INACTIVATION OF RBC ANTIGENS BY TELOS THERMAL DISINFECTION SYSTEM

Applicant:	Telos Germany, co ltd. medical equipment Unter den Linden 26 6303 HUNGEN - OBBORNHOFFEN GERMANY

Test facility:	Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Department of Immune-Haematology Diagnostics Plesmanlaan 125 1066 CX Amsterdam The Netherlands

Date experiment: November 19, 1996

Objective

The Telos Limited Thermal Disinfection System is used for inactivation of contaminants of bone. Here the question is addressed whether this system can reduce the antigenicity of contaminating red blood cells (RBC). Because RBC antigens may immunize the receiver of the bone transplant, the donor and receiver are matched for the A, B and D antigens. Therefore, these antigens are of particular interest for examining inactivation of antigens by the Telos System.

In this pilot study, an agglutination/inhibition test was optimized for examining the antigenicity of A and D for heated (20 min, 80°C) and unheated RBC. The B antigens was left out in this pilot study. Because the biochemical structure of antigen B closely resembles that of antigen A, the results obtained for antigen A are indicative for that of antigen B.

For heating a conventional water bath is used. This enabled the use of a much larger amount of RBC than would be found in e.g. human femoral head allografts. Such large volume was necessary for the experimental design and assay volume used in the agglutination/inhibition test.

The A and D antigens of heated and unheated RBC are determined with an agglutination/inhibition test. The RBC are incubated with anti-A or anti-D monoclonal antibodies. The antibodies that do not react with antigen A or D from the heated or unheated RBC materials, are detected by adding the supernatants of the incubated mixtures with RBC and subsequent reading of agglutination.

Materials

Thirty percent RBC A Dpos and O Dneg suspensions were used for preparing the heated and unheated RBC materials. Anti A and D monoclonal antibodies were derived from CLB.

Experimental design

Preparation of heated and unheated RBC materials. Tubes with 10 ml of a RBC suspension were placed in a water bath preheated at 80°C. Simultaneously a control tube with a thermometer, containing 10 ml of PBS, was placed in the water bath. After the temperature in the control tube had reached 80°C (3 min), the tubes were incubated for 20 min. Although the temperature resistance of a coagulating RBC suspension is not exactly known, it was presumed to be significantly higher than that of PBS. To ensure that the RBC suspension at the centre of the tube was exposed to 80°C for a minimum of 10 min, the tubes were incubated for 20 min. The total time that the tubes were placed in the water bath, was 23 min. Unheated RBC were incubated at room temperature for 20 min.

Agglutination inhibition test. A 100 µl of a two-fold dilution series of the anti-A or anti-D monoclonal antibodies were incubated with 100 µl of RBC material at 16°C or 37°C (anti-A or anti-D, respectively) for 30 min. As a control PBS was used. After incubation, the materials were centrifuged at 2380xg for 5 min and the supernatants were collected. Fifty µl of a 3% RBC A Dpos or O Dneg (negative control) suspension was added to 100 µl of each supernatant, centrifuged at 200xg for 1 min. Then the mixtures were read out for agglutination.

Results and discussion

Heating of RBC A Dpos did not cause inactivation of antigen A (Table 1). The dilution of the anti-A antibodies that caused agglutination was 1:2 for both heated and unheated RBC A Dpos materials, indicating that the amount of A antigen in heated and unheated RBC A Dpos material was similar. Therefore heating of RBC does not cause inactivation of A antigen.

Heating of RBC A Dpos, however, caused inactivation of antigen D (Table 1). The dilution of the anti-D antibodies that caused agglutination was 1:16 for heated RBC A Dpos material, whereas 1:2 for unheated RBC A Dpos material. This result indicates that the amount of D antigen in heated RBC A Dpos material is lower than in unheated RBC A Dpos material. The inhibition of heated RBC A Dpos material was reduced to the same level as that of the PBS control, demonstrating that the amount of D antigen was reduced to the detection limit of the assay. Therefore heating of RBC at 80°C for 20 min causes a significant inactivation of D antigen.

As a control, the experiment was also performed with heated and unheated RBC O Dneg material (Table 2). These materials did not cause inhibition of agglutination, demonstrating that the inhibition observed with RBC A Dpos materials was specific for antigen A and D. Moreover, when RBC O Dneg were used for reading out agglutination, all samples tested negative (Data not shown). This finding further confirms that the assay specifically detects the A and D antigens.

Summary

Heating of RBC for 20 min at 80°C caused a significant inactivation of antigen D, but not of antigen A. Because the Telos Thermal Disinfection System reaches the same temperature for at least 10 min, the results suggest that this system can inactivate antigen D, but not antigen A.

Table 1: Inactivation of RBC antigen A or D by thermal treatment

Treated RBC examined for antigen	Antibody/ target RBC	Dilution of anti A or D antibodies that causes agglutination		
		Control (PBS)	Incubation with treated RBC A Dpos	
			20 min RT	20 min 80°C
A	anti-A/ RBC A	>1:512	1:2	1:2
D	anti-D/ RBC Dpos	1:16	1:2	1:16

Table 2: Controls for establishing the specificity of the agglutination/inhibition test

Treated RBC examined for antigen	Antibody/ target RBC	Dilution of anti A or D antibodies that causes agglutination		
		Control (PBS)	Incubation with treated RBC O Dneg	
			20 min RT	20 min 80°C
A	anti-A/ RBC A	>1:512	>1:512	>1:512
D	anti-D/ RBC Dpos	1:16	1:8	1:16