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(54) **Title**: MICROBIAL CONCENTRATION BY UTILIZING POLY-L-GLUTAMIC ACID (PGA) AS A CENTRIFUGATION BYPASS

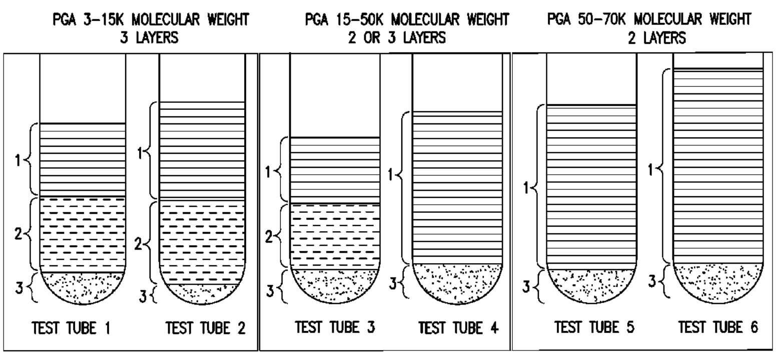


FIG.1A

(57) **Abstract**: The invention pertains to a novel method of using poly-L-glutamic acid polymers of up to and including 100,000 molecular weights to isolate bacteria from a positive blood culture and/or whole blood containing bacteria in less than 60 minutes and without centrifugation. The methods of concentrating bacteria, preferably in the top layer, comprise obtaining a positive blood culture or whole blood cultured with bacteria, adding a PGA, subjecting the solution to about 60 minutes or less of settling time and recovering the microorganisms. The recovered test sample contains high concentration of intact and viable bacteria and low red blood cells for use in downstream sample processing and detection methods.

WO 2013/177277 A1

MICROBIAL CONCENTRATION BY UTILIZING POLY-L-GLUTAMIC ACID (PGA) AS A CENTRIFUGATION BYPASS

CROSS-REFERENCE TO RELATED APPLICATION

[0001] The present application claims the benefit of the filing date of U.S. Provisional Application No. 61/651,239, filed May 24, 2012, entitled Microbial Concentration by Utilizing Poly-L-Glutamic Acid (PGA) as a Centrifugation Bypass, the disclosure of which is hereby incorporated by reference.

BACKGROUND OF THE INVENTION

[0002] Conventional protocols to concentrate bacteria in whole blood consist of high speed centrifugation and recovery of the pellet after the supernatant is decanted. Small clinical microbiology laboratories may not have a high speed centrifuge available for use. Lack of centrifuge access and attendant sharing of centrifuge resources among laboratories (e.g., clinical chemistry, hematology, stat labs) is inconvenient and introduces biohazard safety concerns. Separating microorganisms from peripheral blood components using centrifugation is a function of the physical properties of the sample constituents such as size, shape, density, viscosity of the medium, as well as the rotor speed of the centrifuge. The sheer mechanical force of centrifugation may cause cell lysis or cellular disruption of the microorganisms in the sample. Therefore, centrifugation makes it more difficult to obtain viable and intact bacteria for further processing and identification. Similarly, gross hemolysis of red blood cells (RBC) and damage to other blood components caused by centrifugation can occlude the samples, making visual or optical interrogation of the samples more difficult. Separation of the microorganisms from other blood components such as RBCs is needed to facilitate downstream testing and to detect the presence or absence of target microorganisms in the sample.

[0003] In addition to the above deficiencies, use of high speed centrifugation to concentrate bacteria in whole blood does not provide adequate separation between RBCs and sample bacteria. High RBC content in samples can interfere with Identification (ID) and Antimicrobial Susceptibility Testing (AST) as well as identification by mass spectrometry. Alternative methods used to separate bacteria from peripheral blood components require the use of additives that can inhibit or damage the viability/integrity of the target microorganisms for downstream processing and testing methods. More specifically, it is desirable to provide a test sample that has high concentration of bacteria and low concentration of red blood cells for downstream processing and detection of target microorganisms. Therefore, a need exists for methods that concentrate intact and viable microorganisms in a biological fluid sample (e.g., blood) that do not require centrifugation or additives that adversely affect microorganism viability and that provide a sample from which microorganisms can be recovered that will include a low concentration of sample constituents that have the capacity to interfere with the downstream detection methods.

BRIEF SUMMARY OF THE INVENTION

[0004] Methods are described herein that effectively and efficiently separate bacteria from red blood cells without centrifugation. The described methods concentrate microorganisms without compromising microorganism viability and/or structural integrity for downstream processing and testing methods that require whole/viable microorganisms for testing. The described methods concentrate bacteria using a water-soluble polymer, poly-L-glutamic acid (PGA), and do not require centrifugation or additives that destroy microorganism structure and/or viability. The described methods preferably concentrate microorganisms in the top layer of the biological sample and sediment the RBCs in the bottom layer without compromising the viability/structure of the microorganisms. The top layer of the PGA-treated sample is high in bacterial concentration and, in certain preferred embodiments, low in RBCs by at least two orders of magnitude reduction in the RBC count in the top layer compared to the bottom layer. In preferred embodiments, the desired separation is achieved in a maximum of about 60 minutes, preferably within about 30 minutes or less, and most preferably within about 15 minutes or less. Using PGA, the total time to concentrate and retrieve microorganisms in a biological sample may be reduced.

[0005] In one embodiment of the methods described herein, a biological sample (e.g., blood) suspected of containing at least one microorganism is obtained. Poly-L-glutamic acid (PGA) is added to the sample, which is allowed to settle for about 30 minutes or less. A top layer of the sample is removed, the top layer containing a higher concentration of microorganisms (if present) than the lower portions of the sample. The top layer so obtained is then subjected to additional downstream testing and processing to test for the presence of one or more target microorganisms in the sample.

[0006] Another embodiment of the present invention contemplates obtaining a positive blood culture and adding a PGA solution thereto. The sample is then allowed to sediment for about 30 minutes or less. A fraction of the sample most likely to contain a higher concentration of microorganisms (if present) is observed, after which a portion of the sample is removed from that fraction. That portion of the sample is then subjected to downstream processing and testing to determine the presence or absence of target microorganisms in the sample.

[0007] In the embodiments of the methods described herein, the amount of PGA added to the biological sample is selected so that the desired separation can be achieved within about 30 minutes or less, and most preferably within about 15 minutes or less of sedimentation time. Advantageously, the presence of up to about 0.1 mL of stock PGA solution in the biological sample provides rapid sedimentation of RBCs within about 10-minutes or less of sedimentation time and significantly increases the sedimentation rate compared to samples without PGA added thereto. It will be appreciated by those skilled in the art that the sedimentation time required to obtain a test sample having intact/viable bacteria for downstream processing, preferably in the top layer, will vary based on the biological sample (i.e., degree or hemolysis, presence of an inflammatory response, hematocrit, etc.), as well as the properties of the PGA additive, including molecular weight of PGA, stock PGA concentration, and the amount of stock PGA solution used to segregate/concentrate bacteria preferably in the top layer of the biological sample.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] FIG. 1A illustrates separated layers in the test tubes obtained after approximately 60 minutes or less of sedimentation with PGA to isolate *Staphylococcus aureus* (*S. aureus*) for different molecular weight ranges of PGA polymer. FIG. 1B illustrates the approximate percentage of bacteria within each separated layer of the test tubes measured by Giemsa and Gram Stains.

[0009] FIGS. 2A and 2B are Box & Whisker plot analysis of bacterial concentration (by percentage) in the top and bottom sedimentation layers using PGA of three different molecular weights to isolate *S. aureus* and *Escherichia coli (E. coli)* from the biological sample.

[0010] FIGS. 3A and 3B illustrate visually separated layers and Box & Whisker plot analysis following sedimentation with PGA to isolate bacteria compared to alternative sedimentation additives (i.e., glycerol and sucrose solutions).

[0011] FIGS. 4A and 4B compare the ability of higher molecular weight (50,000-70,000 and 50,000-100,000 molecular weights) PGA to concentrate three microorganisms: *E. coli*, *S. aureus* and *Streptococcus pneumoniae (S. pneumoniae)* in a portion of the sample.

[0012] FIG. 5 compares the ability of PGA with overlapping ranges (50,000-70,000 and 50,000-100,000 molecular weights) to concentrate bacteria in the top portion of a sample at various time intervals for sedimentation.

DETAILED DESCRIPTION

[0013] Described herein are methods of concentrating bacteria in a fraction of a biological sample without centrifugation. Preferably, the fraction is a top fraction of the sample to ensure that this desired fraction can be easily accessed and used for downstream analysis to determine the presence or absence of microorganisms in the sample. The method of concentrating bacteria, preferably in the top layer of a biological sample (e.g., in whole blood), does not require conventional protocols (e.g., centrifugation, additives that kill/dissolve microorganisms) to concentrate microorganisms therein. In one embodiment, a PGA solution is added to a biological sample suspected of containing at least one microorganism. The combined solution is allowed to sediment for approximately 30 minutes or less. The top layer of the sedimented sample (i.e., the fraction of the sample that contains the most accessible/highest concentration of microorganisms) is removed for further processing. The described methods provide rapid sedimentation of a biological sample without the adverse consequences of centrifugation or additives that kill or dissolve the microorganisms. The methods described herein provide for a fast and simple aspiration of the PGA-induced segregation of bacteria from a fraction of the sample that has preferably the greatest concentration of microorganisms (if microorganisms are present in the sample) and which fraction is most easily accessed (e.g., the top layer of the sample). Preferably, the accessed fraction of the sample is the top layer of the sample, which also contains the highest concentration of microorganisms. Simply aspirating a portion of the top sample fraction is easier and less hazardous than decanting a biohazardous supernatant and re-suspending the pellet formed by high speed centrifugation.

[0014] The methods described herein at least partially separate bacteria from other sample components (e.g., red blood cells) and preferably concentrate bacteria in a positive blood culture by adding PGA to a blood sample already determined to contain bacteria. The "test sample" as used herein is the fraction of the liquid sample that is removed therefrom and subjected to downstream processing to determine the presence or absence of bacteria in the sample that is prepared according to the methods of the present disclosure.

[0015] In some embodiments, the test sample is used in methods for downstream detection and/or processing of microorganisms. Downstream processing includes, for example, centrifugation, or exposure of the test sample to a solid phase such as capture beads having a bound ligand that would capture bacteria from the test sample. In some embodiments, a test sample or the microbes in the test sample may be adhered to a solid support. A solid support may include microarrays (e.g., DNA or RNA microarrays), gels, blots, glass slides, beads, or ELISA plates. While centrifugation is preferably avoided when separating the test sample from the liquid sample from which it is obtained, centrifugation can be deployed to further process the test sample once obtained.

[0016] In other embodiments, the test sample is subsequently processed and used for Identification (ID) and Antimicrobial Susceptibility Testing (AST) in an automated system for large scale testing. Similarly, the test samples can be used for ID by Polymerase Chain Reaction (PCR) or in a Mass Spectrophotometer (e.g., matrix-assisted laser desorption/ionization – time-of-light mass spectrometer, MALDI-TOF). In one embodiment, the test sample is directly tested in either a Phoenix ID/AST or MALDI-TOF. In an alternate embodiment, the test sample is subjected to centrifugation and a wash procedure to remove the PGA and extract bacteria from the PGA-concentrated test sample for subsequent testing in a Mass Spectrophotometer (MALDI-TOF). Processes for downstream processing such as ID and antibiotic susceptibility are well known to those skilled in the art and are not described in detail herein. Therefore, identification of bacteria according to the disclosed methods may be performed with or without further processing such as first extracting the bacteria from the PGA solution or extracting nucleic acid from the bacteria.

[0017] The PGA additive to the liquid sample as described herein provides a liquid sample with at least two visually distinct layers stratified by the average molecular weight of the constituents of the liquid sample. In one exemplary embodiment, the bottom layer has a higher concentration of red blood cells and a lower concentration of bacteria than the upper layer. The top layer of the stratified liquid sample therefore has the higher concentration of bacteria and a lower concentration of red blood cells. In other embodiments, the sample is visually stratified into three layers, the top two layers having a higher concentration of bacteria and lower concentration of red blood cells relative to the bottom third layer and the liquid sample prior to PGA-induced sedimentation/stratification. In other embodiments, the concentration of bacteria is not necessarily higher, but one layer has a visibly lower concentration of other sample constituents. It is that layer from which the test sample is drawn for downstream processing (e.g., ID, AST).

[0018] As used herein, "test sample" refers to a liquid sample layer that is same or higher in microbial concentration and lower in the concentration of Red Blood Cells (RBCs) than other portions of the liquid sample from which the test sample remains.

[0019] All numerical values within the detailed description and the claims herein are modified by "about" or "approximately" the indicated value, and take into account experimental error and variations that would be expected by a person having ordinary skill in the art.

[0020] As used herein, the term "biological sample" refers to a sample obtained from a biological subject, including sample of biological tissue or fluid obtained in vivo, for example fresh blood sample or whole banked blood.

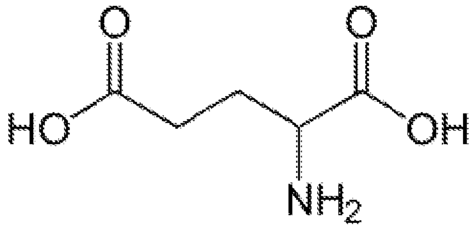
[0021] As used herein, the term "liquid sample" refers to the biological sample from which the test sample is obtained.

[0022] As used herein, "positive blood culture" refers to a biological sample that consists of a growth media and an anticoagulated whole blood determined to contain bacteria.

[0023] As used herein, "Red Blood Cells" are defined as erythrocytes which are formed elements in the peripheral blood. "Peripheral blood components" are defined as the cellular components of blood, consisting of red blood cells, white blood cells, and platelets, which are found within the circulating pool of blood and not sequestered within the lymphatic system, spleen, liver, or bone marrow. Red Blood

Cells exhibit higher density compared to white blood cells and platelets.

[0024] Obtaining a test sample from a liquid sample, where the test sample has a higher concentration of microorganisms (if present) than the overall liquid sample is based, at least in part, on the combination of a water-soluble polymer, such as poly-L-glutamic acid (PGA) with the liquid sample. PGA is an anionic polymer of the amino acid glutamic acid having the following formula:



[0025] Without being bound to any particular theory, it is believed that PGA polymer coats the cellular components of peripheral blood, increasing their respective density, thereby increasing the sedimentation rate of the peripheral blood components in the test sample and decreasing their buoyancy relative to the microorganisms in the sample. Sumida et al., "Platelet Separation From Whole Blood in an Aqueous Two-Phase System With Water-Soluble Polymers", Journal of Pharmacological Sciences, 101(1), 91-97 (2006), proposes two different models (bridging and depletion models) to explain rouleaux (i.e., stacking) of erythrocytes (i.e., red blood cells) in the presence of polymers such as PGA. However, the exact cause of increased sedimentation rate of peripheral blood components relative to microorganisms in the presence of PGA need not be known to practice the invention described herein. The rapid sedimentation rate of peripheral blood components reported herein may also be caused by the formation of a PGA cross-linked structure, similar to that described with gamma poly-glutamic acid in U.S. Patent No. 7,759,088 (issued to Ho et al.). Other polymers having similar characteristics, for example, alpha-poly-D(or L)-glutamic acid, gamma-poly-D(or L)-glutamic acid, alpha-poly-D(or L)-aspartic acid (PAA), beta-poly-D(or L)-aspartic acid(PAA), poly (2-methacryloyloxyethyl phosphorylcholine-co-n-butyl methacrylate) (PBM), polyacrylic acid, poly(methacrylic acid), polysaccharides, polyvinylpyrrolidone, or their analogues, homologues, and their linear, branched or block copolymers are contemplated as suitable in the methods described herein.

[0026] The anti-coagulant used to prepare positive blood is preferably selected from the type that inhibits the antimicrobial systems of blood and is used in blood culture media and in microbiology for clinical specimen processing. For example, acid citrate-dextrose solution is not a preferred anti-coagulant for blood culture because it is primarily used to preserve blood specimens required for tissue typing and blood banking. In the exemplary embodiments, the anti-coagulant is sodium polyanethole sulfonate (SPS) and the growth media is a BD BACTEC™ Standard/10 Aerobic/F media. BD BACTEC™ Standard/10 Aerobic/F media is a commercially available growth media for use with aerobic culture and recovery of microorganisms (e.g., bacteria, yeast, fungi) from blood.

[0027] In one exemplary embodiment, the PGA stock solution of various amounts and concentrations is added to about 1.0 mL of positive blood culture. The volume and concentration of the PGA added to PBC may vary depending on factors such as the density of the biological sample, the molecular weight of the PGA used to prepare a stock concentration, the volume of the stock PGA concentration used, etc. For example, PGA polymers of various molecular weight effect the sedimentation rate, sedimentation quality, visual separation, and viability and intactness of bacteria recovered in the test sample.

[0028] In certain embodiments, PGA powders of a mixture of PGA up to 100,000 molecular weights are used to prepare a stock solution to concentrate/segregate bacteria in a biological sample. Examples of PGA molecular weight ranges for the stock PGA solutions combined with the sample include, but are not limited to about 3,000-15,000, about 15,000-50,000, about 50,000-70,000 and about 50,000-100,000 molecular weights. Consequently, the range of PGA molecular weights that may be combined to form the PGA solution is about 3,000 to 100,000. In other embodiments the ranges are: 1) about 3,000-15,000; 2) about 15,000-50,000; 3) about 50,000-70,000; and 4) about 50,000-100,000 molecular weights. One skilled in the art can determine the molecular weight range most suited for combination with a particular biological sample.

[0029] In one embodiment of the methods described herein, a stock PGA solution having a final concentration of up to about 26 milligrams per milliliter (mg/mL) is prepared. In one exemplary embodiment, about 0.1 mL to about 0.6 mL of the PGA stock solution is combined with the biological sample, which, in certain embodiments, is a positive blood culture. In certain embodiments, the positive blood culture is a biological sample that consists of an anti-coagulated whole blood determined to contain bacteria, upon being combined with a BACTEC™ growth media. In other embodiments, the biological sample is a whole blood sample. In an alternate embodiment, the biological sample is defibrinated blood.

[0030] In the described embodiments, the final concentration of PGA, after being combined with the positive blood culture, is in the range of about 0.99 mg/mL to about 7.00 mg/mL. In other embodiments, the final concentration of PGA, after being combined with the positive blood culture, is in the range of about 1.1 mg/mL to about 5.8 mg/mL. In the described embodiments, after the PGA solution combined with the positive blood culture is allowed to settle for an amount of time described herein, the sample fraction determined to contain a higher concentration of microorganism, is recovered for subsequent testing. Also contemplated herein are kits for providing PGA to a sample as described herein.

EXAMPLES

[0031] In the examples described below, the PGA stock solution used to obtain the test sample was prepared with PGA having molecular weight of up to 100,000. In other examples, a PGA powder having molecular weight in the range of 3,000 to 100,000 was used to prepare the stock solution. In other examples the stock solution was prepared using PGA having a molecular weight of about 50,000-70,000 and about 50,000-100,000.

[0032] The positive blood culture for the examples described below, was prepared by adding sodium polyanethole sulfonate (SPS) anticoagulated whole blood into a culture vial containing growth media and inoculated with fresh bacteria. Various bacteria (Gram-positive, Gram-negative), yeast, etc. can be used to inoculate the culture vial containing a growth media and anti-coagulated whole blood. Specific examples described herein include inoculated samples with *E. coli*, *S. aureus* and *S. pneumoniae*. In certain examples described below, the positive blood culture contained a microbial count of about 1 x 10^8 colony forming units (CFU) per milliliter by plate count of the total suspension. In the described examples, the microbial plate counts were performed in triplicate and the percentage of microbial recovery was calculated for each individual result. The resulting triplicate values for each sample were used to generate a spread in a Box & Whisker plot.

TEST METHOD

[0033] Positive blood culture was prepared by adding 10 mL of anti-coagulated whole blood sample into a culture vial containing 30 mL of growth media and inoculating the vial with up to 8 mL of a solution containing bacteria at a concentration of 2 McFarland. The growth media was a commercially available BACTEC™ Standard/10 Aerobic/F Media (Becton Dickinson) with reactive ingredients consisting of proteins, yeast extracts, amino acids, vitamins and resins to absorb antibiotics. Other growth media having similar reagents/ingredients that are well known to those skilled in the art can similarly be used for blood culture without lysing RBCs.

[0034] The PGA stock solution was made by placing a poly-L-glutamic acid (PGA) sodium salt powder of various molecular weights in a container, adding sterile distilled water to the container with PGA powder, placing the container having PGA powder and water on a Nutator™ (TCS Scientific Corp.) or a rocker to mix and dissolve the PGA powder in the water for about 30 minutes and storing at room temperature. The dissolved solution, having a clear and slightly viscous appearance, was sterilized by passing the solution through a 0.2 µm filter. The poly-L-glutamic acid sodium salt was purchased from Sigma-Aldrich Co. as a solid chemical in various molecular weights and mixed with sterile distilled water in amounts sufficient to achieve the desired stock PGA concentrations.

[0035] Test samples were obtained using the following method. First, the positive blood culture (e.g., 1 mL) was dispensed into a sterile tube. Next, an aqueous PGA solution was added to the sterile tube containing about 1 mL of positive blood culture and capped. The resulting mixture was gently mixed on a flatbed mixer for approximately 5-10 seconds and allowed to rest, undisturbed, in an upright position for about 10 - 30 minutes or until visual separation occurred demarking the PGA-concentrated test sample visually observed in the top layer from the dense bottom layer containing high concentration of red blood cells.

[0036] The test sample containing a PGA-induced segregation of bacteria from other sample constituents (i.e., the microbial concentrate) was removed from the top layer using a pipette. The volume of the test sample varied depending on the percentage of red blood cells in the test sample (i.e., hematocrit) and the sedimentation time.

[0037] The amount of bacteria present in various layers of the test tube containing a PGA solution and positive blood culture was determined by estimating bacteria per microscopic field using both Giemsa and Gram Stains.

[0038] The following are examples of the present disclosure provided for the purposes of illustrating the various methods described herein. They are not to be construed as limiting except within the spirit and scope of the appended claims.

Example 1

[0039] The test samples were prepared and obtained according to the test method described above. The PGA stock solution was prepared using PGA polymers having the following molecular weights: 3,000-15,000 (PGA 1), 15,000-50,000 (PGA 2) and 50,000-70,000 (PGA 3).

[0040] First, 1 mL of positive blood culture (PBC) inoculated with *S. aureus* was added to 6 separate sterile tubes. In Tube 1, 0.2 mL of PGA 1 having a stock concentration of 17.00 mg/mL was added resulting in a test solution having a total volume of 1.2 mL of PGA and PBC combined. Test tube 2 was prepared using the same protocol, except 0.5 mL of PGA 1 was added to the tube containing 1.0 mL of PBC and the total volume of the test solution amounted to

1.5 mL. Test tubes 3 and 4 were prepared by adding 0.2 mL and 0.5 mL of PGA 2 having a stock concentration of 18.7 mg/mL to tubes 3 and 4, respectively. Test tubes 5 and 6 were prepared by adding 0.2 mL and 0.5 mL of PGA 3 having a stock concentration of 12.6 mg/mL to tubes 5 and 6, respectively. The final PGA concentrations relative to the total volume (TV) of positive blood culture and stock PGA solutions are summarized in Table 1.

Table 1: Final PGA Concentration Range

	Tube#	Vol PBC mL	[PGA] mg/mL	Vol PGA mL	TV mL	Final [PGA] mg/mL
PGA 1	1	1.0	17.0	0.2	1.2	2.8
	2	1.0	17.0	0.5	1.5	5.7
PGA 2	3	1.0	18.7	0.2	1.2	3.1
	4	1.0	18.7	0.5	1.5	6.2
PGA 3	5	1.0	12.6	0.2	1.2	2.1
	6	1.0	12.6	0.5	1.5	4.2

[0041] The tested range of final PGA concentration in tubes 1 through 6 varied based on the amount of PGA added to 1.0 mL PBC at various stock PGA concentrations prepared with low molecular weight PGA (PGA 1 and PGA 2) and high molecular weight PGA (PGA 3). The visually separated layers in the test tubes (shown as 1, 2, and 3 for top layer, middle layer and bottom layer, respectively) obtained after approximately 60 minutes of sedimentation time are illustrated in FIG. 1A. Observations based on visually separated layers were recorded at 30 minutes and at 60 minutes. The solution made with PGA having more than 50,000 molecular weight (PGA 3) produced faster separation rate between RBCs and bacteria compared to the solution made with PGA having less than 50,000 molecular weight (PGA 1 and PGA 2). In addition, the gradient layer (i.e., the middle layer, shown as 2) of the liquid sample was absent in test tubes 4, 5 and 6 (FIG. 1A) after approximately

60 minutes of sedimentation. Only two visually distinct layers were observed in test tubes 4-6, indicating that using PGA of higher molecular weight (PGA 3) produced faster separation and efficiently concentrated the test sample in the top layer (shown as 1) of the liquid sample compared to PGA of lower molecular weight (PGA 1 and PGA 2).

[0042] For each visually observed layer within the test tubes 1 through 6, one slide for each stain type (Gram and Giemsa) was prepared and at least 20 high power fields (HPFs) per slide were interpreted under a microscope to determine the presence of bacteria in each layer using an arbitrary scale. The percentage of bacteria within the various layers of the test tubes (induced by the addition of PGA) was calculated to generate a bar chart shown in FIG. 1B using the following formula: % Bacteria = [Plate Count (CFU/mL) ÷ Seeded BACTEC™ Bottle Plate Count (CFU/ml)] x 100.

Example 2

[0043] Using the methods of Example 1, test samples were prepared using PBC inoculated with two different bacteria: *E. coli* and *S. aureus*. The PBC was prepared by adding 10 mL of anti-coagulated whole blood sample into a culture vial containing 30 mL of growth media and inoculating the vial with 8 mL of a solution containing bacteria at a concentration of 2 McFarland to yield an *S. aureus* count of approximately 8 x 10^8 CFU/ml, confirmed by plate count performed in triplicate, and an *E. coli* count of approximately 2 x 10^8 CFU/ml, without incubating the samples. Following PBC preparation, 1.0 mL of PBC with *E. coli* was added to three separated tubes. Next, 0.2 mL of stock PGA solution was added to each test tube. Solution A was prepared using PGA 1. Solution B was prepared using PGA 2. Solution C was prepared using PGA 3. Three additional tubes of PGA-concentrated bacterial test samples were made according to the same methods, except 1.0 mL of PBC prepared with *S. aureus* was used. The stock and final PGA concentrations are summarized in Table 2.

Table 2: PGA solutions containing PBC prepared with *E. coli* and *S. aureus*

PGA Soln	[PGA] mg/mL	Vol PGA mL	Vol Media/Blood	**Final[PGA] mg/mL**
A	11.1	0.2	1.0	1.9
B	20.1	0.2	1.0	3.4
C	25.8	0.2	1.0	4.3

[0044] After approximately 30 minutes of sedimentation time, a Box & Whisker Plot was used to determine the distribution of bacteria within the bottom and top layers of the prepared solution containing PGA and PBC. Data for Box & Whisker Plot analyses was generated as follows. Bacterial plate counts for *E. coli* and *S. aureus* were separately prepared from the seeded BACTEC™ bottle. The test samples that were obtained from the top and bottom layers of the liquid solutions were transferred onto prepared plated media and plate counts were performed to calculate the percentage (%) of bacteria in each layer using the following formula: % Bacteria = [Plate Count (CFU/mL) ÷ Seeded BACTEC™ Bottle Plate Count (CFU/ml)] x 100. The resulting data were imported into STATISTICA V10 data analysis software system (StatSoft, Inc.) to generate median, 25%-75% quartiles, and maximum/minimum values for each plotted category.

[0045] Solutions A, B and C having final PGA concentrations of 1.9 mg/mL, 3.4 mg/mL and 4.3 mg/mL, respectively, and prepared with PBC containing *E. coli*, generally had higher concentration of bacteria in the top layer compared to the control. The control used in the exemplary embodiments was a seeded BACTEC™ bottle without a PGA solution. More specifically, the concentration of *E. coli* in the top layer was highest in Solution A (prepared using the lowest molecular weight range PGA 1) and lowest in Solution C (prepared using the highest molecular weight range PGA 3).

[0046] The experimental results are shown in FIGS. 2A and 2B, which depict the Box & Whisker statistical analysis of the viable bacterial distribution within the top and bottom PGA-concentrated layers achieved after approximately 30 minutes of sedimentation time using seeded BACTEC™ bottle as a control. The Box represents the lower quartile range of 25% and the upper quartile range of 75% (or 25% and 75% quartiles). The Whiskers represent the minimum and maximum data ranges. The values along the Y-axis represent the percentage of bacteria in the top and/or bottom layers. The values along the X-axis represent the final PGA concentrations rounded to the nearest whole number (all CFU/mL values are in $\log_{10}$). Solutions A, B, and C that were prepared with PBC containing *S. aureus* had a higher concentration of *S. aureus* in the bottom layer compared to the control. The concentration of *S. aureus* in the bottom layer was highest in Solution C (prepared using the highest molecular weight range PGA 3) compared to Solution A (prepared using the lowest molecular weight range PGA 1).

<u>Comparative Example</u>

[0047] Solution A from Example 2 was compared to six non-PGA solutions to test alternative sedimentation mechanisms and the visual separation of the sedimentation layers in the absence of PGA (test tube 7). Six test tubes were prepared according to the test methods, except 0.2 mL of glycerol and sucrose solutions were added to the test tubes containing 1 mL of PBC. The PBC was prepared by adding 10 mL of anti-coagulated whole blood sample into a culture vial containing 30 mL of growth media and inoculating the vial with 8 mL of a solution containing *S. aureus* at a concentration of 2 McFarland to yield a microbial count of approximately 2×10^8 CFU/ml by plate count without incubating the samples. Table 3 summarizes the volume and concentrations of glycerol and sucrose used in the test tubes compared to Solution A which was prepared using PGA 1.

Table 3: Sedimentation using glycerol and sucrose as alternative sedimentation additives compared to PGA-concentrated Solution A

Tube#	Additive	Concentration	Units	Vol mL	Vol Media/Blood mL	Final Concentration
1	10% Glycerol	10	% (w/v)	0.2	1	1.7
2	5% Sucrose	5	% (w/v)	0.2	1	0.8
3	5% Glycerol	5	% (w/v)	0.2	1	0.8
4	10% Sucrose	10	% (w/v)	0.2	1	1.7
5	1 % Glycerol	1	% (w/v)	0.2	1	0.2
6	20% Sucrose	20	% (w/v)	0.2	1	3.3
7	PGA Soln "A"	11.1	mg/mL	0.2	1	1.9

[0048] The results of visual separation after approximately 30 minutes of sedimentation are shown in FIG. 3A. In the absence of PGA to segregate bacteria from other sample constituents as in the test tube 7, alternative sedimentation mechanisms did not yield a visual separation demarking the layers of the sample that are highly concentrated in bacteria from the layer that contains a high concentration of red blood cells. Figure 3B is a Box & Whisker Plot analysis of the percentage of bacteria in the top and bottom layers of the test tubes using seeded BACTEC™ bottle as the control. Most of the bacteria were concentrated in the bottom layer for test tubes 1 through 4 and test tube 6. Test tube 5, which was prepared by adding 0.2 mL of 1% glycerol to 1.0 mL of PBC, had a higher bacterial concentration in the top layer; however, there was minimal visual separation between the layers compared to Solution A.

Example 3

[0049] The effects of high molecular weight PGA on the sedimentation and separation rate of the PBC were tested as follows. The PBC was prepared according to the protocol described in Example 1. The tested bacteria in the PBC were

E. coli, *S. aureus* and *S. pneumoniae*. For each tested bacterium, four test tubes (A, B, C, and D) were prepared by first adding 1.0 mL of PBC into each sterile tube. The stock PGA solution added to PBC in tubes A and B was prepared using PGA of 50,000-70,000 molecular weight (PGA 3). The stock PGA solution added to PBC in tubes C and D was prepared using PGA of 50,000-100,000 molecular weight (PGA 4). The final PGA concentrations in tubes A and B were obtained by adding 0.1 mL and 0.3 mL of PGA 3 at a stock concentration of 12.5 mg/mL to each tube, respectively. The final PGA concentrations in tubes C and D were obtained by adding 0.1 mL and 0.3 mL of PGA 4 at a stock concentration of 25 mg/mL into each tube, respectively. Table 4 summarizes the PBC and PGA amounts/concentrations used to obtain the test samples in Example 3.

<u>Table 4: Final concentrations of PGA 3 & PGA 4 having overlapping molecular weight ranges</u>

Tube#	Additive	MW	Concentration	Units	Vol mL	Vol Media/Blood mL	**Final Concentration**
A	PGA 3	50-70K	12.5	mg/mL	0.1	1.0	1.1
B	PGA 3	50-70K	12.5	mg/mL	0.3	1.0	2.9
C	PGA 4	50-100K	25	mg/mL	0.1	1.0	2.3
D	PGA 4	50-100K	25	mg/mL	0.3	1.0	5.8

[0050] The percentages of bacteria in the top and bottom layers after approximately 30 minutes of sedimentation time were analyzed with the Box & Whisker Plot using seeded BACTEC™ bottle as the control. Figures 4A-B show the results of the test samples containing viable *E. coli*, *S. aureus* and *S. pneumoniae* that were concentrated using PGA 3 and PGA 4. Viable bacteria were present in all test samples obtained from the top sedimentation layer of the liquid sample. Use of PGA 4 (50,000-100,000) provided more consistent results across all three tested microorganisms by concentrating more bacteria in the top layer of the sample compared to the bottom layer.

[0051] The test samples prepared according to the methods described in Example 3 using PBC with *E. coli* were analyzed at 5 minute intervals starting at time 0 and up to 30 minutes. The test samples were obtained after 5, 10, 15, 20, 25 and 30 minutes of sedimentation and analyzed using Box & Whisker Plot to compare the effect of PGA having overlapping molecular weight ranges (PGA 3 and PGA 4) on sedimentation rate. The results are summarized in Figure 5. After 5 minutes of sedimentation time, the test sample (250 µL; 0.25mL) prepared with PGA 4 (50,000-100,000 molecular weight) contained viable *E. coli* in higher concentrations compared to the test sample prepared with PGA 3 (50,000-70,000 molecular weight). These results were consistent for test samples analyzed at 10, 15, 20, 25 and 30 minute sedimentation time intervals.

[0052] The test samples prepared according to the methods described herein were tested directly (without first extracting the bacteria) to Phoenix for Antimicrobial Susceptibility Testing (AST). The results are summarized in Table 5.

Table 5: Top layer PGA-concentrated bacterial sample tested directly to Phoenix for AST

Top PGA Layer Directly to Phoenix

Label	Bacteria	PGA	Phx Panel	AST
JR1	E. coli	N/A	G-	Yes
JR2	S. aureus	N/A	G+	Yes
JR3	S. pneumo	N/A	Strep	Yes
JR4	E. coli	A	G-	Yes
JR5	S. aureus	A	G+	Yes
JR6	S. pneumo	A	Strep	Yes
JR7	E. coli	B	G-	Yes
JR8	S. aureus	B	G+	Yes
JR9	S. pneumo	B	Strep	Yes
JR10	E. coli	C	G-	Yes
JR11	S. aureus	C	G+	Yes
JR12	S. pneumo	C	Strep	Yes
JR13	E. coli	D	G-	Yes
JR14	S. aureus	D	G+	Yes
JR15	S. pneumo	D	Strep	Yes

[0053] The use of PGA to concentrate (or at least segregate) bacteria in the top layer did not interfere with AST testing. A small amount of direct test samples were also placed into the Bruker Biotyper instrument and the MALDI-TOF assay was performed to obtain organism profiles. All mass spectrometry (MALDI-TOF) data were recorded on Bruker Microflex LT with MALDI Biotyper software version 2.0.10.0 with the following MALDI score key definitions. A MALDI ID score of greater than 2.299 indicates a highly probable identification to the Species level. A MALDI ID score of 2.000-2.299 indicates a satisfactory identification to the Genus and Species level. A MALDI ID score of 1.700-1.999 indicates a probable identification to the Genus level. A MALDI ID score of less than 1.699 indicates an unacceptable or not reliable identification. There were no peaks when testing direct samples to MALDI-TOF. The MALDI-TOF spectra generated from direct samples did not match mass spectra currently in the Bruker database. The *E. coli* isolates in the control sample achieved a reliable quality identification score of more than or equal to 2.289 (≥2.289), indicating that use of PGA to concentrate bacteria in a top layer interfered with MALDI-TOF identification when testing direct samples.

Example 4

[0054] Five test tubes were prepared according to the methods described in Example 1 using *E. coli* to prepare PBC. The test tubes were prepared by first adding 1.0 mL of PBC into each sterile tube followed by adding 0.1 mL of stock PGA solution prepared using PGA 4 (50,000-100,000 molecular weight) into each tube. All test tubes were subjected to a quick mix using a flatbed mixer to yield a liquid sample having final PGA concentration of 2.27 mg/mL. Following 30 minutes of sedimentation, the test samples were removed from the top layers of the liquid sample in all five test tubes. In order to obtain an adequate sample volume, each test sample was combined (800 µL x 5) and centrifuged at 16000 x g for 3 minutes, followed by two additional cycles of washing with de-ionized water. This pellet was extracted by the Bruker MALDI-TOF extraction protocol. For reproducibility of MALDI-TOF results, duplicate spots from the extracted pellet were used to generate spectra. Recovery of microorganisms was measured by viable plate counts. It would be appreciated by one skilled in the art that methods for identifying bacteria using MALDI-TOF are well known in the art and are not described in detail herein. The results are summarized in Table 6.

Table 6. MALDI-TOF results of *E.coli* PBC sample treated with PGA

Replicate	Best match	Score	2nd best match	Score
1	E. coli MB11464_1 CHB	2.236	E. coli 25922 CHB	2.199
2	E. coli 25922 THL	2.165	E. coli MB11464_1 CHB	2.127

[0055] The methods in accordance with Example 4 resulted in approximately 54% recovery of *E. coli* from the PGA-concentrated test samples. The centrifugation and wash procedure yielded approximately 87% *E. coli* as an isolated pellet. The total yield of *E. coli* recovered from the test samples (which were the top layer of the liquid samples) was approximately 46%. Washing the test samples with de-ionized water followed by centrifugation effectively removed supernatant containing PGA from the pelleted *E. coli* without destroying the suitability of the microorganism for MALDI-TOF identification. The extracted *E. coli* isolates (compared to direct sample testing) achieved a reliable quality identification score of more than or equal to 2.000 (≥2.000).

Example 5

[0056] Using laboratory Neubauer Hemacytometer method and procedure (based upon Clinical and Laboratory Standards Institute, "Body Fluid Analysis for Cellular Composition; Approved Guideline", CLSI doc. H56-A; 26(6) (2006)), the amount of red blood cells were counted in the top and bottom sedimentation layers of a liquid sample to observe the effects of PGA on reducing RBC count in the test sample drawn from the top layer of the liquid sample. A positive blood culture was prepared by adding 10 mL SPS anticoagulated whole blood into a BACTEC™ culture media that was inoculated with about 10-100 CFU of fresh bacteria (*S. aureus*) to yield a final bacterial count of approximately $1x10^8$ CFU/mL. In this example, the BACTEC™ culture bottle inoculated with bacteria, placed in a BACTEC™ FX automated blood culture instrument and incubated for 12-18 hours or until a positive result was indicated. A positive result is indicated when a final bacterial count of approximately $1x10^8$ CFU/mL is detected in each BACTEC™ bottle. Following incubation, 1.0 mL of PBC was placed into a sterile tube with 0.1 mL of stock PGA solution having a final concentration of 25 mg/mL prepared with PGA of 50,000-100,000 molecular weight (PGA 4) resulting in a liquid sample having a final PGA concentration of 2.27 mg/mL. After approximately 30 minutes of sedimentation, the test sample was removed from the top layer of the liquid sample and RBCs were counted in the top layer and the bottom layer of the liquid sample using standard procedures. Compared to the bottom layer (2.89 x 10^6 RBC/µL), there were 3.50 x 10^4 RBC/µL in the top layer indicating that the RBC concentration in the top layer of the liquid sample following PGA-sedimentation was reduced by two logs (all RBC/µL values are in $\log_{10}$). The results are summarized in Table 7 below.

<u>Table 7:</u>

Tube 2 PGA

Bottom	Square	Chamber A	Chamber B	Dilution Factor	Volume Factor
1:200 EryTic	1	38	65	200	1.00E+04
	2	58	59		
	3	74	53		
	4	59	61		
	5	54	57		
	Sum	283	295		
	RBC/mL	2.83E+09	2.95E+09		
Top	Square	Chamber A	Chamber B	Dilution Factor	Volume Factor
1:200 EryTic	1	0	0	200	1.00E+04
	2	2	0		
	3	1	1		
	4	1	1		
	5	1	1		
	Sum	5	3		
	RBC/mL	5.00E+07	3.00E+07		
Top	Square	Chamber A	Chamber B	Dilution Factor	Volume Factor
1:100 Plate Count	1	0	0	100	1.00E+04
	2	2	2		
	3	1	0		
	4	2	2		
	5	1	2		
	Sum	6	6		
	RBC/mL	3.00E+07	3.00E+07		
	RBC/mL	RBC/µL			
Average Bottom	2.89E+09	2.89E+06			
Average Top	3.50E+07	3.50E+04			

[0057] Although the invention herein has been described with reference to particular embodiments, it is to be understood that these embodiments are merely illustrative of the principles and applications of the present invention. It is therefore to be understood that numerous modifications may be made to the illustrative embodiments and that other arrangements may be devised without departing from the spirit and scope of the present invention as defined by the appended claims.

CLAIMS

1. A method of preparing a biological sample without centrifugation, the method comprising:

(a) obtaining a biological sample that may contain at least one microorganism,

(b) adding a poly-L-glutamic acid solution,

(c) allowing the biological sample containing poly-L-glutamic acid to settle,

(d) removing a portion of the solution from a top portion of the settled sample; and

(d) evaluating the removed portion of the sample for the presence or absence of microorganisms.

2. The method of claim 1, wherein the biological sample is a positive blood culture.

3. The method of claim 1, wherein the biological sample is whole blood containing bacteria.

4. The method of claim 1, wherein the settled sample is a stratified solution.

5. The method of claim 1, wherein the solution containing poly-L-glutamic acid is allowed to settle for about 60 minutes or less.

6. The method of claim 1, wherein the solution containing poly-L-glutamic acid is allowed to settle for about 30 minutes or less.

7. The method of claim 1, wherein the solution containing poly-L-glutamic acid is allowed to settle for about 15 minutes or less.

8. The method of claim 1, wherein said at least one microorganism is bacteria.

9. The method of claim 1, wherein the poly-L-glutamic acid solution comprises a poly-L-glutamic acid having at least one molecular weight of about 3,000 to about 100,000.

10. The method of claim 9, wherein the poly-L-glutamic acid is a mixture of poly-L-glutamic acids of different molecular weights, wherein the molecular weights are in the range of about 3,000 to about 100,000.

11. The method of claim 1, wherein the poly-L-glutamic acid solution comprises poly-L-glutamic acid having at least one molecular weight of about 50,000 to about 100,000.

12. The method of claim 11, wherein the poly-L-glutamic acid is a mixture of poly-L-glutamic acids of different molecular weights, wherein the molecular weights are in the range of about 50,000 to about 100,000.

13. The method of claim 1, wherein the poly-L-glutamic acid solution has a poly-L-glutamic acid concentration of about 1 mg/mL to about 26 mg/mL in water.

14. The method of claim 1, wherein the amount of poly-L-glutamic acid solution added to the biological sample is in the amount of about 0.1 mL to about 0.6 mL in about 1 mL of the biological sample.

15. The method of claim 1, wherein the concentration of the poly-L-glutamic acid in the sample is in the range of about 0.99 mg/mL to about 7.00 mg/mL.

16. The method of claim 1, further comprising isolating the microorganisms for use in downstream processing and detection methods.

17. The method of claim 1, wherein the presence or absence of microorganisms is evaluated using a method selected from the group consisting of Antimicrobial Susceptibility Testing, Mass Spectrophotometer, or both.

18. A method of concentrating bacteria in a biological sample without centrifugation, the method comprising:

(a) obtaining a positive blood culture determined to contain at least one bacterium,

(b) adding a poly-L-glutamic acid solution,

(c) allowing the biological sample containing poly-L-glutamic acid to settle for about 60 minutes or less,

(d) removing a portion of the solution from a top portion of the settled sample; and

(d) evaluating the removed portion of the sample for the presence or absence of bacteria.

19. The method of claim 18, wherein the settled sample is a stratified solution.

20. The method of claim 18, wherein the solution containing poly-L-glutamic acid is allowed to settle for about 30 minutes or less.

21. The method of claim 18, wherein the solution containing poly-L-glutamic acid is allowed to settle for about 15 minutes or less.

22. The method of claim 18, wherein the poly-L-glutamic acid solution comprises a poly-L-glutamic acid having at least one molecular weight of about 3,000 to about 100,000.

23. The method of claim 22, wherein the poly-L-glutamic acid is a mixture of poly-L-glutamic acids of different molecular weights, wherein the molecular weights are in the range of about 3,000 to about 100,000.

24. The method of claim 18, wherein the poly-L-glutamic acid solution comprises a poly-L-glutamic acid having at least one molecular weight of about 50,000 to about 100,000.

25. The method of claim 24, wherein the poly-L-glutamic acid is a mixture of poly-L-glutamic acids of different molecular weights, wherein the molecular weights are in the range of about 50,000 to about 100,000.

26. The method of claim 18, wherein the poly-L-glutamic acid solution has a poly-L-glutamic acid concentration of about 1 mg/mL to about 26 mg/mL in water.

27. The method of claim 18, wherein the amount of poly-L-glutamic acid solution added to the positive blood culture is in the amount of about 0.1 mL to about 0.6 mL in about 1 mL of the positive blood culture.

28. The method of claim 18, wherein the concentration of the poly-L-glutamic acid in the sample is in the range of about 0.99 mg/mL to about 7.00 mg/mL.

29. The method of claim 18, further comprising isolating the bacteria for use in downstream processing and detection methods.

30. The method of claim 18, wherein the presence or absence of bacteria is evaluated using a method selected from the group consisting of Antimicrobial Susceptibility Testing, Mass Spectrophotometer, or both.

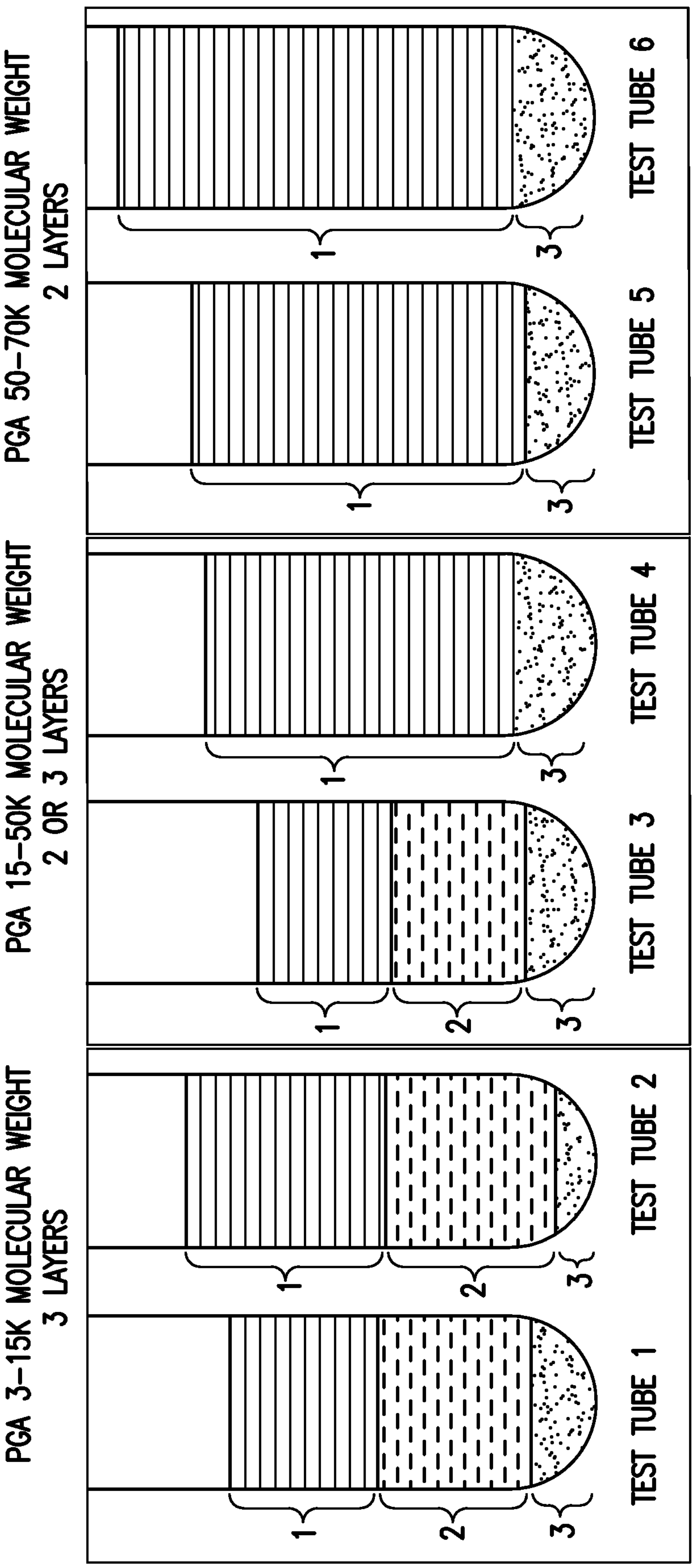

PGA 3-15K MOLECULAR WEIGHT
3 LAYERS
PGA 15-50K MOLECULAR WEIGHT
2 OR 3 LAYERS
PGA 50-70K MOLECULAR WEIGHT
2 LAYERS
TEST TUBE 1
TEST TUBE 2
TEST TUBE 3
TEST TUBE 4
TEST TUBE 5
TEST TUBE 6
1
2
3

FIG.1A

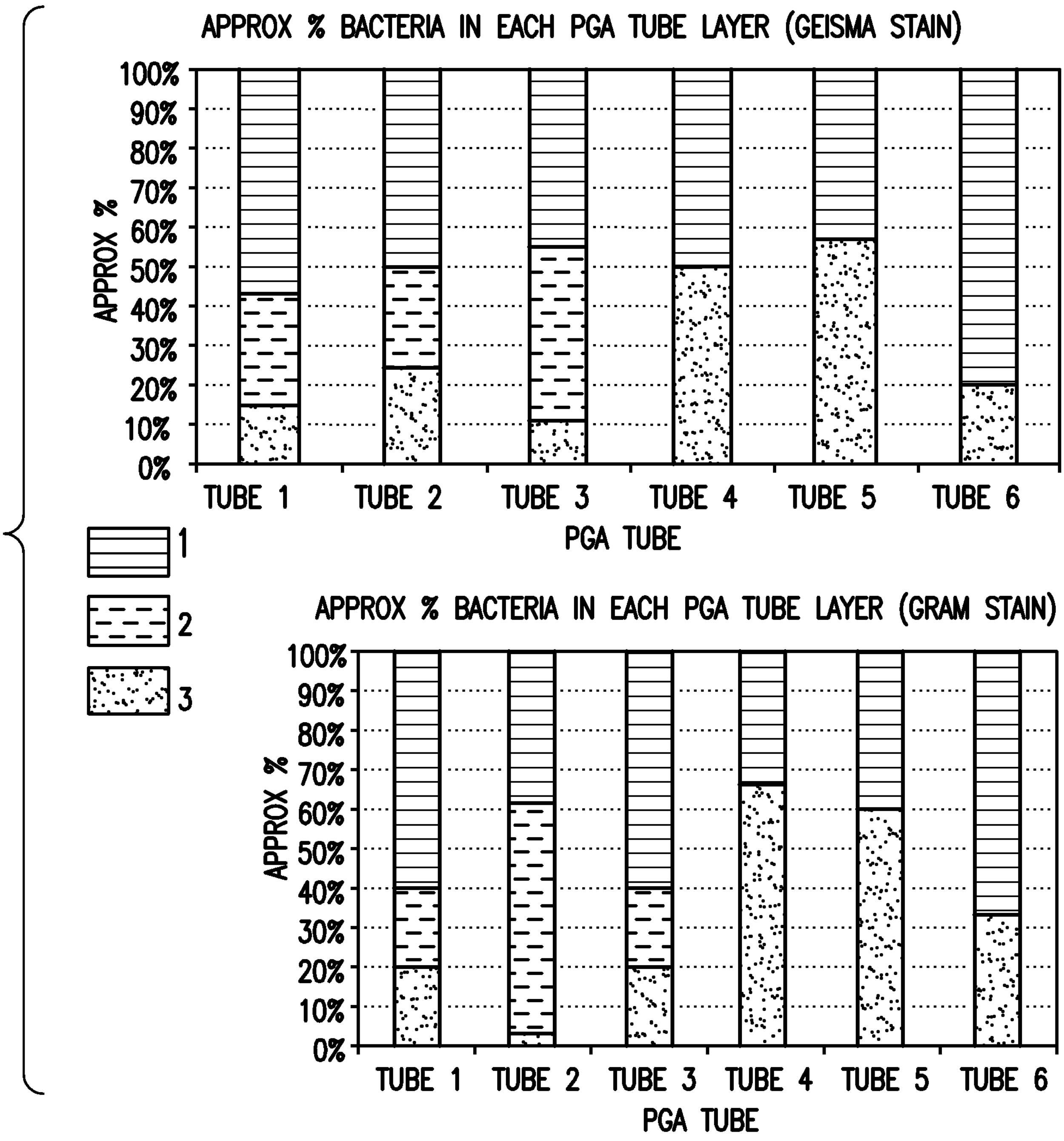

APPROX % BACTERIA IN EACH PGA TUBE LAYER (GEISMA STAIN)
APPROX %
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
TUBE 1
TUBE 2
TUBE 3
TUBE 4
TUBE 5
TUBE 6
PGA TUBE
1
2
3
APPROX % BACTERIA IN EACH PGA TUBE LAYER (GRAM STAIN)
APPROX %
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
TUBE 1
TUBE 2
TUBE 3
TUBE 4
TUBE 5
TUBE 6
PGA TUBE

FIG.1B

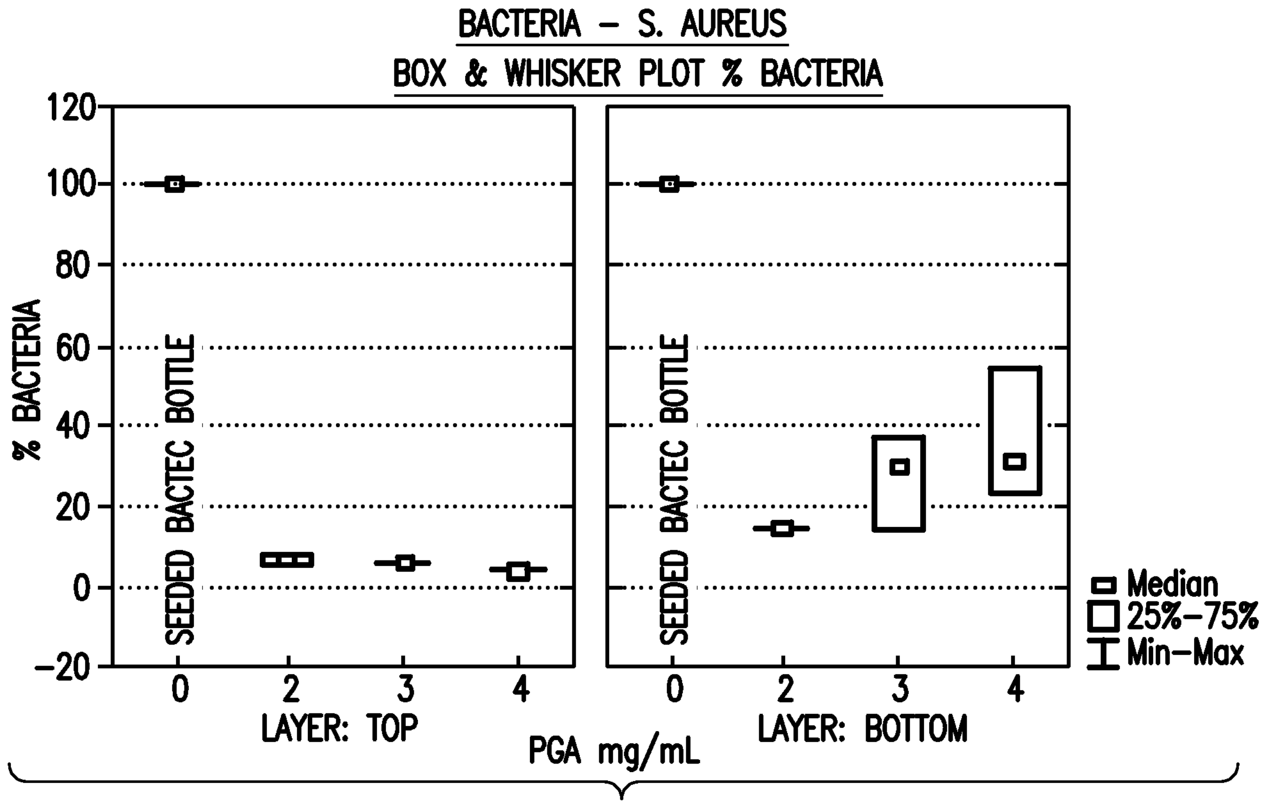

BACTERIA – S. AUREUS
BOX & WHISKER PLOT % BACTERIA
% BACTERIA
120
100
80
60
40
20
0
–20
SEEDED BACTEC BOTTLE
SEEDED BACTEC BOTTLE
0
2
3
4
LAYER: TOP
LAYER: BOTTOM
PGA mg/mL
Median
25%–75%
Min–Max

FIG.2A

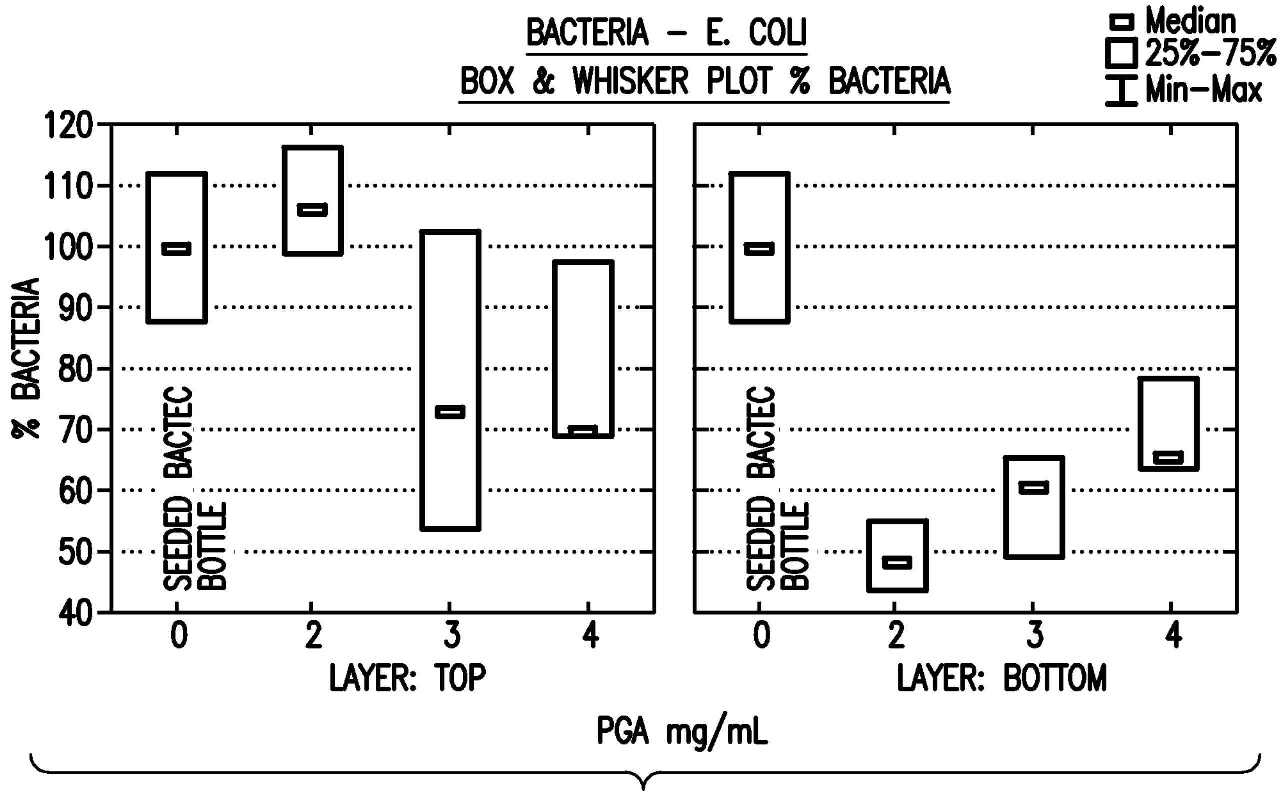

BACTERIA – E. COLI
BOX & WHISKER PLOT % BACTERIA
Median
25%–75%
Min–Max
% BACTERIA
120
110
100
90
80
70
60
50
40
SEEDED BACTEC BOTTLE
SEEDED BACTEC BOTTLE
0
2
3
4
LAYER: TOP
LAYER: BOTTOM
PGA mg/mL

FIG.2B

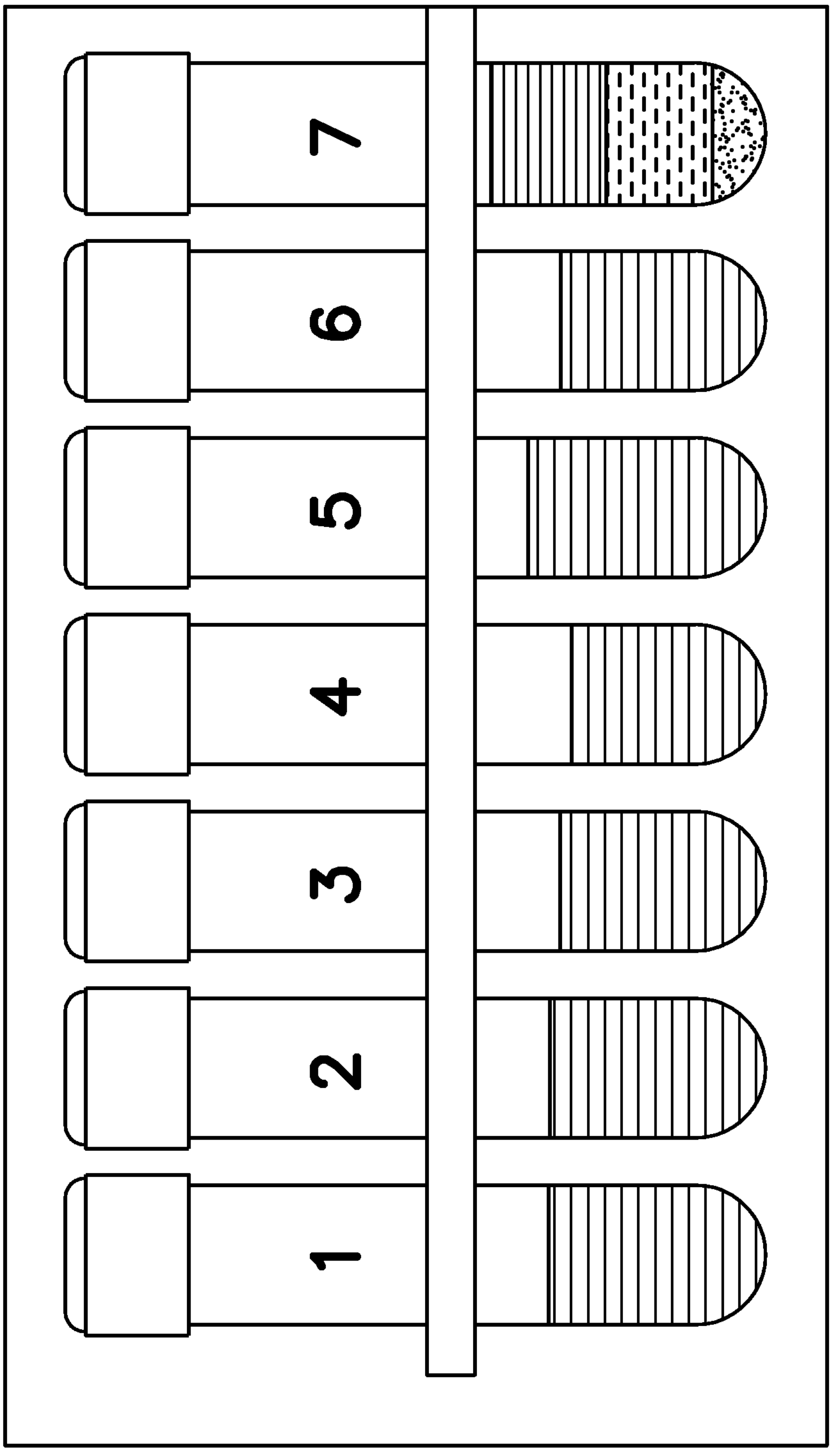


FIG.3A

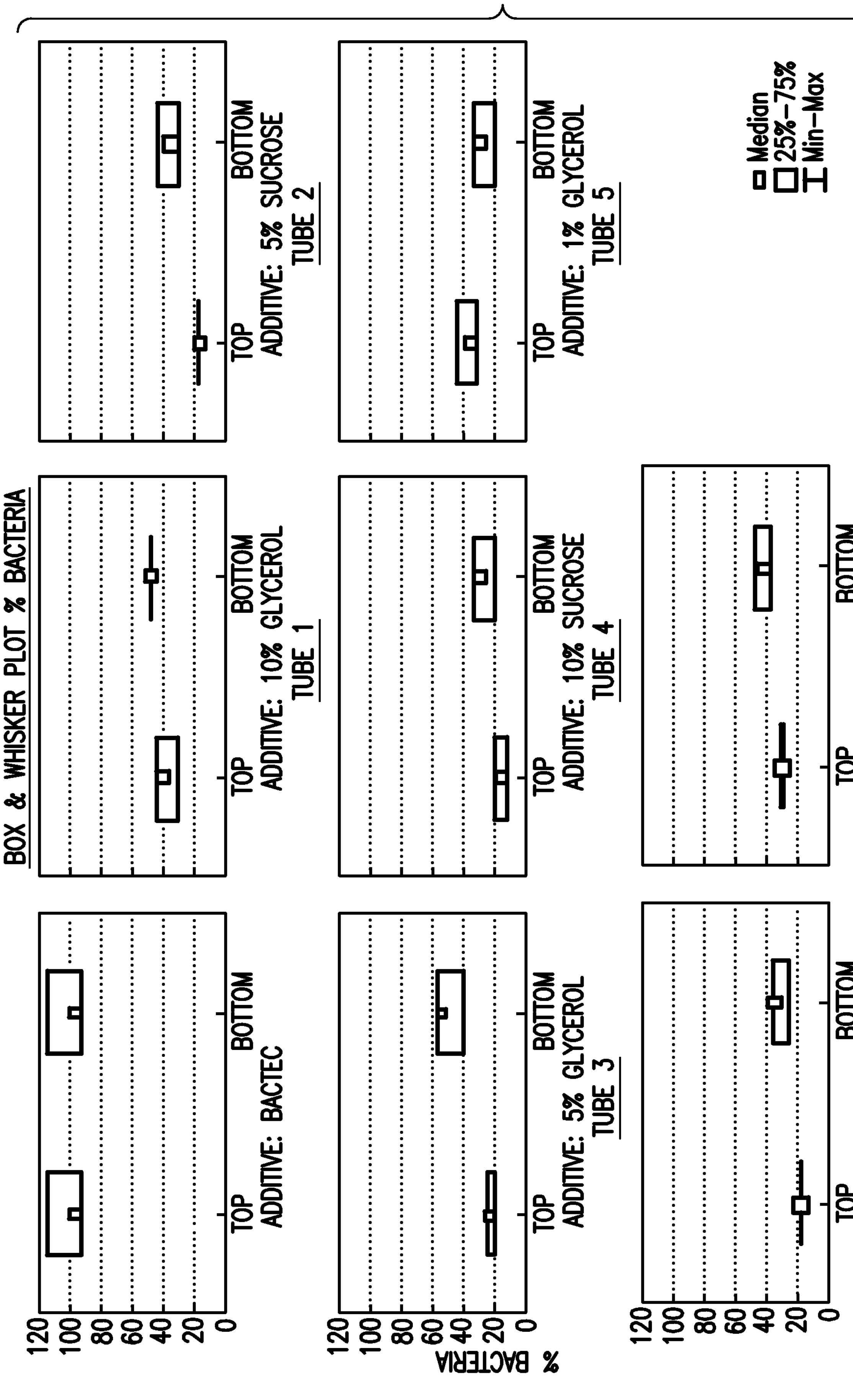

BOX & WHISKER PLOT % BACTERIA
% BACTERIA
120
100
80
60
40
20
0
TOP
BOTTOM
ADDITIVE: BACTEC
ADDITIVE: 10% GLYCEROL
TUBE 1
ADDITIVE: 5% SUCROSE
TUBE 2
ADDITIVE: 5% GLYCEROL
TUBE 3
ADDITIVE: 10% SUCROSE
TUBE 4
ADDITIVE: 1% GLYCEROL
TUBE 5
ADDITIVE: 20% SUCROSE
TUBE 6
ADDITIVE: PGA SOLN A
TUBE 7
Median
25%–75%
Min–Max

FIG.3B

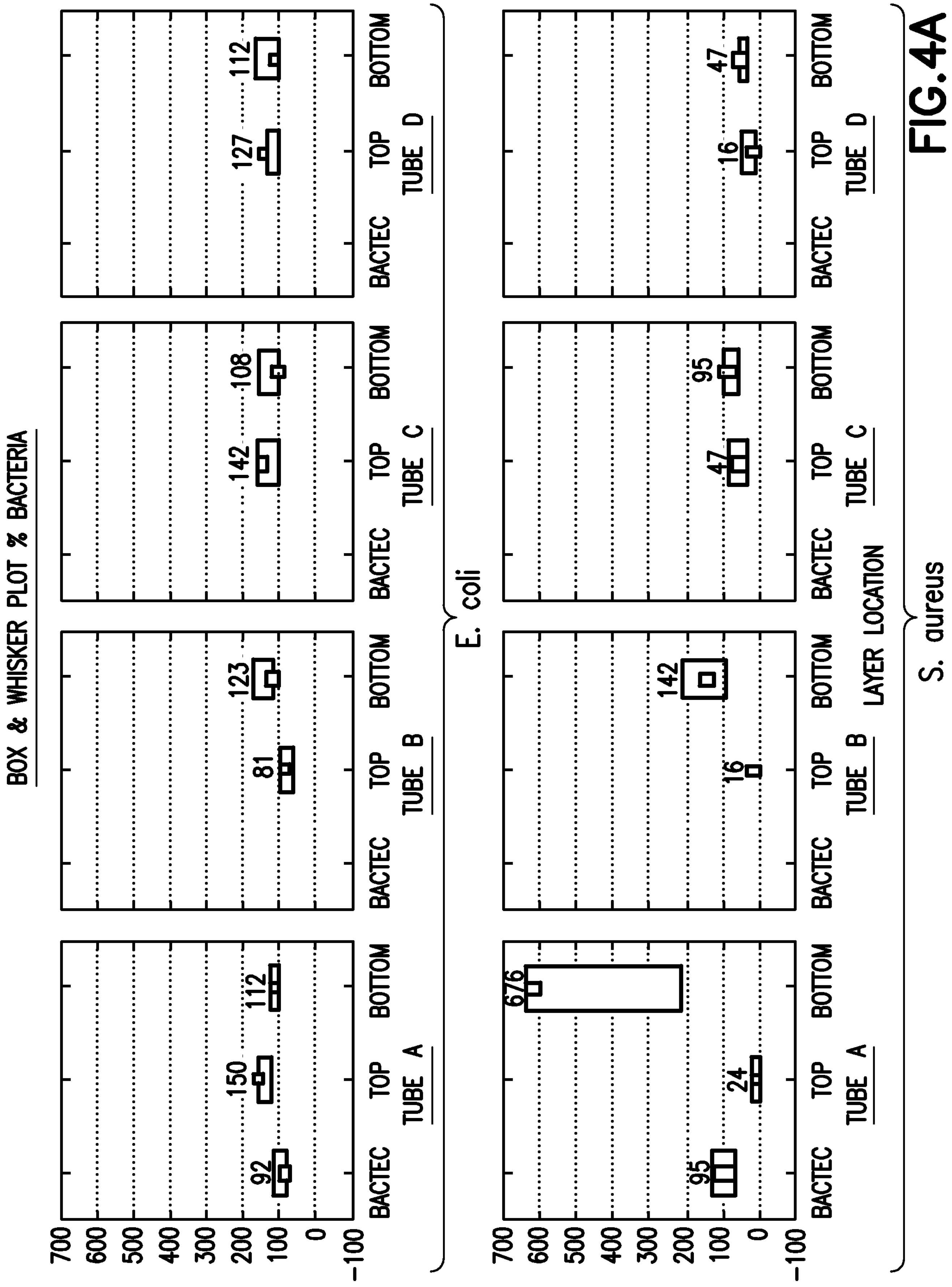
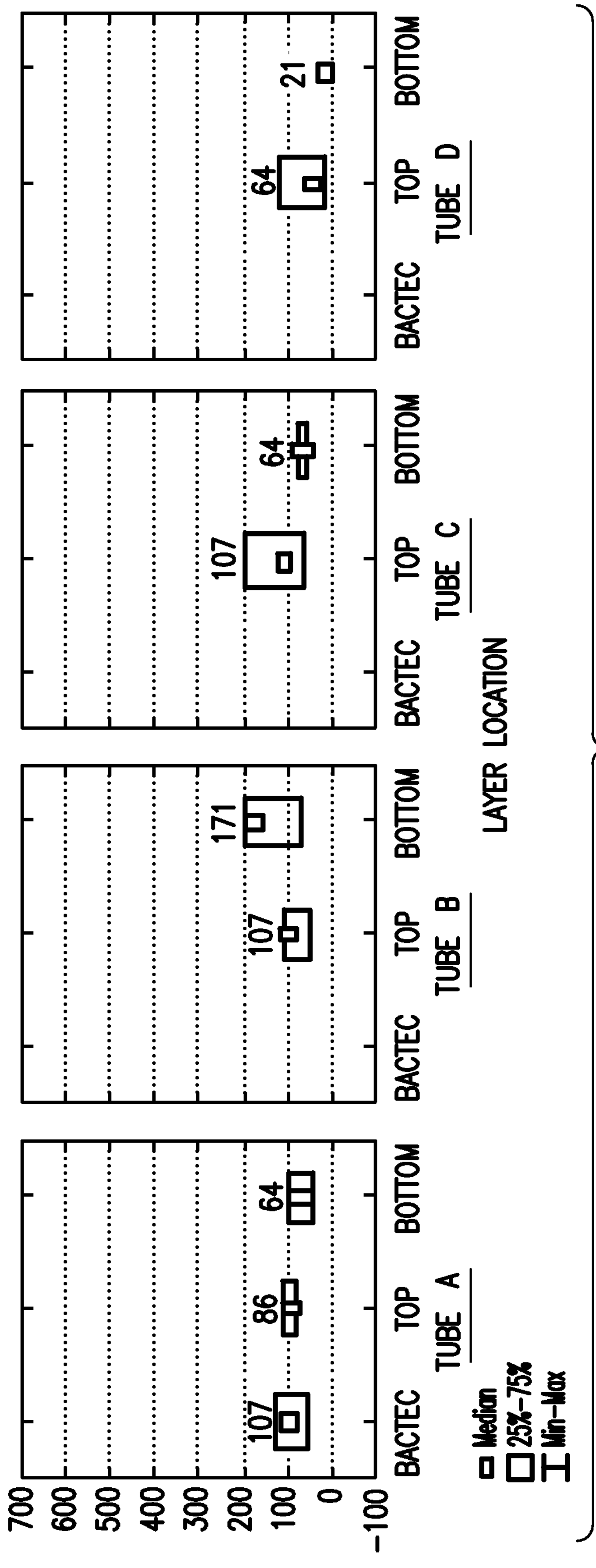

BOX & WHISKER PLOT % BACTERIA
700
600
500
400
300
200
100
0
-100
BACTEC
TOP
BOTTOM
TUBE A
TUBE B
TUBE C
TUBE D
92
150
112
81
123
142
108
127
112
E. coli
95
24
676
16
142
47
95
16
47
LAYER LOCATION
S. aureus

FIG.4A



S. pneumoniae

FIG.4B

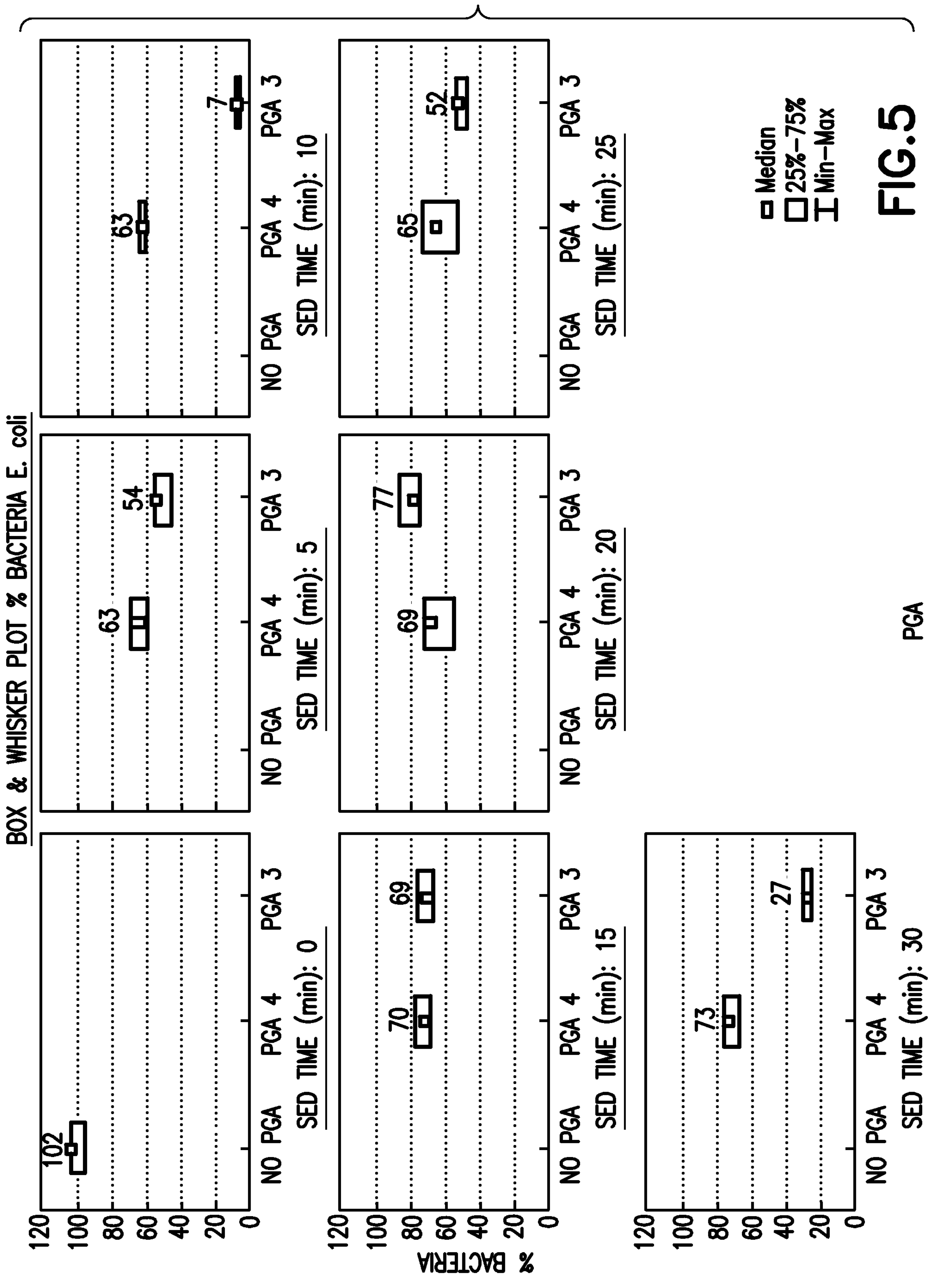

BOX & WHISKER PLOT % BACTERIA E. coli
% BACTERIA
120
100
80
60
40
20
0
NO PGA
PGA 4
PGA 3
SED TIME (min): 0
SED TIME (min): 5
SED TIME (min): 10
SED TIME (min): 15
SED TIME (min): 20
SED TIME (min): 25
SED TIME (min): 30
102
63
54
63
7
70
69
69
77
65
52
73
27
PGA
Median
25%–75%
Min–Max

FIG.5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/042220

A. CLASSIFICATION OF SUBJECT MATTER

G01N 33/49(2006.01)i, G01N 1/40(2006.01)i, C12Q 1/02(2006.01)i

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)
G01N 33/49; A01N 1/02; G01N 33/543; G01N 1/40; C12Q 1/02

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKOMPASS(KIPO internal) & Keywords: concentrate, microorganism, blood, poly-L-glutamic acid, evaluate, presence

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2005-0170327 A1 (SUMIDA et al.) 04 August 2005 See paragraphs [0012]-[0024], [0072]-[0091], [0105]-[0108]; claims 1-8; Figures 6-9(2).	1-30
Y	US 2010-0129836 A1 (GOODNOW, TIMOTHY T.) 27 May 2010 See paragraphs [0012], [0013], [0030], [0059], [0060], [0092]; claims 1-8.	1-30
A	SUMIDA et al., Platelet separation from whole blood in an aqueous two-phase system with water-soluble polymers, Journal of Pharmacological Sciences, 2006, Vol. 101, No. 1, pp. 91-97 See abstract; pages 91-96; Table 1; Figures 1, 3, 4.	1-30
A	BANKS et al., Influences of blood sample processing on low-molecular-weight proteome identified by surface-enhanced laser desorption/ionization mass spectrometry, Clinical Chemistry, 2005, Vol. 51, No. 9, pp. 1637-1649 See pages 1637-1648.	1-30
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☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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