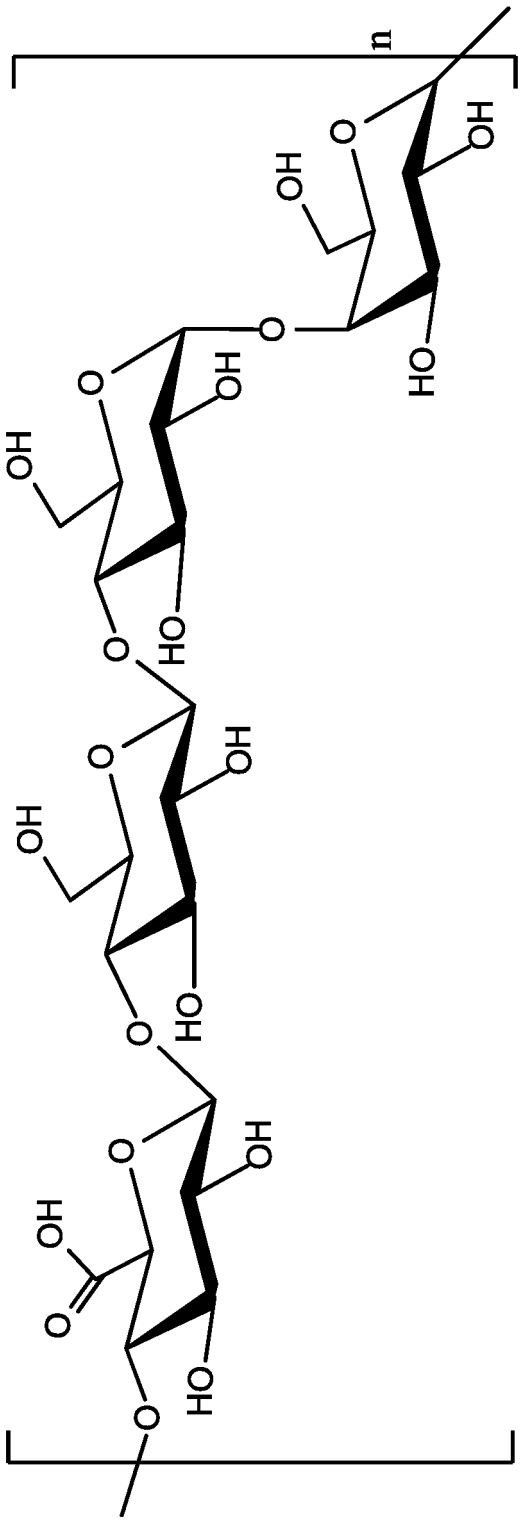
FIGURE 1

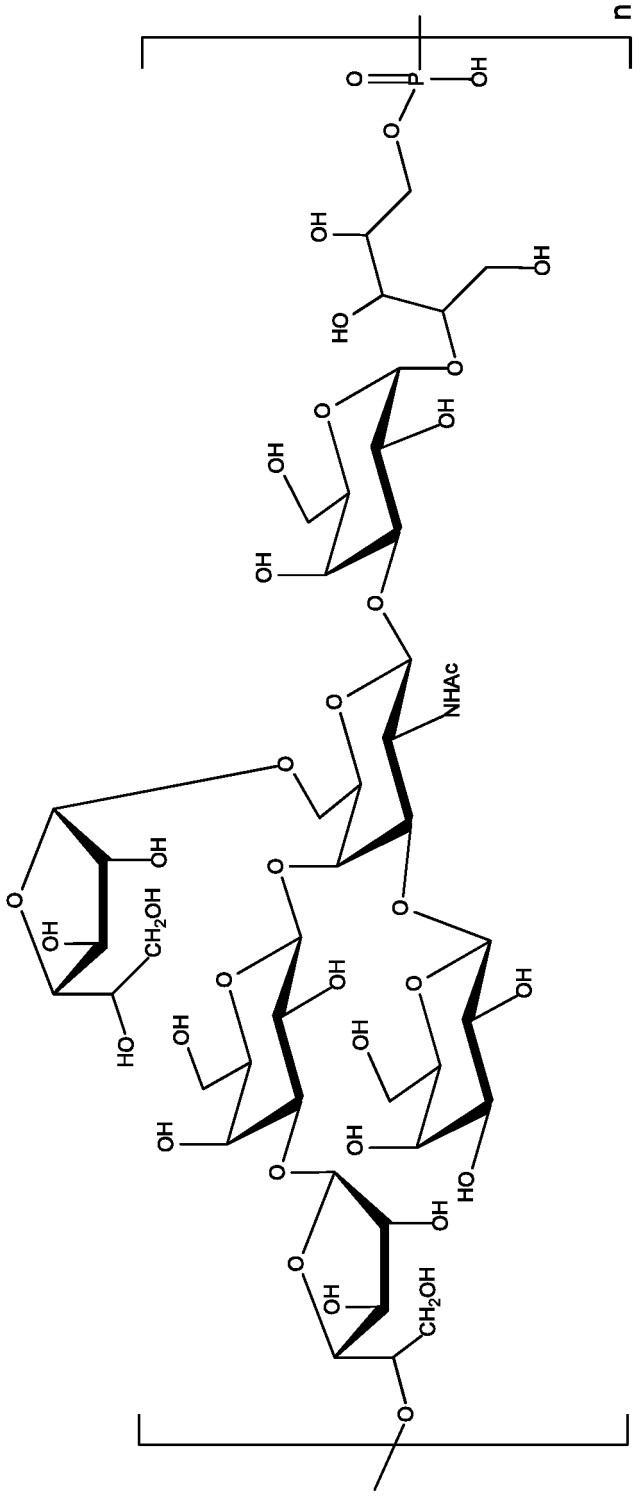


→4)-β-D-Glc*p*A-(1→4)-β-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→4)-α-D-Gal*p*-(1→

MW (H^+) = 662.553 $C_{24}H_{38}O_{21}$

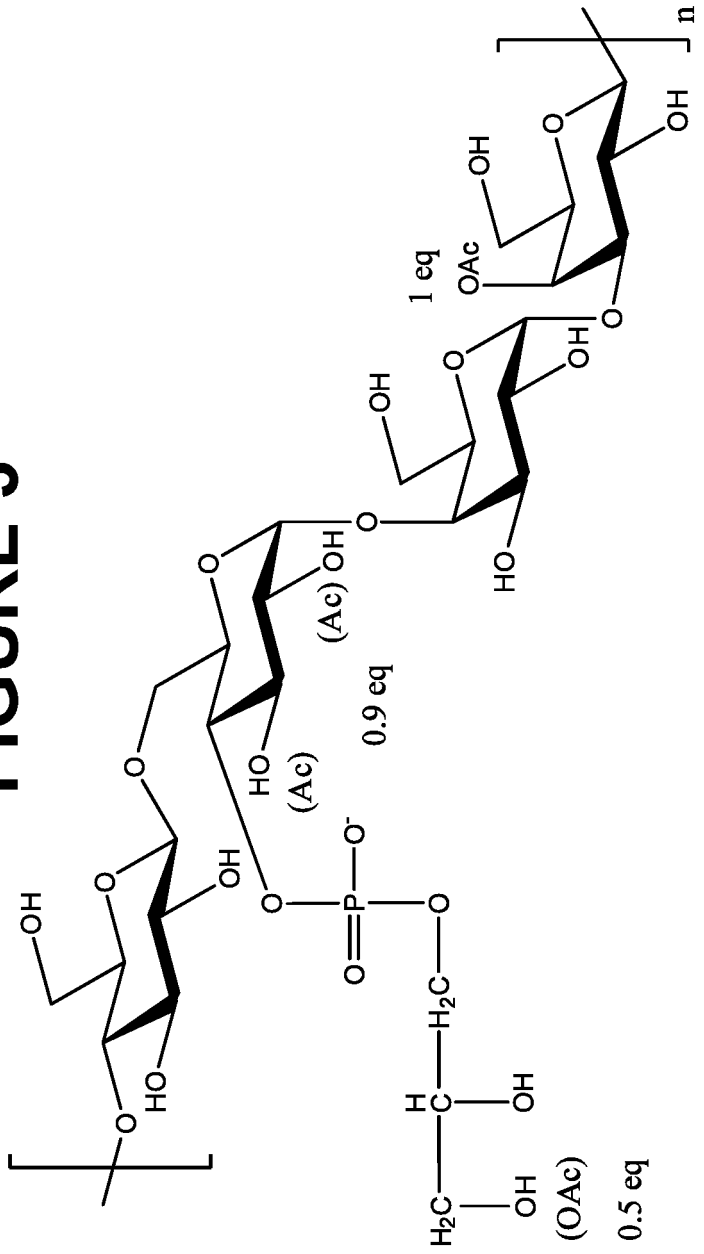
MW (Na^+) = 684.534 $C_{24}H_{37}O_{21}Na_1$

FIGURE 2



β-D-Galf
1
6
5)-β-D-Galf-(1→3)-β-D-Galp-(1→4)-β-D-GalpNAc-(1→3)-α-D-Galp-(1→2)-D-Ribitol-(5-PO4)-]n
3
1
β-D-Galp

FIGURE 3



Mw (Na) = 950.7

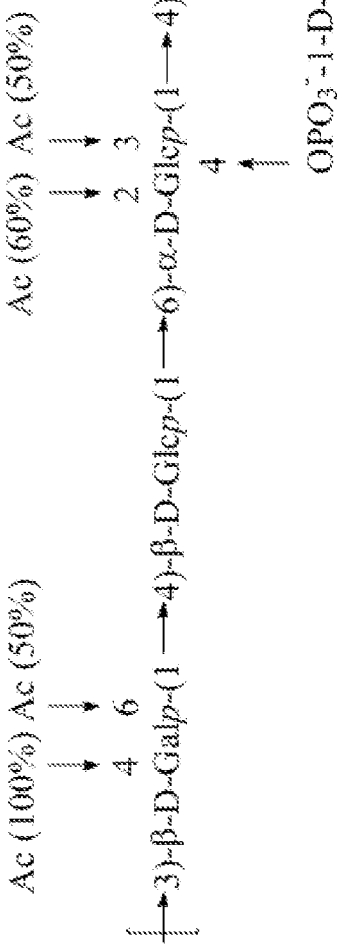


FIGURE 4

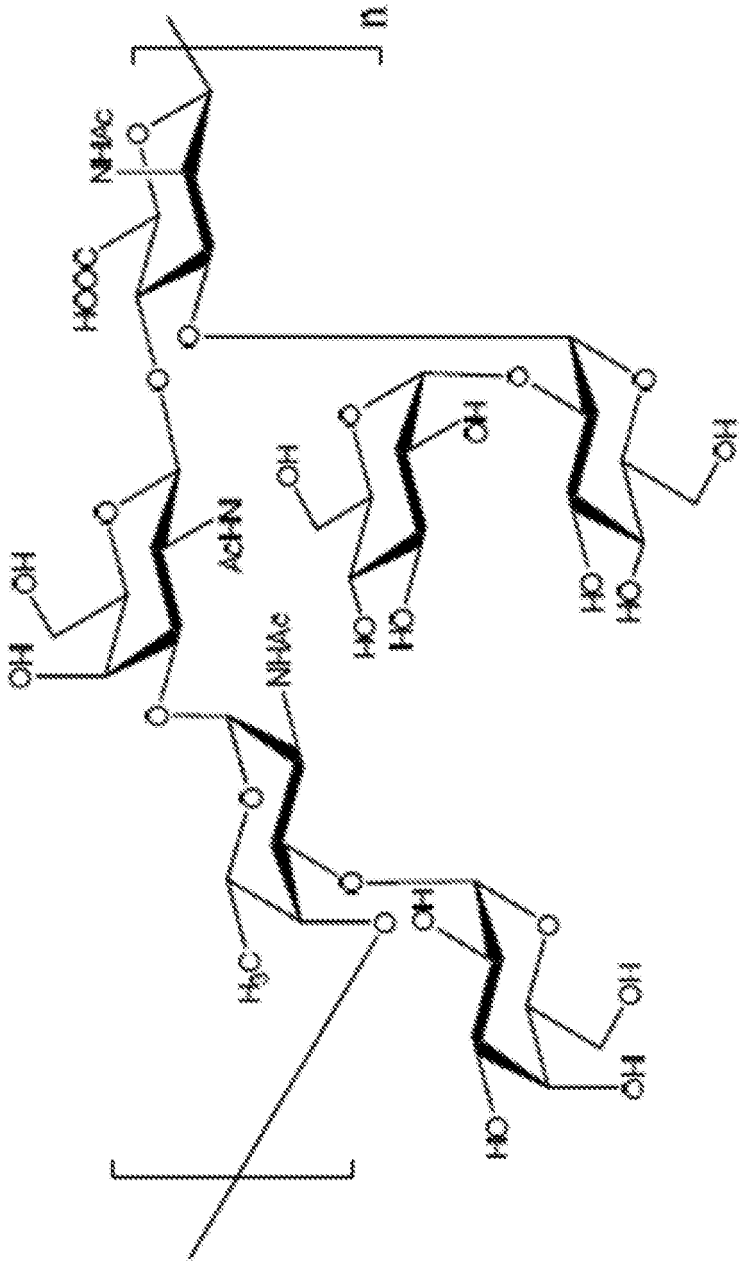
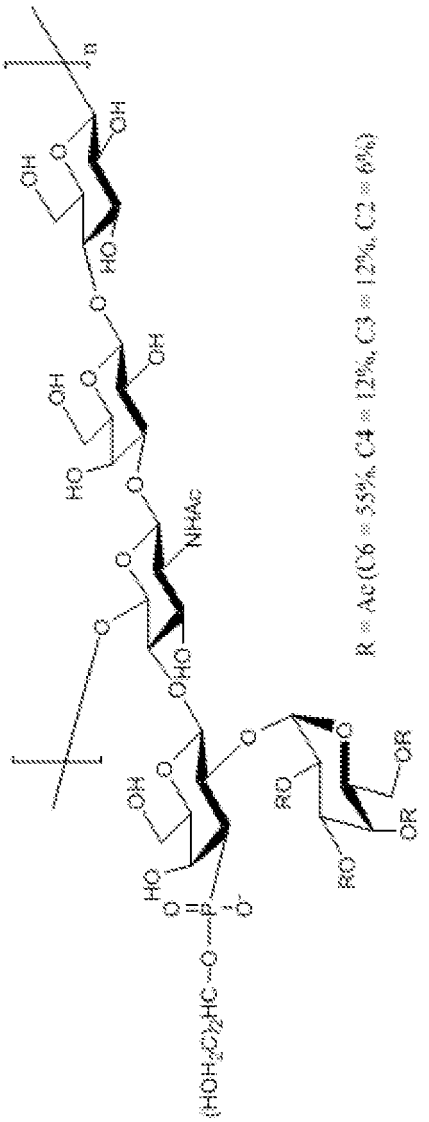

n
HOOC
NHAc
OH
AcHN
NHAc
HO
OH
OH
HO
OH
HO
OH
OH
HO
OH
OH
HO
OH
OH
n
→4)-α-L-FucpNAc-(1→3)-β-D-GalpNAc-(1→4)-β-D-ManpNAcA-(1→
3
1
α-D-Galp
α-D-Glcp-(1→2)-α-D-Glcp

FIGURE 5



R = Ac (C6 = 55%, C4 = 12%, C3 = 12%, C2 = 6%)

Ac= Acetyl

→6)-D-Glcp-NAc-β-(1→3)-D-Galp-β-(1→4)-D-Glcp-β-(1→

D-Galp-α-(1→2)-D-Galp(3-OPO$_3$R^1)-β-(1→4 R^1 = CH(CH$_2$OH)$_2$

$C_{33}H_{61}NNaO_{31}P$; MW=1069.8

FIGURE 6

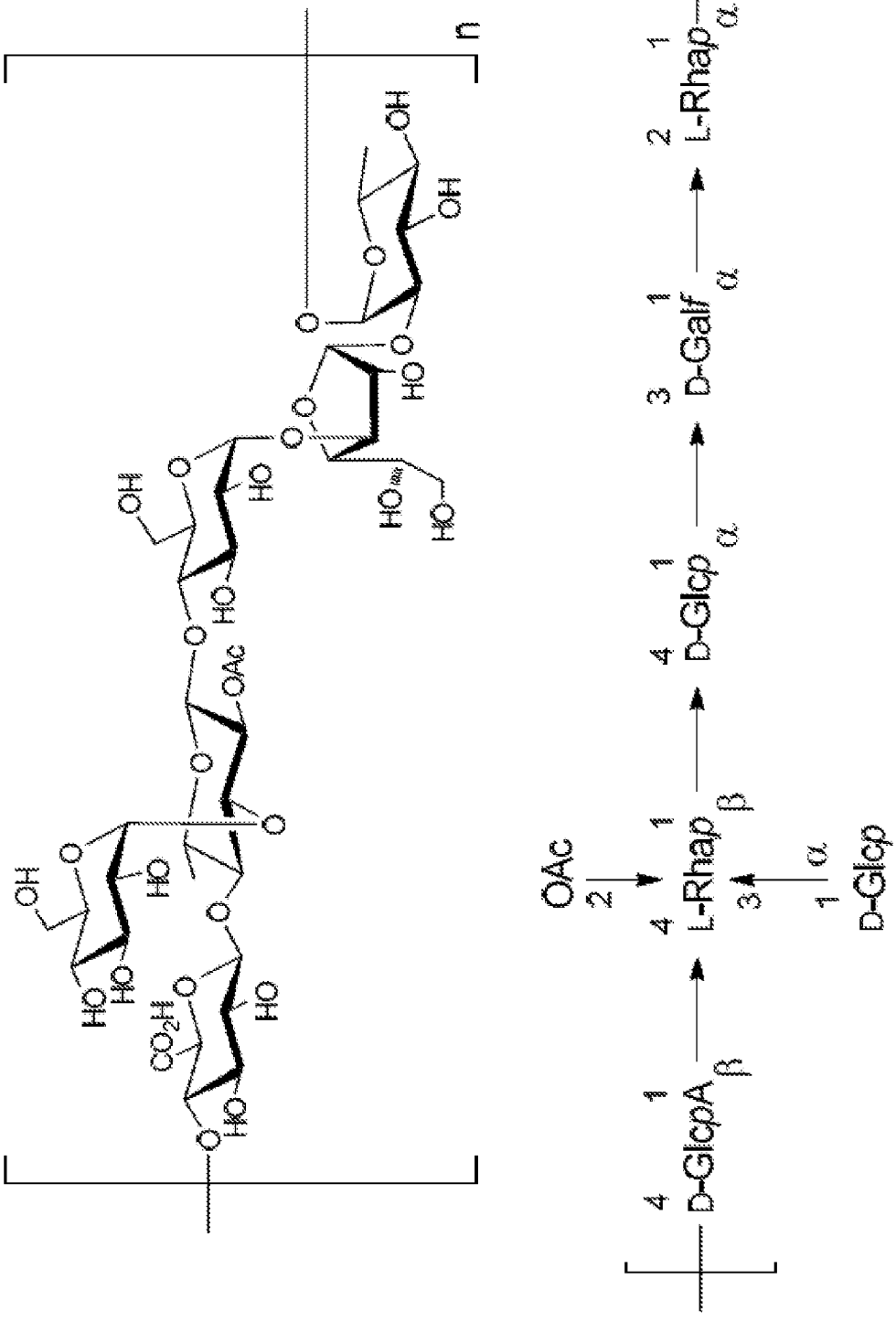


FIGURE 7

Pn 33F

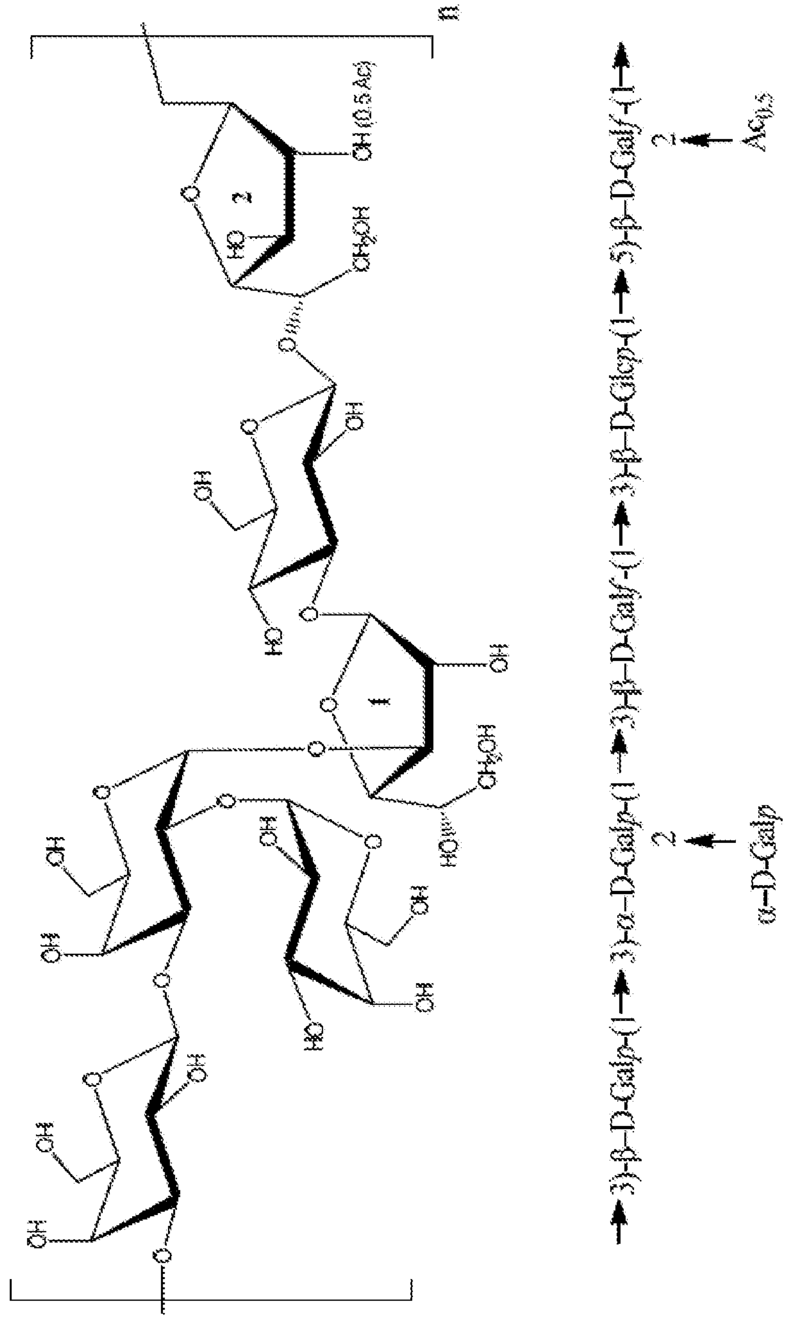


FIGURE 8

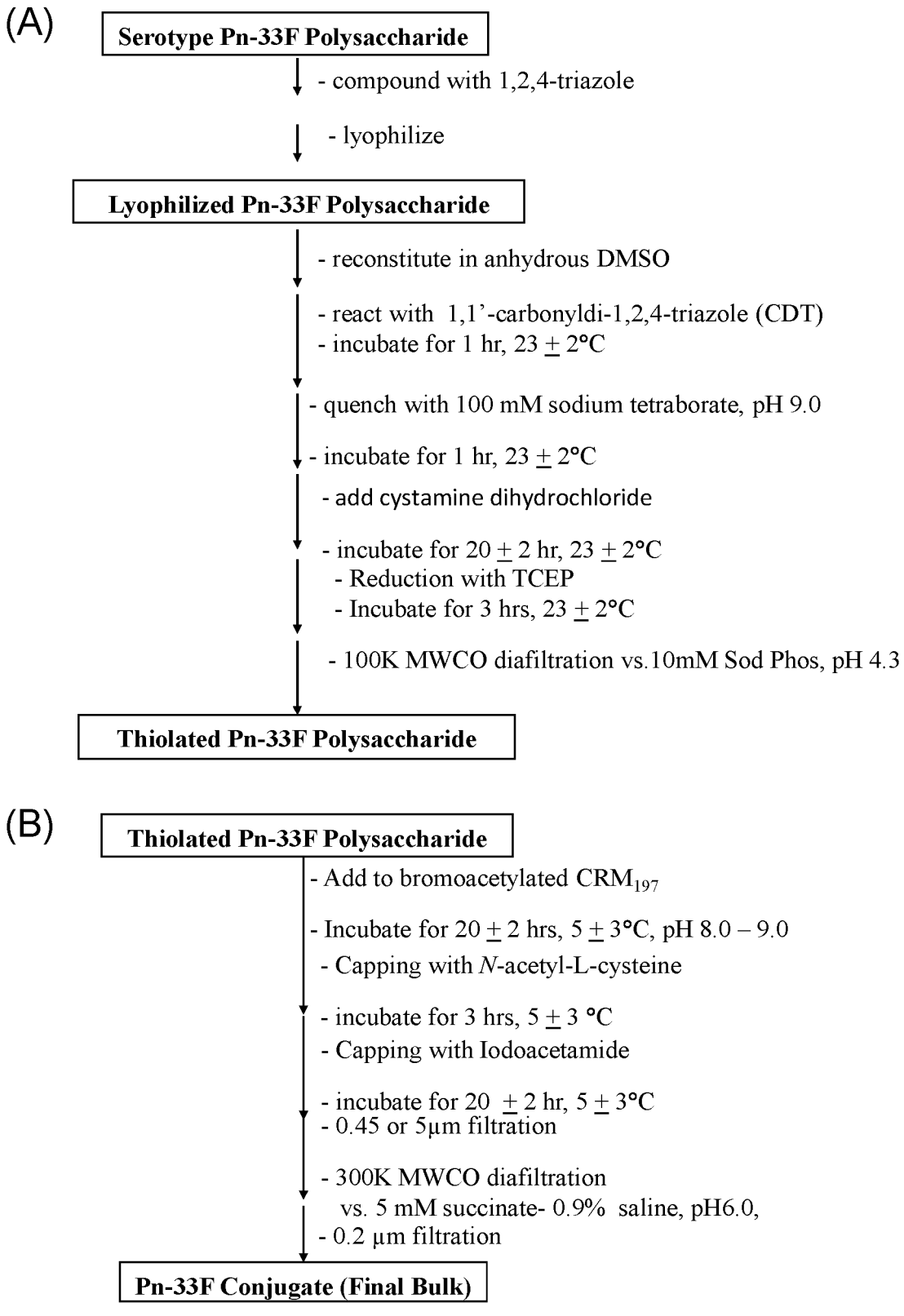

(A)
Serotype Pn-33F Polysaccharide
- compound with 1,2,4-triazole
- lyophilize
Lyophilized Pn-33F Polysaccharide
- reconstitute in anhydrous DMSO
- react with 1,1'-carbonyldi-1,2,4-triazole (CDT)
- incubate for 1 hr, 23 ± 2°C
- quench with 100 mM sodium tetraborate, pH 9.0
- incubate for 1 hr, 23 ± 2°C
- add cystamine dihydrochloride
- incubate for 20 ± 2 hr, 23 ± 2°C
- Reduction with TCEP
- Incubate for 3 hrs, 23 ± 2°C
- 100K MWCO diafiltration vs.10mM Sod Phos, pH 4.3
Thiolated Pn-33F Polysaccharide
(B)
Thiolated Pn-33F Polysaccharide
- Add to bromoacetylated CRM197
- Incubate for 20 ± 2 hrs, 5 ± 3°C, pH 8.0 – 9.0
- Capping with N-acetyl-L-cysteine
- incubate for 3 hrs, 5 ± 3 °C
- Capping with Iodoacetamide
- incubate for 20 ± 2 hr, 5 ± 3°C
- 0.45 or 5µm filtration
- 300K MWCO diafiltration
vs. 5 mM succinate- 0.9% saline, pH6.0,
- 0.2 µm filtration
Pn-33F Conjugate (Final Bulk)

FIGURE 9

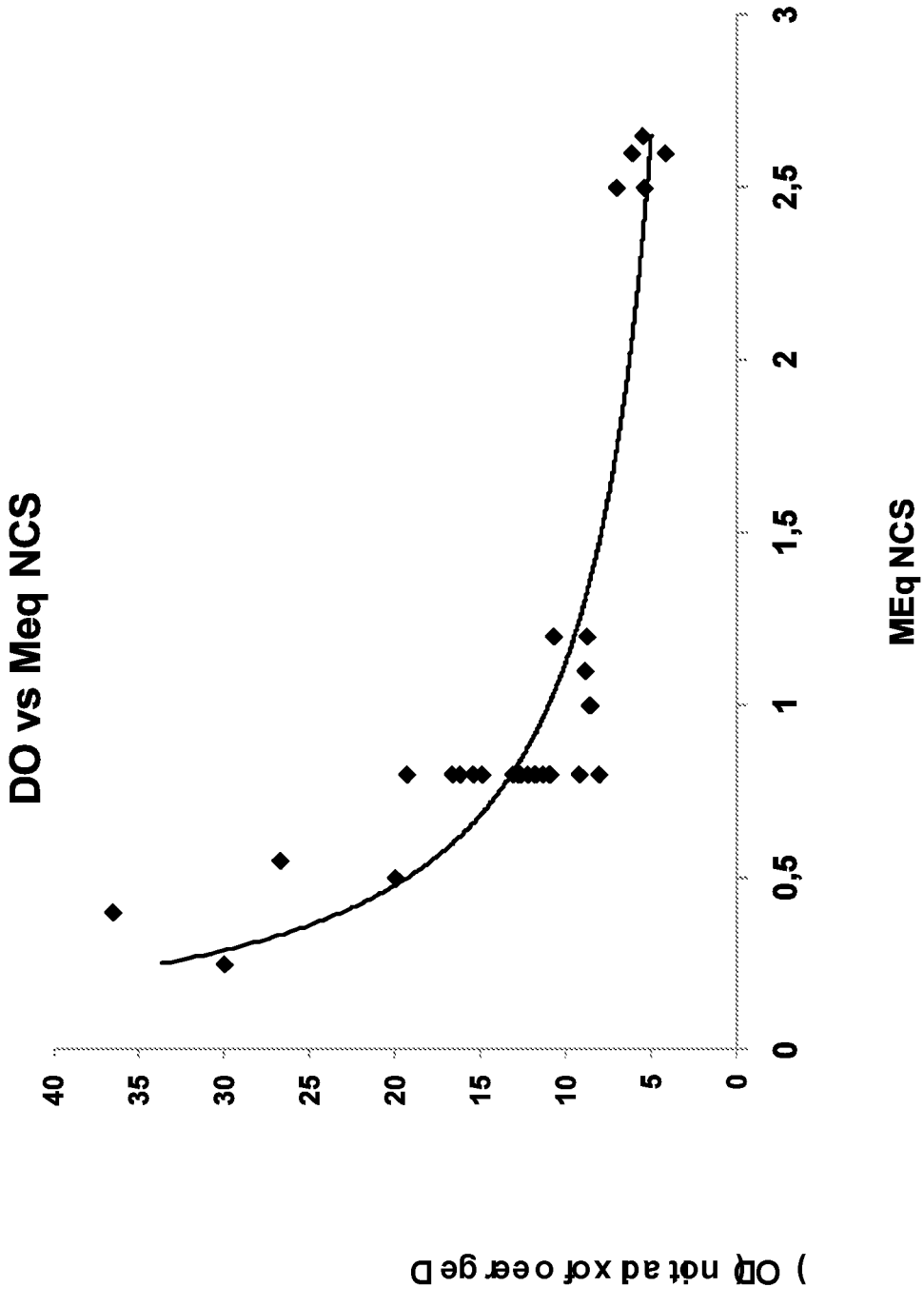

DO vs Meq NCS
DO not adjusted for dilution
MEq NCS
0
0,5
1
1,5
2
2,5
3
0
5
10
15
20
25
30
35
40

FIGURE 10

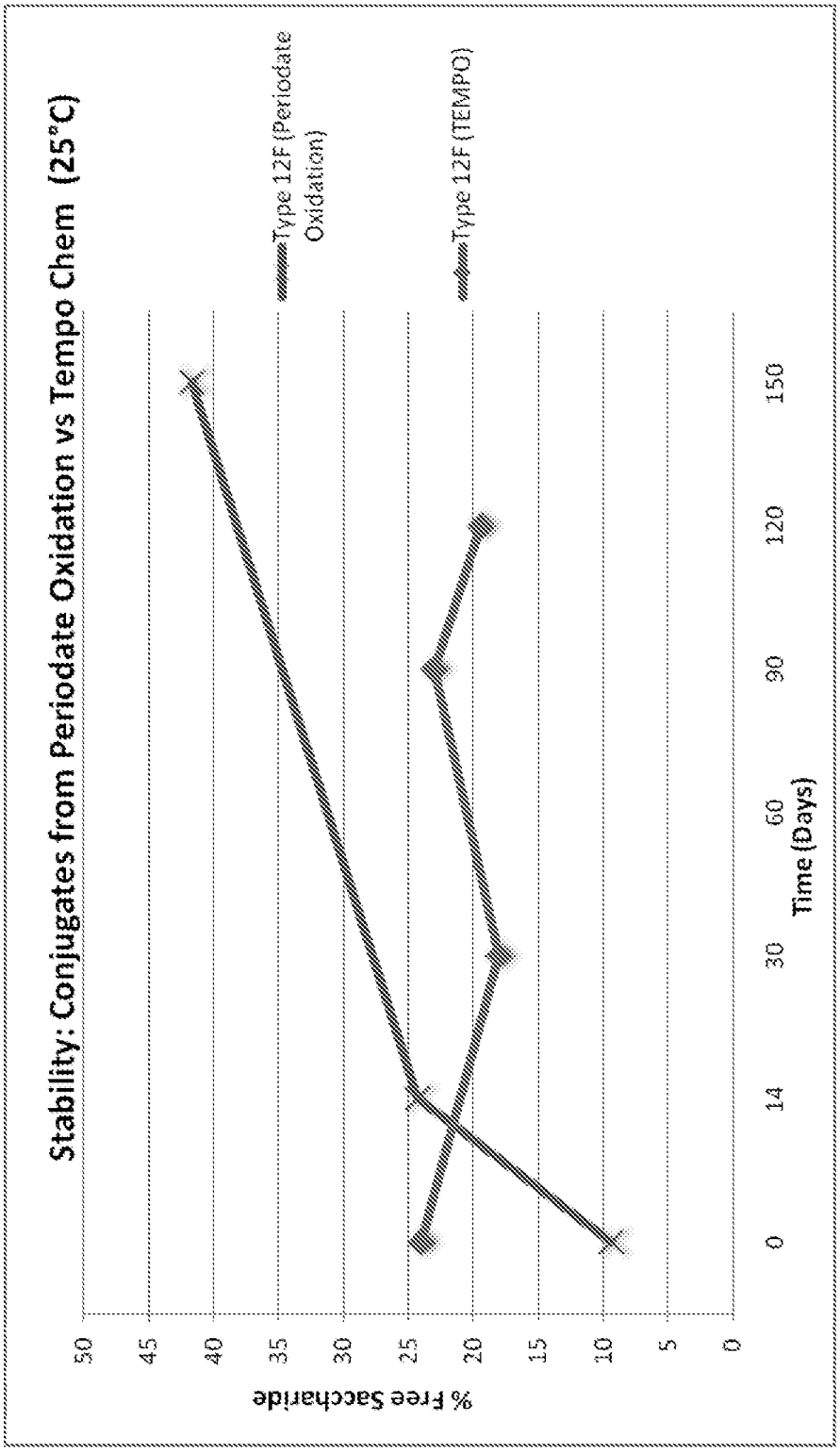

Stability: Conjugates from Periodate Oxidation vs Tempo Chem (25°C)
Type 12F (Periodate Oxidation)
Type 12F (TEMPO)
% Free Saccharide
Time (Days)
0
5
10
15
20
25
30
35
40
45
50
0
14
30
60
90
120
150

FIGURE 11

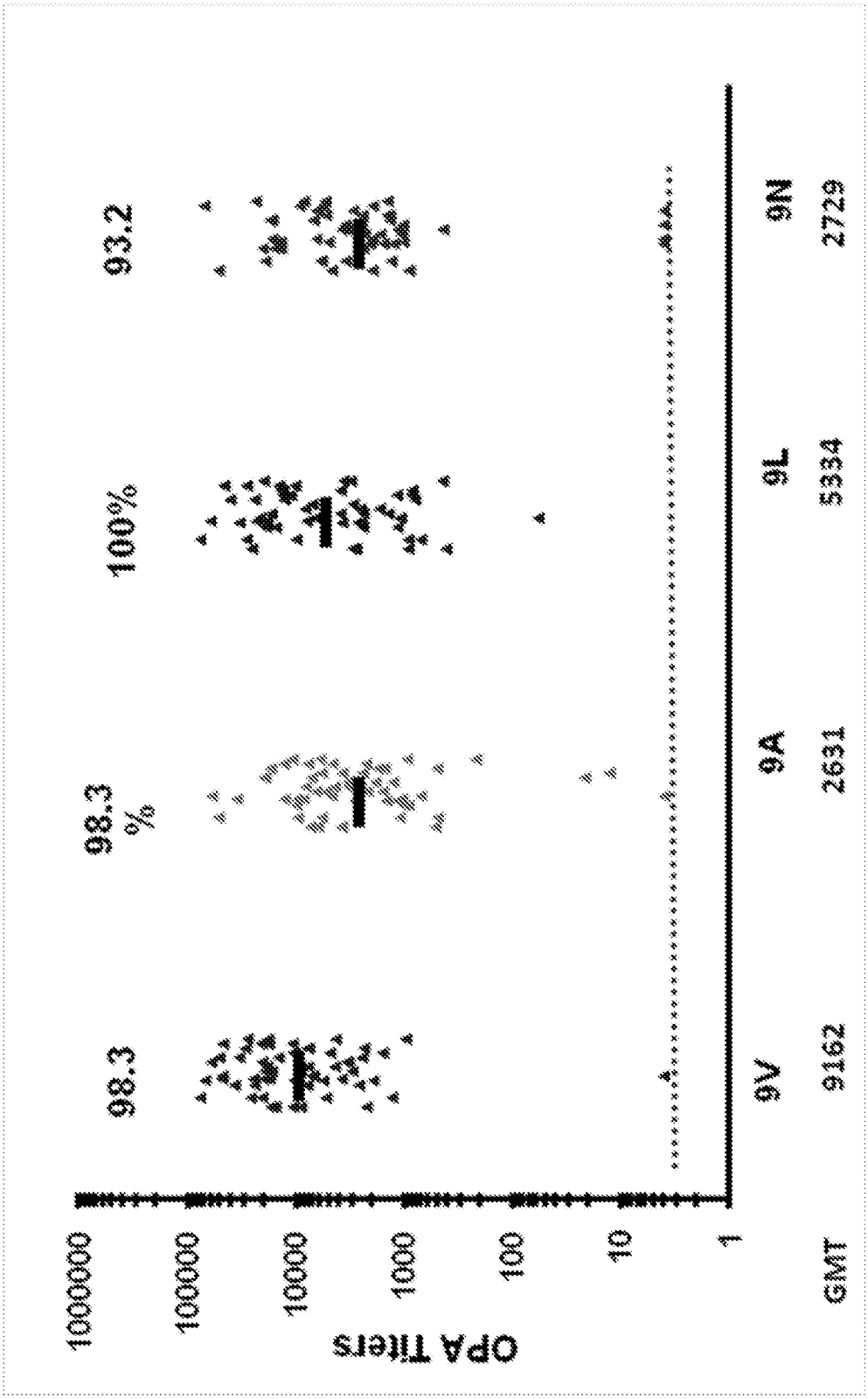

OPA Titers
1000000
100000
10000
1000
100
10
1
98.3
98.3
%
100%
93.2
9V
9A
9L
9N
GMT
9162
2631
5334
2729

FIGURE 12

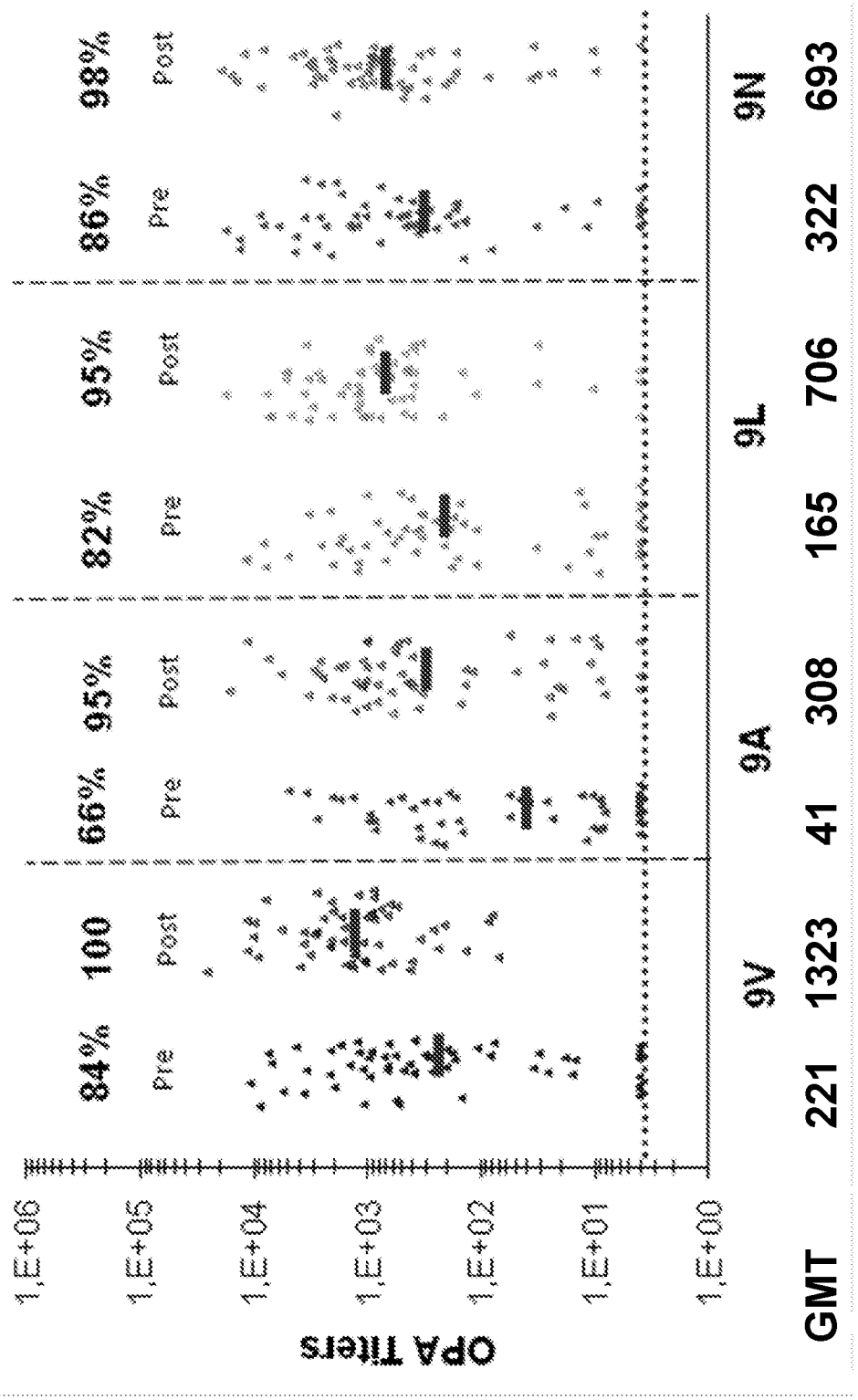

OPA Titers
1,E+06
1,E+05
1,E+04
1,E+03
1,E+02
1,E+01
1,E+00
84%
100
66%
95%
82%
95%
86%
98%
Pre
Post
Pre
Post
Pre
Post
Pre
Post
9V
9A
9L
9N
GMT
221
1323
41
308
165
706
322
693

FIGURE 13

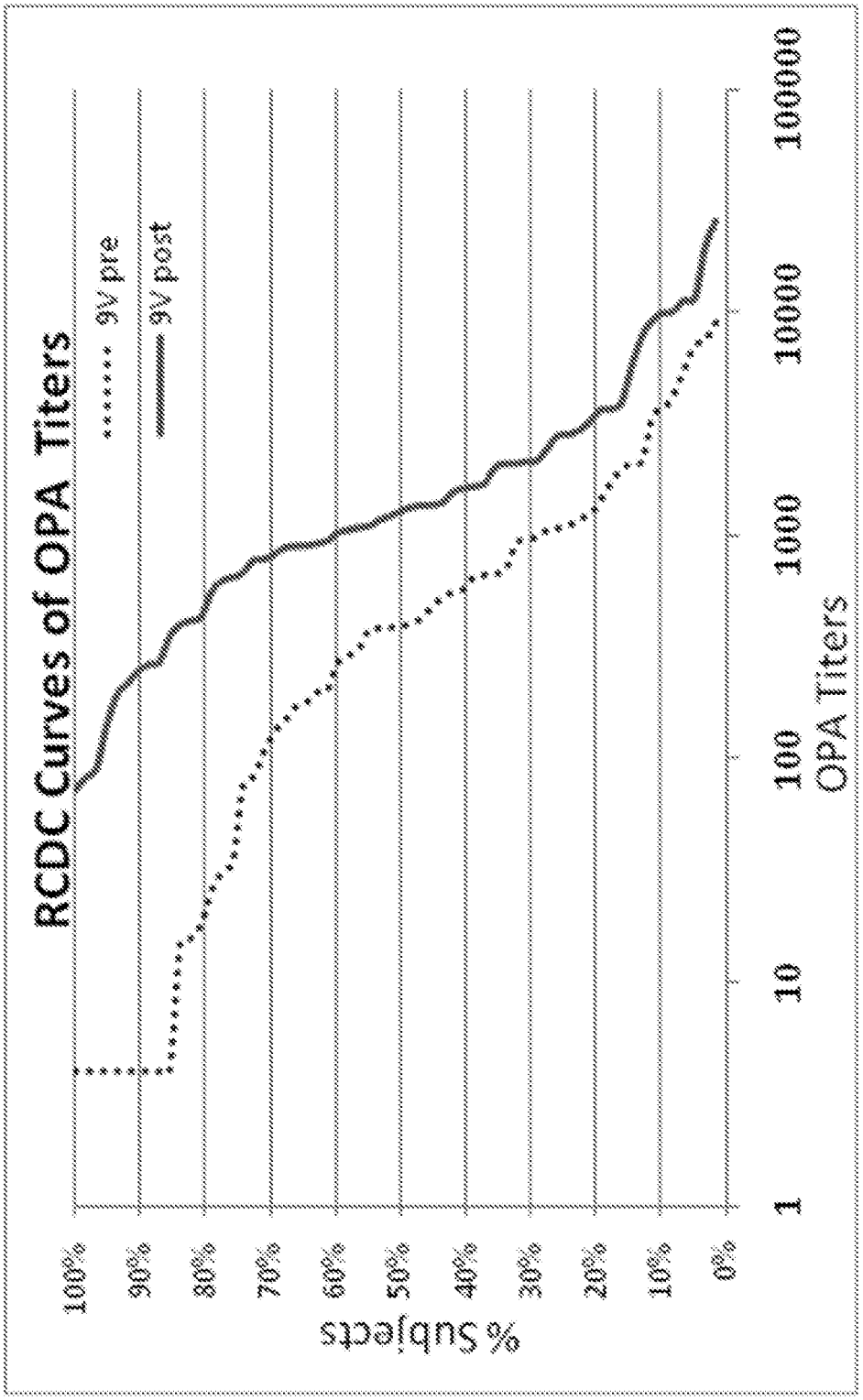

RCDC Curves of OPA Titers
9V pre
9V post
% Subjects
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1
10
100
1000
10000
100000
OPA Titers

FIGURE 14

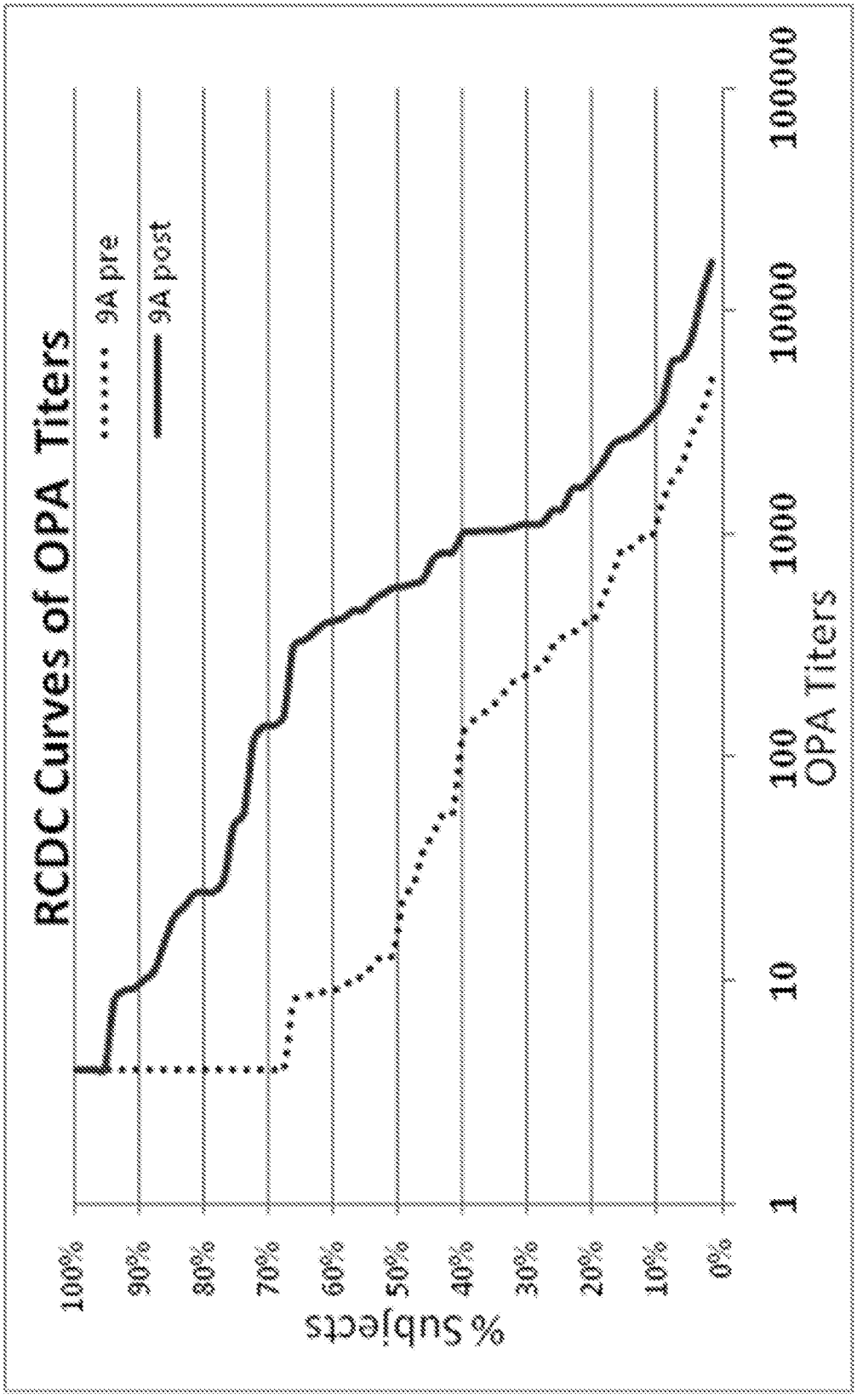

RCDC Curves of OPA Titers
9A pre
9A post
% Subjects
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1
10
100
1000
10000
100000
OPA Titers

FIGURE 15

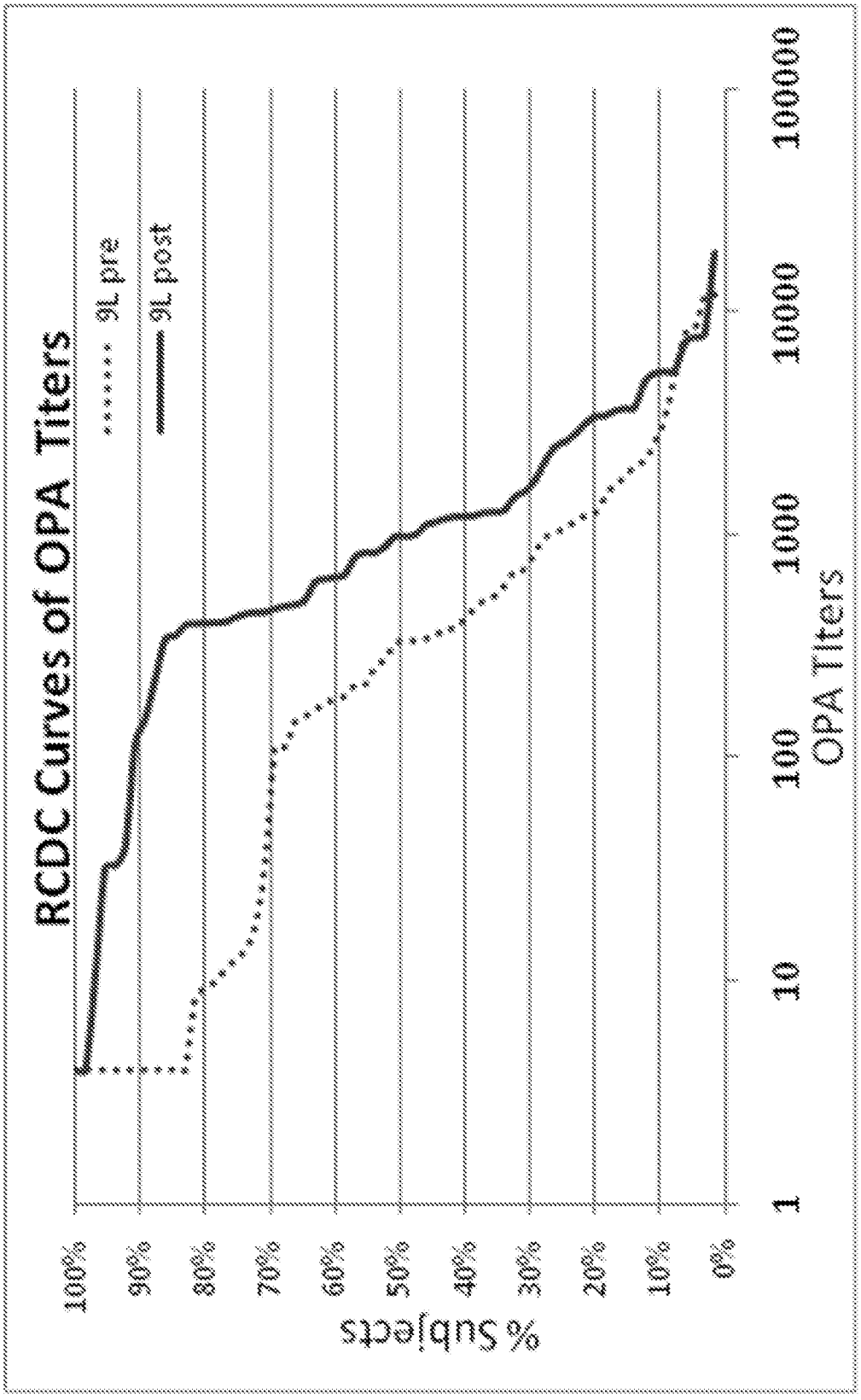
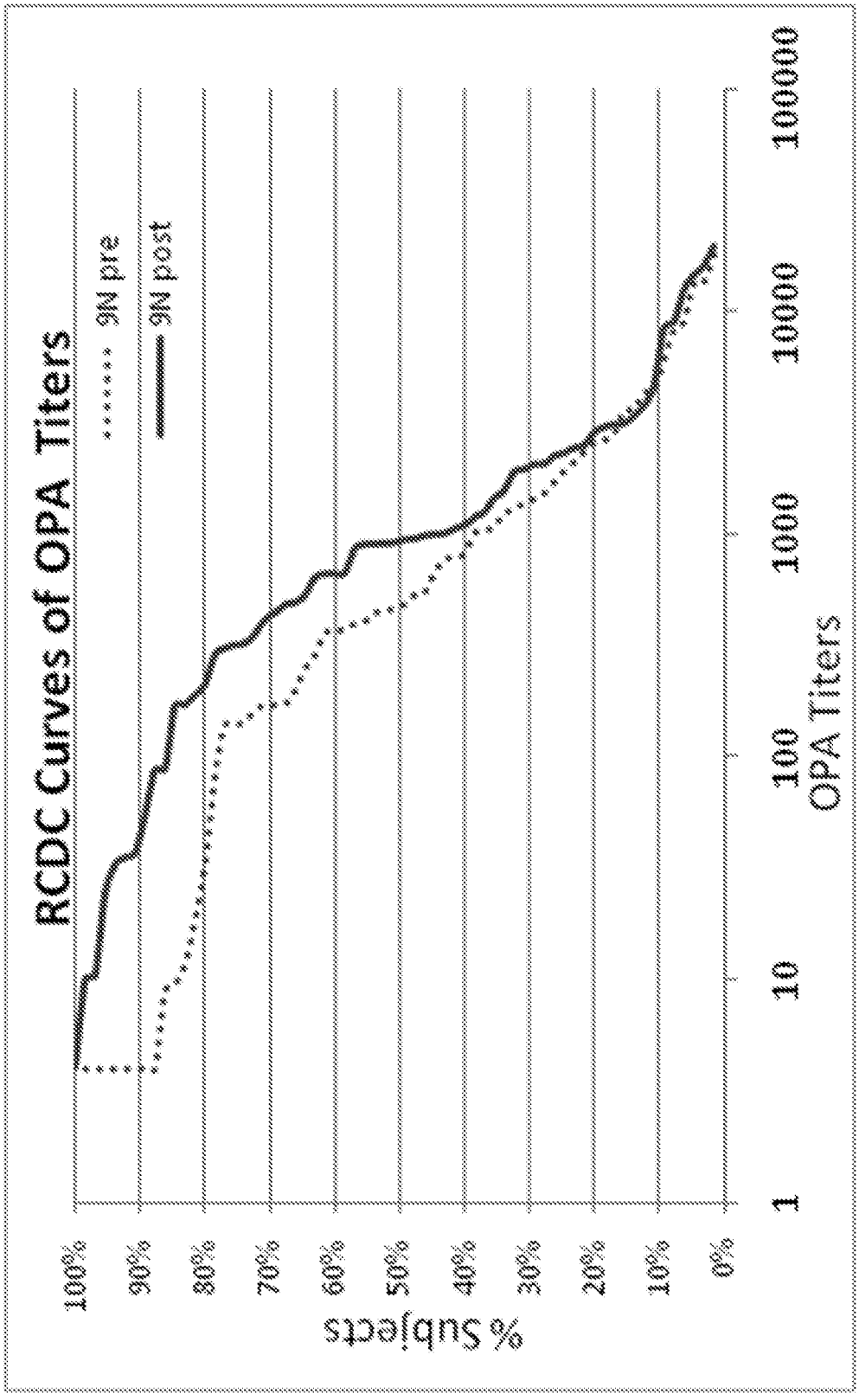

RCDC Curves of OPA Titers
9L pre
9L post
% Subjects
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1
10
100
1000
10000
100000
OPA Titers

FIGURE 16


RCDC Curves of OPA Titers
9N pre
9N post
% Subjects
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1
10
100
1000
10000
100000
OPA Titers