METHODS FOR DETECTING AND MEASURING AGGREGATION

CROSS REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 61/673,215 filed July 18, 2012, which application is incorporated herein by reference in its entirety.

BACKGROUND

[0002] Aggregation or agglutination of molecules or cells forms the basis of various useful biological assays. Hemagglutination, or haemagglutination, is a specific form of agglutination that involves red blood cells (RBCs). This phenomenon is used in the laboratory to determine blood type, the presence and/or quantity of virus in a blood sample, and /or the quantity of certain anti-infectious agent antibodies. Hemagglutination causes RBCs to form macroscopic lattice structures that are stable against moderate agitation. These structures can be distinguished from non-agglutinated RBCs by visual observation, which forms the basis of a variety of traditional assay methods.

[0003] Hemagglutination can be triggered by viruses, bacteria, antibodies, and other factors (e.g. lectins). For example, antibodies that bind to type A antigens will induce hemagglutination in samples containing type A or AB RBCs. Similarly, viruses and viral antigens that bind to cell surface molecules can induce hemagglutination. Viruses may contain the protein "hemagglutinin", which binds to molecules on host cells. For example, hemagglutinin proteins may bind to sialic acid on the membrane of host cells such as RBCs. Viral titer can be approximated by observing hemagglutination at various dilutions of a sample containing virus. Bacteria can also be detected and quantified with these methods.

[0004] Despite the highly useful applications of hemagglutination assays, current methods are relatively slow, subjective, and unreliable for some purposes. Traditional pre-treatment protocols can take as long as 12-24 hours, and visual inspection is not a robust method for making quantitative determinations. Hemagglutination assays are often conducted in settings where time is of the essence (e.g. to prevent spread of a viral outbreak or during an emergency blood transfusion). Therefore, there is a considerable need in the art for improved methods of detecting and measuring aggregation/agglutination.

SUMMARY

[0005] Methods, compositions, systems and devices for improved agglutination assays are provided. In one ~~aspect~~example, improved imaging techniques for analysis of agglutination reactions are provided. In another ~~aspect~~example, methods for reducing the time and/or increasing the accuracy of

agglutination reactions are provided. In another ~~aspect~~example, improved reagents for agglutination reactions are provided. In another ~~aspect~~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.

[0006] In one aspect, 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 another related but separate aspect, 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 ~~embodiments~~examples, a microscopic image of the mixture is obtained.

[0007] In another ~~aspect~~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 (a) – (b) take place in less than one hour. In a related but separate ~~aspect~~example, the method of determining the presence of a viral particle in a biological sample includes: (a) incubating a mixture of erythrocytes, a biological sample suspected of containing the viral particle, 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 the presence of detectable amount of the viral particle, and wherein the absence of the cluster indicates the lack of agglutination and the lack of detectable amount of the viral particle. In some ~~embodiments~~examples, a microscopic image of the mixture is obtained.

[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, under conditions permitting agglutination of the erythrocytes via interaction with said viral particle; 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, and wherein said steps (a) – (b) take place in less than one hour. In another ~~aspect~~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, under conditions permitting agglutination of the erythrocytes via interaction with said viral particle; (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 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. In a related but separate ~~aspect~~example, the method includes (a) obtaining a biological sample from a subject that 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; (c) capturing with the aid of an optical device an image of the mixture; and (d) determining the concentration of an antibody against the viral particle in said biological sample based on the clusters formed by the agglutination of the erythrocytes, 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 detectable amount of the antibody. In some ~~embodiments~~examples, a microscopic image of the mixture is obtained.

[0009] In ~~embodiments~~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 ~~aspects~~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.

[0012] In some ~~embodiments~~examples, the various steps employed in a method disclosed herein can be completed within about 2 hours, 60 minutes, 45 minutes, 30 minutes or less, or between about 30 to about 60 minutes.

[0013] In one ~~aspect~~example, provided herein are methods of analyzing agglutination based on image analysis. In one ~~embodiment~~example, image analysis methods are used to analyze the bulk movement of RBCs or visualization particles in an agglutination assay in a conical well or tube. In another ~~embodiment~~example, image analysis methods are used to analyze microscopic images of RBCs or visualization particles in suspension in an agglutination assay, in order to interrogate the fine structure of the RBC or visualization particle suspension.

[0014] In one ~~aspect~~example, provided herein are methods for image analysis of bulk movement of RBCs and visualization particles. In one ~~embodiment~~example, a method is provided for image analysis of agglutination reactions using designated locations in a reaction vessel (e.g. reaction tube or well). In one ~~embodiment~~example, a method is provided for image analysis of agglutination reactions using scanning of the packed cells of an agglutination reaction. In one ~~embodiment~~example, a method is provided for image analysis of agglutination reactions using determination of the area and/or perimeter of the packed cells an agglutination reaction. In some ~~aspects~~examples, packed cells include a button and/or teardrop region.

[0015] In some ~~embodiments~~examples, the presence of the agglutination is evidenced by formation of erythrocyte-viral particle clusters, and wherein said clusters are captured in an imaging region of an optical device. The optical device can include without limitation a camera, a microscope, an optical scanner, a sensor, a detector, or any other suitable imaging device. Where desired, an analysis of the agglutination reaction can be performed by calculating the size of said clusters based on the center-to-center distance of individual erythrocytes captured in each of said images.

[0016] In some ~~embodiments~~examples, -a method for determining the effective immunization of a subject, can further include the step of administering a second dosage of the first vaccine against the viral particle to the subject if the concentration of the antibody in the biological sample is lower than a predetermined level.

[0017] Further provided is a kit, including: pre-fixed erythrocytes, a viral particle, and instructions for a user to use the kit for antibody or viral detection. Such instructions may be wirelessly transmitted and executed in automated systems.

[0018] In ~~embodiments~~examples, provided herein is a method of assaying for the agglutination of particles in a mixture, comprising: generating a mixture comprising visualization particles and agglutinating particles under conditions permitting agglutination of the visualization particles via interaction with the agglutinating particles; obtaining at least one image of the mixture; and analyzing the image to obtain a measurement of the agglutination of the particles in the mixture.

[0019] In ~~embodiments~~examples, in disclosures provided herein involving a visualization particle, the visualization particle is a RBC or a microsphere.

[0020] In ~~embodiments~~examples, in disclosures provided herein involving an agglutinating particle, the agglutinating particle is a virus, viral particle, or an antibody.

[0021] In ~~embodiments~~examples, in disclosures provided herein involving obtaining an image, the image is obtained with a CCD or CMOS image sensor.

[0022] In ~~embodiments~~examples, in disclosures provided herein involving a obtaining an image, the image is obtained with optical device.

[0023] In ~~embodiments~~examples, in disclosures provided herein involving an agglutinating particle, the agglutinating particle is provided in a biological sample from a subject.

[0024] In ~~embodiments~~examples, in disclosures provided herein involving a biological sample, the biological sample comprises plasma or serum.

[0025] In ~~embodiments~~examples, in disclosures provided herein involving an agglutinating particle, the agglutinating particle is a virus.

[0026] In ~~embodiments~~examples, in disclosures provided herein involving an agglutinating particle, the agglutinating particle is an antibody.

[0027] In ~~embodiments~~examples, in disclosures provided herein involving a method of assaying for agglutination of particles in a mixture comprising a biological sample from a subject, the method permits the determination of the quantity of a virus or an antibody in the sample.

[0028] In ~~embodiments~~examples, in disclosures provided herein involving a method of assaying for agglutination of particles in a mixture comprising agglutinating particles and visualization particles, the mixture further comprises an antibody which specifically binds to the agglutinating particle and inhibits the interaction of the agglutinating particle with the visualization particle. In ~~embodiments~~examples, the antibody is provided to the mixture in a biological sample from a subject. In ~~embodiments~~examples, the biological sample comprises plasma or serum. In ~~embodiments~~examples, the method permits the determination of the quantity of the antibody in the sample.

[0029] In ~~embodiments~~examples, in disclosures provided herein involving a mixture comprising agglutinating particles and visualization particle and obtaining an image of the mixture, the image is obtained within 10 seconds, 30 seconds, 1 minute, 2, minutes, 5 minutes, 10 minutes, 15 minutes 20 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, or 8 hours of generating the mixture.

[0030] In ~~embodiments~~examples, in disclosures provided herein involving analyzing an image of a mixture comprising agglutinating particles and visualization particle to obtain a measurement of agglutination of the particles in the mixture, the analyzing the image comprises identification of a region of interest (ROI) in the image.

[0031] In ~~embodiments~~examples, in disclosures provided herein involving a ROI, the ROI comprises imaged information of the mixture comprising visualization particles and agglutinating particles.

[0032] In ~~embodiments~~examples, in disclosures provided herein involving measurement of agglutination of particles in a mixture comprising agglutinating particles and visualization particles, measurement of the agglutination of the particles in the mixture is a qualitative measurement of agglutination or non-agglutination.

[0033] In ~~embodiments~~examples, in disclosures provided herein involving measurement of agglutination of particles in a mixture comprising agglutinating particles and visualization particles, measurement of the agglutination of the particles in the mixture is a quantitative measurement of degree of agglutination.

[0034] In ~~embodiments~~examples, in disclosures provided herein involving obtaining an image of a mixture comprising agglutinating particles and visualization particles, two or more images of the mixture are obtained.

[0035] In ~~embodiments~~examples, in disclosures provided herein involving obtaining at least one image of a mixture containing agglutinating particles and visualization particles and analyzing the image to obtain a measurement of the agglutination of the particles in the mixture, the analyzing the image comprises identification of clusters in the image.

[0036] In ~~embodiments~~examples, in disclosures provided herein involving methods involving identification of clusters in an image, the methods further comprising determining the size or number of clusters in the image.

[0037] In ~~embodiments~~examples, in disclosures provided herein involving identification of clusters in an image, the identification of clusters in the image comprises determining the distance between visualization particles in the image.

[0038] In ~~embodiments~~examples, in disclosures provided herein involving determining the distance between visualization particles in the image, the determining the distance between visualization particles comprises determining the center-to-center distance between visualization particles in the image.

[0039] In ~~embodiments~~examples, in disclosures provided herein involving obtaining at least one image of a mixture containing agglutinating particles and visualization particles and analyzing the image to obtain a measurement of the agglutination of the particles in the mixture, the analyzing the image comprises determining the center-to-center distance between a first visualization particle and a second visualization particle in the image. In ~~embodiments~~examples, the analyzing the image may further comprising determining the location of the first visualization particle and the second visualization particle in the image.

[0040] In ~~embodiments~~examples, in disclosures provided herein involving methods involving identification of a cluster comprising visualization particles and agglutinating particles in an image, the identification of a cluster comprising visualization particles and agglutinating particles comprises identifying a first visualization particle and a second visualization particle wherein the center-to-center distance between the first visualization particle and the second visualization particle is no greater than 1, 1.1, 1.2, 1.3, 1.4, 1.5, 2, 2.5, 3, or 5 times the diameter of either the first or second visualization particle.

[0041] In ~~embodiments~~examples, in disclosures provided herein involving methods involving identification of a cluster comprising visualization particles and agglutinating particles in an image, the identification of a cluster comprising visualization particles and agglutinating particles comprises identifying a first visualization particle and a second visualization particle wherein the center-to-center distance between the first visualization particle and the second visualization particle is no greater than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 30, 35, 40, or 50 um.

[0042] In ~~embodiments~~examples, in disclosures provided herein involving methods involving identification of a cluster comprising visualization particles and agglutinating particles in an image, the identification of a cluster comprising visualization particle and agglutinating particles comprises identifying a cluster of at least three visualization particles, wherein each of the three visualization particles is positioned such the center of the particle is located no further than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 30, 35, 40, or 50 um from the center of each of the other two visualization particles in the cluster.

[0043] In ~~embodiments~~examples, in disclosures provided herein involving methods involving identification of a cluster comprising visualization particles and agglutinating particles in an image, the identification of clusters in the image comprises selecting a cut-off value distance, wherein particles situated at a distance from each other which is less than the cut-off value distance are considered to be associated with each other.

[0044] In ~~embodiments~~examples, in disclosures provided herein involving selecting a cut-off value distance, the selecting a cut-off value distance comprises calculating a radial distribution function for two or more particles in the image.

[0045] In ~~embodiments~~examples, in disclosures provided herein involving obtaining at least one image of a mixture containing agglutinating particles and visualization particles and analyzing the image to obtain a measurement of the agglutination of the particles in the mixture, the analyzing the image comprises feature extraction from a region of the image comprising imaged information of the mixture comprising visualization particles and agglutinating particles.

[0046] In ~~embodiments~~examples, in disclosures provided herein involving feature extraction from an image, feature extraction comprises analyzing the texture of a mixture comprising visualization particles and agglutinating particles.

[0047] In ~~embodiments~~examples, in disclosures provided herein involving analyzing the texture of an image comprising imaged information of the mixture comprising visualization particles and agglutinating particles, analyzing the texture of the mixture comprises analyzing the imaged information with a local binary patterns operator.

[0048] In ~~embodiments~~examples, in disclosures provided herein involving feature extraction from an image, wherein the feature extraction comprises analyzing the texture of a mixture comprising visualization particles and agglutinating particles, the feature extraction further comprises analyzing the intensity of signal in one or more color channels in a region of the image comprising imaged information of the mixture comprising visualization particles and agglutinating particles.

[0049] In ~~embodiments~~examples, in disclosures provided herein involving analyzing the texture of a mixture comprising visualization particles and agglutinating particles, analyzing the texture of the mixture comprises inputting information obtained from the local binary patterns operator into an algorithm which has been previously trained with local binary patterns information from one or more images of a mixture of known agglutination status comprising visualization particles and agglutinating particles.

[0050] In ~~embodiments~~examples, in disclosures provided herein involving obtaining at least one image of a mixture containing agglutinating particles and visualization particles and analyzing the image to obtain a measurement of the agglutination of the particles in the mixture, the analyzing the image comprises comparing feature extraction information from the image of the mixture with feature extraction information from an image of a control mixture of known agglutination status comprising visualization particles and agglutinating particles.

[0051] As described elsewhere herein, agglutination assays and agglutination inhibitions assays are different but related assays. Thus, in embodiments and examples, descriptions herein of systems, reagents, methods for "agglutination assays" and the like may be applicable to both agglutination assays and agglutination inhibition assays, unless the context clearly dictates otherwise. Similarly, in embodiments examples, descriptions herein of systems, reagents and methods for "agglutination inhibition assays" and the like may be applicable to both agglutination assays and agglutination inhibition assays, unless the context clearly dictates otherwise. For example, both agglutination and agglutination inhibition assays may involve analyzing the

21. The method of claim 19 or 20, wherein said step (a) comprising incubating a mixture of said viral particle and said biological sample prior to adding said erythrocytes .

22. A method for determining the presence of a viral particle in a biological sample, comprising:

(a) incubating a mixture of erythrocytes and a biological sample suspected of containing said viral particle, under conditions permitting agglutination of the erythrocytes via interaction with said viral particle; and

(b) detecting whether said agglutination occurs in said mixture, wherein the presence of said agglutination indicates the presence of said viral particle,

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

23. A method for determining the presence of a viral particle in a biological sample, comprising:

(a) incubating a mixture of erythrocytes and a biological sample suspected of containing said viral particle, under conditions permitting agglutination of the erythrocytes via interaction with said viral particle; and

(b) capturing with the aid of an optical device an image of said mixture, wherein the presence of an erythrocyte –viral particle cluster in said image indicates the occurrence of said agglutination and the presence of detectable amount of said viral particle, and wherein the absence of said cluster indicates the lack of said agglutination and the lack of detectable amount of said viral particle.

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

~~(a) obtaining a biological sample from a subject that has been immunized with a first dosage of a first vaccine against a viral particle~~;

(~~b~~a) incubating a mixture of erythrocytes, ~~the~~ a viral particle, and ~~said~~ a biological sample obtained from the subject that has been immunized with a first dosage of a first vaccine against the viral particle, under conditions permitting agglutination of the erythrocytes via interaction with said viral particle; and

(~~c~~b) determining the concentration of an antibody against said virus in said sample based on the clusters formed by the agglutination of the erythrocytes,

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

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

~~(a) obtaining a biological sample from a subject that has been immunized with a first dosage of a first vaccine against a viral particle;~~

(~~b~~a) incubating a mixture of erythrocytes, ~~said~~ a viral particle, and ~~said~~ a biological sample obtained from the subject that has been immunized with a first dosage of a first vaccine against the viral particle, under conditions permitting agglutination of the erythrocytes via interaction with said viral particle;

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

(~~d~~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 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.

26. The method of claim 23 or 24, further comprising administration of~~administering~~ a second dosage of said first vaccine against said viral particle to said subject if the concentration of said antibody is lower than a predetermined level.

27. The method of claim 23 or 24, further comprising ~~administering~~ administration of a second vaccine against said viral particle to said subject if the concentration of said antibody is lower than a predetermined level.

28. A kit, comprising: pre-fixed erythrocytes, a viral particle, and instructions for a user to use said kit for antibody or viral detection.

29. The method of any of claims 19, 23, or 25, wherein said image is a microscopic image.

30. 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, under conditions permitting agglutination of the erythrocytes via interaction with said viral particle;

(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 position of erythrocytes within the tilted vessel may be used to determine the presence or amount of said antibody in the sample.

(a) incubating a mixture of visualization particles and a biological sample suspected of containing said agglutinating particle, under conditions permitting agglutination of the visualization particle via interaction with said agglutinating particle; and

(b) detecting whether said agglutination occurs in said mixture, wherein the presence of said agglutination indicates the presence of said agglutinating particle,

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

60. A method for determining the presence of an agglutinating particle in a biological sample, comprising:

(a) incubating a mixture of visualization particles and a biological sample suspected of containing said agglutinating particle, under conditions permitting agglutination of the visualization particle via interaction with said agglutinating particle; and

(b) capturing with the aid of an optical device an image of said mixture, wherein the presence of an visualization particle –agglutinating particle cluster in said image indicates the occurrence of said agglutination and the presence of detectable amount of said agglutinating particle, and wherein the absence of said cluster indicates the lack of said agglutination and the lack of detectable amount of said agglutinating particle.

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

~~(a) obtaining a biological sample from a subject that has been immunized with a first dosage of a first vaccine against an agglutinating particle;~~

(~~b~~a) incubating a mixture of visualization particles, ~~the~~ an agglutinating particle, and ~~said~~ a biological sample obtained from the subject that has been immunized with a first dosage of a first vaccine against the agglutinating particle, under conditions permitting agglutination of the visualization particles via interaction with said agglutinating particles; and

(~~c~~b) determining the concentration of an antibody against said agglutinating particle in said sample based on the clusters formed by the agglutination of the visualization particles,

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

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

~~(a) obtaining a biological sample from a subject that has been immunized with a first dosage of a first vaccine against an agglutinating particle;~~

(~~b~~a) incubating a mixture of visualization particles, ~~said~~ an agglutinating particle, and ~~said~~ a biological sample obtained from the subject that has been immunized with a first dosage of a first vaccine against the agglutinating particle, under conditions permitting agglutination of the visualization particles via interaction with said agglutinating particle;

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

(~~d~~c) determining the concentration of an antibody against said agglutinating particle in said biological sample based on the clusters formed by the agglutination of the visualization particles,

wherein the presence of a visualization particle –agglutinating 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.

63. The method of claim 61 or 62, further comprising administration of~~administering~~ a second dosage of said first vaccine against said agglutinating particle to said subject if the concentration of said antibody is lower than a predetermined level.

64. The method of claim 61 or 62, further comprising administration of~~administering~~ a second vaccine against said agglutinating particle to said subject if the concentration of said antibody is lower than a predetermined level.

65. A kit, comprising: visualization particles, an agglutinating particle, and instructions for a user to use said kit for antibody or agglutinating particle detection.

66. The kit of claim 65, wherein the visualization particles are microspheres.

67. The method of any of claims 19, 23, 25, 56, 60, or 62 wherein said image is a microscopic image.

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

(a) incubating a mixture of visualization particles, the agglutinating particle, and the biological sample suspected of containing said antibody, under conditions permitting agglutination of the visualization particle via interaction with said agglutinating particle;

(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 position of visualization particles within the tilted vessel may be used to determine the presence or amount of said antibody in the sample.