CLAIMS

1. A method of isolating disease-related extracellular microvesicles from a biological fluid, in which the disease-related extracellular microvesicles have negatively-charged phosphatidylserine on their surface; the method comprising contacting a sample of the biological fluid with an acetate buffer at a pH and concentration effective to precipitate disease-related extracellular microvesicles from the biological fluid and collecting disease-related extracellular microvesicles from the precipitate, thereby isolating the disease-related extracellular microvesicles.

2. A method according to Claim 1, in which the phosphatidylserine on the surface of the disease-related extracellular microvesicles is present in association with non-lipid membrane components, and in which the non-lipid membrane components comprise membrane proteins.

3. A method according to Claim 1 or 2, in which the acetate buffer neutralizes the surface charge of the phosphatidylserine on the disease-related extracellular microvesicles, thereby precipitating the disease-related extracellular microvesicles from the biological fluid.

4. A method according to any preceding claim, in which the biological fluid contains a mixed population of extracellular microvesicles comprising disease-related and normal extracellular microvesicles, and in which the method selectively precipitates the disease-related extracellular microvesicles from the mixed population, as opposed to the normal extracellular microvesicles.

5. A method according to any preceding claim, in which the disease-related extracellular microvesicles are isolated without substantially damaging their morphological or functional properties or cell surface antigens.

6. A method according to any preceding claim, in which the extracellular microvesicles are exosomes.

7. A method according to any of Claims 1 to 6, in which the disease-related extracellular microvesicles are derived from a cell infected with an intracellular parasite.

8. A method according to any of Claims 1 to 6, in which the disease-related extracellular microvesicles are derived from a virally-infected cell.

9. A method according to Claim 8, in which the method isolates disease-related extracellular microvesicles from a virally-infected cell substantially free from infectious virus.

10. A method according to any of Claims 1 to 6, in which the disease-related extracellular microvesicles are tumor-derived extracellular microvesicles.

11. A method according to Claim 10, in which the tumor-derived extracellular microvesicles are derived from melanoma, colorectal cancer, lung cancer, pancreatic cancer, liver cancer, prostate cancer, breast cancer or ovarian cancer.

12. A method according to Claim 10 or 11, in which the tumor-derived extracellular microvesicles are tumor-derived exosomes.

13. A method according to any preceding claim, in which the disease-related extracellular microvesicles are human extracellular microvesicles.

14. A method according to any preceding claim, in which the acetate buffer has a pH of between about 4.25 and about 5.25.

15. A method according to Claim 14, in which the acetate buffer has a pH of between about 4.5 and about 5.0.

16. A method according to Claim 15, in which the acetate buffer has a pH of about 4.75.

17. A method according to any preceding claim, in which the acetate buffer has a final concentration in the sample of between about 0.05M and about 0.25M.

18. A method according to Claim 17, in which the acetate buffer has a final concentration in the sample of between about 0.05M and about 0.1M.

19. A method according to Claim 17, in which 1/10th volume of 1.0M acetate buffer is added to the sample.

20. A method according to any preceding claim, in which the acetate buffer has a pH of between about 4.25 and about 5.25 and a final concentration in the sample of between about 0.05M and about 0.25M.

21. A method according to any preceding claim, in which the acetate buffer has a pH of between about 4.5 and about 5.0 and a final concentration in the sample of between about 0.05M and about 0.1M.

22. A method according to any preceding claim, in which the acetate buffer has a pH of about 4.75 and a final concentration in the sample of about 0.1M.

23. A method according to any preceding claim, in which the acetate buffer comprises sodium acetate.

24. A method according to any preceding claim, in which the acetate buffer comprises potassium acetate.

25. A method according to any preceding claim, in which the acetate buffer is essentially free from volume excluding polymers.

26. A method according to preceding claim, in which the acetate buffer is essentially free from polyethylene glycol.

27. A method according to any preceding claim, in which the acetate buffer is sodium acetate with a pH of about 4.75 and a final concentration in the sample of about 0.1M.

28. A method according to any preceding claim, in which the biological fluid is a murine, bovine or human biological fluid.

29. A method according to any preceding claim, in which the biological fluid is a cell culture supernatant, whole blood, serum, plasma, ascites fluid, cerebrospinal fluid, bone marrow aspirate, bronco-alveolar washing, urine, semen, vaginal fluid, mucous, saliva, sputum or a clarified lysate from a biological tissue sample.

30. A method according to Claim 29, in which the biological fluid is a cell culture supernatant, serum, plasma or ascites fluid.

31. A method according to Claim 29, in which the biological fluid is a cleared cell culture supernatant that has already been subjected to low-speed centrifugation.

32. A method according to Claim 29, in which the biological fluid is a cell culture supernatant obtained from tumor cells cultured in the presence of serum that contains non-tumor extracellular microvesicles.

33. A method according to Claim 29, in which the biological fluid is a cell culture supernatant obtained from human tumor cells cultured in the presence of non-human serum.

34. A method according to Claim 29, in which the biological fluid is a cell culture supernatant obtained from mouse or human tumor cells cultured in the presence of bovine or fetal bovine serum.

35. A method according to any preceding claim, further comprising the step of subjecting the biological fluid to low-speed centrifugation to remove cells, cell debris and large membrane vesicles prior to contact with the acetate buffer.

36. A method according to any preceding claim, in which the precipitate that contains the disease-related extracellular microvesicles after contact with the acetate buffer is collected by low-speed centrifugation.

37. A method according to any preceding claim, further comprising re-suspending the collected precipitate containing the disease-related extracellular microvesicles in a substantially acetate-free buffer at about neutral pH to provide an isolated population of disease-related extracellular microvesicles.

38. A method according to Claim 37, further comprising centrifuging the isolated population of disease-related extracellular microvesicles to remove contaminating components, thereby providing an essentially pure composition of disease-related extracellular microvesicles.

39. A method according to Claim 12, in which the method comprises the steps of:

obtaining a biological fluid containing a mixed population of exosomes that includes tumor-derived exosomes and non-tumor exosomes;

contacting the biological fluid with an acetate buffer at a pH and concentration effective to selectively precipitate tumor-derived exosomes, but not non-tumor exosomes, from the biological fluid;

collecting the precipitate ~~from step (b)~~; in which the precipitate selectively contains the tumor-derived exosomes; and

re-suspending the precipitate in a substantially acetate-free buffer at about neutral pH, thereby providing a purified population of tumor-derived exosomes essentially free from non-tumor exosomes.

40. A method according to Claim 39, in which the method comprises:

obtaining a biological fluid containing a mixed population of exosomes that includes tumor-derived exosomes and non-tumor exosomes;

performing a first low-speed centrifugation on the biological fluid to provide a clarified fluid essentially free from cells, cell debris and large membrane vesicles;

incubating the clarified fluid with an acetate buffer at a pH and concentration effective, and for a time effective, to provide a turbid suspension comprising selectively precipitated tumor-derived exosomes, but substantially no precipitated non-tumor exosomes;

subjecting the turbid suspension to a low-speed centrifugation to provide a precipitate and a supernatant; in which the precipitate selectively comprises the tumor-derived exosomes;

collecting the precipitate comprising the tumor-derived exosomes;

re-suspending the precipitate in a substantially acetate-free buffer at about neutral pH, thereby providing a purified exosome population that comprises tumor-derived exosomes and is essentially free from non-tumor exosomes; and, optionally

subjecting the purified exosome population to a further centrifugation to provide a pellet comprising non-exosome components and removing the pellet, thereby providing an essentially pure composition of tumor-derived exosomes that is substantially free from both non-tumor exosomes and non-exosome components.

41. A method according to any preceding claim, further comprising identifying or quantifying at least a first biomarker in the disease-related extracellular microvesicles.

42. A method according to Claim 41, in which the biomarker is an RNA, micro RNA, DNA or protein biomarker.

43. A method to obtain disease-related extracellular microvesicles from the supernatant of diseased cells cultured in the presence of serum that contains normal extracellular

microvesicles, in which the disease-related extracellular microvesicles have negatively-charged phosphatidylserine on their surface; the method comprising contacting the supernatant with an acetate buffer at a pH and concentration effective to selectively precipitate disease-related extracellular microvesicles from the supernatant and collecting the precipitate, thereby obtaining disease-related extracellular microvesicles from the supernatant without substantial contamination from normal extracellular microvesicles in the serum.

44. A method according to Claim 43, in which the diseased cells are mouse or human tumor cells cultured in the presence of bovine or fetal bovine serum.

45. A method to prepare serum that is substantially free from disease-related extracellular microvesicles, in which the disease-related extracellular microvesicles have negatively-charged phosphatidylserine on their surface; the method comprising the steps of:

obtaining serum suspected of containing disease-related extracellular microvesicles;

contacting the serum with an acetate buffer at a pH and concentration effective to precipitate disease-related extracellular microvesicles from the serum; and

removing from the serum the precipitate formed ~~in step (b)~~, in which the precipitate contains the disease-related extracellular microvesicles, thereby providing a serum that is substantially free from disease-related extracellular microvesicles.

46. A method according to Claim 43 or 45, in which the disease-related extracellular microvesicles are derived from virally-infected cells.

47. A method according to Claim 43 or 45, in which the disease-related extracellular microvesicles are tumor-derived exosomes.

48. A method to prepare blood, serum or plasma that is substantially free from infectious virus and extracellular microvesicles derived from virally-infected cells, comprising the steps of:

obtaining blood, serum or plasma suspected of containing infectious virus and extracellular microvesicles derived from virally-infected cells;

contacting the blood, serum or plasma with an acetate buffer at a pH and concentration effective to inactivate or precipitate the infectious virus and to precipitate the extracellular microvesicles from the blood, serum or plasma; and

removing from the blood, serum or plasma the precipitate formed ~~in step (b)~~, in which the precipitate contains the inactivated or precipitated virus and the precipitated extracellular microvesicles, thereby providing blood, serum or plasma that is substantially free from infectious virus and extracellular microvesicles derived from virally-infected cells.

49. A method according to Claim 48, in which the method is performed prior to a blood transfusion.

50. A method to detect disease-related extracellular microvesicles in a clarified biological fluid, in which the disease-related extracellular microvesicles have negatively-charged phosphatidylserine on their surface; the method comprising contacting the clarified biological fluid with an acetate buffer at a pH and concentration effective to selectively precipitate disease-related extracellular microvesicles from the clarified biological fluid and determining the presence of turbidity in the resultant biological fluid; in which the presence of turbidity in the resultant biological fluid indicates the detection of disease-related extracellular microvesicles.

51. A method according to Claim 50, in which the presence of turbidity in the resultant biological fluid is detected visually.

52. A method according to Claim 50, in which the disease-related extracellular microvesicles are tumor-derived exosomes.

53. A method according to Claim 52, further comprising isolating the tumor-derived exosomes from the resultant biological fluid with detectable turbidity.

54. A method according to Claim 53, in which the tumor-derived exosomes are isolated from the resultant biological fluid with detectable turbidity by collecting the precipitate using low-speed centrifugation.

55. A method to diagnose a patient having a first disease characterized by the presence of disease-related extracellular microvesicles that have negatively-charged phosphatidylserine on their surface; the method comprising detecting the presence of the disease-related extracellular microvesicles in a biological fluid from the patient, thereby diagnosing the patient as having the first disease; in which the disease-related extracellular microvesicles are detected by a method comprising:

contacting the biological fluid with an acetate buffer at a pH and concentration effective to selectively precipitate the disease-related extracellular microvesicles from the biological fluid; and

determining the presence of the precipitate in the resultant biological fluid; in which the presence of the precipitate in the resultant biological fluid indicates the detection of the disease-related extracellular microvesicles.

56. A method according to Claim 55, in which the biological fluid is a clarified biological fluid and in which the presence of the precipitate in the resultant biological fluid is determined by the presence of turbidity in the resultant biological fluid.

57. A method according to Claim 55, in which the amount of the disease-related extracellular microvesicles is quantified.

58. A method to monitor ~~the~~ a disease ~~burden of~~ in a patient ~~having a first~~, the disease characterized by ~~the~~ an amount of disease-related extracellular microvesicles that have negatively-charged phosphatidylserine on their surface; the method comprising measuring the amount of the disease-related extracellular microvesicles in a biological fluid from the patient; in which the amount of the disease-related extracellular microvesicles is measured by a method comprising:

contacting the biological fluid with an acetate buffer at a pH and concentration effective to selectively precipitate the disease-related extracellular microvesicles from the biological fluid; and

measuring the amount of the disease-related extracellular microvesicles in the precipitate.

59. A method according to Claim 55 or 58, in which the identity of the first disease is confirmed by testing for a biomarker or clinical sign of the first disease.

60. A method according to Claim 55 or 58, in which the first disease is a viral infection.

61. A method according to Claim 55 or 58, in which the first disease is cancer and in which the disease-related extracellular microvesicles are tumor-derived exosomes.

62. A method according to Claim 61, in which the amount of tumor-derived exosomes is measured in a series of biological fluid samples obtained from the patient at a plurality of time

points; and in which an increase in the amount of tumor-derived exosomes is indicative of an increased tumor-burden and a decrease in the amount of the tumor-derived exosomes is indicative of a decreased tumor-burden.