate availability • easy to store • easy to administer • non-immunogenic • non-toxic • no influence on albumin synthesis • no infection risk • cheap/cost-effective 	<ul style="list-style-type: none"> • no oncotic pressure
Colloids	<ul style="list-style-type: none"> • immediate availability • easy to store • easy to administer • non-toxic • no infection risk • oncotic pressure • cheap/cost-effective 	<ul style="list-style-type: none"> • short <i>in vivo</i> survival • some are immunogenic • some influence haemostasis • some affect blood grouping and crossmatching • may slow down albumin synthesis

9. Exchange transfusion

Despite antenatal control, prophylaxis of the mother, and possible intrauterine treatment of the fetus, a newborn may develop haemolysis due to Rhesus antagonism or ABO antagonism. Also other blood group incompatibilities, although rare, may cause haemolytic disease of the newborn, i.e. Kell, Duffy, Kidd, S and s, Lu^b antagonisms.

Haemolytic disease of the newborn should be treated by:

- (a) ultraviolet light exposure;
- (b) albumin infusion, which effectively binds and eliminates bilirubin;
- (c) exchange transfusion.

The reason for exchange transfusion is to:

1. rapidly decrease serum-bilirubin concentration to avoid kernicterus;
2. remove antibody-loaded red blood cells from the neonate;
3. decrease the number of incompatible antibodies circulating in the neonate;
4. administer compatible red blood cells with optimal oxygene-release capacity.

Exchange transfusion is usually done with heparinized blood to prevent citrate toxicity due to the not yet optimally functioning neonatal liver. However, the use of fresh heparin blood has the following serious risks:

1. depends on the availability of the donor;
2. high risk for transmission of viral infections, in particular hepatitis, AIDS and CMV;
3. increased bleeding risk, especially intracranial;
4. potential hypoglycaemia due to the lipolytic effect of heparin (significant increase of free fatty acid concentration).

These risks can easily be avoided without change in the quality of the exchange blood by the use of up to 5-day-old CPDA-1 blood. This is commonly used in exchange transfusion of full term neonates. Possible citrate toxicity can easily be prevented by removing half the citrated plasma, as was already described in 1950 by Mollison. This allows the unit to adapt to the higher haematocrit of the newborn, making the transfusion more physiologically normal.

The importance of readily available CPDA-1 blood for exchange of transfusion should not be underestimated; it allows immediate crossmatching, saving time for donor call-up, selection and collection.

In the severely ill, low-birth-weight premature infant metabolic conditions require a special approach in exchange transfusion. Citrate forms a problem because of the acidity and the inability to metabolize rapidly. This could cause serious hypocalcaemia with tetanus and cardiac problems.

Exchange transfusion in these prematures therefore should preferably be done with a specially composed heparinized blood: O Rhesus D negative red blood cells no older than 5 days, leukocyte-depleted and washed, to which AB heparin plasma is added. AB heparin plasma should be stored, deep-frozen, and thawed at 37°C before use.

The advantage of the transfusion of this composed heparin blood is in the almost physiological composition (**Table 9.1**), the immediate availability of tested and controlled components and the serological neutrality of the mixture.

TABLE 9.1

	Heparin blood (fresh)	CPDA-1 blood	Heparin (composed)
Volume (ml)	±500	±500	±450
Mct (l/l)	0.45	0.40	0.50
pH	7.4	7.0	7.1
Na (mmol/l)	141	174	143
K (mmol/l)	3.5	4.2	3.7
Glucose	48	25	10
ABO/Rh compat.	±	±	±

10. Transfusion sets and infusion fluids

Blood transfusion is practised at the bedside. Ultimately, it treats the transplantation of blood as a living tissue, where the function of the individual components (cells and plasma proteins) is definite. Given the risks related to transfusion practice and the fact that at the bedside a number of things can go wrong, it is of paramount importance to have adequate knowledge and skills and to take optimal precautions for the transfusion of blood and blood components.

The following questions must be raised:

1. Is there a need for transfusion?
2. What are the real needs of the patient? Which blood component should be chosen?
3. How much is needed and how often?
4. How can the transfusion be administered optimally?

These questions demonstrate the need for careful identification of the patient and units of blood or blood components to be transfused, the need for meticulous administration and documentation in transfusion practice, and careful observation of the patient at the beginning of each transfusion as well as during the course of the transfusion.

Be aware of the fact that the major cause of errors and accidents in transfusion practice is within ourselves: inadequate and careless handling and insufficient attention.

A seemingly unimportant clerical error could result in acute haemolysis with lethal results, a risk many times greater than that of a blood transfusion with transmission of a lethal viral infection.

10.1 Administration sets

When deciding upon what donating set to use we should realize that the choice relates to the administration of a precious transplant. Saving a few pennies on a set for the administration of blood may mean the loss of tens to hundreds of litres of precious blood or blood components.

Blood bag systems are designed for collection and processing of human blood in a closed system to secure sterility. They are also designed to create an integral transfusion chain fundamental for optimal transfusion practice.

The choice of an administration set for blood transfusion is therefore defined by the blood bag system.

The inlet ports of a blood bag are sterilely covered and should be connected with the PVC spike of the donating set.

Blood transfusion is best done using a Y-set with an integral 170 micrometre nylon filter and a dripping chamber (**Figure 10.1**). Depending on the use of bottles or PVC bags for infusion fluids one can choose either a Y-set with a spike and a needle or with 2 spikes. The use of a sampling site coupler to connect the set to the bag is incorrect. These couplers are meant for adding medication such as potassium chloride or antibiotics to infusion fluids. Also, their use for blood administration is unsafe (open system) and expensive as the sampling site coupler adds extra costs (**Figure 10.2**).

Figure 10.1 Y- or double infusion set

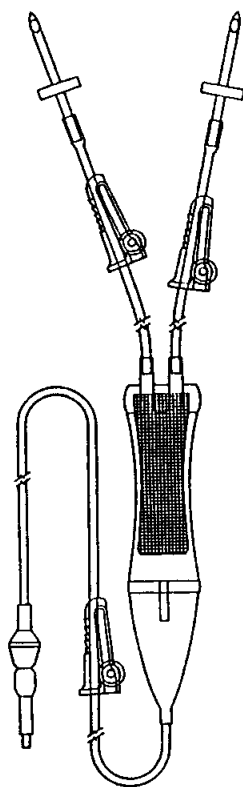
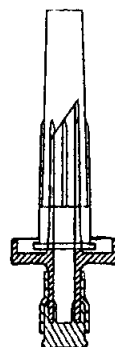


Figure 10.2 Sampling site coupler



The use of microaggregate filters is only justified in open heart surgery during extracorporeal circulation where the pulmonary circulation has been bypassed. This is to prevent cerebral microembolism.

All other indications are questionable, especially when the blood bag is manually shaken before transfusion to disintegrate possible macroaggregates. Microaggregate filters remove leukocytes to a certain extent, but not enough to prevent alloimmunization. Their use may reduce the occurrence of febrile nonhaemolytic transfusion reactions, but only cause delay in further sensitization. Their capacity is limited, so reactions are not prevented, only delayed. In these situations it is advisable to use leukocyte-depleted RBC.

10.2 Infusion fluids

The transfusion of blood and blood components usually parallels the administration of infusion fluids. It is an integral part of the patient's total infusion policy. Not infrequently economical measures are taken during the infusion policy in order to be more cost-effective. However, red blood cells and platelets do not go together with dextrans, glucose-containing solutions and lipid emulsions. Rinsing with 0.9% sodium chloride is essential.

As blood transfusion is an integral part of the total infusion policy, adequate planning and adjustment of the regimen are important. One should also realize that there is a direct correlation between the occurrence of phlebitis and how long a donating set is used before being changed. As a rule-of-thumb each set should be changed every 48-72 hours, and attention should be given to the intravenous cannula and the venepuncture site (**Table 10.1**).

TABLE 10.1

Period of time	Total infusions	Blood transfusions	%
24 hours	197	38	19.3
no complications	107	3	2.8
venous complications	90	35	38.9
48 hours	418	122	29.2
no complications	177	3	1.7
venous complications	241	119	49.9

Prevalence of venous complications after 24 and 48 hours with blood transfusion is considered a part of the infusion policy. The difference is significant at $p < 0.01$ and clearly increases over time.

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BLOOD TRANSFUSION AND BLOOD COMPONENTS

The appropriate transfusion of blood depends on good clinical judgement and a knowledge of the possibilities available to both patient and clinician.

This authoritative work by Dr. C. Th. Smit Sibinga, Director of the Red Cross Blood Bank, Groningen-Drenthe, The Netherlands, describes the various types of transfusion and the possible reactions and risks attached to them.

This publication will be of interest to all doctors and clinicians confronted with the day-to-day decisions in the administering of blood transfusion.