

# Gel Test Assay for IgG Subclass Detection by GM Typing: Application to Hemolytic Disease of the Newborn

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The gel test assay was evaluated for IgG subclass detection by GM typing of antibodies and compared to the classical inhibition agglutination method on slides or microtiter plates. We used a panel of 5 murine monoclonal antibodies directed against G1M(1), G1M(3), G1M(17), G2M(23), and G3M(21) and 1 human polyclonal anti-G3M(5) antibody. Eleven polyclonal antisera (of immunized women) directed against red blood cells were tested for the GM allotypes car-

ried by their alloantibodies. We controlled the specificity of the gel test reaction using a panel of anti-RH(D) monoclonal antibodies. All reagents exhibited a good reactivity and specificity. They can be used for routine typing. The gel test assay for IgG subclass detection is a specific, simple, and low-cost technique for the detection and management of severe forms of diseases in alloimmunized pregnancies. *J. Clin. Lab. Anal.* 14:1–4, 2000. © 2000 Wiley-Liss, Inc.

**Key words:** immunohematology; agglutination assays; maternal antibodies; subclassing IgG alloantibodies; Rh disease

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## INTRODUCTION

The management of hemolytic disease of the newborn (HDN) includes various immunological tests to predict the harmfulness of maternal antibodies, and attempts to establish with obstetrical data the right moment for invasive exploration or treatment. Beside antibody titration and quantitation, and cellular bioassays, IgG subclassing by qualitative agglutination techniques (flow cytometry, ELISA assay, or gel test using rabbit anti-IgG subclasses sera) have been used (1–4). All those techniques sometimes lead to conflicting results that must be interpreted with caution, and this is particularly true for the techniques using polyclonal anti-IgG subclass reagents which, in certain cases, can react with two subclasses.

In our laboratory, we use anti-GM alloantibodies for IgG subclassing (5,6). We previously demonstrated a correlation between the severity of HDN and IgG1 subclasses (40%) versus IgG3 (13%). Among IgG1, severe forms were associated with IgG1 carrying G1M(3) allotype (69.5%), as compared with IgG1 carrying G1M(1) allotype (31%). Moreover, an association of several subclasses correlates with more severe prognosis.

IgG subclasses are marked by GM allotypes (7). GM allotypes are restricted to one subclass and located on the heavy chains of the IgG1: G1M(1,2,3,17), IgG2: G2M(23) and IgG3: G3M(5,6,10,11,13,14,15,16,21,24,26,27,28). Generally, the GM system is not fully explored, due to the reading difficul-

ties in the classical method of agglutination on slides or microtiter plates and to the difficulty in finding good typing reagents for certain specificities. But now, we have at our disposal commercially available murine monoclonal antibodies against Ig allotypes to define, in most of the cases, the IgG subclass for G1M(1), G1M(2), G1M(3), G1M(17), G2M(23), G3M(5,26), G3M(16), and G3M(21) (8). Evaluation of those monoclonal antibodies has been made (9,10).

GM typing with the use of the classical method of agglutination on slides or microtiter plates requires specialised teams. It is very difficult to put into practice as a routine test and to introduce in a biological laboratory of blood transfusion. So, we propose a gel test procedure to define the IgG1, IgG2, and IgG3 subclasses through their allotypic markers and suggest to apply that simple and fast technique to GM typing in HDN (11,12). The aim of our work was to test the feasibility and properties (specificity and sensitivity) of a panel of serumtests (monoclonal and polyclonal anti-GM) using that technique. We compared it with our usual test on slides or microtiter plates. We propose to integrate it for GM allotype

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determination of maternal antibodies in the routine HDN management.

## SUBJECTS AND METHODS

Serum samples from immunized women were not chosen for obstetrical data, but for their GM phenotypes and the GM expressed by the IgG subclasses of their alloantibodies. Most of the cases had high antibody titers and the newborn had a positive direct antiglobulin test. They were previously analyzed in the Foeto-maternal Immunology Laboratory, ETSPG Toulouse (Dr. S. Alié-Daram).

### GM Phenotype of Patients and Their Alloantibodies

Serum samples from each woman immunized against red blood cell (RBC) antigens, mainly RH(D), were typed for GM allotypes using the standard agglutination-inhibition method, as previously described (13,14). Sera were taken during pregnancy (weeks 16–38) and/or after delivery for clinical purpose, aliquoted, and stored at  $-30^{\circ}\text{C}$  (with  $\text{NaN}_3$  at 0.2%). Eleven patients had been chosen. For each GM test, the technique was corroborated by using patient anti-RH (D)s producing both weak and strong reactions according to the classical agglutination method and polyclonal anti-GM allotype sera (standard technique on slides or microtiter plates). We must add that with some antibodies spontaneously agglutinating in saline, a pre-dilution is necessary before GM testing.

### Reagents

Murine monoclonal anti-GM were provided from CLB, Netherlands Red Cross, Amsterdam (Dr. G. de Lange): anti-G1M(1) MG-102-A1, anti-G1M(17) MG-101-A1, anti-G1M(3) MG-104-A1, and anti-G3M(21) MG-305-A1, and obtained from Sigma Immuno chemicals: anti-G2M(23) SH-21. All those reagents are commercially available. Anti-G3M(5) (available from Dr. J.M. Dugoujon by request), from a male blood donor, was chosen after evaluation of its reactivity and specificity using various techniques.

### Gel Test Typing

Gel test typing was performed using neutral gel cards (for NaCl, enzyme and cold agglutinin; DiaMed-ID). The RBCs were sensitized and resuspended as follows: one volume of group O R1R2 (DCe/DcE), washed six times, was sensitized with ten volumes of a one-half saline dilution of maternal (or monoclonal) serum. The washed sensitized RBCs were resuspended at 5% in saline. Twenty-five  $\mu\text{l}$  of RBC suspension was transferred onto the gel and 25  $\mu\text{l}$  of anti-GM was added. The gel cards were incubated at room temperature for 15 min, centrifuged at 84g for 10 min and the reactions were read using an ID-reader (ID-MATIC reader 009903). RBC-Ab complexes were tested with anti-GM reagents according to each

patient's phenotype. Positive and negative GM controls were added to each determination. Agglutination signified that the anti-RH antibody was marked by the corresponding GM allotype, thus leading to the definition of the IgG subclass. The specificity of anti-GMs was tested with RBCs sensitized with human monoclonal anti-Rh (D): MGan. and MLor., gifts of Pr. A. Blancher, Laboratoire d'Immunologie, Toulouse, France; M126 from Dr. D. Goossens, INTS, Paris, France; M10W2, from Dr. B. Kumpel, UK Transplant Service, Bristol, United Kingdom; and MD6DO2, from Dr. M. Uchikawa, C.B.J.R.C., Tokyo, Japan.

## RESULTS

The results of the gel test assay are shown in Table 1. We have identified the IgG subclasses for all the antibodies tested. A comparison with the standard method shows the superiority of the gel test: detection is easier and the results more clearcut. Moreover, some GM allotypes that were not detected with the classical method can be specifically identified.

## DISCUSSION

The aim of this work was not to study the significance of the IgG subclasses in the HDN, but to show the advantages of the gel test technique (compared to the classical test with slides, microtiter plates or tubes). The agglutination reactions of certain polyclonal anti-GM using the classical technique were weak; the reading is eye-dependent and therefore very subjective and cannot be automated. Agglutination in the tube test, widely used in immunohematology, is not appropriate to GM typing because of the small size of the agglutinates, and the high consumption of rare and expensive reagents.

In the gel test assay, all monoclonal anti-GMs exhibited good reactivity and specificity and can be used for routine typing. Monoclonal antibodies were also chosen for their commercial availability, which makes it possible to perform the gel test in all laboratories that are involved in HDN management. However, in the technique, monoclonal anti-G3M(5,26) MG-303-A1 (Netherlands Red Cross, Amsterdam) showed non-specific reactions with other GM allotypes. So, we replaced it for G3M(5) typing with a polyclonal anti-allotype with no cross-reaction. It is interesting to note that the gel test detected an allotype (G1M(17)) not revealed by the classical method on slides using a human polyclonal anti-GM (e.g., Bouk. anti-Rh(D)). The allotype detected was, however, present in the GM phenotype of the patient. This may be due to the high titer of the Amsterdam monoclonal reagents and the gel test technique itself that is known to have higher sensitivity. It should also be stated that with certain monoclonal anti-Rhesus we observed slight nonspecific reactions, but only rarely: such was the case with M10W2 in our gel test assay. The same phenomenon had been previously observed with the same reagent using the classical method (15).

TABLE 1. Gel test<sup>a</sup> assay

RBC- anti-Rh(D)	Gestation weeks	Titer IAT <sup>b</sup>	Serum phenotypes	Anti-Rh allotypes <sup>c</sup>	Anti-GM tested in gel test					
					G1M(1) 1/3,000	G1M(17) 1/3,000	G1M(3) 1/300	G2M(23) 1/3,000	G3M(21) 1/300	G3M(5) 1/10
Monoclonal										
M Gan.		*	1,2,3,17,5* <sup>d</sup> ,21,28	1,2,17	++++	++++	-	-	-	-
M Lor.		*	3 5*	3	-	-	++++	-	-	-
M 126			ND <sup>e</sup>	5*	-	-	-	-	-	++++
M 10W2			ND	21,28	-	+	-	-	++++	-
M D6D02			ND	10,11,13,15,16	-	-	-	-	-	-
Polyclonal										
Del.	NP	32	3 23 5*	23	-	-	-	++++	-	-
Ben.	36	512	3 5*	3	-	-	++++	-	-	-
Ant.	37	128	1,2,17,21,28	21,28	-	-	-	-	+++	-
Adj.	35	64,000	1,3,17 5*	3,5*	-	-	+++	-	-	++++
Boua. <sup>f</sup>	37	512	1,3,17 5*, 21,28	1,17	++++	++++	-	-	-	-
Dau.	34	256	3 5*	3,5	-	-	+++	-	-	++
Gas. <sup>f</sup>	34	512	3 5*	3	-	-	++++	-	-	-
Orf.	36	512	1,17 21, 28	1,17	++++	+++	-	-	-	-
Bouk.	30	2,048	1,3,17 5*, 21,28	1,21	+++	+	-	-	++	-
Lai. <sup>g</sup>	38	128	1,2,3,17 5*, 21,28	Undefined	+	-	+	ND	-	-
SMa.	35	2,048	1,2,3,17 5*, 21,28	Undefined	-	-	-	ND	-	-

<sup>a</sup>Gel test: R1R2 RBC were sensitized with anti-Rh(D). Twenty-five µl of a dilution of anti-GM were incubated with an equal volume of a 5% suspension of sensitized RBC in saline, for 20 mn at 22°C in a neutral card. Cards were then centrifugated at 70g for 10 min. An ID-reader was used to read the reactions.

<sup>b</sup>IAT: Indirect antiglobulin test was performed using serum dilutions in saline and tested by indirect antiglobulin technique using normal O R1R2 RBC.

<sup>c</sup>GM allotypes detected by classical method on slides with polyclonal anti-GM.

<sup>d</sup>5\* = 5,10,11,13,14.

<sup>e</sup>ND, not done.

<sup>f</sup>anti-Rh(D+C+Jka).

<sup>g</sup>anti-Rh(D+C).

The gel test can be easily applied to GM typing of maternal antibodies, allowing a better understanding of the disease and helping to manage the haemolytic disease during pregnancy. The survey of GM typing during alloimmunized pregnancies can give information on the number of clones involved in the maternal antibody population, even if it is possible for several clones of antibodies to carry the same allotypes (1). Particular care is necessary if a new clone appears (even if the Coombs titration or antibody weight is still unchanged). The test makes it also possible to define the maternal GM allotypes on the cord RBC (sensitized in vivo).

The gel test assay for IgG subclass detection is a specific, simple, and low-cost technique, using a very small quantity of serum. It makes it possible to define the subclass of the antirhesus in practically all cases. The technique, automatable, can be introduced in routine transfusion practice in all laboratories involved in the management of HDN; thus, invasive explorations or treatments such as amniocentesis, fetal blood sampling, or intrauterine transfusion (which may cause preterm labor, infection, or lead to a fetal death), can be avoided or at best delayed.

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