

ENTEROVIRUS 71 SPECIFIC ANTIBODIES AND USES THEREOF

FIELD OF THE INVENTION

- 5 The present invention relates generally to Enterovirus 71 (EV71) specific monoclonal antibodies. In particular, but not exclusively the antibodies may be neutralising monoclonal antibodies.

BACKGROUND TO THE INVENTION

- 10 Enteroviruses are a heterogeneous group of pathogens responsible for a broad spectrum of human and nonhuman diseases. Enteroviruses belong to a large genus within the family Picornaviridae; other genera within this family include rhinoviruses, hepatoviruses, cardioviruses, and aphthoviruses. The enterovirus genus encompasses polio viruses, 15 coxsackie A viruses (CAV), coxsackie B viruses (CBV), echoviruses, and enteroviruses 68-71, as well as a number of uncharacterized enteroviruses isolated from humans and other primates. Like other picornaviruses, enteroviral virions comprise an icosahedral capsid with no envelope enclosing a core comprising infectious, single- stranded genomic sense RNA (ssRNA), about 7-8.5 kb in size. Enteroviruses are distinguished from other members of the 20 picornaviridae by their stability in acid and their fecal-oral route of passage and transmission. Virus entry into cells is believed to involve specific cellular receptors. Virion proteins include multiple copies of four capsid proteins (VP1, VP2, VP3 and VP4). A small protein, VPg (Mr about 24×10^3), is linked covalently to the 5' terminus of the genomic RNA. Other proteins of the enteroviruses include the predominantly non-structural proteins, P2 25 and P3.

- Enterovirus 71 (EV71) belongs to the human Enterovirus A species of the Enterovirus genus. EV71 is not a zoonotic agent and is the causative agent of a number of neurological diseases including aseptic meningitis, encephalitis, cranial nerve palsies, Guillan-Barre 30 syndrome, and poliomyelitis-like syndrome.

- EV71 is also associated with large outbreaks of hand foot and mouth disease (HFMD) in human beings including other enteroviruses such as CA16, CA5, CA9 and Echo 7. However, out of all the enteroviruses associated with HFMD, EV71 has raised the most 35 public concern due to its large scale outbreak in Asia-Pacific over the last decade and its ability in causing severe neurological complications and sometimes fatality.

EV71 exhibits a wide variation in clinical presentation. Because of this wide variation in clinical presentation as well as increased public health concerns associated with EV71 and its potential for greater neurovirulence, it is important to be able to rapidly identify the specific strain of EV71 during an HFMD outbreak and to be able to rapidly discriminate between EV71 and CA16 (another leading agent responsible for large outbreaks of HFMD). Since early symptoms are similar for HFMD associated with infections of different strains of EV71 or CA16, the conventional "gold standard" method for EV71 diagnosis is by tissue culture isolation and serotyping using *in vitro* microneutralization tests. This is not only time consuming but also less sensitive and specific (J.L. 1996). Further, the neutralization tests involved use of monospecific antiserum, which are quite limited in their availability. Further, antigenic typing is often hampered by non-neutralizable virus due to aggregation. Enzyme-Linked Immunosorbent Assay (ELISA) is also commonly used. Recombinant protein capture ELISA (Shih, Li et al. 2000) and IgM capture ELISA (Wang, Lin et al. 2004) which are both available as commercial diagnostic kits are limited by the lack of specificity for EV71 (Hovi and Roivainen 1993), often resulting in false positive results.

There are also two diagnostic tests kits on the market, one based on real time RT-PCR and the other based on anti-EV71 IgM detection in patient serum. Both these methods have great disadvantages. For example, real time PCR requires a well maintained laboratory and expensive equipment while detection of serum antibodies is only possible some weeks after infection which is a drawback as severe disease of EV71 has a rapid onset and progression.

Intravenous immunoglobulin (IVIG) is another method that has been commonly used in therapy of viral infection and inflammatory disease showing effective abolishment and modification of the virus (Dwyer 1992). There is thus a potential application of IVIG in EV71 therapy. However, there is no neutralizing monoclonal antibody available in the art that is capable of neutralising EV71 infection.

Since up till now, there is still a lack of an effective antiviral therapy or vaccine against the neurotropic virus, EV71, there is an urgent need to develop effective therapeutics and methods for early and specific diagnostics of EV71.

SUMMARY OF THE INVENTION

5 The present invention addresses the problems above, and in particular provides at least one novel monoclonal antibody that is specific to at least one EV71 and/or EV71 related disease. The monoclonal antibody may be neutralising.

10 According to a first aspect, the present invention provides at least one isolated neutralising monoclonal antibody or a fragment thereof of subclass immunoglobulin M that is capable of specifically binding to at least one epitope of EV71. In particular, the antibody may be selected from the group consisting of:

- (a) antibody produced by hybridoma cell line CBA20110005, or CBA20110007;
- (b) antibody having the immunological binding characteristics of the antibody produced by hybridoma cell line CBA20110005, or CBA20110007; and
- (c) antibody that binds to an antigen capable of binding the antibody produced by the hybridoma cell line CBA20110005, or CBA20110007; .

20 The epitope according to any aspect of the present invention may comprise, consists of or consists essentially of the amino acid sequence KQEKD (SEQ ID NO:1) and/or may be a conformational epitope.

According to another aspect, the present invention provides at least one isolated monoclonal antibody or a fragment thereof that is capable of specifically binding to at least one epitope of EV71. In particular, the antibody may be selected from the group consisting of:

- 25 (a) antibody produced by hybridoma cell line CBA20110004, or CBA20110006;
- (b) antibody having the immunological binding characteristics of the antibody produced by hybridoma cell line CBA20110004, or CBA20110006; and
- (c) antibody that binds to an antigen capable of binding the antibody produced by the hybridoma cell line CBA20110004, or CBA20110006;.

30 The epitope according to any aspect of the present invention may comprise, consists of or consists essentially of the amino acid sequence KQEKD (SEQ ID NO:1) or an epitope to which Mab4 specifically binds.

35 According to another aspect, the present invention provides at least one isolated hybridoma cell line deposited with CellBank Australia (214 Hawkesbury Road, Westmead NSW 2145)

on 27 July 2011 with accession number CBA20110004, CBA20110005, CBA20110006, or CBA20110007.

According to other aspects, the present invention provides a method of detecting and/or quantifying the presence of EV71, a method of treating and/or preventing EV71 and/or at least one EV71-linked disease, the antibody or fragment thereof of the present invention for use as medicine, use of the antibody or fragment thereof of the present invention for the preparation of a medicament, kits, nucleic acids and uses thereof.

As will be apparent from the following description, preferred embodiments of the present invention allow an optimal use of the isolated antibodies to take advantage of their accuracy and specificity to at least one epitope of EV71. This and other related advantages will be apparent to skilled persons from the description below.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a photograph of the results obtained from western blot hybridization of Mab51 and Mab53 during characterization. Lane 1: Rhabdomyosarcoma RD cells total cell lysate. Lane 2: Concentrated C4 (Yamagata) virus. Lane 3: GST-tagged VP1 recombinant protein (~61kDa). Lane 4: GST-tagged VP2 recombinant protein (~56kDa). Lane 5: GST-tagged VP3 recombinant protein (~50kDa).

Figure 2 is a photograph of the results obtained from western blot hybridization of Mab51 and Mab53 during epitope mapping. Lane 1: GST-KQEK fusion protein (~25kDa), Lane 2: GST-HKQEKD fusion protein, Lane 3: GST-HKQEK fusion protein, Lane 4: GST-KQEKD fusion protein, Lane 5: GST fusion protein.

Figures 3A, 3B and 3C are photographs of the results obtained from western blot hybridization of Mab4 epitope mapping. Figure 3A: Lane 1: VP1(1-66) GST recombinant protein protein, Lane 2: VP1(1-132), Lane 3: VP1(1-163), Lane 4: VP1(1-177), Lane 5: VP1(1-208), Lane 6: (1-222), Lane 7: VP1(1-240), Lane 8: VP1(1-260); Figure 3B: Lane 1: GST protein (negative control), Lane 2: A48 (48-297), Lane 3: A32 (32-297), Lane 4: A24 (24-297), Lane 5: A10 (10-297). Figure 3C: Lane 1: GST protein (negative control), Lane 2: A12 (12-297), Lane 3: A14 (14-297), Lane 4: A16 (16-297), Lane 5: A18 (18-297), Lane 6: A18 (20-297), Lane 7: A22 (22-297) and lane 8: A10 (10-297, positive control).

Figure 4 is a photograph of the results obtained from western blot hybridization showing cross reactivity of Mab51 and Mab53 with EV71 subgenotypes. Lane 1: A (BrCr), Lane 2: B2 (7423/MS/87), Lane 3: B4 (HFM41), Lane 4: B5 (NUH0083), Lane 5: C1 (Y90-3761),
5 Lane 6: C4 (75-Yamagata), Lane 7: C5 (3437/SIN/06), Lane 8: RG-C2 (AF286504).

Figure 5 is a photograph of the results obtained from immunofluorescence assay (IFA) of CA16 (isolated in Finland, 1994, U05876) infected vero cells with Mab against 3D, Mab51, Mab53, Mab57 and Mab4. A-E: merged image of bright field and fluorescence microscopy,
10 F-J: Fluorescence microscopy.

Figure 6 is a photograph of the results obtained from IFA of Mab51 to show cross reactivity with EV71 subgenotypes. Vero cells were infected with EV71 virus strains as depicted at the top-left corner of each image.
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Figure 7 is a photograph of the results obtained from IFA of Mab53 to show cross reactivity with EV71 subgenotypes. Vero cells were infected with EV71 virus strains as depicted at the top-left corner of each image.

Figure 8 is a photograph of the results obtained from IFA of Mab4 to show cross reactivity with EV71 subgenotypes. Vero cells were infected with EV71 virus strains as depicted at the top-left corner of each image.
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Figure 9 is a photograph of the results obtained from IFA of Mab57 to show cross reactivity with EV71 subgenotypes. Vero cells were infected with EV71 virus strains as depicted at the top-left corner of each image.
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Figure 10A is a picture of a cross-section of control mice (AG129; challenged with EV71 infection) spinal cord with arrow indicating neuropil vacuolation and neuronal loss without inflammation in the anterior horn (original magnification 100x).
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Figure 10B is a high-power view of Figure 10A showing neuropil vacuolation and loss of neuronal cells without inflammation (original magnification 400x).

Figure 11A is a picture of a cross-section of protected mice (AG129; challenged with EV71 infection and prophylactic protection with Mab 51) spinal cord showing no significant pathology (original magnification 40x).

- 5 Figure 11B is a picture of a cross-section of spinal cord showing no significant pathology (original magnification 100x).

Figure 11C is a high-power view of Figure 11A showing no significant pathology (original magnification 400x).

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DETAILED DESCRIPTION OF THE INVENTION

Bibliographic references mentioned in the present specification are for convenience listed in the form of a list of references and added at the end of the examples. The whole content
15 of such bibliographic references is herein incorporated by reference.

Definitions

For convenience, certain terms employed in the specification, examples and appended claims are collected here.

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As used herein, the term "antibody" refers to any immunoglobulin or intact molecule as well as to fragments thereof that bind to a specific epitope. Such antibodies include, but are not limited to polyclonal, monoclonal, chimeric, humanised, single chain, Fab, Fab', F(ab)' fragments and/or F(v) portions of the whole antibody. The term "monoclonal antibody" may
25 be referred to as "Mab". For example, the antibody "monoclonal antibody 51" may be used interchangeably with "Mab51", and which may be capable of specifically binding to EV71 including but not limited to an epitope of SEQ ID NO:1. The antibody includes monoclonal antibodies, polyclonal antibodies, single-chain antibodies, and fragments thereof which retain the antigen binding function of the parent antibody. Similarly, the antibody "Mab57" is
30 capable of specifically binding to EV71, including but not limited to a conformational epitope comprising at least one capsid protein of EV71 and includes monoclonal antibodies, polyclonal antibodies, single-chain antibodies, and fragments thereof which retain the antigen binding function of the parent antibody. Also, the antibody "Mab53" is capable of specifically binding to EV71 including but not limited to at least one epitope of SEQ ID NO:1
35 and includes monoclonal antibodies, polyclonal antibodies, single-chain antibodies, and fragments thereof which retain the antigen binding function of the parent antibody. The

antibody "Mab4" is capable of specifically binding to EV71 including but not limited to at least one epitope and includes monoclonal antibodies, polyclonal antibodies, single-chain antibodies, and fragments thereof which retain the antigen binding function of the parent antibody.

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The term "antibody fragment" as used herein refers to an incomplete or isolated portion of the full sequence of the antibody which retains the antigen binding function of the parent antibody. Examples of antibody fragments include Fab, Fab', F(ab')₂, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies
10 formed from antibody fragments. Fragments of the Mab51, Mab57, Mab53 and Mab4 are encompassed by the invention so long as they retain the desired affinity of the full-length antibody. In particular, it may be shorter by at least one amino acid. For example, the fragment of Mab51 comprises the antigen binding function that enables it to bind to an epitope of SEQ ID NO:1 of a capsid protein of EV71, the fragment of a Mab57 comprises
15 the antigen binding function that enables it to bind to a conformational epitope of a capsid protein of EV71, the fragment of Mab53 comprises the antigen binding function that enables it to bind to an epitope of SEQ ID NO:1 of a capsid protein of EV71 and the fragment of Mab4 comprises the antigen binding function that enables it to bind to a specific epitope in EV71.

20

The term "antigen" as used herein, refers to a substance that prompts the generation of antibodies and can cause an immune response. It may be used interchangeably in the present invention with the term "immunogen". In the strict sense, immunogens are those substances that elicit a response from the immune system, whereas antigens are defined
25 as substances that bind to specific antibodies. An antigen or fragment thereof may be a molecule (i.e. an epitope) that makes contact with a particular antibody. When a protein or a fragment of a protein is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies (i.e. elicit the immune response), which bind specifically to the antigen (given regions or three-dimensional structures on the protein).
30 The antigen may include but is not limited to a capsid protein and/or non-structural proteins of EV71. In particular, the term "epitope" refers to a consecutive sequence of from about 5 to about 13 amino acids which form an antibody binding site. The epitope in the form that binds to the Mab or binding protein may be in a denatured protein that is substantially devoid of tertiary structure. The epitope may be a conformational epitope. A
35 "conformational epitope" is herein defined as a sequence of subunits (usually, amino acids) composing an antigen that comes in direct contact with a receptor of the immune system.

Whenever a receptor interacts with an undigested antigen, the surface amino acids that come in contact may not be continuous with each other if the protein is unwound. Such discontinuous amino acids that come together in three dimensional conformation and interact with the receptor's paratope are called conformational epitopes. In contrast, if the antigen is digested, small segments called peptides are formed, which bind with major histocompatibility complex molecules, and then later with T cell receptors through amino acids that are continuous in a line. These are known as linear epitopes. A non-limiting example of a linear epitope is SEQ ID NO:1 and/or the epitope to which Mab4 binds.

The term "comprising" is herein defined to be that where the various components, ingredients, or steps, can be conjointly employed in practicing the present invention. Accordingly, the term "comprising" encompasses the more restrictive terms "consisting essentially of" and "consisting of."

The term "derivative," as used herein, refers to the chemical modification of at least one EV71 epitope. Chemical modifications of a polynucleotide sequence can include, for example, replacement of hydrogen by an alkyl, acyl, or amino group. A derivative polynucleotide encodes a polypeptide which retains at least one biological or immunological function of the natural molecule. A derivative polypeptide is one modified by glycosylation, pegylation, or any similar process that retains at least one biological or immunological function of the polypeptide from which it was derived.

The term "humanized antibody," as used herein, refers to at least one antibody molecule in which the amino acid sequence in the non-antigen binding regions has been altered so that the antibody more closely resembles a human antibody, and still retains its original binding ability.

As used herein, the term "hybridoma" refers to cells that have been engineered to produce a desired antibody in large amounts. For example, to produce at least one hybridoma, B cells are removed from the spleen of an animal that has been challenged with the relevant antigen and fused with at least one immortalized cell. This fusion is performed by making the cell membranes more permeable. The fused hybrid cells (called hybridomas), will multiply rapidly and indefinitely and will produce at least one antibody. Examples of hybridomas are the cell lines with accession number CBA20110004, CBA20110005, CBA20110006, or CBA20110007.

"Immortalised cells" as used herein are also known as transformed cells - i.e. cells whose growth properties have been altered. This does not necessarily mean that these are "cancer" or "tumour" cells, i.e. able to form a tumour if introduced into an experimental animal, although in some cases they may do. Immortalised cell lines include but are not limited to NS1, Jurkat, HeLa, HepG2, SP2/0, Hep-3b and the like.

The term "immunological binding characteristics" of a Mab or related binding protein, in all of its grammatical forms, refers to the specificity, affinity and cross-reactivity of the Mab or binding protein for its antigen.

The term "isolated" is herein defined as a biological component (such as a nucleic acid, peptide or protein) that has been substantially separated, produced apart from, or purified away from other biological components in the cell of the organism in which the component naturally occurs, i.e., other chromosomal and extrachromosomal DNA and RNA, and proteins. Nucleic acids, peptides and proteins which have been isolated thus include nucleic acids and proteins purified by standard purification methods. The term also embraces nucleic acids, peptides and proteins prepared by recombinant expression in a host cell as well as chemically synthesized nucleic acids.

The term "neutralising antibody" is herein defined as an antibody that can neutralise the ability of that pathogen to initiate and/or perpetuate an infection in a host. The invention provides a neutralising human monoclonal antibody, wherein the antibody recognises an antigen from EV71.

The term "sample," as used herein, is used in its broadest sense. A biological sample suspected of containing nucleic acids encoding at least one EV71 derived peptide, or fragments thereof, or EV71 itself may comprise a bodily fluid, an extract from a cell, chromosome, organelle, or membrane isolated from a cell, a cell; genomic DNA, RNA, or cDNA (in solution or bound to a solid support), a tissue, a tissue print and the like.

As used herein, the terms "specific binding" or "specifically binding" refer to the interaction between a protein or peptide and an agonist, an antibody, or an antagonist. In particular, the binding is between an antigen and an antibody. The interaction is dependent upon the presence of a particular structure of the protein recognized by the binding molecule (i.e., the antigen or epitope). For example, if an antibody is specific for epitope "A", the presence of a polypeptide containing the epitope A, or the presence of free unlabeled A, in a reaction

containing free labeled A and the antibody will reduce the amount of labeled A that binds to the antibody. For example, Mab51 and Mab53 may specifically bind a linear epitope of SEQ ID NO:1, Mab57 may specifically bind a conformational epitope on the capsid and Mab4 may specifically bind a linear epitope.

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The term "subject" is herein defined as vertebrate, particularly mammal, more particularly human. For purposes of research, the subject may particularly be at least one animal model, e.g., a mouse, rat and the like.

10 A person skilled in the art will appreciate that the present invention may be practised without undue experimentation according to the method given herein. The methods, techniques and chemicals are as described in the references given or from protocols in standard biotechnology and molecular biology text books.

15 According to a first aspect, the present invention provides isolated monoclonal antibodies and related binding proteins that bind specifically to EV71. Monoclonal antibodies also known as "Mabs" may be a substantially homogeneous population of antibodies derivable from a single antibody-producing cell. Thus, all antibodies in the population may be identical and may have the same specificity for a given epitope. The specificity of the Mab
20 responses provides a basis for an effective diagnostic reagent. Monoclonal antibodies and binding proteins derived therefrom also have utility as therapeutic agents.

The antibodies according to any aspect of the present application provide at least one anti-EV71 antibody which is capable of neutralising EV71 infection and inhibiting cell-to-cell
25 spread. These antibodies according to any aspect of the present application may be used as prophylactic and/or therapeutic agent(s) for the treatment of EV71 and EV71 associated diseases.

In particular, the isolated monoclonal antibody or a fragment thereof of subclass
30 immunoglobulin M may be a neutralising monoclonal antibody or a fragment thereof that may be capable of specifically binding to at least one epitope of EV71. The epitope may be a linear one of SEQ ID NO:1 or may be a conformational epitope. The conformational epitope may be an intact virus capsid. More in particular, the capsid protein may be VP1, VP2 VP3, VP4 and/or VP0 precursor.

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According to one embodiment, the antibody may have substantially the immunological binding characteristics of monoclonal antibody Mab51. In particular, the antibody may be selected from the group consisting of:

- (a) antibody produced by hybridoma cell line CBA20110005;
- 5 (b) antibody having the binding characteristics of the antibody produced by hybridoma cell line CBA20110005; and
- (c) antibody that binds to an antigen capable of binding the antibody produced by the hybridoma cell line CBA20110005.

10 According to another embodiment, the antibody may have substantially the immunological binding characteristics of monoclonal antibody Mab57. In particular, the antibody may be selected from the group consisting of:

- (a) antibody produced by hybridoma cell line CBA20110007;
- (b) antibody having the binding characteristics of the antibody produced by hybridoma
15 cell line CBA20110007; and
- (c) antibody that binds to an antigen capable of binding the antibody produced by the hybridoma cell line CBA20110007.

These antibodies may be capable of blocking viral mechanisms to spread within a host.
20 They effectively neutralize cell-free virus particles and inhibit the direct cell-to-cell spread of the virus. Since the antibodies specifically bind to a highly conserved epitopes of EV71 for example SEQ ID NO:1 of VP1 and conformational epitope of an intact virus capsid, which are essential for the virus survival, development of drug resistance is most unlikely. These antibodies of the present invention, provide several advantages including being capable of
25 use as drugs or vaccines for HFMD.

In particular, the antibodies according to any aspect of the present invention may be available in large quantities, prepared either in hybridoma supernatant or ascites fluid. There may also be a constant and renewable source of monoclonal antibodies available,
30 with any one of the hybridoma cell lines according to any aspect of the present invention. The defined epitope of the antibodies according to any aspect of the present invention also allows for mechanistic study of its virus neutralization ability to be easily performed. These antibodies may also be easily purified by affinity chromatography, using any method known in the art. For example, but not limiting, the antibodies according to any aspect of the
35 present invention may be purified using the protocol disclosed in Li, Mao et al., 2009.

In one embodiment, the neutralizing antibodies according to any aspect of the present invention may result in a complete protection from cytopathic effects (CPE) of EV71. These antibodies may be capable of effective *in vivo* protection against EV71 infection. The efficacy and specificity of these antibodies are shown in the Examples.

5

In one embodiment, the neutralizing antibody according to this invention binds an epitope of VP1 of EV71 of SEQ ID NO:1. This region of the epitope may be highly conserved and thus mutations seldom occur.

10 In another embodiment, the neutralizing antibody according to this invention binds to at least one conformational epitope of EV71. This is advantageous as epitopes usually exist in nature in a three dimensional conformation and the antibodies may thus be more efficient and effective in detecting the presence of EV71 and/or subsequently neutralizing the effect of EV71. These antibodies may thus be able to bind and to recognize viral antigens without
15 prior treatments of a tissue section. The conformational epitope may comprise at least one capsid protein and/or at least one non-structural protein. The capsid protein may be an intact virus capsid protein. The capsid protein may comprise one or more proteins selected from the group consisting of VP1, VP2 VP3, VP4 and VP0 precursor.

20 In particular, the antibody according to any aspect of the present invention may comprise the immunological binding characteristics of monoclonal antibody Mab51 or Mab57. These immunological binding characteristics of Mab51 are produced by hybridoma Mab51, deposited with the CellBank Australia, 214 Hawkesbury Road, Westmead NSW 2145, Australia on 27 July 2011, in accordance with the provisions of the Budapest Treaty, and
25 assigned Accession Number CBA20110005. These immunological binding characteristics of Mab57 are produced by hybridoma Mab57, deposited with the CellBank Australia, 214 Hawkesbury Road, Westmead NSW 2145, Australia on 27 July 2011, in accordance with the provisions of the Budapest Treaty, and assigned Accession Number CBA20110007. The hybridoma provides a continuous source of the mAbs and binding proteins of the
30 invention.

According to another aspect, the present invention provides an isolated monoclonal antibody or a fragment thereof that may be capable of specifically binding to at least one linear epitope. In particular, the epitope may be of SEQ ID NO:1 or the epitope to which
35 Mab4 specifically binds or fragment thereof. The antibodies according to any aspect of the present invention may be capable of recognising a whole spectrum of EV71 viruses and at

the same time and/or may be capable of differentiating EV71 from CA16 infection. These antibodies may be of high specificity and sensitivity.

5 In particular, the antibody according to an aspect of the present invention may comprise the immunological binding characteristics of monoclonal antibody Mab53 or Mab4. These immunological binding characteristics of Mab53 are produced by hybridoma Mab53, deposited with the CellBank Australia, 214 Hawkesbury Road, Westmead NSW 2145, Australia on 27 July 2011, in accordance with the provisions of the Budapest Treaty, and assigned Accession Number CBA20110006. The hybridoma provides a continuous source
10 of Mab53 and binding proteins of the invention. These immunological binding characteristics of Mab4 are produced by hybridoma Mab4, deposited with the CellBank Australia, 214 Hawkesbury Road, Westmead NSW 2145, Australia on 27 July 2011, in accordance with the provisions of the Budapest Treaty, and assigned Accession Number CBA20110004. The hybridoma provides a continuous source of Mab4 and binding proteins
15 of the invention.

The antibody according to one aspect of the present invention may be selected from the group consisting of:

- 20 (a) antibody produced by hybridoma cell line CBA20110006 or CBA20110004;
(b) antibody having the binding characteristics of the antibody produced by hybridoma cell line CBA20110006 or CBA20110004; and
(c) antibody that binds to an antigen capable of binding the antibody produced by the hybridoma cell line CBA20110006 or CBA20110004.

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The antibody according to any aspect of the present invention may be capable of recognizing EV71 of any genotype. In particular, the antibody may be capable of recognizing EV71 of a genotype selected from the group consisting of A, B1, B2, B3, B4, B5, C1, C2, C3, C4 and C5.

30

The Mabs of the present invention may be produced by any technique that provides for the production of antibody molecules by continuous cell lines in culture. Such methods include, but are not limited to, the hybridoma technique originally developed in 1975 by Kohler and Milstein, as well as the trioma technique, the human B-cell hybridoma technique and the
35 EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., 1985).

Human antibodies can be used and can be obtained by using human hybridomas (Cote et al., 1983).

Techniques developed for the production of "chimeric antibodies" (Morrison, et al., 1984 incorporated herein by reference in their entirety) by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. For example, the genes from a mouse antibody molecule such as Mab51, Mab57, Mab53 or Mab4 can be spliced together with genes from a human antibody molecule of appropriate biological activity. A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine Mab and a human immunoglobulin constant region. Chimeric antibodies are also those that contain a human Fc portion and a murine (or other non-human) Fv portion.

Techniques have been developed for the production of humanized antibodies (e.g., US 5,585,089 and/or US 5,225,539, which are incorporated herein by reference in their entirety). An immunoglobulin light or heavy chain variable region consists of a "framework" region interrupted by three hypervariable regions, referred to as complementarity determining regions (CDRs). Briefly, humanized antibodies are antibody molecules from non-human species having one or more CDRs from the non-human species and a framework region from a human immunoglobulin molecule. Both chimeric and humanized antibodies may be monoclonal. Such human or humanized chimeric antibodies may be preferred for use in *in vivo* diagnosis or therapy of human diseases or disorders.

Antibody fragments that contain the idiotype of the antibody molecule can be generated by known techniques. For example, such can be produced by pepsin digestion of the antibody molecule; the Fab fragments can be generated by reducing the disulfide bridges of the F(ab)₂ fragment, and the Fab fragments which can be generated by treating the antibody molecule with papain and a reducing agent. Such antibody fragments can be generated from any of the polyclonal or monoclonal antibodies of the invention.

In the production of antibodies, screening for the desired antibody can be accomplished by techniques known in the art. For example, these techniques may include but are not limited to radioimmunoassay, enzyme-linked immunosorbent assay (ELISA), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitin reactions, immunodiffusion assays, *in situ* immunoassays (using colloidal gold, enzyme, radioisotope

labels or the like), western blots, precipitation reactions, agglutination assays (gel agglutination assays, hemagglutination assays or the like), immunofluorescence assays, immunoelectrophoresis assays and the like. For example, the antibody binding may be detected by detecting a label on the primary antibody. In another example, the primary antibody may be detected by detecting binding of a secondary antibody or other reagent to the primary antibody. The secondary antibody may be labeled.

The antibody may be labelled with at least one radionuclide and/or fluorochrome in order to improve targeting of EV71 *in vivo* in at least a diagnostic and/or therapeutic capacity. For example, detecting EV71 by Positron Emission Tomography (PET) may be used. The antibody labeled with the radionuclide may enable better targeting of EV71 for detection and/or treatment. The antibody may be further labelled with at least one drug, anti-viral drug and/or toxin for treatment of EV71. In particular, the antibodies may be used to deliver drugs to the infected cells with a high degree of specificity to suppress viral infections. The drugs may include but are not limited to ribavirin and or other potent inhibitors of EV71 infection such as bovine or human lactoferrins and the like. The antibody of the present invention linked with at least one anti-viral drug, may increase the drug availability for EV71 infected cells which may enhance the efficiency of the drug and also reduce side effects usually caused by the anti-viral drugs. This may lead to a new treatment for EV71 possibly reducing other complications associated with EV71 infection.

Mab51, Mab57, Mab53 or Mab4 may be linked with at least one traceable agent and may be potentially used to quantify EV71 infected cells *in vitro* or *in vivo*. The traceable agent may be any biological or chemical component which is traceable. The traceable agent may include, but is not limited to environmental agents, blood markers, antigens, pesticides, drugs, chemicals, toxins, PCBS, PBBS, lead, neurotoxins, blood electrolytes, metabolites, analytes, NA⁺, K⁺, CA⁺, urea nitrogen, creatinine, biochemical blood markers and components, ChE, AChE, BuChE, tumour markers, PSA, PAP, CA 125, CEA, AFP, HCG, CA 19-9, CA 15-3, CA 27-29, NSE, hydroxybutyrate, acetoacetate, anti-malarial drugs such as amodiaquine, artemether, artemisinin, artesunate, atovaquone, cinchonine, cinchonidine, chloroquine, doxycycline, halofanthne, mefloquine, primaquine, pyrimethamine, quinine, quinidine, and sulfadoxine; anti-biotic drugs such as ampicillin, azithromycin, doxycycline, erythromycin, penicillin, and tetracycline; anti-retroviral drugs such as abacavir, adefovir, didanosine, entecavir, indinavir, lamivudine, nevirapine, remofovir, ritonavir, saquinavir mesylate, telbivudine, tenofovir, zalcitabine, and zidovudine.

The antibodies according to any aspect of the present invention may be used in methods known in the art relating to the detection or localization of EV71. For example, these methods may include but are not limited to Western blotting, ELISA, radioimmunoassay, immunofluorescence assay, immunohistochemical assay, and the like. The method of
5 detecting and/or quantifying according to any aspect of the present invention may be a non-invasive method and may also be useful for studying several immunological aspects of EV71.

In particular, the present invention provides a method of detecting and/or quantifying the
10 presence and distribution of at least one EV71 infected cell in a subject, the method comprising:

- a. contacting at least one antibody or fragment thereof according to any aspect of the present invention with at least one sample obtained from at least one subject; and
- b. detecting and quantifying binding of the antibody to the EV71 infected cell.

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The step of detecting may comprise contacting the sample with a binding protein that contains or is conjugated to a detectable element. In particular, the antibody according to any aspect of the present invention may be immobilized onto a solid surface. More in particular, the binding protein may contain a radioactive atom, may be conjugated to a
20 fluorescent molecule, or may be conjugated to an enzyme.

The present invention also includes assay and test kits for the qualitative and/or quantitative determination of EV71. Such kits may contain at least the Mab or related binding protein according to any aspect of the present invention, means for detecting
25 immunospecific binding of the Mab or related binding protein to EV71 in a biological sample, and instructions for use, depending upon the method selected, e.g., "competitive," "sandwich," "DASP" and the like. The kits may also contain positive and negative controls. They may be configured to be used with automated analyzers or automated immunohistochemical slide staining instruments.

30

In particular, an assay kit of the invention may further comprise a second antibody or binding protein that may be labelled or may be provided for attachment to a solid support (or attached to a solid support). Such an antibody or binding protein may be, for example, one that binds to EV71. Such second antibodies or binding proteins may be polyclonal or
35 monoclonal antibodies.

Mab51, Mab57, Mab53 or Mab4 are highly efficacious in detecting and/or neutralising the effect of EV71. They may be used separately and/or together for efficient and effective early detection of EV71. The antibodies according to any aspect of the present invention provide convenient, highly specific and sensitive means for detecting EV71. One such means is the ELISA format. Each of Mab51, Mab57, Mab53 or Mab4 can be used as a capture antibody, either alone or in combination. If used alone, the selected antibody can be used as the capture antibody and that same antibody conjugated with horseradish peroxidase (HRP) can be used as detecting antibody.

Other immunological methods to detect EV71 viruses include, for example, dot-blot and *in situ* hybridization.

According to another aspect, the present invention provides at least one method of treatment and/or protection of EV71 and/or at least one EV71-linked disease, the method comprising administering to a subject in need thereof at least one antibody or a fragment thereof according to any aspect of the present invention. The antibody of the present invention may be administered in combination with other similar antibodies targeting different EV71 epitopes. The viruses may thus be given no opportunity to adapt to this form of therapy.

According to another aspect, the present invention provides at least one antibody or a fragment thereof according to any aspect of the present invention for use in medicine.

According to yet another aspect, the present invention provides at least one use of the antibody or a fragment thereof according to any aspect of the present invention for the preparation of a medicament for treatment of EV71 and/or at least one EV71-linked disease. EV71-linked diseases include but are not limited to aseptic meningitis, encephalitis, cranial nerve palsies, Guillan-Barre syndrome, poliomyelitis-like syndrome, Hand, Foot and Mouth disease and the like.

According to yet another aspect, the present invention provides at least one pharmaceutical composition comprising an antibody according to any aspect of the present invention and a pharmaceutically acceptable carrier.

The EV71 mAbs according to any aspect of the present invention have advantages over other current methodologies as diagnostic tools. For example, the mAbs are highly specific

for EV71 which is still not well understood in the field of virology. Such highly specific mAbs represent a breakthrough in the field of EV71 diagnosis. The mAbs according to any aspect of the present invention may recognize all, or essentially all, of the EV71 genotypes. These mAbs also provide a safe and convenient diagnostic approach for the detection of EV71.

- 5 The antibodies are useful for diagnosis and for the preparation of recombinant antibodies for treatment, and as such will be very useful tools in restraining a potential EV71 outbreak.

- Also, despite the existence of typing methods, there remains a need for reagents which can be used in a diagnostic assay to rapidly and accurately distinguish EV71 from CA16, and which can be used to identify the strain of EV71 specifically. The mAbs according to any aspect of the present invention may be used to rapidly and accurately identify the strain of EV71 and/or differentiate between EV71 and CA16. Such reagents and methods will allow the clinician to improve the speed and accuracy of processing large numbers of clinical samples. Such reagents and methods will also aid the clinician in patient management, eliminate unnecessary tests, improve the speed and accuracy of diagnosis and prognosis, help control EV71 infection, and reduce the use of unnecessary antibiotics. The Mabs of according to any aspect of the present invention may be used for direct detection of EV71 viral particles from blister or oral swabs.

- 20 Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention.

- A person skilled in the art will appreciate that the present invention may be practised without undue experimentation according to the method given herein. The methods, techniques and chemicals are as described in the references given or from protocols in standard biotechnology and molecular biology text books.

EXAMPLES

- 30 Standard molecular biology techniques known in the art and not specifically described were generally followed as described in Sambrook and Russel, Molecular Cloning: A Laboratory Manual, Cold Springs Harbor Laboratory, New York (2001).

EXAMPLE 1

- 35 **Preparation and purification of Mab51, Mab57, Mab53 or Mab4 from whole EV71 virus.**

Hybridomas secreting specific Mabs were derived from BALB/c mice which had been immunized twice intramuscularly with purified EV71-B5 strain in 0.1 ml of PBS, emulsified with an equal volume of adjuvant (SEPPIC, France). An intraperitoneal booster of the same dose of virus was given three days before splenocytes were fused to the SP2/0 myeloma cells which were purchased from ATCC. Hybridomas identified to produce specific antibody, were cloned by limiting dilution and expanded in 75cm² flasks. One week later, the hybridoma suspension was harvested and cell debris pelleted by centrifugation at 400 g for 10 min, followed by collection of the supernatant and storage at -20 °C. Mab concentrations were determined spectrophotometrically (Nanodrop, DE, USA).

Antigen capture ELISA

96-well, round-bottom microtiter plates (Nunc, Roskilde, Denmark) were coated with 500ng⁻¹ µg/well of capture antibody in 100µl of carbonate buffer (73 mM sodium bicarbonate and 30 mM sodium carbonate, pH 9.7) overnight at 4 °C or 37 °C for 2 h. The plates were washed twice with PBST, followed by two washes with PBS after each incubation with antibody or antigen. The antibody-coated plates were blocked with 100µl of blocking buffer (PBS containing 5% milk) for 1 h at room temperature and then incubated at 37 °C for 1 h with 100µl of virus-containing samples diluted in PBST. Virus binding was detected by incubation with 100µl of horseradish peroxidase-conjugated detection MAb (in-house labeling; Pierce) for 1 h at 37 °C. Chromogen development was mediated by the addition of 100µl of freshly prepared substrate solution (o-phenylenediamine-dihydrochloride; Sigma). The reaction was stopped by adding 0.1 N sulfuric acid, and the optical density at 490 nm was recorded. The detection limit was determined as the optical density value that gave a signal-to-noise ratio of 3.

EXAMPLE 2

Characterization of Mab51 and Mab53

Mab51 and Mab53 belong to subclass IgM and IgG respectively as identified with an isotyping Kit (GE). As shown in Figure 1, Mab51 and Mab53 could detect EV71 viral protein expression in infected vero cells as shown by western blotting. Western blotting of sucrose-gradient purified EV71 virus particles from the C4 strain revealed that the Mab51 and Mab53 are specific for VP1 as shown in Figure 1.

Epitope mapping of Mab51 and Mab53

In order to characterize the Mab51 and Mab53 epitope, the VP1 protein was fragmented into GST-tagged continual overlapping peptides and cloned into pGex-4T-1 vector and expressed in *E. coli* BL21 cells and tested in Western blots using Mab51 and Mab53 separately. Gradual reduction of the fragment length led to the identification of a linear epitope of Mab51 and Mab53 which mapped to amino acid positions 215-219 of the VP1 protein. The minimal epitope consists of the five amino acids KQEKD (SEQ ID NO:1) as shown in Figure 2.

Epitope mapping of Mab4

In order to characterize the Mab4 epitope, the VP1 protein was fragmented into GST-tagged continual overlapping peptides and cloned into pGex-4T-1 vector and expressed in *E. coli* BL21 cells and tested in Western blots using Mab4. The results are shown in Figure 3. VP1 capsid protein was expressed as eight C-terminal truncated proteins. All the eight fragments carrying the intact N-terminal sequence were recognized by Mab4. Thus, the epitope of Mab4 is located at the N-terminal region of VP1 protein. In particular, as shown in Figure 3A, the epitope of Mab4 was found within amino acids 1-66. In continuation, fragment A protein as shown in Figure 3A was expressed as four N-terminal truncated proteins denoted as A10, A24, A32 and A48. From the western blot results shown in Figure 3B, Mab4 could only recognize A10 fragment which encompass amino acid residue 10 to 297. This implied that the epitope of Mab4 was within amino acids 10 to 24.

Another panel of N-terminal truncated proteins, with deletion of 2 amino acids for every consecutive protein was expressed. From the blot, only A12 could be recognized as shown in Figure 3C. This implied that the epitope should begin at either amino acid 12 or 13 as A14 could not be recognized by Mab4. Therefore, the putative epitope of Mab4 should be from amino acid 12-17 (IGDSVS) or 13-18 (GDSVSR). However, to confirm the epitope of Mab4, these putative epitopes must be expressed with GST tag to test for its immunoreactivity with Mab4.

EXAMPLE 3

Specificity of Mab51 and Mab53 to EV71 subgenogroups by Western blotting

To confirm the presence of SEQ ID NO:1 epitope protein in the virus particles, virus particles from the different EV71 subgenogroups were of sucrose purified. ECL reagent was used for developing. RD cells were infected with virus and the supernatant was collected after 48 h when more than 90% of the cells showed cytopathic effect. Cell debris

- were removed by a clarification spin and microfiltration through a 0.2µm cut-off filter, followed by concentration by ultraspin at 100'000g for 3 h. Virus particles were purified by centrifugation in a 20% to 60% sucrose gradient for 3h at 27'000g. Several bands were detected and the virus band at the 40 to 60% sucrose meniscus was collected. Western blotting using Mab51 and Mab53 separately as primary antibodies was performed and SEQ ID NO:1 protein bands were detected in the viral fractions as shown in Figure 4.

EXAMPLE 4

- Immunofluorescence assay (IFA) of CA16 (isolated in Finland, 1994, U05876) infected African green monkey kidney cells (vero) cells with Mab against 3D, Mab 51, Mab 53, Mab 57 and Mab 4 were carried out. The results are shown in Figure 5. As can be seen none of the antibodies, Mab 51, Mab 53, Mab 57 and Mab 4 recognised the presence of CA16.
- To corroborate the findings of the mutational analysis of the epitope, (vero) were infected with wild-type EV71 from different subgenogroups and an IFA conducted. The selected strains are provided in Table 1.

Name	Subgenotype	NCBI (accession number)
BrCr	A	U22521
RG EV71-VP1(B1)	B1	AF135901
7423/MS/87	B2	ETU22522
RG EV71-VP1(B3)	B3	AF376093
HFM41	B4	
NUH0083	B5	FJ461781
Y90-3761	C1	AB433864
NUH0075	C2	FJ172159
RG EV71-VP1(C3)	C3	AY125973
75-Yamagata	C4	AB177813
RG EV71 Fuyang/02/2008	C4	EU703813
3437/SIN/06	C5	GU222654

Table 1. Subgenotypes of EV71 used for infection of Vero cells in IFA

20

All tested strains were positively identified by Mab51 as shown in Figure 6, by Mab53 as shown in Figure 7 and by Mab4 as shown in Figure 8. FITC labeled secondary antibody was used in the assay. Mab51, Mab53 and Mab4 can thus be used as a universal detection antibody for EV71.

The neutralization results for Mab57 and Mab53 against EV71 subgenotypes were confirmed using in vitro microneutralisation methods known in the art. The results are shown in Table 2 below. Mab51 was positive in vitro micro neutralization of all sub genotypes of EV71. And the dilution titer is up to 1:1024. Mab57 was positive in vitro micro neutralization of some different sub genotypes of EV71, B4, B5, C1, C2, C3 and C5. And the dilution titer is up to 1:4096.

AscitesA		RGB1	B2	RGB3	B4	B5	C1	C2	RGC3	C4	C5
#51	2 ¹⁰	2 ¹⁰	2 ¹⁰	2 ¹⁰	2 ¹⁰	2 ¹⁰	2 ⁹	2 ⁹	2 ¹⁰	2 ⁹	2 ¹⁰
#57	-	-	-	-	2 ¹¹	2 ¹²	2 ¹¹	2 ¹¹	2 ¹¹	-	2 ¹²

Table 2. In vitro micro neutralization of different sub genotypes of EV71 by mAb
 -: no activity

10 EXAMPLE 5

This experiment was carried out by using AG129 mice (B&K Universal Ltd, HII, UK).

2-week-old AG129 mice were injected with Mab51 at 10µg/g of body weight one day prior to lethal challenge with 10⁷ plaque forming units (PFU) of EV71 strain HFM41.

15

A control group was injected with an irrelevant mouse IgM antibody (isotype control) before the lethal challenge. While the control animals which received an isotype antibody developed severe limb paralysis as early as day 6 post-infection, mice pre-treated (prophylactic studies) with Mab51 did not display any of the disease manifestations and remained healthy throughout the experiment. 2-week-old immunodeficient AG129 mice were susceptible to infection with the non mouse-adapted EV71 strain HFM 41 via the intraperitoneal route of inoculation and anti-EV71 antibody Mab51 was able to confer 100% protection against the lethal EV71 challenge at a dose of 10µg/g of body weight.

25 The results are shown in Table 3 and Figures 10 and 11. Figure 10 shows the cross-section of the spinal cord with arrow indicating neuropil vacuolation and neuronal loss without inflammation in the anterior horn in AG129 mice challenged with EV71 infection. Figure 11 shows the cross-section of spinal cord showing no significant pathology when AG129 mice challenged with EV71 infection were given prophylactic protection with Mab51.

30

Mab51 was thus capable of conferring protection against EV71 infection and protecting against all pathologic changes in infected mice.

	isotype control	Prophylactic studies
Antibody treatment	Isotype IgM (100µg) 24 hr before challenge	Antibody (100µg) 24 hr before challenge
No. of animal	10	10
Survival as of day (9 days post infection)	Day7: 2 died Day8: 4 died	No death

Table 3. Summary of prophylactic studies with isotype control group

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CLAIMS

1. An isolated neutralising monoclonal antibody or a fragment thereof that is capable of specifically binding to at least one epitope of Enterovirus 71 (EV71), wherein the antibody is of subclass immunoglobulin M, wherein the epitope is of SEQ ID NO: 1 and wherein the antibody is monoclonal antibody Mab51 produced by hybridoma cell line deposited with American Type Culture Collection (ATCC) with accession number CBA20110005.
2. An isolated neutralizing monoclonal antibody or a fragment thereof that is capable of specifically binding to at least one epitope of Enterovirus 71 (EV71), wherein the antibody is of subclass immunoglobulin M, wherein the antibody is monoclonal antibody Mab57 produced by hybridoma cell line deposited with American Type Culture Collection (ATCC) with accession number CBA20110007.
3. The antibody according to claim 1 or 2, wherein the epitope is at least one conformational epitope of EV71.
4. The antibody according to claim 3, wherein the conformational epitope is an intact virus capsid.
5. The antibody according to claim 4, wherein the capsid protein is VP1, VP2 VP3, VP4 and/or VP0 precursor.
6. An isolated monoclonal antibody or a fragment thereof, wherein the antibody is capable of specifically binding to at least one epitope of Enterovirus 71 (EV71) of SEQ ID NO: 1, wherein the antibody is of subclass immunoglobulin G, wherein the antibody is monoclonal antibody Mab53 produced by hybridoma cell line deposited with American Type Culture Collection (ATCC) with accession number CBA20110006.
7. An isolated monoclonal antibody or a fragment thereof that is capable of specifically binding to at least one epitope of Enterovirus 71 (EV71) of sequence IGDSVS or GDSVSR, wherein the antibody is monoclonal antibody Mab4 produced by hybridoma cell line deposited with American Type Culture Collection (ATCC) with accession number CBA20110004.
8. The antibody according to any one of the preceding claims, wherein the antibody is at least one human, humanized or chimeric antibody.

9. The antibody according to any one of the preceding claims, wherein EV71 is of genotype selected from the group consisting of A, B2, B4, B5, C1, C2, C4 and C5.
10. The antibody according to any one of the preceding claims, wherein the antibody is labelled with at least one radionuclide and/or fluorochrome.
11. The antibody according to any one of the preceding claims, wherein the antibody is linked with at least one drug, anti-viral drug, and/or toxin.
12. An isolated hybridoma cell line deposited with American Type Culture Collection (ATCC) with accession number CBA20110004, CBA20110005, CBA20110006 and/or CBA20110007.
13. A method of detecting and/or quantifying the presence and distribution of at least one EV71 infected cell in a subject, the method comprising:
 - a. contacting at least one antibody or fragment thereof according to any one of claims 1 to 11 with at least one sample obtained from at least one subject; and
 - b. detecting and quantifying binding of the antibody to the EV71 infected cell.
14. The method according to claim 13, wherein the step of detecting comprises contacting the sample with a binding protein that contains or is conjugated to a detectable element.
15. The method according to either claim 13 or 14, wherein the antibody is immobilized onto a solid surface.
16. The method according to either claim 14 or 15, wherein the binding protein contains a radioactive atom, is conjugated to a fluorescent molecule, or is conjugated to an enzyme.
17. A kit for diagnosing EV71 and/or at least one EV71-linked disease, the kit comprising at least one antibody or a fragment thereof according to any one of claims 1 to 11.
18. The kit according to claim 17, wherein the EV71-linked disease is at least one disease selected from the group consisting of aseptic meningitis, encephalitis, cranial nerve palsies, Guillan-Barre syndrome, poliomyelitis-like syndrome, and Hand, Foot and Mouth disease.
19. A method of treatment and/or prevention of EV71 and/or at least one EV71-linked disease, the method comprising administering to a subject in need thereof at least one antibody or a fragment thereof according to any one of claims 1 to 11.

20. The method according to claim 19, wherein the EV71-linked disease is at least one disease selected from the group consisting of aseptic meningitis, encephalitis, cranial nerve palsies, Guillan-Barre syndrome, poliomyelitis-like syndrome, and Hand, Foot and Mouth disease.
21. An isolated antibody according to any one of claims 1 to 11 for use in medicine.
22. An isolated antibody according to any one of claims 1 to 11 for use in treatment of and/or prevention of EV71 and/or at least one EV71-linked disease.
23. Use of an isolated antibody according to any one of claims 1 to 11 for the preparation of a medicament for treatment of and/or prevention of EV71 and/or at least one EV71-linked disease.
24. A pharmaceutical composition comprising the antibody of any one of the preceding claims 1 to 11 and a pharmaceutically acceptable carrier.

SEQUENCE LISTING

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