immunogenic, as the D antigen comprises numerous epitopes of the external domains of the
D protein. The D negative phenotype (Rh negative) is associated with the absence of the
whole D protein from the red cell membrane. Numerous variants of D exist and are split into
two types; partial D and weak D. Partial D antigens lack some or most of the D epitopes. If
an individual with a partial D phenotype is immunised by red cells with complete D antigen,
they might make antibodies to those epitopes they lack. Individuals with partial D
phenotypes must be transfused with D negative blood. Weak D antigens appear to express
all epitopes of D, but at a lower site density than normal D. D variants result from amino acid
substitutions in the D protein occurring either as a result of one of more missense mutations
in RHD or from one of more exons of RHD being exchanged for the equivalent exons of
RHCE in a process called gene conversion. Anti-D is produced in D negative individuals due
to transfusion of D positive red cells. Anti-D can cause severe immediate or delayed
haemolytic transfusion reactions (HTRs) and D positive blood must never be transfused to a
patient with anti-D. Anti-D is a common cause of severe haemolytic disease of the fetus or
newborn (HDFN). Anti-c may also cause severe HDFN. Anti-C, anti-E, and anti-e rarely
cause HDFN and when they do the disease is generally mild. Individuals who are Rh
positive can be transfused with Rh positive and Rh negative blood, whereas Rh negative
blood types can only be transfused with Rh negative blood.

All blood groups can be transfused with O Rh D negative blood, thus it is used in emergency
situations.

Laboratory techniques
Agglutination occurs when antibodies on coated cells form cross-linkages between cells
resulting in visible clumping. Agglutination is the formation of cross-linkages between red
blood cell antigens and corresponding antibodies. In the laboratory agglutination indicates a
reaction between antibodies in the patient plasma and antigens in the test reagent. Factors
affecting in vitro detection of agglutination reactions include: the number of antigen sites, as
the more antigen sites available to bind results in more antibodies binding forming
cross-linkages, thus a greater agglutination reaction results; the size of the antibody, as
larger antibodies such as pentameric IgM can cross-link antigens on adjacent cells causing
direct agglutination of red cells, whereas IgG antibodies are monomeric and the distance
between the Fab regions on a single IgG molecule is insufficient to permit the Fab regions of
two IgG molecules to span the distance between two adjacent red cells and cause direct
agglutination, thus IgM antibodies result in a stronger agglutination reaction; the distance
between cells, as positive charge on sodium ions in saline creates an overall positive charge
and red blood cells repel one another (zeta potential), lower ionic strength saline has a lower
sodium concentration, thus a lower positive charge, thus red blood cells agglutinate easier;
and the antigen-antibody ratio.

Column agglutination (gel) systems are used to assess agglutination. Synthetic gel mixtures
of glass microbeads configured into vertical columns on small cards from density barriers,
retaining agglutinated red cells and allowing passage of the non agglutinated cells. Positive
reactions (antibody/antigen interactions) are distinguished by agglutinates near or near the
top of the gel column and negative reactions appear as buttons of red cells at the bottom.
Samples may consist of patient red blood cells and reagent antisera (monoclonal antibody