by the implementation of pathogen reduction steps in the manufacturing processes of blood and plasma-derived products. To develop and demonstrate the effectiveness of such pathogen reduction steps, virus clearance studies are performed. During these studies a known amount of virus is spiked into a blood or plasma product intermediate and then the spiked sample is processed using a bench scale model (a scaled down model) of the manufacturing process comprising a pathogen reduction step. Virus reduction and/or clearance during a pathogen reduction step is determined by comparing the amount of virus before and after the step.

To assure the validity of virus clearance data, the scaled down model must accurately represent a large scale unit operation and the virus spike should be representative of a potential contaminant. For example, cultivation of a virus spike in vitro may require serum, and the presence of serum in the virus spike could affect clearance studies that involve serum-free product intermediates. The presence of non-viral contaminants, such as extraneous host cell membranes, proteins and nucleic acids, could also interfere in the accurate assessment of virus clearance during a downstream step, where product intermediates are presumably highly purified. Therefore, it is important to remove virus spike contaminants that could impact the performance and relevancy of scaled down models.

SUMMARY

In one aspect, the present invention refers to a method of purifying a non-enveloped or a pseudo-enveloped virus propagated in cell culture, the method comprising a step of treating a sample comprising the non-enveloped or the pseudo-enveloped virus with a detergent, wherein the

detergent is selected from the group consisting of Triton X-100 at 0.5% and a mix of Triton X-100 at 0.5% and Lithium Dodecyl Sulfate at 0.1%. In another aspect, the present invention refers to a method of measuring a level of a non-enveloped or a pseudo-enveloped virus in a sample, wherein the sample comprises plasma or blood from a mammal, the method comprising the steps of:a) providing the sample to a mixture comprising a cell line and a culture medium;b) incubating to allow propagation of the non-enveloped or the pseudo-enveloped virus, if present in the sample, to obtain an incubated portion;c) treating with at least one detergent, to obtain a treated portion; wherein the detergent is selected from the group consisting of Triton X-100 at 0.5% and a mix of Triton X-100 at 0.5% and Lithium Dodecyl Sulfate at 0.1%. d) collecting a part of the treated portion, to obtain a collected portion; and e) measuring the level of the non-enveloped or the pseudo-enveloped virus in the collected portion. In some examples, there is disclosed a method of purifying a non-enveloped or a pseudo enveloped virus propagated in cell culture, based on a method of treatment of a sample comprising the non-enveloped or the pseudo enveloped virus with a detergent. In some examples of the method, the virus propagated in cell culture is hepatitis A virus (HAV). In some examples of the method, the virus propagated in cell culture is porcine parvovirus (PPV). In some preferred examples of the method, the virus propagated in cell culture is hepatitis E virus (HEV). In some examples of the method, the virus is produced in an *in vitro* cell culture. In some examples of the method, the *in vitro* cell culture comprises an established cell line. In some examples of the method, the established cell line is selected from a group consisting of HepG2 (ATCC number HB-8065) and HepG2/C3A (ATCC number CRL-10741). In some examples of the method, the detergent is selected from the group consisting of Lithium Dodecyl Sulfate, Triton X-100 and mixtures thereof. In some examples of the method, a concentration of Triton X-100 is 0.5% and a concentration of Lithium Dodecyl Sulfate is 0.1%. In some examples of the method, the step of treating is carried out for 1 hour. In some examples of the method, the sample is a supernatant obtained from ultra-centrifugation of a

clarified virus-infected cell lysate suspension. In some examples of the method, optionally, the sample is a retentate derived from transflow filtration of a clarified virus-infected cell lysate suspension.

A method of measuring a level of a non-enveloped or a pseudo-enveloped virus in a sample, the method comprising the steps of: a) providing the sample to a mixture comprising a cell line and a culture medium; b) incubating to allow propagation of the non-enveloped or the pseudo-enveloped virus, if present in the sample, to obtain an incubated portion; c) treating with at least one detergent, to obtain a treated portion; d) collecting a part of the treated portion, to obtain a collected portion; and e) measuring the level of the non-enveloped or the pseudo-enveloped virus in the collected portion. In some examples of the method, the sample is obtained from a mammal. In some examples of the method, the sample comprises plasma or blood. In some examples of the method, the non-enveloped or the pseudo-enveloped virus is HAV. In some examples of the method, the non-enveloped or the pseudo-enveloped virus is PPV. In some preferred examples of the method, the non-enveloped or the pseudo-enveloped virus is HEV. In some examples of the method, the virus is propagated in an *in vitro* cell culture comprising an established cell line. In some examples of the method, the cell line is selected from the group consisting of HepG2 (ATCC number HB-8065) and HepG2/C3A (ATCC number CRL-10741). In some examples of the method, the sample comprises a first retentate obtained by trans-flow filtering a part of the incubated portion through a membrane. In some examples of the method, optionally, the sample comprises a first supernatant obtained by trans-flow filtering a part of the incubated portion through a membrane to obtain a first retentate and centrifugating the first retentate. In some examples of the method, the sample is treated with at least one detergent or optionally a mixture of detergents to obtain a first solution, the method further comprises: a) providing a first pellet obtained by centrifugating the first solution; b) providing a second solution obtained by resuspending the first pellet in PBS (Phosphate Buffered

Saline); c) providing a third solution obtained by clarifying the second solution; d) providing a first filtrate obtained by filtering the third solution using a membrane; and e) providing a second retentate obtained by ultrafiltering the first filtrate, wherein the second retentate comprises the treated portion. In some examples of the method, measuring the level of the non-enveloped or pseudo-enveloped virus comprises detecting a biological substance, wherein the biological substance comprises a polynucleotide sequence and/or a polypeptide sequence of a virus, and wherein the biological substance is an HEV ribonucleic acid. In some examples of the method, measuring the biological substance comprises: a) providing a first reaction mixture comprising the HEV ribonucleic acid obtained by mixing a part of the treated portion with a lysis solution; b) providing a second reaction mixture obtained by adding a first reagent to the first reaction mixture, wherein the second reaction mixture comprises a deoxyribonucleic acid that is complementary to the HEV ribonucleic acid; c) adding, to the second reaction mixture, a second reagent that is at least partially complementary to a sequence within the deoxyribonucleic acid; d) adding, to the second reaction mixture, a third reagent that is at least partially complementary to a sequence within the deoxyribonucleic acid; e) amplifying the sequence encompassed by the second and the third reagents within the deoxyribonucleic acid; and f) measuring a concentration of the amplified sequence. In some examples of the method, the second reagent and the third reagent comprises an oligonucleotide sequence selected from the group consisting of:

a) 5'-CGGCTATCGGCCAGAAGTT-3' (SEQ ID NO: 1);
b) 5'-CCGTGGCTATAACTGTGGTCT-3' (SEQ ID NO: 2) and
c) 5'-FAM-TTTTTACGC-ZEN-AGGCTGCCAAGGCC-3IABkFQ-3' (SEQ ID NO: 3).

In some examples, the present invention comprises a method of purifying a non-enveloped or a pseudo enveloped virus propagated in cell culture, based on a method of treatment of a sample comprising the non-enveloped or the pseudo enveloped virus with a at least one anionic detergent,

such as lithium dodecyl sulfate (LDS), or at least one non-ionic detergent, such as Triton X-100 or a combination of the two, allowing to obtain virus whose behaviour in clearance studies is presumably the same or nearly the same as those that could potentially be present in highly purified process streams. In some examples, the virus generated by some examples of the method described above would be an appropriate spike to test the virus reduction and/or clearance capacity of downstream steps in manufacturing processes for blood and/or plasma-derived proteins.

In some examples, the method of purifying a virus produced in cell culture, based on a method of treatment, comprises: clarification of a virus-infected material; trans-flow filtering at least part of the mixture through a membrane to obtain a first retentate; and centrifugating the first retentate to obtain a first supernatant. The method of treatment comprises: treating the first supernatant with at least one detergent to obtain a first solution; wherein the method further comprises: after said treating, centrifugating the first solution to obtain a first pellet; resuspending the first pellet to obtain a second solution; clarifying the second solution to obtain a third solution; filtering the third solution using a membrane to obtain a first filtrate; and ultrafiltering the first filtrate to obtain a second retentate which provides at least part of the treated portion to be collected; wherein said at least one detergent is selected from the group consisting of Lithium Dodecyl Sulfate, Triton X-100 and mixtures thereof; wherein a concentration of Triton X-100 is 0.5% and a concentration of LDS is 0.1%; wherein said treating the first supernatant with the at least one detergent is carried out for 1 hour.

In some examples, the method of purifying a virus produced in cell culture, based on a method of treatment, comprises: clarification of virus-infected material; trans-flow filtering at least part of the mixture through a membrane to obtain a first retentate. The method of treatment comprises: treating the first retentate with at least one detergent to obtain a first solution; wherein

the method further comprises: after said treating, centrifugating the first solution to obtain a first pellet; resuspending the first pellet to obtain a second solution; clarifying the second solution to obtain a third solution; filtering the third solution using a membrane to obtain a first filtrate; and ultrafiltering the first filtrate to obtain a second retentate which provides at least part of the treated portion to be collected; wherein said at least one detergent is selected from the group consisting of Lithium Dodecyl Sulfate, Triton X-100 and mixtures thereof; wherein a concentration of Triton X-100 is 0.5% and a concentration of LDS is 0.1%; wherein said treating the first retentate with the at least one detergent is carried out for 1 hour.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows preparation of Trans Flow Filtration HEV (TFF HEV) and TFF Supernatant (Supe) HEV virus spikes according to some embodiments disclosed herein.

Figure 2 shows the relative purity of TFF HEV and TFF Supe HEV virus spikes that were prepared according to some embodiments disclosed herein.

Figure 3 shows preparation of TFF HEV, Triton TFF HEV and Triton/LDS TFF HEV virus spikes according to some embodiments disclosed herein.

Figure 4 shows the nucleotide sequences of primers and probes.

DETAILED DESCRIPTION

Efficient cell culture systems for producing the non-enveloped virus HEV have recently been established and the data indicate that cell culture-derived HEV is similar to HEV found in blood in that they are both associated with lipids. As such, unprocessed clarified cell culture lysates or culture supernatants could be used as virus spikes in clearance studies of upstream steps with relatively crude product intermediates. However, the use of unprocessed virus spikes may not be appropriate for the

evaluation of HEV clearance during downstream steps where process streams are of relatively high purity.

The invention relates to a process for preparing HEV spikes of relatively high purity and high titer that are suitable for use in virus clearance studies for downstream manufacturing process steps.

CLAIMS

1. A method of purifying a non-enveloped or a pseudo-enveloped virus propagated in cell culture, the method comprising a step of treating a sample comprising the non-enveloped or the pseudo-enveloped virus with a detergent, wherein the detergent is selected from the group consisting of Triton X-100 at 0.5% and a mix of Triton X-100 at 0.5% and Lithium Dodecyl Sulfate at 0.1%.

2. The method according to claim 1, wherein the virus propagated in cell culture is Hepatitis E Virus (HEV).

3. The method according to claim 1, wherein the virus propagated in cell culture is either Hepatitis A Virus (HAV) or Porcine Parvovirus (PPV).

4. The method according to claim 1 wherein the virus is produced in an *in vitro* cell culture.

5. The method according to claim 4, wherein the *in vitro* cell culture comprises an established cell line.

6. The method according to claim 5, wherein the established cell line is selected from a group consisting of HepG2 (ATCC number HB-8065) and HepG2/C3A (ATCC number CRL-10741).

7. The method according to claim 1, wherein the step of treating is carried out for 1 hour.

8. The method according to claim 1, wherein the sample is a supernatant obtained from ultra-centrifugation of a clarified virus-infected cell lysate suspension.

9. The method according to claim 1, wherein the sample is a retentate derived from transflow filtration of a clarified virus-infected cell lysate suspension.

10. A method of measuring a level of a non-enveloped or a pseudo-enveloped virus in a sample, wherein the sample comprises plasma or blood from a mammal, the method comprising the steps of:

a) providing the sample to a mixture comprising a cell line and a culture medium;

b) incubating to allow propagation of the non-enveloped or the pseudo-enveloped virus, if present in the sample, to obtain an incubated portion;

c) treating with at least one detergent, to obtain a treated portion; wherein the detergent is selected from the group consisting of Triton X-100 at 0.5% and a mix of Triton X-100 at 0.5% and Lithium Dodecyl Sulfate at 0.1%.

d) collecting a part of the treated portion, to obtain a collected portion; and

e) measuring the level of the non-enveloped or the pseudo-enveloped virus in the collected portion.

11. The method according to claim 10, wherein the non-enveloped or the pseudo-enveloped virus is HEV.

12. The method according to claim 10, wherein the non-enveloped or the pseudo-enveloped virus is either HAV or PPV.

13. The method according to claim 10, wherein the virus is propagated in an *in vitro* cell culture comprising an established cell line.

14. The method according to claim 10, wherein the cell line is selected from the group consisting of HepG2 (ATCC number HB-8065) and HepG2/C3A (ATCC number CRL-10741).

15. The method according to claim 10, wherein the sample comprises a first retentate obtained by trans-flow filtering a part of the incubated portion through a membrane.

16. The method according to claim 10, wherein the sample comprises a first supernatant obtained by trans-flow filtering a part of the incubated portion through a membrane to obtain a first retentate and centrifugating the first retentate.

17. The method according to claim 10, wherein the sample is treated with at least one detergent or a mixture of detergents to obtain a first solution.

18. The method according to claim 17, wherein the method further comprises:

a) providing a first pellet obtained by centrifugating the first solution;

b) providing a second solution obtained by resuspending the first pellet in PBS;

c) providing a third solution obtained by clarifying the second solution;

d) providing a first filtrate obtained by filtering the third solution using a membrane; and

e) providing a second retentate obtained by ultrafiltering the first filtrate, wherein the second retentate comprises the treated portion.

19. The method of claim 10, wherein measuring the level of the non-enveloped or pseudo-enveloped virus comprises detecting a biological substance.

20. The method according to claim 19, wherein the biological substance comprises a polynucleotide sequence and/or a polypeptide sequence of a virus.

21. The method according to claim 20, wherein the biological substance is an HEV ribonucleic acid.

22. The method according to claim 21, wherein measuring the biological substance comprises:

a) providing a first reaction mixture comprising the HEV ribonucleic acid obtained by mixing a part of the treated portion with a lysis solution;

b) providing a second reaction mixture obtained by adding a first reagent to the first reaction mixture, wherein the second reaction mixture comprises a deoxyribonucleic acid that is complementary to the HEV ribonucleic acid;

c) adding, to the second reaction mixture, a second reagent that is at least partially complementary to a sequence within the deoxyribonucleic acid;

d) adding, to the second reaction mixture, a third reagent that is at least partially complementary to a sequence within the deoxyribonucleic acid;

e) amplifying the sequence encompassed by the second and the third reagents within the deoxyribonucleic acid; and

f) measuring a concentration of the amplified sequence.

23. The method according to claim 22, wherein the second reagent and the third reagent comprises an oligonucleotide sequence selected from the group consisting of:

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

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

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