D ANTIGEN DENSITY AND D ZYGOSITY OF REAGENT RED CELLS ARE NOT DETERMINANT FOR ANTI-D SEMIQUANTIFICATION BY STANDARD SEROLOGY TITRATION TECHNIQUES

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Background: Semiquantification of Anti-D alloantibodies (alloD) by titration techniques is widely used for diagnostic and surveillance porposes of individuals carrying alloD. Especially for surveillance of alloD during Rh incompatible pregnancy, the increase of alloD titre may be indicative for interventional decision making. Therefore, reliable laboratory techniques providing comparable results over time are pivotal for alloD surveillance. Since Rh positive reagent red cells (RRC) from the same donor may not be available throughout the entire course of pregnancy, RRC from various donors might be used for alloD surveillance. Therefore, quality issues and inherent differences of D epitope density (DED) of RRC may become an issue. To investigate the contribution of RRC properties on alloD titre, we compared alloD titre of 6 patient's sera assessed by using 8 different RRCs.

Methods: RRC with Rh phenotypes R1R1 (CCDDee, 3 donors) or Ror (CcD.ee, 5 donors) were used to titre 6 patient's sera containing alloD exclusively. Serology testing was carried out by ID-gel sedimentation techniques (DiaMed, Cressier) as well as by tube techniques applying IAT/LISS milieu. DED of RRC was established by FACS (Quanticalc, BectonDickinson) using Anti-D (monoclonal IgG, clone H4111B7, Biotest AG) as 1st antibody and goat-anti-human IgG F(ab)^2, Jackson Laboratory as 2nd antibody. D zygosity status of RRC was evaluated by commercial PCR-SSP (Innotrain) using donor's buffy coat DNA.

Results:

Figure 1: D epitope density of 8 individual RRC donors

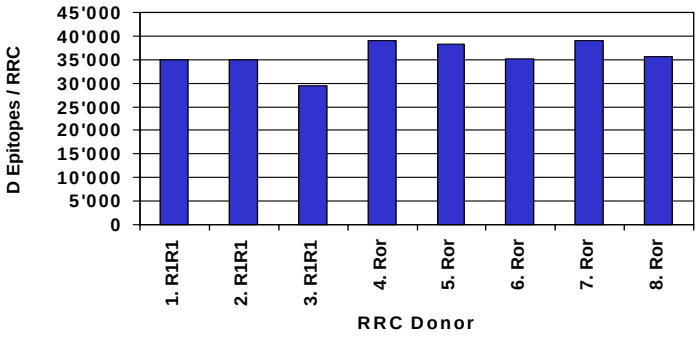
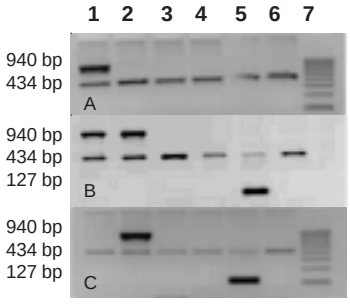


Figure 2: D-Zygosity by PCR-SSP of Rh-Box



Lane 1: Hybrid Rh-box, indicating complete deletion of D gene (d status)
Lane 2: Upstream Rh-box, indicating presence of D gene (D status)
Lane 3: Rare variant of D (PSE)
Lane 4: CE-D^s (305bp) and ψD (142bp), not present
Lane 5: Intronic polymorphism i8-32T
Lane 6: Intronic polymorphism i8-32C, not present. i8-32T and i8-32C confirm the presence of D gene
Lane 7: Molecular marker, 100-1000 bp
434 bp: Amplification control

A: Rhesus dd B: Rhesus Dd C: Rhesus DD

Table 1: D-Zygosity of 8 individual RRC by PCR-SSP

1.R1R1	2.R1R1	3.R1R1	4.Ror	5.Ror	6.Ror	7.Ror	8.Ror
DD	DD	DD	Dd	Dd	Dd	Dd	Dd

Table 2: Anti-D titre of various sera using 8 different RRCs

A: by ID-Gel Techniques

Serum 10005108	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048	1:4056
1.R1R1	4	4	4	4	4	4	3	3	2	1	1*	0	0
2.R1R1	4	4	4	4	4	4	3	3	2	1	1*	0	0
3.R1R1	4	4	4	4	4	4	3	3	3	2	1	1*	0
4.Ror	4	4	4	4	4	4	3	3	2	1	1*	0	0
5.Ror	4	4	4	4	4	4	3	3	2	1	1*	0	0
6.Ror	4	4	4	4	4	4	3	3	2	1	1*	0	0
7.Ror	4	4	4	4	4	4	3	3	2	1	1*	0	0
8.Ror	4	4	4	4	4	4	3	2	2	1	1*	0	0

Serum 10007118	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512
1.R1R1	3	3	3	3	3	3	2	1	1*	0
2.R1R1	3	3	3	3	3	3	2	1	1*	0
3.R1R1	3	3	3	3	3	3	2	1	1*	0
4.Ror	3	3	3	3	3	3	2	1	1*	0
5.Ror	3	3	3	3	3	3	2	1	1*	0
6.Ror	3	3	3	3	3	3	2	1	1*	0
7.Ror	3	3	3	3	3	3	2	1	1*	0
8.Ror	3	3	3	3	3	3	2	1	1*	0

Serum 10004053	1:1	1:2	1:4	1:8	1:16	1:32
1.R1R1	2	2	2	1	1*	0
2.R1R1	3	3	2	1	1*	0
3.R1R1	3	2	2	1	1*	0
4.Ror	2	2	2	1	1*	0
5.Ror	2	2	2	1	1*	0
6.Ror	3	2	2	1	1*	0
7.Ror	2	2	2	1	1*	0
8.Ror	3	2	2	1	1*	0

Anti-D titre of a given serum is not dependent on individual RRC used. Numbers stand for hemagglutination strength of RRC.
By convention: 0 (no agglutination) to 4 (max. agglutination)
*very weak reaction

B: by Tube Techniques

Serum 10006891	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048	1:4096
1.R1R1	3	3	3	2	2	2	2	2	2	1	1*	0	0
2.R1R1	3	3	3	2	2	2	2	2	2	1	1*	0	0
3.R1R1	3	3	3	2	2	2	2	2	2	1	1*	0	0
4.Ror	3	2	2	2	2	2	2	2	2	1	1	1*	0
5.Ror	3	3	2	2	2	2	2	2	1	1	1	1*	0
6.Ror	3	3	3	2	2	2	2	2	2	1	1	1*	0
7.Ror	3	3	2	2	2	2	2	2	1	1	1	1*	0
8.Ror	3	3	2	2	2	2	2	2	1	1	1	1*	0

Serum 10005390	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024
1.R1R1	3	3	3	3	2	2	2	1	1*	0	0
2.R1R1	3	3	3	3	3	3	2	1	1*	0	0
3.R1R1	3	3	3	3	3	3	2	1	1*	0	0
4.Ror	3	3	3	2	2	2	2	2	1	1*	0
5.Ror	3	3	3	2	2	2	2	1	1	1*	0
6.Ror	3	3	3	3	2	2	2	1	1	1*	0
7.Ror	3	3	3	2	2	2	2	1	1	1*	0
8.Ror	3	3	3	3	2	2	2	1	1	1*	0

Serum 10007118	1:1	1:2	1:4	1:8	1:16	1:32	1:64
1.R1R1	3	3	2	1	1*	0	0
2.R1R1	3	2	2	1	1*	0	0
3.R1R1	3	3	2	1	1*	0	0
4.Ror	3	2	2	1	1	1*	0
5.Ror	3	3	2	2	1	1*	0
6.Ror	3	3	2	1	1	1*	0
7.Ror	3	2	2	1	1	1*	0
8.Ror	3	3	2	1	1	1*	0

ID-gel technique results in higher titre level of Anti-D as compared to tube technique (see serum 10007118).

*very weak reaction

Conclusions:

1. **Variable DED of RRC does not influence significantly the titration of Anti-D by standard hemagglutination assays. Therefore, RRCs are interchangeable during follow-up examination of Anti-D**
2. **D homozygosity of R1R1 cells does not lead to higher expression of D epitopes on RRC and is not associated with arteficially high Anti-D titre. This may be explained by the suppression of D epitope expression in the presence of the Ce gene (Cepellini effect[1])**
3. **Major differences of Anti-D titre in the same serum are most likely explained by technically different methods applyed for Anti-D titration. Technical issues have to be considered for data interpretation and comparison**

1) R. Cepellini, L.C. Dunn, M.Turri: An interaction between alleles at the Rh locus in man which weakens the reactivity of Rho factor (Du). PNAS, 1955; 41: 283 - 288

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