

# Further Identification of Anti-CD Antibodies by Adsorption-Elution and Titration

## 1. Abstract

Normally, no further testing is performed after both anti-D and anti-C antibodies have been identified in a patient's serum. If, instead of a real anti-D antibody, there is an anti-G antibody present in the serum, Rh immune globulin (RhIG) has to be administered to prevent immunization against RhD and the resulting haemolytic disease of the foetus and the newborn (HDFN). The adsorption-elution method and antibody titers are ways to test sera containing anti-D and anti-C for the presence of anti-G. Adsorbates and eluates showed that many anti-CD antibodies are not correctly diagnosed during routine antibody identification. Many anti-G antibodies are present, yet not detected. A comparison of antibody titers has also shown that with high anti-C and low anti-D titers the presence of anti-G is more likely- either alone or in combination with anti-C. With pregnant females, high anti-C titers should always be further investigated to make sure that clinically relevant antibody constellations (anti-G alone or in combination with anti-C) are not missed.

## 2. Introduction

The G-antigen belongs to the Rh blood group system. It's an antigen that is present on almost all cells that are either D+, C+ or both D+ and C+. Therefore, only cells that are D- and C- lack the G-antigen.<sup>1</sup>

The G-antigen is not a compound antigen (like e.g. the f-antigen which is present on any cell that has both the c- and the e-antigen). This means that the anti-G antibody reacts with cells that are C+ and D+ (e.g. CcD.ee), but also with cells that are only C+ (e.g. Ccddee) or D+ (e.g. ccD.ee) respectively.<sup>2</sup>

So if exposed to D+ and/or C+ blood during blood transfusion or pregnancy, a D-C- patient might start to make antibodies: anti-D and/or anti-C and/or anti-G (which would appear as anti-D and anti-C combined). If the identification of anti-D and anti-C antibodies is the result of a prenatal screening, further testing is necessary to verify or rule out the presence of a genuine anti-D antibody. The different antibodies in the serum have to be isolated by adsorption and elution procedures. After that, the antibodies in both the eluates and the adsorbates can be identified once more.<sup>1</sup>

According to several studies, an anti-G antibody could also cause mild forms of HDFN. An immunization with anti-D would lead to much more serious complications such as very severe cases of HDFN.

## 3. Aims and Formulation of Questions

The main aim of this project was to find out how many of the collected sera, which were diagnosed with anti-CD antibodies, actually contained anti-G antibodies.

- Is the specificity for anti-CD correct or is there a mix of different specificities?

Additionally, it was also tested if there was a specific connection between the titers of the antibodies and their presence in the sera.

- Is it really useful to test and compare the titers in order to rule out a genuine anti-D antibody and therefore to confirm the presence of an anti-G antibody?

## 4. Materials and Methods

To separate the antibodies in the samples, the adsorption-elution method was used. First, the antibodies were adsorbed to specific RBCs (R2R2 and r'r) and then again removed from the membranes by acid elution.

To identify the antibodies in adsorbates and eluates the gel centrifugation (ID-card system) was used. Three test cells were used: a R2R2 test cell (D+, C-, G+) which reacted with anti-D and anti-G, a r'r test cell (D-, C+, G+) which reacted with anti-C and anti-G and, as a negative control, a rr test cell (D-, C-, G-).

The different antibody combinations could then be identified according to their specific reaction pattern after adsorption and elution (shown in table 4.1).

The ID-card system was also used to determine the anti-D and anti-C titers.

| Antibodies | R2R2           |           | r'r            |           |
|------------|----------------|-----------|----------------|-----------|
|            | Eluate         | Adsorbate | Eluate         | Adsorbate |
| C + D      | D              | C         | C              | D         |
| C + G      | G<br>(= C+D)   | C         | C+G<br>(= C+D) | -         |
| D + G      | D+G<br>(= C+D) | -         | G<br>(= C+D)   | D         |
| C + D + G  | D+G<br>(= C+D) | C         | C+G<br>(= C+D) | D         |
| G          | G<br>(= C+D)   | -         | G<br>(= C+D)   | -         |

Table 4.1 Antibody pattern after adsorption and elution

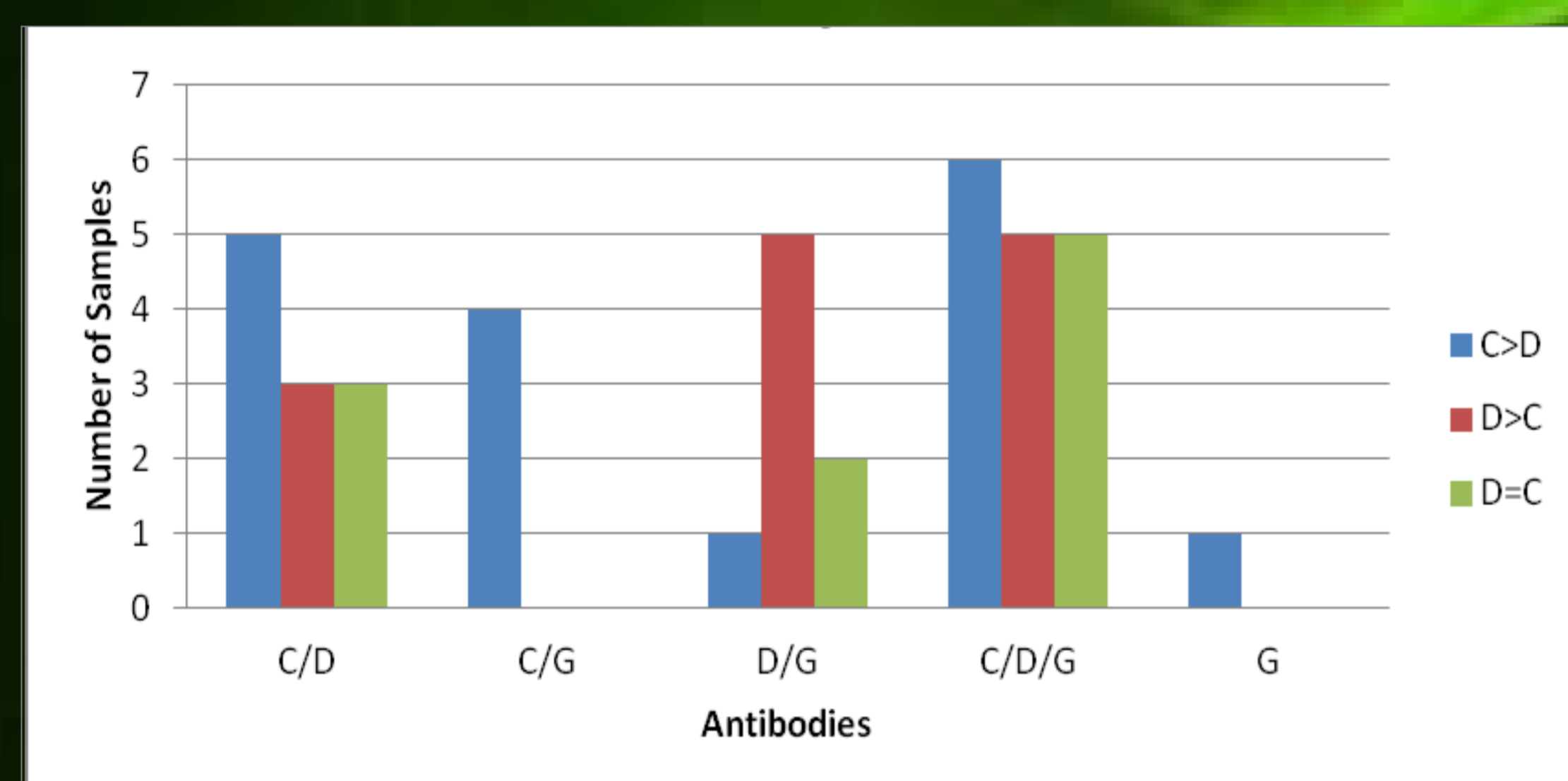


Figure 5.2 Bar chart of antibody titers

## 5. Results

The results of the adsorptions and elutions show that 30 out of the 46 samples (65%) contain an anti-G antibody – either alone or in combination (see table 5.1 and figure 5.1)

The results of the antibody titers showed that a higher anti-C titer is not necessarily an indication for the presence of an anti-G antibody. Higher anti-D titers on the other hand excluded the presence of anti-G alone or in combination with anti-C (see figure 5.2).

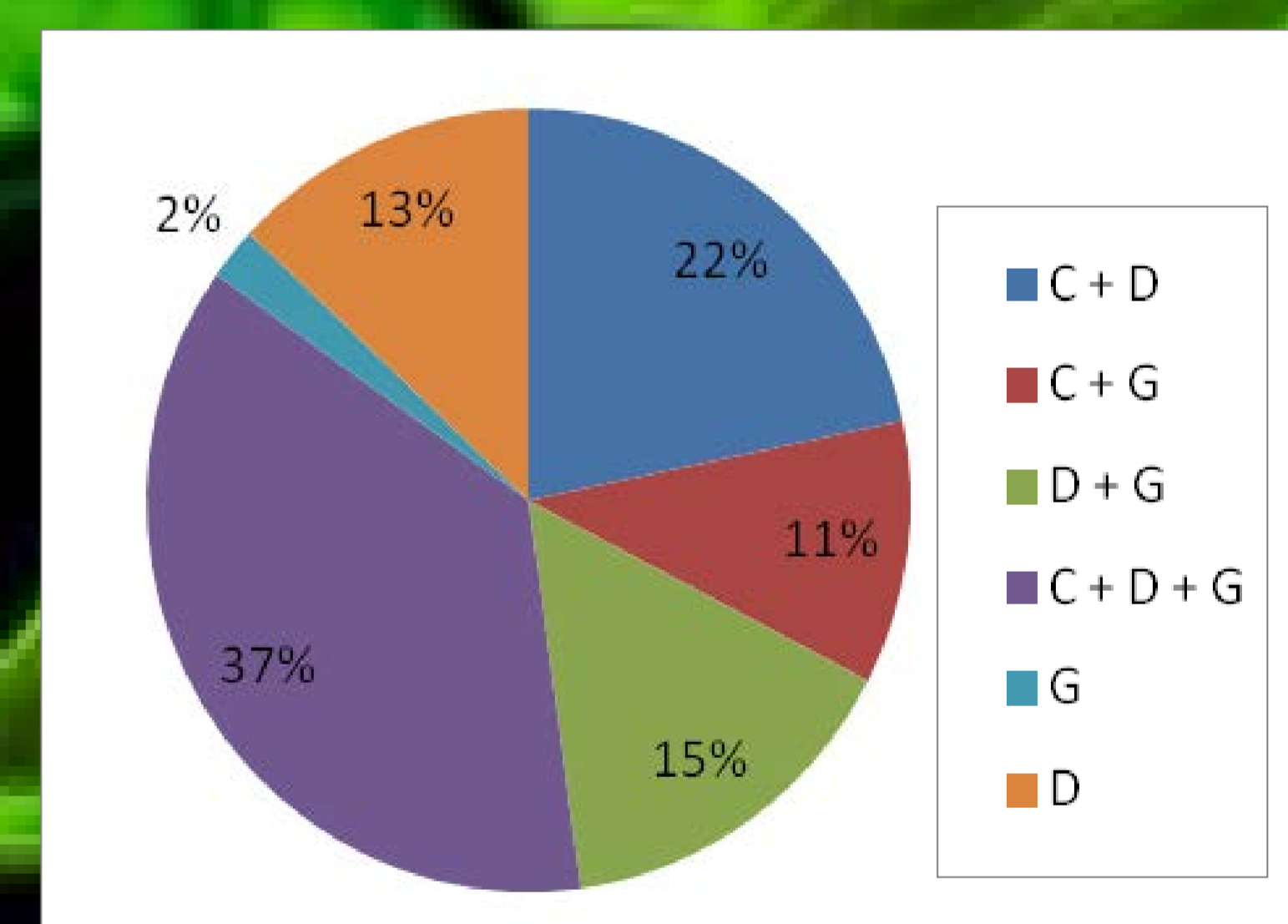


Figure 5.1 Pie chart of results adsorbates / eluates

| C + D | C + G | D + G | C + D + G | G | D |
|-------|-------|-------|-----------|---|---|
| 10    | 5     | 7     | 17        | 1 | 6 |

Table 5.1 Results adsorbates and eluates

## 6. Discussion

According to the results, not even 25% of all the samples were diagnosed correctly. This is not really a problem since D- C- blood would be administered anyway. So the "hidden" anti-G antibodies do not really matter in those cases.

Clinically relevant and misdiagnosed are 13%: samples containing anti-G alone and samples containing anti-C and anti-G combined, with no real anti-D antibody. These constellations are only relevant with pregnant females with a Rh negative blood type. In such a case, it is necessary to administer RhIG to prevent alloimmunization against RhD.

Titer testing is sometimes used to determine the presence of an anti-G antibody in the serum. In patients with anti-C and anti-D antibodies, a high anti-C and a low anti-D titer are supposed to suggest the presence of an anti-G antibody or a combination of anti-G and anti-C antibodies.

The results of this study show that samples that contain clinically relevant antibody constellations for pregnant women (anti-G alone or anti-G and anti-C combined) all have higher anti-C titers. But also samples containing real anti-D antibodies can have high anti-C titers. Therefore no connection between antibody titers and specific antibody combinations in the serum was found.

The results also showed that with anti-D titers that were either higher than or equal to the anti-C titers, genuine anti-D antibodies were present.

### List of References

- <sup>1</sup> Chaffin, J. [Status 13.12.2013] The G antigen and Anti-G. <http://www.bbguy.org/education/gandanti-G.asp>  
<sup>2</sup> Thomas, L. (2005) Labor und Diagnose. Frankfurt/Main. TH-Books Verlagsgesellschaft

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