PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : G01N 33/558, 33/53 — A1

(11) International Publication Number: **WO 95/13542**

(43) International Publication Date: 18 May 1995 (18.05.95)

(21) International Application Number: PCT/EP94/03701

(22) International Filing Date: 8 November 1994 (08.11.94)

(30) Priority Data:
93309054.0 12 November 1993 (12.11.93) EP
(34) Countries for which the regional or international application was filed: GB et al.

(71) Applicant *(for all designated States except US)*: UNIPATH LIMITED [GB/GB]; Wade Road, Basingstoke, Hampshire RG24 0PW (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: MUNDILL, Paul, Henry, Charles [GB/GB]; 44 Ashby Drive, Rushden, Northampton NN10 9HH (GB). PRIOR, Michael, Evans [GB/GB]; 330 Newton Road, Rushden, Northampton NN10 0SY (GB).

(74) Agent: BUTLER, David, John; Unilever plc, Patent Division, Colworth House, Sharnbrook, Bedford MK44 1LQ (GB).

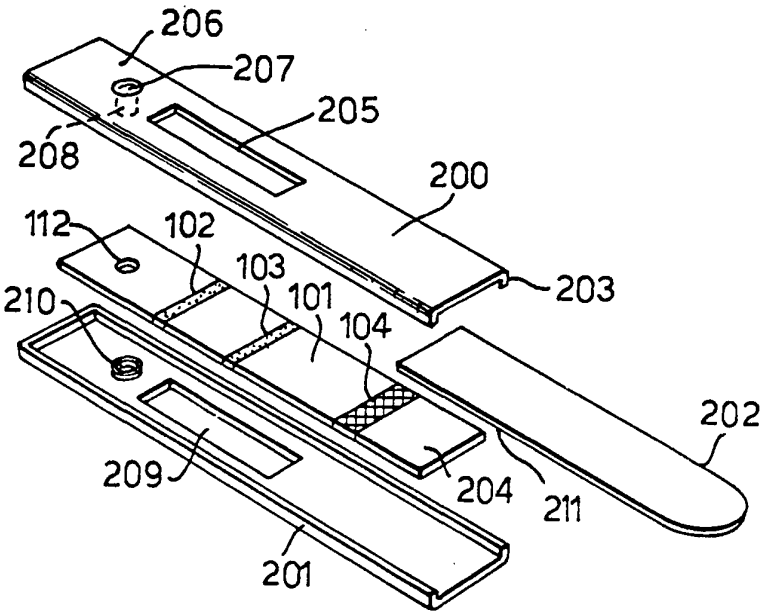
(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ).

Published
With international search report.

(54) Title: ASSAY DEVICES AND THE MANUFACTURE THEREOF

(57) Abstract

An assay sample testing device comprises a porous liquid-permeable carrier strip within a casing, the carrier including a detection zone in which an assay result is revealed by specific binding of a detectable material directly or indirectly to a binding agent immobilised in the detection zone, the casing including internal registration means which cooperatively engages with corresponding registration means associated with the carrier, for example a pin and hole, such that said detection zone within the casing is precisely located in relation to the interlocking means on the casing. During manufacture, the corresponding registration means is used to control accurate formation of the detection zone on the carrier, and accurate placement of the carrier within the casing.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**GB**	United Kingdom	**MR**	Mauritania
AU	Australia	**GE**	Georgia	**MW**	Malawi
BB	Barbados	**GN**	Guinea	**NE**	Niger
BE	Belgium	**GR**	Greece	**NL**	Netherlands
BF	Burkina Faso	**HU**	Hungary	**NO**	Norway
BG	Bulgaria	**IE**	Ireland	**NZ**	New Zealand
BJ	Benin	**IT**	Italy	**PL**	Poland
BR	Brazil	**JP**	Japan	**PT**	Portugal
BY	Belarus	**KE**	Kenya	**RO**	Romania
CA	Canada	**KG**	Kyrgystan	**RU**	Russian Federation
CF	Central African Republic	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CG	Congo			**SE**	Sweden
CH	Switzerland	**KR**	Republic of Korea	**SI**	Slovenia
CI	Côte d'Ivoire	**KZ**	Kazakhstan	**SK**	Slovakia
CM	Cameroon	**LI**	Liechtenstein	**SN**	Senegal
CN	China	**LK**	Sri Lanka	**TD**	Chad
CS	Czechoslovakia	**LU**	Luxembourg	**TG**	Togo
CZ	Czech Republic	**LV**	Latvia	**TJ**	Tajikistan
DE	Germany	**MC**	Monaco	**TT**	Trinidad and Tobago
DK	Denmark	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**US**	United States of America
FI	Finland	**ML**	Mali	**UZ**	Uzbekistan
FR	France	**MN**	Mongolia	**VN**	Viet Nam
GA	Gabon				

ASSAY DEVICES AND THE MANUFACTURE THEREOF

This invention relates to assay devices and to improved ways of manufacturing such devices.

Many assay devices are now available which comprise a porous carrier material, such as a strip, through which applied sample liquid such as urine can permeate and wherein the assay result occurs by means of specific binding of a detectable material in a precisely-defined region (detection zone) of the carrier, such as a narrow line or small dot, containing an immobilized specific binding reagent. Home-use versions of such devices for the analysis of urine, for example in pregnancy tests and ovulation prediction tests, are now widely available commercially. Many such devices are based on the principles of immunochromatography, and typically comprise a hollow casing constructed of plastics material containing a porous assay strip carrying pre-dosed reagents. The reagents within the device can include, for example, one or more reagents labelled with a direct label, such as a dye sol, a metallic (e.g. gold) sol, a non-metallic element (e.g. selenium, carbon) sol, or a coloured latex (polystyrene) microparticle, which are visible to the eye when concentrated in a comparatively small test area of the strip. The user merely needs to apply a urine sample to one part of the casing to initiate the assay. The assay result becomes visible by eye within a few minutes without further action by the user. Examples of such devices are described in EP-A-291194 and EP-A-383619, the disclosures of which are incorporated herein by reference. Sample collection can conveniently be achieved by means of a bibulous member which forms part of the device and which can readily take up sample liquid, eg. from a urine stream. Optionally the bibulous member can protrude from the casing of the device to facilitate sample application.

A particular objective of the invention is to improve the manufacture of assay devices by facilitating the precise locating of a relatively small detection zone within the device. This will have the advantages that less wastage may occur during manufacture by the production of fewer defective devices in which the detection zone is misplaced. Also, the subsequent reading of an assay result in the detection zone is facilitated, because for example an associated optical reading device may not need to "scan" to identify the exact location of the detection zone prior to determining an assay result.

In one embodiment, the present invention provides an assay sample testing device comprising a porous liquid-permeable carrier within a casing, said carrier including a detection zone thereof in which an assay result is revealed by specific binding of a detectable material directly or indirectly to a binding agent immobilised in said detection zone and said device casing includes internal registration means which cooperatively engage with corresponding registration means associated with said carrier such that said detection zone within said device casing is precisely located in relation to said registration means on said device casing. For example, said internal registration means comprises a peg or the like, engagable with a hole or depression in said carrier, said detection zone being situated at a precise predetermined location on said carrier relative to said hole or depression.

During manufacture of said assay device, said corresponding registration means may be used to facilitate or control accurate formation, e.g. by means of reagent printing techniques, of said detection zone on said carrier. In addition, or alternatively, accurate placement of said carrier within said device casing can be facilitated or controlled by said registration.

The invention particulaarly provides a process for the manufacture of an assay device comprising a porous strip within a casing, the strip including at least one detection zone formed by depositing a reagent onto a sheet of porous material prior to subdivision of the sheet into a plurality of identical strips each bearing a portion of the printed reagent, wherein:

a) prior to reagent deposition, the sheet of porous material is provided with a plurality of holes or depressions, at least one hole or depression lying in each region of the sheet which will form an individual strip on subdivision of the sheet;

b) during reagent deposition, the sheet is held by means of the holes or depressions in registration with a printing means such that deposition of the reagent on the sheet is inn a predetermined position relative to the holes or indentations; and

c) following subdivision of the sheet, the assay device is assembled by locating an individual strip within a casing by means of cooperative engagement between said at least one hole or depression and locating means within said casing.

The holes or indentations preferably lie along the longitudinal periphery of the sheet. Reagent deposition can involve printing reagent in one or more lines substantially parallel to an array of said holes or indentations.

A benefit of providing an internal registration system which ensures precise location of the detection zone within the test device, is that automated manufacture and quality control of the testing devices can be facilitated. If the testing devices are intended to be disposable after

individual use, they may need to be manufactured in large numbers at low cost. Internal registration can facilitate automated manufacture and high throughput. Other zones requiring critical placement, such as a control zone, can also be located more precisely if desired by means of the invention.

By way of example only, an assay device in accordance with the invention will now be described with reference to the accompanying drawings, of which:

Figure 1 shows a general view of a sheet of carrier material, e.g. nitrocellulose paper, during the course of reagent deposition on the sheet and subdivision of the sheet into assay strips.

Figure 2 shows an "exploded" view of an assay device of the invention incorporating an assay strip made as shown in Figure 1.

Referring to Figure 1, the sheet 100 of porous carrier material is intended to be divided into a plurality of identical assay strips 101 by cutting along central axis A-A and the lateral axes B-B.

Parallel lines (102-107) of assay reagents are placed on sheet 100 prior to sub-division. For the purposes of example only, the reagents involved are a first immobilised antibody in lines 102 and 107, and a second different immobilised antibody in lines 103 and 106. Reagent deposition can be by means of a "pen" 108 or the like operated on a computer-controlled "x-y" plotting mechanism (not shown) and fed with appropriate buffered reagent solution via a metered flexible tube 109. If the material of sheet 100 is nitrocellulose, reagents such as antibodies or antigens can be immobilised by simple direct application onto the nitrocellulose, followed by blocking of the sheet

material with albumen or polyvinyl alcohol. Following reagent deposition and blocking, two lines 104 and 105 of mobile labelled reagent, such as antigen (e.g. E3G, LH) labelled for example with a particulate direct label such as coloured latex, can be deposited. This deposition can be for example by means of an other pen (not shown). Alternatively, the labelled reagent(s) can be held in a separate porous pad or the like, rather than being applied directly to the test strip material.

In order to achieve precise location of the reagent-containing lines, each longitudinal periphery 110, 111 of sheet 100 is pierced with a plurality of identical small holes 112 each one being situated within the width of a designated strip 113. Holes 112 are made in sheet 100 prior to the deposition of any reagents. The untreated sheet is located on a frame (not shown) or similar operating surface by means of a bar 114 pressed downwardly onto each lateral periphery of the sheet. Only one of these bars is (partially) shown. Each bar has a plurality of downwardly projecting pegs 115, each of which locates precisely into one of the holes 112. The tracking of the reagent-depositing pen 108 is registered with the position of the bars holding the sheet, and accordingly the reagent deposition is made in a predetermined precise line relative to the perforations in the sheet.

Following all necessary reagent depositions and other treatments of the sheet, the sheet is subdivided by cutting means (not shown) into individual identical strips 101. Each individual strip therefore contains one locating hole 112 with two reagent-containing lines or reaction zones (e.g. 102 and 103) located relative to hole 112 in precise predetermined positions extending across the width of each strip. At a location remote from hole 112 is a region (e.g. 104) of the strip bearing the mobile labelled reagent. The exact position of the labelled reagent

relative to the hole is not critical.

Referring to Figure 2, an assay device of the invention comprises a plastics casing having upper and lower halves 200 and 201 adapted to contain the assay strip 101 and also an optional bibulous sample receiving member 202 which can extend out of one end 203 of the assembled casing. In the assembled device as shown the bibulous receiving member 202 overlaps the end 204 of the assay strip adjacent to the deposited labelled reagent. The upper half 200 of the casing includes an observation window 205 through which both detection zones 102 and 103 can be observed from outside the casing. On the inside of the upper half of the casing is a downwardly extending pin or peg 206. The diameter of the downwardly extending pin or peg 206 matches that of the hole 112 in the assay strip 101, so that the strip can be positively located within the assembled device on the peg.

Lower half 201 of the casing also includes a result observation window 207 which, in the assembled device lies, directly opposite to the result window 205 in the upper half of the casing. Lower half of the casing also contains means, such as a depression 208, which can accommodate the bottom end of the pin or peg 206 when the two halves of the casing are placed together to make an enclosure.

In the assembled device, the act of enclosing the strip and bibulous member between the upper and lower halves of the casing causes the overlapping portions 204 and 209 of the strip and bibulous member to be crimped together to provide a good moisture-conductive junction.

CLAIMS

1. An assay sample testing device comprising a porous liquid-permeable carrier within a casing, said carrier including a comparatively small detection zone in which an assay result is revealed by specific binding of a detectable material directly or indirectly to a binding agent immobilised in said detection zone, and said device casing includes internal registration means which cooperatively engage with corresponding registration means associated with said carrier such that said detection zone within said device casing is precisely located in relation to said registration means on said device casing.

2. A device according to claim 1, wherein said internal registration means comprises a peg or the like, engagable with a hole or depression in said carrier, said detection zone being situated at a precise predetermined location on said carrier relative to said hole or depression.

3. Process for the manufacture of an assay device according to claim 1 or claim 2, during which said corresponding registration means is used to control accurate formation of said detection zone on said carrier, and accurate placement of said carrier within said device casing.

4. A process for the manufacture of an assay device comprising a porous strip within a casing, the strip including at least one detection zone formed by depositing a reagent onto a sheet of porous material prior to subdivision of the sheet into a plurality of identical strips each bearing a portion of the printed reagent, wherein:

a) prior to reagent deposition, the sheet of porous material is provided with a plurality of holes or

depressions, at least one hole or depression lying in each region of the sheet which will form an individual strip on subdivision of the sheet;

b) during reagent deposition, the sheet is held by means of the holes or depressions in registration with a printing means such that deposition of the reagent on the sheet is inn a predetermined position relative to the holes or indentations; and

c) following subdivision of the sheet, the assay device is assembled by locating an individual strip within a casing by means of cooperative engagement between said at least one hole or depression and locating means within said casing.

5. A process according to claim 4, wherein said holes or indentations lie along the longitudinal periphery of the sheet.

6. A process according to claim 4 or claim 5, wherein reagent deposition involves printing reagent in one or more lines substantially parallel to an array of said holes or indentations.

7. A process according to any one of claims 4 to 6, wherein said casing is provided with an observation window through which said detection zone can be observed from outside the casing, location of the strip within the casing ensuring that said detection zone is in a predetermined position relative to said observation window.

8. A process according to any one of claims 4 to 7, wherein the strip carries more than one detection zone.

9. A process according to any one of claims 4 to 8, wherein said locating means within said casing is a peg.

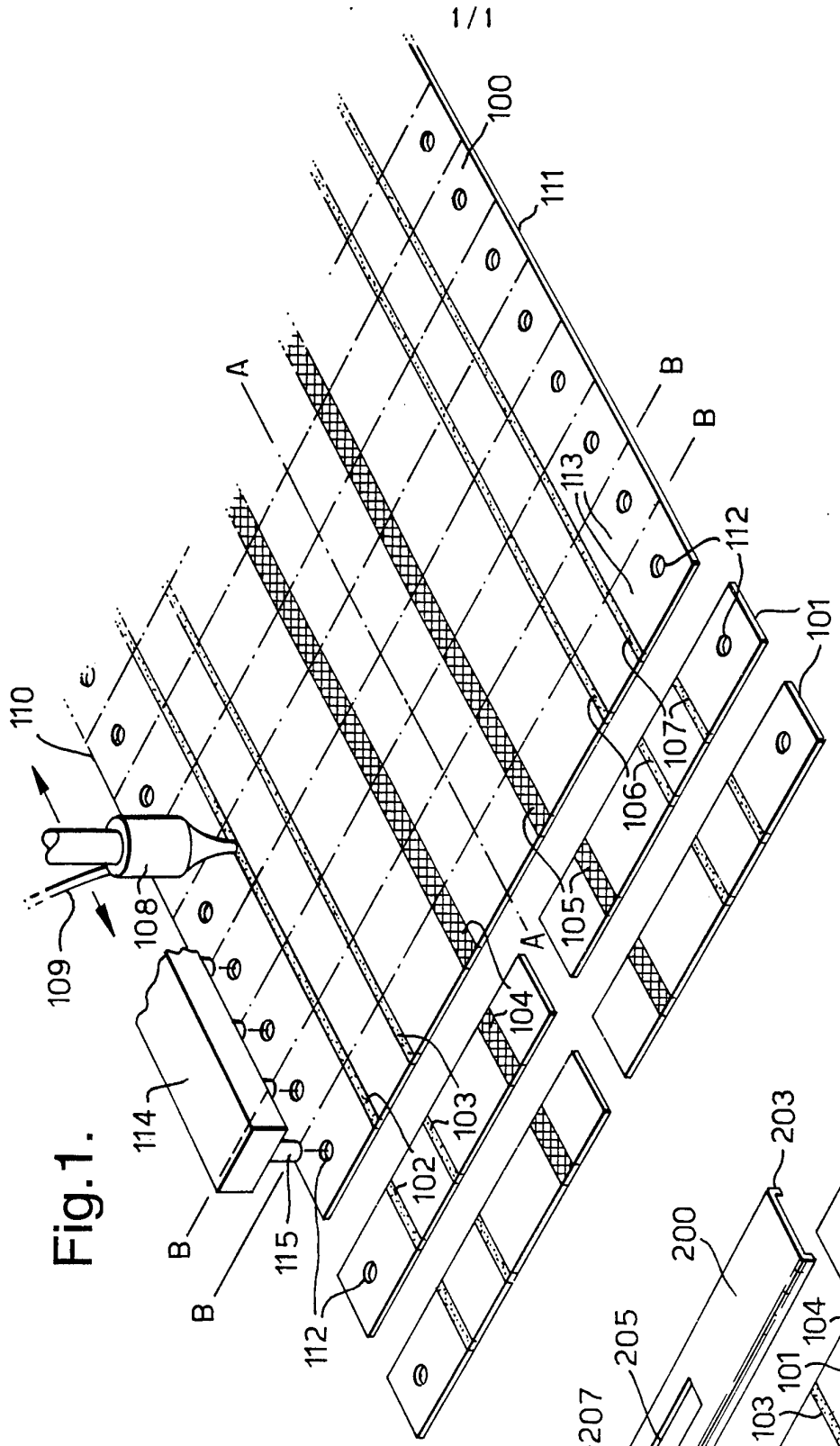

100
111
A
B
B
113
112
101
107
106
105
A
104
103
102
112
B
B
115
114
108
109
110

Fig.1.

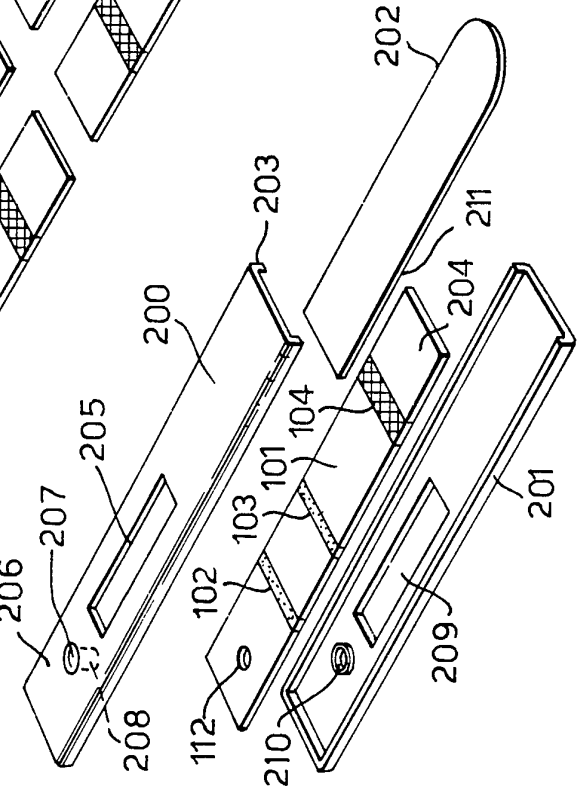

202
203
211
200
204
205
104
101
207
103
206
102
201
209
208
112
210

Fig.2.

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/EP 94/03701

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/558 G01N33/53

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N B01L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 492 326 (BOEHRINGER MANNHEIM GMBH) 1 July 1992 see column 6, line 31 - line 54; figures 2,4	1-3
Y		4-9
X	EP,A,0 259 797 (HOECHST AKTIENGESELLSCHAFT) 16 March 1988 see the whole document	1-3
X	WO,A,91 11700 (GDS TECHNOLOGY, INC.) 8 August 1991 see page 4 - page 24; figures 1-24	1,3

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 January 1995	17. 02. 95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	De Kok, A

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE WPI Week 8849, Derwent Publications Ltd., London, GB; AN 88-349753 & JP,A,63 261 161 (FUJI PHOTO FILM KK) 27 October 1988 see abstract	1-3
X	GB,A,1 218 749 (XEROX CORPORATION) 13 January 1971 see the whole document	3
Y		4-9
A	EP,A,0 560 410 (UNILEVER N.V.) 15 September 1993 see the whole document & EP,A,0 291 194 cited in the application	1,4-9
A	EP,A,0 174 247 (B.GUERIN ET AL.) 12 March 1986 see page 6, line 10 - page 7, line 37; figure 3	4-9

2

INTERNATIONAL SEARCH REPORT

...information on patent family members

Intern ...al Application No
PCT/EP 94/03701

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0492326	01-07-92	DE-A-	4041905	02-07-92
		US-A-	5281395	25-01-94
EP-A-0259797	16-03-88	DE-A-	3630777	24-03-88
		AU-B-	594499	08-03-90
		AU-A-	7821387	17-03-88
		CA-A-	1302118	02-06-92
		DE-A-	3777924	07-05-92
		JP-A-	63075644	06-04-88
		US-A-	4867946	19-09-89
WO-A-9111700	08-08-91	US-A-	5104619	14-04-92
		AU-A-	7253491	21-08-91
		EP-A-	0512059	11-11-92
GB-A-1218749	13-01-71	AU-B-	452281	12-08-74
		AU-A-	5037569	13-08-70
		BE-A-	728248	11-08-69
		CA-A-	950341	02-07-74
		CH-A-	501922	15-01-71
		DE-A-	1798423	02-08-73
		DE-A,B,C	1673340	24-02-72
		FR-A-	1584397	19-12-69
		GB-A-	1218745	13-01-71
		NL-A-	6902107	13-08-70
		US-A-	3526480	01-09-70
EP-A-0560410	15-09-93	AU-B-	626207	23-07-92
		AU-A-	1622888	02-12-88
		DE-D-	3887771	24-03-94
		DE-T-	3887771	26-05-94
		DE-U-	8805565	18-08-88
		EP-A-	0291194	17-11-88
		EP-A-	0560411	15-09-93
		ES-T-	2050704	01-06-94
		FR-A-	2614423	28-10-88
		WO-A-	8808534	03-11-88
		GB-A-	2204398	09-11-88
		JP-A-	6180320	28-06-94
		JP-A-	6160388	07-06-94

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter... al Application No
PCT/EP 94/03701

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0560410		JP-T-	1503174	26-10-89
		AU-A-	1704992	27-08-92
		AU-A-	1705092	27-08-92
EP-A-0291194	17-11-88	AU-B-	626207	23-07-92
		AU-A-	1622888	02-12-88
		DE-D-	3887771	24-03-94
		DE-T-	3887771	26-05-94
		DE-U-	8805565	18-08-88
		EP-A-	0560410	15-09-93
		EP-A-	0560411	15-09-93
		ES-T-	2050704	01-06-94
		FR-A-	2614423	28-10-88
		WO-A-	8808534	03-11-88
		GB-A-	2204398	09-11-88
		JP-A-	6180320	28-06-94
		JP-A-	6160388	07-06-94
		JP-T-	1503174	26-10-89
		AU-A-	1704992	27-08-92
		AU-A-	1705092	27-08-92
EP-A-0174247	12-03-86	FR-A-	2569478	28-02-86
		JP-A-	61062866	31-03-86