Methods and compositions are disclosed for determining canine proBNP or fragments thereof in a sample. In one method, canine proBNP or fragments thereof are determined by providing a canine sample, contacting the sample with at least one antibody that binds an epitope in the region from amino acids 1 to 22 of canine proBNP, and determining the presence of the canine proBNP or fragments thereof present in the sample. Antibodies that bind canine proBNP and kits comprising such antibodies are also disclosed.
Fig. 1A

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<td>Eptope 1, AA 1-22</td>
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</tr>
</tbody>
</table>

Fig. 1B
### Recognition factors

**Fig. 2A**

| Epitope 1, AA 1-20 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
|--------------------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
|                    | L | L | G | P | E | P | A | E | A | S | A | I | F | E | L | L | D |   |   |   |   |   |
| Epitope 2, AA 35-45| M | A | L | G | A | L | G | T | G | H | S |   |   |   |   |   |   |   |   |   |   |   |   |
| Epitope 3, AA 45-60| H | S | P | A | E | S | E | A | Q | E | E | P | P | A | R |   |   |   |   |   |   |   |
| Epitope 4, AA 68-80| V | L | A | P | H | D | N | E | R | A | L | R | L | G | S | S | L |   |   |   |   |   |

**Fig. 2B**
Recombinant Canine NT-proBNP
Epitopes: AA 25-41 and 74 - 86

Fig. 3A

Recombinant Feline NT-proBNP
Epitopes: AA 35-45 and AA 68-80

Fig. 3B
NT-proBNP Signal Intensity of Feline Samples vs. Severity of Heart Disease

Fig. 4
DETERMINING CANINE PROBNP
CROSS-REFERENCE TO RELATED APPLICATIONS


BACKGROUND

[0002] The present invention relates to a method of determining proBNP or fragments thereof in mammals.

[0003] Heart diseases play an important part not only in humans, but also in animals, in particular pets, such as dogs or cats, which are afflicted with these diseases. Studies have shown that, e.g., each tenth canine heart has a functional impairment. The heart diseases occurring in dogs, for example, the cardiac valves and the cardiac muscle. Since at first the heart is capable of compensating functional impairment by working harder, such a disease in most cases remains hidden, with the consequence that the state of the heart will deteriorate due to the increased load on the heart. The symptoms resulting from heart diseases, such as fatigue, circulatory insufficiency, lenguor, can mostly be recognized when the pet’s heart is no longer able to compensate the weakness. In such a case, the heart disease has already progressed so much that complete curing is hardly possible any more.

[0004] As a rule, chronic cardiac valve and cardiac muscle changes are not curable, yet by the use of medicaments, the further progress of the heart disease can be slowed down. Therefore, an early diagnosis must be made for the occurring heart diseases. By way of routine, mainly physical methods are used for this purpose, such as auscultation of the heart sounds, the recording of an electrocardiogram, X-ray and ultrasonic examinations. These examination methods mainly have the disadvantage that they can be carried out only when already visible or audible defects can be directly recognized on the heart. Furthermore, physical examination methods require suitable and, as a rule, expensive devices in order to carry out a respective diagnosis.

[0005] The heart diseases most frequently occurring in dogs, e.g., are heart decompensation and dilated cardiomyopathy, which mainly afflict big animals. Dilated cardiomyopathy is a heart disease which causes an enlargement of the ventricles of the heart with normal wall thickness, such enlargement quickly causing cardiac insufficiency in the afflicted animal. By admixing taurine to the feed, the risk of falling ill with dilated cardiomyopathy could be reduced significantly. In an illness related to dilated cardiomyopathy, the restrictive cardiomyopathy which frequently is found in older cats, a continuous decrease in the heart function with a reduced ability for pumping can be observed. The heart disease most frequently occurring in cats is hypertrophic cardiomyopathy. This disease of the cardiac muscle causes thickening of the heart wall and a resultant reduced ability to fill the ventricles of the heart with blood. This leads to an accumulation of blood in the left ventricle and to a greatly reduced amount of blood being pumped through the body.

[0006] In many heart diseases, such as, e.g., cardiac insufficiency, dilated cardiomyopathy, hypertrophic cardiomyopathy, left-ventricular hypertrophy and dysfunction, a peptide hormone, the so-called BNP (brain natriuretic peptide) is secreted. This hormone causes the excretion of liquid via the kidneys and thus regulates the cardiovascular system. Since this peptide is produced in the heart and is increasingly produced in case of an overstretch and congestion of the heart, determining the BNP level in blood is a suitable means for evaluating cardiac insufficiency.

[0007] BNP as well as other natriuretic peptides play an important part in regulating the water balance and the blood pressure. If the cardiac wall is dilated, it secretes BNP in increasing amounts, causing an excretion of sodium and liquid via the kidneys and a dilution of the blood vessels, which in sum can lower the blood pressure and the filling level of the heart. BNP is synthesized by the cells of the cardiac muscle as proBNP which finally is cleaved into N-terminal proBNP and BNP. Both parts of the BNP are delivered to the blood and can be determined therein.


[0009] A large number of methods are already known in the prior art which assist in the detection of human proBNP or the fragments thereof, respectively, in the serum of an individual. By way of example, here EP 0 648 228 B1, WO 03/87819 and FR 2 843 396 should be mentioned.

[0010] In US 2004/0018577, an immunoonasay is disclosed which comprises at least three antibodies which all are capable of binding to different epitopes of an analyte. The analytes to be detected particularly concern the detection of markers regarding heart diseases, wherein i.a. also BNP and proBNP can be detected.

[0011] Biondo A. W. et al. (Vet. Pathol. 2003, 40(5):501-506) describe a method of detecting ANP and BNP in cats by means of polyclonal antibodies which are directed against a peptide of the ANP which comprises the amino acids 1 to 28, and against a peptide which comprises the amino acids 43 to 56 of proBNP, respectively.

[0012] In EP 1 016 867 A1, an immunoonasay is described for the detection of proproBNP in mammals. There, antibodies are used which are directed against peptides comprising the amino acids 27 to 102, 73 to 102 and 27 to 64 of human BNP.

[0013] Jortani S. A. et al. (Clin. Chem. 2003, 50(2):265-278) describe the use of BNP and its prepro- and pro-forms as possible markers for heart diseases. In this article, no preferred peptide regions of BNP are mentioned which would be suitable for detecting heart diseases in dogs and cats.

[0014] In WO 2000/35951 several peptides are disclosed against which antibodies can be prepared, which are suitable in a method for diagnosing heart diseases. Three peptides comprising the amino acids 1 to 13, 37 to 49, and 65 to 76 of human Nt-pro-BNP protein are disclosed there, which may also be used for preparing antibodies that are directed against these peptides.
Moreover, several test kits for detecting human proBNP or the fragments thereof, respectively, are commercially available (e.g., from Roche and Biomedica). Nevertheless, there is no known method with whose assistance specifically proBNP in animal samples can be determined. Therefore, and because of the costly and complicated physical examinations of animals it is an object of the present invention to provide suitable means for determining proBNP or the fragments thereof, respectively.

**SUMMARY OF THE INVENTION**

Therefore, the present invention provides a method of determining feline or canine proBNP or fragments thereof, comprising the steps of:

- providing a feline or canine sample,
- contacting the sample with at least one antibody which, when determining feline proBNP, or fragments thereof, binds to at least one epitope in the region comprising the amino acids 20 to 42 and/or in the region comprising the amino acids 57 to 80 of feline proBNP, and when determining canine proBNP, or fragments thereof, binds to at least one epitope in the region comprising the amino acids 20 to 86 of canine proBNP, and
- determining the presence and/or concentration of the feline or canine proBNP, or fragments thereof, present in the sample.

It has been found that an antibody which can bind to an epitope in the disclosed regions of the feline, or canine, proBNP, respectively, is very well suited to specifically determine proBNP.

It is pointed out that the feline and canine proBNP sequences herein disclosed have been used by way of example for the family of the felidae, or canidae, respectively, and that therefore individual amino acids which differ from the sequences disclosed herein, in the proBNP sequences of animals of other species of these families also fall within the scope of the sequences disclosed herein as long as these differing amino acids do not relate to the epitopes of the anti-bodies disclosed herein in a manner that a specific binding is no longer rendered possible. The amino acid sequences disclosed herein have been published in public data bases (e.g. Swiss-Prot: canine BNP-P16859 and feline BNP-Q9GKL4).

The samples used in the method according to the invention comprise fluid samples, such as, e.g., blood, urine, as well as tissue samples, such as, e.g., tissue sections of the cardiac muscle or of the brain. As required, the samples may be processed accordingly, so as to facilitate or render possible the later contacting of the sample with the antibodies according to the invention. Thus, fractions containing proBNP or fragments thereof, respectively, can be provided from blood samples, or also tissue samples may, e.g., be homogenized and likewise be separated from non-proteinaceous fractions.

The binding of at least one antibody to an epitope of the feline or canine proBNP in the sample means that the antibody is capable of binding an epitope in a defined sequence region of a specific protein, the at least one antibody not being capable of specifically binding epitopes of the protein outside of the defined region.

According to the invention, an antibody which is capable of binding an epitope may be used to determine proBNP or fragments thereof. Nevertheless, it may be advantageous to use several (e.g., two, three, four or five) antibodies which are capable of binding different epitopes of the proBNP.

The determination of the presence, or concentration, respectively, of the feline or canine proBNP, or fragment thereof, present in the sample can be effected by methods known in the prior art. By way of example, the carrying out of enzyme immunoassays (e.g., ELISA) may be mentioned in case of liquid samples or immunohistochemical methods in case of tissue samples.

“Antibodies” according to the present invention also comprise fragments of antibodies which are capable of recognizing an epitope according to the invention. Thus, an antibody may, e.g., merely consist of the (Fab) portion which exhibits the antigen-binding side. These antibody fragments may furthermore be part of a bispecific antibody or of a heteroaminobody (cf., e.g., EP 1 100 830 B1).

“proBNP or their fragments” according to the invention comprise all the proBNP fragments which are formed in vivo (e.g., Nt-proBNP) or in vitro (e.g. by mixing a sample with protease or with chemical substances, such as CNBr), and which have the epitopes according to the invention.

According to a preferred embodiment, the at least one antibody binds to at least one epitope in the region comprising the amino acids 25-35 and/or in the region comprising the amino acids 45-55 and/or in the region comprising the amino acids 60-80 of the feline proBNP.

It has been shown that primarily the above-indicated amino acid regions of the feline proBNP have epitopes which allow for a specific binding of antibodies.

In a method according to the present invention, several antibodies can be used which are capable of specifically binding different epitopes on feline, or canine, respectively, proBNP. For this reason, at least one antibody which is capable of binding to at least one epitope can be used according to the invention. Furthermore, it should be mentioned that the amino acid regions indicated here may comprise not only one epitope, but, depending on their size, may comprise several epitopes. Thus, the method according to the invention comprises the use of a combination of several antibodies which are capable of specifically binding to at least one epitope.

According to the invention, when determining canine proBNP or fragments thereof, at least one antibody binds to at least one epitope in the region comprising the amino acids 25-41 and/or in the region comprising the amino acids 55-65 and/or in the region comprising the amino acids 74-86 of the canine proBNP.

Antibodies which recognize epitopes in these regions are particularly well suited for determining proBNP or the fragments thereof in a sample of canine origin.

According to a preferred embodiment, the at least one epitope comprises at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten amino acids.

According to a preferred embodiment, the at least one antibody is polyclonal and/or monoclonal.

The antibodies employed in a method according to the invention may be polyclonal as well as monoclonal. For preparing these antibodies, peptide fragments comprising the amino acid regions disclosed herein of the feline and/or of the canine proBNP are used. These peptide fragments may be produced either synthetically, (Merrifield R. P., 1963, J Am Chem Soc 85, 2000, 149), recombinantly, or by chemical or
enzymatic degradation of proBNP of recombinant or native origin. Depending on their size, the peptides recovered therefrom will be bound to an immunogenic carrier (e.g. KLH) or directly be used for preparing polyclonal or monoclonal antibodies (e.g. Köhler G. and Milstein C., 1975, Nature 256:495; Galfre et al., 1977, Nature 266:559). According to the invention, the antibodies may also be recombinantly prepared. Method for preparing recombinant antibodies are sufficiently known to the person skilled in the art (cf., e.g., Sambrook et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor, Laboratory Press, 2001).  

According to a further preferred embodiment, at least one further antibody binds to the at least one antibody or to the at least one epitope, whereby, for instance, it is rendered possible to carry out the inventive test as a sandwich assay.  

The binding of a further antibody to the at least one antibody makes it possible to determine the latter and indirectly, the epitope bound to the at least one antibody qualitatively and quantitatively, respectively. If the at least one further antibody binds to the at least one epitope, it is possible to determine the binding of the at least one antibody to the at least one epitope qualitatively and quantitatively, respectively, via an enzyme immuno assay, e.g. if the at least one antibody is immobilized on a solid phase.  

Preferably, the at least one antibody and/or the at least one further antibody is labelled.  

In doing so, the at least one antibody and/or the at least one further antibody is labelled with an enzyme, such as peroxidase, in particular horseradish peroxidase, biotin, fluorescent dye, in particular fluorescein (FITC, DTF), R-phycocerythrin (PE), peridinin chlorophyll protein (PerCP) and tandem conjugates, such as PE-Cy5 or PE-Texas Red, gold colloid or radionuclides.  

By labelling one of the two antibodies, it is possible to determine in a secondary reaction, or also directly, the presence and/or the concentration of the labelled antibody bound to the at least one epitope. The antibodies themselves again can be detected by protein A conjugates (e.g., protein A gold conjugate).  

According to a preferred embodiment, the at least one antibody or the at least one further antibody is bound to a solid phase.  

By the binding of the at least one antibody or of the at least one further antibody, it is possible to produce, e.g., antibody chips, coated microtiter plates or lateral flow devices which can be used in a great number of methods.  

Preferably, the determination of feline or canine proBNP or of fragments thereof is effected by a method selected from the group consisting of radioimmunoassay, immune binding assay, Western blot, immunohistochemistry, enzyme immunoassay, lateral flow device (LFD, test strips), and combinations thereof.  

The above-mentioned methods are sufficiently known to the person skilled in the art. A survey of these methods is given in “Bioanalytic” (Lotspeich and Zorbas, Spektrum Verlag 1998). Lateral flow devices (LFD, test strips) are disclosed, e.g., in WO 02/059567.  

According to a further aspect, the present invention relates to antibodies or antibody mixtures binding to at least one epitope in the region comprising the amino acids 25-35 and/or in the region comprising the amino acids 45-55 and/or in the region comprising the amino acids 60-80 of the feline proBNP.  

According to a further aspect, the present invention relates to an antibody or to an antibody mixture which binds to at least one epitope in the region comprising the amino acids 20-42 of the canine proBNP.  

Preferably, the antibody or the antibody mixture binds to at least one epitope in the region comprising the amino acids 25-41 and/or in the region comprising the amino acids 55-65 and/or in the region comprising the amino acids 74-86 of the canine proBNP.  

According to a preferred embodiment, the antibody or the antibody mixture binds to an epitope which comprises at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten amino acids. The epitopes according to the invention preferably have a length of 40 amino acids at the most, 35 amino acids at the most, 30 amino acids at the most, in particular 25 amino acids at the most, 20 amino acids at the most, or 15 amino acids at the most.  

A further aspect of the present invention relates to a peptide comprising three amino acids in the region of the amino acids 20-42 and/or in the region comprising the amino acids 57-80 of the canine proBNP.  

According to a further embodiment, the peptide comprises at least three amino acids in the region of the amino acids 25-35 and/or in the region of the amino acids 45-55 and/or in the region of the amino acids 60-80 of the feline proBNP.  

According to a preferred embodiment, the peptide is chemically synthesized or isolated from a sample, or prepared recombinantly, respectively.  

In order to appropriately prepare the epitope from a peptide which has been isolated from a sample or has been recombinantly produced, respectively, the latter may be further processed by enzymatic or chemical methods known per se.  

A further aspect of the present invention relates to the use of an inventive antibody or of an antibody mixture for determining feline or canine proBNP or fragments thereof in the method according to the invention.  

The peptides according to the invention can be used in competitive immunoassays in labelled form.  

Preferably, the peptides according to the present invention are used for preparing an anti-body or an antibody mixture.  

Furthermore, the peptides according to the present invention are used as positive control or as a standard, respectively, for concentration determinations in a method according to the invention.  

A further aspect of the present invention relates to a kit for determining feline or canine proBNP or fragments thereof, comprising at least one antibody according to the invention or at least one antibody mixture according to the invention, means for the qualitative and/or quantitative detec-
tion of a binding of the at least one antibody or of the at least one antibody mixture to feline or canine proBNP or fragments thereof, and, optionally, peptides according to the invention and/or feline or canine proBNP or fragments thereof as a positive control or as a standard for a concentration determination.

[0061] According to the invention, the kit may comprise at least one further antibody.

[0062] This additional antibody has an avidity to at least one antibody, or also to the at least one epitope.

[0063] According to a preferred embodiment, the at least one antibody and/or the at least one further antibody is labelled.

[0064] Preferably, the labelling comprises enzymes, such as peroxidases, in particular horseradish peroxidase, biotin, fluorescent dye, in particular fluorescein (FITC, DTTF), R-phycoerythin (PE), peridinin chlorophyll protein (PerCP) and tandem conjugates, such as PE-Cy5 or PE-Texas Red, gold colloid or radionuclides.

[0065] A further aspect of the present invention relates to the use of a kit according to the invention in a method of determining feline or canine proBNP.

[0066] With the method of the present invention, it is possible not only to detect proBNP and its fragments in cats and dogs, but also in other mammals, such as horses, cattle, elephants, mice (Swiss-Prot: P40753), pigs (Swiss-Prot: P07634), rats (Swiss-Prot: P13205), camels (Swiss-Prot: Q61723) and sheep (Swiss-Prot: 046541) and fish, such as perch (Swiss-Prot: Q80558), sturgeon (Swiss-Prot: P83965) and pufferfish (Swiss-Prot: Q80557).

[0067] In order to detect proBNP in the above-mentioned animals, antibodies which bind to at least one epitope in an amino acid region of amino acid residue 1 to amino acid residue 80 of the corresponding proBNP are preferred. In particular antibodies are preferred which bind to at least one epitope comprising the amino acid regions 1-15, 15-30, 20-30, 25-35, 30-40, 35-50, 35-55, 45-55, 50-70, 60-70, 60-80 and 70-80. The following particularly preferred specific epitopes have also been found with the scheme lying at the basis of the present invention (cf. Examples).

<table>
<thead>
<tr>
<th>Swiss-Prot No.</th>
<th>AA-Region</th>
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<tbody>
<tr>
<td>Mouse P40753</td>
<td>1-15, 20-30, 50-70</td>
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<tr>
<td>Pig P07634</td>
<td>20-30, 35-50, 60-70</td>
</tr>
<tr>
<td>Rat P13205</td>
<td>1-13, 30-40, 45-55, 60-80</td>
</tr>
<tr>
<td>Camel Q61723</td>
<td>20-30, 55-65, 70-80</td>
</tr>
<tr>
<td>Sheep 046541</td>
<td>1-15, 20-30, 45-55, 60-80</td>
</tr>
<tr>
<td>Perch Q80558</td>
<td>15-30, 35-50, 70-80</td>
</tr>
<tr>
<td>Sturgeon P83965</td>
<td>1-20, 25-35, 70-80</td>
</tr>
<tr>
<td>Pufferfish Q80557</td>
<td>15-30, 55-65, 80-80</td>
</tr>
</tbody>
</table>

[0068] As listed above for dogs and cats, the preferred lengths of the epitopes are also given for the above-mentioned animals.

[0069] The present invention is further illustrated by the following Examples and Figures without, however, being restricted thereto.

**DETAILED DESCRIPTION OF THE ILLUSTRATIVE EMBODIMENTS**

**Examples**

**Example 1**

**Preparing the Antibodies**


[0075] The peptide fragments were chosen from those regions of the amino acid sequence of the canine NT-proBNP, in which a maximum of the epitope recognition factors (corresponding to the results of the ProtScale program) was obtained, since these epitopes proved to be particularly immunogenic and readily accessible for antibodies. The selected peptides of the canine or canine proBNP were chemically synthesised and conjugated to a suitable carrier protein (KLH, e.g.).

[0076] One peptide/epitope each, conjugated to KLH, was injected into three sheep. For the first immunisation, each sheep received 0.5 mg of the corresponding antigen, mixed with Freund's Adjuvant (Guilhay, UK) and BCG (Bacillus Calmette-Guérin) and 0.25 mg of the immunogens to further increase the immune response.

[0077] According to the invention, it has been shown that the use of polyclonal antibodies yields good and reproducible results. Nevertheless, the use of monoclonal antibodies in this method such as described in the present invention is also possible. Monoclonal antibodies against peptides/epitopes of the canine or canine proBNP can be prepared by standard methods known to the person skilled in the art (cf. in this respect e.g. Köhler G and Milstein C, Nature, 1975, 256:495-497).

**Example 2**

**Determining the Antibody Reactivity by Means of ELISA**

[0078] The reactivity of antibodies or sera, respectively, against peptides/epitopes of the proBNP which are recovered from the blood of the sheep was assayed by means of an ELISA test. At first, the microtiter plates were coated over night at 4°C with Streptavidin (0.5 μg/ml, 200 μl per well),
washed, blocked with 1% BSA in 0.1 M PBS, pH 7.5, containing 0.25% Tween 20, washed once more and incubated for 3 h at 4°C with synthetic proBNP peptide sequences conjugated to biotin (0.25 μg/ml, 200 μl per well). After a further washing step, the serum samples were diluted 1:1000/1:10000/1:100000 with 0.1 M phosphate buffer containing 3% BSA and applied to the microtiter plate. The binding of the antibodies to the peptides/epitopes on the plate was determined by the addition of anti-sheep-IgG antibodies which are conjugated with horseradish peroxidase, and of a substrate solution comprising TMB (tetramethyl-benzidine). The reaction of the horseradish peroxidase with TMB was stopped by adding 0.9% sulfuric acid. The colour development was monitored with a photometer capable of analyzing microtiter plates.

Example 3

**Nt-proBNP Measurement in Samples of Healthy and Sick Animals**

[0079] Into the wells of a microtiter plate coated with one of the inventive antibodies, 20 μl of feline or canine serum were pipetted and incubated for 4-16 h at room temperature with 200 μl of a second, further peroxidase-labelled inventive antibody in 0.1 M phosphate buffer, pH 7. Subsequently, the microtiter plate was washed with 5×300 μl of 0.1 M phosphate buffer, pH 7, with 0.1% tritonx100, and 200 μl of tetramethyl benzidine were added as substrate. After a colour development of 20-30 minutes, the reaction is stopped by adding 50 μl of 0.9% sulfuric acid, and the colour intensity which is directly proportional to the amount of Nt-proBNP is measured with a microtiter plate photometer. The exact concentration is determined by a comparison with a calibration curve from recombinant feline or canine Nt-proBNP.

[0080] By way of example, in 8 healthy dogs and in 15 dogs suffering from heart disease the concentration of Nt-pro-BNP was determined by means of antibodies against the epitopes in the region of the amino acids 25-41 and 74-86 of the canine Nt-proBNP (Table 2):

<table>
<thead>
<tr>
<th>State of Health</th>
<th>Canine Nt-proBNP precl/1</th>
</tr>
</thead>
<tbody>
<tr>
<td>healthy</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>862</td>
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<td>2</td>
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**Example 4**

**Cross Reactivity**

[0083] Recombinant feline, canine and human Nt-proBNP were coated on microtiter plates (250 ng/ml, 200 μl/well). Subsequently, the plates were washed and contacted with a dilution of the anti-human, anti-feline and anti-canine antisera (10-100 μg/ml, in 0.1 M phosphate buffer, pH 7). After a washing step, the amount of bound antibodies was measured with a suitable secondary antibody (peroxidase-labelled anti-sheep antibody). It has been shown that the respective antibodies react very well with the corresponding Nt-proBNP molecules (i.e., anti-feline antisera with feline Nt-proBNP), but surprisingly do not react or react to a very slight extent with the Nt-proBNP molecules of the respective other species.

[0084] It could be demonstrated that the antibodies which were produced against the epitopes of the feline Nt-proBNP exhibit a high specificity and can bind to the corresponding human sequence to a very slight extent only. Since in the measurement of the antibody specificity Nt-proBNP was used as entire polypeptide as the binding partner and not the peptides which were used for producing the antibodies, it could impressively be demonstrated that the antibodies to feline epitopes of the Nt-proBNP do not exhibit any cross-reaction over the entire sequence region of the human Nt-proBNP. An exception is only that antibody which binds in the region of the amino acids 1 to 20 of feline Nt-proBNP. When binding to feline Nt-proBNP, this antibody shows only twice as high a relative reactivity than when binding to human Nt-proBNP.

[0085] Moreover, it could be demonstrated that antibodies against human epitopes of the Nt-proBNP also have a low reactivity relative to feline Nt-proBNP. Thus, it could be impressively be demonstrated that antibodies which are directed against epitopes of the human Nt-proBNP, cannot bind to feline Nt-proBNP and, thus, cannot be employed for determining Nt-proBNP in cats (cf. Table 3).

<table>
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<tr>
<th>Antiserum No.</th>
<th>Antibody Specificity</th>
<th>Relative Reactivity</th>
<th>Rel. Reactivity Relative to the Corresponding Human Sequences</th>
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TABLE 3-continued

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<td>AA 60-80 human</td>
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[0086] The cross-reactivity was also tested with antibodies against epitopes of the canine Nt-proBNP and with antibodies against epitopes of the human Nt-proBNP. Also with canine sequences, a result comparable to the tests with feline sequences could be achieved (cf. Table 4)

### TABLE 4

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<th>Antiserum No.</th>
<th>Antibody Specificity</th>
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1. A method of detecting canine proBNP or a fragment thereof in a canine sample comprising
   (a) providing a canine sample;
   (b) contacting the sample with at least one antibody that
       binds an epitope in the region from amino acids 1 to 22
       of canine proBNP; and
   (c) detecting the binding of the antibody to the epitope
       whereby the canine proBNP or the fragment thereof in
       the sample is detected.
2. The method of claim 1, wherein the epitope comprises at
   least 3 amino acids.
3. The method of claim 1, wherein the at least one antibody
   is polyclonal.
4. The method of claim 1, wherein the at least one antibody
   is monoclonal.
5. The method of claim 1, wherein the sample is a blood
   sample or a urine sample.
6. The method of claim 1, wherein detecting the binding of
   the antibody to the epitope comprises radioimmunoassay,
   immune binding assay, Western blot, immunohistochemistry,
   or enzyme immunoassay.
7. The method of claim 1, further comprising determining
   the concentration of the canine proBNP or fragment thereof
   in the sample.
8. The method of claim 7, further comprising comparing
   the concentration of the canine proBNP or fragment thereof
   in the sample with the concentration of canine proBNP or
   fragment thereof in a sample from a healthy canine.
9. The method of claim 8, further comprising diagnosing
   cardiac insufficiency in a canine from which the sample was
   obtained if the concentration of the canine proBNP or frag-
   ment thereof in the sample is higher than the concentration
   of canine proBNP or fragment thereof in the sample from the
   healthy canine.
10. The method of claim 1, wherein contacting the sample
    with the at least one antibody occurs on a solid phase.
11. The method of claim 10, wherein the at least one
    antibody is bound to a solid phase.
12. The method of claim 10, wherein the canine proBNP or
    fragment thereof is bound to a solid phase.
13. An isolated antibody that binds an epitope in the region
    from amino acids 1 to 22 of canine proBNP.
14. The antibody of claim 13, wherein the epitope com-
    prises at least 3 amino acids in the region from amino acids 1
    to 22 of canine proBNP.
15. A kit for detecting canine proBNP or a fragment thereof
    comprising an antibody that binds an epitope in the region
    from amino acids 1 to 22 of canine proBNP and a label.
16. The kit of claim 15, wherein the label is peroxidase,
    biotin, fluorescent dye, gold colloid, or a radionuclide.
17. The kit of claim 15, further comprising a peptide com-
    prising amino acids 1 to 22 of canine proBNP.
18. The kit of claim 15, further comprising at least one
    further antibody.
19. The kit of claim 18, wherein the at least one further
    antibody is labeled.
20. The kit of claim 15, wherein the antibody is bound to a
    solid phase.

* * * * *