**Title:** METHOD TO DETECT IgE

The present invention includes a method to detect IgE using a human Fc epsilon receptor (FcεR) to detect IgE antibodies in a biological sample from a cat, a dog, or a horse. The present invention also relates to kits to perform such methods.
For the purposes of information only

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

<table>
<thead>
<tr>
<th>AL</th>
<th>Albania</th>
<th>ES</th>
<th>Spain</th>
<th>LS</th>
<th>Lesotho</th>
</tr>
</thead>
<tbody>
<tr>
<td>AM</td>
<td>Armenia</td>
<td>FI</td>
<td>Finland</td>
<td>LT</td>
<td>Lithuania</td>
</tr>
<tr>
<td>AT</td>
<td>Austria</td>
<td>FR</td>
<td>France</td>
<td>LU</td>
<td>Luxembourg</td>
</tr>
<tr>
<td>AU</td>
<td>Australia</td>
<td>GA</td>
<td>Gabon</td>
<td>LV</td>
<td>Latvia</td>
</tr>
<tr>
<td>AZ</td>
<td>Azerbaijan</td>
<td>GE</td>
<td>Georgia</td>
<td>MC</td>
<td>Monaco</td>
</tr>
<tr>
<td>BA</td>
<td>Bosnia and Herzegovina</td>
<td>GB</td>
<td>United Kingdom</td>
<td>MD</td>
<td>Republic of Moldova</td>
</tr>
<tr>
<td>BB</td>
<td>Barbados</td>
<td>GH</td>
<td>Ghana</td>
<td>MG</td>
<td>Madagascar</td>
</tr>
<tr>
<td>BE</td>
<td>Belgium</td>
<td>GN</td>
<td>Guinea</td>
<td>MK</td>
<td>The former Yugoslav Republic of Macedonia</td>
</tr>
<tr>
<td>BF</td>
<td>Burkina Faso</td>
<td>GR</td>
<td>Greece</td>
<td>ML</td>
<td>Mali</td>
</tr>
<tr>
<td>BG</td>
<td>Bulgaria</td>
<td>HU</td>
<td>Hungary</td>
<td>MN</td>
<td>Mongolia</td>
</tr>
<tr>
<td>BJ</td>
<td>Benin</td>
<td>IE</td>
<td>Ireland</td>
<td>MR</td>
<td>Mauritania</td>
</tr>
<tr>
<td>BR</td>
<td>Brazil</td>
<td>IL</td>
<td>Israel</td>
<td>MW</td>
<td>Malawi</td>
</tr>
<tr>
<td>BY</td>
<td>Belarus</td>
<td>IS</td>
<td>Iceland</td>
<td>MX</td>
<td>Mexico</td>
</tr>
<tr>
<td>CA</td>
<td>Canada</td>
<td>IT</td>
<td>Italy</td>
<td>NE</td>
<td>Niger</td>
</tr>
<tr>
<td>CF</td>
<td>Central African Republic</td>
<td>JP</td>
<td>Japan</td>
<td>NL</td>
<td>Netherlands</td>
</tr>
<tr>
<td>CG</td>
<td>Congo</td>
<td>KE</td>
<td>Kenya</td>
<td>NO</td>
<td>Norway</td>
</tr>
<tr>
<td>CH</td>
<td>Switzerland</td>
<td>KG</td>
<td>Kyrgyzstan</td>
<td>NZ</td>
<td>New Zealand</td>
</tr>
<tr>
<td>CI</td>
<td>Côte d’Ivoire</td>
<td>KP</td>
<td>Democratic People’s Republic of Korea</td>
<td>PL</td>
<td>Poland</td>
</tr>
<tr>
<td>CM</td>
<td>Cameroon</td>
<td>KR</td>
<td>Republic of Korea</td>
<td>PT</td>
<td>Portugal</td>
</tr>
<tr>
<td>CN</td>
<td>China</td>
<td>KZ</td>
<td>Kazakhstan</td>
<td>RO</td>
<td>Romania</td>
</tr>
<tr>
<td>CU</td>
<td>Cuba</td>
<td>LC</td>
<td>Saint Lucia</td>
<td>RU</td>
<td>Russian Federation</td>
</tr>
<tr>
<td>CZ</td>
<td>Czech Republic</td>
<td>LI</td>
<td>Liechtenstein</td>
<td>SD</td>
<td>Sudan</td>
</tr>
<tr>
<td>DE</td>
<td>Germany</td>
<td>LK</td>
<td>Sri Lanka</td>
<td>SE</td>
<td>Sweden</td>
</tr>
<tr>
<td>DK</td>
<td>Denmark</td>
<td>LR</td>
<td>Liberia</td>
<td>SG</td>
<td>Singapore</td>
</tr>
<tr>
<td>EE</td>
<td>Estonia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SI | Slovenia
SK | Slovakia
SN | Senegal
SZ | Swaziland
TD | Chad
TG | Togo
TJ | Tajikistan
TM | Turkmenistan
TR | Turkey
TT | Trinidad and Tobago
UA | Ukraine
UG | Uganda
US | United States of America
UZ | Uzbekistan
VN | Viet Nam
YU | Yugoslavia
ZW | Zimbabwe
METHOD TO DETECT IgE

Field of the Invention

The present invention relates to a novel method to detect epsilon immunoglobulin (IgE). The present invention also includes novel kits to detect IgE as well as methods to produce the detection reagent.

Background of the Invention

Diagnosis of disease and determination of treatment efficacy are important tools in medicine. In particular, detection of IgE production in an animal can be indicative of disease. Such diseases include, for example, allergy, atopic disease, hyper IgE syndrome, internal parasite infections and B cell neoplasia. In addition, detection of IgE production in an animal following a treatment is indicative of the efficacy of the treatment, such as when using treatments intended to disrupt IgE production.

Until the discovery of the present invention, detection of IgE in samples obtained from non-human animals has been hindered by the absence of suitable reagents for detection of IgE. Various compounds have been used to detect IgE in IgE-containing compositions. In particular, antibodies that bind selectively to epsilon idiotype antibodies (i.e., anti-IgE antibodies) have been used to detect IgE. These anti-IgE antibodies, however, can cross-react with other antibody idiotypes, such as gamma isotype antibodies. The discovery of the present invention includes the use of a Fc epsilon receptor (FcεR) molecule to detect the presence of IgE in a putative IgE-containing composition. A FcεR molecule provides an advantage over, for example anti-IgE antibodies, to detect IgE because a FcεR molecule can bind to an IgE with more specificity (i.e., less idiotype cross-reactivity) and more sensitivity (i.e., affinity) than anti-IgE binding antibodies.

Lowenthal et al., 1993, Annals of Allergy 71:481-484, dog serum can transfer cutaneous reactivity to a human. While it is possible that Lowenthal et al. properly teach the binding of human FcεR to canine IgE. Lowenthal et al., however, do not provide data defining the particular cellular proteins responsible for the transfer of cutaneous reactivity. As such, a skilled artisan would conclude that the transfer of cutaneous reactivity taught by Lowenthal et al. could be due to a variety of different molecular interactions and that the conclusion drawn by Lowenthal et al. is merely an
interpretation. In addition, Lowenthal et al. do not teach the use of purified human Fc,R to detect canine IgE. The subunits of human Fc,R have been known as early as 1988 and have never been used to detect canine, feline or equine IgE. Indeed, U.S. Patent No. 4,962,035, to Leder et al., issued October 9, 1990, discloses human Fc,R but does not disclose the use of such a human Fc,R to detect human or non-human IgE. The use of purified human Fc,R avoids complications presented by use of Fc,R bound to a cell, such as non-specific binding of the Fc,R-bearing cell due to additional molecules present on the cell membrane. That purified human Fc,R detects non-human IgE is unexpected because inter-species binding between a Fc,R and an IgE is not predictable. For example, human Fc,R binds to rat IgE but rat Fc,R does not bind to human IgE.


Thus, methods and kits are needed in the art that will provide specific detection of non-human IgE.

Summary of the Invention

The present invention includes detection methods and kits that detect IgE. One embodiment of the present invention is a method to detect IgE comprising: (a) contacting an isolated human Fc,R receptor (Fc,R) molecule with a putative IgE-containing composition under conditions suitable for formation of a Fc,R molecule:IgE complex, wherein the IgE is selected from the group consisting of canine IgE, feline IgE and equine IgE; and (b) determining the presence of IgE by detecting the Fc,R molecule:IgE complex, the presence of the Fc,R molecule:IgE complex indicating the presence of IgE. A preferred Fc,R molecule in which a carbohydrate group of the Fc,R molecule is conjugated to biotin.

Another embodiment of the present invention is a method to detect IgE comprising: (a) contacting a recombinant cell with a putative IgE-containing composition under conditions suitable for formation of a recombinant cell:IgE complex,
in which the recombinant cell includes: a recombinant cell expressing a human \( \text{Fc}_c \)R molecule; and a recombinant cell expressing an antibody that binds selectively to an IgE including canine IgE, feline IgE and equine IgE; and (b) determining the presence of IgE by detecting the recombinant cell:IgE complex, the presence of the recombinant cell:IgE complex indicating the presence of IgE. A preferred recombinant cell includes a RBL-hFc\(_c\)R cell.

Another embodiment of the present invention is a method to detect flea allergy dermatitis comprising: (a) immobilizing a flea allergen on a substrate; (b) contacting the flea allergen with a putative IgE-containing composition under conditions suitable for formation of an antigen:IgE complex bound to said substrate; (c) removing non-bound material from the substrate under conditions that retain antigen:IgE complex binding to the substrate; and (c) detecting the presence of the antigen:IgE complex by contacting the antigen:IgE complex with a \( \text{Fc}_c \)R molecule. Preferably, the flea allergen is a flea saliva antigen and more preferably flea saliva products and/or flea saliva proteins.

The present invention also includes a kit for performing methods of the present invention. One embodiment is a kit for detecting IgE comprising a human \( \text{Fc}_c \) receptor (\( \text{Fc}_c \)R) molecule and a means for detecting an IgE including canine IgE, feline IgE and equine IgE. Another embodiment is a general allergen kit comprising an allergen common to all regions of the United States and a human \( \text{Fc}_c \) receptor (\( \text{Fc}_c \)R) molecule.

Another embodiment is a kit for detecting flea allergy dermatitis comprising a human \( \text{Fc}_c \) receptor (\( \text{Fc}_c \)R) molecule and a flea allergen.

Another embodiment of the present invention is an isolated human \( \text{Fc}_c \) receptor (\( \text{Fc}_c \)R) alpha chain protein, in which a carbohydrate group of the \( \text{Fc}_c \)R alpha chain protein is conjugated to biotin. A preferred \( \text{Fc}_c \)R alpha chain protein comprises PhFc\(_c\)Re\(_{172}\)-BIOT.

Brief Description of the Figures

Fig. 1 depicts ELISA results using biotinylated alpha chain of human \( \text{Fc}_c \)R to detect canine IgE antibodies.

Fig. 2 depicts ELISA results using biotinylated alpha chain of human \( \text{Fc}_c \)R to detect plant allergen-specific canine IgE antibodies.
Fig. 3 depicts ELISA results using biotinylated alpha chain of human FcεR to detect human or canine IgE antibodies.

Fig. 4 depicts ELISA results using biotinylated alpha chain of human FcεR to detect flea allergen-specific canine IgE antibodies.

Fig. 5 depicts ELISA results using biotinylated alpha chain of human FcεR to detect flea allergen-specific and heartworm antigen-specific canine IgE antibodies.

Fig. 6 depicts ELISA results using biotinylated alpha chain of human FcεR to detect flea saliva-specific canine IgE antibodies.

Fig. 7 depicts ELISA results using biotinylated alpha chain of human FcεR to detect heartworm antigen-specific feline IgE antibodies.

Fig. 8 depicts ELISA results using biotinylated alpha chain of human FcεR to detect heartworm antigen-specific feline IgE antibodies.

Fig. 9 depicts ELISA results using biotinylated alpha chain of human FcεR to detect antigen-specific equine IgE antibodies.

Fig. 10 depicts ELISA results using basophilic leukemia cells expressing alpha chain of human FcεR to detect canine IgE antibodies in sera from heartworm-infected dogs.

Fig. 11 depicts ELISA results using basophilic leukemia cells expressing alpha chain of human FcεR to detect canine IgE antibodies in sera from flea saliva sensitized dogs.

Detailed Description of the Invention

The present invention relates to the discovery that purified high affinity human Fc epsilon receptor (i.e., FcεRI; referred to herein as FcεR) can be used in certain non-human (i.e., canine, feline or equine) epsilon immunoglobulin (referred to herein as IgE or IgE antibody)-based detection (e.g., diagnostic, screening) methods and kits. The use of human FcεR to detect non-human IgE is unexpected because canine, feline and equine immune systems are different from the human immune system, as well as from each other (i.e., molecules important to the immune system usually are species specific).

One embodiment of the present invention is a method to detect a non-human IgE using an isolated human FcεR molecule. It is to be noted that the term "a" entity or "an" entity refers to one or more of that entity; for example, a protein refers to one or more
proteins or at least one protein. As such, the terms "a" (or "an"), "one or more" and "at least one" can be used interchangeably herein. It is also to be noted that the terms "comprising", "including", and "having" can be used interchangeably. It is also to be noted that the terms "comprising", "including", and "having" can be used interchangeably. Furthermore, a compound "selected from the group consisting of" refers to one or more of the compounds in the list that follows, including mixtures (i.e., combinations) of two or more of the compounds.

According to the present invention, an isolated, or biologically pure, FcɛR molecule, is a molecule that has been removed from its natural milieu. As such, "isolated" and "biologically pure" do not necessarily reflect the extent to which the molecule has been purified. An isolated human FcɛR molecule of the present invention can be obtained from its natural source (e.g., from a human mast cell), can be produced using recombinant DNA technology or can be produced by chemical synthesis.

A FcɛR molecule (also referred to herein as FcɛR or FcɛR protein) of the present invention can be a full-length protein, a portion of a full-length protein or any homolog of such a protein. As used herein, a protein can be a polypeptide or a peptide. A FcɛR molecule of the present invention can comprise a complete FcɛR (i.e., alpha, beta and gamma FcɛR chains), an alpha FcɛR chain (also referred to herein as FcɛR α chain) or portions thereof. Preferably, a FcɛR molecule comprises at least a portion of a FcɛR α chain that binds to IgE, i.e., that is capable of forming an immunocomplex with an IgE constant region. Preferably, a FcɛR molecule of the present invention binds to IgE with an affinity of about Kₘ = 10⁴, more preferably with an affinity of about Kₘ = 10⁹ and even more preferably with an affinity of about Kₘ = 10¹⁰.

An isolated FcɛR molecule of the present invention, including a homolog, can be identified in a straightforward manner by the FcɛR molecule's ability to form an immunocomplex with an IgE. Examples of FcɛR homologs include FcɛR proteins in which amino acids have been deleted (e.g., a truncated version of the protein, such as a peptide), inserted, inverted, substituted and/or derivatized (e.g., by glycosylation, phosphorylation, acetylation, myristoylation, prenylation, palmitoylation, amidation and/or addition of glycerophosphatidyl inositol) such that the homolog includes at least one epitope capable of forming an immunocomplex with an IgE.
FcR homologs can be the result of natural allelic variation or natural mutation. FcR homologs of the present invention can also be produced using techniques known in the art including, but not limited to, direct modifications to the protein or modifications to the gene encoding the protein using, for example, classic or recombinant DNA techniques to effect random or targeted mutagenesis.

According to the present invention, a human FcR α chain of the present invention is encoded by at least a portion of the nucleic acid sequence of the coding strand of a cDNA encoding a full-length FcR α chain protein represented herein as SEQ ID NO:1, the portion at least encoding the IgE binding site of the FcR α chain protein. The double-stranded nucleic acid molecule including both the coding strand having SEQ ID NO:1 and the complementary non-coding strand (the nucleic acid sequence of which can be readily determined by one skilled in the art and is shown herein as SEQ ID NO:3) is referred to herein as FcR nucleic acid molecule nhFcRα1198. Translation of SEQ ID NO:1 suggests that nucleic acid molecule nhFcRα1198 encodes a full-length FcR α chain protein of about 257 amino acids, referred to herein as PhFcRα257, represented by SEQ ID NO:2, assuming an open reading frame having an initiation (start) codon spanning from nucleotide 107 through nucleotide 109 of SEQ ID NO:1 and a termination (stop) codon spanning from nucleotide 878 through nucleotide 880 of SEQ ID NO:1. The coding region encoding PhFcRα257, including the stop codon, is represented by nucleic acid molecule nhFcRα774, having a coding strand with the nucleic acid sequence represented herein as SEQ ID NO:4. The compliment of SEQ ID NO:4 is represented herein as SEQ ID NO:5. SEQ ID NO:1 encodes a signal peptide of about 25 amino acids as well as a mature protein of about 232 amino acids, denoted herein as PhFcRα232, the amino acid sequence of which is represented herein as SEQ ID NO:6. The nucleic acid molecule encoding the apparent mature protein is referred to as nhFcRα699, the nucleic acid sequence of the coding strand of which is denoted herein as SEQ ID NO:7. SEQ ID NO:1 also encodes a hydrophobic transmembrane domain and a cytoplasmic tail which as a group extend from amino acid 205 to amino acid 257 of SEQ ID NO:2. Knowledge of these nucleic acid and amino acid sequences allows one skilled in the art to make modifications to the respective nucleic acid molecules and proteins to, for example, develop a FcR α chain protein with increased solubility and/or a truncated
protein (e.g., a peptide) capable of detecting IgE, e.g., PhFc_e Rα_{197} and PhFc_e Rα_{172}.
Preferred Fc_e R molecules include PhFc_e Rα_{257}, PhFc_e Rα_{197}, PhFc_e Rα_{232} and PhFc_e Rα_{172}.
Preferred nucleic acid molecules to encode a Fc_e R molecule include nhFc_e Rα_{774},
nhFc_e Rα_{1198}, nhFc_e Rα_{612}, nhFc_e Rα_{591}, nhFc_e Rα_{699} and/or nhFc_e Rα_{516}.

Isolated Fc_e R molecule protein of the present invention can be produced by
5 culturing a cell capable of expressing the protein under conditions effective to produce
the protein, and recovering the protein. A preferred cell to culture is a recombinant cell
that is capable of expressing the protein, the recombinant cell being produced by
transforming a host cell with one or more nucleic acid molecules of the present
10 invention. Transformation of a nucleic acid molecule into a cell can be accomplished by
any method by which a nucleic acid molecule can be inserted into the cell.
Transformation techniques include, but are not limited to, transfection, electroporation,
microinjection, lipofection, adsorption, and protoplast fusion. A recombinant cell may
remain unicellular or may grow into a tissue, organ or a multicellular organism.

15 Transformed nucleic acid molecules of the present invention can remain
extrachromosomal or can integrate into one or more sites within a chromosome of the
transformed (i.e., recombinant) cell in such a manner that their ability to be expressed is
retained. Suitable and preferred nucleic acid molecules with which to transform a cell
are as disclosed herein for suitable and preferred Fc_e R nucleic acid molecules per se.

20 Particularly preferred nucleic acid molecules to include in recombinant cells of the
present invention include nhFc_e Rα_{774}, nhFc_e Rα_{1198}, nhFc_e Rα_{612}, nhFc_e Rα_{591}, nhFc_e Rα_{699}
and/or nhFc_e Rα_{516}.

Suitable host cells to transform include any cell that can be transformed with a
nucleic acid molecule of the present invention. Host cells can be either untransformed
25 cells or cells that are already transformed with at least one nucleic acid molecule. Host
cells of the present invention either can be endogenously (i.e., naturally) capable of
producing a Fc_e R molecule protein of the present invention or can be capable of
producing such proteins after being transformed with at least one nucleic acid molecule
of the present invention. Host cells of the present invention can be any cell capable of
producing at least one protein of the present invention, and include bacterial, fungal
(including yeast), parasite (including protozoa and ectoparasite), insect, other animal and plant cells.

Preferably, a recombinant cell is transfected with a recombinant molecule of the present invention is a molecule that can include at least one of any nucleic acid molecule heretofore described operatively linked to at least one of any transcription control sequence capable of effectively regulating expression of the nucleic acid molecule(s) in the cell to be transformed, examples of which are disclosed herein. A particularly preferred recombinant molecule includes pVL-nhFc\textsubscript{e}R\alpha\textsubscript{612}. Details regarding the production of Fc\textsubscript{e}R molecule nucleic acid molecule-containing recombinant molecules are disclosed herein. Particularly preferred recombinant cell of the present invention includes *Trichoplusia ni*-pVL-nhFc\textsubscript{e}R\alpha\textsubscript{612}.

A Fc\textsubscript{e}R molecule of the present invention can include chimeric molecules comprising a portion of a Fc\textsubscript{e}R molecule that binds to an IgE and a second molecule that enables the chimeric molecule to be bound to a substrate in such a manner that the Fc\textsubscript{e}R portion binds to IgE in essentially the same manner as a Fc\textsubscript{e}R molecule that is not bound to a substrate. An example of a suitable second molecule includes a portion of an immunoglobulin molecule.

A Fc\textsubscript{e}R molecule of the present invention can be contained in a formulation, herein referred to as a Fc\textsubscript{e}R formulation. For example, a Fc\textsubscript{e}R can be combined with a buffer in which the Fc\textsubscript{e}R is solubilized, and/or a carrier. Suitable buffers and carriers are known to those skilled in the art. Examples of suitable buffers include any buffer in which a Fc\textsubscript{e}R can function to selectively bind to IgE, such as, but not limited to, phosphate buffered saline, water, saline, phosphate buffer, bicarbonate buffer, HEPES buffer (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid buffered saline), TES buffer (Tris-EDTA buffered saline), Tris buffer and TAE buffer (Tris-acetate-EDTA). Examples of carriers include, but are not limited to, polymeric matrices, toxoids, and serum albumins, such as bovine serum albumin. Carriers can be in mixed with Fc\textsubscript{e}R or conjugated (i.e., attached) to Fc\textsubscript{e}R in such a manner as to not substantially interfere with the ability of the Fc\textsubscript{e}R to selectively bind to IgE.

A Fc\textsubscript{e}R of the present invention can be bound to the surface of a cell expressing the Fc\textsubscript{e}R. A preferred Fc\textsubscript{e}R-bearing cell includes a recombinant cell expressing a nucleic
acid molecule encoding a human FcγR alpha chain of the present invention. A more preferred recombinant cell of the present invention expresses a nucleic acid molecule that encodes at least one of the following proteins: PhFcγRα_{257} and PhFcγRα_{232}. An even more preferred recombinant cell expresses a nucleic acid molecule including nhFcγRα_{612}, nhFcγRα_{591}, nhFcγRα_{699} and/or nhFcγRα_{516} with a recombinant cell expressing a nucleic acid molecule comprising a nucleic acid sequence including SEQ ID NO:1 or SEQ ID NO:4, or a nucleic acid molecule comprising an allelic variant of a nucleic acid molecule comprising SEQ ID NO:1 or SEQ ID NO:4, being even more preferred. An even more preferred recombinant cell is a RBL-hFcγR cell.

In addition, a FcγR formulation of the present invention can include not only a FcγR but also one or more additional antigens or antibodies useful in detecting IgE. As used herein, an antigen refers to any molecule capable of being selectively bound by an antibody. As used herein, specific binding of a first molecule to a second molecule refers to the ability of the first molecule to preferentially bind (e.g., having higher affinity higher avidity) to the second molecule when compared to the ability of a first molecule to bind to a third molecule. The first molecule need not necessarily be the natural ligand of the second molecule. Examples of such antibodies include, but are not limited to, antibodies that bind selectively to the constant region of an IgE heavy (i.e., anti-IgE isotype antibody) or antibodies that bind selectively to an IgE having a specific antigen specificity (i.e., anti-IgE idiotypic antibody). Examples of such antigens include any antigen known to induce the production of IgE. Preferred antigens include allergens and parasite antigens. Allergens of the present invention are preferably derived from fungi, trees, weeds, shrubs, grasses, wheat, corn, soybeans, rice, eggs, milk, cheese, bovines (or cattle), poultry, swine, sheep, yeast, fleas, flies, mosquitos, mites, midges, biting gnats, lice, bees, wasps, ants, true bugs or ticks. A suitable flea antigen includes an allergen derived from a flea, in particular flea saliva antigen. A preferred flea allergen includes a flea saliva antigen Preferred f_{sala} saliva antigens include antigens such as those disclosed in PCT Patent Publication No. WO 96/11271, published April 18, 1996, by Frank et al. (this publication is incorporated by reference herein in its entirety), with flea saliva products and flea saliva proteins being particularly preferred. According to the present invention, a flea saliva protein includes a protein produced by
recombinant DNA methods, as well as proteins isolated by other methods disclosed in

Preferred general allergens include those derived from grass, Meadow Fescue, Curly Dock, plantain, Mexican Firebush, Lamb’s Quarters, pigweed, ragweed, sage, elm, cocklebur, Box Elder, walnut, cottonwood, ash, birch, cedar, oak, mulberry, cockroach, Dermatophagoides, Alternaria, Aspergillus, Cladosporium, Fusarium, Helminthosporium, Mucor, Penicillium, Pullularia, Rhizopus and/or Trichophyton. More preferred general allergens include those derived from Johnson Grass, Kentucky Blue Grass, Meadow Fescue, Orchard Grass, Perennial Rye Grass, Redtop Grass, Timothy Grass, Bermuda Grass, Brome Grass, Curly Dock, English Plantain, Mexican Firebush, Lamb’s Quarters, Rough Pigweed Short Ragweed, Wormwood Sage, American Elm, Common Cocklebur, Box Elder, Black Walnut, Eastern Cottonwood, Green Ash, River Birch, Red Cedar, Red Oak, Red Mulberry, Cockroach, Dermatophagoides farinae, Alternaria alternata, Aspergillus fumigatus, Cladosporium herbarum, Fusarium vasinfectum, Helminthosporium sativum, Mucor recemosus, Penicillium notatum, Pullularia pullulans, Rhizopus nigricans and/or Trichophyton spp. Preferred tropical allergens include those derived from Bermuda Grass, June Bluegrass, Annual Bluegrass, Orchard Grass, Perennial Rye Grass, Timothy Grass, Meadow Fescue, Common Cocklebur, Yellow Dock, Sheep Sorrel, English Plantain, Lamb’s Quarters, Rough Pigweed, Russian Thistle, Short Ragweed, Red Cedar, Cat Epithelium, Arizona Cypress, Bald Cypress, Date Palm, Australian Pine, Eucalyptus, Mango, Acacia, Grama Grass, Nettle, Western Cottonwood, Saltgrass, Dermatophagoides pteronyssinus, Aureobasidium pullans, Penicillium notatum, Penicillium chrysogenum, Drechslera sorokiniana, Fusarium roseum, Cladosporium sphaerospermum, Aspergillus fumigatus, Alternaria tenuis Dermatophagoides farinae and Stemphylium sarciniforme. Preferred desert allergens include those derived from Bahia Grass, Smooth Brome, Johnson Grass, Redtop Grass, Falf* e Ragweed, Carelessweed, Greasewood, Rough Marsh Elder, Kochia, Tall Ragweed, Western Ragweed, Slender Ragweed, Common Sage, Prairie Sage, Mugwort Sage and Shadscale. Preferred parasite antigens include, but are not limited to, helminth antigens, in particular heartworm antigens, such as Di33 (described in U.S. Patent Application Serial No. 08/715,628, filed September 18, 1996, to Grieve et al.).
The term "derived from" refers to a natural allergen of such plants or organisms (i.e., an allergen directly isolated from such plants or organisms), as well as, non-natural allergens of such plants or organisms that possess at least one epitope capable of eliciting an immune response against an allergen (e.g., produced using recombinant DNA technology or by chemical synthesis).

The present invention also includes human FcεR mimetopes and use thereof to detect IgE. In accordance with the present invention, a "mimotope" refers to any compound that is able to mimic the ability of a FcεR molecule to bind to IgE. A mimotope can be a peptide that has been modified to decrease its susceptibility to degradation but that still retains IgE-binding activity. Other examples of mimetopes include, but are not limited to, carbohydrate-based compounds, lipid-based compounds, nucleic acid-based compounds, natural organic compounds, synthetically derived organic compounds, anti-idiotypic antibodies and/or catalytic antibodies, or fragments thereof. A mimotope can be obtained by, for example, screening libraries of synthetic compounds for compounds capable of binding to IgE. A mimotope can also be obtained by, for example, rational drug design. In a rational drug design procedure, the three-dimensional structure of a compound of the present invention can be analyzed by, for example, nuclear magnetic resonance (NMR) or x-ray crystallography. The three-dimensional structure can then be used to predict structures of potential mimetopes by, for example, computer modeling. The predicted mimotope structures can then be produced by, for example, chemical synthesis, recombinant DNA technology, or by isolating a mimotope from a natural source. Specific examples of FcεR mimetopes include anti-idiotypic antibodies, oligonucleotides produced using Selex technology, peptides identified by random screening of peptide libraries and proteins identified by phage display technology.

One embodiment of the present invention is a method to detect non-human IgE which includes the steps of: (a) contacting an isolated human Fcε receptor (FcεR) molecule with a putative IgE-containing composition under conditions suitable for formation of an FcεR molecule:IgE complex; and (b) determining levels of IgE by detecting said FcεR molecule:IgE complex. Presence of such a FcεR molecule:IgE complex indicates that the animal is producing IgE. Preferred non-human IgE to detect
using a human Fc\_R molecule include canine IgE, feline IgE and equine IgE. The present method can further include the step of determining whether an IgE complexed with a Fc\_R molecule is heat labile. Methods to determine heat lability of IgE are disclosed in the Examples section. Preferably, an IgE is heat labile when incubated at about 56°C for about 4 hours. Without being bound by theory, Applicants believe that heat labile forms of IgE bind to certain allergens and non-heat labile forms of IgE bind to other types of allergens. As such, detection of heat labile IgE compared with non-heat labile IgE can be used to discriminate between allergen sensitivities. For example, Applicants believe that IgE antibodies that bind to certain flea allergens and heartworm allergens are heat labile while IgE antibodies that bind to certain plant allergens are not heat labile. Thus, the presence of non-heat labile IgE can indicate that an animal is sensitive to certain plant allergens but not to certain flea or heartworm allergens. Moreover, Applicants believe that identification of heat labile IgE and non-heat labile IgE using a human Fc\_R suggests the presence of different sub-populations of IgE that may or may not have substantially similar structures to known IgE. As such, a Fc\_R molecule of the present invention may be useful for detecting molecules bound by the Fc\_R molecule but not identical to a known IgE.

As used herein, canine refers to any member of the dog family, including domestic dogs, wild dogs and zoo dogs. Examples of dogs include, but are not limited to, domestic dogs, wild dogs, foxes, wolves, jackals and coyotes. As used herein, a feline refers to any member of the cat family, including domestic cats, wild cats and zoo cats. Examples of cats include, but are not limited to, domestic cats, lions, tigers, leopards, panthers, cougars, bobcats, lynx, jaguars, cheetahs, and servals. As used herein, equine refers to any member of the horse family, including horses, donkeys, mules and zebras.

As used herein, the term “contacting” refers to combining or mixing, in this case a putative IgE-containing composition with a human Fc\_R molecule. For nation of a complex between a Fc\_R and an IgE refers to the ability of the Fc\_R to selectively bind to the IgE in order to form a stable complex that can be measured (i.e., detected). As used herein, the term selectively binds to an IgE refers to the ability of a Fc\_R of the present invention to preferentially bind to IgE, without being able to substantially bind to
other antibody isotypes. Binding between a Fc, R and an IgE is effected under conditions suitable to form a complex; such conditions (e.g., appropriate concentrations, buffers, temperatures, reaction times) as well as methods to optimize such conditions are known to those skilled in the art, and examples are disclosed herein. Examples of complex formation conditions are also disclosed in, for example, in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Labs Press, 1989, the reference Sambrook et al., ibid., is incorporated by reference herein in its entirety.

As used herein, the term "detecting complex formation" refers to determining if any complex is formed, i.e., assaying for the presence (i.e., existence) of a complex. If complexes are formed, the amount of complexes formed can, but need not be, determined. Complex formation, or selective binding, between Fc, R and any IgE in the composition can be measured (i.e., detected, determined) using a variety of methods standard in the art (see, for example, Sambrook et al. ibid.), examples of which are disclosed herein.

In one embodiment, a putative IgE-containing composition of the present method includes a biological sample from an animal. A suitable biological sample includes, but is not limited to, a bodily fluid composition or a cellular composition. A bodily fluid refers to any fluid that can be collected (i.e., obtained) from an animal, examples of which include, but are not limited to, blood, serum, plasma, urine, tears, aqueous humor, central nervous system fluid (CNF), saliva, lymph, nasal secretions, milk and feces. Such a composition of the present method can, but need not be, pretreated to remove at least some of the non-IgE isotypes of immunoglobulin and/or other proteins, such as albumin, present in the fluid. Such removal can include, but is not limited to, contacting the bodily fluid with a material, such as Protein G, to remove IgG antibodies and/or affinity purifying IgE antibodies from other components of the body fluid by exposing the fluid to, for example, Concanavalin A. In another embodiment, a composition includes collected bodily fluid that is precipitated to concentrate immunoglobulin contained in the fluid. For example, immunoglobulin contained in a bodily fluid can be precipitated from other proteins using ammonium sulfate. A preferred composition of the present method is serum.
In another embodiment, a composition of the present method includes an IgE-producing cell. Such a cell can have IgE bound to the surface of the cell and/or can secrete IgE. Examples of such cells include basophil cells and myeloma cells. IgE can be bound to the surface of a cell either directly to the membrane of a cells or bound to a molecule (e.g., an antigen) bound to the surface of the cell.

A complex can be detected in a variety of ways including, but not limited to use of one or more of the following assays: an enzyme-linked immunoassay, a radioimmunoassay, a fluorescence immunoassay, a chemiluminescent assay, a lateral flow assay, an agglutination assay, a particulate-based assay (e.g., using particulates such as, but not limited to, magnetic particles or plastic polymers, such as latex or polystyrene beads), an immunoprecipitation assay, a BioCore™ assay (e.g., using colloidal gold) and an immunoblotting assay (e.g., a western blot). Such assays are well known to those skilled in the art. Assays can be used to give qualitative or quantitative results depending on how they are used. Some assays, such as agglutination, particulate separation, and immunoprecipitation, can be observed visually (e.g., either by eye or by a machines, such as a densitometer or spectrophotometer) without the need for a detectable marker. In other assays, conjugation (i.e., attachment) of a detectable marker to the FcR or to a reagent that selectively binds to the FcR or to the IgE being detected (described in more detail below) aids in detecting complex formation. Examples of detectable markers include, but are not limited to, a radioactive label, a fluorescent label, a chemiluminescent label, a chromophoric label or a ligand. A ligand refers to a molecule that binds selectively to another molecule. Preferred detectable markers include, but are not limited to, fluorescein, a radioisotope, a phosphatase (e.g., alkaline phosphatase), biotin, avidin, a peroxidase (e.g., horseradish peroxidase) and biotin-related compounds or avidin-related compounds (e.g., streptavidin or ImmunoPure® NeutrAvidin). Preferably, biotin is conjugated to an alpha chain of a FcR. Preferably a carboxy-terminal group of the FcR alpha chain is conjugated to biotin. A preferred FcR molecule conjugated to biotin comprises PhFcRα172-BIOT (the production of which is described in the Examples section).

In one embodiment, a complex is detected by contacting a putative IgE-containing composition with a FcR molecule that is conjugated to a detectable marker.
A suitable detectable marker to conjugate to a Fc, R molecule includes, but is not limited to, a radioactive label, a fluorescent label, a chemiluminescent label or a chromophoric label. A detectable marker is conjugated to a Fc, R molecule or a reagent in such a manner as not to block the ability of the Fc, R or reagent to bind to the IgE being detected. Preferably, a carbohydrate group of a Fc, R is conjugated to biotin.

In another embodiment, a Fc, R molecule: IgE complex is detected by contacting a putative IgE-containing composition with a Fc, R molecule and then contacting the complex with an indicator molecule. Suitable indicator molecules of the present invention include molecules that can bind to either the Fc, R molecule or to the IgE antibody. As such, an indicator molecule can comprise, for example, a Fc, R molecule, an antigen, an antibody and a lectin, depending upon which portion of the Fc, R molecule: IgE complex being detected. Preferred identifying labeled compounds that are antibodies include, for example, anti-IgE antibodies and anti-Fc, R antibodies. Preferred lectins include those lectins that bind to high-mannose groups. More preferred lectins bind to high-mannose groups present on a Fc, R molecule of the present invention produced in insect cells. An indicator molecule itself can be attached to a detectable marker of the present invention. For example, an antibody can be conjugated to biotin, horseradish peroxidase, alkaline phosphatase or fluorescein.

In one preferred embodiment, a Fc, R molecule: IgE complex is detected by contacting the complex with a reagent that selectively binds to a Fc, R molecule of the present invention. Examples of such a reagent includes, but are not limited to, an antibody that selectively binds to a Fc, R molecule (referred to herein as an anti-Fc, R antibody) or a compound that selectively binds to a detectable marker conjugated to a Fc, R molecule. Fc, R molecules conjugated to biotin are preferably detected using streptavidin, more preferably using ImmunoPure® NeutrAvidin (available from Pierce, Rockford, IL).

In another preferred embodiment, a Fc, R molecule: IgE complex is detected by contacting the complex with a reagent that selectively binds to an IgE antibody (referred to herein as an anti-IgE reagent). Examples of such an anti-IgE reagent include, but are not limited to, a secondary antibody that is an anti-isotype antibody (e.g., an antibody that selectively binds to the constant region of an IgE), an antibody-binding bacterial
surface protein (e.g., Protein A or Protein G), an antibody-binding cell (e.g., a B cell, a T cell, a natural killer cell, a polymorphonuclear leukocyte cell, a monocyte cell or a macrophage cell), an antibody-binding eukaryotic cell surface protein (e.g., an Fc receptor), and an antibody-binding complement protein. Preferred anti-IgE reagents include, but are not limited to, D9, and CMI antibody #9, CMI antibody #19, CMI antibody #59 and CMI antibody #71 (available from Custom Monoclonal International, West Sacramento, CA). In particular, as used herein, an anti-IgE antibody includes not only a complete antibody but also any subunit or portion thereof that is capable of selectively binding to an IgE heavy chain constant region. For example, a portion of an anti-IgE reagent can include an Fab fragment or a F(ab')2 fragment, which are described in detail in Janeway et al., in Immunobiology, the Immune System in Health and Disease, Garland Publishing, Inc., NY, 1996 (which is incorporated herein by this reference in its entirety).

In one embodiment a complex can be formed and detected in solution. In another embodiment, a complex can be formed in which one or more members of the complex are immobilized on (e.g., coated onto) a substrate. Immobilization techniques are known to those skilled in the art. Suitable substrate materials include, but are not limited to, plastic, glass, gel, celluloid, paper, PVDF (poly-vinylidene-fluoride), nylon, nitrocellulose, and particulate materials such as latex, polystyrene, nylon, nitrocellulose, agarose and magnetic resin. Suitable shapes for substrate material include, but are not limited to, a well (e.g., microtiter dish well), a plate, a dipstick, a bead, a lateral flow apparatus, a membrane, a filter, a tube, a dish, a celluloid-type matrix, a magnetic particle, and other particulates. A particularly preferred substrate comprises an ELISA plate, a dipstick, a radioimmunoassay plate, agarose beads, plastic beads, latex beads, immunoblot membranes and immunoblot papers. In one embodiment, a substrate, such as a particulate, can include a detectable marker.

A preferred method to detect IgE is an immunosorbent assay. An immunoabsorbent assay of the present invention comprises a capture molecule and an indicator molecule. A capture molecule of the present invention binds to an IgE in such a manner that the IgE is immobilized to a substrate. As such, a capture molecule is preferably immobilized to a substrate of the present invention prior to exposure of the
capture molecule to a putative IgE-containing composition. An indicator molecule of
the present invention detects the presence of an IgE bound to a capture molecule. As
such, an indicator molecule preferably is not immobilized to the same substrate as a
capture molecule prior to exposure of the capture molecule to a putative IgE-containing
composition.

A preferred immunoabsorbent assay method includes a step of either: (a) binding
an Fc\(\varepsilon\)R molecule to a substrate prior to contacting a Fc\(\varepsilon\)R molecule with a putative IgE-
containing composition to form a Fc\(\varepsilon\)R molecule-coated substrate; or (b) binding a
putative IgE-containing composition to a substrate prior to contacting a Fc\(\varepsilon\)R molecule
with a putative IgE-containing composition to form a putative IgE-containing
composition-coated substrate. Preferably, the substrate includes of a non-coated
substrate, a Fc\(\varepsilon\)R molecule-coated substrate, an antigen-coated substrate or an anti-IgE
antibody-coated substrate.

Both a capture molecule and an indicator molecule of the present invention are
capable of binding to an IgE. Preferably, a capture molecule binds to a different region
of an IgE than an indicator molecule, thereby allowing a capture molecule to be bound to
an IgE at the same time as an indicator molecule. The use of a reagent as a capture
molecule or an indicator molecule depends upon whether the molecule is immobilized to
a substrate when the molecule is exposed to an IgE. For example, a Fc\(\varepsilon\)R molecule of
the present invention is used as a capture molecule when the Fc\(\varepsilon\)R molecule is bound to
a substrate. Alternatively, a Fc\(\varepsilon\)R molecule is used as an indicator molecule when the
Fc\(\varepsilon\)R molecule is not bound to a substrate. Suitable molecule for use as capture
molecules or indicator molecules include, but are not limited to, a Fc\(\varepsilon\)R molecule of the
present invention, an antigen reagent or an anti-IgE antibody reagent of the present
invention.

An immunoabsorbent assay of the present invention can further comprise one or
more layers and/or types of secondary molecules or other binding molecules capable of
detecting the presence of an indicator molecule. For example, an untagged (i.e., not
conjugated to a detectable marker) secondary antibody that selectively binds to an
indicator molecule can be bound to a tagged (i.e., conjugated to a detectable marker)
tertiary antibody that selectively binds to the secondary antibody. Suitable secondary
antibodies, tertiary antibodies and other secondary or tertiary molecules can be selected by those of skill in the art. Preferred secondary molecules of the present invention include, an antigen, an anti-IgE idiotypic antibody and an anti-IgE isotypic. Preferred tertiary molecules can be selected by a skilled artisan based upon the characteristics of the secondary molecule. The same strategy is applied for subsequent layers.

In one embodiment, a desired antigen is used as a capture molecule by being immobilized on a substrate, such as a microtiter dish well or a dipstick. Preferred antigens include those disclosed herein. A biological sample collected from an animal is applied to the substrate and incubated under conditions suitable (i.e., sufficient) to allow for antigen:IgE complex formation bound to the substrate (i.e., IgE in a sample binds to an antigen immobilized on a substrate). Excess non-bound material (i.e., material from the biological sample that has not bound to the antigen), if any, is removed from the substrate under conditions that retain antigen:IgE complex binding to the substrate. Preferred conditions are disclosed herein in the Examples section and generally in

Sambrook et al., *ibid*. An indicator molecule that can selectively bind to an IgE bound to the antigen, the indicator molecule can be conjugated to a detectable marker (preferably to an enzyme label, to a colorimetric label, to a fluorescent label, to a radioisotope, or to a ligand such as of the biotin or avidin family), is added to the substrate and incubated to allow formation of a complex between the indicator molecule and the antigen:IgE complex. Excess indicator molecule is removed, a developing agent is added if required, and the substrate is submitted to a detection device for analysis. A preferred indicator molecule for this embodiment is a FcR molecule, preferably conjugated to biotin, to a fluorescent label or to an enzyme label.

In one embodiment, a FcR molecule is used as a capture molecule by being immobilized on a substrate, such as a microtiter dish well or a dipstick. A biological sample collected from an animal is applied to the substrate and incubated under conditions suitable to allow for FcR molecule:IgE complex formation bound to the substrate. Excess non-bound material, if any, is removed from the substrate under conditions that retain FcR molecule:IgE complex binding to the substrate. An indicator molecule that can selectively bind to an IgE bound to the FcR is added to the substrate and incubated to allow formation of a complex between the indicator molecule and the
FcεR molecule-IgE complex. Preferably, the indicator molecule is conjugated to a detectable marker (preferably to an enzyme label, to a colorimetric label, to a fluorescent label, to a radioisotope, or to a ligand such as of the biotin or avidin family). Excess indicator molecule is removed, a developing agent is added if required, and the substrate is submitted to a detection device for analysis. A preferred indicator molecule for this embodiment is an antigen that will bind to IgE in the biological sample or an anti-IgE isotype or idiotype antibody, either preferably being conjugated to fluorescein or biotin.

In one embodiment, an anti-IgE antibody (e.g., isotype or idiotype specific antibody) is used as a capture molecule by being immobilized on a substrate, such as a microtiter dish well or a dipstick. A biological sample collected from an animal is applied to the substrate and incubated under conditions suitable to allow for anti-IgE antibody-IgE complex formation bound to the substrate. Excess non-bound material, if any, is removed from the substrate under conditions that retain anti-IgE antibody-IgE complex binding to the substrate. A FcεR molecule is added to the substrate and incubated to allow formation of a complex between the FcεR molecule and the anti-IgE antibody-IgE complex. Preferably, the FcεR molecule is conjugated to a detectable marker (preferably to biotin, an enzyme label or a fluorescent label). Excess FcεR molecule is removed, a developing agent is added if required, and the substrate is submitted to a detection device for analysis.

In one embodiment, an immunosorbent assay of the present invention does not utilize a capture molecule. In this embodiment, a biological sample collected from an animal is applied to a substrate, such as a microtiter dish well or a dipstick, and incubated under conditions suitable to allow for IgE binding to the substrate. Any IgE present in the bodily fluid is immobilized on the substrate. Excess non-bound material, if any, is removed from the substrate under conditions that retain IgE binding to the substrate. A FcεR molecule is added to the substrate and incubated to allow formation of a complex between the FcεR molecule and the IgE. Preferably, the FcεR molecule is conjugated to a detectable marker (preferably to biotin, an enzyme label or a fluorescent label). Excess FcεR molecule is removed, a developing agent is added if required, and the substrate is submitted to a detection device for analysis.
Another preferred method to detect IgE is a lateral flow assay, examples of which are disclosed in U.S. Patent No. 5,424,193, issued June 13, 1995, by Pronovost et al.; U.S. Patent No. 5,415,994, issued May 16, 1995, by Imrich et al.; WO 94/29696, published December 22, 1994, by Miller et al.; and WO 94/01775, published January 20, 1994, by Pawlak et al.; each of these patent publications is incorporated by reference herein in its entirety. In one embodiment, a biological sample is placed in a lateral flow apparatus that includes the following components: (a) a support structure defining a flow path; (b) a labeling reagent comprising a bead conjugated to an antigen, the labeling reagent being impregnated within the support structure in a labeling zone; and (c) a capture reagent comprising an IgE-binding composition. Preferred antigens include those disclosed herein. The capture reagent is located downstream of the labeling reagent within a capture zone fluidly connected to the labeling zone in such a manner that the labeling reagent can flow from the labeling zone into the capture zone. The support structure comprises a material that does not impede the flow of the beads from the labeling zone to the capture zone. Suitable materials for use as a support structure include ionic (i.e., anionic or cationic) material. Examples of such a material include, but are not limited to, nitrocellulose (NC), PVDF, carboxymethylcellulose (CM). The support structure defines a flow path that is lateral and is divided into zones, namely a labeling zone and a capture zone. The apparatus can further comprise a sample receiving zone located along the flow path, more preferably upstream of the labeling reagent. The flow path in the support structure is created by contacting a portion of the support structure downstream of the capture zone, preferably at the end of the flow path, to an absorbent capable of absorbing excess liquid from the labeling and capture zones.

In this embodiment, the biological sample is applied to the sample receiving zone which includes a portion of the support structure. The labeling zone receives the sample from the sample receiving zone which is directed downstream by the flow path. The labeling zone comprises the labeling reagent that binds IgE. A preferred labeling reagent is an antigen conjugated, either directly or through a linker, to a plastic bead substrate, such as to a latex bead. The substrate also includes a detectable marker, preferably a colorimetric marker. Typically, the labeling reagent is impregnated to the support structure by drying or lyophilization. The sample structure also comprises a
capture zone downstream of the labeling zone. The capture zone receives labeling reagent from the labeling zone which is directed downstream by the flow path. The capture zone contains the capture reagent, in this case a Fc_eR molecule, as disclosed above, that immobilizes the IgE complexed to the antigen in the capture zone. The capture reagent is preferably fixed to the support structure by drying or lyophilizing. The labeling reagent accumulates in the capture zone and the accumulation is assessed visually or by an optical detection device.

In another embodiment, a lateral flow apparatus used to detect IgE includes: (a) a support structure defining a flow path; (b) a labeling reagent comprising a Fc_eR molecule as described above, the labeling reagent impregnated within the support structure in a labeling zone; and (c) a capture reagent comprising an antigen, the capture reagent being located downstream of the labeling reagent within a capture zone fluidly connected to the labeling zone in such a manner that the labeling reagent can flow from the labeling zone into the capture zone. The apparatus preferably also includes a sample receiving zone located along the flow path, preferably upstream of the labeling reagent. The apparatus preferably also includes an absorbent located at the end of the flow path.

One embodiment of the present invention is an inhibition assay in which the presence of IgE in a putative IgE-containing composition is determined by adding such composition to a Fc_eR molecule of the present invention and an isolated IgE known to bind to the Fc_eR molecule. The absence of binding of the Fc_eR molecule to the known IgE indicating the presence of IgE in the putative IgE-containing composition.

The present invention also includes kits to detect IgE based on each of the disclosed detection methods. One embodiment is a kit to detect IgE comprising a human Fc_e receptor (Fc_eR) molecule and a means for detecting an IgE including canine IgE, feline IgE and/or equine IgE. Suitable and preferred Fc_eR molecules are disclosed herein. Suitable means of detection include compounds disclosed herein that bind to either the Fc_eR molecule or to an IgE. A preferred kit of the present invention further comprises a detection means including one or more antigens disclosed herein, an antibody capable of selectively binding to an IgE disclosed herein and/or a compound capable of binding to a detectable marker conjugated to a Fc_eR molecule (e.g., avidin, streptavidin and ImmunoPure® NeutrAvidin when the detectable marker is biotin).
Such antigens preferably induce IgE antibody production in animals including canines, felines and/or equines.

A preferred embodiment of a kit of the present invention is a flea allergen kit comprising a flea allergen such as those disclosed herein. A particularly preferred flea allergen for use with a flea allergen kit includes a flea saliva product or a flea saliva protein.

Another preferred kit of the present invention is a general allergen kit comprising an allergen common to all regions of the United States and a human FcεR molecule of the present invention. As used herein, a “general allergen” kit refers to a kit comprising allergens that are found substantially throughout the United States (i.e., essentially not limited to certain regions of the United States). A general allergen kit provides an advantage over regional allergen kits because a single kit can be used to test an animal located in most geographical locations on the United States. Suitable and preferred general allergens for use with a general allergen kit of the present invention include those general allergens disclosed herein.

Another preferred kit of the present invention is a food allergen kit comprising a food allergen including beef, chicken, pork, a mixture of fish, such as cod, halibut or and tuna, egg, milk, Brewer’s yeast, whole wheat, corn, soybean, cheese and rice, and a human FcεR molecule of the present invention. Preferably, the beef, chicken, pork, fish, corn and rice, are cooked.

A preferred kit of the present invention includes those in which the allergen is immobilized to a substrate. If a kit comprises two or more antigens, the kit can comprise one or more compositions, each composition comprising one antigen. As such, each antigen can be tested separately. A kit can also contain two or more diagnostic reagents for IgE, additional isolated IgE antigens and/or antibodies as disclosed herein.

Particularly preferred are kits used in a lateral flow assay format. It is within the scope of the present invention that a lateral flow assay kit can include one or more lateral flow assay apparatuses. Multiple lateral flow apparatuses can be attached to each other at one end of each apparatus, thereby creating a fan-like structure.

In particular, a method and kit of the present invention are useful for diagnosing abnormal conditions in animals that are associated with changing levels of IgE.
Particularly preferred conditions to diagnose include allergies, parasitic infections and neoplasia. For example, a method and kit of the present invention are particularly useful for detecting flea allergy dermatitis (FAD), when such method or kit includes the use of a flea saliva antigen. FAD is defined as a hypersensitive response to fleabites.

Preferably, a putative IgE-containing composition is obtained from an animal suspected of having FAD. Preferred animals include those disclosed herein, with dogs and cats being more preferred. In addition, methods and kits of the present invention are particularly useful for detecting helminth infection, in particular heartworm infection, when such methods or kits include the use of a helminth antigen, such as Di33.

Preferably, a putative IgE-containing composition is obtained from an animal suspected of having a helminth infection. Preferred animals include those disclosed herein, with dogs and cats being more preferred.

The following examples are provided for the purposes of illustration and are not intended to limit the scope of the present invention.

Examples

Example 1.

This example describes the construction of a recombinant baculovirus expressing a truncated portion of the α-chain of the human Fcγ receptor.

Recombinant molecule pVL-nhFcγRα612, containing a nucleic acid molecule encoding the extracellular domain of the FcγR α chain, operatively linked to baculovirus polyhedron transcription control sequences was produced in the following manner. A cDNA clone encoding the full-length alpha chain (α chain) of the human Fcγ receptor was obtained from Dr. Jean-Pierre Kinet (Harvard University, Cambridge, MA). The cDNA clone included an about 1198 nucleotide insert, referred to herein as nhFcγRα1198.

The nucleic acid sequence of the coding strand of nhFcγRα1198 is denoted herein as SEQ ID NO:1. Translation of SEQ ID NO:1 indicates that nucleic acid molecule nhFcγRα1198 encodes a full-length human Fcγ receptor α chain protein of about 257 amino acids, referred to herein as PhFcγRα257, having amino acid sequence SEQ ID NO:2, assuming an open reading frame in which the initiation codon spans from nucleotide 107 through nucleotide 109 of SEQ ID NO:1 and the termination codon spans from nucleotide 878 through nucleotide 880 of SEQ ID NO:1. The complement of SEQ ID NO:1 is
represented herein by SEQ ID NO:3. The proposed mature protein (i.e., Fc,RA chain from which the signal sequence has been cleaved), denoted herein as PhFc,Rα_{232}, contains about 232 amino acids which is represented herein as SEQ ID NO:6. The nucleic acid molecule encoding PhFc,Rα_{232} is denoted herein as nhFc,Rα_{699}, the coding strand of which is represented by SEQ ID NO:7.

To produce a secreted form of the extracellular domain of the Fc, R α chain, the hydrophobic transmembrane domain and the cytoplasmic tail of the Fc, R α chain encoded by nhFc,Rα_{1198} were removed as follows. A Fc,R α chain extracellular domain nucleic acid molecule-containing fragment of about 612 nucleotides was PCR amplified from nhFc,Rα_{1198} using a forward primer EJH 040 containing a BamHI site, having the nucleic acid sequence 5' CGC GGA TCC TAT AAA TAT GGC TCC TGC CAT GG 3' (denoted SEQ ID NO:8) and a reverse primer IgE ANTI-SENSE containing an EcoRI site, having the nucleic acid sequence 5' GGC GAA TTC TTA AGC TTT TAT TAC AG 3' (denoted herein as SEQ ID NO:9). The resulting PCR product was digested with BamHI and EcoRI to produce nhFc,Rα_{612}. Nucleic acid molecule nhFc,Rα_{612} contained an about 591 nucleotide fragment encoding the extracellular domain of the human Fc, R α chain, extending from nucleotide 107 to nucleotide 697 of SEQ ID NO 1, denoted herein as nucleic acid molecule nhFc,Rα_{591}, the coding strand of which has a nucleic acid sequence denoted SEQ ID NO:10. Translation of SEQ ID NO:10 indicates that nucleic acid molecule nhFc,Rα_{612} encodes a Fc,R protein of about 197 amino acids, referred to herein as PhFc,Rα_{197}, having amino acid sequence SEQ ID NO:11. Nucleic acid molecule nhFc,Rα_{612} encodes a secretable form of the human Fc,R α chain which does not possess a leader sequence, which is denoted herein as PhFc,Rα_{172} having amino acid sequence SEQ ID NO:13. The coding region for PhFc,Rα_{172} is denoted nhFc,Rα_{516}, the coding strand of which has a nucleic acid sequence denoted SEQ ID NO:12. The complement of SEQ ID NO:12 is represented herein by SEQ ID NO:14.

In order to produce a baculovirus reovirus binant molecule capable of directing the production of PhFc,Rα_{197}, the nucleic acid molecule nhFc,Rα_{612} was subcloned into unique BamHI and EcoRI sites of pVL1392 baculovirus shuttle plasmid (available from Pharmingen, San Diego, CA) to produce a recombinant molecule referred to herein as
pVL-nhFc\textsubscript{c}R\alpha\textsubscript{612}. The resultant recombinant molecule pVL-nhFc\textsubscript{c}R\alpha\textsubscript{612} was verified for proper insert orientation by restriction mapping.

Example 2.

This example describes the production of PhFc\textsubscript{c}R\alpha\textsubscript{172} protein.

The recombinant molecule pVL-nhFc\textsubscript{c}R\alpha\textsubscript{612} was co-transfected with a linear Baculogold baculovirus DNA (available from Pharmingen) into Trichoplusia ni cells (available from Invitrogen Corp., San Diego, CA; High Five\textsuperscript{TM} cells) using the following method. About 1.5 liter cultures of serum-free ex-Cell Medium (available from Invitrogen) were seeded with about 1 x 10\textsuperscript{6} cells per ml of medium. The Trichoplusia ni cells were infected with recombinant molecule pVL-nhFc\textsubscript{c}R\alpha\textsubscript{612} at a multiplicity of infection (MOI) of about 2 to about 5 particle forming units (pfu) per cell to produce recombinant cell Trichoplusia ni-pVL-nhFc\textsubscript{c}R\alpha\textsubscript{612}. The infection was allowed to proceed at a controlled temperature of 27°C for 48 hours, to produce recombinant protein PhFc\textsubscript{c}R\alpha\textsubscript{172}. Following infection, cells were separated from the medium by centrifugation, and the medium was frozen at -70°C.

PhFc\textsubscript{c}R\alpha\textsubscript{172} was purified from the culture medium described immediately above by affinity chromatography using an IgE antibody produced by the myeloma cell line U266DI (American Tissue Type Catalogue No. TIB196) linked to sepharose 4B. The amino acid composition and N-terminal amino acid sequence of the affinity purified PhFc\textsubscript{c}R\alpha\textsubscript{172} were determined using methods standard in the art. The results indicated that PhFc\textsubscript{c}R\alpha\textsubscript{172} was properly processed by the Trichoplusia ni cells.

Example 3.

This example describes the biotinylation of a recombinant human Fc\textsubscript{c}R alpha chain protein.

Affinity purified recombinant protein PhFc\textsubscript{c}R\alpha\textsubscript{172}, prepared as described above in Example 2, was biotinylated as follows. About 440 micrograms (\mu g) of PhFc\textsubscript{c}R\alpha\textsubscript{172} were diluted in 1.5 milliliter (ml) of acetate buffer (0.1 M NaAc, pH 5.5) containing about 200 microliter (\mu l) of 0.1 M NaIO\textsubscript{4}. The mixture was incubated for about 20 minutes, on ice, and about 2 \mu l of glycerol was added following the incubation. The mixture was then dialyzed against about 2 liters of acetate buffer in a 3 ml Slide-A-Lyzer cassette (available from Pierce, Rockford, IL), 2 times for about 2 hours each
time. About 3.72 µg of biotin-LC-hydrazide (available from Pierce) was dissolved in about 200 µl of dimethylsulfoxide (DMSO) and injected into the cassette. The cassette was then rocked at room temperature for about 2 hours. Following the incubation, the mixture containing recombinant protein and biotin dialyzed 3 times, a first time for about 18 hours and two times for about 2 hours, each time at 5°C against phosphate buffered saline. The biotinylated protein was recovered from the dialysis, and is referred to herein as PhFc₅Rα₁₇₂-BIOT.

Example 4.

This example describes detection of canine IgE in a solid-phase ELISA using PhFc₅Rα₁₇₂-BIOT.

Wells of two Immulon II microtiter plates (available from Dynatech, Alexandria, VA) were coated with duplicate samples of about 100 µl/well of various concentrations of purified canine IgE as denoted in Fig. 1. The canine IgE was obtained from a canine IgE producing hybridoma, such as heterohybridoma 2.39 (described in Gebhard et al., *Immunology* 85:429-434, 1995) and was diluted in a CBC buffer (15 mM Na₂CO₃ and 34.8 mM NaHCO₃, pH 9.6. The coated plates were incubated overnight at 4°C. Following incubation, the canine IgE-containing solution was removed from each plate, and the plates were blotted dry. The plates were then blocked using about 200 µl/well of 0.25% bovine serum albumin (BSA) contained in phosphate buffered saline (PBSB) for about 1 hour at room temperature. The plates were then washed four times with 0.05% Tween-20 in PBS (PBST) using an automatic washer (available from Dynatech). Experimental samples consisting of about 100 µl/well of a 1:4000 dilution of 40 µg/ml PhFc₅Rα₁₇₂-BIOT (about 145 µg/ml; described in Example 3), contained in PBSB with 0.05%Tween-20 (PBSBT) were added to each well of one plate coated with canine IgE. Control samples consisting of about 100 µl of biotinylated anti-canine IgE monoclonal antibody D9 (supplied by Dr. DeBoer, U. of Wisconsin, Madison, WI) were added to each well of the other plate coated with canine IgE. The plates were incubated for 1 hour at room temperature and then washed four times with PBST. About 100 µl of about 0.25 ug/ml streptavidin conjugated to horseradish peroxidase (available from Kirkegaard and Perry Laboratories (KPL), Gaithersburg, MD; diluted in PBST) was added to each well that received experimental or control samples. The plates were then
incubated for 1 hour at room temperature and washed four times with PBST. About 100 μl of TMB substrate (available from available from KPL), that had been pre-warmed to room temperature, was added. Plates were then incubated for 10 minutes at room temperature and then about 100 μl/well of Stop Solution (available from KPL) was added. Optical densities of wells were read on a Spectramax Microtiter Plate (available from Molecular Devices Inc.) reader at 450 nm within 10 minutes of adding the stop solution.

The results shown in Fig. 1 indicate that the alpha chain of human Fc,γR detects the presence of canine IgE (closed circles) in a solid-phase assay in a similar manner as the control antibody that binds specifically to canine IgE (D9; open circles).

Example 5.

This example describes detection of plant allergen-specific canine IgE using PhFcγRα172-BIOT.

Multiple wells of an Immulon II microtiter plate (available from Dynatech) were coated with either about 100 μl/well of 1 μg/ml of Kentucky Blue Grass allergen or about 100 μl/well of about 1 μg/ml of Green Ash allergen (both available from Greer Inc., Lenoir, NC) both diluted in CBC buffer. The plate was incubated overnight at 4°C. The plate was blocked and washed as described in Example 4. Two different pools of canine sera were then added to the antigen-coated wells. The first pool consisted of sera isolated from 8 dogs reported to be allergen reactive. The second pool consisted of sera isolated from 8 dogs reported to be allergen non-reactive. Each pool of sera was diluted 1:10 or 1:100 in PBST. About 100 μl of each concentration of each diluted sera sample was added to the wells and incubated for 1 hour at room temperature. The plate was then washed four times with PBST. About 100 μl/well of a 1:4000 dilution of 40 μg/ml PhFcγRα172-BIOT (described in Example 3), contained in PBSBT was added to the antigen-coated wells. The plate was incubated for 1 hour at room temperature. The plate was then washed four times with PBST. About 100 μl/well of about 0.25 μg/ml of neutravidin conjugated to horseradish peroxidase (available from Pierce) contained in PBSBT, was added. The plate was incubated for 1 hour at room temperature. The plate was then washed and the presence of neutravidin bound to the plate detected using the method described in Example 4.
The results shown in Fig. 2 indicate that the alpha chain of human FcεRI detects the presence of canine IgE antibodies that bind specifically to a common grass allergen or to a common tree allergen. In addition, detection of canine IgE antibodies is dose dependent.

5 Example 6.

This example describes detection of total canine IgE using PhFcεRIα172-BIOT.

Multiple wells of an Immulon II microtiter plate (available from Dynatech) were coated with about 100 μl/well of about 1 μg/ml CMI anti-canine IgE antibody #6 (available from Custom Monoclonals International, West Sacramento, CA) diluted in CBC buffer. The plate was incubated overnight at 4°C. The plate was blocked and washed as described in Example 4. About 100 μl/well of a 1:60 dilution in PBSBT of sera samples from a variety of sources were then added to multiple wells coated with anti-IgE antibody. The samples included: (1) serum from a dog known to be allergic to flea saliva; (2) serum from dogs infected with *D. immitis*; (3) and (4) a pool of dog sera from dogs known as canine allergy calibrators (available from BioProducts DVM, Tempe, AZ); (5) pools of dog sera containing antibodies that have low binding to Kentucky Blue Grass allergen; (6) pools of dog sera that have high binding to Kentucky Blue Grass allergen; (7) a pool of dog sera from dogs known to be allergic to flea saliva, the sample was heat inactivated (at 56°C for 4 hours); (8) a pool of dog sera from dogs known to be allergic to flea saliva; or (9) a pool of dog sera from dogs raised in a barrier facility (i.e., negative control). A set of positive control samples consisting of IgE derived from the canine heterohybridoma described in Example 4 were also added to the plate to generate a standard curve. The plate was incubated for 1 hour at room temperature and then washed four times with PBST. The presence of canine IgE was detected using either about 100 μl/well of a 1:4000 dilution of 40 μg/ml PhFcεRIα172-BIOT (described in Example 3) or about 100 μl/well of about 1 μg/ml CMI anti-canine IgE antibody #19 (available from Cusom Monoclonals International), both contained in PBSBT. The plate was incubated for 1 hour at room temperature. The plate was then washed, contacted with about 0.25 μg/ml streptavidin conjugated to horseradish peroxidase, washed again, and the presence of streptavidin bound to the plate was detected using the method described in Example 4. The optical density readings
obtained for the control samples were used to generate a standard curve that was used to determine the total IgE bound to wells that had received test samples.

The results shown in Fig. 3 indicate that canine IgE from a variety of dog sera are detected using the alpha chain of human Fc_eR in a manner similar to using an antibody that binds specifically to canine IgE. The absence of detectable amounts of IgE in the heat treated sample (Sample 7) indicates that the antibody detected by PhFc_eRα_{172}-BIOT is IgE. In addition, the results indicate that PhFc_eRα_{172}-BIOT is an effective reagent for detecting IgE that binds to allergen Kentucky Blue Grass, Samples 5 and 6), as well as a parasite antigen (D. immitis, Sample 2).

Example 7.

This example describes detection of canine IgE in dog sera isolated from dogs known to be allergic to flea saliva, using PhFc_eRα_{172}-BIOT.

Multiple wells of an Immulon II microtiter plate were coated with about 100 μl/well of varying concentrations of flea saliva recombinant protein fspN (described in PCT Patent Publication No. WO 96/11271, ibid.; concentrations shown in Fig. 4) diluted in CBC buffer. The plate was incubated overnight at 4°C. The plate was then blocked and washed as described in Example 4. About 100 μl/well of a 1:10 dilution in PBSBT of a pool of sera isolated from dogs known to produce IgE that binds specifically to flea saliva. Some wells did not receive dog sera so that background binding levels could be determined. The plate was incubated for 1 hour at room temperature and then washed four times with PBST. About 100 μl/well of a 1:4000 dilution of 40 μg/ml PhFc_eRα_{172}-BIOT (described in Example 3) contained in PBSBT was added. The plate was incubated for 1 hour at room temperature. The plate was then washed, contacted with about 0.25 μg/ml streptavidin-conjugated to horseradish peroxidase, washed again, and the presence of streptavidin bound to the plate was detected using the method described in Example 4.

The results shown in Fig. 4 indicate that canine IgE that binds specifically to a flea saliva antigen is detected using the alpha chain of human Fc_eR.
Example 8.

This example describes detection of total canine IgE in dog sera isolated from dogs known to be allergic to flea saliva, heartworm-infected dogs and specific pathogen free (SPF) dogs, using PhF_{a}Rα_{172}-BIOT.

Multiple wells of an Immulon II microtiter plate were coated with about 100 μl/well of about 1 μg/ml CMI anti-canine IgE antibody #6 (available from Custom Monoclonals International) in CBC buffer. The plate was incubated overnight at 4°C. The plate was blocked and washed as described in Example 4. About 100 μl/well of different samples of IgE-containing fluids in PBSBT were added to multiple wells coated with the anti-canine IgE antibody. The samples included: (1) 100 μg/ml of canine IgE purified from the heterohybridoma described in Example 4; (2) a 1:10 dilution of a pool of sera from dogs known to be allergic to flea saliva, (3) a 1:10 dilution of the same sera pool as in (2) but heat inactivated; (4) a 1:10 dilution of serum from a dog known to have clinical flea allergy dermatitis (dog CPO2); (5) a 1:10 dilution of heat inactivated CPO2 serum; (6) a 1:10 dilution of serum from a heartworm-infected dog (dog 417); (7) a 1:10 dilution of heat inactivated 417 serum; (8) a 1:10 dilution of a pool of sera from heartworm-infected dogs; (9) a 1:10 dilution of the same sera pool as in (8) but heat inactivated; and (10) a pool of sera from dogs raised in a barrier facility. Each sample was diluted in PBSBT. The plate was incubated for 1 hour at room temperature and then washed four times with PBST. About 100 μl/well of a 1:4000 dilution of 40 μg/ml PhF_{a}Rα_{172}-BIOT (described in Example 3) in PBSBT was added. The plate was incubated for 1 hour at room temperature. The plate was then washed, contacted with about 0.25 μg/ml streptavidin-conjugated to horseradish peroxidase, washed again, and the presence of streptavidin bound to the plate was detected using the method described in Example 4.

The results shown in Fig. 5 indicate that canine IgE from dogs allergic to flea saliva and from dogs infected with heartworm are detected using the alpha chain of human Fc_{a}R. In addition, the absence of colorimetric signal in samples of heat inactivated sera indicates that antibody bound to the anti-IgE antibody and detected by Fc_{a}R alpha chain is an epsilon isotype antibody and not another isotype.
Example 9.

This example describes detection of IgE that specifically binds to flea saliva, using PhFc_e\alpha_{172}-BIOT.

Multiple wells of an Immulon II microtiter plate were coated with about 100 µl/well of about 0.1 µg/ml of flea saliva collected using the method described in PCT Patent Publication No. WO 96/11271, *ibid.*, in CBC buffer. The plate was incubated, blocked and washed as described in Example 4. The IgE-containing samples described in Example 8 were then applied to the flea saliva coated plate. The plate was then treated using the method described in Example 8.

The results shown in Fig. 6 indicate that canine IgE that binds specifically to flea saliva, contained in serum, is detected using the alpha chain of human Fc.R. In addition, the absence of colorimetric signal in samples of heat inactivated serum indicates that antibody bound to the flea saliva protein and detected by Fc.R alpha chain is an epsilon isotype antibody.

Example 10.

This example describes the detection of feline IgE using PhFc_e\alpha_{172}-BIOT.

Multiple wells of an Immulon II microtiter plate were coated with about 100 µl/well of about 10 µg/ml Di33 protein (described in U.S. Patent Application Serial No. 08/715,628, *ibid.*) or 10 µg/ml crude homogenate of heartworm, both in CBC buffer.

Crude homogenate of heartworm is the clarified supernatant of adult heartworms homogenized in PBS. The plate was incubated overnight at 4°C. The plate was blocked and washed as described in Example 4. Serum samples from 2 heartworm infected cats were then added to Di33-coated wells and to heartworm antigen-coated wells. About 100 µl/well of a 1:10 dilution in PBSBT of sera from heartworm-infected cat # AXH3 or from cat #MGC2 were added to the plate. Negative control samples consisting of serum from pre-infection bleeds of cat #AXH3 and cat# MGC2 were also added to the plate at a dilution of 1:10 in PBSBT. A positive control sample consisting of a pool of sera from heartworm-infected dogs was also added to the plate at a dilution of 1:10 in PBSBT.

The plate was incubated for 1 hour at room temperature and then washed four times with PBST. About 100 µl/well of a 1:4000 dilution of 40 µg/ml PhFc_e\alpha_{172}-BIOT (described in Example 3) in PBSBT was added. The plate was incubated for 1 hour at room
temperature. The plate was then washed, contacted with 1:4000 dilution of a 0.5 mg/ml solution of streptavidin-conjugated to horseradish peroxidase, washed again, and the presence of streptavidin bound to the plate was detected using the method described in Example 4.

The results shown in Fig. 7 indicate that feline IgE that binds specifically to crude homogenate of heartworm or Di33 protein is detected using the alpha chain of human Fc, R.

**Example 11.**

This example describes detection of feline IgE using PhFc,Rα172-BIOT.

Multiple wells of an Immulon II microtiter plate were coated with Di33 as described in Example 10, in CBC buffer. The plate was incubated overnight at 4°C. The plate was blocked and washed as described in Example 4. Serum samples from 2 heartworm infected cats were then added to Di33-coated wells. About 100 µl/well of a 1:10 dilution in PBSBT of serum from heartworm-infected cat # MGC2 and a pool of sera from heartworm-infected cats, as well as heat inactivated samples of each of these sera, were added to the plate. A positive control sample consisting of a pool of sera from heartworm-infected dogs was also added to the plate at a dilution of 1:10 in PBSBT. The plate was incubated for 1 hour at room temperature and then washed four times with PBST. About 100 µl/well of a 1:4000 dilution of 40 µg/ml PhFc,Rα172-BIOT (described in Example 3) in PBSBT was added. The plate was incubated for 1 hour at room temperature. The plate was then washed, contacted with streptavidin-conjugated to horseradish peroxidase, washed again, and the presence of streptavidin bound to the plate was detected using the method described in Example 4.

The results shown in Fig. 8 indicate that feline IgE from heartworm-infected cats that specifically binds to the heartworm antigen Di33 is detected using the alpha chain of human Fc,R. In addition, the absence of colorimetric signal in samples of heat inactivated sera indicates that antibody bound to the Di33 protein and detected by Fc,R alpha chain is an epsilon isotype antibody.

**Example 12**

This example describes detection of equine IgE in a solid-phase ELISA using PhFc,Rα172-BIOT.
Horse sera from a horse known to be allergic to certain allergens and horse sera from a horse known not to be allergic the same allergens, were assayed for the presence of IgE using PhFc\textsubscript{R}a\textsubscript{172}-BIOT as follows. A North Atlantic/Ohio Valley Regional Panel plate of a Canitec\textsuperscript{TM} Allergen-Specific IgE Kit (available from BioProducts DVM) was blocked and washed as described in Example 4. Two samples of about 1:10 dilutions of the two horse sera were prepared using PBSBT. The two samples were added to the blocked plate and the plate was incubated for 1 hour at room temperature. The plate was washed as described in Example 4. About 100 \mu l/well of a 1:4000 dilution of 40 \mu g/ml PhFc\textsubscript{R}a\textsubscript{172}-BIOT (described in Example 3), contained in PBSBT was added to each well. The plate was then washed, contacted with 1:4000 dilution of a 0.5 mg/ml solution of streptavidin-conjugated to horseradish peroxidase, washed again, and the presence of streptavidin bound to the plate was detected using the method described in Example 4.

The results shown in Fig. 9 indicate that equine IgE from a horse known to be allergic to certain allergens specifically binds to certain plant and mite allergens is detected using the alpha chain of human Fc\textsubscript{R}.

Example 13

This example describes detection of canine IgE in a solid-phase ELISA using basophilic cells transfected with human Fc\textsubscript{R} alpha chain.

Rat basophilic leukemia (RBL) cells transfected with a nucleic acid molecule encoding a human Fc\textsubscript{R} alpha chain (referred to herein as RBL-hFc\textsubscript{R} cells; described in Miller et al., Science 244:334-337, 1989) were used to detect canine IgE as follows. About 4 x 10\textsuperscript{4} RBL-hFc\textsubscript{R} cells contained in Earles Modified Eagles Medium containing 10% fetal bovine serum (EMEM-FBS) were added to each well of 96-well flat bottom tissue culture plates. The RBL-hFc\textsubscript{R} cells were incubated overnight at 37\textdegree C.

Following the incubation the plates were washed 4 times with PBST. The cells were then fixed for about 2 minutes using about 200 \mu l per well of absolute alcohol at room temperature. The plates were then washed 8 times with PBST to remove residual alcohol.

Serial dilutions in EMEM-FBS (concentrations shown in Fig. 10) were prepared using a pool of sera from dogs infected with heartworm. Serial dilutions in EMEM-FBS
(concentrations shown in Fig. 11) were prepared using a pool of sera from dogs sensitized to flea saliva. Additional samples were prepared in which both pools of sera were heat inactivated for about 4 hours at 56°C. The heat treated samples were diluted as described above.

About 100 μl of each dilution of each serum sample was added to separate wells containing fixed RBL-hFcR cells and the plates were incubated at 37°C for about 1 hour. Following the incubation, the plates were washed 4 times with PBST. About 5 μg of a murine IgG monoclonal antibody anti-canine IgE antibody (i.e., Custom Monoclonal Antibody #71; available from Custom Monoclonal International) in 100 μl of EMEM-FBS was added to each well. The plates were incubated for about 30 minutes at 37°C. Following the incubation, the plates were washed 4 times with PBST. About 100 ng of horseradish peroxidase labelled donkey anti-murine IgG (available from Jackson Laboratories, Westgrove, PA) in 100 μl of EMEM-FBS was added to each well, and the plates were incubated for about 30 minutes at room temperature. Following the incubation, the plates were washed 4 times with PBST. The presence of anti-murine IgG bound to the plates thereby indicating the ability of RBL-hFcR cells to bind to canine IgE was detected using the method described in Example 4.

The results shown in Fig. 10 indicate that canine IgE from heartworm-infected dogs (♦) is detected using RBL-h FcR cells expressing the alpha chain of human FcR. In addition, the absence of colorimetric signal in samples of heat inactivated samples of such sera (■) indicates that antibody detected by the FcR alpha chain on the RBL-h FcR cells is an epsilon isotype antibody. Similarly, the results shown in Fig. 11 indicate that canine IgE from dogs sensitized with flea saliva (♦) is detected using RBL-h FcR cells expressing the alpha chain of human FcR. In addition, the absence of colorimetric signal in samples of heat inactivated samples of such sera (■) indicates that antibody detected by the FcR alpha chain on the RBL-h FcR cells is an epsilon isotype antibody.
SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
   (A) NAME: Heska Corporation
   (B) STREET: 1825 Sharp Point Drive
   (C) CITY: Fort Collins
   (D) STATE: CO
   (E) COUNTRY: US
   (F) POSTAL CODE (ZIP): 80525
   (G) TELEPHONE: (970) 493-7272
   (H) TELEFAX: (970) 484-9505

(ii) TITLE OF INVENTION: METHOD TO DETECT IGE

(iii) NUMBER OF SEQUENCES: 13

(iv) CORRESPONDENCE ADDRESS:
   (A) ADDRESSEE: LAHIVE & COCKFIELD, LLP
   (B) STREET: 28 STATE STREET
   (C) CITY: BOSTON
   (D) STATE: MA
   (E) COUNTRY: US
   (F) ZIP: 02109

(v) COMPUTER READABLE FORM:
   (A) MEDIUM TYPE: Floppy disk
   (B) COMPUTER: IBM PC compatible
   (C) OPERATING SYSTEM: Windows 95
   (D) SOFTWARE: ASCII DOS TEXT

(vi) CURRENT APPLICATION DATA:
   (A) APPLICATION NUMBER: 08/756,387
   (B) FILING DATE: November 26, 1996
   (C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
   (A) APPLICATION NUMBER:
   (B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:
   (A) NAME: Rothenberger, Scott D.
   (B) REGISTRATION NUMBER: 41,277
   (C) REFERENCE/DOCKET NUMBER:

(ix) TELECOMMUNICATION INFORMATION:
   (A) TELEPHONE: (617) 227-7400
   (B) TELEFAX: (617) 742-4214

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 1198 nucleotides
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) FEATURE:
   (A) NAME/KEY: CDS
   (B) LOCATION: 107..877
<table>
<thead>
<tr>
<th>Met</th>
<th>Ala</th>
<th>Pro</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>15</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ala</th>
<th>Met</th>
<th>Glu</th>
<th>Ser</th>
<th>Pro</th>
<th>Thr</th>
<th>Leu</th>
<th>Leu</th>
<th>Leu</th>
<th>Leu</th>
<th>Phe</th>
<th>Phe</th>
<th>Ala</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>10</td>
<td>5</td>
<td>25</td>
<td>30</td>
<td>35</td>
<td>40</td>
<td>45</td>
<td>50</td>
<td>55</td>
<td>60</td>
<td>65</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pro</th>
<th>Asp</th>
<th>Gly</th>
<th>Val</th>
<th>Ala</th>
<th>Val</th>
<th>Pro</th>
<th>Gln</th>
<th>Lys</th>
<th>Pro</th>
<th>Lys</th>
<th>Val</th>
<th>Ser</th>
<th>Leu</th>
<th>Asn</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>25</td>
<td>5</td>
<td>55</td>
<td>70</td>
<td>75</td>
<td>80</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cys</th>
<th>Thr</th>
<th>Asn</th>
<th>Ser</th>
<th>Leu</th>
<th>Gly</th>
<th>Val</th>
<th>Glu</th>
<th>Thr</th>
<th>Asn</th>
<th>Ser</th>
<th>Leu</th>
<th>Asn</th>
<th>Ile</th>
<th>Val</th>
<th>Asn</th>
</tr>
</thead>
<tbody>
<tr>
<td>85</td>
<td>90</td>
<td>95</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lys</th>
<th>Phe</th>
<th>Glu</th>
<th>Asp</th>
<th>Ser</th>
<th>Gly</th>
<th>Glu</th>
<th>Tyr</th>
<th>Lys</th>
<th>Cys</th>
<th>Gln</th>
<th>His</th>
<th>Gln</th>
<th>Gln</th>
<th>Val</th>
<th>Asn</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>105</td>
<td>110</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cys</th>
<th>His</th>
<th>Gly</th>
<th>Trp</th>
<th>Arg</th>
<th>Asn</th>
<th>Trp</th>
<th>Asp</th>
<th>Val</th>
<th>Tyr</th>
<th>Lys</th>
<th>Val</th>
<th>Ile</th>
<th>Tyr</th>
<th>Tyr</th>
<th>Lys</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>135</td>
<td>140</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Asp</th>
<th>Gly</th>
<th>Glu</th>
<th>Ala</th>
<th>Leu</th>
<th>Lys</th>
<th>Tyr</th>
<th>Trp</th>
<th>Tyr</th>
<th>Glu</th>
<th>Asn</th>
<th>His</th>
<th>Asn</th>
<th>Ile</th>
<th>Ser</th>
<th>Ile</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>150</td>
<td>155</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Thr</th>
<th>Asn</th>
<th>Ala</th>
<th>Thr</th>
<th>Val</th>
<th>Glu</th>
<th>Asp</th>
<th>Ser</th>
<th>Gly</th>
<th>Thr</th>
<th>Tyr</th>
<th>Tyr</th>
<th>Cys</th>
<th>Thr</th>
<th>Gly</th>
<th>Lys</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>165</td>
<td>170</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gly</th>
<th>Trp</th>
<th>Gln</th>
<th>Leu</th>
<th>Asp</th>
<th>Tyr</th>
<th>Ser</th>
<th>Glu</th>
<th>Pro</th>
<th>Leu</th>
<th>Asn</th>
<th>Thr</th>
<th>Val</th>
<th>180</th>
</tr>
</thead>
<tbody>
<tr>
<td>45</td>
<td>190</td>
<td>195</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lys</th>
<th>Ala</th>
<th>Pro</th>
<th>Arg</th>
<th>Glu</th>
<th>Lys</th>
<th>Tyr</th>
<th>Trp</th>
<th>Leu</th>
<th>Gln</th>
<th>Phe</th>
<th>Phe</th>
<th>Ile</th>
<th>Pro</th>
<th>Leu</th>
<th>Leu</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>200</td>
<td>205</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Thr</th>
<th>Arg</th>
<th>Lys</th>
<th>Gly</th>
<th>Phe</th>
</tr>
</thead>
<tbody>
<tr>
<td>220</td>
<td>225</td>
<td>230</td>
<td>235</td>
<td>240</td>
</tr>
</tbody>
</table>
### INFORMATION FOR SEQ ID NO:2:

**SEQUENCE CHARACTERISTICS:**

- **LENGTH:** 257 amino acids
- **TYPE:** amino acid
- **TOPOLOGY:** linear

**MOLECULE TYPE:** protein

**SEQUENCE DESCRIPTION:**

<table>
<thead>
<tr>
<th>Position</th>
<th>Amino Acid(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Met Ala Pro Ala Met Glu Ser Pro Thr Leu Leu Cys Val Ala Leu Leu</td>
</tr>
<tr>
<td>20</td>
<td>Phe Phe Ala Pro Asp Gly Val Leu Ala Val Pro Gln Lys Pro Lys Val</td>
</tr>
<tr>
<td>30</td>
<td>Ser Leu Asn Pro Pro Trp Asn Arg Ile Phe Lys Gly Glu Asn Val Thr</td>
</tr>
<tr>
<td>40</td>
<td>Leu Thr Cys Asn Gly Asn Asn Phe Phe Glu Val Ser Ser Thr Lys Trp</td>
</tr>
<tr>
<td>50</td>
<td>Phe His Asn Gly Ser Leu Ser Glu Glu Thr Asn Ser Ser Leu Asn Ile</td>
</tr>
<tr>
<td>60</td>
<td>Val Asn Ala Lys Phe Glu Asp Ser Gly Glu Tyr Lys Cys Gln His Gln</td>
</tr>
<tr>
<td>70</td>
<td>Gln Val Asn Glu Ser Glu Pro Val Tyr Leu Glu Val Phe Ser Asp Trp</td>
</tr>
<tr>
<td>80</td>
<td>Leu Leu Leu Gln Ala Ser Ala Glu Val Val Met Gly Glu Gln Pro Leu</td>
</tr>
<tr>
<td>90</td>
<td>Phe Leu Arg Cys His Gly Trp Arg Asn Trp Asp Val Tyr Lys Val Ile</td>
</tr>
<tr>
<td>100</td>
<td>Tyr Tyr Lys Asp Gly Glu Ala Leu Lys Tyr Trp Tyr Glu Asn His Asn</td>
</tr>
<tr>
<td>110</td>
<td>Ile Ser Ile Thr Asn Ala Thr Val Glu Asp Ser Gly Thr Tyr Tyr Cys</td>
</tr>
<tr>
<td>120</td>
<td>Thr Gly Lys Val Trp Gln Leu Asp Tyr Glu Ser Glu Pro Leu Asn Ile</td>
</tr>
<tr>
<td>130</td>
<td>Thr Val Ile Lys Ala Pro Arg Glu Lys Tyr Trp Leu Gln Phe Phe Ile</td>
</tr>
<tr>
<td>140</td>
<td>Pro Leu Leu Val Ile Phe Ala Val Asp Thr Gly Leu Phe Ile</td>
</tr>
<tr>
<td>150</td>
<td>Ser Thr Gln Glu Gln Val Thr Phe Leu Leu Lys Ile Lys Arg Thr Arg</td>
</tr>
</tbody>
</table>

**Sequence:**

```
AGA CTT CTG AAC CCA CAT CTC AAG CCA AAC CCC AAA AAC AAC TGA
Arg Leu Leu Asn Pro His Pro Lys Pro Asn Pro Lys Asn Asn

TATAATTACT CAAGAAATAT TGCCACACATT AGTTTTTTTC CAGCATCAGC AATTGCTACT

CAATTGTCAA ACACACCTTG CAATTATCAT AGAAACGCTT GTGCTCAAGG ATTTATGAA

ATGCCATATT AAACCTGAGT AAACCTGTTA AGTGGCAGTG AAATAGTAAGT GCTCAATTAA

CATTGGTGCA ATAAATGAGA GAATGAAGAT ATCCATTAT TAGCATTGT AAAAGAGATG

TTCAATTTCA ATAAAAATTA TATAAAAACCA TGTTACAGAA TGCTTCTGAG TAAAA AAAA

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 257 amino acids
   (B) TYPE: amino acid
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ala Pro Ala Met Glu Ser Pro Thr Leu Leu Cys Val Ala Leu Leu

Phe Phe Ala Pro Asp Gly Val Leu Ala Val Pro Gln Lys Pro Lys Val

Ser Leu Asn Pro Pro Trp Asn Arg Ile Phe Lys Gly Glu Asn Val Thr

Leu Thr Cys Asn Gly Asn Asn Phe Phe Glu Val Ser Ser Thr Lys Trp

Phe His Asn Gly Ser Leu Ser Glu Glu Thr Asn Ser Ser Leu Asn Ile

Val Asn Ala Lys Phe Glu Asp Ser Gly Glu Tyr Lys Cys Gln His Gln

Gln Val Asn Glu Ser Glu Pro Val Tyr Leu Glu Val Phe Ser Asp Trp

Leu Leu Leu Gln Ala Ser Ala Glu Val Val Met Gly Glu Gln Pro Leu

Phe Leu Arg Cys His Gly Trp Arg Asn Trp Asp Val Tyr Lys Val Ile

Tyr Tyr Lys Asp Gly Glu Ala Leu Lys Tyr Trp Tyr Glu Asn His Asn

Ile Ser Ile Thr Asn Ala Thr Val Glu Asp Ser Gly Thr Tyr Tyr Cys

Thr Gly Lys Val Trp Gln Leu Asp Tyr Glu Ser Glu Pro Leu Asn Ile

Thr Val Ile Lys Ala Pro Arg Glu Lys Tyr Trp Leu Gln Phe Phe Ile

Pro Leu Leu Val Ile Phe Ala Val Asp Thr Gly Leu Phe Ile

Ser Thr Gln Glu Gln Val Thr Phe Leu Leu Lys Ile Lys Arg Thr Arg
```
Lys Gly Phe Arg Leu Leu Leu 245
Asn Pro His Pro Lys Pro Asn Pro Lys 250
Asn

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1198 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TTTTTTTTTT TTTTTTTTT TTTTTTTACT CAGAGCCATT CTGGTACATG GTTTATATT 60
TATTTTAGGG AATTTGACA CTCCTTTTAC AATGCATAAT AATGAACTT ATCTATTCTC 120
TCTATTATTC ACCAATTTG GATGAGCAG CCTCTTTTTA ATGTTTTTGA TATATACCA 180
CTCAGTTTAA TGAAGCATT CTATTTAATG TGGAGCAAG AGGTCTTCTAT ATGATTACCA 240
AGCTTTGATT GCAATTGAG TACAAATGCA GTATGCTAG AAAAACATTA TGTTGCAAT 300
ATTTCCGAG AATAAATGAG AGTTGTGTTT GGGGTGTGGC TTAGATGCT GGTTCAGAG 360
TCTCAAGGCT TCTCTGTGGC TCTTAATGTC CAAGAGAAA CAGCTGCTGCT GTCTGAGTG 420
GATAAATAT CTTGTCGCCA CAGCAAACAG AATCACCACC AACAATTGGG TAAAAATGG 480
20
TAGACCTGAC TCTCTCAGGG GAGCTTTAT TACAGATAAT TCTAGGGCGT CAGCTACATA 540
GTCACGCTGC CACACTTGG CCGTACAGA GTAGGCCTCA CTGTTCTCAA CTGTTGCAAT 600
TGGTAACTGAG ATGCTTGGTT TCTCTACCCA GTATGAGAG GCTTCACAT CTTATAATG 660
GATCAGCCTG TACACATCCC AGGTCTTCTCA ACATGACACC GTAGAGAAG GGGCTCGGCC 720
25
CTCAGCTACC ATCACTGAG AGGCGCTGAG GACAGCCGCA TACTGACAA GCCCTACGTA 780
CCAGCCTTCA CTCCTCATTAA CTTTTGATTG TGCACTATTT TCTTCCCACA TGTTTTCAAA 840
TTGCTGCATC ACCAATTTCA AACTGGAATT TGTGCTCTCT GAAGGGCTCG TATGTTGGA 900
CCATTTGATG GAACTGACCT AAAAGAAATT GTTCCCACATT AATGAAAGAG TCACATCCAT 960
TCTCTTAAAT ATCTTATTTCA ATGGAGGGTTT CAAGAGACC TTAGTCTTCC GAGGACTGCG 1020
TAACAGCCCA TCTGGACGGA AGAACAAGTAA GGCTACACAC AGTAGAGTAG GGGATCTCAT 1080
GCGAGGAGCC ATCTCTTCCA TGAGACTCTG TGCTTACTCG TGCTGGAGAG ATCTAAGGCT 1140
TCAATATAG GCCCATGCTC GGTGTTAGAC AGGTGGAGGA TGCTGTGAGAC TCTTATAG 1198

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 774 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..774

(iv) SEQUENCE DESCRIPTION: SEQ ID NO:4:

ATG GCT CCT GCC ATG GAA TCC CCT ACT CTA CTG GTA GCC TTA CTG Met Ala Pro Ala Met Glu Ser Pro Thr Leu Leu 48
1 5

10

45

20

50

TTC TTC GCT CCT ACA GAT GCC GTG TTA GCA GTC CCT CAG AAA CCT AAG GTC Phe Phe Ala Pro Asp Gly Val Leu Ala Val Pro Gln Lys Pro Lys Val 96
25

30

144

TCC TTG AAC CCT CCA TGG AAT AGA ATA TTT AAA GGA GAG AAT GTG ACT Ser Leu Asn Pro Pro Trp Asn Arg Ile Phe Lys Gly Glu Asn Val Thr 144
35

40

45
CTT ACA TGT AAT GGG AAC AAT TCT TTT GAA GTC AGT TCC ACC AAA TGG
Leu Thr Cys Asn Gly Asn Asn Phe Phe Glu Val Ser Ser Thr Lys Trp
50 55
TTC CAC AAT GCC AGC CTT GCA GAG ACA AAT TCA AGT TGG AAT ATT
Phe His Asn Gly Ser Leu Ser Glu Glu Thr Asn Ser Ser Leu Asn Ile
65 70 75 80
GTG AAT GCC AAA TTT GAA GAC AGT GGA GAA TAC AAA TGT CAG CAC CAA
Val Asn Ala Lys Phe Glu Asp Ser Gly Glu Tyr Lys Cys Gln His Gin
85 90 95
CAA GGT AAT GAG AGT GAA CCT GTG TAC CTG GAA GTC TTC AGT GCC
Gln Val Asn Glu Ser Glu Pro Val Tyr Leu Glu Val Phe Ser Asp Trp
100 105 110
CTG CTC CTT CAG GCC TCT GCT GAG GTG TGT AGT GAG GCC CAG CCC CTC
Leu Leu Leu Gin Ala Ser Ala Glu Val Val Met Glu Gly Gin Pro Leu
115 120 125
TTC CTC AGG TGC CAT GAT TGG AGG AAC TGG GAT TCG TAC AAG GTG ATC
Phe Leu Arg Cys His Gly Trp Arg Asn Trp Asp Val Tyr Lys Val Ile
130 135 140
TAT TAT AAG GAT GGG ACA CTT GCA GCT CAC AAG TAC TGG TAT GAG AAC CAC AAC
Tyr Tyr Lys Asp Gly Glu Ala Leu Tyr Trp Tyr Glu Asn His Asn
145 150 155 160
ATC TCC ATT ACA AAT GCC ACA GTT GAA GAC AGT GGA ACC TAC TAC TGT
Ile Ser Ile Thr Asn Ala Thr Val Glu Asp Ser Gly Thr Tyr Tyr Cys
165 170 175
ACG GGC AAA GTG TGG CAG CTG GAC TAT GAG TCT GAG CCC CTC AAC ATT
Thr Gly Lys Val Trp Gin Leu Asp Tyr Gly Ser Glu Pro Leu Asn Ile
180 185 190
ACT GTA ATA AAA GCT CGT GAG AAG TAC TGG CTA CAA TTT TTT ATC
Thr Val Ile Lys Ala Pro Arg Glu Tyr Lys Trp Leu Gin Phe Phe Ile
195 200 205
CCA TTG TGG GTG ATT CTG TTT GCT GTG GAC ACA GGA TTA TTT ATC
Pro Leu Val Leu Val Ile Leu Phe Ala Val Asp Thr Gly Leu Phe Ile
210 215 220
TCA ACT CAG CAG CAG GTC TAT GAC AGT TGG AAG ATT AGA AGA ACC AGG
Ser Thr Gin Gin Gin Val Thr Phe Leu Leu Lys Ile Lys Arg Thr Arg
225 230 235 240
AAA GGC TTC AGA CTG CAG CCA CAT CCT AAG CCA AAC CCC AAA AAC
Lys Gly Phe Arg Leu Leu Asn Pro His Pro Lys Pro Asn Pro Lys Gin
245 250 255

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 774 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
SEQUENCE DESCRIPTION: SEQ ID NO:5:

TCAGTGTGTT TTGGGGTTTG GCTTAGGAGT TGTTCTCAGA AGTCTGAAGC CTTCCTTTGC
TCTCTTAATC TTCAAGAGAA ATGTGACTTG CTGCTGAGTT GAGATAAAAT TGGTACAGT
CACAGCAAAA AGAATCACA CCAACAATGG GATAAAAAAT TGTAGGCCAT ACTTCTCAAG
CGAGCCTTTT ATTCACGTA TGGTGAGGGG CTCAAGCTCA TAGTCCAGCT GCGACACTTT
GCCGCTACAG TAGTGTTTCC ACTGCTTCAC AACTTGGAAC TTGGTAATGG AGATGGTGGG
GTTGTCATCT CAGTACTTTG GAGCCTTCAG ATCTCTTATAA TAGATCCACT GTGTCACATC
CCAGCTTCCC CAACATGGCC AACTGAGGAA GAGGGGCTGG CCGTTCACTCA CCACCTCAGC
ACGGCCTTTGA AGGGACGAGG AGYCTGAGA GACTTCAGGG TACACAGATT CACTTCTATT
AACCTTGAGG TGGTGACATT TGGATTTCTCC ACTTGCTTCA AATTTGGCAT TCACAATATT
CAAACCTGAA TTTGTCTCTT CGAAAGGCT GCCATGGTGG AACACTTGGG TGGAACTGAC
TTCAAGAGAA TTTGTTTTTT CATCTGTAAG AGTCACATT TCATTTTAA ATATTCTATT
CCATGGAGGC TTCAAGAGGA CCGTAGATTT CGTGGGAGCT GCTAACACGC CAGTCGGACC
GAAGAACAGT AAGGCTACAC AGCAGAGACT AGGGATTTCC ATGGGACGC CCAT

INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 232 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Val Pro Gln Lys Pro Lys Val Ser Leu Asn Pro Pro Trp Asn Arg Ile
1  5 10 15
Phe Lys Gly Glu Asn Val Thr Leu Thr Cys Asn Gly Asn Asn Phe Phe
20 25 30
Glu Val Ser Ser Thr Lys Trp Phe His Asn Gly Ser Leu Ser Glu Glu
35 40 45
Thr Asn Ser Ser Leu Asn Ile Val Asn Ala Lys Phe Glu Asp Ser Gly
50 55 60
Glu Tyr Lys Cys Gln His Glu Gln Glu Val Glu Ser Glu Pro Val Tyr
65 70 75 80
Leu Glu Phe Ser Asp Trp Leu Leu Gln Ala Ser Ala Glu Val
85 90 95
Val Met Glu Gly Glu Pro Leu Phe Leu Arg Cys His Gly Trp Arg Asn
100 105 110
Trp Asp Val Tyr Lys Val Ile Tyr Tyr Lys Asp Gly Glu Ala Leu Lys
115 120 125
Tyr Tyr Glu Asn His Asn Ile Ser Ile Thr Asn Ala Thr Val Glu
130 135 140
Asp Ser Gly Thr Tyr Tyr Cys Thr Gly Lys Val Trp Gln Leu Asp Tyr
145 150 155 160
Glu Ser Glu Pro Leu Asn Ile Thr Val Ile Lys Ala Pro Arg Glu Lys
165 170 175
Tyr Trp Leu Glu Phe Phe Ile Pro Leu Leu Val Val Ile Leu Phe Ala
180 185 190
Val Asp Thr Gly Leu Phe Ile Ser Thr Gln Gln Glu Val Thr Phe Leeu
195 200 205
Leu  Lys  Ile  Lys  Arg  Thr  Arg  Lys  Gly  Phe  Arg  Leu  Leu  Asn  Pro  His
   210                        215

Pro  Lys  Pro  Asn  Pro  Lys  Asn  Asn
   225                        230

---

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 699 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..699

(iii) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GTC  CCT  CAG  AAA  CCT  AAG  GTC  TCC  TGG  AAC  CCT  CCA  TGG  AAT  AGA  ATA
Val  Pro  Gln  Lys  Pro  Lys  Val  Ser  Leu  Asn  Pro  Pro  Trp  Asn  Arg  Ile
   1                         5                      10                        15

TTT  AAA  GGA  GAG  AAT  GTG  ACT  CTT  ACA  TGT  AAT  GGG  AAC  AAT  TTC  TTT
Lys  Gly  Glu  Asn  Val  Thr  Leu  Thr  Cys  Phe  Asn  Gly  Asn  Phe  Phe
   20                        25                   30

GAA  GTC  AGT  TCC  ACC  AAA  TGG  TTC  CAC  AAT  GCC  AGC  CTT  TCA  GAA  GAG
Glu  Val  Ser  Ser  Thr  Lys  Trp  Phe  His  Asn  Gly  Ser  Leu  Ser  Leu  Glu
   35                        40                   45

ACA  AAT  TCA  AGT  TTG  AAT  ATT  GTG  AAT  GCC  AAA  TTT  GAA  GAC  AGT  GGA
Thr  Asn  Ser  Ser  Leu  Asn  Ile  Val  Asn  Ala  Lys  Phe  Glu  Asp  Ser  Gly
   50                        55                   60

GAA  TAC  AAA  TGT  CAG  CAC  CA  CAA  GAA  GAT  GAG  ACT  GAA  CCT  GTG  TAC
Glu  Tyr  Lys  Cys  Gln  His  Gln  Gln  Val  Asn  Glu  Ser  Glu  Pro  Val  Tyr
   65                        70                   75                   80

CTG  GAA  GTC  TTC  AGT  GAC  TGG  CTG  CTC  CTT  CAG  GCC  TCT  GCT  GAG  GTG
Leu  Glu  Val  Phe  Asp  Trp  Leu  Leu  Leu  Gln  Ala  Ser  Ala  Glu  Val
   85                        90                   95

GTG  ATG  GAG  GCC  CAG  CCC  CTC  TCC  CTC  AGG  TGC  CAT  GGT  TGG  AGG  AAC
Val  Met  Glu  Gly  Gln  Pro  Leu  Arg  Cys  His  Gly  Trp  Arg  Asn
  100                       105                   110

TGG  GAT  GTG  TAC  AAG  GTG  ATC  TAT  TAT  AAG  GAT  GGT  GAA  GCT  CTC  AAG
Trp  Asp  Val  Tyr  Lys  Val  Ile  Tyr  Tyr  Lys  Asp  Gly  Glu  Ala  Leu  Lys
  115                       120                  125

TAC  TGG  TAT  GAG  AAC  CAC  AAC  ATC  TCC  ATT  ACA  AAT  GGC  ACA  GTT  GAA
Tyr  Trp  Tyr  Glu  Asn  His  Asn  Ile  Ser  Ile  Thr  Asn  Ala  Thr  Val  Glu
  130                       135                  140

GAC  AGT  GGA  ACC  TAC  TAC  TGG  GCC  AAA  GTC  TGG  CAG  CTG  GAC  TAT
Asp  Ser  Gly  Thr  Tyr  Tyr  Cys  Thr  Gly  Lys  Val  Trp  Glu  Leu  Asp  Tyr
  145                       150                  155                   160

GAG  TCT  GAG  CCC  CTC  AAC  ATT  ACT  GTA  ATA  AAA  GCT  CCG  GTG  GAG  AAG
Glu  Ser  Glu  Pro  Leu  Asn  Ile  Thr  Val  Ile  Lys  Ala  Pro  Arg  Glu  Lys
  165                       170                  175
(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: primer

(iii) SEQUENCE DESCRIPTION: SEQ ID NO:8:
CGCCGATCCT ATAAATATGG CTCCGCACAT GG

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: primer

(iii) SEQUENCE DESCRIPTION: SEQ ID NO:9:
GCCGAATTCT TAAGCCTTTA TTACAG

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 591 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..591

(iv) SEQUENCE DESCRIPTION: SEQ ID NO:10:
ATG GCT CCT GCC ATG GAA TCC CCT ACT CTA CTG TGT GTA GCC TTA CTG
Met Ala Pro Ala Met Glu Ser Pro Thr Leu Leu Cys Val Ala Leu Leu
1 5 10 15

ATTAC TGG CTA CAA TTT TTT ATC CCA TGG TGG GTG ATG CTG TTT GCT
Tyr Trp Leu Gln Phe Phe Ile Pro Leu Leu Val Val Ile Leu Phe Ala
180 185 190

GTG GAC ACA GGA TTA TTT ATC TCA ACT CAG CAG CAG GTG ACA TTT CTC
Val Asp Thr Gly Leu Phe Ile Ser Thr Gln Gln Gln Val Thr Phe Leu
195 200 205

TTG AAG ATT AAG AGA ACC AGG AAA GGC TTC AGA CCT CTG AAC CCA CAT
Leu Lys Ile Lys Arg Thr Arg Lys Gly Phe Arg Leu Leu Asn Pro His
210 215 220

CCT AAG CCA AAC CCC AAA AAC AAC TGA
Pro Lys Pro Asn Pro Lys Asn Asn
225 230

699
TTC TCC CCA GAT GGC GTG TTA GCA GTC AAG GTC
Phe Leu Ala Pro Asp Gly Val Leu Ala Val Pro Gln Lys Pro Lys Val
20 25 30

TCC TTG AAC CCT CCA TGG AAT AGA ATA TTT AAA GGA GAG AAT GTG ACT
Ser Leu Asn Pro Pro Trp Arg Ile Phe Lys Gly Glu Asn Val Thr
35 40 45

CTT ACA TGT AAT GGG AAC AAT TTC TTT GAA GTC AGT TCC ACC AAA TGG
Leu Thr Cys Asn Gly Asn Asn Phe Phe Glu Val Ser Ser Thr Lys Trp
50 55 60

TTC CAC AAT GGC AGC CTT TCA GAA GAG ACA AAT TCA AGT TTG AAT ATT
Phe His Asn Gly Ser Leu Ser Glu Glu Thr Asn Ser Ser Leu Asn Ile
65 70 75 80

GTG AAT GCC AAA TTT GAA GAC AGT GGA GAA TAC AAA TGT CAG CAC CAA
Val Asn Ala Lys Phe Glu Asp Ser Gly Glu Tyr Lys Cys Gln His Gln
85 90 95

CAA GTT AAT GAG AGT GAA CTT GTG TAC AGT GAA GTC TTC AGT GAC TGG
Gln Val Asn Glu Ser Ala Glu Pro Val Tyr Leu Glu Val Phe Ser Asp Trp
100 105 110

CTG CTC CTT CAG GCC TCT GCT GAG GTG AGT GAG GCC CAG CCC CTC
Leu Leu Leu Gln Ala Ser Ala Glu Val Met Glu Gly Gln Pro Leu
115 120 125

TTC CTC AGG TGC CAT GTG TGG AGG AAC TGG GAT GTG TAM AAG GTG ATC
Phe Leu Arg Cys His Gly Trp Arg Asn Trp Asp Val Tyr Lys Val Ile
130 135 140

TAT TAT AAG GAT GGT GAA GCT CTC AAG TAC TGG TAT GAG AAC CAC
Tyr Tyr Lys Asp Gly Ala Leu Lys Tyr Trp Tyr Glu Asn His Asn
145 150 155 160

ATC TCC ATT ACA AAT GCC ACA GTT GAA GAC AGT GGA ACC TAC TAC TGG
Ile Ser Ile Thr Asn Ala Thr Val Glu Asp Ser Gly Thr Tyr Cys
165 170 175

ACG GGC AAA GTG TGG CAG CTG GAC TAT GAG TCT GAG CCC CTC ACC ATT
Thr Gly Lys Val Trp Gln Leu Asp Tyr Glu Ser Glu Pro Leu Asn Ile
180 185 190

ACT GTA ATA AAA GCT
Thr Val Ile Lys Ala
195

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
  (A) LENGTH: 197 amino acids
  (B) TYPE: amino acid
  (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Met Ala Pro Ala Met Glu Ser Pro Thr Leu Leu Cys Val Ala Leu Leu
1 5 10 15

Phe Phe Ala Pro Asp Gly Val Leu Ala Val Pro Gln Lys Pro Lys Val
20 25 30
Ser Leu Asn Pro Pro Trp Asn Arg Ile Phe Lys Gly Glu Asn Val Thr  35
    40
Leu Thr Cys Asn Gly Asn Phe Phe Glu Val Ser Ser Thr Lys Trp  50
    55
70
Phe His Asn Gly Ser Leu Ser Glu Glu Thr Asn Ser Ser Leu Asn Ile 65
    75
Val Asn Ala Lys Phe Glu Asp Ser Gly Glu Tyr Lys Cys Gln His Gln  85
    90
Gln Val Asn Gly Ser Glu Pro Val Tyr Leu Glu Val Phe Ser Asp Trp 100
    105
110
Leu Leu Leu Gln Ala Ser Ala Glu Val Val Met Glu Gly Gln Pro Leu 115
    120
125
Phe Leu Arg Cys His Gly Trp Arg Asn Trp Asp Val Tyr Lys Val Ile 130
    135
Tyr Tyr Lys Asp Gly Glu Ala Leu Lys Tyr Trp Tyr Glu Asn His Asn 140
    145
150
Ile Ser Ile Thr Asn Ala Thr Val Glu Asp Ser Gly Thr Tyr Cys  155
    160
    165
170
175
Thr Gly Lys Val Trp Gln Leu Asp Tyr Glu Ser Glu Pro Leu Asn Ile 180
    185
190
Thr Val Ile Lys Ala  195

(2) INFORMATION FOR SEQ ID NO:12:

(i)  SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 516 nucleotides
    (B) TYPE: nucleic acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) FEATURE:
    (A) NAME/KEY: CDS
    (B) LOCATION: 1..516

(xi) SEQUENCE DESCRIPTION:  SEQ ID NO:12:

GTC CCT CAG AAA CCT AAG GTC TCC TTG AAC CCT CCA TGG AAT AGA ATA  35
Val Pro Gln Lys Pro Lys Val Ser Leu Asn Pro Pro Trp Asn Arg Ile  1
    5
    10
    15
TTT AAA GGA GAG GAT GCT ACT CTT ACA TGG AAT GGG AAC AAT TCC TTT 96
Phe Lys Gly Glu Asp Val Thr Leu Thr Cys Asn Gly Asn Phe Phe  20
    25
    30
40
GAA GTC ACT TCC ACC AAA TGG TTC CAG AAT GCC AGC CTT TCA GAA GAG
Glu Val Ser Ser Thr Lys Trp Phe His Asn Gly Ser Leu Ser Glu Glu  35
    40
    45
ACA AAT TCA AGT TTG AAT ATT GTG AAT GCC AAA TTT GAA GAC AGT GGA
Thr Asn Ser Ser Leu Asn Ile Val Asn Ala Lys Phe Glu Asp Ser Gly  50
    55
    60
(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 172 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Val Pro Gln Lys Pro Lys Val Ser Leu Asn Pro Pro Trp Asn Arg Ile
1      5      10     15
Phe Lys Gly Glu Asn Val Thr Leu Thr Cys Asn Gly Asn Asn Phe Phe
20     25     30
Glu Val Ser Ser Thr Lys Trp Phe His Asn Gly Ser Leu Ser Glu Glu
35     40     45
Thr Asn Ser Ser Leu Asn Ile Val Asn Ala Lys Phe Glu Asp Ser Gly
50     55     60
Glu Tyr Lys Cys Gln His Gln Gln Val Asn Glu Ser Glu Pro Val Tyr
65     70     75     80
Leu Glu Val Phe Ser Asp Trp Leu Leu Leu Gln Ala Ser Ala Glu Val
85     90     95
Val Met Glu Gly Glu Pro Leu Phe Leu Arg Cys His Gly Trp Arg Asn
100    105    110
Trp Asp Val Tyr Lys Val Ile Tyr Tyr Lys Asp Gly Glu Ala Leu Lys
115    120    125
GAG TCT GAG CCC CTC AAC ATT ACT GTA ATA AAA GCT
165    170

(2) INFORMATION FOR SEQ ID NO:13:

Val Pro Gln Lys Pro Lys Val Ser Leu Asn Pro Pro Trp Asn Arg Ile
1      5      10     15
Phe Lys Gly Glu Asn Val Thr Leu Thr Cys Asn Gly Asn Asn Phe Phe
20     25     30
Glu Val Ser Ser Thr Lys Trp Phe His Asn Gly Ser Leu Ser Glu Glu
35     40     45
Thr Asn Ser Ser Leu Asn Ile Val Asn Ala Lys Phe Glu Asp Ser Gly
50     55     60
Glu Tyr Lys Cys Gln His Gln Gln Val Asn Glu Ser Glu Pro Val Tyr
65     70     75     80
Leu Glu Val Phe Ser Asp Trp Leu Leu Leu Gln Ala Ser Ala Glu Val
85     90     95
Val Met Glu Gly Glu Pro Leu Phe Leu Arg Cys His Gly Trp Arg Asn
100    105    110
Trp Asp Val Tyr Lys Val Ile Tyr Tyr Lys Asp Gly Glu Ala Leu Lys
115    120    125
Tyr Trp Tyr Glu Asn His Asn Ile Ser Ile Thr Asn Ala Thr Val Glu
130    135    140
Asp Ser Gly Thr Tyr Tyr Cys Thr Gly Lys Val Trp Gln Leu Asp Tyr
145 150 155 160

Glu Ser Glu Pro Leu Asn Ile Thr Val Ile Lys Ala
165 170
While various embodiments of the present invention have been described in detail, it is apparent that modifications and adaptations of those embodiments will occur to those skilled in the art. It is to be expressly understood, however, that such modifications and adaptations are within the scope of the present invention, as set forth in the following claims.
What is claimed is:

1. A method to detect IgE comprising:
   (a) contacting an isolated human Fc_e receptor (Fc_eR) molecule with a
       putative IgE-containing composition under conditions suitable for formation of a Fc_eR
       molecule:IgE complex, wherein said IgE is selected from the group consisting of canine
       IgE, feline IgE and equine IgE; and
   (b) determining the presence of IgE by detecting said Fc_eR
       molecule:IgE complex, the presence of said Fc_eR molecule:IgE complex indicating the
       presence of IgE.

2. A method to detect IgE comprising:
   (a) contacting a recombinant cell with a putative IgE-containing
       composition under conditions suitable for formation of a recombinant cell:IgE complex,
       wherein said recombinant cell is selected from the group consisting of: a recombinant
       cell expressing a human Fc_eR molecule; and a recombinant cell expressing an antibody
       that binds selectively to an IgE selected from the group consisting of canine IgE, feline
       IgE and equine IgE; and
   (b) determining the presence of IgE by detecting said recombinant
       cell:IgE complex, the presence of said recombinant cell:IgE complex indicating the
       presence of IgE.

3. A kit for detecting IgE comprising a human Fc_e receptor (Fc_eR) molecule
   and a means for detecting an IgE selected from the group consisting of canine IgE, feline
   IgE and equine IgE.

4. A general allergen kit comprising an allergen common to all regions of
   the United States and a human Fc_e receptor (Fc_eR) molecule.

5. A method to detect flea allergy dermatitis comprising:
   (a) immobilizing a flea allergen on a substrate;
   (b) contacting said flea allergen with a putative IgE-containing
       composition under conditions suitable for formation of an allergen:IgE complex bound
       to said substrate;
   (c) removing non-bound material from said substrate under
       conditions that retain allergen:IgE complex binding to said substrate; and
(d) determining the presence of said allergen-IgE complex by contacting said allergen-IgE complex with a \( \text{Fc}_c \text{R} \) molecule.

6. A kit for detecting flea allergy dermatitis comprising a human \( \text{Fc}_c \) receptor (\( \text{Fc}_c \text{R} \)) molecule and a flea allergen.

7. An isolated human \( \text{Fc}_c \) receptor (\( \text{Fc}_c \text{R} \)) alpha chain protein, wherein a carbohydrate group of said \( \text{Fc}_c \text{R} \) alpha chain protein is conjugated to biotin.

8. The invention of Claim 1, 2, 3, 4, 5, 6 or 7, wherein said \( \text{Fc}_c \text{R} \) molecule comprises at least a portion of a \( \text{Fc}_c \text{R} \) alpha chain that binds to IgE.

9. The invention of Claim 1, 3, 4, 5, 6 or 7, wherein said \( \text{Fc}_c \text{R} \) molecule comprises a protein selected from the group consisting of \( \text{PhFc}_c \text{R}_\alpha_{257} \), \( \text{PhFc}_c \text{R}_\alpha_{197} \), \( \text{PhFc}_c \text{R}_\alpha_{235} \) and \( \text{PhFc}_c \text{R}_\alpha_{172} \).

10. The invention of Claim 1, 3, 4, 5, 6 or 7, wherein said \( \text{Fc}_c \text{R} \) molecule is encoded by a nucleic acid molecule selected from the group consisting of \( \text{nhFc}_c \text{R}_\alpha_{774} \), \( \text{nhFc}_c \text{R}_\alpha_{1198} \), \( \text{nhFc}_c \text{R}_\alpha_{612} \), \( \text{nhFc}_c \text{R}_\alpha_{594} \), \( \text{nhFc}_c \text{R}_\alpha_{699} \) and \( \text{nhFc}_c \text{R}_\alpha_{516} \).

11. The invention of Claim 1, 3, 4, 5, 6 or 7, wherein said \( \text{Fc}_c \text{R} \) molecule is encoded by a nucleic acid molecule selected from the group consisting of a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 7, SEQ ID NO: 10 and SEQ ID NO: 12, and a nucleic acid molecule comprising an allelic variant of a nucleic acid molecule comprising any of said nucleic acid sequences.

12. The invention of Claim 1, 3, 4, 5 or 6, wherein said \( \text{Fc}_c \text{R} \) molecule is conjugated to a detectable marker.

13. The invention of Claim 1, 3, 4, 5 or 6, wherein said \( \text{Fc}_c \text{R} \) molecule is conjugated to a detectable marker selected from the group consisting of a radioactive label, a fluorescent label, a chemiluminescent label, a chromophoric label and a ligand.

14. The invention of Claim 1, 3, 4, 5 or 6, wherein a carbohydrate group of said \( \text{Fc}_c \text{R} \) molecule is conjugated to biotin.

15. The method of Claim 1, 2 or 5, wherein said putative IgE-containing composition comprises a composition selected from the group consisting of blood, serum, plasma, urine, tears, aqueous humor, central nervous system fluid (CNF), saliva, lymph, nasal secretions, milk and feces.
16. The method of Claim 1, 2 or 5, wherein said putative IgE-containing composition comprises serum.

17. The method of Claim 1, 2 or 5, wherein said putative IgE-containing composition comprises a cell that produces IgE.

18. The method of Claim 1, 2 or 5, wherein said putative IgE-containing composition comprises a cell selected from the group consisting of a myeloma cell and a basophil cell.

19. The method of Claim 1, further comprising the step selected from the group consisting of binding said FcεR molecule to a substrate prior to performing step (a) to form a FcεR molecule-coated substrate; and binding said putative IgE-containing composition to a substrate prior to performing step (a) to form a putative IgE-containing composition-coated substrate, wherein said substrate is selected from the group consisting of a non-coated substrate, a FcεR molecule-coated substrate, an antigen-coated substrate and an anti-IgE antibody-coated substrate.

20. The method of Claim 19, wherein said antigen is selected from the group consisting of an allergen and a parasitic antigen.

21. The method of Claim 19, further comprising removing non-bound material from said antigen-coated substrate or said antibody-coated substrate under conditions that retain antigen or antibody binding to said substrate.

22. The method of Claim 5 or 19, wherein said substrate comprises a material selected from the group consisting of plastic, glass, gel, celluloid, paper and particulate material.

23. The method of Claim 1, 2 or 5, wherein said step of determining comprises performing assays selected from the group consisting of enzyme-linked immunoassays, radioimmunoassays, immunoprecipitations, fluorescence immunoassays, chemiluminescent assay, immunoblot assays, lateral flow assays, agglutination assays and particulate-based assays.

24. The method of Claim 1, wherein said step of determining comprises:

(a) contacting said FcεR molecule:IgE complex with an indicator molecule that binds selectively to said FcεR molecule:IgE complex;
(b) removing substantially all of said indicator molecule that does not selectively bind to \( \text{Fc}_\varepsilon \text{R molecule: IgE complex} \); and

(c) detecting said indicator molecule, wherein the presence of said indicator molecule is indicative of the presence of IgE.

25. The method of Claim 24, wherein said indicator molecule comprises a compound selected from the group consisting of a \( \text{Fc}_\varepsilon \text{R molecule} \), an antigen, an antibody and a lectin.

26. The method of Claim 1, said method comprising the steps of:
   
   (a) immobilizing said \( \text{Fc}_\varepsilon \text{R molecule} \) on a substrate;

   (b) contacting said \( \text{Fc}_\varepsilon \text{R molecule} \) with said putative IgE-containing composition under conditions suitable for formation of an \( \text{Fc}_\varepsilon \text{R molecule: IgE complex} \) bound to said substrate;

   (c) removing non-bound material from said substrate under conditions that retain \( \text{Fc}_\varepsilon \text{R molecule: IgE complex} \) binding to said substrate; and

   (d) determining the presence of said \( \text{Fc}_\varepsilon \text{R molecule: IgE complex} \).

27. The method of Claim 26, wherein the presence of said \( \text{Fc}_\varepsilon \text{R molecule: IgE complex} \) is detected by contacting said \( \text{Fc}_\varepsilon \text{R molecule: IgE complex} \) with a compound selected from the group consisting of an antigen and an antibody that binds selectively to IgE.

28. The method of Claim 27, wherein said compound comprises a detectable marker.

29. The method of Claim 1, said method comprising the steps of:
   
   (a) immobilizing a desired antigen on a substrate;

   (b) contacting said antigen with said putative IgE-containing composition under conditions suitable for formation of an antigen: IgE complex bound to said substrate;

   (c) removing non-bound material from said substrate under conditions that retain antigen: IgE complex binding to said substrate; and

   (d) determining the presence of said antigen: IgE complex by contacting said antigen: IgE complex with said \( \text{Fc}_\varepsilon \text{R molecule} \).
30. The method of Claim 1, said method comprising the steps of:
   (a) immobilizing an antibody that binds selectively to IgE on a
   substrate;
   (b) contacting said antibody with said putative IgE-containing
   composition under conditions suitable for formation of an antibody:IgE complex bound
   to said substrate;
   (c) removing non-bound material from said substrate under
   conditions that retain antibody:IgE complex binding to said substrate; and
   (d) determining the presence of said antibody:IgE complex by
   contacting said antibody:IgE complex with said Fc\_R molecule.

31. The method of Claim 1, said method comprising the steps of:
   (a) immobilizing said putative IgE-containing composition on a
   substrate;
   (b) contacting said composition with said Fc\_R molecule under
   conditions suitable for formation of an Fc\_R molecule:IgE complex bound to said
   substrate;
   (c) removing non-bound material from said substrate under
   conditions that retain Fc\_R molecule:IgE complex binding to said substrate; and
   (d) determining the presence of said Fc\_R molecule:IgE complex.

32. The invention of Claim 1, 3, 4, 5, 6, 29, 30 or 31, wherein said Fc\_R molecule
   is conjugated to a detectable marker selected from the group consisting of
   fluorescein, a radioisotope, a phosphatase, biotin, biotin-related compounds, avidin,
   avidin-related compounds and a peroxidase.

33. The method of Claim 32, wherein the presence of said Fc\_R molecule:IgE
   complex is determined by contacting said Fc\_R molecule:IgE complex with an indicator
   molecule selected from the group consisting of an antibody, an antigen and a lectin.

34. The method of Claim 32, wherein said Fc\_R molecule comprises a
detectable marker.

35. The method of Claim 1, wherein said putative IgE-containing
   composition is obtained from an animal, wherein said animal is selected from the group
   consisting of a dog and a cat.
36. The method of Claim 1, wherein said method is performed in solution.

37. The method of Claim 2, wherein said recombinant cell expresses a FcεR molecule comprising a protein selected from the group consisting of PhFcεRα257 and PhFcεRαα232.

38. The method of Claim 2, wherein said recombinant cell expresses a FcεR molecule encoded by a nucleic acid molecule selected from the group consisting of nhFcεRα612, nhFcεRαα91, nhFcεRαα099 and nhFcεRαα516.

39. The method of Claim 2, wherein said recombinant cell expresses a FcεR molecule encoded by a nucleic acid molecule selected from the group consisting of a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:1 and SEQ ID NO:4, and a nucleic acid molecule comprising an allelic variant of a nucleic acid molecule comprising SEQ ID NO:1 and SEQ ID NO:4.

40. The method of Claim 2, wherein said recombinant cell is a RBL-hFcεR cell.

41. The kit of Claim 3, wherein said detection means further comprises an antigen selected from the group consisting of an allergen and a parasite antigen, wherein said antigen induces IgE antibody production in animals selected from the group consisting of canines, felines and equines.

42. The kit of Claim 3, wherein said detection means comprises an antibody that selectively binds to an IgE.

43. The kit of Claim 3, wherein said detection means detects said FcεR molecule.

44. The kit of Claim 3, wherein said FcεR molecule is on the surface of a recombinant cell that expresses said FcεR molecule.

45. The kit of Claim 41, wherein said antigen is immobilized on a substrate.

46. The kit of Claim 45, wherein said substrate comprises material selected from the group consisting of plastic, glass, gel, celluloid, paper, magnetic resin, polyvinylidene-fluoride, nylon, nitrocellulose and particulate material.
47. The invention of Claim 5, 19 or 45, wherein said substrate material is selected from the group consisting of latex, polystyrene, nylon, nitrocellulose, agarose and magnetic resin.

48. The invention of Claim 19 or 45, wherein said substrate comprises a shape selected from the group consisting of a well, a plate, a dipstick, a bead, a lateral flow apparatus, a membrane, a filter, a tube, a dish, a celluloid-type matrix and a magnetic particle.

49. The invention of Claim 5, 19 or 45, wherein said substrate comprises an ELISA plate, a dipstick, a radioimmunoassay plate, agarose beads, plastic beads, latex beads, immunoblot membranes and immunoblot papers.

50. The kit of Claim 45, wherein said substrate is latex beads.

51. The kit of Claim 41, wherein said allergen is derived from material selected from the group consisting of fungi, trees, weeds, shrubs, grasses, wheat, corn, soybean, rice, eggs, milk, cheese, bovine, poultry, swine, sheep, yeast, fleas, flies, mosquitos, mites, midges, biting gnats, lice, bees, wasps, ants, true bugs and ticks.

52. The invention of Claim 5 or 51, wherein said flea allergen is a flea saliva antigen.

53. The kit of Claim 3, wherein said parasite antigen is a heartworm antigen.

54. The kit of Claim 3, further comprising an apparatus comprising:

(a) a support structure defining a flow path;

(b) a labeling reagent comprising a bead conjugated to said antigen, wherein said labeling reagent is impregnated within the support structure in a labeling zone; and

(c) a capture reagent comprising said Fe₃R molecule, wherein said capture reagent is located downstream of said labeling reagent within a capture zone fluidly connected to said labeling zone in such a manner that said labeling reagent can flow from said labeling zone into said capture zone.

55. The kit of Claim 54, wherein said apparatus further comprises a sample receiving zone located along said flow path.

56. The kit of Claim 54, wherein said apparatus further comprises an absorbent located at the end of said flow path.
57. The kit of Claim 55, wherein said sample receiving zone is located upstream of said labeling reagent.

58. The kit of Claim 54, wherein said support structure comprises a material that does not impede the flow of said bead from said labeling zone to said capture zone.

59. The kit of Claim 54, wherein said support structure comprises an ionic material.

60. The kit of Claim 54, wherein said support structure comprises a material selected from the group consisting of nitrocellulose, PVDF and carboxymethylcellulose.

61. The kit of Claim 54, wherein said bead comprises a latex bead.

62. The kit of Claim 54, wherein said labeling reagent is dried within said labeling zone and said capture reagent is dried within said capture zone.

63. The kit of Claim 4, wherein said allergen is selected from the group consisting of grass, Meadow Fescue, Curly Dock, plantain, Mexican Firebush, Lamb’s Quarters, pigweed, ragweed, sage, elm, cocklebur, Box Elder, walnut, cottonwood, ash, birch, cedar, oak, mulberry, cockroach, *Dermatophagoides, Alternaria, Aspergillus, Cladosporium, Fusarium, Helminthosporium, Mucor, Penicillium, Pullularia, Rhizopus* and *Tricophyton*.

64. The kit of Claim 4, wherein said allergen is selected from the group consisting of Johnson Grass, Kentucky Blue Grass, Meadow Fescue, Orchard Grass, Perennial Rye Grass, Redtop Grass, Timothy Grass, Bermuda Grass, Brome Grass, Curly Dock, English Plantain, Mexican Firebush, Lamb’s Quarters, Rough Pigweed Short Ragweed, Wormwood Sage, American Elm, Common Cocklebur, Box Elder, Black Walnut, Eastern Cottonwood, Green Ash, River Birch, Red Cedar, Red Oak, Red Mulberry, Cockroach, *Dermatophagoides farinae, Alternaria alternata, Aspergillus fumigatus, Cladosporium herbarum, Fusarium vasinfectum, Helminthosporium sativum, Mucor recemosus, Penicillium notatum, Pulularia pullulans, Rhizopus nigricans* and *Tricophyton* spp.

65. The kit of Claim 4, wherein said kit comprises one or more compositions, each composition comprising one allergen.

66. The kit of Claim 4, wherein allergen is immobilized to said substrate.
67. The invention of Claim 5 or 6, wherein said flea allergen is selected from the group consisting of flea saliva products and flea saliva proteins.

68. The Fc_eR alpha chain protein of Claim 7, wherein said Fc_eR alpha chain protein is encoded by a nucleic acid molecule selected from the group consisting of a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:10 and SEQ ID NO:12, and a nucleic acid molecule comprising an allelic variant of a nucleic acid molecule comprising any of said nucleic acid sequences.

69. The Fc_eR alpha chain protein of Claim 7, wherein said Fc_eR alpha chain protein comprises PhFc_eRα_{172}-BIOT.
Fig. 1

[Graph showing optical density at 450 nm against canine IgE concentration in ug/ml. The graph includes two lines: one for D9 anti-canine IgE Mab and another for HuFcERI alpha chain.]
Fig. 2

Optical Density @ 450nm

Kentucky Blue Grass  Green Ash

Samples

- Positive Canine serum pool 1:10
- Positive Canine serum pool 1:100
- Negative Canine serum pool 1:10
- Negative Canine serum pool 1:100
Fig. 4
Fig. 5
Fig. 6
Fig. 7
Fig. 8
Fig. 9
### A. CLASSIFICATION OF SUBJECT MATTER

| IPC   | G01N33/68 | G01N33/566 |

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

### B. FIELDS SEARCHED

- **Minimum documentation searched** (classification system followed by classification symbols):
  - IPC 6 G01N C07K

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

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

### C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>P,X</td>
<td>WO 97 24617 A (NOVARTIS/SANOD) 10 July 1997&lt;br&gt;see claims&lt;br&gt;see page 2, paragraph 2&lt;br&gt;see page 5, paragraph 2</td>
<td>1-69</td>
</tr>
<tr>
<td>X</td>
<td>PATENT ABSTRACTS OF JAPAN&lt;br&gt;vol. 095, no. 007, 31 August 1995&lt;br&gt;&amp; JP 07 092167 A (KINKI UNIV; OTHERS: 01),&lt;br&gt;7 April 1995,&lt;br&gt;see abstract</td>
<td>1-69</td>
</tr>
<tr>
<td>X</td>
<td>PATENT ABSTRACTS OF JAPAN&lt;br&gt;vol. 095, no. 006, 31 July 1995&lt;br&gt;&amp; JP 07 072150 A (TONEN CORP; OTHERS: 01),&lt;br&gt;17 March 1995,&lt;br&gt;see abstract</td>
<td>1-69</td>
</tr>
</tbody>
</table>

### Date of the actual completion of the international search

12 March 1998

### Date of mailing of the international search report

19/03/1998

### Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel: (+31-70) 340-2040, Tx: 31 651 epo nl, Fax: (+31-70) 340-3016

Authorized officer:

Routledge, B
<table>
<thead>
<tr>
<th>Patent document cited in search report</th>
<th>Publication date</th>
<th>Patent family member(s)</th>
<th>Publication date</th>
</tr>
</thead>
<tbody>
<tr>
<td>WO 9724617 A</td>
<td>10-07-97</td>
<td>AU 1305897 A</td>
<td>28-07-97</td>
</tr>
</tbody>
</table>