(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
24 November 2005 (24.11.2005)

PCT

(10) International Publication Number
WO 2005/110602 A1

(51) International Patent Classification[7]: B01L 3/00, A61B 10/00

(21) International Application Number: PCT/US2005/016400

(22) International Filing Date: 10 May 2005 (10.05.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
10/843,863 11 May 2004 (11.05.2004) US

(71) Applicant *(for all designated States except US)*: **INPRO BIOTECHNOLOGY, INC.** [US/US]; 870 Dubuque Avenue, South San Francisco, California 94080 (US).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **PRUSINER, Stanley, B.** [US/US]; 2615 Divisadero, San Francisco, California 94123 (US).

(74) Agent: **BENZ, William, H.**; Foley & Lardner LLP, 1530 Page Mill Road, Palo Alto, California 94304-1125 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ,*

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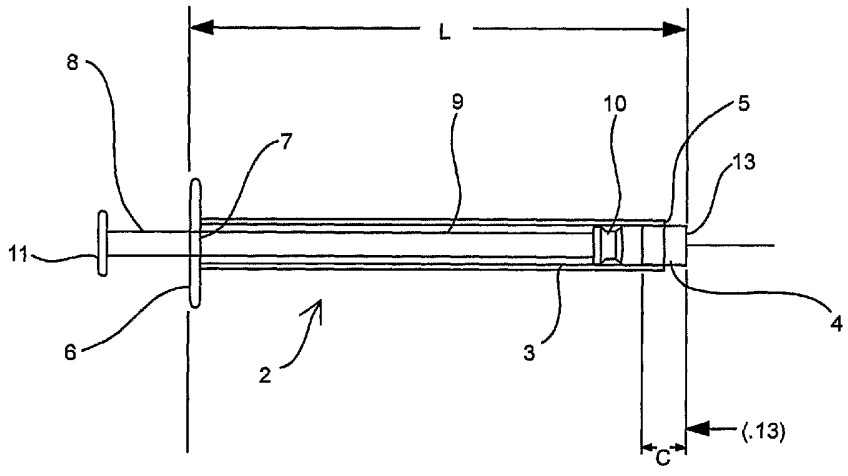
(54) Title: DEVICE, SYSTEM AND METHOD FOR EXTRACTING AND PREPARING ANIMAL TISSUE



(57) Abstract: A syringe type tissue extraction device is disclosed. The device comprises a transparent graduated tube having a sharp circumferential edge at one end which is pushed into the tissue and a plunger slidably disposed within the tube. Once the tube is inserted, the plunger is pulled back and by observation through the transparent tube and comparison with the graduations on the tube a predetermined amount of tissue is extracted. The sharp edge is then used to separate the sample of tissue within the cylinder from the bulk of the tissue. The tube is withdrawn from the bulk of the tissue and the device is then used to place the sample in a single homogenization tube or in, for example, a well of a 96 well plate. Each well of the plate (or the single tube) typically holds tissue-homogenization aids and once tissue is inserted the plate is shaken to homogenize the tissue in each well in order to prepare the sample for assaying.

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CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for all designations*

Published:

— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

DEVICE, SYSTEM AND METHOD FOR EXTRACTING AND PREPARING ANIMAL TISSUE

FIELD OF THE INVENTION

[0001] This invention relates to the field of devices for the removal of animal tissue and particularly brain tissue and to methods for preparing such tissue for assay.

BACKGROUND OF THE INVENTION

[0002] A range of different biopsy devices utilizing needle aspiration to extract tissue samples are known to those skilled in the art. Such devices are useful to obtain samples of tissue, such as tissue suspected of being cancerous. The tissue samples may be examined in order to confirm such suspected diagnosis. Such devices are frequently used when sampling suspected cancerous tissue in the lungs, liver, adrenal glands, kidneys, breasts, and prostate.

[0003] Biopsy devices may extract a sample of tissue from a tissue mass by either drawing a tissue sample into a hollow needle via an external vacuum force or by severing and containing a tissue sample within a notch formed in a stylet. Typical of such devices utilizing a vacuum force are U.S. Pat. No. 5,246,011 issued to Cailouette and U.S. Pat. No. 5,183,052 issued to Terwilliger. Such devices contemplate advancing a hollow needle into a tissue mass and applying a vacuum force to draw a sample into the needle and hold the same therein while the tissue is extracted.

[0004] Biopsy devices may utilize a biopsy needle with a tissue sample recovery notch formed thereon to extract a specimen, such as described in U.S. Pat. No. 3,477,423 issued to Griffith, often referred to as the TRU-CUT needle and U.S. Pat. No. 4,776,423 issued to Beraha et al.

[0005] Specially designed cannulas may be used to enhance the cutting and recovery of tissue samples, as well as combining the application of a vacuum force to draw a tissue sample into a biopsy cavity prior to cutting the tissue contained therein. U.S. Pat. No.

4,708,147 issued to Haaga discloses a cannula for a biopsy needle designed to cut a sample of tissue and then apply a vacuum to the cannula such that the tissue is drawn into the cannula and thus retained therein for recovery. Additionally, U.S. Pat. No. 3,844,272 issued to Banko discloses a biopsy device wherein a suction force, created by a vacuum, draws a sample of tissue into a receiving compartment whereby two coaxial members are rotated relative to each other so that the members essentially coact to cut off the specimen and place it into a compartment.

[0006] Many of these devices are complex and expensive to produce and are often reused which requires sterilization of the device after each use. Accordingly, there exists a substantial need in the art to provide a tissue sample extractor capable of effectively and efficiently drawing in a suitable sample of tissue and isolating the tissue sample within the biopsy device. Additionally, there is a need for a biopsy device that is easy to use and can effectively be manipulated by one hand. Furthermore, there is a need in the art to provide a tissue sample extractor that not only provides tissue samples of sufficient size, but allows the sample to be readily placed in a container for treatment and later analysis. Further, it would be of even greater advantage to provide a tissue sample extractor having the above-mentioned features which is also a completely disposable device which can be easily and cheaply manufactured.

SUMMARY OF THE INVENTION

[0007] A device for extracting samples of animal tissue (and in preferred embodiments brain tissue) and systems and methods for extracting such samples and for preparing the samples for assay are disclosed.

[0008] In a preferred application this device allows the cutting of a cylinder of brain tissue from the region of the obex without distorting the contralateral obex tissue. In preferred embodiments the tissue cylinder measures approximately 5 mm in diameter and 18 mm in length yielding ~350 mg. Because the device cuts through the brainstem without distorting the surrounding tissue, the cylinder of obex tissue can be removed without compromising the remaining tissue for confirmatory testing. Obtaining reproductible small samples of the obex where the highest number of prions is found is an important step in the

screening procedure for neurological disorders especially the disorder commonly referred to as "Mad Cow Disease."

[0009] The device, which makes up a first aspect of this invention, includes a transparent, graduated, hollow, cylindrical tube having an axis and having a sharp, tissue-cutting circumferential edge positioned perpendicular to the axis at a first end and a flange or protruding rim at the opposite end. A plunger having a first and a second end has its first end slidably positioned inside the hollow, cylindrical tube such that it can be partially withdrawn from the tube to create suction within the tube and draw sample into the tube and then can be further inserted into the tube to create pressure upon sample drawn into the tube and expel the sample from the tube. The plunger may have a flange, protruding rim or other means at its second end to facilitate manual movement of the plunger relative to the cylindrical tube. In one embodiment, the sharp, tissue-cutting circumferential edge can be provided by circumferentially sharpening the first end of the cylindrical tube.

[0010] In an alternate embodiment of this device, the sharp, tissue-cutting circumferential edge can be provided by a tubular metal cuff which at one end is coaxially connected to the first end of the hollow tube and at its second end extends from the first end of the hollow tube. The inside diameter of this tubular metal cuff is similar to the inside diameter of the hollow tube. The second end of the cuff extending from the tube provides the tissue-cutting circumferential edge and is optionally sharpened around its circumference. The tissue-cutting circumferential edge can be easily forced into animal tissue such as brain tissue to cut out a sample of about 0.1 to 10 ml, 0.1 to 2 ml, or 0.1 to 1.0 ml in volume which sample may be pulled into the tube and expelled from the tube with the aid of the plunger.

[0011] Another aspect of this invention is a method for extracting a sample from the tissue, and especially brain tissue, of an animal. This method includes the steps of:

(a) inserting into the tissue to be sampled the tissue-cutting first end of the cylindrical tube of the tissue extraction device of this invention to cut out a sample of tissue of about 0.1 to 10 ml, 0.1 to 2 ml, or 0.1 to 1.0 ml in volume. This tissue extracting device includes a transparent, graduated, hollow, cylindrical tube having an axis and having a

sharp, tissue-cutting circumferential edge positioned perpendicular to the axis at a first end and a flange or protruding rim at the opposite end. A plunger having a first and a second end has its first end slidably positioned inside the hollow, cylindrical tube such that it can be partially withdrawn from the tube to create suction within the tube and draw sample into the tube and then can be further inserted into the tube to create pressure upon sample drawn into the tube and expel the sample from the tube. The plunger may have a flange, protruding rim or other means at its second to facilitate manual movement of the plunger relative to the cylindrical tube so that sample may be pulled into the tube and expelled from the tube with the aid of the plunger.

(b) withdrawing the plunger to draw the sample of tissue into the tube.

(c) withdrawing the tube from the tissue, and

(d) moving the plunger forward to expel the tissue sample from the tube into a sample-receiving container.

[0012] In one preferred embodiment of this method of extracting a sample aspect of this invention, the sample-receiving container is a simple individual sample vial. In a second preferred embodiment of this aspect of the invention the sample container is a well of a multiple sample container such as a 96 well plate and especially such a plate wherein the internal diameter of the wells is substantially the same as the external diameter of the transparent cylindrical tube of the extraction device.

[0013] Yet another aspect of the invention is achieved by repeating steps (a), (b), (c) and (d) a plurality of times wherein a new tissue extraction device is used in each set of repeated steps to extract tissue from another animal which is expelled into a different sample-receiving container such as a different well of a multi-well plate.

[0014] In accordance with the above method in step (d) the tissue sample, such as a brain tissue sample, may be expelled into an individual sample container such as a tube or vial or the like having a volume of about 1.5 to 3 ml or more particularly about 2 ml in volume. These tubes are most commonly made of glass or polypropylene or other plastic. Such a method can be used in accordance with a low through-put system which does not

require obtaining large numbers of samples which would be used with the high through-put 96 well plate system.

[0015] Once the tissue is extracted it is expelled from the tube of the extraction device by forcing the plunger forward. The expelled tissue is placed in a sample container generally in combination with tissue-breaking beads which aid in homogenizing the tissue sample placed therein.

[0016] An advantage of the invention is that the tissue extraction device it provides is simple and easy to use for the rapid extraction of tissue. In addition, with a minimum of practice a user of this device can become proficient at taking multiple samples which are reproducible and virtually identical in size to one another and to a predetermined standard.

[0017] Another advantage of the invention is that the tissue can be easily and rapidly extruded from the device into the next available well or other container for later treatment.

[0018] Yet another advantage of the device of the invention is that it is inexpensive and as such can be disposed of after a single use.

[0019] These and other objects, advantages, and features of the invention will become apparent to those persons skilled in the art upon reading the details of the device, system and method as more fully described below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] The invention is best understood from the following detailed description when read in conjunction with the accompanying drawings. It is emphasized that, according to common practice, the various features of the drawings are not to-scale. On the contrary, the dimensions of the various features are arbitrarily expanded or reduced for clarity. Included in the drawings are the following figures:

[0021] Fig. 1 is a cross-sectional view of an embodiment of a tissue extraction device of the invention;

[0022] Fig. 2 is a cross-sectional view of another embodiment of a tissue extraction device of the invention with a biasing means;

[0023] Fig. 3 is a cross-sectional view of yet another embodiment of a tissue extraction device of the invention;

[0024] Fig. 4 is a cross-sectional view of still another embodiment of a tissue extraction device of the invention;

[0025] Figs. 5 and 6 are perspective views of two embodiments of a 96 well plate which may be a component of the invention;

[0026] Figs. 7 and 8 are detail views of the sharpened metal cuff which is used as the tissue-cutting surface in several embodiments of this invention;

[0027] Fig. 9 is a top plan view (dorsal view) sketch showing a bovine brainstem pointing out the obex region;

[0028] Fig. 10 is a side elevational view (lateral view) of the bovine brainstem of Fig. 9.

[0029] Fig. 11 is a sketch of a bovine brainstem in the hand of a gloved researcher with a device of the invention positioned thereon;

[0030] Fig. 12 is a sketch of a bovine brainstem being held while a device of the invention is being used to extract a sample; and

[0031] Fig. 13 is a graph showing the high degree of sample size reproductivity achievable using the sampling device of this invention.

DETAILED DESCRIPTION OF THE INVENTION

[0032] Before the present device, system and method is described, it is to be understood that this invention is not limited to a particular embodiment described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the

purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0033] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limits of that range is also specifically disclosed. Each smaller range between any stated value or intervening value in a stated range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included or excluded in the range, and each range where either, neither or both limits are included in the smaller ranges is also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

[0034] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

[0035] It must be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a sample" includes a plurality of such samples and reference to "the assay" includes reference to one or more assays and equivalents thereof known to those skilled in the art, and so forth.

[0036] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of

prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

[0037] Fig. 1 shows a schematic cross-sectional view of a device of this invention 2. The device 2 is comprised of a transparent cylindrical tube 3 and has a cuff 4 coaxially fitted to its first end 5 and has a flange 6 at its second end 7. As shown in detail in Figs. 7 and 8, cuff 4 is a hollow cylinder open at both ends 36 and 38. End 36 is the end that is fitted into tube 3 and end 38 provides a tissue-cutting circumferential surface 13. If the wall thickness of cuff 4 is less than about 0.1 mm end 38 may be inherently sharp enough to provide the desired tissue-cutting function. Alternatively, and preferably, end 38 is ground to provide a circumferential sharpened edge 40. Several additional alternative configurations for the tissue-cutting surface 13 will be described with reference to other figures. However, it should be understood that in all cases the goal is to provide a circumferential edge 13 at the end 5 of the cylindrical tube 3 which is sharp enough to permit the person using the device to sample tissue to easily, smoothly and confidently insert end 5 of the device into the tissue being sampled.

[0038] A plunger 8 sealably slides within cylinder 3 between its ends 5 and 7. Plunger 8 may be a conventional syringe plunger which would typically include a shaft 9 with a rubber or polymeric stopper 10 on its end inserted inside the tube 3. The shaft 9 has a flange or handle 11 at its other end which can be easily pushed to move the stopper 10 toward the end 5 of the cylindrical tube 3 and easily pulled to move the stopper 10 toward the end 7 of the tube 3 and create a tissue-sample-drawing vacuum inside the tube 3.

[0039] In additional embodiments of the device which is shown in Fig. 2 a biasing means such as an elastic body or spring 12 is provided to bias flange 11 toward or away from flange 7 thereby moving the stopper 10 within the tube 3 to a set, predetermined position. In one embodiment, the biasing means 12 is designed to position the stopper at a preset point and to provide a preset volume sample to be reproducibly extracted. In use, the user manually moves flange 11 toward flange 6 the maximum extent possible, thus moving stopper 10 toward end 5 of tube 3. At this point, the sharp edge of cuff 4 is used to cut into the tissue being sampled and the tube 3 is forced into the tissue. As this occurs the manual pressure on the flange 11 is released and the biasing means 12 forces the flange 11

away from the flange 7 by a predetermined amount. This predetermined amount causes the stopper 10 to move a predetermined distance thereby causing a predetermined volume of tissue (e.g. 0.3 ml ± 5%) to be drawn into the tube 3.

[0040] In the embodiments shown in Figs. 1 and 2 the cuff 4 has an external diameter which is substantially the same as the internal diameter of the tube 3. Accordingly, end 36 of cuff 4 fits snugly into end 5 of tube 3 and may be held in position by the snugness of the fit between the internal diameter of the tube 3 and the external diameter of the cuff 4 alone or in combination with an adhesive between the outside of the cuff 4 and the inside of the tube 3.

[0041] It will be noted that in the embodiments shown in Figs. 1 and 2 the external diameter of the tube 3 and the external diameter of the cuff 4 are different with the tube 3 having a larger diameter. This is generally not a problem but the step up in diameter may present undesired additional resistance as the device is inserted into a body of tissue to take a sample.

[0042] However, in the embodiments of the extractor shown in Figs. 3 and 4 this step up is not present. In Fig. 3 the internal and external diameter of the cuff 4 matches the internal and external diameter of the tube 3. When the external diameters of both the tube 3 and cuff 4 match each other the device can be more easily inserted into tissue without any interference caused by the step in diameter at end 5 of tube 3. In this embodiment the end 5 of tube 3 and end 36 of cuff 4 may be machined to mate with one another and facilitate their attachment while maintaining the desired overall constant internal and external diameters at their point of joining. In the embodiment of Fig. 4, the cuff 4 may not be present as a separate part but rather is an integral part of tube 3 and the sharpened circumferential edge 40 essentially becoming a sharpened portion of the end 5 of tube 3.

[0043] Device 2 can be simply manufactured from a commercial syringe lumen which is cut and modified by the addition of a cuff 4 or, in the case of the devices of Fig. 4, by circumferentially sharpening end 5. This simplifies the manufacture and reduces the cost of the devices and thus reduces the cost of the entire procedure in which the devices are

used. When a commercial syringe is used the syringe has a body made of a transparent polymeric material.

[0044] The device of the invention can be used for the extraction of many types of tissue. It is preferably used to extract tissue and especially brain tissue from dead animals, including for example avian species and other nonmammals but particularly mammals, and in particular farm animals including cattle, pigs, horses, sheep, goats and the like. In a most preferred application the device of this invention is used to extract brain tissue from these animals and especially cattle which are being assayed for the presence of prions. The device can readily be used for the extraction of a sample of brain tissue from a deceased human or other primate, as well.

[0045] The device of the invention can be produced in various sizes such as in the range of 0.1 to 10 ml. In its preferred application extracting samples of brain tissue, the devices are generally sized to extract samples in the range of 0.1 to 5 ml and especially are sized to extract a sample of about 0.2 to about 0.4 ml and especially about 0.30 ml, as this is a sample size (0.25-0.35g) that works well in brain tissue assays. Thus, a preferred syringe body is one graduated to show a measured volume of 1ml (± 5% or ±1% or ±0.1%). A preferred syringe body includes graduation markings on its cylinder 3 to indicate when the desired sample volume of say 0.3 ml is reached and also preferably should have various fractional points indicated thereon such as ½, ¼ , 1/5, 1/3, or 1/10 markings which markings show the sample volume with a very small (e.g. ± 0.01 ml) volume of error.

[0046] The inside diameter of the tube 3 should be approximately 3/16 of an inch (0.4 cm). The length of the tube 3 plus the cuff 4, if present, "L" should be approximately three inches (75-80 mm) and the length "C" of the cuff 4 should be approximately ¼ inch (6-7 mm) with about half 36 of the cuff inserted into the tube 3 and half 38 extending outside of the tube 3 with the cutting edge 13 (or 40) of the cuff 4 extending outward.

[0047] The system of the invention comprises the device 2 as shown in any of Figs. 1-4 in combination with a sample-containing device such as 96 well plate 21 as shown in Figs. 5 and 6. The plate 21 is comprised of a plurality of wells and in preferred

embodiments includes 96 wells. The volume of each well should be large enough to accommodate the sample of the size extracted by the devices of this invention and generally, in addition, some amount of liquid and tissue-breaking beads. The liquid and beads aid in homogenization of the tissue sample which is often part of the assay of the tissue. The liquid and beads are entirely optional. However, in most assays, the tissue is dispersed such as by sonication, use of a homogenization device or mixer or the aforemention beads. The well volume (1-3 ml) should accommodate this.

[0048] The top 22 of the wells is optionally covered with a breakable seal 23 as shown in Fig. 5. Fig. 6 shows a 96 well plate 21 without seal 23. The seal 23, if present, may be made of any material which does not adversely affect the sample and which is breakable using the sharp end 13 of the sampling device. It has been found convenient to make the breakable seal 23 of a metal foil such as aluminum foil, which may be adhered to the top surface 22 of the wells, such as by adhesive or more preferably without adhesive but rather by pressure and by conforming in shape to the top surface 22.

[0049] If a breakable seal 23 is present, in use it is punctured by the sharp end 13 of device 2 shown in any of Figs. 1-4. Once the seal is punctured the plunger 8 is moved forward in order to expel the sample out of the tube 3 and into the well. By having the breakable seal 23 in place it is possible to easily determine that the well has been filled and keep the well sterile prior to breaking the seal. The next sample is then placed in the next available well for which the seal has not been broken. The process is repeated up to a total of 96 times and with each tissue extraction a new device 2 is used. This avoids the necessity for sterilization of the device which would be necessary to avoid contamination from one sample to the next if a single sampling device was used.

[0050] Alternatively, the 96 well plate may employ a conventional removable plastic plate cover in place of the breakable seal 23 just described. This plate cover would be removed to add samples and replaced for storage.

[0051] As previously noted, each well of the 96 well plate may contain one or a plurality of beads such as 2, 3, 4, 5 or 10 or more etc. small beads approximately 1 to 3 mm in diameter. The well may also contain reagent materials to aid in dispersing the tissue for

assay or for conducting the assay. These include, for example, water or other liquid, protease, detergent, buffers and the like. Once all of the wells (or as many of the 96 wells as are needed) are filled, the 96 well plate is shaken vigorously so that the beads aid in homogenizing the sample and mixing the reagent into the sample. When all the samples have been sufficiently homogenized the entire 96 well plate may be subjected to centrifugation.

[0052] It will be appreciated that a high through-put configuration such as a 96 well plate is excellent when a large number of samples need to be run in the system. It will, however, be equally appreciated that there are settings such as where a small number of animals are being assayed where the 96 well plate would be inefficient. In these cases, single or multiple individual sample tubes, or the like can more efficiently and more easily be used. A more detailed description of the sample preparation is provided in the examples. After the sample is prepared the prepared sample is used such as in an assay in order to determine if the sample contains prions. A more detailed description of the assay process is also provided in the examples.

[0053] In the most preferred application where the tissue extractor is used in conjunction with an assay for prions in bovine brain tissue the first step is to isolate the brainstem. Such an isolated brainstem 31 is shown in Figs. 9 and 10. Figs. 9 and 10 specifically identify the obex region 33 of the brainstem 31 and show it in the context of other regions of the brainstem including the medulla through caudel peduncles region 35 at the midbrain through rostral colliculi region 37.

[0054] When extracting the sample from the brainstem it is important to follow a set procedure in order to extract tissue from the appropriate area and in order to minimize damage to any un-sampled section of the brainstem in that another section of the brainstem may be used for confirmatory testing. It is recommended that a user of the device extract away approximately ten samples in order to ensure that accurate and consistent tissue samples are obtained. It is desirable to obtain a tissue sample which as a weight in a range of about 0.25 to about 0.35 grams.

[0055] In order to extract the sample the brainstem 31 shown in Fig. 11 is placed in the palm of the user's gloved hand 30. To ensure additional protection and minimize cross-contamination the gloved hand may be placed inside a clear plastic bag or plastic glove which is discarded and replaced between samplings. The sample is then oriented such that the spinal cord 32 in the hand 30 faces toward the user and the anterior portion 39 extends towards the thumb 34.

[0056] A sampling device 2 of a type as shown in Figs. 1-4 is then held at an angle of about 10 to 15° with respect to the sample as is shown within Fig.11. The plunger 8 is pushed into the device 2 such that its end is at a consistent position at or near the sharp end 40 of cuff 4 (if present) or at or near the otherwise sharp end 13 of the device 2. The sharp end 13 of device 2 is inserted into the sample approximately 1 to 2 cm (0.5 to 0.75 inch) at an area posterior to the obex region 33 which is shown in Figs. 9 and 10. As shown in Fig. 11 the end 13 of device 2 is inserted into one side of the brainstem. Failure to limit the sampling to one side may make it difficult to check the results if a confirmation test is necessary. After inserting the end 13 of the device 2 into the brainstem as shown in Fig. 12 the user simultaneously begins to pull back, slowly, on plunger 8 decreasing the angle of the device relative to the sample slightly and continuing to push the end 13 of the device 2 into the obex region 33.

[0057] As shown in Fig. 12, end 13 of the device 2 is pushed into the center of the obex region while pulling back on plunger 8. A trained user will be able to consistently withdraw plunger 8 to a consistent volume as the sharp end 13 is pushed to a consistent position within the obex 33. Once the end 13 of device 2 has reached the center of the obex region 33 the device 2 should be twisted one complete turn and the sharp end 13 (40) of the device 2 is then pushed against the wall of the obex 33 in order to release, cut or break the tissue sample away. With training, and watching the position of the plunger 8 relative to the graduations on the wall of the cylinder 3 the user can become quite proficient at breaking away samples which are consistently sized and sized to conform to the assay procedures to follow. When the sample has broken away from the brainstem tissue the sample within the device 2 should be pulled further up into the cylinder 3 by withdrawing

the plunger 8 slightly. After the sample is moved further up into the cylinder 3 of device 2 the device can be removed from the brainstem 31.

[0058] After the device 2 is taken out of the brainstem 31 the plunger 8 should be pushed forward slowly until the end 10 of plunger 8 contacts the tissue sample present within cylinder 3 of the device 2 and moves the tissue sample to a consistent position relative to the end 13 of the device. At this point the user should confirm that the end 10 of the plunger 8 is positioned at or near the desired volume markings on the cylinder 3, such as between the 0.3 and 0.35 ml marks on a 1 ml device. If there is too much tissue in the device it is possible for the user to push out tissue into a waste container until a proper measurement is achieved. If there is too little tissue in the device then the sample material may be extruded into a weighing device and reinserted into the same side of the brainstem where the process can be repeated in order to extract additional material with the device 2.

[0059] The sample should be dispensed for example into a tubular opening 23 in the 96 well plate 21 of Figs. 5 or 6, if a substantial number of samples are being taken from a substantial number of tissue specimens. If only one or a few samples are being taken, the sample may be conveniently dispensed into an individual sample tube or the like.

[0060] The sampling device 2 should then be discarded into a receptacle where all materials will be properly disposed of. It should be kept in mind that prions are infectious and that they are particularly difficult to inactivate. The brain stem sample should be returned to an appropriate container with appropriate marking indices in order to associate that brainstem with the sample deposited into the opening of the 96 well plate. By doing this it is possible to confirm a test. The process may be repeated multiple times until up to all of the openings within the 96 well plate are filled.

[0061] In general the sample is treated to concentrate any prions it may contain. This is carried out by homogenizing the sample and mixing the sample with a complexing agent which selectively complexes with any prions within the sample. The complexing agent may be any compound or material which selectively binds to prions and forms a complex which has a higher specific gravity as compared to the prion itself or the remainder of the sample. A preferred complexing agent is sodium phosphotungstate. Those skilled in the

art will recognize a range possible complexing agents which include other salts of phosphotungstic acid, antibodies, and other agents which selectively bind to prions forming a complex which has the desired higher specific gravity. The complexing agent is mixed with the sample for a time sufficient to form complexes between the agent and any prions which might be present in the sample. After the mixing has been completed the sample is subjected to centrifugation to separate and concentrate the high specific gravity prion complexes.

[0062] The sample may be treated with a proteinase which selectively degrades other proteins present within the sample, i.e. proteins other than prions. The treatment process may be eliminated in some circumstances and when used with Proteinase K or other suitable protease or other agent which degrades other proteins present in the sample e.g., heat, pressure or agitation. This treatment may be carried out prior to, during or after centrifugation. A more detailed description of sample preparation is provided within U.S. Patent 5,977,324 issued November 2, 1999 incorporated here by reference to disclose and describe a sample preparation method.

[0063] Once the sample has been properly prepared, individual samples within the individual wells or sample vials are then subjected to an assay method in order to determine if the prepared sample contains prions. In one embodiment, this is carried out by extracting the sample to localize any prions and then dividing the sample into a first portion and a second portion. The first portion of the sample is contacted with an antibody such as an antibody disclosed within U.S. Patent 6,537,548 issued March 25, 2003 incorporated herein by reference to disclose and describe antibodies, labeled antibodies, and methods of using such. The antibody binds to PrP proteins in their non-disease conformation with a higher degree of affinity than to PrP proteins in their disease conformation.

[0064] The second portion of the sample is then subjected to further treatment. This treatment causes any PrP protein which is present in the second disease-related conformation (prions) to assume a different conformation which has a higher degree of binding affinity for the antibody than the untreated protein. Thereafter, the treated second portion is brought into contact with a labeled antibody of the same type used in contacting the first portion of the sample.

[0065] The level of binding of labeled antibody to PrP protein in the first portion is determined as is the level of binding to the treated PrP protein in the second sample. The level of binding of the labeled antibody to PrP protein in the first portion of the sample is then compared with the level of binding in the second portion. If an increase is seen in the second sample it indicates that it is likely that the second sample contained PrP protein in the second disease conformation, i.e. that the sample included prions. In essence the assay is carried out by relaxing the constricted conformation of prions so that they bind to the antibody. If there are no prions present then there will be no constricted conformation to relax. Those skilled in the art will understand that the treatment of the second portion may cause some increase in signal even if no prions are present. However, this increase can be accounted for and appropriate adjustments made so as to not assume a positive result when no prions are present. A more detailed description of such an assay is provided within U.S. Patent 5,891,641 issued April 6, 1999 incorporated herein to disclose and describe this and other assay methods. In addition, other assays for the detection of prions could be used to test the samples prepared in accordance with the present invention.

EXAMPLES

[0066] The following examples provide those of ordinary skill in the art with a complete disclosure and description of how to practice the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

EXAMPLE 1

[0067] Devices 2 as shown in Fig. 1 are used to extract tissue samples from bovine brainstems in a manner as described above. Three hundred examples of this device 2 were produced. In order to test the accuracy of the device nine of those devices were selected.

The nine devices were in three categories with category 1 being the least sharp, category 2 being of medium sharpness and category 3 being the most sharp. Each device 2 was used to extract three samples. This would not be done in practice in that a device would be discarded after extracting a single sample in order to avoid the possibility of contamination. The result of the 27 different tests are set forth below in terms of the weight of the samples obtained.

	Weights (milligrams)		
1	245	345	338
1	308	350	289
1	345	300	256
2	315	319	276
2	311	215	350
2	350	348	366
3	355	323	335
3	320	342	250
3	347	321	329

Ave.	316.5926
Std.dev.	38.63464
%CV	12.203327
Min	215
Max	366

[0068] As shown in the above example the device 2 makes it possible to repeatedly obtain substantially the same amount of brainstem sample. Those skilled in the art will understand that it is somewhat difficult to obtain multiple samples from a bovine brainstem and will appreciate the accuracy and convenience of the device described here.

[0069] Thereafter this experiment was repeated until over 1000 samples had been taken and weighed. The results of these further experiments are provided in Fig. 13 and demonstrate that the devices of this invention provide very consistently-sized samples.

EXAMPLE 2

[0070] A tissue extraction device 2 is used to extract 300 milligrams of bovine brain tissue from the brain of a slaughtered animal. The extracted 300 milligrams of tissue is placed in a well of a 96 well plate of the type as shown in Figure 5. The well has therein a plurality of beads and proteinase K. After depositing the sample in the well the device 2 is discarded. Thereafter, a new device 2 is used to extract a sample from the brain of a different animal and the same process is repeated. The process is repeated until each well of the 96 well plate has a sample of about 300 milligrams therein and 96 different devices 2 as shown in any of Figs. 1-4 have been used and discarded. The 96 well plate is then shaken vigorously in order to homogenize the sample within each of the wells and intermix the proteinase K into the homogenized sample.

[0071] This example can also be carried out without the use of the 96 well plate. As indicated above it is possible to use individual sample containers such as 2 ml tubes which may include beads into which the sample is placed and shaken in order to bring about homogenization of the brain tissue. Similarly other sample containers such as 24 well plates or the like could be used.

EXAMPLE 3

Purification of Hamster PrP^{c} from Normal and PrP^{Sc} From Scrapie Infected Hamster Brains

[0072] The PrP^{c} protein can be purified as described in Pan, Stahl et al. (1992) Protein Sci 1:1343-1352; Pan, Baldwin et al. (1993) Proc Natl Acad Sci USA 90:10962-10966. Protein content can be determined by the BCA method. The purity of PrP^{c} protein, can be determined on SDS PAGE followed by silver staining and Western Blot.

[0073] Standard Syrian hamster PrP^{Sc} can be purified from a standard pool of scrapie strain Sc237 infected hamster brains as described in Turk, Teplow et al. (1988) Eur J Biochem 176:21-30. The infectivity of this standard, as determined by an incubation time assay on Syrian hamsters after intracerebral inoculation, should be $10^{7.3}$ ID_{50}/ml and specific infectivity $10^{8.2}$ ID_{50}/mg of PrP^{Sc} protein. However, the specific infectivity may

vary from lot to lot ± $10^{0.5}$ ID_{50}/mg. The protein content can be determined by BCA assay using bovine serum albumin as a standard. The preparation can be considered homogeneous with one major band on SDS PAGE after silver staining and Western Blots. The PrP proteins of the brain of other animals can be obtained in the same manner.

EXAMPLE 4

Isolation of PrP^{Sc} from bovine brain

[0074] PrP^{Sc} is isolated from a fresh bovine brain sample of an animal that exhibited symptoms of a neurological disorder consistent with the presence of PrP^{Sc}. Approximately 10 g of brain tissue is used to produce a homogenate. The brain tissue is flash-frozen in liquid nitrogen, and then homogenized using a standard mortar and pestle technique to dissociate the tissue for further extraction procedures. Phosphate buffered saline (PBS) pH 7.4 containing 4% (w/v) sodium dodecylsarcosinate (sarcosyl), an ionic surfactant, is added to the brain homogenate in a 1:5 (v/v) ratio to the brain homogenate. A solution of 4% sodium phosphotungstic acid (PTA) and 170 mM $MgCl_2$, pH 7.4, is added to the buffered homogenate solution to a final concentration of 0.2% PTA. The sample is exposed to the PTA for 16 hours at 30°C on a rocking platform. At the end of 16 hours, Proteinase K is added to the solution providing a final concentration of 25 μg/ml, and the sample is incubated for one additional hour at 37°C. The addition of Proteinase K generally increases the efficiency of the precipitation of PrP^{Sc}, in part by degrading other remaining proteins including PrP^{c}.

[0075] Following incubation, the sample is transferred to 1.5 ml sterile tubes, with approximately 1 ml aliquots of the PTA-homogenate solution per tube. The sample is centrifuged at 10,000 x g in a table top centrifuge (Eppendorf) for 40 minutes at room temperature. The supernatant is decanted from the tubes, and each pellet is resuspended in sterile water to the desired overall protein concentration. Protease inhibitors are added to the solution: PMSF to a concentration 0.5 mM, Aprotinin to a final concentration of 2 mg/ml, and Leupeptin to a final concentration of 2 mg/ml. The protease inhibitors protect the sample from degradation under certain storage conditions. An aliquot of protein for current use is stored at 4°C. The remaining protein is aliquoted, and stored at -20°C.

[0076] The total protein content of the pellet is reduced 100-fold as compared to similar procedures known in the art. The PrP^{Sc} or PrP 27-30 content of this pellet represents approximately 40-60% of the total protein. This procedure thus results in a protein sample highly enriched in PrP^{Sc} protein species.

EXAMPLE 5

Isolation of PrP^{Sc} from human brain

[0077] PrP^{Sc} is isolated from brain samples of a deceased individual suspected of being affected with a prion-based neurological disorder. Approximately 5 g of human brain tissue is used to produce a homogenate. The homogenate is produced using a dounce homogenizer to dissociated the tissue for protein extraction. A solution of 0.24 mM Triton-X, a non-ionic surfactant, in PBS pH 7.4 is added to a final 1:1 (v/v) ratio. A solution of 4% sodium phosphotungstic acid (PTA) and 170 mM $MgCl_2$, pH 7.4, is added to the buffered homogenate solution to a final concentration of 0.3% PTA. The sample is exposed to the PTA for 8 hours at 37°C on a rocking platform.

[0078] Following incubation, the sample is transferred to 2.0 ml sterile tubes, with approximately 1 ml aliquots of the PTA-homogenate solution per tube. The sample is centrifuged at 14,000 x g in a table top centrifuge (Brinkmann) for 30 minutes at room temperature. The supernatant is decanted from the tubes, and each pellet is resuspended in a 25 μg/ml Proteinase K solution. The tubes are incubated for one additional hour, rocking, at 37°C. The sample is again centrifuged at 14,000 x g in a table top centrifuge for 30 minutes at room temperature. The supernatant is decanted, and the sample resuspended in 100 μl of sterilized water. Concentration can be determined using spectrophotometric techniques. The PrP^{Sc} or PrP 27-30 content of this pellet represents approximately 40-60% of the total protein.

EXAMPLE 6

Isolation of PrP^{Sc} from sheep brain

[0079] A brain sample from a sheep exhibiting neurological disorders is isolated such as by using a device of this invention and homogenized using a Polytron automated homogenizer. Approximately 1 gm of protein is homogenized in this fashion. PBS pH 7.4 containing 1% (w/v) sodium dodecyl sulfate (SDS) is added to the brain homogenate in a 5:1 (v/v) ratio. A solution of 4% sodium phosphotungstic acid (PTA) and 170 mM $MgCl_2$, pH 7.4, is added to the buffered homogenate solution to a final concentration of 0.25% PTA. The sample is exposed to the PTA for 1 hour at 45°C on a rocking platform.

[0080] Following incubation, the sample is transferred to 1.5 ml sterile tubes, with approximately 1 ml aliquots of the PTA-homogenate solution per tube. The sample is centrifuged at 20,000 x g in a table top centrifuge (Eppendorf) for 20 minutes at room temperature. The supernatant is decanted from the tubes, and each pellet is resuspended in sterile water to the desired overall protein concentration. An aliquot of protein for current use is stored at 4°C. The remaining protein is aliquoted, and stored at -20°C. The PrP^{Sc} or PrP 27-30 content of this pellet represents approximately 40-60% of the total protein.

[0081] The preceding merely illustrates the principles of the invention. It will be appreciated that those skilled in the art will be able to devise various arrangements which, although not explicitly described or shown herein, embody the principles of the invention and are included within its spirit and scope. Furthermore, all examples and conditional language recited herein are principally intended to aid the reader in understanding the principles of the invention and the concepts contributed by the inventors to furthering the art, and are to be construed as being without limitation to such specifically recited examples and conditions. Moreover, all statements herein reciting principles, aspects, and embodiments of the invention as well as specific examples thereof, are intended to encompass both structural and functional equivalents thereof. Additionally, it is intended that such equivalents include both currently known equivalents and equivalents developed in the future, i.e., any elements developed that perform the same function, regardless of structure. The scope of the present invention, therefore, is not intended to be limited to the

exemplary embodiments shown and described herein. Rather, the scope and spirit of present invention is embodied by the appended claims.

WHAT IS CLAIMED IS:

1. A tissue extraction device comprising:

a transparent, graduated, hollow, cylindrical tube having an axis and having a sharp, tissue-cutting circumferential edge positioned perpendicular to the axis at a first end and a flange or protruding rim at the opposite end;

plunger having a first and a second end with its first end slidably positioned inside the hollow, cylindrical tube such that it can be partially withdrawn from the tube to create suction within the tube and draw sample into the tube and then can be further inserted into the tube to create pressure upon the sample drawn into the tube and expel the sample from the tube.

2. The device of claim 1 having a flange, handle, protruding rim or other means at its second end to facilitate manual movement of the plunger relative to the cylindrical tube.

3. The device of claim 1 wherein the sharp, tissue-cutting circumferential edge is provided as a circumferentially sharpened first end of the cylindrical tube.

4. The device of claim 1 wherein the sharp, tissue-cutting circumferential edge is provided by the second end of a tubular metal cuff having a first end and a second end and which at its first end is coaxially connected to the first end of the hollow tube and at its second end extends from the first end of the hollow tube.

5. The device of claim 4 wherein the inside diameter of the tubular metal cuff is similar to the inside diameter of the hollow tube.

6. The device of claim 4 wherein the second end of the cuff is sharpened around its circumference.

7. A tissue extraction device, comprising:

a graduated, cylindrical, transparent tube having a first end and a second end connected to a tube flange;

a metal cuff inserted into the first end of the tube and extending outward from the first end, the cuff comprising a circular metal cylinder comprising a sharpened end extending from the tube; and

a plunger comprised of a shaft connected to a stopper at a first end and a plunger flange at a second end.

8. The device of claim 7, wherein the tube has an internal volume in the range of 0.1 ml to 10 ml.

9. The device of claim 8, wherein the cylinder has a volume in a range of 0.1 ml to 2 ml.

10. The device of claim 9, wherein the tube has a volume in a range of about 1 ml ± 5%.

11. The device of claim 7, further comprising:

a biasing means positioned between the tube flange and the plunger flange.

12. The device of claim 11, wherein the biasing means is chosen from a spring and an elastic polymeric material.

13. A tissue extraction system, comprising:

a tissue extraction device comprising a graduated, cylindrical, transparent tube having a first end and a second end, a metal cuff inserted into the first end of the tube and extending outward from the first end, the cuff comprising a circular metal cylinder having a sharpened end extending from the first end of the tube and a plunger comprised of a shaft connected to a stopper at a first end and a flange at a second end; and

a 96 well plate having 96 wells, wherein the internal diameter of each well of the 96 well plate is substantially the same as the external diameter of the cylindrical, transparent tube of the extraction device.

14. The system of claim 13, further comprising:

a plurality of beads in each of the wells.

15. The system of claim 14, further comprising:

a protease in each of the wells.

16. The system of claim 15, further comprising:

an agent which binds PrP protein in each of the wells.

17. The system of claim 16, comprising:

Proteinase K in each of the wells; and

a salt of phosphotungstic acid in each of the wells.

18. A method for extracting a sample from the tissue of an animal comprising the steps of:

(a) inserting into the tissue to be sampled the tissue-cutting first end of the cylindrical tube of the tissue extraction device to cut out a sample of tissue of about 0.1 to 10 ml, 0.1 to 2 ml, or 0.1 to 1.0 ml in volume, said tissue extracting device comprising

(i) a transparent, graduated, hollow, cylindrical tube having an axis and having a sharp, tissue-cutting circumferential edge positioned perpendicular to the axis at a first end and a flange or protruding rim at the opposite end; and

(ii) a plunger having a first and a second end with its first end slidably positioned inside the hollow, cylindrical tube such that it can be partially withdrawn from the tube to create suction within the tube and draw sample into the tube and then can be further inserted into the tube to create pressure upon sample drawn into the

tube and expel the sample from the tube, said plunger having a flange, protruding rim or other means at its second to facilitate manual movement of the plunger relative to the cylindrical tube so that sample may be pulled into the tube and expelled from the tube with the aid of the plunger;

(b) withdrawing the plunger a distance to draw the sample of tissue into the tube;

(c) withdrawing the tube from the tissue; and

(d) moving the plunger forward to expel the tissue sample from the tube into a sample-receiving container.

19. The method of claim 18 wherein the distance is a predetermined distance.

20. The method of claim 19 wherein steps (a) through (d) are repeated using a new extraction device and wherein the distance on each repeat is consistent and related to the predetermined distance.

21. A method comprising the steps of:

(a) inserting into a brain of an animal a tissue extraction device comprising a graduated, cylindrical, transparent tube comprising a first end and a second end, a metal cuff inserted into the first end of the tube and extending outward from the first end, the cuff comprising a circular metal cylinder comprising a sharpened end extending from the first end of the tube and a plunger comprised of a shaft connected to a stopper at a first end and a flange at a second end;

(b) withdrawing the plunger to draw about 0.3 ml of brain tissue into the tube; and

(c) moving the plunger forward to expel the brain tissue from the tube into a well of a 96 well plate wherein the internal diameter of each well of the 96 well plate is substantially the same as the external diameter of the cylindrical, transparent tube of the extraction device which well has therein a plurality of beads.

22. The method of claim 21, further comprising:

repeating steps (a), (b) and (c) whereby brain tissue from another animal is inserted into another well in the repeated steps.

23. The method of claim 21, wherein a new tissue extraction device is used in each set of repeated steps to extract tissue from another animal.

24. The method of claim 23, further comprising:

(d) shaking the 96 well plate to homogenize tissue in the wells.

25. The method of claim 24, wherein steps (a), (b) and (c) are carried out 96 times to expel tissue into all 96 wells.

26. The method of claim 25, further comprising:

(e) centrifuging the 96 well plate.

27. A method comprising the steps of:

(a) inserting into a brain of an animal a tissue extraction device comprising a graduated, cylindrical, transparent tube comprising a first end and a second end, a metal cuff inserted into the first end of the tube and extending outward from the first end, the cuff comprising a circular metal cylinder comprising a sharpened end extending from the first end of the tube and a plunger comprised of a shaft connected to a stopper at a first end and a flange at a second end;

(b) withdrawing the plunger to draw about 0.3 ml of brain tissue into the tube; and

(c) moving the plunger forward to expel the brain tissue from the tube into a container.

28. The method of claim 27, wherein the container is a cylindrical tube having a containment volume of approximately 2 ml and the container has therein a plurality of beads.

FIG. 1

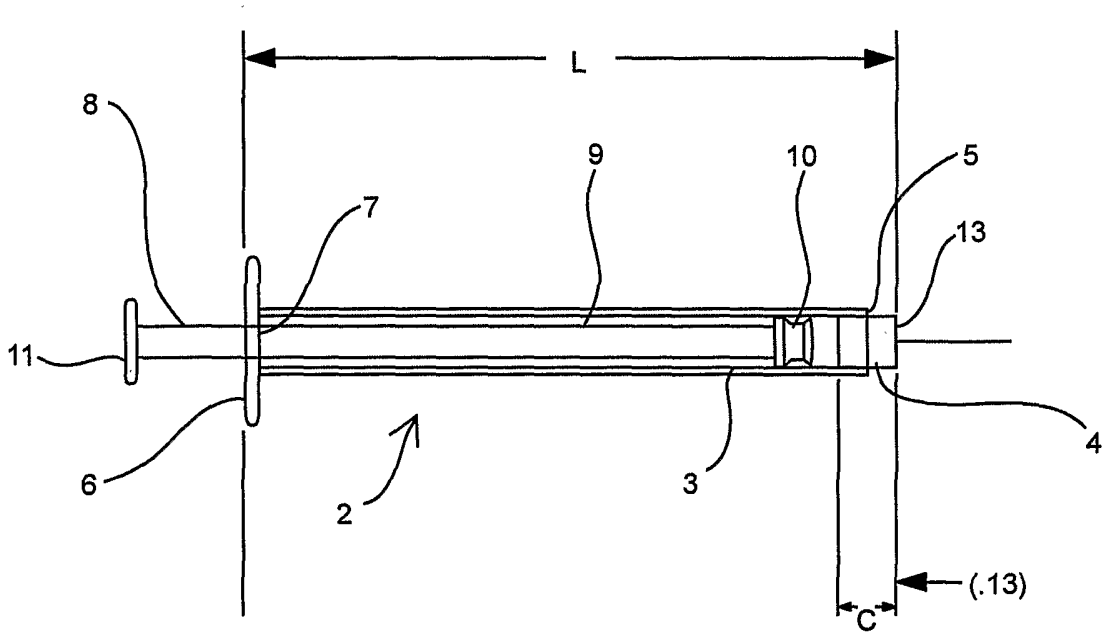


FIG. 2

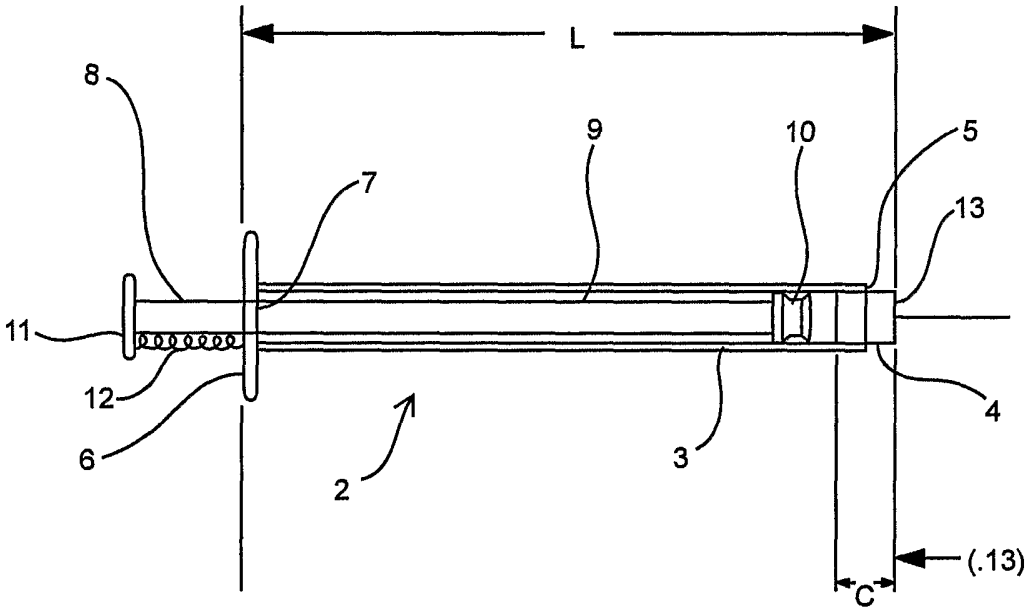


FIG. 3

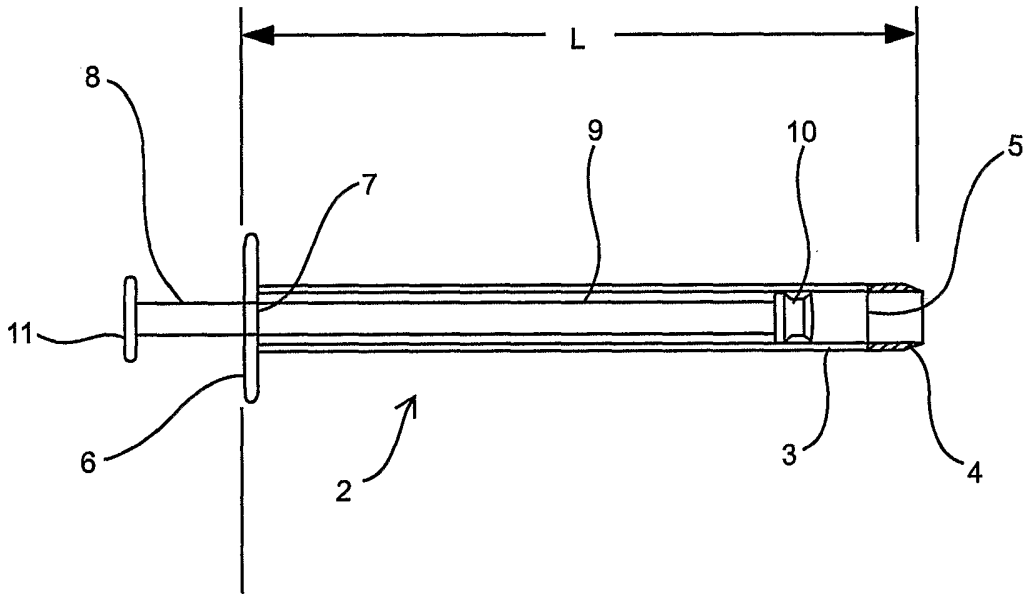
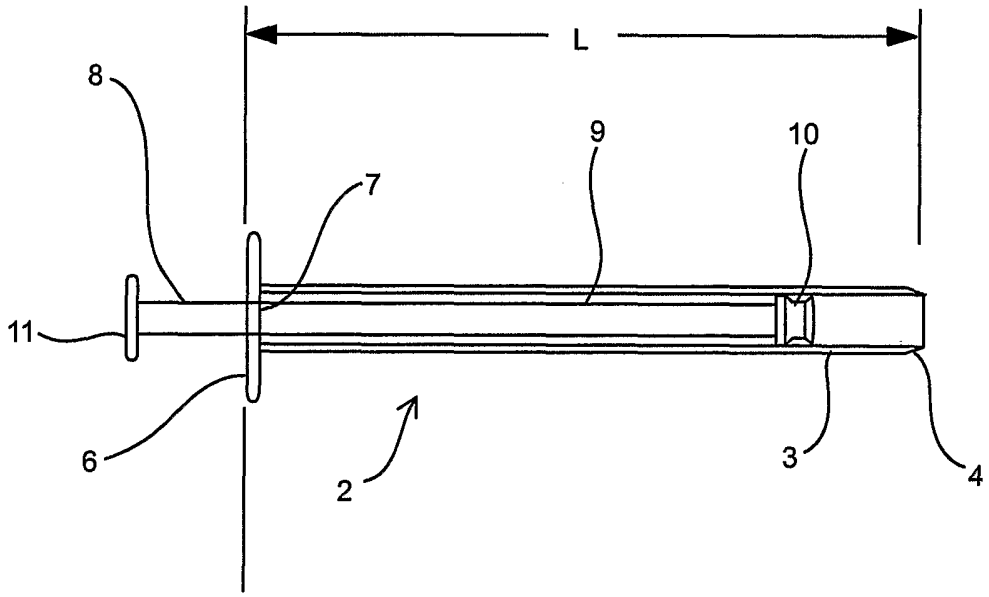


FIG. 4



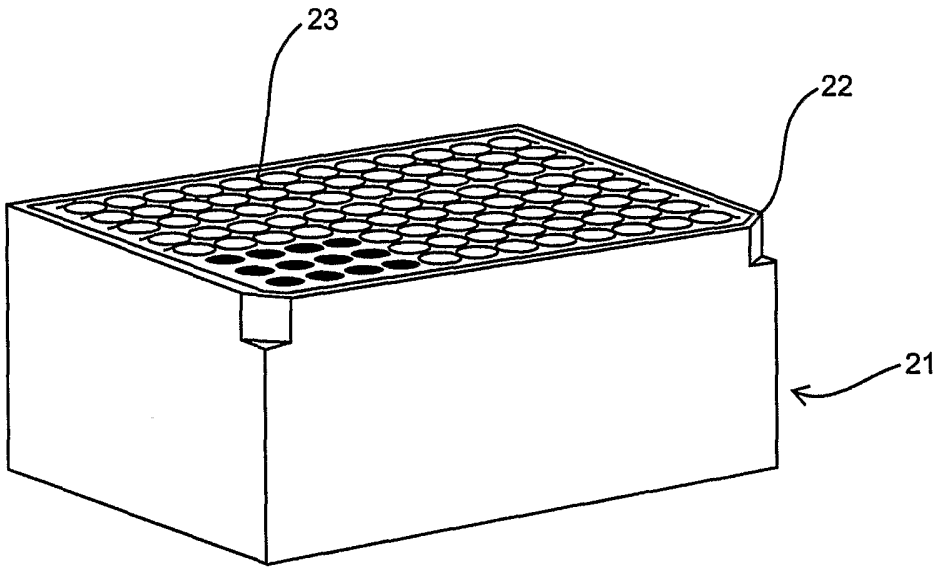


FIG. 5

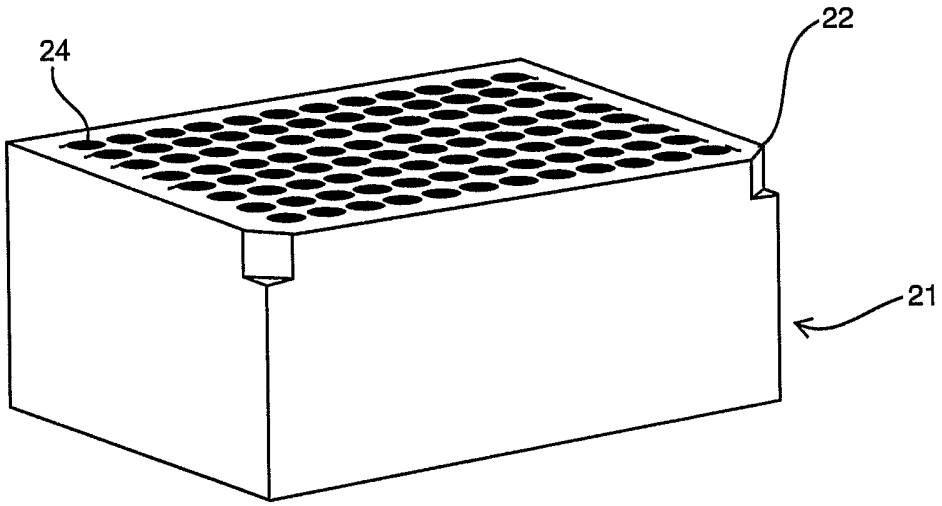


FIG. 6

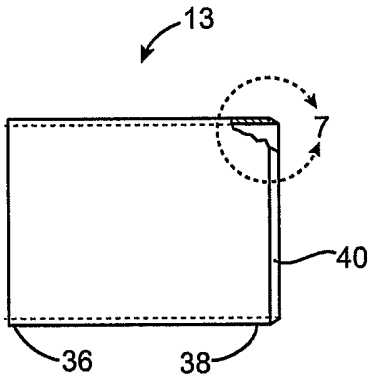


FIG. 7

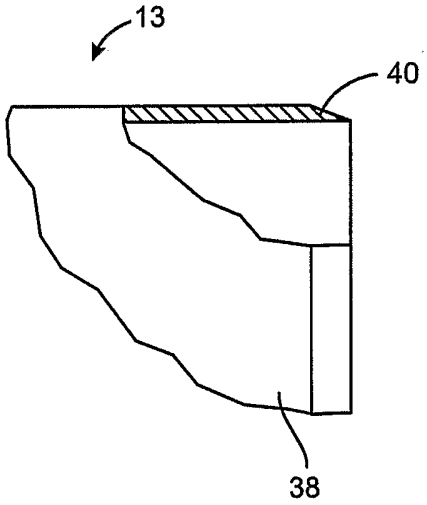


FIG. 8

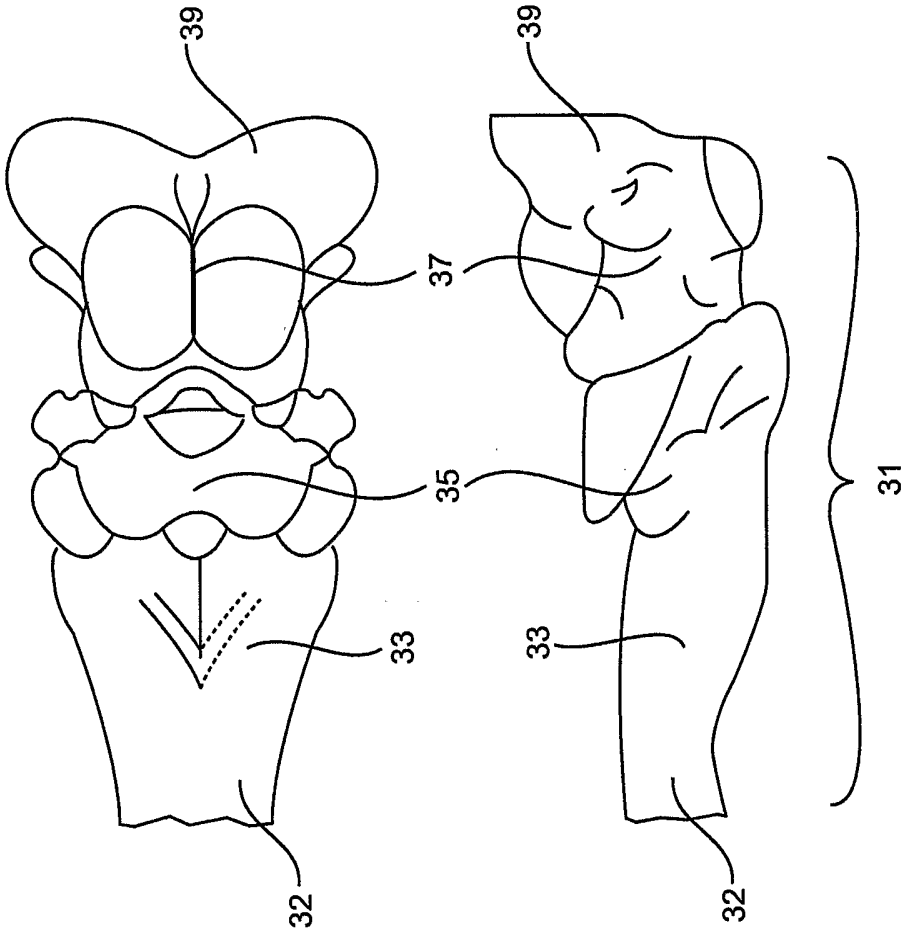


FIG. 9

FIG. 10

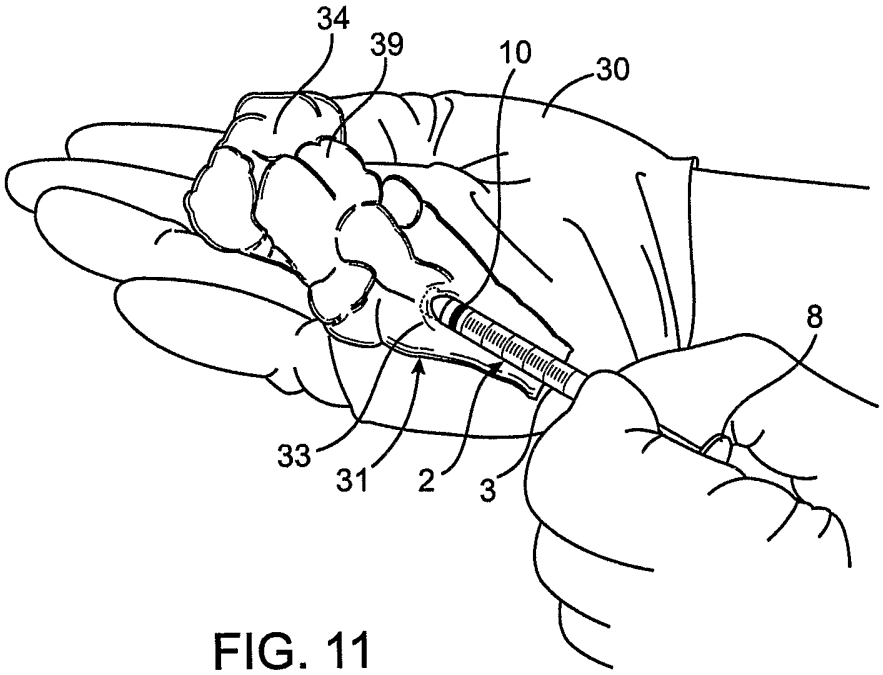


FIG. 11

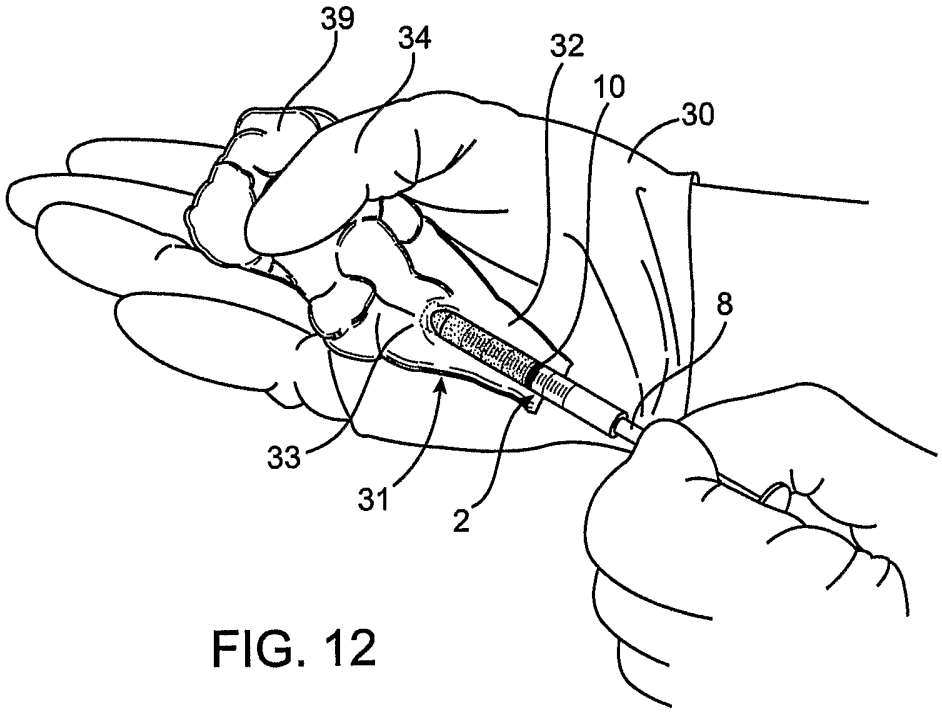


FIG. 12

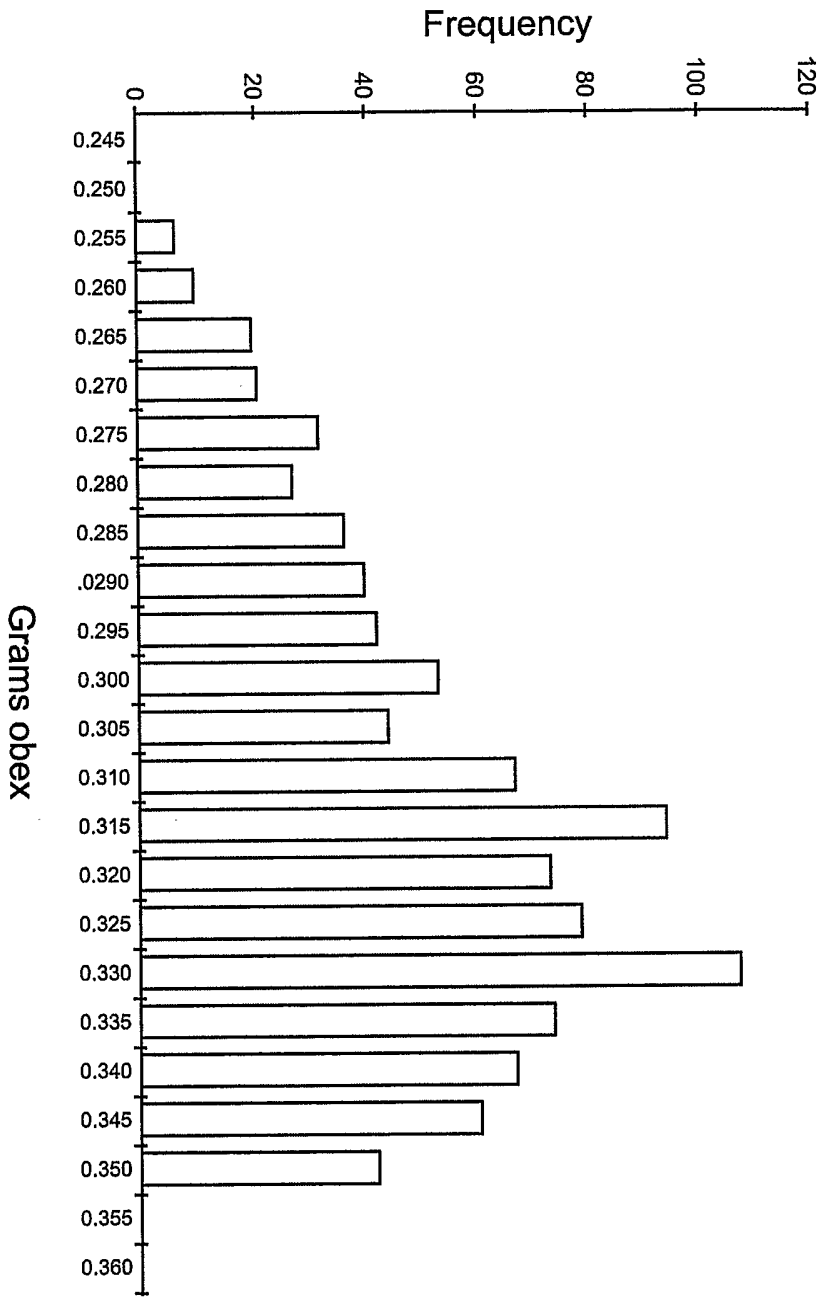

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FIG. 13

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US2005/016400

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 B01L3/00 A61B10/00

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 7 B01L A61B

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)

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	US 2003/082797 A1 (RASTORGOUEFF MICHEL ET AL) 1 May 2003 (2003-05-01) the whole document	1-3 4-7
X Y	US 6 325 806 B1 (FOX WILLIAM CASEY) 4 December 2001 (2001-12-04) column 10, line 27 - column 11, line 57 table 1 figures 7,8	1-12 13
Y	WO 2004/032750 A (BECTON DICKINSON AND COMPANY; RAINER, LYNNE; LIEBMANN-VINSON, ANDREA) 22 April 2004 (2004-04-22) page 18, paragraph 54 - page 20, paragraph 59 page 8, paragraph 32 - page 9, paragraph 33 figures 2,3a,3b	13

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[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
8 August 2005	16/08/2005

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	Compos, F

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US2005/016400

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 6 440 086 B1 (HOHENBERG HEINZ) 27 August 2002 (2002-08-27) page 1, line 38 - line 60 figures 1-3	4-7
A	US 5 515 861 A (SMITH ET AL) 14 May 1996 (1996-05-14) column 2, line 35 - column 3, line 6 figure 1	11,12

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2005/016400

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 18-28
because they relate to subject matter not required to be searched by this Authority, namely:

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by surgery

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US2005/016400

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