FIG. 1A

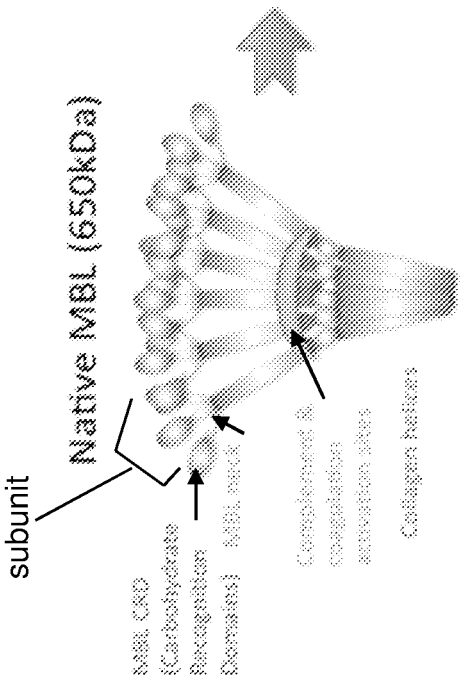


FIG. 1B

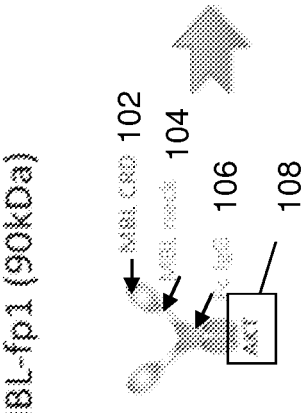


FIG. 1C

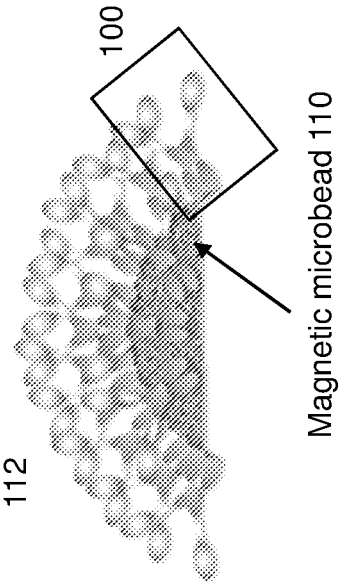


FIG. 2

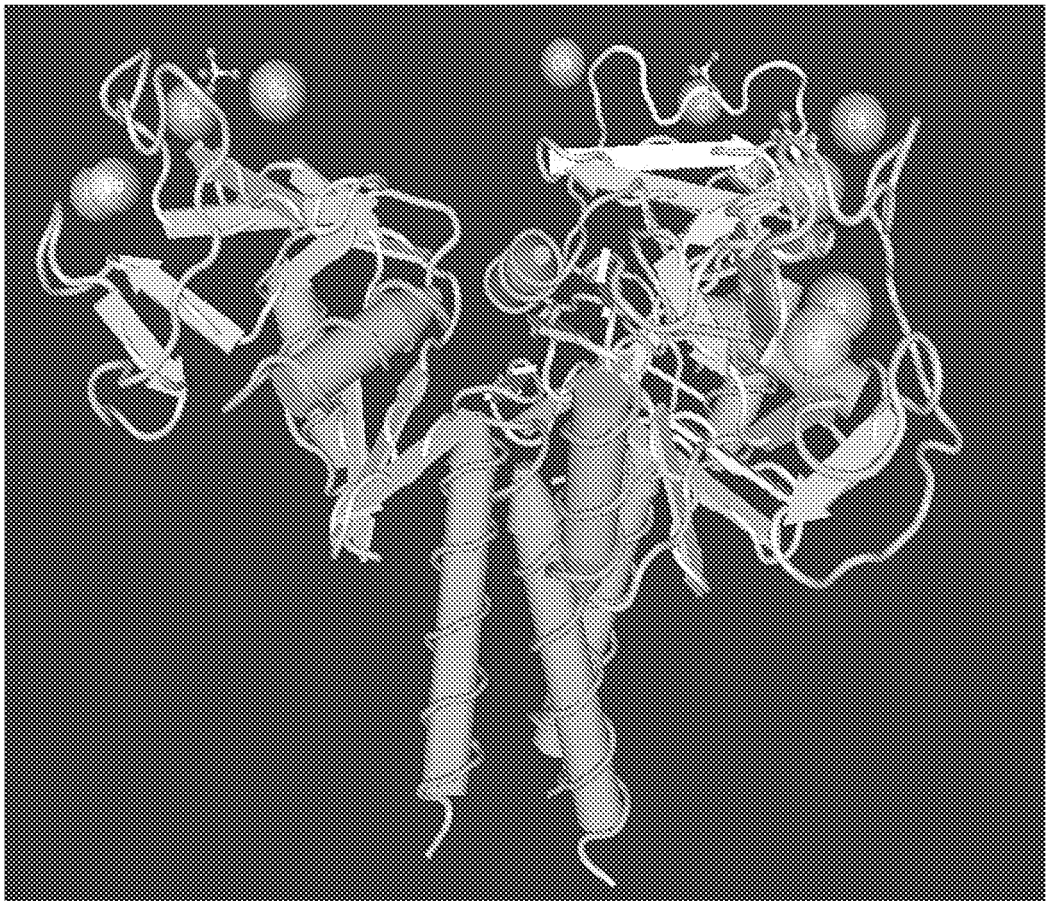


FIG. 3

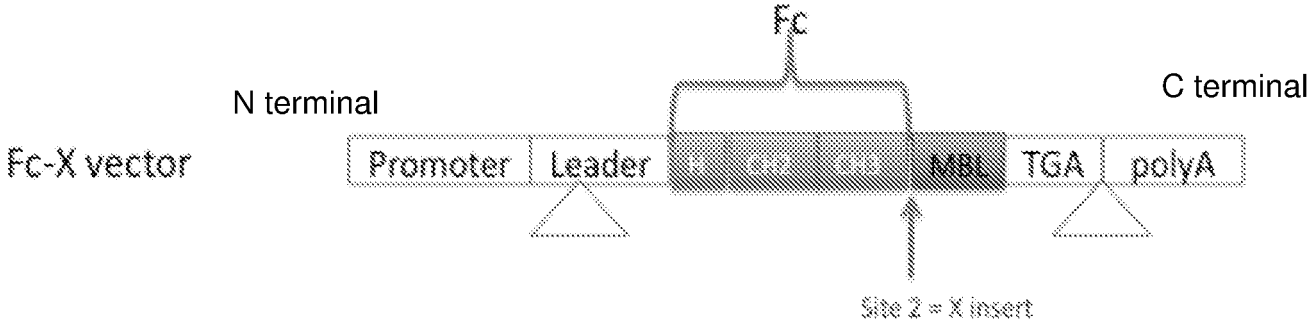

Fc
N terminal
C terminal
Fc-X vector
Promoter
Leader
MBL
TGA
polyA
Site 2 = X insert

FIG. 4

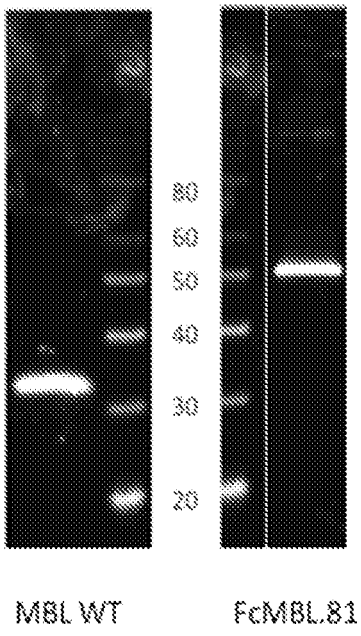

80
60
50
40
30
20
MBL WT
FcMBL81

FIG. 5

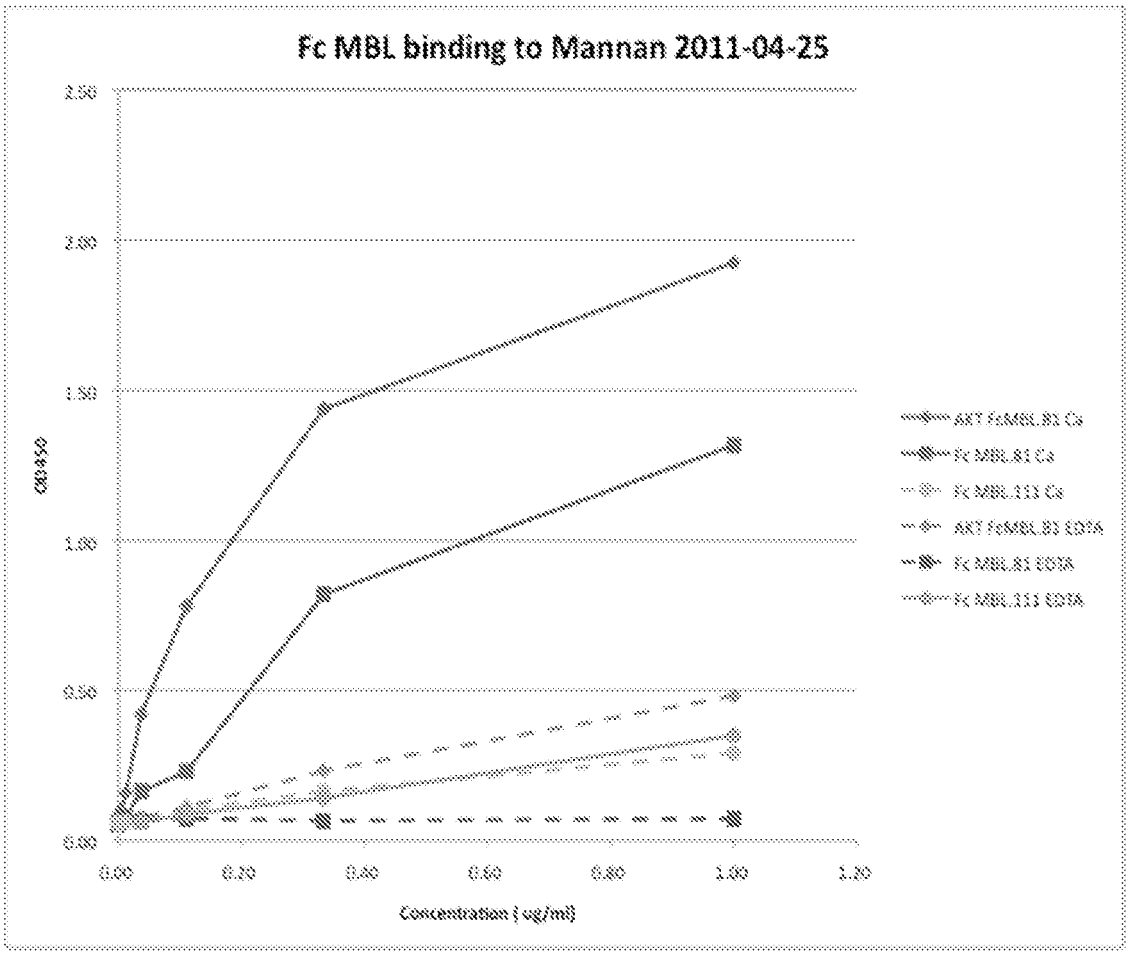

Fc MBL binding to Mannan 2011-04-25
OD450
Concentration (ug/ml)
0.00
0.20
0.40
0.60
0.80
1.00
1.20
0.00
0.50
1.00
1.50
2.00
2.50
ART FcMBL81 Ca
Fc MBL81 Ca
Fc MBL111 Ca
ART FcMBL81 EDTA
Fc MBL81 EDTA
Fc MBL111 EDTA

FIG. 6A

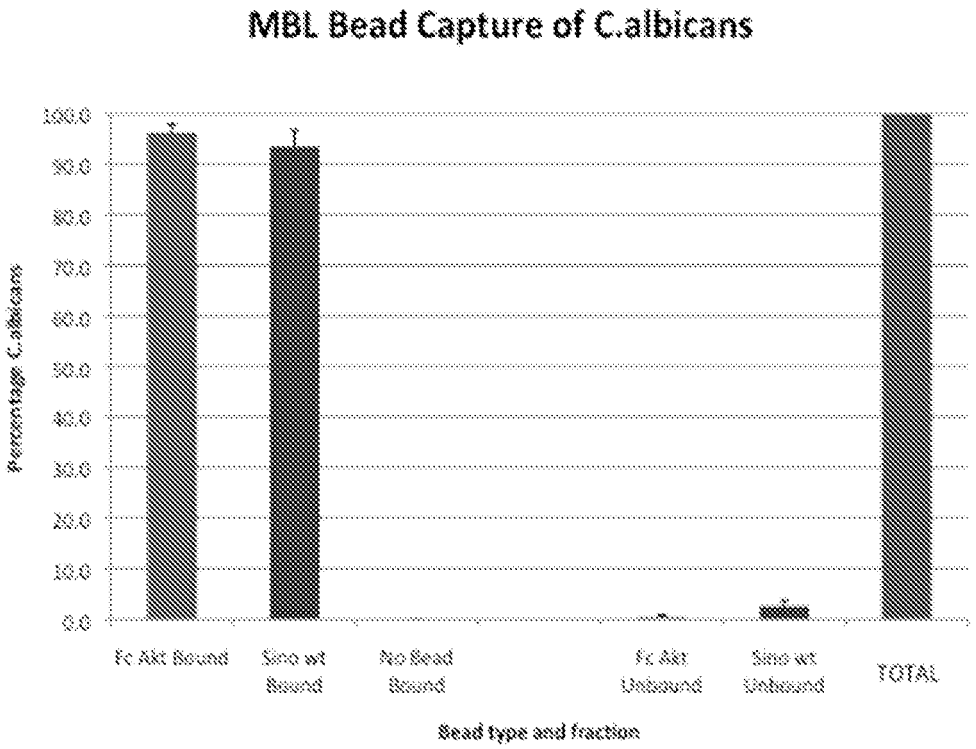
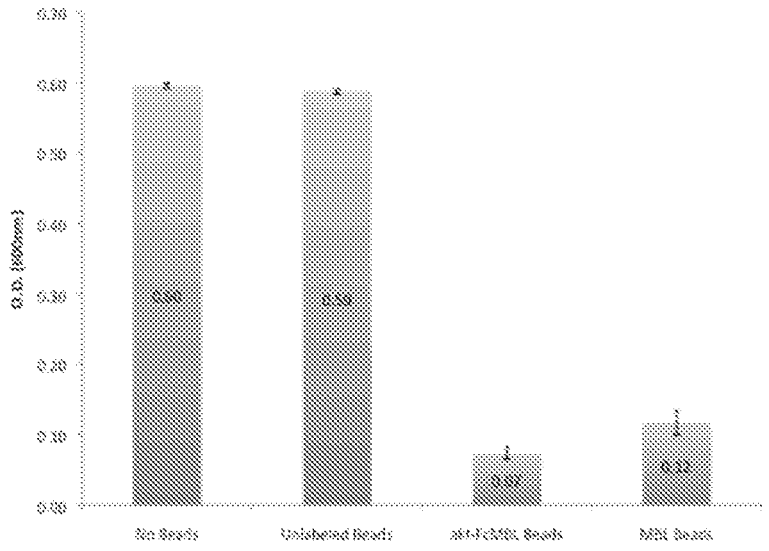
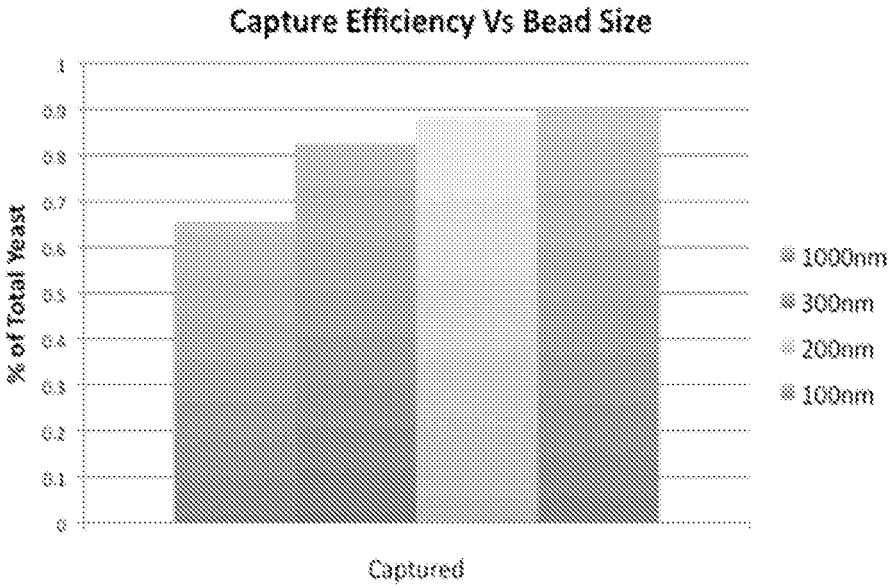


FIG. 6B



* difference is statistically significant ($p < 0.01$) by a two-tailed, unpaired, T-test.

FIG. 7



AKT-FcMBL81 beads
1um = Dynal T1
300nm,200nm,100nm = Ademtech Streptavadin Plus (C1)

FIG. 8

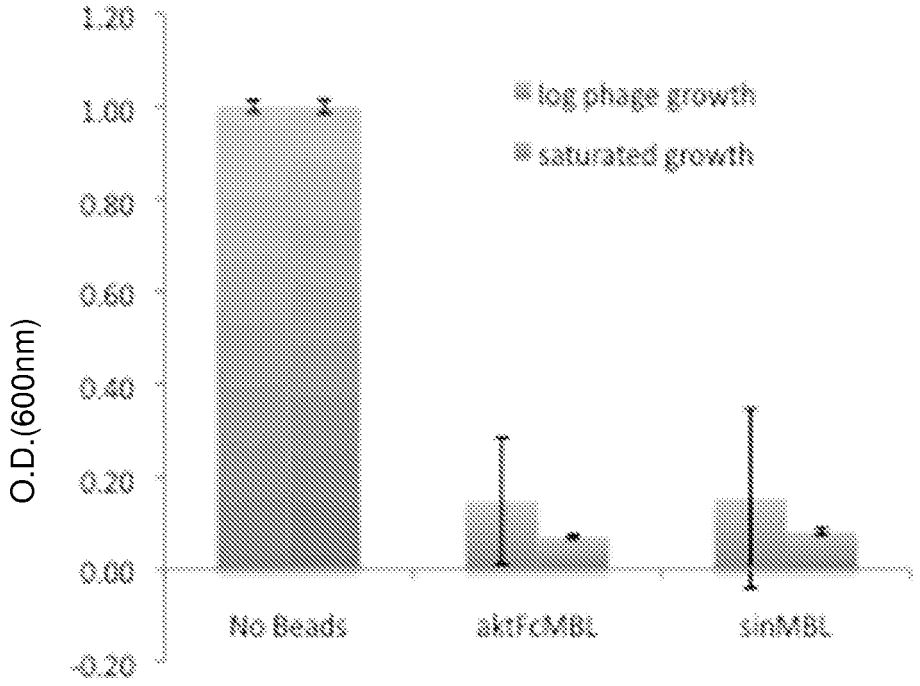


FIG. 9

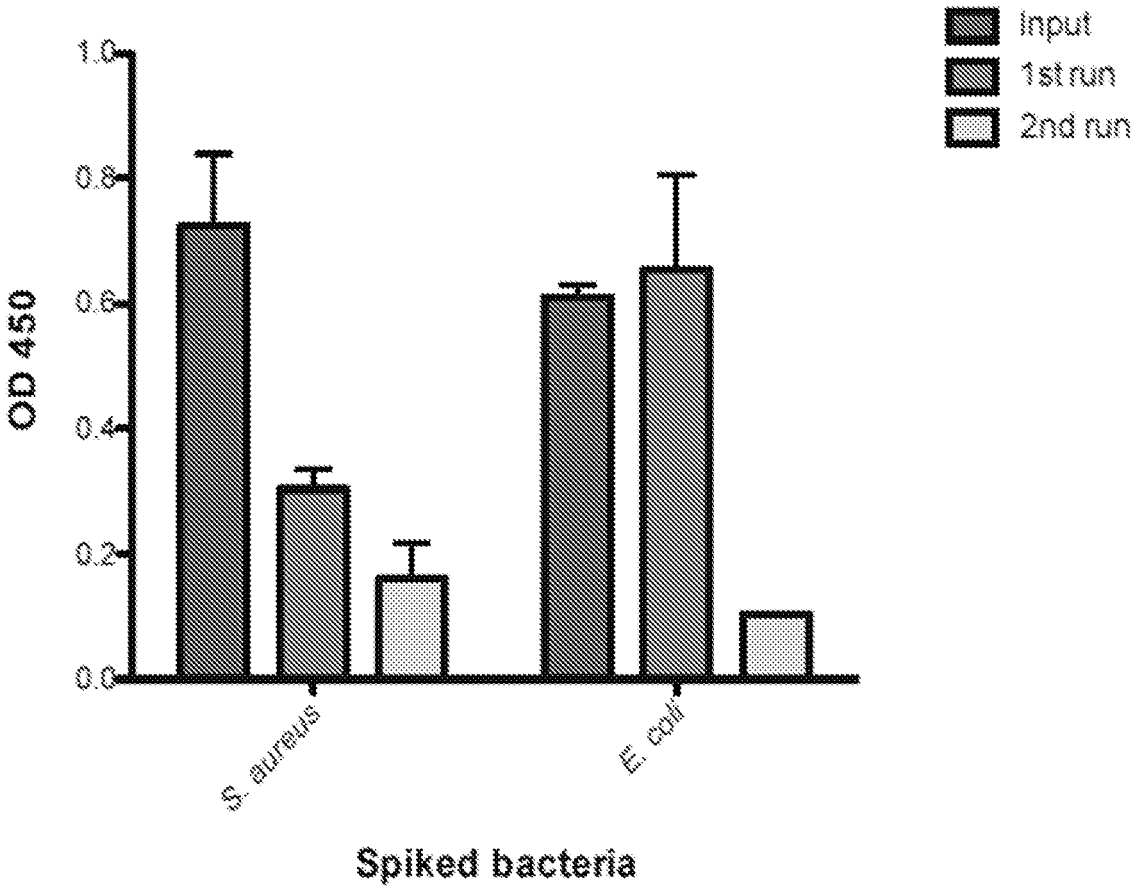

Input
1st run
2nd run
OD 450
1.0
0.8
0.6
0.4
0.2
0.0
S. aureus
E. coli
Spiked bacteria

FIG. 10

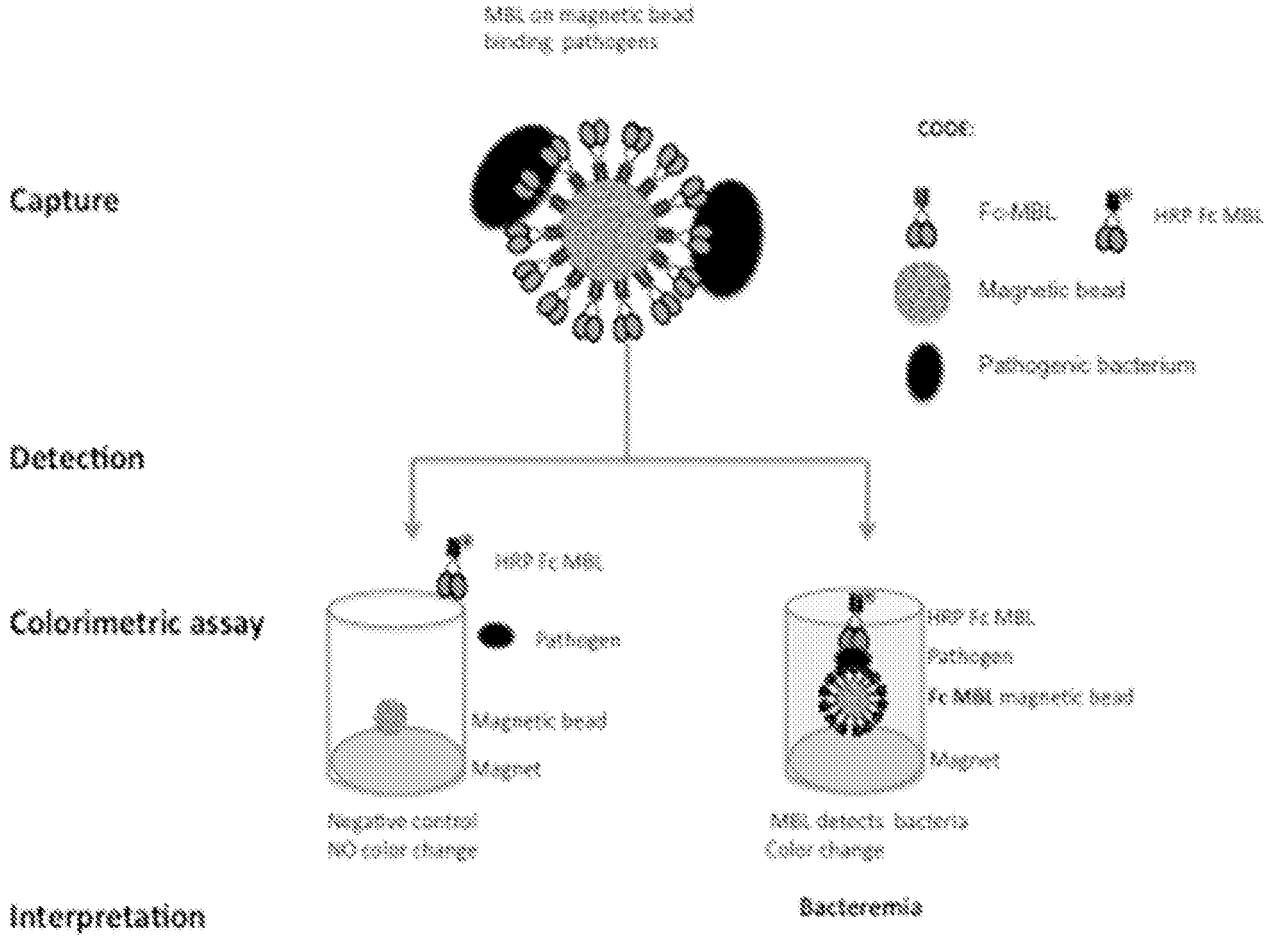

MBL on magnetic bead
binding pathogens
Capture
Detection
Colorimetric assay
Interpretation
Fc MBL
Magnetic bead
Pathogenic bacterium
Pathogen
Magnet
Negative control
NO color change
MBL detects bacteria
Color change
Bacteremia

FIG. 11

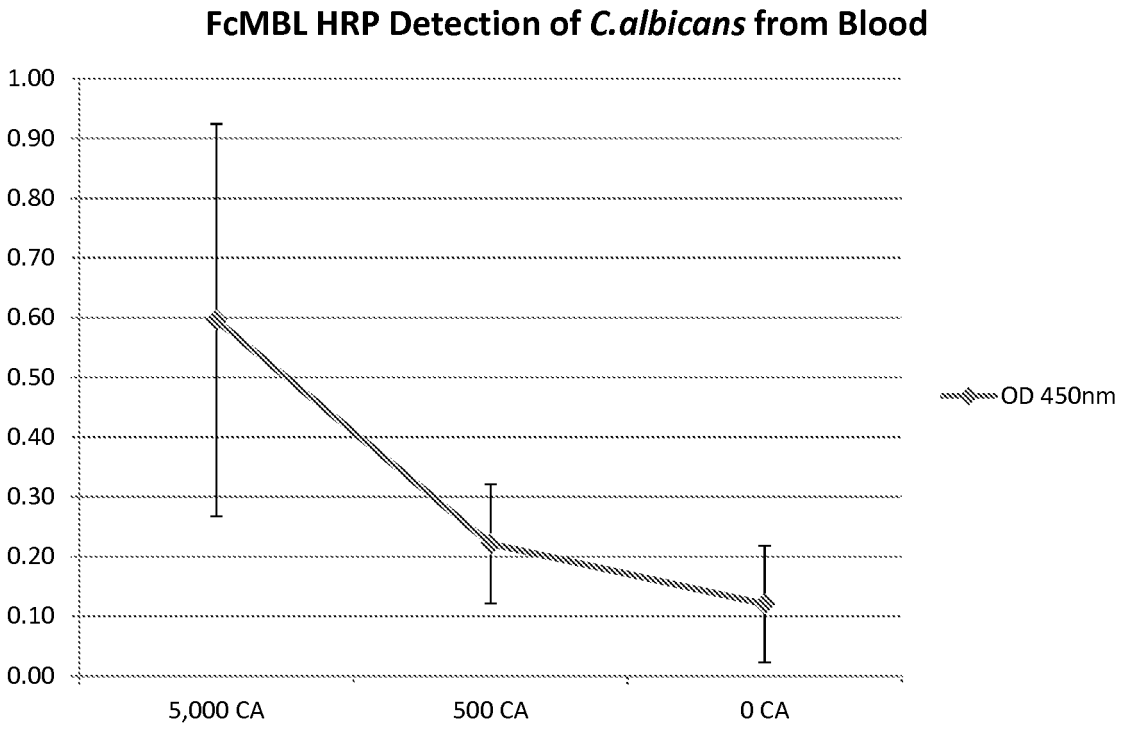

FcMBL HRP Detection of C.albicans from Blood
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
5,000 CA
500 CA
0 CA
OD 450nm

FIG. 12

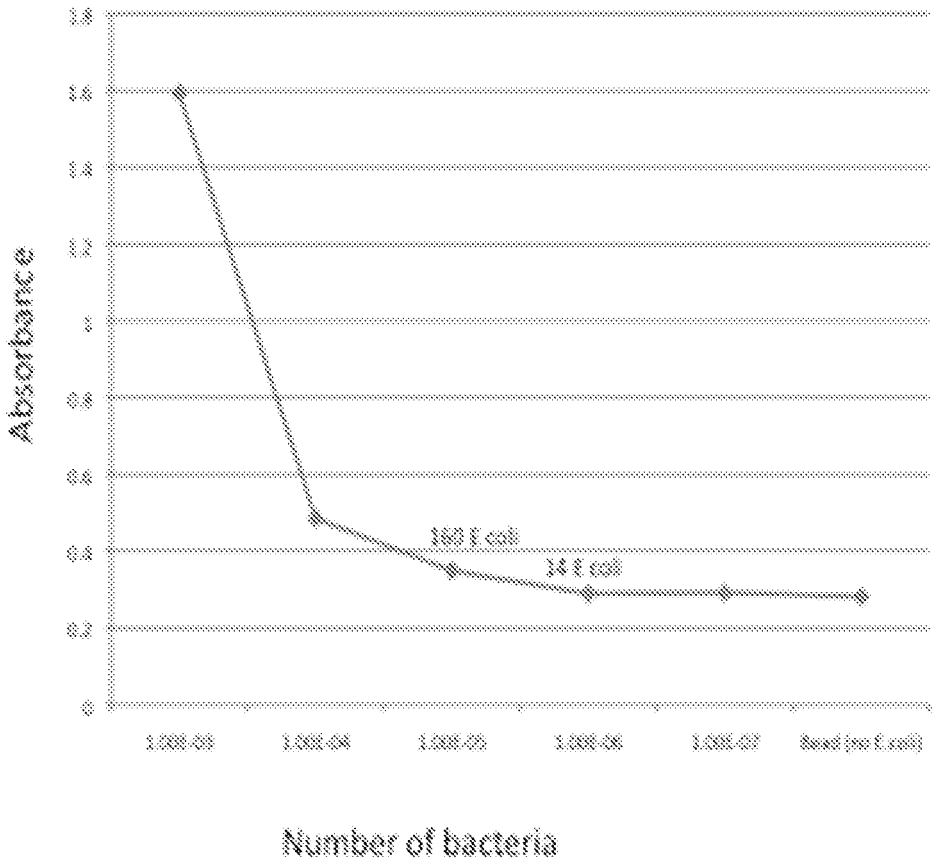

Absorbance
Number of bacteria

FIG. 13

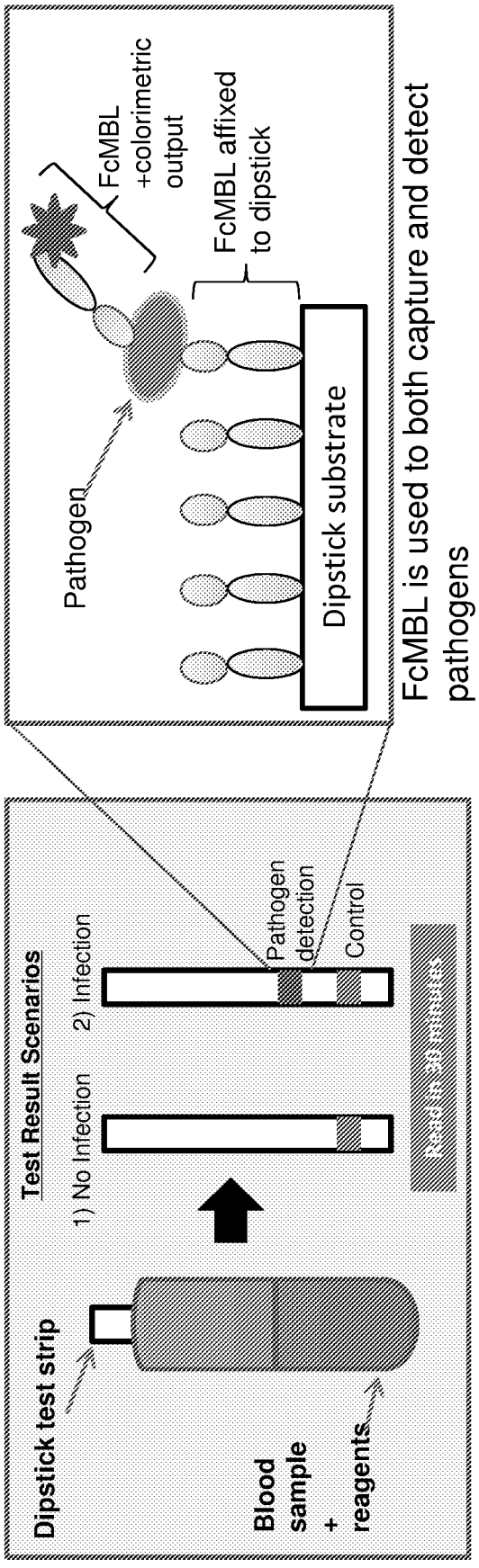

FcMBL is used to both capture and detect pathogens
Dipstick substrate
FcMBL affixed to dipstick
FcMBL +colorimetric output
Pathogen
Dipstick test strip
Blood sample + reagents
Test Result Scenarios
1) No Infection
2) Infection
Pathogen detection
Control

FIG. 14

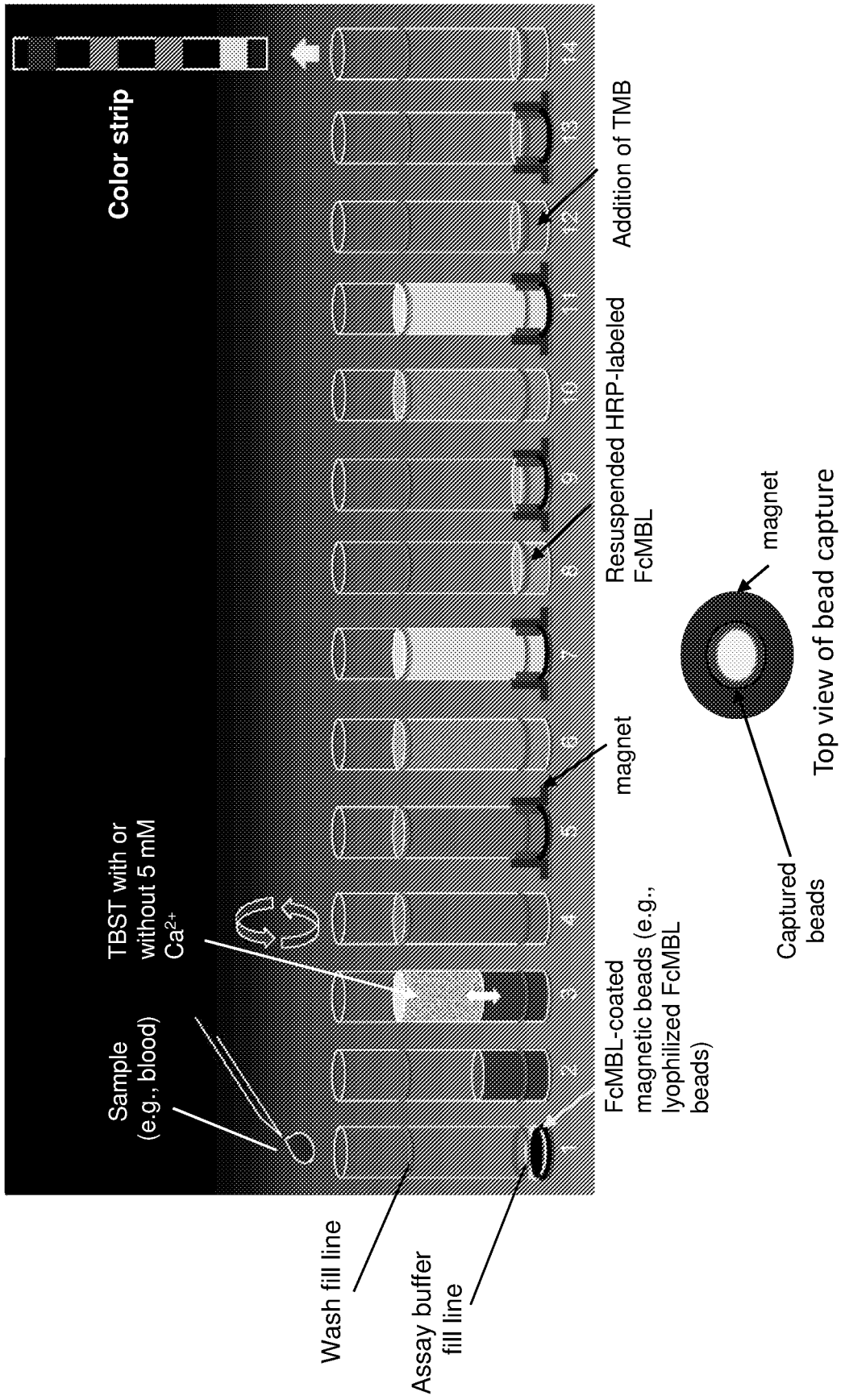

Color strip
Addition of TMB
Resuspended HRP-labeled FcMBL
magnet
FcMBL-coated magnetic beads (e.g., lyophilized FcMBL beads)
TBST with or without 5 mM Ca^{2+}
Sample (e.g., blood)
Wash fill line
Assay buffer fill line
1
2
3
4
5
6
7
8
9
10
11
12
13
14
magnet
Captured beads
Top view of bead capture

FIG. 15

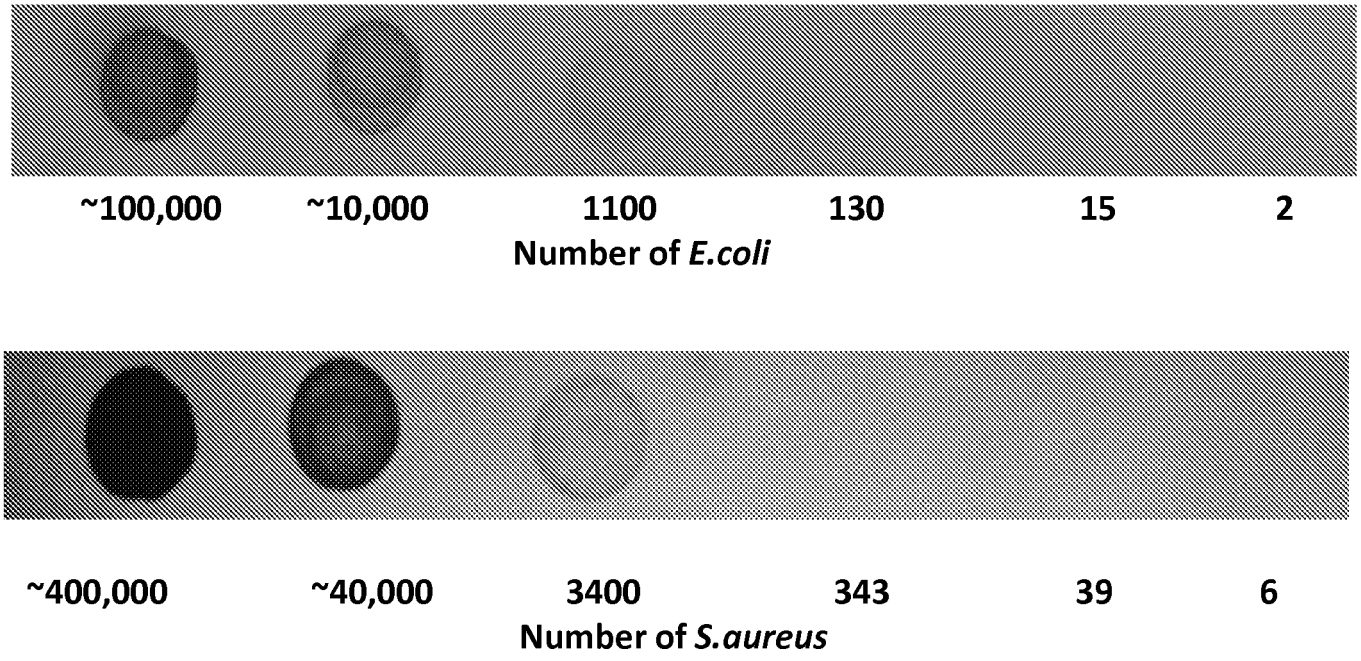

~100,000
~10,000
1100
130
15
2
Number of E.coli
~400,000
~40,000
3400
343
39
6
Number of S.aureus

FIG. 16

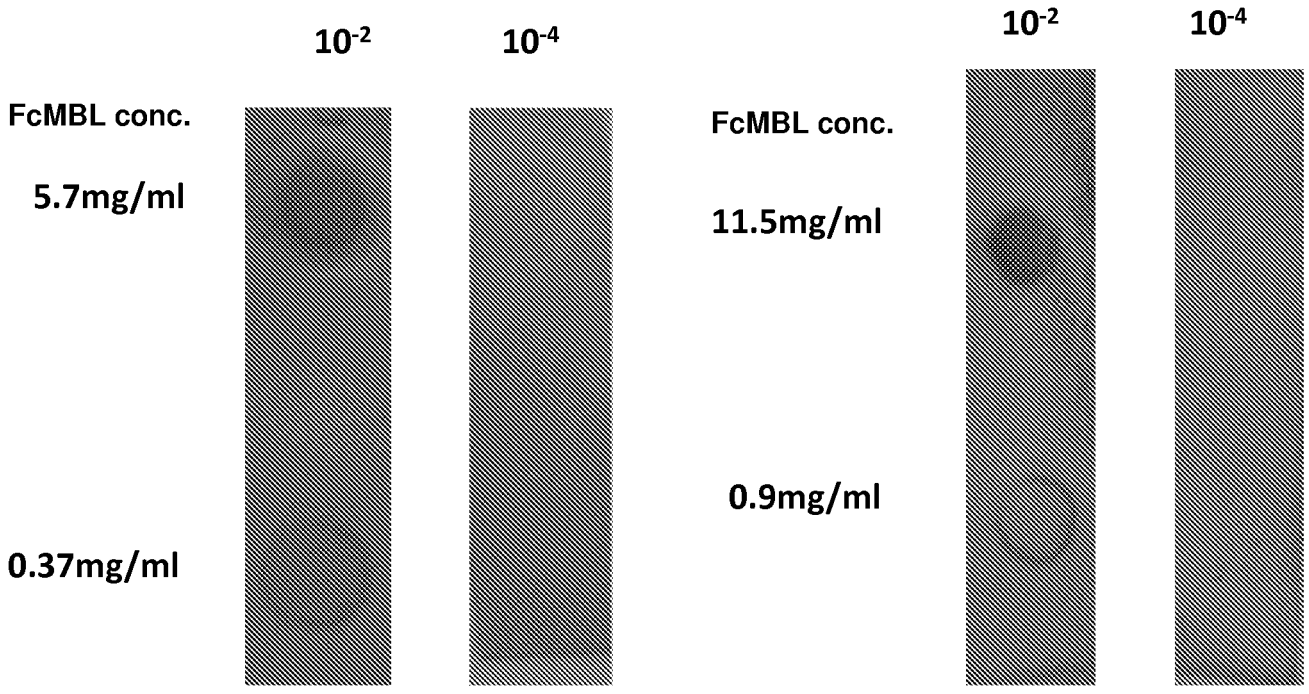

10-2
10-4
FcMBL conc.
5.7mg/ml
0.37mg/ml
10-2
10-4
FcMBL conc.
11.5mg/ml
0.9mg/ml

FIG. 17

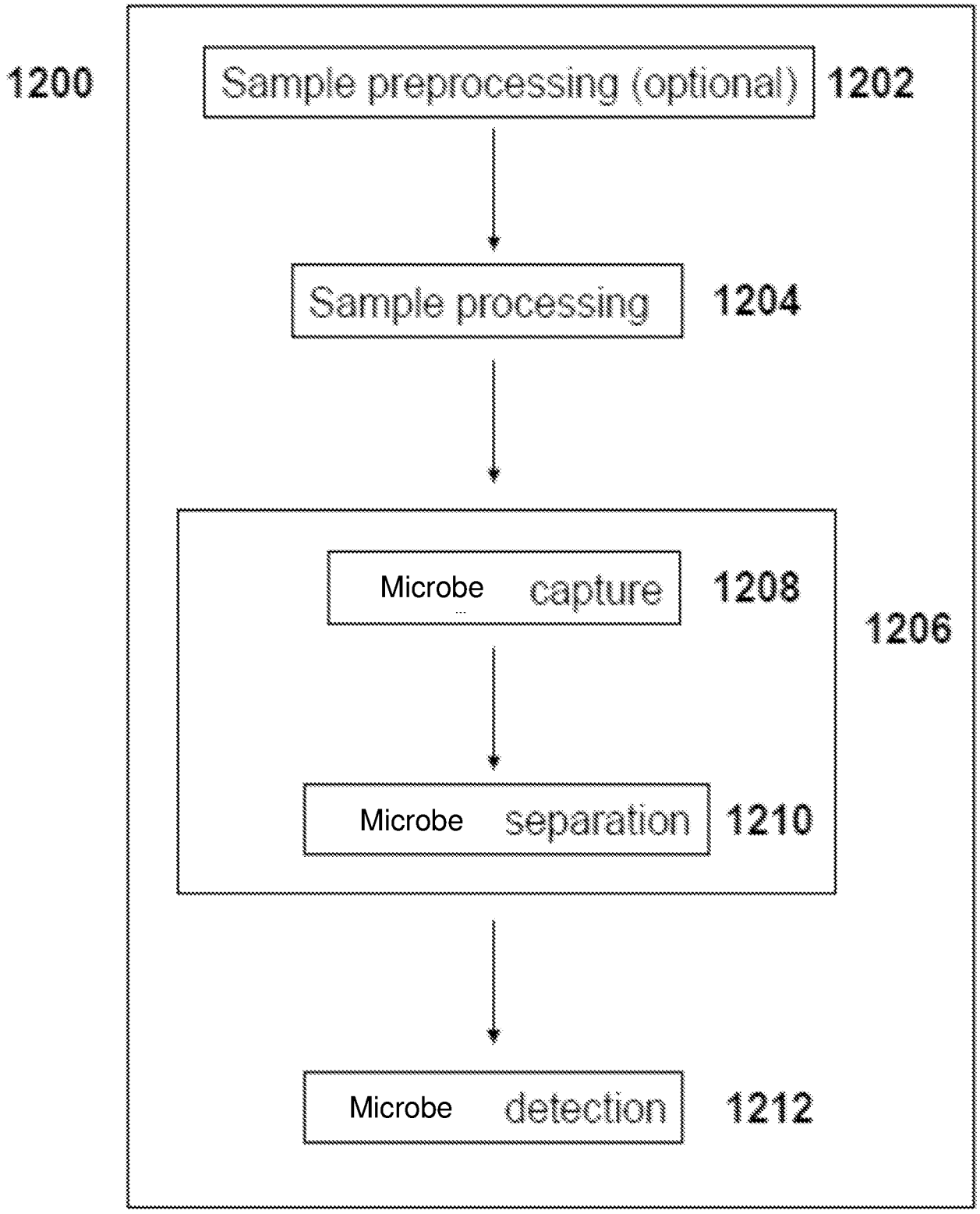

1200
Sample preprocessing (optional)
1202
Sample processing
1204
Microbe capture
1208
1206
Microbe separation
1210
Microbe detection
1212

FIG. 18A

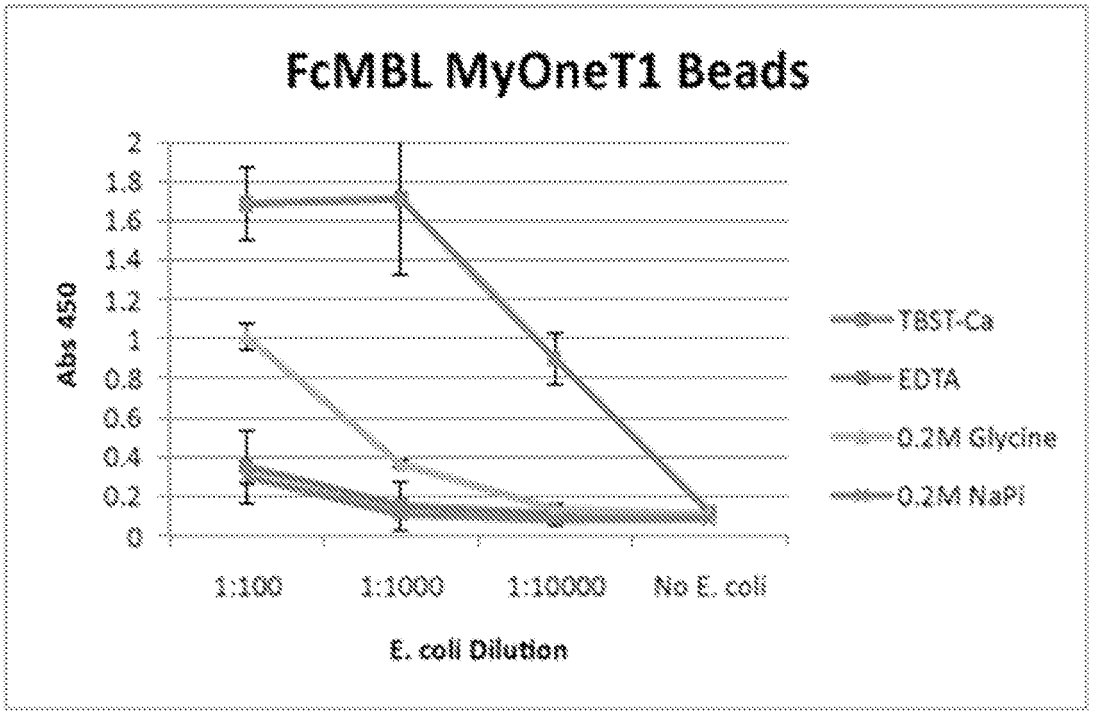


FIG. 18B

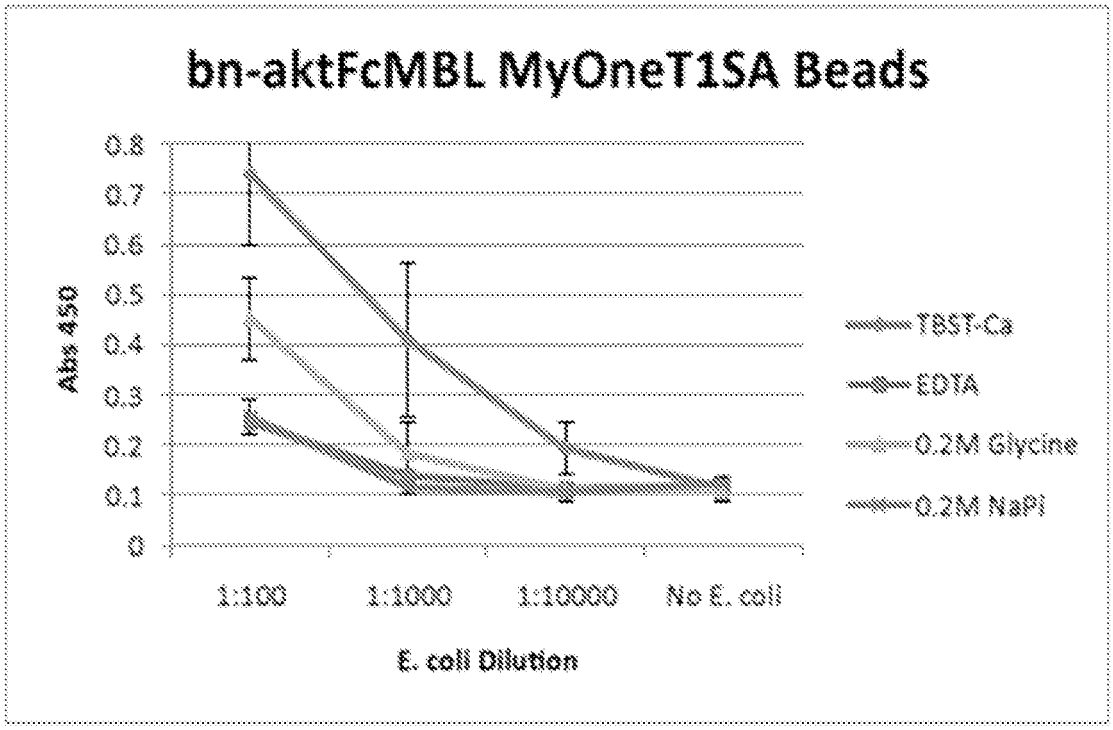


FIG. 19

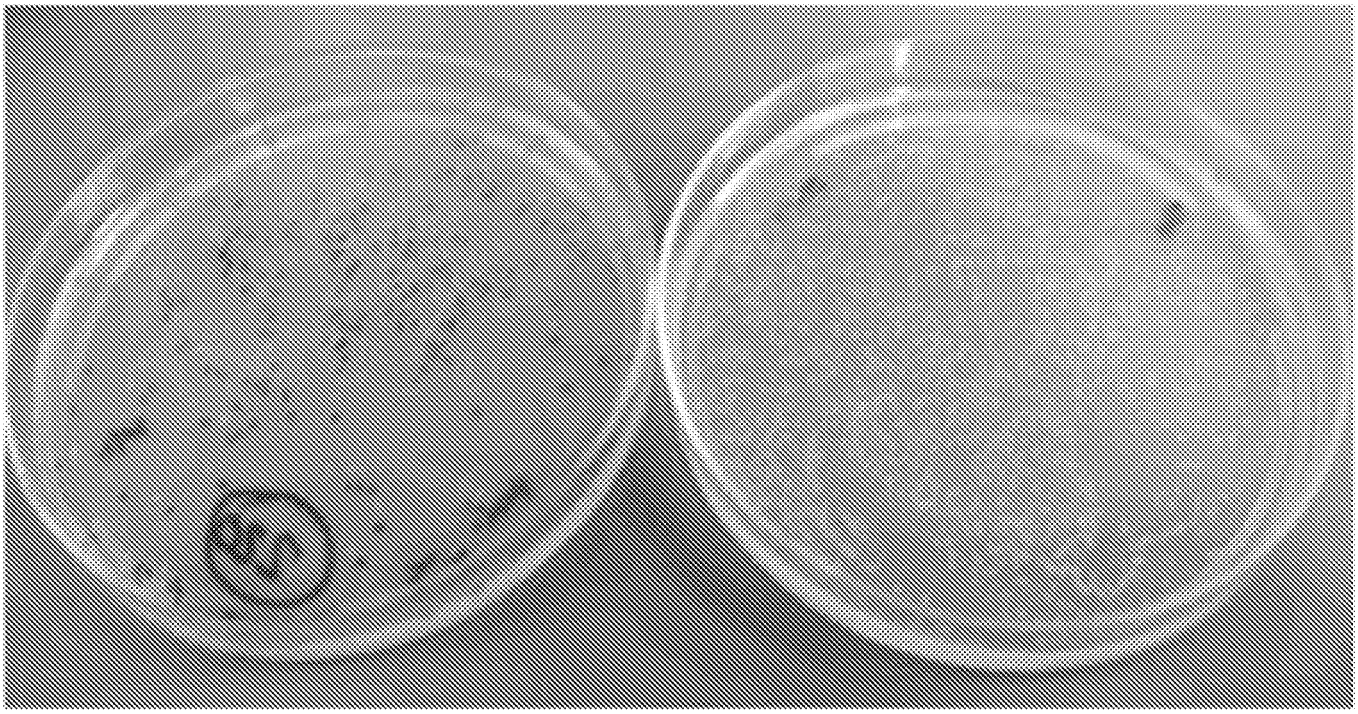


FIG. 20A

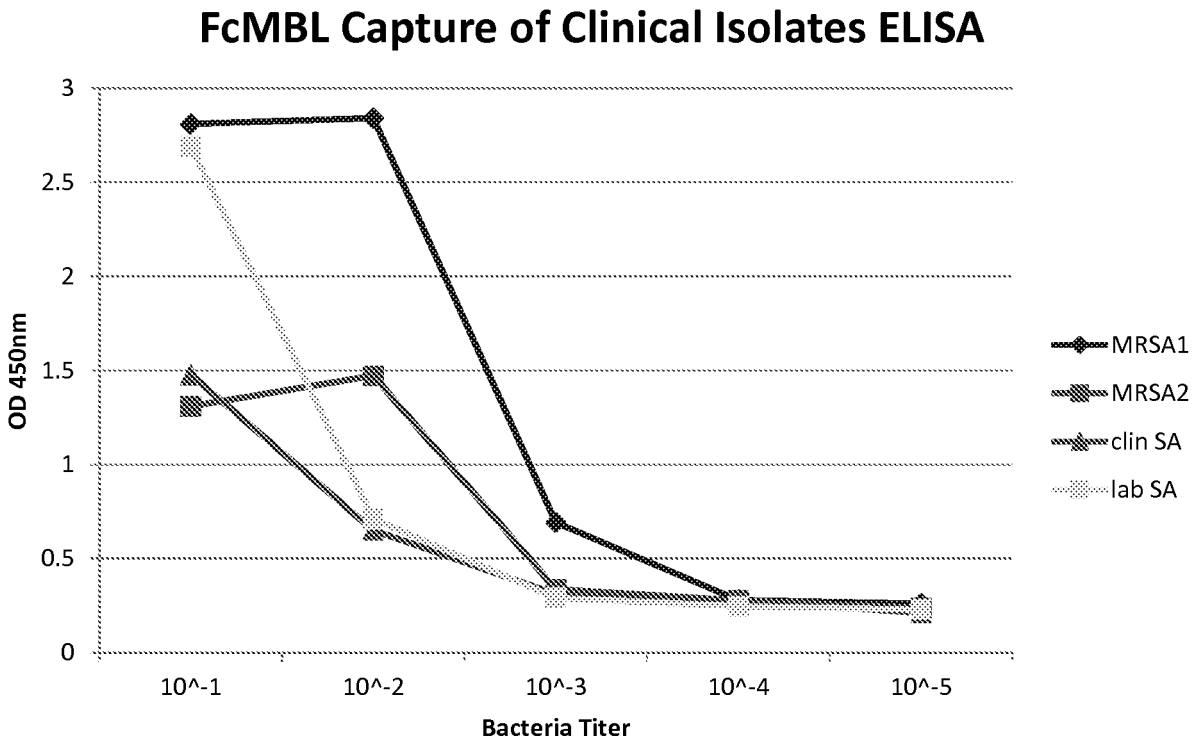


FIG. 20B

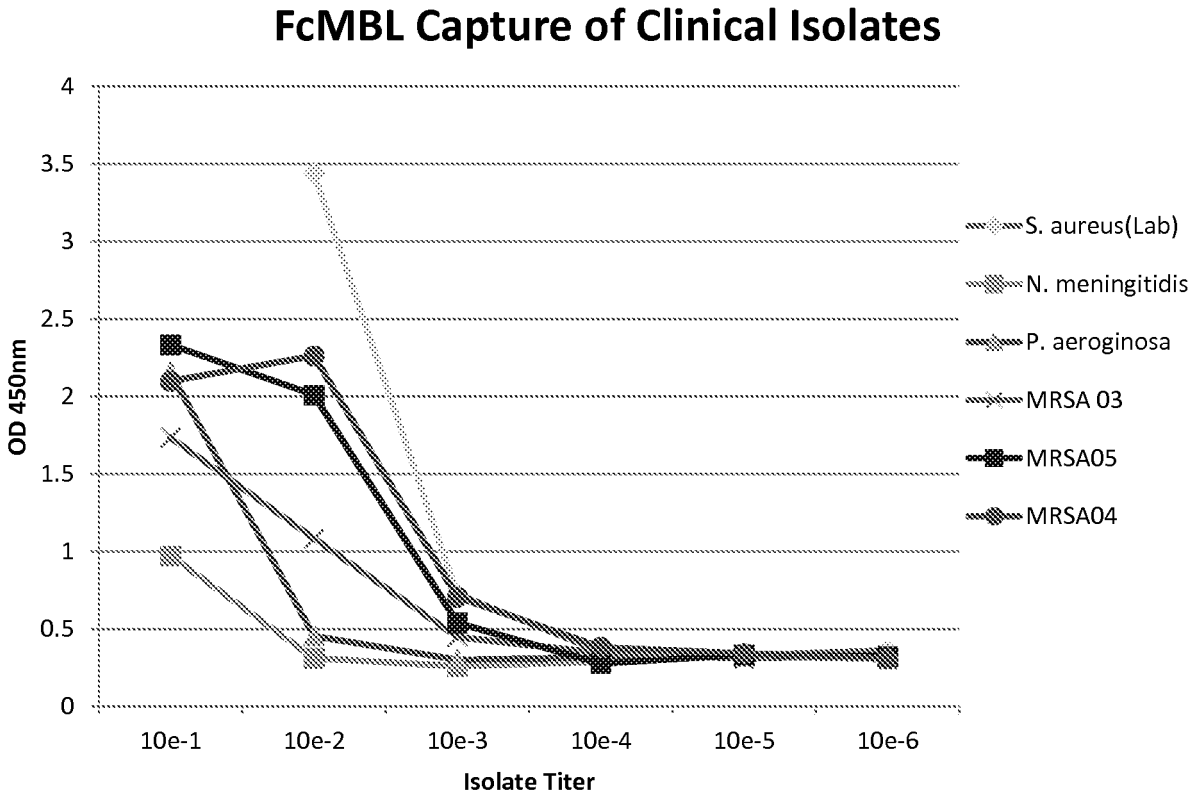


FIG. 21A

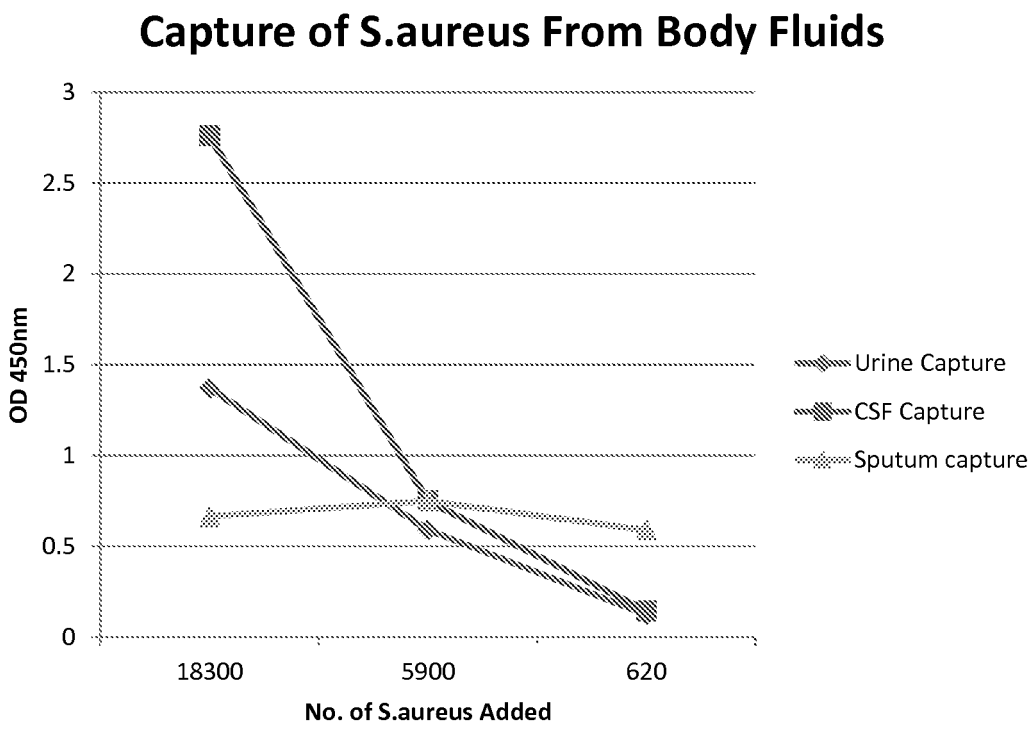

Capture of S.aureus From Body Fluids
OD 450nm
3
2.5
2
1.5
1
0.5
0
18300
5900
620
No. of S.aureus Added
Urine Capture
CSF Capture
Sputum capture

FIG. 21B

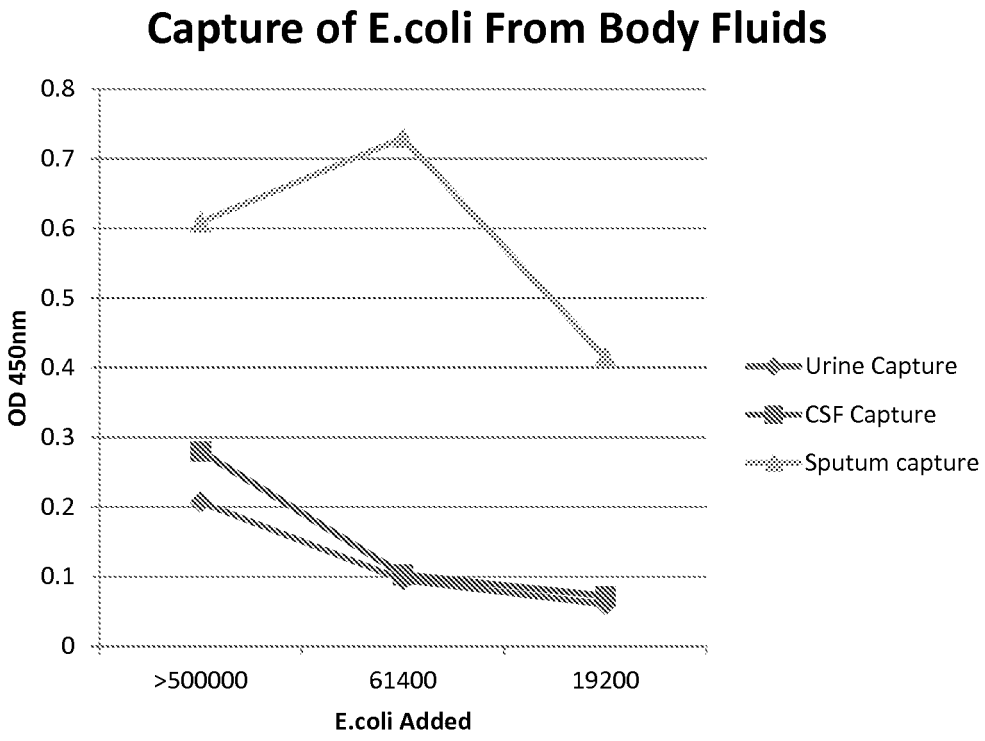


FIG. 22

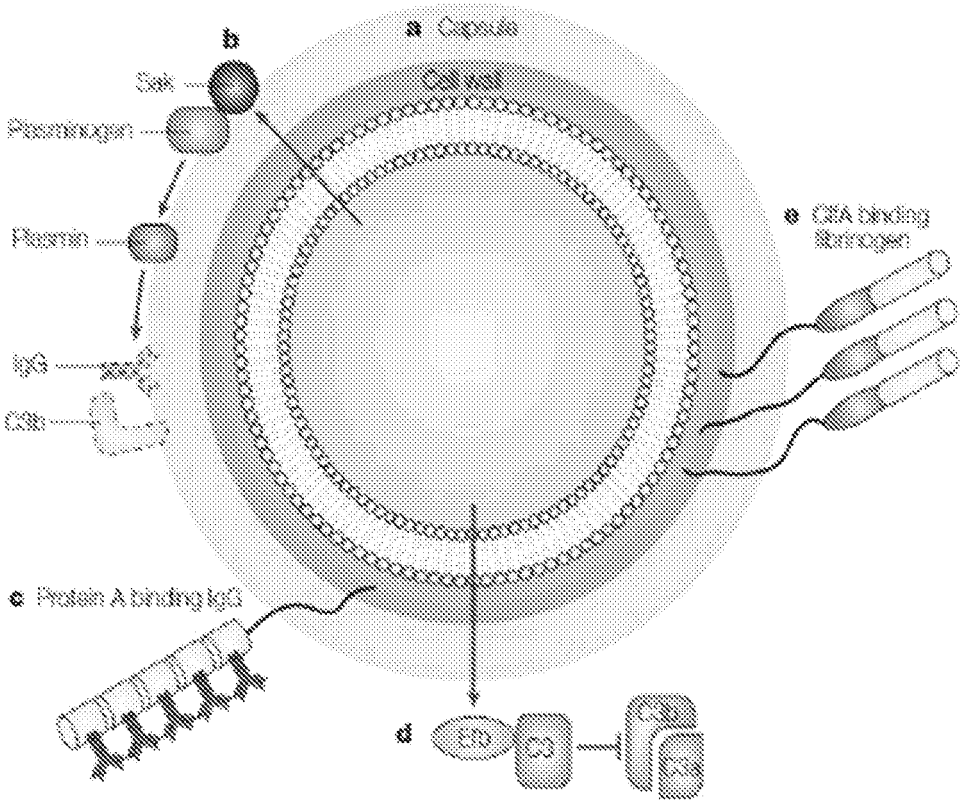


FIG.23

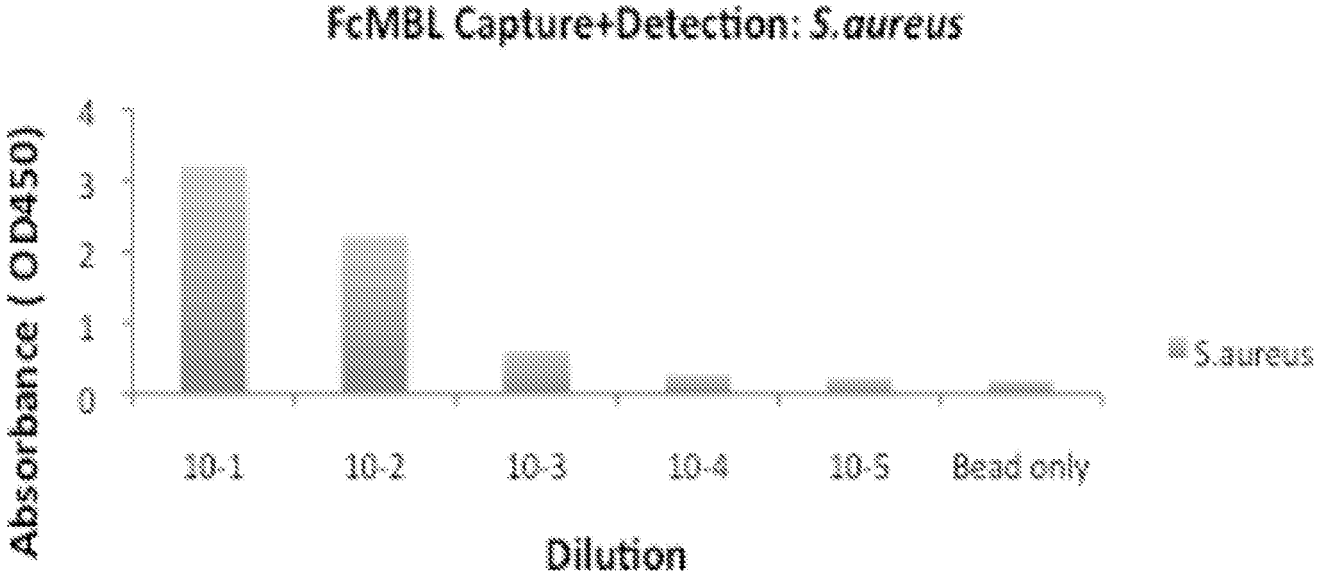

FcMBL Capture+Detection: S.aureus
Absorbance (OD450)
4
3
2
1
0
10-1
10-2
10-3
10-4
10-5
Bead only
Dilution
S.aureus

FIG.24

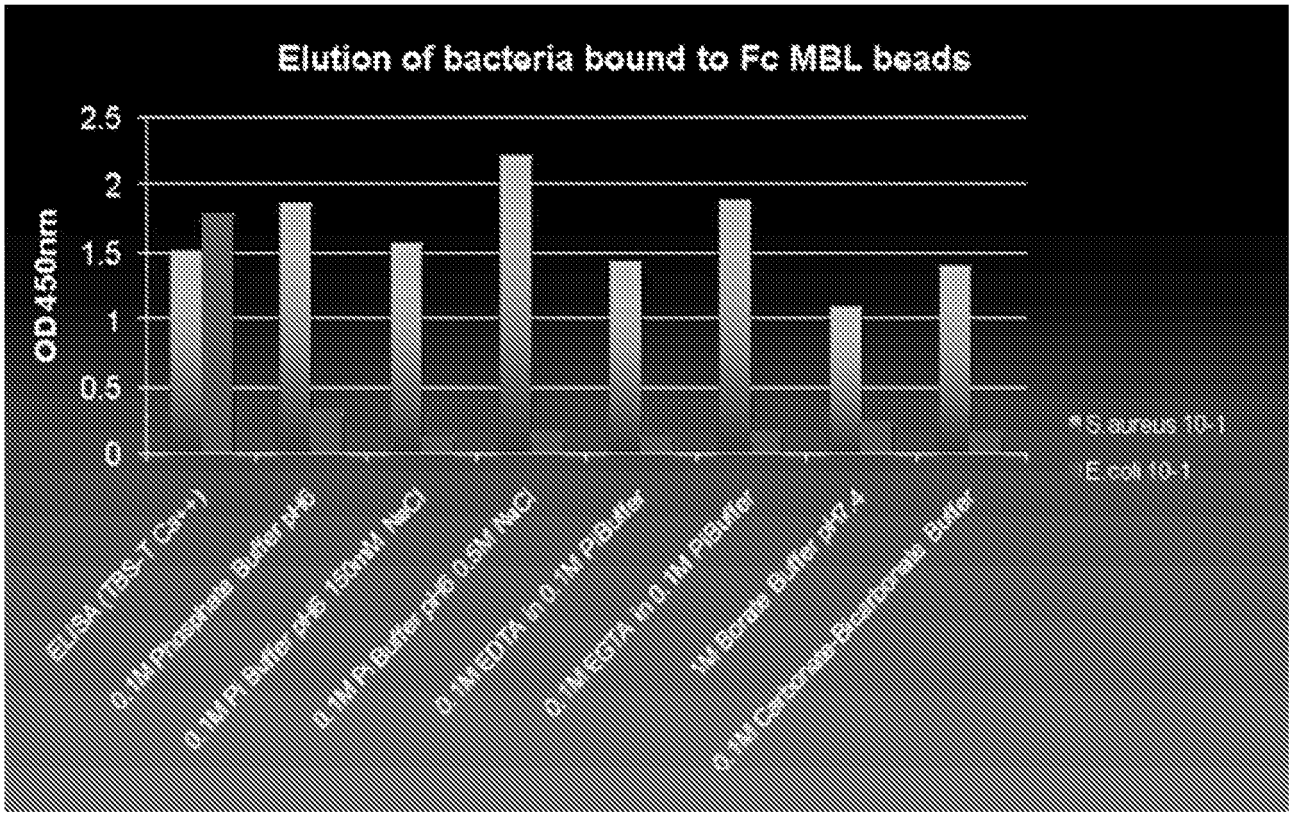

Elution of bacteria bound to Fc MBL beads
OD450nm
2.5
2
1.5
1
0.5
0
S.aureus 10-1
E.coli 10-1

FIG. 25A

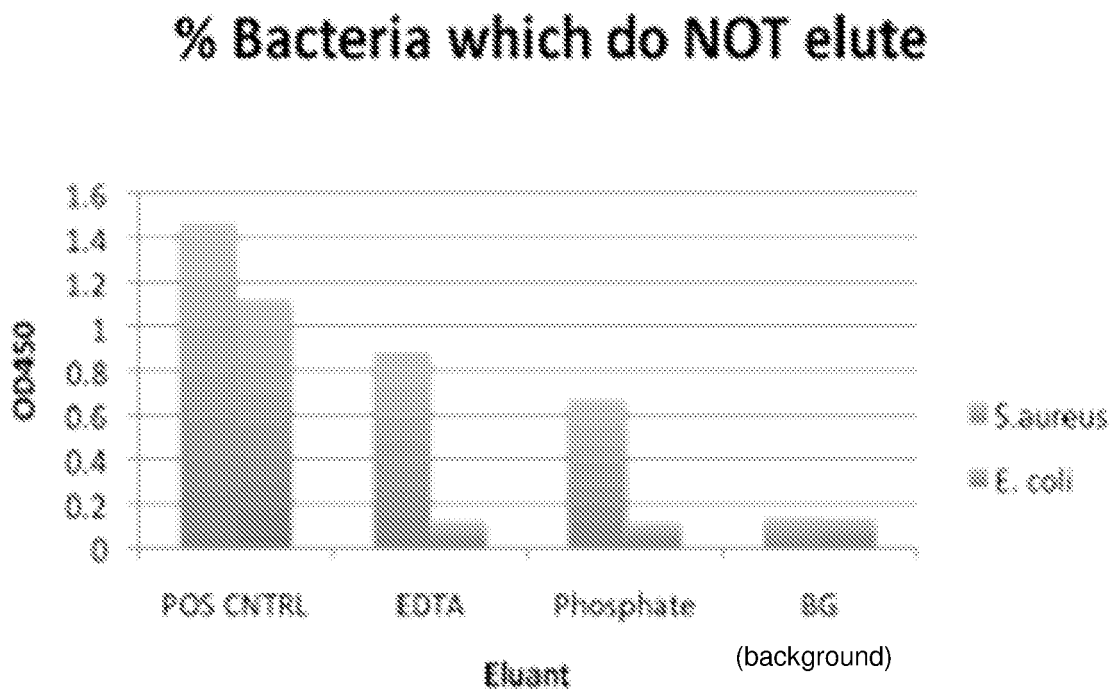


FIG. 25B

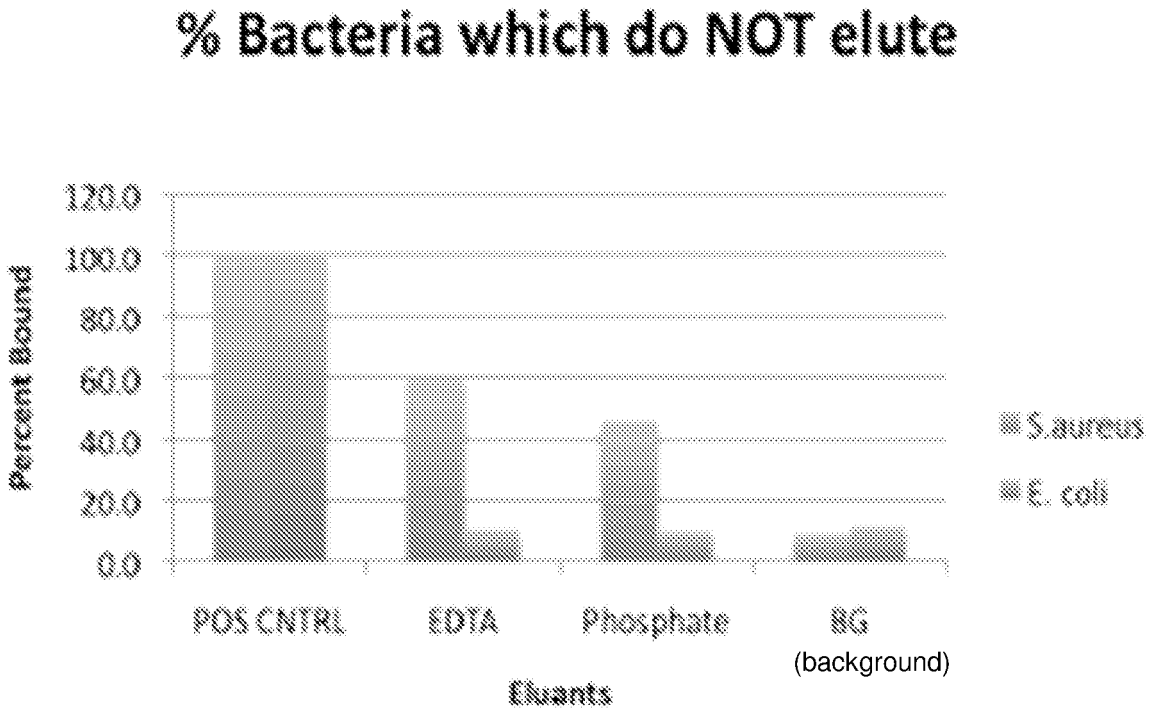


FIG. 26A

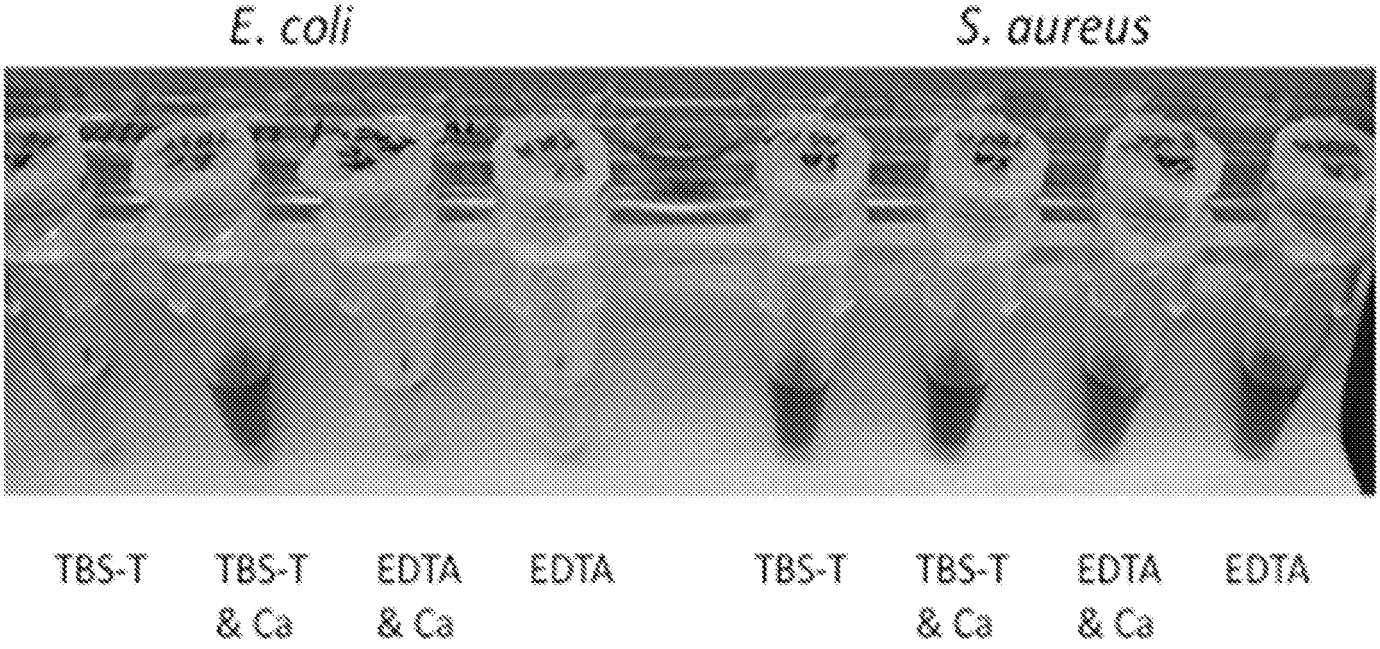


FIG. 26B

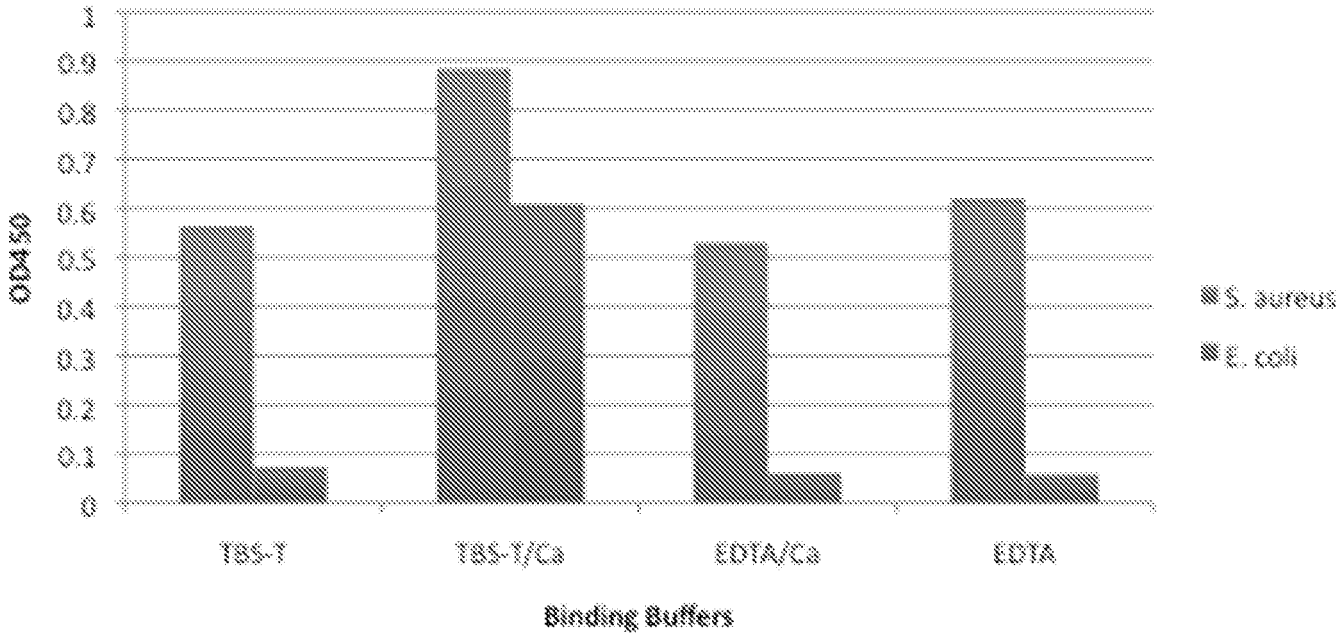


FIG. 27

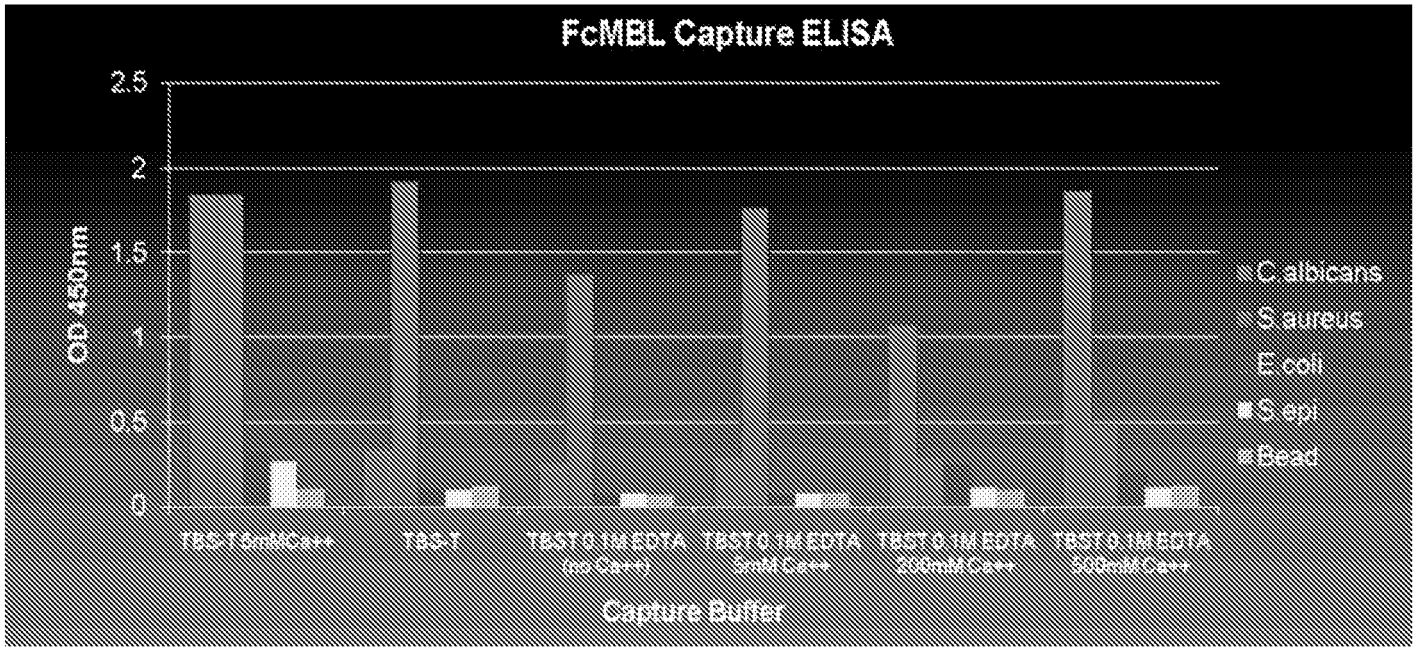

FcMBL Capture ELISA
OD 450nm
2.5
2
1.5
1
0.5
0
C albicans
S aureus
E coli
S epi
Bead
TBS-T 5mMCa++
TBS-T
TBST 0.1M EDTA 5mMCa++
TBST 0.1M EDTA 200mMCa++
TBST 0.1M EDTA 500mMCa++
Capture Buffer

FIG. 28

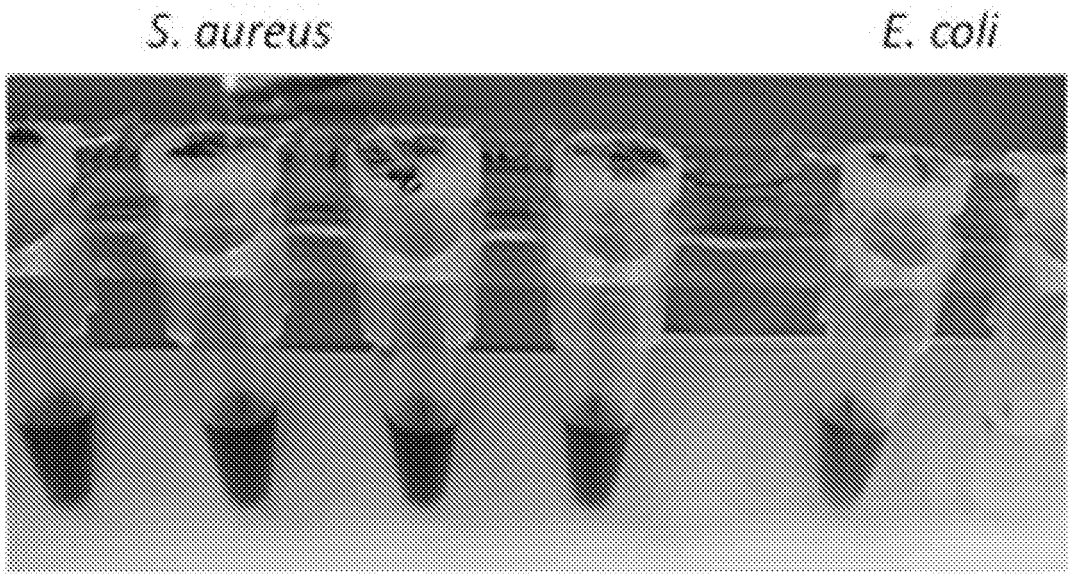

S. aureus
E. coli
TBS-T & Ca2+
Phos pH3
EDTA
EDTA & Phos pH3
TBS-T & Ca2+
EDTA

FIG. 29

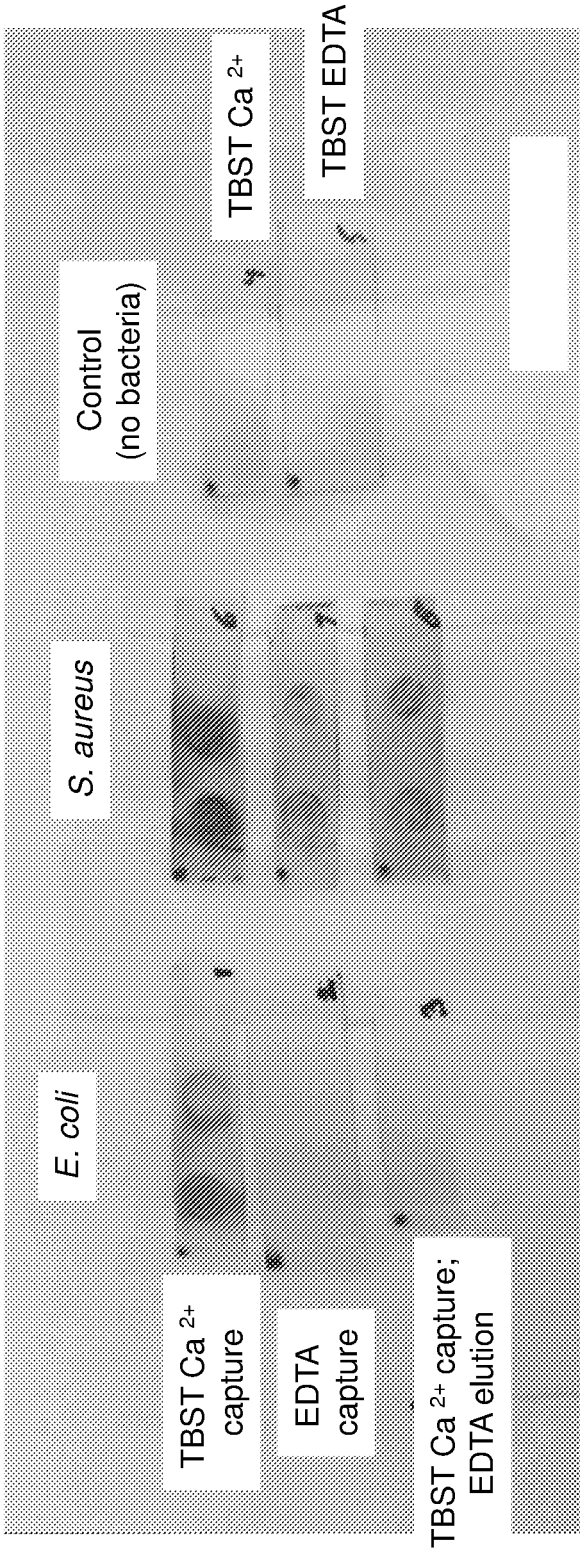
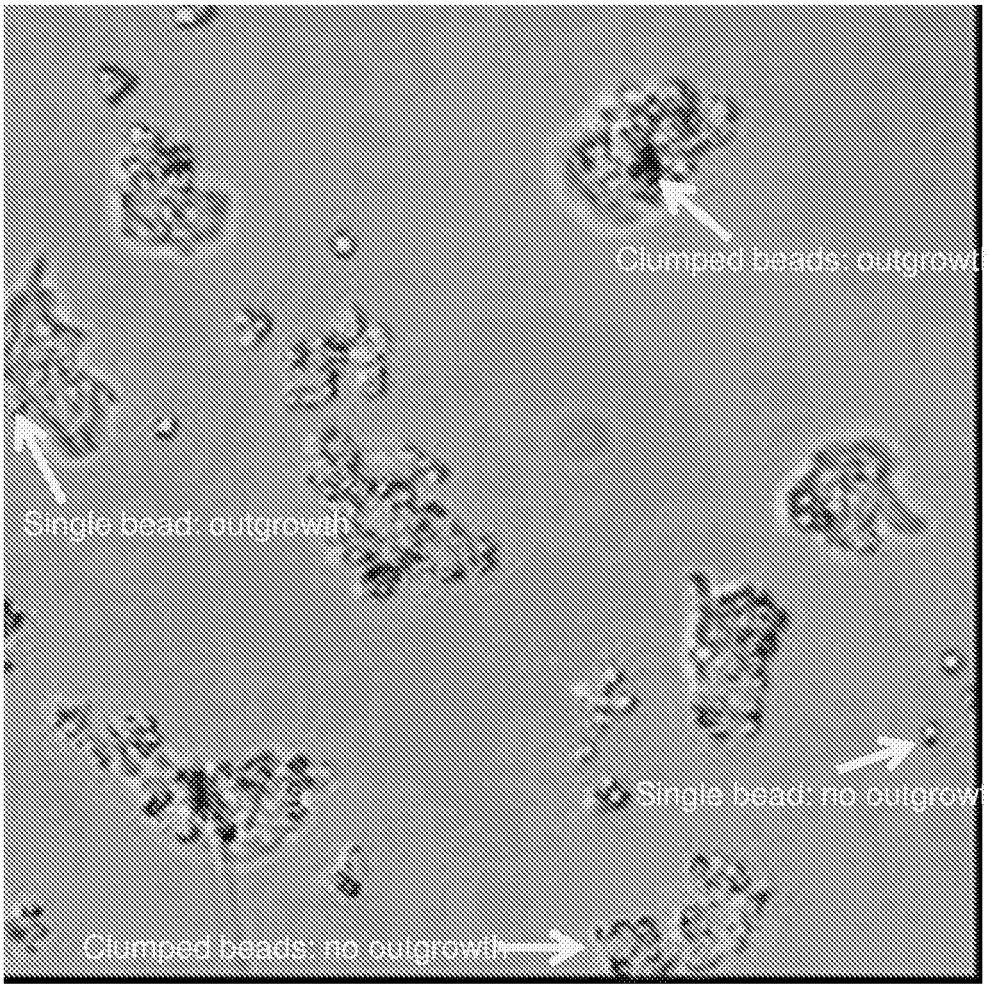

TBST Ca 2+
TBST EDTA
Control (no bacteria)
S. aureus
E. coli
TBST Ca 2+ capture
EDTA capture
TBST Ca 2+ capture; EDTA elution

FIG. 30A


Clumped beads: outgrowth
Single bead: outgrowth
Single bead: no outgrowth
Clumped beads: no outgrowth

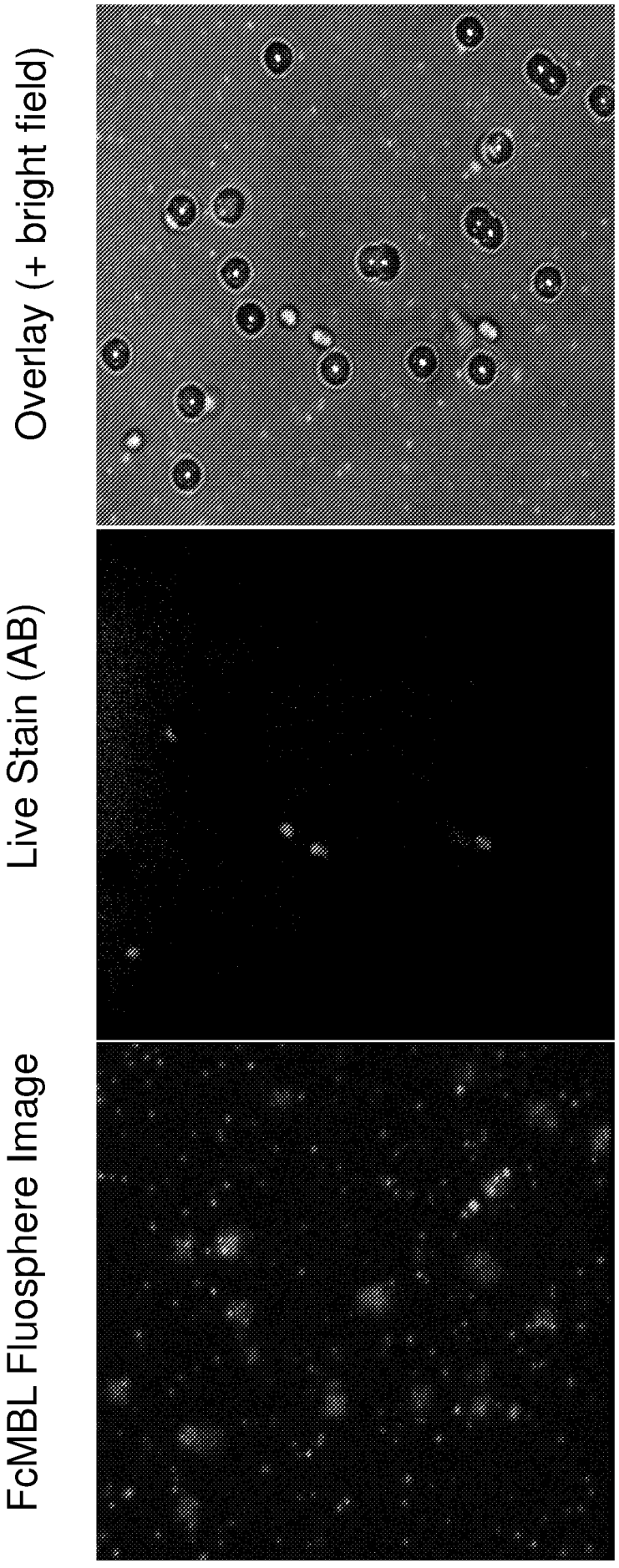


FIG. 30B

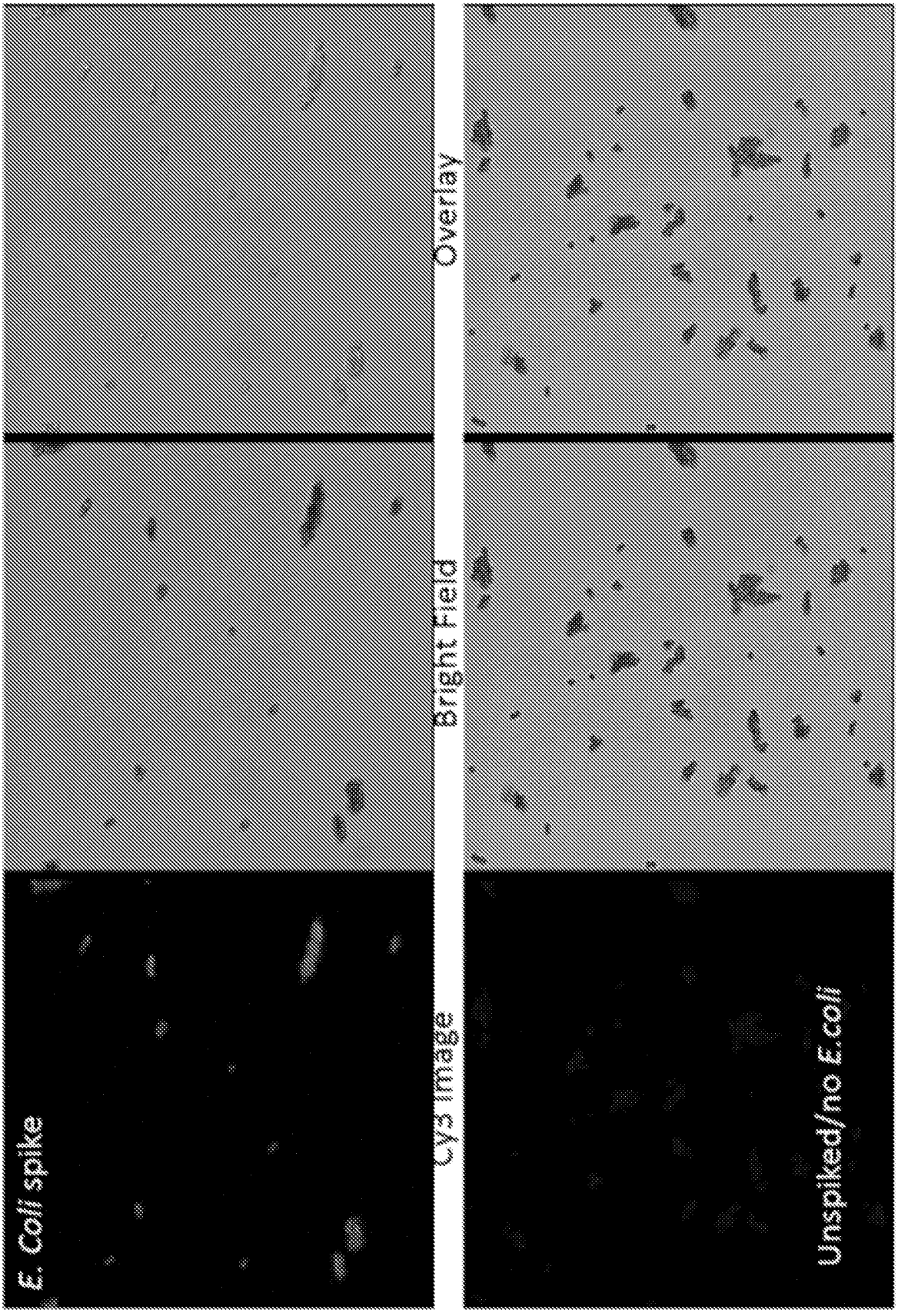

E. Coli spike
Unspiked/no E.coli
Cy3 image
Bright Field
Overlay

FIG. 31A

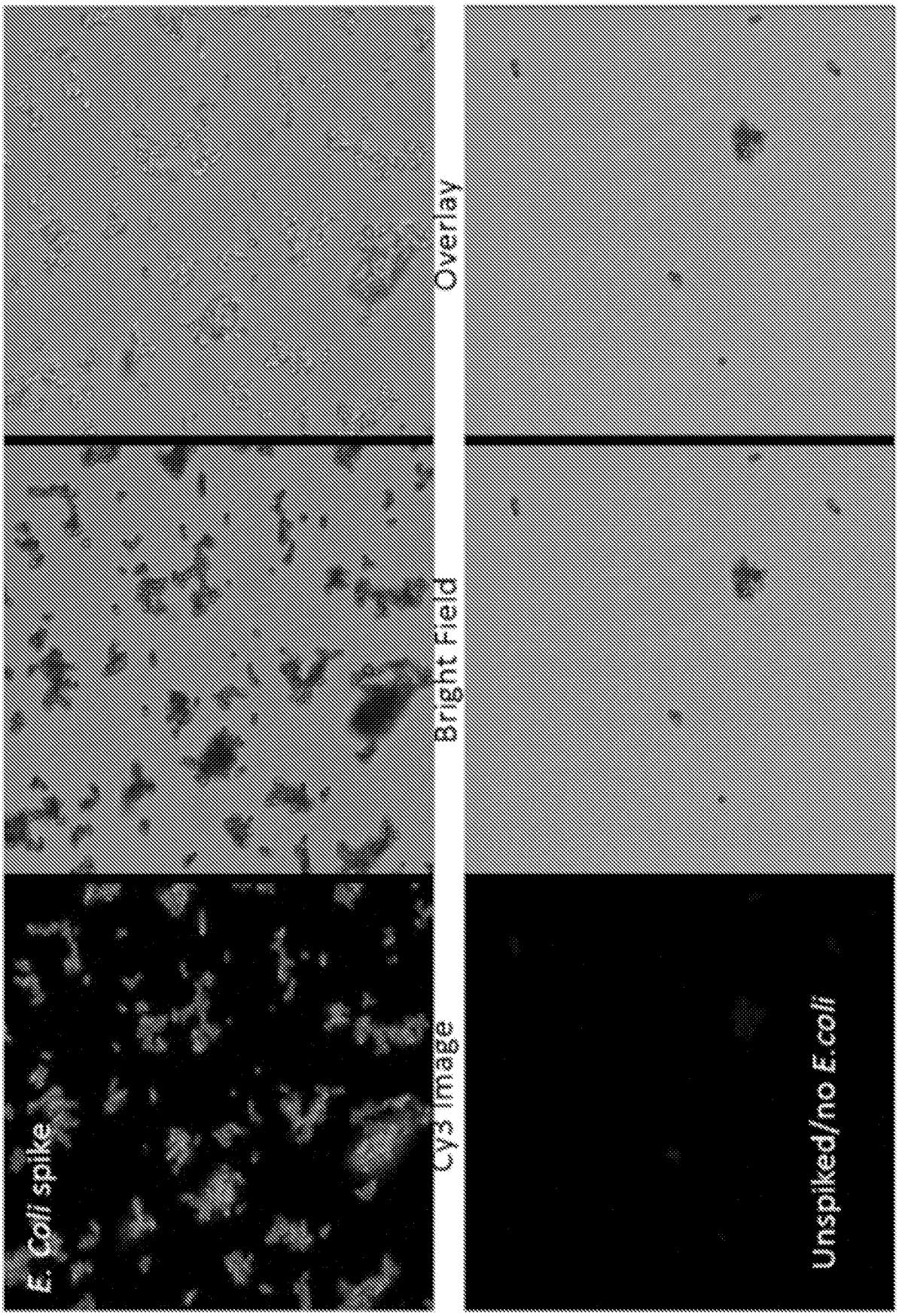

E. Coli spike
Unspiked/no E.coli
Cy3 Image
Bright Field
Overlay

FIG. 31B

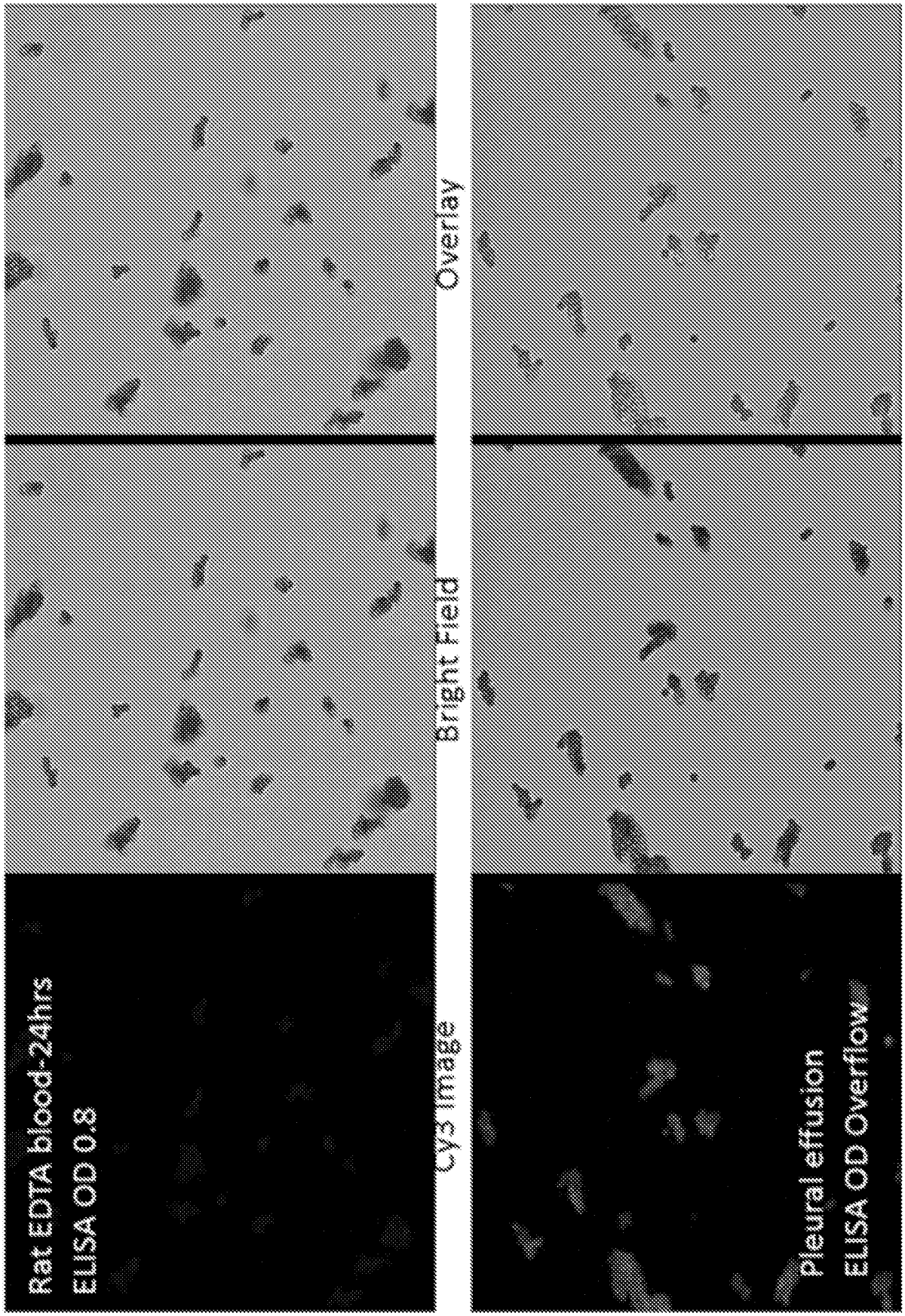

Rat EDTA blood-24hrs
ELISA OD 0.8
Pleural effusion
ELISA OD Overflow
Cy5 Image
Bright Field
Overlay

FIG. 32A

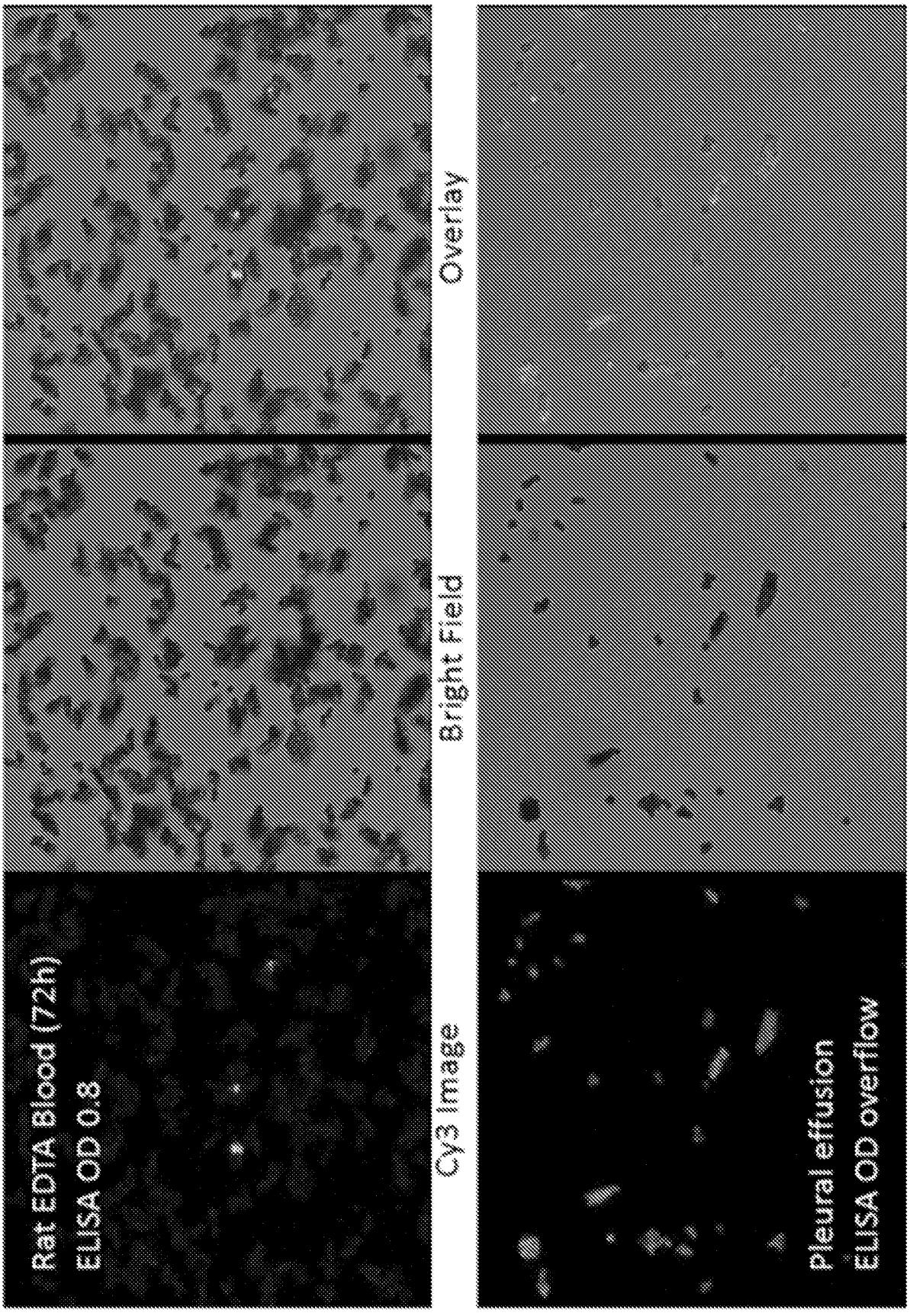

Rat EDTA Blood (72h)
ELISA OD 0.8
Cy3 Image
Bright Field
Overlay
Pleural effusion
ELISA OD overflow

FIG. 32B

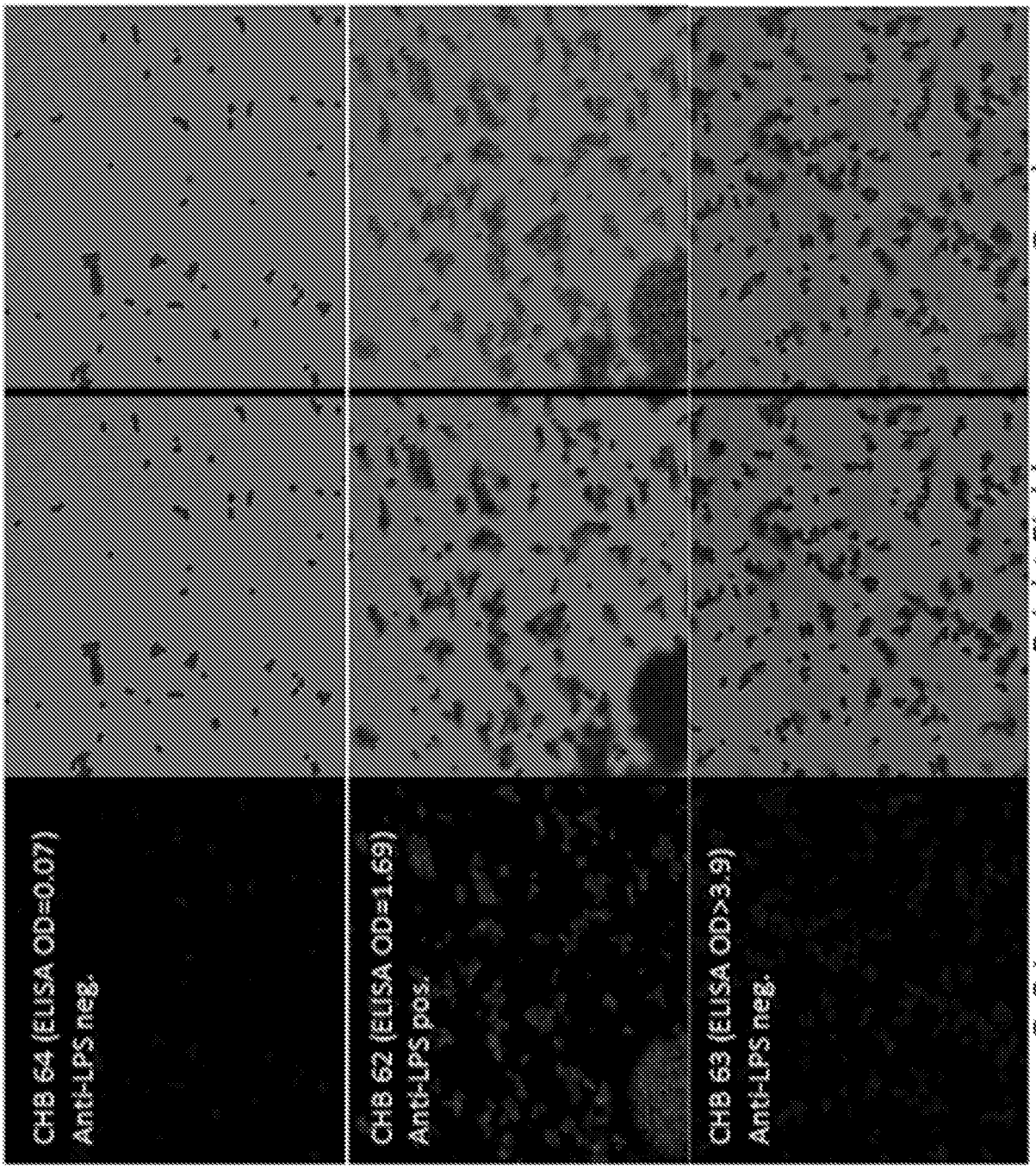

CHB 64 (ELISA OD=0.07)
Anti-LPS neg.
CHB 62 (ELISA OD=1.69)
Anti-LPS pos.
CHB 63 (ELISA OD>3.9)
Anti-LPS neg.
Cy3 Image
Bright Field
Overlay

FIG. 33

FIG. 34A

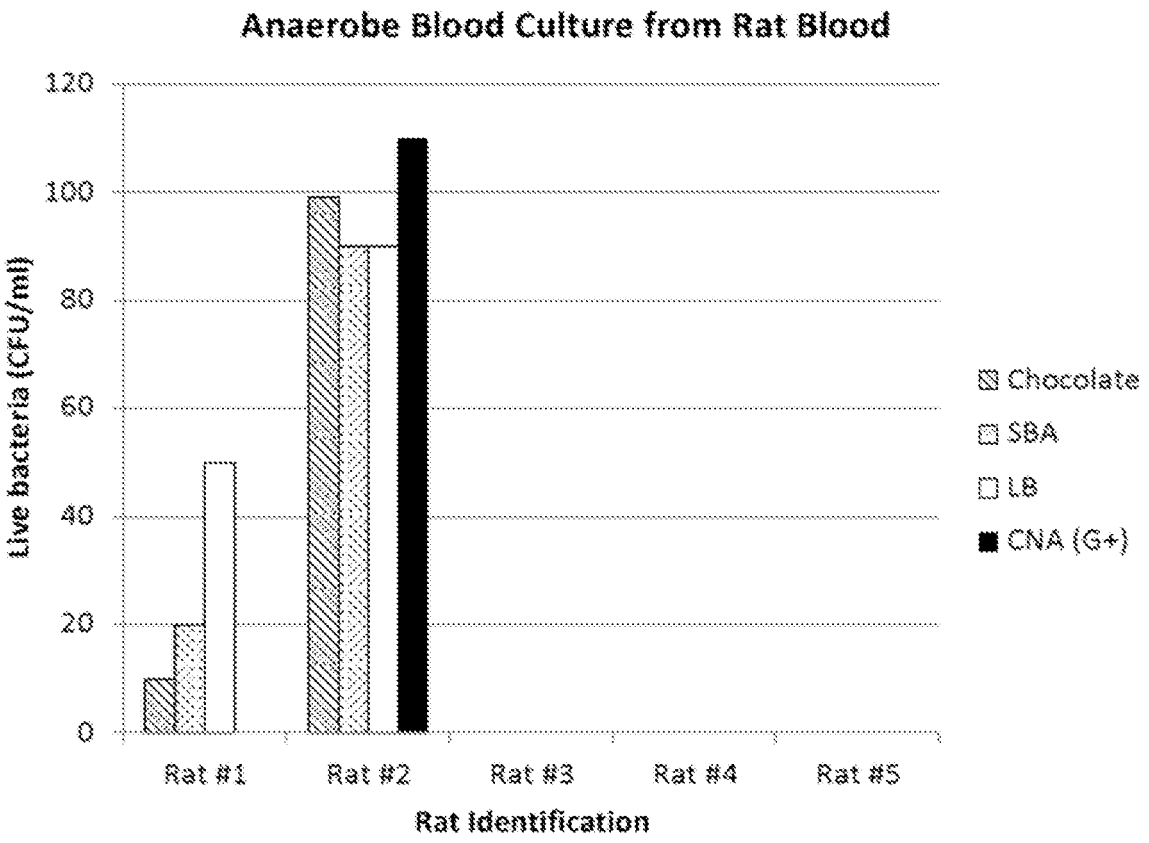


FIG. 34B

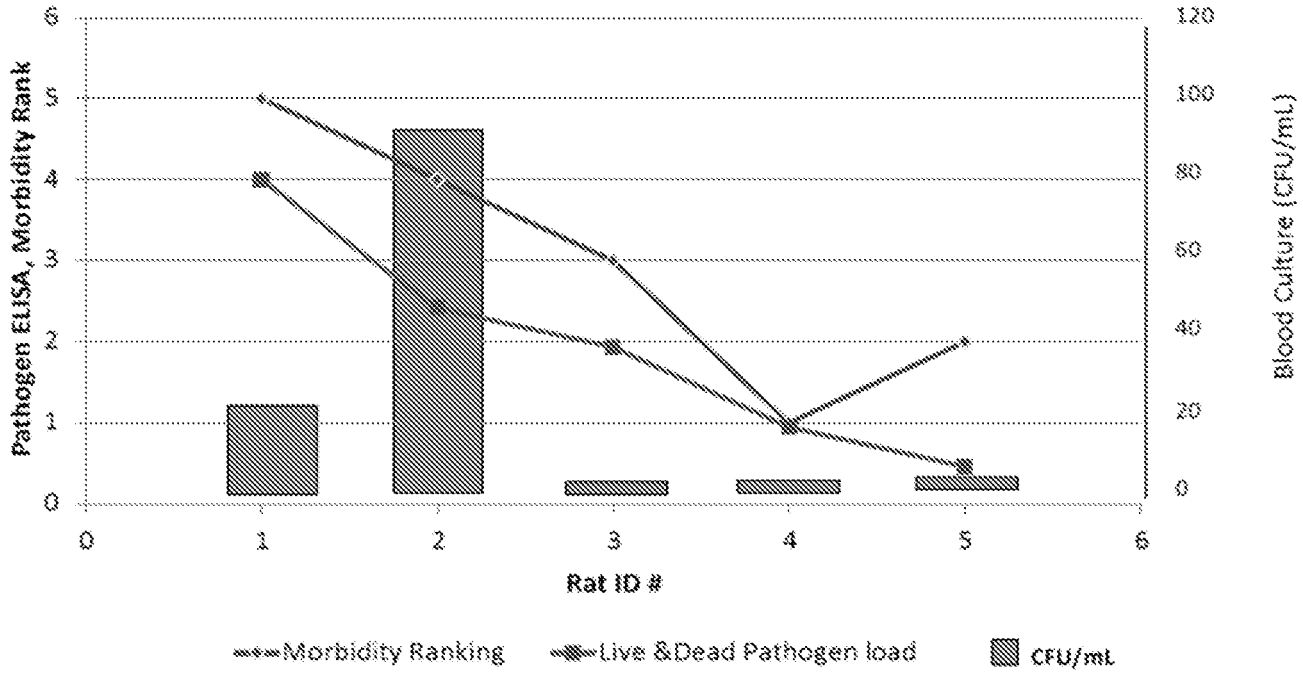


FIG. 34C

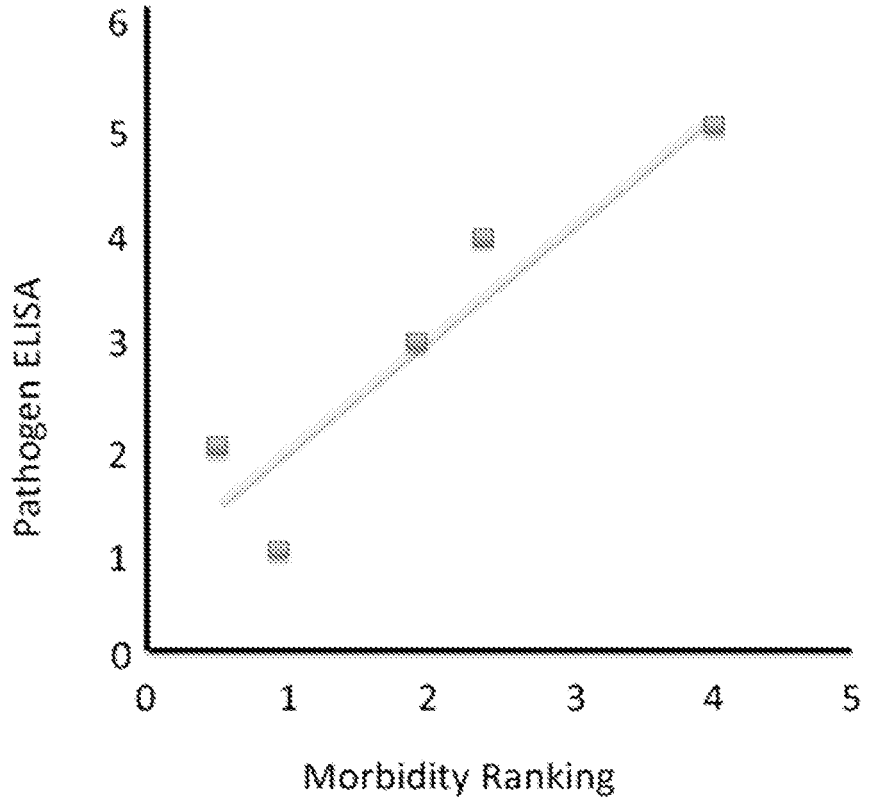


FIG. 34D

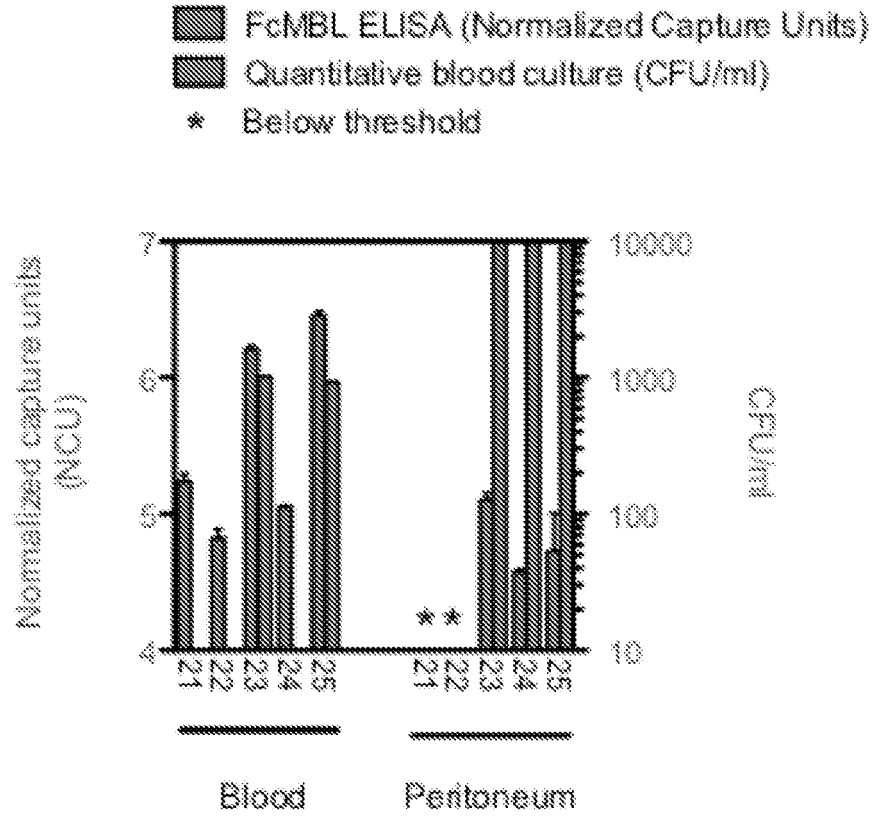
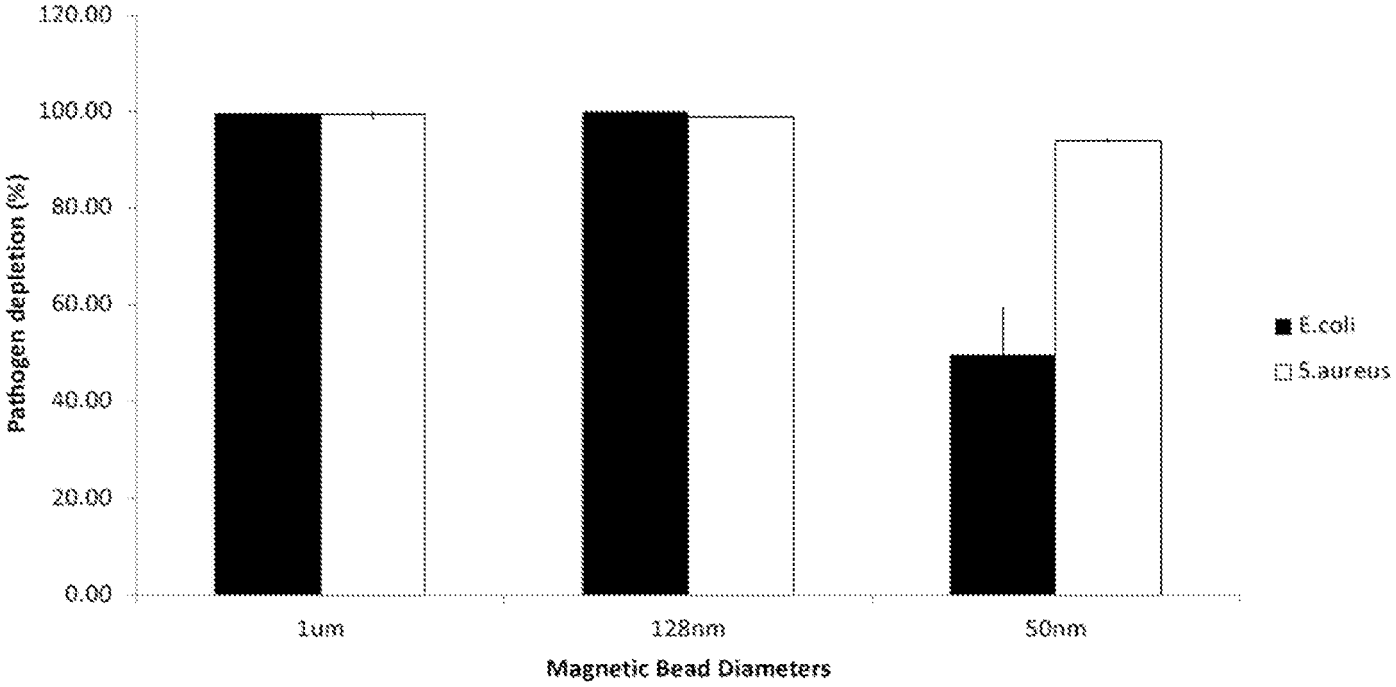


FIG. 35


120.00
100.00
80.00
60.00
40.00
20.00
0.00
Pathogen depletion (%)
1um
128nm
50nm
Magnetic Bead Diameters
E.coli
S.aureus