

Process for demonstrating erythrocyte agglutination for the analysis of blood compatibility.

- 5 The invention concerns a process for demonstrating erythrocyte agglutination as used in transfusion immunohaemotology, in pregnancy follow up and in diagnostic, which provides a significant increase of sensitivity and reliability as compared with other known methods.
- 10 The decrease of the frequency of blood transfusion and mother-foetus incompatibility accidents results from the large amount of compatibility tests performed and from the constant improvement of their specificity and reliability.
- 15 Those tests whose number and implementation conditions are fixed by law include ABO and standard Rhesus cross-matching, search for irregular anti-erythrocyte antibodies, compatibility tests and precise determination of erythrocyte phenotypes.
- 20 - Tests for ABO and standard Rh blood cross-matching include a "corpuscle test" for the detection of antigens on the erythrocyte (or red blood cells or corpuscles) surface with standardized antisera and, for the ABO group, a "serum test" for the detection of antibodies present in the blood and directed against the erythrocyte antigens using test-
- 25 erythrocytes.
- 30 - Search for irregular antibodies, i.e. antibodies other than anti-A and anti-B, and which are not common to the global population, represents the basis of immunological security of transfusions and of prevention of mother-foetus allo-immunization accidents.
- 35 - Precise determination of erythrocyte phenotypes of donor and receptor is required when irregular antibodies have been detected in the receptor ; it is recommended for patients who need frequent transfusions ; it is mandatory for pregnant women. Indeed in case of transfusion need, the patient must imperatively be transfused with phenotyped blood (donor's blood) i.e. devoid of the antigen



corresponding to the detected antibody and compatibilized in the laboratory.

These various tests are performed according to conventional immunohaematology techniques that include demonstrating erythrocyte agglutination (due to antigen-antibody reaction),

- either direct, in saline medium, optionally after enzymatic treatment of the erythrocytes (with trypsin, papain or bromelin) whose effect on the membranes decreases the spontaneous repulsion between erythrocytes (due to negative charges of the carboxylic residues of sialic acid molecules present on the erythrocyte surfaces),
- or indirect, i.e. in two steps, first by contacting the erythrocytes with the antibodies (which are not necessarily agglutinating), followed by their agglutination by adding polyvalent antiglobulin. This reaction is performed in the presence of anti-complement. This technique known as "indirect Coombs" is very time consuming and tricky and involves many possible errors during implementation or reading of the results.

The field of use of these tests includes the search for irregular agglutinins, the tests for pre-transfusion compatibility and mother-foetus compatibility, various diagnostics of hemolytic accidents (hemolytic disease of the new born, auto-immune hemolytic anemia, post-transfusion hemolysis...).

The large number of tests performed, at present, and legally required for pre-transfusion safety as well as for pregnancy follow-up renders highly desirable further search for technical improvements in order to facilitate implementing and reading of the test and to increase the reliability of the result, at the same time.

A significant improvement has already been brought by the use of gellified medium and of gentle centrifugation in order to separate corpuscles from serum (Mollison - Blood transfusion in clinical medicine - ed Blackwell - Oxford - 1983 - p.512).

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A further improvement was obtained by applying the same principle to the selective retention of erythrocyte agglutinates, by analogy with a conventional technique of immunology to demonstrate the presence of agglutinated antigen-antibody networks within gel.

This process, which is described in French patent 2 577 321, consists of a gentle centrifugation of test samples in a cone-shaped bottom tube filled with dextran gel ; it allows an easy reading, even by poorly skilled technician, because non-agglutinated erythrocytes (negative test) clearly sediment at the bottom of the tube whereas the agglutinated erythrocytes linked by the antibodies are slowed down and held back into the gel, the more positive the reaction the more they are held back. This kind of test is known and abbreviated as "gel-centrifugation".

The patented process has been developed and marketed under the form of kits by Dia-Med-(SA-Morat CH 3280-Switzerland or 75002 Paris- France). With these kits it is possible to perform a large number of tests in a short time and with easy reading. Nevertheless their reliability is not as good as that of conventional tube tests and some significant discrepancies are observed with the detection of some irregular agglutinins which may lead to transfusion related accidents.

The applicant has thus tried to improve the specificity of this kind of "gel-centrifugation" test in order to guarantee the reliability of the result.

More precisely the Applicant has observed that it was possible to increase the discriminating capacity of the gel by incorporating a second gel, with thinner cross-linkage. As a preferred embodiment a mixture of Sephadex G100 (with a particle diameter of 20 to 50 μm) and Sephasorb HP Ultrafine (with a particle diameter of 10 to 23 μm) will be used ; both products are dextrans, sold by Pharmacia (Pharmacia Fine Chemicals - Uppsala - Sweden). Other similar materials routinely used for size exclusion chromatographic separations may also be used.

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Various reactants specifically adapted for each type of test are preferably incorporated into the gel.

- Thus - for performing the test in saline medium and the test with previous enzymatic treatment of the erythrocytes, a previously controlled human AB serum is incorporated, or purified immunoglobulins, in order to facilitate the sedimentation of non agglutinated erythrocytes ;
- for performing the Coombs direct or indirect agglutination tests (or any test derived from said ones), polyvalent or specific antiglobulins and anti-complement are incorporated into the gel.
- The antiglobulins induce agglutination of the erythrocytes sensitized by an immunologic treatment.
 - The human antiglobulins, of the IgG type, play the same role as the human AB serum or the purified human IgG, which is to improve the sedimentation of non agglutinated erythrocytes.

As in conventional tests, the antiglobulins to be used for indirect agglutination are polyvalent antiglobulins and/or rabbit polyclonal anti-IgG and/or rabbit anti-complement.

The Applicant has significantly improved the results of the direct and indirect Coombs test by replacing the standard anti-complement with a monoclonal antibody with anti-C3d specificity, which is produced by a hybridoma selected according to criteria chosen with a view to the said test.

Conditions for carrying out each test have been specially adjusted for the "gel-centrifugation" technique. Thus the Applicant has observed that the reaction in the Coombs test with low ionic strength buffer could be improved by using an erythrocyte preparation of better quality (they are conventionally suspended in low ionic strength solution, as described by Löw and Messeter - Vox Sang. 26, 1974- 53- 61), and more precisely by adding 0.26% disodium EDTA in said suspension, as a chelating agent for calcium ion ; it thus protects erythrocytes against hemolysis (which would



result from non specific sensitization of the erythrocytes by the low ionic strength medium in the presence of complement fractions from the serum sample). It must be noted that a too high EDTA concentration prevents detection of complement fixing antibodies. This effect can be compensated by boosting the polyvalent antiglobulin with purified and concentrated monoclonal anti-C3d in order to make it easier to detect complement fixing antibodies such as anti-Jka, without however decreasing the anti-IgG activity.

The Applicant has also improved the results of the test by choosing particularly efficient standard antibodies, most of which are monoclonal antibodies.

In one specific case, particularly difficult to identify, like Fya phenotyping, the Applicant has shown that the addition of methylcellulose in the antibody solution increased the sensitivity of the reaction.

The present invention thus concerns a process for demonstrating erythrocyte agglutination characterized by a series of steps, each of which being especially suited to the gel-centrifugation process. More precisely the invention concerns :

- the use as a gel of a mixture of 2 dextrans having different thinness of reticulation ;
- the addition, in said gel, of reactants adapted to each type of test to be performed, i.e. AB serum or (polyvalent or specific) antiglobulin-anti-complement mixture ;
- the use of disodium-EDTA containing medium to form the erythrocyte suspension to be used in the test in order to increase the discriminating capacity of the test by avoiding hemolysis of the erythrocytes.

The invention also covers the use of a monoclonal antibody with anti-C3d specificity, high affinity and very high titer instead of a standard anti-complement antibody, as well as the selection of the hybridoma that produces said monoclonal antibody.

More precisely, the screening of the hybridomas



has been performed by titration in order to preferably select a cell line that produces a monoclonal antibody with very high titer, i.e. still positive at a dilution of 1/512.

The invention will be better understood in the following examples, which illustrate the invention without however limiting its scope.

Figure 1 shows a practical result of the invention in the form of a Table with comparative results of detection and identification of 106 irregular antibodies, according to 3 processes : the reference process, in tubes (headline: tube), the process sold as a kit by DiaMed (headline : DiaMed gel) and the process according to the present invention (headline : Lille gel) ; 2 types of tests are performed in parallel : the enzymatic test (headline : papain) and the indirect Coombs test in low ionic strength medium (headline : IC-LIS).

Example I - Gel preparation and filling into microtubes

I.a. Composition of the basic gel, identical for all tests

The gel is prepared with 2 dextrans having different thinness of reticulation (cross-linking) :

- Sephadex® G100 Superfine (20-50 µm)
- Sephasorb® HP Ultrafine (10-23 µm)

Both products are sold by Pharmacia Fine Chemicals (Uppsala-Sweden).

The relative amount of the 2 products is adapted to each test :

- for Coombs tests one uses

5 g Sephadex and 10 mg Sephasorb

- for tests in saline medium or enzymatic tests one uses

5 g Sephadex and 5 mg Sephasorb

This mixture is suspended in 90 ml low ionic strength (LIS) saline solution (conventional medium adapted by Löw and Messeter - Vox Sang. 26, 1974, 53-61) which contains 0,03 M NaCl.

I.b. Specific reactants

For Coombs tests with antiglobulin, 15ml polyvalent antiglobulin (rabbit polyclonal anti-IgG) and



anti-complement (mouse monoclonal anti C3d - see below) are incorporated in the gel.

For enzymatic tests (i.e. when erythrocytes pretreated with enzymes are used) 10 ml human AB serum, previously controlled, or possibly purified immunoglobulins, are incorporated in the gel.

I.c. Filling of the gel into microtubes

Microtubes for centrifugation, with cone shaped bottom, are used (for example of the type Elkay® having a content of 250 µl). The gel is dispensed by automatic pipetting of 100 µl per tube.

Tubes are centrifuged for 2 minutes at 1500 g in order to pack down the gel and to eliminate bubbles.

15 Example II - Carrying out the tests

IIa - Erythrocyte preparation

The test samples are centrifuged for 15 minutes at 2000 g.

For Coombs tests, a 0.8% erythrocyte suspension (or 1% for some tests) is prepared in low ionic strength saline solution (as described before), supplemented with 0.26% disodium EDTA. This modification of the medium significantly increases the erythrocyte sedimentation through the gel when the result is negative (i.e. serum devoid of antibodies directed against the erythrocyte antigens).

For tests in saline medium and enzymatic tests (papain, trypsin, bromelin, according to conventional methods), a 0.8% erythrocyte suspension (or 1% for some tests) is prepared in physiological water.

II b. Carrying out the tests

The samples are placed on the top of the gel in each microtube :

- first, 50 µl erythrocyte suspension (as described in IIa)
- 35 - second, 25 µl serum

After 15 minutes incubation in a water bath at 37°C the microtubes are centrifuged for 9 minutes at 430g



and the reading is performed macroscopically.

- in case of negative result the erythrocytes form a dense pellet at the bottom of the tube.

- in case of positive result the agglutinated erythrocytes are more or less retarded into the gel ; the results are noted as +, in a range of 1+ to 4+.

- when the antibodies have fixed the complement, the enzymatically treated erythrocytes undergo some hemolysis (light red supernatant).

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Example III - Selection of an anti-complement monoclonal antibody with anti-C3d specificity, with strong affinity and very high titer.

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The conventional method of Kohler and Milstein for inducing hybridomas by fusing splenocytes (spleen cells) of immunized mouse with murine myeloma cells (of immunoglobulin non secreting type) was adapted for producing hybridomas which secrete anti-complement antibodies with anti-C3d specificity.

20

1) Chosen immunogen

Mice were immunized with healthy donor erythrocytes, controlled and coated with the C3d fraction of complement. This preparation of C3d-erythrocytes is made in vitro according to the following procedure :

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1.a - Prepare C3-erythrocytes according to cold low ionic strength method

- prepare solution A as follows :

1,0 M K_2HPO_4	1,25 ml
0,2 M $Na_2 EDTA$	5,25 ml
saccharose	23,1 g
distilled water	250 ml

30

- prepare solution B as follows :

1,0 M KH_2PO_4	1,1 ml
0,2 M $Na_2 EDTA$	5,25 ml
saccharose	23,1 g
distilled water	250 ml

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- prepare solution C as follows :

2,0 M $MgCl_2 \cdot 6H_2O$ 10 ml

distilled water 50 ml

- Prepare phosphate/saccharose buffer, pH 5.1 by mixing
5 solution A with B until the desired pH is reached

- Put 19 ml phosphate/saccharose buffer, pH 5.1, in a 50 ml beaker placed in a thawing ice bath. Mix until the buffer temperature reaches 0-1°C.

- Add 0.5 ml centrifuged erythrocytes of Group 0, after 3
10 times washing.

- Add 0.5 ml serum or fresh plasma 1/50 diluted with isotonic saline solution and 0.1 ml of solution C.

- Incubate for 30 minutes at 0°C

- Centrifuge, discard the supernatant, wash the erythrocytes
15 4 times with 20 ml PBS buffer and discard the last washing supernatant.

- Add 14.5 ml of PBS on the cell pellet.

This provides a 3% erythrocyte suspension, coated on their surface with the C3b determinant of the complement
20 (said erythrocytes are called EC3b)

1.b. Preparation of C3d-erythrocytes by trypsin treatment of EC3b erythrocytes

- Dilute 2.5 ml 1N Hcl in 50 ml distilled water

- Dissolve 0.1 g cristallized trypsin (Sigma-ref 8253) in
25 10 ml 0.05 N HCl.

- Aliquot in 0.1 ml fractions and store at -20°C

- Prepare solution C as follows :

1,0 M K_2HPO_4 5,0 ml

distilled water 50 ml

30 - Prepare solution D as follows :

1,0 M KH_2PO_4 1,0 ml

distilled water 10 ml

- Add solution D in solution C under stirring until a pH of 7.7 is reached.

35 - Aliquot in 10 ml fractions and store at -20°C.

- Prepare 1 ml 0.1% trypsin by adding 0.9 ml phosphate buffer pH 7.7 to the 1% trypsin stock solution.

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- Add 1 ml 0.1% trypsin to 0.5 ml EC3b or EC3 erythrocytes prepared as described in a, in a 25 ml beaker ; stirr and incubate for 30 minutes at 37°C.
- Wash 4 times the erythrocytes with 20 ml PBS buffer and discard the washing supernatant.
- Add 14.5 ml PBS buffer to the cell pellet.

This provides a 3% erythrocyte suspension, coated on their surface with the C3d determinant of the complement (said erythrocytes are called EC3d).

10 2) Mice immunization schedule

An immunization schedule was chosen to favour the induction of antibodies with high affinity (i.e. a very long lasting repetitive immunization : for 6 months).

15 Mice are injected twice a week intraperitoneally with 300 µl of the 3% EC3d erythrocyte suspension as described previously. The last injection is performed intraveinously.

Three days after the last injection, the spleen is taken from the mice and homogenized ; the bulk of cells are put together with $2 \cdot 10^7$ myeloma cells to obtain their fusion. Hybridoma selection is further performed under conventional conditions in HAT medium. The specific screening is performed 15 days later.

25 3) Selection of hybridomas that secrete a high level of anti-C3d antibodies

Hybridoma screening is performed in microplates with U-shaped wells, in the presence of erythrocytes coated with the C3d fraction of complement.

30 Two tricks are used to favour the selection of clones that produce high yields of antibodies :

3.a. choice of the revealing antigen

35 In order to detect clones that produce anti-C3d monoclonal antibodies, one uses as revealing agent the erythrocytes of a patient chosen for that goal, i.e. a patient with "chronic hemolytic anemia with cold agglutinins" which has been shown, in the laboratory, to bear



erythrocytes coated with the C3d fraction of complement.

3.d Screening by titration

The screening of the hybridomas is not only performed on the basis of positive clones but also at various dilutions in order to select clones which produce the highest antibody titer.

After the first selection, the hybridomas are recloned by titration in limiting dilution. Afterwards they are amplified and rechecked for their antibody production and for their stability.

One particularly efficient clone has been selected and used to prepare a pilot batch and a master bank.

This cell line produces a monoclonal anti-C3d antibody with a titer of 1/512 (i.e. the last dilution which gives a positive response).

To the contrary of common opinion, the high titer of said antibody does not provoke false positive results in blood compatibility tests (see below) but it significantly improves the accuracy of the results.

Example IV - Detection and identification of irregular antibodies in donors and pregnant women serum.

The gel centrifugation process for indirect Coombs test and papain enzymatic test has been carried out with 2050 serum samples with the panel of erythrocytes required by law.

Among these samples, 453 contain irregular antibodies (single or associated). A total of 615 irregular antibodies have been detected and identified.



TABLE I

5 SPECIFICITY OF 615 IRREGULAR ALLO-ANTIBODIES DETECTED (COMBS
AND PAPAIN).

	ANTIBODIES	NUMBERS	ANTIBODIES	NUMBERS
10	ANTI-D	106	ANTI-Lea	52
	ANTI-C	72	ANTI-Leb	27
	ANTI-E	101	ANTI-H	15
	ANTI-c	32	ANTI-i	1
	ANTI-Cw	22	ANTI-U	1
15	ANTI-e	5	ANTI-GE	2
	ANTI-Ce	1	ANTI-LW	1
	ANTI-K	63	ANTI-JMH	1
	ANTI-k	2	ANTI-Tja	2
	ANTI-Kpa	5	ANTI-Vel	2
20	ANTI-Fya	28	ANTI-Lub	3
	ANTI-Fyb	1	ANTI-Wra	3
	ANTI-Jka	25	ANTI-Vw	2
	ANTI-Jkb	4	ANTI-M	14
	ANTI-S	5	ANTI-N	1
25	ANTI-s	1	ANTI-Pl	14
			ANTI-P	1

30

The sensitivity and reliability of the test as described were compared with those of the same tests performed either according to conventional method in tubes or in gel centrifugation with the kit sold by DiaMed®.

In the following tables the 3 tests will be noted as "tube" "DiaMed" and "Lille" (for the present invention).

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- Among the total 615 irregular antibodies detected,
- only one was only detected by conventional tube test (antibody anti-H),
 - 614 were identified with the "Lille" gel, thus 99.84%,
 - 5 - 581 were identified with the tube test, thus 94.47%,
 - 566 were identified with "Dia Med" test, thus 92.03%.

With one exception the process according to the present invention performs better than the reference method and significantly better than the "DiaMed" test.

- 10 A more detailed identification of the antibodies (106 among those previously detected) shows important discrepancies between the 3 processes. Detailed results are presented in appendix as Figure 1.

Discrepancies can be summarized as follows :

- 15 - among the anti-Rhesus, which are better detected with the papain enzymatic test, 21 reactions show discrepancies, thus 6.2 % :

4 anti-D, 3 anti-C, 2 anti-e, a anti-Cw, 11 anti-E.

- if one looks at table anti-E of Figure 1, it appears
20 that :

• among the 19 anti-E detected with the "Lille"-gel (with a papain test),

• 11 are not detected with "DiaMed" (whereas 3 of those were detected in tube)

- 25 • and 8 are not detected in tube

- among the 125 antibodies detectable by conventional indirect Coombs method (anti-Kell, anti-Duffy, anti-Kidd, anti-S, etc..), 19 give rise to discrepancies :

8 anti-Kell, 4 anti-Fya, 5 anti-Jka,

- 30 1 anti-Jkb, 1 anti-S

(it must be noted that anti-Kell, Duffy and Kidd antibodies, that can fix the complement, are especially dangerous during transfusion).

- among 122 antibodies with other specificities, 19 (thus
35 15,5%) give rise to discrepancies :

5 anti-Lea, 3 anti-Leb, 8 anti-M,

1 anti-P₁, 2 anti-H

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Among all those discrepancies it appears that the "DiaMed" test never detects an antibody which is undetected with at least one of the 2 other methods.

5 Example V - Use of the test for erythrocyte phenotyping

Va - The Rhesus phenotyping was performed in the presence of human Ab serum, (as described in I), with bromelin enzymatic test. In this particular case a 1% erythrocyte suspension in 5% bromelin solution in physiological water is used.

10 Incubation is performed at room temperature for 10 minutes.

The following reactants are used and incorporated into the gel :

- monoclonal antibodies (selected by the Applicant) :

anti-D, anti-C, anti-E

15 - controlled polyclonal human antibodies :

anti-c, anti-Cw, anti-e

V.b Other erythrocyte phenotyping were performed in "gel with antiglobulin" (human anti-IgG or polyvalent antiglobulin, standardized and controlled, and anti-complement).

20 This phenotyping more precisely concerns the Kell, Duffy and S antigens.

For this test a 1% erythrocytes suspension is used.

25 For the Fya phenotyping which is particularly delicate, the saline solution of antibodies is supplemented with 2 g/l methylcellulose.

V.c Results

30 The process carried out with the "Lille-gel"-centrifugation is particularly efficient and this more strikingly appears in the study of double populations :

- the anti-D detects 4% of D⁺ erythrocytes among 96% D⁻
and 6% of D⁻ among 94% D⁺

35 - the anti-C detects 10% of C⁺ erythrocytes among 90% C⁻
and 10% of C⁻ among 90% of C⁺

- the anti-E detects 8% of E⁺ erythrocytes among 92% E⁻

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- and 10% of E^- among 90% E^+
- the anti-c detects 6% of c^+ erythrocytes among 94% of c^-
- the anti-e detects 8% of e^+ erythrocytes among 92% of e^-
and 10% of e^- among 90% of e^+
- 5 - the anti-Cw detects 8% of erythrocytes Cw^+ among 92% Cw^-
- the anti-Kell detects 5% of erythrocytes K^+ among 95% of K^-
and 10% of K^- among 90% of K^+
- the anti-Duffy detects 10% of erythrocytes Fy^+ among 90%
of Fy^-
- 10 and 10% of Fy^- among 90% of Fy^+
- the anti-S detects 10% of erythrocytes S^+ among 90% of S^- .

Example VI - Direct Coombs test

The process according to the present invention may
15 also be carried out to perform a direct Coombs test. This
test allows the detection of antibodies and/or complement
that have been fixed in vivo on the patient erythrocytes.

This test is particularly useful for diagnosis of
auto-immune hemolytic anemia (resulting from the presence of
20 anti-erythrocyte auto-antibodies directed against antigens
that are normally present) or of hemolytic anemia caused by
mother-foetus allo-immunization or post-transfusion allo
immunization.

For the direct Coombs test, the process is carried
25 out under the following conditions :

- The Coombs reactant is incorporated into the gel
(15 ml/100 ml gel), i.e. :
 - either polyvalent antiglobulin
 - or anti-IgG antiglobulin
 - 30 - or monoclonal anti-complement antibody with
anti-C3d specificity (see ex.III)
- A neutral gel, devoid of Coombs reactant is used as
negative control.
- The patient's erythrocyte suspension is prepared at 1%
35 concentration and, as in previous tests, 50 μ l are placed on
the top of the gel.
- Centrifugation is performed for 9 minutes at 430 g.



Among the 150 tests that were carried out, the sensitivity of the test was demonstrated to be at least similar or better than that of the same method in tube.

5 Example VII - Diagnosis of transfusion hemolytic accident

The process of the present invention can also be carried out for the diagnosis of transfusion accidents upon which rely the safety of future transfusions.

10 This diagnosis is performed after "direct elution", i.e. the antibody which caused the accident is unbound (eluted) from the incompatible erythrocytes mistakenly transfused, by a physico-chemical mechanism. In particular, the Applicant has improved the acid pH elution procedure (pH3) described by Rekvig and Hannestd (Vox Sang.
15 33, 280-285, 1977) which avoids hemolysis of sensitized erythrocytes.

The elution is performed by contacting one volume of erythrocyte pellet with 1,5 volume of HCl-glycine buffer, at pH2, which increases the efficacy of elution.

20 In order to avoid hemolysis of the erythrocytes at pH2, EDTA is added to the HCl-glycine buffer.

As a result, later identification of the antibody is easier, after pH neutralization and reimplantation of the eluate.

25 The eluted antibodies are much better visualized by the gel-centrifugation process than by conventional tube test.

More precisely the following eluted antibodies have been revealed :

30 Anti-D, anti-C, anti-c, anti-K, anti-E, anti-e, anti-Fya, anti-Jka, anti-Fyb, anti-Jkb, anti-S, anti-s and anti-Lea.

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CLAIMS

- 1.- Process for demonstrating erythrocyte
5 agglutination by the selective retention of the agglutinated
products after centrifugation in a gelled medium, in
microtubes, adapted to direct or indirect conventional
immunohaematology test, characterized in that
- the gelled medium comprises 2 gels having different
 - 10 thickness of reticulation,
 - the gelled medium contains one or more reactants
specifically adapted to each type of test,
 - the conditions for carrying out each test are specially
adapted to that method of centrifugation in gelled medium.
- 15 2.- Process according to claim 1, characterised in
that the gelled medium comprises a mixture of one dextran of
which the particles have a diameter of from 20 to 50 μm and
another dextran of which the particles have a diameter of
from 10 to 23 μm .
- 20 3.- Process according to claim 1 or claim 2,
characterised in that the gelled medium contains a human AB
serum.
- 4.- Process according to claim 1 or claim 2,
characterised in that the gelled medium contains
- 25 antiglobulins.
- 5.- Process according to any one of claims 1, 2
and 4, characterised in that the gelled medium contains a
monoclonal anticomplement antibody.
- 6.- Process according to claim 5, characterised in
- 30 that the monoclonal antibody is an antibody having anti C3d
specificity and high affinity.
- 7.- Process according to claim 5 or claim 6,
characterised in that the anti-C3d antibody is produced by a
hybridoma induced and selected with a view to the said
- 35 process.
- 8.- Process according to claim 7, characterised in
that the selection of the hybridoma is carried out by



titration in order to obtain a hybridoma that produces high titer monoclonal antibodies.

9.- Process according to anyone of claims 1, 2, 4, 5 and 6, characterised in that carrying out the test comprises the addition of disodium EDTA to the suspension of erythrocytes.

10.- Process according to claim 9, characterised in that the concentration of disodium EDTA is from 0.15 to 0.30%.

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