Gel Test and column agglutination technology – Comparative study of two red cell antibody screening and identification systems

Geltest und Säulenagglutinations-Technik – Vergleichsuntersuchung zweier Testsysteme zur Erfassung und Differenzierung erythrozytärer Antikörper

H. Eichler, U. Klinge, V. Kretschmer

Summary

We describe the results of a study comparing the gel test (ID) and column agglutination technology (CAT) for red cell antibody (AB) screening. Both systems were tested under controlled routine conditions with the enzyme (bromelin) test (ET) and the LISS indirect antiglobulin test (IAT). Three thousand unselected patient blood samples were studied in parallel tests under strictly defined conditions.

A total of 64 AB (2.1 %) were detected, 63 by ID an 56 by CAT. The CAT-ET proved to be of much lower sensitivity than the ID-ET (36 to 50 AB detected). Less distinct differences were observed in the IAT: 6 of 53 AB found by the ID-IAT were not detected by CAT-IAT (anti-D 3, Jk(a)+E 1, e 1, Le(b) 1), while only one anti-Le(b) was found by CAT-IAT alone. Regarding the strength of the reaction, the ID system achieved better results than the CAT method. Positive reactions due to unspecificity or irrelevant cold antibodies were less frequent with ID than with CAT (2.3 % vs. 4.0 %). This occurred mainly with the enzyme test in both test systems.

In conclusion, the sensitivity (98.4 %) and specificity (97.7 %) of the ID gel test clearly exceeds that of the CAT test (87.5 %/96.2 %). However, after improvement and elimination of some disadvantages (e.g. more complicated handling and less stable agglutinates), CAT should be able to provide results comparable to those of the gel test.

Key words

gel test – column agglutination technology – red cell antibody

Zusammenfassung

Wir berichten über einen direkten Vergleich des Geltestes ID MicroTyping® (ID) mit dem Säulenagglutinationstest BioVue® (CAT) zur Erfassung irregulärer erythrozytärer Antikörper (AK) unter kontrollierten Routinebedingungen. 3000 unselektierte Patientenblutproben wurden parallel in beiden Systemen sowohl im Enzym(-Bromelin)test (ET) als auch im indirekten Antiglobulintest LISS-Technik (IAT) unter streng definierten Bedingungen untersucht.

Von den insgesamt 64 gefundenen AK (2,1 %) waren 63 in ID und 56 in CAT nachweisbar. Insbesondere der CAT-ET erwies sich als deutlich weniger sensitiv als der ID-ET (36 vs. 50 erkannte AK). Im IAT waren die Unterschiede weniger deutlich: 6 der 53 im ID-IAT gefundenen AK waren im CAT-IAT nicht nachweisbar (Anti-D 3, -Jk(a)+E 1, -e 1 und Le(b) 1), während ein Anti-Le(b) allein im CAT-IAT positiv reagierte. Auch im Vergleich der Reaktionsstärken zeigte sich ID empfindlicher als CAT: 44 %

Anschriften der Autoren:

Department of Transfusion Medicine, Marburg University Hospital, Germany

Korrespondenz-Adresse:

Prof. Dr. Volker Kretschmer, Klinikum der Phillips-Universität, Abt. für Transfusionsmedizin und Gerinnungsphysiologie, Conradistraße, D-35033 Marburg

Ullrich Klinge, Abt. für Transfusionsmedizin und Gerinnungsphysiologie, Phillips-Universität, Marburg

der AK-haltigen Seren reagierten um mehr als eine Reaktionsstärke-Stufe deutlicher im ID-IAT (36 % im ID-ET), der entsprechende Test in CAT reagierte dagegen nur in je 4 % stärker positiv. Positive Befunde, welche unspezifisch oder durch irrelevante Kälteantikörper bedingt waren, traten weniger häufig in ID als in CAT auf (2,3 vs. 4,0 %), wobei in beiden Systemen in erster Linie der Enzymtest betroffen war.

Mit dem Säulenagglutinationstest BioVue ist nun ein weiteres Testverfahren erhältlich, das in seiner Sensitivität dem konventionellen Röhrchentest deutlich überlegen zu sein scheint. Der direkte Vergleich mit dem ID-System zeigt jedoch eine deutlich geringere Sensitivität (ID: 98,4 %, BV: 87,5 %) und Spezifität (ID: 97,7 %, BV: 96,2 %). Nach einer methodischen Ausreifung des Systems – insbesondere bezüglich einer vereinfachten Handhabung und größeren Stabilität der Agglutinate im Gradienten – sollten jedoch gleichwertige Ergebnisse gegenüber dem ID-System erreichbar sein.

Schlüsselwörter

Geltest – Säulenagglutinations-Technik – erythrozytäre Antikörper

Introduction

The gel test has become a widely accepted method for identification of red blood cell antibodies (AB). In several comparative studies performed under routine conditions, the gel test proved to be more sensitive and more specific than the standard tube test [1, 2].

Column agglutination technology (CAT) is an alternative antibody screening method based on the same principle as the gel test [3]. In both systems serum samples are incubated with test cells in the top of a plastic microtube with a gradient and a diluent such as buffer or a high-density polymer mixed with antiglobulin serum. After centrifugation non-agglutinated cells form a discrete pellet at the bottom of the tube, whereas agglutinated cells are trapped in the gradient. The two systems utilize different gradients, i.e., sephadex gel in the gel test and glass bead microparticles in the CAT test.

In this study we screened a large number of fresh patient sera in parallel tests under controlled routine conditions in order to compare the sensitivity and specifity of the gel test and column agglutination technology.

Abkürzungen:

ID	= Geltest ID Micro-Typing®
CAT	= Säulenagglutinationstest BioVue®
AK	= Antikörper
ET	= Enzym(-Bromelin)test
IAT	= indirekter Antiglobintest LISS-Technik

Materials and Methods

Three thousand random serum samples from all patients who had received blood typing and compatibility testing at the Marburg University Transfusion Center were screened for the presence of irregular red cell antibodies. For this purpose, parallel ID gel tests (ID Micro Typing™, DiaMed, Bensheim, Germany) and column agglutination tests (BioVue™, Ortho Diagnostics, Neckargemünd, Germany) were performed. The sera were separated from fresh coagulated blood, stored at 4° to 8 °C and tested within 24 hours. The same cell populations were studied in all tests (Selectogen™, two test cell populations, Ortho Diagnostics). Additional patient cells were used as auto-controls. Antibody screening was performed by means of both the (bromelin) enzyme test (ET) with neutral test cards and the LISS indirect antiglobulin test (IAT) with cards containing polyspecific antiglobulin serum. After incubation at 37 °C for 15 minutes the samples were centrifuged in special equipment supplied with the two systems (ID gel test: 10 min at 70 × g, CAT: 2 min at 55 × g and 3 min at 199 × g). Positive results were graded on a scale of 1+ to 4+.

ID Micro Typing Screening Conditions

The serum samples were added to a 1 % LISS suspension of washed test cells and patient cells (auto-controls) according to the test instructions (sample volumes: see Table 1). An ID-specific bromelin solution was also used for the enzyme test (diluent 1, DiaMed).

BioVue Screening Conditions

Untreated test cells and a 3 % saline suspension of washed patient cells (autocontrols) were diluted 1:5 with LISS (OAES™, Ortho Diagnostics). An MT-6™ (bromelin) enzyme solution (Ortho Diagnostics) was used for the enzyme test. The manufacturer's instructions for antibody screening and identification

Table 1. Sample volumes used in the gel test and the CAT screening test (µl)

Gel test		IAT	ET
	cell suspension	50	50
	serum samples	25	25
	enzyme solution	–	25
CAT		IAT	ET
	cell suspension	40	40
	serum samples	40	40
	enzyme solution	–	40

were modified to make the procedure easier (sample volumes: see Table 1). A pilotstudy on 23 titred serum samples with irregular antibodies showed no affect on the sensitivity or specificity of the test system.

Retesting of positive samples

In order to identify the antibodies, all samples with positive screening in the ID gel test and/or CAT test were retested with 11 to 33 panel cells (Ortho Diagnostics, Baxter, Munich, Germany) as described above. Samples positive in both ID and CAT were retested via ID and the standard tube technique (TT), whereas samples positive in either ID or CAT alone were retested via both ID and CAT plus TT.

Antibody titration

45 sera containing the various antibodies (anti-D 15, –C 1, –C+D 1, –E 3, –c 1, –K 8, –Fy(a) 3, Le(a) 2, –Le(b) 1, –P_1 3, –S 2, –M 2, –N 1, –k 1, –Jk(a) + K 1) were titred geometrically with the sera of blood donors of blood group AB. ID gel tests and column agglutination tests via the indirect antiglobulin test (IAT) and the enzyme test (ET) were then performed as described above.

Results

Antibody screening

255 sera (8.5 %) tested positive by at least one test method. In 64 cases (2.1 %) relevant and possibly relevant antibodies (AB) were clearly identified, whereas 191 samples (6.4 %) tested positive due to unspecific reactions or irrelevant cold antibodies. Table 2 gives an overview of the 55 antibodies detected in both systems.

All anti-Kell, anti-Duffy and anti-Le(a) antibodies and most combinations of these tested positive in both systems. The antibodies detected by one system only are listed in Table 3.

Table 2. Relevant and possibly relevant antibodies (AB) detected in 55 sera by gel tests as well as by CAT (85.9 % from a total of 64 AB)

33 Rhesus:	D 20; E 8; C 1; C, D 2; D, E 1; C, D, E 1
2 Kell:	K 2
2 Duffy:	Fy(a) 2
4 Kidd:	Jk(a), E 2 Jk(a), E, S 1; Jk(a), K 1
4 MnSs*:	M 2; S 1; S, E 1
4 Lewis*:	Le(a) 4
4 P*:	$P_1$4
2 auto-AB:	warm auto-AB 2

* possibly relevant AB

Eight antibodies or antibody combinations were detected by the ID gel test alone. Six of these (mostly relevant antibodies) were detected in the IAT. Two antibodies were identified exclusively by ID-ET (anti-C 1, -E+S 1) while one anti-Le(b) was found only in the CAT-IAT.

Table 3. Antibodies (AB) only detected by one test system

Eight sera with AB detected by gel test only			
AB	detected in	IAT	ET
D 3		3	2
Jk(a), E 1		1	1
Le(b) 1		1	–
e 1		1	–
C 1		–	1
E, S 1		–	1
One serum with an AB detected by CAT only			
AB	detected in	IAT	ET
Le(b) 1		1	–

Regarding the strength of the reaction observed in both systems, 43 % (IAT) and 36 % (ET) of the samples were graded at least one point higher in ID than in CAT. The score of the CAT reaction was higher than that of the ID reaction in only 4 % of the samples. 45 sera with antibodies that tested positive by both CAT and ID were titred geometrically. In CAT score was at least one grade higher in 44 % of the IAT titers, whereas the ID test score was higher in 34 %. The ID test was much more sensitive in the enzyme test: 75 % of the samples showed titer endpoints more than one grade higher than those of the CAT test.

Unspecific reactions

Seventy (2.3 %) of the positive reactions in the ID gel test were shown to be unspecific or were caused

by irrelevant cold AB (Table 4). Of those reactions 69 % were obtained only in the enzyme test. 121 sera (4 %) were unspecifically positive in CAT tests. The number of sera testing positive even in the IAT was much higher in CAT (n = 51) than in ID (n = 22).

Table 4. Unspecific reactions and irrelevant cold antibodies (AB) observed by gel test and CAT

	n	%
Gel test	70	2.3
only ET	48	1.6
only IAT	12	0.4
IAT and ET	10	0.3
CAT	121	4.0
only ET	70	2.3
only IAT	33	1.1
IAT and ET	18	0.6

Sensitivity and specificity

The ID system proved to be more sensitive than the CAT system with respect to the total number of antibodies and the number of clinically relevant antibodies detected (Table 5). The higher sensitivity (ET+IAT) of the ID system correlated with a lower rate of false-positive results due to unspecific reactions or irrelevant cold antibodies.

Table 5. Sensitivity and specifity of the both test systems

ET + IAT	Sensitivity %	Specifity %
1. All AB (n = 64)		
Gel test	98.4	97.7
CAT	87.5	96.2
2. Only relevant AB (n = 48)		
Gel test	100	
CAT	85.4	

Particular problems

Such artefacts as annular test cell clots on top of the gradient were observed in both CAT and ID (1.6 to 0.5 %). These clots presumably consist of cells attached to fibrin that cannot pass through the gradient during centrifugation. Fortunately, this kind of reaction was easily identified by technician, and no retesting was required. In both systems false-positive results can also occur in very highly concentrated test cell suspensions. With a 7 % saline cell suspension, unspecific positive results were observed in 29 % (n = 100) of the auto-controls in CAT – IAT, as compared to less than 2 % in ID – IAT.

Discussion

Since Lapierre et al. [4] first introduced the gel test as a new method for detecting irregular red cell antibodies, comparative studies in which a large number of unselected fresh patient sera have been studied in parallel gel tests and standard tube tests (albumin and LISS methods) have been performed [1, 2]. In these studies the gel test proved to be significantly more sensitive than the tube test. The superior detection of relevant antibodies by the gel test was a particular advantage. In other studies using stored sera with antibodies of known specifity, the sensitivity of the gel test was found to be nearly the same or slightly lower than that of the tube test [5, 6]. It is relatively difficult to evaluate such studies correctly, because the test method by which the antibodies were detected for the first time enjoys certain advantages. Similar studies where stored sera were tested via column agglutination technology (CAT) and the tube test show that the sensitivity of the two systems is almost identical [3, 7]. Because of the methodological similarity of the gel test and CAT, a basic comparative investigation seemed to be necessary to answer the question of which test method is the most sensitive. In order to give both methods an equal chance to detect unknown antibodies, we decided to screen unselected and unfrozen sera in parallel tests.

The data show that the sensitivity of the gel test to detect red cell antibodies clearly exceedes that of the CAT test. Five relevant antibodies which reacted in the IAT (anti-D 3, – Jk(a), E 1, – e 1) were detected only by the gel test. However, in another study designed along similar lines (1), the CAT test seems to be clearly more sensitive than the standard tube test (for all antibodies detected: 87.5 % compared to 63.3 % [enzyme test 37 °C+IAT]).

New findings suggest an important role of socalled "enzyme only" red cell antibodies in the safety of transfusions [8]. Thus, the detection of "enzyme-only" antibodies as an evaluation criterion for screening systems becomes less important. On the other hand, a test able to detect such antibodies should not automatically be considered a bad test, especially when it does not cause too many artefacts. Noting the presence of such antibodies does not usually cause problems in the finding of compatible blood. Furthermore this could possibly even avoid the shift towards IAT-reactive immune antibodies. Tests (i.e. the solid phase Capture R™ test) which detect only IgG antibodies and possibly show a higher sensitivity are already available [9]. So far, it is not known if this kind of test can detect relevant complement-dependent IgG antibodies and thus prevent dangerous boosts, or if the portion of unspecific reactions is tolerable. Further detailed information will be necessary to answer these questions.

The titer endpoints of 75 % of tested sera in the enzyme test were higher by at least by one grade level in the gel test than in the CAT. Because such a difference in sensitivity was not observed in the IAT, the discrepancy in the results in the enzyme test is presumably attributable to the different enzyme solutions used. Thus, better results in the CAT system may be expected in the future. The distinct advantages of the ID gel test over the CAT test are, in our opinion, that weak positive reactions are more easily noted since the gradient medium is clearer, and that agglutinates are more stable (ID: days; CAT: less than two hours). Furthermore, the CAT system becomes more complicated when carrying out the recommended standard test, i.e. the twostep pipetting of LISS and cell suspension (only 10 µl!). Futhermore, the incidence of false-positive results in very highly concentrated test cell suspensions is lower in the gel test. In this case, some test cells do not travel fully through the gradient to reach the tube bottom during centrifugation. Thus, these "trapped" cells can easily be mistaken for agglutinates. Pilottests have shown that the gel test is better able to cope with highly concentrated cell suspensions than the CAT test, probably because its centrifugation procedure is twice als long. When using column agglutination technology the cell concentration must be kept strictly constant to avoid artefacts and time-consuming retests. Nevertheless, the CAT test should be able to produce results comparable to those of the gel test after some of the above-mentioned disadvantages have been overcome.

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