botic risk factors have a higher risk of recurrence is important in order to design appropriate therapeutic trials.

A family history of obstetric complications is significantly associated with IUFD.

Our data seem to indicate that there is not a higher risk of recurrence in women with a previous IUFD and an inherited thrombophilia. Moreover, this study suggests that recording the family history has an important role in choosing women who need screening for thrombophilia.

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The gel enzyme technique in pretransfusion antibody screening

Some authors report that the sensitivity and specificity of the enzyme gel technique are comparable to those of enzyme tube tests,[1] while others present contrasting results.[2] However, studies using gel enzyme techniques have not been commonly performed. The aim of this study was to report our experience using a gel enzyme test as a supplementary method in a routine setting.

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We studied 12,789 sequential samples submitted to our laboratory for pretransfusion testing. Sera were tested by a gel indirect antiglobulin test (G-IAT) and a gel papain technique (G-PT) according to the manufacturer's procedure (Diagnostic Grifols S.A., Spain). Antibody screening was performed using a commercial panel of two untreated and papain-treated red blood cell (RBC) samples. A commercial 11-cell panel was used for antibody identification studies.

Positive results were found in 283 (2.2%) of the 12,789 samples. Irregular red blood cell antibodies were identified in 160 (1.2%) samples. Positive results were due to autoantibodies in 66 (0.5%) samples. Non-specific reactions (positive screening test followed by inconclusive results using an identification method) or false-positive results (positive screening test followed by a negative investigation) were found in 68 (0.5%) cases.

The 160 positive samples were from 91 patients and contained 192 alloantibodies: one antibody in 80 patients, two anti-

Table 1. Cumulative specificity of RBC alloantibodies.

Single antibody	*Number of patients*	*Number of samples*	*Two antibodies*	*Number of patients*	*Number of samples*
Anti-C	1*	2 (2*)	Anti-C+D	8	13
Anti-D	17	25	Anti-D+E	1	1
Anti-E	21	38	Anti-D+Le[a]	1	1
Anti-c	2	2	Anti-c+E	1	1
Anti-e	1	1	Anti-E+K	5 (1*)	12 (6*)
Anti-K	19	32	–	–	–
Anti-Jk[a]	8 (1*)	16 (2*)	–	–	–
Anti-Fy[a]	1	1	–	–	–
Anti-S	2 (1*)	2 (1*)	–	–	–
Anti-Le[a]	4	6	–	–	–
Anti-Le[b]	1	2	–	–	–
Anti-M	2	2	–	–	–
Anti-P	1	1	–	–	–

Multiple samples	*Number of antibodies*	*Number of patients*
Anti-C+D+E	2	2

**Autoantibody in association with alloantibody.*

Table 2. Serologic behavior of alloantibodies.

	G-IAT + G-PT	G-IAT	G-PT
Anti-D	40	1	1
Anti-C	14	–	3
Anti-E	28	–	26
Anti-c	3	–	–
Anti-e	1	–	–
Anti-K	10	34	–
Anti-Jk^a	4	12	–
Anti-Fy^a	–	1	–
Anti-S	–	2	–
Anti-Le^a	1	–	6
Anti-Le^b	–	2	–
Anti-M	–	2	–
Anti-P^1	–	–	1
	101	54	37

bodies in 16 patients and three antibodies in 2 patients. Additional alloantibodies were identified in six patients during the study period. The cumulative antibody specificities are presented in Table 1. Table 2 depicts the specificities of the antibodies identified by G-IAT and/or G-PT. As shown, except in 7 cases, all the alloantibodies that were active only by the enzyme test, were in the Rh blood group system. In the patient in whom the anti-D antibody reacted only in the G-PT, the antibody later became reactive by G-IAT as well, despite the fact that only ccddee units had been transfused. Two of the three anti-C that were active only by the enzyme test came from the same patient, a woman on a chronic transfusion regimen who had an anti-D antibody upon entering the study. She formed an additional anti-C antibody that initially reacted only in the G-PT, but later, after two transfusion episodes, became reactive in the G-IAT. Seventeen samples of enzyme-only anti-E antibodies were from 5 patients.

Red cell autoantibodies were identified in 66 serum samples from 27 patients who presented a positive direct antiglobulin test (11 IgG+C', 14 IgG and 2 C'). Four autoantibodies reacted in the G-IAT, 34 in the G-PT and 28 in both tests. Antibodies active in the G-PT and/or G-IAT were warm autoantibodies and most of them reacted as a panagglutinin. Antibodies that were only detected by G-PT were either cold or warm autoantibodies.

A total of 38 non-specific reactions were observed in 32 patients, 31 were obtained with the G-PT, 3 with the G-PT and G-IAT and 4 only with the G-IAT. Retrospective review failed to identify cases of delayed hemolytic reaction in transfused patients. Moreover, in 7 patients with non-specific reactions in whom follow-up studies were undertaken, no antibodies were detected one year later. False-positive screening tests were obtained in 30 sera. Nine of them occurred only with the G-IAT and the rest only with the G-PT.

Eighty-six unwanted positive reactions (34 autoantibodies, 31 non-specific reactions and 21 false positive results) were exclusively detected by G-PT.

Additional antibodies revealed by enzyme-treated RBCs often have Rh and Kell system specificities. These antibodies have been shown to be of virtually no clinical significance in the transfusion setting. The only exception would be the rare occasion when an enzyme only antibody represented the initiation of an immune response.[3] Our testing of 12,789 pretransfusional samples revealed 37 alloantibodies that reacted with papain-treated red cells but not by G-IAT. Almost all were in the Rh blood group system; the rest were antibodies not usually considered clinically significant. Enzyme-only Kell system antibodies were not encountered (27/36 anti-K antibodies reacted only in G-IAT). This may be explained by the fact that anti-K antibodies tend to react poorly in LISS. Our findings did not vary significantly from reported results using enzyme tube testing,[3] nevertheless, some differences deserve mention. The G-PT detected fewer Kell system antibodies and antibodies of minor clinical significance, and registered fewer unwanted positive reactions than the conventional serologic methodology.

The outcome of performing approximately 13,000 enzyme screening tests identified four enzyme-only antibodies (1 anti-D, 3 anti-C) that oblige the use of antigen-negative units since they were potentially clinically significant. Nevertheless, if routine enzyme screening had not been performed, patients with such antibodies might have received compatible blood, anyway, by chance. Instead of routine enzyme screening, a measure of safety for polytransfused and alloimmunized RhD-negative recipients is to provide units that are phenotype-matched for Rh system antigens. According to our findings, by so doing, the workload would be decreased without compromising the patients' safety.

Gel enzyme testing in our hands does not have the disadvantage of conventional enzyme testing in detecting many unwanted positive reactions but, as in the case of the tube test, the sensitivity of pretransfusional screening is only marginally improved.

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