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(71) Applicant *(for all designated States except US)*: **PRIONICS AG** [CH/CH]; Wagistrasse 27a, CH-8952 Schlieren (CH).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **BUHOLZER, Patrik** [CH/CH]; Im Oberen Gern 61, CH-8409 Winterthur (CH). **RAEBER, Alex** [CH/CH]; Glaubtenstrasse 89, CH-8046 Zürich (CH). **PRICE, Paul** [CH/CH]; In Stelzenacker 13, CH-8049 Zürich (CH). **ZWALD, Daniel** [CH/CH]; Löffelgraben 274, CH-5237 Mönthal (CH). **BONILLA PINSCHEWER, Weldy** [CH/CH]; Witikonstrasse 68, CH-8032 Zürich (CH). **MARTI, Roger** [CH/CH]; Höhenweg 6, CH-1717 Ursen (CH). **WEIDMANN, Jürg** [CH/CH]; Schulstrasse 16, CH-8400 Winterthur (CH). **STAMM, Christoph** [CH/CH]; Im Zollikof 8, CH-8260 Stein am Rhein (CH).

(74) Agent: **EMMEL, Thomas**; Schaefer Emmel Hausfeld, Gehölzweg 20, 22043 Hamburg (DE).

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(54) **Title:** DIAGNOSTIC COMPOSITION AND METHOD FOR THE DETECTION OF A TRICHINELLA INFECTION

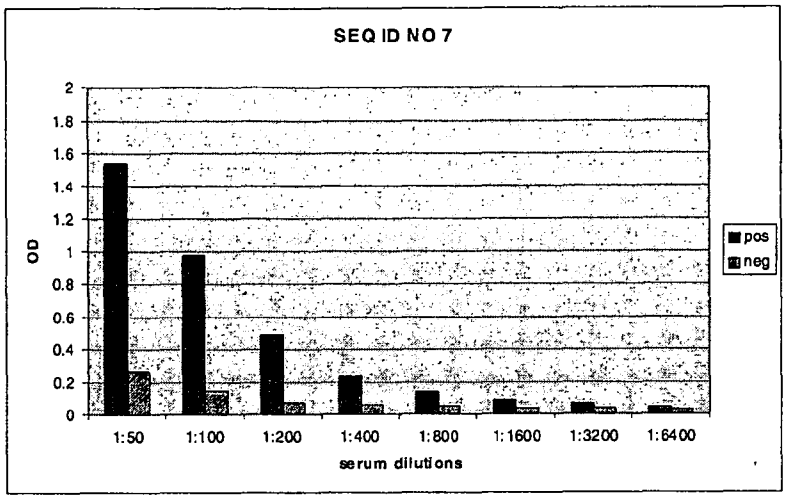

Fig. 8a

(57) **Abstract:** A diagnostic composition to detect antibodies in a sample obtained from an animal or human being infected by Trichinella, comprising at least one peptide containing a series of amino acids that form a continuous or discontinuous epitope recognized by sera from pigs infected with Trichinella.

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DIAGNOSTIC COMPOSITION AND METHOD FOR THE DETECTION OF A TRICHINELLA INFECTION

The present invention relates to a diagnostic composition and method for the detection of an infection by the nematode *Trichinella* spp in a body fluid sample from animals or humans, preferably in serum or meat juice of pigs or other susceptible organisms and to diagnostic kits that can be used for monitoring and surveillance for *Trichinella* infections or for testing of individual carcasses for food safety.

Trichinella spp. is a group of nematodes that can occur worldwide. There are eight different species; the species of main importance in Europe are *Trichinella spiralis*, *Trichinella britovi*, *Trichinella pseudospiralis*, and *Trichinella nativa*. *Trichinella* spp. can infect a wide variety of species including humans, pigs, rats, bears, horses and birds.

The parasitic nematode *Trichinella* undergoes a distinct live cycle. Larvae are ingested by eating raw or undercooked meat. They are released from nurse cells in the stomach from where they enter the small intestine, where adults mature. Females shed newborn larvae that enter the lymph, reach the venous blood and settle in voluntary muscles, especially those of the diaphragm, tongue and masseters. Larvae mature and form nurse cell-larvae complexes that calcify.

Trichinella infection in production animals is important because of the risk for humans to contract trichinellosis following consumption of raw or undercooked meat from infected animals. Human trichinellosis is a serious disease that can cause much suffering and may result in death.

There are three different diagnostic methods to detect *Trichinella* spp. in animals. Two methods are based on the detection of the parasite; the third method is based on the detection of antibodies against this nematode.

The first method called "Trichinoscopy" or the compressorium method is a direct detection method in which tissue is compressed between two glass plates and studied microscopically. This method is rather insensitive and labour-intense. More important, non-encapsulating *Trichinella* species such as *T. pseudospiralis* may not be detected with this method.

The second method called "digestion method" is a more sensitive direct detection method in which muscle tissue surrounding the larvae is artificially digested to release the larvae. Subsequently, the number of larvae can be determined microscopically. The detection limit by artificial digestion of 1 g tissue is 3-5 larvae per gram.

The third method is an indirect detection method using serology to detect antibodies against *Trichinella* spp. As antigens the excretory/secretory (E/S) antigens can be used. Although this method is currently not recommended for meat inspection or food safety programs, it is an important tool for surveillance programs and epidemiological investigations in animal populations. Serology can be based on blood serum or meat juice. This method has a limit of detection of 0.01 larvae per gram tissue.

For the purpose of ensuring food safety, all pig carcasses must be tested as part of the post-mortem examination. Carcasses may be cut up in a maximum of six parts, or be cut up in a cutting plant adjacent to the slaughterhouse before the results of the *Trichinella* tests are available, but further processing can only take place after the *Trichinella* tests have shown negative results.

According to EU Regulation Trichinoscopy will no longer be permitted as a standard method of examination, although it may be used as a transitional measure during a period of maximum four years following the date of application of the new EU Regulation.

The prescribed method for *Trichinella* examinations will be the digestion method with the magnetic stirrer method for pooled sample digestion. Equivalent methods are the mechanically assisted (Stomacher) pooled sample digestion method using 'sedimentation technique', the mechanically assisted (Stomacher) pooled sample digestion method using 'on filter isolation technique', and the automatic digestion method (Trichomatic 35) for pooled samples of up to 35 gram.

Currently used *Trichinella* digestion tests have a number of disadvantages.

All digestion methods are labour intensive as they require manual inspection of the filtered digestion mixture by microscopy performed by an experienced investigator. No routine training of investigators or testing of proficiency panels to assure validity of the inspection procedure is provided by current EU regulation. As a rule the investigators performing the digestion method know positive test results only from their initial training.

Finally currently approved test methods do not allow tracking of the samples and documentation of the results.

At present as stated above serological methods are considered useful for monitoring purposes but are not considered suitable for the detection of *Trichinella* infection in individual animals intended for human consumption. In most cases problems in known serological methods result from the diagnostic antigens used. The presently used crude antigens or E/S antigens include antigenic components that may react non-specifically and thus produce false positive results. Additionally the diagnostic window and selectivity of known serological tests using e.g. E/S antigens is considered to be too small. Finally, known serological methods require special technical and personal equipment which at present is not available in slaughterhouses.

When comparing both systems it appears that on principle serological tests have a number of advantages over digestion methods and that if improved they could be an interesting alternative also in routine testing.

Some of the disadvantages mentioned above can be met by using different antigens. In this context Ting-Xian et al. e.g. describe (Chin. J. Parasitol. Dis.; June 2005, No. 3; 143) the use of T668 antigen as diagnostic antigen in immunoassays. However, also here the problems regarding the narrow diagnostic window remain.

The object of the invention is to provide compositions and methods which allow the design of improved serological tests.

The object of the invention is realised by a diagnostic composition and a method to detect antibodies in a sample obtained from an animal or human being infected by *Trichinella.* Additionally covered by the invention are kits, specific peptides, fusion peptides and tracer complexes especially for use in fluorescence polarisation.

A diagnostic composition according to the invention comprises at least one peptide containing a series of amino acids that form a continuous or discontinuous epitope recognized by sera from pigs infected with *Trichinella.*

The term "peptide" comprises native forms of peptides as well as recombinant peptides and chemically synthesized peptides. Unless explicitly mentioned the term peptide when used in this application will always denote a series of amino acids that form a continuous or discontinuous epitope recognized by sera from pigs infected with *Trichinella*

The term "sample" shall include any material in which antibodies against *Trichinella* can be detected. Typical sample materials are body fluids like blood, serum, plasma, urine and saliva to give only some examples. Further sample materials which can be used in animal testing are meat and meat juice.

By mapping experiments a number of different peptides were identified by the applicants, which can be used either alone or in combination in the diagnostic composition or methods. Each peptide according to the invention corresponds to a part of an antigen expressed by *Trichinella.*

In the mapping experiments sera from *Trichinella* infected animals, especially pigs were used to identify different series of amino acids that form a continuous or discontinuous epitope on *Trichinella* antigens. Especially the excretory secre-

tory antigenic proteins but also further antigens related to different stages of the infection cycle were mapped.

A number of preferred peptides which can be used with the invention and the proteins to which they belong are identified in the following:

Trichinella spiralis newborn larvae-specific protein SS1 (SEQ ID NO 1):

MFISIIVILI SLKTCIAQVA TCKNDNDANV DWYFVYKPPN VLSSKILQSG
VNPAWAASRA NINQGAGHSI IRTMASFVVH HAQINVLAYS DDPPNLPPRN
EKSKTKGVLL VNNAADEAAW FVHTVPNFLA YLNAYSWPPA ETPKGHMFLC
VSFNKAHLNS VGKAIRYQEP YVYANNLPAA ILNQNMELFN LINGIDVRVT
SFLAHETFAT KSVQAVANIQ AFGKHSKSFA DMYARILRNR FAASIMVWSP
ADARSKSICK GQHKLQKITS IQLDGVQVSR EADSAKWALI DGKNTVCFTT
NDYTATEKRT PGAAVCLENA GVYNAFRTAA LNVEACNN

Preferred peptides having an amino acid sequence of SS1 are:

SEQ ID NO 2: QINVLAYSDD PPNLPPRNEK SKTKG

and

SEQ ID NO3: IRYQEPYVYA NNLPAAILNQ N

Trichinella spiralis newborn larvae-specific protein SS2 (SEQ ID NO 4):

MHKITHKSIV SRHTFAVYLL VSGQKLQYIY IFICKMIRRL FQYTSMTFAW
ILLFLSAASP SLGAFECGVP HFKPYIWKSG RIVGGTDVRP HSHPWQIQLL
KSETGGYSSL CGGSLVHFGK PSNGTRFVLT AAHCITTSNM YPRTSRFTVV
TGAHNIKMHE KEKKRIPITS YYVQHWNPVM TTNDIALLRL AETVYYNEYT
RPVCLPEPNE ELTPGDICVV TGWGDTTENG TTSNTLKQVG VKIMKKGTCA
NVRSEVITFC AGAMEGGKDS CQGDSGGPLI CKKNGKSVQF GVVSYGTGCA
RKGYPGVYAK VPSYVTWLNK AAKELENSPE GTVKWASKED SPVDLSTASR
PTNPYTGSRP TSPSSGSRPT YPSSGSRPTS PSSGSRPTYP SSGSRPTYPS
SGSRPTYPYT GSRPTPQKPV FPSYQKYPPA VQKYIDSLPS GTQGTLEYTV
TQNGVTTTTY YHFSK

Preferred peptides having an amino acid sequence of SS2 are:

SEQ ID NO 5: MHKITHKSIV SRHTFAVYLL VSGQK

SEQ ID NO 6: VGGTDVRPHSH PWQIQLLKSET G

SEQ ID NO 7: LSTASRPTNP YTGSRPTSPS SGSRP

SEQ ID NO 8: PTYPSSGSRP TYPSSGSRPT YPYTG

and

SEQ ID NO 9: PTYPSSGSRP TYPSSGSRP

Trichinella spiralis glutamic acid-rich protein cNBL1700 (SEQ ID NO 10):

MWLFRCPIYF VLLQLFFLTF LTVTSSNAIP GRSSSRLRLL ERYDSLPSLR
SHSEDRYDDG VDRKWKKREG NSDDICTEDE TTVIEKESEN GVDKEKPTSK
EESGEKTSQE KESEEKSSQE KDEDKSESEA SEEKDVSQEQ NSKEEKGASE
EDEDTPEEQN SKEENGSSEE DDEDASEEQA SNEEKEASEE KNTVSEERKG
ASEEEDEEKD DGHESEVESQ ASEEQTTEEG ASEEEDEESA SEEQTSEGEE
KGASQEEEED EGNEQESEVE SQASEEQTSE EEESASEEED EENESKEQTT
EEEESASEEE DEESASEREE KNASQEEEED EGNESKEQTT EEEESASEEE
DEESVSEEQT SEGEEKGASQ EEEEDEGNDQ ESEVESQASE EQTSEEEGAS
EEEDEENESE EQTTEEESAS EEEDEESASE GEEKNASQEE EEDEGNEQES
EVESQASEEQ TSEEEEKEGA SQEEDEENES EEQTSEEEEE GASEEEDEES
AFEEQTSEEE EEKGASQEEE EDEENEQESE VESQASEEQT SEEEGASEEG
QDASEEEDED ESEEEESDES V

Preferred peptides having an amino acid sequence of cNBL1700 are:

SEQ ID NO 11: EKESENGVDK EKPTSKE

and

SEQ ID NO 12: NEQESEVESQ ASEEQTS

Trichinella spiralis 43 kDa secreted glycoprotein (SEQ ID NO 13):

MRIYIFLSAF WVILHNCLQI HAANCTCRTA TDDTEWFLLF KPVGLLKAKI
ISPANAGWAN DGANMNTDSG HALVQTLAEW MGPILDDMTA LGYSNTPPKS
TITSQTTSSK GILMFGNETT DGFWLLHTFE RAFPNSVAWS WPSKFTSEGH
MALCLSISED NVPLIVPALQ YQEVVIYFGQ VSSEKATEFA DLTSLIDGSL
PTITPPLWNQ QTITTLNSAL STVVYSKTSS SRLEMYGSFL AKVMVVNMRI
WAVTDNTLQT TCGGKIGFVK VVKSPVTIDG TQNDRSKDKS QWAVIDDSLP
KPVFCFTTNG YSTKQRTVAG SATCITQQVV SNLFATSAAN FIPCPYS

Preferred peptides having an amino acid sequence of 43kDa secreted glycoprotein are:

SEQ ID NO 14: FLLFKPVGLL KAKIISPANA G

SEQ ID NO 15: SEKATEFADL TSLIDGSLP

SEQ ID NO 16: VVYSKTSSSR LEMYGSF

and

SEQ ID NO 17: TIDGTQNDRS KDKSQWA

Trichinella spiralis 53kDa excretory/secretory antigen (SEQ ID NO 18):

MFSITLNLFI IAFVNFQLCT CSTDNENVAM KEMTFSVPIS VLQNERQFDE
NKLKKLLKPL GKLYKTPSDK GIPISRTEAT LSVEKMMVEL NRLIQKEYSF
LYKQYQKLKT VQQAEKCDDT TNVYTVTLQN TDCESKPIIE GSPATNCSDV
ENKHPLSCSI LSKVASAEEK IIGAYCSVHL EESFPKKKSI CKLSRYPGEE
KFKTFVPEDV SSWFHDAIVY VPTGNRPQSN SKHSNNYRGR QGIAGLGMLP
HLGAVQMNVV TIFRKNGKTT EVLSLINAND SIEIPKVFVT NPIQKPFGDE
IDRILRKAFD TMELSNSDKE DKLQKLYNAT ISTKVKHRAT PYDTDDAYVI
TEVAGVFDEN KEHIGSIDKF PSDGNLQIGW KEADKSALRL KRFAKPPKGF
FQHVFSELQL LF

Preferred peptides having an amino acid sequence of 53kDa excretory/secretory antigen are:

SEQ ID NO 19: QNERQFDENK LKKLLKPLGK LYKTPSD

SEQ ID NO 20: QNERQFDENK LKKLLKPLG

SEQ ID NO 21: LSRYPGEEKF KTFVPEDVS

SEQ ID NO 22: PGEEKFKTFV P

SEQ ID NO 23: NNYRGRQGIA GLGML

SEQ ID NO 24: VVTIFRKNGK TTEVLSL

SEQ ID NO 25: TIFRKNGKTT EVL

SEQ ID NO 26: KPFGDEIDRI LRKAF

SEQ ID NO 27: KEADKSALRL KRFAKPPKGF F

SEQ ID NO 28: ALRLKRFAK

Trichinella pseudospiralis 21 kDa excretory/secretory protein (SEQ ID NO 29):

QNMHCQYILS LLLLSLNVVF FAAGDSLDSV DDKSRRCTDE QTEVCAKTEC
KAEDAAMTEL LLEGESDITE HPDFVYYTRC MQRCCAKLNG AKVAPLKEEE

KRRGPTKLPF QSIFDVADQQ TVERCDATMC KSQRMKYESL VARTTSYKKL RASQELRDYK ECIESCDAKL NGRQ

A preferred peptide derived from the amino acid sequence of the 21 kDa excretory/secretory antigen is:

SEQ ID NO 30: KSQRMKYESL VARTTSYKKL R

Trichinella pseudospiralis 28 kDa excretory/secretory protein (SEQ ID NO 31):

MVHFKVMNIN ITLLFAIILL QFISNASTER FRKLKKESMP AAVKEHLKKL MKNSIVQQSG HESEGGIVEE TKQVLQKSHD SFYHLEGTIH KLEEKLEKEK KLYDPWDKKD NSAKRLALGF FVRVAKQYRE GLLNESGMMA GIRQPRKKCF VKYSMLDEYS ATTEEDDKIL MKIERKFYKC ESQCQSNTKM KDFYTKDLCI LKCFEKKLDK FAEKLGVPFD EAKVNEGVNQ LQDLDKSVVP FTSI

A preferred peptide having an amino acid sequence of the 21kDa excretory/secretory antigen is:

SEQ ID NO 32: EKKLDKFAEK LGVPFDEAKV N

The sequences of the peptides indicated above and their position relative to the full sequences of the antigenic *Trichinella* proteins are illustrated by figures. Further figures show the results of tests performed on the peptides with respect to their properties to discriminate between negative and positive sera:

Fig. 1 shows the sequence of *Trichinella spiralis* newborn larvae-specific protein SS1 (SEQ ID NO 1). The sequences (SEQ IDs NO 2 and 3) shown in italics relate to preferred peptides which can be used in the diagnostic composition according to the invention.

Fig. 2 shows the sequence of *Trichinella spiralis* newborn larvae-specific protein SS2 (SEQ ID NO 4). The sequences (SEQ IDs NO 5-9) shown in italics

relate to preferred peptides which can be used in the diagnostic composition according to the invention.

Fig. 3 shows the sequence of *Trichinella spiralis* glutamic acid-rich protein cNBL1700 (SEQ ID NO 10). The sequences (SEQ IDs NO 11 and 12) shown in italics relate to preferred peptides which can be used in the diagnostic composition according to the invention.

Fig. 4 shows the sequence of *Trichinella spiralis* 43 kDa secreted glycoprotein (SEQ ID NO 13). The sequences (SEQ IDs NO 14-17) shown in italics relate to preferred peptides which can be used in the diagnostic composition according to the invention.

Fig. 5 shows the sequence of *Trichinella spiralis* 53 kDa excretory/secretory antigen (SEQ ID NO 18). The sequences (SEQ IDs NO 19-28) shown in italics relate to preferred peptides which can be used in the diagnostic composition according to the invention.

Fig. 6 shows the sequence of *Trichinella pseudospiralis* 21 kDa excretory/secretory protein (SEQ ID NO 29). The sequence (SEQ ID NO 30) shown in italics relates to a preferred peptide which can be used in the diagnostic composition according to the invention.

Fig. 7 shows the sequence of *Trichinella pseudospiralis* 28 kDa excretory/secretory protein (SEQ ID NO 31). The sequence (SEQ ID NO 32) shown in italics relates to a preferred peptide which can be used in the diagnostic composition according to the invention.

Fig. 8a and b show the results of 2 of the preferred SS2 peptides (SEQ IDs NO 7 and 8) tested in ELISA assays. It was shown that both peptides are able to discriminate between negative and positive sera. Serial two fold dilutions of the serum sample were tested.

Fig. 9a and b show the results of a FPA of 2 peptides (SEQ ID NO 14 (9a); SEQ ID NO 24 (Fig. 9b).

Diagnostic compositions according to the invention can e.g. be employed in usual immunoassays in which the at least one peptide of the composition is reacted with antibodies possibly present in a sample obtained from an animal or a human. In case antibodies are present an immune complex is formed which can be detected by methods known in the art. As stated above the invention does not only cover diagnostic compositions but also especially immunoassay methods in which the compositions are used as antigenic substance.

On basis of the compositions according to the invention containing one or more of the peptides diagnostic tests can be designed which allow individual animal/carcass testing in a more rapid, standardized and automatic manner than currently used digestion methods. The tests allow full documentation with a short time to result and internal controls will show the reliability of the test procedure. It will be suitable for low and high throughput testing with little equipment required. Since the same sample (serum/meat juice) can be used for other diagnostic tests (e.g. for Salmonellosis in pigs), the sampling efficiency can be increased. The diagnostic tests used with the invention are immunological assay systems which quantitatively or qualitatively detect the presence of antibodies in samples taken from animals or human and preferably allow the determination of a status with regard to *Trichinella* infection. Preferably the peptides including the amino

acid sequences that are identified as epitopes of *Trichinella* antigenic proteins are synthesized or made by recombinant techniques.

The assay system can be either a homogeneous type of immunoassay or a heterogeneous type of an immunoassay.

Heterogeneous immunoassays involve binding of the at least one peptide either as a mixture or covalently linked to a solid support. In this embodiment the sample containing the antibodies is brought into contact with the peptide(s) and the bound antibodies are detected with a second antibody which is specific to the species from which the first antibody originates and is conjugated with an enzyme to allow detection by means of a chemical reaction.

Homogeneous assays use at least one peptide as probe for the detection of antibodies in solution. In this aspect of the invention, the peptides are e.g. chemically directly or indirectly via a chemical linker linked with fluorescent dyes and can be used either as mixture or covalently linked as tracers in a fluorescent assay system.

In a particularly preferred embodiment of the invention said method is a fluorescence polarization assay to detect antibodies in a sample by contacting said sample with the tracer molecule, allowing the mixture to interact for a certain period of time and then measuring the polarization value which indicates whether antibodies were present in the sample fluid.

The invention is not limited to the mentioned polarisation assays. Further methods which can be applied as well are e.g. immunoblot techniques, ELISA, RIA, SPR (surface plasmon resonance) or agglutination assays to list only some examples.

One main advantage of the invention is that the peptides selected for the compositions or methods due to their limited length can be easily linked to further peptides or immobilised or incorporated into tracer molecules for special test systems. A further advantage is that the peptides can be selected from different antigens present at different stages of the infection cycle which allows the design of tests with a broad diagnostic window if desired.

E.g. it is possible to provide a composition including at least one peptide corresponding to an amino acid sequence from new-born larvae specific antigenic proteins such as e.g. the SS1, SS2 (Niu et al. 2005) protein or NBL1700 antigen (Zarlenga et al. 2002).

Such compositions allow detection of antibodies in the early stage of infection of an organism with *Trichinella* and thus overcome current limitations of serologic methods to detect antibodies in the sera within the first 15 days following infection by the parasite.

If later stages of the infection are to be detected one can use a composition containing a peptide including a series of amino acids that form a continuous or discontinuous epitope recognized by sera from pigs infected with *Trichinella*. Especially preferred are compositions containing peptides corresponding to an epitope of one of the following ES-antigen components: 43kDa, 53kDa, 28kDa and 21kDa.

As stated above the selection of peptides as diagnostic antigens provides a number of advantages over the use of whole antigens. One advantage is that the peptides used according to the invention in general are much shorter compared to the whole antigens. The risk of cross-reactions etc. is diminished and it is no problem

to combine 2 or more different peptides in a composition as is provided by a preferred embodiment of the invention. By using different peptides one can optimise the test systems with respect to selectivity etc.

It is possible to include peptides in the composition in form of a mixture. Especially preferred is to provide the peptides in form of a fusion peptide or fusion peptides.

A further preferred embodiment provides that the composition includes peptides corresponding to epitopes of different antigens which are expressed by *Trichinella* at different stages of the infection's cycle. As stated above this embodiment allows the design of test systems with a broad diagnostic window. Such test systems may at the same time detect early and late infections.

One further embodiment of the invention intended for heterogeneous assays provides a composition wherein at least one peptide or fusion peptide is linked to solid support.

Independent on the form of the assay a further embodiment provides that the composition includes a tracer complex composed of at least one peptide or fusion peptide linked to a marker.

As a rule the peptide or fusion peptide is linked with a marker via a linker and it is especially preferred that the marker whether bound directly or via a linker to the peptide or fusion peptide respectively is a fluorophore.

The use of fluorescence polarisation assay is a preferred option and in this context a further preferred embodiment of the invention provides that the tracer

complex composed of peptide or fusion peptide respectively with a fluorophore is adapted to such fluorescence polarisation assay.

In this context an especially preferred embodiment provides that the linker linking the peptide or fusion peptide to the marker is adapted to reduce or inhibit rotation of the marker relative to the peptide or fusion peptide. By this embodiment the so called propeller-effect which often negatively influences the result of fluorescence polarisation assays can be avoided or reduced. The propeller-effect and its implications for the preparation of fluorescent probes is described in 'The Handbook — A Guide to Fluorescent Probes and Labeling Technologies Invitrogen Corp., 10th edition, Richard P. Haugland, ISBN 0-9710636-4-8.

In a preferred embodiment of the invention, linkers which contain amino acids with sterically bulky side chains such as phenyalanin, tyrosin or tryptophan or with side chains that reduce the rotational freedom of the peptide chain when incorporated into a oligopeptide such a proline or histidine are used. In a particularly preferred embodiment of the invention, linkers which contain one or several amino acids with tryptophan as side chains are used.

A general model how to reduce the "propeller effect" is lined out in Example 1

Table 2 referred to under Example lists a number of tracer complexes consisting of a peptide and a marker which can be used with the invention in fluorescence polarisation assay

The invention is not only directed to a composition but also to a method for the diagnosis of *Trichinella* infection.

The method according to the invention comprises in vitro-detection of antibodies against at least one peptide from *Trichinella* in a tissue sample taken from an animal or a human. As a rule the sample is contacted with diagnostic compositions according to the invention. The presence of antibodies in the sample is detected by testing whether or not a binding reaction of antibodies in the sample with the immunogenic peptide in the diagnostic composition has occurred.

It is possible to detect the binding reaction by homogenous assays like ELISA, immunoblot techniques, RIA etc. or by heterogeneous assays.

Especially preferred is a homogenous assay in form of a fluorescence polarisation assay.

The tissue sample analysed can be taken from an animal selected from a group comprising mammals and reptiles, including specifically swine, wild boars, equines, carnivores, aquatic animals, bear, fox, marten, sheep, cattle and from humans.

The tissue used as sample is selected from body fluids including blood, serum, plasma and urine, saliva and as far as animals are concerned also from meat juice, carcasses and meat.

The invention further covers specific peptides including fusion peptides and tracer complexes made from the peptides and a marker.

Finally the invention covers kits for the diagnosis of a *Trichinella* infection in a sample taken from a susceptible animal or human, which comprise the diagnostic composition according to the invention together with the necessary reagents to

perform a usual homogenous or heterogeneous immunoassay to detect antibodies against the at least one peptide included in the composition.

EXAMPLES

EXAMPLE 1: Development of a labelling strategy using a model peptide

The FLAG peptide (a well known tag, e.g. used for immuno affinity purification) was used as a model peptide to develop a labelling strategy for peptide tracers which allows chemical linking of a fluorophore to the peptide to reduce or inhibit rotation of the fluorophore relative to the peptide. The FLAG peptide and an elongated FLAG peptide were used to and coupled to 5-Carboxyfluorescein (5-CF) with a linker containing the following amino acid composition

FLAG: Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys (SEQ ID NO 33)
$Gly_{(9)}$-FLAG: $Gly_{(9)}$-Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys (SEQ ID NO 34)
$Gly_{(9)}$-FLAG: $Gly_{(9)}$-Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys
5-CF- $Gly_{(9)}$-FLAG: 5-CF-$Gly_{(9)}$-Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys
5-CF-Pro-$Gly_{(9)}$-FLAG: 5-CF-Pro-$Gly_{(9)}$-Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys (SEQ ID NO 35)
5-CF-Trp-$Gly_{(9)}$-FLAG: 5-CF-Trp-$Gly_{(9)}$-Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys (SEQ ID NO 36)
5-CF-$Trp_{(2)}$-$Gly_{(9)}$-FLAG: 5-CF-$Trp_{(2)}$-$Gly_{(9)}$-Asp-Tyr-Lys-Asp-Asp-Asp-Asp -Lys (SEQ ID NO 37)

FPA analysis of the FLAG Model peptide shown in Table 1 was performed by the following procedure.

The FLAG Model peptide tracers were diluted in TBS (containing 25mM Tris, 0.15M NaCl, NP40 0.05%, pH 7.2) at a concentration of 8.1 nM or 2.7 nM, re-

spectively in a single well of a black colored 96-well plate. The plate was incubated for 2 minutes at room temperature on an orbital shaker set at 1350 rpm and then incubated for another minute without shaking. Blank measurement of the sample was then performed by reading the fluorescence polarisation in a fluorescence reader (Safir2, Tecan, Switzerland) with excitation and emission wavelength set at 470 nm and 520 nm, respectively.

Then, 1, 3 or 10 molar equivalence of the anti-FLAG M2 monoclonal antibody (Sigma , Switzerland), respectively, were added and the plate was incubated at room temperature with shaking (1350 rpm) for 3 minutes and then for an additional minute without shaking. Then the plate was measured.

The mP values were calculated according to the following formula:

$$mP = \frac{(parallel\ light - perpendicular\ light)}{(parallel\ light + perpendicular\ light)} * 1000$$

The ΔmP values were obtained by subtracting the mP values of the negative serum from the positive sera:

$$\Delta mP = mP_{positive\ serum} - mP_{negative\ serum}$$

ΔmP values		5-CF-Pro-Gly$_{(9)}$-FLAG		5-CF-Trp-Gly$_{(9)}$-FLAG		5-CF-Trp$_{(2)}$-Gly$_{(9)}$-FLAG	
		8.1 nM	2.7 nM	8.1 nM	2.7 nM	8.1 nM	2.7 nM
anti-FLAG antibody	10 equiva-	62	33	86	55	124	97
	3 equiva-	33	15	50	27	78	35
	1 equiva-	14	5	19	9	37	5

Table 1

The labelled FLAG peptides show the influence of the bulky amino acids in the linker . The amino acids Trp-Trp in the linker resulted in higher ΔmP values than one Trp or one Pro and therefore showing a higher hindrance of the rotation of the fluorophore due to the bulky amino acids.

EXAMPLE 2: Discrimination between *Trichinella* positive and *Trichinella* negative serum in FPA using different peptides according to the invention.

Table 2 depicts for some of the *Trichinella* peptides their ability to discriminate between a *Trichinella* positive and a *Trichinella* negative serum sample using fluorescence polarisation assay (FPA). Shown are the DmP values of the tracers and the buffer composition which resulted in DmP values = 20 mP.

Tracer	Sequence	Buffer	ΔmP
SEQ ID NO 5	MHKITHKSIV SRHTFAVYLL VSGQK	PBS with 0.05% Glucopyranosid	39
SEQ ID NO 6	VGGTDVRPHSH PWQIQLLKSET G	PBS with 0.1% Sarcosyl	35
SEQ ID NO 7	LSTASRPTNP YTGSRPTSPS SGSRP	PBS with 0.1% Sarcosyl	20
SEQ ID NO 8	PTYPSSGSRP TYPSSGSRPT YPYTG	PBS with 0.001% Pluronic	66
SEQ ID NO 9	PTYPSSGSRP TYPSSGSRP	PBS with 0.001% Pluronic	66
SEQ ID NO 16	VVYSKTSSSR LEMYGSF	PBS	20
SEQ ID NO 19	QNERQFDENK LKKLLKPLGK LYKTPSD	PBS with 0.05% DOC	33
SEQ ID NO 20	QNERQFDENK LKKLLKPLG	PBS with 0.05% Chaps	28
SEQ ID NO 23	NNYRGRQGIA GLGML	PBS with 0.003% Triton X-100	23
SEQ ID NO 24	VVTIFRKNGK TTEVLSL	PBS with 0.1% Sarcosyl	57
SEQ ID NO 26	KPFGDEIDRI LRKAF	PBS with 0.05% Glucopyranosid	31
SEQ ID NO 27	KEADKSALRL KRFAKPPKGF F	PBS with 0.02% SDS	31
SEQ ID NO 28	ALRLKRFAK	PBS with 0.02% SDS	41

Table 2

FPA analysis of the tracers shown in Table 2 was performed by the following procedure. A 15 ml aliquot of the positive or negative serum was diluted with 185 ml of buffer (PBS with detergent as indicated in Table 2) in a single well of a black coloured 96-well plate. The plate was incubated for 2 minutes at room temperature on an orbital shaker set at 1350 rpm and then incubated for another minute without shaking. Blank measurement of the sample was then performed by

reading the fluorescence polarisation in a fluorescence reader (Safire2, Tecan, Switzerland) with excitation and emission wavelength set at 470 nm and 520 nm, respectively. Then, 5 ml tracer was added to the reaction mixture to a final concentration of 5 nM and the plate was incubated at room temperature with shaking (1350 rpm) for 15 minutes and then for an additional minute without shaking. Then the plate was measured.

The mP values were calculated according to the following formula:

$$mP = \frac{(parallel\ light - perpendicular\ light)}{(parallel\ light + perpendicular\ light)} * 1000$$

The DmP values were obtained by subtracting the mP values of the negative serum from the positive sera:

$$\Delta mP = mP_{positive\ serum} - mP_{negative\ serum}$$

EXAMPLE 3: Discrimination between *Trichinella* positive and *Trichinella* negative serum in Elisa Assays using SS2 peptides SEQ ID NO 7 and 8

Single wells of a 96-well microtitre plate were coated with the SS2 peptides (SEQ ID No 5 and 6) at a concentration of 10 mg ml-1 in carbonate buffer 0.1 M (pH 9.5) overnight at 4°C. The plate was washed 4 times with 0.05% Tween 80 and then blocked with a PBS buffer containing 2% I-block and 0.1% Tween 20 for 90 minutes at 25°C ± 3°C. Pig sera were diluted 50-fold in phosphate buffered saline and serial 2-fold dilutions were incubated in the microtitre

plate with shaking (500 rpm) for 2 hours at room temperature. The plates were washed 4 times with 0.05% Tween 80 Detection of the bound antibodies was performed by incubation with a goat anti-pig IgG(Fc)-POD conjugate (Bethyl Laboratories, USA) at a concentration of 1.3 ug/ml for 1 h at RT with shaking (500 rpm). Following four more washes, 2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) substrate solution was added and incubated for 30 minutes, colour development was measured in a ELISA reader (Tecan, Switzerland) at a wavelength of 405 nm.

EXAMPLE 4: Discrimination between *Trichinella* positive and *Trichinella* negative serum in FPA using tracer complexes including peptides SEQ ID NO 14 and 24

FPA analysis of the tracers shown in Figure 9a and 9b was performed by the following procedure. A 15 ml aliquot of the positive or negative serum was diluted with 185 ml of buffer (PBS with 0.02% sodium dodecyl sulphate (SDS)) in a single well of a black coloured 96-well plate. The plate was incubated for 2 minutes at room temperature on an orbital shaker set at 1350 rpm and then incubated for another minute without shaking. Blank measurement of the sample was then performed by reading the fluorescence polarisation in a fluorescence reader (Safire2, Tecan, Switzerland) with excitation and emission wavelength set at 470 nm and 520 nm, respectively. Then, 5 ml tracer was added to the reaction mixture to a final concentration of 5 nM and the plate was incubated at room temperature with shaking (1350 rpm) for 3 minutes and then for an additional minute without shaking. Then the plate was measured.

The mP values were calculated according to the following formula:

$$mP = \frac{(parallel\ light - perpendicular\ light)}{(parallel\ light + perpendicular\ light)} * 1000$$

The DmP values were obtained by subtracting the mP values of the negative serum from the positive sera:

$$\Delta mP = mP_{positive\ serum} - mP_{negative\ serum}$$

CLAIMS:

1. A diagnostic composition to detect antibodies in a sample obtained from an animal or human being infected by *Trichinella*, comprising at least one peptide containing a series of amino acids that form a continuous or discontinuous epitope recognized by sera from pigs infected with Trichinella.

2. Composition according to claim 1, wherein the peptide contains an epitope of an antigen expressed by new born larvae..

3. Composition according to claim 2, wherein the peptide contains an epitope of one of the following antigens: nbl 1700, SS1 and SS2 antigens.

4. Composition according to claim 1, wherein the peptide contains an epitope of an antigen expressed and/or secreted or excreted by muscle larvae.

5. Composition according to claim 4, wherein the peptide contains an epitope of one of the following ES-antigen components: 43kDa, 53kDa, 28kDa and 21kDa.

6. Composition according to any one of the preceding claims, comprising at least 2 peptides.

7. Composition according to claim 6, wherein the peptides are included in form of a mixture.

8. Composition according to any one of the claims 1-7, comprising peptides in form of a at least one fusion peptide.

9. Composition according to any one of the claims 6-8, wherein the peptides contain epitopes of different antigens from *Trichinella* expressed at different stages of the infection cycle.

10. Composition according to any one of the preceding claims, wherein at least one peptide or fusion peptide is linked to a solid support.

11. Composition according to any one of the preceding claims, comprising a tracer complex of at least one peptide or fusion peptide linked to a marker.

12. Composition according to claim 11, wherein at least one peptide or fusion peptide is linked with the marker via a linker.

13. Composition according to any one of the claims 11 or 12, wherein the marker is a fluorophore.

14. Composition according to claim 11, wherein the tracer complex is adapted to a fluorescence polarisation assay.

15. Composition according to claim 11, wherein the at least one peptide or fusion peptide is linked to the marker via a linker, which reduces or inhibits rotation of the marker relative to the peptide or fusion peptide,

16. Composition according to claim 15, wherein the linker is composed of one or more bulky molecules, which reduce or inhibit rotation of the marker relative to the peptide or fusion peptide.

17. Composition according to claim 15, wherein the linker is composed of one or more bulky amino acids, which reduce or inhibit rotation of the marker relative to the peptide or fusion peptide.

18. Composition according to claim 17, wherein the linker is an amino-acid linker having the sequence Trp-Trp.

19. Composition according to claim 17, wherein the linker is an amino-acid linker containing 1 to 3 prolines.

20. A method for the diagnosis of a *Trichinella* infection in a susceptible animal or human being, which comprises in vitro-detection of antibodies against at least one peptide from *Trichinella* in a tissue sample taken from the animal or human being, with the immunogenic peptide containing a series of amino acids (amino acid sequence) that form a continuous or discontinuous epitope recognized by sera from pigs infected with *Trichinella*.

21. Method according to claim 14, wherein the tissue sample is contacted with a diagnostic composition according to claims 1-13.

22. Method according to claim 15, wherein the animal is selected from a group comprising mammals and reptiles, including swine, wild swine, equines, carnivores, bear, fox, marten, sheep, cattle and humans.

23. Method according to claim 15, wherein the tissue used as sample is selected from body fluids, especially blood, serum, plasma, urine, saliva and meat juice.

24. Method according to claim 15, wherein the presence of antibodies in the sample is detected by testing whether or not a binding reaction of antibodies in the sample with the immunogenic peptide in the diagnostic composition has occurred.

25. Method according to claim 18, wherein the binding reaction is detected by a homogenous assay.

26. Method according to claim 16, wherein the homogenous assay is a fluorescence polarisation assay.

27. A kit for the diagnosis of a *Trichinella* infection in a sample taken from a susceptible animal or human being, which comprises the diagnostic composition according to claims 1-19.

Trichinella spiralis newborn larvae-specific protein SS1 (AF331159)

```
1          10          20          30          40          50          60          70          80          90
MFISIIVILISLKTCIAQVATCKNDNDANVDWYFVYKPPNVLSSKILQSGVNPAWAASRANINQGAGHSIIRTMASFVVHHAQINVLAYSDDPPNLPPR
                                                                                  (SEQ ID NO: 2) QINVLAYSDDPPNLPPR

100         110         120         130         140         150         160         170         180         190
NEKSKTKGVLLVNNAADEAAWFVHTVPNFLAYLNAYSWPPAETPKGHMFLCVSFNKAHLNSVGKAIRYQEPYVYANNLPAAILNQNMELFNLINGIDVR
NEKSKTKG                                                                (SEQ ID NO: 3) IRYQEPYVYANNLPAAILNQN

 200         210         220         230         240         250         260         270         280         290
VTSFLAHETFATKSVQAVANIQAFGKHSKSFADMYARILRNRFAASIMVWSPADARSKSICKGQHKLQKITSIQLDGVQVSREADSAKWALIDGKNTVC

  300         310         320         330
FTTNDYTATEKRTPGAAVCLENAGVYNAFRTAALNVEACNN
```

Fig. 1

Trichinella spiralis newborn larvae-specific protein SS2 (AF331160)

```
                    20                  40                  60                  80
MHKITHKSIVSRHTFAVYLLVSGQKLQYIYIFICKMIRRLFQYTSMTFAWILLFLSAASPSLGAFECGVPHFKPYIWKSGRIVGGTDVRPHSHPWQIQLL
MHKITHKSIVSRHTFAVYLLVSGQK (SEQ ID NO 5)                          (SEQ ID NO 6) VGGTDVRPHSHPWQIQLL
                   120                 140                 160                 180

KSETGGYSSLCGGSLVHFGKPSNGTRFVLTAAHCITTSNMYPRTSRFTVVTGAHNIKMHEKEKKRIPITSYYVQHWNPVMTTNDIALLRLAETVYYNEYT
KSETG
                   220                 240                 260                 280

RPVCLPEPNEELTPGDICVVTGWGDTTENGTTSNTLKQVGVKIMKKGTCANVRSEVITFCAGAMEGGKDSCQGDSGGPLICKKNGKSVQFGVVSYGTGCA

                   320                 340                 360                 380
RKGYPGVYAKVPSYVTWLNKAAKELENSPEGTVKWASKEDSPVDLSTASRPTNPYTGSRPTSPSSGSRPTYPSSGSRPTSPSSGSRPTYPSSGSRPTYPS
                                  (SEQ ID NO 7) LSTASRPTNPYTGSRPTSPSSGSRP      (SEQ ID NO 8) PTYPSSGSRPTYPS
                                                                               (SEQ ID NO 9) PTYPSSGSRPTYPS

                   420                 440                 460
SGSRPTYPYTGSRPTPQKPVFPSYQKYPPAVQKYIDSLPSGTQGTLEYTVTQNGVTTTTYYHFSK
SGSRPTYPYTG
SGSRP
```

Fig. 2

Trichinella spiralis glutamic acid-rich protein cNBL1700 (AY092820)

```
1          10         20         30         40         50         60         70         80         90
MWLFRCPIYFVLLQLFFLTFLTVTSSNAIPGRSSSRLRLLERYDSLPSLRSHSEDRYDDGVDRKWKKREGNSDDICTEDETTVIEKESENGVDKEKPTS
                                                                                 (SEQ ID NO:11) EKESENGVDKEKPTS

100        110        120        130        140        150        160        170        180        190
KEESGEKTSQEKESEEKSSQEKDEDKSESEASEEKDVSQEQNSKEEKGASEEDEDTPEEQNSKEENGSSEEDDEDASEEQASNEEKEASEEKNTVSEER
KE

  200        210        220        230        240        250        260        270        280        290
KGASEEEDEEKDDGHESEVESQASEEQTTEEGASEEEDEESASEEQTSEGEEKGASQEEEEDEGNEQESEVESQASEEQTSEEEESASEEEDEENESKE
                                                                  (SEQ ID NO:12) NEQESEVESQASEEQTS

  300        310        320        330        340        350        360        370        380        390
QTTEEEESASEEEDEESASEREEKNASQEEEEDEGNESKEQTTEEEESASEEEDEESVSEEQTSEGEEKGASQEEEEDEGNDQESEVESQASEEQTSEE

    400        410        420        430        440        450        460        470        480        490
EGASEEEDEENESEEQTTEEESASEEEDEESASEGEEKNASQEEEEDEGNEQESEVESQASEEQTSEEEEKEGASQEEDEENESEEQTSEEEEEGASEE

      500        510        520        530        540        550        560        570
EDEESAFEEQTSEEEEEKGASQEEEEDEENEQESEVESQASEEQTSEEEGASEEGQDASEEEDEDESEEEESDESV
```

Fig. 3

Trichinella spiralis 43 kDa secreted glycoprotein (M95499)

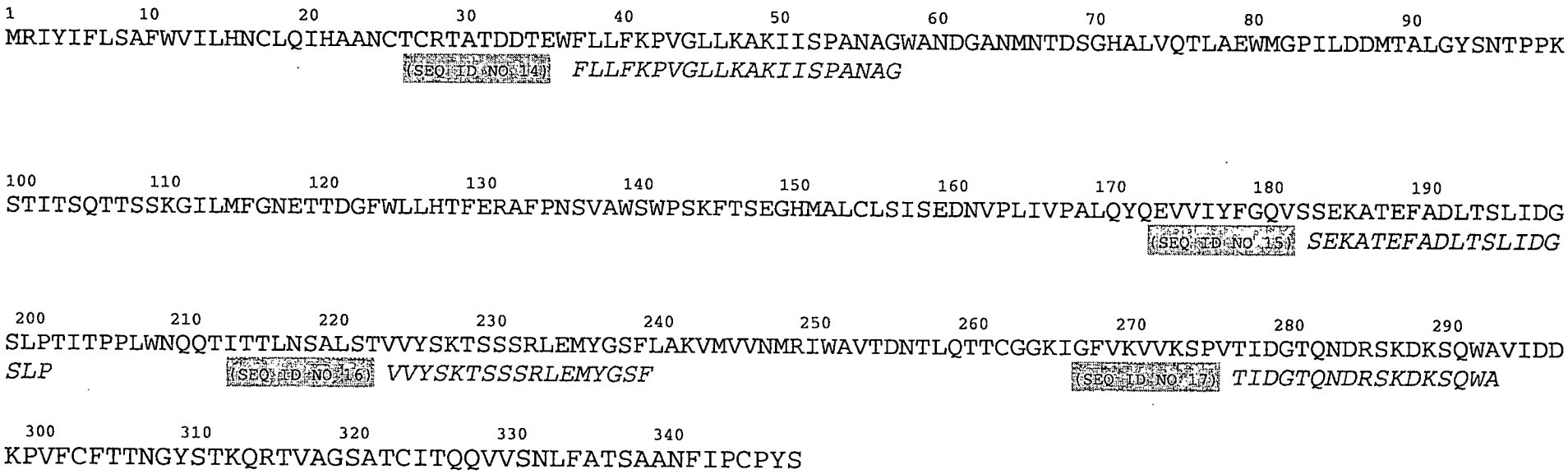

Fig. 4

Trichinella spiralis 53kDa excretory/secretory antigen (U25127)

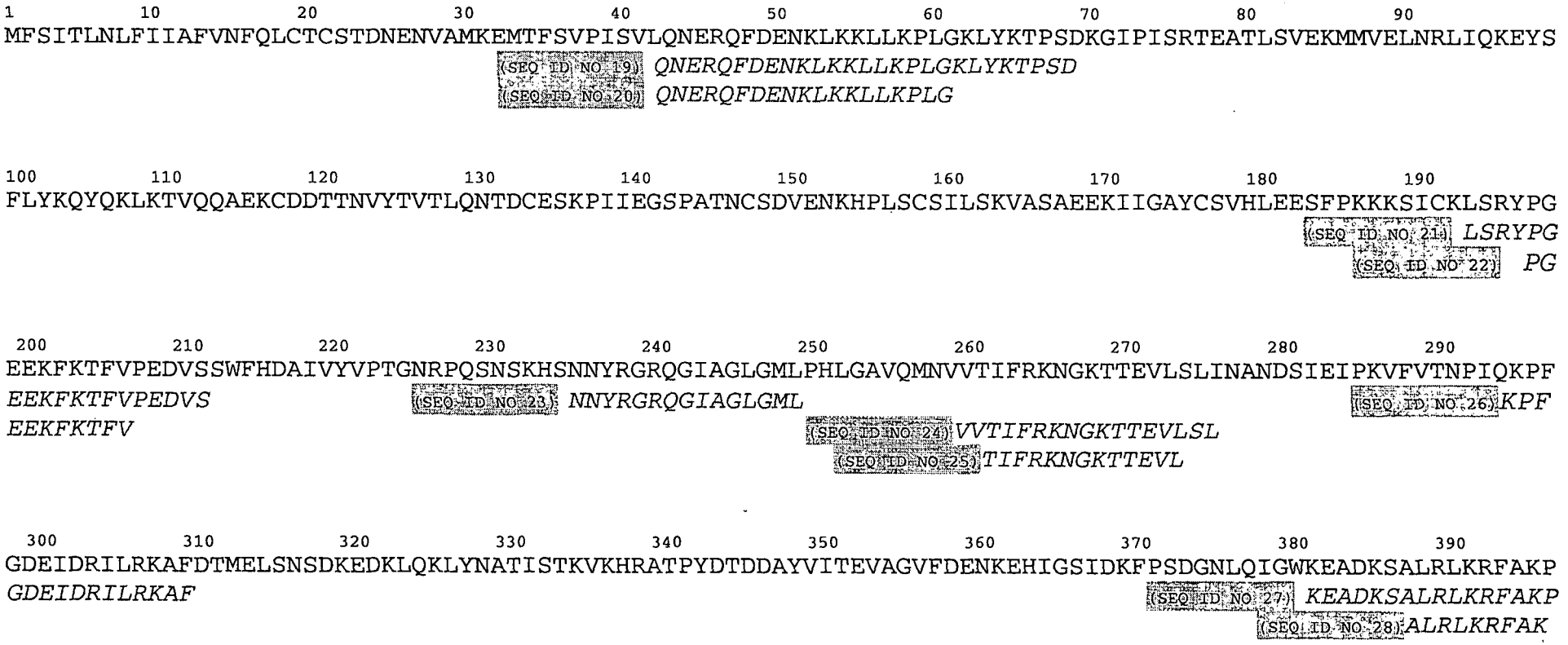

Fig. 5

Trichinella pseudospiralis 21 kDa excretory/secretory protein (AF269089)

```
1          10          20          30          40          50          60          70          80          90
QNMHCQYILSLLLLSLNVVFFAAGDSLDSVDDKSRRCTDEQTEVCAKTECKAEDAAMTELLLEGESDITEHPDFVYYTRCMQRCCAKLNGAKVAPLKEE

100         110         120         130         140         150         160         170
EKRRGPTKLPFQSIFDVADQQTVERCDATMCKSQRMKYESLVARTTSYKKLRASQELRDYKECIESCDAKLNGRQ
                        (SEQ ID NO:30) KSQRMKYESLVARTTSYKKLR
```

Fig. 6

Trichinella pseudospiralis 28 kDa excretory/secretory protein (AF348087)

```
1          10          20          30          40          50          60          70          80          90
MVHFKVMNINITLLFAIILLQFISNASTERFRKLKKESMPAAVKEHLKKLMKNSIVQQSGHESEGGIVEETKQVLQKSHDSFYHLEGTIHKLEEKLEKE

100         110         120         130         140         150         160         170         180         190
KKLYDPWDKKDNSAKRLALGFFVRVAKQYREGLLNESGMMAGIRQPRKKCFVKYSMLDEYSATTEEDDKILMKIERKFYKCESQCQSNTKMKDFYTKDL

200         210         220         230         240
CILKCFEKKLDKFAEKLGVPFDEAKVNEGVNQLQDLDKSVVPFTSI
      EKKLDKFAEKLGVPFDEAKVN (SEQ ID NO 32)
```

Fig. 7

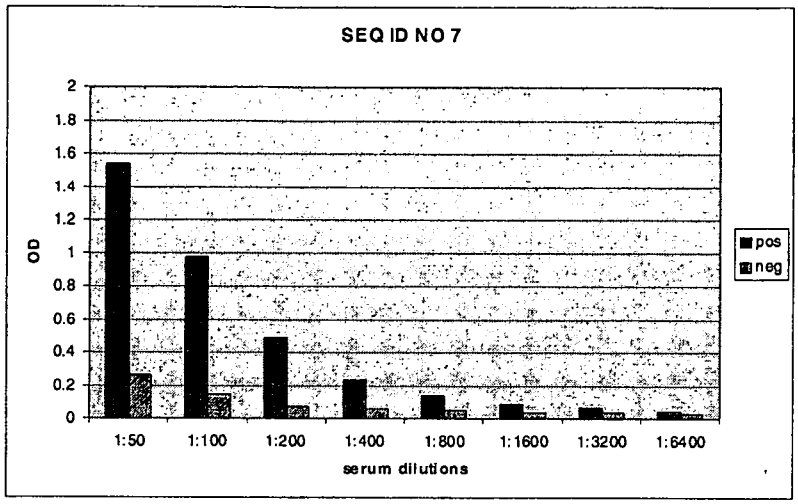
SEQ ID NO 7
OD
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1:50
1:100
1:200
1:400
1:800
1:1600
1:3200
1:6400
serum dilutions
pos
neg

Fig. 8a

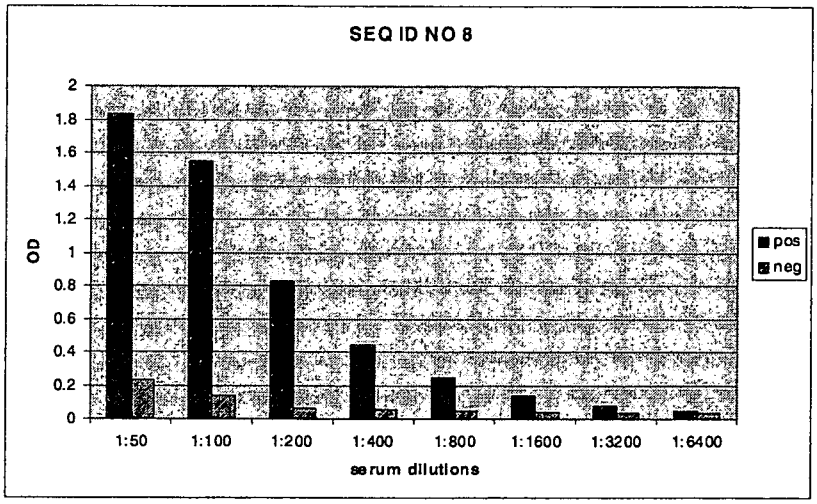
SEQ ID NO 8
OD
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1:50
1:100
1:200
1:400
1:800
1:1600
1:3200
1:6400
serum dilutions
pos
neg

Fig. 8b

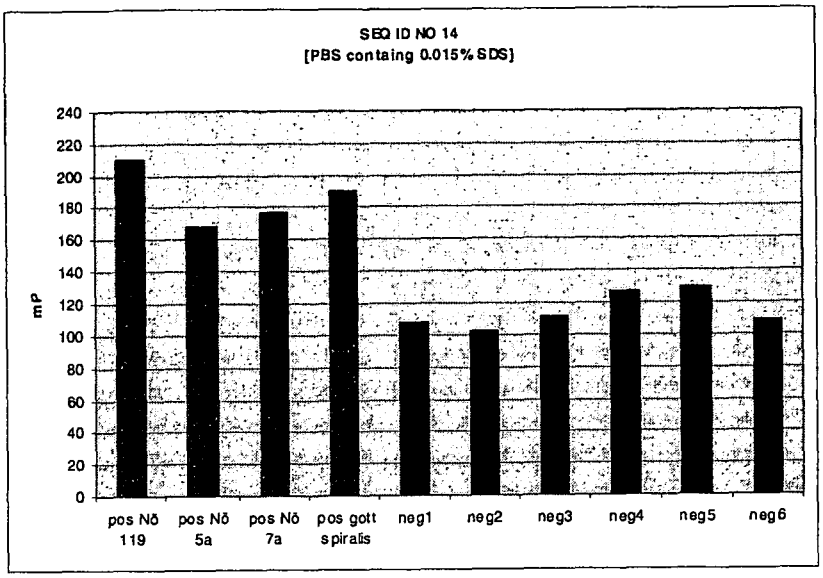
SEQ ID NO 14
[PBS containg 0.015% SDS]
mP
240
220
200
180
160
140
120
100
80
60
40
20
0
pos Nõ 119
pos Nõ 5a
pos Nõ 7a
pos gott spiralis
neg1
neg2
neg3
neg4
neg5
neg6

Fig. 9a

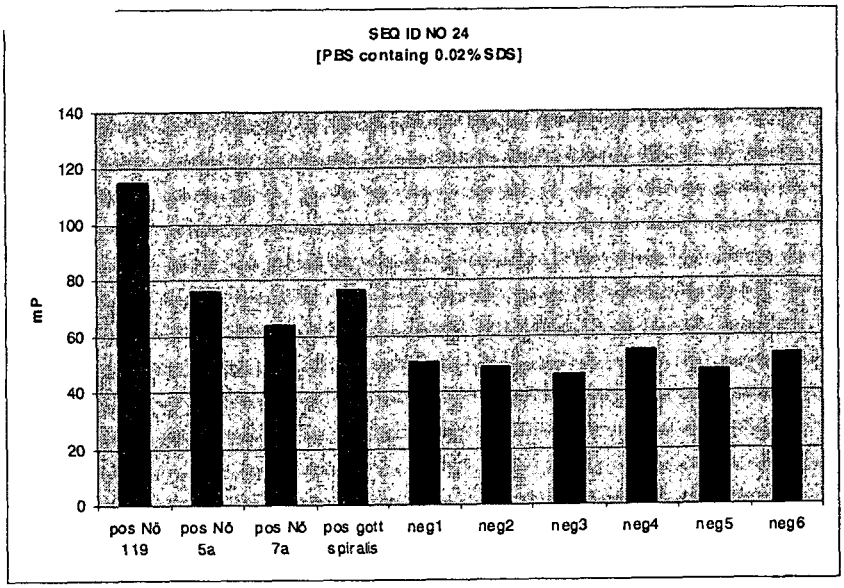
SEQ ID NO 24
[PBS containg 0.02% SDS]
mP
140
120
100
80
60
40
20
0
pos Nõ 119
pos Nõ 5a
pos Nõ 7a
pos gott spiralis
neg1
neg2
neg3
neg4
neg5
neg6

Fig. 9b

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2007/005774

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/68 G01N33/569 G01N33/53 C07K14/435 C12N9/64
A61K39/00

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N C07K C12N A61K

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BIOSIS, EMBASE, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96/30044 A (UNIV COLORADO RES [US]) 3 October 1996 (1996-10-03) page 7, line 20 - page 9, line 3 page 11, line 19 - page 12, line 8 page 18, lines 7-23 page 21, lines 13-25 page 25, line 1 - page 26, line 24 page 27, line 15 - page 28, line 15 page 30, lines 23-28 page 32, lines 13-21 example 9 claims 34,41-59 ----- -/--	1-27

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

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 October 2007	26/10/2007

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

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2007/005774

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 94/17824 A (UNIV COLORADO RES [US]) 18 August 1994 (1994-08-18) page 8, line 21 - page 10, line 23 page 11, lines 4-18 page 12, lines 4-16 page 27, line 8 - page 28, line 11 page 29, lines 11-25 page 33, lines 3-21 claims 50,51,56,62-64,67-76	1-27
X	ZHANG ET AL: "Development and evaluation of an immunochromatographic strip for trichinellosis detection" VETERINARY PARASITOLOGY, ELSEVIER SCIENCE, AMSTERDAM, NL, vol. 137, no. 3-4, 30 April 2006 (2006-04-30), pages 286-293, XP005364351 ISSN: 0304-4017 abstract page 287, right-hand column, paragraph 3 - page 290, left-hand column, paragraph 2 page 290, right-hand column, paragraph 3.4. - page 291, left-hand column, paragraph 1 page 292, left-hand column, paragraph 2 - right-hand column, paragraph 1	1-27
Y	DENKERS E Y ET AL: "THE MOUSE ANTIBODY RESPONSE TO TRICHINELLA SPIRALIS DEFINES A SINGLE, IMMUNODOMINANT EPITOPE SHARED BY MULTIPLE ANTIGENS" JOURNAL OF IMMUNOLOGY, THE WILLIAMS AND WILKINS CO. BALTIMORE, US, vol. 144, no. 8, 15 April 1990 (1990-04-15), pages 3152-3159, XP002027466 ISSN: 0022-1767 abstract	1-27
Y	DEA-AYUELA MARIA A ET AL: "TRICHINELLA ANTIGENS: A REVIEW" VETERINARY RESEARCH, ELSEVIER, PARIS, NL, vol. 30, no. 6, November 1999 (1999-11), pages 559-571, XP008069175 ISSN: 0928-4249 abstract	1-27
E	WO 2007/090960 A (AGRONOMIQUE INST NAT RECH [FR]; AGENCE FRANCAISE DE SECURITE S [FR]; J) 16 August 2007 (2007-08-16) the whole document	1-27

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2007/005774

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9630044 A	03-10-1996	AU 5379396 A	16-10-1996
		CA 2215529 A1	03-10-1996
WO 9417824 A	18-08-1994	AT 215383 T	15-04-2002
		AU 6129794 A	29-08-1994
		DE 69430301 D1	08-05-2002
		EP 0682527 A1	22-11-1995
		US 5541075 A	30-07-1996
		US 5707817 A	13-01-1998
		US 5686256 A	11-11-1997
WO 2007090960 A	16-08-2007	FR 2897063 A1	10-08-2007

专利名称(译)	用于检测旋毛虫感染的诊断组合物和方法		
公开(公告)号	EP2160608A1	公开(公告)日	2010-03-10
申请号	EP2007764944	申请日	2007-06-29
[标]申请(专利权)人(译)	普利奥尼克斯股份公司		
申请(专利权)人(译)	Prionics公司		
当前申请(专利权)人(译)	Prionics公司		
[标]发明人	BONILLA PINSCHEWER WELDY STAMM CHRISTOPH BUHOLZER PATRIK RABER ALEX PRICE PAUL ZWALD DANIEL MARTI ROGER WEIDMANN JURG		
发明人	BONILLA PINSCHEWER, WELDY STAMM, CHRISTOPH BUHOLZER, PATRIK RÄBER, ALEX PRICE, PAUL ZWALD, DANIEL MARTI, ROGER WEIDMANN, JÜRG		
IPC分类号	G01N33/68 G01N33/569 G01N33/53 C07K14/435 C12N9/64 A61K39/00		
CPC分类号	G01N33/5308 C07K14/4354 G01N33/569 G01N33/6854 G01N2333/4353		
外部链接	Espacenet		

摘要(译)

一种诊断组合物，用于检测从被旋毛虫感染的动物或人获得的样品中的抗体，其包含至少一种含有一系列氨基酸的肽，所述氨基酸形成由感染旋毛虫的猪的血清识别的连续或不连续表位。