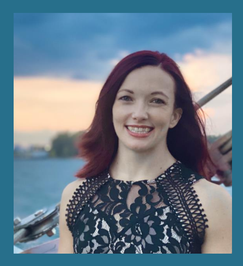
Use of DTT treatment in antibody screening methods



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Key words: Dithiothreitol (DTT), Daratumumab, Ortho BioVue, CD38, antibody screening, haemagglutination tube technique, false positive

Introduction

Daratumumab is an anti-CD38 monoclonal antibody treatment that targets the CD38 antigens expressed on multiple myeloma cells. CD38 is expressed at low levels on the surface of red blood cells. As a result, it has been observed that Daratumumab causes interference with blood compatibility testing, producing a pan reactive antibody screen. The Daratumumab in the patient plasma binds to the CD38 on the red cells used in the antibody screen causing positive agglutination reactions for all antibodies. The effect of Daratumumab can be overcome by treating the red cells with dithiothreitol (DTT). DTT is a reducing agent that breaks the CD38 disulphide bonds on the red cells, thereby preventing daratumumab from binding to them.

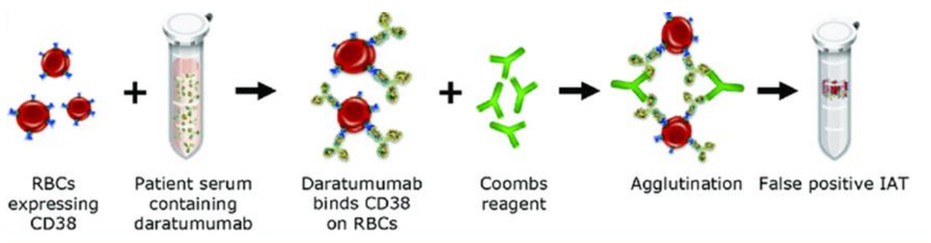


Diagram showing how daratumumab interferes with the antibody screen (Chari et al., 2018)

DTT treatment of reagent red cells has been proven to be effective in antibody screens for patients on Daratumumab treatment. DTT is easily available, cost effective and has been thoroughly researched and validated as a reliable method in removing the interference caused by the CD38 monoclonal antibody treatments. Chapuy et al., 2016, conducted validation testing for the DTT method to be used for gel column and tube testing, proving it to be successful. However, many laboratories use Ortho BioVue cassettes, which use glass micro beads instead of gel. If antibody screens could be performed in the hospital laboratories using the equipment already in use, it would be of great benefit to the patient. Blood products would then be issued in a timelier manner, and it would also be more cost effective for the laboratories.

Aim

To investigate the feasibility of using Ortho BioVue cassettes with DTT treated Surigiscreen and NHSBT panel red cells to perform antibody screens. This method was compared with the gold standard method of the haemagglutination tube technique.

Method

The red cells were treated with DTT following the procedure in the product insert.

Nine known antisera were used to determine the feasibility of DTT treatment in anti-IgG Ortho BioVue cassettes.

The protocol was repeated using the gold standard haemagglutination tube method for comparison of results.

A three-panel screen was performed using Surgiscreen cells for four of the antisera

AB serum was used as a negative control.

The full antibody panel screen was performed using NHSBT reagent panel cells for all nine of the antisera.

The results were interpreted and recorded on the relevant antigram using the agglutination grading system.

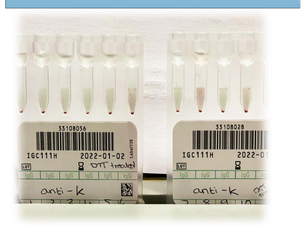
Results

The DTT treated Surgiscreen cells did not have the expected reactions when added to each known antisera, showing weak agglutination reactions when no reactions should have taken place. Table 1 shows a comparison between the results obtained from the DTT treated NHSBT panel cells with the Ortho BioVue CAT method, the haemagglutination technique in tube and the expected results for that batch according to the antigram. CAT using ortho BioVue did not always produce the results as expected, however, the haemagglutination tube technique had the expected reactions against all the antisera.

Table 1: Results obtained from the DTT treated NHSBT panel cell carried out using the ortho BioVue column technique and the haemagglutination tube technique. The expected results from the antigram are shown. The results are recorded using the agglutination grading system 0 – 4+, H-haemolysis, W-weak reaction.

	Cell #	Anti-sera test results									Control
		Weak anti-D	Weak anti-K	Weak anti-Fya	Weak anti-c	Weak anti-E	Weak anti-e	Weak anti-C	Weak anti-Fyb	Weak anti-Jka	AB serum
Expected antigram results	1	3+	0	0	0	0	3+	3+	3+	3+	0
	2	3+	3+	3+	0	0	3+	3+	0	0	0
	3	3+	0	0	3+	3+	0	0	3+	0	0
	4	0	0	0	3+	0	3+	3+	3+	0	0
	5	0	0	3+	3+	3+	3+	0	0	3+	0
	6	0	3+	0	3+	0	3+	0	3+	3+	0
	7	0	3+	0	3+	0	3+	0	3+	0	0
	8	0	0	3+	3+	0	3+	0	0	0	0
	9	0	0	0	3+	0	3+	0	3+	3+	0
	10	0	0	3+	3+	0	3+	0	0	3+	0
DTT treated cells in ortho cards	1	2+	0	0	0	W	3+	3+	2+	H	0
	2	2+	0	2+	0	0	3+	3+	0	0	0
	3	2+	0	0	4+	3+	0	0	1+	0	0
	4	0	0	0	3+	0	3+	3+	2+	0	0
	5	W	W	2+	4+	3+	3+	0	0	2+	0
	6	W	1	0	4+	W	3+	0	3+	3+	0
	7	0	0	0	3+	0	3+	W	2+	0	0
	8	1	1	3+	4+	0	3+	0	0	0	0
	9	0	0	0	3+	0	3+	0	2+	H	0
	10	W	W	3+	4+	0	3+	0	0	H	0
DTT treated cells in tube	1	4+	0	0	0	0	4+	4+	3+	2+	
	2	4+	0	2+	0	0	4+	4+	0	0	
	3	4+	0	0	4+	4+	0	0	3+	0	
	4	0	0	0	3+	0	4+	3+	3+	0	
	5	0	0	3+	4+	3+	4+	0	0	3+	
	6	0	0	0	4+	0	4+	0	3+	3+	
	7	0	0	0	3+	0	4+	0	3+	0	
	8	0	0	2+	4+	0	4+	0	0	0	
	9	0	0	0	4+	0	4+	0	3+	2+	
	10	0	0	2+	4+	0	4+	0	0	2+	
	Pos	4+	4+	4+	4+	4+	4+	4+	4+	4+	
	Neg	0	0	0	0	0	0	0	0	0	

DTT treated cells tested against anti-k antisera in BioVue cassettes



DTT treated cells tested against anti-k antisera using the haemagglutination tube technique

For the DTT treated cells in Ortho BioVue the sensitivity is 100% and the specificity is 77.6%. For the DTT treated cells in tube, the sensitivity and specificity are 100%. These values can be used to calculate the Positive predictive value (PPV) and Negative predictive value (NPV). For the DTT treated cells in Ortho BioVue, the PPV is 78.4% and the NPV is 100%. For the DTT treated cells in tube, the PPV and NPV are 100%.

Table 2: The statistical values calculated from the results obtained from the CAT and haemagglutination tube technique showing the True positives, false positives, true negative and false negatives.

Test type:	True positive	False Positives	True Negatives	False Negatives
CAT (ortho BioVue)	40	11	30	0
Haemagglutination tube technique	40	0	50	0

Conclusion

This study tested the feasibility of using DTT red cells in Ortho BioVue cassettes and was found to be unreliable in completely removing the false positive results. Although it was proven that the DTT treatment was successful in denaturing the CD38 antigen, as was demonstrated using the haemagglutination tube technique, there was interference caused by potentiators in the Ortho BioVue cassettes that generated false positive results. The DTT treated Surgiscreen cells were found to be completely unreliable and should not be used. Therefore, from the results obtained in this study, it would not be recommended to use 0.2M DTT treated red cells with Ortho BioVue cassettes. However, the use of the haemagglutination tube technique with DTT treated NHSBT reagent cells provided satisfactory and expected results.

References and Acknowledgements

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