

Table 7: Summary of TVCC-1 Downstream Process

Dev Run	Dev 2	Dev 3	Dev 4	Dev 5	Dev 6	Dev 7	Dev 8	Dev 9	CTM #2
Strain	A/Wis	A/Wis	A/NC	B/Mal	B/Mal	A/NC	A/Wis	B/Mal	B/Mal
Harvest (hr)	60 – 65	60 – 65	60 – 65	60 – 65	60 – 65	60 – 65	48	48	48
Column	BRG200	BPG200	BPG200	BPG200	BPG100	BPG100	BPG100	BPG100	BPG100
Loading (log10/mL)	8.32	8.73	8.76	8.40	9.18	9.44	9.33	8.97	9.56
Process*	TVCC-1a	TVCC-1a	TVCC-1a	TVCC-1b	TVCC-1b	TVCC-1b	TVCC-1b	TVCC-1b	TVCC-1b
VH Titer (CCD)	8.4	8.4	8.4	8.3	8.6	8.4	8.6	8.0	8.3
VH Titer (PD)	7.7 [^]	8.4	8.6	8.1	8.3	8.3	8.4	7.9	8.3
Final Titer	8.3	8.5	8.5	8.5	8.9	8.6	9.2	9.0	9.7
Overall Yield §	60.9%	27.0%	6.3%	25.4%	22.0%	21.2%	30.4%	56.3%	57.5%
VH DNA (ng/mL)	n/a	7200	5480	n/a	5720	10800	3440	2070	2050
Bulk DNA (ng/dose)	76.7	n/a	36.4	31.9	n/a	n/a	0.4	0.3	0.06
PicoGreen									
Bulk DNA (ng/dose)	n/a	n/a	0.92	n/a	0.23	0.18	0.032	0.164	n/a
PCR									
VH HCP (µg/mL)	278	392	269	231	250	233	135	174	74
Bulk HCP (µg/dose)	1.10	n/a	n/a	n/a	n/a	n/a	0.13	0.29	0.14
Benzonase® (ng/mL)	0.9	n/a	0.69	2.1	6.6	3.6	0.27	0.52	LOD

Note: * TVCC-1a: Benzonase® treatment in bag; TVCC-1a: Benzonase® treatment on column

[^] FFA assay based on Anti-NA instead of Anti-HA

§ Based on VH-PD titer

CLAIMS

1. A Madin-Darby Canine Kidney (MDCK) cell, wherein a cell culture composition comprising a plurality of the MDCK cells supports replication of a cold-adapted influenza virus to a base 10 logarithm of the median tissue culture infection dose per milliliter ($\log_{10}$ TCID₅₀/mL) of at least about 7.8 or to a base 10 logarithm of fluorescent focus units per milliliter ($\log_{10}$ FFU/mL) of at least about 7.8.
2. The MDCK cell of claim 1, wherein the cell culture composition is serum free.
3. The MDCK cell of claim 1, wherein the MDCK cell is adherent.
4. The MDCK cell of claim 1, wherein the MDCK cell is non-tumorigenic and/or non-oncogenic.
5. The MDCK cell of claim 1, wherein the MDCK cell is identified by ATCC Accession No. PTA-7909 or PTA-7910.
6. The MDCK cell of claim 1, wherein the influenza virus is also attenuated and temperature sensitive.
7. The MDCK cell of claim 1, wherein the influenza virus comprises one or more gene segments of influenza strain A/Ann Arbor/6/60 or B/Ann Arbor/1/66.
8. A method for proliferating the MDCK cell of claim 1 to a cell density of at least about 1×10^6 cells/ml in a Single Use Bioreactor (SUB) system comprising inoculating a serum free cell culture medium with the MDCK cell of claim 1 at a seeding density of between about 1×10^4 to about 12×10^4 cells/mL and culturing the cells while maintaining one or more culture conditions selected from the group consisting of:
 - a. an agitation rate of between about 50 to 150 rpm;
 - b. a pH of between about 6.0 to about 7.5;
 - c. dissolved oxygen (DO) between about 35% to about 100%; and
 - d. a temperature of between about 33°C to about 42°C.
9. The method of claim 8, wherein the cell culture medium is selected from the group consisting of MediV-105, MediV-105 supplemented with glucose, M-32, M-32 supplemented with glucose, MediV-107, MediV-107 supplemented with glucose.

10. The method of claim 8, wherein the MDCK cell is an adherent MDCK cell and wherein a microcarrier is used for culturing the cells.

11. A method for producing cold adapted influenza viruses to a $\log_{10}$ TCID₅₀/mL of at least about 7.8 in cell culture, comprising:

- a. proliferating the MDCK cell of claim 1 to a cell density of at least about 1×10^6 cells/ml in a Single Use Bioreactor (SUB) system comprising inoculating an animal protein free cell culture medium with the MDCK cell of claim 1 at a seeding density of between about 1×10^4 to about 12×10^4 cells/mL and culturing the cells while maintaining one or more culture conditions selected from the group consisting of an agitation rate of between about 50 to 150 rpm, a pH of between about 6.0 to about 7.5, and dissolved oxygen (DO) between about 35% to about 100%;
- b. infecting the proliferated MDCK cells with an influenza virus;
- c. incubating the infected proliferated MDCK cells under conditions that permit replication of the influenza virus; and
- d. isolating influenza viruses from the cell culture composition.

12. The method of claim 11, wherein fresh medium or additional medium components are added to the cell culture during step (a).

13. The method of claim 11, wherein none or some of the cell culture medium is removed and replaced with fresh medium prior to or during step (b).

14. The method of claim 11, wherein the cell culture medium is selected from the group consisting of MediV-105, MediV-105 supplemented with glucose, M-32, M-32 supplemented with glucose, MediV-107, MediV-107 supplemented with glucose.

15. The method of claim 11, wherein the MDCK cell is an adherent MDCK cell and wherein a microcarrier is used for culturing the cells.

16. The method of claim 11, wherein step (b) is carried out at a Multiplicity Of Infection (MOI) of between about 0.00001 to about 0.003 FFU/cell.

17. The method of claim 11 , wherein the conditions of step (c) are selected from the group consisting of an agitation rate of between about 50 to 150 rpm, a pH of between about 6.0 to about 7.5, dissolved oxygen (DO) between about 35% to about 100%, and a temperature of between about 30°C to about 35°C.
18. The method of claim 11, wherein the MDCK cell is identified by ATCC Accession No. PTA-7909 or PTA-7910.
19. The method of claim 11, wherein the influenza virus is also attenuated and temperature sensitive.
20. The method of claim 11, wherein the influenza virus comprises one or more gene segments of influenza strain A/Ann Arbor/6/60 or B/Ann Arbor/1/66.
21. A method of eliminating DNA contaminants from a viral preparation comprising:
- a. passing the viral preparation over an affinity chromatography media under conditions wherein the DNA contaminants are not retained on the affinity chromatography media and the virus present in the viral preparation are retained;
 - b. washing the affinity chromatography media to remove the DNA contaminants; and
 - c. eluting the virus present in the viral preparation from the affinity chromatography media.
22. The method of claim 21, wherein between steps (a) and (b) a non-specific endonuclease preparation is passed over the affinity chromatography media.
23. The method of claim 21, wherein the viral preparation is an influenza virus preparation.
24. The method of claim 23, wherein the influenza virus preparation was prepared from mammalian cells.
25. The method of claim 21 , wherein the affinity chromatography media is Cellufine™ Sulfate resin, and the conditions used in step (a) are IX SP buffer at about pH 7.2, and the virus are eluted in step (c) in IX SP buffer containing about 1 M NaCl at about pH7.2.

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Column	BPG200	BPG200	BPG200	BPG200	BPG100	BPG100	BPG100	BPG100	BPG100
Loading (log10/mL)	8.32	8.73	8.76	8.40	9.18	9.44	9.33	8.97	9.56
Process*	TVCC-1a	TVCC-1a	TVCC-1a	TVCC-1b	TVCC-1b	TVCC-1b	TVCC-1b	TVCC-1b	TVCC-1b
VH Titer (CCD)	8.4	8.4	8.4	8.3	8.6	8.4	8.6	8.0	8.3
VH Titer (PD)	7.7 [^]	8.4	8.6	8.1	8.3	8.3	8.4	7.9	8.3
Final Titer	8.3	8.5	8.5	8.5	8.9	8.6	9.2	9.0	9.7
Overall Yield §	60.9%	27.0%	6.3%	25.4%	22.0%	21.2%	30.4%	56.3%	57.5%
VH DNA (ng/mL)	n/a	7200	5480	n/a	5720	10800	3440	2070	2050
Bulk DNA (ng/dose) PicoGreen	76.7	n/a	36.4	31.9	n/a	n/a	0.4	0.3	0.06
Bulk DNA (ng/dose) PCR	n/a	n/a	0.92	n/a	0.23	0.18	0.032	0.164	n/a
VH HCP (µg/mL)	278	392	269	231	250	233	135	174	74
Bulk HCP (µg/dose)	1.10	n/a	n/a	n/a	n/a	n/a	0.13	0.29	0.14
Benzonase® (ng/mL)	0.9	n/a	0.69	2.1	6.6	3.6	0.27	0.52	LOD

Note: * TVCC-1a: Benzonase® treatment in bag; TVCC-1a: Benzonase® treatment on column

[^] FFA assay based on Anti-NA instead of Anti-HA

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CLAIMS

1. A Madin-Darby Canine Kidney (MDCK) cell identified by ATCC Accession No. PTA-7909 or ATCC Accession No. PTA-7910, wherein a cell culture composition comprising a plurality of the MDCK cells supports replication of an influenza virus to a base 10 logarithm of the median tissue culture infection dose per milliliter ($\log_{10}$ TCID₅₀/mL) of 7.8 or greater or to a base 10 logarithm of fluorescent focus units per milliliter ($\log_{10}$ FFU/mL) of 7.8 or greater.
2. The MDCK cell of claim 1, wherein the MDCK cells are adherent, non-tumorigenic and/or non-oncogenic.
3. The MDCK cell of claim 1, wherein the influenza virus is cold-adapted, attenuated and/or temperature sensitive.
4. The MDCK cell of claim 1, wherein the influenza virus comprises one or more gene segments of influenza strain A/Ann Arbor/6/60 or B/Ann Arbor/1/66.
5. The MDCK cell of claim 1, wherein the composition comprises a serum-free cell culture medium.
6. The MDCK cell of claim 5, wherein the cell culture medium is selected from the group consisting of MediV-105, MediV-105 supplemented with glucose, M-32, M-32 supplemented with glucose, MediV-107 and MediV-107 supplemented with glucose.
7. A method for replicating an influenza virus in a bioreactor, comprising:
 - (a) culturing the Madin-Darby Canine Kidney (MDCK) cells of claim 1 in a bioreactor in the presence of microcarriers under culture conditions that include agitation, thereby producing cultured cells;
 - (b) infecting the cultured cells with a cold-adapted influenza virus, thereby producing infected cells; and
 - (c) incubating the infected cells under conditions that permit replication of the influenza virus.

8. The method of claim 7, wherein the culture conditions are serum-free.
9. The method of claim 8, wherein the culture conditions include a cell culture medium selected from the group consisting of MediV-105, MediV-105 supplemented with glucose, M-32, M-32 supplemented with glucose, MediV-107 and MediV-107 supplemented with glucose.
10. The method of claim 7, wherein the agitation is at a rate of 80 rpm to 120 rpm.
11. The method of claim 10, wherein the agitation rate is 90 rpm to 100 rpm.
12. The method claim 7, wherein fresh medium or additional medium components are added to the cell culture prior to, during, or after step (b).
13. The method of claim 12, wherein the fresh medium or additional medium components comprise a protease.
14. The method of claim 13, wherein the protease is a serine protease.
15. The method of claim 14, wherein the protease is trypsin.
16. The method of claim 7, wherein none or some of the cell culture medium is removed and replaced with fresh medium prior to or during step (b).
17. The method of claim 7, wherein step (b) is carried out at a Multiplicity Of Infection (MOI) of between about 0.00001 FFU/cell to about 0.003 FFU/cell.
18. The method of claim 17, wherein step (b) is carried out at a MOI of between about 0.001 FFU/cell to about 0.003 FFU/cell.
19. The method of claim 7, wherein a protease is added to the bioreactor before, simultaneously with, or after step (b).
20. The method of claim 19, wherein the protease is a serine protease.

21. The method of claim 20, wherein the protease is trypsin.
22. The method of claim 7, wherein the bioreactor is a single-use bioreactor (SUB).
23. The MDCK cell of claim 1, wherein the cell is identified by ATCC Accession No. PTA-7909.
24. The MDCK cell of claim 1, wherein the cell is identified by ATCC Accession No. PTA-7910.