agglutination reactions are provided. In another example, improved reagents for agglutination reactions are provided. In another example, improved devices for performing or analyzing agglutination reactions are provided. Additional improvements are also provided and the disclosure includes additional aspects. Multiple improvements disclosed herein may be used together.

In one example, a method for determining the presence of an antibody in a biological sample is provided, wherein the antibody binds selectively to a viral particle, and the method includes the steps of: (a) incubating a mixture of erythrocytes, the viral particle and the biological sample suspected of containing the antibody, under conditions permitting agglutination of the erythrocytes via interaction with the viral particle; and (b) detecting whether agglutination occurs in the mixture, wherein the absence of agglutination indicates the presence of the antibody, and wherein said steps (a) – (b) take place in less than one hour. In one aspect, there is provided a method for determining the presence of an antibody in a biological sample, wherein said antibody binds selectively to a viral particle, the method comprising: (a) incubating a mixture of erythrocytes, the viral particle, and the biological sample suspected of containing said antibody in a single incubation step, under conditions permitting agglutination of the erythrocytes via interaction with said viral particle without, fresh unfixed erythrocytes and without red cell reagent preparation; and (b) detecting whether said agglutination occurs in said mixture, wherein the absence of said agglutination indicates the presence of said antibody, wherein the presence of said agglutination is evidenced by formation of erythrocyte-viral particle clusters, wherein a cluster comprises a contiguous distribution of two or more erythrocytes having center-to-center distances between said erythrocytes of up to two times erythrocyte diameter, and wherein said clusters are captured in an imaging region of an optical device, and wherein said detecting step comprises: (i) capturing a plurality of images of said clusters at different locations of said imaging region with said optical device; (ii) calculating the size of said clusters based on the center-to-center distances between individual erythrocytes captured in each of said images; and (iii) detecting the occurrence of said agglutination based on analysis of said cluster sizes calculated from the plurality of images obtained using the optical device, wherein said steps (a) – (b) take place in less than one hour, and wherein the viral particle is hemagglutinating viral particle. In another aspect, there is provided a method for determining the presence of an antibody in a biological sample, wherein said antibody binds selectively to a viral particle, the method comprising: (a) incubating a mixture of erythrocytes, the viral particle, and the biological sample suspected of containing said antibody in a single incubation step, under conditions permitting agglutination of the erythrocytes via interaction with said viral particle without, fresh unfixed erythrocytes and without red cell reagent preparation; (b) concurrent with or subsequent to (a), providing the mixture in a vessel having an opening at the top of the vessel and interior conical shape at the bottom of the vessel; (c) tilting the vessel such that after tilting, at least a portion of the mixture is closer to the opening at the top of the vessel than prior to the tilting, and wherein at least a portion of the sample remains in the vessel after the tilting; and (d) capturing with the aid of an optical device an image of said mixture, wherein the image of erythrocytes within the tilted vessel may be used to determine the presence or amount of said antibody in the sample, wherein the presence of said agglutination is evidenced by formation of erythrocyte-viral particle clusters, wherein a cluster comprises a contiguous

distribution of two or more erythrocytes having center-to-center distances between said erythrocytes of up to two times erythrocyte diameter, and wherein said clusters are captured in an imaging region of an optical device, and wherein said detecting step comprises: (i) capturing a plurality of images of said clusters at different locations of said imaging region with said optical device; (ii) calculating the size of said clusters based on the center-to-center distances between individual erythrocytes captured in each of said images; and (iii) detecting the occurrence of said agglutination based on analysis of said cluster sizes calculated from the plurality of images obtained using the optical device, and wherein the viral particle is hemagglutinating viral particle. In another related but separate example, the method includes (a) incubating a mixture of erythrocytes, a viral particle, and a biological sample suspected of containing the antibody, under conditions permitting agglutination of the erythrocytes via interaction with the viral particle; and (b) capturing with the aid of an optical device an image of the mixture, wherein the presence of an erythrocyte –viral particle cluster in the image indicates the occurrence of agglutination and lack of detectable amount of the antibody, and wherein the absence of the cluster indicates the lack of agglutination and the presence of a detectable amount of the antibody. In some examples, a microscopic image of the mixture is obtained.

In another example, a method for determining the presence of a viral particle in a biological sample is provided, wherein the method includes: (a) incubating a mixture of erythrocytes or modified red blood cells, a biological sample suspected of containing the viral particle, under conditions permitting agglutination of the erythrocytes via interaction with the viral particle; and (b) detecting whether agglutination occurs in the mixture, wherein the presence of agglutination indicates the presence of the viral particle, and wherein steps

[0008] In another aspect, the present invention refers to a method for determining the effective immunization of a subject, comprising: (a) incubating a mixture of erythrocytes, a viral particle, and a biological sample obtained from the subject that has been immunized with a first dosage of a first vaccine against the viral particle in a single incubation step, under conditions permitting agglutination of the erythrocytes via interaction with said viral particle without, fresh unfixed erythrocytes and without red cell preparation; and (b) determining the concentration of an antibody against said virus in said sample based on the clusters formed by the agglutination of the erythrocytes, wherein the presence of said agglutination is evidenced by formation of erythrocyte-viral particle clusters, wherein a cluster comprises a contiguous distribution of two or more erythrocytes having center-to-center distances between said erythrocytes of up to two times erythrocyte diameter, and wherein said clusters are captured in an imaging region of an optical device, and wherein a detecting step comprises: (i) capturing a plurality of images of said clusters at different locations of said imaging region with said optical device; (ii) calculating the size of said clusters based on the center-to-center distances between individual erythrocytes captured in each of said images; and (iii) detecting the occurrence of said agglutination based on analysis of said cluster sizes calculated from the plurality of images obtained using the optical device, and wherein said steps (a) – (b) take place in less than one hour, and wherein the viral particle is hemagglutinating viral particle. In another example, a method for determining the effective immunization of a subject is provided, including: (a) obtaining a biological sample from a subject who has been immunized with a first dosage of a first vaccine against a viral particle; (b) incubating a mixture of erythrocytes, the viral particle, and the biological sample, under conditions permitting agglutination of the erythrocytes via interaction with the viral particle; and (c) determining the concentration of an antibody against the virus in the sample based on the clusters formed by the agglutination of the erythrocytes, and wherein said steps (b) – (c) take place in less than one hour. In another aspect, the present invention refers to a method for determining the effective immunization of a subject, comprising: (a) incubating a mixture of erythrocytes, a viral particle, and a biological sample obtained from the subject that has been immunized with a first dosage of a first vaccine against the viral particle in a single incubation step, under conditions permitting agglutination of the erythrocytes via interaction with said viral particle without, fresh unfixed erythrocytes and without red cell preparation; (b) capturing with the aid of an optical device an image of said mixture; and (c) determining the concentration of an antibody against said viral in said biological sample based on the clusters formed by the agglutination of the erythrocytes, wherein the presence of said agglutination is evidenced by formation of erythrocyte-viral particle clusters, wherein a cluster comprises a contiguous distribution of two or more erythrocytes having center-to-center distances between said erythrocytes of up to two times erythrocyte diameter, and wherein said clusters are captured in an imaging region of an optical device, and wherein a detecting step comprises: (i) capturing a plurality of images of said clusters at different locations of said imaging region with said optical device; (ii) calculating the size of said clusters based on the center-to-center distances between individual erythrocytes captured in each of said images; and (iii) detecting the occurrence of said agglutination based on analysis of said cluster sizes calculated from the plurality of images obtained using the optical device, wherein the presence of an erythrocyte – viral particle cluster in said image indicates the occurrence of said agglutination and lack of detectable amount of said antibody, and wherein the absence of said cluster indicates the lack of said agglutination and the presence of detectable amount of said antibody, and wherein the viral particle is hemagglutinating viral particle. In one aspect, there is provided a method for determining the effective immunization of a subject, comprising: (a) incubating a mixture of visualization particles, an agglutinating viral particle, and a biological sample obtained from the subject that has been immunized with a first dosage of a first vaccine against the agglutinating viral particle in a single incubation step, under conditions permitting agglutination of the visualization particles via interaction with said agglutinating viral particles without, fresh unfixed erythrocytes and without visualization particle reagent preparation; wherein said visualization particles are selected from erythrocytes and microspheres; and (b) determining the concentration of an antibody against said agglutinating viral particle in said sample based on the clusters formed by the agglutination of the visualization particles, wherein the presence of said agglutination is evidenced by formation of visualization particle-agglutinating viral particle clusters, wherein a cluster comprises a contiguous distribution of two or more visualization particles having center-to-center distances between said visualization particles of up to two times erythrocyte diameter, and wherein said clusters are captured in an imaging region of an optical device, and wherein said detecting step comprises: (i) capturing a plurality of images of said clusters at different locations of said imaging region with said optical device; (ii) calculating the size of said clusters based on the center-to-center distances between individual visualization particles captured in each of said images; and (iii) detecting the occurrence of said agglutination based on analysis of said cluster sizes

calculated from the plurality of images obtained using the optical device, and wherein said steps (a) – (b) take place in less than one hour.

[0009] In examples, the erythrocytes can be pre-fixed, e.g., by treatment with glutaraldehyde. Where desired, the erythrocytes may include turkey red blood cells or red cells from other non-human RBCs.

[0010] The viral particles detected by any of the subject methods can include any type of agglutinating virus. For example and without limitation, hepatitis B virus (HBV), hepatitis C virus (HCV), and any strain of influenza virus may be detected by methods disclosed herein. In some examples, antibodies detected by any of the methods disclosed herein can be against any type of agglutinating particle, such as agglutinating viruses. For example and without limitation, antibodies against HBV, HCV and any strains of influenza virus may be detected.

[0011] The biological sample utilized in any of the methods disclosed herein can be any suitable bodily fluid, processed or unprocessed, including but not limited to fresh or anti-coagulated whole blood, plasma and serum. In some instances, the plasma or serum is from a subject that has been administered with a vaccine against the viral particle. Where desired, the biological sample can be pre-treated with neuraminidase, for example, by incubating said biological sample with neuraminidase, for a suitable period of time such as less than 2 hours, 1 hour, 30 minutes, 20 minutes, 10 minutes, 5 minutes or even less.

CLAIMS

WHAT IS CLAIMED IS:

1. A method for determining the presence of an antibody in a biological sample, wherein said antibody binds selectively to a viral particle, the method comprising:

(a) incubating a mixture of erythrocytes, the viral particle, and the biological sample suspected of containing said antibody in a single incubation step, under conditions permitting agglutination of the erythrocytes via interaction with said viral particle without, fresh unfixed erythrocytes and without red cell reagent preparation; and

(b) detecting whether said agglutination occurs in said mixture, wherein the absence of said agglutination indicates the presence of said antibody, wherein the presence of said agglutination is evidenced by formation of erythrocyte-viral particle clusters, wherein a cluster comprises a contiguous distribution of two or more erythrocytes having center-to-center distances between said erythrocytes of up to two times erythrocyte diameter, and wherein said clusters are captured in an imaging region of an optical device, and wherein said detecting step comprises:

(i) capturing a plurality of images of said clusters at different locations of said imaging region with said optical device;

(ii) calculating the size of said clusters based on the center-to-center distances between individual erythrocytes captured in each of said images; and

(iii) detecting the occurrence of said agglutination based on analysis of said cluster sizes calculated from the plurality of images obtained using the optical device,

wherein said steps (a) – (b) take place in less than one hour, and wherein the viral particle is hemagglutinating viral particle.

2. The method of claim 1, wherein said erythrocytes are pre-fixed.

3. The method of claim 2, wherein said erythrocytes are pre-fixed by treatment with glutaraldehyde.

4. The method of any of claims 1-3, wherein said erythrocytes comprise turkey red blood cells.

5. The method of any of claims 1-4, wherein said viral particle comprises an influenza virus.

6. The method of any of claims 1-5, wherein said biological sample comprises plasma or serum.

7. The method of claim 6, wherein said plasma or serum is from a subject that has been administered with a vaccine against said viral particle.

8. The method of any of claims 1-7, wherein said biological sample is pre-treated with neuraminidase.

9. The method of claim 8, wherein said treatment is carried out by incubating said biological sample with neuraminidase, and wherein the final concentration of the neuraminidase in the mixture is between 0.1 and 1.5 U/L.

10. The method of claim 8, wherein said treatment is carried out by incubating said sample with active neuraminidase for less than 30 minutes.

11. The method of any of claims 1-10, wherein said biological sample is serially diluted prior to step (a).

12. The method of any of claims 1-11, wherein said steps (a) – (b) take place between about 30 to 60 minutes.

13. The method of claim 1, wherein said optical device comprises a camera.

14. The method of claim 13, wherein said optical device comprises a microscope.

15 The method of claim 13 or 14, wherein said imaging region resides in a microfluidic channel.

16. A method for determining the effective immunization of a subject, comprising:

(a) incubating a mixture of erythrocytes, a viral particle, and a biological sample obtained from the subject that has been immunized with a first dosage of a first vaccine against the viral particle in a single incubation step, under conditions permitting agglutination of the erythrocytes via interaction with said viral particle without, fresh unfixed erythrocytes and without red cell reagent preparation; and

(b) determining the concentration of an antibody against said virus in said sample based on the clusters formed by the agglutination of the erythrocytes, wherein the presence of said agglutination is evidenced by formation of erythrocyte-viral particle clusters, wherein a cluster comprises a contiguous distribution of two or more erythrocytes having center-to-center distances between said erythrocytes of up to two times erythrocyte diameter, and wherein said clusters are captured in an imaging region of an optical device, and wherein a detecting step comprises:

(i) capturing a plurality of images of said clusters at different locations of said imaging region with said optical device;

(ii) calculating the size of said clusters based on the center-to-center distances between individual erythrocytes captured in each of said images; and

(iii) detecting the occurrence of said agglutination based on analysis of said cluster sizes calculated from the plurality of images obtained using the optical device

and wherein said steps (a) – (b) take place in less than one hour, and wherein the viral particle is hemagglutinating viral particle.

17. A method for determining the effective immunization of a subject, comprising:

(a) incubating a mixture of erythrocytes, a viral particle, and a biological sample obtained from the subject that has been immunized with a first dosage of a first vaccine against the viral particle in a single incubation step, under conditions permitting agglutination of the erythrocytes via interaction with said viral particle without, fresh unfixed erythrocytes and without red cell reagent preparation;

(b) capturing with the aid of an optical device an image of said mixture; and

(c) determining the concentration of an antibody against said viral in said biological sample based on the clusters formed by the agglutination of the erythrocytes, wherein the presence of said agglutination is evidenced by formation of erythrocyte-viral particle clusters, wherein a cluster comprises a contiguous distribution of two or more erythrocytes having center-to-center distances between said erythrocytes of up to two times erythrocyte diameter,

and wherein said clusters are captured in an imaging region of an optical device, and wherein a detecting step comprises:

(i) capturing a plurality of images of said clusters at different locations of said imaging region with said optical device;

(ii) calculating the size of said clusters based on the center-to-center distances between individual erythrocytes captured in each of said images; and

(iii) detecting the occurrence of said agglutination based on analysis of said cluster sizes calculated from the plurality of images obtained using the optical device

wherein the presence of an erythrocyte –viral particle cluster in said image indicates the occurrence of said agglutination and lack of detectable amount of said antibody, and wherein the absence of said cluster indicates the lack of said agglutination and the presence of detectable amount of said antibody, and wherein the viral particle is hemagglutinating viral particle.

18. A method for determining the presence of an antibody in a biological sample, wherein said antibody binds selectively to a viral particle, the method comprising:

(a) incubating a mixture of erythrocytes, the viral particle, and the biological sample suspected of containing said antibody in a single incubation step, under conditions permitting agglutination of the erythrocytes via interaction with said viral particle without, fresh unfixed erythrocytes and without red cell reagent preparation;

(b) concurrent with or subsequent to (a), providing the mixture in a vessel having an opening at the top of the vessel and interior conical shape at the bottom of the vessel;

(c) tilting the vessel such that after tilting, at least a portion of the mixture is closer to the opening at the top of the vessel than prior to the tilting, and wherein at least a portion of the sample remains in the vessel after the tilting; and

(d) capturing with the aid of an optical device an image of said mixture, wherein the image of erythrocytes within the tilted vessel is used to determine the presence or amount of said antibody in the sample, wherein the presence of said agglutination is evidenced by formation of erythrocyte-viral particle clusters, wherein a cluster comprises a contiguous distribution of two or more erythrocytes having center-to-center distances between said erythrocytes of up to two times erythrocyte diameter, and wherein said clusters are captured in an imaging region of an optical device, and wherein a detecting step comprises:

(i) capturing a plurality of images of said clusters at different locations of said imaging region with said optical device;

(ii) calculating the size of said clusters based on the center-to-center distances between individual erythrocytes captured in each of said images; and

(iii) detecting the occurrence of said agglutination based on analysis of said cluster sizes calculated from the plurality of images obtained using the optical device,

wherein the viral particle is hemagglutinating viral particle.

19. A method for determining the presence of an antibody in a biological sample, wherein said antibody binds selectively to an agglutinating viral particle, the method comprising:

(a) incubating a mixture of visualization particles, the agglutinating viral particle, and the biological sample suspected of containing said antibody in a single incubation step, under conditions permitting agglutination of the visualization particle via interaction with said agglutinating viral particle without, fresh unfixed erythrocytes and without visualization particle reagent preparation; wherein said visualization particles are selected from erythrocytes and microspheres; and

(b) detecting whether said agglutination occurs in said mixture, wherein the absence of said agglutination indicates the presence of said antibody, wherein the presence of said agglutination is evidenced by formation of visualization particle-agglutinating viral particle clusters, wherein a cluster comprises a contiguous distribution of two or more visualization particles having center-to-center distances between said visualization particles of up to two times visualization particle diameter, and wherein said clusters are captured in an imaging region of an optical device, and wherein said detecting step comprises:

(i) capturing a plurality of images of said clusters at different locations of said imaging region with said optical device;

(ii) calculating the size of said clusters based on the center-to-center distances between individual visualization particles captured in each of said images; and

(iii) detecting the occurrence of said agglutination based on analysis of said cluster sizes calculated from the plurality of images obtained using the optical device,

wherein said steps (a) – (b) take place in less than one hour.

20. The method of claim 19, wherein said visualization particles are microspheres.

21. The method of claim 19 or 20, wherein said biological sample comprises plasma or serum.

22. The method of claim 21, wherein said plasma or serum is from a subject that has been administered with a vaccine against said agglutinating viral particle.

23. The method of any of claims 19-22, wherein said biological sample is pre-treated with neuraminidase.

24. The method of claim 23, wherein said treatment is carried out by incubating said sample with active neuraminidase for less than 30 minutes.

25. The method of any of claims 19-24, wherein said biological sample is serially diluted prior to step (a).

26. The method of any of claims 19-25, wherein said steps (a) – (b) take place in between about 30 to 60 minutes.

27. The method of any of claims 19-26, wherein the presence of said agglutination is evidenced by formation of visualization particle-agglutinating viral particle clusters, and wherein said clusters are captured in an imaging region of an optical device, and wherein said detecting step comprises:

(i) capturing a plurality of images of said clusters at different locations of said imaging region with the optical device; and

(ii) detecting the occurrence of said agglutination based on analysis of said images.

28. The method of claim 27, wherein said optical device comprises a camera.

29. The method of claim 27, wherein said optical device comprises a microscope.

30. The method of any of claims 27-29, wherein said imaging region resides in a microfluidic channel.

31. A method for determining the effective immunization of a subject, comprising:

(a) incubating a mixture of visualization particles, an agglutinating viral particle, and a biological sample obtained from the subject that has been immunized with a first dosage of

a first vaccine against the agglutinating viral particle in a single incubation step, under conditions permitting agglutination of the visualization particles via interaction with said agglutinating viral particles without fresh, unfixed erythrocytes and without visualization particle reagent preparation; wherein said visualization particles are selected from erythrocytes and microspheres; and

(b) determining the concentration of an antibody against said agglutinating viral particle in said sample based on the clusters formed by the agglutination of the visualization particles, wherein the presence of said agglutination is evidenced by formation of agglutinating viral particle clusters, wherein a cluster comprises a contiguous distribution of two or more visualization particles having center-to-center distances between said visualization particles of up to two times visualization particle diameter, and wherein said clusters are captured in an imaging region of an optical device, and wherein a detecting step comprises:

(i) capturing a plurality of images of said clusters at different locations of said imaging region with said optical device;

(ii) calculating the size of said clusters based on the center-to-center distances between individual visualization particles captured in each of said images; and

(iii) detecting the occurrence of said agglutination based on analysis of said cluster sizes calculated from the plurality of images obtained using the optical device

and wherein said steps (a) – (b) take place in less than one hour.

32. The method of claim 31, further comprising that a second dosage of said first vaccine against said agglutinating viral particle is to be administered if the concentration of said antibody is lower than a predetermined level.

33. The method of claim 31, further comprising that a second vaccine against said agglutinating viral particle is to be administered if the concentration of said antibody is lower than a predetermined level.

34. The method of claim 17, wherein said image is a microscopic image.

35. A method for determining the presence of an antibody in a biological sample, wherein said antibody binds selectively to an agglutinating viral particle, the method comprising:

(a) incubating a mixture of visualization particles, the agglutinating viral particle, and the biological sample suspected of containing said antibody in a single incubation step, under conditions permitting agglutination of the visualization particle via interaction with said agglutinating viral particle without fresh, unfixed erythrocytes and without visualization particle reagent preparation; wherein said visualization particles are selected from erythrocytes and microspheres;

(b) concurrent with or subsequent to (a), providing the mixture in a vessel having an opening at the top of the vessel and interior conical shape at the bottom of the vessel;

(c) tilting the vessel such that after tilting, at least a portion of the mixture is closer to the opening at the top of the vessel than prior to the tilting, and wherein at least a portion of the sample remains in the vessel after the tilting; and

(d) capturing with the aid of an optical device an image of said mixture, wherein the image of visualization particles within the tilted vessel is used to determine the presence or amount of said antibody in the sample, wherein the presence of said agglutination is evidenced by formation of visualization particles -agglutinating viral particle clusters, wherein a cluster comprises a contiguous distribution of two or more visualization particles having center-to-center distances between said visualization particles of up to two times visualization particle diameter, and wherein said clusters are captured in an imaging region of an optical device, and wherein a detecting step comprises:

(i) capturing a plurality of images of said clusters at different locations of said imaging region with said optical device;

(ii) calculating the size of said clusters based on the center-to-center distances between individual visualization particles captured in each of said images; and

(iii) detecting the occurrence of said agglutination based on analysis of said cluster sizes calculated from the plurality of images obtained using the optical device.