Controlling the Coombs Test: Are Strong Reactions Better or Worse?

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BACKGROUND

Anti-IgG can be neutralized or partially neutralized by the presence of IgG. This may occur as contamination of the reagent or inadequate washing of red cells in the anti-human globulin (AHG) procedure. Neutralization is undesirable as it can cause weak or false negative reactions in the AHG assay. IgG sensitized red cells (IgG cells) are used as a control reagent to show reactivity of anti-IgG. We set out to assess the efficacy of various commercially available IgG cells and their ability to detect partially neutralized AHG reagent.

INTRODUCTION

Manufacturers have a challenge when designing and building commercial control cells. A strongly reacting IgG control cell is more likely to be commercially successful and also more likely to remain strongly sensitized through its expiry period. However, this feature needs to be balanced with a responsible goal of providing an efficacious red cell control product that suitably meets the purpose of an IgG control cell, i.e. it detects an inactivated or neutralized AHG reagent.

METHODS

A. Determination of plasma dilution for neutralization of anti-IgG

1. Two-fold serial dilutions of normal adult plasma in saline were prepared.
2. 100ul anti-IgG + 100ul of each dilution of plasma + 1 drop IgG-sensitized red cells were mixed.
3. Tubes were centrifuged, cells resuspended and read .

	Dilution of Plasma in Saline						
	1 in 256	1 in 512*	1 in 1024*	1 in 2048*	1 in 4096	1 in 9192	1 in 18384
IgG cells	0	0	2+	3+	3+	3+	4+

B. Neutralization of anti-IgG

- 1 part normal saline or diluted plasma (1 in 500, 1 in 1000, 1 in 2000)* + 4 parts anti-IgG.

C. Detection of anti-IgG neutralization

- 5 manufacturers' IgG cells were tested in parallel in the following blind study:

1. 1 drop of 2-4% saline-suspended DAT-negative red cells were washed x4 in cell washer.
2. 100ul of neutralized anti-IgG was added, tubes centrifuged, cells resuspended and read.
3. 50ul of IgG cells were added, tubes centrifuged, cells resuspended and read.

RESULTS

4 Parts Anti-IgG Neutralized with 1 Part Plasma Diluted 1 in 500

Manufacturer	Tech 1	Tech 2	Tech 3	Tech 4	Tech 5	Tech 6	Tech 7
Medion	0	0	0	0	0	0	0
Immucor†	0	0	0	0	0	0	0
Biotest	0	0	0	0	0	0	0
Ortho	0	0	0	0	0	0	0
ARC	0	0	0	0	0	0	0

4 Parts Anti-IgG Neutralized with 1 Part Plasma Diluted 1 in 1000

Manufacturer	Tech 1	Tech 2	Tech 3	Tech 4	Tech 5	Tech 6	Tech 7
Medion	+	1+	2+	1+	1+	+	+
Immucor†	+	1+	3+	2+	1+	1+	+
Biotest	+	1+	3+	2+	2+	2+	+
Ortho	0	+	1+	2+	+	+	1+
ARC	0	0	1+	0	0	0	+

4 Parts Anti-IgG Neutralized with 1 Part Plasma Diluted 1 in 2000

Manufacturer	Tech 1	Tech 2	Tech 3	Tech 4	Tech 5	Tech 6	Tech 7
Medion	3+	3+	3+	3+	2+	3+	2+
Immucor†	3+	3+	4+	3+	3+	3+	3+
Biotest	3+	3+	4+	3+	3+	2+	2+
Ortho	2+	1+	2+	2+	1+	1+	3+
ARC	+	1+	1+	2+	2+	1+	1+

4 Parts Anti-IgG Neutralized with 1 Part Saline

Manufacturer	Tech 1	Tech 2	Tech 3	Tech 4	Tech 5	Tech 6	Tech 7
Medion	3+	3+	4+	3+	3+	2+	3+
Immucor†	3+	3+	4+	3+	3+	2+	3+
Biotest	3+	3+	4+	3+	4+	3+	3+
Ortho	2+	1+	4+	2+	2+	2+	3+
ARC	2+	1+	3+	1+	2+	1+	3+

SUMMARY

Reaction Grade Totals per Dilution of Plasma or Saline Added to Anti-IgG‡

Manufacturer	1 in 500	1 in 1000	1 in 2000	Saline
Medion	0	6.5	19	21
Immucor†	0	9	22	21
Biotest	0	10	20	23
Ortho	0	5.5	12	16
ARC	0	2.5	8.5	13

CONCLUSION

All of the commercial control cells tested are effective at detecting a gross contamination of the AHG test resulting in inactivation/neutralization of the AHG reagent. However, the ARC and Ortho IgG cells are better at detecting partial anti-IgG neutralization which can occur because of poor washing technique or a defective automated cell washer. While strong reactions with IgG cells are comforting to see and may make a more commercially attractive product, they may hide a partially neutralized anti-IgG.

†Checkcell (weak)

‡4+=4, 3+=3, 2+=2, 1+=1, +=0.5