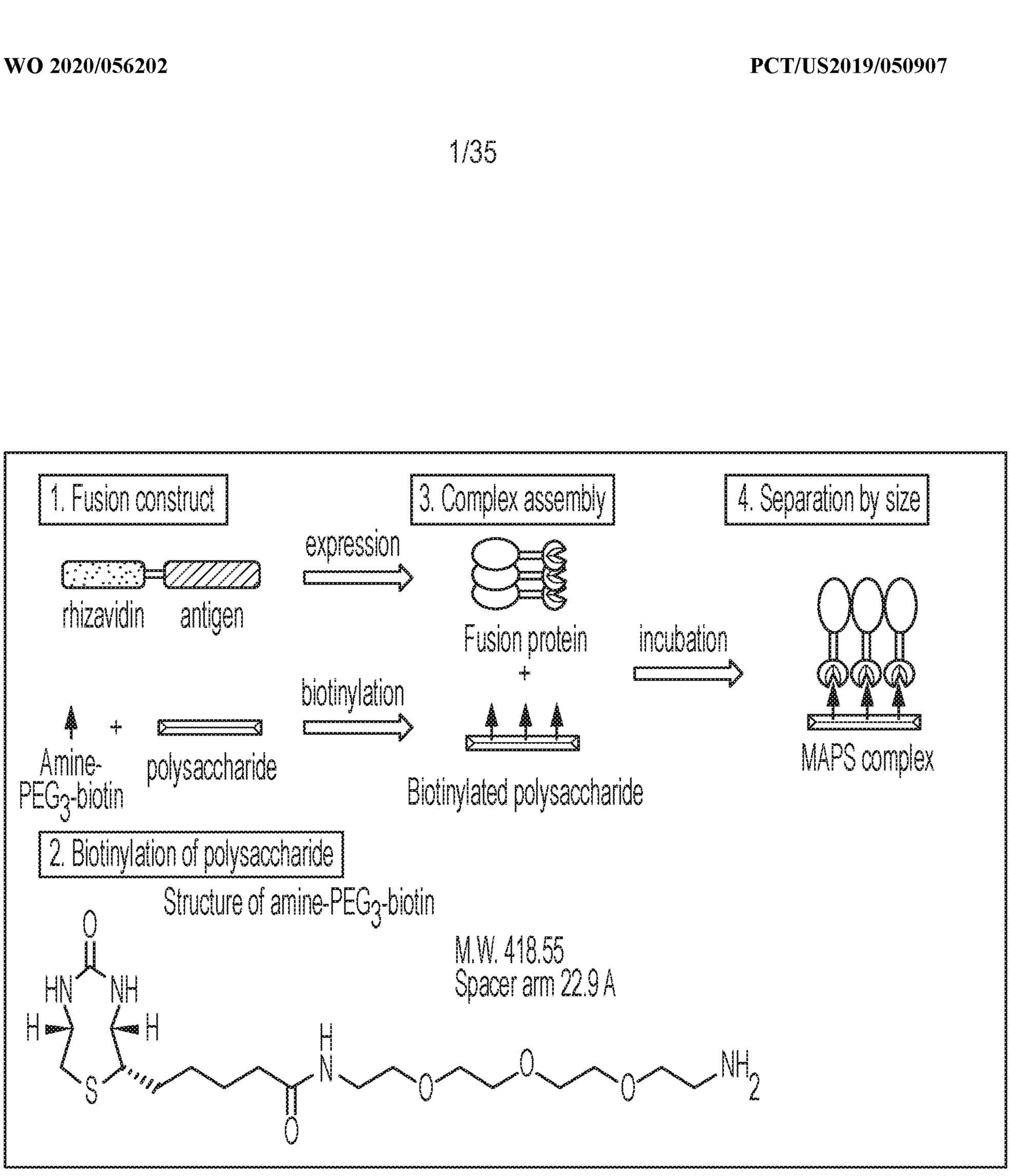

1. Fusion construct
rhizavidin
antigen
expression
3. Complex assembly
Fusion protein
+
Biotinylated polysaccharide
incubation
4. Separation by size
MAPS complex
Amine-PEG3-biotin
+
polysaccharide
biotinylation
2. Biotinylation of polysaccharide
Structure of amine-PEG3-biotin
M.W. 418.55
Spacer arm 22.9 A

FIG. 1

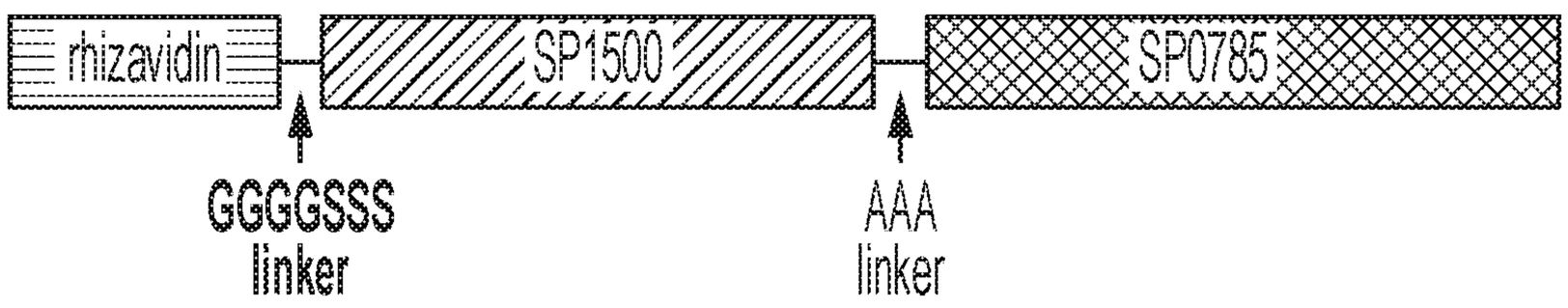


FIG. 2

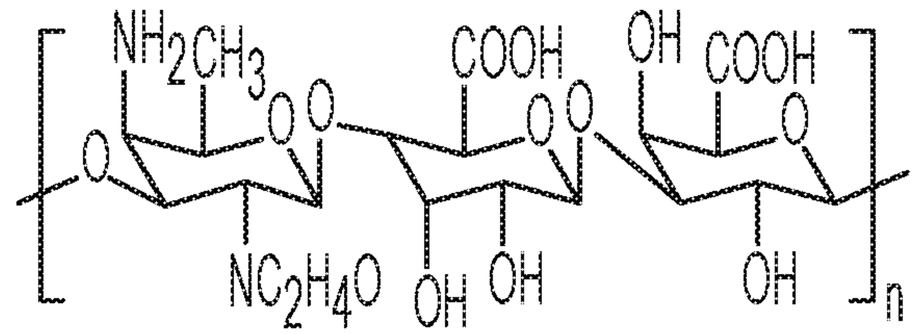
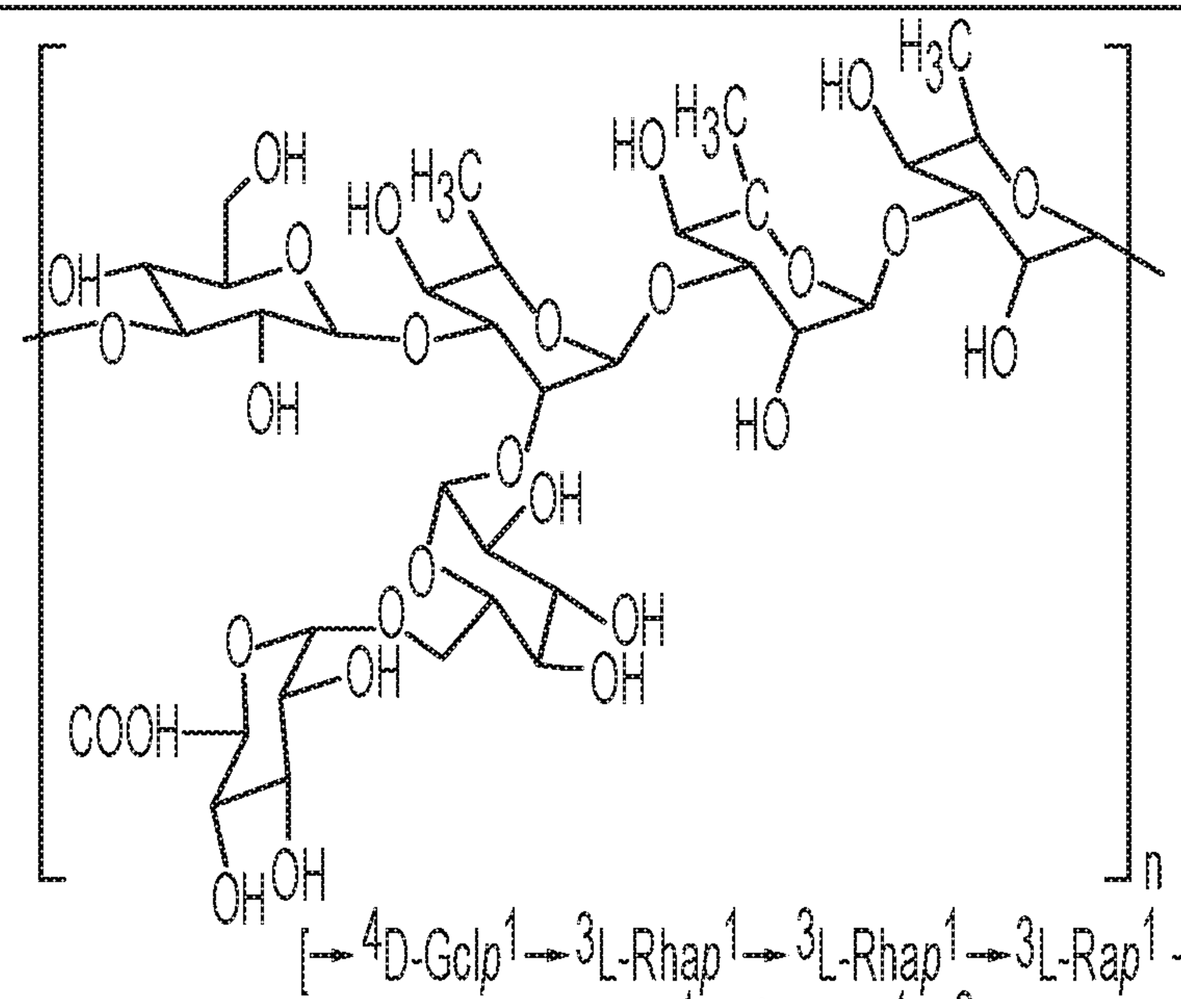
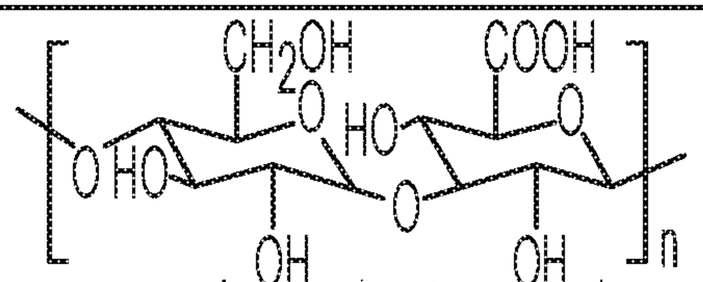
TYPE	STRUCTURE	ADDITIONAL INFORMATION
1	 [→3AATp1→4D-GalpA1→3D-GalpA1→]n	MW: 625.147 Monosaccharides: 1 AAT, 2 D-GalA AAT: 2-acetamido-4-amino-2,4,6-tridexoy- D-galactopyranose Structure: Linear Net Charge: -1 O-Acetate: 1
2	 [→4D-Glcp1→3L-Rhap1→3L-Rhap1→3L-Rap1→]n D-GlcpA1→6D-Glcp1→2	MW: 960.811 Monosaccharides: 2 D-Glc, 3 L-Rha, 1 D-GlcA Structure: Branched Net Charge: -1
3	 [→4D-Glcp1→3D-GlcpA1→]n	MW: 360.067 Monosaccharides: 1 D-Glc, 1 D-GlcA Structure: Linear Net Charge: -1

FIG. 3

SUBSTITUTE SHEET (RULE 26)

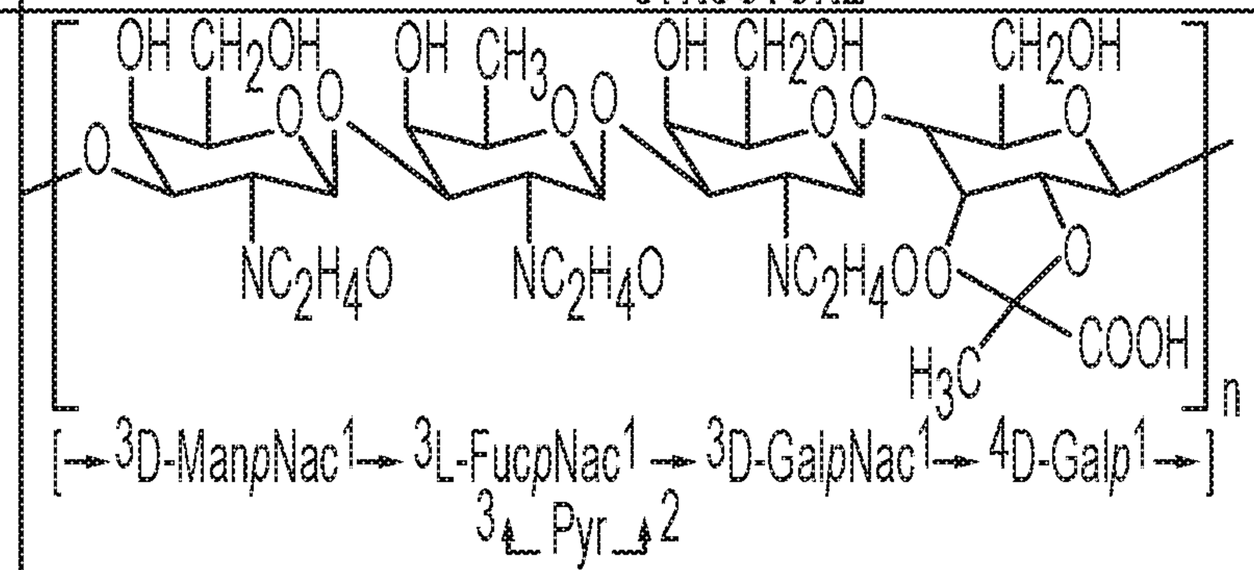
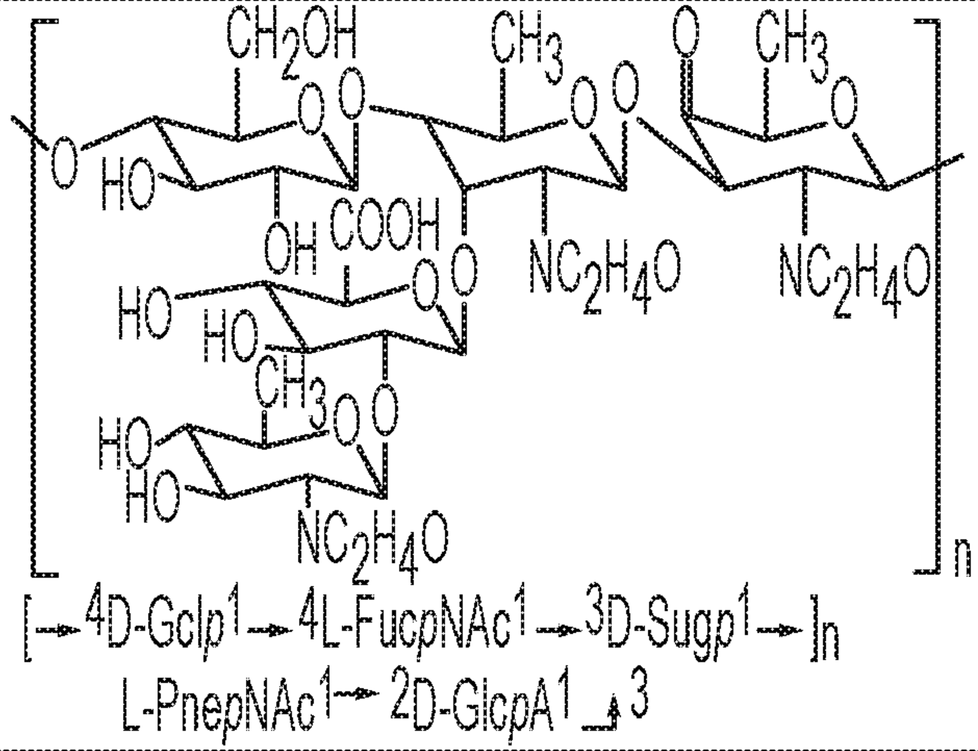
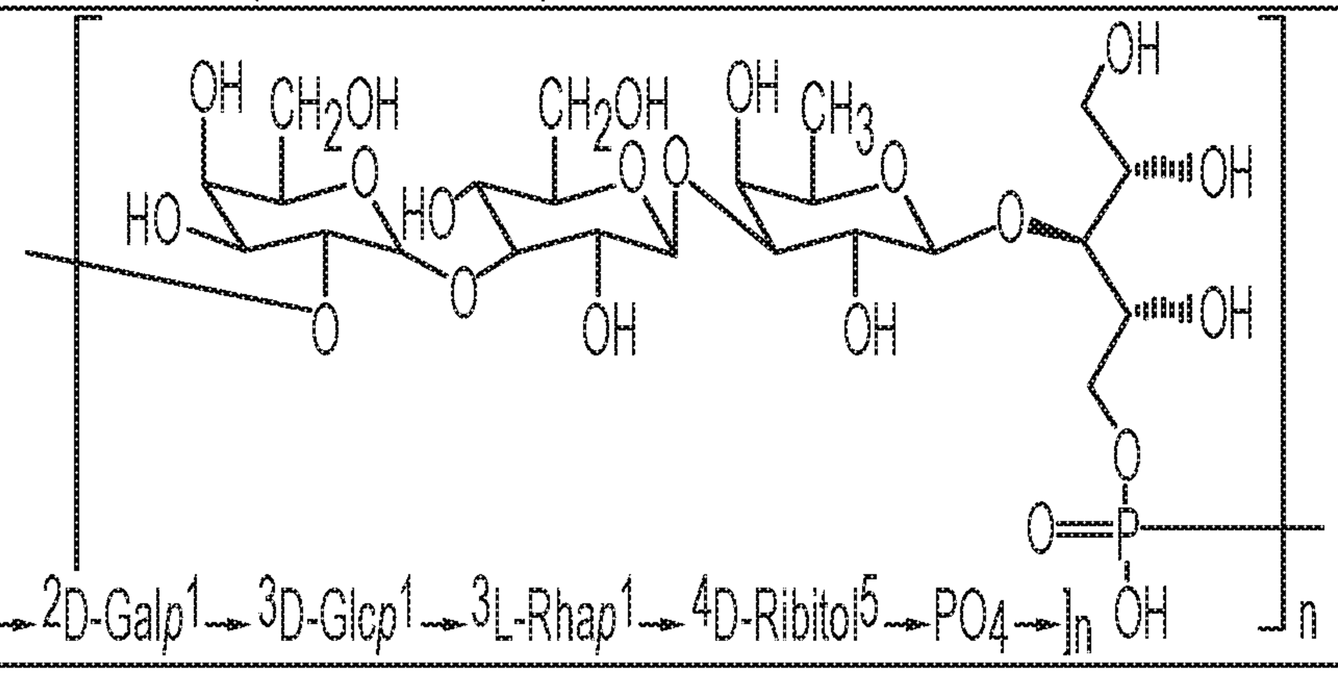
TYPE	STRUCTURE	ADDITIONAL INFORMATION
4		MW: 847.283 Monosaccharides: 1 D-Gal, 1 D-GalNac, 1 D-ManNac, 1 L-FucNac Structure: Linear Net Charge: -1 Pyruvate: 1
5		MW: 919.305 Monosaccharides: 1 D-Glc, 1 L-FucNac, 1 D-GlcA, 1 D-Sug, 1 L-PneNAc D-Sug: 2-acetamido-2,6,-dideoxy-D- xylohexos-4-ulose L-PneNac: 2-acetamido-2,6,-dideoxy-L-talose (pneumosamine) Structure: Branched Net Charge: -1
6A		MW: 706.170 Monosaccharides: 1 D-Glc, 1 D-Gal, 1 L-Rha, 1 Ribitol Structure: Linear Net Charge: -1 Phosphorous: 1 in polysaccharide backbone

FIG. 3
Continued

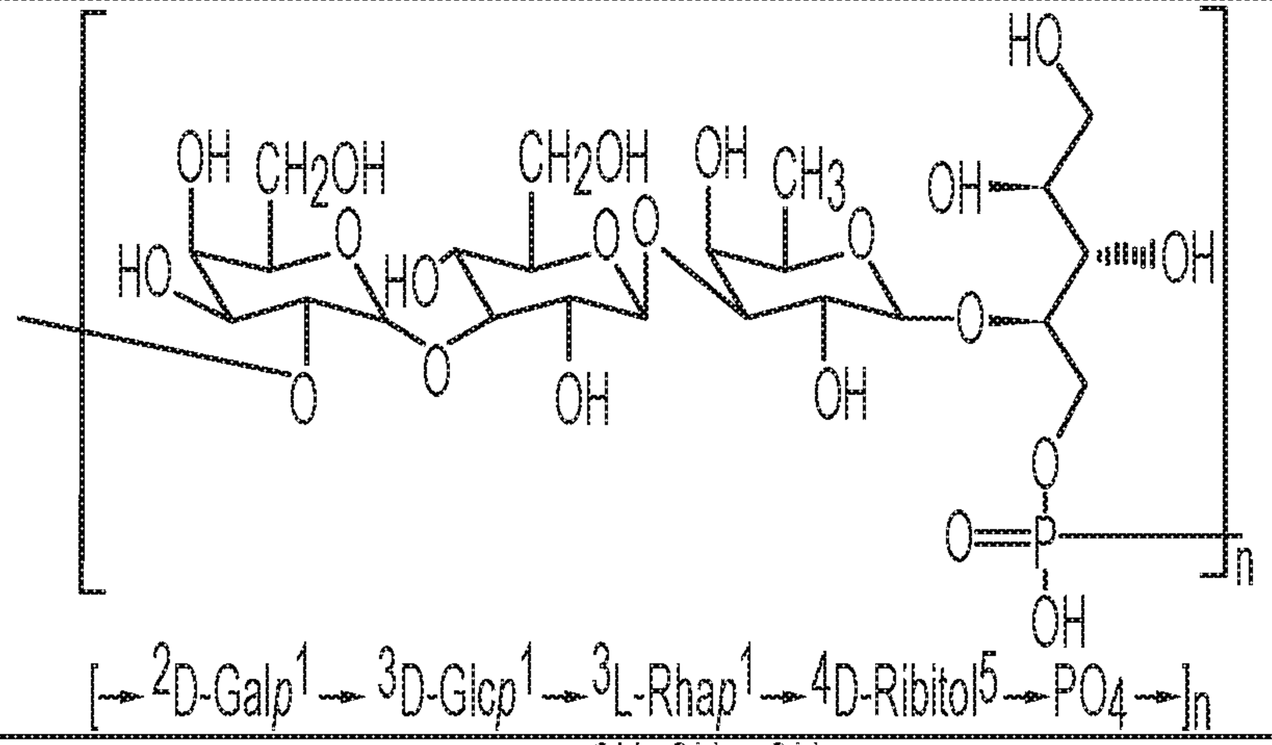
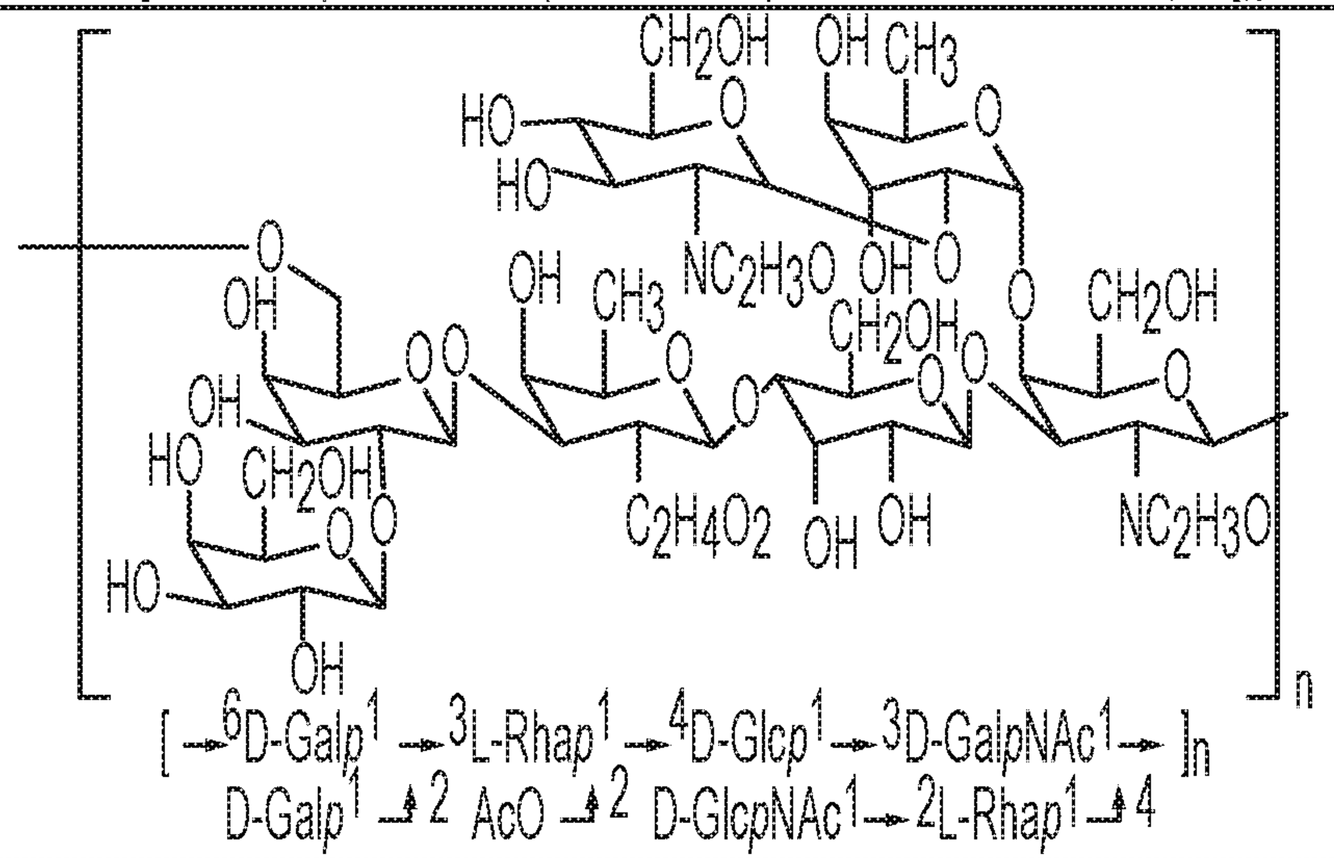
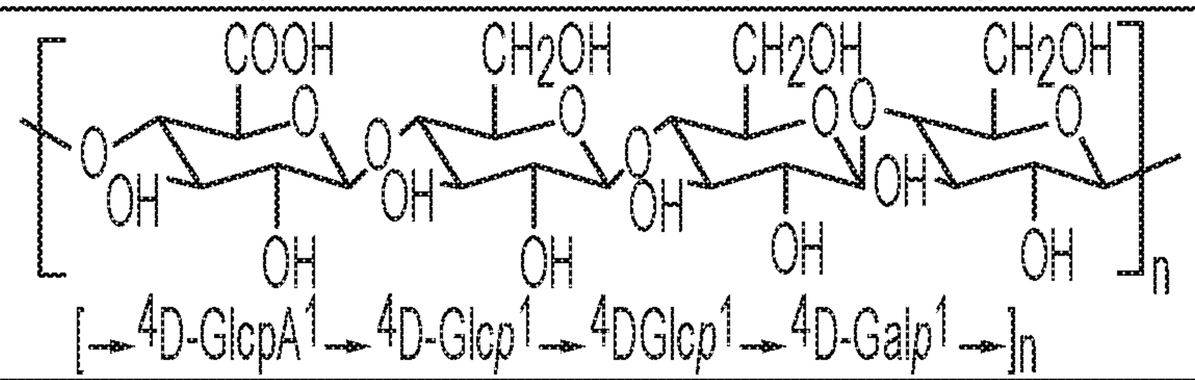
TYPE	STRUCTURE	ADDITIONAL INFORMATION
6B		MW: 706.170 Monosaccharides: 1 D-Glc, 1 D-Gal, 1 L-Rha, 1 Ribitol Structure: Linear Net Charge: -1 Phosphorous: 1 in polysaccharide backbone
7F		Monosaccharides: 1 D-Glc, 2 D-Gal, 2 L-Rha, 1 D-GlcNac, 1 D-GalNac Structure: Branched Net Charge: 0 O-Acetate: 1 MW: 1,226.444
8		MW: 684.528 Monosaccharides: 2 D-Glc, 1 D-Gal, 1 D-GlcA Structure: Linear Net Charge: -1

FIG. 3
Continued

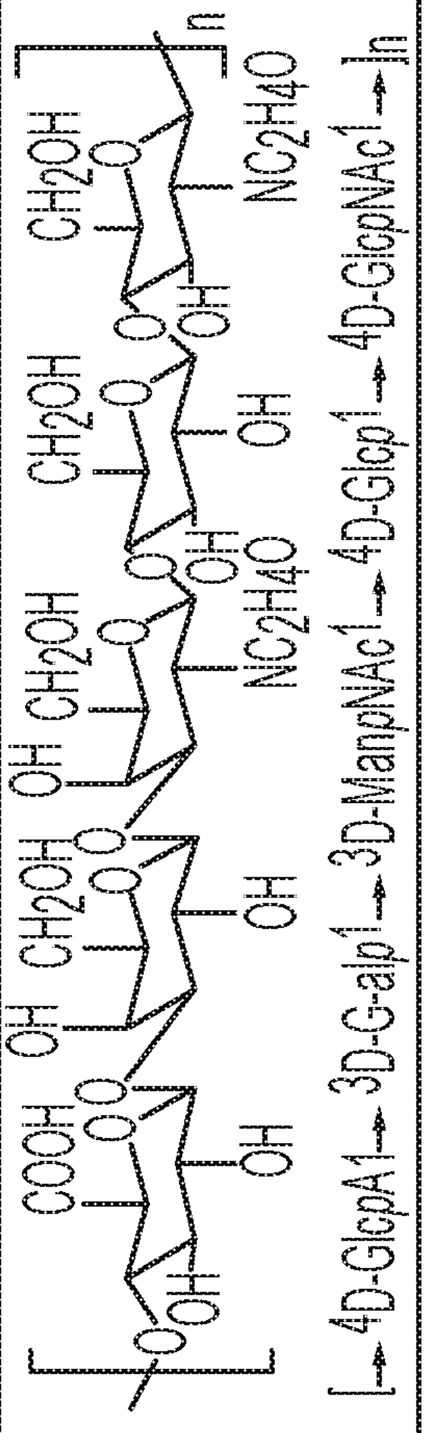
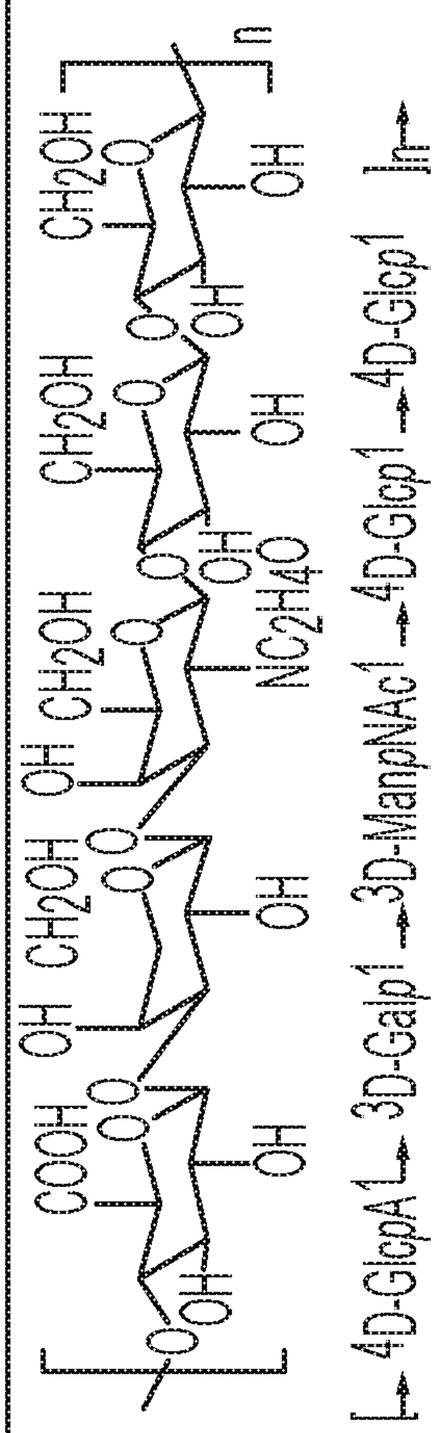
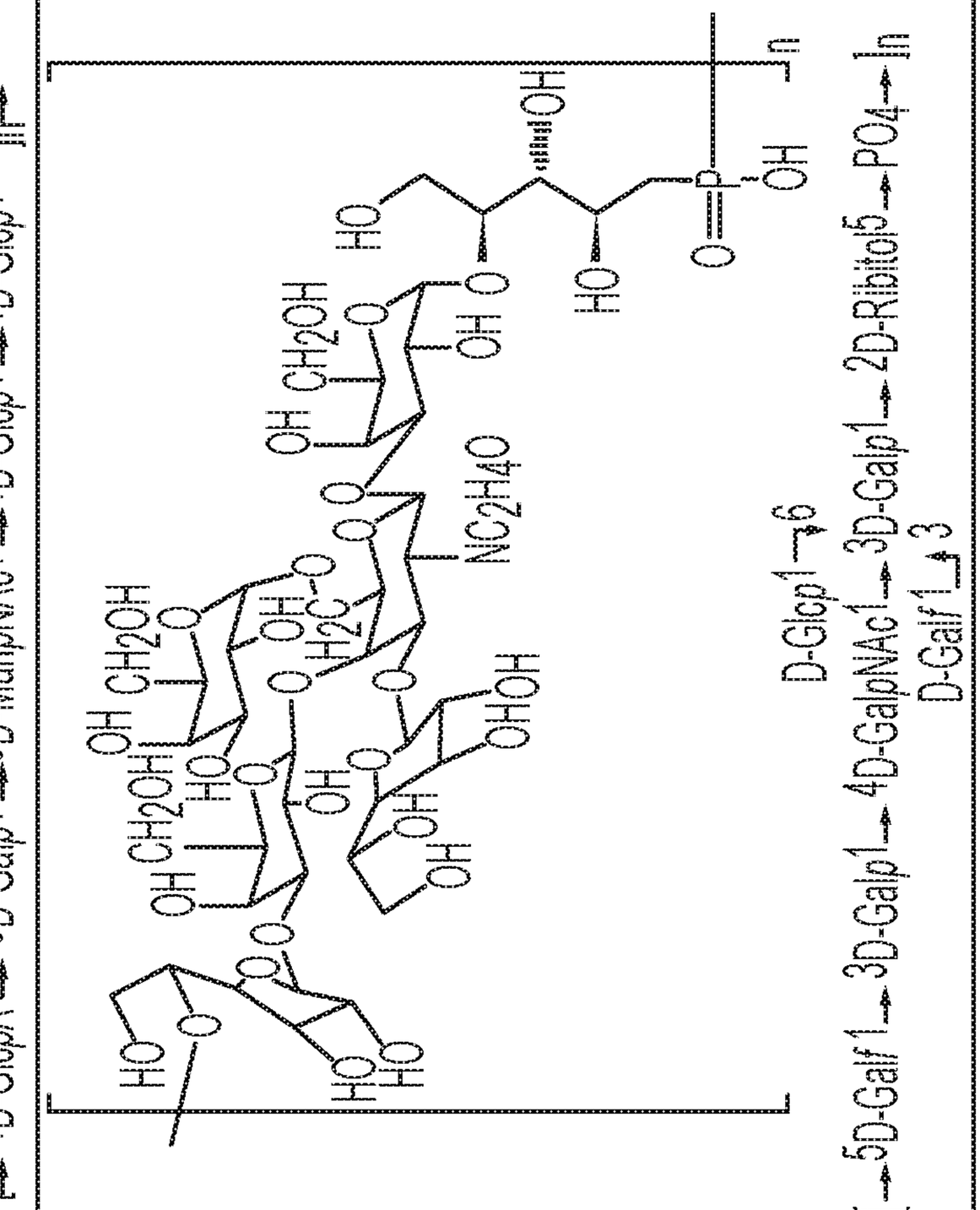
TYPE	STRUCTURE	ADDITIONAL INFORMATION
9N		MW: 928.772 Monosaccharides: 1 D-Glc, 1 D-Gal, 1D-GlcNac, 1 D-ManNac, 1 D-GlcA Structure: Linear Net Charge: -1
9V		MW: 971.794 Monosaccharides: 2 D-Glc, 1 D-Gal, 1 D-ManNac, 1 D-GlcA Structure: Linear Net Charge: -1 O-Acetate: 2
10A		MW: 1,249.989 Monosaccharides: 5 D-Gal, 1 D-GalNac, 1 Ribitol Structure: Branched Net Charge: -1 Phosphorous: 1 in polysaccharide backbone

FIG. 3
Continued

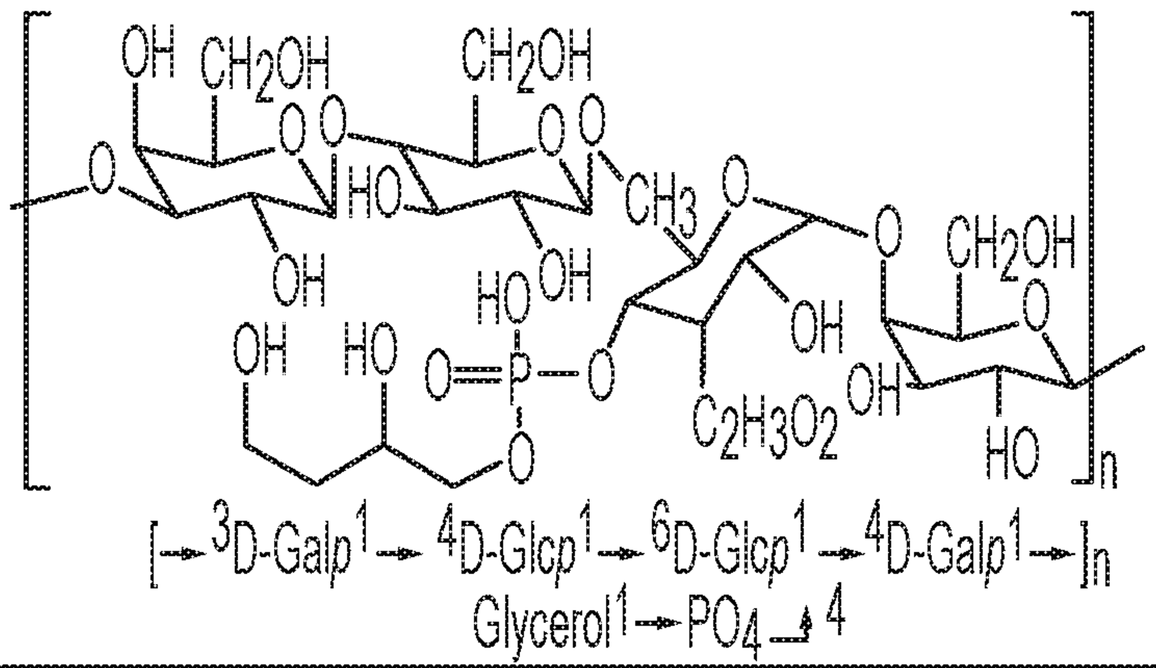
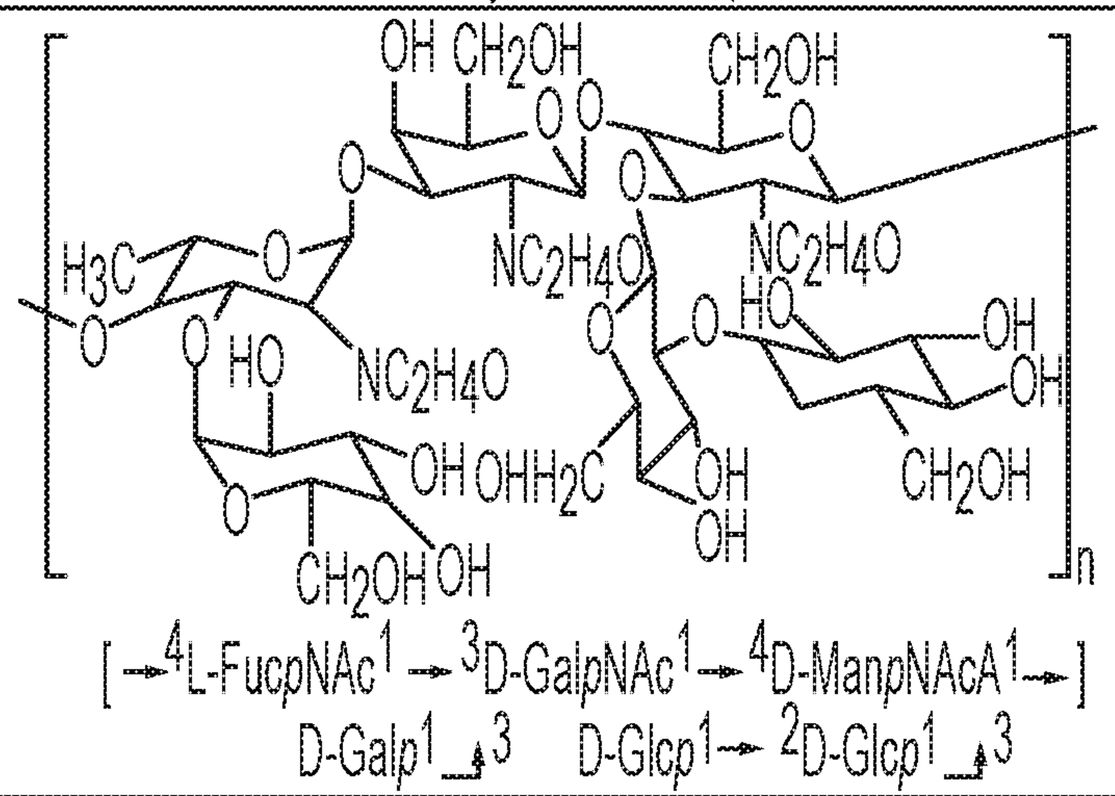
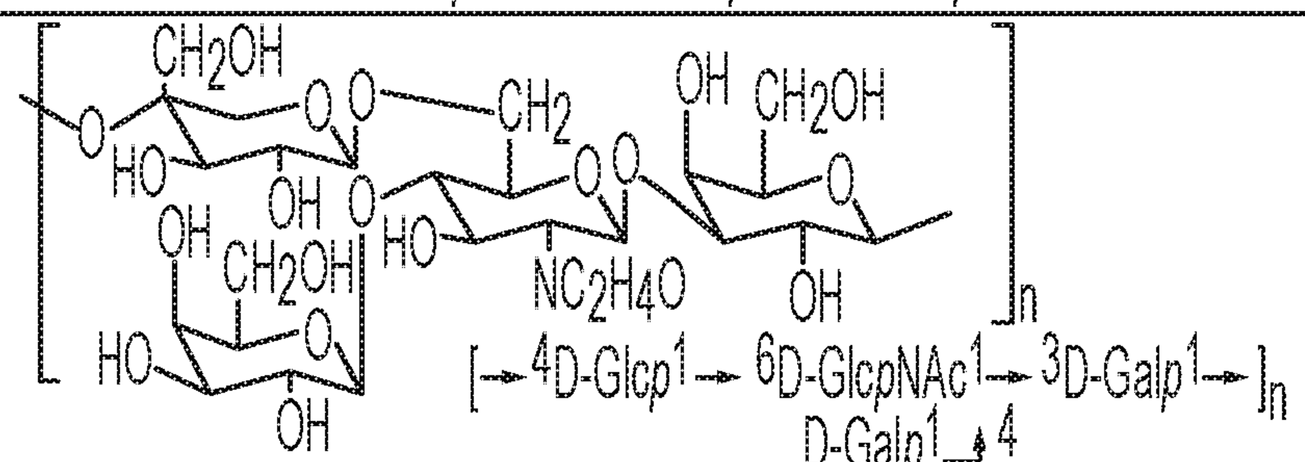
TYPE	STRUCTURE	ADDITIONAL INFORMATION
11A		MW: 933.900 Monosaccharides: 2 D-Glc, 2 D-Gal, 1 Glycerol Structure: Linear Net Charge: -1 Phosphorous: 1 in polysaccharide sidechaine O-Acetate: 1
12F		Monosaccharides: 2 D-Glc, 1 D-Gal, 1 D-GalNac, 1 L- FucNac, 1D-ManNacA Structure: Branched Net Charge: -1 MW: 1,116.02
14		Monosaccharides: 1 D-Glc, 2 D-Gal, 1 D-GlcNac Structure: Branched Net Charge: 0 MW: 689.238

FIG. 3
Continued

FIG. 3 cont'd

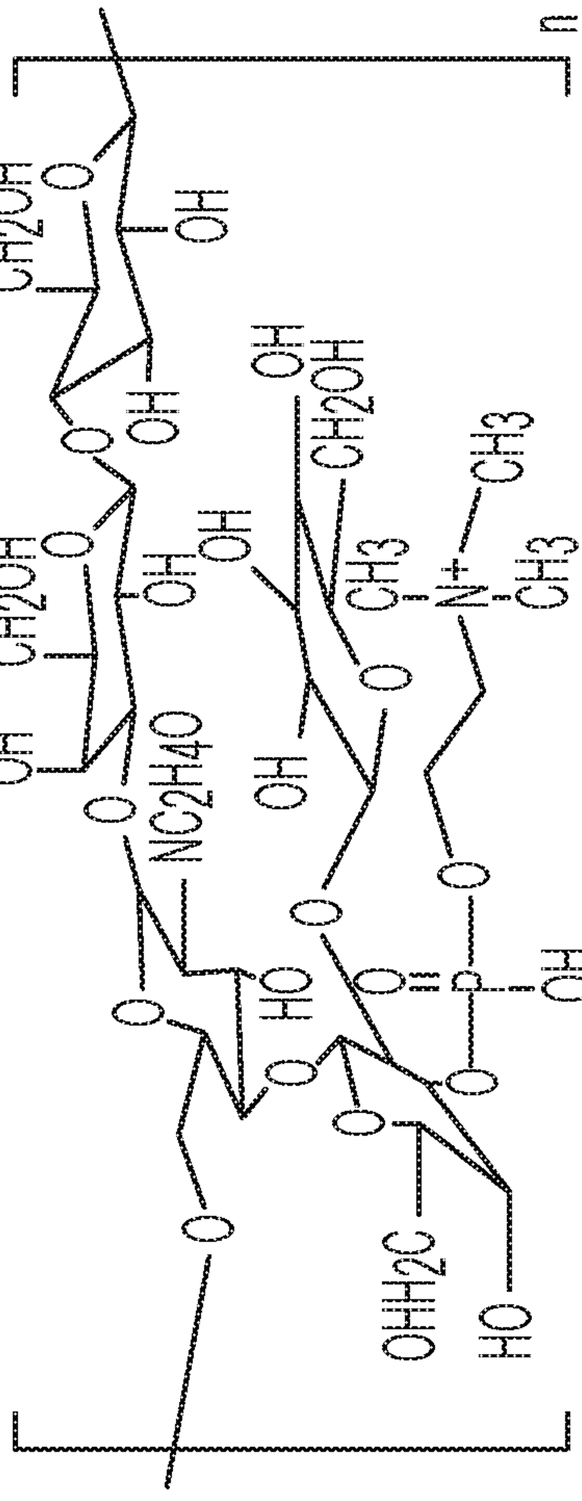
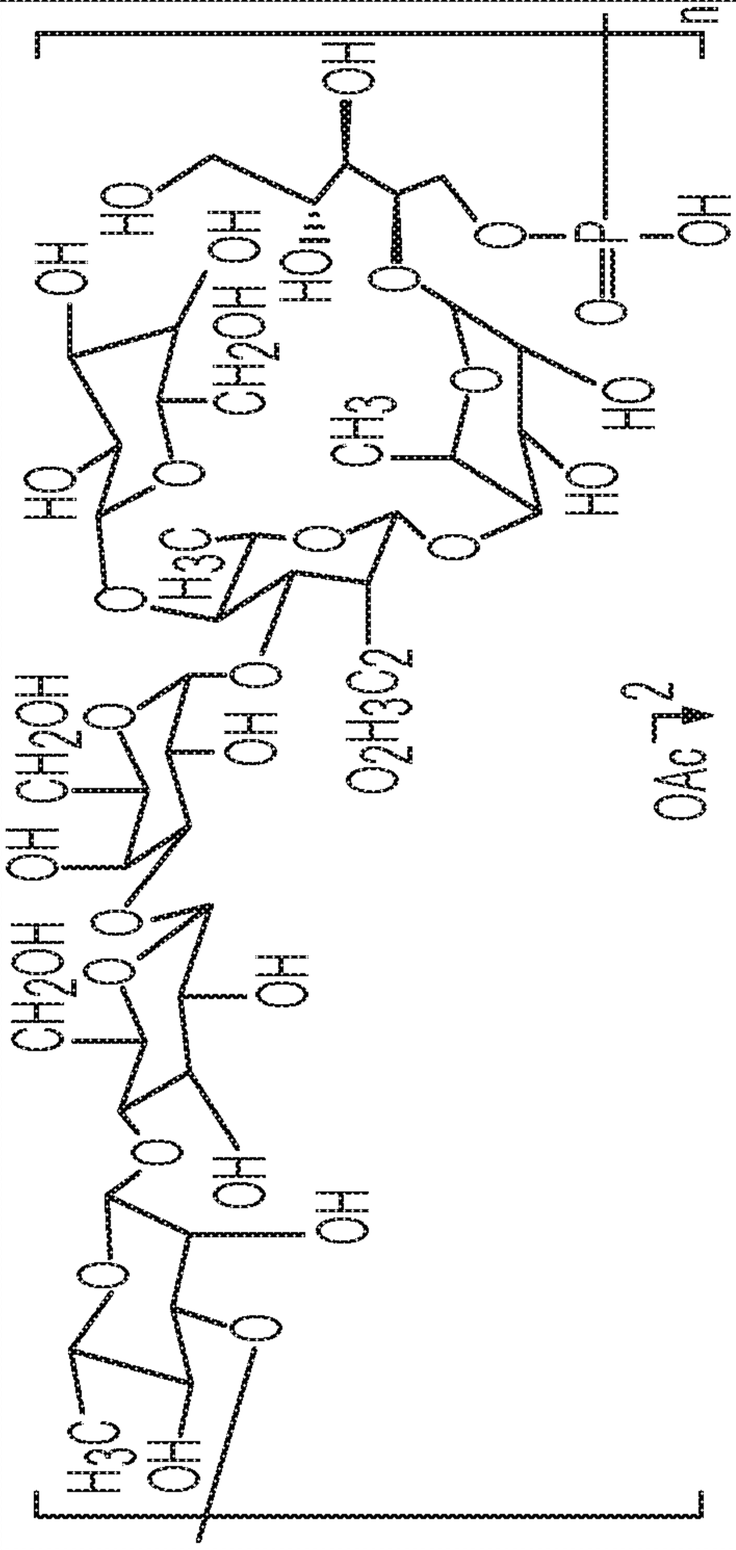
TYPE	STRUCTURE	ADDITIONAL INFORMATION
15B		MW: 1,011.420 Monosaccharides: 1 D-Glc, 3 D-Gal, 1 D-GlcNac Structure: Branched Net Charge: -1 Phosphorous: 1 in polysaccharide sidechain O-Acetate: 1 Pyruvate: 1 Note: Published stucture indicates 0.2 mol of phosphocholine per mol of repeat unit. Nuclear magnetic resonance analysis at Merck suggests that phosphocholine is not present.
17F		MW: 1,202.920 Monosaccharides: 1 D-Glc, 2 D-Gal, 3 L-Rha, 1 Arabinitol Structure: Branched Net Charge: -1 Phosphorous: 1 in polysaccharide backbone O-Acetate: 1 Pyruvate: 1

FIG. 3 cont'd

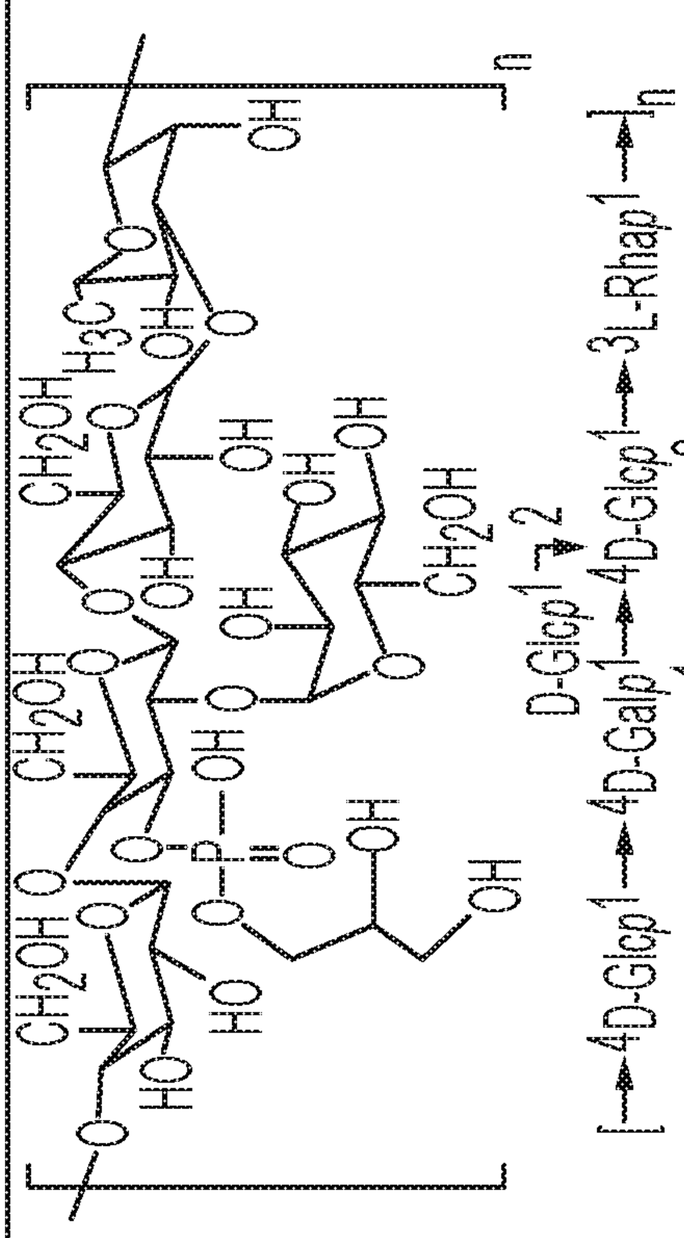
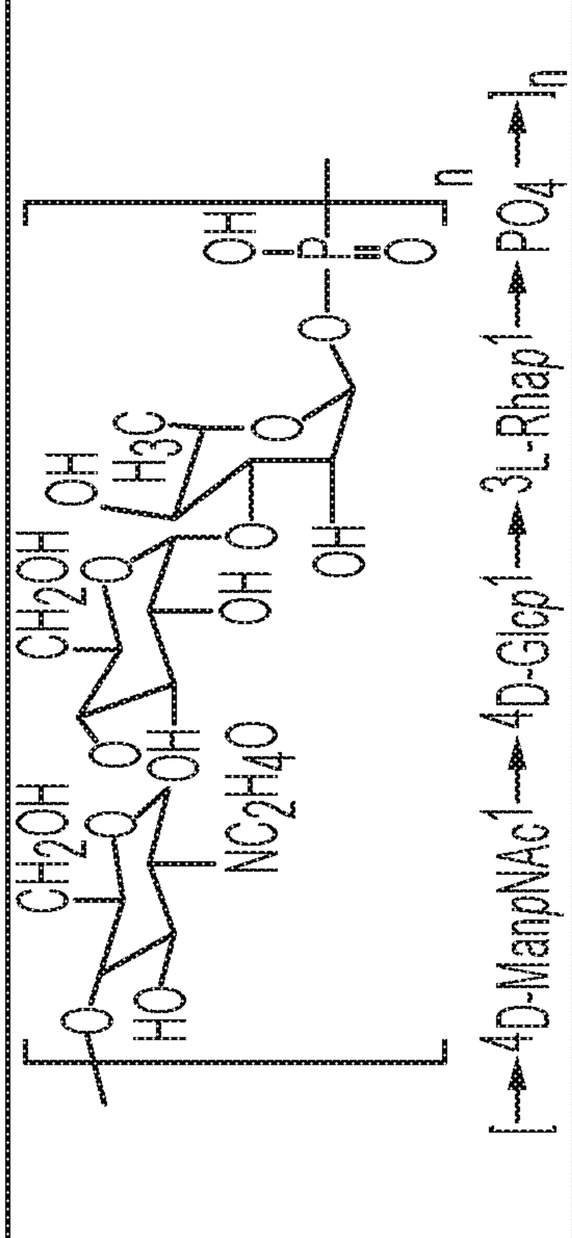
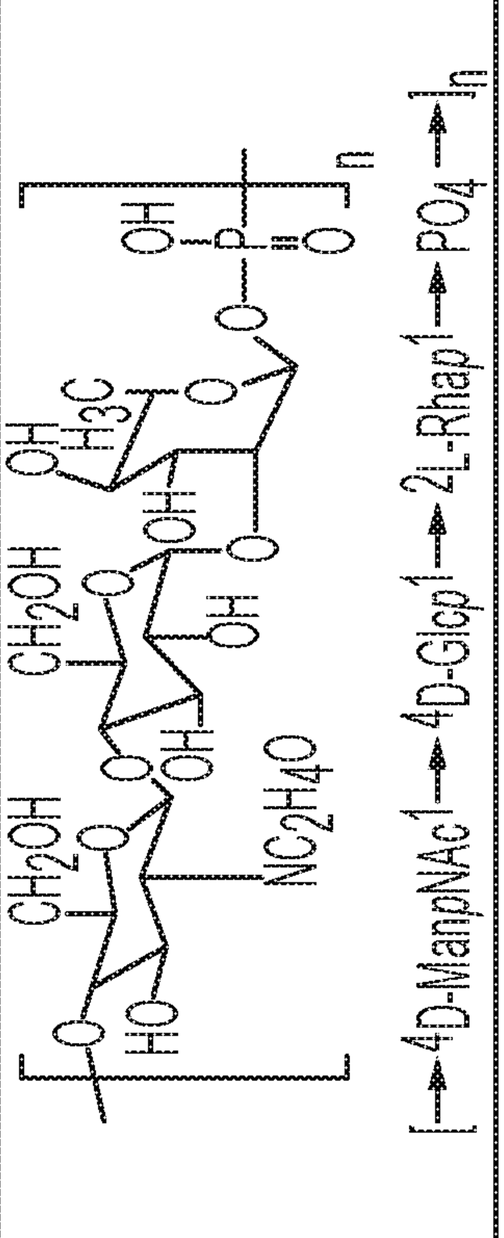
TYPE	STRUCTURE	ADDITIONAL INFORMATION
18C		MW: 1,012.265 Monosaccharides: 3 D-Glc, 1 D-Gal, 1 L-Rha, 1 Glycerol Structure: Branched Net Charge: -1 Phosphorous: 1 in polysaccharide sidechain O-Acetate: 1
19A		MW: 613.138 Monosaccharides: 1 D-Glc, 1 L-Rha 1 D-ManNac Structure: Linear Net Charge: -1 Phosphorous: 1 in polysaccharide backbone
19F		MW: 613.138 Monosaccharides: 1 D-Glc, 1 L-Rha 1 D-ManNac Structure: Linear Net Charge: -1 Phosphorous: 1 in polysaccharide backbone

FIG. 3 cont'd

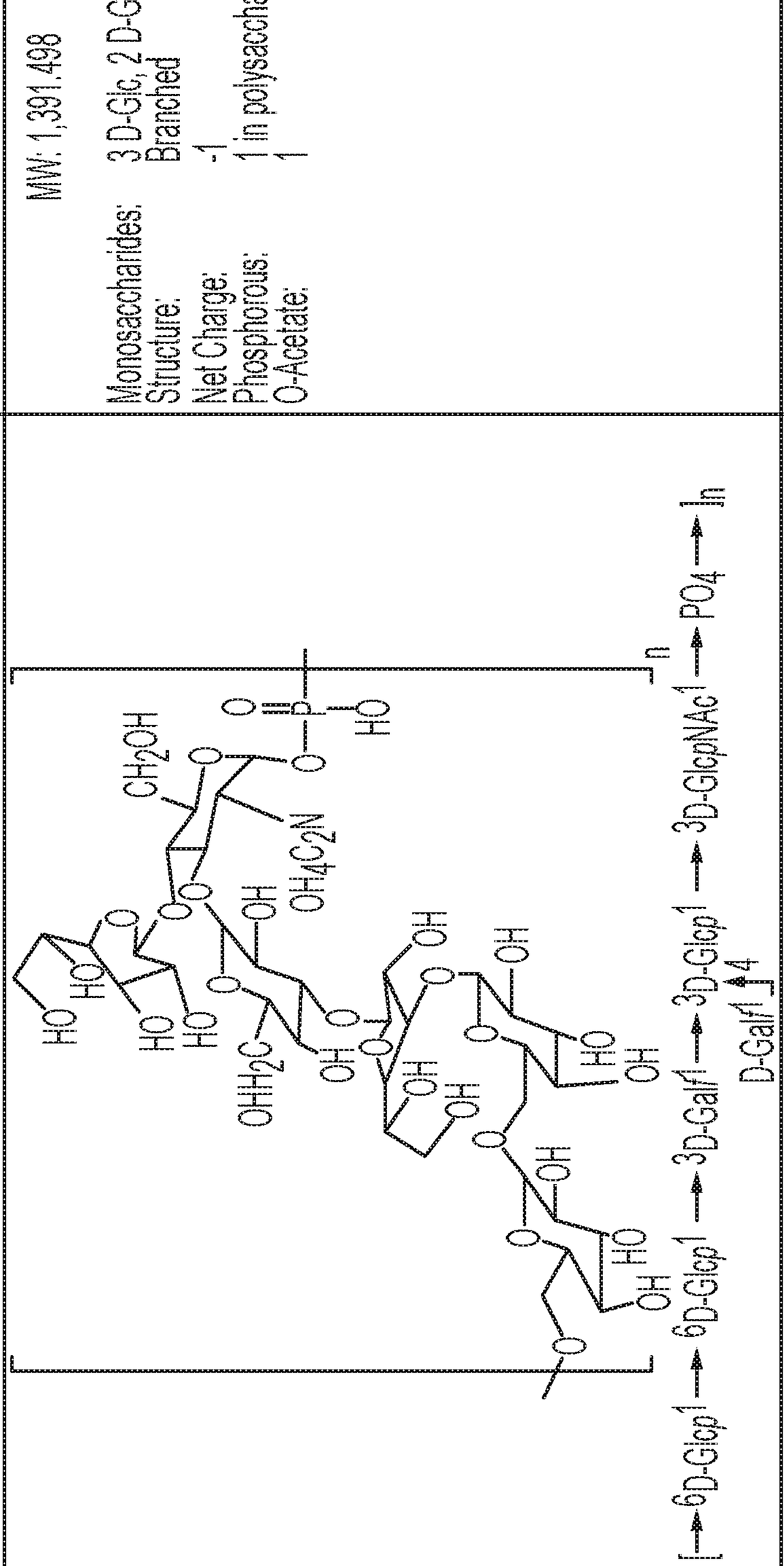
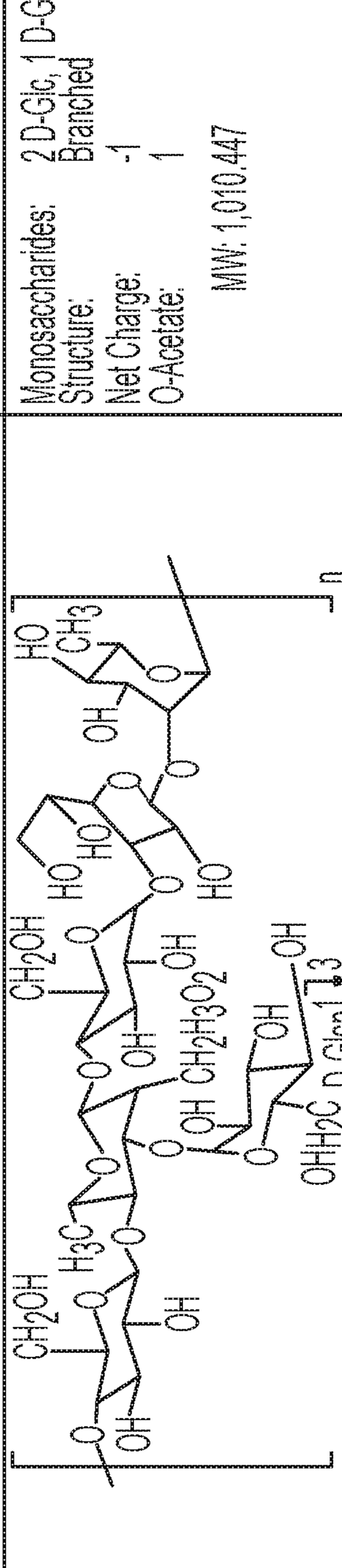
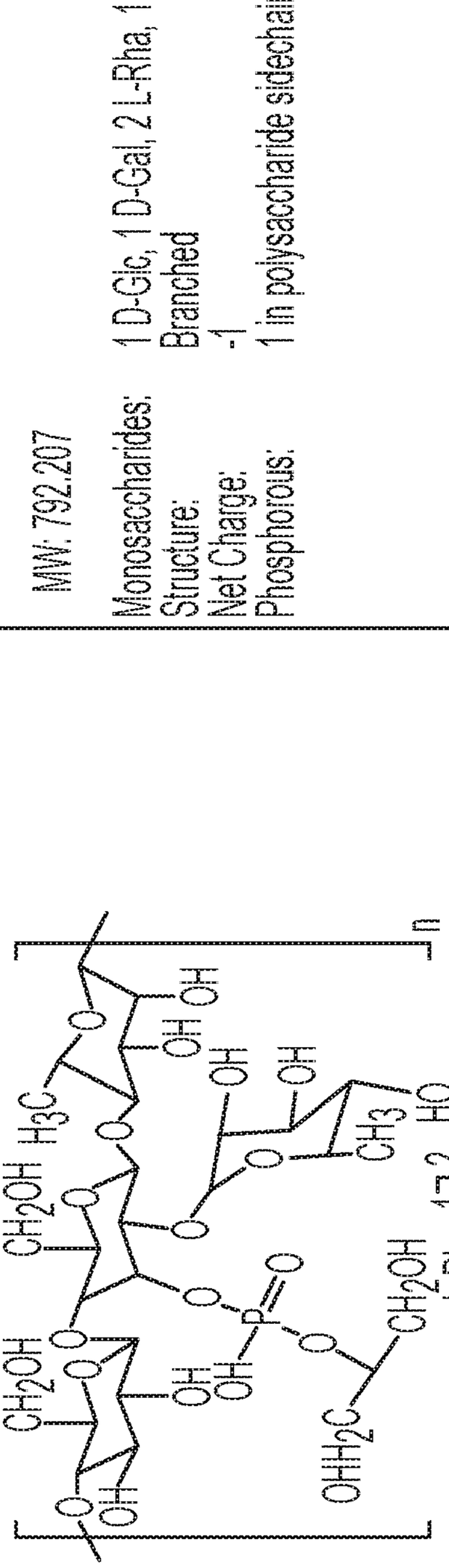
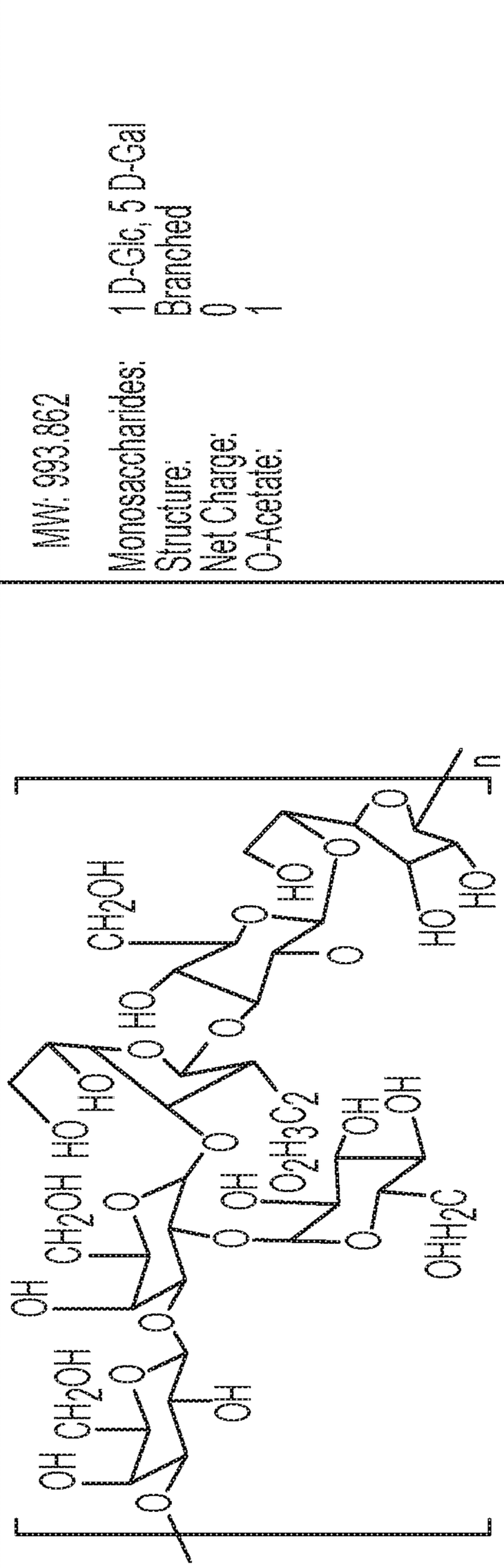
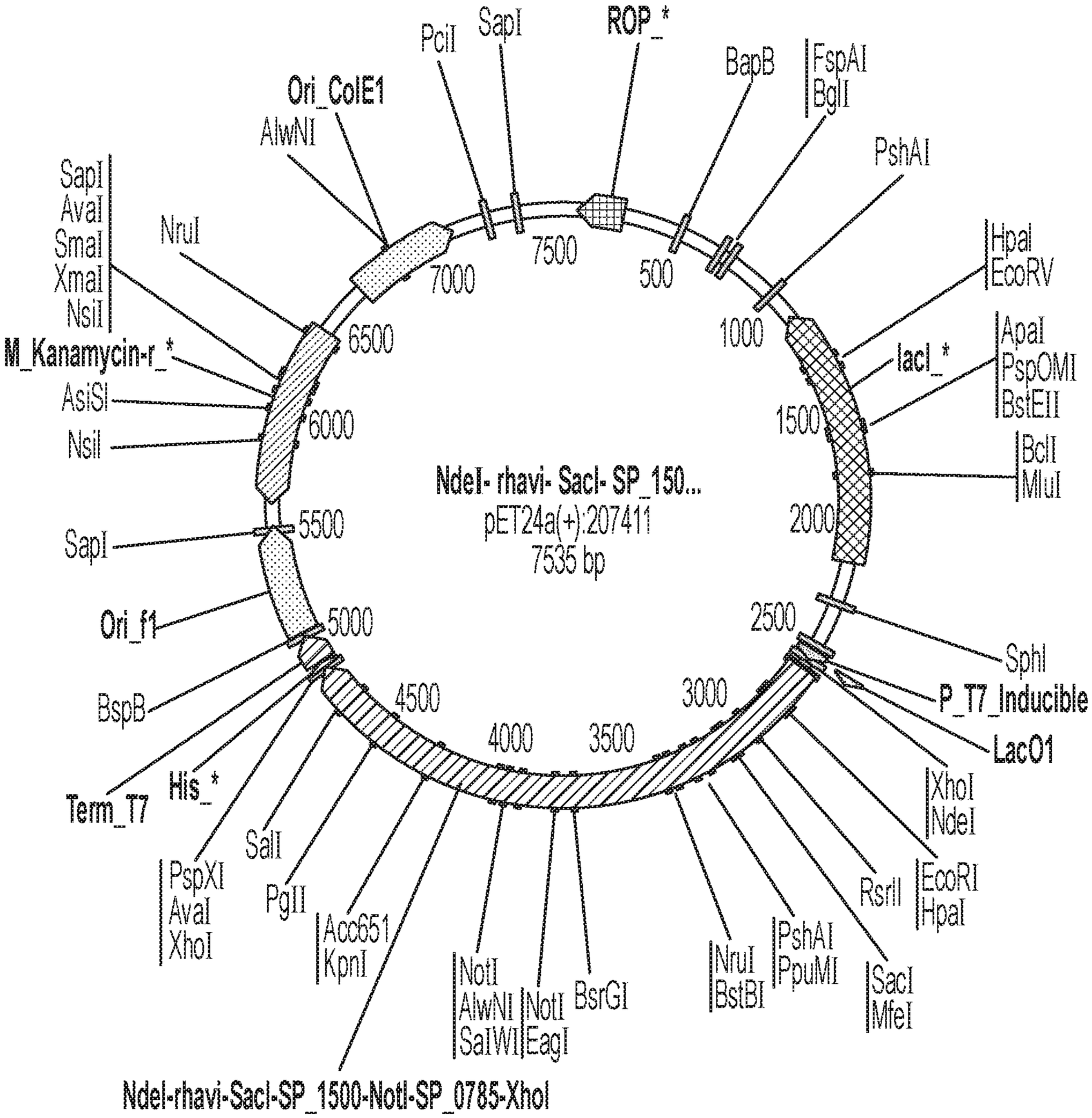
TYPE	STRUCTURE	ADDITIONAL INFORMATION
18C		MW: 1,391.498 Monosaccharides: 3 D-Glc, 2 D-Gal, 1 D-GlcNac Structure: Branched Net Charge: -1 Phosphorous: 1 in polysaccharide backbone O-Acetate: 1
22F		Monosaccharides: 2 D-Glc, 1 D-Gal, 2 L-Rha, 1 D-GlcA Structure: Branched Net Charge: -1 O-Acetate: 1 MW: 1,010.447

FIG. 3 cont'd

TYPE	STRUCTURE	ADDITIONAL INFORMATION
23F		MW: 792.207 Monosaccharides: 1 D-Glc, 1 D-Gal, 2 L-Rha, 1 Glycerol Structure: Branched Net Charge: -1 Phosphorous: 1 in polysaccharide sidechain
33F		MW: 993.862 Monosaccharides: 1 D-Glc, 5 D-Gal Structure: Branched Net Charge: 0 O-Acetate: 1



NdeI-rhavi-SacI-SP_1500-NotI-SP_0785-XhoI

FIG. 4

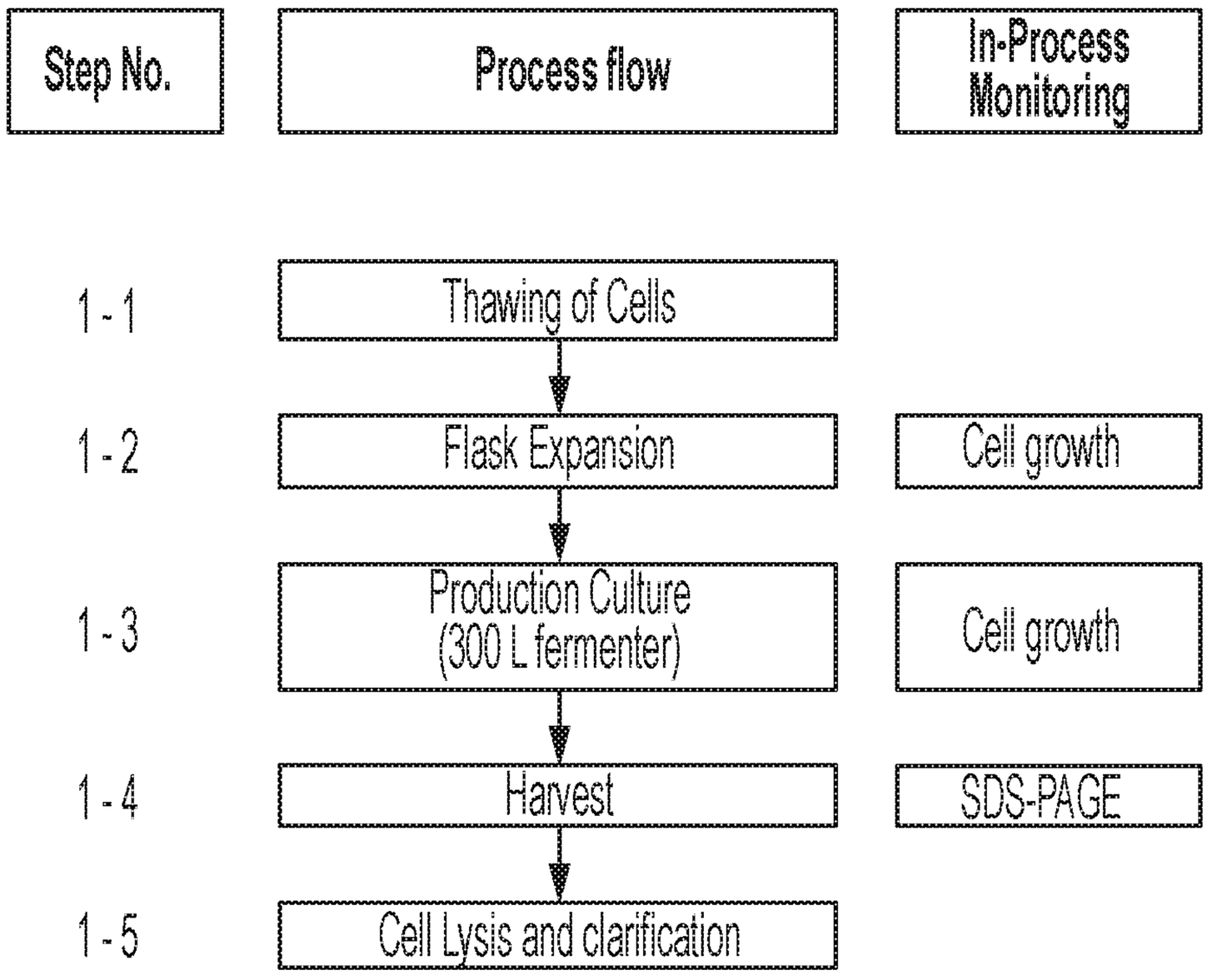

Step No.
Process flow
In-Process Monitoring
1 - 1
Thawing of Cells
1 - 2
Flask Expansion
Cell growth
1 - 3
Production Culture (300 L fermenter)
Cell growth
1 - 4
Harvest
SDS-PAGE
1 - 5
Cell Lysis and clarification

FIG. 5

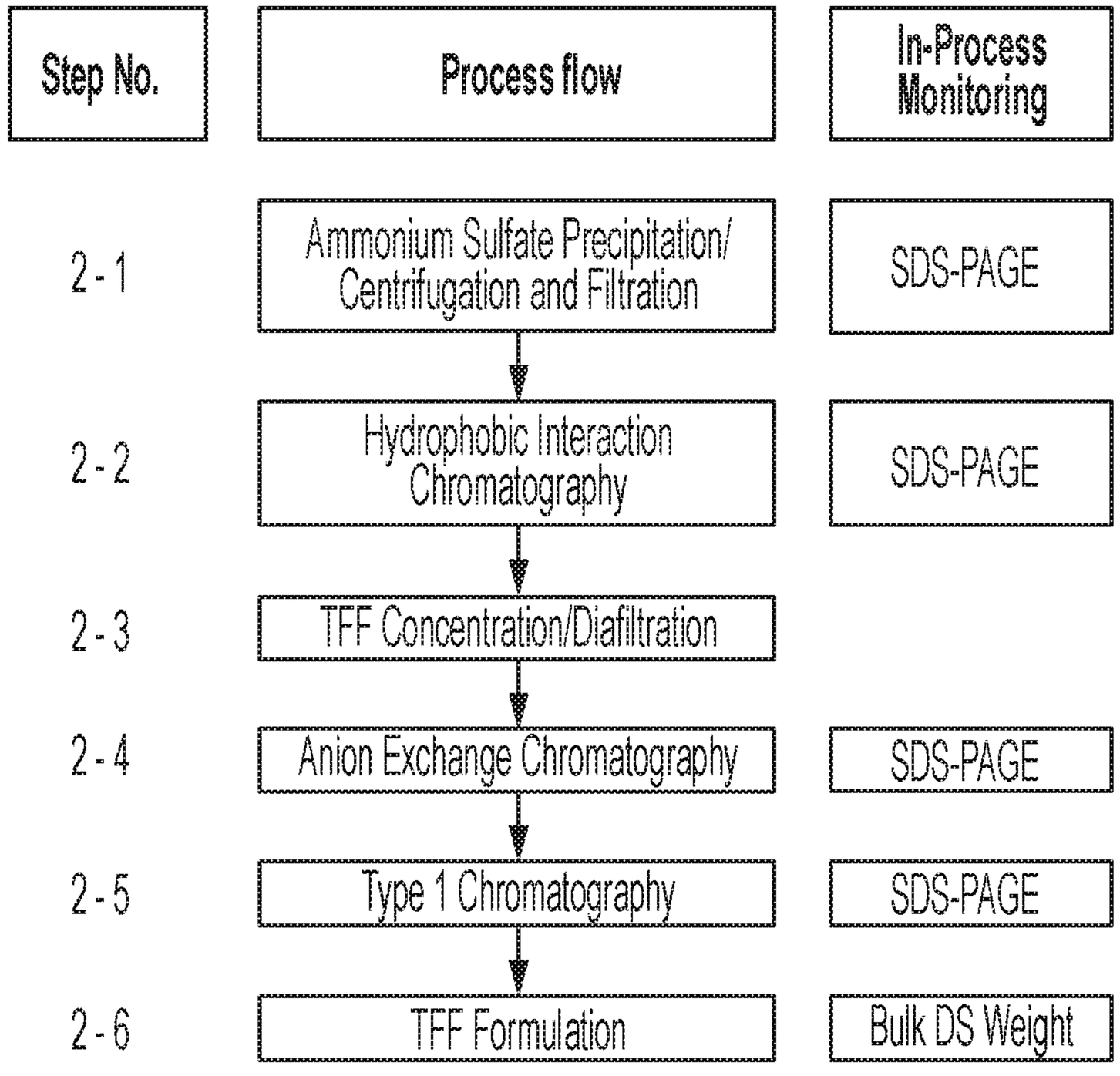

Step No.
Process flow
In-Process Monitoring
2 - 1
Ammonium Sulfate Precipitation/ Centrifugation and Filtration
SDS-PAGE
2 - 2
Hydrophobic Interaction Chromatography
SDS-PAGE
2 - 3
TFF Concentration/Diafiltration
2 - 4
Anion Exchange Chromatography
SDS-PAGE
2 - 5
Type 1 Chromatography
SDS-PAGE
2 - 6
TFF Formulation
Bulk DS Weight

FIG. 6

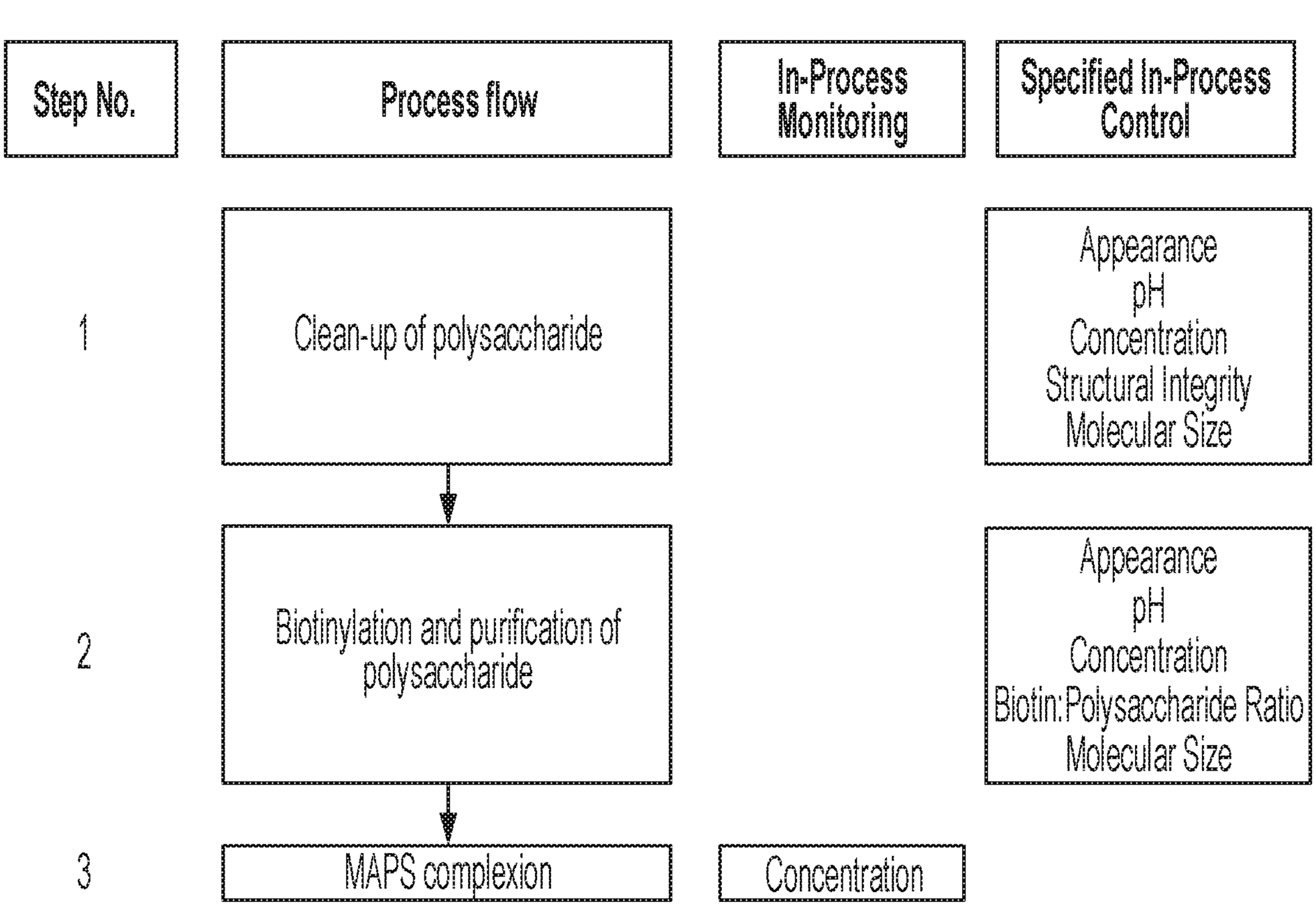

Step No.
Process flow
In-Process Monitoring
Specified In-Process Control
1
Clean-up of polysaccharide
Appearance
pH
Concentration
Structural Integrity
Molecular Size
2
Biotinylation and purification of polysaccharide
Appearance
pH
Concentration
Biotin:Polysaccharide Ratio
Molecular Size
3
MAPS complexion
Concentration

FIG. 7

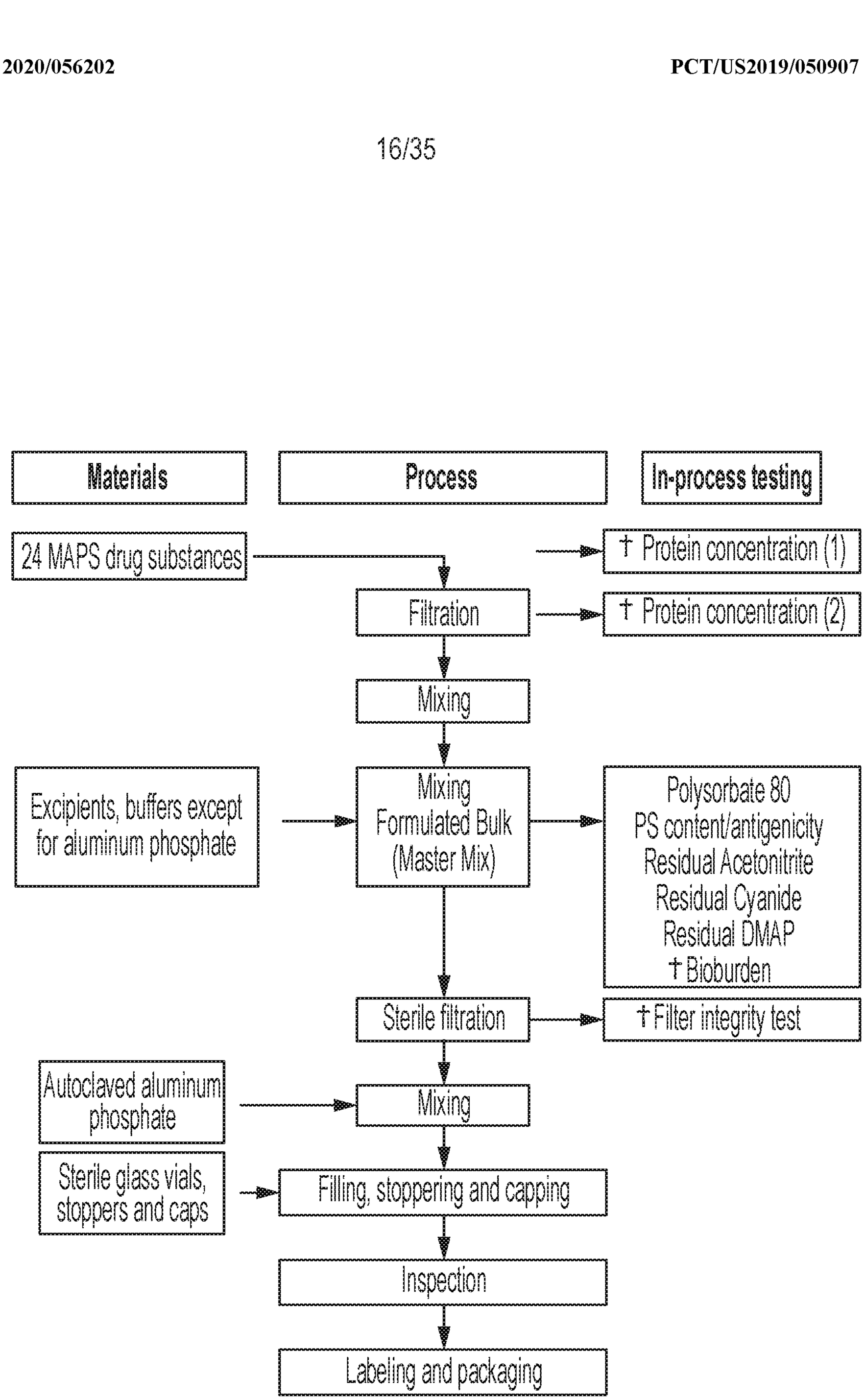

Materials
Process
In-process testing
24 MAPS drug substances
† Protein concentration (1)
Filtration
† Protein concentration (2)
Mixing
Excipients, buffers except for aluminum phosphate
Mixing Formulated Bulk (Master Mix)
Polysorbate 80
PS content/antigenicity
Residual Acetonitrite
Residual Cyanide
Residual DMAP
† Bioburden
Sterile filtration
† Filter integrity test
Autoclaved aluminum phosphate
Mixing
Sterile glass vials, stoppers and caps
Filling, stoppering and capping
Inspection
Labeling and packaging

FIG. 8
Panel A

Process Flow Diagram

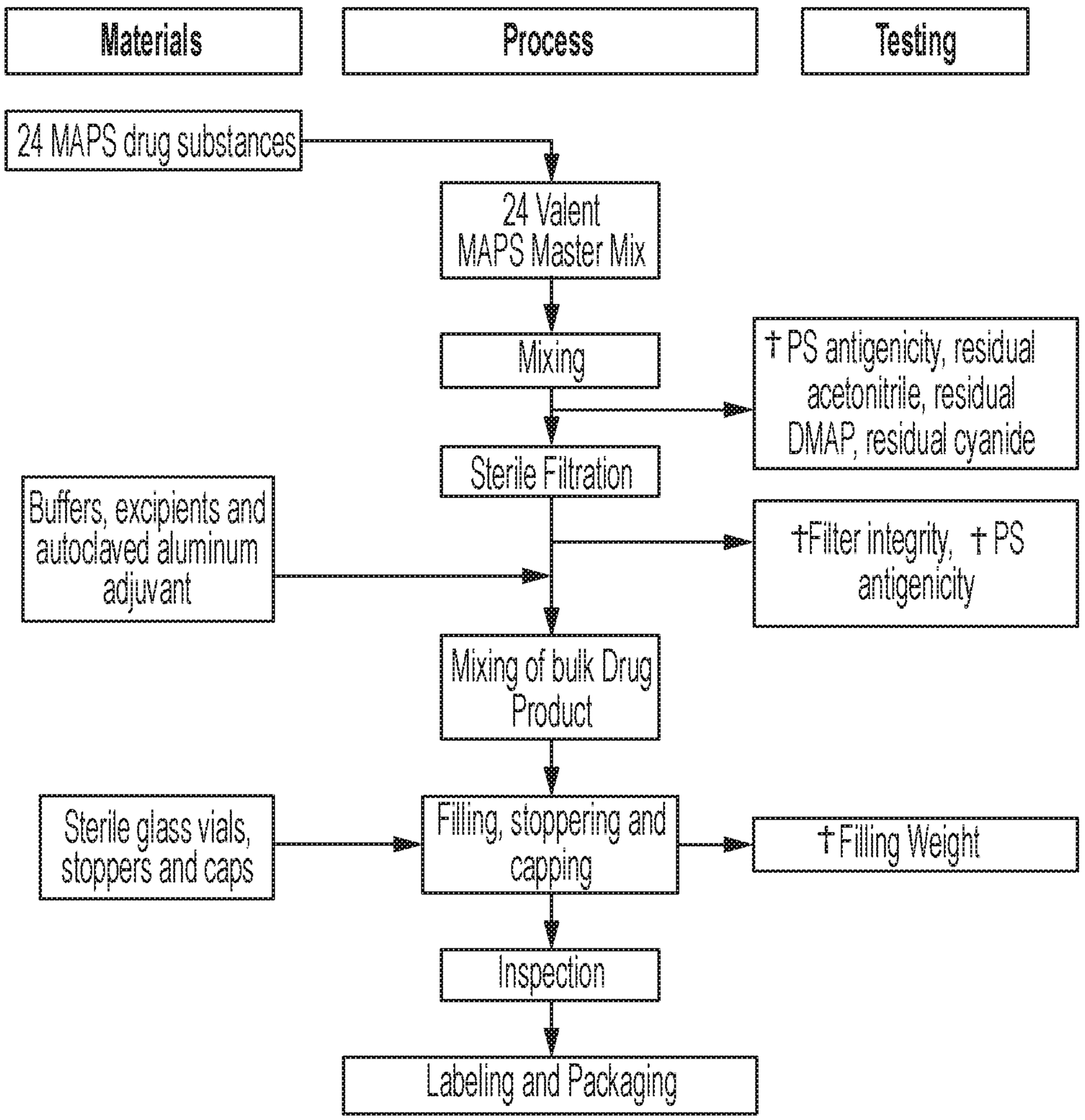


FIG. 8
Panel B

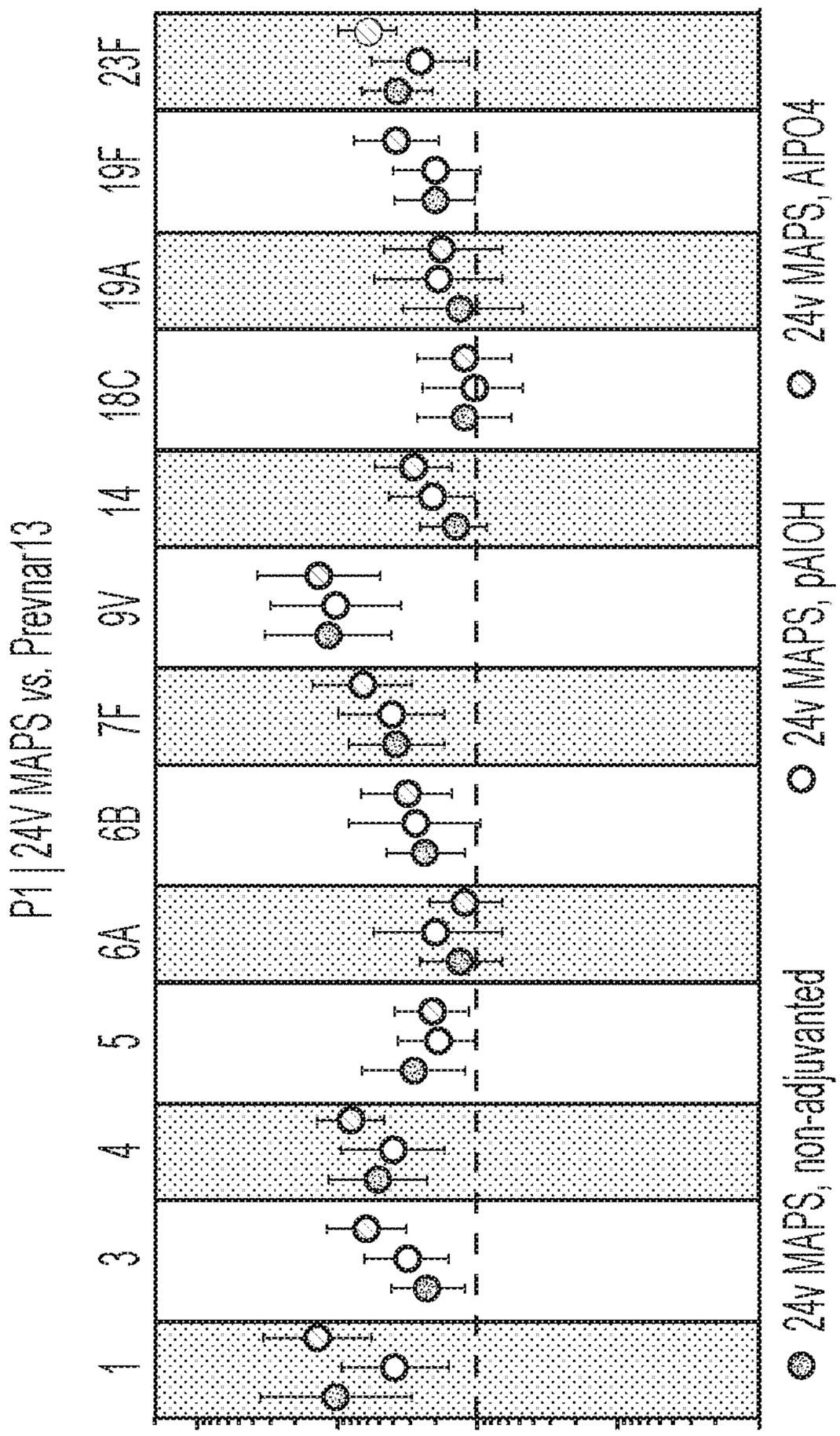

P1 | 24V MAPS vs. Prevnar13
1
3
4
5
6A
6B
7F
9V
14
18C
19A
19F
23F
24v MAPS, non-adjuvanted
24v MAPS, pAIOH
24v MAPS, AlPO4
GMT Ratio (95% CI)
10
1
0.1
0.01
0.001

FIG. 9

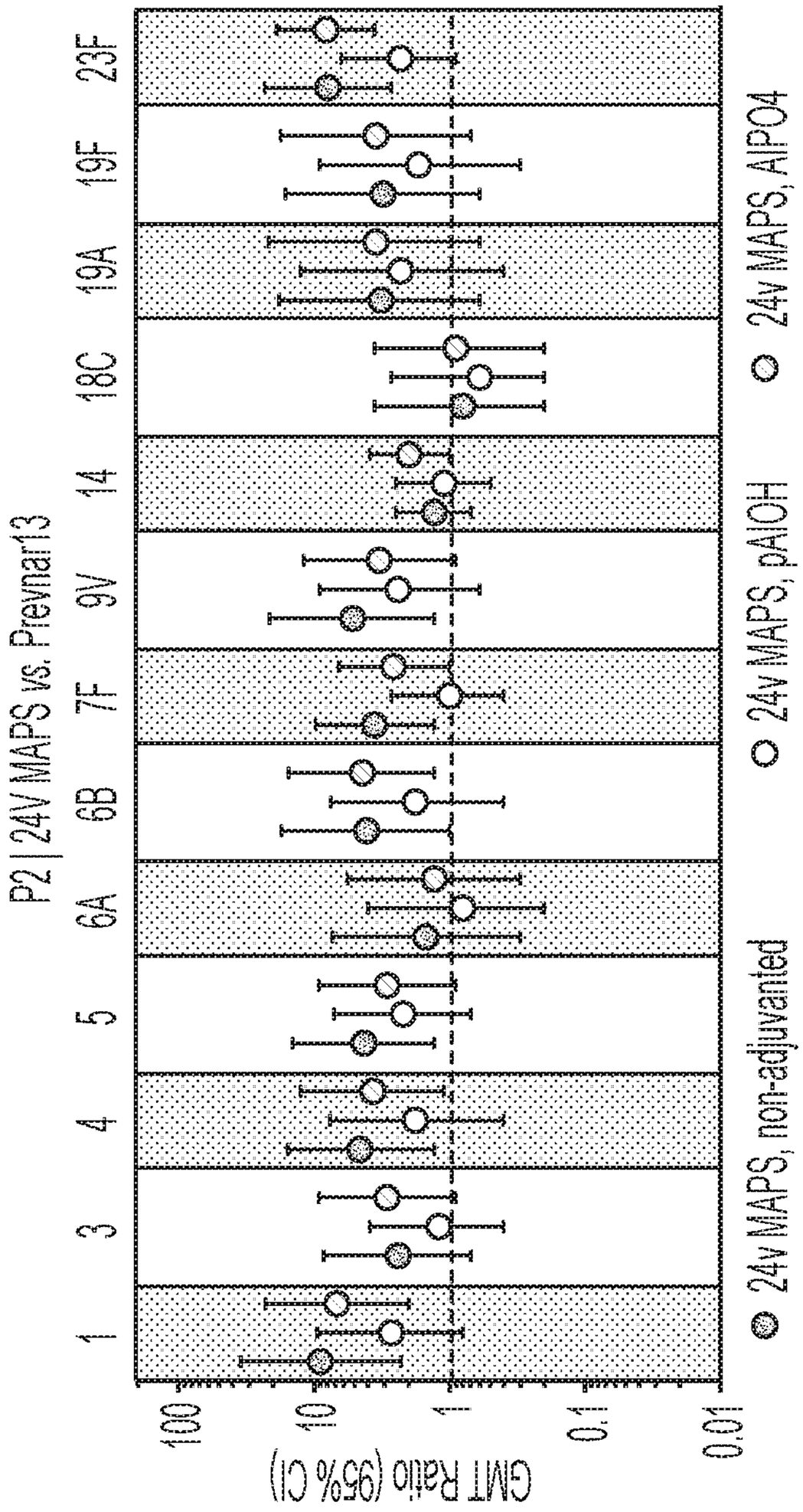

P2|24V MAPS vs. Prevnar13
1
3
4
5
6A
6B
7F
9V
14
18C
19A
19F
23F
GMT Ratio (95% CI)
0.01
0.1
1
10
24v MAPS, non-adjuvanted
24v MAPS, pAIOH
24v MAPS, AIPO4

FIG. 10

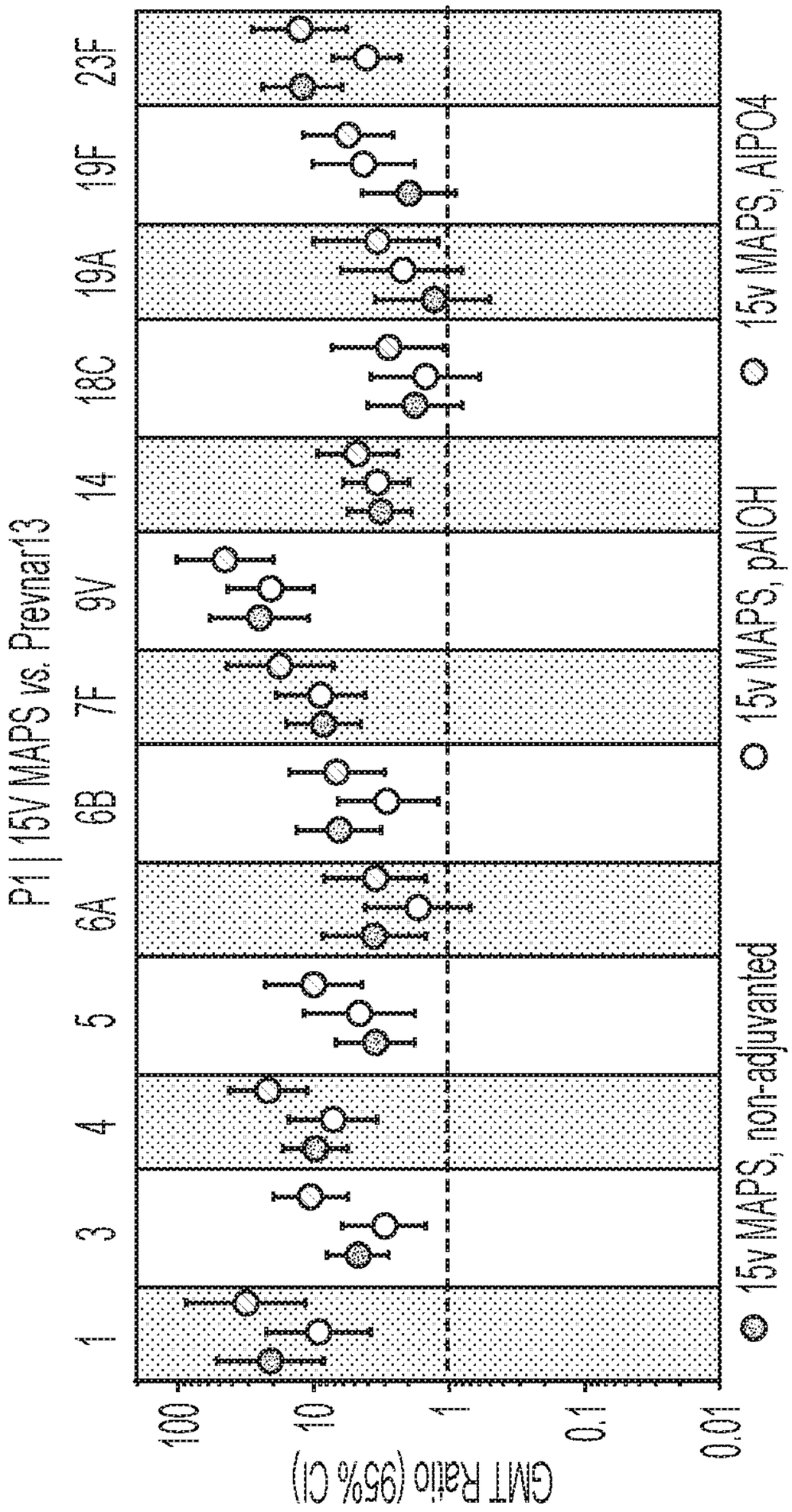

P1 | 15V MAPS vs. Prevnar13
1
3
4
5
6A
6B
7F
9V
14
18C
19A
19F
23F
GMT Ratio (95% CI)
0.01
0.1
1
10
100
15v MAPS, non-adjuvanted
15v MAPS, pAlOH
15v MAPS, AlPO4

FIG. 11

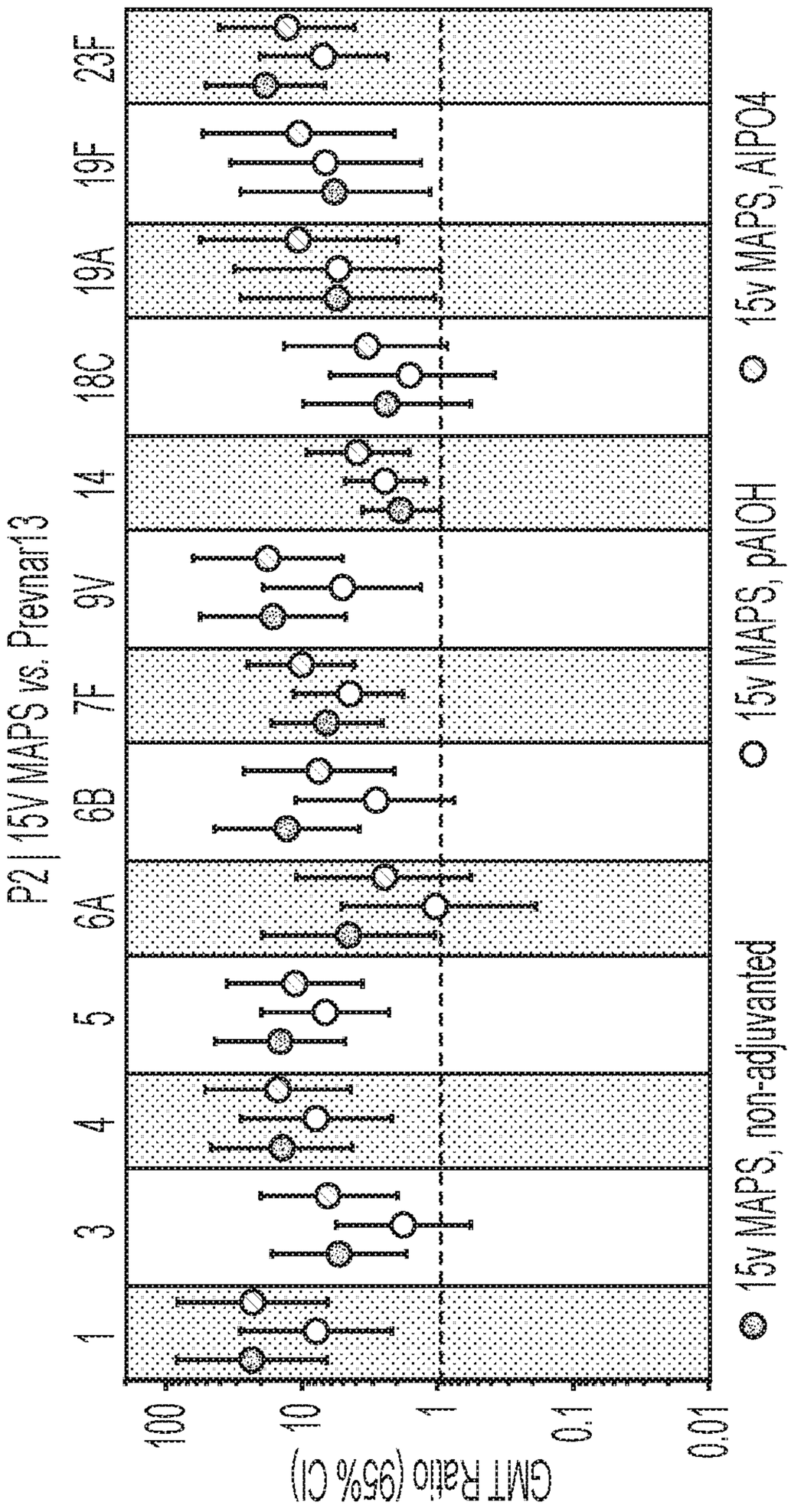

P2 | 15V MAPS vs. Prevnar13
1
3
4
5
6A
6B
7F
9V
14
18C
19A
19F
23F
100
10
1
0.1
0.01
GMT Ratio (95% CI)
15v MAPS, non-adjuvanted
15v MAPS, pAlOH
15v MAPS, AlPO4

FIG. 12

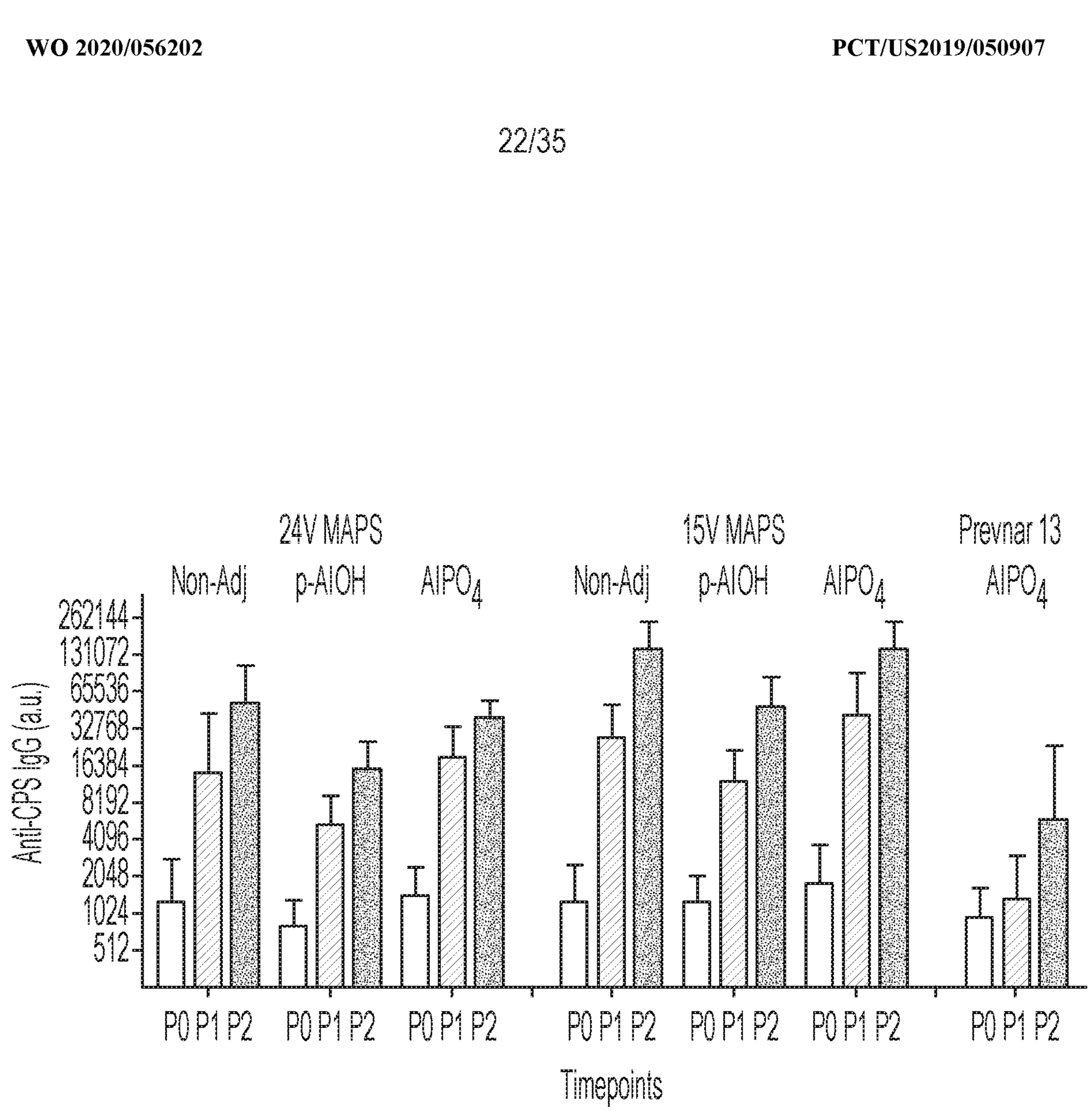

24V MAPS
15V MAPS
Prevnar 13
Non-Adj
p-AlOH
AlPO4
Non-Adj
p-AlOH
AlPO4
AlPO4
Anti-CPS IgG (a.u.)
262144
131072
65536
32768
16384
8192
4096
2048
1024
512
P0 P1 P2 P0 P1 P2 P0 P1 P2 P0 P1 P2 P0 P1 P2 P0 P1 P2 P0 P1 P2
Timepoints

FIG. 13

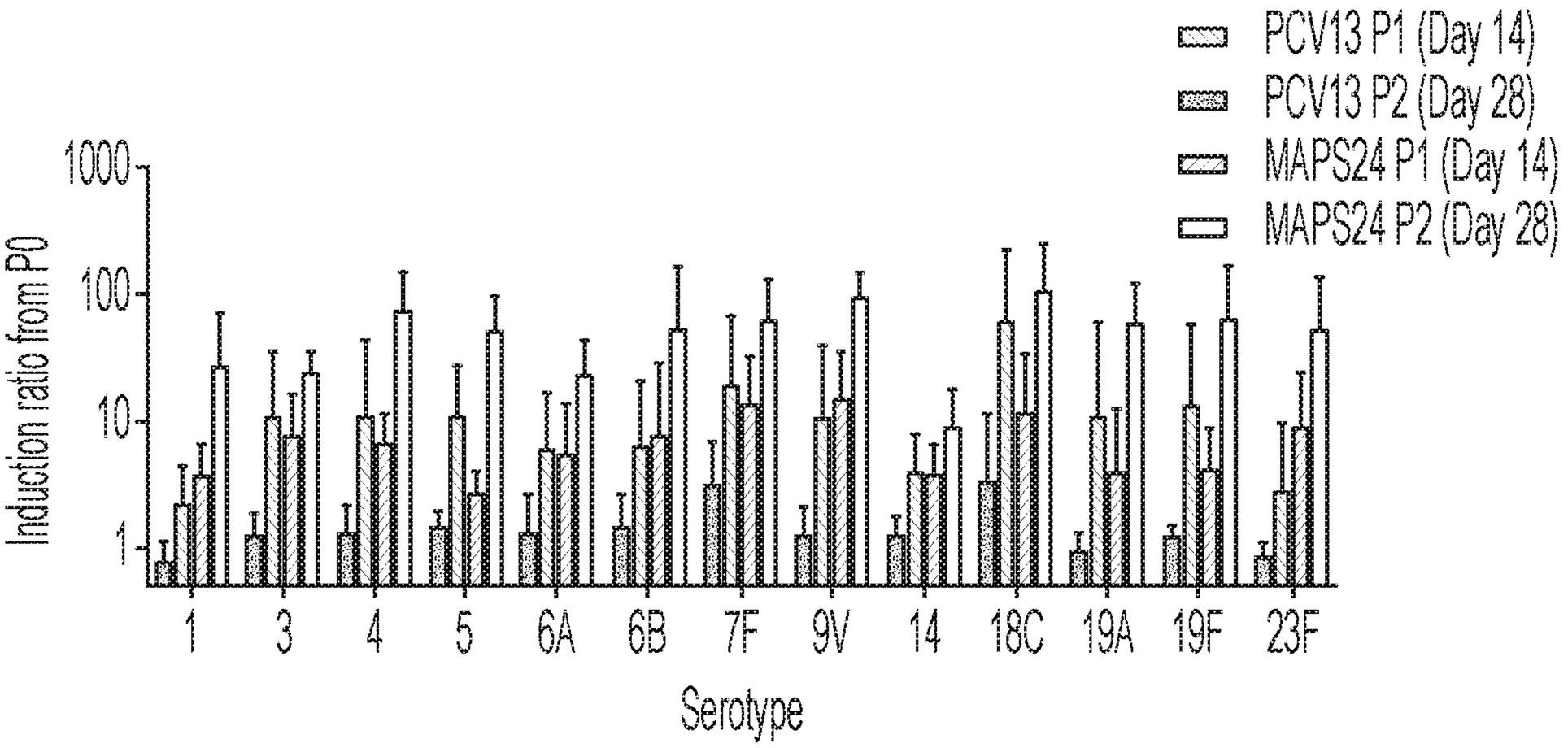

PCV13 P1 (Day 14)
PCV13 P2 (Day 28)
MAPS24 P1 (Day 14)
MAPS24 P2 (Day 28)
Induction ratio from P0
1000
100
10
1
1 3 4 5 6A 6B 7F 9V 14 18C 19A 19F 23F
Serotype

FIG. 14

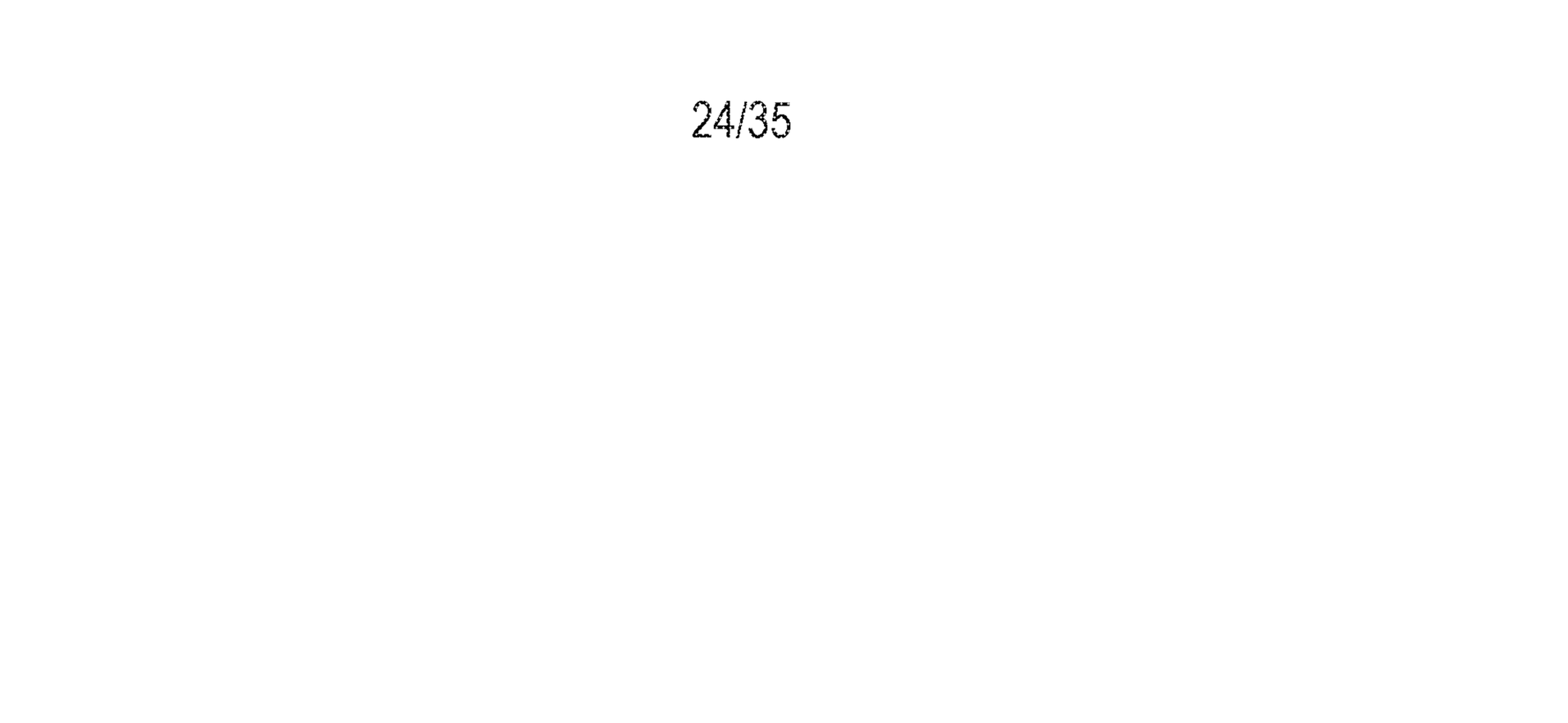

PCV13 P1 (Day 14)
PCV13 P2 (Day 28)
MAPS24 P1 (Day 14)
MAPS24 P2 (Day 28)
Induction ratio from P0
1000
100
10
1
2
8
9N
10A
11A
12F
13B
17F
20B
22F
33F
Serotype

FIG. 15

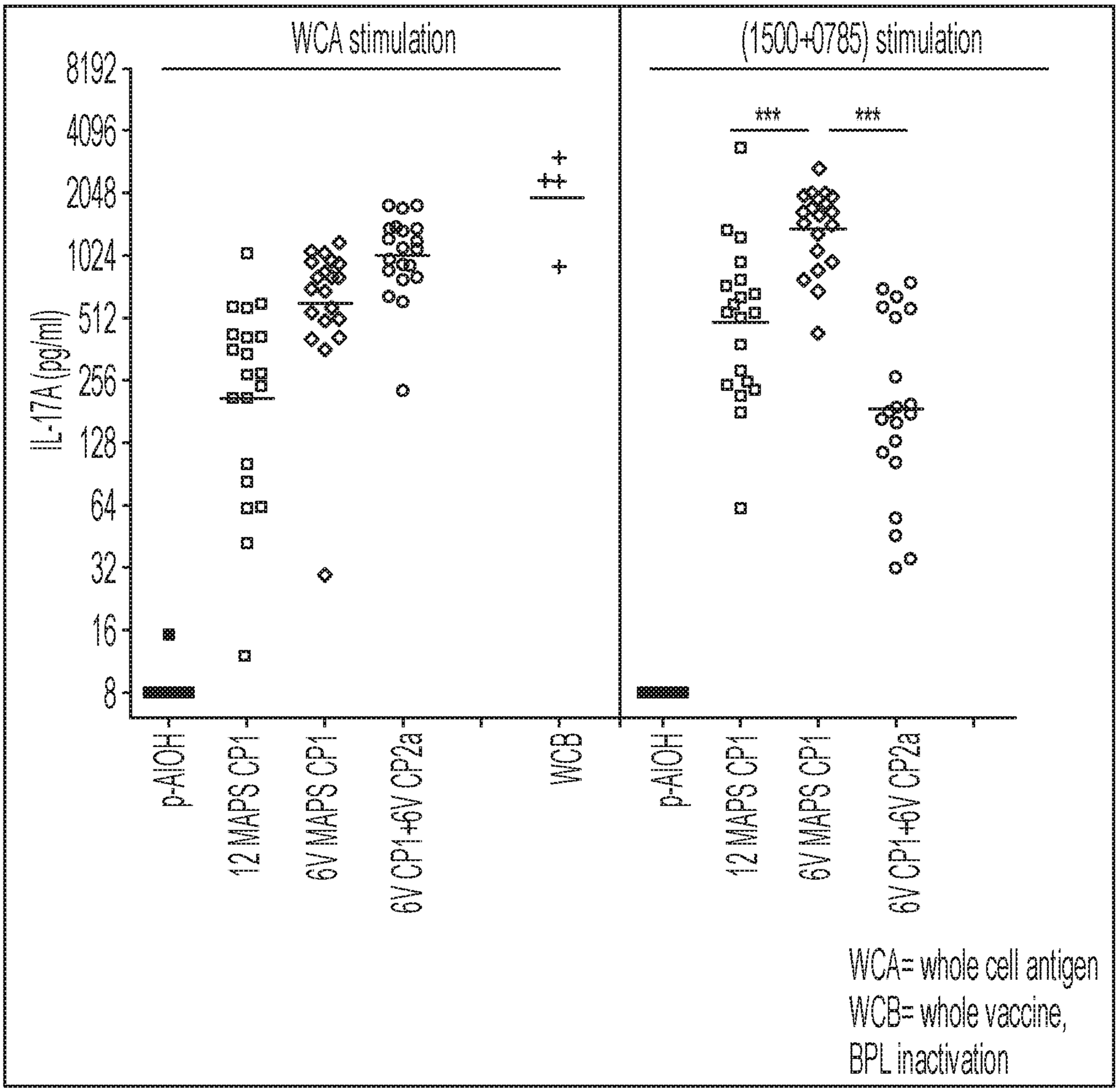

WCA stimulation
(1500+0785) stimulation
IL-17A (pg/ml)
8192
4096
2048
1024
512
256
128
64
32
16
8
***
***
p-AIOH
12 MAPS CP1
6V MAPS CP1
6V CP1+6V CP2a
WCB
p-AIOH
12 MAPS CP1
6V MAPS CP1
6V CP1+6V CP2a
WCA= whole cell antigen
WCB= whole vaccine,
BPL inactivation

FIG. 16

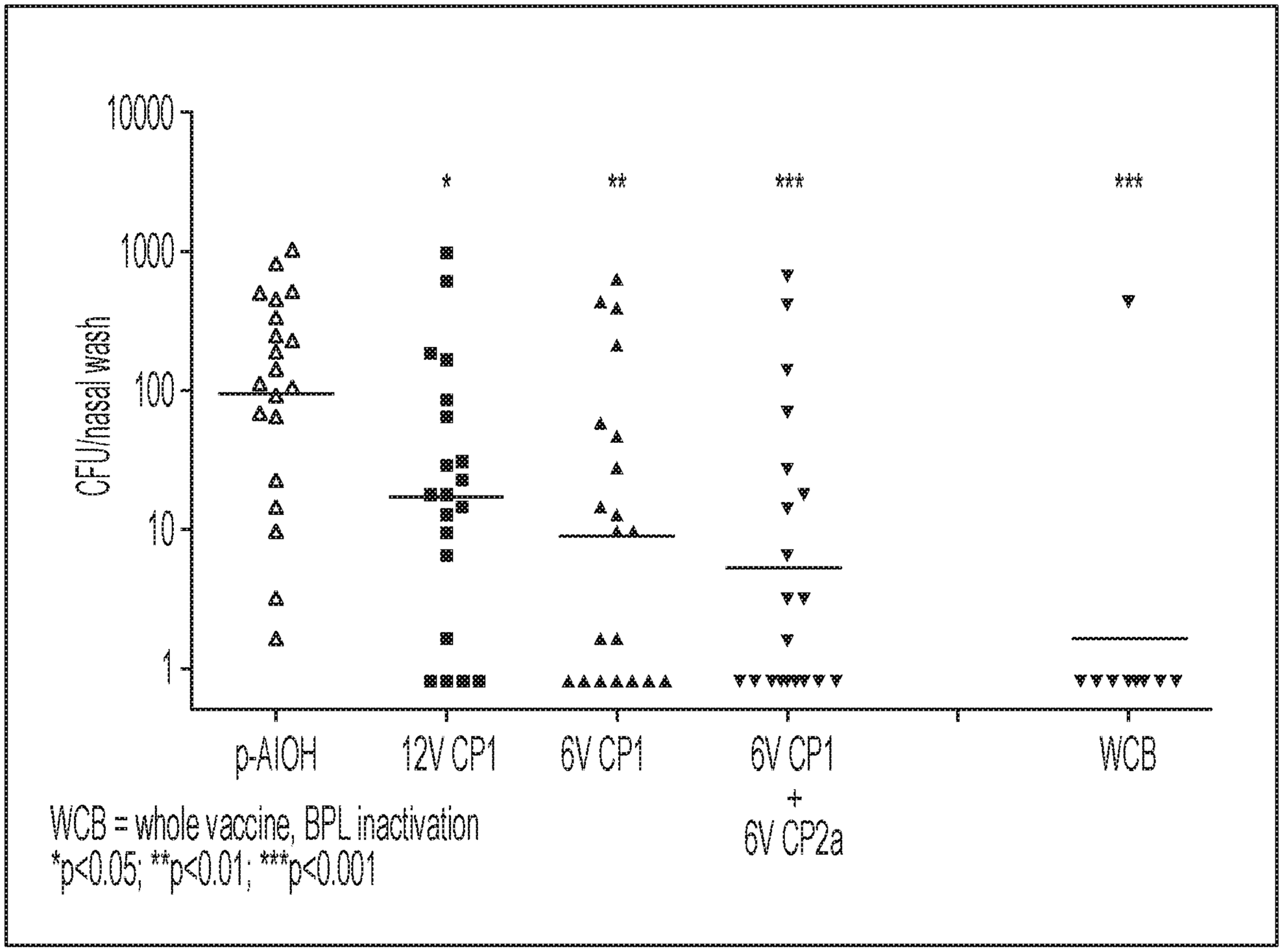

10000
1000
100
10
1
CFU/nasal wash
*
**
***
***
p-AlOH
12V CP1
6V CP1
6V CP1
+
6V CP2a
WCB
WCB = whole vaccine, BPL inactivation
*p<0.05; **p<0.01; ***p<0.001

FIG. 17

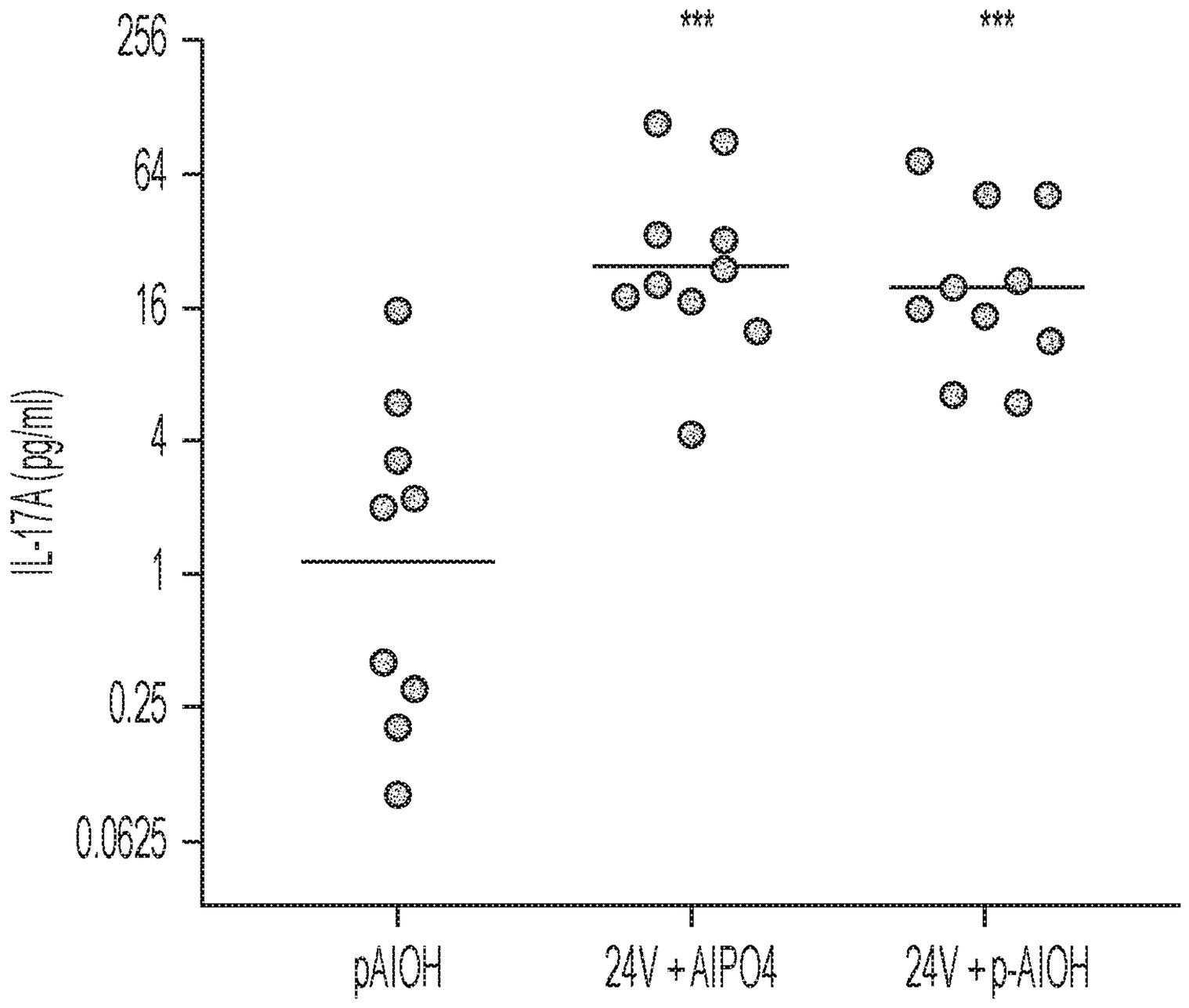


FIG. 18

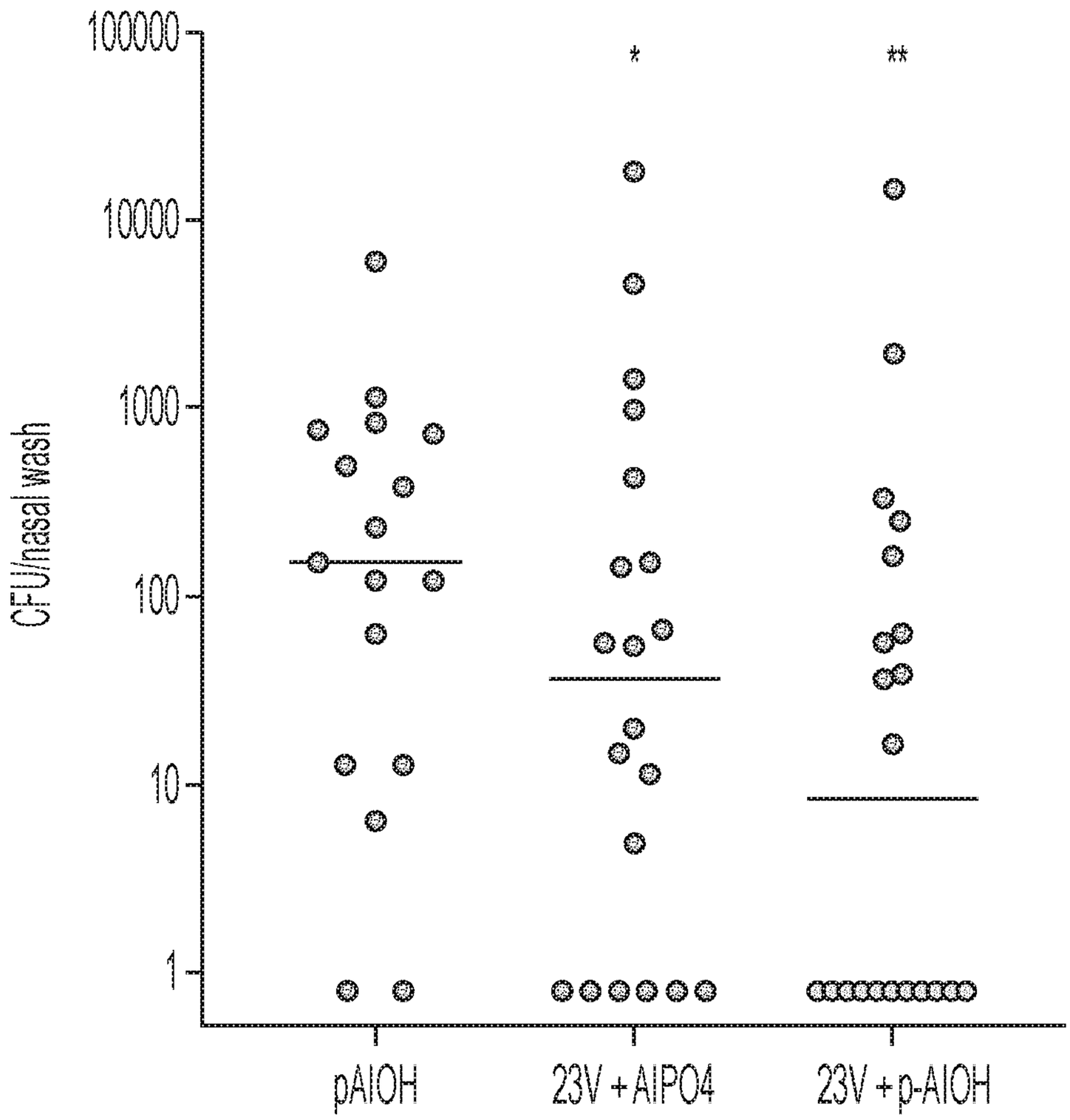

100000
10000
1000
100
10
1
CFU/nasal wash
*
**
pAlOH
23V + AlPO4
23V + p-AlOH

FIG. 19

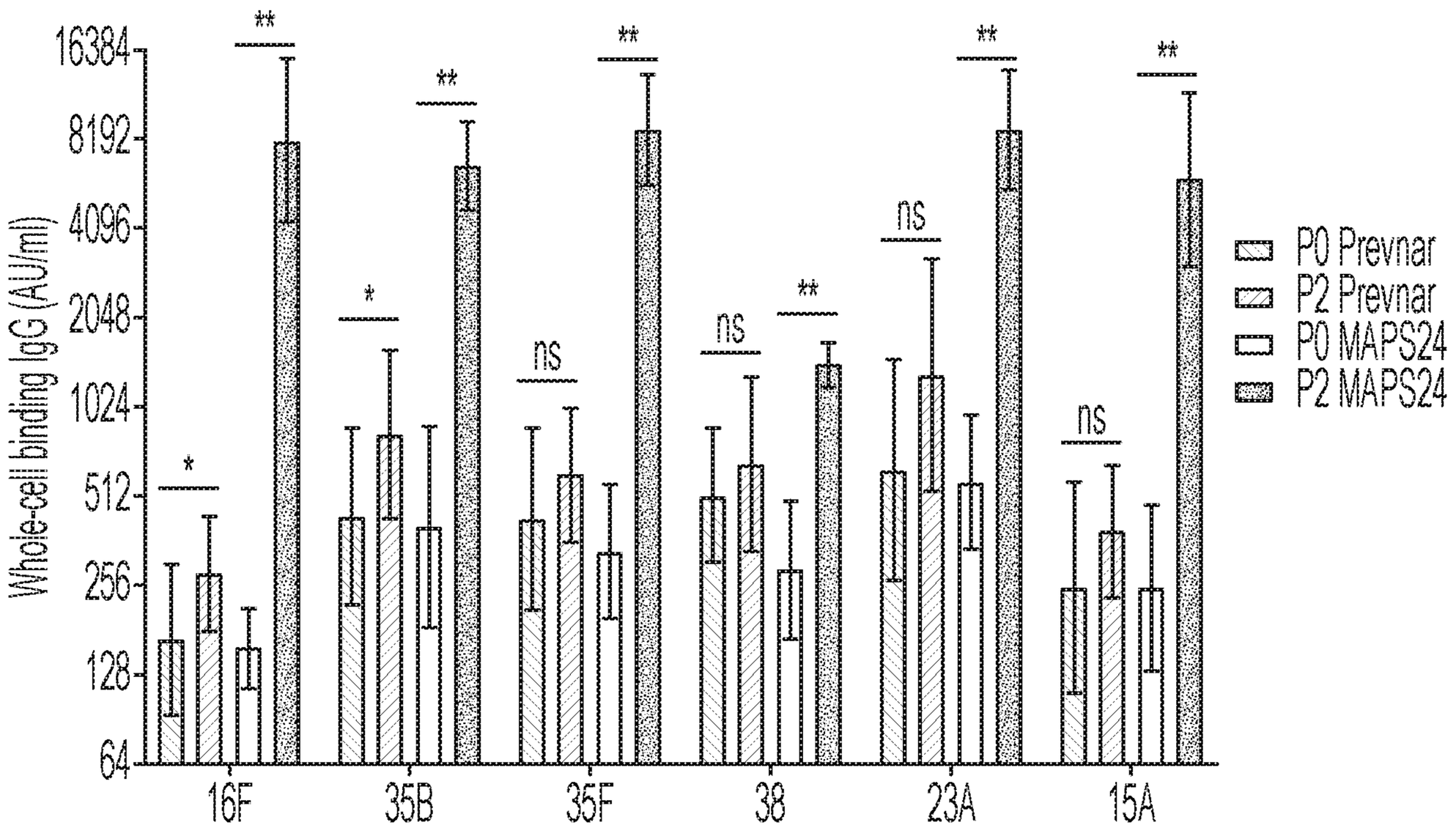

Whole-cell binding IgG (AU/ml)
16384
8192
4096
2048
1024
512
256
128
64
*
**
*
**
ns
**
ns
**
ns
**
ns
**
16F
35B
35F
38
23A
15A
P0 Prevnar
P2 Prevnar
P0 MAPS24
P2 MAPS24

FIG. 20

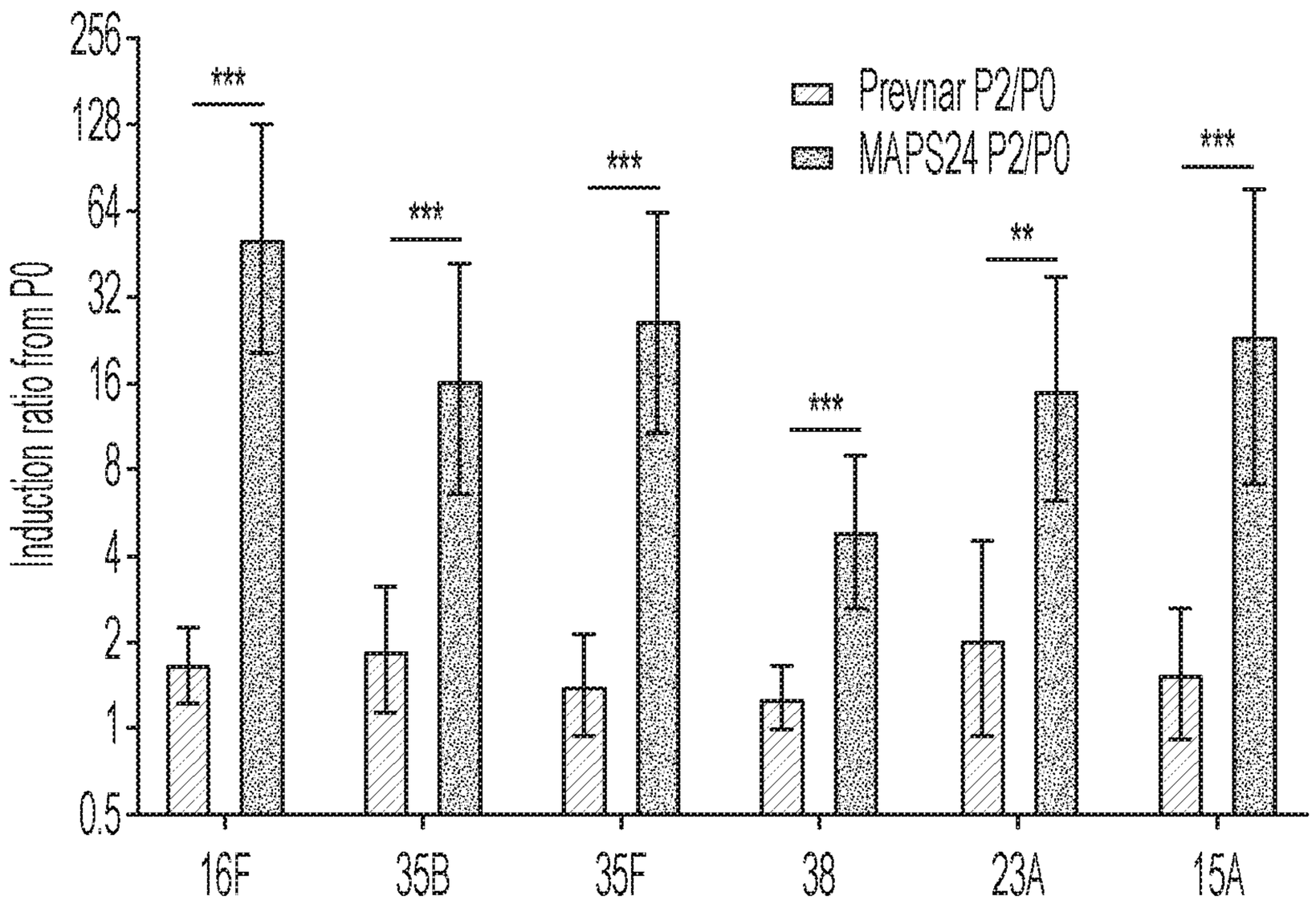

Induction ratio from P0
256
128
64
32
16
8
4
2
1
0.5
Prevnar P2/P0
MAPS24 P2/P0
***
***
***
***
**
***
16F
35B
35F
38
23A
15A

FIG. 21

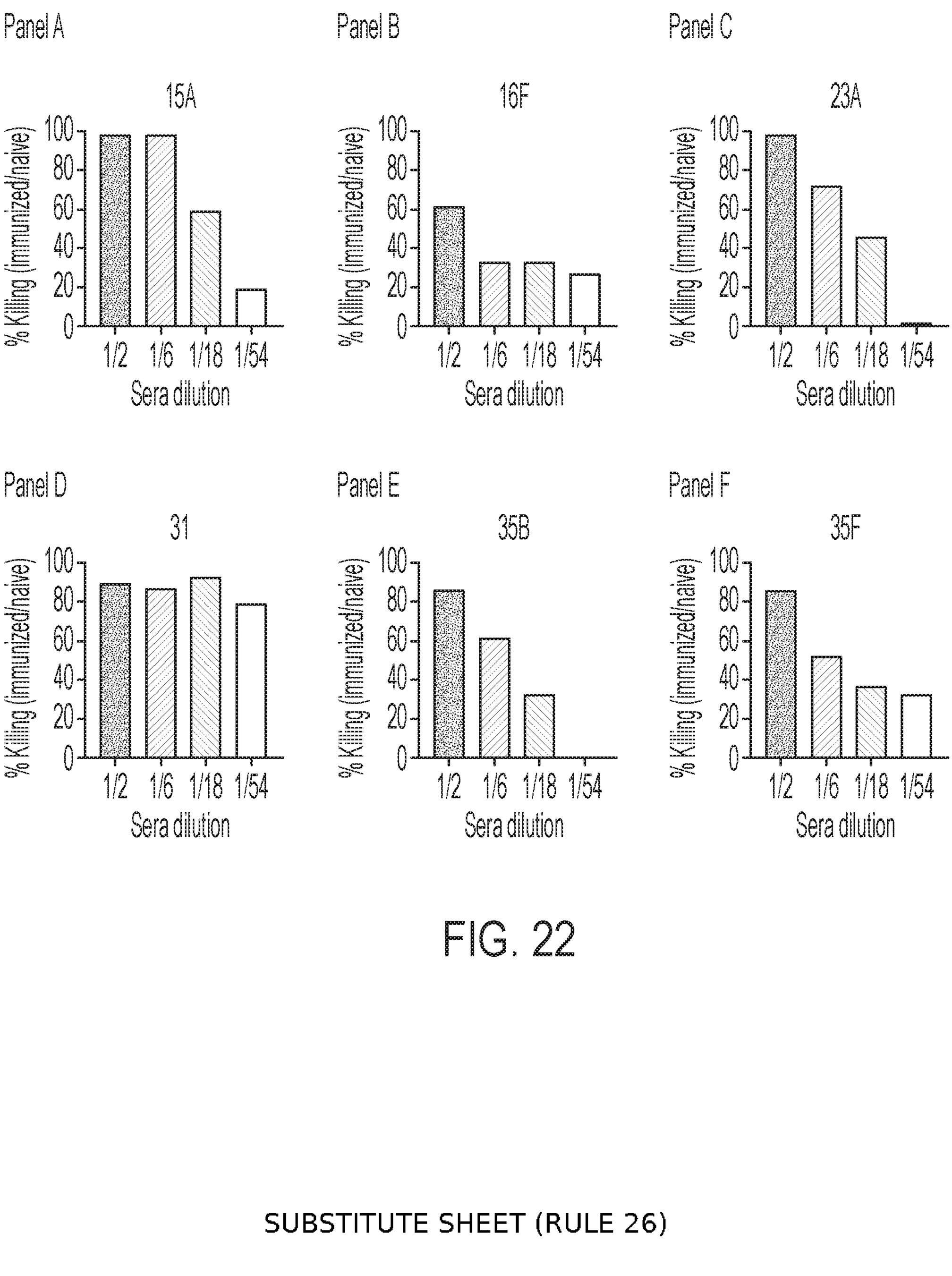
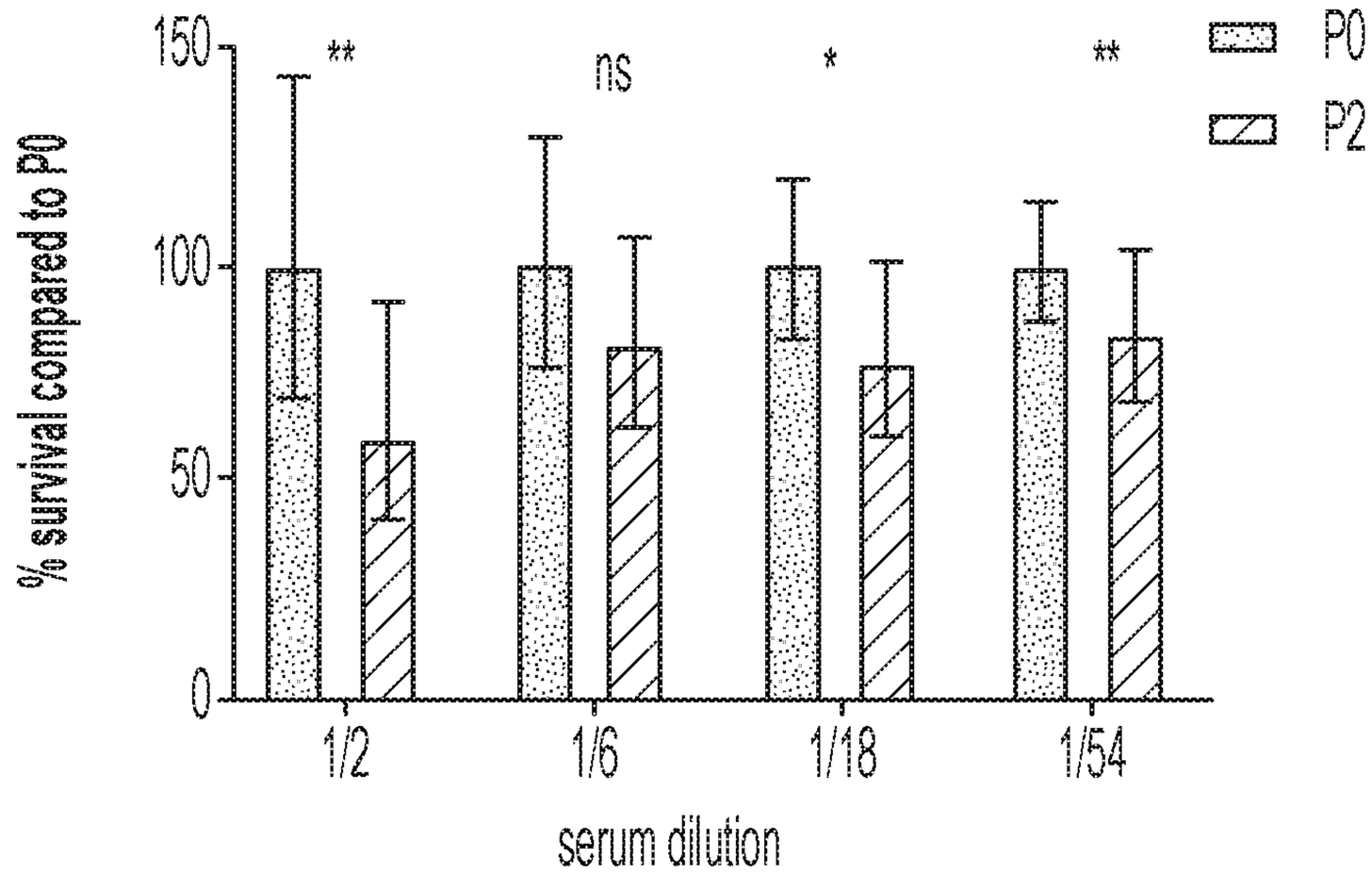

Panel A
15A
% Killing (immunized/naive)
100
80
60
40
20
0
1/2
1/6
1/18
1/54
Sera dilution
Panel B
16F
% Killing (immunized/naive)
100
80
60
40
20
0
1/2
1/6
1/18
1/54
Sera dilution
Panel C
23A
% Killing (immunized/naive)
100
80
60
40
20
0
1/2
1/6
1/18
1/54
Sera dilution
Panel D
31
% Killing (immunized/naive)
100
80
60
40
20
0
1/2
1/6
1/18
1/54
Sera dilution
Panel E
35B
% Killing (immunized/naive)
100
80
60
40
20
0
1/2
1/6
1/18
1/54
Sera dilution
Panel F
35F
% Killing (immunized/naive)
100
80
60
40
20
0
1/2
1/6
1/18
1/54
Sera dilution

FIG. 22


Panel A
16F - MAPS24
150
100
50
0
% survival compared to P0
**
ns
*
**
P0
P2
1/2
1/6
1/18
1/54
serum dilution

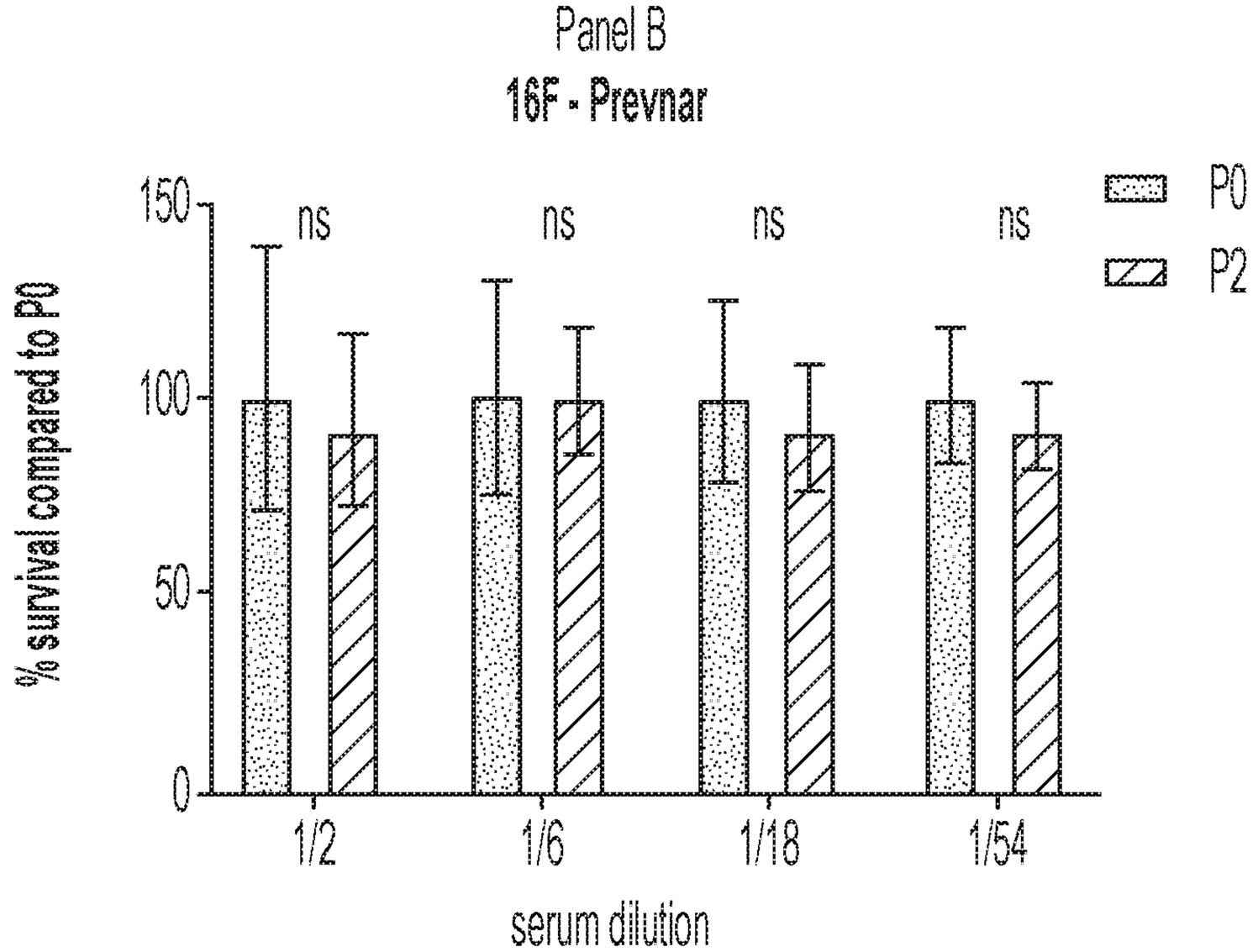
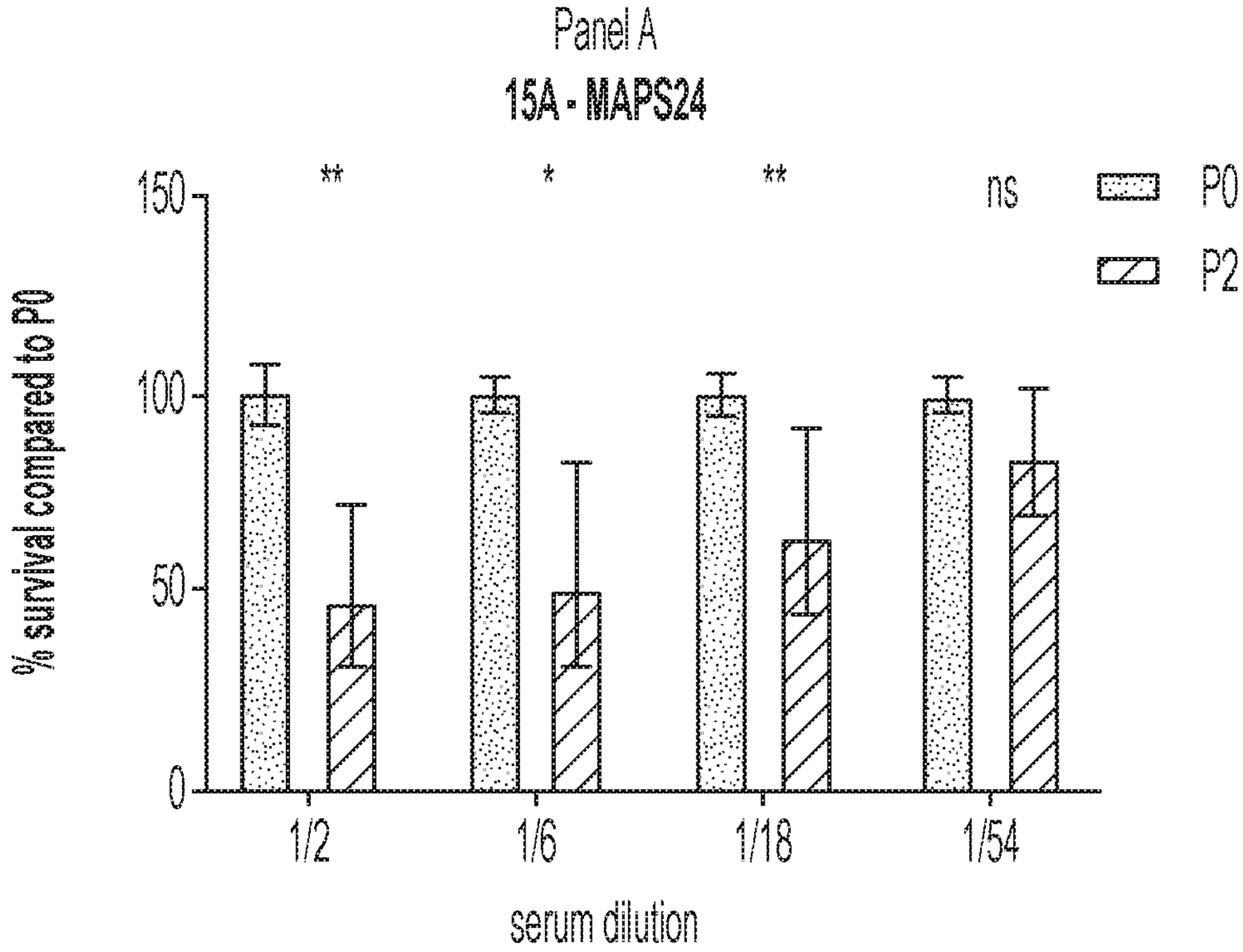

Panel B
16F - Prevnar
150
100
50
0
% survival compared to P0
ns
ns
ns
ns
P0
P2
1/2
1/6
1/18
1/54
serum dilution

FIG. 23


Panel A
15A - MAPS24
**
*
**
ns
P0
P2
150
100
50
0
% survival compared to P0
1/2
1/6
1/18
1/54
serum dilution

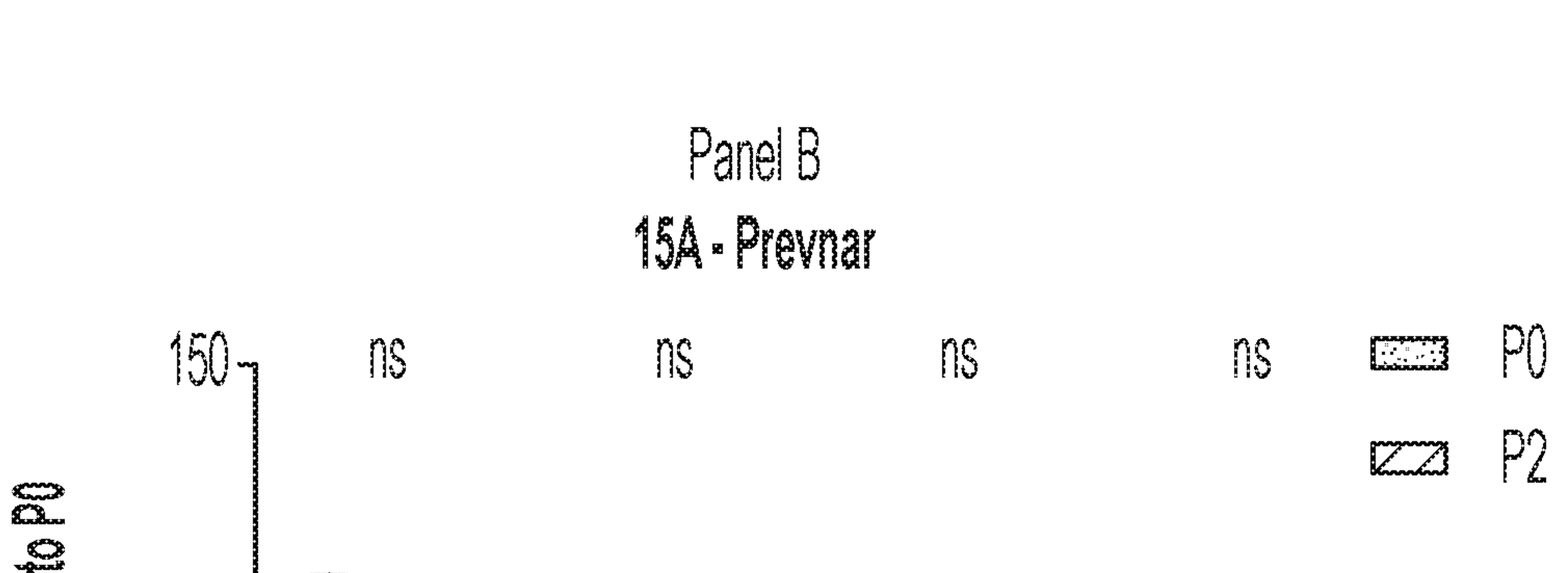

Panel B
15A - Prevnar
ns
ns
ns
ns
P0
P2
150
100
50
0
% survival compared to P0
1/2
1/6
1/18
1/54
serum dilution

FIG. 24

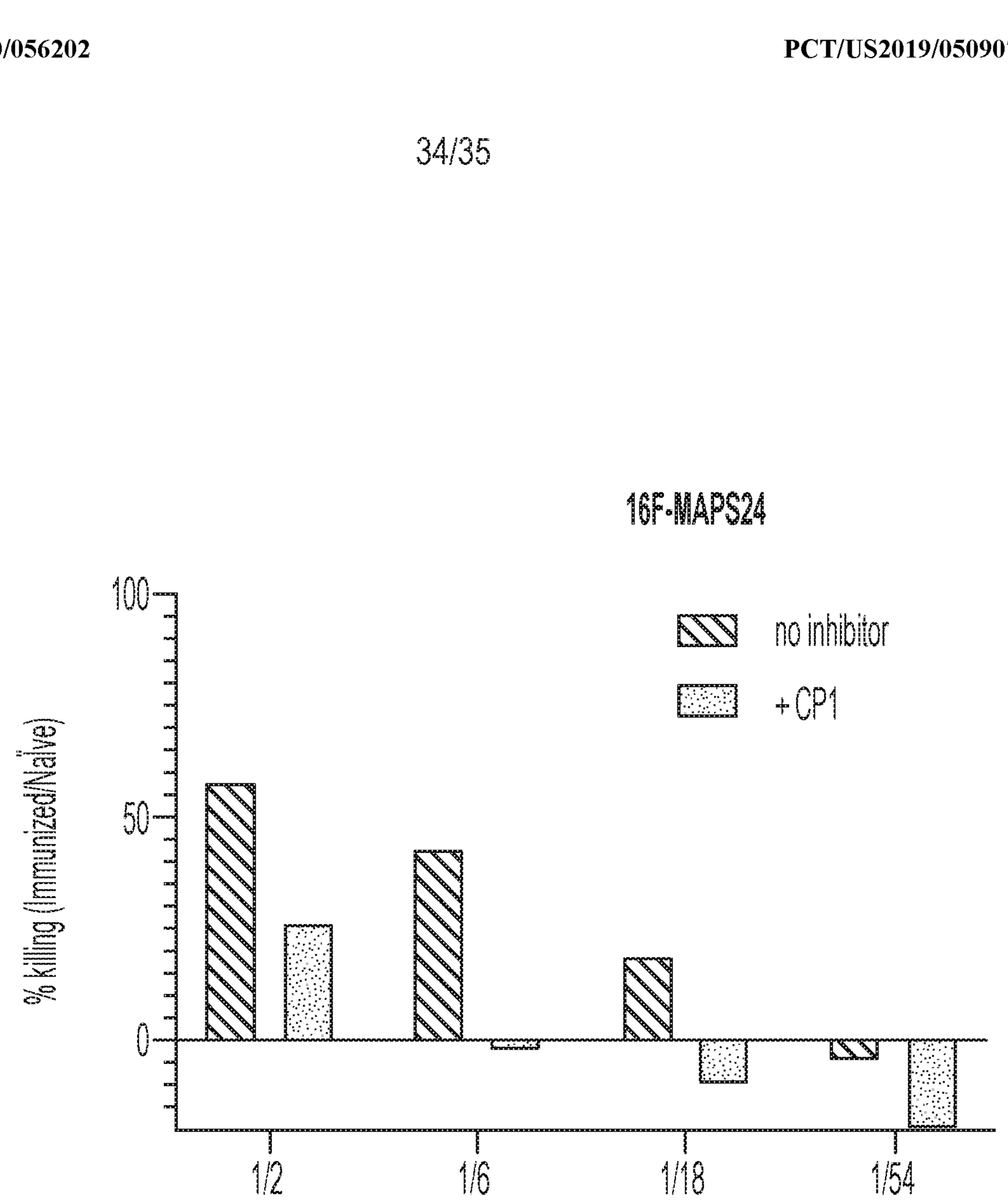

16F-MAPS24
100
50
0
% killing (Immunized/Naïve)
no inhibitor
+ CP1
1/2
1/6
1/18
1/54
Sera dilution

FIG. 25

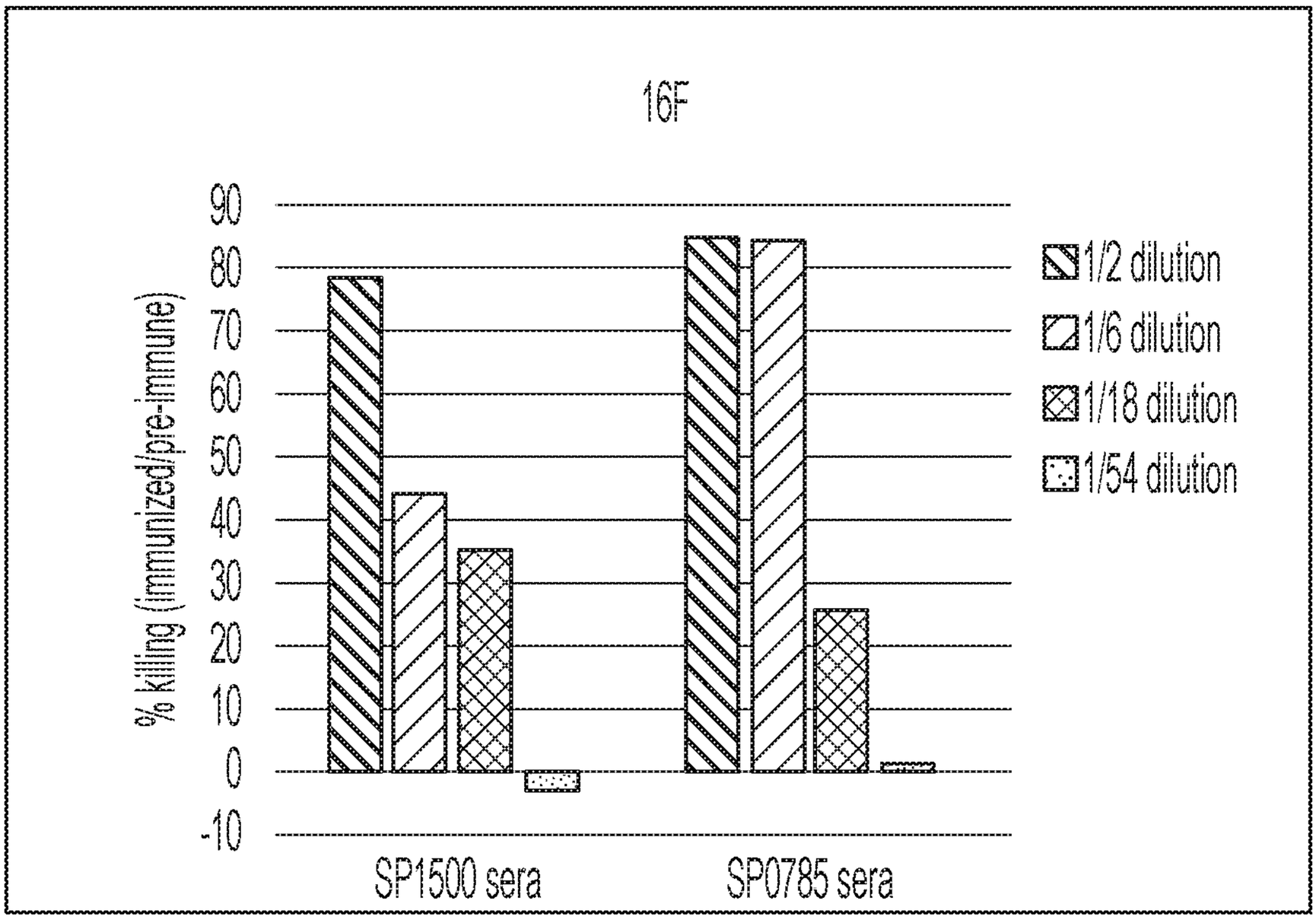

16F
% killing (immunized/pre-immune)
90
80
70
60
50
40
30
20
10
0
-10
SP1500 sera
SP0785 sera
1/2 dilution
1/6 dilution
1/18 dilution
1/54 dilution

FIG. 26