CLAIMS

What is currently claimed is:

1. An assay system configured to at least one of detect, enumerate, and characterize one or more parasite oocyst or egg from at least one fecal sample or a plurality of samples, comprising:

a ferrofluidic assay device configured to receive a ferrofluidic cartridge;

the ferrofluidic cartridge including a plurality of windows;

an imager configured to image each window of the cartridge either separately or together, wherein detecting, enumerating, and characterizing one or more parasite oocyst or egg is determined by an analysis of one or more images;

a controller configured to control at least one of the ferrofluidic assay device, the ferrofluidic cartridge, and the imager;

and

assay processing components comprising at least one of reagents, and controls

wherein the system is configured to at least one of: separate any and all of parasite oocysts or eggs; move or otherwise locate the parasite oocysts or eggs to one or more of the windows; detect, enumerate/count, and/or characterize the parasite oocysts or eggs.

2. The system of claims 1, wherein any parasite oocyst or egg contained within the plurality of samples are at least one of:

labeled and visualized by fluorescence, and

unlabeled and visualized by respective intrinsic auto-fluorescence.

3. The system of any of claims 1-2, wherein:

the samples are mixed with ferrofluid prior to being flowed past at least one window; and

the device establishes at least one electromagnetic field to act on the ferrofluid so as to direct at least one of parasite oocysts or eggs contained in the sample to a specific area or location.

4. The system of any of claims 1-4, wherein the ferrofluid includes at least one density modifying agents.

5. The system of claim 4, wherein the density modifying agent comprises at least one of a concentrated sugar solution, and Sheather's solution.

6. The system of any of claims 1-5, where each sample is at least one of processed and mixed with one or more of sodium hydroxide, a sodium hydroxide solutionand/or detergents.

7. The system of claim 6, wherein the sodium hydroxide is mixed with each sample by mixing equal volumes of sample and sodium hydroxide solution, mixing a 1:2 volume:volume of sample and sodium hydroxide solution, mixing a 1:3 volume:volume of sample and sodium hydroxide solution, mixing a 1:4 volume:volume of sample and sodium hydroxide solution, mixing a 1:5 volume:volume of sample and sodium hydroxide solution, mixing a 2:1 volume:volume of sample and sodium hydroxide solution, mixing a 3:1 volume:volume of sample and sodium hydroxide solution, mixing a 4:1 volume:volume of sample and sodium hydroxide solution, or mixing a 5:1 volume:volume of sample and sodium hydroxide solution, and optionally incubating the mixture prior to flowing the mixture over a capture region of the system.

8. The system of claims 6 or 7, wherein the concentration of sodium hydroxide is: between 0.01 molar (M) and 2M, between 0.2M and 1.2M, or at about 1M.

9. The system of claims 7 or 8, wherein mixture is incubated for less than 45 minutes, between 1-45 minutes, less than 60 minutes, between 1-60 minutes, about 15 minutes, about 30 minutes, about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 12 hours, or between 1-24 hours prior to the samples being analyzed.

10. The system of any of claims 3-9, wherein the samples are added to ferrofluid at a predetermined ratio, e.g., 1 part ferrofluid to 14 parts sample, 1 part ferrofluid to 10 parts sample, 1 part ferrofluid to 9 parts sample, 1 part ferrofluid to 8 parts sample, 1 part ferrofluid to 7 parts sample, 1 part ferrofluid to 6 parts sample, 1 part ferrofluid to 5 parts sample, 1 part ferrofluid to 4 parts sample, 1 part ferrofluid to 3 parts sample, 1 part ferrofluid to 2 parts sample, 1 part ferrofluid to 1 part sample, or 2 parts ferrofluid to 1 part sample.

11. The system of any of claims 1-10, further comprising at least one of a hemocytometer and a McMaster chamber.

12. The system of claim 11, where at least one of the hemocytometer and McMaster chamber are configured to count the number of parasite oocysts and/or eggs, characterize the parasite oocysts and/or eggs, and/or permit the fluorescence excitation/emission of labeled parasite oocysts and/or eggs in the samples using wavelengths specific to the fluorophore used.

13. The system of any of claims 1-12, wherein the system further comprises a capture region adjacent to each window, configured to capture one or more predetermined parasites and/or oocysts; wherein each capture region is coated with a binding agent configured to bind with surface features on at least one of the parasite oocysts and/or eggs.

14. The system of claims 12 or 13, wherein the binding agent comprises at least one of an antibody, aptamer, lectin, and mucin.

15. The system of any of claims 12-14, wherein the binding agent is bound to the capture region via at least one of avidin, streptavidin, neutravidin, and any other modified binder.

16. The system of any one of the preceding claims, wherein the system is configured to flow at least one of the plurality of samples into cartridge for a predetermined period of time.

17. The system of claim 16, wherein after the predetermined period of time, the system is configured to stop the flow of the at least one sample into the cartridge, and the captured parasite oocysts and/or eggs are counted.

18. The system of claim 17, wherein the system is additionally configured to characterize the counted parasite oocysts and/or eggs.

19. The system of any of claims 1-18, wherein each sample is exposed to at least one fluorophore or fluorophore labeled agent.

20. The system of claim 19, wherein exposing each sample to at least one fluorophore or fluorophore labeled agent is configured such that at least one of SYBR, fluorescent labeled lectins, fluorescent labeled antibodies, acid fast stains, membrane stains, and fluorophore labeled in-situ-hybridization probes can be visualized.

21. The system of any of claims 13-20, wherein parasite oocysts and/or eggs are labeled after capture in a capture region/zone.

22. The system of any claims 1-21 wherein parasite oocysts and/or eggs are pre-labeled prior to processing.

23. The system of any of claims 1-22, wherein the system is configured to characterize parasites by at least one of size, shape, and other morphological parameter.

24. The system of any of claims 1-23, wherein the system is further configured to characterize a level of sporulation of individual oocyst contained in at least one sample.

25. The system of any of claims 1-24, wherein the system is further configured to determine a state of oocyst sporulation.

26. The system of any of claims 1-25, wherein the system is further configured to visualize a level of sporulation of individual oocysts contained in at least one sample.

27. The system of claim 26, wherein sporulation is visualized by exposing the at least one sample with at least one staining agent, the at least one staining agent may comprise a DNA intercalating dye, and wherein the DNA intercalating dye can comprise SYBR.

28. The system of any of claims 1-27, wherein the oocysts and/or eggs are from the parasite Coccidia.

29. The system of claim 28, wherein the coccidia comprises at least one of genera Toxoplasma, Cryptosporidium, Cyclospora, Eimeria, and Cyclospora.

30. The system of claim 29, wherein the coccidia comprises Eimeria.

31. A method for detecting parasitic infection in a sample in an assay system comprising:

preparing a plurality of fecal samples for analysis, wherein preparing comprises, for each sample:

labeling at least each of a first tube and a second tube

placing a predetermined amount of sample within the first tube;

placing a predetermined amount of NaOH at a pre-determined concentration in the first tube;

vortexing the first tube for a first predetermined period of time;

placing a predetermined amount of Sheather's sugar solution to the first tube;

vortexing the first tube for a second predetermined period of time;

optionally removing an amount of the sample from the first tube and determining a reference count of oocysts;

transferring a third predetermined amount of each fecal-NaOH mixture of each first tube into a respective second tube;

adding a fourth predetermined amount of ferrofluid and a fifth predetermined amount of SYBR into each respective second tube;

vortexing each respective second tube for a third predetermined amount of time

add the vortexed samples to lanes of an assay cartridge having a plurality of channels/lanes, each channel configured with at least one capture region/zone having at least one unique agent for binding with at least one specific parasite oocyst or egg;

flowing the mixture over at least one of the channels of the cartridge, wherein electromagnetic fields produced by the system and acting on the ferrofluid push up oocysts contained in the at least one sample to a capture region proximate a channel window provided for each channel;

scanning each window of each channel to produce a respective scan image thereof;

processing each respective scan image so as to at least one of identify and count each oocyst imaged from a respective channel window;

converting each count from each processed image to oocysts per gram of original fecal sample; and

optionally identifying and measuring the major axis of each counted oocyst such that the number of small (<18 µm), medium (18-25µm), and large (>25µm) oocysts are counted for each sample and optionally converted to oocysts per gram of original fecal sample.

32. The method of claim 31, wherein the sample is a fecal sample.

33. The method of claim 31 or 32, wherein the first tube is a vortexing tube, wherein the first tube is a 1 mL to 20 mL vortexing tube, a 5 mL to 10 mL vortexing tube, a 5 mL vortexing tube, or a 10 mL vortexing tube.

34. The method of any one of claim 31-33 wherein the second tube is a 1.7 mL to 2 mL vortexting tube.

35. The method of any one of claim 31-34, wherein the predetermined amount of sample is 0.1 g, 0.2 g, 0.3 g, 0.4 g, 0.5 g, 0.6 g, 0.7 g, 0.8 g, 0.9 g, 1g, or is between 0.1 g and 2 g, between 0.1 g and 1 g, or between 0.2 g and 0.5 g.

36. The method of any one of claim 31-35, wherein the predetermined amount NaOH is 1 mL, 2 mL, 3 mL, 4 mL, 5 mL, or is between 1 mL and 5 mL.

37. The method of any one of claim 31-36, wherein the pre-determined concentration of NaOH is 0.1M, 0.2M, 0.3M, 0.4M, 0.5M, 0.6M, 0.7M, 0.8M, 0.9M, 1M, is between 0.2M and 1M, is between 0.1M and 1M.

38. The method of any one of claims 31-37, wherein the first predetermined period of time is 30 seconds, 45 seconds, or 60 seconds, or is 30 seconds to 60 seconds.

39. The method of any one of claims 31-38, wherein the predetermined amount of Sheather's sugar solution is 1 mL, 2 mL, 3 mL, 4 mL, 5 mL, or is between 1 mL and 5 mL.

40. The method of any one of claims 31-39, wherein the second predetermined period of time is 30 seconds, 45 seconds, or 60 seconds, or is from 30 seconds to 60 seconds.

41. The method of any one of claims 31-40, wherein the third predetermined amount is 200 µL, 210 µL, 220 µL, 230 µL 240 µL, 250 µL, 260 µL, 270 µL, 280 µL, 290 µL, 300 µL, between 200-300 µL, or between 260-300 µL.

42. The method of any one of claims 31-41, wherein the fourth predetermined amount is 10 µL 20 µL, 30 µL, 40 µL, 50 µL, 60 µL or between 10-60 µL of ferrofluid.

43. The method of any one of claims 31-42, wherein the fifth predetermined amount is 1 µL, 2 µL, 3 µL, 4 µL, 5 µL or between 1-5 µL.

44. The method of any one of claims 31-43, wherein the third predetermined amount of time is 30 seconds, 45 seconds, or 60 seconds, or is from 30 seconds to 60 seconds.

45. The method of any one of claims 31-44, wherein 200 µL, 210 µL, 220 µL, 230 µL 240 µL, 250 µL, 260 µL, 270 µL, 280 µL, 290 µL, 300 µL, between 200-300 µL, or between 260-300 µL of the vortexed samples are added to lanes of the assay cartridge.

46. A method for detecting parasitic infection in fecal samples in an assay system comprising:

optionally placing a predetermined amount of animal fecal matter into a first side ("fecal side") of at least a two-sided sample filter bag ("sample bag");

adding a predetermined amount of NaOH to the first side of the sample bag forming a first mixture;

performing a first homogenization of the mixture, via, for example, massaging the mixture for a first predetermined period of time;

incubating the mixture for a second predetermined period of time;

adding in a predetermined amount of Sheather's solution to a second side of the sample bag ("filtered side");

performing a second homogenization of the mixture, via, for example, massaging the mixture of a second predetermined period of time;

removing an aliquot of a predetermined amount from the filtered side of the bag;

transferring the aliquot to a tube of predetermined size;

adding a predetermined amount of ferrofluid to the tube;

vortexing the tube for a predetermined period of time;

transferring the contents of the tube to a single sample well of the assay cartridge;

flowing each sample from each sample well into an imaging window;

push all cells to the surface of the imaging zone;

optionally repeating the above-noted steps a plurality of times so as to utilize a corresponding number of sample wells, such that, each sample corresponds to a unique sample well,

optionally flowing each sample from each sample well into at least one imaging/capture zone of the assay cartridge so as to capture at least one specific parasite oocyst or egg contained in the sample;

acquiring image of each imaging zone;

determining a number of parasite oocysts or eggs present in the imaging zone; and

determining a size distribution of the parasite oocysts or eggs captured in the at least one imaging zone.

47. The method of claim 46, wherein the method further comprises adding in a predetermined amount a fluorophore or fluorophore labeled agent to the tube.

48. The method of claim 46 or 47, wherein the parasite ocysts and/or eggs are labeled with at least one fluorophore or fluorophore labeled agent, wherein the fluorophore or fluorophore labeled agent is selected from the group consisting of SYBR, fluorescent labeled lectins, fluorescent labeled antibodies, acid fast stains, membrane stains, or fluorophore labeled in-situ-hybridization probes.

49. The method of any one of claims 46-48, wherein the fluorophore or fluorophore labeled agent is added prior to mixing the sample with ferrofluid.

50. The method of claim 46 where no fluorescent labeling agent is used, and the parasite oocysts and/or eggsare monitored by their intrinsic fluorescence.

51. The method of claim 46-50 wherein the predetermined amount of animal fecal matter is between 0.1 g and 5 g.

52. The method of claim 46 or 51, wherein the predetermined amount of NaOH is 1 mL, 2 mL, 3 mL, 4 mL, 5 mL, or is between 1 mL and 5 mL and the concentration of NaOH is 0.1M, 0.2M, 0.3M, 0.4M, 0.5M, 0.6M, 0.7M, 0.8M, 0.9M, 1M, is between 0.1M and 2M, is between 0.1M and 1M.

53. The method of any one of claims 46-52, wherein the first predetermined period of time is 30 seconds, 1 minute, 5 minutes, 10 minutes, 15 minutes, 30 minutes, or between 30 seconds and 2 minutes, between 1-15 minutes, or between 1 and 30 minutes.

54. The method of any one of claims 46-53, wherein the second predetermined period of time is less than 30 minutes, 1-30 minutes, less than 45 minutes, between 1-45 minutes, less than 60 minutes, between 1-60 minutes, about 15 minutes, about 30 minutes, or about 1 hour.

55. The method of any one of claims 46-54, wherein the predetermined amount of Sheather's sugar solution is 1 mL, 2 mL, 3 mL, 4 mL, 5 mL, or is between 1 mL and 5 mL.

56. The method of any one of claims 46-55, wherein the second predetermined period of time is 30 seconds, 1 minute, 5 minutes, 10 minutes, 15 minutes, 30 minutes, or between 30 seconds and 2 minutes, between 1-15 minutes, or between 1 and 30 minutes.

57. The method of any one of claims 46-56, wherein aliquot of a predetermined amount is 200 µL, 210 µL, 220 µL, 230 µL 240 µL, 250 µL, 260 µL, 270 µL, 280 µL, 290 µL, 300 µL, between 200-300 µL, or between 260-300 µL.

58. The method of any one of claims 46-57, wherein the tube of predetermined size is a 1.7 mL to 2 mL tube.

59. The method of any one of claims 46-58, the predetermined amount of ferrofluid added to the tube is 10 µL 20 µL, 30 µL, 40 µL, 50 µL, 60 µL or between 10-60 µL.

60. The method of any one of claims 46-49 or 51-59, the predetermined amount of concentrated SYBR to the tube is 1 µL, 2 µL, 3 µL, 4 µL, 5 µL or between 1-5 µL.

61. The method of claims 46-60, wherein the method is performed using a microfluidic device.

62. The method of claims 46-61, wherein the method is performed using a ferrofluid-based microfluidic device.

63. The method of claims 31-62 wherein the parasite oocysts or eggs are counted using at least one of a hemocytometer and McMaster chamber.

64. The method of claim 63, where at least one of the hemocytometer and McMaster chamber are configured to cause fluorescence excitation/emission of fluorophore labeled parasite oocysts or eggs in the samples using wavelengths specific to the fluorophore used.

65. The method claims 31-64, wherein the method further comprises flowing at least one of the plurality of samples into cartridge for a predetermined period of time.

66. The method of any one of claims 31-65, wherein the method further comprises counting the oocysts and/or eggs without capture.

67. The method of any one of claims 31-66, wherein the method further comprises characterizing the counted parasite oocysts and/or eggs.

68. The method of any one of claims 31-67, wherein the method further comprises characterizing a level of sporulation of individual oocysts and/or eggs contained in at least one sample.

69. The method of any one of claims 31-68, wherein the method further comprises determining a state of oocyst and/or egg sporulation.

70. The method of any one of claims 31-69, wherein the method further comprises visualizing a level of sporulation of individual oocysts contained in at least one sample.

71. The method of claim 70, wherein sporulation is visualized by exposing the at least one sample with at least one staining agent, the at least one staining agent may comprise a DNA intercalating dye, and wherein the DNA intercalating dye can comprise SYBR.

72. The method, system or device of any one of the preceding claims, further configured to assess a size distribution of Eimeria oocysts in vaccine preparation.

CLAIMS

What is currently claimed is:

1. A method for detecting a parasitic infection in a sample, the method comprising:

adding NaOH to a first side of a two-sided sample filter bag that includes a sample in the first side to form a first mixture;

performing a first homogenization of the first mixture for a first period of time;

incubating the first mixture for a second period of time;

adding a Sheather's solution to a second side of the two-sided sample filter bag;

performing a second homogenization of the first mixture for a third period of time;

removing an aliquot of the first mixture from the second side of the two-sided sample filter bag;

transferring the aliquot to a tube;

adding a ferrofluid to the tube to provide a second mixture;

vortexing the tube for a fourth period of time;

transferring the second mixture from the tube into a single sample well of an assay cartridge;

flowing the second mixture from the single sample well into an imaging window;

pushing cells, if present in the second mixture, to the surface of the imaging window;

acquiring an image of the imaging window;

determining a number of parasite oocysts or eggs present in the imaging window; and

determining a size distribution of parasite oocysts or eggs present in the imaging window.

2. The method of claim 1, further comprising adding in a fluorophore or fluorophore labeled agent to the tube.

3. The method of claim 1 or 2, wherein the parasite ocysts and/or eggs are labeled with at least one fluorophore or fluorophore labeled agent, wherein the fluorophore or

fluorophore labeled agent is selected from the group consisting of SYBR, fluorescent labeled lectins, fluorescent labeled antibodies, acid fast stains, membrane stains, and fluorophore labeled in-situ-hybridization probes.

4. The method of claim 2 or 3, wherein the fluorophore or fluorophore labeled agent is added prior to adding the ferrofluid to the tube.

5. The method of claim 1, wherein no fluorescent labeling agent is used, and the parasite oocysts and/or eggs are monitored by their intrinsic fluorescence.

6. The method of any one of claims 1 to 5, wherein the adding step comprises adding NaOH to the first side of the two-sided sample filter bag that comprises 0.1 g to 5 g of the sample.

7. The method of any one of claims 1 to 6, wherein the adding step comprises adding 1 mL to 5 mL of NaOH having a concentration of 0.1M to 1M to the first side.

8. The method of any one of claims 1 to 7, wherein the first period of time is in a range of 1 minute to 30 minutes.

9. The method of any one of claims 1 to 8, wherein the second period of time is in a range of 1 minute to 60 minutes.

10. The method of any one of claims 1 to 9, wherein adding the Sheather's solution comprises adding 1 mL to 5 mL of the Sheather's solution to the second side of the two-sided sample filter bag.

11. The method of any one of claims 1 to 10, wherein the third period of time is in a range of 1 to 30 minutes.

12. The method of any one of claims 1 to 11, wherein the aliquot has a volume of 200 µL to 300 µL.

13. The method of any one of claims 1 to 12, wherein the tube is a 1.7 mL to 2 mL tube.

14. The method of any one of claims 1 to 13, wherein adding the ferrofluid to the tube comprises adding 10 µL to 60 µL of the ferrofluid to the tube.

15. The method of any one of claims 2-14, wherein the fluorophore or fluorophore labeled agent is added to the tube in an amount of 1-5 µL.

16. The method of any one of claims 1 to 15, wherein the method is performed using a microfluidic device.

17. The method of any one of claims 1 to 16, wherein determining the number of parasite oocysts or eggs comprising counting the parasite oocysts or eggs using a hemocytometer and/or a McMaster chamber.

18. The method of claim 17, wherein the hemocytometer and/or the McMaster chamber are configured to cause fluorescence excitation/emission of fluorophore labeled parasite oocysts or eggs in the samples using wavelengths specific to the fluorophore used.

19. The method of any one of claims 1 to 18, further comprising flowing a plurality of samples into the assay cartridge for a predetermined period of time.

20. The method of any one of claims 1 to 19, wherein determining the number of parasite oocysts or eggs comprises counting the parasite oocysts and/or eggs without capture.

21. The method of claim 20, further comprising characterizing the counted parasite oocysts and/or eggs.

22. The method of any one of claims 1 to 21, further comprising characterizing a level of sporulation of individual parasite oocysts and/or eggs contained in the sample.

23. The method of any one of claims 1 to 22, further comprising determining a state of oocyst and/or egg sporulation.

24. The method of any one of claims 1 to 23, further comprising visualizing a level of sporulation of individual oocysts contained in the sample.

25. The method of claim 24, wherein sporulation is visualized by exposing the sample with at least one staining agent.

26. The method of any one of claims 1 to 25, wherein the sample is a vaccine preparation and determining the size distribution of parasite oocysts or eggs comprises determining a size distribution of Eimeria oocysts.

27. The method of any one of claims 1 to 26, wherein the sample comprises animal fecal matter.

28. An assay system configured to at least one of detect, enumerate, and characterize one or more parasite oocyst or egg from at least one fecal sample or a plurality of samples, comprising:

a ferrofluidic assay device configured to receive a ferrofluidic cartridge;

the ferrofluidic cartridge including a plurality of windows;

an imager configured to image each window of the cartridge either separately or together, wherein detecting, enumerating, and characterizing one or more parasite oocyst or egg is determined by an analysis of one or more images;

a controller configured to control at least one of the ferrofluidic assay device, the ferrofluidic cartridge, and the imager;

and

assay processing components comprising at least one of reagents, and controls

wherein the system is configured to at least one of: separate any and all of parasite oocysts or eggs; move or otherwise locate the parasite oocysts or eggs to one or more of the windows; detect, enumerate/count, and/or characterize the parasite oocysts or eggs.

29. A method for detecting parasitic infection in a sample in an assay system comprising:

preparing a plurality of samples for analysis, wherein preparing comprises, for each sample of the plurality of samples:

labeling each of a first tube and a second tube;

placing a predetermined amount of sample within the first tube;

placing a predetermined amount of NaOH at a pre-determined concentration in the first tube;

vortexing the first tube for a first predetermined period of time;

placing a predetermined amount of Sheather's sugar solution in the first tube to provide a NaOH mixture;

vortexing the first tube for a second predetermined period of time;

optionally removing an amount of the sample from the first tube and determining a reference count of oocysts;

transferring a third predetermined amount of the NaOH mixture from the first tube into a respective second tube;

adding a fourth predetermined amount of ferrofluid and a fifth predetermined amount of SYBR into the second tube;

vortexing the second tube for a third predetermined amount of time to provide a vortexed sample;

adding a portion of the vortexed sample to a lane of an assay cartridge having a plurality of channels/lanes;

flowing the NaOH mixture over at least one of the channels of the cartridge, wherein electromagnetic fields produced by the system and acting on the ferrofluid push up oocysts contained in the at least one sample to a channel window provided for each of the channels;

scanning each window of each channel to produce a respective scan image thereof;

processing each respective scan image so as to at least one of identify and count each oocyst imaged from a respective channel window;

converting each count from each processed image to oocysts per gram of original sample; and

optionally identifying and measuring the major axis of each counted oocyst such that the number of small (<18 μm), medium (18-25μm), and large (>25μm) oocysts are counted for each sample and optionally converted to oocysts per gram of original sample.