CLAIMS

1. A method for purifying virus like particles (VLPs), comprising:

   generating or obtaining a cell lysate, culture supernatant, or filtrate containing said VLPs; wherein said VLPs comprise at least a first subpopulation of VLPs comprising VLPs with a full length VP1 subunit and a second subpopulation of VLPs comprising VLPs with a truncated VP1 subunit;

   adjusting the pH of the lysate, supernatant, or filtrate to a pH value less than 5 to generate a pH-adjusted solution; and

   removing non-VLP particulates/aggregates and the second subpopulation of VLPs with a truncated VP1 subunit from the pH-adjusted solution to generate a purified solution, wherein the purified solution substantially retains the first subpopulation of VLPs comprising VLPs with a full length VP1 subunit.

2. The method of claim 1, wherein the VLPs of the cell lysate or culture supernatant/filtrate are norovirus VLPs, wherein the first subpopulation comprises norovirus VLPs with a full length VP1 subunit.

3. The method of claim 2, wherein the ratio of the first subpopulation of VLPs to residual contaminating protein is increased at least two-fold.

4. The method of claim 1, wherein said adjusting the pH is carried out for a duration between 30 minutes and 48 hours.

5. The method of claim 1, wherein the VLPs comprise acid-stable VLPs.

6. The method of claim 1, wherein the adjusted pH of the lysate, supernatant or filtrate is less than 3.

7. The method of claim 1, wherein the adjusted pH of the lysate, supernatant or filtrate is less than 4.

8. The method of claim 1, wherein said removing is done via one or more of the following types of processes: centrifugation, precipitation, flocculation, settling, and filtration.

9. The method of claim 8, wherein said removing includes clarifying the pH-adjusted solution by depth filtration followed by additional filtration to generate the purified solution.

10. The method of claim 1, wherein at least 65% of residual contaminating nucleic acid is removed from the lysate, supernatant, or filtrate as a result of said purifying.

11. The method of claim 1, wherein at least 98% of residual contaminating nucleic acid is removed from the lysate, supernatant, or filtrate as a result of said purifying.

12. The method of claim 1, further comprising:

purifying the VLPs using a multistep chromatographic process, wherein at least one chromatographic process of the multistep chromatographic process comprises:

contacting the solution with a chromatographic material, wherein the VLPs bind to said chromatographic material;

treating the chromatographic material with a solvent and/or detergent; and

eluting, after said treating, the VLPs from the chromatographic material.

13. The method of claim 12, wherein the solvent and/or detergent comprises one or more of the following: TnBP, octylphenol, ethyleneoxide condensate, polyoxyethylene sorbitan monooleate, and sodium cholate, Triton X-100, and tributyl phosphate.

14. The method of claim 12, wherein the VLPs are one or more of: norovirus genogroup I VLPs, norovirus genogroup II VLPs, norovirus genogroup IV VLPs, chimeric norovirus VLPs, and sapovirus VLPs.

15. The method of claim 12, wherein the ratio of the VLPs to residual contaminating protein is increased at least ten-fold as compared to the lysate, supernatant or filtrate.

16. The method of claim 12, wherein the ratio of the VLPs to residual contaminating protein is increased at least ninety-fold as compared to the lysate, supernatant or filtrate.

17. The method of claim 12, wherein at least 50% of residual contaminating protein is removed from the lysate, supernatant or filtrate as a result of said purifying.

18. The method of claim 12, wherein up to 99% of residual contaminating protein is removed from the lysate, supernatant or filtrate as a result of said purifying.

19. The method of claim 12, wherein no more than 50% of the VLPs are lost during said purification.

20. The method of claim 12, wherein at least 65% of the VLPs are recovered as a result of said purification.

21. The method of claim 12, wherein the multistep chromatographic process comprises the sequential application of cation exchange chromatography and hydroxyapatite chromatography.

22. The method of claim 21 wherein said treating step is performed on a cation exchange column of the cation exchange chromatography process.

23. The method of claim 12, wherein the lysate, supernatant or filtrate is generated using recombinant methodologies.

24. The method of claim 23, wherein the VLPs are produced in insect cells.

25. A method of purifying virus like particles (VLPs), comprising:

generating or obtaining a cell lysate, culture supernatant, or filtrate containing VLPs, wherein said VLPs comprises at least a first subpopulation comprising VLPs

with a full length VP1 subunit and a second subpopulation of the VLPs comprising VLPs with a truncated VP1 subunit;

adjusting the pH of the lysate, supernatant or filtrate to a pH of less than 5 to generate a pH adjusted solution;

removing the second subpopulation of VLPs from the pH-adjusted solution using a filtration process to generate a filtered, purified solution, wherein the filtered, purified solution substantially retains the first subpopulation of VLPs; and

separating the first subpopulation of VLPs from the filtered, purified solution using a multistep chromatographic process.

26. The method of claim 25, wherein at least one chromatographic process of the multistep chromatographic process comprises:

contacting the filtered, purified solution with a chromatographic material, wherein the first subpopulation of VLPs bind to said chromatographic material;

treating the chromatographic material with a solvent and/or detergent; and

eluting the first subpopulation of VLPs from the chromatographic material.

27. The method of claim 26, wherein the ratio of the first population of VLPs to residual contaminating protein is increased at least about two-fold compared to the lysate, supernatant or filtrate.

28. The method of claim 25, wherein the multistep chromatographic process comprises the sequential application of cation exchange chromatography and hydroxyapatite chromatography.

29. The method of claim 25, wherein the VLPs are one or more of: norovirus genogroup I VLPs, norovirus genogroup II VLPs, norovirus genogroup IV VLPs, chimeric norovirus VLPs, and sapovirus VLPs.

30. The method of claim 29, wherein the VLPs are norovirus genogroup I VLPs, wherein the first subpopulation comprises norovirus VLPs with full length VP1 subunit, and wherein the second subpopulation comprises norovirus VLPs with truncated VP1 subunit.

31. The method of claim 25, wherein the lysate, supernatant or filtrate is generated using recombinant methodologies.

32. The method of claim 25, wherein the VLPs are produced in insect cells.

33. The method of claim 25, wherein the adjusted pH of the lysate, supernatant or filtrate is less than 3.

34. The method of claim 25, wherein the adjusted pH of the lysate, supernatant or filtrate is less than 4.

35. The method of claim 25, wherein said adjusting the pH is carried out for a duration between 30 minutes and 48 hours.

36. The method of claim 25, wherein the filtration process includes a depth filtration process.

37. The method of claim 25, further comprising one or more of the following processes following filtration: centrifugation, precipitation, flocculation, settling, and additional filtration.

38. A vaccine comprising the first subpopulation of VLPs prepared by the method of claim 25.

39. A method for purifying virus like particles (VLPs), comprising:

generating or obtaining a cell lysate, culture supernatant, or filtrate containing said VLPs, wherein said VLPs comprise at least a first subpopulation comprising VLPs with a full length VP1 subunit and a second subpopulation comprising VLPs with a truncated VP1 subunit;

adjusting the pH of the lysate, supernatant or filtrate to a pH value less than 5 to generate a pH adjusted solution; and

removing non-VLP particulates/aggregates and the second subpopulation of VLPs from the pH-adjusted solution to generate a purified solution, said removing including clarifying the pH-adjusted solution by a depth filtration process to generate

a purified, depth-filtered solution, wherein the purified solution substantially retains the first subpopulation of VLPs comprising VLPs with a full length VP1 subunit.

40. The method of claim 39, wherein the depth filtration process comprises contacting the pH-adjusted solution with one or more depth filters.

41. The method of claim 39, wherein at least one of the depth filters is a diatomaceous earth depth filter.

42. The method of claim 40, wherein the one or more depth filters are a plurality of diatomaceous earth depth filters each having a filter capacity independently selected from a range of about 50 liters/m^2 to about 1000 liters/m^2.

43. The method of claim 39, wherein the one or more depth filters is a single depth filter each having a filter capacity selected from a range of 150 liters/m^2 to 400 liters/m^2.

44. The method of claim 39, wherein at least 99% of residual contaminating nucleic acid is removed from the pH-adjusted solution as compared to the lysate, supernatant or filtrate.

45. The method of claim 39, wherein at least 95% of residual contaminating nucleic acid is removed from the pH-adjusted solution as compared to the lysate, supernatant or filtrate.

46. The method of claim 39, wherein at least 75% of the VLPs are recovered from the pH-adjusted solution as compared to the lysate, supernatant or filtrate.

47. The method of claim 39, wherein 100% of the VLPs are recovered from the pH-adjusted solution as compared to the lysate, supernatant or filtrate.

48. The method of claim 39, said removing further comprising separating the first subpopulation of VLPs from the depth-filtered, purified solution using the sequential application of cation exchange chromatography and hydroxyapatite chromatography.

49. The method of claim 47, wherein the VLPs are one or more of: norovirus genogroup I VLPs, norovirus genogroup II VLPs, norovirus genogroup IV VLPs, chimeric norovirus VLPs, and sapovirus VLPs.

50. The method of claim 49 wherein the VLPs are norovirus genogroup I VLPs, wherein the first subpopulation comprises norovirus VLPs with full length VP1 subunit, and wherein the second subpopulation comprises norovirus VLPs with truncated VP1 subunit.

51. The method of claim 39, wherein the cell lysate or the culture supernatant/filtrate is generated using recombinant methodologies.

52. The method of claim 39, wherein the VLPs are produced in insect cells.

53. A solution comprising a purified population of norovirus VLPs, wherein said solution contains no more than 80% residual contaminating protein relative to VLP as measured by size-exclusion chromatography and ELISA, and wherein said solution is generated by a process comprising:

generating or obtaining a cell lysate, culture supernatant, or filtrate containing the VLPs, wherein said norovirus VLPs comprise at least a first subpopulation of VLPs comprising VLPs with a full length VP1 subunit and a second subpopulation of VLPs comprising VLPs with a truncated VP1 subunit;

adjusting the pH of the lysate, supernatant or filtrate to a pH of less than 5 to generate a pH-adjusted solution;

removing the second subpopulation with truncated VP1 subunit of VLPs from the pH-adjusted solution to generate a purified solution, wherein the purified solution substantially retains the first subpopulation of VLPs comprising VLPs with a full length VP1 subunit; and separating the first subpopulation of VLPs from the filtered, purified solution.

54. A vaccine comprising the norovirus VLPs prepared from the solution of claim 53.

55. The solution of claim 53, containing no more than 35% residual contaminating nucleic acid relative to the lysate, supernatant or filtrate.

56. The solution of claim 53, wherein at least 50% of the norovirus VLPs are recovered from the lysate, supernatant or filtrate.

57. The solution of claim 53, wherein the ratio of the norovirus VLPs to residual contaminating protein is increased at least ten-fold as compared to the lysate, supernatant or filtrate.

58. The solution of claim 53, wherein the solution is for use in a commercial process, said commercial process having a processing volume of at least 20 liters.

59. The solution of claim 53, generated by one or more of the following processes: pH treatment, centrifugation, precipitation, flocculation, settling, filtration, and chromatography.

60. The solution of claim 59, wherein the process further comprises:

separating the first subpopulation of norovirus VLPs from the filtered, purified solution using a multistep chromatographic process.

61. The solution of claim 60, wherein the norovirus VLPs are norovirus genogroup I VLPs, wherein the first subpopulation comprises norovirus genogroup I VLPs with full length VP1 subunit, and wherein the second subpopulation comprises norovirus genogroup I VLPs with truncated VP1 subunit.

62. The solution of claim 60, wherein the norovirus VLPs are norovirus genogroup II VLPs, wherein the first subpopulation comprises norovirus genogroup II VLPs with full length VP1 subunit, and wherein the second subpopulation comprises norovirus genogroup II VLPs with truncated VP1 subunit.

63. A solution comprising a purified population of VLPs, wherein said solution contains at least 50% VLPs with a full length VP1 subunit, and less than 10% VLPs with a truncated VP1 subunit.

64. The solution of claim 63, containing up to about 100% VLPs with the full length VP1 subunit.

65. The solution of claim 63, wherein the solution is substantially free of VLPs with a truncated VP1 subunit.

66. The solution of claim 63, wherein the truncated VP1 subunit is generated from the unpurified solution includes cell lysate, culture supernatant, or filtrate containing the VLPs.

67. The solution of claim 63, wherein the solution is generated from the unpurified solution by:

adjusting the pH of the unpurified solution to a pH value less than 5 to generate a pH-adjusted solution; and

removing VLPs with a truncated VP1 subunit from the pH-adjusted solution to generate the solution as a purified solution, wherein the pH adjusted solution substantially retains the VLPs with the full length VP1 subunit.

68. The solution of claim 67, wherein said removing is done via one or more of the following processes: centrifugation, precipitation, flocculation, settling, and filtration.

69. A purified population of VLPs with a full length VP1 subunit, generated by applying one or more chromatographic processes to the solution of claim 63.

70. A method of clearing and/or inactivating live virus in a solution containing the live virus and further containing VLPs generated by the live virus, wherein said VLPs comprise at least a first subpopulation comprising VLPs with a full length VP1 subunit and a second subpopulation comprising VLPs with a truncated VP1 subunit, the method comprising

adjusting the pH of the solution containing the live virus to a pH value less than 5 to generate a pH adjusted solution; and

removing non-VLP particulates/aggregates and the second subpopulation of VLPs with a truncated VP1 subunit from the pH-adjusted solution to generate a

purified solution comprising the VLPs, wherein the purified solution substantially retains the first subpopulation of VLPs with a full length VP1 subunit.

71. The method of claim 70, wherein the level of live virus in the pH-adjusted solution is reduced at least about 10^4 fold.

72. The method of claim 70, wherein the level of live virus in the pH-adjusted solution is reduced at least about 10^5 fold.

73. The method of claim 70, wherein the VLPs are selected from norovirus genogroup I VLPs and norovirus genogroup II VLPs.

74. The method of claim 70, wherein the live virus includes baculovirus.

75. The method of claim 70, further comprising:

purifying the purified solution using at least one chromatographic process, wherein the at least one chromatographic process comprises:

contacting the pH-adjusted solution with a chromatographic material, wherein the VLPs bind to said chromatographic material;

treating the chromatographic material with a solvent and/or detergent; and

eluting, after said treating, the VLPs from the chromatographic material to generation an eluate,

wherein the level of live virus in the eluate is reduced by at least about 10^2 fold compared to the solution containing the live virus.

76. The method of claim 75, wherein the level of live virus in the eluate is reduced at least 10^3 fold compared to the solution containing the live virus.

77. The method of claim 75, wherein the VLPs are selected from norovirus genogroup I VLPs and norovirus genogroup II VLPs.

78. The method of claim 75, wherein the live virus includes baculovirus.

79. A purified VLP composition generated from the eluate of claim 75.

80. A vaccine generated from the eluate of claim 75.

81. A method of clearing and/or inactivating live virus in a solution containing the live virus and further containing VLPs generated by the live virus, wherein said VLPs comprise at least a first subpopulation of VLPs comprising VLPs with a full length VP1 subunit and a second subpopulation of VLPs comprising VLPs with a truncated VP1 subunit, the method comprising adjusting the pH of the solution to a pH value less than 5 to generate a pH-adjusted solution, wherein the second subpopulation of VLPs with truncated VP1 subunit are removed to generate a purified solution comprising VLPs with a full length VP1 subunit, wherein the method further comprising purifying the solution containing the live virus using at least one chromatographic process, wherein the at least one chromatographic process comprises:

contacting the solution containing the live virus with a chromatographic material, wherein the VLPs bind to said chromatographic material;

treating the chromatographic material with a solvent and/or detergent; and

eluting, after said treating, the VLPs from the chromatographic material to generation an eluate.

82. The method of claim 81, wherein the level of live virus in the eluate is reduced at least 10^5 fold compared to the solution containing the live virus.

83. The method of claim 81, wherein the level of live virus in the eluate is reduced at least 10^7 fold compared to the solution containing the live virus.

84. The method of claim 81, wherein the VLPs are selected from norovirus genogroup I VLPs and norovirus genogroup II VLPs.

85. The method of claim 81, wherein the live virus includes baculovirus.