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(54) METHODS AND KITS FOR DETECTING CEREBROSPINAL FLUID IN A SAMPLE

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(57) **ABSTRACT**

The present invention relates to detection of the presence or absence of cerebrospinal fluid (CSF) in a sample, in particular to the analysis of the CSF protein lipocalin-type prostaglandin D2 synthase (PGDS). The present invention provides assays for the analysis of PGDS indicating the presence or absence of CSF in a sample.

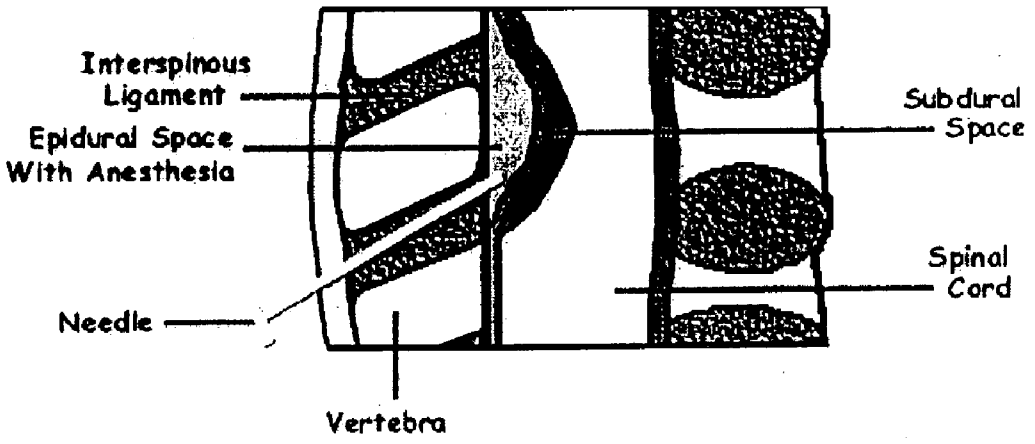


Figure 1

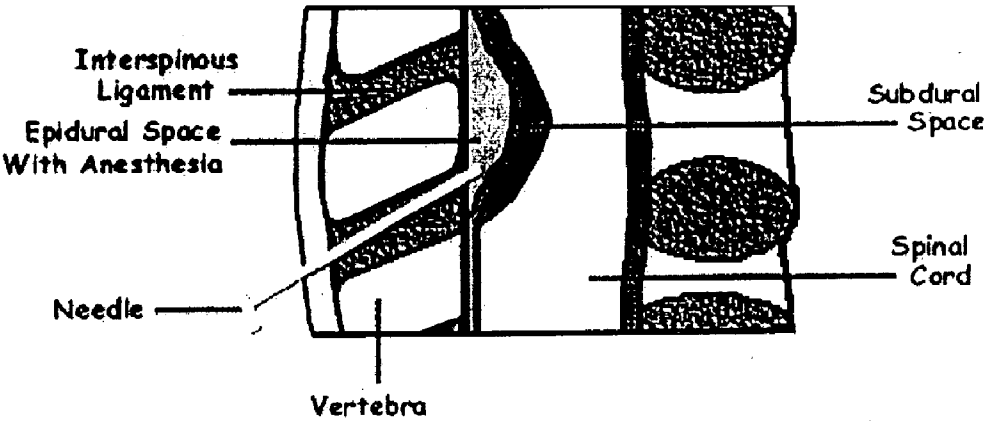

Interspinous Ligament
Epidural Space With Anesthesia
Needle
Vertebra
Subdural Space
Spinal Cord

FIGURE 2A

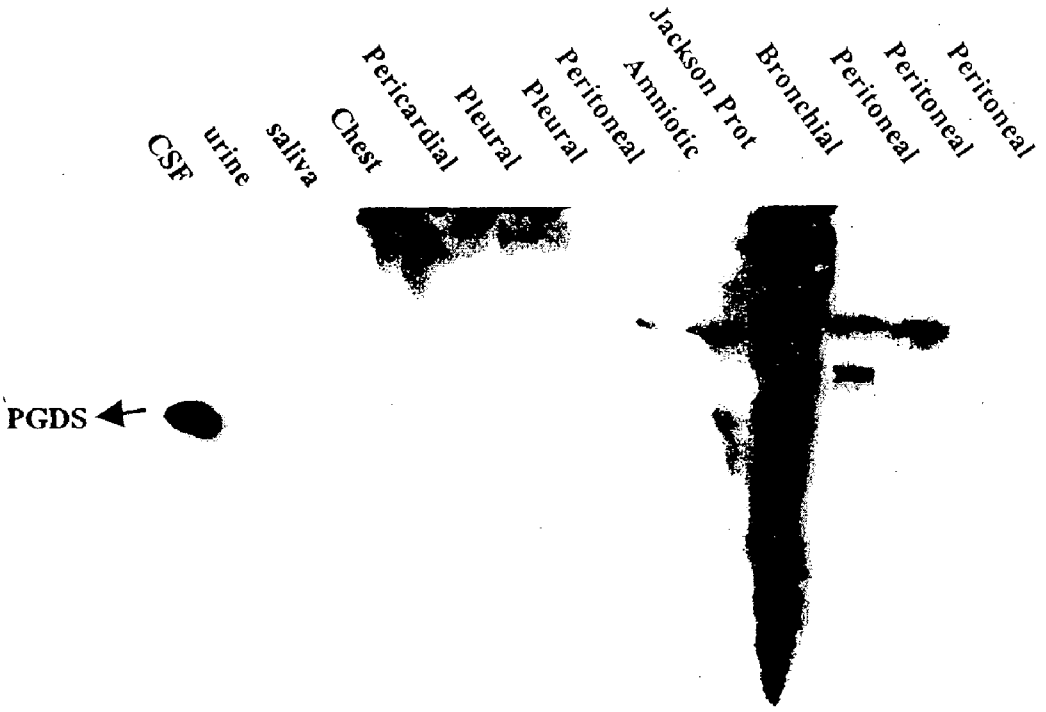

CSF
urine
saliva
Chest
Pericardial
Pleural
Pleural
Peritoneal
Amniotic
Jackson Prot
Bronchial
Peritoneal
Peritoneal
Peritoneal
PGDS

FIGURE 2B

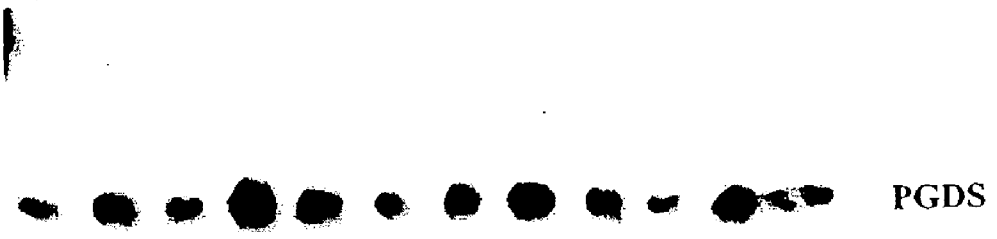


FIGURE 2C

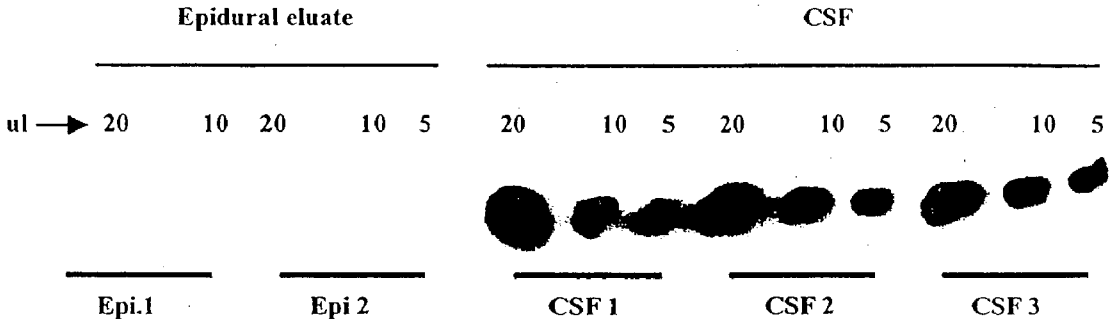
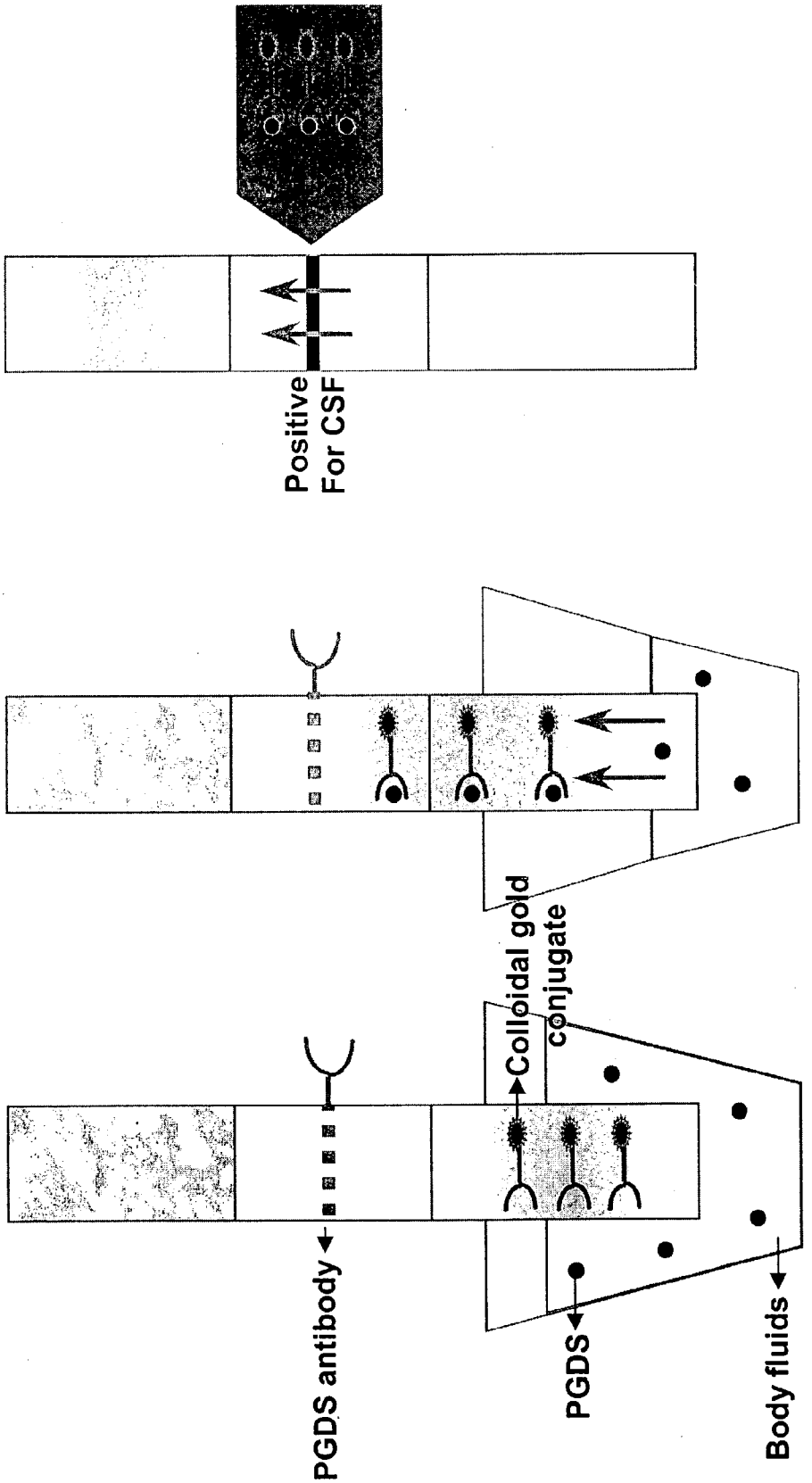

Epidural eluate
CSF
ul
20
10
20
10
5
20
10
5
20
10
5
20
10
5
Epi.1
Epi 2
CSF 1
CSF 2
CSF 3

Figure 3


Positive
For CSF
Colloidal gold
conjugate
PGDS antibody
PGDS
Body fluids

METHODS AND KITS FOR DETECTING CEREBROSPINAL FLUID IN A SAMPLE

FIELD OF THE INVENTION

[0001] The present invention relates to detection of the presence or absence of cerebrospinal fluid (CSF) in a sample, in particular to the analysis of the CSF protein lipocalin-type prostaglandin D2 synthase (PGDS). The present invention provides assays for the analysis of PGDS indicating the presence or absence of CSF in a sample.

BACKGROUND OF THE INVENTION

[0002] Neural blockade is associated with many complications. Among the most feared is accidental, unrecognized penetration of nervous system compartments containing cerebrospinal fluid (CSF). If puncture into the CSF is not noted immediately, or if it is incorrectly diagnosed, subsequent drug administration may lead to paralysis or death. Despite widespread use during surgery, childbirth and pain relief, current methods to detect CSF during and after neural blockade are unreliable, costly and time-consuming.

[0003] Patients undergoing epidural anesthesia and analgesia are at particular risk for needle puncture into the CSF. The epidural space is identified prior to blockade by loss of resistance to syringe injection of air, or of water solutions containing salt or sugar. Placement of the needle in the epidural space is followed by injection of drugs dissolved in sugar- or salt-containing water solutions in serial increments. If the epidural blockade is to be maintained for a long interval, a catheter may be threaded through the needle for both continuous and intermittent injection of drug-containing solutions. Because penetration of the dura and unintended entry into the CSF is possible at any step, the needle and catheter are routinely observed for passive drainage of CSF, and aspirated for fluid return before each manipulation and drug administration. If CSF is present, repositioning of the needle or catheter may be required to avoid spinal rather than epidural blockade upon drug injection.

[0004] Although unambiguous identification of drained or aspirated CSF is essential for the safe conduct of epidural anesthesia, caregivers are often uncertain over the origin of fluid that may be present. CSF is clear and watery, thereby closely resembling injected sugar- or salt-containing solutions and drug mixtures. In the past, efforts to discriminate CSF from injected or accumulated fluids have relied on physical properties such as temperature, or in vitro precipitation with second compounds, or on measurement of the possible chemical constituents of the fluid in question, such as glucose, protein, or ion levels. However, because of sample admixture, variability in test precision and inconsistent test thresholds, these methods are rarely helpful and little used.

[0005] At present, measurement of beta-2 transferrin in a sample is the only laboratory test to reach clinical practice capable of unequivocal discrimination of CSF, but its use is hampered in many regards. Each sample must be provided in high volume requiring as much as 1-2 mL of sample/assay. Turn-around time for results to reach the caregiver takes up to 4 days. Because immunofixation electrophoresis is necessary to detect beta-2 transferrin, the assay is expensive ($230-300/sample), and carries multiple added costs for specimen handling, archiving, shipping and storage. Moreover, special technical skills and experienced technicians are required to assure test precision and reliability, mandating that beta-2 transferrin assays be performed by specialty laboratories.

[0006] Clearly there is a great need for a rapid, robust, cost effective and accurate method to unambiguously identify CSF in samples obtained at the bedside during and after neural blockade.

SUMMARY OF THE INVENTION

[0007] The present invention relates to detection of the presence or absence of cerebrospinal fluid in a sample, in particular to the analysis of the CSF protein lipocalin-type prostaglandin D2 synthase. The present invention provides assays for the analysis of PGDS indicating the presence or absence of CSF in a sample.

[0008] Accordingly, in some embodiments, the present invention provides a method for the detection of cerebrospinal fluid in a sample, comprising providing a sample from a subject, and detecting the presence or absence of prostaglandin D2 synthase in said sample. In some embodiments, one or more additional compounds are detected, alone or in combination with, prostaglandin D2 synthase. In some embodiments, beta-2 transferrin is detected. In some embodiments the subject has spontaneous otorrhea or rhinorrhea. In other embodiments, the subject has undergone trauma. In further embodiments, the subject has undergone surgery. In preferred embodiments, the subject is undergoing, or has undergone, neural blockade.

[0009] The present invention also provides a method wherein the amount of prostaglandin D2 synthase is correlated to a known value to determine the presence or absence of cerebrospinal fluid in the sample. In preferred embodiments the presence or absence of cerebrospinal fluid in a sample comprises determining the amount of prostaglandin D2 synthase in the sample. In some embodiments prostaglandin D2 synthase is present at a concentration of less than approximately 2.0 mg/L. In other embodiments, prostaglandin D2 synthase is present at concentration of between approximately 2.0 mg/L and approximately 6.0 mg/L. In yet other embodiments, prostaglandin D2 synthase is present at a concentration of between approximately 6.0 and approximately 10.0 mg/L. In still other embodiments, prostaglandin D2 synthase is present at a concentration of greater than approximately 10.0 mg/L.

[0010] The present invention also provides a method of determining the presence or absence of cerebrospinal fluid in a sample comprising determining the ratio of prostaglandin D2 synthase in the serum, to prostaglandin D2 synthase in a sample. In some embodiments the ratio is less than 10. In other embodiments, the ratio is between 10 and 20. In still other embodiments, the ratio is greater than 20.

[0011] The present invention further provides a method for detection of cerebrospinal fluid in a sample when the sample is obtained from a superficial opening on the body. In other embodiments, the sample is obtained from a needle in the body. In still other embodiments, the sample is free flowing. In yet other embodiments, the sample is aspirated. In further embodiments, the sample is obtained from a catheter. In additional embodiments, the sample is free flowing from the catheter. In further embodiments, the sample is aspirated

from a catheter. In additional embodiments, the sample is obtained at serial intervals for the duration the catheter is in place. In preferred embodiments, the sample is obtained before catheter placement. In particularly preferred embodiments, the sample is obtained after catheter placement.

[0012] In some embodiments, the sample is obtained before administration of fluid. In other embodiments, the sample is obtained after administration of fluid. In preferred embodiments, the sample is obtained before administration of a drug. In particularly preferred embodiments, the sample is obtained after administration of a drug.

[0013] In some embodiments, the subject is a mammal. In other embodiments, the subject is a human. In further embodiments, the subject is undergoing surgery. In still further embodiments, the subject is undergoing acute pain management. In preferred embodiments, the subject is undergoing pain management after surgery. In other embodiments the subject is undergoing pain management after trauma. In particularly preferred embodiments, the subject is undergoing pain management during labor and delivery. In some embodiments, the subject is undergoing chronic pain management. In further embodiments, the pain comprises malignant pain. In still further embodiments, the pain comprises non-malignant pain.

[0014] The present invention also provides a method for detecting the presence or absence of cerebrospinal fluid in a sample from a subject undergoing neural blockade when the neural blockade comprises regional anesthesia. In some embodiments the regional anesthesia comprises spinal anesthesia. In some embodiments, the spinal anesthesia comprises a single drug dose. In other embodiments, the spinal anesthesia is continuous through a catheter. In preferred embodiments, the regional anesthesia comprises epidural anesthesia. In some embodiments, the epidural anesthesia comprises a single drug dose. In other embodiments, the epidural anesthesia is continuous through a catheter. In additional embodiments, the regional anesthesia is combined spinal and epidural anesthesia. In other embodiments, the regional anesthesia comprises a peripheral nerve block. In still other embodiments, the regional anesthesia comprises a plexus neural blockade.

[0015] The present invention also provides a method for detecting the presence or absence of cerebrospinal fluid in a sample from a subject undergoing neural blockade when the neural blockade comprises regional analgesia. In some embodiments, the regional analgesia comprises spinal analgesia. In some embodiments, the spinal analgesia comprises a single drug dose. In other embodiments, the spinal analgesia is continuous through a catheter. In preferred embodiments, the regional analgesia comprises epidural analgesia. In some embodiments, the epidural analgesia comprises a single drug dose. In other embodiments, the epidural analgesia is continuous through a catheter. In additional embodiments, the regional analgesia is combined spinal and epidural analgesia. In other embodiments, the regional analgesia comprises a peripheral nerve block. In still other embodiments, the regional analgesia comprises a plexus neural blockade. In further embodiments, the regional analgesia comprises an implanted drug delivery system. In still further embodiments, the regional analgesia comprises a nerve stimulator.

[0016] The present invention provides a method for the detection of cerebrospinal fluid in a sample, comprising providing a sample from a subject, and detecting the presence or absence of prostaglandin D2 synthase in said sample using any suitable method. In some embodiments, detection comprises differential antibody binding. In a preferred embodiment, detection comprises an in situ immunoassay. In a particularly preferred embodiment, detection comprises an in situ immunoassay using a colloidal gold label. In other embodiments, differential antibody binding comprises a Western blot. In still other embodiments, differential antibody binding comprises a nephelometric assay. In further embodiments, the presence or absence of prostaglandin D2 synthase a sample is detected by a chromatographic assay. In other embodiments, the presence or absence of prostaglandin D2 synthase in a sample is detected by an enzymatic assay. In still other embodiments, the presence or absence of prostaglandin D2 synthase in a sample is detected by a spectroscopic assay.

[0017] The present invention further provides a kit comprising a reagent for detecting the presence or absence of prostaglandin D2 synthase in a sample before, during, or after neural blockade. In some embodiments, the kit further comprises instructions for using the kit for detecting the presence or absence of prostaglandin D2 synthase in a sample. In some embodiments, the instructions comprise instructions required by the U.S. Food and Drug Agency for in vitro diagnostic kits. In some embodiments, the kit further comprises instructions for diagnosing the presence or absence of cerebrospinal fluid in a sample based on the presence or absence of prostaglandin D2 synthase in said sample. In some embodiments, the reagent comprises one or more antibodies. In other embodiments, the reagent comprises one or more enzymes, enzyme inhibitors or enzyme activators. In still other embodiments, the reagent comprises one or more chromatographic compounds. In yet other embodiments, the reagent comprises one or more compounds used to prepare the sample for spectroscopic assay. In further embodiments, the kit comprises comparative reference material to interpret the presence or absence of prostaglandin D2 synthase according to intensity, color spectrum, or other physical attribute of an indicator.

DESCRIPTION OF THE FIGURES

[0018] **FIG. 1** shows spinal anatomy and placement of a needle tip within the epidural space. CSF surrounds and cushions the spinal cord and conus medullaris. Presence of CSF in catheter or needle drainage or aspirate indicates penetration of the dural membrane. Identical drugs and dosages administered into the epidural and spinal compartments have widely divergent kinetics (e.g. time to onset, duration of effects), and physiologic consequences (e.g. extent of motor, sensory and autonomic blockade) because of differential distribution of the pharmacologically active agents.

[0019] **FIG. 2** shows antibody-mediated detection of PGDS in CSF and other body fluids. **FIG. 2A** shows specific binding of anti-PGDS antibody to PGDS from a CSF source, and absence of PGDS in samples from other sources. **FIG. 2B** shows anti-PGDS antibody detection of PGDS in samples of CSF from 12 distinct human sources. **FIG. 2C** shows absence of PGDS in epidural eluates, but presence of PGDS in CSF detected by anti-PGDS antibody binding in samples from 2 and 3 subjects, respectively.

[0020] **FIG. 3** shows bedside anti-PGDS in situ antibody-mediated detection of PGDS configured as an absorbent

dipstick. Presence of a colloidal gold indicator band in the solid phase following immersion indicates presence of CSF in the test sample.

GENERAL DESCRIPTION OF THE INVENTION

[0021] CSF is a clear liquid similar in appearance to water, and in composition to plasma. The brain and spinal cord are rendered buoyant and protected by the CSF. Clinical, surgical and accidental events may cause CSF to breach its physiologic barriers. CSF leaks may occur with the placement of needles and catheters for anesthesia and analgesia, trauma, skull fractures, intracranial surgical procedures, infection, hydrocephalus, congenital malformations, neoplasms, and spontaneous rhinorrhea, and otorrhea.

[0022] The risk of dural puncture and entry into the CSF is particularly high in epidural anesthesia and analgesia. Epidural opioids and local anesthetics are most commonly injected in the lumbar or thoracic region. The needle for epidural neural blockade passes between the vertebrae of the spinal column into the epidural space. The epidural space is normally devoid of fluids, and the distance across it is small (**FIG. 1**). If the epidural needle is advanced too far, it will pierce the dura which contains the spinal cord, spinal nerves and CSF. A hole in the dura may allow CSF to leak into the epidural space causing severe headaches. In patients with increased intracranial pressure, accidental dural puncture made while locating the epidural space risks cerebellar or tentorial herniation due to loss of CSF. Other serious complications include permanent paralysis, cardiac arrest and death.

[0023] Spinal block arising from undesired drug administration into the CSF is of major concern to anesthesiologists. This may occur if the medication intended for the epidural space is mistakenly administered into the spinal fluid, causing sudden and profound hypotension, lower extremity paralysis, impaired ventilation and cardiac rhythm disorders. To prevent and diagnose these conditions, the anesthesiologist carefully evaluates and monitors the patient's reaction to test doses of the anesthetic or analgesic drugs. However, at present there is no rapid and reliable method to evaluate the incidence of dural puncture and CSF leakage during procedures such as these, which are performed by the thousands every day.

[0024] The optimal method to detect dural puncture is to test for the presence or absence of CSF. Chemical analysis of fluid for glucose, protein, potassium or sodium is unreliable as a means of determining if the fluid is CSF. Radiographic studies are only intermittently successful in demonstrating small or delayed CSF leaks, are cumbersome, costly, time consuming, and carry risks of exposure to radiation and to radioisotopic dyes. Electrophoresis of fluid from a suspected CSF source combined with immunofixation to detect the CSF protein constituent beta-2 transferrin has been shown to be a specific and generally accepted method of detecting CSF. However, the beta-2 transferrin assay requires the coordination of multiple acquisition and handling steps, is prohibitively time consuming for use during and after neural blockade (4 days), and is expensive ($300/assay).

[0025] Prostaglandin D_2 synthase (PGDS), also referred to as the beta-trace protein, is secreted into the CSF after production in the choroid plexus, leptomeninges, and oligodendrocytes of the central nervous system. PGDS catalyzes the isomerization of prostaglandin H2 to prostaglandin D2 (PGD), which serves as an endogenous sleep-promoting compound. In comparison to transferrin, for which only the beta-2 isoform is specific to CSF, the entire structure of PGDS is specific to CSF. PGDS is therefore preferred for use in the present invention as a rapid and specific indicator of the presence or absence of CSF.

[0026] Experiments conducted during the course of development of the present invention demonstrate that PGDS is a reliable marker for CSF, and is less expensive and less time-consuming than conventional methods. The present invention provides novel methods and kits for rapid detection of CSF in samples obtained before, during and after neural blockade. Use of the present invention assures safer performance of neural blockade, and reduces the incidence and severity of life-threatening complications.

[0027] Definitions

[0028] To facilitate understanding of the invention, a number of terms are defined below.

[0029] As used herein, the term "subject" encompasses humans and animals, whether or not hospitalized.

[0030] As used herein, the term "PGDS" or "lipocalin-type prostaglandin D2 synthase" is used in reference to a specific protein which is secreted into the CSF after production in the choroid plexus, leptomeninges, and oligodendrocytes of the central nervous system, which catalyzes the isomerization of prostaglandin H2 to prostaglandin D2, and which is correlated with the presence or absence of CSF.

[0031] As used herein, "neural blockade" refers to administration of cellular receptor agonists and antagonists in direct proximity to targeted neuronal structures for the purposes of interfering with neuronal transmission. Neural blockade includes, but is not limited to, regional anesthesia (e.g. spinal, epidural, peripheral or plexus anesthesia), and regional analgesia (e.g. spinal, epidural, peripheral or plexus analgesia).

[0032] As used herein, the term "is undergoing" is used in reference to being subjected to neural blockade.

[0033] As used herein, the term "malignant pain" refers to pain arising from a cancerous or neoplastic origin including pressure, ischemia, necrosis, obstruction and other consequences of tumor location and growth.

[0034] As used herein, the term "non-malignant pain" refers to pain arising from causes other than cancer.

[0035] As used herein, the term "regional anesthesia" is used in reference to neural blockade to refer to the agents and procedures used to render a region of the body insensible without direct interference with consciousness.

[0036] As used herein, the term "regional analgesia" is used in reference to neural blockade to refer to agents and procedures used to reduce or eliminate pain in a region of the body without direct interference with consciousness.

[0037] As used herein, the term "continuous" is used in reference to neural blockade wherein drugs are infused through a catheter to the desired site of action without interruption.

[0038] As used herein, the term "spinal" is used in reference to neural blockade to refer to administration of a drug or drugs beneath the dura and into the cerebrospinal fluid.

[0039] As used herein, the term "epidural" is used in reference to neural blockade to refer to administration of a drug or drugs within the epidural space external to the dural membrane of the nervous system.

[0040] As used herein, the term "peripheral" is used in reference to neural blockade to refer to administration of a drug or drugs directly in proximity to a peripheral nerve external to the central nervous system.

[0041] As used herein, the term "plexus" is used in reference to neural blockade to refer to administration of a drug or drugs directly in proximity to a nerve plexus external to the central nervous system.

[0042] As used herein, the term "implanted drug delivery system" is used to refer to a device indwelling in the body capable of providing a drug or drugs to the nervous system (e.g. through a catheter) either as a continuous infusion or in response to patient demand.

[0043] As used herein, the term "nerve stimulator" is used in reference to a device indwelling in the body capable of providing constant or intermittent electrical current or voltage in relief of malignant and non-malignant pain.

[0044] Where "amino acid sequence" is recited herein to refer to an amino acid sequence of a naturally occurring protein molecule, "amino acid sequence" and like terms, such as "polypeptide" or "protein" are not meant to limit the amino acid sequence to the complete, native amino acid sequence associated with the recited protein molecule.

[0045] The terms "fragment" or "portion" as used herein refer to a polypeptide that has an amino-terminal and/or carboxy-terminal deletion as compared to the native protein, but where the remaining amino acid sequence is identical to the corresponding positions in the amino acid sequence deduced from a full-length cDNA sequence. Fragments typically are at least 4 amino acids long, preferably at least 20 amino acids long, usually at least 50 amino acids long or longer, and span the portion of the polypeptide required for intermolecular binding of the compositions (claimed in the present invention) with its various ligands and/or substrates.

[0046] As used herein, the term "purified" or "to purify" refers to the removal of contaminants from a sample. For example, PGDS antibodies are purified by removal of contaminating non-immunoglobulin proteins; they are also purified by the removal of immunoglobulin that does not bind PGDS. The removal of non-immunoglobulin proteins and/or the removal of immunoglobulins that do not bind PGDS results in an increase in the percent of PGDS-reactive immunoglobulins in the sample. In another example, recombinant PGDS polypeptides are expressed in bacterial host cells and the polypeptides are purified by the removal of host cell proteins; the percent of recombinant PGDS polypeptides is thereby increased in the sample. The term "recombinant DNA molecule" as used herein refers to a DNA molecule that is comprised of segments of DNA joined together by means of molecular biological techniques.

[0047] The term "recombinant protein" or "recombinant polypeptide" as used herein refers to a protein molecule that is expressed from a recombinant DNA molecule.

[0048] The term "native protein" as used herein to indicate that a protein does not contain amino acid residues encoded by vector sequences; that is the native protein contains only those amino acids found in the protein as it occurs in nature. A native protein may be produced by recombinant means or may be isolated from a naturally occurring source.

[0049] The term "Western blot" refers to the analysis of protein(s) (or polypeptides) immobilized onto a support such as nitrocellulose or a membrane. The proteins are run on acrylamide gels to separate the proteins, followed by transfer of the protein from the gel to a solid support, such as nitrocellulose or a nylon membrane. The immobilized proteins are then exposed to antibodies with reactivity against an antigen of interest. The binding of the antibodies may be detected by various methods, including the use of radiolabeled antibodies.

[0050] The term "antigenic determinant" as used herein refers to that portion of an antigen that makes contact with a particular antibody (i.e., an epitope). When a protein or fragment of a protein is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies that bind specifically to a given region or three-dimensional structure on the protein; these regions or structures are referred to as antigenic determinants. An antigenic determinant may compete with the intact antigen (i.e., the "immunogen" used to elicit the immune response) for binding to an antibody.

[0051] As used herein, the term "nephelometric assay" is used to refer to an assay developed by DADE BEHRING, (Liederbach, Germany), consisting or polystryene particles coated with immunoaffinity-purified polyclonal antibodies from rabbit against human PGDS. The increase in light scattering caused by agglutination is measured by laser absorption.

[0052] The term "sample" as used herein is used in its broadest sense. A sample suspected of containing a protein may comprise a cell or can be cell-free, a portion of a tissue, an extract containing one or more proteins, body fluid, and the like. A "free flowing" sample refers to passive drainage of body fluid from a needle, catheter or other instrument penetrating a body compartment.

[0053] As used herein, the term "response," when used in reference to an assay, refers to the generation of a detectable signal (e.g., accumulation of reporter protein, increase in ion concentration, accumulation of a detectable chemical product).

[0054] As used herein, the term "instructions for using said kit for said detecting the presence or absence of PGDS polypeptide in a said biological sample" includes instructions for using the reagents contained in the kit for the detection of PGDS polypeptides. In some embodiments, the instructions further comprise the statement of intended use required by the U.S. Food and Drug Administration (FDA) in labeling in vitro diagnostic products. The FDA classifies in vitro diagnostics as medical devices and requires that they be approved through the 510(k) procedure. Information required in an application under 510(k) includes: 1) The in vitro diagnostic product name, including the trade or proprietary name, the common or usual name, and the classification name of the device; 2) The intended use of the product; 3) The establishment registration number, if appli-

cable, of the owner or operator submitting the 510(k) submission; the class in which the in vitro diagnostic product was placed under section 513 of the FD&C Act, if known, its appropriate panel, or, if the owner or operator determines that the device has not been classified under such section, a statement of that determination and the basis for the determination that the in vitro diagnostic product is not so classified; 4) Proposed labels, labeling and advertisements sufficient to describe the in vitro diagnostic product, its intended use, and directions for use. Where applicable, photographs or engineering drawings should be supplied; 5) A statement indicating that the device is similar to and/or different from other in vitro diagnostic products of comparable type in commercial distribution in the U.S., accompanied by data to support the statement; 6) A 510(k) summary of the safety and effectiveness data upon which the substantial equivalence determination is based; or a statement that the 510(k) safety and effectiveness information supporting the FDA finding of substantial equivalence will be made available to any person within 30 days of a written request; 7) A statement that the submitter believes, to the best of their knowledge, that all data and information submitted in the premarket notification are truthful and accurate and that no material fact has been omitted; 8) Any additional information regarding the in vitro diagnostic product requested that is necessary for the FDA to make a substantial equivalency determination. Additional information is available at the Internet web page of the U.S. FDA.

[0055] As used herein, the term "superficial opening" refers to a natural or acquired aperture on the surface of the body.

DETAILED DESCRIPTION OF THE INVENTION

[0056] The present invention relates to detection of the presence or absence of cerebrospinal fluid (CSF) in a sample, in particular to the analysis of the CSF protein lipocalin-type prostaglandin D2 synthase (PGDS). The present invention provides assays for the analysis of PGDS indicating the presence or absence of CSF in a sample.

[0057] Detection of PGDS Protein

[0058] As described above, a new method of detecting the presence or absence of CSF during and after neural blockade has been developed. Accordingly, the present invention provides methods for detecting PGDS in a sample from a subject undergoing, or having undergone, neural blockade. Any suitable method may be used to detect PGDS polypeptides. For example, in some embodiments a chromatographic method wherein the presence of PGDS produces a detectable color change is utilized. In other embodiments an enzymatic method wherein the presence of PGDS initiates detectable enzymatic activation is utilized. In further embodiments the presence of PGDS in a sample is detected by atomic absorption or atomic emission spectroscopy.

[0059] In certain embodiments of the present invention, the PGDS protein (prostaglandin D synthase, also referred to as "prostaglandin-H2 D-isomerase", and "beta-trace protein") is a glycoprotein with a molecular mass of about 26 kDa (EC 5.3.99.2) belonging to the lipocalin family of secretory proteins. In preferred embodiments the PGDS protein catalyzes the isomerization of PGH_2 to produce PGD_2. In further embodiments the cDNA of human PGDS encodes 190 amino acid residues with an N-terminal signal peptide of 22 amino acid residues. In particularly preferred embodiments, the PGDS protein is the "brain-type" isoform with brain-specific N-glycosylation oligosaccharide chains.

[0060] In a preferred embodiment of the present invention, antibodies are used to determine if a sample contains PGDS indicating the presence or absence of CSF (see Generation of PGDS Antibodies below). Antibody binding is detected by techniques known in the art (e.g., radioimmunoassay, ELISA (enzyme-linked immunosorbant assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, in situ immunoassays (e.g., using colloidal gold, enzyme or radioisotope labels, for example), Western blots, precipitation reactions, agglutination assays (e.g., gel agglutination assays, hemagglutination assays, etc.), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc.

[0061] In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many methods are known in the art for detecting binding in an immunoassay and are within the scope of the present invention.

[0062] In some embodiments, an automated detection assay is utilized. Methods for the automation of immunoassays include those described in U.S. Pat. Nos. 5,885,530, 4,981,785, 6,159,750, and 5,358,691, each of which is herein incorporated by reference. In some embodiments, the analysis and presentation of results is also automated. For example, in some embodiments, software that generates a score correlating to the presence of PGDS and likelihood of CSF in a sample based on the result of the immunoassay is utilized.

[0063] In other embodiments, the immunoassay is as described in U.S. Pat. Nos. 5,599,677 and 5,672,480, each of which is herein incorporated by reference.

[0064] Generation of PGDS Antibodies

[0065] The present invention provides isolated antibodies or antibody fragments (e.g., Fab fragments, Fab_2 fragements, and the like). Antibodies can be generated to allow for the detection of PGDS protein. The antibodies may be prepared using various immunogens. In one embodiment, the immunogen is a human PGDS peptide to generate antibodies that recognize human PGDS. Such antibodies include, but are not limited to polyclonal, monoclonal, chimeric, single chain, Fab fragments, Fab expression libraries, or recombinant (e.g., chimeric, humanized, etc.) antibodies, as long as it can recognize the protein. Antibodies can be produced by using a protein of the present invention as the antigen according to a conventional antibody or antiserum preparation process.

[0066] Various procedures known in the art may be used for the production of polyclonal antibodies directed against PGDS. For the production of antibody, various host animals can be immunized by injection with the peptide corresponding to the PGDS epitope including but not limited to rabbits, mice, rats, sheep, goats, etc. In a preferred embodiment, the peptide is conjugated to an immunogenic carrier (e.g.,

diphtheria toxoid, bovine serum albumin (BSA), or keyhole limpet hemocyanin (KLH)). Various adjuvants may be used to increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels (e.g., aluminum hydroxide), surface active substances (e.g., lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (Bacille Calmette-Guerin) and *Corynebacterium parvum*).

[**0067**] For preparation of monoclonal antibodies directed toward PGDS, it is contemplated that any technique that provides for the production of antibody molecules by continuous cell lines in culture will find use with the present invention (See e.g., Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.). These include but are not limited to the hybridoma technique originally developed by Kohler and Milstein (Kohler and Milstein, Nature 256:495-497 [1975]), as well as the trioma technique, the human B-cell hybridoma technique (See e.g., Kozbor et al., Immunol. Tod., 4:72 [1983]), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96 [1985]).

[**0068**] In an additional embodiment of the invention, monoclonal antibodies are produced in germ-free animals utilizing technology such as that described in PCT/US90/02545). Furthermore, it is contemplated that human antibodies will be generated by human hybridomas (Cote et al., Proc. Natl. Acad. Sci. USA 80:2026-2030 [1983]) or by transforming human B cells with EBV virus in vitro (Cole et al., in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, pp. 77-96 [1985]).

[**0069**] In addition, it is contemplated that techniques described for the production of single chain antibodies (U.S. Pat. No. 4,946,778; herein incorporated by reference) will find use in producing PGDS specific single chain antibodies. An additional embodiment of the invention utilizes the techniques described for the construction of Fab expression libraries (Huse et al., Science 246:1275-1281 [1989]) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity for PGDS.

[**0070**] In other embodiments, the present invention contemplates recombinant antibodies or fragments thereof to PGDS. Recombinant antibodies include, but are not limited to, humanized and chimeric antibodies. Methods for generating recombinant antibodies are known in the art (See e.g., U.S. Pat. Nos. 6,180,370 and 6,277,969 and "Monoclonal Antibodies" H. Zola, BIOS Scientific Publishers Limited 2000. Springer-Verlay New York, Inc., New York; each of which is herein incorporated by reference).

[**0071**] It is contemplated that any technique suitable for producing antibody fragments will find use in generating antibody fragments that contain the idiotype (antigen binding region) of the antibody molecule. For example, such fragments include but are not limited to: F(ab')2 fragment that can be produced by pepsin digestion of the antibody molecule; Fab' fragments that can be generated by reducing the disulfide bridges of the F(ab')2 fragment, and Fab fragments that can be generated by treating the antibody molecule with papain and a reducing agent.

[**0072**] In the production of antibodies, it is contemplated that screening for the desired antibody will be accomplished by techniques known in the art (e.g., radioimmunoassay, ELISA (enzyme-linked immunosorbant assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, in situ immunoassays (e.g., using colloidal gold, enzyme or radioisotope labels, for example), Western blots, precipitation reactions, agglutination assays (e.g., gel agglutination assays, hemagglutination assays, etc.), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc.

[**0073**] In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention. As is well known in the art, the immunogenic peptide should be provided free of the carrier molecule used in any immunization protocol. For example, if the peptide was conjugated to KLH, it may be conjugated to BSA, or used directly, in a screening assay.)

[**0074**] The foregoing antibodies can be used in methods known in the art relating to the localization and structure of PGDS (e.g., for Western blotting), measuring levels thereof in appropriate biological samples, etc. The antibodies can be used to detect PGDS in a biological sample from an individual. The biological sample can be a biological fluid, such as, but not limited to, blood, serum, plasma, interstitial fluid, urine, cerebrospinal fluid, and the like.

[**0075**] The biological samples can then be tested directly for the presence of human PGDS using an appropriate strategy (e.g., ELISA or radioimmunoassay) and format (e.g., microwells, dipstick (e.g., as described in International Patent Publication WO 93/03367), etc. Alternatively, proteins in the sample can be size separated (e.g., by polyacrylamide gel electrophoresis (PAGE), in the presence or not of sodium dodecyl sulfate (SDS), and the presence of PGDS detected by immunoblotting (Western blotting). Immunoblotting techniques are generally more effective with antibodies generated against a peptide corresponding to an epitope of a protein, and hence, are particularly suited to the present invention.

[**0076**] Kits for Analyzing Presence or Absence of CSF

[**0077**] The present invention also provides kits for determining whether a sample contains PGDS. The diagnostic kits are produced in a variety of ways. In some embodiments, the kits contain at least one reagent for specifically detecting a PGDS protein. In preferred embodiments, the kits contain multiple reagents for detecting the PGDS protein. In particularly preferred embodiments, the reagents are antibodies that preferentially bind PGDS proteins.

[**0078**] In some embodiments, the kit contains instructions for determining whether the sample contains PGDS. In preferred embodiments, the instructions specify that presence or absence of CSF is determined by detecting the presence or absence of PGDS in a sample from the subject, wherein subjects are undergoing or have undergone neural blockade.

[0079] The presence or absence of a PGDS in a sample can be used to make therapeutic or other medical decisions. For example, deciding whether to use the existing catheter in place, or to replace the catheter during or after neural blockade, may be based on the presence or absence of PGDS-containing CSF in a sample obtained from the catheter.

[0080] In some embodiments, the kits include ancillary reagents such as buffering agents, protein stabilizing reagents, and signal producing systems (e.g., fluorescence generating systems such as Fret systems). The test kit may be packaged in any suitable manner, typically with the elements in a single container or various containers as necessary, along with a sheet of instructions for carrying out the test. In some embodiments, the kits also preferably include a positive control sample. In further embodiments, the kit contains comparative reference material to interpret the presence or absence of prostaglandin D2 synthase according to intensity, color spectrum, or other physical attribute of an indicator.

[0081] Experimental

[0082] The following examples are provided in order to demonstrate and further illustrate certain preferred embodiments and aspects of the present invention, and are not to be construed as limiting the scope thereof.

[0083] Methodology

[0084] Epidural eluates and CSF samples were obtained from patients in the labor and delivery unit of SUNY Stony Brook University Hospital. The body fluids were obtained from the Hematology and Chemistry labs of SUNY Stony Brook University Hospital. The samples were analyzed for PGDS with use of a polyclonal antibody specific for lipocalin PGDS (CAYMAN CHEMICAL CO. Ann Arbor Mich.).

[0085] Epidural eluates

[0086] Following signed informed consent (IRB approved), and permission of the attending obstetrician, an epidural catheter was placed and dosed with routine epidural medications (3 cc of lidocaine-1.5% with epinephrine-1:200,000; and 10 cc of bupivacaine-0.125% with fentanyl-50 μg). The patient was then placed on a continuous epidural infusion of bupivacaine 0.0625% with fentanyl 1.6 ug/cc at 10 cc/hr. For the purpose of this investigation, the epidural catheter was aspirated twice between one and four hours after the epidural placement. Aspirated fluid was tested for the presence of CSF by PGDS immunoblotting.

[0087] Spinal Fluid

[0088] Following signed informed consent (IRB approved), and permission of their obstetrician, patients having routine spinal anesthetic placement for elective cesarean section volunteered to donate a small volume of spinal fluid. After locating the subarachnoid space with 25 G pencan spinal needle, 1 mL of CSF was aspirated before injection of medications intended for anesthesia. Thus, CSF that is normally either discarded or injected back into the patient, was used to evaluate the presence of PGDS by immunoblotting.

[0089] Body Fluids

[0090] Other body fluids that were tested in this study were procured from the Clinical Loaboratories of SUNY Brook University Hospital, Stony Brook, N.Y. After clinical analysis of the fluids as requested by the attending physician, these body fluid samples are routinely discarded. Rather than their destruction, with IRB approval these samples were for evaluation of the presence of the CSF marker PGDS by immunoblotting.

[0091] Immunoblotting

[0092] Samples (5-20 ul) of CSF, and body fluids from other sources, were subjected to SDS-PAGE and electrotransferred to PVDF membranes. The membranes were blocked with 5% dry milk in TBS, and probed with anti-prostaglandin D2 synthase (anti-PGDS) antibody (CAYMAN CHEMICAL CO. Ann Arbor Mich.), followed by a secondary antibody labeled with HRP. The blots were then developed by enhanced chemiluminescence (ECL-Amersham).

RESULTS

EXAMPLE 1

[0093] Polyclonal anti-PGDS antibody reliably and specifically detects the presence of PGDS in CSF upon immunoblotting, and its absence in other body fluids (**FIG. 2A**). Sample volumes as small as 5 uL are suitable for accurate analysis.

EXAMPLE 2

[0094] Polyclonal anti-PGDS antibody reliably detects PGDS in CSF samples from 12 distinct human sources, indicating the antibody-mediated detection of PGDS in CSF is antigen-specific but not patient-specific (**FIG. 2B**).

EXAMPLE 3

[0095] Polyclonal anti-PGDS antibody reliably discriminates aspirates of fluid from different body compartments. Strong anti-PGDS binding is observed in fluid samples from spinal sources, whereas no PGDS binding is seen in eluates from catheters in the epidural space (**FIG. 2C**).

[0096] These results indicate that the presence or absence of PGDS in body fluid samples reliably and specifically predicts the source of the fluid, and thereby discriminates between spinal, epidural and other body fluid compartment origins.

[0097] All publications and patents mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention that are obvious to those skilled in the relevant fields are intended to be within the scope of the following claims.

What is claimed is:

1. A method for detection of cerebrospinal fluid in a sample, comprising: providing a sample from a subject, wherein said subject is undergoing neural blockade; and

a) detecting the presence or absence of prostaglandin D2 synthase in said sample to detect cerebrospinal fluid in said sample.

2. The method of claim 1, wherein determining the presence or absence of cerebrospinal fluid in a sample comprises determining the amount of prostaglandin D2 synthase in the sample.

3. The method of claim 1, wherein said sample is obtained from a device selected from the group consisting of a needle and a catheter.

4. The method of claim 1, wherein said sample is obtained before catheter placement.

5. The method of claim 1, wherein said sample is obtained after catheter placement.

6. The method of claim 1, wherein said sample is obtained before administration of fluid.

7. The method of claim 1, wherein said sample is obtained after administration of fluid.

8. The method of claim 1, wherein said sample is obtained before administration of a drug.

9. The method of claim 1, wherein said sample is obtained after administration of a drug.

10. The method of claim 1, wherein said subject is undergoing surgery.

11. The method of claim 1, wherein said subject is undergoing pain management.

12. The method of claim 11, wherein said pain management is after surgery.

13. The method of claim 11, wherein said pain management is after trauma.

14. The method of claim 11, wherein said pain management is during labor and delivery.

15. The method of claim 1, wherein said neural blockade comprises regional anesthesia.

16. The method of claim 1, wherein said neural blockade comprises regional analgesia.

17. The method of claim 1, wherein said detecting comprises differential antibody binding.

18. The method of claim 17, wherein said detecting comprises an in situ immunoassay

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