In addition, not all of these coatings work well for all cell types.

SUMMARY

In one aspect, the present invention refers to a method of producing a high titer of Hepatitis E Virus (HEV), the method comprising:

a) culturing a cell line *in vitro*, wherein the cell line is HepG2 deposited under ATCC number HB-8065 or HepG2/C3A deposited under ATCC number CRL-10741, in a medium comprising polybrene in a concentration of about 1 µg/ml - about 5 µg/ml, and;

b) infecting the cell line with HEV.

In another aspect, the present invention refers to a method of determining a presence and/or a level of HEV in a sample, the method comprising the steps of:

a) providing the sample to a mixture comprising a cell line, wherein the cell line is HepG2 deposited under ATCC number HB-8065 or HepG2/C3A deposited under ATCC number CRL-10741, and a culture medium, wherein the culture medium comprises polybrene in a concentration of about 1 µg/ml - about 5 µg/ml;

b) incubating the mixture comprising the sample from step a), to allow for the propagation of HEV, if present in the sample;

c) collecting a portion of step b), the portion comprising HEV, if present and propagated during step b); and

d) measuring the presence and/or the level of a biological substance associated with HEV in the collected portion.

In yet another aspect, the present invention refers to a HEV titration assay comprising the use of a culture medium

comprising polybrene in the range of about 1 µg/ml - about 5 µg/ml and a cell line wherein the cell line is HepG2 deposited under ATCC number HB-8065 or HepG2/C3A deposited under ATCC number CRL-10741.

In some examples, a method of producing a high- titer of Hepatitis E Virus is provided, the method comprising: culturing a cell line *in vitro* in a medium comprising a concentration of polybrene, and infecting the cell line with HEV. In some examples of the method, the cell line used is HepG2 (ATCC number HB-8065) or HepG2/C3A (ATCC number CRL-10741). In some examples of the method, the concentration of polybrene is about 1 µg/ml - about 5 µg/ml. In some examples of the method, the high titer is about 10^8 copies/mL - about 10^{10} copies/mL. In some examples of the method, the high titer is about 10^7 copies/mL - about 10^{10} copies/mL. In some examples, the method further comprises the steps of adding a concentration of polybrene to the medium; a passaging the HEV-infected cell line in the medium comprising polybrene; and collecting the medium and/or infected cells. In some examples of the method, the concentration of polybrene is about 1 µg/ml - about 5 µg/ml. In some examples of the method, the high titer of HEV obtained in the medium and/or infected cells is about 10^8 copies/mL - about 10^{10} copies/mL. In some examples of the method, the high titer of HEV obtained in the medium and/or infected cells is about 10^7 copies/mL - about 10^{10} copies/mL.

In some examples, a method of determining a presence and/or a level of HEV in a sample is provided, the method comprising the steps of: providing the sample to a mixture comprising a cell line and a culture medium, wherein said

culture medium comprises polybrene; incubating the mixture comprising the sample from the previous step to allow for the propagation of the HEV, if present in the sample; collecting a portion of the previous step, said portion comprising HEV, if present and propagated during the previous step; and measuring the presence and/or the level of a biological substance associated with HEV in the collected portion. In some examples of the method, the cell line is selected from the group consisting of cell lines of HepG2 (ATCC number HB-8065) and HepG2/C3A (ATCC number CRL-10741). In some examples of the method, said biological substance comprises a polynucleotide and/or a polypeptide sequence of HEV. In some examples of the method, said measuring comprises the steps of: providing a first reaction mixture by mixing the collected portion with a first solution so as to expose a polynucleotide of HEV, if HEV is present in the collected portion, wherein the polynucleotide is an RNA of HEV; providing a second reaction mixture by adding, to the first reaction mixture, a first reagent so as to produce a complementary deoxyribonucleic acid (cDNA) that is at least partially complementary to the RNA of HEV; providing a third reaction mixture by adding, to the second reaction mixture, a second reagent comprising a pair of polynucleotides to amplify a sequence of the cDNA that is at least partially complementary to each of the pair of polynucleotides; providing a fourth reaction mixture by amplifying the sequence; and determining a concentration of the amplified sequence in the fourth reaction mixture. In some examples of the method, said determining the concentration comprises: providing the fourth reaction mixture; providing a one or more controls comprising a predetermined amount of the cDNA of HEV; adding an agent

to the fourth reaction mixture and the one or more controls, wherein said agent is at least partially specific to the amplified sequence in the fourth reaction mixture and the predetermined amount of the cDNA of HEV in the one or more controls; and calculating a level of recognition of HEV by the agent in the fourth reaction mixture relative to the one or more controls. In some examples of the method, said pair of polynucleotides comprises:

a) 5′-CGGCTATCGGCCAGAAGTT-3′ (SEQ ID NO: 1)

b) 5′-CCGTGGCTATAACTGTGGTCT-3′ (SEQ ID NO: 2)

In some examples of the method, the agent comprises:

5′-FAM-TTTTTACGC-ZEN-AGGCTGCCAAGGCC-3IABkFQ-3′ (SEQ ID NO: 3)

In some examples, a culture medium for generating a high-titer HEV comprising polybrene in the range of about 1 µg/ml – about 5 µg/ml is provided. In some examples, the high-titer HEV is about 10^8 copies/mL – about 10^{10} copies/mL. In some examples, the high-titer HEV is about 10^7 copies/mL – about 10^{10} copies/mL.

In some examples, an HEV titration assay comprising the use of polybrene is provided. In some examples of the assay, the concentration of polybrene used is in the range of about 1 µg/ml – about 5 µg/ml.

CLAIMS

1. A method of producing a high titer of Hepatitis E Virus (HEV), the method comprising:

   a) culturing a cell line *in vitro*, wherein the cell line is HepG2 deposited under ATCC number HB-8065 or HepG2/C3A deposited under ATCC number CRL-10741, in a medium comprising polybrene in a concentration ofabout 1 µg/ml - about 5 µg/ml, and;
   b) infecting the cell line with HEV.

2. The method according to claim 1, wherein the high titer of HEV is about 10^8 copies/mL - about 10^{10} copies/mL.

3. The method according to claim 1, wherein the high titer of HEV is about 10^7 copies/mL - about 10^{10} copies/mL.

4. The method according to claim 1, wherein the method further comprises the steps of passaging the HEV-infected cell line of step b) in a culture medium comprising polybrene in a concentration of about 1 µg/ml - about 5 µg/ml.

5. The method according to claim 4, wherein the high titer of HEV obtained in the medium and/or infected cells is about 10^8 copies/mL - about 10^{10} copies/mL.

6. The method according to claim 4, wherein the high titer of HEV obtained in the medium and/or infected cells is about 10^7 copies/mL - about 10^{10} copies/mL.

7. A method of determining a presence and/or a level of HEV in a sample, the method comprising the steps of:

   a) providing the sample to a mixture comprising a cell line, wherein the cell line is HepG2 deposited under ATCC number HB-8065 or HepG2/C3A deposited under ATCC number CRL-10741, and a culture medium, wherein the culture medium comprises polybrene in a concentration of about 1 µg/ml - about 5 µg/ml;
   b) incubating the mixture comprising the sample from step a), to allow for the propagation of HEV, if present in the sample;
   c) collecting a portion of step b), the portion comprising HEV, if present and propagated during step b); and
   d) measuring the presence and/or the level of a biological substance associated with HEV in the collected portion.

8. The method according to claim 7, wherein the biological substance comprises a polynucleotide and/or a polypeptide sequence of HEV.

9. The method according to claim 7, wherein measuring the presence and/or the level of a biological substance comprises the steps of:

   a) providing a first reaction mixture by mixing the collected portion with a first solution so as to expose a polynucleotide of HEV, if HEV is present in the collected portion, wherein the polynucleotide is an RNA of HEV;

b) providing a second reaction mixture by adding, to the first reaction mixture, a first reagent so as to produce a complementary deoxyribonucleic acid (cDNA) that is at least partially complementary to the RNA of HEV;

c) providing a third reaction mixture by adding, to the second reaction mixture, a second reagent comprising a pair of polynucleotides to amplify a sequence of the cDNA that is at least partially complementary to each of the pair of polynucleotides;

d) providing a fourth reaction mixture by amplifying the sequence; and

e) determining a concentration of the amplified sequence in the fourth reaction mixture.

10. The method according to claim 9, wherein said determining the concentration comprises:

a) providing the fourth reaction mixture;

b) providing a one or more controls comprising a predetermined amount of the cDNA of HEV;

c) adding an agent to the fourth reaction mixture and the one or more controls, wherein said agent is at least partially specific to the amplified sequence in the fourth reaction mixture and the predetermined amount of the cDNA of HEV in the one or more controls; and

d) calculating a level of recognition of HEV by the agent in the fourth reaction mixture relative to the one or more controls.

11. The method according to claim 9, wherein said pair of polynucleotides comprises:

a) 5'-CGGCTATCGGCCAGAAGTT-3' (SEQ ID NO: 1)
b) 5'-CCGTGGCTATAACTGTGGTCT-3' (SEQ ID NO: 2)

12. The method according to claim 10, wherein the agent comprises:

5'-FAM-TTTTTACGC-ZEN-AGGCTGCCAAGGCC-3IABkFQ-3' (SEQ ID NO: 3)

13. A HEV titration assay comprising the use of a culture medium comprising polybrene in the range of about 1 µg/ml - about 5 µg/ml and a cell line wherein the cell line is HepG2 deposited under ATCC number HB-8065 or HepG2/C3A deposited under ATCC number CRL-10741.