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(54) **ANTIBODIES HAVING SPECIFICITY FOR THE ORF2I PROTEIN OF HEPATITIS E VIRUS AND USES THEREOF FOR DIAGNOSTIC PURPOSES**

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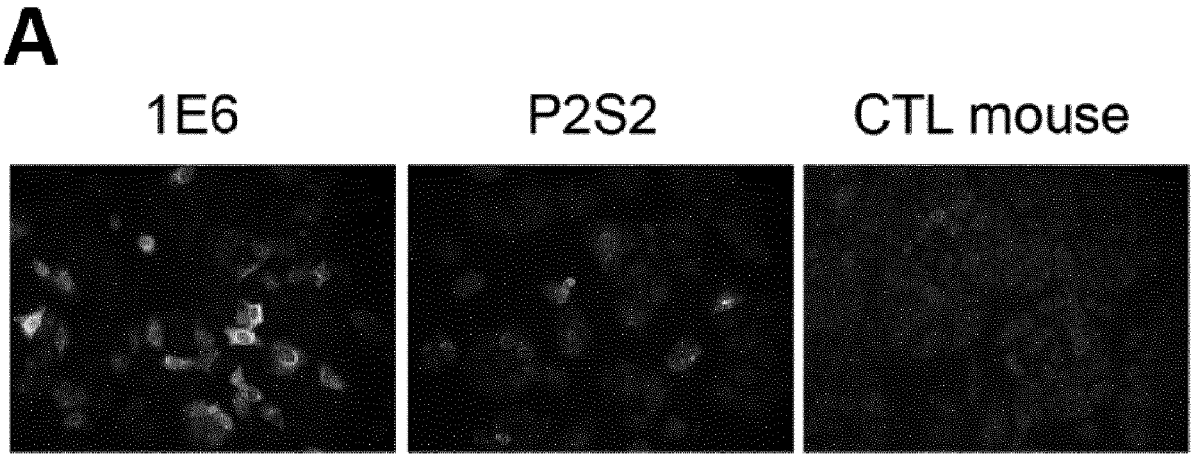
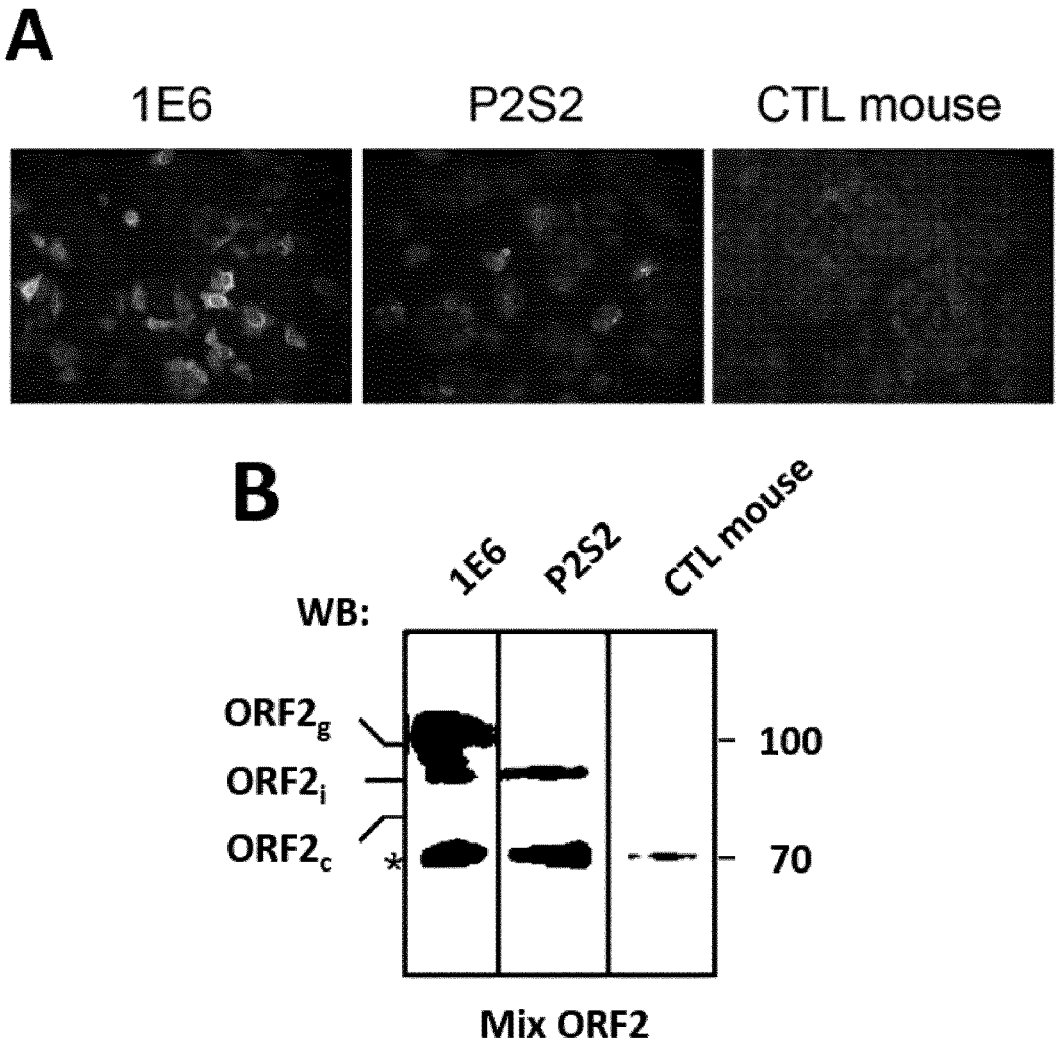
(57) **ABSTRACT**

Hepatitis E virus (HEV) is annually responsible for 20 million infections with 3.4 million symptomatic cases and 70,000 deaths mainly occurring in less developed regions of the world. HEV is a non-enveloped virus containing a linear, single-stranded, positive-sense RNA genome that contains three open reading frames (ORFs), namely, ORF1, ORF2 and ORF3. ORF2 encodes the ORF2 viral capsid protein, which is involved in particle assembly, binding to host cells and eliciting neutralizing antibodies. Recently, 3 different forms of the ORF2 capsid protein were identified: infectious/intracellular ORF2 (ORF2i), glycosylated ORF2 (ORF2g), and cleaved ORF2 (ORF2c). The ORF2i protein, for which the precise sequence has been identified, is the form that is associated with infectious particles and thus antibodies having specificity for the ORF2i protein would be suitable for the diagnosis of HEV. The present fulfills this need by providing an antibody which binds to the ORF2i protein of hepatitis E virus and wherein said antibody does not bind to the ORF2g protein nor to the ORF2c of hepatitis E virus, and wherein the epitope of said antibody comprises at least one amino acid residue from amino acid residues 542 to 555 of SEQ ID NO: 1.

Specification includes a Sequence Listing.

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1E6
P2S2
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B
1E6
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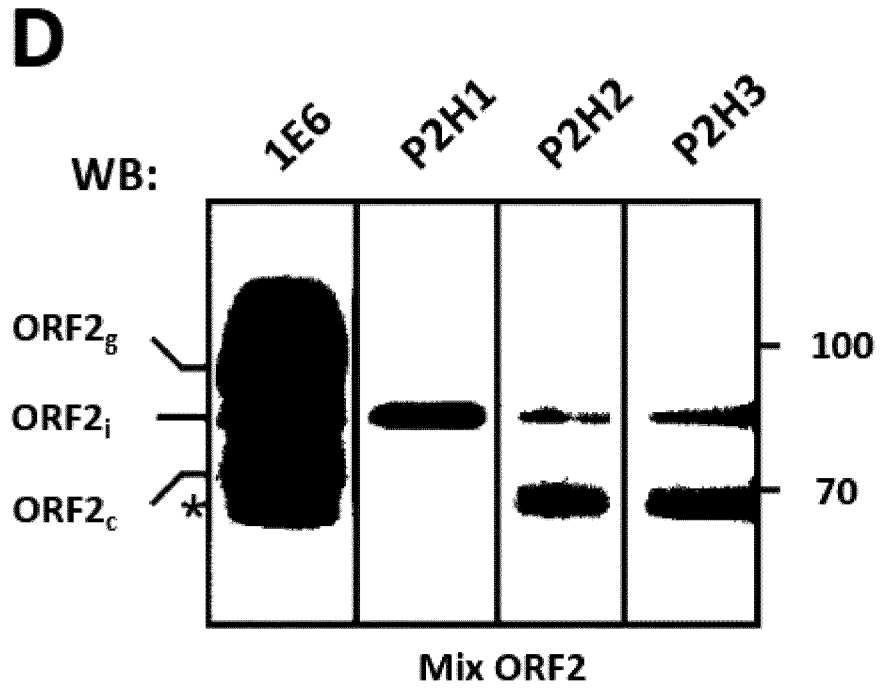
WB:
ORF2g
ORF2i
ORF2c
*
100
70
Mix ORF2
C
1E6
P2H1
P2H2
P2H3

Figure 1A-1C



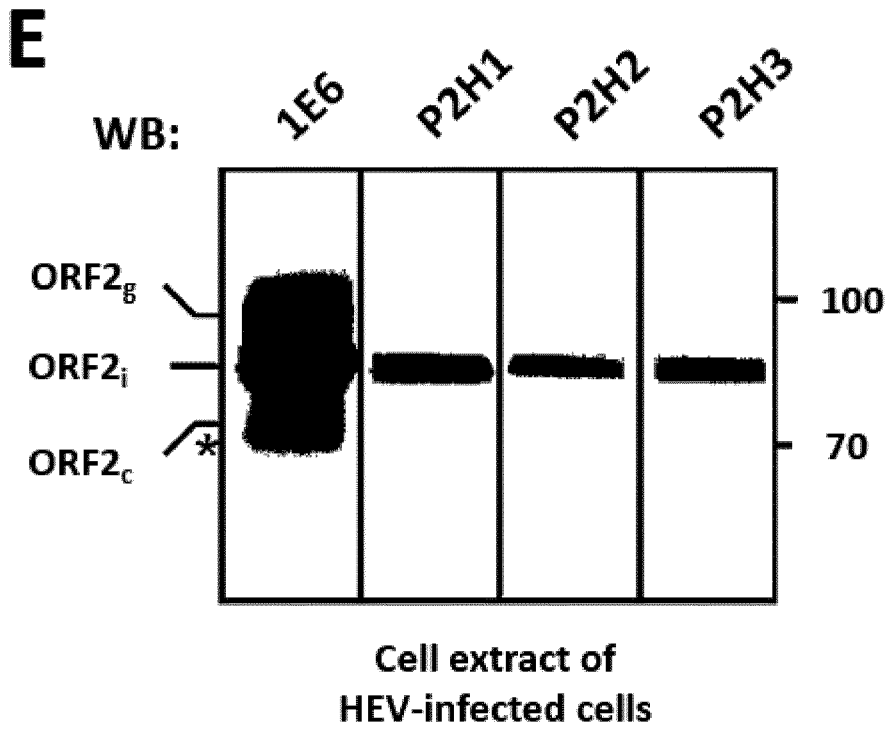


Figure 1D and 1E

ANTIBODIES HAVING SPECIFICITY FOR THE ORF2I PROTEIN OF HEPATITIS E VIRUS AND USES THEREOF FOR DIAGNOSTIC PURPOSES

FIELD OF THE PRESENT INVENTION

[0001] The present invention relates to antibodies having specificity for the ORF2i protein of hepatitis E virus and uses thereof for diagnostic purposes.

BACKGROUND OF THE PRESENT INVENTION

[0002] Hepatitis E virus (HEV) is annually responsible for 20 million infections with 3.4 million symptomatic cases and 70,000 deaths mainly occurring in less developed regions of the world. It causes acute hepatitis either as water-borne outbreaks or as sporadic cases through the fecal-oral route (Debing Y, Moradpour D, Neyts J, et al. *Update on Hepatitis E Virology:Implications for Clinical Practice. Journal of Hepatology* 2016; 65:200-212). Though infection by HEV is usually self-resolving, severe forms or chronic infections have been described, mainly in immunocompromised patients. A high rate of mortality has also been reported among pregnant women. HEV infection has also been associated with extrahepatic disorders (Pischke S, Hartl J, Pas S D, et al. *Hepatitis E virus infection beyond the liver? Journal of Hepatology* 2016. 10.1016/j.jhep.2016.11.016). Four genotypes (gt) are pathogenic in humans. Gt1 and gt2 exclusively infect humans, whereas gt3 and gt4 are zoonotic and mainly infect mammalian animals with occasional transmission to humans. Recently, gt3 infections have been emerging in the Western world likely due to the consumption of contaminated food and blood transfusion (Debing Y, Moradpour D, Neyts J, et al. *Update on Hepatitis E Virology:Implications for Clinical Practice. Journal of Hepatology* 2016; 65:200-212). The diagnosis of hepatitis E is based on detecting anti-HEV antibodies and/or viral RNA in patient serum (Khuroo M S, Khuroo M S. *Hepatitis E: an emerging global disease—from discovery towards control and cure. Journal of Viral Hepatitis* 2016; 23:68-79). Recently, a new assay based on detection of the HEV antigen capsid protein was developed (Wantaï Biologicals) notably for laboratories with no molecular diagnosis facilities.

[0003] HEV is a quasi-enveloped virus containing a linear, single-stranded, positive-sense RNA genome that contains three open reading frames (ORFs), namely, ORF1, ORF2 and ORF3 (Debing Y, Moradpour D, Neyts J, et al. *Update on Hepatitis E Virology:Implications for Clinical Practice. Journal of Hepatology* 2016; 65:200-212). ORF1 is the largest gene that encodes a non-structural polyprotein (ORF1 protein) that contains several functional domains essential for viral replication. ORF2 encodes the ORF2 viral capsid protein, which is involved in particle assembly, binding to host cells and eliciting neutralizing antibodies. ORF3 encodes a small multifunctional phosphoprotein that is involved in virion morphogenesis and egress. Although HEV is a non-enveloped virus in bile and feces, patient serum and cell culture-produced particles have been described to be associated with cellular lipids and display the ORF3 protein at their surface (Okamoto H. *Culture systems for hepatitis E virus. J Gastroenterol* 2012; 48:147-158).

[0004] Recently, 3 different forms of the ORF2 capsid protein were identified: infectious/intracellular ORF2 (ORF2i), glycosylated ORF2 (ORF2g), and cleaved ORF2 (ORF2c) (Montpellier C., et al. "*Hepatitis E virus lifecycle and identification of 3 forms of the ORF2 capsid protein.*" *Gastroenterology* 154.1 (2018): 211-223). The ORF2i protein, for which the precise sequence has been identified, is the form that is associated with infectious particles. The ORF2i protein is not glycosylated and is likely not translocated into the endoplasmic reticulum (ER) lumen and stays in the cytosolic compartment. In contrast, ORF2g and ORF2c proteins are secreted in large amounts in cell culture and infected patient sera, sialylated, N- and O-glycosylated but are not associated with infectious virions. Importantly, ORF2g and ORF2c proteins are the main antigens present in HEV-infected patient sera. Accordingly antibodies having specificity for the ORF2i protein would be suitable for the diagnosis of HEV.

SUMMARY OF THE PRESENT INVENTION

[0005] The present invention relates to antibodies having specificity for the ORF2i protein of hepatitis E virus and uses thereof for diagnostic purposes. In particular, the present invention is defined by the claims.

DETAILED DESCRIPTION OF THE PRESENT INVENTION

[0006] The first object of the present invention relates to an antibody which binds to the ORF2i protein of hepatitis E virus and wherein said antibody does not bind to the ORF2g protein nor to the ORF2c of hepatitis E virus, and wherein the epitope of said antibody comprises at least one amino acid residue from amino acid residues 542 to 555 of SEQ ID NO: 1.

[0007] The terms "peptide", "polypeptide", and "protein" are used interchangeably, and refer to a compound comprised of amino acid residues covalently linked by peptide bonds. A polypeptide is not limited to a specific length: it must contain at least two amino acids, and no limitation is placed on the maximum number of amino acids that can comprise a polypeptide's sequence. Peptides, oligopeptides, and proteins are included within the definition of polypeptide, and such terms may be used interchangeably herein unless specifically indicated otherwise. As used herein, the term refers to both short chains, which also commonly are referred to in the art as peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, of which there are many types. In one embodiment, as used herein, the term "peptides" refers to a linear polymer of amino acids linked together by peptide bonds, preferably having a chain length of less than about 50 amino acids residues; a "polypeptide" refers to a linear polymer of at least 50 amino acids linked together by peptide bonds; and a protein specifically refers to a functional entity formed of one or more peptides or polypeptides, optionally glycosylated, and optionally of non-polypeptides cofactors. This term also does exclude post-expression modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations and the like, as well as other modifications known in the art, both naturally occurring and non-naturally occurring. A polypeptide may be an entire protein, or a subsequence thereof. "Polypeptides" include, for example, biologically active

fragments, substantially homologous polypeptides, oligopeptides, homodimers, heterodimers, variants of polypeptides, modified polypeptides, derivatives, analogs, fusion proteins, among others. A polypeptide includes a natural peptide, a recombinant peptide, or a combination thereof.

[0008] As used herein, the term "ORF2i protein" refers to the hepatitis E virus ORF2 capsid protein (ORF2i) comprising an amino acid sequence having at least 90% of identity with the amino acid sequence as set forth in SEQ ID NO:1 and wherein said protein is not glycosylated.

SEQ ID NO: 1:

[1]LPMLPAPPAGQPSGRRRGRRSGGAGGGFWGDRVDSQPFALPYIHPTNPF

AADIVSQSGAGTRPRQPPRPLGSAWRDQSQRPSAAPRRRSAPAGAAPLTA

VSPAPDTAPVPDVDSRGAILRRQYNLSTSPLTSSVASGTNLVLYAAPLNP

LLPLQDGTNTHIMATEASNYAQYRVVRATIRYRPLVPNAVGGYAISISFW

PQTTTTPTSVDMNSITSTDVRILVQPGIASELVIPSERLHYRNQGWRSVE

TTGVAEEEATSGLVMLCIHGSPVNSYTNTPYTGALGLLDFALELEFRNLT

PGNTNTRVSRYTSTARHRLRRGADGTAELTTTAATRFMKDLHFTGTNGVG

EVGRGIALTLFNLADTLLGGLPTELISSAGGQLFYSRPVVSANGEPTVKL

YTSVENAQQDKGITIPHDIDLGDSRVVIQDYDNQHEQDRPTPSPAPSRPF

SVLRANDVLWLSLTAAEYDQATYGSSTNPMYVSDTVTFVNVATGAQAVAR

SLDWSKVTLDGRPLTTIQQYSKTFYVLPLRGKLSFWEAGTTR[542]**AGYPY**

NYNTTASDQ[555]ILIENAAGHRVAISTYTTSLGAGPASISAVGVLAPHSA

LAVLEDTVDYPARAHTFDDFCPECRTLGLQGCAFQSTIAELQRLKTEVGK

TRES

[0009] According to the invention, the mass of the ORF2i protein of the present invention is approximately 80 kDa.

[0010] As used herein, the term "ORF2g protein" refers to the hepatitis E virus ORF2 capsid protein (ORF2g) comprising an amino acid sequence having at least 90% of identity with the amino acid sequence as set forth in SEQ ID NO:2 and wherein said protein is glycosylated.

SEQ ID NO: 2:

SGGAGGGFWGDRVDSQPFALPYIHPTNPFAADIVSQSGAGTRPRQPPRPL

GSAWRDQSQRPSAAPRRRSAPAGAAPLTAVSPAPDTAPVPDVDSRGAILR

RQYNLSTSPLTSSVASGTNLVLYAAPLNPLLPLQDGTNTHIMATEASNYA

QYRVVRATIRYRPLVPNAVGGYAISISFWPQTTTTPTSVDMNSITSTDVR

ILVQPGIASELVIPSERLHYRNQGWRSVETTGVAEEEATSGLVMLCIHGS

PVNSYTNTPYTGALGLLDFALELEFRNLTPGNTNTRVSRYTSTARHRLRR

GADGTAELTTTAATRFMKDLHFTGTNGVGEVGRGIALTLFNLADTLLGGL

PTELISSAGGQLFYSRPVVSANGEPTVKLYTSVENAQQDKGITIPHDIDL

GDSRVVIQDYDNQHEQDRPTPSPAPSRPFSVLRANDVLWLSLTAAEYDQA

TYGSSTNPMYVSDTVTFVNVATGAQAVARSLDWSKVTLDGRPLTTIQQYS

KTFYVLPLRGKLSFWEAGTTRAGYPYNYNTTASDQILIENAAGHRVAIST

-continued

YTTSLGAGPASISAVGVLAPHSALAVLEDTVDYPARAHTFDDFCPECRTL

GLQGCAFQSTIAELQRLKTEVGKTRES

[0011] According to the invention, the mass of the ORF2g protein of the present invention is approximately 90 kDa.

[0012] As used herein, the term "ORF2c protein" refers to the hepatitis E virus ORF2 capsid protein (ORF2c) comprising an amino acid sequence having at least 90% of identity with the amino acid sequence as set forth in SEQ ID NO:3 and wherein said protein is glycosylated.

SEQ ID NO: 3

SAPAGAAPLTAVSPAPDTAPVPDVDSRGAILRRQYNLSTSPLTSSVASGT

NLVLYAAPLNPLLPLQDGTNTHIMATEASNYAQYRVVRATIRYRPLVPNA

VGGYAISISFWPQTTTTPTSVDMNSITSTDVRILVQPGIASELVIPSERL

HYRNQGWRSVETTGVAEEEATSGLVMLCIHGSPVNSYTNTPYTGALGLLD

FALELEFRNLTPGNTNTRVSRYTSTARHRLRRGADGTAELTTTAATRFMK

DLHFTGTNGVGEVGRGIALTLFNLADTLLGGLPTELISSAGGQLFYSRPV

VSANGEPTVKLYTSVENAQQDKGITIPHDIDLGDSRVVIQDYDNQHEQDR

PTPSPAPSRPFSVLRANDVLWLSLTAAEYDQATYGSSTNPMYVSDTVTFV

NVATGAQAVARSLDWSKVTLDGRPLTTIQQYSKTFYVLPLRGKLSFWEAG

TTRAGYPYNYNTTASDQILIENAAGHRVAISTYTTSLGAGPASISAVGVL

APHSALAVLEDTVDYPARAHTFDDFCPECRTLGLQGCAFQSTIAELQRLK

TEVGKTRES

[0013] According to the invention, the mass of the ORF2c protein of the present invention is approximately 75 kDa.

[0014] As used herein, the term "glycosylated" with respect to a protein means that a carbohydrate moiety is present at one or more sites of the protein molecule. In particular, a glycosylated protein refers to a protein that is typically modified by N-glycan or O-glycan addition.

[0015] According to the invention a first amino acid sequence having at least 90% of identity with a second amino acid sequence means that the first sequence has 90; 91; 92; 93; 94; 95; 96; 97; 98; 99 or 100% of identity with the second amino acid sequence. Sequence identity is frequently measured in terms of percentage identity (or similarity or homology); the higher the percentage, the more similar are the two sequences. Methods of alignment of sequences for comparison are well known in the art. Various programs and alignment algorithms are described in: Smith and Waterman, Adv. Appl. Math., 2:482, 1981; Needleman and Wunsch, J. Mol. Biol., 48:443, 1970; Pearson and Lipman, Proc. Natl. Acad. Sci. U.S.A., 85:2444, 1988; Higgins and Sharp, Gene, 73:237-244, 1988; Higgins and Sharp, CABIOS, 5:151-153, 1989; Corpet et al. Nuc. Acids Res., 16:10881-10890, 1988; Huang et al., Comp. Appls Biosci., 8:155-165, 1992; and Pearson et al., Meth. Mol. Biol., 24:307-31, 1994). Altschul et al., Nat. Genet., 6:119-129, 1994, presents a detailed consideration of sequence alignment methods and homology calculations. By way of example, the alignment tools ALIGN (Myers and Miller, CABIOS 4:11-17, 1989) or LFASTA (Pearson and Lipman, 1988) may be used to perform sequence comparisons (Inter-

net Program® 1996, W. R. Pearson and the University of Virginia, fasta20u63 version 2.0u63, release date December 1996). ALIGN compares entire sequences against one another, while LFASTA compares regions of local similarity. These alignment tools and their respective tutorials are available on the Internet at the NCSA Website, for instance. Alternatively, for comparisons of amino acid sequences of greater than about 30 amino acids, the Blast 2 sequences function can be employed using the default BLOSUM62 matrix set to default parameters, (gap existence cost of 11, and a per residue gap cost of 1). When aligning short peptides (fewer than around 30 amino acids), the alignment should be performed using the Blast 2 sequences function, employing the PAM30 matrix set to default parameters (open gap 9, extension gap 1 penalties). The BLAST sequence comparison system is available, for instance, from the NCBI web site; see also Altschul et al., J. Mol. Biol., 215:403-410, 1990; Gish. & States, Nature Genet., 3:266-272, 1993; Madden et al. Meth. Enzymol., 266:131-141, 1996; Altschul et al., Nucleic Acids Res., 25:3389-3402, 1997; and Zhang & Madden, Genome Res., 7:649-656, 1997.

[0016] As used herein, the term "antibody" has its general meaning in the art and refers to any antibody-like molecule that has an antigen binding region, and this term includes antibody fragments that comprise an antigen binding domain such as Fab', Fab, F(ab')2, and single domain antibodies (DABs). In natural antibodies, two heavy chains are linked to each other by disulfide bonds and each heavy chain is linked to a light chain by a disulfide bond. There are two types of light chain, lambda (l) and kappa (κ). There are five main heavy chain classes (or isotypes) which determine the functional activity of an antibody molecule: IgM, IgD, IgG, IgA and IgE. Each chain contains distinct sequence domains. The light chain includes two domains, a variable domain (VL) and a constant domain (CL). The heavy chain includes four domains, a variable domain (VH) and three constant domains (CHI, CH2 and CH3, collectively referred to as CH). The variable regions of both light (VL) and heavy (VH) chains determine binding recognition and specificity to the antigen. The constant region domains of the light (CL) and heavy (CH) chains confer important biological properties such as antibody chain association, secretion, transplacental mobility, complement binding, and binding to Fc receptors (FcR). The Fv fragment is the N-terminal part of the Fab fragment of an immunoglobulin and consists of the variable portions of one light chain and one heavy chain. The specificity of the antibody resides in the structural complementarity between the antibody combining site and the antigenic determinant. Antibody combining sites are made up of residues that are primarily from the hypervariable or complementarity determining regions (CDRs). Occasionally, residues from nonhypervariable or framework regions (FR) influence the overall domain structure and hence the combining site. Complementarity Determining Regions or CDRs refer to amino acid sequences which together define the binding affinity and specificity of the natural Fv region of a native immunoglobulin binding site. The light and heavy chains of an immunoglobulin each have three CDRs, designated L-CDR1, L-CDR2, L-CDR3 and H-CDR1, H-CDR2, H-CDR3, respectively. An antigen-binding site, therefore, includes six CDRs, comprising the CDR set from each of a heavy and a light chain V region. Framework Regions (FRs) refer to amino acid sequences interposed between CDRs.

[0017] The term "antibody fragment" refers to at least one portion of an intact antibody, preferably the antigen binding region or variable region of the intact antibody, that retains the ability to specifically interact with (e.g., by binding, steric hindrance, stabilizing/destabilizing, spatial distribution) an epitope of an antigen. Examples of antibody fragments include, but are not limited to, Fab, Fab', $F(ab)_2$, Fv fragments, single chain antibody molecules, in particular scFv antibody fragments, disulfide-linked Fvs (sdFv), a Fd fragment consisting of the VH and CHI domains, linear antibodies, single domain antibodies such as, for example, sdAb (either VL or VH), camelid VHH domains, multispecific antibodies formed from antibody fragments such as, for example, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region, and an isolated CDR or other epitope binding fragments of an antibody. An antigen binding fragment can also be incorporated into single domain antibodies, maxibodies, minibodies, nanobodies, intrabodies, diabodies, triabodies, tetrabodies, v-NAR and bis-scFv (see, e.g., Hollinger and Hudson, Nature Biotechnology 23:1126-1136, 2005). Antigen binding fragments can also be grafted into scaffolds based on polypeptides such as a fibronectin type III (see U.S. Pat. No. 6,703,199, which describes fibronectin polypeptide minibodies). Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, and a residual "Fc" fragment, a designation reflecting the ability to crystallize readily.

[0018] The term "Fab" denotes an antibody fragment having a molecular weight of about 50,000 and antigen binding activity, in which about a half of the N-terminal side of H chain and the entire L chain, among fragments obtained by treating IgG with a protease, papaine, are bound together through a disulfide bond.

[0019] The term "F(ab')2" refers to an antibody fragment having a molecular weight of about 100,000 and antigen binding activity, which is slightly larger than the Fab bound via a disulfide bond of the hinge region, among fragments obtained by treating IgG with a protease, pepsin.

[0020] The term "Fab'" refers to an antibody fragment having a molecular weight of about 50,000 and antigen binding activity, which is obtained by cutting a disulfide bond of the hinge region of the F(ab')2.

[0021] A single chain Fv ("scFv") polypeptide is a covalently linked VH:VL heterodimer which is usually expressed from a gene fusion including VH and VL encoding genes linked by a peptide-encoding linker. "dsFv" is a VH::VL heterodimer stabilised by a disulfide bond. Divalent and multivalent antibody fragments can form either spontaneously by association of monovalent scFvs, or can be generated by coupling monovalent scFvs by a peptide linker, such as divalent sc(Fv)2.

[0022] According to the present invention, the antibody of the present invention has specificity for the ORF2i protein. As used herein, the term "specificity" refers to the ability of an antibody to detectably bind to an epitope presented on the ORF2i protein, while having relatively little detectable reactivity with the ORF2g protein and the ORF2c protein.

[0023] As used herein, the term "epitope" refers to a specific arrangement of amino acids located on a protein to which an antibody binds. Epitopes often consist of a chemi-

cally active surface grouping of molecules such as amino acids or sugar side chains, and have specific three dimensional structural characteristics as well as specific charge characteristics. Epitopes can be linear or conformational, i.e., involving two or more sequences of amino acids in various regions of the antigen that may not necessarily be contiguous. The epitopes of the present invention thus comprise at least one amino acid residue from amino acid residues 542 to 555 of SEQ ID NO: 1 (i.e. SEQ ID NO:4).

SEQ ID NO: 4

AGYPYNYNTTASDQ

[0024] In some embodiments, the antibody of the invention binds to an epitope comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 amino acid residues from SEQ ID NO:4, or from a sequence sharing at least 90% of identity over SEQ ID NO: 4.

[0025] In one embodiment, the antibody of the invention binds to an epitope comprising the amino acid sequence as set forth in SEQ ID NO: 4 or an amino acid sequence sharing at least 90% of identity over SEQ ID NO: 4.

[0026] As used herein, the term "binding" as used herein refers to a direct association between two molecules, due to, for example, covalent, electrostatic, hydrophobic, and ionic and/or hydrogen-bond interactions, including interactions such as salt bridges and water bridges. In particular, as used herein, the term "binding" in the context of the binding of an antibody to a predetermined antigen or epitope typically is a binding with an affinity corresponding to a K_D of about 10^{-7} M or less, such as about 10^{-8} M or less, such as about 10^{-9} M or less, about 10^{-10} M or less, or about 10^{-11} M or even less.

[0027] Specificity can be relatively determined by binding or competitive binding assays, using, e.g., Biacore instruments, as described elsewhere herein. Specificity can be exhibited by, e.g., an about 10:1, about 20:1, about 50:1, about 100:1, 10.000:1 or greater ratio of affinity/avidity in binding to the specific antigen versus nonspecific binding to other irrelevant molecules. The term "affinity", as used herein, means the strength of the binding of an antibody to an epitope. The affinity of an antibody is given by the dissociation constant Kd, defined as [Ab]×[Ag]/[Ab-Ag], where [Ab-Ag] is the molar concentration of the antibody-antigen complex, [Ab] is the molar concentration of the unbound antibody and [Ag] is the molar concentration of the unbound antigen. The affinity constant Ka is defined by 1/Kd. Preferred methods for determining the affinity of mAbs can be found in Harlow, et al., Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1988), Coligan et al., eds., Current Protocols in Immunology, Greene Publishing Assoc. and Wiley Interscience, N.Y., (1992, 1993), and Muller, Meth. Enzymol. 92:589-601 (1983), which references are entirely incorporated herein by reference. One preferred and standard method well known in the art for determining the affinity of mAbs is the use of Biacore instruments.

[0028] In some embodiments, the antibody is a polyclonal antibody or a monoclonal antibody. The term "polyclonal antibody" as used herein refers to multiple immunoglobulins in antiserum produced to an antigen following immunization, and which may recognize and bind to one or more epitopes to that antigen. The terms "monoclonal antibody", "monoclonal Ab", "monoclonal antibody composition", "mAb", or the like, as used herein refer to a preparation of antibody molecules of single molecular composition. Monoclonal antibodies may be generated using the method of Kohler and Milstein (Nature, 256:495, 1975). To prepare monoclonal antibodies useful in the invention, a mouse or other appropriate host animal (e.g. mouse, goat, camelid . . .) is immunized at suitable intervals (e.g., twice-weekly, weekly, twice-monthly or monthly) with a relevant viral antigenic form. Typically, the immunogenic form consists of the peptide having the amino acid sequence ranging from the amino residue at position 542 to 555 in SEQ ID NO: 1 (i.e. SEQ ID NO:4). The animal may be administered a final "boost" of the antigenic form within one week of sacrifice. It is often desirable to use an immunologic adjuvant during immunization. Suitable immunologic adjuvants include Freund's complete adjuvant, Freund's incomplete adjuvant, alum, Ribi adjuvant, Hunter's Titermax, saponin adjuvants such as QS21 or Quil A, or CpG-containing immunostimulatory oligonucleotides. Other suitable adjuvants are well-known in the field. The animals may be immunized by subcutaneous, intraperitoneal, intramuscular, intravenous, intranasal or other routes. Following the immunization regimen, lymphocytes are isolated from the spleen, lymph node or other organ of the animal and fused with a suitable myeloma cell line using an agent such as polyethylene glycol to form a hydridoma. Following fusion, cells are placed in media permissive for growth of hybridomas but not the fusion partners using standard methods, as described (Coding, Monoclonal Antibodies: Principles and Practice: Production and Application of Monoclonal Antibodies in Cell Biology, Biochemistry and Immunology, 3rd edition, Academic Press, New York, 1996). Following culture of the hybridomas, cell supernatants are analyzed for the presence of antibodies of the desired specificity, i.e., that selectively bind the antigen. Suitable analytical techniques include ELISA, immunofluorescence, flow cytometry, immunoprecipitation, and western blotting. Other screening techniques are well-known in the field. Preferred techniques are those that confirm binding of antibodies to conformationally intact, natively folded antigen, such as non-denaturing ELISA, flow cytometry, and immunoprecipitation.

[0029] In some embodiments, the antibody of the present invention is conjugated with a detectable label. Suitable detectable labels include, for example, a radioisotope, a fluorescent label, a chemiluminescent label, an enzyme label, a bio luminescent label or colloidal gold. Methods of making and detecting such detectably-labeled immunoconjugates are well-known to those of ordinary skill in the art, and are described in more detail below. For instance, the detectable label can be a radioisotope that is detected by autoradiography. Isotopes that are particularly useful for the purpose of the present invention are 3H, 125I, 131I, 35S and 14C. The antibody of the present invention can also be labeled with a fluorescent compound. The presence of a fluorescently-labeled antibody of the present invention is determined by exposing the immuno conjugate to light of the proper wavelength and detecting the resultant fluorescence. Fluorescent labeling compounds include fluorescein isothiocyanate, rhodamine, phycoerytherin, phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine and Alexa Fluor dyes. Alternatively, the antibody of the present invention can be detectably labeled by coupling said antibody to a chemiluminescent compound. The presence of the chemiluminescent-tagged immuno conjugate is determined

by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of chemiluminescent labeling compounds include luminol, isoluminol, an aromatic acridinium ester, an imidazole, an acridinium salt and an oxalate ester. Similarly, a bio luminescent compound can be used to label the antibody of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent protein is determined by detecting the presence of luminescence. Bioluminescent compounds that are useful for labeling include luciferin, luciferase and aequorin. Typically, when the antibody is conjugated to an enzyme then, the antibody is incubated in the presence of the appropriate substrate, the enzyme moiety reacts with the substrate to produce a chemical moiety which can be detected, for example, by spectrophotometric, fluorometric or visual means. Examples of enzymes that can be used to detectably label polyspecific immunoconjugates include β-galactosidase, glucose oxidase, peroxidase and alkaline phosphatase. Those of skill in the art will know of other suitable labels which can be employed in accordance with the present invention. The binding of marker moieties to anti-the antibody of the present invention is accomplished using standard techniques known to the art. Typical methodology in this regard is described by Kennedy et al., Clin. Chim. Acta 70: 1, 1976; Schurs et al., Clin. Chim. Acta 81: 1, 1977; Shih et al., Int'U. Cancer 46: 1101, 1990; Stein et al, Cancer Res. 50: 1330, 1990; and Coligan, supra. Moreover, the convenience and versatility of immunochemical detection can be enhanced by using antibodies of the present invention that have been conjugated with avidin, streptavidin, and biotin. {See, e.g., Wilchek et al. (eds.), "Avidin-Biotin Technology," Methods In Enzymology (Vol. 184) (Academic Press 1990); Bayer et al., "Immunochemical Applications of Avidin-Biotin Technology," in Methods In Molecular Biology (Vol. 10) 149-162 (Manson, ed., The Humana Press, Inc. 1992).).

[0030] In some embodiments, the antibody of the present invention is conjugated with a detectable label. Suitable detectable labels include, for example, a radioisotope, a fluorescent label, a chemiluminescent label, an enzyme label, a bio luminescent label or colloidal gold. Methods of making and detecting such detectably-labeled immunoconjugates are well-known to those of ordinary skill in the art, and are described in more detail below. For instance, the detectable label can be a radioisotope that is detected by autoradiography. Isotopes that are antibody of the present invention can also be labeled with a fluorescent compound. The presence of a fluorescently-labeled antibody of the present invention is determined by exposing the immuno conjugate to light of the proper wavelength and detecting the resultant fluorescence. Fluorescent labeling compounds include fluorescein isothiocyanate, rhodamine, phycoerytherin, phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine and Alexa Fluor dyes. Alternatively, the antibody of the present invention can be detectably labeled by coupling said antibody to a chemiluminescent compound. The presence of the chemiluminescent-tagged immuno conjugate is determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of chemiluminescent labeling compounds include luminol, isoluminol, an aromatic acridinium ester, an imidazole, an acridinium salt and an oxalate ester. Similarly, a bio luminescent compound can be used to label the antibody of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent protein is determined by detecting the presence of luminescence. Bioluminescent compounds that are useful for labeling include luciferin, luciferase and aequorin. Typically, when the antibody is conjugated to a fluorescent label as described above, the presence of the fusion protein can be detected with any means well known in the art such as a microscope or microscope or automated analysis system. Typically, when antibody is conjugated to an enzyme then, the enzyme moiety reacts with the substrate to produce a chemical moiety which can be detected, for example, by spectrophotometric, fluorometric or visual means. Examples of enzymes that can be used to detectably label polyspecific immunoconjugates include β-galactosidase, glucose oxidase, peroxidase (e.g. horseradish perodixase) and alkaline phosphatase. Those of skill in the art will know of other suitable labels which can be employed in accordance with the present invention. The binding of marker moieties to anti-the antibody of the present invention is accomplished using standard techniques known to the art. Typical methodology in this regard is described by Kennedy et al., Clin. Chim. Acta 70: 1, 1976; Schurs et al., Clin. Chim. Acta 81: 1, 1977; Shih et al., Int'U. Cancer 46: 1101, 1990; Stein et al, Cancer Res. 50: 1330, 1990; and Coligan, supra. Moreover, the convenience and versatility of immunochemical detection can be enhanced by using antibodies of the present invention that have been conjugated with avidin, streptavidin, and biotin. {See, e.g., Wilchek et al. (eds.), "Avidin-Biotin Technology," Methods In Enzymology (Vol. 184) (Academic Press 1990); Bayer et al., "Immunochemical Applications of Avidin-Biotin Technology," in Methods In Molecular Biology (Vol. 10) 149-162 (Manson, ed., The Humana Press, Inc. 1992).).

[0031] The antibodies of the present invention are particularly of interest for diagnostic purposes. In particular, the antibodies of the present invention are suitable for determining presence of infectious particles of hepatitis E virus in a sample. More particularly, the antibodies of the present invention are suitable for diagnosing hepatitis E virus infection in a subject.

[0032] Accordingly a further object of the present invention relates to a method for detecting the presence of the ORF2i protein in a sample comprising contacting the sample with the antibodies of the present invention under conditions that allow an immunocomplex of the protein and the antibodies to form wherein detection of the immunocomplex indicates the presence of the ORF2i protein in the sample (for instance: immunoprecipitation, immunofluorescence and western blotting).

[0033] As used herein, the term "sample" includes any solid or fluid sample, liable to contain infectious particles of hepatitis E virus. In some embodiments, the sample is selected from the group consisting of ascites; urine; saliva; sweat; milk; synovial fluid; peritoneal fluid; amniotic fluid; percerebrospinal fluid; lymph fluid; lung embolism; cerebrospinal fluid; and pericardial fluid. In some embodiments, the sample is a faeces samples. In some embodiments, the sample is a urine sample. In some embodiments, the sample is a saliva sample. In some embodiments, the sample is a blood sample. As used herein the term "blood sample"

means any blood sample derived from the subject. Collections of blood samples can be performed by methods well known to those skilled in the art. For example, the subject's blood can be drawn by trained medical personnel directly into anti-coagulants such as citrate and EDTA. The whole blood can be separated into the plasma portion, the cells, and platelets portion by refrigerated centrifugation at 3500×G for 2 minutes. After centrifugation, the supernatant is the plasma.

[0034] More particularly, a further object of the present invention relates to a method for detecting the presence of infectious particles of hepatitis E virus in a sample comprising contacting the sample with the antibodies of the present invention under conditions that allow an immunocomplex of the antibody and the infectious particles to form wherein detection of the immunocomplex indicates the presence of the infectious particles in the sample.

[0035] In some embodiments, the detecting methods of the present invention may further comprises contacting the sample with antibodies recognizing the ORF2g and/or ORF2c proteins.

[0036] The detecting methods of the present invention are particularly suitable for diagnosing acute HEV infection, recent HEV infection, chronic HEV infection, weak active HEV infection or cleared HEV infection.

[0037] Assays and conditions for the detection of immunocomplexes are known to those of skill in the art. Such assays include, for example, competition assays, direct reaction assays sandwich-type assays and immunoassays (e.g. ELISA). The assays may be quantitative or qualitative. There are a number of different conventional assays for detecting formation of an antibody-peptide complex comprising a protein of the present invention. For example, the detecting step can comprise performing an ELISA assay, performing a lateral flow immunoassay, performing an agglutination assay, analyzing the sample in an analytical rotor, or analyzing the sample with an electrochemical, optical, or opto-electronic sensor. These different assays are well-known to those skilled in the art.

[0038] For example, any of a number of variations of the sandwich assay technique may be used to perform an immunoassay. Briefly, in a typical sandwich assay, a first antibody of the present invention is immobilized on a solid surface and the sample to be tested is brought into contact with the immobilized antibody for a time and under conditions allowing formation of the immunocomplex. Following incubation, a second antibody of the present invention that is labeled with a detectable moiety is added and incubated under conditions allowing the formation of a ternary complex between any immunocomplex and the labeled antibody. Any unbound material is washed away, and the presence of polypeptide in the sample is determined by observation/detection of the signal directly or indirectly produced by the detectable moiety. Detection may be either qualitative or quantitative. Methods for labeling biological molecules such as antibodies are well-known in the art (see, for example, "Affinity Techniques. Enzyme Purification: Part B", Methods in EnzymoL, 1974, Vol. 34, W. B. Jakoby and M. Wilneck (Eds.), Academic Press: New York, N.Y.; and M. Wilchek and E. A. Bayer, Anal. Biochem., 1988, 171: 1-32). The most commonly used detectable moieties in immunoassays are enzymes and fluorophores. In the case of an enzyme immunoassay (EIA or ELISA), an enzyme such as horseradish perodixase, glucose oxidase, beta-galactosidase, alkaline phosphatase, and the like, is conjugated to the second antibody, generally by means of glutaraldehyde or periodate. The substrates to be used with the specific enzymes are generally chosen for the production of a detectable color change, upon hydrolysis of the corresponding enzyme. In the case of immunofluorescence, the second antibody is chemically coupled to a fluorescent moiety without alteration of its binding capacity. After binding of the fiuorescently labeled antibody to the immunocomplex and removal of any unbound material, the fluorescent signal generated by the fluorescent moiety is detected, and optionally quantified. Alternatively, the second antibody may be labeled with a radioisotope, a chemiluminescent moiety, or a bio luminescent moiety. In some embodiments, the assay utilizes a solid phase or substrate to which the antibody of the present invention is directly or indirectly attached. Accordingly in some embodiments, the antibody of the present invention is attached to or immobilized on a substrate, such as a solid or semi-solid support. The attachment can be covalent or non-covalent, and can be facilitated by a moiety associated with the protein that enables covalent or non-covalent binding, such as a moiety that has a high affinity to a component attached to the carrier, support or surface. In some embodiments, the substrate is a bead, such as a colloidal particle (e.g., a colloidal nanoparticle made from gold, silver, platinum, copper, metal composites, other soft metals, core-shell structure particles, or hollow gold nanospheres) or other type of particle (e.g., a magnetic bead or a particle or nanoparticle comprising silica, latex, polystyrene, polycarbonate, polyacrylate, or PVDF). Such particles can comprise a label (e.g., a colorimetric, chemiluminescent, or fluorescent label) and can be useful for visualizing the location of the proteins during immunoassays. In some embodiments, the substrate is a dot blot or a flow path in a lateral flow immunoassay device. For example, the antibody of the present invention can be attached or immobilized on a porous membrane, such as a PVDF membrane (e.g., an Immobilon™ membrane), a nitrocellulose membrane, polyethylene membrane, nylon membrane, or a similar type of membrane. In some embodiments, the substrate is a flow path in an analytical rotor. In some embodiments, the substrate is a tube or a well, such as a well in a plate (e.g., a microtiter plate) suitable for use in an ELISA assay. Such substrates can comprise glass, cellulose-based materials, thermoplastic polymers, such as polyethylene, polypropylene, or polyester, sintered structures composed of particulate materials (e.g., glass or various thermoplastic polymers), or cast membrane film composed of nitrocellulose, nylon, polysulfone, or the like. A substrate can be sintered, fine particles of polyethylene, commonly known as porous polyethylene, for example, 0.2-15 micron porous polyethylene from Chromex Corporation (Albuquerque, N. Mex.). All of these substrate materials can be used in suitable shapes, such as films, sheets, or plates, or they may be coated onto or bonded or laminated to appropriate inert carriers, such as paper, glass, plastic films, or fabrics. Suitable methods for immobilizing peptides on solid phases include ionic, hydrophobic, covalent interactions and the like.

[0039] A further object of the present invention relates to a kit or device for identifying the presence of infectious hepatitis E viral particles in a sample comprising at least one antibody of the present invention (immobilized or not on a solid support as described above). In some embodiments, the

kit can include a second antibody of the present invention which produces a detectable signal. In some embodiments, the kit further comprises antibodies having specificity for ORF2g and/or ORF2c proteins. Examples of kits include but are not limited to ELISA assay kits, and kits comprising test strips and dipsticks. In some embodiments, the kits described herein further comprise reference values of the levels of the protein or infectious particles. The reference values are typically average levels in samples from a population of healthy individuals. In some embodiments, the kits described herein further comprise at least one sample collection container for sample collection. Collection devices and container include but are not limited to syringes, lancets, BD VACUTAINER® blood collection tubes. In some embodiments, the kits described herein further comprise instructions for using the kit and interpretation of results.

[0040] The invention will be further illustrated by the following FIGURES and examples. However, these examples and FIGURES should not be interpreted in any way as limiting the scope of the present invention.

FIGURES

[0041] FIG. **1**: Generation of specific antibodies directed against the ORF2i protein using the peptide AGYPYNYNTTASDQ (SEQ ID NO:4) Reactivity and specificity of P2S2 and control (CTL) mouse sera and 1E6 antibody were analyzed (A) by immunofluorescence on fixed HEV-infected cells and (B) by western blotting on a mixture of ORF2 proteins (ORF2i and ORF2g/ORF2c). The asterisk indicates a non-specific band. Reactivity and specificity of P2H1, P2H2 and P2H3 hybridoma supernatants and 1E6 antibody were analyzed (C) by immunofluorescence on fixed HEV-infected cells and by western blotting on a mixture of ORF2 proteins (D) or an extract of HEV-infected cells (E).

RESULTS: GENERATION OF SPECIFIC ANTIBODIES DIRECTED AGAINST THE ORF2I PROTEIN USING THE PEPTIDE AGYPYNYNTTASDQ (SEQ ID NO:4)

[0042] The ORF2 protein sequence contains three highly conserved N-glycosylation sites represented by the sequon Asn-X-Ser/Thr (N—X—S/T). The ORF2i protein is not glycosylated whereas the ORF2g and ORF2c proteins are highly N-glycosylated (Montpellier et al. *Gastroenterology*, 2018). Among the three sites, the third site (^{549}NTT, N3) is highly (at least 95%) occupied by N-glycans in ORF2g and ORF2c proteins (Ankavay et al., submitted). Therefore, a peptide covering the N3 site can represent a good strategy for obtaining highly specific antibodies of the ORF2i form. Such antibodies will recognize the non-glycosylated ORF2i protein but not the glycosylated ORF2g and ORF2c proteins. In order to verify the specificity between strains/genotypes, sequence alignment between amino acids (a.a) 510 to 580 was carried out (data not shown). The prediction of secondary structure, prediction of immunogenicity, hydrophilicity, and accessibility were performed to define the best sequence for the immunogen. Finally, the peptide AGYPYNYNTTASDQ (SEQ ID NO:4) was selected for the immunization. In order to make this sequence more immunogenic during murine immunizations, a coupling to the protein carrier KLH was carried out via a maleimide function by adding a cysteine at the N-terminal position. Immunization was performed according a routine protocol. Five mice were immunized three times at three weeks intervals with the peptide (SEQ ID NO:4). Freund's complete and incomplete adjuvants were used during immunisation. The animals were immunized by subcutaneous and intraperitoneal routes. Ten days after the third immunisation, mice have been bleeded and their sera tested for immunoreactivity. Sera were first assayed by indirect ELISA on plates coated with the peptide (SEQ ID NO:4) (data not shown). Their reactivity was analyzed by immunofluorescence (IF) on fixed HEV-infected cells (FIG. **1**A). The 1E6 monoclonal antibody (antibody registry #AB-827236), which recognizes the three forms of ORF2 proteins (Montpellier et al., Gastroenterology, 2018), was used as a positive control. The serum of a PBS-immunized mouse was used as a negative control (CTL mouse). As shown in FIG. **1**A, serum of the P2S2 mouse showed a specific reactivity in IF. Specificity of the P2S2 serum was next analyzed in western blotting experiments with a mixture of ORF2 proteins (ORF2i and ORF2g/ORF2c) as antigens (FIG. **1**B). The P2S2 serum showed a highly specific recognition of the ORF2i protein, with no cross-reaction with the ORF2g/c proteins, as compared to the 1E6 antibody that recognizes the three forms.

[0043] After a final boost, the P2S2 mouse was sacrificed. Lymphocytes were isolated from the spleen and fused with a myeloma cell line using polyethylene glycol to form a hydridoma. Following fusion, cells were placed in media permissive for growth of hybridomas. Following culture of the hybridomas, cell supernatants were first screened by indirect ELISA on plates coated with the peptide (SEQ ID NO:4) (data not shown) and then by immunofluorescence. As shown in FIG. **1**C, the P2H1, P2H2 and P2H3 hybridoma supernatants displayed an ORF2i-specific staining. Specificity of these hybridomas was next analyzed in western blotting experiments with a mixture of ORF2 proteins (ORF2i and ORF2g/ORF2c) (FIG. **1**D) or an extract of HEV-infected cells (FIG. **1**E), as antigens. The P2H1, P2H2 and P2H3 clones showed a highly specific recognition of the ORF2i protein, with no cross-reaction with the ORF2g/c proteins, as compared to the 1E6 antibody that recognizes the three forms.

[0044] Together, these results indicate that antibodies specifically directed against the ORF2i polypeptides (SEQ ID NO:4) can be produced. Such antibodies will be very suitable for determining presence of infectious particles of hepatitis E virus in a sample. More particularly, detection of the ORF2i polypeptide of the present invention is suitable for diagnosing hepatitis E virus infection in a subject.

REFERENCES

[0045] Throughout this application, various references describe the state of the art to which this invention pertains. The disclosures of these references are hereby incorporated by reference into the present disclosure.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 4

<210> SEQ ID NO 1
<211> LENGTH: 647
<212> TYPE: PRT
<213> ORGANISM: Hepatitis E virus

<400> SEQUENCE: 1

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Leu Pro Met Leu Pro Ala Pro Pro Ala Gly Gln Pro Ser Gly Arg Arg
1               5                   10                  15

Arg Gly Arg Arg Ser Gly Gly Ala Gly Gly Gly Phe Trp Gly Asp Arg
            20                  25                  30

Val Asp Ser Gln Pro Phe Ala Leu Pro Tyr Ile His Pro Thr Asn Pro
        35                  40                  45

Phe Ala Ala Asp Ile Val Ser Gln Ser Gly Ala Gly Thr Arg Pro Arg
    50                  55                  60

Gln Pro Pro Arg Pro Leu Gly Ser Ala Trp Arg Asp Gln Ser Gln Arg
65                  70                  75                  80

Pro Ser Ala Ala Pro Arg Arg Arg Ser Ala Pro Ala Gly Ala Ala Pro
                85                  90                  95

Leu Thr Ala Val Ser Pro Ala Pro Asp Thr Ala Pro Val Pro Asp Val
            100                 105                 110

Asp Ser Arg Gly Ala Ile Leu Arg Arg Gln Tyr Asn Leu Ser Thr Ser
        115                 120                 125

Pro Leu Thr Ser Ser Val Ala Ser Gly Thr Asn Leu Val Leu Tyr Ala
    130                 135                 140

Ala Pro Leu Asn Pro Leu Leu Pro Leu Gln Asp Gly Thr Asn Thr His
145                 150                 155                 160

Ile Met Ala Thr Glu Ala Ser Asn Tyr Ala Gln Tyr Arg Val Val Arg
                165                 170                 175

Ala Thr Ile Arg Tyr Arg Pro Leu Val Pro Asn Ala Val Gly Gly Tyr
            180                 185                 190

Ala Ile Ser Ile Ser Phe Trp Pro Gln Thr Thr Thr Thr Pro Thr Ser
        195                 200                 205

Val Asp Met Asn Ser Ile Thr Ser Thr Asp Val Arg Ile Leu Val Gln
    210                 215                 220

Pro Gly Ile Ala Ser Glu Leu Val Ile Pro Ser Glu Arg Leu His Tyr
225                 230                 235                 240

Arg Asn Gln Gly Trp Arg Ser Val Glu Thr Thr Gly Val Ala Glu Glu
                245                 250                 255

Glu Ala Thr Ser Gly Leu Val Met Leu Cys Ile His Gly Ser Pro Val
            260                 265                 270

Asn Ser Tyr Thr Asn Thr Pro Tyr Thr Gly Ala Leu Gly Leu Leu Asp
        275                 280                 285

Phe Ala Leu Glu Leu Glu Phe Arg Asn Leu Thr Pro Gly Asn Thr Asn
    290                 295                 300

Thr Arg Val Ser Arg Tyr Thr Ser Thr Ala Arg His Arg Leu Arg Arg
305                 310                 315                 320

Gly Ala Asp Gly Thr Ala Glu Leu Thr Thr Thr Ala Ala Thr Arg Phe
                325                 330                 335

Met Lys Asp Leu His Phe Thr Gly Thr Asn Gly Val Gly Glu Val Gly
            340                 345                 350
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Arg Gly Ile Ala Leu Thr Leu Phe Asn Leu Ala Asp Thr Leu Leu Gly
        355                 360                 365

Gly Leu Pro Thr Glu Leu Ile Ser Ser Ala Gly Gly Gln Leu Phe Tyr
    370                 375                 380

Ser Arg Pro Val Val Ser Ala Asn Gly Glu Pro Thr Val Lys Leu Tyr
385                 390                 395                 400

Thr Ser Val Glu Asn Ala Gln Gln Asp Lys Gly Ile Thr Ile Pro His
                405                 410                 415

Asp Ile Asp Leu Gly Asp Ser Arg Val Val Ile Gln Asp Tyr Asp Asn
            420                 425                 430

Gln His Glu Gln Asp Arg Pro Thr Pro Ser Pro Ala Pro Ser Arg Pro
        435                 440                 445

Phe Ser Val Leu Arg Ala Asn Asp Val Leu Trp Leu Ser Leu Thr Ala
    450                 455                 460

Ala Glu Tyr Asp Gln Ala Thr Tyr Gly Ser Ser Thr Asn Pro Met Tyr
465                 470                 475                 480

Val Ser Asp Thr Val Thr Phe Val Asn Val Ala Thr Gly Ala Gln Ala
                485                 490                 495

Val Ala Arg Ser Leu Asp Trp Ser Lys Val Thr Leu Asp Gly Arg Pro
            500                 505                 510

Leu Thr Thr Ile Gln Gln Tyr Ser Lys Thr Phe Tyr Val Leu Pro Leu
        515                 520                 525

Arg Gly Lys Leu Ser Phe Trp Glu Ala Gly Thr Thr Arg Ala Gly Tyr
    530                 535                 540

Pro Tyr Asn Tyr Asn Thr Thr Ala Ser Asp Gln Ile Leu Ile Glu Asn
545                 550                 555                 560

Ala Ala Gly His Arg Val Ala Ile Ser Thr Tyr Thr Thr Ser Leu Gly
                565                 570                 575

Ala Gly Pro Ala Ser Ile Ser Ala Val Gly Val Leu Ala Pro His Ser
            580                 585                 590

Ala Leu Ala Val Leu Glu Asp Thr Val Asp Tyr Pro Ala Arg Ala His
        595                 600                 605

Thr Phe Asp Asp Phe Cys Pro Glu Cys Arg Thr Leu Gly Leu Gln Gly
    610                 615                 620

Cys Ala Phe Gln Ser Thr Ile Ala Glu Leu Gln Arg Leu Lys Thr Glu
625                 630                 635                 640

Val Gly Lys Thr Arg Glu Ser
                645


<210> SEQ ID NO 2
<211> LENGTH: 627
<212> TYPE: PRT
<213> ORGANISM: Hepatitis E virus

<400> SEQUENCE: 2

Ser Gly Gly Ala Gly Gly Gly Phe Trp Gly Asp Arg Val Asp Ser Gln
1               5                   10                  15

Pro Phe Ala Leu Pro Tyr Ile His Pro Thr Asn Pro Phe Ala Ala Asp
            20                  25                  30

Ile Val Ser Gln Ser Gly Ala Gly Thr Arg Pro Arg Gln Pro Pro Arg
        35                  40                  45

Pro Leu Gly Ser Ala Trp Arg Asp Gln Ser Gln Arg Pro Ser Ala Ala
    50                  55                  60
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Pro Arg Arg Arg Ser Ala Pro Ala Gly Ala Ala Pro Leu Thr Ala Val
65                  70                  75                  80

Ser Pro Ala Pro Asp Thr Ala Pro Val Pro Asp Val Asp Ser Arg Gly
                85                  90                  95

Ala Ile Leu Arg Arg Gln Tyr Asn Leu Ser Thr Ser Pro Leu Thr Ser
            100                 105                 110

Ser Val Ala Ser Gly Thr Asn Leu Val Leu Tyr Ala Ala Pro Leu Asn
        115                 120                 125

Pro Leu Leu Pro Leu Gln Asp Gly Thr Asn Thr His Ile Met Ala Thr
    130                 135                 140

Glu Ala Ser Asn Tyr Ala Gln Tyr Arg Val Val Arg Ala Thr Ile Arg
145                 150                 155                 160

Tyr Arg Pro Leu Val Pro Asn Ala Val Gly Gly Tyr Ala Ile Ser Ile
                165                 170                 175

Ser Phe Trp Pro Gln Thr Thr Thr Thr Pro Thr Ser Val Asp Met Asn
            180                 185                 190

Ser Ile Thr Ser Thr Asp Val Arg Ile Leu Val Gln Pro Gly Ile Ala
        195                 200                 205

Ser Glu Leu Val Ile Pro Ser Glu Arg Leu His Tyr Arg Asn Gln Gly
    210                 215                 220

Trp Arg Ser Val Glu Thr Thr Gly Val Ala Glu Glu Glu Ala Thr Ser
225                 230                 235                 240

Gly Leu Val Met Leu Cys Ile His Gly Ser Pro Val Asn Ser Tyr Thr
                245                 250                 255

Asn Thr Pro Tyr Thr Gly Ala Leu Gly Leu Leu Asp Phe Ala Leu Glu
            260                 265                 270

Leu Glu Phe Arg Asn Leu Thr Pro Gly Asn Thr Asn Thr Arg Val Ser
        275                 280                 285

Arg Tyr Thr Ser Thr Ala Arg His Arg Leu Arg Arg Gly Ala Asp Gly
    290                 295                 300

Thr Ala Glu Leu Thr Thr Thr Ala Ala Thr Arg Phe Met Lys Asp Leu
305                 310                 315                 320

His Phe Thr Gly Thr Asn Gly Val Gly Glu Val Gly Arg Gly Ile Ala
                325                 330                 335

Leu Thr Leu Phe Asn Leu Ala Asp Thr Leu Leu Gly Gly Leu Pro Thr
            340                 345                 350

Glu Leu Ile Ser Ser Ala Gly Gly Gln Leu Phe Tyr Ser Arg Pro Val
        355                 360                 365

Val Ser Ala Asn Gly Glu Pro Thr Val Lys Leu Tyr Thr Ser Val Glu
    370                 375                 380

Asn Ala Gln Gln Asp Lys Gly Ile Thr Ile Pro His Asp Ile Asp Leu
385                 390                 395                 400

Gly Asp Ser Arg Val Val Ile Gln Asp Tyr Asp Asn Gln His Glu Gln
                405                 410                 415

Asp Arg Pro Thr Pro Ser Pro Ala Pro Ser Arg Pro Phe Ser Val Leu
            420                 425                 430

Arg Ala Asn Asp Val Leu Trp Leu Ser Leu Thr Ala Ala Glu Tyr Asp
        435                 440                 445

Gln Ala Thr Tyr Gly Ser Ser Thr Asn Pro Met Tyr Val Ser Asp Thr
    450                 455                 460
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Val Thr Phe Val Asn Val Ala Thr Gly Ala Gln Ala Val Ala Arg Ser
465                 470                 475                 480

Leu Asp Trp Ser Lys Val Thr Leu Asp Gly Arg Pro Leu Thr Thr Ile
                485                 490                 495

Gln Gln Tyr Ser Lys Thr Phe Tyr Val Leu Pro Leu Arg Gly Lys Leu
            500                 505                 510

Ser Phe Trp Glu Ala Gly Thr Thr Arg Ala Gly Tyr Pro Tyr Asn Tyr
        515                 520                 525

Asn Thr Thr Ala Ser Asp Gln Ile Leu Ile Glu Asn Ala Ala Gly His
    530                 535                 540

Arg Val Ala Ile Ser Thr Tyr Thr Thr Ser Leu Gly Ala Gly Pro Ala
545                 550                 555                 560

Ser Ile Ser Ala Val Gly Val Leu Ala Pro His Ser Ala Leu Ala Val
                565                 570                 575

Leu Glu Asp Thr Val Asp Tyr Pro Ala Arg Ala His Thr Phe Asp Asp
            580                 585                 590

Phe Cys Pro Glu Cys Arg Thr Leu Gly Leu Gln Gly Cys Ala Phe Gln
        595                 600                 605

Ser Thr Ile Ala Glu Leu Gln Arg Leu Lys Thr Glu Val Gly Lys Thr
    610                 615                 620

Arg Glu Ser
625


<210> SEQ ID NO 3
<211> LENGTH: 559
<212> TYPE: PRT
<213> ORGANISM: Hepatitis E virus

<400> SEQUENCE: 3

Ser Ala Pro Ala Gly Ala Ala Pro Leu Thr Ala Val Ser Pro Ala Pro
1               5                   10                  15

Asp Thr Ala Pro Val Pro Asp Val Asp Ser Arg Gly Ala Ile Leu Arg
            20                  25                  30

Arg Gln Tyr Asn Leu Ser Thr Ser Pro Leu Thr Ser Ser Val Ala Ser
        35                  40                  45

Gly Thr Asn Leu Val Leu Tyr Ala Ala Pro Leu Asn Pro Leu Leu Pro
    50                  55                  60

Leu Gln Asp Gly Thr Asn Thr His Ile Met Ala Thr Glu Ala Ser Asn
65                  70                  75                  80

Tyr Ala Gln Tyr Arg Val Val Arg Ala Thr Ile Arg Tyr Arg Pro Leu
                85                  90                  95

Val Pro Asn Ala Val Gly Gly Tyr Ala Ile Ser Ile Ser Phe Trp Pro
            100                 105                 110

Gln Thr Thr Thr Thr Pro Thr Ser Val Asp Met Asn Ser Ile Thr Ser
        115                 120                 125

Thr Asp Val Arg Ile Leu Val Gln Pro Gly Ile Ala Ser Glu Leu Val
    130                 135                 140

Ile Pro Ser Glu Arg Leu His Tyr Arg Asn Gln Gly Trp Arg Ser Val
145                 150                 155                 160

Glu Thr Thr Gly Val Ala Glu Glu Glu Ala Thr Ser Gly Leu Val Met
                165                 170                 175

Leu Cys Ile His Gly Ser Pro Val Asn Ser Tyr Thr Asn Thr Pro Tyr
            180                 185                 190
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Thr Gly Ala Leu Gly Leu Leu Asp Phe Ala Leu Glu Leu Glu Phe Arg
        195                 200                 205

Asn Leu Thr Pro Gly Asn Thr Asn Thr Arg Val Ser Arg Tyr Thr Ser
    210                 215                 220

Thr Ala Arg His Arg Leu Arg Arg Gly Ala Asp Gly Thr Ala Glu Leu
225                 230                 235                 240

Thr Thr Thr Ala Ala Thr Arg Phe Met Lys Asp Leu His Phe Thr Gly
                245                 250                 255

Thr Asn Gly Val Gly Glu Val Gly Arg Gly Ile Ala Leu Thr Leu Phe
            260                 265                 270

Asn Leu Ala Asp Thr Leu Leu Gly Gly Leu Pro Thr Glu Leu Ile Ser
        275                 280                 285

Ser Ala Gly Gly Gln Leu Phe Tyr Ser Arg Pro Val Val Ser Ala Asn
    290                 295                 300

Gly Glu Pro Thr Val Lys Leu Tyr Thr Ser Val Glu Asn Ala Gln Gln
305                 310                 315                 320

Asp Lys Gly Ile Thr Ile Pro His Asp Ile Asp Leu Gly Asp Ser Arg
                325                 330                 335

Val Val Ile Gln Asp Tyr Asp Asn Gln His Glu Gln Asp Arg Pro Thr
            340                 345                 350

Pro Ser Pro Ala Pro Ser Arg Pro Phe Ser Val Leu Arg Ala Asn Asp
        355                 360                 365

Val Leu Trp Leu Ser Leu Thr Ala Ala Glu Tyr Asp Gln Ala Thr Tyr
    370                 375                 380

Gly Ser Ser Thr Asn Pro Met Tyr Val Ser Asp Thr Val Thr Phe Val
385                 390                 395                 400

Asn Val Ala Thr Gly Ala Gln Ala Val Ala Arg Ser Leu Asp Trp Ser
                405                 410                 415

Lys Val Thr Leu Asp Gly Arg Pro Leu Thr Thr Ile Gln Gln Tyr Ser
            420                 425                 430

Lys Thr Phe Tyr Val Leu Pro Leu Arg Gly Lys Leu Ser Phe Trp Glu
        435                 440                 445

Ala Gly Thr Thr Arg Ala Gly Tyr Pro Tyr Asn Tyr Asn Thr Thr Ala
    450                 455                 460

Ser Asp Gln Ile Leu Ile Glu Asn Ala Ala Gly His Arg Val Ala Ile
465                 470                 475                 480

Ser Thr Tyr Thr Thr Ser Leu Gly Ala Gly Pro Ala Ser Ile Ser Ala
                485                 490                 495

Val Gly Val Leu Ala Pro His Ser Ala Leu Ala Val Leu Glu Asp Thr
            500                 505                 510

Val Asp Tyr Pro Ala Arg Ala His Thr Phe Asp Asp Phe Cys Pro Glu
        515                 520                 525

Cys Arg Thr Leu Gly Leu Gln Gly Cys Ala Phe Gln Ser Thr Ile Ala
    530                 535                 540

Glu Leu Gln Arg Leu Lys Thr Glu Val Gly Lys Thr Arg Glu Ser
545                 550                 555


<210> SEQ ID NO 4
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Hepatitis E virus
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-continued

<400> SEQUENCE: 4

Ala Gly Tyr Pro Tyr Asn Tyr Asn Thr Thr Ala Ser Asp Gln
1 5 10

1. An antibody which binds to the ORF2i protein of hepatitis E virus and wherein said antibody does not bind to the ORF2g protein nor to the ORF2c of hepatitis E virus, and wherein the epitope of said antibody comprises at least one amino acid residue from amino acid residues 542 to 555 of SEQ ID NO: 1 (SEQ ID NO:4).

2. The antibody of claim **1** wherein the antibody binds to an epitope comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 amino acid residues from SEQ ID NO:4, or from a sequence sharing at least 90% of identity over SEQ ID NO: 4.

3. The antibody of claim **1** wherein the antibody binds to an epitope comprising the amino acid sequence as set forth in SEQ ID NO: 4 or an amino acid sequence sharing at least 90% of identity over SEQ ID NO: 4.

4. The antibody of claim **1** which is a polyclonal antibody or a monoclonal antibody.

5. The antibody of claim **1** which is a Fab', Fab, F(ab')2, scFv or a single domain antibody.

6. The antibody of claim **1** which is conjugated with a detectable label.

7. The antibody of claim **6** wherein the detectable label is a radioisotope, a fluorescent label, a chemiluminescent label, an enzyme label, or a bio luminescent label.

8. The antibody of claim **6** wherein the detectable label is selected from the group consisting of β-galactosidase, glucose oxidase, peroxidase and alkaline phosphatase.

9. (canceled)

10. A method for detecting the presence of the ORF2i protein in a sample comprising contacting the sample with the antibody of claim **1** under conditions that allow an immunocomplex of the protein and antibody to form wherein detection of the immunocomplex indicates the presence of the ORF2i protein in the sample.

11. A method for detecting the presence of infectious particles of hepatitis E virus in a sample comprising contacting the sample with the antibody of claim **1** under conditions that allow an immunocomplex of the antibody and the infectious particles to form wherein detection of the immunocomplex indicates the presence of the infectious particles in the sample.

12. The method of claim **10** wherein the sample is selected from the group consisting of faeces, blood, ascites; urine; saliva; sweat; milk; synovial fluid; peritoneal fluid; amniotic fluid; cerebrospinal fluid; lymph fluid; lung embolism; cerebrospinal fluid; and pericardial fluid.

13. A method for diagnosing and treating an acute HEV infection, a recent HEV infection, a chronic HEV infection, a weak active HEV infection or a cleared HEV infection in a subject in need thereof, comprising

contacting a sample from the subject with the antibody of claim **1**, wherein the step of contacting is performed under conditions that allow formation of immunocomplexes of the antibody with i) ORF2i protein; and/or ii) infectious particles of hepatitis E virus; and

treating the subject when immunocomplexes are detected.

14. A kit or device for identifying the presence of infectious hepatitis E viral particles in a sample, the kit or device comprising at least one antibody of claim **1**.

15. The kit or device of claim **14** which is a flow immunoassay device.

16. The kit or device of claim **14**, wherein the at least one antibody is immobilized on a solid support.

17. The method of claim **11** wherein the sample is selected from the group consisting of faeces, blood, ascites; urine; saliva; sweat; milk; synovial fluid; peritoneal fluid; amniotic fluid; cerebrospinal fluid; lymph fluid; lung embolism; cerebrospinal fluid; and pericardial fluid.

18. The antibody of claim **8**, wherein the peroxidase is horseradish perodixase.

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