

Daratumumab F(ab')₂ fragments in use for gel card and solid phase IAT – practical aspects.

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Aim: Evaluation of DARA-F(ab')₂ fragments stability and their application in IAT gel card and solid phase assays

Methods

DARA-F(ab')₂ fragment was prepared by pepsin digestion of whole DARA antibody. 24 plasmas were investigated by gel card and 18 plasmas were investigated by solid phase IAT. Both IAT techniques were performed using alloantibody containing plasmas with and without spiking with DARA (500 µg/mL); with and without DARA-F(ab')₂ fragments addition (500 µg/mL).

DARA-F(ab')₂ fragments were stored at 4°C, -20°C and -80°C and tested after 7, 14, 30, 45, 60, 90 and 180 days by IAT gel card technique.

Background

Daratumumab (DARA) is a humanized antibody directed against CD38 for treatment of multiple myeloma. CD38 is also expressed on red cell membranes enabling free daratumumab in patient serum to bind to test erythrocytes during pretransfusion compatibility testing, resulting in panreactive agglutination in the standard indirect antihuman globulin (IAT) test used for antibody screening and crossmatching. Consequently, clinically relevant red cell alloantibodies may not be recognized in patients treated with daratumumab. The addition of DARA-F(ab')₂ fragments to red cells can mask the CD38 binding epitope of DARA and re-establish recognition of known red cell antibodies by IAT.

Results

		Plasma without anti-RBC abs			Anti-K			Anti-E			Anti-Fy ^a		
RBC phenotype					Kk	Kk	kk	EE	EE	ee	Fy ^a +	Fy ^a +	Fy ^a -
DARA		-	+	+	-	+	+	-	+	+	-	+	+
F(ab') ₂ fragments		-	-	+	-	+	+	-	+	+	-	+	+
IAT result	Gel card												
	Solid phase												

Fig. 1: Results of gel card and solid phase IAT using RBC alloantibody containing plasmas in the presence (+) and absence (-) of DARA and F(ab')₂ fragments

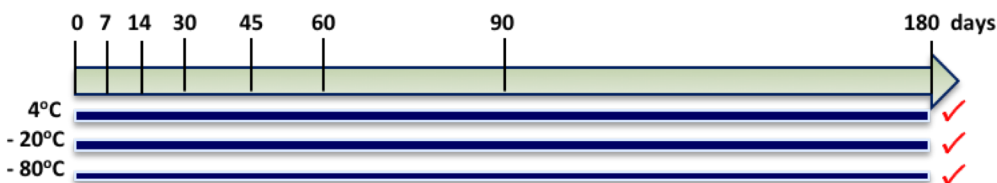


Fig. 2: DARA-F(ab')₂ fragments can be stored at 4°C, -20°C and -80°C up to 180 days.

Conclusion

- Preincubation of RBCs with DARA-F(ab')₂ fragments completely blocked binding of free DARA up to 500 µg/mL
- No loss of sensitivity or specificity compared to native plasma was observed in both, gel card and solid phase IAT
- Freeze-thawing and storage of DARA-F(ab')₂ fragments for up to 180 days (4°C, -20°C and -80°C) did not influence the results of IAT