APPLICATION NOTE

Using the Thermo Scientific CW3 cell washer designed for efficient and reliable blood cell washing procedures in blood bank and clinical laboratories

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Abstract

The purpose of pretransfusion compatibility testing is to prevent transfusion of incompatible donor red blood cells (RBCs) that may result in immune mediated hemolytic transfusion reaction. The aim is to have donor RBCs survive in the recipient without destruction.[1]

Some of this compatibility testing procedures require a step in which cells are washed with physiologic saline to remove residual plasma and cellular debris. This washing may be done manually or with the aid of an automated cell washing centrifuge.

This study compares the performance of the new Thermo Scientific™ CW3 automated cell washer with its predecessor, the Thermo Scientific™ Sorvall™ CW2+ system. The CW3 cell washer is a fully automated unit with durable stainless steel rotors and is designed to shorten cycle time, to simplify disassembling of the chamber for cleaning, and to provide better decanting from the lid to minimize sample residue in the chamber.

The comparison was made by running laboratory methods used during pretransfusion compatibility testing on both the CW3 and Sorvall CW2+ cell washers. Samples were compared for reaction grade, cell button size and hemolysis. The comparison analysis demonstrated that the CW3 cell washer is suitable for routine blood bank testing in blood bank and clinical laboratories.

Introduction

Safe transfusion of red blood cells is possible because donor red blood cell units can be specifically selected for their compatibility with the recipient's blood type by running pretransfusion testing. Each compatibility test has one or more purposes and varying level of importance in different situations. In most blood banks and clinical laboratories, pretransfusion testing involves the following two tests: Antibody screen test and Direct Antiglobulin Test (DAT).

The Antibody screen test is performed to detect the presence of unexpected antibodies by incubating patient plasma with commercially available red blood cells in the presence of Low-ionic strength solutions (LISS) or Polyethylene glycol (PEG). LISS and PEG are reagents used for the optimization of agglutination reactions.[2]

Patients requiring blood transfusion, or with suspected transfusion reactions, pregnant women and blood/plasma donor undergo an antibody screen.

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A negative antibody screen means there is no agglutination and therefore no significant antibodies present. Any degree of agglutination indicates a positive antibody screen which requires the identification of antibodies.

The aim of the DAT test is to detect the presence of antibodies bound to red blood cells by incubating patient red cells with the antiglobulin (Coombs) reagent.

DAT is used to help diagnose transfusion-related hemolysis, hemolytic disease of the fetus and newborn, autoimmune hemolytic anemia, and drug-induced immune hemolysis.[3, 4]

A negative DAT means there is no detectable antibody attached to the patient's red cell antigen and a positive one means patient's red blood cells are coated with antibodies.

Figure 1. Thermo Scientific CW3 automated cell washer.

Material and methods
Materials and reagents

Table 1. Equipment

Equipment	Description	Supplier	Product number
Cell washer	CW3 cell washer	Thermo Fisher Scientific	75007405
Cell washer	24-place rotor	Thermo Fisher Scientific	75000020
Cell washer	Sorvall CW2+ with 12-place rotor	Thermo Fisher Scientific	80300566
Tubes	12 mm x 75 mm	Thermo Fisher Scientific	48270
Incubator	MTS incubator	Ortho™ Clinical Diagnostics	5150-60

Table 2. Reagents

Material	Supplier	Location
Antibody screen test		
Screen cells	Bio-Rad™	California, US
Low-ionic strength saline (LISS)	Immucor™	Georgia, US
Polyethylene glycol (PEG)	Immucor	Georgia, US
Anti-human globulin (AHG)	Bio-Rad	California, US
Control cells (CC)	Bio-Rad	California, US
Saline (0.9%)	Thermo Fisher Scientific	Maryland, US
Direct antiglobulin test		
AHG	Bio-Rad	California, US
Coombs control cells	Immucor	Georgia, US
Saline (0.9%)	Thermo Fisher Scientific	Maryland, US

Operational conditions

Cell washer	Method	Centrifugation speed [rpm]	Wash cycles	Decant speed [rpm]	Agitation time [s]	Centrifugation time [s]
Table 3. Operational conditions						
CW3	Antibody screen test	3000	4	350	30	35
CW2+	Antibody screen test	3400	4	-	-	35
CW3	Direct antiglobulin test	3000	4	350	30	35
CW2+	Direct antiglobulin test	2800	4	-	-	35

Grading system

The degree of red cell agglutination is important because it
gives an indication of the amount of antigen or antibody present.

| Grade	Definition
Table 4. Grading system	
Macroscopic	
4+	One solid aggregate or clump of cells
3+	Several large aggregates, clear background
2+	Small- to medium-sized aggregates, clear background
1+	Small aggregates, turbid reddish background
W+	Tiny aggregates, turbid reddish background
M+	Mixed field – Any degree of agglutination in a sea of unagglutinated cells
HEM	Hemolysis
Microscopic	
POS.	Aggregates of at least 3–5 cells
NEG.	No agglutination
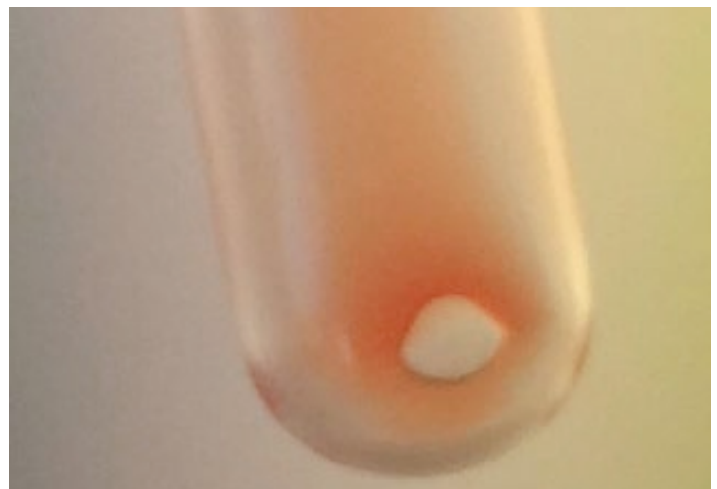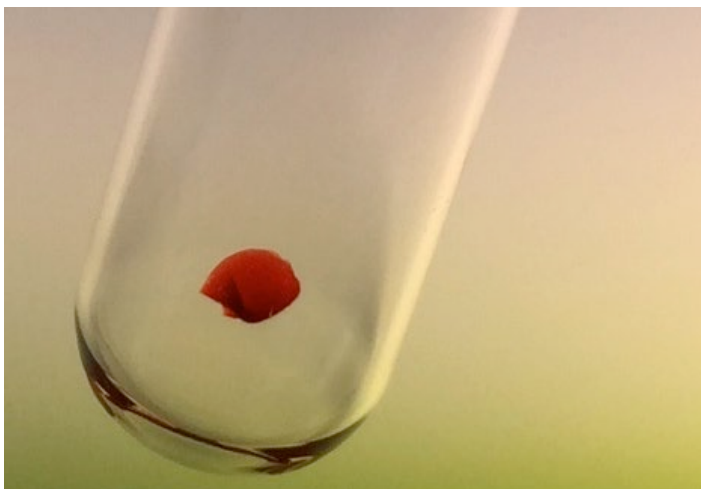
Figure 2. Grading system (left: agglutination; right: no agglutination).

Pass criteria

Acceptable: The reaction results have to be within one grade of the CW3 and the Sorvall CW2+ cell washers.

Unacceptable: The reaction results are greater than one reaction grade difference between the CW3 and the Sorvall CW2+ cell washers.

Methods

Antibody screen test 50 samples (patient plasma) were used for the comparison of the CW3 and Sorvall CW2+ cell washers. 50 test tubes were labeled for each of the two systems.

As shown in Figure 3, two drops of each patient plasma was added to the appropriate test tubes. One drop of screening cells was added to all tubes. The tubes were centrifuged, then resuspended by gentle agitation and read for agglutination or hemolysis.

Two drops of LISS or PEG was added to the tubes and the tubes were incubated in the heat block at 37°C for 15 or 30 minutes. During the incubation, antibody in the patient plasma will bind to antigens on the reagent red blood cells. After incubation the tubes were centrifuged, then resuspended by gentle agitation and examined for agglutination or hemolysis.

The cell mixture was carefully washed using either the CW3 or Sorvall CW2+ cell washer to remove unbound IgG that neutralizes the AHG reagent.

Two drops of AHG was added to the dry cell button in the tubes. The tubes were centrifuged, then resuspended by agitation and read macroscopically.

To all negative tests, one drop of Coombs reagent control cells was added. The tubes were centrifuged and examined for agglutination.[5]

Direct antiglobulin test 25 samples (EDTA-blood) were used for the comparison of the CW3 and Sorvall CW2+ cell washers (Figure 4). Two drops of patient cells were added to tubes. Patient cells were washed using either the CW3 or Sorvall CW2+ cell washer to remove residual plasma and cellular debris. After washing, the cells were resuspended in 2 mL saline to get a 3–5% suspension. The 3–5% suspension was compared with the commercially prepared one. Commercially prepared 3-5% red cell suspensions served as a good guide for comparison in the preparation of cell suspensions.

One drop of the washed 3–5% suspensions was added to the appropriate labeled test tubes. The tubes were washed using one of the two cell washers. One drop of AHG was added to the dry cell button in the tubes. The tubes were centrifuged, then resuspended by agitation and read for agglutination or hemolysis. The tubes were incubated for 5 minutes. After incubation the tubes were centrifuged, then resuspended by gentle agitation and examined macroscopically.

To all negative tests, one drop of Coombs control cells was added. The tubes were centrifuged and examined for agglutination.[5]
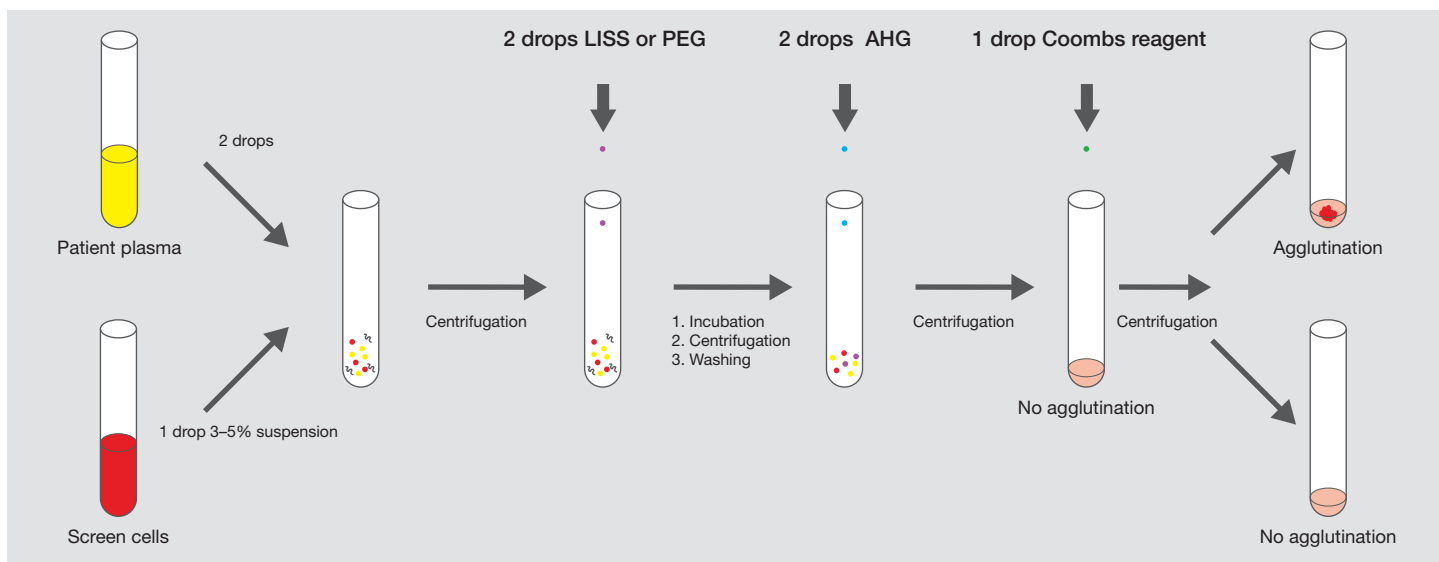

Figure 3. Antibody Screen Test.

1 drop AHG   **1 drop Coombs reagent**

Patient blood → 1 drop 3–5% suspension → Washing → 1. Centrifugation 2. Incubation 3. Centrifugation → No agglutination → Centrifugation → Agglutination / No agglutination
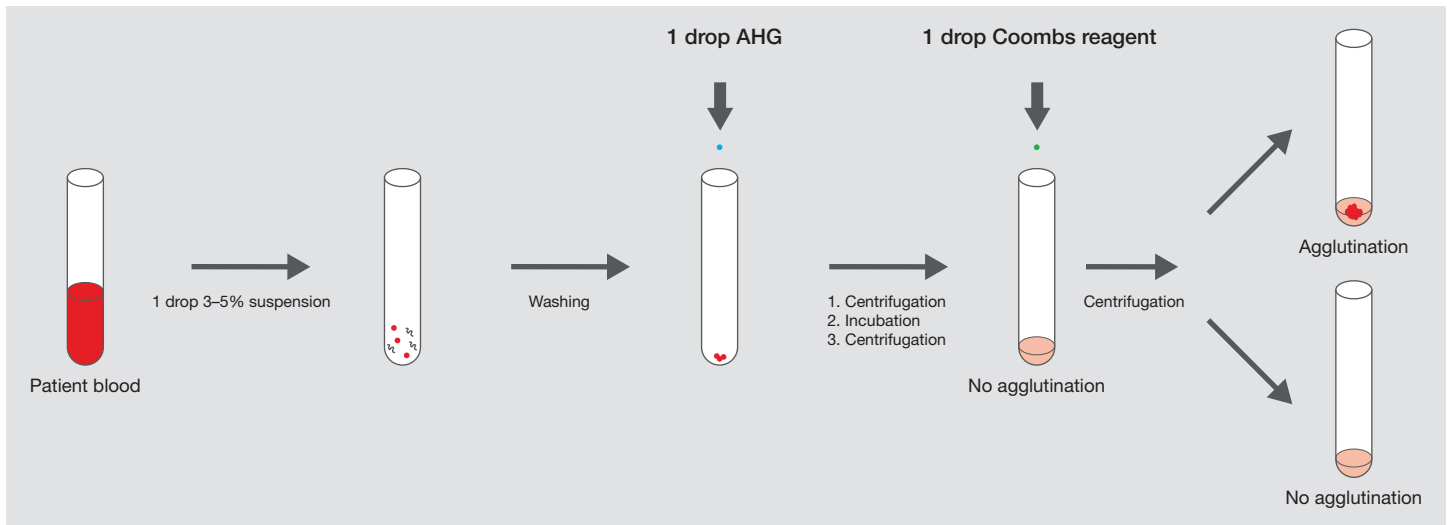
Figure 4. Direct Antiglobulin Test.

Results

A total of 25 antibody screens using LISS were performed. 19 had same reaction results in the CW3 and Sorvall CW2+ cell washers; See table 5 and 6. 6 samples had 1 reaction difference between the CW3 and Sorvall CW2+ observed.

A total of 25 antibody screens using PEG were carried out. 22 samples had same reaction results. 3 had a 1 reaction grade difference between the CW3 and Sorvall CW2+ cell washers; See Table 7 and 8. A total of 25 blood samples underwent a DAT. 20 samples had the same reaction results. 5 samples had a 1 reaction difference between the CW3 and Sorvall CW2+ cell washers; See table 10 and 11.

For all procedures the cell button was consistent through testing and no hemolysis was observed.

Conclusion

The objective of this note was to compare the Thermo Scientific CW3 automated cell washer with its predecessor, the Thermo Scientific Sorvall CW2+ cell washer system by performing pretransfusion compatibility testing.

Antibody screen test and DAT were carried out with agglutination reactions read macroscopically and microscopically in tubes and graded from 0 to 4+. Samples were also compared for cell button size and hemolysis.

All reaction results were within one grade difference between the CW3 and the Sorvall CW2+ cell washers. The cell button was consistent through testing and no hemolysis was observed.

The CW3 cell washer performed as predicted in the acceptable criteria and with reproducibility, without impacting quality or increasing reagent costs. Compared to its predecessor, the new CW3 cell washer is designed to simplify operation which was observed during the analysis, increasing the efficiency of general laboratory personnel or blood bank technologists.

REFERENCES
1. Shulman IA, Downes KA, Sazama K, Maffei LM, Pretransfusion compatibility testing for red blood cell administration, Curr Opin Hematol, Nov; 8(6): 397–404, 2001.
2. Shirey RS, Boyd JS, Ness PM, Polyethylene glycol versus low-ionic-strength solution in pretransfusion testing: a blinded comparison study, Transfusion, May, 34(5), 368–370, 1994.
3. Zantek ND, Koepsell SA, Tharp Jr.DR, Cohn CS, The direct antiglobulin test: A critical step in the evaluation of hemolysis, Am. J. Hematol. 87:707–709, 2012.
4. Tissot JD, Kiener C, Burnand B, Schneider P, The direct antiglobulin test: still a place for the tube technique?, Vox Sang, 77(4):223–226, 1999.
5. All tests were done by Alison Leigh Burgess Rankin at Wake Forest Baptist Health, NC, USA.

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Table 5. Antibody screen test using LISS—incubation time 15 min.

PATIENT	SCR	CW3						CW2+					
		IS	37°C	AHG	CC	INTERP	TECH	IS	37°C	AHG	CC	INTERP	TECH
1	1	-	-	2+				-	-	2+			
	2	-	-	2+		POS.	AB	-	-	2+		POS.	AB
	3	-	-	-	2+			-	-	-	2+		
2	1	-	-	-	2+			-	-	-	2+		
	2	-	-	1+		POS.	AB	-	-	1+		POS.	AB
	3	-	-	1+				-	-	1+			
3	1	-	-	3+				-	-	3+			
	2	-	-	-	2+	POS.	AB	-	-	-	2+	POS.	AB
	3	-	-	-	2+			-	-	-	2+		
4	1	-	-	-	2+			-	-	-	2+		
	2	-	-	-	2+	NEG.	AB	-	-	-	2+	NEG.	AB
	3	-	-	-	2+			-	-	-	2+		
5	1	-	-	4+				-	-	4+			
	2	-	-	4+		POS.	AB	-	-	4+		POS.	AB
	3	-	-	-	2+			-	-	-	2+		
6	1	-	-	-	2+			-	-	-	2+		
	2	-	-	W+		POS.	AB	-	-	W+		POS.	AB
	3	-	-	W+				-	-	1+			
7	1	-	-	-	2+			-	-	-	2+		
	2	-	-	1+		POS.	AB	-	-	1+		POS.	AB
	3	-	-	-	2+			-	-	-	2+		
8	1	-	-	-	2+			-	-	-	2+		
	2	-	-	-	2+	POS.	AB	-	-	-	2+	POS.	AB
	3	-	-	W+				-	-	W+			
9	1	-	-	-	2+			-	-	-	2+		
	2	-	-	M+		POS.	AB	-	-	1+		POS.	AB
	3	-	-	M+				-	-	M+			
10	1	-	-	-	2+			-	-	-	2+		
	2	-	-	4+		POS.	AB	-	-	4+		POS.	AB
	3	-	-	-	2+			-	-	-	2+		
21	1	-	-	-	2+			-	-	-	2+		
	2	-	-	W+		POS.	AB	-	-	M+		POS.	AB
	3	-	-	M+				-	-	M+			
22	1	-	-	2+				-	-	2+			
	2	-	-	2+		POS.	AB	-	-	2+		POS.	AB
	3	-	-	2+				-	-	2+			
23	1	-	-	W+				-	-	W+			
	2	-	-	-	2+	POS.	AB	-	-	-	2+	POS.	AB
	3	-	-	-	2+			-	-	-	2+		
24	1	-	-	1+				-	-	1+			
	2	-	-	-	2+	POS.	AB	-	-	-	2+	POS.	AB
	3	-	-	-	2+			-	-	-	2+		
25	1	-	-	-	2+			-	-	-	2+		
	2	-	-	2+		POS.	AB	-	-	2+		POS.	AB
	3	-	-	-	2+			-	-	-	2+		

Appendix, continued

PATIENT	SCR	CW3						CW2+					
		IS	37°C	AHG	CC	INTERP	TECH	IS	37°C	AHG	CC	INTERP	TECH
11	1	-	-	-	2+			-	-	-	2+		
	2	-	-	4+		POS.	AB	-	-	4+		POS.	AB
	3	-	-	4+				-	-	4+			
12	1	-	-	1+				-	-	2+			
	2	-	-	-	2+	POS.	AB	-	-	-	2+	POS.	AB
	3	-	-	-	2+			-	-	-	2+		
13	1	-	-	-	2+			-	-	-	2+		
	2	-	-	3+		POS.	AB	-	-	3+		POS.	AB
	3	-	-	-	2+			-	-	-	2+		
14	1	-	-	-	2+			-	-	-	2+		
	2	-	-	-	2+	POS.	AB	-	-	-	2+	POS.	AB
	3	-	-	2+				-	-	2+			
15	1	-	-	M+				-	-	M+			
	2	-	-	M+		POS.	AB	-	-	W+		POS.	AB
	3	-	-	-	2+			-	-	-	2+		
16	1	-	-	1+				-	-	1+			
	2	-	-	-	2+	POS.	AB	-	-	-	2+	POS.	AB
	3	-	-	2+				-	-	2+			
17	1	-	-	4+				-	-	4+			
	2	-	-	-	2+	POS.	AB	-	-	-	2+	POS.	AB
	3	-	-	-	2+			-	-	-	2+		
18	1	-	-	-	2+			-	-	-	2+		
	2	-	-	W+		POS.	AB	-	-	1+		POS.	AB
	3	-	-	-	2+			-	-	-	2+		
19	1	-	-	-	2+			-	-	-	2+		
	2	-	-	-	2+	POS.	AB	-	-	-	2+	POS.	AB
	3	-	-	2+				-	-	2+			
20	1	-	-	-	2+			-	-	-	2+		
	2	-	-	4+		POS.	AB	-	-	4+		POS.	AB
	3	-	-	-	2+			-	-	-	2+		

Table 6. Antibody screen test using LISS—incubation time 30 min.

Table 7. Antibody screen test using PEG—incubation time 15 min.												
PATIENT	SCR	CW3						CW2+					
		IS	37°C	AHG	CC	INTERP	TECH	IS	37°C	AHG	CC	INTERP	TECH
26	1	-	-	2+		POS.	AB	-	-	2+		POS.	AB
	2	-	-	-	2+			-	-	-	2+		
	3	-	-	-	2+			-	-	-	2+		
27	1	-	-	3+		POS.	AB	-	-	3+		POS.	AB
	2	-	-	3+				-	-	3+			
	3	-	-	-	2+			-	-	-	2+		
28	1	-	-	-	2+	POS.	AB	-	-	-	2+	POS.	AB
	2	-	-	1+				-	-	1+			
	3	-	-	-	2+			-	-	-	2+		
29	1	-	-	-	2+	POS.	AB	-	-	-	2+	POS.	AB
	2	-	-	2+				-	-	2+			
	3	-	-	2+				-	-	2+			
30	1	-	-	-	2+	POS.	AB	-	-	-	2+	POS.	AB
	2	-	-	W+				-	-	W+			
	3	-	-	-	2+			-	-	-	2+		
31	1	-	-	-	2+	NEG.	AB	-	-	-	2+	NEG.	AB
	2	-	-	-	2+			-	-	-	2+		
	3	-	-	-	2+			-	-	-	2+		
32	1	-	-	M+		POS.	AB	-	-	M+		POS.	AB
	2	-	-	M+				-	-	M+			
	3	-	-	-	2+			-	-	-	2+		
33	1	-	-	-	2+	POS.	AB	-	-	-	2+	POS.	AB
	2	-	-	-	2+			-	-	-	2+		
	3	-	-	3+				-	-	3+			
34	1	-	-	-	2+	POS.	AB	-	-	-	2+	POS.	AB
	2	-	-	1+				-	-	1+			
	3	-	-	2+				-	-	2+			
35	1	-	-	4+		POS.	AB	-	-	4+		POS.	AB
	2	-	-	-	2+			-	-	-	2+		
	3	-	-	4+				-	-	4+			
36	1	-	-	-	2+	NEG.	AB	-	-	-	2+	NEG.	AB
	2	-	-	-	2+			-	-	-	2+		
	3	-	-	-	2+			-	-	-	2+		
46	1	-	-	-	2+	POS.	AB	-	-	-	2+	POS.	AB
	2	-	-	2+				-	-	2+			
	3	-	-	-	2+			-	-	-	2+		
47	1	-	-	-	2+	POS.	AB	-	-	-	2+	POS.	AB
	2	-	-	M+				-	-	W+			
	3	-	-	-	2+			-	-	-	2+		
48	1	-	-	1+		POS.	AB	-	-	1+		POS.	AB
	2	-	-	-	2+			-	-	-	2+		
	3	-	-	1+				-	-	1+			
49	1	-	-	-	2+	POS.	AB	-	-	-	2+	POS.	AB
	2	-	-	4+				-	-	4+			
	3	-	-	4+				-	-	4+			
50	1	-	-	-		NEG.	AB	-	-	-		NEG.	AB
	2	-	-	-				-	-	-			
	3	-	-	-				-	-	-			

PATIENT	SCR	CW3						CW2+					
		IS	37°C	AHG	CC	INTERP	TECH	IS	37°C	AHG	CC	INTERP	TECH
37	1	-	-	-	2+			-	-	-	2+		
	2	-	-	-	2+	POS.	AB	-	-	-	2+	POS.	AB
	3	-	-	W+				-	-	1+			
38	1	-	-	-	2+			-	-	-	2+		
	2	-	-	2+		POS.	AB	-	-	2+		POS.	AB
	3	-	-	-	2+			-	-	-	2+		
39	1	-	-	3+				-	-	3+			
	2	-	-	3+		POS.	AB	-	-	3+		POS.	AB
	3	-	-	3+				-	-	3+			
40	1	-	-	1+				-	-	2+			
	2	-	-	2+		POS.	AB	-	-	2+		POS.	AB
	3	-	-	-	2+			-	-	-	2+		
41	1	-	-	-	2+			-	-	-	2+		
	2	-	-	-	2+	POS.	AB	-	-	-	2+	POS.	AB
	3	-	-	1+				-	-	1+			
42	1	-	-	W+				-	-	W+			
	2	-	-	-	2+	POS.	AB	-	-	-	2+	POS.	AB
	3	-	-	-	2+			-	-	-	2+		
43	1	-	-	-	2+			-	-	-	2+		
	2	-	-	M+		POS.	AB	-	-	M+		POS.	AB
	3	-	-	M+				-	-	M+			
44	1	-	-	3+				-	-	3+			
	2	-	-	3+		POS.	AB	-	-	3+		POS.	AB
	3	-	-	-	2+			-	-	-	2+		
45	1	-	-	-	2+			-	-	-	2+		
	2	-	-	2+		POS.	AB	-	-	2+		POS.	AB
	3	-	-	2+				-	-	2+			

Table 8. Antibody screen test using PEG—incubation time 30 min.

Table 9. Direct antiglobulin test

PATIENT		CW3				TECH	CW2+				TECH
		IS	5°	CC/ccc[1]	INTERP		IS	5°	CC/ccc[1]	INTERP	
51	Poly	-	1+		POS.	AB	-	1+		POS.	AB
	IgG	2+			POS.		2+			POS.	
	C3d	-	-	W+	NEG.		-	-	W+	NEG.	
	SC	-	-		NEG.		-	-		NEG.	
52	Poly	2+			POS.	AB	2+			POS.	AB
	IgG	2+			POS.		2+			POS.	
	C3d	-	-	W+	NEG.		-	-	W+	NEG.	
	SC	-	-		NEG.		-	-		NEG.	
53	Poly	-	-	2+	NEG.	AB	-	-	2+	NEG.	AB
	IgG	-		2+	NEG.		-		2+	NEG.	
	C3d	-	-	W+	NEG.		-	-	W+	NEG.	
	SC	-	-	NT	NEG.		-	-	NT	NEG.	
54	Poly	1+			POS.	AB	1+			POS.	AB
	IgG	1+			POS.		1+			POS.	
	C3d	W+			POS.		W+			POS.	
	SC	-			NEG.		-			NEG.	
55	Poly	3+			POS.	AB	2+			POS.	AB
	IgG	2+			POS.		2+			POS.	
	C3d	-	-	W+	NEG.		-	-	W+	NEG.	
	SC	-	-		NEG.		-	-		NEG.	
56	Poly	4+			POS.	AB	4+			POS.	AB
	IgG	4+			POS.		4+			POS.	
	C3d	1+			POS.		1+			POS.	
	SC	-	-		NEG.		-	-		NEG.	
57	Poly	-	W+		POS.	AB	-	W+		POS.	AB
	IgG	1+			POS.		1+			POS.	
	C3d	-	-	W+	NEG.		-	-	W+	NEG.	
	SC	-	-		NEG.		-	-		NEG.	
58	Poly	2+			POS.	AB	2+			POS.	AB
	IgG	2+			POS.		2+			POS.	
	C3d	-	-	W+	NEG.		-	-	W+	NEG.	
	SC	-	-		NEG.		-	-		NEG.	
59	Poly	3+			POS.	AB	3+			POS.	AB
	IgG	3+			POS.		3+			POS.	
	C3d	-	-	W+	NEG.		-	-	W+	NEG.	
	SC	-	-		NEG.		-	-		NEG.	
60	Poly	-	-	2+	NEG.	AB	-	-	2+	NEG.	AB
	IgG	-		2+	NEG.		-		2+	NEG.	
	C3d	-	-	1+	NEG.		-	-	1+	NEG.	
	SC	-	-		NEG.		-	-		NEG.	
61	Poly	1+			POS.	AB	1+			POS.	AB
	IgG	2+			POS.		2+			POS.	
	C3d	-	-	W+	NEG.		-	-	W+	NEG.	
	SC	-	-		NEG.		-	-		NEG.	
62	Poly	-	W+		POS.	AB	-	W+		POS.	AB
	IgG	W+			POS.		W+			POS.	
	C3d	-	-	1+	NEG.		-	-	1+	NEG.	
	SC	-	-		NEG.		-	-		NEG.	
63	Poly	1+			POS.	AB	1+			POS.	AB
	IgG	1+			POS.		1+			POS.	
	C3d	1+			POS.		1+			POS.	
	SC	-	-		NEG.		-	-		NEG.	

1. NT: Not tested

Table 10. Direct antiglobulin test

PATIENT		CW3				TECH	CW2+				TECH
		IS	5°	CC/ccc[1]	INTERP		IS	5°	CC/ccc[1]	INTERP	
64	Poly	-	2+		POS.	AB	-	2+		POS.	AB
	IgG	2+			POS.		2+			POS.	
	C3d	-	-	1+	NEG.		-	-	1+	NEG.	
	SC	-	-		NEG.		-	-		NEG.	
65	Poly	-	1+		POS.	AB	-	2+		POS.	AB
	IgG	1+			POS.		1+			POS.	
	C3d	-	-	1+	NEG.		-	-	1+	NEG.	
	SC	-	-		NEG.		-	-		NEG.	
66	Poly	-	-	2+	NEG.	AB	-	-	2+	NEG.	AB
	IgG	-		2+	NEG.		-		2+	NEG.	
	C3d	-		1+	NEG.		-		1+	NEG.	
	SC	-	-		NEG.		-	-		NEG.	
67	Poly	3+			POS.	AB	4+			POS.	AB
	IgG	4+			POS.		3+			POS.	
	C3d	1+			POS.		1+			POS.	
	SC	-	-		NEG.		-	-		NEG.	
68	Poly	4+			POS.	AB	4+			POS.	AB
	IgG	4+			POS.		4+			POS.	
	C3d	4+			POS.		4+			POS.	
	SC	-	-		NEG.		-	-		NEG.	
69	Poly	-	1+		POS.	AB	-	1+		POS.	AB
	IgG	2+			POS.		2+			POS.	
	C3d	-	-	1+	NEG.		-	-	1+	NEG.	
	SC	-	-		NEG.		-	-		NEG.	
70	Poly	1+			POS.	AB	1+			POS.	AB
	IgG	1+			POS.		1+			POS.	
	C3d	-	-	W+	NEG.		-	-	W+	NEG.	
	SC	-	-		NEG.		-	-		NEG.	
71	Poly	W+			POS.	AB	W+			POS.	AB
	IgG	W+			POS.		W+			POS.	
	C3d	-	-	1+	NEG.		-	-	1+	NEG.	
	SC	-	-		NEG.		-	-		NEG.	
72	Poly	M+			POS.	AB	M+			POS.	AB
	IgG	W+			POS.		W+			POS.	
	C3d	-	-	1+	NEG.		-	-	1+	NEG.	
	SC	-	-		NEG.		-	-		NEG.	
73	Poly	1+			POS.	AB	W+			POS.	AB
	IgG	1+			POS.		1+			POS.	
	C3d	-	-	W+	NEG.		-	-	W+	NEG.	
	SC	-	-		NEG.		-	-		NEG.	
74	Poly	-	-	2+	NEG.	AB	-	-	2+	NEG.	AB
	IgG	-		2+	NEG.		-		2+	NEG.	
	C3d	-		1+	NEG.		-		1+	NEG.	
	SC	-	-		NEG.		-	-		NEG.	
75	Poly	2+			POS.	AB	2+			POS.	AB
	IgG	2+			POS.		2+			POS.	
	C3d	-	-	1+	NEG.		-	-	1+	NEG.	
	SC	-	-		NEG.		-	-		NEG.	