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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 3 : G01N 33/80	A1	(11) International Publication Number: WO 83/ 03477 (43) International Publication Date: 13 October 1983 (13.10.83)

(21) International Application Number: PCT/GB83/00081

(22) International Filing Date: 18 March 1983 (18.03.83)

(31) Priority Application Numbers: 8209235, 8305364

(32) Priority Dates: 29 March 1982 (29.03.82), 25 February 1983 (25.02.83)

(33) Priority Country: GB

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(81) Designated States: AT (European patent), BE (European patent), CH (European patent), DE (European patent), FR (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US.

Published
With international search report.

(54) Title: BLOOD CELL GROUPING REAGENT

(57) Abstract

An ABO blood cell grouping reagent comprising, in admixture, one or more anti-A monoclonal antibodies and one or more anti-B monoclonal antibodies. An anti-A monoclonal antibody incorporates an anti-A, B monoclonal antibody, provided that the anti-A, B is potent enough to cause a macroscopic reaction with red blood cells possessing one or more A-type antigenic determinants. Similary, an anti-B monoclonal antibody incorporates an anti-A, B monoclonal antibody provided the anti-A, B is potent enough to cause a macroscopic reaction with red blood cells having one or more B-type antigens. Any mixture of monoclonal anti-A and anti-B antibodies that has sufficient agglutinating quality to give reliable results (for the presence of agglutinogens A and B) by the routine blood typing methods may be used. Preferably the monoclonal antibodies are derived from hybrid myelomas produced by the fusion of a parent cell line, especially NSI (abbreviated from P3-NS1/1-Ag 4-1) or NSO/1, with spleen or other immunocyte cells from mice immunised with human agglutinogen A or B or A, B. In one particularly preferred embodiment a monoclonal anti-A (6D4 or 3D3) and a monoclonal anti-b (NB1/19), derived respectively from the hybrid myeloma 6D4.12.3, A15 3D3.92.00 and NB1/19.112.28 are mixed. The present reagent may also contain a certain proportion of conventional Group O serum. The addition of this serum is to provide the present reagent with A_x activity especially of the monoclonal mixture lacks this activity. In a particularly preferred embodiment a one-third volume of Group O serum is added.

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BLOOD CELL GROUPING REAGENT

The present invention relates to ABO blood cell grouping reagents.

ABO incompatibility is a potentially lethal barrier in transfusion therapy and in tissue transplantation. All donor blood and potential recipients for transfusion or tissue transplantation are therefore routinely ABO grouped as this is the only way to ensure maximum safety for these patients.

ABO blood cell grouping is conventionally performed by testing the red cells of the blood to be typed for the presence or absence of antibodies and antigens. The presence of the latter is generally determined by adding an anti-serum (a blood serum containing known antibodies to the A and B red cell antigens) to the red cells in saline suspension and looking for agglutination. The anti-sera employed in the grouping of ABO blood contain either anti- A antibodies (Group B serum) or anti-B antibodies (Group A serum) or both anti-A and anti-B antibodies (Group O serum). Of these, Group O serum is used to check that the anti-A and anti-B tests, that are used in all routine ABO groupings of blood donations and patients, are correct.

The main factors affecting the choice of ABO reagents are,

(a) The specificity of the reagent for the appropriate antigen.
(b) The stability of the reagent under the conditions of use.
(c) The potency or agglutinating quality which must be sufficient to give reliable results by the routine methods employed.
(d) The guaranteed availability of sufficient volumes to meet the commitments of the transfusion service, and
(e) The cost of production which must be as low as possible.

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At present the National Health Service obtains anti-sera either by screening the sera of donor blood samples or by hyperimmunisation of volunteer donors. By contrast commercial sources and most European and North American countries obtain sera only from
5 hyperimmunised donors. In both cases the supply of potent group O serum is limited, and in the latter case expensive. This limited availability cannot be remedied by blending the more readily obtainable group A and group B sera, since this leads to the loss of some anti-A and anti-B activity by cross neutralisation.

10 It is the aim of the present invention to provide a new ABO blood cell grouping reagent that may be used in place of conventional group O serum and that is both readily available and cheap to produce.

According to the present invention there is provided an ABO
15 blood cell grouping reagent comprising, in admixture, one or more anti-A monoclonal antibodies and one or more anti-B monoclonal antibodies.

The present ABO blood cell grouping reagent is an anti-A/B grouping reagent which detects A antigenic determinants and B
20 antigenic determinants on red blood cells.

In the present specification an anti-A monoclonal antibody incorporates an anti-A,B monoclonal antibody, provided the anti-A,B monoclonal antibody is potent enough to cause a macroscopic reaction (by spin-tube, tube sedimentation, or tile/slide methods) with red
25 blood cells possessing one or more A-type antigenic determinants. Moreover, an anti-B monoclonal antibody incorporates an anti-A,B monoclonal antibody, provided the anti-A,B monoclonal, antibody is potent enough to cause a macroscopic reaction (by spin-tube, tube sedimentation or tile/slide methods) with red blood cells possessing
30 one or more B-type antigenic determinants.

Any mixture of monoclonal anti-A and anti-B antibodies that has sufficient agglutinating quality to give reliable results (for the presence of antigens A and B) by the routine blood.typing methods may be used as an ABO reagent according to this invention.
35 A 'sufficient agglutinating quality' will be an agglutinating quality as good as or better than that displayed by current UK National Health Service group O reagents. 'Routine blood typing methods' are set out

in full in "Techniques in blood grouping" by I Dunsford and C C Bowley, 2nd Edn., 1967.

In a further aspect of the present invention the reagent may also incorporate one or more monoclonal antibodies (in particular monoclonal anti-A,B antibodies) that are specific for the antigenic determinants on Ax-type red blood cells (Ax activity) and Bx-type red blood cells (Bx activity)(or other weak B variants). A reagent that possesses Ax activity is particularly useful since such a reagent should prevent the mistyping of certain weak A-type blood cells as group O.

Preferably the monoclonal antibodies are derived from hybrid myelomas produced by the fusion of mouse myeloma cells, especially NSI (abbreviated from P3-NSI/1-Ag4-1, G Kohler, S C Howe and C Milstein, Eur J Immunol,1976, 6, 292) or NSO/1(G.Galfre and C Milstein, Methods in Enzymology, 1981,73,3), both available on request from the MRC Laboratory of Molecular Biology, Hills Road, Cambridge), with spleen or other immunocyte cells from mice immunised with human antigen A,human antigen B, or human antigen A,B.In one particularly preferred embodiment a monoclonal anti-A(6D4) and a monoclonal anti-B (NB1/19), derived respectfully from the hybrid myeloma 6D4.12.3 and NB1/19.112.28 are mixed. In another preferred embodiment a monoclonal anti-A (3D3), derived from the hybrid myeloma A15.3D3.92.00, and monoclonal anti-B (NB1/19) are mixed.

The monoclonal antibodies used to form the present reagent may be separated either from the ascitic fluids of tumours produced in animals, generally mice, or from tissue culture supernatants.

It will be seen from the above that the present reagent is not an anti-serum as previously understood since the antibodies are not contained in a serum. However the medium surrounding the monoclonal antibodies will still, in many cases, contain a certain proportion of serum. In the former case the serum will often be present in the ascitic fluid as collected, whilst in the latter case the serum will be added to the tissue culture to provide nutrients for cell growth.

In addition to this serum the present reagent may also contain a certain proportion of conventional Group O serum. The addition of this serum is to provide the present reagent with A_X activity, especially in cases where the mixture of monoclonal antibodies alone lack this activity.

In a particularly preferred embodiment a one-third volume of Group O serum is added.

The present reagent will be used primarily to check that anti-A and anti-B tests (for antigens A and B) are correct. In order to facilitate this test there is also provided a process for the grouping of ABO blood cells comprising adding to a suspension of cells in sodium chloride solution, a blood cell grouping reagent which comprises, in admixture, one or more anti-A monoclonal antibodies and one or more anti-B monoclonal antibodies. Once again the terms anti-A and anti-B monoclonal antibodies incorporate certain anti-A,B monoclonal antibodies, as defined above. Moreover the preferred monoclonal antibodies for use in this process are also listed above. Further, the reagent may also contain a certain proportion of conventional group O serum, especially if none of the monoclonal antibodies employed exhibit Ax activity.

The ABO blood cell blended grouping reagents of the present invention are as stable as the individual antibodies used in the blend when stored at 4°C or lower temperatures. Once the present blood cell grouping reagent has been added to the blood cell suspension in sodium chloride solution, the cell grouping test is generally continued by observing whether or not agglutination of the cells takes place. Generally, if the present reagent contains an antibody specific for one or more of the antigens of the cells under test, then agglutination will take place. Such agglutination is a visible reaction and therefore may be followed visually in a qualitative or quantitative manner. On the other hand, if the present reagent does not contain an antibody specific for the blood cells antigens then generally, unless a cross reaction takes place, no agglutination will occur. (Cross reaction refers to both false reactions and auto antibody sensitised cells).

For convenience, the present reagent may be provided for use in the above process in the form of a blood cell grouping kit. In one embodiment of this kit the anti-A monoclonal antibodies and the anti-B monoclonal antibodies are provided in the form of a mixture. In a second embodiment the anti-A monoclonal antibodies and the anti-B monoclonal antibodies are provided in separate containers together with instructions on the method of mixing. In a third embodiment either of the previous kits also contains group O serum, either in admixture with or separate from the monoclonal antibodies.

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The reagent, process and kit of the present invention will now be described by way of example only,

Materials and Methods

Analysis to determine antibody heavy and light chains

5 The myeloma NS1 produces K light chains which, although not secreted by the myeloma, can be 'mixed' with the light chains of spleen cell origin and the antibody secreted as mixed chain types called HLK. Full antibody activity requires both light and heavy chains, hence HLK antibody molecules are defective as some of the 10 light chains are non-antibody myeloma K chains and thus reduce the antibody binding efficiency of the molecules. Therefore it is obviously desirable to select HL variants to obtain the best agglutinating antibodies. Recenctly myelomas which do not produce K chains, e.g. NSO/1, have become available and this eliminates the 15 HLK problem. Composition is stablished by radioautograph of electrophoretic runs detecting the C^{14} lysine incorporated into the antibodies secreted by the hybrid-myeloma cells. There are two types of procedure for separating the antibody compartments in polyacrylamide gel slabs for subsequent radioautograph analysis:

20 (i) Iso-electric focussing (IEF). The separation is based on pH gradient separating components having different iso-electric points with prior reduction by 2-mercapto ethanol to break the interchain links.

(ii) Electrophoresis of the reduced antibodies with sodium 25 dodecyl sulphate polyacrylamide slab gel electrophoresis (SDS-PAGE). The separation is based on molecular sieving of the different sized components that have a similar negative charge by being coated with SDS.

Serum reagents

30 NHS anti-sera prepared by the Blood Group Reference Laboratory (BGRL) from sera referred by regional transfusion centres. Commercial antisera from several sources.

Haemagglutination tests

These were tube tests as set out in Dunsford and Bowley. Red cells were from ACD or clotted samples received in the transfusion centre. Cells were washed X4 in isotonic saline for comparative 5 titration studies, but otherwise used by preparing a 2-3% suspension in saline. Cells were used at 20% for slide tests and 5% for spin tube tests.

Antibody dilutions were made in saline and titration results scored.

10 Elution was by the heat method, and enhancement tests used 2% pre-papainised cell suspensions or 20% bovine albumin by the displacement method.

Example 1. Preparation of monoclonal anti-A (6D4)

C3H/He-mg mice (OLAC) were immunised with tissue culture cells 15 (HT-29 from Dr J Fogh, Sloan Kettering Institute) from a human group A colon carcinoma case. Spleen cells (10^8) from the immunised mice were fused with mouse myeloma cells (10^7) by use of the polyethylene glycol method (Weir, Handbook of experimental immunology, 3rd Edn, vol II). The myeloma cells were non-secretor P3-NS1/1-Ag4-1 selected 20 enzyme deficient cells that die in HAT medium.

The culture supernatants were tested 2 weeks post fusion and those cells with antibody binding activity on HT-29 cells were cloned on soft agar and grown to larger volumes, recloned, regrown and divided into aliquots for larger scale antibody production and liquid 25 nitrogen storage of the selected cell lines.

The established anti-A secreting clone (6D4) was grown in Dulbecco's modified Eagle's medium (DMEM, Gibco Biocult) supplemented with feotal calf serum (FCS, Sera-Lab). The concentration of FCS is gradually reduced from 15 to 5%. The supplemented tissue culture 30 medium was free of anti-A or anti-B activity and A and B substances (antigens).

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The tissue culture supernatants were centrifuged to remove cells and debris, supplemented with 10mM Hepes and 0.1% sodium azide and stored at 4°, -20° or -40°C. Aliquots were concentrated 5 fold, 8 fold and 18 fold by ultra-filtration on an Amicon filter (PM 10) 5 and stored at 4° and -20°C.

Example 2. Preparation of monoclonal anti-A (3D3)

Male B10 BR mice (OLAC) were immunised with A_1 cells followed by several injections of human blood group A substance. Spleen cells (10^8) from the immunised mice were fused with mouse myeloma cells 10 (10^7) by the use of the polyethylene glycol method. The myeloma cells were non-secretor NSO/1 derived from P3-NS1/1/Ag4/1 selected enzyme deficient cells that die in HAT medium.

The culture supernatants were tested, beginning 2 week post fusion and those cells producing anti-A were selected by agglutina- 15 tion tests, then cloned on soft agar, grown to larger volumes, recloned, regrown and divided into aliquots for larger scale antibody production and liquid nitrogen storage of the selected cell lines.

To prepare culture supernatants for testing, the established anti-A secreting clone (3D3) was grown in Dulbecco's modified 20 Eagle's medium (DMEM, Gibco Biocult), supplemented with feotal calf serum (FCS, Serolab). The concentration of FCS was gradually reduced from 15% to 2.5%.

The supplemented tissue culture medium was free of anti-A or anti-B activity and A or B substances.

25 Example 3. Preparation of monoclonal anti-B (NB1-19).

To find suitable mice, serum samples were assayed for anti-B activity after absorption with group O cells to remove anti-species antibodies. A mouse with an anti-B agglutination titre of 1:8 after absorption was chosen and injected intraperitoneally with 100 μg 30 group B substance (donated by Dr W Watkins, MRC Clinical Research Centre, Harrow) in 0.1ml complete Freund's adjuvant (Difco Bacto). This injection was repeated after 5 weeks and boosted after a further 9 weeks with 200μg B substance in 0.1ml saline injected intravenously. 3 days later, the spleen was removed and a cell 35 suspension was prepared.

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Spleen cells (10^8) were fused with mouse myeloma NS1 cells (10^7) using polyethylene glycol. Growing cell hybrids were selected by their ability to produce specific anti-B activity, detected in the culture supernatant by agglutination assays (I Dunsford and 5 C C Bowley, Techniques in blood grouping, 2nd Edn, 1967) using human A, B and O erythrocytes. Anti-B secreting hybrids were cloned twice on soft agar, and grown up into 1 litre spinner cultures in Dulbecco's Modified Eagle's Medium (DMEM, Gibco Biocult) supplemented with 5% (v/v) feotal calf serum (FCS, Sera-Lab). The cloned hybrids 10 were stored in liquid nitrogen.

Tissue culture supernatant containing the monoclonal antibody was prepared by centrifugation to remove cells and debris, filtration through Millipore filters and addition of 10mM Hepes buffer and 0.1% sodium azide. Aliquots of each batch were then 15 stored at 4°C for routine use or -20°C for stocks.

Example 4. Preparation of monoclonal anti-A,B (A15/1A4.7)

Male B10 BR mice (OLAC) were immunised with A_1 cells followed by several injections of human blood group A substance. Spleen cells 20 (10^8) from the immunised mice were fused with mouse myeloma cells(10^7) by the use of the polyethylene glycol method. The myeloma cells were non-secretor NSO/1 derived from P3-NS1/1/Ag4/1 selected enzyme deficient cells that die in HAT medium.

The culture supernatants were tested, beginning 2 week post 25 fusion and those cells producing anti- A,B were selected by agglutination tests, then cloned on soft agar, grown to larger volumes, recloned, regrown and divided into aliquots for larger scale antibody production and liquid nitrogen storage of the selected cell lines.

To prepare culture supernatants for testing, the established anti-30 A,B secreting clone (A15/1A4.7) was grown in Dulbecco's modified Eagle's medium (DMEM, Gibco Biocult), supplemented with feotal calf serum (FCS, Serolab). The concentration of FCS was gradually reduced from 15% to 2.5%.

The supplemented tissue culture medium was free of anti-A or 35 anti-B activity and A or B substances.

Example 5. Blending monoclonal anti-A and anti-B reagents.

An ABO blood cell grouping reagent was prepared by mixing (in a 1:1:1 (v/v/v)ratio) monoclonal anti-A supernatant (5 fold concentrate, Example 1), monoclonal anti-B supernatant(Example 3) and group O serum (from a Hyperimmunised group O donor). Titration scores and avidity times for this mixture were compared with those of a group O serum obtained from a hyperimmunised group O donor (and in the latter case with commercial and NHS group O sera). Results are given in Tables 1 and 2.

TABLE 1

Blending monoclonal anti-A, anti-B and group O serum-titrations

Equal parts anti-A 6D4 X 5 + anti-B NB1/19 + group O serum

Dilutions

2% cells	1	2	4	8	16	32	64	128	256	512	1024	2048
A_1	C	C	C	C	+++	++	+	(+)	(+)	GW	GW	-
A_2	C	C	+++	++	+	(+)	(+)	GW	GW	-	-	-
B	C	C	C	+++	+	+	(+)	GW	GW	W	W	-
A_3	+	+	+	+	(+)	(+)	GW	W	-	Free cell picture		
A_x	W	W	W	W	W	-	-	-	-	-	-	-
O	-	-	-	-	-	-	-	-	-	-	-	-

Group O serum

	1	2	4	8	16	32	64	128	256	512
A_1	C	C	C	+++	++	+	GW	W	W	-
A_2	C	+++	+++	++	+	+	GW	-	-	-
B	C	C	C	+++	+++	+	(+)	GW	W	-
A_3	+	+	(+)	(+)	(+)	GW	W	-	free cell picture	
A_x	GW (+)	GW	W	W	W	-	-	-	-	-
O	-	-	-	-	-	-	-	-	-	-

NB C = Complete

GW = Good/Weak, requires microscope to determine.

W = Weak

TABLE 2

Blending monoclonal anti-A, anti-B and group O serum - avidity

Anti-A + Anti-B reagent	Avidity tests (sec) X 20% cells A_2	B	O control
Commercial	5	5	0
NHS (1750)	11	12	0
Hyperimmunised Group O serum	10	11	0
Blend 6D4 X 5 + NB1/19 + Group O serum, equal volumes	9	10	0

Example 6. Blending monoclonal anti-A and anti-B reagents

An ABO blood cell grouping reagent was prepared by mixing (in a 1 : 1 (v/v) ratio) monoclonal anti-A supernatant (2 fold concentrate, Example 2) and monoclonal anti-B supernatant (2 fold concentrate, Example 3). Titration scores for this mixture are given in Table 3. The avidity scores for the mixture, which were compared to commercial sera, are given in Table 4.

TABLE 3

1) Equal parts anti-A 3D3 x 2 + anti-B NB1/19 x 2

2% cells	Dilutions 1	2	4	8	16	32	64	128	256	512
A_1	C	C	+++	+++	++	++	+	(+)	(+)	GW
A_2	C	C	+++	+++	++	+	+	(+)	GW	GW
A_1B	C	C	C	C	++	+	+	+	(+)	(+)
A_2B	C	C	+++	+++	++	+	+	+	(+)	GW
B	C	C	+++	+++	++	+	+	+	(+)	W
A_3	++	+	+	(+)	(+)	(+)	GW	W	W	Free cell picture
A_x	-	-	-	-	-	-	-	-	-	-
O	-	-	-	-	-	-	-	-	-	-

TABLE 4

Anti-A/Anti-B reagents	Avidity tests (sec) X 20% cells		
	A_2	B	0 control
Commercial	5	5	0
Blend 3D3 x 2 + NB1/19 x 2 equal volumes	4	5	0

Agglutination reactions were all 4+ after two minutes with A_2 and B cells.

Example 7. Blending monoclonal anti-A, anti-B and anti-A, B reagents

An ABO blood cell grouping reagent was prepared by mixing (in a 1 : 1 : 1 (v/v/v) ratio) monoclonal anti-A supernatant(two fold concentrate, Example 2), monoclonal anti-B supernatant (two fold concentrate, Example 3) and monoclonal anti-A, B supernatant (Example 4). Titration scores for this mixture are given in Table 5. The avidity scores for the mixture, which were compared to commercial sera, are given in Table 6.

TABLE 5

2) Equal parts anti-A 3D3 x 2 + anti-B NB1/19 x 2 + anti-A,B A15/1A4.7

	1	2	4	8	16	32	64	128	256	512
A_1	C	C	+++	+++	++	++	+	+	+	(+)
A_2	C	C	+++	+++	++	++	+	+	(+)	W
A_1B	C	C	C	+++	+++	++	+	+	(+)	W
A_2B	C	C	+++	+++	++	+	+	+	(+)	GW
B	C	C	+++	+++	++	++	+	(+)	GW	-
A_3	++	+	+	(+)	(+)	GW	GW	W	Free cell picture	
A_x	-	-	-	-	-	-	-	-	-	-
0	-	-	-	-	-	-	-	-	-	-

TABLE 6

Anti-A/Anti-B reagents	Avidity Tests (sec). x 20% cells		
	A	B	O control
Commercial	5	5	0
Blend 3D3 x 2 +NBI/19 + A15/1A4.7 equal volumes	4	6	0

Agglutination reactions were all 4 + after two minutes with A_2 and B cells.

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CLAIMS (PCT) JX/6188

1. An ABO blood grouping reagent comprising, in admixture, one or more anti-A monoclonal antibodies and one or more anti-B monoclonal antibodies.

2. An ABO blood grouping reagent according to claim 1 wherein one or more of the monoclonal antibodies has Ax activity.

3. An ABO blood grouping reagent according to claim 1 wherein one or more of the monoclonal antibodies has Bx activity.

4. An ABO blood grouping reagent according to claim 1 wherein the one or more monoclonal antibodies are derived from hybrid myelomas prepared by fusion of spleen cells with mouse myeloma cells selected from NSI cells and NSO cells.

5. An ABO blood grouping reagent according to claim 1 further comprising Group O serum.

6. A process for the grouping of ABO blood cells comprising adding to a suspension of cells in sodium chloride solution a blood cell grouping reagent according to claim 1 and detecting the agglutination or non-agglutination of the cells within the solution.

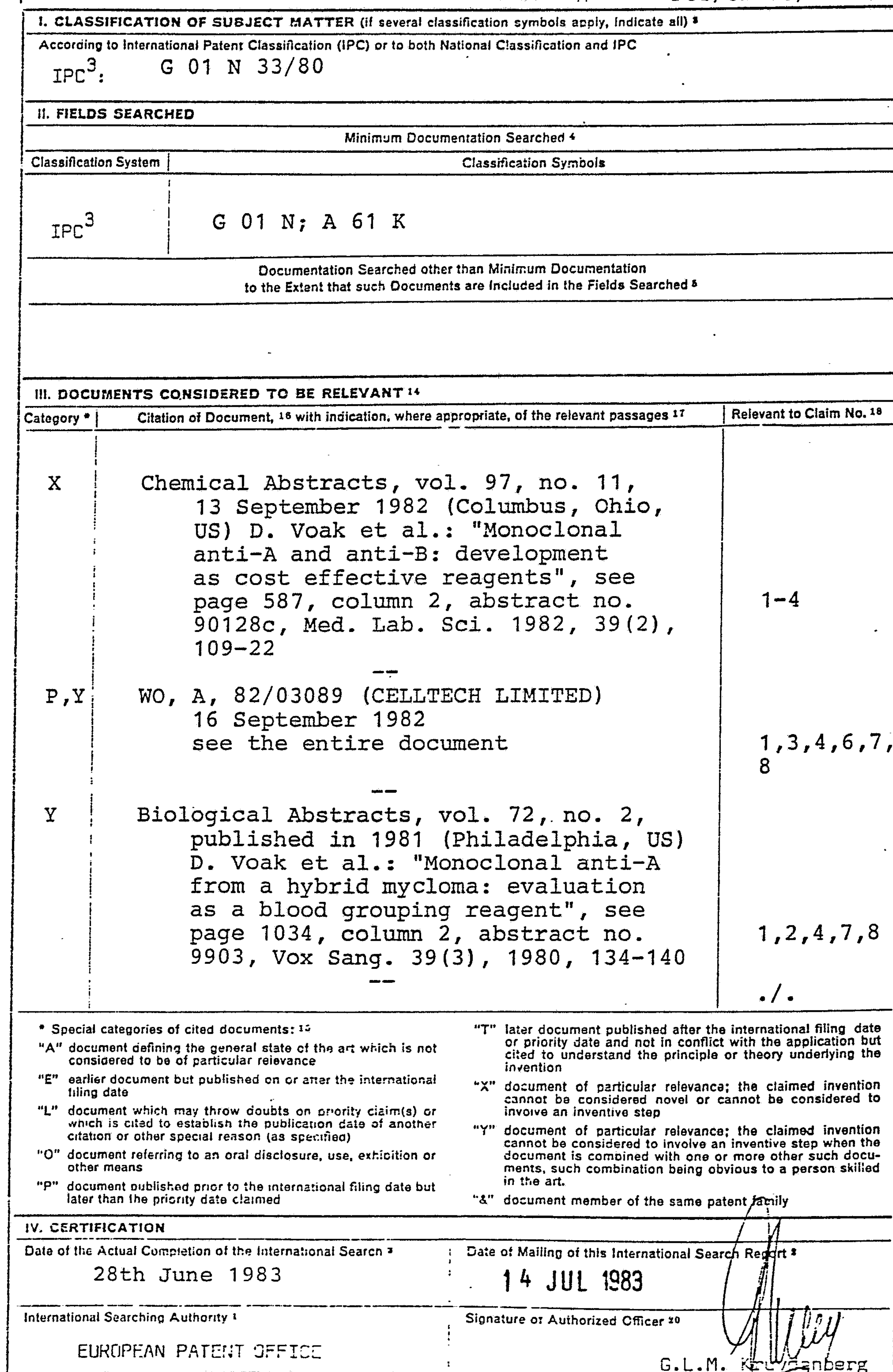
7. A blood cell grouping kit for use in the process of claim 6 comprising an ABO blood grouping reagent according to claim 1.

8. A blood cell grouping kit for use in the process of claim 6 comprising, in separate containers,

 (a) one or more anti-A monoclonal antibodies, and

 (b) one or more anti-B monoclonal antibodies.

9. A blood cell grouping kit according to claim 8 further comprising Group O serum.



INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 83/00081

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC^3: G 01 N 33/80

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
IPC^3	G 01 N; A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
X	Chemical Abstracts, vol. 97, no. 11, 13 September 1982 (Columbus, Ohio, US) D. Voak et al.: "Monoclonal anti-A and anti-B: development as cost effective reagents", see page 587, column 2, abstract no. 90128c, Med. Lab. Sci. 1982, 39(2), 109-22 --	1-4
P,Y	WO, A, 82/03089 (CELLTECH LIMITED) 16 September 1982 see the entire document --	1,3,4,6,7, 8
Y	Biological Abstracts, vol. 72, no. 2, published in 1981 (Philadelphia, US) D. Voak et al.: "Monoclonal anti-A from a hybrid mycloma: evaluation as a blood grouping reagent", see page 1034, column 2, abstract no. 9903, Vox Sang. 39(3), 1980, 134-140 --	1,2,4,7,8
		./.

* Special categories of cited documents: [15]

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"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
28th June 1983	14 JUL 1983
International Searching Authority [1]	**Signature of Authorized Officer [20]**
EUROPEAN PATENT OFFICE	G.L.M. Kruydenberg

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No [18]
Y	Vox Sanguinis, vol. 40, published in 1981, S.H. Sarks et al.: "Monoclonal anti-B as a new blood-typing reagent", see pages 99-104	1,3,4,6-8
P,Y	The Journal of Immunology, vol. 129, no. 2, published in August, 1982 (Baltimore, US) D.R. Bundle et al.: "Hybridomas specific for carbohydrates; synthetic human blood group antigens for the production, selection and characterization of monoclonal typing reagents", see pages 678-682	1,3,4,6-8
A	Medical Laboratory Sciences, vol. 36, published in 1979, A.D. Blann: "Cell hybrids: an important new source of antibody production", see pages 329-338	
A	Clinical Chemistry, vol. 27, no. 11, published in November 1981 (Easton, US) E.D. Sevier et al.: "Monoclonal antibodies in clinical immunology", see pages 1797-1806	
A	WO, A, 81/02104 (S.E. SVENSSON) 6 August 1981 see the entire document	

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON

INTERNATIONAL APPLICATION NO. PCT/GB 83/00081 (SA 4948)

This Annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 07/07/83

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see Official Journal of the European Patent Office, No. 12/82