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(54) **Title**: METHODS FOR DETECTING AND MEASURING AGGREGATION

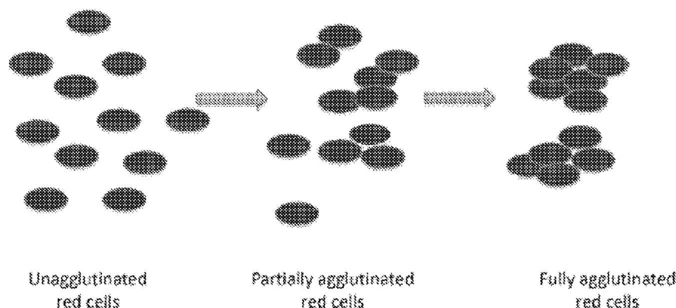

FIG. 1

(57) **Abstract**: Methods, compositions, systems, and devices are provided for performing and analyzing agglutination assays. In one aspect, methods for image analysis of agglutination assays are provided. In another aspects, methods for performing agglutination assays are provided. In one aspect, the methods may be used for the detection of various molecules, including viruses or antibodies against a virus. In another aspect, the methods can be used to determine effective immunization of a subject.

INTERNATIONAL SEARCH REPORT **PCT/US2013/051165 13.03.2014**

International application No.
PCT/US13/51165

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G01N 33/53, 33/556 (2014.01)
USPC - 435/5, 7.25; 436/521, 539, 164; 422/73
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): G01N 33/53, 33/563, 33/556, 33/555, 33/554, 33/536; A61K 35/12, 35/14, 35/18, 35/76, 39/00, 39/395, 39/12, 38/36 (2014.01)
USPC: 435/7.2, 7.1, 4, 5, 7.21, 7.25, 7.8, 40.5, 40.51; 436/512, 521, 522, 539, 540, 8, 17, 63, 164, 166, 174; 422/73

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
MicroPatent (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-C,B, DE-A, DE-T, DE-U, GB-A, FR-A); Google/Google Scholar; ProQuest; ScienceDirect; agglutination, hemagglutination, red blood cells, RBCs, erythrocytes, glutaraldehyde, pentanedial, glutardialdehyde, glutaric acid dialdehyde, glutaric aldehyde, glutaric dialdehyde, '1,5-pentanedial', 'pentane-1,5-dial, detect, distinguish, presence, test

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 4298346 A (ITO, H) November 3, 1981; abstract; column 1, line 32-62; column 1, line 66-column 2, line 17; column 3, line 22-48; column 6, lines 24-35	1-3, 4/1-4/3 -- 19, 20, 21/19, 21/20, 29/19, 58/56, 58/57, 67/19, 78/19
X -- Y	EP 0435246 B1 (KANO, T et al.) August 9, 1995; abstract; column 3, lines 13-21; column 4, line 54-column 5, line 2; column 6, line 44-column 7, line 5; column 8, lines 24-39, column 9, lines 17-43; figures 2, 5c, 11	23, 29/23, 56, 57, 59, 60, 67/23, 67/56, 67/60, 68, 69, 70/68, 70/69, 71/70/68, 71/70/69, 72/68, 72/69, 73/72/68, 73/72/69, 75/68, 75/69, 76/75/68, 76/75/69 ----- 19, 20, 21/19, 21/20, 26/23, 27/23, 29/19, 29/25, 30, 31, 32/30, 32/31, 33/32/30, 33/32/31, 34/30, 34/31, 35/34/30, 35/34/31, 37/30, 37/31, 38/37/30, 38/37/31, 58/56, 58/57, 67/19, 67/25, 67/62, 78/19, 78/23, 78/30, 78/31, 78/56, 78/60, 78/68, 78/69

☒ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 2009/0325148 A1 (KACHURIN, A et al.) December 31, 2009; abstract; paragraphs [0003], [0005]-[0009], [0013], [0028], [0044], [0045]	24, 25, 61, 62 -- 26/24, 27/24, 29/25, 63/61, 63/62, 64/61, 64/62, 67/25, 67/62, 78/25, 78/62
X -- Y	US 4829011 A (GIBBONS, I) May 9, 1989; abstract; column 12, lines 39-67; column 4, line 64-column 5, line 19; column 11, lines 16-24	28, 65 ----- 66
X -- Y	US 4176174 A (RUSSELL, SR et al.) November 27, 1979; abstract; column 1, line 51-column 2, line 2; column 6, lines 53-60	40, 42/40 ------------ 41, 42/41
X	US 4403037 A (COATES, SR) September 6, 1983; abstract; column 1, lines 47-63; column 2, lines 10-23; Claim 7	22
Y	US 2011/0229914 A1 (LEE, FE et al.) September 22, 2011; abstract; paragraphs [0006], [0029]	26/23, 26/24, 27/23, 27/24, 63/61, 63/62, 64/61, 64/62
Y	US 8007999 B2 (HOLMES, EA et al.) August 30, 2011; abstract; column 2, lines 25-67; column 4, lines 20-36; column 5, lines 34-64; column 8, lines 4-15	30, 31, 32/30, 32/31, 33/32/30, 33/32/31, 34/30, 34/31, 35/34/30, 35/34/31, 37/30, 37/31, 38/37/30, 38/37/31, 78/30, 78/31
Y	McHUGH, TM et al. Simultaneous Detection Of Antibodies To Cytomegalovirus And Herpes Simplex Virus By Using Flow Cytometry And A Microsphere-Based Fluorescence Immunoassay. October 1988. Journal of Clinical Microbiology, Vol. 26, No. 10, pp. 1957-1961; abstract; page 1957, left column, second paragraph. DOI: 0095-1137/88/101957-05$02.00/0	41, 42/41, 66
Y	US 2001/0034068 A1 (SPIVEY, RJ et al.) October 25, 2001; abstract; figure 3; paragraph [0047]	78/19, 78/23, 78/25, 78/30, 78/31, 78/56, 78/60, 78/62, 78/68, 78/69

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 5-18, 36, 39, 43-55, 74, 77, 79, 83-117
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

-***-Please See Supplemental Page-***-

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Group I: Claims 1-4, 19-35, 37, 38, 40-42, 56-73, 75, 76, 78

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

-***-Continuation of Box No. III - Observations where unity of invention is lacking:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: Claims 1-4, 19-35, 37, 38, 40-42, 56-73, 75, 76 and 78 are directed towards 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; and (b) detecting whether said agglutination occurs in said mixture, wherein the absence of said agglutination indicates the presence of said antibody, wherein said steps (a)- (b) take place in less than one hour; a method for determining the presence of an antibody in a biological sample, where 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; 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 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; 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; 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; 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) incubating a mixture of erythrocytes, the viral particle, and said biological sample, under conditions permitting agglutination of the erythrocytes via interaction with said viral particle; and (c) 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) - (c) take place in less than one hour; 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) incubating a mixture of erythrocytes, said viral particle, and said biological sample, under conditions permitting agglutination of the erythrocytes via interaction with said viral particle; (c) capturing with the aid of an optical device an image of said mixture; and (d) 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; a kit, comprising: pre-fixed erythrocytes, a viral particle, and instructions for a user to use said kit for antibody or viral detection; 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 method for determining the presence of a viral particle in a biological sample, wherein said viral particle may be selectively bound by an antibody, the method comprising: (a) incubating a mixture of erythrocytes, the antibody, and the biological sample suspected of containing said viral particle, 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 viral particle in the sample; 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; and (b) detecting whether said agglutination occurs in said mixture, wherein the absence of said agglutination indicates the presence of said antibody, wherein said steps (a)- (b) take place in less than one hour; a method for determining the presence of an antibody in a biological sample, where 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; and (b) capturing with the aid of an optical device an image of said mixture, 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; 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) 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; 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; 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... Continued from Previous Supplemental Page ... 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; 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) incubating a mixture of visualization particles, the agglutinating particle, and said biological sample, under conditions permitting agglutination of the visualization particles via interaction with said agglutinating particles; and (c) 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) - (c) take place in less than one hour; 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) incubating a mixture of visualization particles, said agglutinating particle, and said biological sample, under conditions permitting agglutination of the visualization particles via interaction with said agglutinating particle; (c) capturing with the aid of an optical device an image of said mixture; and (d) 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; a kit, comprising: visualization particles, an agglutinating particle, and instructions for a user to use said kit for antibody or agglutinating particle detection; 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; and a method for determining the presence of an agglutinating particle in a biological sample, wherein the agglutinating particle may be selectively bound by an antibody, the method comprising: (a) incubating a mixture of visualization particle, the antibody, and the biological sample suspected of containing said agglutinating particle, under conditions permitting agglutination of the visualization particles 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 agglutinating particle in the sample.

Group II: Claims 80-82 are directed toward a method of assaying for the agglutination of visualization particles in a mixture, comprising: a) generating a mixture comprising the visualization particles and agglutinating particles under conditions permitting agglutination of the visualization particles via interaction with the agglutinating particles; b) obtaining at least one image of the mixture; and c) analyzing the image to obtain a measurement of the agglutination of the visualization particles in the mixture.

The inventions listed as Groups I-II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the special technical features of Group I include a method for determining the presence of an antibody in a biological sample, wherein said antibody binds selectively to a viral particle, wherein the absence of agglutination indicates the presence of said antibody, wherein said steps (a)-(b) take place in less than one hour; a method for determining the presence of an antibody in a biological sample, where said antibody binds selectively to a viral particle, wherein the absence of a cluster indicates the lack of agglutination and the presence of detectable amount of said antibody; a method for determining the presence of a viral particle in a biological sample, wherein steps (a)-(b) take place in less than one hour; a method for determining the presence of a viral particle in a biological sample, wherein the absence of a cluster indicates the lack of agglutination and the lack of detectable amount of said viral particle; 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; 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) - (c) take place in less than one hour; 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; and (d) 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 absence of a cluster indicates the lack of said agglutination and the presence of detectable amount of said antibody; a kit, comprising: pre-fixed erythrocytes, a viral particle, and instructions for a user to use said kit for antibody or viral detection; 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: providing a 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;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 method for determining the presence of a viral particle in a biological sample, wherein said viral particle may be selectively bound by an antibody, the method comprising: providing a 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; wherein the position of erythrocytes within the tilted vessel may be used to determine the presence or amount of said viral particle in the sample;... Continued on Next Supplemental Page ...

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... Continued from Previous Supplemental Page ...a method for determining the presence of an antibody in a biological sample, wherein said antibody binds selectively to an agglutinating particle, wherein said steps (a)-(b) take place in less than one hour; a method for determining the presence of an antibody in a biological sample, wherein the lack of detectable amount of said antibody, and wherein the absence of a cluster indicates the lack of agglutination and the presence of detectable amount of said antibody; a method for determining the presence of an agglutinating particle in a biological sample, wherein the presence of agglutination indicates the presence of said agglutinating particle, and wherein steps (a)-(b) take place in less than one hour; a method for determining the presence of an agglutinating particle in a biological sample, wherein the absence of a cluster indicates the lack of agglutination and the lack of detectable amount of said agglutinating particle; 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; wherein said steps (b) - (c) take place in less than one hour; 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; wherein the absence of a cluster indicates the lack of said agglutination and the presence of detectable amount of said antibody; a kit, comprising: visualization particles, an agglutinating particle, and instructions for a user to use said kit for antibody or agglutinating particle detection; 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: providing a 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; 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; and a method for determining the presence of an agglutinating particle in a biological sample, wherein the agglutinating particle may be selectively bound by an antibody, the method comprising: 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; wherein the position of visualization particles within the tilted vessel may be used to determine the presence or amount of said agglutinating particle in the sample, which are not present in Group II, the special technical features of Group II including a method of assaying for the agglutination of visualization particles in a mixture, and analyzing the image to obtain a measurement of the agglutination of the visualization particles in the mixture.

Groups I--II share the technical features including a method comprising: a) generating a mixture comprising visualization particles and agglutinating particles under conditions permitting agglutination of the visualization particles via interaction with the agglutinating particles; b) obtaining at least one image of the mixture.

However, these shared technical features are previously disclosed by US 4,829,011 A (GIBBONS) in view of US 4,556,641 to Kano, et al. (hereinafter 'Kano'). Gibbons discloses a method comprising (a method comprising: column 2, lines 15-18): a) generating a mixture (forming a reaction medium (generating a mixture); column 2, lines 17-19) comprising visualization particles (comprising a plurality of particles having a binding pair member (comprising visualization particles); column 2, lines 19-20) and agglutinating particles (and a polyvalent receptor (agglutinating particles); column 2, lines 24-28) under conditions permitting agglutination of the visualization particles via interaction with the agglutinating particles (under conditions permitting agglutination of the visualization particles via interaction with the agglutinating particles; column 2, lines 23-37). Gibbons does not disclose obtaining at least one image of the mixture. Kano discloses obtaining at least one image of a mixture of an agglutination reaction (obtaining at least one image of a mixture of an agglutination reaction; column 3, lines 52-60). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Gibbons regarding the agglutination of particles enabling visualization and agglutinating particles, in combination with the imaging method of Kano, for enabling the real-time image tracking of the agglutination process, whereby a rapid and reliable determination of the results of the agglutination reaction would have been possible using the various images previously disclosed by the method of Kano, without undue experimentation or testing.

Since none of the special technical features of the Groups I-II inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by a combination of the Gibbons and Kano references, unity of invention is lacking.