



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : C12Q 1/70	A2	(11) International Publication Number: WO 00/58524 (43) International Publication Date: 5 October 2000 (05.10.00)
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(54) Title: TYPING OF HUMAN ENTEROVIRUSES (57) Abstract The present invention discloses a method for detecting the presence of an enterovirus in a clinical sample. The invention additionally discloses a method for typing an enterovirus in a clinical sample. Both methods employ a set of primer oligonucleotides for reverse transcription and amplification that hybridize to conserved regions of the enterovirus genome, and that provide amplicons that include significant portions of the VP1 region that are characteristic of the various serotypes. In the typing method, the invention further provides a database consisting of nucleotide sequences from prototypical enteroviral serotypes, which is used to type the clinical sample by comparing the sequence of its amplicon with each prototypical sequence in the database. The invention additionally provides mixtures of primer oligonucleotides, and a kit for use in conducting the typing method that includes a mixture of the primer oligonucleotides.		

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TYPING OF HUMAN ENTEROVIRUSES

FIELD OF THE INVENTION

The present invention relates to methods of detecting the presence, and of establishing the serotype, or serovar, of an enterovirus that may be present in a clinical sample or a biological sample, as well as to a kit that includes primers that may be used in the methods. The methods include amplification of viral RNA, and sequencing of the resulting amplicons.

BACKGROUND OF THE INVENTION

Enteroviruses constitute a broad range of pathogens etiologically responsible for a wide range of diseases in humans, as well as in other animals. The genus *Enterovirus* is a member of the family *Picornaviridae*. As the family name indicates, enteroviruses are small RNA viruses; they contain positive single stranded RNA as the genome. Five groups are found within the enteroviruses: coxsackievirus A (CA), coxsackievirus B (CB), echovirus (E), and numbered enteroviruses (EV), as well as poliovirus (PV). There are 66 serotypes currently classified among the human enteroviruses, although two serotypes, E22 and E23, are to be reclassified in a different genus.

The viral genome is shown schematically in Figure 1. The single stranded RNA comprises a 5' nontranslated region (single line), which is followed by an open reading frame coding for a polyprotein precursor of Mr 240-250 x 10³ Da (boxed portion), followed by a 3' noncoding sequence and a poly (A) tract (single line). In the polyprotein, the sequence of gene products begins 1A, 1B, 1C, 1D, and 2A. 1A through 1D are, respectively, the structural proteins VP4, VP2, VP3, and VP1 of the viral capsid; VP1 is followed in the open reading frame by a nonstructural protein 2A.

The various members of the human enteroviruses cause a wide range of symptoms, syndromes and diseases. These include acute benign pericarditis, acute flaccid paralysis, acute hemorrhagic conjunctivitis, aseptic meningitis, various exanthemas, carditis, croup, encephalitis, enanthema, gastrointestinal disease,

hepatitis, hand-foot-and-mouth disease, various respiratory diseases, myocarditis, neonatal disease including multi-organ failure, pericarditis, pleurodynia, rash, and undifferentiated fever. In general, the syndromes are not correlated with particular enterovirus serotypes, nor does a serotype specifically correlate with a particular disease, although in certain cases serotypes do correlate with particular diseases.

Enteroviruses are responsible for large numbers of infections. There may be between 30 million to 50 million illnesses that are ascribable to enteroviruses each year in the United States (CDC; MMWR 46:748-750; Strikas et al. J. Infect. Dis. 146:346-351 (1986); Rotbart in Human Enterovirus Infections, H. A. Rotbart (ed.) ASM Press, Washington, DC, pp. 401-418 (1995)). After rhinoviruses, enteroviruses are the most common viral infection in humans. Enteroviral infections lead to 30,000 to 50,000 hospitalizations each year for aseptic meningitis, myocarditis, encephalitis, acute hemorrhagic conjunctivitis, nonspecific febrile illnesses, and upper respiratory infections (Melnick, Biologicals 21:305-309 (1993); Morens et al. in Human Enterovirus Infections, H. A. Rotbart (ed.) ASM Press, Washington, DC, pp. 3-23 (1995); Melnick in Fields Virology (B. N. Fields et al. (eds.) 3rd ed., Lippincott-Raven Publishers, Philadelphia, pp. 655-712 (1996)). Enteroviruses are also implicated in acute flaccid paralysis in animal models, as well as in dilated cardiomyopathy. The six serotypes of coxsackie B viruses are implicated in a variety of clinical diseases, such as meningitis, myocarditis and severe neonatal disease. Recently, enterovirus infection has been linked to chronic fatigue syndrome (Clements et al., J. Med. Virol. 45:156-161 (1995)).

Poliovirus is also an enterovirus that infects humans. Three serotypes, PV1, PV2, and PV3 are known. A nonenteroviral picornavirus that also afflicts humans is human rhinovirus (HRV), responsible for many common cold infections; several serotypes have been identified. Additionally, picornaviruses affect mammals other than humans, including viruses such as bovine enterovirus (BEV) and simian picornavirus (SPV).

It is important to identify the serotype of an enterovirus infection in a subject. Knowledge of the serotype can provide useful guidance to a physician in determining

a course of treatment of the disease in the subject. For example, the appropriately identified immune globulin having a sufficient titer may be administered to immunocompromised patients. Furthermore, an antiviral drug such as Pleconaril (Viropharma) may differ in its relative efficacy against different serotypes.

5 Additionally, an understanding of the geographic and chronological development of an enterovirus infection in a population can influence preventive measures among the members of the population to minimize the spread of the disease. Furthermore, it is useful from a broader perspective to track the incidence and distribution of an enterovirus disease from an epidemiological point of view. In earlier practice, it was
10 found that the various serotypes could be grown in different cell culture hosts, and in different animal model hosts. In the animal hosts, furthermore, different symptomology also provided typing information. These classical assays provide ways of distinguishing the serotypes. Nevertheless, some enterovirus serotypes, especially in the coxsackievirus A group, do not grow in cell culture. It has been observed that
15 25% to 35% of patient specimens are not identified by cell culture for a variety of reasons (Rotbart, 1995). Furthermore, such culturing and classification procedures are costly, time-consuming, subject to experimental variation, and not amenable to repetitive or extensive application in the field.

The serotypes of non-polio enteroviruses have been identified during the past
20 several decades using classical immunological neutralization assays based on a panel of specific antibodies. Application of such a determination to a clinical sample is generally impractical and inconvenient. Although a number of neutralization sites have been localized to the VP1 protein of enteroviral particles, the exact identity of the epitopes responsible for serotype specificity remain unknown; VP2 and VP3 may
25 also contain specific neutralizing epitopes. Serotyping has generally been carried out using intersecting pools of antisera, the Lim and Benyesh-Melnick (LBM) pools, which were originally defined in 1960 (Lim et al., J. Immunol. 84:309-317 (1960)). The antiserum pools currently distributed by the World Health Organization cover 42 serotypes in 8 pools (Melnick et al., Bull. WHO 48:263-268 (1973)). Analysis of the
30 neutralization pattern affords an identification of serotype. (See Rotbart, 1995).

Clearly, this is a cumbersome and painstaking process. Additionally, the supply of the antisera is limited or difficult to maintain. Problems in serotyping more recent isolates have been ascribed to pronounced intratypic antigenic variation (Melnick, Enteroviruses: polioviruses, coxsackie viruses, echoviruses, and newer enteroviruses. In Fields Virology (Fields et al., (Eds.) 3rd Ed., Lippincott-Raven Publishers, Philadelphia, 1996, pp. 655-712; Melnick et al., Bull. W.H.O. 63:453-550 (1985); Wigand et al., Arch. Ges. Virusforsch. 12:29-41 (1962); Wenner et al., Am J. Epidemiol. 85:240-249 (1967); Duncan, Arch. Ges. Virusforsch. 25:93-104 (1968)). This has been explained by pointing out that enteroviruses, being RNA viruses, undergo spontaneous mutation at a very high rate. This can lead to antigen drift, with the potential of producing antigenic variants such that a neutralization assay would produce a false negative result. For example, escape mutants in picornaviruses are discussed in detail in Mateu (Virus Res. 38:1-24 (1995)). For all these reasons there is a need to supplant neutralization assays for serotyping non-polio enteroviruses.

More recently assays based on nucleic acid detection have been developed. Probe hybridization assays directed either to RNA or to cDNA have been used to detect non-polio enteroviruses (Rotbart et al., Mol. Cell. Probes 2:65-73 (1988); Rotbart, J. Clin. Microbiol. 28:438-442 (1990); Chapman et al., J. Clin. Microbiol. 28:843-850 (1990); Hyypia et al., J. Gen. Virol. 70:3261-3268 (1989); Olive et al. J. Gen. Virol. 71:2141-2147 (1990); Gilmaker et al., J. Med. Virol. 38:54-61 (1992); Yang et al., Virus Res. 24:277-296 (1992); Zoll et al., J. Clin. Microbiol. 30:160-165 (1992); Muir et al., J. Clin. Micro. 31:31-38 (1993); Drebot et al., J. Med. Virol. 44:340-347 (1994); Rotbart et al., J. Clin. Microbiol. 32:2590-2592 (1994)). In the absence of nucleic acid sequence information for the non-polio enteroviruses, most of these probes have targeted the highly conserved 5' non-coding region of the viral genomes. Additionally, RNA probes directed to the VP1 capsid gene have been used on a limited basis to identify some of the CBs and a few closely related CAs (Cova et al., J. Med. Virol. 24:11-18 (1988); Alksnis et al., Mol. Cell. Probes 3:103-108 (1989); Petitjean et al., J. Clin. Microbiol. 28:307-311 (1990)). More recently, oligonucleotides having sequences based on the VP4-VP2 junction have been applied

as diagnostic and epidemiologic tools (Drebot et al., J. Med. Virol. 44:340-347 (1994); Arola et al., J. Clin. Microbiol. 34:313-318 (1996); Kim et al., Arch. Virol. 142:853-860 (1997); Oberste et al., Virus Res. 58:35-43 (1998)). The sequences in these regions, however, do not always correlate with serotype (Kopecka et al., Virus Res. 38:125-136 (1995); Arola et al., J. Clin. Microbiol. 34:313-318 (1996)). Furthermore, sequences of only certain prototypes were available with which to compare and classify clinical samples (Arola et al., (1996)). A generic probe-based assay for nucleic acids in the presence of chaotropic agents is described in U.S. Patent 5,726,012. An assay for a target nucleic acid sequence wherein two separate probes are hybridized to the same strand of a nucleic acid, and then joined, for example by a polymerase activity, is disclosed in U.S. Patent 5,516,641.

Reverse transcription (RT) coupled with the polymerase chain reaction (PCR) (RT-PCR) has been developed using enterovirus universal primers or broadly selective primers. Such primers are intended to amplify nucleotide regions from a large number of enterovirus serotypes in one diagnosis. One set of primers (Rotbart, J. Clin. Microbiol. 28:438-442 (1990)) has been reported to amplify 60 of the 66 serotypes tested. (Among the nonreactive serotypes, two are atypical enteroviruses and may be reclassified.) A comparison of sequence identities of the various sets of universal primers with serotype sequences is given in Rotbart et al. (1995). Many of the universal primer sets are designed to amplify regions of the 5' untranslated region of the genome (see, for example, Drebot et al. (1994); Diedrich et al., J. Med. Virol. 46:148-152 (1995); Arola et al. (1996); Bailly et al., Virology 215:83-96 (1996); and U.S. Patent 5,075,212 to Rotbart). A comparison of base sequences in coxsackievirus B5 was reported for isolates from three different outbreaks of disease, based on amplicons obtained using primers in the VP1/2A region of the genome (Kopecka et al., (1995)). Variations in sequence occurred even within the same outbreak, and somewhat greater variations were found among isolates from the different outbreaks, and between serotypes. International application WO 98/14611 discloses degenerate primers directed to the VP1 gene, which, when used in certain defined pairs, provide PCR amplification of enterovirus nucleic acids. Use of the specific primer pairs

permits ascertaining whether a sample belongs to an enterovirus serotype, or to a small group of cognate serotypes, based on correlation of the pattern of the presence or absence of an amplicon with priming by the various primer pairs. This method does not rely on obtaining nucleotide sequences for accomplishing the serotyping.

5 Oberste et al. developed a database of homologous sequences for a portion of the VP2 gene of all 66 human enterovirus serotypes (Virus Res. 58:35-45 (1998a)). They found that the sequences of many antigenic variants failed to cluster with their respective prototype strains as determined by serotyping. This finding suggested that the portion of VP2 examined may not prove to be useful for consistent molecular
10 inference of serotype.

 According to Holland et al. (J. Clin. Microbiol. 36:1588-1594 (1998)) neither cell culture growth, nor PCR can successfully type enterovirus infections. They report an alternative typing protocol based on polyacrylamide gel electrophoretic fingerprinting of whole virus radiolabeled proteins. However, the database of viral
15 protein profiles contains data for less than one-third of the known EV serotypes. Therefore its general applicability remains unknown.

 In the case of poliovirus, U.S. Patents 5,585,477 and 5,691,134 to Kilpatrick disclose methods and oligonucleotide primers that are specific and sensitive for detecting all genotypes of poliovirus, as well as primers that are specific and sensitive
20 for distinguishing the three serotypes of poliovirus, and methods for detecting poliovirus and/or distinguishing among the serotypes based on the use of the disclosed primers. Additionally WO 98/14611 discloses an extensive set of degenerate oligonucleotide primers for use in detecting the presence or absence of a non-polio enterovirus in a sample and to identify non-polio enterovirus serotypes. The primers
25 are combined in pairs that detect various groupings of serotypes, and several amplification procedures are carried out in order to detect the presence or absence of an amplicon in each case. A pooled grid of the results provides information useful in typing a non-polio enterovirus in a sample.

 In summary, immunological methods for serotyping enteroviral infections are
30 cumbersome and time consuming. They rely on an antigen-antibody reaction between

antiserum pools established more than two decades ago, and whose supply may become limited. As explained, for example in Mateu (1995), antigen drift among RNA viruses such as the enteroviruses leads to a high probability that escape mutants will arise, and thereby escape not only serotyping, but perhaps detection as well. A second classical approach, cell culture coupled with whole animal host growth and use of antisera for typing, is extremely cumbersome, expensive, and labor-intensive. Modern molecular biological methods similarly have important deficiencies as currently implemented. Probe assays generally tend to lack sensitivity. Furthermore, a probe directed to a conserved region, such as the 5' non-coding region of the non-polio enteroviruses, lacks specificity, and so cannot be readily applied in typing a viral infection. RT-PCR has been implemented as a generic enteroviral diagnostic assay. In general, these assays fail to implement serotype-specific detection, so that typing is not currently available using RT-PCR. Holland et al. (1998) state that all typing methods in use or then currently under development are limited by virtue of the large number of different enteroviral serotypes, and as a consequence, the need for virus-specific reagents that would discriminate among them.

For these reasons, there remains a need for a typing procedure that avoids the necessity of infecting live animals, animal tissue homogenates, or cell cultures. There further remains a need to implement a nucleic acid-based enteroviral typing procedure that optimizes the specificity required for a typing protocol. There additionally persists a need for a typing procedure that avoids a requirement for a plethora of reagents directed toward the specificity of the various serotypes. There still further remains the need for an enteroviral typing procedure that does not require extended periods of time or complicated procedures to carry out. Thus, there remains a need for an operationally elegant and efficient typing procedure that utilizes the specificity that resides, for example, in the VP1 region. The present invention recognizes these needs, and addresses them.

SUMMARY OF THE INVENTION

As noted above, the determinants of serotype identity are understood to reside primarily in VP1. This amino acid sequence specificity should be reflected in the corresponding VP1 gene sequences. The present invention discloses a method, based on reverse transcription and amplification of a characteristic enteroviral nucleic acid segment, for detecting the presence of an enterovirus in a clinical sample. The method includes the steps of

- (i) obtaining a clinical sample from a subject;
- (ii) purifying RNA contained in the sample;
- 10 (iii) reverse transcribing the RNA with primers effective to reverse transcribe enteroviral RNA to provide a cDNA;
- (iv) contacting at least a portion of the cDNA with
 - (a) a composition that promotes amplification of a nucleic acid and
 - (b) an oligonucleotide mixture wherein the mixture comprises at least one oligonucleotide that hybridizes to a highly conserved sequence of the sense strand of an enterovirus nucleic acid and at least one
 - 15 oligonucleotide that hybridizes to a highly conserved sequence of the antisense strand of an enterovirus nucleic acid, thereby providing an amplification mixture, such that, upon hybridizing, the
 - 20 oligonucleotides direct amplification of at least a portion of the nucleotide sequence of the VP1 gene of the enterovirus genome;
- (v) carrying out an amplification procedure on the amplification mixture, such that, if an enterovirus is present in the sample, an enterovirus amplicon is produced whose sequence includes a nucleotide sequence of at least a portion
- 25 of the VP1 region of the enterovirus genome; and
- (vi) detecting whether the amplicon is present.

The presence of the amplicon, of course, indicates that an enterovirus is present in the sample.

In important embodiments of the method, the highly conserved sequences occur within the VP1 gene or within about 100 nucleotides from a terminus of the

VP1 gene. Advantageously, at least one oligonucleotide of the mixture includes, at the 3' end thereof, a sequence that hybridizes to a sequence encoding the amino acid motif given by the sequences of either SEQ ID NO:80 or SEQ ID NO:81, and at least one oligonucleotide includes, at the 3' end thereof, a sequence that hybridizes to a
5 sequence encoding a motif given by SEQ ID NO:82. Still more advantageously, the oligonucleotide mixture includes an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:3, and at least one oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:4, or an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence
10 given by SEQ ID NO:9. In a highly advantageous embodiment, the sequences of these three oligonucleotides are given respectively by SEQ ID NO:3, SEQ ID NO:4, and SEQ ID NO:9.

In a further important embodiment of the method of detection, at least one oligonucleotide of the mixture includes, at the 3' end thereof, a sequence that
15 hybridizes to a sequence encoding a motif given by SEQ ID NO:86, and at least one oligonucleotide includes, at the 3' end thereof, a sequence that hybridizes to a sequence encoding the amino acid motif given by the sequences of either SEQ ID NO:83, SEQ ID NO:84, or SEQ ID NO:85. In a further important embodiment, the oligonucleotide mixture contains an oligonucleotide whose sequence includes, at the
20 3' end thereof, the sequence given by SEQ ID NO:22, and at least one oligonucleotide chosen from among an oligonucleotide whose sequence includes, at the 3' end thereof, the sequence given by SEQ ID NO:19, an oligonucleotide whose sequence includes, at the 3' end thereof, the sequence given by SEQ ID NO:20, and an oligonucleotide whose sequence includes, at the 3' end thereof, the sequence given by SEQ ID NO:21.
25 In a still more important embodiment, the oligonucleotide mixture contains an oligonucleotide whose sequence is given by SEQ ID NO:22, and at least one oligonucleotide chosen from among oligonucleotides whose sequences are given by SEQ ID NOs:19, 20, and 21.

In further significant embodiments of the method, the amplification procedure
30 includes a polymerase chain reaction, and the sample is obtained from among whole

blood or a fraction thereof, a bronchial wash, cerebrospinal fluid, an eye swab, a conjunctival swab, a swab or scraping from a lesion, a nasopharyngeal swab, an oral or buccal swab, pericardial fluid, a rectal swab, serum, sputum, saliva, stool, a stool extract, a throat swab, urine, brain tissue, heart tissue, intestinal tissue, kidney tissue, liver tissue, lung tissue, pancreas tissue, spinal cord tissue, skin tissue, spleen tissue, thymus tissue, cells from a tissue culture, a supernatant from a tissue culture, and tissue from an experimentally infected animal. In still other significant embodiments, the detection is carried out by a procedure chosen from among gel electrophoresis and visualization of amplicons contained in a resulting gel, capillary electrophoresis and detection of the emerging amplicon, probing for the presence of the amplicon using a labeled probe, and labeling a PCR primer employed in the method and detecting the label.

The invention additionally discloses a method for typing an enterovirus in a clinical sample that includes the steps of

- (i) obtaining a clinical sample from a subject;
- (ii) purifying RNA contained in the sample;
- (iii) reverse transcribing the RNA with primers effective to reverse transcribe enteroviral RNA to provide a cDNA;
- (iv) contacting at least a portion of the cDNA with
 - (a) a composition that promotes amplification of a nucleic acid and
 - (b) an oligonucleotide mixture wherein the mixture comprises at least one oligonucleotide that hybridizes to a highly conserved sequence of the sense strand of an enterovirus nucleic acid and at least one oligonucleotide that hybridizes to a highly conserved sequence of the antisense strand of an enterovirus nucleic acid, thereby providing an amplification mixture, such that, upon hybridizing, the oligonucleotides direct amplification of at least a portion of the nucleotide sequence of the VP1 gene of the enterovirus genome;
- (v) carrying out an amplification procedure on the amplification mixture, such that, if an enterovirus is present in the sample, an enterovirus sample amplicon

is produced whose sequence includes a nucleotide sequence of at least a portion of the VP1 region of the enterovirus genome;

(vi) determining that the sample amplicon is present;

(vii) determining at least a partial nucleotide sequence of the sample amplicon;

(viii) providing a database consisting of prototypical nucleotide sequences, wherein each prototypical sequence is the sequence of a standard amplicon obtained from a member of a set of prototypical enterovirus serotypes by carrying out the procedure of steps (ii) through (v) on each prototypical enterovirus serotype, wherein each prototypical sequence comprises at least a portion of the sequence of the VP1 gene, and wherein the sequence of each prototypical VP1 gene is different from the sequence of every other prototypical VP1 gene in the database;

(ix) comparing the sequence of the sample amplicon with each prototypical sequence in the database; and

(x) identifying the prototypical sequence that has the highest extent of identity to the sequence of the sample amplicon, thereby providing an identified serotype;

wherein the type of the sample is the serotype of the identified serotype.

In important embodiments of this method, the highly conserved sequences occur within the VP1 gene or within about 100 nucleotides from a terminus of the VP1 gene. More importantly, at least one oligonucleotide of the mixture includes, at the 3' end thereof, a sequence that hybridizes to a sequence encoding the amino acid motif given by the sequences of either SEQ ID NO:80 or SEQ ID NO:81, and at least one oligonucleotide includes, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a motif given by SEQ ID NO:82. In significant embodiments of the method, the oligonucleotide mixture includes an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:3, at least one oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:4 or an oligonucleotide whose sequence contains, at the 3' end thereof,

the sequence given by SEQ ID NO:9. In a highly advantageous embodiment, the sequences of the oligonucleotides are given by SEQ ID NO:3, SEQ ID NO:4, and SEQ ID NO:9.

In an additional important embodiment, at least one oligonucleotide of the mixture includes, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a motif given by SEQ ID NO:86, and at least one oligonucleotide includes, at the 3' end thereof, a sequence that hybridizes to a sequence encoding the amino acid motif given by the sequences of either SEQ ID NO:83, SEQ ID NO:84, or SEQ ID NO:85. In a further important embodiment, the oligonucleotide mixture contains an oligonucleotide whose sequence includes, at the 3' end thereof, the sequence given by SEQ ID NO:22, and at least one oligonucleotide chosen from among an oligonucleotide whose sequence includes, at the 3' end thereof, the sequence given by SEQ ID NO:19, an oligonucleotide whose sequence includes, at the 3' end thereof, the sequence given by SEQ ID NO:20, and an oligonucleotide whose sequence includes, at the 3' end thereof, the sequence given by SEQ ID NO:21. In a still more important embodiment, the oligonucleotide mixture contains an oligonucleotide whose sequence is given by SEQ ID NO:22, and at least one oligonucleotide chosen from among oligonucleotides whose sequences are given by SEQ ID NOs:19, 20, and 21.

In a further important aspect, the amplification procedure includes a polymerase chain reaction, and the resulting sample amplicon encompasses at least a portion of the nucleotide sequence for the VP1 gene of an enterovirus. The method furthermore importantly provides that the set of prototypical enterovirus serotypes comprises serotypes of coxsackie A viruses, coxsackie B viruses, echoviruses, and numbered enteroviruses. In advantageous aspects of the method, comparing the sequence of the sample amplicon with each sequence in the database employs a sequence alignment and comparison algorithm.

In further important aspects of the method, the sample is chosen from among whole blood or a fraction thereof, a bronchial wash, cerebrospinal fluid, an eye swab, a conjunctival swab, a swab or scraping from a lesion, a nasopharyngeal swab, an oral or buccal swab, pericardial fluid, a rectal swab, serum, sputum, saliva, stool, a stool

extract, a throat swab, urine, brain tissue, heart tissue, intestinal tissue, kidney tissue, liver tissue, lung tissue, pancreas tissue, spinal cord tissue, skin tissue, spleen tissue, thymus tissue, cells from a tissue culture, a supernatant from a tissue culture, and tissue from an experimentally infected animal.

5 The present invention further provides an oligonucleotide containing, at the 3' end thereof, a sequence that hybridizes to a nucleotide sequence encoding an amino acid motif chosen from among the sequences given by SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, and SEQ ID NO:86, or an oligonucleotide complementary to any of these oligonucleotides. In
10 an advantageous embodiment, the complete sequence of the oligonucleotide is a sequence that hybridizes to a sequence encoding a motif whose sequence is chosen from among SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, and SEQ ID NO:86, or is an oligonucleotide complementary to any of them.

15 In particularly important embodiments, such an oligonucleotide is one whose sequence contains, at the 3' end thereof, a sequence chosen from among the sequences given by SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:9, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, and SEQ ID NO:22, or an oligonucleotide whose sequence is complementary to any of these oligonucleotides. In still more important
20 embodiments, the sequence of the oligonucleotide consists of a sequence chosen from among SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:9, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, and SEQ ID NO:22, or an oligonucleotide that is complementary to any of them.

 The present invention further discloses a mixture of oligonucleotides including
25 at least two oligonucleotides, wherein at least one of the oligonucleotides hybridizes to a sense strand of a double stranded nucleic acid and at least one of the oligonucleotides hybridizes to an antisense strand of the nucleic acid. The nucleic acid to which the oligonucleotides hybridize encodes the VP1 gene of an enterovirus, and the oligonucleotides hybridize to sequences that are highly conserved among the
30 group of enteroviruses. The oligonucleotides, when hybridized to the nucleic acid, are

bound in the correct orientation on their respective strands to direct the synthesis of an amplicon encoding at least a portion of the VP1 protein of enteroviruses when they are employed in an amplification procedure using the nucleic acid.

In important embodiments of the mixture, each oligonucleotide includes, at the 3' end thereof, a sequence that hybridizes to the nucleic acid. In still more important embodiments, the highly conserved sequences occur within the VP1 gene or within about 100 nucleotides from a terminus of the VP1 gene. Advantageously, at least one oligonucleotide includes, at the 3' end thereof, a sequence that hybridizes to a sequence encoding the amino acid motif given by the sequences of either SEQ ID NO:80 or SEQ ID NO:81, and at least one oligonucleotide includes, at the 3' end thereof, a sequence that hybridizes to a sequence encoding an amino acid motif given by SEQ ID NO:82. Still more advantageously, the mixture includes an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:3, an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:4, and an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:9. In a highly advantageous embodiment, the sequences of the oligonucleotides are given by SEQ ID NO:3, SEQ ID NO:4, and SEQ ID NO:9.

In an important embodiment, at least one oligonucleotide of the mixture includes, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a motif given by SEQ ID NO:86, and at least one oligonucleotide includes, at the 3' end thereof, a sequence that hybridizes to a sequence encoding the amino acid motif given by the sequences of either SEQ ID NO:83, SEQ ID NO:84, or SEQ ID NO:85.

In additional significant embodiments, the oligonucleotide mixture includes an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:22, and at least one oligonucleotide chosen from among an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:19, an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:20, and an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:21. In a still more significant

embodiment, the oligonucleotide mixture includes an oligonucleotide whose sequence is given by SEQ ID NO:22, and at least one oligonucleotide chosen from among an oligonucleotide whose sequence is given by SEQ ID NO:19, an oligonucleotide whose sequence is given by SEQ ID NO:20, and an oligonucleotide whose sequence is given by SEQ ID NO:21.

The present invention additionally provides a kit for use in conducting the typing method that includes a mixture of oligonucleotides, the mixture containing an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:3, an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:4, and an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:9. In important embodiments of the kit, the oligonucleotide sequences are given by SEQ ID NO:3, SEQ ID NO:4, and SEQ ID NO:9.

In additional significant embodiments, the kit includes an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:22, and at least one oligonucleotide chosen from among an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:19, an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:20, and an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:21. In a still more significant embodiment, the oligonucleotide mixture includes an oligonucleotide whose sequence is given by SEQ ID NO:22, and at least one oligonucleotide chosen from among an oligonucleotide whose sequence is given by SEQ ID NO:19, an oligonucleotide whose sequence is given by SEQ ID NO:20, and an oligonucleotide whose sequence is given by SEQ ID NO:21.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic diagram of the non-polio enterovirus genome.

Figure 2 illustrates RT-PCR amplification of all enterovirus prototype strains using primer pairs given by SEQ ID NOs:3 and 4, and by SEQ ID NOs: 3 and 9. PCR

products were resolved by 1% agarose gel electrophoresis and visualized by ethidium bromide staining and UV transillumination. Panel A: Coxsackie A viruses, Coxsackie B viruses, and polioviruses amplified with primer pair given by SEQ ID NOs:3 and 4; Panel B: Coxsackie A viruses, Coxsackie B viruses, and polioviruses amplified with primer pair given by SEQ ID NOs: 3 and 9; Panel C: Echoviruses and numbered enteroviruses amplified with primer pair given by SEQ ID NOs: 3 and 4; Panel D: Echoviruses and numbered enteroviruses simplified with primer pair given by SEQ ID NOs: 3 and 9.

DETAILED DESCRIPTION OF THE INVENTION

The present invention advantageously provides methods for serotyping enteroviruses obtained from clinical samples. The methods are easily extended to human poliovirus, human picornaviruses such as human rhinovirus, and nonhuman picornaviruses such as bovine enterovirus and simian picornavirus. The procedures are easily and rapidly implemented using common laboratory procedures and instrumentation. They avoid the need for cumbersome, time-consuming and resource-intensive methods such as cell culture and/or host animal infection. They furthermore avoid reliance on prototypical antiserum pools which may fail to identify an enterovirus in a contemporary clinical sample because of antigen drift and escape from immunological reactivity. The methods of the present invention further advantageously permit identifying a serotype as being the most probable serotype even in the case of antigen drift, since nucleotide sequences are matched to provide a most probable serotype match, or, failing a unique match, a set of most probable serotype matches, even in the absence of a high extent of identity.

As used herein, the non-polio enteroviruses refer to the species/subgroups and serotypes, shown in Table 1, that are known in the field at the present time.

Table 1. Non-polio Enterovirus Species/Subgroups and Serotypes.

Species/Subgroup	Serotypes ^a
Coxsackievirus A	CA1 to CA22, CA24
Coxsackievirus B	CB1-CB6
Echovirus	E1-E7, E9, E11-E27, E29-
Enterovirus (Numbered)	EV68-EV71

(a). Serotypes CA-23, E-10, E-28, and EV-72 have been reclassified (Miller, Clin. Infect. Dis. 16:612-613 (1993)). E-8 has been reclassified (Committee on the Enteroviruses, Virology 16:501-504 (1962); Harris et al., J. Infect. Dis. 127:63-68 (1973)).

As used herein, a "clinical sample" or a "clinical isolate" relates to any sample obtained from a subject for use in carrying out the procedures of the present invention. In a principal aspect, the subject is suspected of suffering from a disease or syndrome that is at least partially caused by an enterovirus. The subject may also be an asymptomatic individual considered to be at risk of enterovirus infection. The sample may be a cellular sample such as a tissue sample, for example, a sample of lung tissue obtained as a biopsy or post-mortem, a fluid sample such as blood, saliva, sputum, urine, cerebrospinal fluid, or a swabbed sample obtained by swabbing a mucus membrane surface such as a nasal surface, a pharyngeal surface, a buccal surface, and the like, or it may be obtained from an excretion such as feces, or it may be obtained from other bodily tissues or body fluids commonly used in clinical diagnostic testing. In its broadest sense, a "clinical sample" or a "clinical isolate" as used herein is obtained from a human subject or a non-human mammalian subject, and is directed to suspected symptoms or syndromes ascribable to a picornavirus or enterovirus infection.

As used herein, purification of RNA as a step in the methods of the invention, in particular, as a step leading up to a RT-PCR procedure, relates to releasing RNA

from a latent or inaccessible form in a virion or a cell and allowing the RNA to become freely available. In such a state, it is suitable for effective amplification by reverse transcription and use of the polymerase chain reaction. Releasing RNA may include steps that achieve the disruption of virions containing viral RNA, as well as
5 disruption of cells that may harbor such virions. Purification of RNA is generally carried out under conditions that rigorously and effectively exclude or inhibit any ribonuclease activity that may be present. Additionally, purification of RNA may include steps that achieve at least a partial separation of the RNA dissolved in an aqueous medium from other cellular or viral components, wherein such components
10 may be either particulate or dissolved.

As used herein, "reverse transcription" or "RT" relates to a procedure catalyzed by an enzyme activity, reverse transcriptase, that synthesizes a cDNA from a single stranded RNA molecule, with the use of oligonucleotide primers having free 3'-hydroxyl groups. As used herein the term "polymerase chain reaction" or "PCR"
15 relates to a procedure whereby a limited segment of a nucleic acid molecule, which frequently is a desired or targeted segment, is amplified repetitively to produce a large amount of DNA molecules which consist only of that segment. The procedure depends on repetition of a large number of priming and transcription cycles. In each cycle, two oligonucleotide primers bind to the segment, and define the limits of the
20 segment. A primer-dependent DNA polymerase then transcribes, or replicates, the strands to which the primers have bound. Thus, in each cycle, the number of DNA duplexes is doubled.

As used herein the term "primer" or "oligonucleotide primer" relates to an oligonucleotide having a specific or desired nucleotide sequence which is
25 complementary to a particular sequence on one of the strands of a DNA duplex. When the primer is caused to hybridize to the specific sequence in a DNA duplex to which it is complementary, it may serve as the priming position, or the initiation position, for the action of a primer-dependent DNA polymerase activity. The primer, once hybridized, acts to define the 5' end of the operation of the transcription activity
30 of the polymerase on the duplex. Commonly in PCR, a specific pair of primers is

employed, wherein one of the primers hybridizes to one of the strands and the second primer hybridizes to the complementary strand. The primers hybridize in such an orientation that transcription, which proceeds in the direction from 5'- to 3'-, is in the direction leading from each primer toward the site of hybridization of the other primer. After several rounds of hybridization and transcription the amplified DNA produced is a segment having a defined length whose ends are defined by the sites to which the primers hybridize.

The oligonucleotide primers of the invention are intended for use in a RT-PCR-based amplification of a target segment of a nucleic acid from an enterovirus.

Both RT and PCR rely on the action of a DNA polymerase activity to extend the new DNA strands beyond the 3' termini of the primers. Since DNA polymerases extend a chain in the direction from 5' to 3', an oligonucleotide that contains sequences in addition to those nucleotides that hybridize to the target nucleic acid and serve as the primer must have the primer sequence at the 3' end of the oligonucleotide.

Additionally, any complements of the oligonucleotides contemplated in the invention must have the sequence complementary to the hybridizing sequence at the 5' end of the molecule such that action of a DNA polymerase will generate a primer oligonucleotide having its complementary sequence at its 3' end.

As used herein the terms "specific to" or "specific for" a target sequence, in relation to a nucleic acid sequence such as an oligonucleotide sequence, relate to a nucleotide sequence that hybridizes, under conditions used in given experimental circumstances, to the target but does not hybridize under those circumstances to sequences that are not target sequences. Nucleotide sequences that are specific for a particular target, such as the enteroviral target sequences that are included in the subject matter of the present invention, are those that include bases all of which are complementary to the corresponding base on the target.

Further as used herein, "specificity" of a nucleic acid sequence for a target sequence also encompasses nucleic acids and oligonucleotides having a small number of nucleotides which may not be complementary to the corresponding nucleotides of the target sequence. Such sequences are still "specific" for the target sequence, as

used herein, as long as the extent of deviation from complementarity remains functionally of no consequence. In particular, such a sequence is "specific" for the target sequence as long as it hybridizes effectively to the target sequence but does not hybridize to any sequence that is not a target sequence, under the conditions used in
5 given experimental circumstances.

As used herein, an "amplicon" relates to a double stranded nucleic acid segment having a defined size and sequence that results from an amplification procedure, such as a PCR procedure. The size of the amplicon is governed by the sites on the two strands of a nucleic acid duplex to which the primers bind. As
10 explained in U.S. Patent 4,683,195, that segment of the product nucleic acid becomes the prevalent product of the amplification procedure after a small number of cycles of amplification.

As used herein, the terms "prototype", "prototypical sequence", "prototypical amplicon", and "prototypical enterovirus serotype" relate, insofar as the root
15 "prototyp-" occurs in each of these terms, to the enterovirus serotypes which were used to establish the classical antisera defined against each serotype. These were originally obtained several decades ago, as described in Lim et al. (1960) and subsequently, for example, in Melnick et al. (Bull. Wld. Hlth. Org. 48:2163-268 (1973)), and Melnick et al. (1985). As used herein, these terms are distinguished from
20 variants of a given prototypical serotype, wherein a variant represents a phenotype resulting from antigenic drift, such as a phenotype that may represent an escape mutant. Such variants may occur in the field among contemporary clinical isolates of enteroviruses.

As used herein, a "motif" relates to a short sequence of amino acid residues
25 that is highly conserved among a family of proteins from different species or variants.

Developing a Database of Nucleotide Sequences Characteristic of the Prototypical Enteroviruses. In order to practice the methods of the present invention, a database of sequences characteristic of the prototypical enteroviruses is needed. In order to prepare such a database, a region of the enteroviral genome is
30 selected that has within its nucleotide sequence sufficient variation among the

different serotypes that the sequence from each serotype may be considered to be unique. In the present invention, the VP1 region of the viral RNA was identified as having the requisite sequence uniqueness from one serotype to another. Among the entries in Table 2, below, direct comparison of results based on VP1 versus those
5 obtained with VP2 for the following variants of the respective serotypes provided evidence that VP1 affords the selectivity required for this invention, whereas VP2 does not. The variants are CA24v strain EH24/70, E4 strain Du Toit, E4 strain Shropshire, E6 strain Charles, E6' strain Cox, E6" strain Burgess, E8 strain Bryson, E9 strain Barty, E11' strain Silva, E30 strain Frater, E30 strain Giles, E30 strain PR-
10 17, E34 strain DN-19, PV1 strain Sabin, PV2 strain Sabin, and PV3 strain Sabin. Once such a region is identified, the nucleotide sequences from this region are determined for each virus among the set of prototypical serotypes. The serotype prototypes of interest in the present invention are listed in Tables 1 and 2; Table 2 includes entries for additional enteroviruses and picornaviruses as well. The viruses
15 may be obtained from publicly available deposits made at the American Type Culture Collection (Rockville, MD).

Table 2. Enterovirus and Picornavirus VP1 Sequences Used in Establishing a Sequence Database

Serotype	Strain	GenBank Accession Number	SEQ ID NO:
CA1	Tompkins	AF081293	23
CA2	Fleetwood	L28146 (a)	
CA3	Olson	AF081294	24
CA4	High Point	AF081295	25
CA5	Swartz	AF081296	26
CA6	Gdula	AF081297	27
CA7	AB-IV	AF061298	28
CA8	Donovan	AF081299	29
CA9	Griggs	D00627 (b)	

Serotype	Strain	GenBank Accession Number	SEQ ID NO:
CA10	Kowalik	AF081300	30
CA11	Belgium-1	AF081301	31
CA12	Texas-12	AF081302	32
CA13	Flores	AF081303	33
CA14	G-14	AF081304	34
CA15	G-9	AF081305	35
CA16	G-10	U05876 (c)	
CA17	G-12	AF081306	36
CA18	G-13	AF081307	37
CA19	8663	AF081308	38
CA20	IH-35	AF081309	39
CA21	Kuykendall	D00538 (d)	
CA22	Chulman	AF081310	40
CA24	Joseph	AF081311	41
CA24v	EH24/70	D90457 (e)	
CB1	Conn-5	M16560 (f)	
CB2	Ohio-1	AF081312	42
CB3	Nancy	M16572 (g)	
CB4	JVB	D00149 (h)	
CB5	Faulkner	X67706 (i)	
CB6	Schmitt	AF081313	43
E1	Farouk	AF081314	44
E2	Cornelis	AF081315	45
E3	Morrissey	AF081316	46
E4	Pesacek	AF081317	47
E4	Du Toit	AF081318	48
E4	Shropshire	AF081319	49
E5	Noyce	AF081320	50
E6	Charles	U16283 (j)	

Serotype	Strain	GenBank Accession Number	SEQ ID NO:
E6	D'Amori	AF081321	51
E6'	Cox	AF081322	52
E6"	Burgess	AF081323	53
E7	Wallace	AF081324	54
E8	Bryson	AF081325	55
E9	Hill	X84981 (k)	
E9	Barty	X92886 (l)	
E11	Gregory	X80059 (m)	
E11'	Silva	AF081326	56
E12	Travis	X79047 (n)	
E13	Del Carmen	AF081327	57
E14	Tow	AF081328	58
E15	CII96-51	AF081329	59
E16	Harrington	X89545 (o)	
E17	CHHE-29	AF081330	60
E18	Metcalf	AF081331	61
E19	Burke	AF081332	62
E20	JV-1	AF081333	63
E21	Farina	AF081334	64
E22	Harris	S45208 (o)	
E23	Williamson	AF055846 (p)	
E24	De Camp	AF081335	65
E25	JV-4	AF081336	66
E26	Coronel	AF081337	67
E27	Bacon	AF081338	68
E29	JV-10	AF081339	69
E30	Bastianni	AF081340	70
E30	Frater	AF081341	71
E30	Giles	AF081342	72

Serotype	Strain	GenBank Accession Number	SEQ ID NO:
E30	PR-17	AF081343	73
E31	Caldwell	AF081344	74
E32	PR-10	AF081345	75
E33	Toluca-3	AF081346	76
E34a	DN-19	AF081347	77
EV68	Fermon	AF081348	78
EV69	Toluca-1	AF081349	79
EV70	J670/71	D00820 (q)	
EV71	BrCr	U22521 (r)	
PV1	Mahoney	J02281(s)	
PV1	Sabin	V01150 (t)	
PV2	Lansing	M12197 (u)	
PV2	Sabin	X00595 (v)	
PV3	Leon	K01392 (w)	
PV3	Sabin	X00596 (v)	
BEV1	VG-5-27	D00214 (x)	
BEV2a	RM-2	X79369 (y)	
BEV2b	PS-87	X79368 (y)	
HRV3	Unknown	U60874	
PEV9	UKG/410/73	Y14459 (z)	
SVDV	H/3'76	D00435 (h)	
HRV1b	Unknown	D00239(dd)	
HRV2	Unknown	X02316 (aa)	
HRV3	Unknown	U60874	
HRV14	Unknown	K02121, X01087 (bb)	
HRV16	Unknown	L24917(ee)	
HRV89	41467 Gallo	M16248(ff)	
HAV	HM-175	M14707 (cc)	

Notes for Table 2:

- PEV, porcine enterovirus; SVDV, swine vesicular disease virus; HRV, human rhinovirus; HAV, hepatitis A virus.
- a) Pulli, T., et al., *Virology* 211:30-38 (1995).
 - b) Chang, K., et al., *J. Gen. Virol.* 70:3269-3280 (1989).
 - c) Poyry, T., et al., *Virology* 202:982-987 (1994).
 - d) Hughes, P.J., et al. *J. Gen. Virol.* 70:2943-2952 (1989).
 - e) Supanaranond, K., et al., *Virus. Genes* 6:149-158 (1992).
 - f) Iizuka, N., et al. *Virology* 156:64-73 (1987).
 - g) Lindberg, A. M., et al., *Virology* 156:50-63 (1987).
 - h) Jenkins, O., et al., *J. Gen. Virol.* 68:1835-1848 (1987).
 - i) Zhang, G., et al., *J. Gen. Virol.* 74:845-853 (1993).
 - j) Harris, L.F., et al., *J. Infect. Dis.* 127:63-68 (1973).
 - k) Zimmermann, H., et al., *Virus Res.* 39:311-319 (1995).
 - l) Zimmermann, H., et al., *Virus Genes* 12:149-154 (1996).
 - m) Dahllund, L., et al., *Virus Res.* 35:215-223 (1995).
 - n) Kraus, W., et al. *J. Virol.* 69:5853-5858 (1995).
 - o) Huttunen, P., et al., *J. Gen. Virol.* 77:715-725 (1996).
 - p) Oberste, M.S., et al., *Virus. Res.* 56:217-223 (1998).
 - q) Ryan, M.D., et al., *J. Gen. Virol.* 71:2291-2299 (1990).
 - r) Brown, B.A., et al., *Virus. Res.* 39:195-205 (1995).
 - s) Kitamura, N.B., et al., *Nature* 291:547-553 (1981); Racaniello, V.R., et al. *Proc. Natl. Acad. Sci. USA* 78:4887-4891 (1981).
 - t) Dorner, A.J., et al., *J. Virol.* 42:1017-1028 (1982); Emini, E. A., et al., *J. Virol.* 42:194-199 (1982); Nomoto, A., et al. *Proc. Natl. Acad. Sci. USA* 79:5793-5797 (1982).
 - u) La Monica, N., et al., *J. Virol.* 57:515-525 (1986).
 - v) Toyoda, H., et al. *J. Mol. Biol.* 174:561-585 (1984).
 - w) Stanway, G., et al. *Proc. Natl. Acad. Sci. USA* 81:1539-1543 (1984).
 - x) Earle, J. A., et al., *J. Gen. Virol.* 69:253-263 (1988).
 - y) McNally, R.M., et al., *Arch. Virol.* 139:287-299 (1994).
 - z) Peng, J., et al., Unpublished data.
 - aa) Skern, T., et al., *Nucl. Acids Res.* 13:2117-2126 (1985).
 - bb) Callaghan, P.L., et al., *Proc. Natl. Acad. Sci. USA* 82:732-736 (1985); Stanway, G., et al., *Nucl. Acids Res.* 12:7859-7875 (1984).
 - cc) Cohen, J. L., et al., *J. Virol.* 61:50-59 (1987).
 - dd) Hughes, P.J., et al., *J. gen. Virology* 69:49-58 (1988).
 - ee) Lee, W.M., et al., *Virus Genes* 9:177-181 (1995).
 - ff) Duechler, M., et al., *Proc Natl. Acad. Sci. USA* 84:2605-2609 (1987).

The virus specimens are used to infect any enterovirus-susceptible cell line in culture, including, by way of nonlimiting example, RD (human rhabdomyosarcoma) cells, HLF (human embryonic lung fibroblast) cells, LLC-MK₂ (monkey kidney) cells, or BGM (buffalo green monkey kidney) cells; alternatively, a tissue homogenate in

5 tissue culture medium may be prepared from mouse brain after infection of the mouse with the virus. In the case of cell cultures, the culture supernatant is used. In the case of the brain homogenate, the whole homogenate, after growth of the virus, is used. Viral RNA is extracted from the growth media containing the enterovirus prototypes

by any method that releases the RNA from the virion and/or the cell components and provides a purified preparation of the RNA. By way of nonlimiting example, the RNA may be extracted using guanidinium isothiocyanate, such as the single-step isolation by acid guanidinium thiocyanate-phenol-chloroform extraction of
5 Chomczynski et al. (Anal. Biochem. 162:156-159 (1987)). Alternatively, the virion may be disrupted by a suitable detergent in the presence of proteases and/or inhibitors of ribonuclease activity. The RNA released from the virion is isolated or purified, using, for example, methods such as precipitation with an alcohol (e.g., ethyl alcohol or isopropyl alcohol) or banding in a suitable density gradient using an
10 ultracentrifuge.

The purified viral RNA is then subjected to a reverse transcription to prepare a cognate cDNA that encompasses the region of the genome chosen for discriminating between serotypes (i.e., the region encoding VP1). An advantageous way of achieving this is to use a set of random oligonucleotide primers in the reverse
15 transcription, such that certain of the primers in the set will hybridize to the RNA and yield one or more cDNA molecules from the virus encompassing the required serotype-specific nucleotide sequence. Alternatively, gene-specific primers based on a viral RNA-specific sequence from a suitable cDNA may be employed for reverse transcription. Subsequently, the cDNA is amplified using a suitable amplification
20 protocol. By way of nonlimiting example, a polymerase chain reaction (PCR) protocol may be employed for this purpose. PCR is described in operational detail in, for example, "Molecular Cloning: A Laboratory Manual," 2nd ed., Sambrook, Fritsch and Maniatis, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989; "Current Protocols in Molecular Biology," Ausubel et al., John Wiley and Sons, New
25 York 1987 (updated quarterly); and "PCR Protocols: A Guide to Methods and Applications," Innis et al., Academic Press, San Diego, CA 1990; and in U.S. Patents 4,683,195; 4,683,202; 4,965,188; 5,578,467; 5,545,522; and 5,624,833, all of which are incorporated herein by reference.

For the PCR of the cDNA to yield an amplicon containing a sequence from the
30 VP1 region, primers such as those provided in Table 3 (SEQ ID NOs:1-22) may be

employed. In Table 3, nucleotide sequence positions are given relative to the sequence of poliovirus1-Mahoney (Kitamura, N.B., et al., Nature 291:547-553 (1981); Racaniello, V.R., et al. Proc. Natl. Acad. Sci. USA 78:4887-4891 (1981)).

Table 3. Primers Used for PCR Amplification of the VP1 Region of Enteroviruses

Primer	Sequence	Gene	Position	SEQ ID NO
008	GCRTGCAAGAYTTCTCWGT	VP3	2411-2430	1
009	NGCNCCDGAPPTTGNTGSCC	2A	3409-3391	2
011	GCICIGAYTGITGICCRAA	2A	3408-3389	3
012	ATGTAYGTICCCICGIGG	VP1	2951-2970	4
013	GGIGCRTTICCYTGIGTCCA	VP1	3051-3032	5
019	ACRTGICIIGTYTGCATIGT	VP1	2676-2657	6
035	AWITTYTAYGAYGGITGG	VP1	3098-3115	7
036	TAIAIIGTICCCATRTTRTT	VP1	3201-3182	8
040	ATGTAYRTICCIMCIGGIGC	VP1	2951-2970	9
041	GGIGGIGGRTCIGTJAKYTT	VP1	3054-3035	10
045	GAIGARAAYCTIATIGARAC	VP1	2648-2667	11
046	CCCATIAKRTCIASTRTCCC	VP1	2820-2801	12
050	GTRCTYACIAIAGRTCYCT	2A	3513-3494	13
051	TSAARYTGTGCAARGACAC	VP3	2429-2448	14
052	STGYCCAGATTCAGTGT	VP3	2413-2430	15
053	GGNACNCAYRTNATHTGGA	VP3	2216-2235	16
054	GCCITRTTITGRTGICCRAA	2A	3408-3389	17
055	GGIACICAYRTIRTITGGA	VP3	2216-2235	18
187	ACIGCIGYIGARACIGGNCA	VP1	2612-2631	19
188	ACIGCIGTIGARACIGGNG	VP1	2612-2630	20
189	CARGCIGCIGARACIGGNGC	VP1	2612-2631	21
222	CICGIGGIGGIAYRWACAT	VP1	2969-2951	22

These primers were designed to amplify a broad range of cDNA fragments drawn from the set of enteroviruses (see Example 2). The primers of SEQ ID NOs:1-22 were designed based on information available regarding known sequences of non-polio enteroviruses, as well as sequences in the VP1 region obtained as part of the development of the present invention (see Example 1; see Table 2 for GenBank accession numbers of the sequences). Additional information used to design the primers of SEQ ID NOs:1-22, especially the primers of SEQ ID NOs:19-22, was obtained from known sequences of other members of the *Picornaviridae* family, as provided in Table 2.

10 The amplicons obtained from the PCR protocol applied to each prototype virus are sequenced to obtain the nucleotide sequence in each. Procedures that may be used for sequencing include the methods of Maxam and Gilbert (Meth. Enzymol. 65, 499-566 (1980)) and Sanger et al., (Proc. Natl. Acad. Sci. USA 74:5463-5467 (1977)) (see also Sambrook et al., (1989)). The method of Maxam and Gilbert involves random
15 chemical degradation reactions carried out on a nucleic acid labeled at one end. Each of four separate degradation reactions is specific for a different one of the four bases in the nucleic acid. The method of Sanger et al. involves use of a different 2',3'-dideoxynucleotide chain terminator in each of four template-driven DNA polymerase reactions. The Sanger method is readily implemented in automated sequencing
20 instruments, such as those of PE-Biosystems, Foster City, CA. The VP1 sequences that were obtained with the above procedures were incorporated into the non-polio enterovirus database of the present invention (see Table 2).

Typing of Clinical Isolates Obtained in the Field. A clinical sample is obtained from a subject suspected of harboring an enterovirus. Any suitable clinical
25 specimen may be used for this purpose. Commonly, and by way of nonlimiting example, such a sample may be whole blood or a fraction thereof, a bronchial wash, cerebrospinal fluid, an eye swab, a conjunctival swab, a swab or scraping from a lesion, a nasopharyngeal swab, an oral or buccal swab, pericardial fluid, a rectal swab, serum, sputum, saliva, stool, a stool extract, a throat swab, urine, brain tissue, heart
30 tissue, intestinal tissue, kidney tissue, liver tissue, lung tissue, pancreas tissue, spinal

cord tissue, skin tissue, spleen tissue, thymus tissue, cells from a tissue culture, a supernatant from a tissue culture, or tissue from an experimentally infected animal.

Viral RNA may be isolated from a clinical sample either directly or after inoculating a cell culture with the clinical sample and cultivating a larger virus
5 population. Direct isolation is rapid but may result in low virus titer, whereas inoculation and cell culture will provide a higher titer but may take several days.

In order to obtain amplicons from viral RNA, the RNAs from the virus isolates are treated with a reverse transcriptase primer preparation that contains a random oligonucleotide RT primer, such as a library of random hexanucleotides. The
10 resulting cDNA is amplified in a PCR procedure using a mixture of oligonucleotide primers that hybridize to motifs that are highly conserved throughout the enteroviruses, or more generally, motifs that are highly conserved among the picornaviruses. As used herein, the notion of hybridizing specifically to a highly conserved region encoding a highly conserved amino acid motif relates to identifying
15 at least two nucleotide sequences in the viral genomes which display minimal variation across both the complete spectrum of prototypical enterovirus serotypes, as well as the variants that may be present in clinical samples at any given time. Thus, at least two relatively constant amino acid sequences, or motifs, encoded by these nucleotide sequences, occur phenotypically in all or most of the viruses of the
20 enteroviral species and variants, and the corresponding coding sequences in the nucleic acid are likewise relatively constant across the prototypes and variants. Such conserved or invariant sequences, or motifs, are required in order that a single pair of oligonucleotide primers, or as small a set of such primers as is practical, suffices to prime the amplification of all or the maximum possible number of prototypical
25 viruses and all or the maximum number of viral variants infecting the population at any given time.

In important embodiments of the invention, the primers used are a mixture of oligonucleotides whose use in a PCR amplification provides an amplicon encompassing most or all of the VP1 gene. By way of nonlimiting example, such a
30 mixture may include an oligonucleotide chosen from among an oligonucleotide whose

sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:4, an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:9, and a mixture thereof, as well as an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:3 (see Table 3); in particularly important embodiments the oligonucleotides employed according to the above mixtures are primer 011 (SEQ ID NO:3), primer 012 (SEQ ID NO:4), and primer 040 (SEQ ID NO:9). The use of either or both of the primers (012, SEQ ID NO:4 and 040, SEQ ID NO:9) provides specific hybridization to target sequences in the 5' region of the VP1 gene of most or all of the non-polio enteroviruses. The third primer, 011 (SEQ ID NO:3), specifically hybridizes to a target sequence in the 2A region of most or all the non-polio enteroviruses. Each of the primers is disclosed in PCT application WO 98/14611, which is incorporated herein by reference.

More generally, primer sets that include a mixture of oligonucleotides that contain the sequences given by SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, or SEQ ID NO:22 may be employed in amplifying a broad range of picornaviruses. Specifically, oligonucleotides chosen from among an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:19, an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:20, an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:21, and mixtures thereof, may be combined with an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:22 (see Table 3) for use in the present method. Advantageously, the oligonucleotides included in the above mixtures are primer 187 (SEQ ID NO:19), primer 188 (SEQ ID NO:20), primer 189 (SEQ ID NO:21), and primer 222 (SEQ ID NO:22).

Using the mixtures of oligonucleotide primers set forth in the preceding paragraphs leads to preparation of the enteroviral PCR amplicons according to the method of this invention. The amplicons are then either detected or isolated for sequence analysis. They may be isolated by any of a variety of amplicon purification procedures that serve to provide a purified preparation of the amplicon. These

include, by way of nonlimiting example, gel electrophoresis coupled with visualization using a fluorescent dye and extraction of the detected amplicon from the gel, and extraction from the amplification solution using an immobilized derivative of one or more of the PCR primers to bind a strand of the amplicon after it has been
5 denatured. The purified amplicons may be sequenced using conventional sequencing techniques or procedures.

The nucleotide sequence obtained for the amplicon derived from a particular clinical sample of an enterovirus is then matched with the sequences in the database of prototypical sequences describing the known serotypes of enteroviruses. The
10 sequence matching may be carried out by any suitable sequence matching algorithm designed to determine the extent of identity or similarity between a query sequence in its entirety and a standard or reference sequence. By way of nonlimiting example, such an algorithm may be that of Needleman and Wunsch (J. Mol. Biol. 48:443-453 (1970) implemented in the program Gap in the Wisconsin Sequence Analysis
15 Package, version 9.1), and the like. Such algorithms provide a result that the query sequence most resembles a particular one, and (in most cases) only one, of the reference sequences drawn from the database. According to the present method, the serotype of the enterovirus in the clinical sample is the serotype of the sequence from the database identified as most closely resembling the sequence of the sample.

20 Numerous advantages result upon implementation of the present invention. Typing of an enterovirus in a clinical sample may be done avoiding the necessity of culturing the sample in a cell culture or in a whole animal host (e.g., mouse). Such procedures are cumbersome, labor-intensive and resource-intensive, and pose dangers of infection to the workers conducting the assay. The typing likewise avoids the
25 necessity of conducting a standardized serotyping assay. Serotyping is labor-intensive, and requires the availability of the antiserum pools that are specific or selective for the various enterovirus serotypes. Furthermore, serotyping using these procedures is not very effective because numerous variants and escape mutants in field samples of enteroviruses avoid detection and provide, therefore, a false negative
30 result. The present invention additionally avoids the disadvantages of known PCR

amplification procedures employed with non-polio enteroviruses, which are largely based on the conserved 5' untranslated region of the non-polio enterovirus genome, and thereby lack a means for typing the samples found.

In contrast, the present invention provides the only PCR-based means for
5 typing a clinical sample of an enterovirus available at the present time. The procedure is easy to carry out and provides an unambiguous, and accurate, typing of a clinical sample in a large fraction of test cases that were also typed by standard serotype pools. Typing of cases of enterovirus-caused diseases or syndromes permits an appropriate therapy to be chosen in suitable cases. Such therapy should lead to
10 amelioration of the severity of the disease or syndrome and, hopefully, a complete recovery. Typing furthermore provides important public health and epidemiological information that could lead to protective and/or preventive measures being taken among a population at risk of contracting such a disease or syndrome.

The following examples are intended to illustrate the invention and not to limit
15 it.

Example 1. Establishing a Database of Sequences Corresponding to Standard Non-polio Enterovirus Serotypes. The viruses used for sequence analysis are listed in Table 2, above. The prototypical virus samples were obtained from the American Type Culture Collection. The viruses were propagated in RD cells, HLF cells, LLC-
20 MK₂ cells, or primary monkey kidney cells using Eagle's MEM supplemented with 2% fetal bovine serum or by intracerebral inoculation of newborn mice (see Grandien, M., et al., "Enteroviruses and Reoviruses", in Diagnostic procedures for viral, rickettsial, and chlamydial infections, 6th Ed. (Schmidt, N.J., et al., eds.) 1989, Amer. Public Health Assoc., Washington, DC, pp. 513-578) . The isolation of the viral
25 RNA, and the RT-PCR amplification was conducted as described by Oberste et al. (Am. J. Trop. Med. Hyg. 58:41-46 (1998b)). In summary, in this procedure, viral RNA was extracted from infected cell culture supernatants, or from 10% infected mouse brain homogenate with Trizol LS™ (Life Technologies, Inc., Gaithersburg, MD), and cDNA was obtained by use of a set of random hexanucleotide primers
30 (Boehringer Mannheim Biochemicals, Indianapolis, IN), and a SuperScript™

preamplification kit (Life Technologies, Inc.). Reverse transcription was performed in a solution containing 20 mM Tris chloride pH 8.3, 50 mM KCl, 2.5 mM MgCl₂, 0.1 M dithiothreitol, 0.5 mM each of dATP, dCTP, dGTP, and TTP, 0.8 μM random hexamer primer, 5 μL RNA, and 10 U SuperScript II™ reverse transcriptase (Life Technologies, Inc.). The reaction proceeded for 1 h at 42°C.

The resulting cDNAs were amplified by PCR using primers for VP3 and 2A shown in Table 3 (SEQ ID NOs:1-18), in a reaction containing 20 mM Tris chloride pH 8.3, 50 mM KCl, 2.5 mM MgCl₂, 0.2 mM each of dATP, dCTP, dGTP, and TTP, 1 μM sense-orientation primer, 1 μM antisense-orientation primer 1 μL cDNA from the reverse transcription step, above, and 1.25 U *Thermus aquaticus* DNA polymerase (Life Technologies, Inc.). The reaction was incubated at 94°C for 3 min, then followed by 35 cycles of 94°C for 30 s, 42°C for 30 s, and 72°C for 30 s, followed by incubation at 72°C for 5 min. The specific primer pairs used differed from one virus to another in order to obtain satisfactory yields of the amplicons. For some viruses, VP1 was amplified as two overlapping fragments with internal VP1 primers as well as the VP3 and 2A primers. The PCR products were gel isolated and purified in preparation for sequencing with the QIAquick™ gel extraction kit (QIAGEN, Inc., Santa Clarita, CA), in which DNA is selectively adsorbed to a silica gel membrane at pH below 7.5 at high salt concentration. The impurities are separated from the membrane, then the DNA is eluted therefrom using Tris buffer or water. Sequencing was carried out on an automated DNA sequencer (Applied Biosystems Division, Perkin Elmer, Inc., Foster City, CA) using 2',3'-dideoxynucleotide chain terminators (Sanger et al. (1977)) that carried fluorescent labels.

Complete VP1 PCR products of viruses for which VP1 primers were not available were obtained by cloning the viral cDNA into the plasmid pGEM-T (Promega Corp., Madison, WI). Nested-deletion subclones were constructed from the resulting plasmid with an Erase-a-Base™ kit (Promega Corp.). In this procedure, the plasmid is first digested with a restriction nuclease providing either a blunt end or a 5' overhang. The opened plasmid is then digested with a 3'-5' exonuclease, *E. coli* exonuclease III, to remove plasmid sequences unrelated to the viral VP1 gene. The

extended 5' overhang is then removed using S1 nuclease, and the plasmid is resealed by first repairing the ends with DNA polymerase, then ligating with DNA ligase. The resulting shortened plasmid is propagated in a suitable host to provide larger amounts of the plasmid, including the VP1 sequence. For each virus, at least two independent clones were sequenced by automated methods as described above.

Using these procedures, complete VP1 nucleotide sequences were determined for 57 human non-polio enterovirus strains for which VP1 sequences had not previously been determined. These are summarized in Table 2, which shows both the GenBank accession numbers (numbers AF081293 to AF081349) and the corresponding SEQ ID NOs, 23-79. Forty-seven of the strains were prototype strains for recognized human enterovirus serotypes (Melnick (1996)). The other ten sequenced strains were well-characterized antigenic variants which, while antigenically distinct from their respective prototype strains, were similar enough to them to have been considered to be the same serotype (Committee on Enteroviruses of the National Foundation for Infantile Paralysis, *Am. J. Public Health* 47:1556-1566 (1957); Melnick (1996)). Combined with the 21 previously available complete enterovirus VP1 sequences, of which 19 are prototypes and 2 are variants, the database constructed for use in the present method includes 66 prototype VP1 sequences and 16 variants or other enteroviruses, including the three poliovirus Sabin strains and the Barty variant of E9.

The boundaries of the newly sequenced VP1 genes were predicted by comparison of the nucleotide and deduced amino acid sequences with those of previously characterized enteroviruses. Human enterovirus VP1 sequences varied in length from 834 to 951 nucleotides (278 to 317 amino acid residues). The CB group has the shortest predicted VP1 amino acid sequences (278 to 298 residues), while EV68 and EV70 had the longest ones (312 and 317 residues, respectively).

Each of the enterovirus VP1 sequences developed in this work is characteristic of the serotype from which it arises, and differs from the sequence of every other serotype. For this reason, the VP1 sequences can be used as markers for the prototypical serotypes of the non-polio enteroviruses. The 66 prototype and 16

variant sequences identified above are used in the method of the present invention to form the content of a database for use in typing an enterovirus obtained in a clinical sample.

5 Example 2. Design of Non-Polio Enterovirus PCR Primers and Assessment of the Breadth of Their Specificity.

Design of PCR primers. Since the VP1 sequence was found to correlate with serotype (Example 1), this region was targeted for development of sequence-based molecular diagnostics, namely, generic PCR primers to amplify and sequence a portion of the VP1 gene. Degenerate deoxyinosine-containing PCR primers were
10 designed which specifically recognize regions within or near the termini of the VP1 gene of non-polio enteroviruses. Primers with the broadest specificity within the non-polio enterovirus genus were chosen by searching for regions in the genome that encode amino acid motifs within VP1 and those immediately C-terminal to VP1, in 2A, that are the most conserved across the prototypes. (Echoviruses E22 and E23
15 were excluded, because it is likely that they will be reclassified as members of a new Picornavirus genus, *Parechovirus* (Mayo et al., J. Gen. Virol. 79:649-657 (1997)). The motif MYVPPG (Met-Tyr-Val-Pro-Pro-Gly) was present in the deduced VP1 amino acid sequences of 44 enterovirus prototype strains whose nucleotide sequences are provided in Example 1. Thirteen prototypes had Ile substituted for Val and CA7
20 contained Ala instead of Val. CA12, CA14, and EV71 contain the motif, MFVPPG (Met-Phe-Val-Pro-Pro-Gly). In EV68 and 70, a slightly different motif was present, MYVPTG (Met-Tyr-Val-Pro-Thr-Gly). For viruses in the CB-like phylogenetic group the M(Y/F)(V/I)PPG motif is followed by Gly, whereas in all other
25 enteroviruses, the motif is followed by Ala (A). To account for differences between the virus groups and for codon degeneracy, two different inosine-containing primers were designed to anneal to this region. Primer 012 (ATGTAYGTICCCICGIGG) is based on the amino acid sequence, MYVPPGG (SEQ ID NO:80). Primer 040 (ATGTAYRTICCMCIGGIGC) is based on the amino acid sequence, MY(V/I)P(P/T)GA (SEQ ID NO:81). The selectivity of these two primers is

primarily due to the first position at the 3' end of each primer (i.e., in primer 012, the base at the 3' end is G, and in primer 040, the base at the 3' end is C) (see Table 3.) In addition, primer 040 contains increased degeneracy at positions 8 and 14 from the 3' end of the primer in order to detect those viruses which encode an isoleucine (position 8) or a threonine (position 14) in these positions. For PCR, primers 012 and 040 were each paired with primer 011 (GCICIGAYTGITGICCRAA), which corresponds to the amino acid motif FG(Q/H)QSGA (Phe-Gly-(Gln/His)-Gln-Ser-Gly-Ala; SEQ ID NO:82), present near the 5' end of the 2A gene and which is conserved among most enteroviruses for which the 2A sequence is available.

10 Specificity of PCR Primers. To assess the breadth of specificity and thereby the general applicability of the 012/011 and 040/011 primer pairs, both pairs were tested in RT-PCR reactions with template RNA derived from each of the human non-polio enterovirus prototype strains (see Figure 2). Primer pair 012/011 amplified 23 of 30 echovirus prototypes (Figure 2C), as well as CA2, CA7, CA9, CA11, CB1, 15 CB2, CB3, CB6, and PV1 (Poliovirus 1) (Figure 2A). Primer pair 040/011 amplified 14 of 23 CA prototypes and PV1 (Figure 2B), as well as E2, E6, E14, E16, E18, E19, E20, E24, E25, E27, E30, and E31 (Figure 2D). Twenty-two prototypes were not amplified by either primer pair (CA10, CA13, CA15, CA16, CA20, CA21, CA22, CB4, CB5, E1, E7, E9, E21, E22, E23, E32, EV68, EV 69, EV70, EV71, as well as 20 PV2 and PV3, where PV signifies poliovirus).

Example 3. Typing of Clinical Isolates Obtained in the Field.

25 Viruses. Fifty-one virus isolates of 24 different serotypes were chosen from those processed in the inventors' laboratory at the Centers for Disease Control and Prevention (CDC) during the period 1991-1998 for routine non-polio enterovirus reference testing. The viruses were from 19 different states in the United States and two other countries, and were chosen to be representative of the serotypes in the collection for the period surveyed. To avoid the effects of sampling bias in the interpretation of sequence comparisons, no more than four isolates of any given

serotype were chosen for sequencing. The isolates included examples of coxsackievirus A, coxsackievirus B, echovirus, and numbered enteroviruses.

Virus isolation and neutralization. The virus strains were isolated from a wide range of clinical specimens, including blood (n=1), cerebrospinal fluid (n=7), conjunctival swab (n=1), "lesion" (n=1), postmortem lung (n=1), nasopharyngeal swab (n=2), sputum (n=1), stool (n=18), throat swab (n=8), and tissue not specified (n=11). Forty-four of the 51 strains were originally isolated by the submitting laboratory, most of which were state public health laboratories in the United States. The remaining seven strains were isolated from original stool specimens at CDC. All isolates were typed antigenically using WHO-standard antiserum pools (Melnick et al., 1973), supplemented with additional pooled and monospecific antisera such that all human enterovirus serotypes, as well as antigenic variants of E4, E6, E11, and E30, could be identified (P. Feorino, personal communication to the inventors).

RNA extraction and RT-PCR. Viral RNA was extracted from infected cell culture supernatant using the QIAamp™ Viral RNA Kit (QIAGEN, Inc.). Reverse-transcription polymerase chain reaction (RT-PCR) was carried out as described previously (Oberste et al., (1998a,b)). From each viral cDNA, an amplicon of approximately 450 bp, encompassing the 3' half of VP1 and the 5' end of 2A, was amplified by PCR using the primers 012/011 or 040/011 (Table 3). Primer specificity was tested by PCR amplification of the prototype strain of each human enterovirus serotype with both primer pairs. Amplification products were visualized by agarose gel electrophoresis and ethidium bromide staining. PCR products from clinical isolates were gel-isolated and purified for sequencing using the QIAquick™ Gel Extraction Kit (QIAGEN, Inc.) and sequenced on an automated DNA sequencer using fluorescent dideoxy-chain terminators as in Example 1 (Applied Biosystems Division, Perkin Elmer, Inc.). The sequences obtained for the clinical samples were deposited in the GenBank sequence database (Accession Numbers AF081595-AF081645).

Sequence analysis. The sequences were compared to the enterovirus VP1 sequence database developed in Example 1 by sequential pairwise alignment of the query sequence with each sequence in the database, using the algorithm of Needleman

and Wunsch (1970), implemented in the program Gap (Wisconsin Sequence Analysis Package, version 9.1). The results of the pairwise comparisons were compiled and sorted in descending order by percent identity with the query sequence.

PCR-amplification of clinical isolates. In order to establish the utility of using viral sequence analysis as an enterovirus typing tool, typing by partial sequencing of VP1 was compared with the conventional serological typing method using 52 clinical isolates typed in the inventors' laboratory from 1991 to 1997. Partial VP1 sequences relate to obtaining sequences in a region of approximately 400 nucleotides at the 3' end of the VP1 gene. Despite the failure of primer pair 012/011 to amplify the E7, E9, E21, CB4 and CB5 prototype strains (see Example 2), 012/011 successfully amplified recent clinical isolates of each these serotypes. Likewise, primer pair 040/011 amplified recent isolates of CA16, CA21, and EV71, but not the prototype strains of these serotypes (see Example 2). Taken together, these two primer pairs failed to amplify only one clinical isolate of the 52 tested, a 1993 EV6 isolate from Texas (TX93-1673). The presence of amplifiable RNA in the latter specimen was confirmed by amplification of 5'-specific sequences by pan-enterovirus primers (data not shown). For the other 51 isolates, a VP1-specific fragment was amplified from purified RNA by RT-PCR using primer pairs 012/011 or 040/011. In most cases, only one of the two primer pairs produced an amplicon of the expected size (data not shown).

Typing of clinical isolates by nucleotide sequence analysis. The PCR products were gel isolated and sequenced. The sequences were compared to the complete enterovirus VP1 database developed in Example 1 by pairwise alignment of the isolate sequence to each sequence in the database using the program Gap. These comparisons produced, for each clinical isolate, a set of values of the percent identity giving the extent of identity between the sequence of the given clinical isolate and each of the prototype sequences in the database. Typing was obtained as that prototype whose extent of identity to the clinical sample was the highest of all the prototypes. In general, as implemented in this study, if the highest global identity is >75%, the clinical sample and the prototype are of the same serotype. If the highest score is 70%-75%, the identification is presumptive and should be confirmed by

neutralization using monospecific antisera specific for each of the four highest scoring prototypes. If the highest score is <70%, the clinical sample is considered to be of no known serotype; for example, it may be from a picornavirus for which a sequence is not yet available, or it may be a new enterovirus serotype. For each clinical isolate,

5 the matches with the highest and second highest pairwise identity score were identified. Table 4 shows the serotype as obtained from the classical neutralization test, as well as the types of the highest and next highest scoring prototypes obtained in this way (with entries giving the extent of identity of both the nucleotide sequences (nt) and the translated amino acid sequences(aa)). Strains in Table 4 are identified by

10 U.S. state (two letter code) or country (three letter code) of origin, year of isolation, and lab identifier number. For example, WA91-0374 indicates that the strain was isolated in the state of Washington in 1991 and the lab sample number was 0374. The abbreviations DOR and PER in Table 4 designate the Dominican Republic and Peru, respectively.

Table 4. Correspondence Between Typing by Sequence and by Neutralization.

Strain	Neut. Type	Highest Scoring Prototype			Second Highest Scoring Prototype(s)			
		Type	nt (%)	aa (%)	Type	nt (%)	Type	aa (%)
WA91-0374	E6	E6	83.3	95.6	E1	69.7	E29	74.3
OR91-1426	E30	L30	85.8	92.9	E21	69.5	E21	81.7
CT92-1465	E16	E16	81.4	93.6	E5	72.2	E5	78.6
FL92-1512	CB2	CB2	86.5	98.5	CB4	68.3	CB4	75.2
WA92-1516	E11'	E11	77.1	90.1	E11	72.9	E19	83.0
NC92-1612	E9	E9	77.8	94.6	E17	70.2	E16	72.9
GA92-1616	E11	E11	77.6	89.4	E19	72.2	E19	82.3
TX92-1647	CA14	CA14	86.8	91.1	CA7	63.4	CA7	67.9
MD92-1649	E25	E25	77.1	91.5	E1	68.5	E21	77.6
DOR93-1657	CA24v	CA24	77.4	92.8	CA20	67.6	CA17	75.9
FL93-1763	E11'	E11	78.5	90.1	E19	72.6	E19	83.0
GA93-1763	CA9	CA9	93.8	95.3	E4	68.6	E4	70.8
GA93-1765	E7	E7	79.7	95.7	E32	68.8	E32	77.1
M093-1808	E25	E25	77.6	91.5	E33	67.5	E21	76.9
ME93-1814	CB5	CB5	95.2	98.5	CB1	71.3	CB1	77.7
NM93-1816	CB3	CB3	90.3	97.7	CB6	69.9	CB1	81.5
OR93-1817	E25	E25	77.9	91.5	E1	68.5	E21	76.9
WA93-1821	E4	E4	81.1	96.1	E1	73.1	E1	80.9
MN94-1828	E25	E25	76.9	92.2	E29	67.9	E21	77.6
WA94-1849	E3	E3	79.6	93.0	E7	68.2	E12	80.0
AR94-1884	E30	E30	96.0	93.6	E21	70.0	E21	82.4
GA93-2460	CB5	CB5	95.8	93.5	CB1	70.8	CB1	77.7
GA93-1892	E30	E30	85.5	93.6	E21	69.5	E21	83.4
GA93-1994	E7	E7	79.7	95.7	E32	69.1	E32	77.1
NM94-1919	EV71	EV71	80.6	93.4	CA16	66.9	CA16	76.6
AZ94-1925	CA14	CA14	86.5	97.0	CA7	63.8	CA7	68.2
RI94-1959	E21	E21	78.3	93.7	E30	69.6	E30	80.0
CT94-2006	EV71	EV71	80.3	93.4	CA16	66.0	CA16	76.6

Strain	Neut. Type	Highest Scoring Prototype			Second Highest Scoring Prototype(s)			
		Type	nt (%)	aa (%)	Type	nt (%)	Type	aa (%)
MD95-2037	EV71	EV71	79.9	92.7	CA16	67.0	CA16	76.6
AZ94-2060	CA21	CA21	90.9	98.6	CA24	68.7	CA24	75.5
PA94-5753	CA16	CA16	77.9	94.7	EV71	68.7	EV71	83.0
NM95-2070	E6	E6	76.8	94.1	E29	68.1	E29	75.5
TX95-2089	E13	E13	72.4	88.7	EV69	71.5	EV69	93.0
GA95-2093	CA21	CA21	91.4	98.6	CA24	67.5	CA24	75.5
GA95-2095	CA16	CA16	77.9	94.9	EV71	69.4	EV71	77.4
NC95-2135	CB2	CB2	83.2	99.2	CB4	68.3	CB4	76.2
AR95-2139	E9	E9	75.7	92.8	E17	70.0	E1	71.8
TX95-2147	CA16	CA16	76.5	94.9	EV71	70.4	EV71	77.4
VA95-2154	E11'	E11	78.3	90.8	E19	71.7	E19	83.7
WT95-7151	E9	E9	75.7	93.5	E17	69.4	E16	71.4
VA95-2157	E30	E30	85.3	92.1	E21	70.0	E21	82.1
GA96-2175	CA9	CA9	81.5	92.6	E19	68.4	E11	72.3
CT96-2181	E5	E5	86.5	92.9	E31	71.5	E31	82.1
CT96-2181	E18	E18	75.7	93.6	E17	69.9	E4	75.4
TX96-2184	CA21	CA21	91.6	98.6	CA24	68.2	CA24	75.5
TX97-2320	E18	E18	78.8	92.9	E17	69.7	E17	74.5
NH97-2342	CB3	CB3	77.4	98.5	CB5	67.9	CB1	84.6
PER98-2528	E6	E6	86.0	95.6	CB1	71.6	E29	74.3
PER98-2533	E7	E7	80.4	95.7	E32	68.1	E12	78.6
PER98-2537	E11	E11	78.5	94.3	E19	71.9	E19	82.3
PER98-2558	E33	E33	79.3	96.9	CB1	70.3	E4	75.4

The typing results for the 51 isolates shown in Table 4, fully correlate with the serotype as determined by the conventional neutralization test (Table 4). The nucleotide sequences of the various clinical isolates ranged from 72.4% identity to 95.2% identity with the sequences of the respective prototype strains and only from 5 63.4% identity to 73.1% identity to the sequences of the second highest scoring

prototypes. The predicted amino acid sequences of the clinical isolates ranged from 88.7% identity to 98.5% identity with that of the cognate prototype strain and from 67.7% identity to 84.6% identity to that of the second highest scoring prototype strain. With one exception, the difference between percent nucleotide sequence identity to the highest scoring prototype and the percent identity to the second highest scoring prototype was 4.2%. In the exception (TX95-2089), typed antigenically as E13, the highest-to-second-highest difference was only 0.9% (72.4% identical to E13 vs. 71.5% identical to EV69), suggesting that either TX95-2089 has diverged significantly from E13 or EV69, or that the E13 prototype strain (Del Carmen) is not representative of the serotype as a whole. When the complete VP1 nucleotide sequence of TX95-2089 was examined, it was found to be 72.6% identical to that of the E13 prototype, 70.1% identical to that of the EV69 prototype (second highest score), and 64.7% identical to that of the E12 prototype (third highest score). The predicted complete VP1 amino acid sequence of TX95-2089 was 88.2% identical to that of E13, 80.8% identical to that of EV69 (second highest score), and 70.0% identical to that of CB1 (third highest score), suggesting that TX95-2089 is probably a strain of E13 which has diverged in nucleotide sequence by accumulating mutations in the third codon position. TX95-2089 was neutralized by monospecific anti-E13 antisera but not by monospecific anti-EV69 antisera (data not shown).

The typing procedure described in this invention contravenes the evaluation of the state of the art in Holland et al. (J. Clin. Microbiol. 36:1588-1594 (1998)), which states that PCR is not able successfully to type enterovirus infections. Furthermore, Oberste et al. (1998a) conducted sequence and phylogenetic analyses of all human enterovirus serotypes based on a portion of the VP2 gene. They determined that this portion of VP2 may be inappropriate for consistent molecular inference of serotype. For these reasons, the method of the present invention, as described above and exemplified in Examples 1-3, provides results that are unexpected by workers in the field.

Example 4. Detection of a Broad Range of Picornaviruses.

The present method has been applied to the detection of a broad range of picornaviruses that afflict both human and nonhuman subjects, according to the procedures generally followed in Example 2.

5 In addition to the primers 011, 012, and 040, additional primers directed to the detection of human and nonhuman picornaviruses were devised. These are provided as Primer 187 (ACIGCIGYIGARACIGGNCA) (SEQ ID NO:19) that hybridizes to a sequence encoding the amino acid motif TA(A/V)ETGH (SEQ ID NO:83), Primer 188 (ACIGCIGTIGARACIGGNG) (SEQ ID NO:20) that hybridizes to a sequence
10 encoding the amino acid motif TAVETG(A/V) (SEQ ID NO:84), Primer 189 (CARGCIGCIGARACIGGNGC) (SEQ ID NO:21) that hybridizes to a sequence encoding the amino acid motif QAAETGA (SEQ ID NO:85), and Primer 222 (CICCIGGIGGIAYRWACAT) (SEQ ID NO:22) that hybridizes to a sequence encoding a motif M(F/Y)(I/V)PPG(A/G) (SEQ ID NO:86) (see Table 3). Primer 187
15 is directed to amplification of the CB and E groups in the forward direction (i.e., it hybridizes to the sense strand of the cDNA), Primer 188 is directed to amplification of the poliovirus (PV) group, EV68 and EV70 in the forward direction, Primer 189 is directed to amplification of the group of CA16-like viruses (Oberste et al., J. Virol. 73:1941-1948 (1999)) in the forward direction, and Primer 222 is directed to
20 amplification of all enteroviruses in the reverse direction (i.e., it hybridizes to the antisense strand of the cDNA).

In this example, prototypical serotypes of human enteroviruses were subjected to RT-PCR using, in separate experiments, primer pairs 012/011 (SEQ ID NOs:3 and 4), 040/011 (SEQ ID NOs:3 and 9), 187/222 (SEQ ID NOs:19 and 22), 188/222 (SEQ
25 ID NOs:20 and 22), and 189/222 (SEQ ID NOs:21 and 22). The results are shown in Table 5. Additionally several serotypes from a selection of human and nonhuman picornaviruses, namely bovine enterovirus, human rhinovirus, and simian picornavirus, were examined according to the present method. For simian picornaviruses and HRV2, actual experiments were done. For the other serotypes
30 considered, provision of an amplicon was predicted by comparison of the primer

sequences to each of the viral VP1 sequences. The results of this experiment are shown in Table 6.

Table 5. Amplification of Human Enterovirus Serotypes by Specific Primer Pairs.

Virus	012/011	040/011	187/222	188/222	189/222
CA1	—	—	—	■	□
CA2	□	■	□	□*	■
CA3	—	■	—	□	■
CA4	—	■	—	—	■
CA5	—	■	□	□*	■
CA6	—	■	—	□*	■*
CA7	—	—	±	—	■
CA8	—	□	—	□	■
CA9	■	—	■*	□	—
CA10	—	—	—	□	■
CA11	—	±	—	■	□
CA12	—	■	—	□*	■
CA13	—	—	□*	■	□
CA14	—	■	—	□	■
CA15	—	—	□	■	□
CA16	—	■	—	—	■
CA17	—	±	±	■	□
CA18	—	■	—	(±)	—
CA19	—	±	—	■	□
CA20	—	—	—	■	±
CA21	—	■	—	■	□
CA22	—	—	—	■	□
CA24	—	■	—	■	□
CB1	■	—	■	—	—
CB2	■	—	■	□*	±
CB3	■	±	■*	—	±
CB4	—	—	■*	—	±
CB5	■	—	■	□	□
CB6	■	—	■	□*	□*
PV1	—	■	□	■	□
PV2	—	—	□	■	□*
PV3	—	—	—	■	□
E1	—	—	■	—	—
E2	■	□	■	—	±

Virus	012/011	040/011	187/222	188/222	189/222
E3	■	—	■	—	±
E4	■	—	■*	□	□*
E5	■	—	■	—	±
E6	■	□	■	—	±
E7	■	—	(±)	—	□
E9	■	—	■	—	±
E11	■	—	■*	—	±
E12	■	—	■*	—	□*
E13	■	—	■	—	□
E14	■	□	■	—	□*
E15	—	—	■	—	—
E16	■	—	■	—	±
E17	■	—	■*	—	±
E18	■	□	■	□	□
E19	■	—	■	—	±
E20	■	□	■	□	±
E21	■	—	■	—	—
E24	■	□	■	—	±
E25	■	□	■	—	±
E26	■	—	■	—	±
E27	■	□	■*	—	±
E29	—	—	■	—	—
E30	■	□	■	—	±
E31	■	□	■*	—	±
E32	—	—	■	—	±
E33	■	—	■	—	—
EV68	—	—	□	■	□
EV69	—	—	■	—	—
EV70	—	—	—	■	□
EV71	—	■	—	—	■

CA, coxsackie A virus; CB, coxsackie B virus; PV, poliovirus; E, echovirus; EV, numbered enterovirus. Results are for amplification of prototype strains and/or clinical isolates of the indicated serotypes, based on testing in a standard RT-PCR assay for human enteroviruses (Oberste et al., 1999).

□ and ■ : strong amplification, single band on gel; ■ indicates the primer pair giving optimal amplification for a particular serotype.

± and (±) : weak amplification, single band on gel; (±) indicates the primer pair giving optimal amplification for a particular serotype.

□* and ■* : strong amplification, multiple bands on gel; ■* indicates the primer pair giving optimal amplification for a particular serotype.

– : No amplification observed.

Table 6. Predicted and Observed Results of Amplification of Picornavirus Serotypes by Specific Primer Pairs.

Virus	012/011	040/011	187/222	188/222	189/222
BEV1				[■]	
BEV2a				[■]	
BEV2b				[■]	
HRV1b			[■]		
HRV2			■		
HRV3				[■]	
HRV14				[■]	
HRV16			[■]		
HRV89			[(±)]		
SPV2		■			
SPV9	–	–	–	–	–
SPV10		■			
SPV11	–	–	–	■	–
SPV12	–	–	–	–	■
SPV13		■			
SPV15	–	–	–	■	–
SPV16	–	–	–	–	■
SPV17			■		□

BEV, bovine enteroviruses; HRV, human rhinovirus; SPV, simian picornavirus.

Results are for amplification of prototype strains and/or clinical isolates of the indicated serotypes, based on testing in a standard RT-PCR assay (Oberste et al., 1999) for HRV2, and simian picornaviruses. For the other viruses (indicated by square brackets []), the entry provides a predicted result based on comparison of the primer sequences with the available VP1 nucleotide sequences found in the GenBank database.

□ and ■ : strong amplification, single band on gel; ■ indicates the primer pair giving optimal amplification for a particular serotype.

(±) : weak amplification, single band on gel, optimal amplification for a particular serotype.

– : No amplification observed.

Empty cells indicate primer-template combinations that have not yet been tested.

The results for 012/011 and 040/011 in Table 5 tabulate the observations already discussed with respect to Figure 2 in Example 2.

5 Taking the results for primer pairs 187/222, 188/222, and 189/222 in Tables 5 and 6 together, it is seen that these primer pairs amplify all human enteroviruses, and five of the six simian picornaviruses tested. They should also amplify the three bovine enteroviruses and all six human rhinoviruses for which VP1 sequences are available in GenBank; other than HRV2, these have not yet been directly tested. Furthermore, the three simian picornaviruses that were not tested using primer pairs 187/222, 188/222, and 189/222 were successfully amplified by primer pair 040/011
10 (see Table 6).

CLAIMS

We claim:

1. A method for detecting the presence of an enterovirus in a clinical sample comprising the steps of:

- (i) obtaining a clinical sample from a subject;
 - (ii) purifying RNA contained in the sample;
 - (iii) reverse transcribing the RNA with primers effective to reverse transcribe enteroviral RNA to provide a cDNA;
 - (iv) contacting at least a portion of the cDNA with
 - (a) a composition that promotes amplification of a nucleic acid and
 - (b) an oligonucleotide mixture wherein the mixture comprises at least one oligonucleotide that hybridizes to a highly conserved sequence of the sense strand of an enterovirus nucleic acid and at least one oligonucleotide that hybridizes to a highly conserved sequence of the antisense strand of an enterovirus nucleic acid, thereby providing an amplification mixture, such that, upon hybridizing, the oligonucleotides direct amplification of at least a portion of the nucleotide sequence of the VP1 gene of the enterovirus genome;
 - (v) carrying out an amplification procedure on the amplification mixture, such that, if an enterovirus is present in the sample, an enterovirus amplicon is produced whose sequence comprises a nucleotide sequence of at least a portion of the VP1 gene of the enterovirus genome; and
 - (vi) detecting whether the amplicon is present;
- wherein the presence of the amplicon indicates that an enterovirus is present in the sample.

2. The method as described in claim 1, wherein the highly conserved sequences occur within the VP1 gene or within about 100 nucleotides from a terminus of the VP1 gene.

3. The method as described in claim 2, wherein at least one oligonucleotide comprises, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a motif chosen from the group consisting of the sequences given by SEQ ID NO:80 and SEQ ID NO:81, and at least one oligonucleotide comprises, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a motif given by SEQ ID NO:82.

4. The method as described in claim 3, wherein the oligonucleotide mixture comprises an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:3, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:4 and an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:9.

5. The method as described in claim 4, wherein the oligonucleotide mixture comprises an oligonucleotide whose sequence is given by SEQ ID NO:3, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence is given by SEQ ID NO:4 and an oligonucleotide whose sequence is given by SEQ ID NO:9.

6. The method as described in claim 2, wherein at least one oligonucleotide comprises, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a motif chosen from the group consisting of the sequences given by SEQ ID NO:83, SEQ ID NO:84, and SEQ ID NO:85, and at least one oligonucleotide comprises, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a motif given by SEQ ID NO:86.

7. The method as described in claim 6, wherein the oligonucleotide mixture comprises an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:22, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:19, an oligonucleotide whose sequence

comprises, at the 3' end thereof, the sequence given by SEQ ID NO:20, and an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:21.

8. The method as described in claim 7, wherein the oligonucleotide mixture comprises an oligonucleotide whose sequence is given by SEQ ID NO:22, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence is given by SEQ ID NO:19, an oligonucleotide whose sequence is given by SEQ ID NO:20, and an oligonucleotide whose sequence is given by SEQ ID NO:21.

9. The method as described in claim 1, wherein the amplification procedure comprises a polymerase chain reaction.

10. The method as described in claim 1, wherein the sample is chosen from the group consisting of whole blood or a fraction thereof, a bronchial wash, cerebrospinal fluid, an eye swab, a conjunctival swab, a swab or scraping from a lesion, a nasopharyngeal swab, an oral or buccal swab, pericardial fluid, a rectal swab, serum, sputum, saliva, stool, a stool extract, a throat swab, urine, brain tissue, heart tissue, intestinal tissue, kidney tissue, liver tissue, lung tissue, pancreas tissue, spinal cord tissue, skin tissue, spleen tissue, thymus tissue, cells from a tissue culture, a supernatant from a tissue culture, and tissue from an experimentally infected animal.

11. The method as described in claim 1, wherein the detection is carried out by a procedure chosen from the group consisting of gel electrophoresis and visualization of amplicons contained in a resulting gel, capillary electrophoresis and detection of the emerging amplicon, probing for the presence of the amplicon using a labeled probe, and labeling a PCR primer employed in the method and detecting the label.

12. A method for typing an enterovirus in a clinical sample comprising the steps of:

- (i) obtaining a clinical sample from a subject,
- (ii) purifying RNA contained in the sample,
- (iii) reverse transcribing the RNA with primers effective to reverse transcribe enteroviral RNA to provide a cDNA;
- (iv) contacting at least a portion of the cDNA with
 - (a) a composition that promotes amplification of a nucleic acid and
 - (b) an oligonucleotide mixture wherein the mixture comprises at least one oligonucleotide that hybridizes to a highly conserved sequence of the sense strand of an enterovirus nucleic acid and at least one oligonucleotide that hybridizes to a highly conserved sequence of the antisense strand of an enterovirus nucleic acid, thereby providing an amplification mixture, such that, upon hybridizing, the oligonucleotides direct amplification of at least a portion of the nucleotide sequence of the VP1 gene of the non-polio enterovirus genome;
- (v) carrying out an amplification procedure on the amplification mixture, such that, if an enterovirus is present in the sample, an enterovirus sample amplicon is produced whose sequence comprises a nucleotide sequence of at least a portion of the VP1 region of the enterovirus genome;
- (vi) determining that the sample amplicon is present;
- (vii) determining at least a partial nucleotide sequence of the sample amplicon;
- (viii) providing a database consisting of prototypical nucleotide sequences, wherein each prototypical sequence is the sequence of a standard amplicon obtained from a member of a set of prototypical enterovirus serotypes by carrying out the procedure of steps (ii) through (v) on each prototypical enterovirus serotype, wherein each prototypical sequence comprises at least a portion of the sequence of the VP1 gene, and wherein the sequence of each prototypical VP1 gene is different from the sequence of every other prototypical VP1 gene in the database;

(ix) comparing the sequence of the sample amplicon with each prototypical sequence in the database; and

(x) identifying the prototypical sequence that has the highest extent of identity to the sequence of the sample amplicon to provide an identified serotype; wherein the type of the sample is the serotype of the identified serotype.

13. The method as described in claim 12, wherein the highly conserved sequences occur within the VP1 gene or within about 100 nucleotides from a terminus of the VP1 gene.

14. The method as described in claim 13, wherein at least one oligonucleotide comprises, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a motif chosen from the group consisting of the sequences given by SEQ ID NO:80 and SEQ ID NO:81, and at least one oligonucleotide comprises, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a motif given by SEQ ID NO:82.

15. The method as described in claim 14, wherein the oligonucleotide mixture comprises an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:3, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:4 and an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:9.

16. The method as described in claim 15, wherein the oligonucleotide mixture comprises an oligonucleotide whose sequence is given by SEQ ID NO:3, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence is given by SEQ ID NO:4 and an oligonucleotide whose sequence is given by SEQ ID NO:9.

17. The method as described in claim 13, wherein at least one oligonucleotide comprises, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a

motif chosen from the group consisting of the sequences given by SEQ ID NO:83, SEQ ID NO:84, and SEQ ID NO:85, and at least one oligonucleotide comprises, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a motif given by SEQ ID NO:86.

18. The method as described in claim 17, wherein the oligonucleotide mixture comprises an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:22, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:19, an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:20, and an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:21.

19. The method as described in claim 18, wherein the oligonucleotide mixture comprises an oligonucleotide whose sequence is given by SEQ ID NO:22, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence is given by SEQ ID NO:19, an oligonucleotide whose sequence is given by SEQ ID NO:20, and an oligonucleotide whose sequence is given by SEQ ID NO:21.

20. The method as described in claim 12, wherein the sample is chosen from the group consisting of whole blood or a fraction thereof, a bronchial wash, cerebrospinal fluid, an eye swab, a conjunctival swab, a swab or scraping from a lesion, a nasopharyngeal swab, an oral or buccal swab, pericardial fluid, a rectal swab, serum, sputum, saliva, stool, a stool extract, a throat swab, urine, brain tissue, heart tissue, intestinal tissue, kidney tissue, liver tissue, lung tissue, pancreas tissue, spinal cord tissue, skin tissue, spleen tissue, thymus tissue, cells from a tissue culture, a supernatant from a tissue culture, and tissue from an experimentally infected animal.

21. The method as described in claim 12, wherein the amplification procedure comprises a polymerase chain reaction.

22. The method as described in claim 12, wherein an amplicon encompasses at least a portion of the nucleotide sequence for the VP1 gene of an enterovirus.

23. The method as described in claim 12, wherein the set of prototypical enterovirus serotypes comprises serotypes of coxsackie A viruses, coxsackie B viruses, echoviruses, and numbered enteroviruses.

24. The method as described in claim 23, wherein the serotypes of coxsackie A viruses (CA) comprise CA1 through CA22 and CA24.

25. The method as described in claim 23, wherein the serotypes of coxsackie B viruses (CB) comprise CB1 through CB6.

26. The method as described in claim 23, wherein the serotypes of echoviruses (E) comprise E1 through E7, E9, and E11 through E27, and E29 through E33.

27. The method as described in claim 23, wherein the serotypes of numbered enteroviruses (EV) comprise EV68 through EV71.

28. The method as described in claim 12, wherein determining at least a partial nucleotide sequence of the sample amplicon comprises a sequencing method chosen from the group consisting of a method using 2',3'-dideoxynucleotide chain terminators and a method using chemical degradation of terminally-labeled amplicons.

29. The method as described in claim 12, wherein comparing the sequence of the sample amplicon with each sequence in the database employs a sequence alignment and comparison algorithm.

30. An oligonucleotide comprising, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a motif chosen from the group consisting of a sequence given by SEQ ID NO:80, a sequence given by SEQ ID NO:81, a sequence given by SEQ ID NO:82, a sequence given by SEQ ID NO:83, a sequence given by SEQ ID NO:84, a sequence given by SEQ ID NO:85, and a sequence given by SEQ ID NO:86, or an oligonucleotide complementary to any of them.

31. The oligonucleotide described in claim 30 wherein the oligonucleotide consists of a sequence that hybridizes to a sequence encoding a motif whose sequence is chosen from the group consisting of SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, and SEQ ID NO:86, or an oligonucleotide complementary to any of them.

32. An oligonucleotide whose sequence comprises, at the 3' end thereof, a sequence chosen from the group consisting of the sequence given by SEQ ID NO:3, the sequence given by SEQ ID NO:4, the sequence given by SEQ ID NO:9, the sequence given by SEQ ID NO:19, the sequence given by SEQ ID NO:20, the sequence given by SEQ ID NO:21, and the sequence given by SEQ ID NO:22, or an oligonucleotide complementary to any of them.

33. The oligonucleotide described in claim 32 whose sequence consists of a sequence chosen from the group consisting of SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:9, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, and SEQ ID NO:22, or an oligonucleotide complementary to any of them.

34. A mixture of oligonucleotides comprising at least two oligonucleotides, wherein at least one of the oligonucleotides hybridizes to a sense strand of a double stranded nucleic acid and at least one of the oligonucleotides hybridizes to an antisense strand of the nucleic acid, the nucleic acid encoding at least a portion of the VP1 gene of an enterovirus, wherein the oligonucleotides hybridize to sequences that are highly conserved among enteroviruses, and wherein the oligonucleotides, when

hybridized to the nucleic acid, direct the synthesis of an amplicon encoding at least a portion of the VP1 protein of enteroviruses when the oligonucleotides are employed in an amplification procedure using the nucleic acid.

35. The mixture of oligonucleotides as described in claim 34, wherein each oligonucleotide comprises, at the 3' end thereof, a sequence that hybridizes to the nucleic acid.

36. The mixture of oligonucleotides as described in claim 34, wherein the highly conserved sequences occur within the VP1 gene or within about 100 nucleotides from a terminus of the VP1 gene.

37. The mixture of oligonucleotides as described in claim 34, wherein at least one oligonucleotide comprises, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a motif chosen from the group consisting of the sequences given by SEQ ID NO:80 and SEQ ID NO:81, and at least one oligonucleotide comprises, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a motif given by SEQ ID NO:82.

38. The mixture of oligonucleotides as described in claim 37, the mixture comprising an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:3, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:4 and an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:9.

39. The mixture of oligonucleotides as described in claim 38, wherein the mixture comprises an oligonucleotide whose sequence is given by SEQ ID NO:3, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence is given by SEQ ID NO:4 and an oligonucleotide whose sequence is given by SEQ ID NO:9.

40. The mixture of oligonucleotides as described in claim 34, wherein at least one oligonucleotide comprises, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a motif given by SEQ ID NO:86, and at least one oligonucleotide comprises, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a motif whose sequence is chosen from the group consisting of SEQ ID NO:83, SEQ ID NO:84, and SEQ ID NO:85.

41. The mixture of oligonucleotides as described in claim 40, wherein the oligonucleotide mixture comprises an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:22, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:19, an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:20, and an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:21.

42. The mixture of oligonucleotides as described in claim 41, wherein the oligonucleotide mixture comprises an oligonucleotide whose sequence is given by SEQ ID NO:22, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence is given by SEQ ID NO:19, an oligonucleotide whose sequence is given by SEQ ID NO:20, and an oligonucleotide whose sequence is given by SEQ ID NO:21.

43. A kit comprising a mixture of oligonucleotides, wherein the oligonucleotide mixture comprises an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:3, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:4 and an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:9.

44. The kit as described in claim 43, wherein the oligonucleotide mixture comprises an oligonucleotide whose sequence is given by SEQ ID NO:3, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence is given by SEQ ID NO:4 and an oligonucleotide whose sequence is given by SEQ ID NO:9.

45. A kit comprising a mixture of oligonucleotides, wherein the oligonucleotide mixture comprises an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:22, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:19, an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:20, and an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:21.

46. The kit described in claim 45 wherein the mixture comprises an oligonucleotide whose sequence is given by SEQ ID NO:22, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence is given by SEQ ID NO:19, an oligonucleotide whose sequence is given by SEQ ID NO:20, and an oligonucleotide whose sequence is given by SEQ ID NO:21.

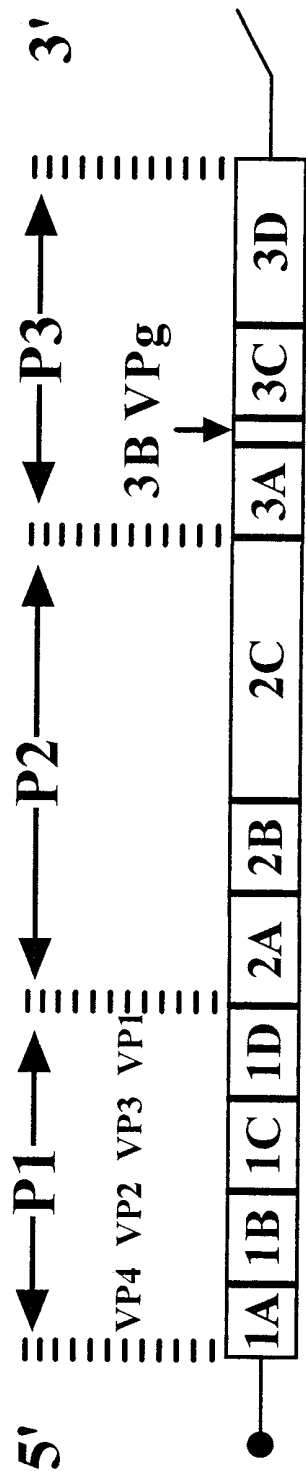
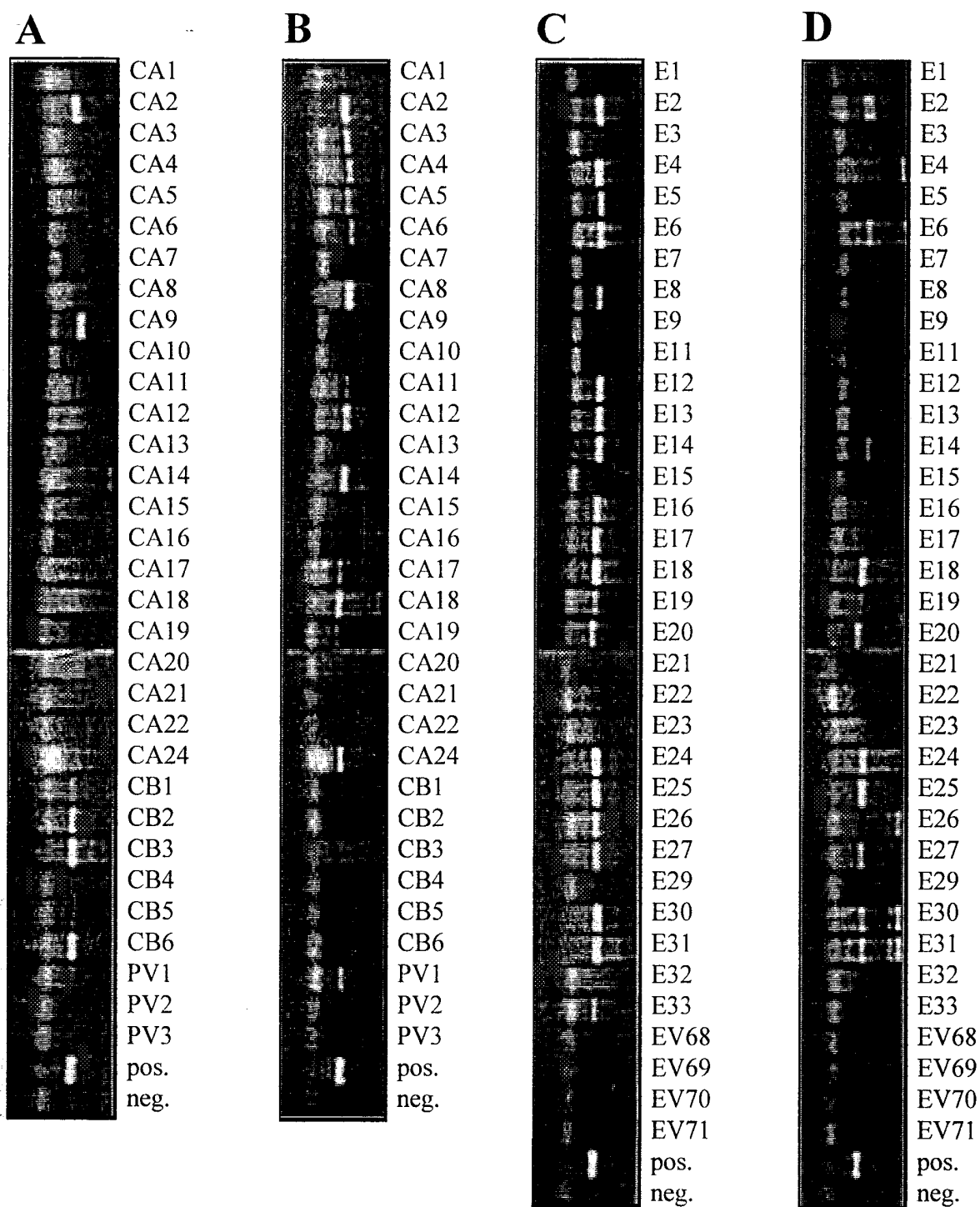


Figure 1

**Figure 2**

SEQUENCE LISTING

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 BY THE SECRETARY OF THE DEPARTMENT OF HEALTH AND HUMAN
 SERVICES CENTERS FOR DISEASE CONTROL AND PREVENTION
 Oberste, M. Steven
 Maher, Kaija
 Kilpatrick, David R.
 Pallansch, Mark A.

<120> TYPING OF HUMAN ENTEROVIRUSES

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 <141> 2000-03-24

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gcncngayt gntgnccraa

20

<210> 4
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 <213> Artificial Sequence

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 <223> Inosine

<221> modified_base
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<221> modified_base
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<221> modified_base
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<223> UNKNOWN

<400> 4

atgtaygtnc cncngnggg

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 <223> UNKNOWN

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 ggngcrttnc cytcngtcca 20

 <210> 6
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 <213> Artificial Sequence

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 <222> (6)...(0)
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 <222> (8)...(0)
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 <223> Inosine

 <221> modified_base
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 <223> Inosine

 <223> UNKNOWN

 <400> 6
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 <210> 7
 <211> 18
 <212> DNA
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 <221> modified_base
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 <223> UNKNOWN

 <400> 7
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 <210> 8
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    <223> Inosine

    <221> modified_base
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    <223> UNKNOWN

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    <223> Inosine

    <221> modified_base
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    <223> UNKNOWN

    <400> 9
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    <223> Inosine

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    <223> Inosine

    <221> modified_base
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<221> modified_base
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 <223> Inosine
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 <221> modified_base
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 <223> UNKNOWN

 <400> 11
 gangaraayc tnatngarac 20

 <210> 12
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 <223> UNKNOWN

 <400> 12
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 <210> 14
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 <220>
 <223> UNKNOWN
 <400> 14
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 <210> 15
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 <222> (4)...(0)
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 <222> (15)...(0)
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<400> 17
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<210> 18
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<220>
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<223> UNKNOWN

<400> 18
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20

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<221> modified_base
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<221> modified_base
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<223> UNKNOWN

<400> 19
acngcngyng aracngnca

20

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 <221> modified_base
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 <223> UNKNOWN

 <400> 20
 acngcngtng aracnggng 19

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 <222> (9)...(0)
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 <223> UNKNOWN

 <400> 21
 cargcngcng aracnggngc 20

 <210> 22
 <211> 19
 <212> DNA
 <213> Artificial Sequence

 <220>
 <221> modified_base
 <222> (2)...(0)
 <223> Inosine

 <221> modified_base
 <222> (5)...(0)
 <223> Inosine

 <221> modified_base
 <222> (8)...(0)
 <223> Inosine

 <221> modified_base

<222> (11)...(0)

<223> Inosine

<223> UNKNOWN

<400> 22

cncncngngg nayrwacat

19

<210> 23

<211> 888

<212> DNA

<213> Enterovirus

<220>

<221> misc_feature

<222> (0)...(0)

<223> CA1, strain Tomkins

<400> 23

ggattggg	cgc	attctattga	ggctgccatt	gacagcatca	cacaaaatgc	actaaccact	60
gtacaaaata	caacacaatc	aggacctact	cattcaaaaag	aagttccagc	attaacagca		120
gtggaaacag	gtgctactag	tcaagtagaa	ccaggtgact	tgattgaaac	cagacatggt		180
ataaacatga	gacaaaagatc	tgaagcatct	atcgaatctt	tctttggccg	atccgcatgt		240
gttgcgatac	ttggtttgtc	aaacgccaaa	ccaactgaca	caaacaccaa	acaattgttc		300
aaaacatgga	gaatatcata	tttagaaaact	caccaactca	gaagaaaact	tgagttcttt		360
acgtactcaa	ggtttgattt	ggaaatgacc	atagtaatta	cagagagggt	tttcaatgca		420
gtcaatgtcc	cattgcgcaa	ttatgtgtac	caaataatgt	acgttcccc	aggtgctcca		480
gaaccacaat	catgggatga	ttacacgtgg	caatcttcta	ccaacccatc	aatattctac		540
accactggaa	atgctcctcc	cagagtgtca	attccatttg	ttggaatagg	gtctgcatat		600
tcacactttt	atgatggttt	ctcacagatt	cctcttgact	caatcagtg	tgagcaagt		660
aataagtatg	gttaccttc	aatcaatgac	tttggtaccc	tggaattag	aatagtaaat		720
gaatatgacc	cagtgcgaagt	ggatgcaaag	gcccagagtgt	atattaaacc	caaacatggt		780
cgcatgtggt	gccccagacc	accacggggc	atgccttaca	agaatagcac	agtggatttc		840
gacccatcag	caactgtaat	gacccaagtc	gcagacatca	ggacgtat			888

<210> 24

<211> 882

<212> DNA

<213> Enterovirus

<220>

<221> misc_feature

<222> (0)...(0)

<223> CA3, strain Olson

<400> 24

ggagatccag	tggaagactt	aatcgccaat	acagttgcta	ggactctaga	gagaataacc	60
tctccaactc	ataatacaac	ggcaggcaac	accaccgtta	gcgagcacag	catcggtacc	120
ggttcagtg	ctgcgttgca	agctgctgag	actggggctt	cgtctaacac	cacagatgag	180
agtatgatag	aaacacgggtg	tggtgtcaat	aggaatggag	tgattgagac	tagcatcaac	240
catttcttct	cccagcgagg	gcttgtggga	gtgctgaaca	tacttgatgg	aggcacctca	300
aaaggctttg	aagtttgga	tatagacatc	atgggctttg	ttcagcttcg	cagaaagcta	360
gagatgttca	cctacatg	gttcaacgct	gaattcacct	ttgtcgcgac	tttgagtgc	420
ggaacaactc	cccatataat	gttgcaatac	atgtatgtgc	cccctggagc	tcccaaacc	480
caggaaagag	attcattcca	atggcagact	gcaaccaacc	catccgtgtt	tgcgaaaatg	540
agtgaccctc	ctccgcaagt	ttcagtacct	ttcatgtctc	ctgctagcgc	ctaccagtgg	600
ttttatgatg	ggtacccaac	atgtgatgat	agaccacaga	cctctaatac	tccctacgga	660
caatgcccc	ataacatggt	gggcacattc	gcggtgcgca	ttgttagcaa	gacgctcg	720
gagagagact	tgcgcgctccg	tggttacatg	aaactgaagc	atgtgcgagc	atgggtaccg	780
cgaccataa	ggtcacagcc	ttacgtcttg	aagaactacc	ccaactatga	tggaacccaa	840
atcgtgcccc	gtgccaaga	tcgagaagac	ataaagaaca	ca		882

<210> 25

<211> 915
 <212> DNA
 <213> Enterovirus
 <220>
 <221> misc_feature
 <222> (0)...(0)
 <223> CA4, strain High Point

<400> 25
 ggtgatgcaa tcgctgatgc tatacaaaac acagttacat ctactataca gagagtcaca 60
 accaactactg ttgggcaaga tgcaacagct gctaacacag caccagctc tcatagtttg 120
 aactactggcc tagtccccgc gcttcaagct gctgagacag gagcttcac cacagccacg 180
 gatgggaatt tgattgagac tagatgtgtt gtaaactcca atgggtacac tgaaaccac 240
 attgagcatt tcttctctag gtcagggctg gtgggagtta tggaggtaga tgatacgggt 300
 actagtggca agggattctc aaactgggac attgacatca tggcgtttgt gcaactgcgc 360
 cgtaaaactcg aggcatttac atatatgcgg ttcgacgcag agtttacctt tgtcaccaat 420
 ttggagaacg ggctcacgaa taatagtgtg atacagtaca tgatgtacc acctggagcg 480
 cctaaacccg atgcccggga atcattccag tggcaaactg caaccaatcc gtcagtcttt 540
 caaaaaatgg acagtccgcc acctcaagtt tcagtaccct tcatgtcacc agccagtgcc 600
 tatcaatggt tctatgacgg ttaccccacc tttgggcccc actcggagac atctaatacta 660
 tcttacgggc aatgtcccaa taatatgctg ggaacattct cggccagggt tgttagcaag 720
 caaatcacca atcagaaatt ccagatccgt atttatctac ggctgaagag ggtgagggcg 780
 tggatcccca gacctttgag atcgcagccg tacatttaca gaaactaccc cacctatggt 840
 actaccatcc aatacctggc caaagatagg cgcaagatca ctgaaactga ttataatgct 900
 gaacagcgca cgcac 915

<210> 26
 <211> 885
 <212> DNA
 <213> Enterovirus

<220>
 <221> misc_feature
 <222> (0)...(0)
 <223> CA5, strain Swartz

<400> 26
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 agtggaccaa ttcagccagt gacagcggcc aacacctctc ccagttcaca tcggcttggt 120
 acggggcaag tgccagcttt gcaagcagca gaaacgggag ccacctcgaa tgcgaccgac 180
 gagagtttga ttgaaaccag gtgtgtggtc aacagacatg gagtcatgga aactagcatt 240
 gaacacttct tttcacgctc aggccttgga ggaattttga taattgagga ctccggtact 300
 tccacgaaag gctacgccac ttgggaaatc gatgttatgg gatttgtcca gctgaggcgt 360
 aaactagaga tgttcacata catgcgattt gatgcagagt tcacctttat cacagcagaa 420
 aggaatggca acaccagccc aatacccatc cagtacatgt atgtcccacc cggagcccca 480
 gtccctactg gtagggagac attccaatgg caaacagcga ccaatccatc cgtgatctca 540
 aagatgactg atccaccagc ccaggtgtct gtaccattta tgagcccagc cagtacttat 600
 caatggttct acgatggcta cccacgttc ggagaagttc cagtgactac gaacttgaac 660
 tatggacagt gcccaaacaa caaaatgggc actttctgca tccgcatggt ctccaggtgta 720
 tctacaggca aggacgtcac tgtgcgcatt ttcattgaagt tgaagcatgt gcgcgcctgg 780
 gtgccaaggc ccacagggag ccagccttac ttgttaaaga attatcccaa ctttgacaag 840
 tcaaataattg tagacgcac atcgaacagg acatatacca ccact 885

<210> 27
 <211> 915
 <212> DNA
 <213> Enterovirus

<220>
 <221> misc_feature
 <222> (0)...(0)
 <223> CA6, strain Gdula

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<400> 27
aatgacccca tttcaaagtc aatagaaaat gctgtgagca cactcgcgtga caccacgata      60
tcacgtgtta cagcggccaa cactgctgct agctcccatt cccttggtac tggacgcgtg      120
ccggcggttg aaggctgcga gacaggggca agttccaacg ctacgcgatga gaacctgatt      180
gaaactcgtt gtgtgatgaa tagaaaatgga gttaacgaag caagtgtaga acacttctac      240
tcccgtgcag ggctagtagg agttgtggag gtgaaagact caggcactag tcaggacggg      300
tacacggtgt ggcccataga tgtgatgggc tttgtgcaac agcggcgcaa gttagagcta      360
tctacttaca tgcgctttga cgctgaattt acctttgtgt ccaatctcaa tgacagcaca      420
acaccgggca tgctattgca gtacatgtac gtgcccggcg gtgcgcccaa accagacggt      480
aggaagtcat atcaatggca aacagccacc aacccttcaa tattcgcaaa gttgagtgc      540
ccaccgcccc aagtgtctgt cccattcatg tcaccggcgt cagcctacca gtggttctac      600
gatgggtacc ccacgttttg cgaacacaag caagctacta atttacaata cggtcagtgc      660
cctaacaaca tgatggggga ttttgctatt cggacagtta gtgaatccac caccgggaaa      720
aatgtccatg tccgggtgta catgagaatt aagcacgtaa gagcatgggt gccagacct      780
ttcagatccc aagcttacat ggtcaaaaac taccgcacat acagccaaac aatatccaat      840
actgcagccg atcgtgcgag cataaccact acggactatg aggggtggcg accagcaaac      900
ccgcagagaa cttttt

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<210> 28

<211> 888

<212> DNA

<213> Enterovirus

<220>

<221> misc_feature

<222> (0)...(0)

<223> CA7, strain AB-IV

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<400> 28
ggagacgaaa tactcgacct aatcgagagt gctgtacaga ataccactaa agccattacc      60
agctcaatcg acaccaaacc tgggtgctaac actcaagcta gccaacatcg tataggcttg      120
ggggagggtt ccgctcttca agctgctgag acaggatcgt cttcgctcgt ttcggacaag      180
aacatgatag aaacaagggtg tgtcgtaaac aaacacagca cagaggaaac cagcattaca      240
aacttctact ccagggcggg cctagtgggg gttgtgaaca tgccagtaca aggaaccagc      300
aacacaaagg gtttcgcaaa gtgggggata gatataatgg gctttgtgca gatgaggcgc      360
aaacttgagc tcatgacata catgagattc tccgcccagt ttacgttcgt acccagcact      420
cctgggggag agactactaa ctttatactg caatacatgt atgcacctcc cggagctccg      480
ctgccaacca ggcgggattc atacgaatgg caaacatcca ctaacccctc tattatcagc      540
aagatggcgg acccaccgcg tcaggtatcg gttccattcc tttctcctgc atcagcatat      600
cagtggttct atgatggcta cccacattt gggaaacacc caatagatca ggacttccaa      660
tatggcatgt gcccaaaca catgatgggc acattctgtg tgcgcatgat cgggtggggc      720
aaaccgaccc aatcagttac catacgtata tacatgagat taaagcatat cgtgcatgg      780
gtgccccggc cactgaggag tcagaattac actatgagga attaccgaa ctacaacggg      840
ggcgcaataa aatgtacatc aaaaagcaga gctaccataa caacctta

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<210> 29

<211> 882

<212> DNA

<213> Enterovirus

<220>

<221> misc_feature

<222> (0)...(0)

<223> CA8, strain Donovan

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<400> 29
ggagattcca ttgaagacat aataagcaac actgtcaccc gtacactgca acaaatcagt      60
gccccatcac acgacactac agcagccaac acctcagtga gtaatcataa aattggtagc      120
ggggatgtcc cagctctcca agctgcagag actggcgcta cttccaatgc ctcagacgag      180
aacatgattg agacacgatg tgtgttaaag cgcaatgggg ttgtggaaac tagtttggac      240
catttctttt caagagcagg ccttgtggga gtgatcaatg tgcaagatgg cggcactcag      300
aagggttttg aagtgtggga catagatgtc atgggggttg ttcaactcag gaggaagttg      360
gagatgttca cgtacatgag gttcaacgcc gagttcacat tcgtatccac actcgcggat      420

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ggcacaactc	ccagagtgat	gttgacgtac	atgtacgttc	cacctggtgc	ccccaaacct	480
caggagagag	attcgtttca	gtggcaaact	gcaaccaacc	catcagtatt	ttgcaaaatg	540
agtgaccctc	ctccacaggt	ttccgttcct	ttcatgtcac	cagctagtgc	ctaccaatgg	600
ttctacgatg	ggtacccaac	attcgatgat	cgaccggcca	cctcaaacca	cccgtacggt	660
cagtgcacca	ataacatgat	gggcacattc	gcagtgcggt	ttgtcagcaa	gaccccagcc	720
acacgggatc	tgcgtgtcag	agtgtacatg	cgcctgaaac	acgtgcgcgc	atgggtaccg	780
agacctatcc	gatctcaacc	ctatatattg	aaaaactacc	caaattatga	tggcacaaag	840
ataacgtcga	catctaagga	taggcaaagc	atcaaaaaca	ca		882

<210> 30

<211> 894

<212> DNA

<213> Enterovirus

<220>

<221> misc_feature

<222> (0)...(0)

<223> CA10, strain Kowalik

<400> 30

ggcgaccccc	tggaggacat	catccacgac	gctttgagca	gcactgtgcg	gcgggccata	60
actagtggtc	aagatgtcaa	cacagcggcc	ggtaccgctc	ctagctctca	caggttgagg	120
actggtcgtg	ttcccgccct	acaagcagca	gaaactggag	ccacttctaa	cgctacagat	180
gagaacatga	tagaaacgcg	gtgtgtcatg	aacagaaatg	gagtgttgga	ggcgactata	240
agtcattttc	tctcacgctc	aggtttggtg	ggtgttgctc	atctaactga	cggaggcacc	300
gatacaacgg	gatatgcagt	gtgggacatt	gacatcatgg	gttttgtgca	actgcggcgg	360
aaatgtgaga	tgttcacata	catgagattc	aacgctgagt	tcacattcgt	cactacaaca	420
gaaaatggcg	aggcaaggcc	atztatgtta	cagtatatgt	atgtacctcc	aggtgcccct	480
aagccaacgg	gtagagatgc	ttttcagtgg	caaacagcga	caaatccatc	cgttttcgtt	540
aagctcacag	atccacctgc	tcaggatatca	gtccccttca	tgtcacctgc	tagtgectac	600
caatggttct	atgacgggta	tccaacattt	ggacaacacc	cggaaacatc	taatacaaca	660
tatggacagt	gccctaacaa	catgatgggg	acctttgctg	tgagagtagt	gagtagagt	720
gctagccagc	tcaaactaca	gacacgagtg	tatatgaagc	ttaagcatgt	gagagcatgg	780
atccctaggc	caataagatc	ccagccttac	ctcctaaaga	attttccaaa	ttatgatagt	840
agtaagatca	catacagcgc	aagagatcgt	gccagcataa	aacaagctaa	tatg	894

<210> 31

<211> 912

<212> DNA

<213> Enterovirus

<220>

<221> misc_feature

<222> (0)...(0)

<223> CA11, strain Belgium-1

<400> 31

ggggcaatag	aagaaatcat	ctcaactgtt	gccagtaacg	cgttggcgct	cagtcaaccc	60
aagccagtgg	acaactctgt	acaaaacacc	caacaaagtg	ctccagtgcg	tagccaggag	120
gtgccagcat	tgaccgcagt	ggagacaggg	gcgacaagtg	atgtgggtcc	atctgacctg	180
attcagacta	gacacgtatt	gaatgttaaa	tccaggtctg	aatccaccat	cgagtcattt	240
tttgcaagag	ctgcatgtgt	aaccattatg	caggtggaca	atttcaacgc	aacctctgtg	300
gaagacaaaa	gaaagtgtgt	tgctaaatgg	gcaatcacct	acactgatac	cgtccagctg	360
agacggaaat	tagagttttt	cacttattct	agatttgact	tagagatgac	ttttgtgcta	420
actgagagat	actactccca	aagctcaggg	catgctagat	ctcaggtgta	ccaaattatg	480
tatgttccac	caggggcacc	cacgcctagt	gcatgggacg	actacacatg	gcaaacatcc	540
tccaacccat	ccattttctt	taccaccggc	aatgcaccac	cgcgcatttc	aattccattt	600
gttggaatcg	ccaatgcata	ctcacacttt	tatgatggct	ttagtagagt	acctttggag	660
ggagaaacaa	cagacacagg	agacgcttac	tacgggtcca	cttcaataaa	cgattttggt	720
acacttgcag	tcagggtagt	taatgactac	aaccagacca	gggtggagac	aaggattaga	780
gtatacatga	agcccaaaca	tgtgagagtc	tggtgcccgc	gacctccaag	agcggtaagc	840
tacagaggac	ctggagtcga	cctcctatca	acatcagtaa	cacctttatc	caaacatgac	900

ctagcgacat ac

912

<210> 32
 <211> 888
 <212> DNA
 <213> Enterovirus

<220>
 <221> misc_feature
 <222> (0)...(0)
 <223> CA12, strain Texas-12

<400> 32
 ggagatacag tgagtgatat gatcgaaaat tccatcaacc gaattaccag tgcaatttcc 60
 actaccaga cacaccagac agcagctgac actagagtta gtacacacag gttaggcacg 120
 ggggaggtgc cacctttaca agcagcagag acagggtgcc cctccaacgc aaccgacgag 180
 aacatgattg aaacacgctg tgtcgtcaac aggacgggg tgagcgagac cagcgtggaa 240
 tacttcttct ctcgctcttg tttggcagga atagtcacgc tggaggatgc aactgccact 300
 aataagggtt atgccacatg ggagattgat gtcattgggt tcgcgcaact gcgtcgcaag 360
 ctggagatct tcacatacat gcgcttcgat gcagagttca cttttgtggc aacagaacgc 420
 aatgggagca ccagcccgtt catgatgcag tacatgttcg tgccccctgg cgccccgtt 480
 ccaacaggga gagatacctt ccaatggcaa tctgtacta acccttcagt gctagtaaaa 540
 atgacggatc caccggccca agttgccatc ccttttatgt ctccagctag tgcataccaa 600
 tggttctatg atggatatcc tacctttgga gaaagaccag ttacaaccaa catgaattat 660
 ggacagtgtc ccaacaacaa aatgggaact tttgtatac gcactgtctc cggatgaagcg 720
 tcagggaaaa acatcactat acgtatcttt atgaggttga agcatgtaag agcgtgggtg 780
 cctcgcccaa ttagaagcca gctatatctg cttaaaaatt accccaactt tgataaact 840
 aagatcctca acgcctccca caacagagct tctatcacat caaacaca 888

<210> 33
 <211> 927
 <212> DNA
 <213> Enterovirus

<220>
 <221> misc_feature
 <222> (0)...(0)
 <223> CA13, strain Flores

<400> 33
 ggggttgaag atctaataca acaagttgct tctaacgcat tacaattgtc ccagccaaca 60
 agaccggcac tcccaccagc cgagcagagt gtccccaaca ctaaccaaac aactccagaa 120
 cactccaagg aagtcccagc gttaacggca gttgaaactg gcgccacgaa tcctctagag 180
 cctggcgaca cagttcagac tagacatgtg atacaaacta gaagtagaag tgaaagtaca 240
 gtggagtctt tctttgcgct aggtgcatgt gtaaccatta tgggagtggg caactataat 300
 gagacattga aaggagacca gaagtctact ctatttacia cctggaacat cacctacact 360
 gacacagtcc agctacggag aaaactggaa atgttcactt actccaggtt tgacatcgag 420
 tttacttttg tggtgactga acgctactac tcatcaaaca gtgggcatgc tctgaaccaa 480
 gtgtacaaa ttatgtatgt accacctgga gcaccagtgc caaagaaatg ggatgattac 540
 acctggcaaa cctcttcaaa cccgtccata ttctacactt atgggtcagc accaccagag 600
 atatccatac cttttgtggg tatagcaaac gcttactccc acttctatga tgggtatgct 660
 acagtgcctt tgaaaactga caccacagac tcaggagcag cctactatgg agcagtatcc 720
 ataaacgact tcggactgct tgcagttcgc gtcgtcaatg aacataatcc agtcagagta 780
 tcatccaaaa ttagagtgtg tatgaaacca aaacatgtca gggtatggtg tcccagacct 840
 ccaagggctg tagagtatta tggaccagga gtggactaca aggcaaacac tttaacaccg 900
 ttgccaataa agaatttgac tacttat 927

<210> 34
 <211> 888
 <212> DNA
 <213> Enterovirus

<220>

<221> misc_feature
 <222> (0)...(0)
 <223> CA14, strain G-14

<400> 34

ggtgacaaag	tggcagacat	gattgagacc	gcagtggaga	agaccgtgtc	ctcactaact	60
tcccctattc	aaacccccac	agccgccaac	acaaacgtga	gtaatcatcg	aattgagctg	120
ggggaagtcc	cggcttttga	agctgctgaa	accggcgcg	cgtctcttgt	gtctgatgaa	180
tacttgatag	agactcgttg	tgtagtgaat	agccatagta	cagaggaaac	tacagtgggg	240
cacttctttt	caagagcggg	gttggtggga	gtgattgacc	tcccattaca	gggaacagtc	300
aacacaggag	gattcgcttc	gtgggatatt	gatgtaatgg	gatatgttca	gatgagaagg	360
aaacttgagc	tgttcacata	tgcccgttcc	gatgcggagt	ttaccttcat	agcttccacc	420
ccagatggcg	aggtgaagcc	agtgttctta	cagtacatgt	tcgtcccccc	tggtgcacca	480
aaaccaacag	ggcgcaacac	ctacgaatgg	caaactgcaa	caaacccttc	tgtgttggtc	540
aagagcacag	atcctccagc	acaagtctct	gtaccgttca	tgtcaccagc	cagcgcatat	600
cagtgtttct	atgacgggta	cccaaccttt	ggaaagcacc	tgcttgctga	tgactttcag	660
tacggtatga	ccccaaataa	catgatggga	tcgttctgtg	ccaggatagt	gggggaagga	720
gcgcctagt	tacacttggt	tatccgtatc	tacatgcgca	tgaaacacgt	gcgggtgtgg	780
attccacgac	ctatgcgcag	ccagccatac	gttgcgaaaga	attaccctaa	ctacaagggg	840
tctgagatca	agtgcgcac	atctagtcgt	aagtcaatca	ccacatta		888

<210> 35
 <211> 912
 <212> DNA
 <213> Enterovirus

<220>

<221> misc_feature
 <222> (0)...(0)
 <223> CA15, strain G-9

<400> 35

gggccaatag	aggagatcat	ctcgaccgtc	gccagcaatg	cacttgccct	cagtcagcct	60
aaaccggtgg	ataattctgt	acaaaacacc	caacagagcg	cgcccgtgca	cagccaagag	120
gttccagcat	taacagcagt	agagactgga	gcaacaagt	atgtggtgcc	agctgatcta	180
gtgcaaacca	ggcatgtagt	gaatgtcaag	tccagatctg	agtcactat	cgagtcgttc	240
tttgcaagag	ctgcctgcgt	gactattatg	cagggtgata	actttaatgc	caccaccacg	300
gaggacaaga	ggaagttatt	tgccaaatgg	gccatcacat	acacagacac	agtacaattg	360
aggaggaaat	tggaattttt	cacgtactcc	aggttcgatc	ttgagatgac	tttcgtgcta	420
actgaaagat	actattctca	gagctcgagg	cacgctagat	cgcaggtgta	tcaaatacatg	480
tacgtccctc	caggagcacc	aacaccaa	gcatgggatg	attacacgtg	gcagacgtct	540
tctaaccctc	caattttctt	caccactggg	aacgcacccc	cacgggtttc	aatcccattt	600
gtgggcattg	caaatgctta	ctcacacttt	tatgatgggt	tcagcagggg	acctttggaa	660
ggagagacca	ctgactcagg	tgacgcttat	tatggcctca	cttctatcaa	tgactttgga	720
acacttgacg	taagagtggg	caatgactac	aaccagcgca	gagtgagagc	aaggatcaga	780
gtctacatga	aacctaagca	tgtgagagt	tggtgtccac	gacccctag	ggctgtgagc	840
tacagaggac	ccggtgtgga	cctactgtcc	acctcagtga	cgccctatc	taagcatgaa	900
ttgacaacgt	ac					912

<210> 36
 <211> 918
 <212> DNA
 <213> Enterovirus

<220>

<221> misc_feature
 <222> (0)...(0)
 <223> CA17, strain G-12

<400> 36

ggcattgaag	acttgatcca	acaggttgca	tcgaatgcgc	tgcaaattctc	acagccgacg	60
cgtccggcac	tgccctctac	agaaagtctt	cccaacacac	aacaatcggc	accttcgcat	120
tctcaagagg	tcccggcgct	gacagcagtt	gagacaggcg	cgacaaatcc	attggagccc	180

tctgacacgg	tacaaacaag	gcatgtttatc	cagactagat	ccagggtcaga	gtccacaata	240
gagtccttct	tcgcgcgtgg	tgcatgtgtg	acaatcatga	cagtggaaaa	ttttaacgcg	300
actgaggcgg	cagacaagaa	aaagttgttc	gccacttgga	atattacata	cacagacaca	360
gtgcagctca	gaaggaagtt	ggagatgttc	acttactctc	gatttgacat	tgaattttacc	420
tttgtcacca	cagaaaggta	ctacgccagt	aactcaggcc	atgcgcgtaa	tcagggtttac	480
caactcatgt	atgtaccccc	aggagcccct	gtgccacaac	aatgggatga	ttacacgtgg	540
caaacttcct	ccaacccatc	ggtgttttac	acatacggtg	acgctccagc	gcgcattttcc	600
ataccatttg	tagggatagc	taatgcctat	tcccactttt	atgacggcta	tgcagtgggtg	660
ccattgaaag	attccaccca	ggatgctggt	gctgcctatt	atggtgcaac	ctcaattaat	720
gattttggaa	tggtggcggt	gagagtagtc	aacgaattca	acccagccag	aatcacatct	780
aaattgagag	tgtacatgaa	accaaagcat	gttaggggtg	ggtgtcctag	accaccaagg	840
gtgggtgccg	acttcggacc	cggtgttgat	tataaggata	gtttgacacc	gctttctaca	900
aaagcactca	acacttat					918

<210> 37

<211> 927

<212> DNA

<213> Enterovirus

<220>

<221> misc_feature

<222> (0)...(0)

<223> CA18, strain G-13

<400> 37

ggcttggaag	acctcatcca	acaagtggcc	acgaatgcat	tgagtctgtc	gcagcccaca	60
agacccgcac	ttccaccagc	agaacaaagt	gtgcaaaca	ccagtcagac	caccccagaa	120
cattcaaagg	aagtacccgc	actcactgca	gtggagaccg	gtgcaacca	cccattggaa	180
ccagggtgaca	cagtgcacaac	tagacatggt	gttcaaaca	gatcaaggag	cgaaagtacg	240
gtggaatctt	tctttgcaag	aggggcgtgt	gtcacgatta	tgggagttga	caattacaat	300
gaaagcttga	ccagtagtca	aaaatccacc	ctattcgcca	cttggaatat	tacatacact	360
gatacagtac	agttgaggag	aaaattggaa	atgttcacct	actccagatt	tgacattgaa	420
tttaccttcg	tagtaactga	acgttactac	tcgtcaaaca	gtggccatgc	cttgaatcag	480
gtgtatcaaa	tcatgtatgt	gccaccaggc	gtcccaattc	ctaagaagtg	ggatgattat	540
acctggcaaa	catcatcaaa	cccctcaata	ttctacacct	atggaacagc	accacccaga	600
atttcgatcc	cttttgtggg	cattacaac	gcgtactcac	atTTTTatga	cggatattgcg	660
actgtaccac	tcaagacaga	cactacggat	ccgggggcgg	ccttctatgg	agcagtttcc	720
atcaatgact	ttggtttgtt	ggcgggtgca	gtttgtcaacg	agcacaaccc	ggtaagagtg	780
tcttcaaaga	taagagtgtg	catgaagcct	aaacatgtca	gagtggtgtg	cccacgacca	840
ccacgtgccg	tggagtacta	cggaccaggg	gtagattaca	aggcaaacac	attgacacct	900
ctccctacca	agaacttaac	tacttat				927

<210> 38

<211> 888

<212> DNA

<213> Enterovirus

<220>

<221> misc_feature

<222> (0)...(0)

<223> CA19, strain 8663

<400> 38

ggtattgatg	atatcataga	taatgttgta	accaatgctt	tgaagggtgc	catgccacaa	60
gttcaagata	cgcaatctag	tggaccagtt	aactcaaaag	aagtacctgc	attaacagct	120
gttgaacag	gggctactag	tcaagttgac	ccatcagacc	taatagaaac	tagacatggt	180
attaataacc	gcctcagatc	tgagtgcaca	atagaatcat	tctttgggag	gtcagcatgt	240
gtggccataa	ttgggtttatc	taaccaaana	cccaccagtg	acaatgcagc	caagctcttt	300
gctacatgga	agattagtta	tcttgatatg	tatcaattga	gaagaaaatt	ggaattcttc	360
acatactcca	gatttgatct	tgagttaacc	tttgtaattt	cagaaagatt	cttcacctca	420
acttcagctg	ctgcaagaga	ttatgtatac	cagatcatgt	acattccccc	aggagcccct	480
atccctcagg	tatgggatga	ttacacatgg	caatcatcca	caaaccctc	aatattctac	540
accacaggaa	atgcatgccc	tagagtgtcc	atcccttttg	ttgggatcgg	tgacgcatac	600

tctcacttct	atgatggatt	ctcttttagta	cctttcaata	ccatcgatgc	tgggtgcttca	660
aacaggtagc	ggtacaccac	cataaatgat	tttgggacta	tggcaatcag	gatagttaat	720
gaatacgacc	cagtcacaat	tgatgcaaaa	gtcaggggtt	acatgaaacc	aaagcatatt	780
aaggtgtggt	gccccagacc	tccacgggca	gtagcataca	atgggccaac	agtgaatttt	840
aatgaaaacc	cccatgtaat	gacagcagtt	gctgatatta	gaacttat		888

<210> 39
 <211> 909
 <212> DNA
 <213> Enterovirus

<220>
 <221> misc_feature
 <222> (0)...(0)
 <223> CA20, strain IH-35

<400> 39						
ggtatcgaag	atcttatcac	cgaagttgca	agcaacgctc	tgaagttgtc	acaaccaaaaa	60
cccagcacac	aacagagttt	accaaact	agtagctcag	aaccaactca	ctctcaggaa	120
gcgccggcat	tgaccgcagt	agaaacagga	gcaactagta	gcgtagtacc	agctgatctg	180
gtccagacgc	ggcatgtgat	acaaacacgt	agccgaagtg	agtctacagt	tgagtcattc	240
tttgctcggg	gggcgtgtgt	aacaatcatg	tcagtggaaa	attacaatga	aaccgctatc	300
gcagagtcca	aattattttac	caagtggaac	attacctaca	cagacacagt	ccagttgaga	360
agaaaaactag	agatgttcac	atactccaga	tttgatattg	agttcacatt	tgtggtgact	420
gagcgttacc	actccgcaaa	ctcagggtcat	gcactaaatc	aagtttacca	gatcatgtat	480
gttcctccag	gtgcaccagt	gccacaaaga	tgggacgact	acacatggca	aacgtcatcc	540
aaccctcag	tctttttatac	ctatggtaca	gcaccagcca	gaatatcgat	tccatatgta	600
ggcatagcca	atgcctactc	gcatttttat	gatggcttcg	ccaaagtgcc	cattgaaggc	660
gagacgtcag	atccagggtga	tgcatactat	ggtgcaacgt	ccatcaatga	tttcggcatc	720
ttagccatac	gtgtggtcaa	cgaacacaat	ccagtgcgaag	tttcttccaa	gattagagtg	780
tacatgaaac	ctaaacatgt	gcgcgttttg	tgtcccagac	cacctagagc	tgttccatac	840
tttggtcccg	gggttgatta	taaaggtgac	gccctcacac	cactatcacg	caaggattta	900
accacctat						909

<210> 40
 <211> 888
 <212> DNA
 <213> Enterovirus

<220>
 <221> misc_feature
 <222> (0)...(0)
 <223> CA22, strain Chulman

<400> 40						
gggattgagg	atacaatcga	aaaagtgggt	ggtgatgctc	taagggtctc	aatgccacaa	60
gttgccaaca	cccagccatc	aggacccgta	aattctaagg	aagttccagc	actgacagca	120
gtggaaacag	gtgcaaccag	tcaagtcacc	cctgaagatt	tgatcgaaac	caggcatgtt	180
attaacaata	gactaagatc	tgagtgcact	gtggaggcct	tctttggaag	gtctgcatgt	240
gttgccatcc	ttggtaggt	aaacaaaaag	ccagacacca	caaatgccaa	agacctcttt	300
acaacatgga	ggatctta	cctgcaaact	tatcaactga	ggaggaaact	cgaactcttc	360
acgtattcta	gattcttt	ggaattaacg	tttgtcatta	cagaaagata	cttttcaggg	420
acagcagcca	caaccagaga	ttatgtttac	caaataatgt	atgtaccacc	aggagccccc	480
ataccaaata	cctgggacga	ctacacctgg	cagtcattcta	ccaaccctc	tgtcttctac	540
accacaggca	atgccagccc	acgcattgtc	ataccctttg	ttgggtattgg	tgcgcctat	600
gctcactttt	atgacgggtt	cagtgtggta	ccattcaatc	aaatagatgc	aggagcatcc	660
aacaaatatg	gctactcatc	aatcaaagac	tttggtacat	tggcagttag	aattgttaat	720
gagtttgatc	cagtgcacat	agaggctaaa	gtcagagtgt	acatgaaacc	caaacatgtc	780
aggggtgtgt	gtccaagacc	acctcgtgca	gtacatatc	aaaactcatc	agttgatttc	840
gcccacaaacg	cagtagcaat	gaaccaagta	gccacaatta	ggacgtat		888

<210> 41
 <211> 915

<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> CA24, strain Joseph

<400> 41

ggtatcgaag	ataccattga	cactgtcatt	aacaatgccc	tacaactatc	tcaaccacag	60
ccaaataagc	agttgacagc	tcagtctacc	ccctccacaa	gtggagtaaa	ctcccaggag	120
gttccagctc	tgaccgctgt	ggaaaccggt	gcctcgggac	aagcagtgcc	cagtgatgtg	180
attgagacca	gacacgtggt	taattataag	acccgatctg	aatctactct	tgagtctttc	240
tttgggaagg	cagcttgtgt	caccataatt	gaggtcgaga	acttcaatgc	cactagttaa	300
gcagacaaga	ggaaacagtt	caccacttgg	ccaatcacat	acaccaatac	cgtgcaattg	360
cgcaggaaac	tagaattctt	cacttactcc	aggtttgacc	tagagatgac	ctttgtagtg	420
acagaaagat	attatgccag	caacacaggt	cacgccagaa	accaagtgtg	tcaaataatg	480
tacattcctc	ctggtgcacc	acaaccacac	gcatgggatg	attacacgtg	gcaaagctct	540
tcgaatccgt	cagtctttta	cacttatggg	agtgtccac	ccaggatgtc	tataccgtat	600
gtcggtatcg	caaatgcata	ctctcttttt	tatgatgggt	ttgcacgagt	accactgaag	660
gacgaaacag	cggactcagg	tgatactttt	tacgggctag	tcaccatcaa	tgattttgga	720
accttagcaa	taagagtagt	gaatgaattt	aaccagcta	ggattacatc	aaaaattaga	780
gtgtatatga	aaccaaagca	tgtaagatgc	tggtgcccta	gaccaccacg	tgcagtgccg	840
taccgtggtg	aaggagtaga	ttttaattca	agttcaatca	caccactaac	agcagtcgca	900
aacatcaaca	cattc					915

<210> 42
<211> 852
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> CB2, strain Ohio-1

<400> 42

agcccagtg	aggaatccat	tgagagaagc	attggcagag	ttgctgacac	cattggtagt	60
ggaccatcca	attcggaggc	aataccggca	ctcacagcag	tagaaacagg	acacacatca	120
caggttacac	ctagtgcac	gatgcaaaca	agacatgtgc	acaactacca	ttcaaggctc	180
gaatccagcg	tagagaactt	cctggcacgc	tcggcttgtg	tgttttatac	aacatacacc	240
aacggtaaaa	aaaaaaatgc	cgccaaagag	aagaagtttg	caacgtggaa	agtgagtgtt	300
agacaagccg	cccaactaag	aagaaagcta	gagttattca	catacttacg	ctgtgacatc	360
gaattaacat	tcgtcatcac	cagtgcacaa	gatccatcga	ccgctaccaa	cttggatgtg	420
ccagtgttga	cccatacaat	aatgtacgtc	ccacctgggtg	gtccagtccc	tgaaaccgtg	480
gacgattaca	actggcaaac	atctacaaat	cccagccttt	tttggactga	aggggaatgca	540
cctccacgca	tgtcaattcc	attcatgagc	ataggcaatg	cctatagtat	gttctatgat	600
ggttggtccg	agtttaggca	tgacgggtgtg	tacggcctga	atacccttaa	caatatgggc	660
acaatatatg	ctaggcacgt	caacgctgac	aaccaggtg	gcatcaccag	cacagtgaga	720
atatacttca	aacccaaaca	tgtcaaggca	tgattcctc	gcccgcctcg	tttggcacag	780
tatcttaaag	ccaataatgt	gaattttgag	atcaccgatg	tgacagaaaa	gagagatagt	840
ctcacgacca	cg					852

<210> 43
<211> 846
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> CB6, strain Schmitt

<400> 43

agcccagtg	g	agggcgccat	agagagagcc	attgcacggg	tcgctgacac	tatgccaaagt	60
ggcccaacca		attcagaagc	agtgcctgcc	ctgacagcag	tggaaacggg	ccacacctcc	120
caagtcgtcc		ccagtgataa	catgcaaacc	aggcacgtga	agaagtacca	ttcacgctcc	180
gaaaccagcg		tcgagaactt	tctgtgtagg	tctgcatgtg	tatatattac	cacatataag	240
aaccagacag		gggcgaaaaa	tagatttgct	tcttgggtaa	tcaccacaag	acaagtggcc	300
cagctcagga		gaaaactaga	aatgtttacg	tacttgcggt	tcgacattga	actcaccttt	360
gtcattacaa		gtgcgcaaga	ccaatccact	atttcccaag	acgcccctgt	gcagacacat	420
cagataatgt		acgtgccacc	gggaggccca	gtgccaaacca	aagttgacga	gtatgtgtgg	480
caaacatcca		ccaaccccag	cgtcttttgg	accgagggtg	acgctccacc	acgtatgtca	540
gttcccttta		tgagtatcgg	taatgcttat	agcacatttt	atgacgggtg	gtctgatttt	600
tcaaacaaaag		gaatatatgg	gttgaacacc	ttgaacaaca	tgggaacatt	gtacatccgc	660
cacgttaacg		ggcccaaccc	agtaccaatt	accagcacag	tgaggatata	ctttaagccc	720
aagcatgtta		aggcctgggt	gcctaggcct	ccaaggcttt	gccagtacaa	aacgtttagg	780
caagtcaact		ttacagtgcg	tggagtgcac	gagagttagg	caaataatac	caccatgaat	840
actaca							846

<210> 44

<211> 852

<212> DNA

<213> Enterovirus

<220>

<221> misc_feature

<222> (0)...(0)

<223> E1, strain Farouk

<400> 44

ggtgatgtgc	agaatgctgt	cgaaggggct	atggtcaggg	tggcagatac	agtgcaaact	60
tcagccacaa	actcagagag	ggtgcctaac	ttgacagcag	tagaaactgg	tcacacttcg	120
caggtagtac	ctggtgatac	catgcagact	agacatgtga	tcaacaatca	cgtgagggtca	180
gaatctacaa	ttgagaactt	ccttgccaga	tcagcgtgtg	ttttcttctt	agagtacaag	240
acagggacca	aagaggattc	caatagcttc	aacaattggg	tgattacaac	caggcgagtg	300
gctcaactac	gtagaaaact	ggaaatgttt	acttacctac	ggtttgacat	ggaaatcacc	360
gtggtcatta	caagctcgca	agatcagtct	acatcacaaa	accagaatgc	accagtgcata	420
acacaccaga	taatgtatgt	accaccaggg	ggacccatac	ccataagcgt	ggatgattac	480
agctggcaaa	catccaccaa	cccagtatc	ttttggaccg	aagggaacgc	tccggcacgc	540
atgtcaattc	cattttattag	cataggcaat	gcgtatagta	atttctacga	tgggtggtct	600
cacttctccc	agactggcgt	gtatggcttc	actactctga	acaacatggg	tcaattgttc	660
ttccggcacg	taaacaagcc	caaccagcc	gctattacaa	gtgtggcgcg	catttacttc	720
aaaccgaaac	atgtacgcgc	ttgggtgcct	agaccaccgc	gcttgtgtcc	atacatcaat	780
agcacgaatg	tcaactttga	acccaagcca	gtgactgaag	tacgtaccaa	cataataaca	840
acgggtgcct	tc					852

<210> 45

<211> 882

<212> DNA

<213> Enterovirus

<220>

<221> misc_feature

<222> (0)...(0)

<223> E2, strain Cornelis

<400> 45

ggagatgagg	tgaagcatga	acccacagtg	gccaacacaa	cagcaagtgg	accatcaaatt	60
tcacaacaag	taccggcact	cacagcagtg	gagactgggc	acacctcaca	ggtggttcca	120
agcgatacca	tacaaaccag	acatgttcac	aattaccata	gtagaactga	atccaccctg	180
gagaacttcc	tcggaagatc	agcatgcgtg	cacattgact	cgtataagac	caagggagtg	240
accggcgaga	gcaccgggta	cgcatcatgg	gagatcacca	ctcgcgagat	ggtgcagctg	300
cggagggaagt	gtgaactctt	cacctacatg	cgatatgac	tagaaatcac	gtttgtgatt	360
acaagtgcgc	aggagcaagg	ggccaaactg	tcgcagaaca	tgccagtatt	aacacatcag	420
atcatgtatg	tcccaccggg	cgggcctata	ccaaccagca	acgagagtta	cgcttgccaa	480

```

acgtcaacga acccaagcgt gttttggaca gaaggaagct cgccaccacg aatgtcaata 540
ccgtttgtta gcataggaaa cgcatacagc aatttctatg atgggtgggtc gcacttctca 600
caaaacgggtg cgtatgggta cacggcacta aacaagatgg gtaggatatt cgtgcgccat 660
gtaaacaagg agacaccact gcaagtcata agcacaatac ggatgtatat gaagcccaaa 720
cacgtgcggg cttgggtgcc aagaccacca cgcctgtgtc catacctgcg ggcgggtgat 780
ataaactttg aagtgactga tgttacagaa aaacgaaata acatcaatta tgtcccaacc 840
ccatcccaca gcagcagtggt gcacatgcgc ttgaacaacc at 882

```

```

<210> 46
<211> 879
<212> DNA
<213> Enterovirus

```

```

<220>
<221> misc_feature
<222> (0)...(0)
<223> E3, strain Morrisey

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```

<400> 46
ggggacgtcg aagaggcaat tgatagggca gttgcgaggg tggctgacac aatgccaaacc 60
ggtccacgaa acactgagag cgtgcctgcc ctgacagcag tagagacagg ccacacctca 120
caggctcgttc ctggtgacac aatgcagacg aggcattgta agaactatca ctccaggaca 180
gagtcacaa ttgaaaactt cctgtgcagg gctgcgtgcg tgtatataac aacatacaaa 240
tcagctgggtg gaacaccac agagcgatat gcaagttgga ggataaacac caggcaaattg 300
gtgcagctca ggaggaaatt tgagctcttc acatacttgc gctttgacat ggaaatcaca 360
tttgtgatca caagcacaca agatcctggg acacaattgg cacaagatat gcctgtacta 420
actcatcagc tcatgtatat cccacctggg ggccctgttc ctaacagtgc cacagatttt 480
gcatggcaat catcaactaa tccaagtata ttttggacgg aaggctgtgc tccagcacga 540
atgtcgggtgc cgttcacacg cattggcaat gcctacacca atttttacga tgggtgggtcg 600
catttcaccc aagaaggggt ttatgggttt aactcactga acaacatggg ccacatatat 660
gtgaggcacg tcaatgagca aagcctgggt gtctcgacca gcaccgttcg cgtgtatttt 720
aaacccaaac atgtgcgtgc ttgggtacca agaccacca gactgtgccc atacactaag 780
agttcaaattg tgaatttcaa accgaccgct gtcactgatg agcgaaagga tatcaacgat 840
gtaggcaccc ttcgaccaac agtgtacact aaccttgtg 879

```

```

<210> 47
<211> 843
<212> DNA
<213> Enterovirus

```

```

<220>
<221> misc_feature
<222> (0)...(0)
<223> E4, strain Pesacek

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```

<400> 47
ggagacgtgc aagatgcagt gacaggtgct atagtacgtg tcgctgacac tctcccaaca 60
ggtccctcaa ataatgaagc tatacccaat ttaacagcag tggagactgg ccatacctcg 120
caagtgcacac caggcgacac aatgcaaaaca cgccatgtgg tgaacatgca caccgcgtct 180
gagtcgtcca tcgagaattt cctggcacgt tcagcatgcg tgtactacct tgattaccaa 240
acgggagaag ggccggcgca tcagtatttt ggccagtgga ccattaccac gaggaggggtt 300
gcgcaattgc gtcgaaagct ggagatgttc acttatctaa gatttgacat ggaaatcaca 360
atcgtgatta ctagtccaca ggatcaatct accatctcga accagatac accagttttg 420
acgcacaaaa ttatgtatgt accaccagga ggaccaatcc cagcaaaaagt cgatgattac 480
agttggcaaa catccacgaa tcccagcgta ttctggactg aagggaatgc gcctgcccgr 540
atatccatcc cattcattag cgttggaaat gcatacagta gcttttatga cgggtgggtcg 600
aacttctcac aaaacgggcy gtatggctac aataccctca acaacatggg acaattgttc 660
tttaggcacg ttaacaaacc cagccctaact actgtcacaac gcgtcgcccg catatacttc 720
aagcctaagc acgtgagagc ttggatcccc cgaccaccgc ggttgtgtcc atacataaat 780
gcgggagacg tgaacttcac tccgacacca gtgactgaaa agcgaaagga cctaataaac 840
acg 843

```

```

<210> 48

```

<211> 843
 <212> DNA
 <213> Enterovirus

<220>
 <221> misc_feature
 <222> (0)...(0)
 <223> E4, strain DuToit

<400> 48
 ggagatgtgc aggacgcagt ggctggggcc atagtgcgtg tggctaatac tctcccatca 60
 ggccccctcaa acaatgaggc tatacccaac ttaacagccg tagaaactgg acacacctcg 120
 caggtgacac cgggtgatac aatgcagacg cgccacgtag tgaacatgca cactcgttct 180
 gagtcgtcaa tcgagaactt cctggcgcggt tcagcatgtg tatactacct cgattaccga 240
 acaggaacgg ggccctggcaa tcaatacttt agccagtgga ctattaccac aagacgagtt 300
 gcgcagctgc gtcgaaaatt ggagatgttc acctatctaa ggttcgacat ggagatcacg 360
 attgtaataa cgagttcaca agatcagcct accgtccgaa acccagacac accggtcttg 420
 acacaccaa tcatgtatgt gccaccagga gggccaatcc cagcaaagggt cgacgattac 480
 tggttgcaaa catccacaaa cccagtggtc ttctggactg aagggaacgc accagcccgg 540
 atatccatcc cgttcacacg tgctcggaat gcataatagta gtttctacga tggatgggtca 600
 aattttctcgc aaaatggggt gtatggctac aacaccctga acaacatggg gcaattgttt 660
 ttcaggcatg tcaataaacc cagtcaccaac actgtcacaa gtgttgcccg catatacttc 720
 aagcccaaac acgtgaaggc atgggtcccg cgaccaccgc gattgtgccc ttacattaat 780
 gctggagatg taaatttcac cccacatcgt gtcactgaga agcgagcgag cctgataacc 840
 aca 843

<210> 49
 <211> 843
 <212> DNA
 <213> Enterovirus

<220>
 <221> misc_feature
 <222> (0)...(0)
 <223> E4, strain Shropshire

<400> 49
 ggggacgtgc aagatgccgt gactggagcc atagtgcgtg tcgccgacac actgcacacg 60
 ggaccctcga acaacgaagc aatacccaat ttgacggccg tggaaacagg gcatacatcg 120
 caagtgcacac caggcgatac aatgcagacg cgtcacgtgg tcaacatgca caccggttca 180
 gagtcatcaa ttgagaactt cctagctcga tctgcgtgtg tgtattacct cgactatcaa 240
 acagggtcag gacctggcac ccaatacttc ggccagtgga ccatctccac aaggagagtt 300
 gcgcaactgc gccggaagtt ggaaatgttc acctaccta gatttgacat ggaaataaca 360
 atcgtgatca ccagttcgca agatcactcc acctctcaa atccagatac accaatcatg 420
 acgcaccaa ttatgtacgt accaccaggg ggtccaatcc cggcgaagggt cgacgactat 480
 agctggcaaa catctacaaa ccctagtgtg ttttggacag aagggaacgc accgcccgc 540
 atatccattc cattcattag tgctcgaaat gcctatagca gcttctacga cgggtgggtca 600
 aattttctcgc aaaacggccg atatggatac aacactttga acaacatggg acaactattc 660
 ttcagacacg tgaataagcc cagccccaac accttcacaa gtgttgcccg tgtatacttc 720
 aagccaaaac acgtgaaggc gtggattcca cgaccaccgc gattatgtcc atacataaat 780
 gcgggagacg tgaatttcaa accaacaccc gtgaccgaaa agagggcgag cttaatcacc 840
 aca 843

<210> 50
 <211> 876
 <212> DNA
 <213> Enterovirus

<220>
 <221> misc_feature
 <222> (0)...(0)
 <223> E5, strain Noyce

<400> 50

ggagactcag	agcacgcagt	ggaaagcgcc	gtatctaggg	tggcagatac	aattatgagt	60
ggcccgctcaa	actcccaaca	ggcccccgct	cttactgcag	ttgaaactgg	acacacatcg	120
caagttgttc	caagtgtatc	catccaaacc	agacatgtgc	agaatttcca	ctctaggtcc	180
gagtcgacca	ttgaaaattt	cctgagtagg	tcagcatgtg	tgcataatcg	caattacaac	240
gcgaagggcg	ataagacgga	tgtggacagg	tttgacaggt	gggagatcaa	cattcgtgaa	300
atggtgcaac	tacgtaaaaa	gtgtgagatg	ttcacatatc	tacgctatga	tattgaagtt	360
acatttgtta	taaccagcaa	acaggatcag	ggccccaac	taaaccagga	tatgcctgtt	420
cttaccacc	aaattatgta	cgtaccccca	ggagggttcag	tacctagcac	cgttgagagc	480
tatgcgtggc	aaacatcaac	aaaccctagc	gtgttttgga	ccgaggggaa	cgctccagct	540
agaatgtcca	taccctttat	cagcataggg	aacgcttata	gtagcttcta	tgatggatgg	600
tcacacttta	ctcaaaaagg	ggtctacgga	tacaacacat	taaacaagat	ggggcagcta	660
tttgtcagac	atgtgaacaa	acagaccccc	acgccagtta	ctagtaccat	aagggtttac	720
ttcaaaccaa	agcacattag	agcttggggtc	cctaggcccc	cgcggttatg	cccctatgtg	780
aacaagacaa	atgtaaactt	catcaccaca	caggtaacag	aacctacaaa	tgacctcaat	840
gacgtgcccc	agtctgagca	taacatgcac	acatat			876

<210> 51

<211> 867

<212> DNA

<213> Enterovirus

<220>

<221> misc_feature

<222> (0)...(0)

<223> E6, strain D'Amori

<400> 51

aacgacgttc	agaacgcggt	ggaacgggtca	attgttcgtg	tagcggacac	attaccagct	60
gggccaaagca	actcagaaaag	cataccagca	ctcacagcag	ccgagactgg	acatacctcg	120
caggtcgtcc	ccagcgacac	catccagacg	cgacatgtga	ggaattttca	cgttcgggtct	180
gagtcacg	tagagaattt	tcttagcagg	tcagcttgcg	tgtacatcgt	ggagtacaaa	240
accgggaca	cgactcccga	caagatgtat	gatagctgga	ttatcaatac	caaacaagtg	300
gcgcagttga	gaagggaagct	ggagttcttt	acctatgtca	gattcgacgt	ggaagttacc	360
tttgtcataa	ccagcgtgca	agatgactcc	acaaaacgga	acaccgacac	cccagtgcta	420
actcatcaaa	ttatgtatgt	gccgccagga	gggcccatac	cacaagcggt	ggacgattat	480
aactggcaaa	cttccacca	ccccagcgta	ttttggactg	aggggaacgc	gccaccaagg	540
atgtctatcc	cgttcatgag	tggtggcaat	gcatacagta	acttctacga	cgggtgggtcc	600
cactttttctc	aaactgggggt	ttacgggttt	aacaccctaa	acaacatggg	taagttatat	660
ttcaggcatg	taaacgacag	gactattagc	ccaatcaaaa	gtaaggtcag	aatatatttc	720
aaacccaaac	acgtgaaggc	atgggtaccc	agaccgccga	gattgtgtga	atacaccac	780
aaggataacg	tggactatga	accaaagggg	gtcacaacat	cacgcacttc	aatcaccatc	840
accaactcca	cacacatgga	gacgcac				867

<210> 52

<211> 867

<212> DNA

<213> Enterovirus

<220>

<221> misc_feature

<222> (0)...(0)

<223> E6, strain Cox

<400> 52

aatgacgttc	aaaatgcagt	cgagcaatca	attgttcgtg	tggctgacac	gttaccagct	60
ggaccagta	attcagagag	cataccggca	ctgacggccg	ccgagactgg	ccatacttct	120
caagttgtgc	ccagtgtatc	tatacagaca	cgccacgtaa	aaaactttca	tgtgaggtcg	180
gagtcgtcag	tagagaactt	tctcagtagg	tccgcttgcg	tgtatatagt	gggatacaag	240
accacagatg	cgacccctga	caaaatgtat	gacagctggg	ttatcaacac	aaggcaggtg	300
gcgcagctaa	ggagaaaatt	agagttcttc	acctatgtta	ggtttgatgt	tgaggtcacc	360
tttgtgataa	caagcgtgca	agacgattca	actagacgga	acacagacac	ccccgttcta	420

accacacaaa	tcatgtacgt	acccccaggt	gggcccattcc	cgcaggcagt	ggacgactac	480
aattggcaaa	cttccacaaa	tcccagtgta	ttttggacag	aagggaatgc	cccaccaaga	540
atgtccatac	cattcatgag	cgtaggtaac	gcatacagca	atttctatga	tgggtggtct	600
cactttcttc	aaactgggggt	gtacggtttc	aacaccctga	acaacatggg	caagctatac	660
ttcaggcatg	tgaacggcaa	gacaataagc	cctatcgcaa	gcaagggttag	gatttacttc	720
aaaccaaagc	atgtgaaggc	atgggtgccc	agaccaccgc	gattgtgtga	atacaccac	780
aaggacaatg	tggattacga	accaaaggga	gtcacaacat	cccgtacatc	tatcacaatt	840
agcaattcca	ctcatatgga	aacatat				867

<210> 53

<211> 867

<212> DNA

<213> Enterovirus

<220>

<221> misc_feature

<222> (0)...(0)

<223> E6, strain Burgess

<400> 53

aacgacgttc	agaacgcggt	ggaacggtca	attgttcgtg	tagcggacac	attacccagt	60
gggccaagca	actcagaaag	cataccagca	ctcacagcag	ctgagactgg	acatacctcg	120
caggtcgtcc	ccagcgacac	catccagacg	cgacatgtga	agaattttca	cgttcgggtct	180
gagtcacggg	tagagaattt	tcttagcagg	tcagcttgcg	tgtacatcgt	ggagtacaaa	240
acccatgaca	cgactcccga	cgagatgtat	gatagctgga	ttatcaatac	cagacaagtg	300
gcgcagttga	gaaggaagct	ggagttcttt	acctatgtca	gattcgacgt	ggaagttacc	360
tttgtcataa	ccagcgtgca	agatgactcc	acaagacaga	acaccgacac	cccagtgtta	420
actcatcaaa	ttatgtatgt	gccgccagga	gggcccatac	cacaagcggg	ggacgattat	480
aactggcaaa	cttccaccaa	ccccagcgta	ttttggactg	aggggaacgc	gccaccaagg	540
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cacttttctc	aaactgggggt	ttacgggttt	aacaccctaa	acaacatggg	taagttatat	660
ttcaggcatg	taaacgacag	gactattagc	ccaatcacaa	gcaagggtcag	aataatatttc	720
aaacccaac	acgtgaaggc	atgggtaccc	agaccgccga	gattgtgtga	gtacacccac	780
aaggataacg	tggactatga	accaaagggg	gtcacaacat	cacgcacttc	aatcaccatc	840
accaactcca	cacacatgga	gacgcac				867

<210> 54

<211> 876

<212> DNA

<213> Enterovirus

<220>

<221> misc_feature

<222> (0)...(0)

<223> E7, strain Wallace

<400> 54

ggcgacaccg	aaacggctat	tgacaatgca	atcgccaggg	tagcagatac	ggtggcgagc	60
ggtcctagta	attcgaccag	tatcccagca	ctcacagcag	ttgagacagg	tcacacgtca	120
caagtcgagc	ccagcgatac	agtgc aaact	agacatgtca	aaaactacca	ctcgcgttct	180
gagtcaaccg	tggaaaactt	tctaagtcgc	tccgcttggtg	tgtacatcga	agagtactac	240
accaaggacc	aagacaatgt	taataggtac	atgtcgtgga	caataaatgc	cagaagaatg	300
gtgcaattga	ggagaaagtt	tgagctggtt	acatacatga	gatttgatat	ggaaatcacg	360
tttghtaatca	caagtagaca	actacctggg	actagcatag	cacaagatat	gccgccactc	420
accaccaga	tcatgtacat	accaccagggt	ggcccgggtac	caaacagcgt	aacagatttt	480
gcgtggcaga	catcaacaaa	ccccagtatt	ttctggacag	aaggaaacgc	gccacctcgc	540
atgtctattc	cattcatcag	tattggcaat	gcataatagca	acttctatga	cgggtggtca	600
cacttttccc	aaaacgggtgt	gtacggatac	aacgccctga	acaacatggg	caagctgtac	660
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aaacccaagc	acatccgagt	atgggtccca	cggccgcctc	gactgagccc	gtacatcaaa	780
tcaagtaatg	taaattttta	ccccacgaac	ctgacggacg	agcgggtcatc	catcacatat	840
gtgcccagaca	ctatacgtcc	agatgtgcgc	accaac			876

<210> 55
 <211> 843
 <212> DNA
 <213> Enterovirus

<220>
 <221> misc_feature
 <222> (0)...(0)
 <223> E8, strain Bryson

<400> 55
 ggtgatgtcc agaatgcagt tgagggggcga atggtttagag ttgcagatac cgtgagcact 60
 agcgccacca actccgaaca agtgccgaac ctgaccgcgg tggagaccgg tcacacatcg 120
 caggtagtgc ccggcgacac tatgcagacc aggcacgtag tgaacaagca tgtgcatct 180
 gaatctacaa ttgaaaattt cctcgcacgt tcagcctgtg tgtactttct tgagtacaag 240
 actggtagca agactgactc caacgccttc agcaattggg tcatcacaac gcgcaagggt 300
 gcgcagctga ggcgcaagtt ggagatgttt acatacttaa ggtttgatat ggagattact 360
 gtgggtcatta ctagctccca agaccagtcc acatcacaaa atcaaaatgc gcccgctctg 420
 actcaccaga ttatgtatgt accacctggt ggcccagtgc ccactagcgt tgatgattat 480
 tgctggcaaa catccacaaa cccaagcata ttttggacgg aaggaaacgc acctgccaga 540
 atgtccatcc cctttatcag cattggaaat gcttatagca acttttatga tgggtggtca 600
 catttctcac agaacggagt ctatggtttt accaccttaa acaacatggg ccagctgttt 660
 tttaggcatg ttaacaagcc taaccggcgg acaataacca gtgtggcccg catttacttc 720
 aagccaaaac atgtgagggc ctgggtgcct agaccgccac ggttgtgccc ttacatcaac 780
 agtagcaacg tgaacttcga cccaaaacct gtggcagagg tcaggtctag catcatcacc 840
 acc 843

<210> 56
 <211> 876
 <212> DNA
 <213> Enterovirus

<220>
 <221> misc_feature
 <222> (0)...(0)
 <223> E11, strain Silva

<400> 56
 ggtgatgtgg ttgaagccat tgagggcgca gttgctagag tagcagacac tatcagcagc 60
 ggcccaacaa atttctcaagc agtcccagca ctcacagcgg tggagactgg acacacctcg 120
 caagttgtac cagggtgatac catgcagacc agacacgtaa agaattacca ctcacgatca 180
 gaatcgacca ttgaaaattt tctgagtagg gcggccttgtg tctacatggg tgagtattac 240
 actacaaaata cagatgagac caagagattt gctaattgga caatcagcgc aaggcgcatg 300
 gtacaaatga ggaggaagct tgaaatgttc acgtacgtcc gtttcgacgt ggaggtgaca 360
 ttcgtaatta ccagcaaaaca ggaccaaggg aatcggttgg gacaagatat gccccgctc 420
 acacaccaga taatgtacat cccgccaggt ggtcgtatac ccaaatccac cacagattac 480
 gcatggcaaa cgtcgacaaa ccccagcatc ttttggacgg agggtaacgc gccccccagg 540
 atgtccattc ctttcatgag cattggaaac gcatatagca atttttatga cggttggtct 600
 cacttctctc aaaatggcgt gtacggatat aacacactaa accacatggg tcaattatac 660
 atgcgccatg taaatggacg atcacctctt ccaatgacca gcacggtgag ggtgtacttc 720
 aaacccaaac atgtgaaaac atgggtgccca cgaccccaa gattgtgccca atacaaaaac 780
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 attccagaca ccgtgaaacc cgacatgaca acatat 876

<210> 57
 <211> 861
 <212> DNA
 <213> Enterovirus

<220>
 <221> misc_feature
 <222> (0)...(0)
 <223> E13, strain Del Carmen

<400> 57

ggggatgaga	gtgcaaaggc	tacagtttcc	aacacacagc	ctagcgggtcc	aagtaattct	60
gtcagcgtgc	caatgcttac	tgctgctgag	accggggcaca	catctcaagc	agtacccagt	120
gacactatac	agaccagggtg	cgtagtgaac	caacacaagc	ggtcggaatc	atccgtggaa	180
aatttcctgt	gtcgtccgc	ttgctatac	tacacaacct	atgacactca	cggggatgca	240
gccgacgcaa	agtacgccag	ttggacgata	accacccgaa	aagctgcaca	gctgcgagaga	300
aaactagaga	tggtcacata	cttgagggtt	gatttagaag	tgacattcgt	tataacaagt	360
gcacaagtaa	catctaccaa	taaacgtcag	gacacgcctg	ttctcacgca	tcaagtcattg	420
tacgtgccac	caggtgggtgc	agtacccgct	agtgtggacg	attatgcgtg	gcagacgtcc	480
acaaacccaa	gtatcttctg	gacggaaggg	aatgcaccag	cacgcattgtc	tatacccttt	540
atcagcgtgg	gcaacgcata	cagtagcttc	tatgatgggt	ggtccaactt	tacacagaat	600
ggagtttaag	ggttcaaacac	gctaaacaac	atgggaaagc	tatacgtacg	acacgtcaat	660
ggagctagcc	ccggccctgt	gaagagtacc	atacggtttt	acatgaagcc	caaacacgtg	720
aaggcttggg	tacccagacc	tcctcgcttc	tgcgagtacg	aaaaatcagg	caatgtaaac	780
ttcaaaccga	agggcgtgac	agagagccgg	acgtctatca	aattagaaaa	accaaaccct	840
gcgtccaaat	taatgaacca	c				861

<210> 58

<211> 894

<212> DNA

<213> Enterovirus

<220>

<221> misc_feature

<222> (0)...(0)

<223> E14, strain Tow

<400> 58

aatgatccag	agcaagctat	aaatcggggc	ctagcgaggg	tggcagacac	agttcgtagt	60
gggcccgtcta	actctgaaca	aattcccgc	ctgacagccg	tggagacagg	gcatacatca	120
caagtcgtcc	ccagtgcac	aatgcaaacc	cggcatgtga	agaattacca	ctccagggtca	180
gagtcaacaa	tagagaactt	tttgtgtaga	tcggcttgcg	tgacatcgc	aacatacaag	240
gctaaaggcg	gagctggaga	cgctgaccgg	tacgacagct	gggacataaa	cataaaagag	300
ctggtacagt	tgcgacgcaa	gtgcgagatg	tttacgtacc	taaggtttga	tatggagggtc	360
acctttgtga	ttaccagcat	acaggagcag	ggcaaagcac	tgaccagga	catgccggtg	420
ctaacgcacc	aaataatgta	cgttccaccg	ggcgggtgccg	tgctagtgg	tgacagaaagc	480
tttgctgtgg	agtcacaaac	gaatcccagt	gtgttctgga	cagaaggcaa	tgccacagca	540
cgtatgtcta	taccctttat	aagtattggg	aacgcttaca	gtaattttcta	tgatgggtgg	600
tcccacttta	cccagaacgg	tggttacggg	tacaacacac	taaacaaact	gggtaagatc	660
tacgtcaggc	atgtgaacaa	acaaaccccc	acggatgtca	ccagcaccgt	gcgaattttac	720
ttcaagccca	aacacgtgcg	agcttgggtg	cctcgcccg	ctagactatg	tccttataag	780
aacaaggcaa	atgtaaactt	tgaagttact	agtgtaacca	ctgccagaa	gagtcctta	840
gatgtcccca	ctccaacca	cagtagtagc	gtgcacctgc	gcatgcacac	gcac	894

<210> 59

<211> 882

<212> DNA

<213> Enterovirus

<220>

<221> misc_feature

<222> (0)...(0)

<223> E15, strain CH96-51

<400> 59

ggtgatgacc	aacacaagac	caatacagtg	acagacacag	agcagagtgg	cccgtcaa	60
tccgaacgcg	tcccagccct	cacagcagtg	gagactggcc	acacttcgca	ggtcgtaccc	120
agcgacacag	tgcaaaactcg	ccacgtacgc	aattaccact	caaggacaga	gtctacctta	180
gagaatttttc	ttggtagggtc	agcatgtgtg	cacatcgaca	catacaaggc	taagggtgaa	240
aaaggatcctt	ctgagaggta	cgcgtcatgg	gagataacta	acagggagat	gggtgcaattg	300
cgccgaaaaat	gtgagatgtt	cacatatatg	aggtatgacg	tggaataaac	atgtgtgata	360
accagctacc	aggagcaggg	cacacgattg	gcccaggaca	tgctgtact	aacacaccaa	420
atcatgtacg	tgcccccggg	tgggcctgtg	ccaacaagca	cggagagcta	tgcatggcag	480

acctcaacga	accctagcgt	cttttggact	gagggcaacg	caccaccgcg	tattttccata	540
cccttcatca	gcataggaaa	tgcgtactgc	aactttttatg	atgggtgggtc	acattttctca	600
caagatgggt	cctatggcta	cacagcgctc	aatagaatgg	ggaaaatata	tatttagacat	660
gtaaataagg	agacccccac	acaggtcatt	agtaccgtga	ggatgtacat	gaaacccaaaa	720
cacattcgcg	catgggtgcc	cagaccccc	cggctgtgca	aatacctaca	ctcaggcaac	780
atgaacttca	acgtggagga	cattacagag	gagcggaaacg	atataaacca	tgtaccacc	840
cccagccaca	gcagtagtgt	gcgtgtgcgt	cttggcacca	ca		882

<210> 60

<211> 867

<212> DNA

<213> Enterovirus

<220>

<221> misc_feature

<222> (0)...(0)

<223> E17, strain CHHE-29

<400> 60

ggtgatgttg	aggactcagt	aaacagagca	gtggtttaggg	tagcagacac	catgccaaagt	60
ggaccatcca	attcgcaggc	agtacctgcc	ttgacagccg	ctgagacagg	tcacacgtct	120
caagtgggtg	ctgggtgataa	catccaaaca	cgtcatgtgc	acaactacca	ctccagaact	180
gaatccagta	tcgaaaattt	cttcggggcgt	tccgcatgtg	tagtgggtcaa	aacatataaa	240
atgggtcaaa	aagttgtagc	tacagacaga	tatgatagtt	ggatgatttc	cattagggac	300
atggtacaac	taagacggaa	gtgtgaaatg	ttcacgtaca	tgagatttga	tttagagatc	360
accttcgtgg	tcacgagtta	ccaacaatat	agtacatcct	tgacacagga	catgccagtg	420
atcacgcatc	agttcatgta	tgtgccgcct	gggggtccgg	ttcctgagag	tgtaaatagc	480
tacgcttggc	aaacgtcaac	caatcccagt	atattctgga	ctgagggtaa	tgccccagca	540
aggatgtcca	ttcccttcat	cagtgttggg	aacgcatata	gctgcttcta	cgatggctgg	600
tcacacttca	cacagaaggg	ggtttatgg	tataacactc	tcaacaacat	gggcaaattg	660
tacatgcgac	acgtgaacaa	aaatagcccc	acagagatca	taagcactct	tcgtgtgtat	720
ttcaagccaa	agcacgtgaa	agcgtgggta	cccagaccac	ccaggctatg	tccatacaaa	780
tataaggcaa	atgttgactt	tgaagtgact	ccaatcacag	acaagcgaga	ctccataacc	840
agcataccag	tcccacagca	cactcat				867

<210> 61

<211> 861

<212> DNA

<213> Enterovirus

<220>

<221> misc_feature

<222> (0)...(0)

<223> E18, strain Metcalf

<400> 61

ggggataacc	aggatcggac	ggtcgccaac	acacagccta	gcgggtccgtc	caactccacg	60
gaaattccag	ccttaacagc	ggtggaaacg	gggcacacct	cacaagtga	tcccagtgac	120
actatccaga	ccaggcacgt	ggtaaacttc	cactcacgtt	ctgagtccac	tatagaaaat	180
ttcatggggc	gtgcagcatg	tgtgttcatg	gatcagtata	aaatcaatgg	agaagagacg	240
tccactgata	ggttcgcagt	gtggaccata	aacataaggg	agatggccca	attaagaagg	300
aagtgtgaaa	tgttcacgta	catgcgtttt	gatatcgaga	tgacaatgg	cattaccagc	360
tgtcaagacc	agggaaacgat	actagatcag	gacatgcctg	ttttgacgca	tcaaattatg	420
tacgtccac	cagggggccc	aatcccagcc	aaagtagata	gttacgagtg	gcagacatca	480
acaaacccca	gcgtcttctg	gacggaagg	aatgcaccac	cgcgtatgtc	tattccattc	540
attagcgtcg	gcaatgctta	tagctcattt	tacgatgggt	ggtcacactt	cacacaggac	600
ggtacctatg	ggtatacaac	ccttaatgca	atggggaaac	tgtacattag	gcatgtgaat	660
aggagcagcc	ctcatcagat	aaccagcacg	atcagagtat	acttcaaacc	caaacacatc	720
aaggcatggg	tgccccgacc	accacgattg	tgcccgata	taaacaaaag	ggacgtaaac	780
tttgtagtca	cggagataac	agactcaagg	acttccatca	ctgatacacc	acaccagaa	840
catagtgtcc	tggcaacgca	t				861

<210> 62

<211> 879
 <212> DNA
 <213> Enterovirus

<220>
 <221> misc_feature
 <222> (0)...(0)
 <223> E19, strain Burke

<400> 62
 ggagacatcg tggaggctgt ggagggagcc atctcgcgag tggcagatac tgtagtagt 60
 gggcccagta actctcaagc agtaccagcc ctccacagcag tcgaaacggg tcacacttct 120
 caagtcaatc ctagtgacac catgcagacc agacacgtga caaattacca ctgcggtca 180
 gaatccagca tagaaaattt ccttagccgc tctgcttggtg tgtatatggg cgaatacagc 240
 acacaagcat cagatgagac caaaaagtac atgtcatgga ccataagccc aaggaggatg 300
 gttcaaatgc gcaggaagt ttagctcttc acttacctgc gttttgatgt ggagattact 360
 tttgtaatac ccagcagaca agtcaaggta gggacacaat taggccaaga tgcccccccg 420
 ctaactcacc aagtcattga tatacccccga ggaggccag tacctgattc agttggtgat 480
 tacgcatggc agacttccac taaccctagt atcttttgga ccgaaggtaa tgcattcccc 540
 aggatgtcaa tacccttcat tagcataggt aacgcctata gcaactttta tgacgggtgg 600
 tcgcattttc accagaatgg cgtctatgga tacaacacgc tgaaccatat ggggcaactg 660
 tacgtgcggc atgttaacgg cccttcacca ttaccagtga caagcacagt cagggtctac 720
 tttaaaccca aacacgtgaa ggcttgggta ccgagggcac ccaggctatg tcaatatgta 780
 aatgcattca ctgtgaactt cgagccaaca gacatcactg agtcacgcac tgacatcaac 840
 catgttcacg acaccgtgaa gccagatctc caaacatac 879

<210> 63
 <211> 843
 <212> DNA
 <213> Enterovirus

<220>
 <221> misc_feature
 <222> (0)...(0)
 <223> E20, strain JV-1

<400> 63
 ggggacgtgc acgatgcggt ggttggggcc atgacccgtg ttgcagacac gataagtagt 60
 gggccaagca attcagaaag cgtgccagca ttgactgcag ccgagacagg acacacatca 120
 caggtagtac cgagtgtatc catgcagacc agacatgtgc ggaatttcca cacaagatca 180
 gagtcttcaa tagaaaattt catgagtcgc tccgcctgtg tctactatac taagtataag 240
 accaaagacc cggacccaac ggagatgtac tctagttgga aggttaccac caggcaagtg 300
 gcacaactca ggaggaagat ggagatgttc acttatttgc gctttgacgt agaagtgaac 360
 tttgtaataa ctagctcgca agatcagtc acgagtgttg cacaggacgc acctgttctc 420
 actcaccaaa tcatgtacat cccaccggga ggcccgggtc ccaaatacagg tagggattac 480
 tcatggcaat cctgtactaa cccaagtgtt ttctggactg agggtaaatgc accaccacgc 540
 atgtgtattc cgttcattag tattggaggg gcatatagtt cattctatga cgggtggtcc 600
 cactttaacc aacaagggtc gtacgggtat aacactctca atgacatggg tcaactgtat 660
 tttaggcatg tgaacgaggg tagcccaggg gcggtaacaa gctacatcag aatatacttc 720
 aaacctaacc atattagagc atgggtgccc agaccaccta gattgtgtca gtatgagaaa 780
 caagggagcg ttgacttcaa ggtgcaggga gtaactgatg ctctacctc gctcaccact 840
 aca 843

<210> 64
 <211> 885
 <212> DNA
 <213> Enterovirus

<220>
 <221> misc_feature
 <222> (0)...(0)
 <223> E21, strain Farina

<400> 64

aatgacccag	cacaagccgt	gttgagtgcg	atcggtcgtg	tcgctgacac	cgctcgctagc	60
gggccatcga	attcagagag	agttccagtt	ctaaccgctg	cggagacagg	tcatacctca	120
caggtggttc	ccagcgatac	cattcagacg	cgccacgtcg	tcaacttcca	cacaagatcg	180
gagtcaacaa	ttgaaaattt	tatgtgtcgc	tccgcctgcg	tgtacatcgc	ccggtacggt	240
actgaaaagc	aaggggaaca	aatatccaga	tacaccaagt	ggaagatcac	cactaggcag	300
gtggcgcaac	tgcgcaggaa	gatggagatg	ttcacatata	tgcgatttga	tttggaatg	360
acatttgtaa	tcacaagctc	ccagcgatatg	tcaacggcat	atgattcaga	cacaccagcc	420
ctcaccacc	aaataatgta	cgtgccacct	ggggggcccg	agccccgtca	ttatgaggat	480
ttcgcctggc	agacatccac	aaatccaagc	atatttttga	ccgaaggtaa	cgcaccacca	540
cgcttatcaa	tcccatttat	gagtgtggga	aatgcctatt	gcaattttta	tgatgggtgg	600
tctcactttt	cacaaagtgg	agtgtatggg	tttaccacct	taaataacat	gggacaactg	660
ttcatgccc	atgtcaataa	gtcaacagcg	cacccattg	atagtgtggt	gcgagtttat	720
tttaaaccaa	agcatgttaa	ggcgtgggtt	ccaagacctc	cccgttgtg	cccatacatc	780
tatgcaagga	acgtggattt	tgagccacaa	ggtgtcactg	aatcaagaga	aaagataaca	840
ctagataggg	atactcacac	ccctatgcgc	acatgcgggc	cgttc		885

<210> 65

<211> 882

<212> DNA

<213> Enterovirus

<220>

<221> misc_feature

<222> (0)...(0)

<223> E24, strain De Camp

<400> 65

ggagatgtct	gtgaggaagt	agagagggct	attgtcaggg	ttgcagatac	tgtcggacgc	60
ggtcctgcta	acactgagag	tgtaccagcg	ctgactgcag	ttgaaactgg	acacacttca	120
caagttgtac	ccggggacac	catgcaaacc	agacatgtta	aaaactttca	cacgcggtca	180
gaatcatctg	tggaaaattt	catgtgcaga	gcagcggtg	tgtattatgt	ggattaccac	240
acacaaaatg	acagtggagga	tgaaaaatat	gcattcttga	ttatcaacac	gagacaggta	300
gcacagctac	gcaggaaaat	tgagctgttc	acatacacta	ggtttgatgt	cgaaatcaca	360
ttcgtgatca	ccaccacaca	gcagcaatcc	acagctccca	accccgacac	tcctctgctg	420
acacaccaaa	tcatgtatgt	gccccgggt	ggcccagtgc	caaatagtgc	taccgattat	480
tggttggaat	catccacaaa	tcccagtata	ttctggaccg	agggtagcgc	accacccaaa	540
atgtcaatac	cctttataag	tgtgggaaat	gcatacagca	gtttttatga	tgggtgggtca	600
catttctactc	aaaacggggt	gtacgggttc	aacactctga	acaatatggg	caaattatac	660
ttcaggcacg	taaatgacaa	caccgtaggg	ccatatgtga	gcaaagcccc	catttatattc	720
aaaccaaaag	atgtgcgtgc	gtgggttccc	aaacctccca	ggctctgtga	atacaacaat	780
cgagccaaag	tgaactttga	accacgaggg	gttaccgatg	ccaggtctag	tatcacggcc	840
acaaccgaca	cgatcactga	gagcacaggg	atgcaaacga	ct		882

<210> 66

<211> 876

<212> DNA

<213> Enterovirus

<220>

<221> misc_feature

<222> (0)...(0)

<223> E25, strain JV-4

<400> 66

aatgatccag	caactgccat	agttagatcg	gttgagagag	tggctgatac	catagcaagt	60
ggacccacta	actcagagag	agtgccagca	ctaaccgccc	ttgaaacagg	tcacacctca	120
caggtagtcc	cgagcgacac	catgcaaact	aggcatgttg	tgaaccatca	cattagatca	180
gagtcctcta	ttgaaaactt	cctgagcagg	tccgcctgcg	tgtacatcga	catgtatggg	240
acaaaagaga	atggtgacat	caagcgcttc	accaactgga	gaataaacac	acgtcaggtc	300
gtgcagctaa	ggcgcaagct	ggaaatgttt	acatacatta	gatttgatgt	tgaatacact	360
tttgtaatac	ctagcacaca	gggaacaccg	actcaaaaaga	acaaggatac	cccagttcct	420
acacaccaaa	tcatgtatgt	gccaccaggg	ggcccaatcc	ctgtatctta	tgaagattat	480

tottggcaga	cctctacaaa	tcctagtgtt	ttctggacag	aaggggaatgc	cccagcccgt	540
atgtcaattc	ccttcatgag	cgtagggaac	gcctattgta	acttttacga	cggttggtca	600
cacttctcac	aatcggtgt	gtatgggttc	actacactca	ataacatggg	tcagttgtac	660
tttcgacacg	tgaacaagga	cacccttgga	ccatacaata	gcacgggttcg	ggtttacttc	720
aaacccaaac	atgtgaaggc	atgggtaccc	agaccaccgc	gcctgtgcga	ctacgtttac	780
gcacataatg	ttgacttcac	acaaaaaggg	gttactgaca	gcagggacaa	gatcacccgt	840
gaccgtgatg	aacacgtgcc	gtcagtggtt	aaccac			876

<210> 67

<211> 870

<212> DNA

<213> Enterovirus

<220>

<221> misc_feature

<222> (0)...(0)

<223> E26, strain Coronel

<400> 67

ggagatgatc	caccgcattc	gatctcaaac	acggttgcaa	acaccaaccc	tagtgggtcca	60
accaactcag	aaaggatccc	agcgctcaca	gcagcggaaa	ctggtcacac	ctcgaggtg	120
gtcccagagt	ataccgtaca	aactcgttgt	gtgaaaaact	tccacactcg	atcggagtca	180
tcaattgaga	actttttgtg	cagatcagct	tgcgcacaca	tgtcatcgta	tgaggccttc	240
ccaacaacaa	cacaagacgg	tacacaaagg	ttcgccaatt	ggacgattag	tgtgaaagac	300
atggtgcagt	tgaggaggaa	atgtgagatg	ttcacgtact	taagatttga	catggaggtg	360
actttttgtg	taactagtgt	gatcgaaact	acaaaaggga	aagtaccggc	accagcagtc	420
acacaccaag	taatgtacat	tccaccaggc	ggacctattc	cagctagcgt	tgaaagtatt	480
gcctggcaaa	catccaccaa	ccaagcgtg	ttttggacag	aaggggaatgc	tccccacgc	540
atgtctatac	catttatcgg	cattggtaat	gcctacagca	tgttctatga	cggatgggcc	600
agtttcagac	aatcggttgg	atatggatac	agcacctga	accacatggg	ccagatattc	660
gtaagacacg	tgaatgcaac	cataccaaac	ttgatcagca	cagtcaggat	atattttcaag	720
cccaagcacg	ttagggttgg	gattcctaga	ccgcccaggg	tgtgtcagta	catttacaag	780
gcaaatgtag	actacgcagt	gtcaaatatc	actgaaaagc	gagatagtat	aagatggaca	840
ccaacaaccg	gtccgtcaat	gacatcccac				870

<210> 68

<211> 855

<212> DNA

<213> Enterovirus

<220>

<221> misc_feature

<222> (0)...(0)

<223> E27, strain Bacon

<400> 68

ggtgacgacg	caaggactgt	tagcgacaca	caaaagagcc	agccatctaa	ctctgagcaa	60
gtgcctgcct	taacagcggg	tgagactgga	cacacctctc	aagttgagcc	cagtgatata	120
gtacagacac	gacatgttgt	caactcacac	agtaggacag	agtcgacaat	tgagaatttc	180
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tgtgagatgt	tcacatacat	gaggtttgac	ttggaagtga	cgctagtcac	caccagctat	360
caagaaccag	ggacaatcac	caccagcatg	atgcccgtcc	taaccaccca	gattatgtat	420
gtgccgccag	gagggcccgg	cccagccaag	gctgacagtt	acgcgtggca	aacgtcaaca	480
aatcccagta	tattctggac	cgaaggcaac	gctccacctc	ggatgtctat	cccatacatt	540
ggcatcgcca	atgcataatg	cagcttttat	gacgggtggt	cgagcttcaa	caactcgggt	600
gtgtatggct	acacaaccct	gaataacatg	ggtaaactgt	acttcagaca	cgtgaacaaa	660
cacagcccaa	acactattaa	gagcactgtg	aggatatatt	tcaagcccaa	gcacgtccag	720
gcgtgggtcc	caagaccacc	gcgcttgtgc	ccgtatctga	ataagaggga	tgtcaacttt	780
gaagtgtcaac	ccgttacgag	caagagagac	agtattaact	gggtgccaca	aacaaaccgc	840
caagtgtaca	atcat					855

<210> 69

<211> 876
 <212> DNA
 <213> Enterovirus

<220>
 <221> misc_feature
 <222> (0)...(0)
 <223> E29, strain JV-10

<400> 69
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 gggcctgcaa actcagagca aatccctgcc ctaaccgctg ctgagactgg tcacacctcg 120
 caagtgggtc ccagcgacac tatgcaaacc cgccatgtat gtaactacca caccagatct 180
 gaatcatcga tcgagaactt cctatgcagg gctgcatgtg tctacatagt gagttacaaa 240
 acacagggcg acgaacaaac cgacaaatac gctagtggg agatcaacac gcggcagggtg 300
 gcacagttaa ggagaaaatt ggaattcttt acttacataa gatttgacat ggaggtaaca 360
 tttgtgatca ctggttcaca agacaccagc acacagacta acacggatac gccagtgcta 420
 acccatcaaa ttatgtatgt gcctcccgtt ggtccagtac cgacatcagc cacagattac 480
 agctggcaga catctacaaa tcccagtggtg ttctggacag aagggaatgc gcctcccgtt 540
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 cacttttagc agtcaggggt gtatggttac accacactca ataatatggg taccctgtat 660
 ttcaggcacg tgaacaactc gaccatcggg ccttacacca gtgcagttag gatataattt 720
 aagccaaagc acgtcaaagc gtgggtgccg cgaccgccac ggttgtgcga ttacaaacac 780
 aaaaagaacg tagactttac tcccacaggt gtgaccacaa ctagagacaa gataaccttg 840
 gacaagggga ctcacgtgcc gagcgtatgg aacaca 876

<210> 70
 <211> 876
 <212> DNA
 <213> Enterovirus

<220>
 <221> misc_feature
 <222> (0)...(0)
 <223> E30, strain Bastianni

<400> 70
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 gggcccgtca atacagagca aattcctgca ttgacagcag tggagacagg gcatacatct 120
 caagtgggtac ctagtgcacac aatgcaaacc cgacacgtgg tcaacttcca tactagatca 180
 gagtcactgt tacagaactt catggggaga gcggcatgtg tatatatcgc ccactatgcc 240
 acagaaaagg ctaatgatga tttggacaga tacactaact gggagatcac aactaggcag 300
 gtggcacagt tgaggcgcaa gttggagatg tttacgtata tgagatttga cctcgagatt 360
 acattcgtaa tcaccagctc ccagcgtact tccaacaggt atgcgtcaga ctcccccca 420
 ttaacacatc aaataatgta cgtgccgccc ggggggtccaa ttcccaaggg ttatgaagac 480
 tttgcctggc agacgtccac caaccgaagt gtgttttgga ccgaaggtaa cggccctcct 540
 aggatgtcaa taccattcat gagegttggc aacgcataat gtaactttta tgatggatgg 600
 tcccatttca gtcagagcgg tgtgtacggg tacactacat tgaacaacat ggggcgctta 660
 tatttttagc atgtaaacaa atcaacagga taccagtaa atagtgtcgc ccgcgtctat 720
 ttcaagccca agcatgtgaa ggcattgggt cctcgcgcgc cagccttatg tccatatttg 780
 tatgtctaaa atgtcaactt tgatgtgcaa ggcgtgaccg agtcccgggg taagatcact 840
 ctgcaccgtt cgactcacia ccccggttta accact 876

<210> 71
 <211> 876
 <212> DNA
 <213> Enterovirus

<220>
 <221> misc_feature
 <222> (0)...(0)
 <223> E30, strain Frater

```

<400> 71
aatgaccctg aaggtgcgct caacaaggcg gtgggcagag tggctgatac aatagccagt      60
gggcccgtca acactgagca aattcccgcg ttgacagcag tggaaacagg gcacacatct      120
caagtagtac ctagtgtatc aatgcaaact cgacacgtgg tcaacttcca caccagatca      180
gaatcatcgt tggagaactt catgggaaga gcagcgtgtg tgtatatcgc tcattatgct      240
acagagaagg ctaatgatga tttagacaga tacaccaact gggaggtcac aaccaggcag      300
gtagcacagt tgaggcgtaa actggagatg ttcacgtaca tgaggtttga cctcgagatc      360
acatttgtaa tcaccagctc ccagcgcact tcaaccaagt atgcgtcaga tccccccca      420
ctaacacacc agataatgta tgtaccgccg gggggcccga tccccagggt ttatgaagat      480
tttgccctggc agacgtccac caacccaagt gtatttttga cggaaggtaa cggccccct      540
aggatgtcga taccattcat gagcgttggg aacgcatact gcaactttta cgacggatgg      600
tcccatttca gccagagcgg tgtgtacggg tacactacat tgaacaacat ggggcacttg      660
tatttcagac atgtaaaaca atcaactgca taccagttta acagtgttgc ccgcgtctac      720
ttcaagccca agcacgtaaa ggcttgggtg cctcgcgcgc cagcgttatg tccatatttg      780
tatgcaaaaa atgtcaattt tgatgtacaa ggtgtgaccg agtctcgggg aaaaatcact      840
cttgatcgat cgactcaca cctgtgtca accacg

```

```

<210> 72
<211> 877
<212> DNA
<213> Enterovirus

```

```

<220>
<221> misc_feature
<222> (0)...(0)
<223> E30, strain Giles

```

```

<400> 72
aacgaccccg aacatgcgtt aaacaacgcc attggtagag tggcagatac gatcgccagt      60
gggcccgtga actcggaacg catacctgca ctaaccgcag tggagacagg acacacgtct      120
caagtgggtg caagcgacac catgcaaaca aggcacgtag tcaacatgca tacaagatcc      180
gaatccacca tcgaaaattt catgggaagg gctgcttgtg tatacattgc gcaatacgcc      240
actgataagg ccagtgatga tctggacagg tacaccagct gggagatcac tacgagacag      300
gttgcgcaat tgaggagaaa gctggagctg tttacataca tgaggatatga cttagaagtt      360
acctttgtca ttaccagttc ccagcgcact tcgactacat atgcatcaga ctccccgcca      420
ttgacccacc aaattatgta tgtgcctccc gggggcccta tttccatagg acacgaagac      480
ttcgccctggc agacttcaac aaaccccagt gtcttttga ctgaaggaaa tgccccacca      540
cgtatgtcca taccattcat gagtgtgggc aatgcctact gcaattttta cgatgggtgg      600
tcacatttta accagagtgg ggtgtatgga tacactacac taaacaacat gggtcgctta      660
tatttcaggc atgtaaaacag atctactgcc taccagttta atagtgttgc acgtgtttac      720
tttaaaccce aacacgtcaa agcctgggtc ccacgagcac cagcattgtg cccatacttg      780
tatgctaaga acgtgaactt taatgtgcaa ggtgtgactg actcccgaga caagataacc      840
gtagaccgaa ccaaccatgt acgtatgcgc accacag

```

```

<210> 73
<211> 876
<212> DNA
<213> Enterovirus

```

```

<220>
<221> misc_feature
<222> (0)...(0)
<223> E30, strain PR-17

```

```

<400> 73
aacgaccccg aacacgtggt aaacaatgcc gttggcagag tggcagatac aatcgccagc      60
gggcccgtga actcggaacg cgtacctgca ctaactgcag tggagacagg gcatacgtct      120
caagtgggtg caagcgatac tatgcaaaca agacacgtag tcaacatgca cacaagatct      180
gaatccacta tcgaaaattt catgggaagg gctgcttgtg tatacatcgc acaatacgtc      240
actgacaaaag ccagtgcaga tttggatagg tacaccagct gggaaatcac cacgagacag      300
gttgcgcaat tgaggagaaa gttggaaatg ttcacataca tgaggatatga cctggaagtc      360
acctttgtta tcaccagttc ccagcgcacc tcgactacat atgcatcaga tttccccacca      420
ttgactcatc agatcatgta cgtgcctccc gggggcccca ttcctatagg atacgaggac      480

```

ttcgcctggc	aaacatcgac	taaccctagt	gtcttttggg	ctgaaggaaa	tgccccacca	540
cgcatgtcca	ttccatttat	gagtgtgggc	aatgcctact	gcaattttta	cgatgggtgg	600
tcacacttta	gccagagtgg	ggtgtacgga	tacactacac	taaataatat	gggtcgtctg	660
tatttcaggc	atgtaaacaa	atctactgcg	taccgggtta	atagtgttgc	acgtattttac	720
ttcaaaccga	aacatgttaa	agcctgggtc	ccgcgagcac	cacgactgtg	cccatatttg	780
tatgcaagga	acgtgaactt	taatgtgcaa	ggtgtgactg	actcccgaga	aaagataacc	840
atagaccgaa	ccaacatgt	gccccatgct	aacaca			876

<210> 74
 <211> 876
 <212> DNA
 <213> Enterovirus

<220>
 <221> misc_feature
 <222> (0)...(0)
 <223> E31, strain Caldwell

<400> 74						
ggggacacgg	aacatgcagt	tgagtcagct	atctccaggg	tagcagatac	cattagctca	60
ggtcctagta	acactgttgc	tataccagcg	ctcaccgcg	cagaaacggg	ccacacatcg	120
caagtcaccc	ccagcgacaa	tcttcagacg	cgccatgtta	agaactatca	ctcccgtctt	180
gagtcaacta	ttgaaaactt	cctgtgtaaa	tccgcgtgtg	tgcataattgc	gtcatacaac	240
gcatacggtg	atgttgatc	agacagtaga	tatgatagtt	gggagatcaa	catcaggga	300
atggtgcagt	taaggaggaa	gtgcgaaatg	ttcacctatc	tcagatttga	catggagggtg	360
acatttgtca	tcactagcaa	gcaagatcaa	gggacttcgc	tatcacaaga	catgccagtg	420
ctaacacatc	agatcatgta	cgtgccgcca	ggcggatccg	tgcccactag	cgtccagagc	480
tacgcatggc	aaacatccac	caacccgagc	gtgttttggg	cagagggcaa	tgccccctgct	540
agaatgtcca	tcccattcat	tagcataggg	aatgcataca	gcagcttcta	cgacgggttg	600
tcacatttca	cccaacaagg	tggctatggc	tataatacac	tgaacaagat	gggtaagttg	660
tttghtaaggc	atgtgaataa	agaaacacca	acccatgtga	cgagcacgat	acgtgtatat	720
tttaaaccga	agcatgttag	agcgtgggtg	ccaaggccac	ctagattgtg	cccgtacatc	780
aataaagcgg	actgtaactt	cgtgtttaca	ccactcacca	aacagcggtt	aggaatcaac	840
gatgtcccg	ggcccagcca	cacattacat	actcat			876

<210> 75
 <211> 875
 <212> DNA
 <213> Enterovirus

<220>
 <221> misc_feature
 <222> (0)...(0)
 <223> E32, strain PR-10

<400> 75						
aacgaccccc	caaccgctat	tgaaggagca	gtccggcgag	tggcggacac	gatccagagc	60
ggaccgagca	attcggagcg	ggttccagcg	ttaacggccg	ttgagacagg	tcacacagca	120
caggttaccc	cgagtgtatc	aatgcaaact	agacatgtac	acaacttcca	caccagatcg	180
gagtctagca	tcgagaactt	cctcagtaga	gcagcttgtg	tgtacatagg	gaaatatagt	240
agcaatgcaa	caacacaaga	tgaacaatac	atgtcatgga	caattaatac	cagacagatg	300
gtgcagctga	gacgcaaatt	cgaaatgttc	acctacctac	gcttcgacgt	agaagtcact	360
tttataataa	catcgcacca	agatcaagg	acacagttca	accaggatgc	gcccgtaatg	420
tgccacaaaa	tcatgtatgt	gccacctggt	ggcccgggtg	ctaagagtgt	tgatgacttc	480
acatggcaaa	cctctactaa	ccctagtgtc	ttttggtcag	aaggcaatgc	accaccgaga	540
atgaccattc	cattcattag	tatagggaac	gcctacagca	gcttttatga	tggctgggtca	600
cacttctctc	aaaatgggg	ttacgggttt	aatgcactca	ataacatggg	taaactgtat	660
gtgagacaag	tgaacctaaa	agcccctatg	ccagtcagca	gtacagttag	gatctatttc	720
aaacccaagc	atatcaaagc	ttgggtaccc	agaccaccgc	gtctatgtaa	gtacctgaag	780
tctgggagtg	tcaattttga	gcccactgat	ttgacagaaa	aacggaaatc	cagaaagtac	840
atccccaaaa	ctttcagacc	agatgtgaga	accat			875

<210> 76

<211> 843
 <212> DNA
 <213> Enterovirus

<220>
 <221> misc_feature
 <222> (0)...(0)
 <223> E33, strain Toluca-3

<400> 76
 ggtgatgtgc atgatgcagt tgtgggtgcg atgtcgcgcg tcgctgatac agtagcaagt 60
 ggccctgcaa actctgagag cgtgcctgct ctactgcgg tagaaactgg acacacgtca 120
 caggtgacac caagtgatac aatgcagacc agacacgtac acaacttcca cacacggtcc 180
 gaatcgtcaa tcgagaactt cttaagccgc tctgcatgtg tctattatgc aacgtacaaa 240
 acaacagcca gcagaccgga agaccaattc gttagggtgt ccatttcata ccgccagggtg 300
 gcccaactgc gcaggaaaat ggaaatgttc acctacctgc gctacgatgt ggaggtcact 360
 tttgtgatta caagttctca ggacccatcg accaacgtaa gccaggatgc tcctgtactc 420
 acacatcagt taatgtacgt acccccggg ggtccagtgc ccaaaaattc aagagactat 480
 gcatggcaaa catccaccaa cccgagtgtg ttctggaccg aggggaacgc accaccaagg 540
 atatccatcc cttttatcag tgtgggcaac gcatacagtt gcttttatga tggatggtcc 600
 cactactcac agacgggggt gtatggttac aacaccttaa acgacatggg ccaattatct 660
 gtcaggcacg tgaatgaggc aagcccgggt gcggtgtcaa gtgtagttag gatttacttc 720
 aaacccaaac atgtgaaggc atgggtcccg agaccaccac ggttgtgcca atatgttaac 780
 gcagcaacgg tgaacttcac tcctgaaggg gtcactaagg cacgtactga tctcatgaca 840
 aca 843

<210> 77
 <211> 915
 <212> DNA
 <213> Enterovirus

<220>
 <221> misc_feature
 <222> (0)...(0)
 <223> E34, strain DN-19

<400> 77
 ggaatagaag aaactattga cacagtgatc accaacgctt tacaactgtc tcagcccaaa 60
 ccgcagaaac aactcactgc tcaatccacc gcctcatcca gcggagtcaa ttcacaagaa 120
 gtgccagcat tgactgctgt ggagacggga gcttctggtc aagccatacc cagcgacgtg 180
 attgagacca gacatgtcgt caattacaaa actagatctg aatcaaccct tgagtcattc 240
 tttggtagat cagcatgcgt aaccatactg gaagtagaga acttcaatgc cactaccgaa 300
 tcggacaaga aaaagcaatt caccacctgg ccaatcacat acaccaaacac agtccagttg 360
 cgcaggaaat tgggaattctt tacatactcc agatttgatc tggaaatgac ttttgtcata 420
 actgagagggt accacacaag taatacagga catgctagaa atcaagtgtg ccaaataatg 480
 tacataccac cgggtgcgcc aaggcccaca gcacgggatg attacacctg gcaaagttca 540
 tccaatccat cagtgtttta cacatatggt agcgcgcctc ccagaatgtc tatcccatat 600
 gttggcattg ccaatgcata ctacactttt tatgacgggt ttgcccgagt tcccctgaaa 660
 gatgatacaa ctgactccgg tgacactttt tatggattgg tcaccatcaa tgacttttga 720
 acattggctg tgaggggtgt gaatgagttc aaccctgcaa ggataacatc aaaggtcaga 780
 gtttatatga agcccaaaac tgtgagggtg tgggtgtccta ggccaccgcg cgcagtgccc 840
 tatcgtgggtg aagggttga tttcaaaaca gattcaatca cgccaataac agcagtcacc 900
 aatattaata ccttc 915

<210> 78
 <211> 936
 <212> DNA
 <213> Enterovirus

<220>
 <221> misc_feature
 <222> (0)...(0)
 <223> EV68, strain Fermon

<400> 78

tcaaaccact	tacatggagc	agaggcagcc	tatcaggtgg	agagtatcat	caaaacagca	60
actgatactg	tgaagagtga	gattaacgcc	gaacttggtg	tggtccctag	tctaaatgca	120
gttgaaactg	gtgcaacttc	caacactgaa	ccagaagaag	ccatacaaac	tcgcacagta	180
ataaatcagc	atgggtgtgc	ggagacgtta	gtggagaatt	ttcttggtag	ggcagcccta	240
gtgtcaaaga	aaagttttga	atacaagaat	catgcctcat	ccagcgcagg	gacacacaaa	300
aactttttta	aatggacaat	taataactaag	tcttttgtcc	agttaagaag	aaagctggaa	360
ttattcacat	accttaggtt	tgatgctgaa	atcaccatac	tcacaactgt	ggcagtaaat	420
ggtaataatg	acagcacata	catgggtctc	cctgacttga	cactccaagc	aatgtttgta	480
ccaactgggtg	ctcttactcc	aaaggagcag	gattcatttc	attggcaatc	aggcagtaat	540
gctagtgtgt	tctttaaaat	ttctgatccc	ccagctagaa	tgactatacc	ttttatgtgc	600
atcaactcag	catattcagt	tttttatgat	ggctttgctg	gatttgagaa	aaatgggtcta	660
tatggaataa	acccagctga	cactattggc	aacttgtgtg	tcagaatagt	gaatgaacat	720
caaccagttg	gttttacagt	gaccgttagg	gtttacatga	agcctaaaca	tataaaagca	780
tgggctccac	gaccaccgcg	aaccatgcca	tacatgagca	ttgctaattgc	aaattacaaa	840
ggtagagata	cagcaccaaa	cacacttaat	gccataattg	gtaatagagc	gagtgtcaca	900
actatgcctc	acaacatagt	aaccaccggt	ccgggt			936

<210> 79

<211> 861

<212> DNA

<213> Enterovirus

<220>

<221> misc_feature

<222> (0)...(0)

<223> EV69, strain Toluca-1

<400> 79

aatgaccagc	acaatggggc	gatcgttgcc	aacacaacag	ctagcggacc	ttctaattcg	60
gaaagcatat	cggcacttac	tgcggctgag	actggccaca	catcgcaggt	tgtccctagc	120
gacaccatcc	agacaagaca	tgtgaaaaac	taccactcgc	gttcagagtc	caccatagag	180
aacttcctgt	gtagatctgc	ctgtgtgtac	tacaccacgt	acaacactca	gggcgagcaa	240
gcacatgata	aatacgcaag	ttggccaatc	acgactagaa	aagttgccca	actgcgcagg	300
aagctggagt	tctttaccta	cctgcggttt	gatctcgaga	tcacgttcgt	gatcacgagc	360
gccagatca	catccacgaa	ccaaaaccag	gatgccccag	tactcacaca	tcaggtgatg	420
tatgtacccc	cagggggggg	ggtaccgcgc	agtgtggatg	actatagttg	gcagacttcc	480
accaatccca	gcatcttctg	gacagaaggg	aacgcacctc	ctcgtatgtc	aataccattc	540
attagtgtgg	gcaacgccta	cagcagcttt	tacgacgggt	ggtcacactt	tgaacaaacc	600
gggggtatat	gattcaatac	ccttaataat	atggggactt	tgtacgccag	gcacgttaac	660
ggtgctagtc	ccgggccagt	caagagcacc	attaggatat	atatgaaacc	taaacatgtg	720
aaagcgtgga	tacctaggcc	cccacggttg	tgcgactatg	tgaaatctgg	caacgtcaac	780
tttgaaccaa	aaggagtcac	cgaagcaga	ccatctataa	agttagaaaa	gacctcaagt	840
gggcacaggc	tgacaaccca	c				861

<210> 80

<211> 7

<212> PRT

<213> Enterovirus

<400> 80

Met	Tyr	Val	Pro	Pro	Gly	Gly
1				5		

<210> 81

<211> 7

<212> PRT

<213> Enterovirus

<220>

<223> Xaa(Position 3) = Val or Ile

<223> Xaa(Position 5) = Pro or Thr

<400> 81
Met Tyr Xaa Pro Xaa Gly Ala
1 5

<210> 82
<211> 7
<212> PRT
<213> Enterovirus

<220>
<223> Xaa(Position 3) = Gln or His

<400> 82
Phe Gly Xaa Gln Ser Gly Ala
1 5

<210> 83
<211> 7
<212> PRT
<213> Enterovirus

<220>
<223> Xaa (Position 3) = Ala or Val

<400> 83
Thr Ala Xaa Glu Thr Gly His
1 5

<210> 84
<211> 7
<212> PRT
<213> Enterovirus

<220>
<223> Xaa (Position 7) = Ala or Val

<400> 84
Thr Ala Val Glu Thr Gly Xaa
1 5

<210> 85
<211> 7
<212> PRT
<213> Enterovirus

<400> 85
Gln Ala Ala Glu Thr Gly Ala
1 5

<210> 86
<211> 7
<212> PRT
<213> Enterovirus

<220>
<223> Xaa (Position 2) = Phe or Tyr

<223> Xaa (Position 3) = Ile or Val

<223> Xaa (Position 7) = Ala or Gly

<400> 86
Met Xaa Xaa Pro Pro Gly Xaa

1

5