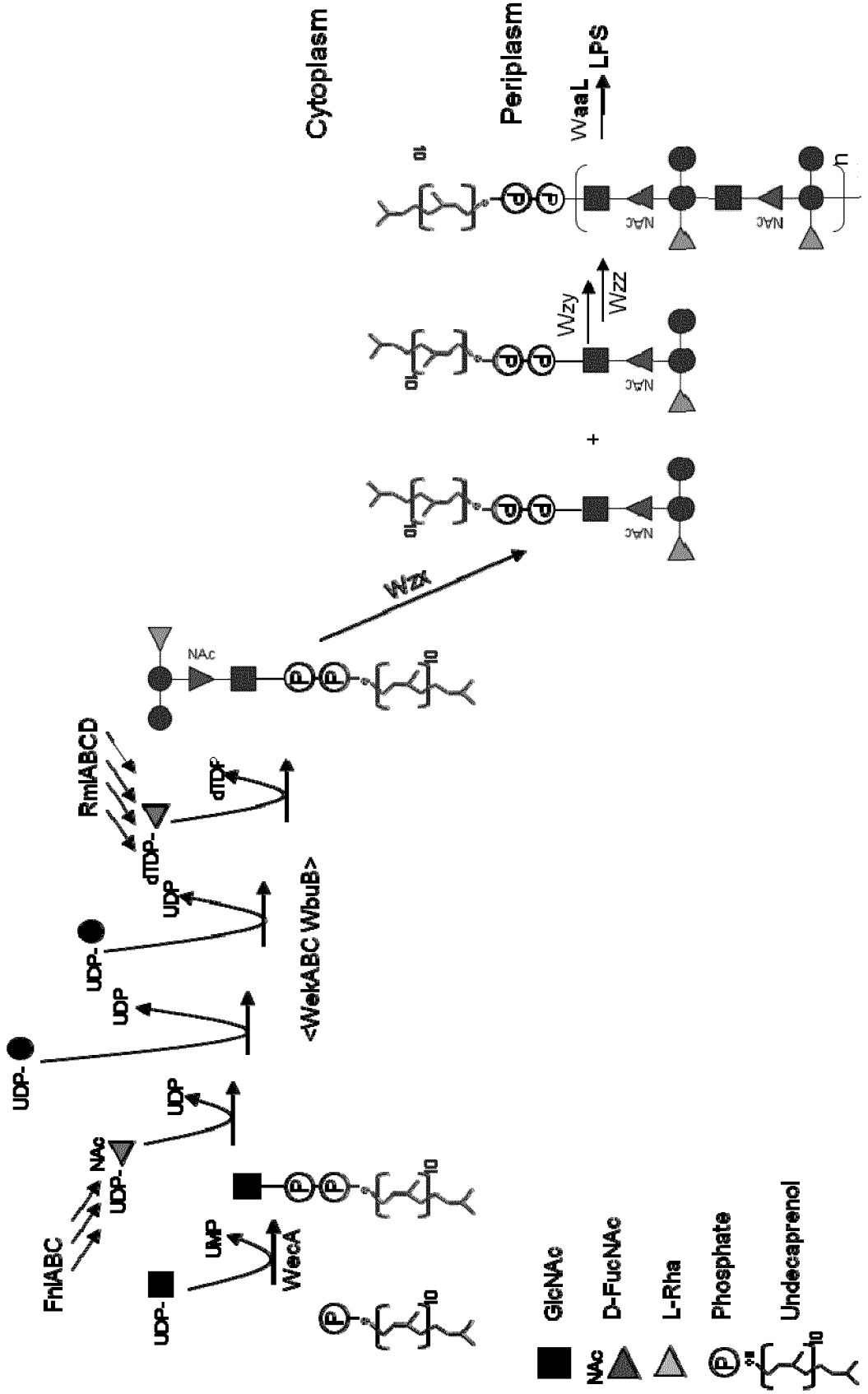

Cytoplasm
Periplasm
WaaL
LPS
Wzy
Wzz
Wzx
NAc
RmlABCD
dTDP-
dTDP
UDP-
UDP
<WekABC WbuB>
FnlABC
NAc
UMP
WecA
GlcNAc
D-FucNAc
L-Rha
Phosphate
Undecaprenol

FIG. 1

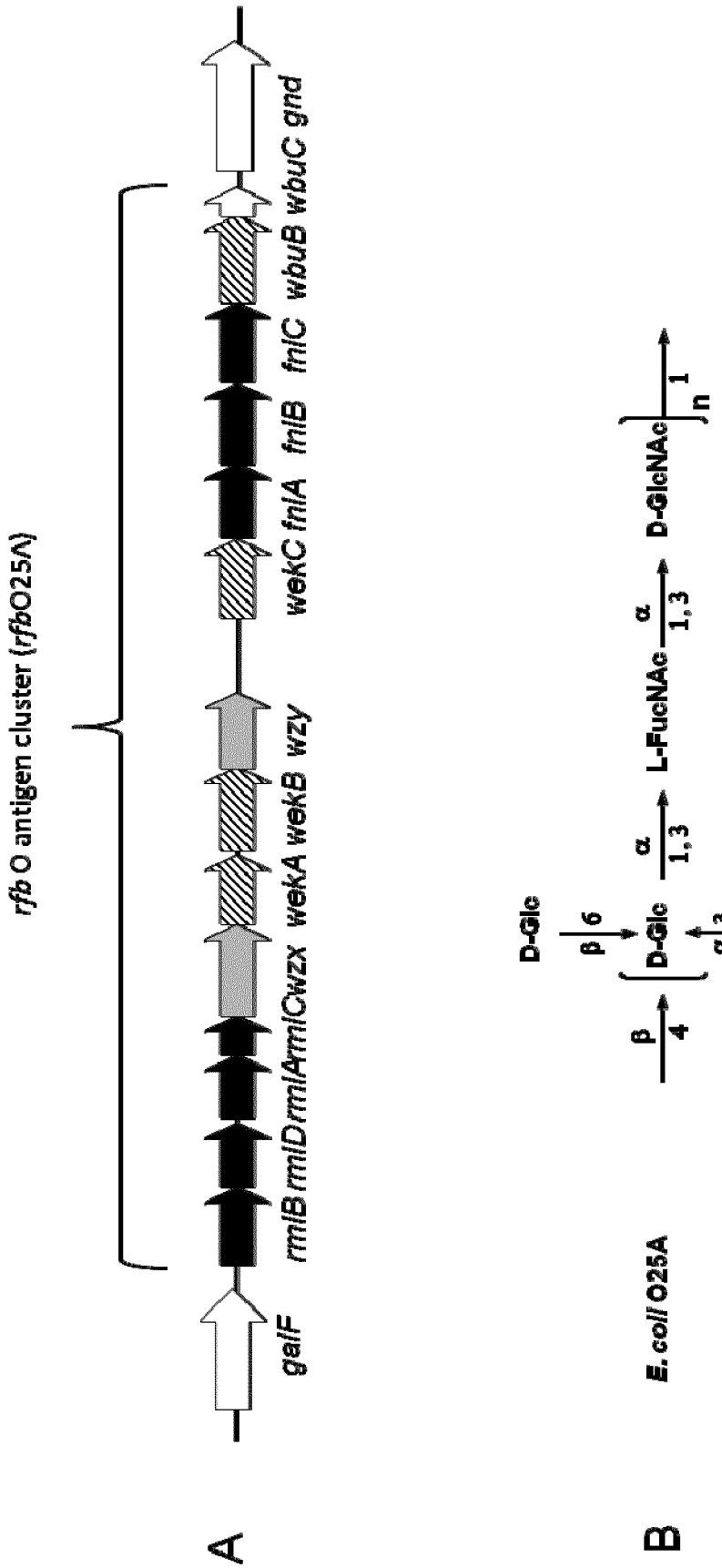

A
rfb O antigen cluster (rfbO25A)
galF
rmlB rmlD rmlA rmlC wzx
wekA wekB wzy
wekC fnlA fnlB fnlC wbuB wbuC gnd
B
E. coli O25A
D-Glc
β 6
β 4
D-Glc
α 1,3
α 3
L-Rha
L-FucNAc
α 1,3
D-GlcNAc
n 1

FIG. 2

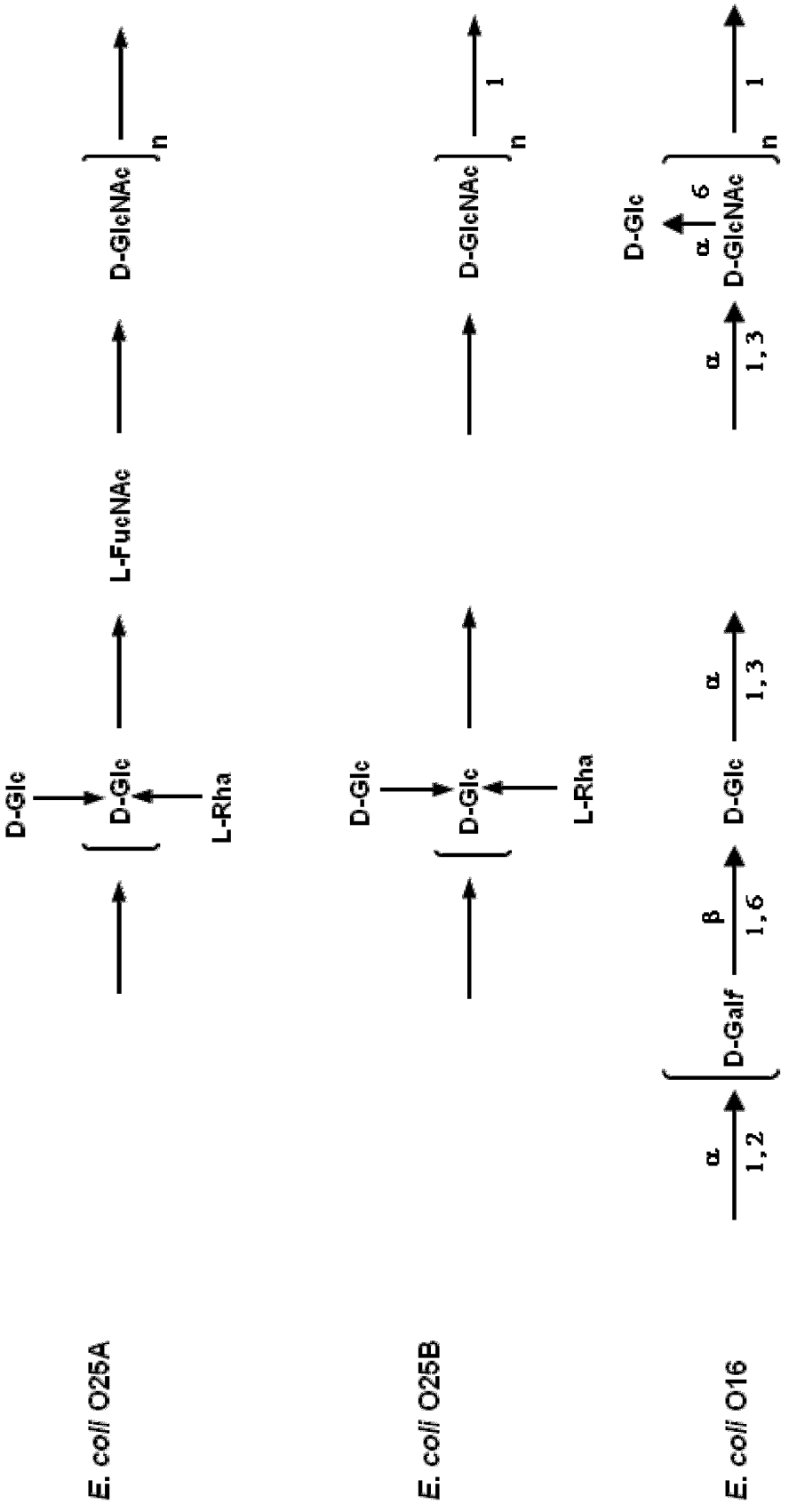

E. coli O25A
D-Glc
D-Glc
L-Rha
L-FucNAc
D-GlcNAc
n
E. coli O25B
D-Glc
D-Glc
L-Rha
D-GlcNAc
n
1
E. coli O16
α
1, 2
D-Galf
β
1, 6
D-Glc
α
1, 3
D-Glc
α
6
D-GlcNAc
α
1, 3
D-GlcNAc
n
1

FIG. 3A

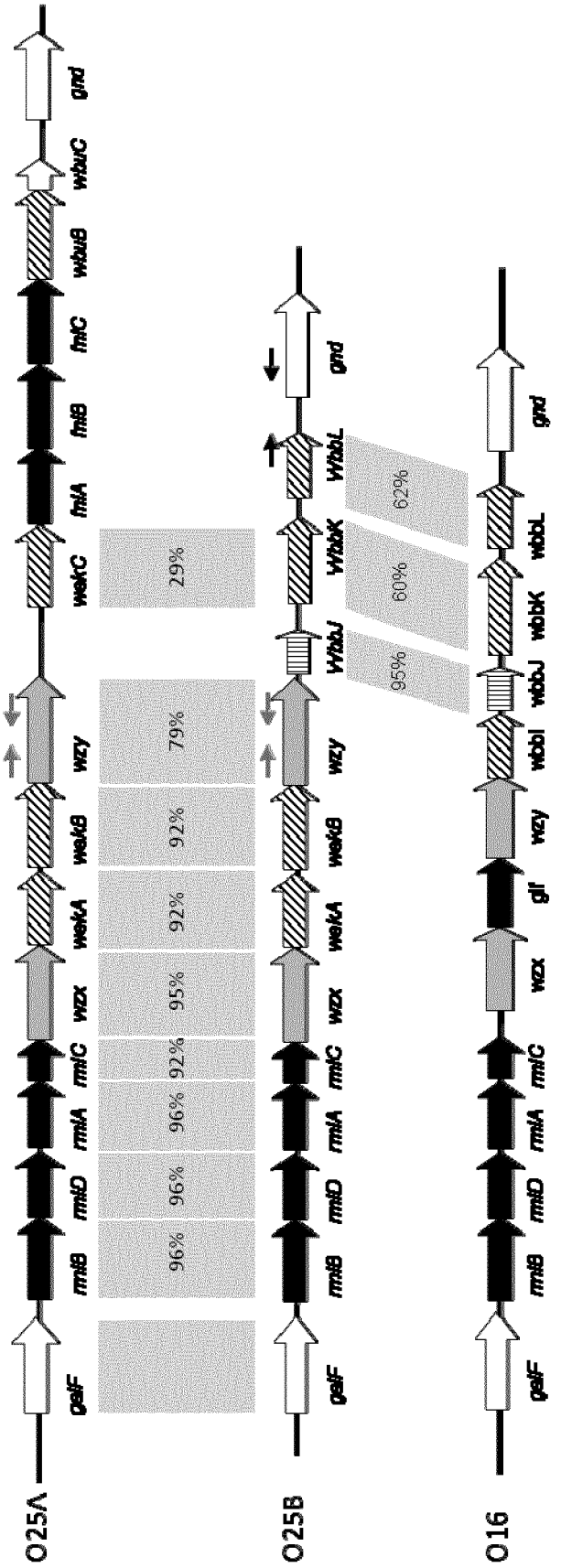

O25A
galF
rmlB
rmlD
rmlA
rmlC
wzx
wekA
wekB
wzy
wekC
fmlA
fmlB
fmlC
wbuB
wbuC
gnd
96%
96%
96%
92%
95%
92%
92%
79%
29%
O25B
galF
rmlB
rmlD
rmlA
rmlC
wzx
wekA
wekB
wzy
WbbJ
WbbK
WbbL
gnd
95%
60%
62%
O16
galF
rmlB
rmlD
rmlA
rmlC
wzx
gll
wzy
wblI
wbbJ
wblK
wblL
gnd

FIG. 3B

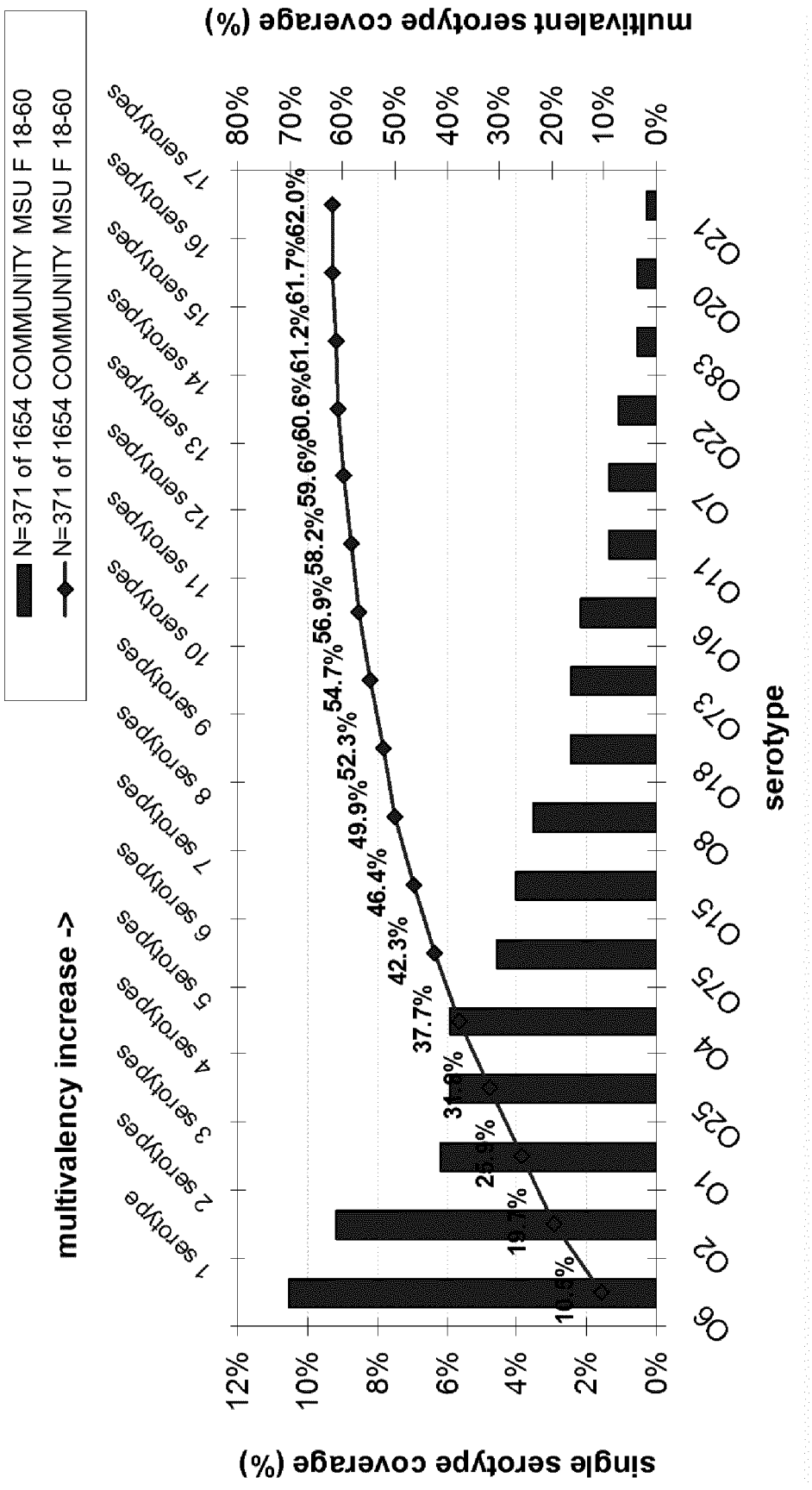

N=371 of 1654 COMMUNITY MSU F 18-60
N=371 of 1654 COMMUNITY MSU F 18-60
multivalent serotype coverage (%)
single serotype coverage (%)
serotype
multivalency increase ->
1 serotype
2 serotypes
3 serotypes
4 serotypes
5 serotypes
6 serotypes
7 serotypes
8 serotypes
9 serotypes
10 serotypes
11 serotypes
12 serotypes
13 serotypes
14 serotypes
15 serotypes
16 serotypes
17 serotypes
10.4%
19.1%
25.9%
31.6%
37.7%
42.3%
46.4%
49.9%
52.3%
54.7%
56.9%
58.2%
59.6%
60.6%
61.2%
61.7%
62.0%

FIG. 4

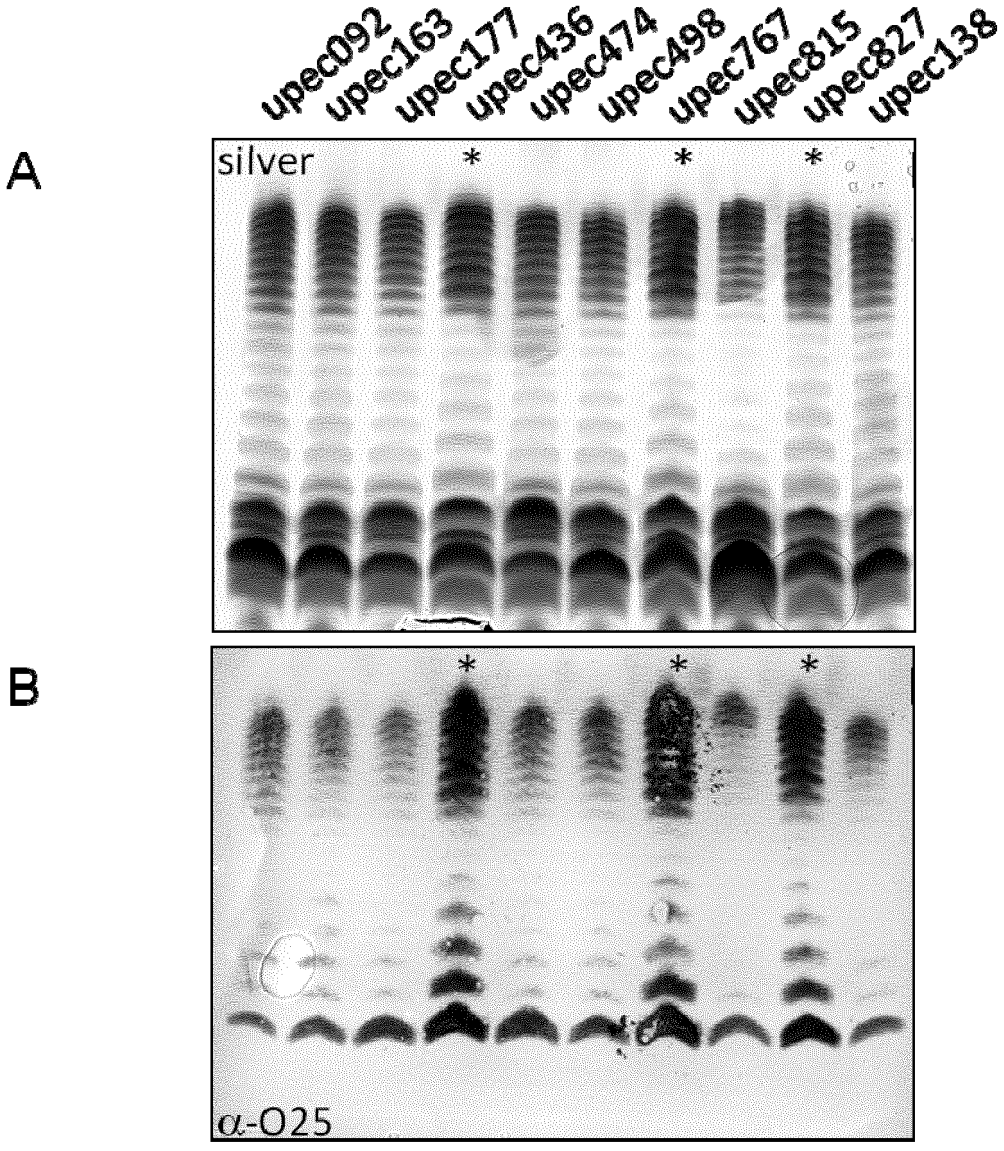

upec092
upec163
upec177
upec436
upec474
upec498
upec767
upec815
upec827
upec138
A
silver
*
*
*
B
*
*
*
α-O25

FIG. 5 A-B

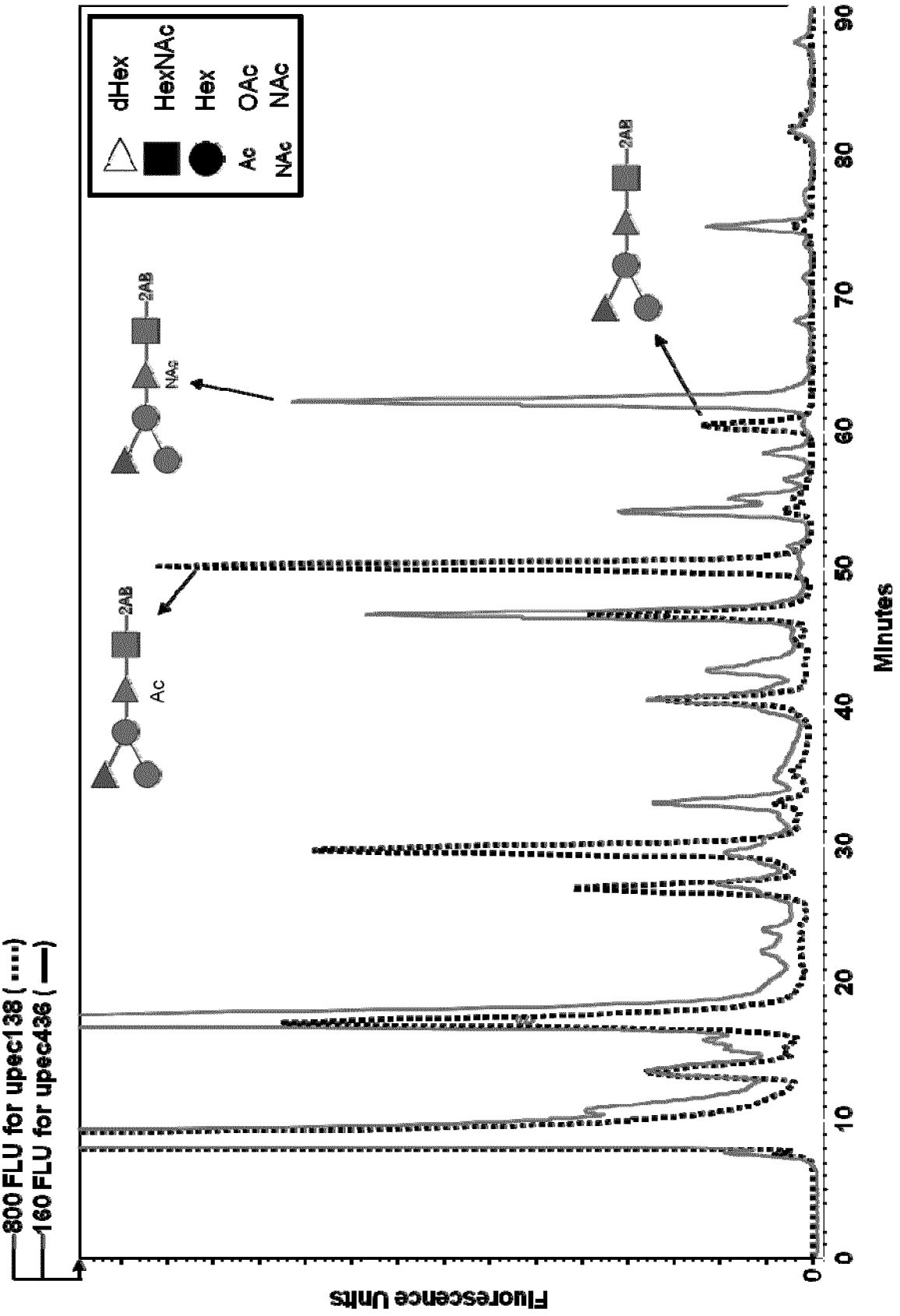

800 FLU for upec138 (.....)
160 FLU for upec436 (——)
Fluorescence Units
Minutes
0
10
20
30
40
50
60
70
80
90
dHex
HexNAc
Hex
OAc
Ac
NAc
2AB
Ac
NAc

FIG. 6

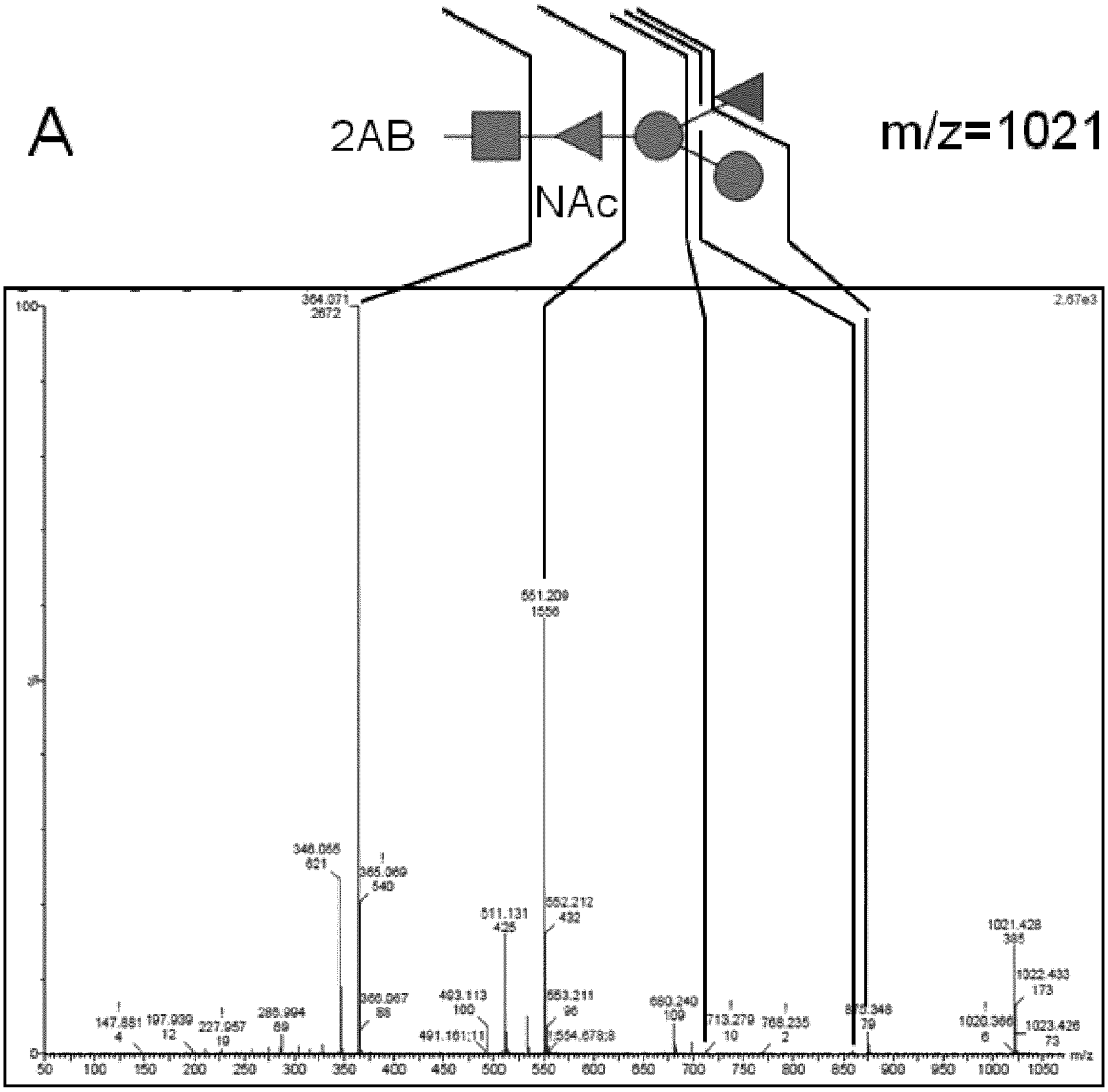

A
2AB
NAc
m/z=1021
364.071
2672
551.209
1556
346.055
621
365.069
540
511.131
425
552.212
432
1021.428
385
1022.433
173
1023.426
73
366.067
88
493.113
100
553.211
96
660.240
109
713.279
10
768.235
2
875.348
79
1020.366
6
147.881
4
197.939
12
227.957
19
286.994
69
491.161;11
554.678;8
2.67e3
100
%
0
50
100
150
200
250
300
350
400
450
500
550
600
650
700
750
800
850
900
950
1000
1050
m/z

FIG. 7A

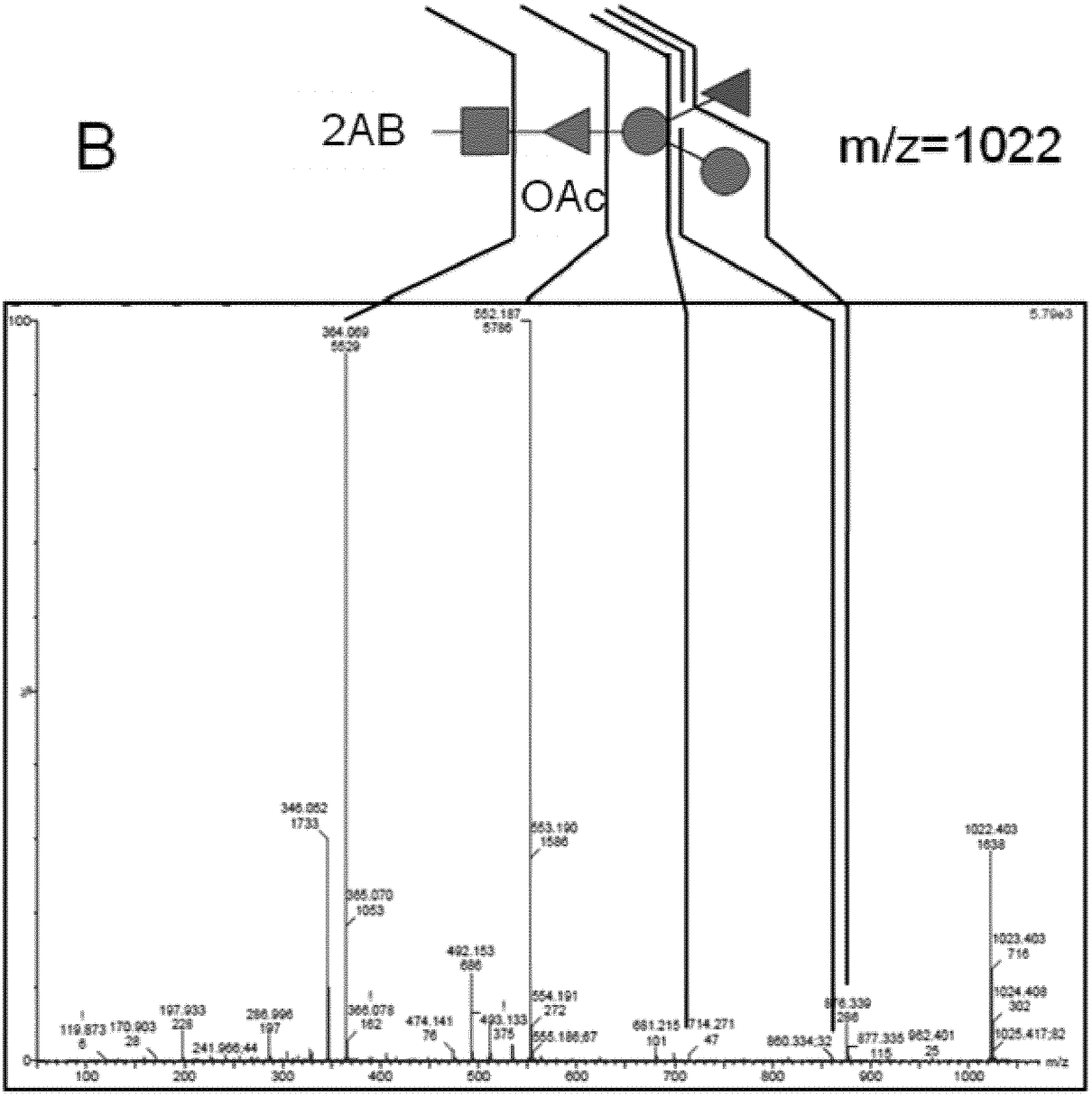

B
2AB
OAc
m/z=1022
100
%
0
364.069
5529
552.187
5786
5.79e3
346.052
1733
553.190
1586
1022.403
1638
366.070
1053
492.153
686
1023.403
716
1024.408
302
1025.417
82
554.191
272
876.339
286
119.873
6
170.903
28
197.933
228
241.966
44
286.996
197
366.078
162
474.141
76
493.133
375
555.186
87
681.215
101
714.271
47
860.334
32
877.335
115
962.401
25
100
200
300
400
500
600
700
800
900
1000
m/z

FIG. 7B

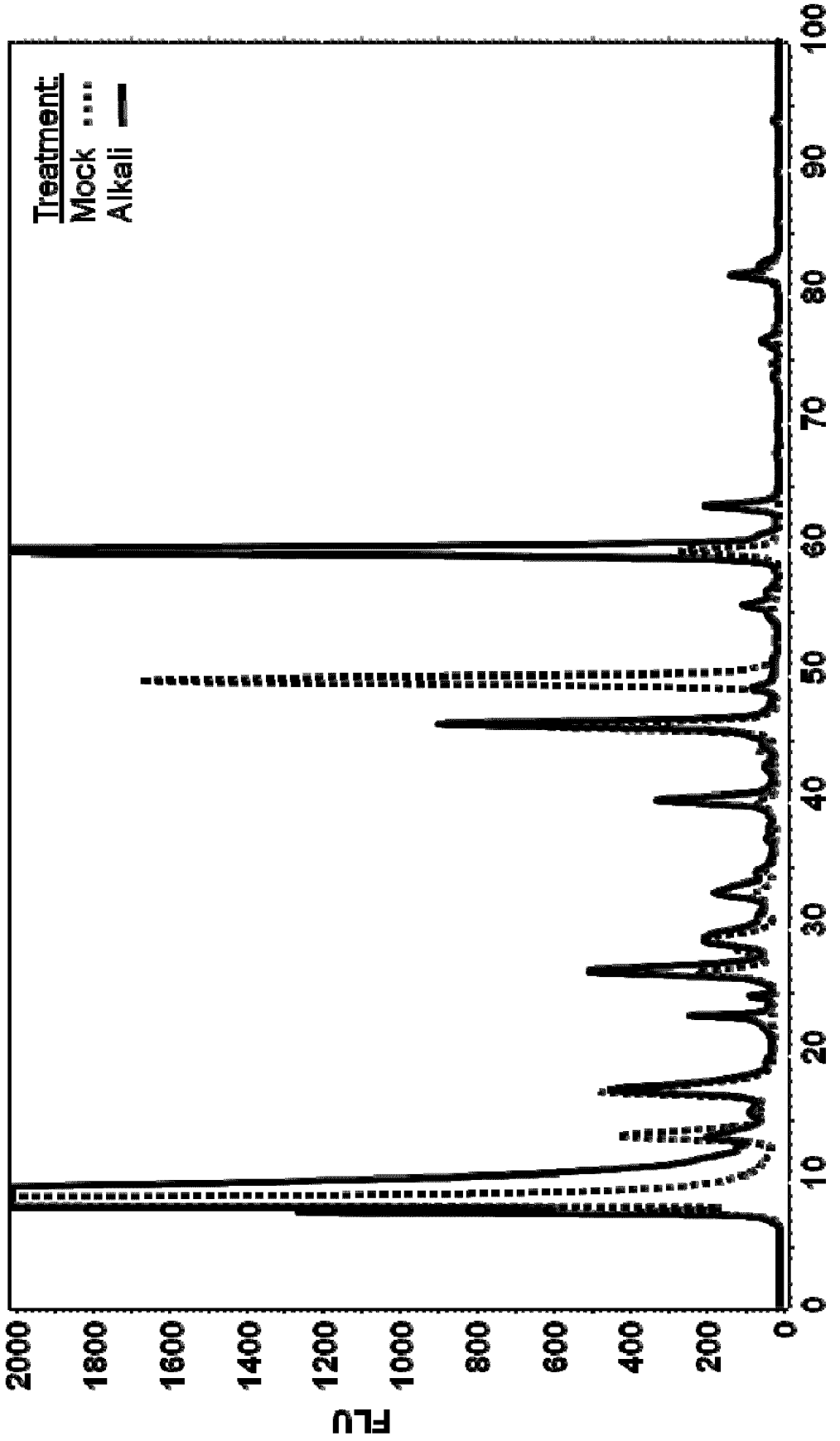

Treatment:
Mock
Alkali
FLU
Minutes
0
200
400
600
800
1000
1200
1400
1600
1800
2000
0
10
20
30
40
50
60
70
80
90
100

FIG. 8

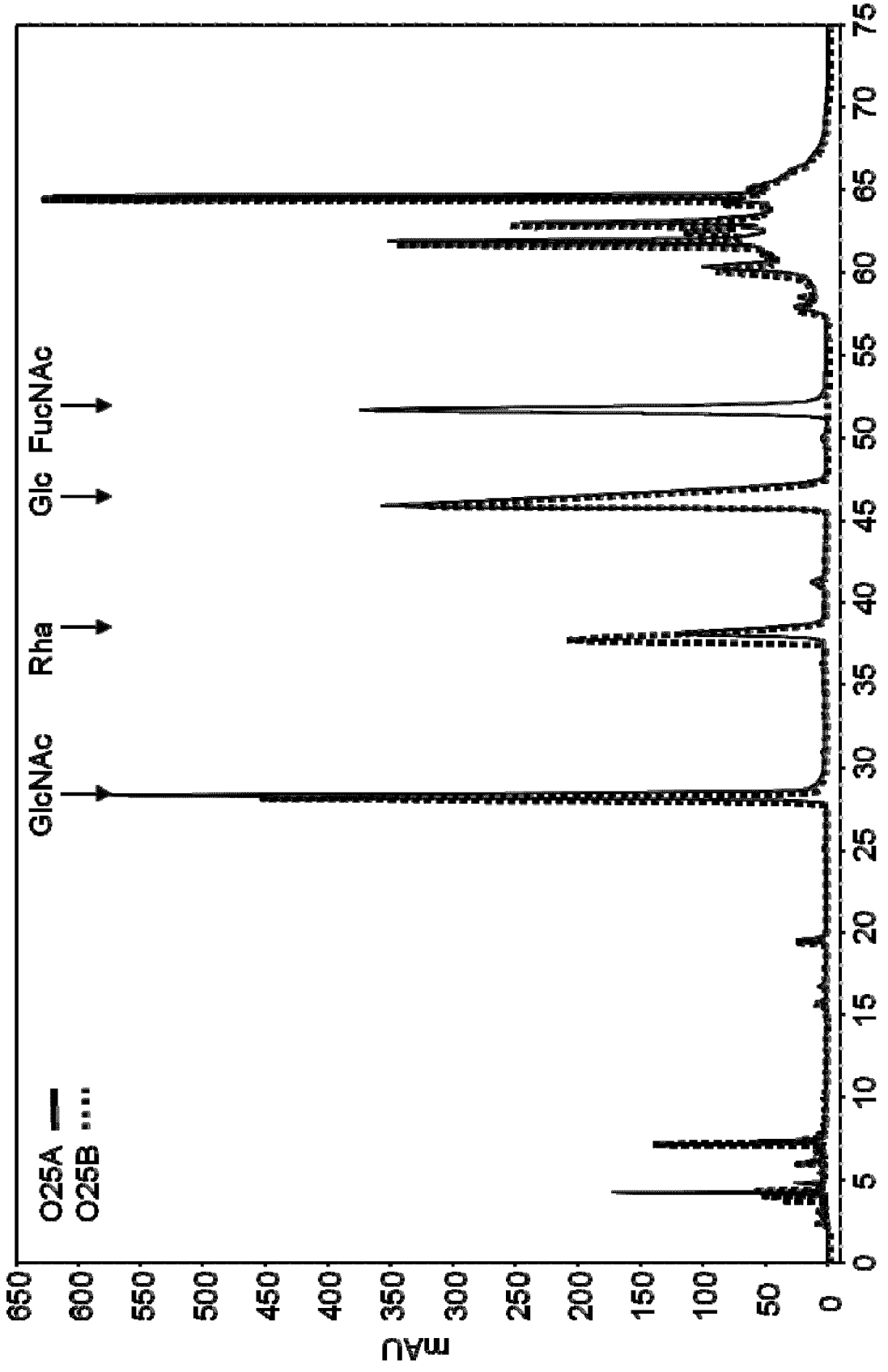

O25A
O25B
GlcNAc
Rha
Glc
FucNAc
mAU
Minutes
650
600
550
500
450
400
350
300
250
200
150
100
50
0
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75

FIG. 9

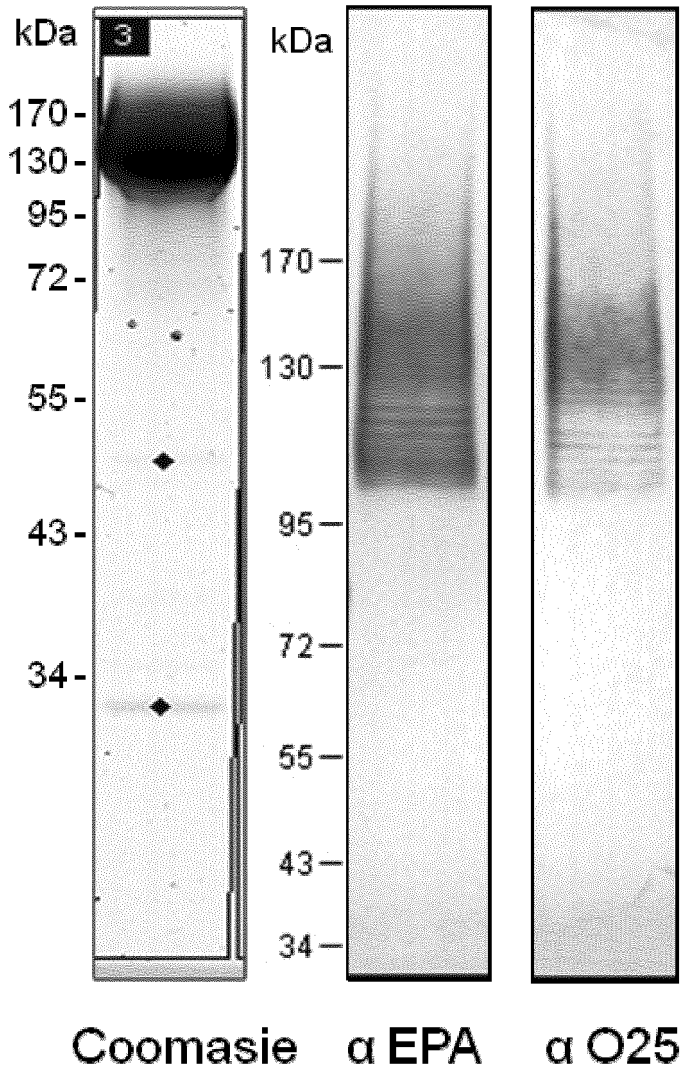

kDa
3
170
130
95
72
55
43
34
kDa
170
130
95
72
55
43
34
Coomasie
α EPA
α O25

FIG. 10

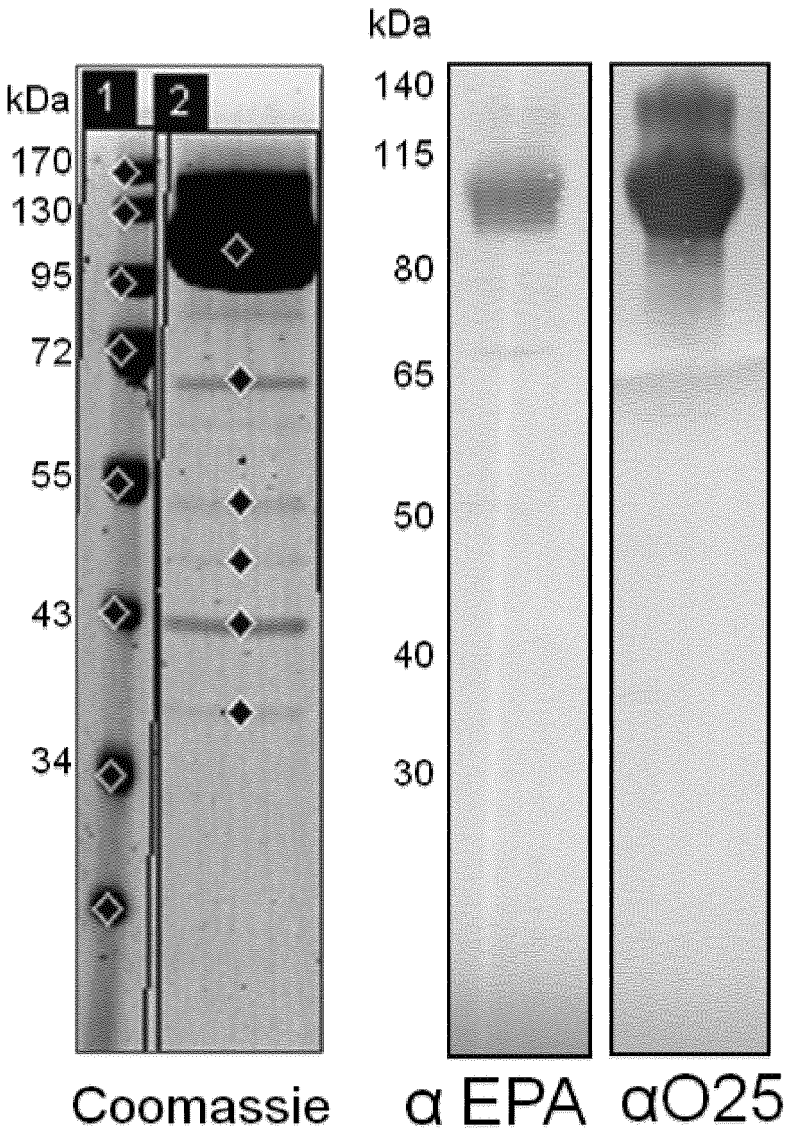

kDa
1
2
170
130
95
72
55
43
34
kDa
140
115
80
65
50
40
30
Coomassie
α EPA
αO25

FIG. 11

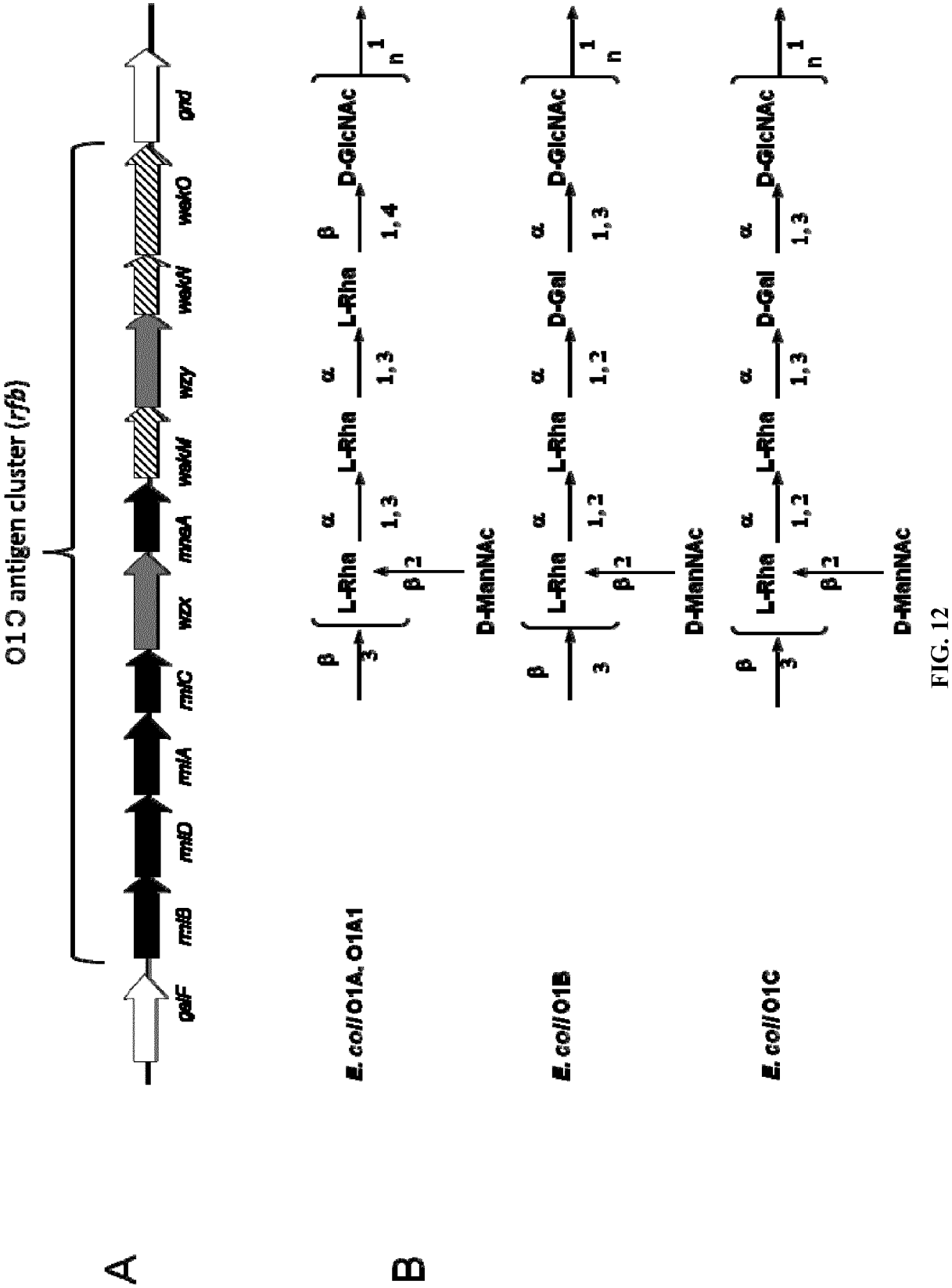

A
O1 O antigen cluster (rfb)
galF
rmlB
rmlD
rmlA
rmlC
wzx
mseA
wekM
wzy
wekN
wekO
gnd
B
E. coli O1A, O1A1
β
3
L-Rha
α
1,3
L-Rha
α
1,3
L-Rha
β
1,4
D-GlcNAc
1
n
β
2
D-ManNAc
E. coli O1B
β
3
L-Rha
α
1,2
L-Rha
α
1,2
D-Gal
α
1,3
D-GlcNAc
1
n
β
2
D-ManNAc
E. coli O1C
β
3
L-Rha
α
1,2
L-Rha
α
1,3
D-Gal
α
1,3
D-GlcNAc
1
n
β
2
D-ManNAc

FIG. 12

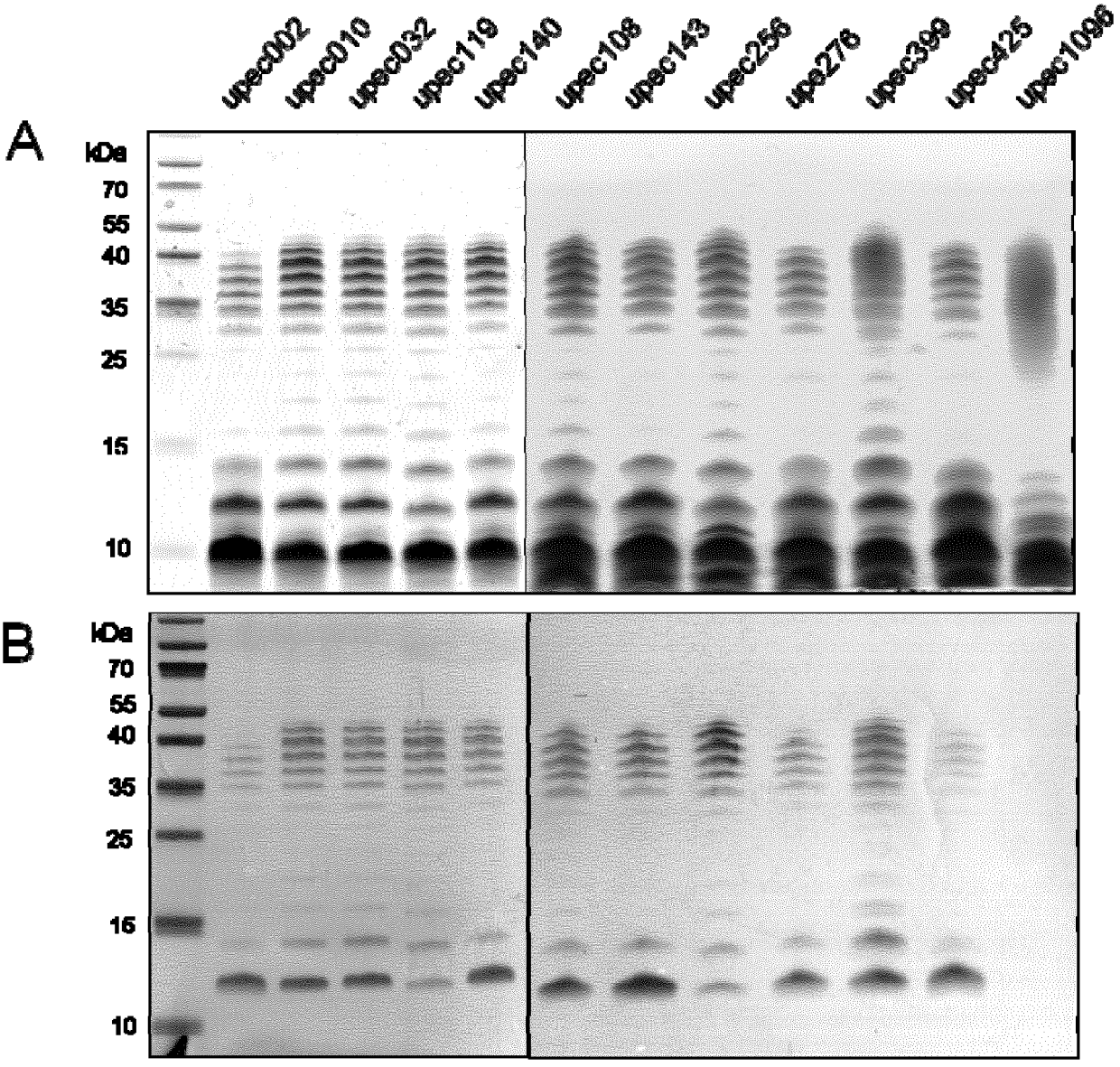

upec002
upec010
upec032
upec119
upec140
upec108
upec143
upec256
upe278
upec399
upec425
upec1096
A
kDa
70
55
40
35
25
15
10
B
kDa
70
55
40
35
25
15
10

FIG. 13

A

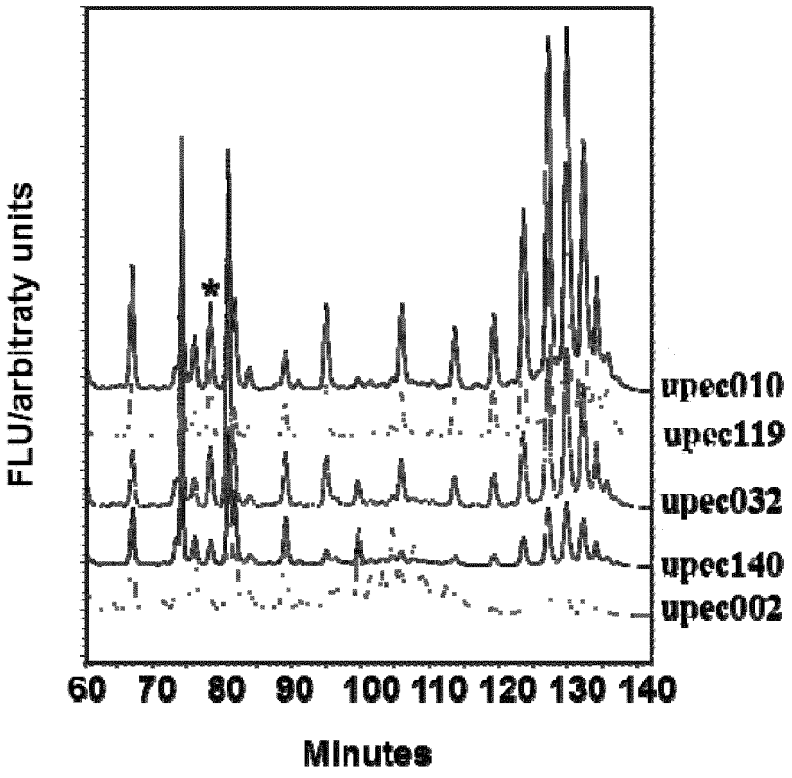

FLU/arbitraty units
*
upec010
upec119
upec032
upec140
upec002
60 70 80 90 100 110 120 130 140
Minutes

FIG. 14A

B

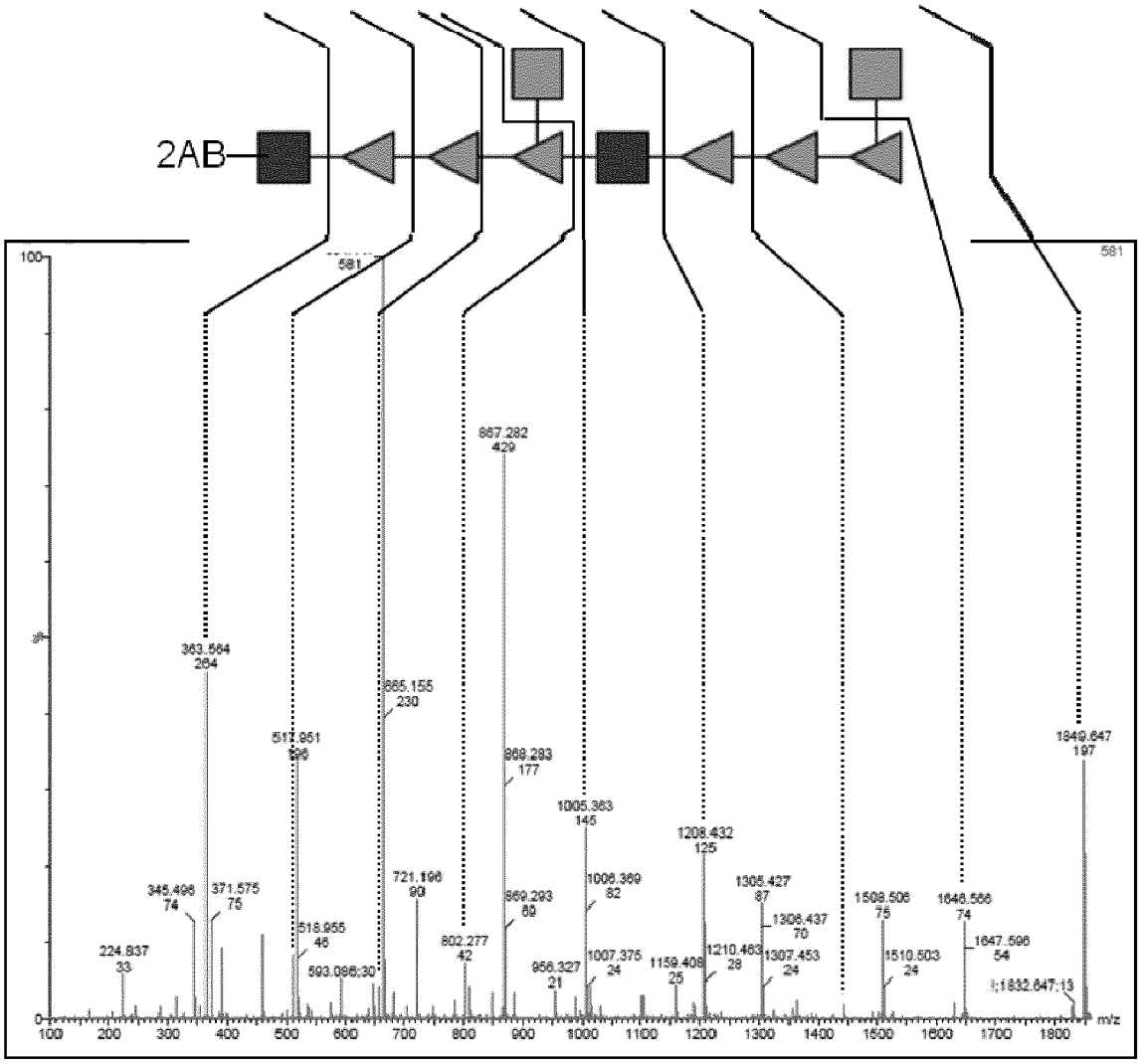

2AB
100
%
0
581
867.282
429
363.564
264
885.155
230
517.951
990
969.293
177
1005.363
145
1208.432
125
721.196
90
969.293
89
1006.369
82
1305.427
87
1306.437
70
1508.506
75
1646.556
74
1647.596
54
1510.503
24
1307.453
24
1210.463
28
1159.408
25
1007.375
24
956.327
21
802.277
42
518.955
46
593.086;30
345.496
74
371.575
75
224.837
33
1949.647
197
1832.647;13
100 200 300 400 500 600 700 800 900 1000 1100 1200 1300 1400 1500 1600 1700 1800
m/z

FIG. 14B

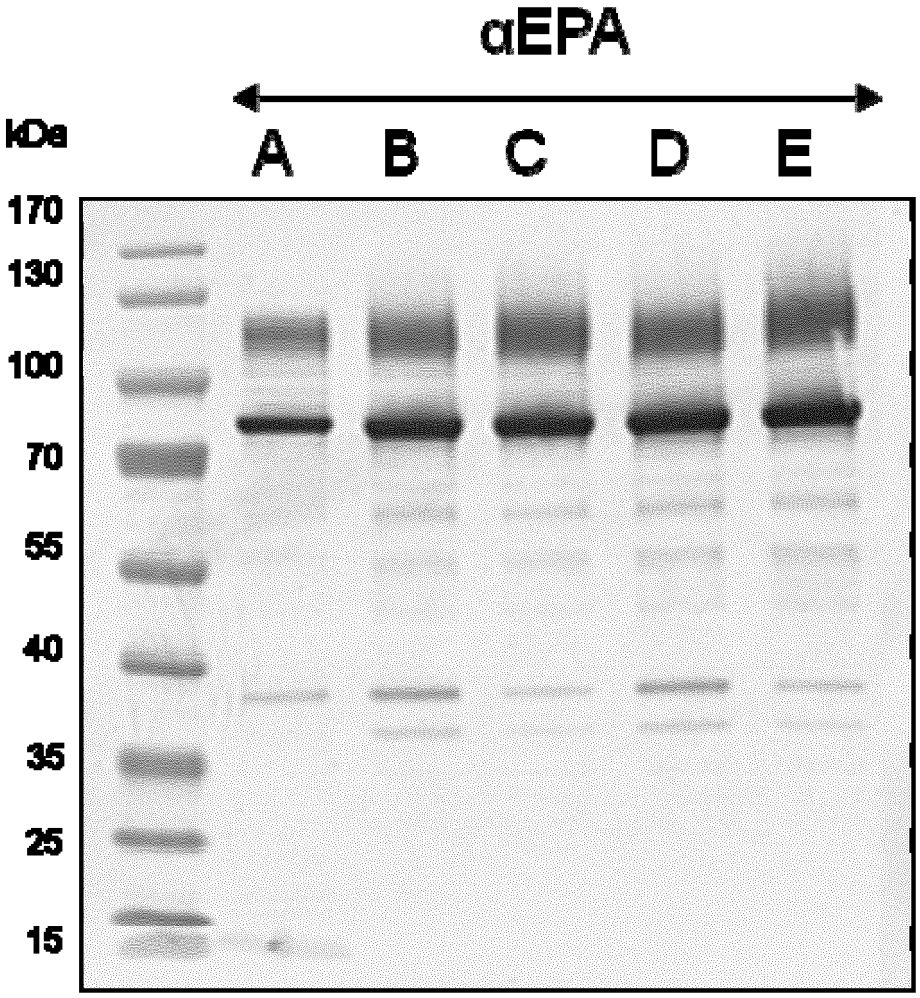


FIG. 15

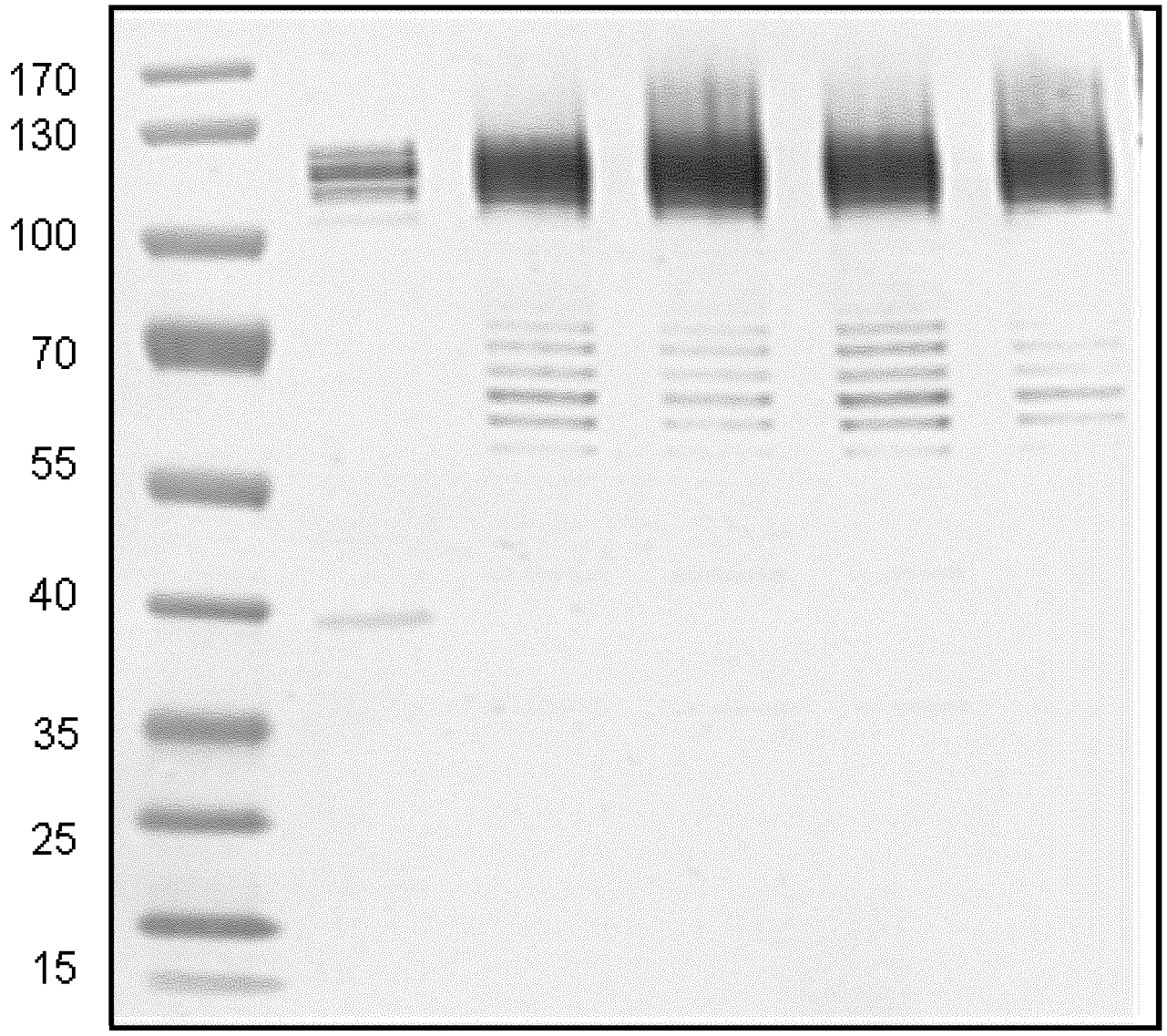


FIG. 16

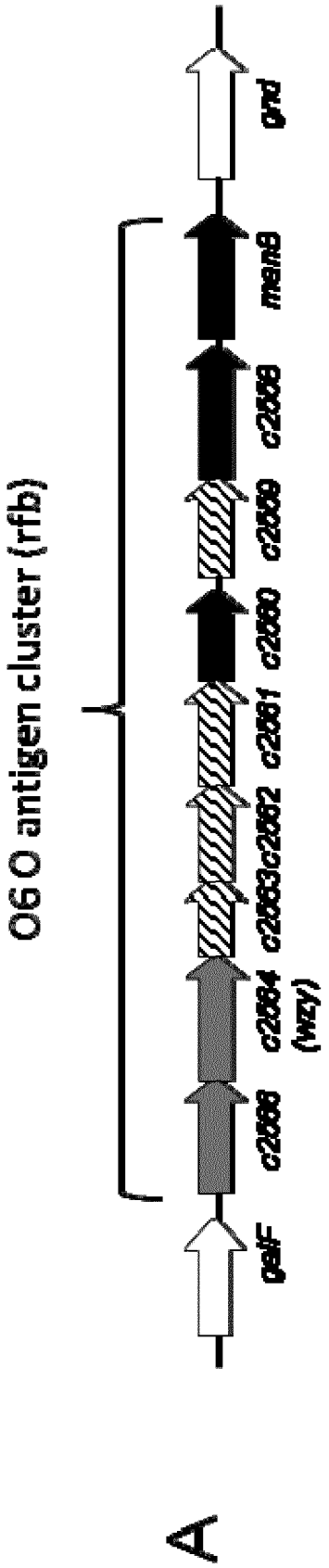

A
O6 O antigen cluster (rfb)
gnd
manB
c2358
c2359
c2360
c2361
c2362
c2363
c2364 (wzy)
c2365
galF

FIG. 17A

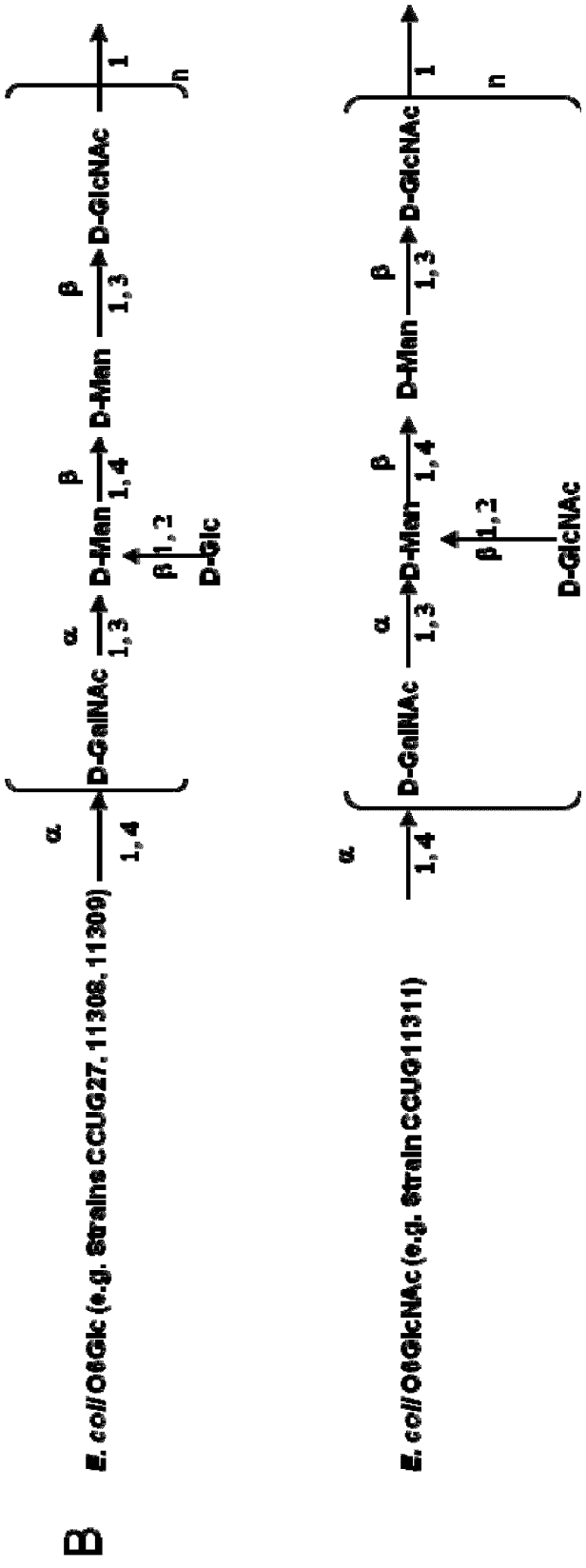

B
E. coli O6Glc (e.g. Strains CCUG27, 11308, 11309)
α 1,4
D-GalNAc
α 1,3
D-Man
β 1,4
D-Man
β 1,3
D-GlcNAc
n
1
β 1,2
D-Glc
E. coli O6GlcNAc (e.g. Strain CCUG11311)
α 1,4
D-GalNAc
α 1,3
D-Man
β 1,4
D-Man
β 1,3
D-GlcNAc
n
1
β 1,2
D-GlcNAc

FIG. 17B

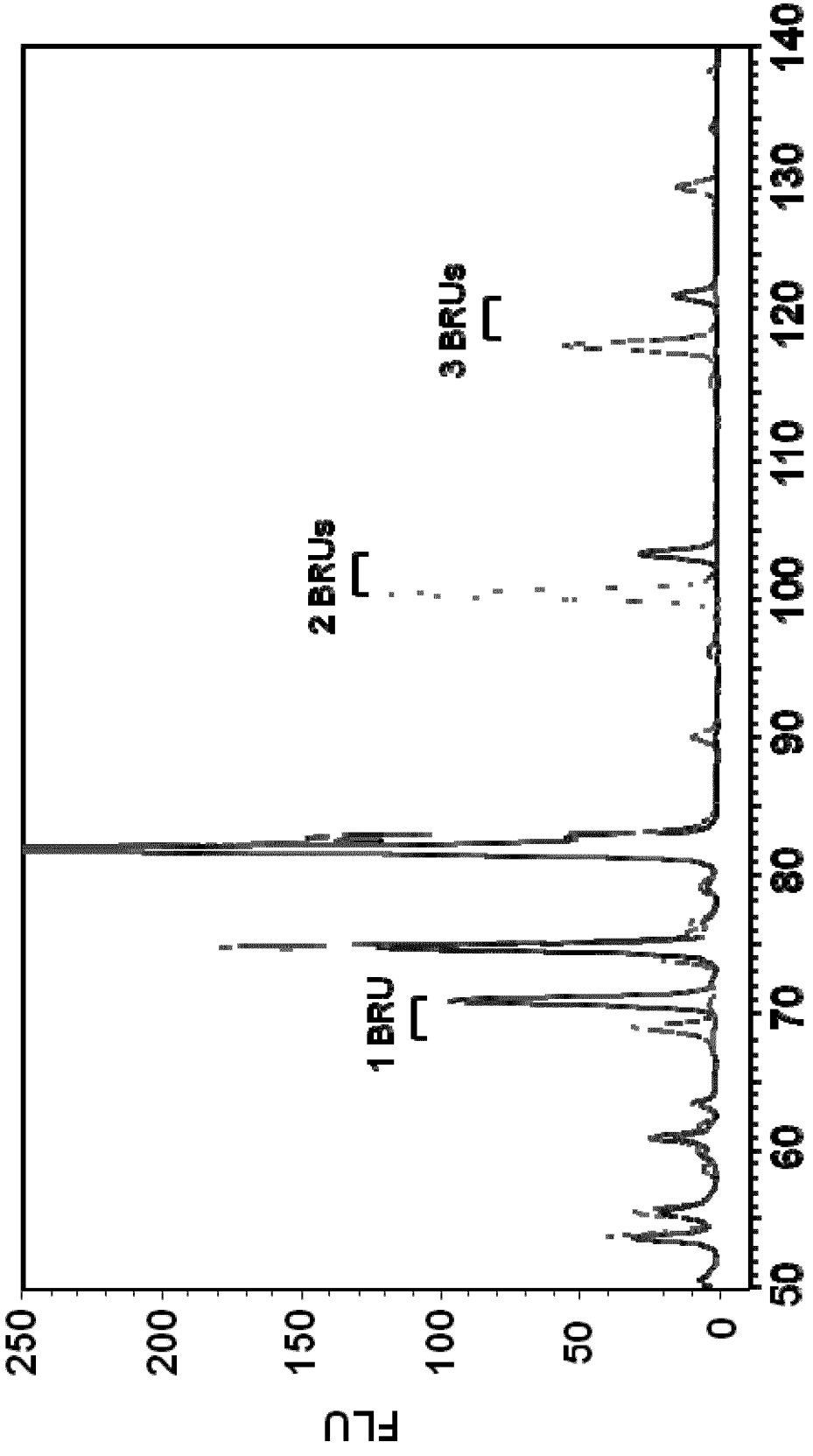

FLU
250
200
150
100
50
0
50
60
70
80
90
100
110
120
130
140
Minutes
1 BRU
2 BRUs
3 BRUs

FIG. 18A

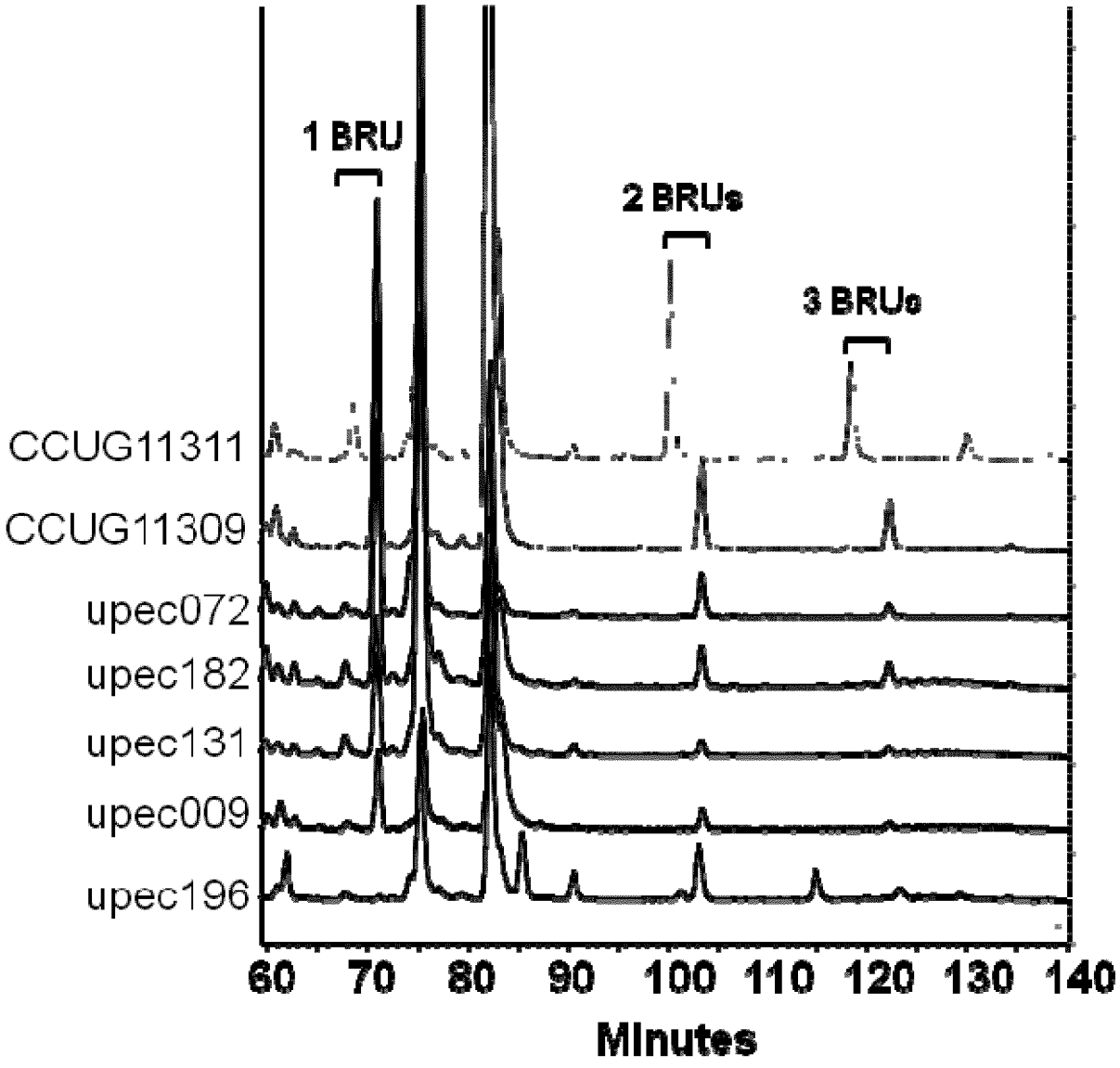

1 BRU
2 BRUs
3 BRUs
CCUG11311
CCUG11309
upec072
upec182
upec131
upec009
upec196
60
70
80
90
100
110
120
130
140
Minutes

FIG. 18B

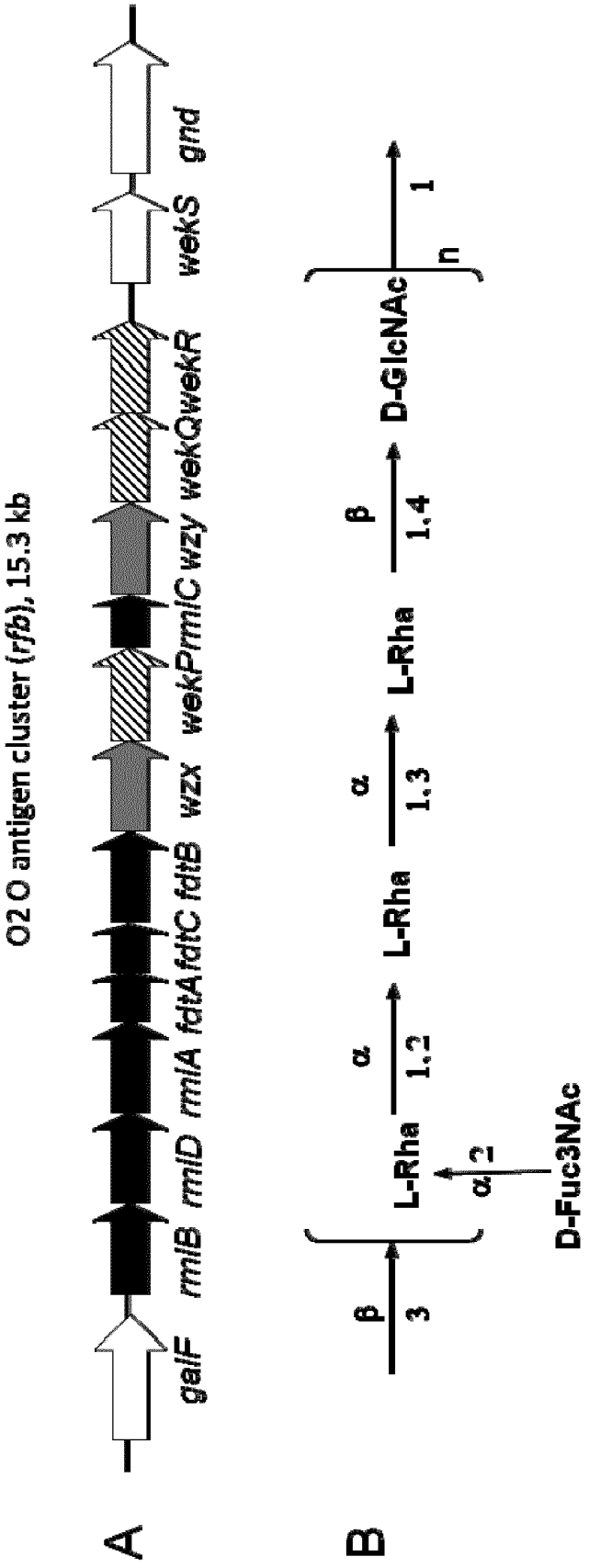

A
O2 O antigen cluster (rfb), 15.3 kb
galF
rmlB
rmlD
rmlA
fdtA
fdtC
fdtB
wzx
wekP
rmlC
wzy
wekQ
wekR
wekS
gnd
B
β
3
L-Rha
α
1,2
L-Rha
α
1,3
L-Rha
β
1,4
D-GlcNAc
1
n
α
2
D-Fuc3NAc

FIG. 19 A-B

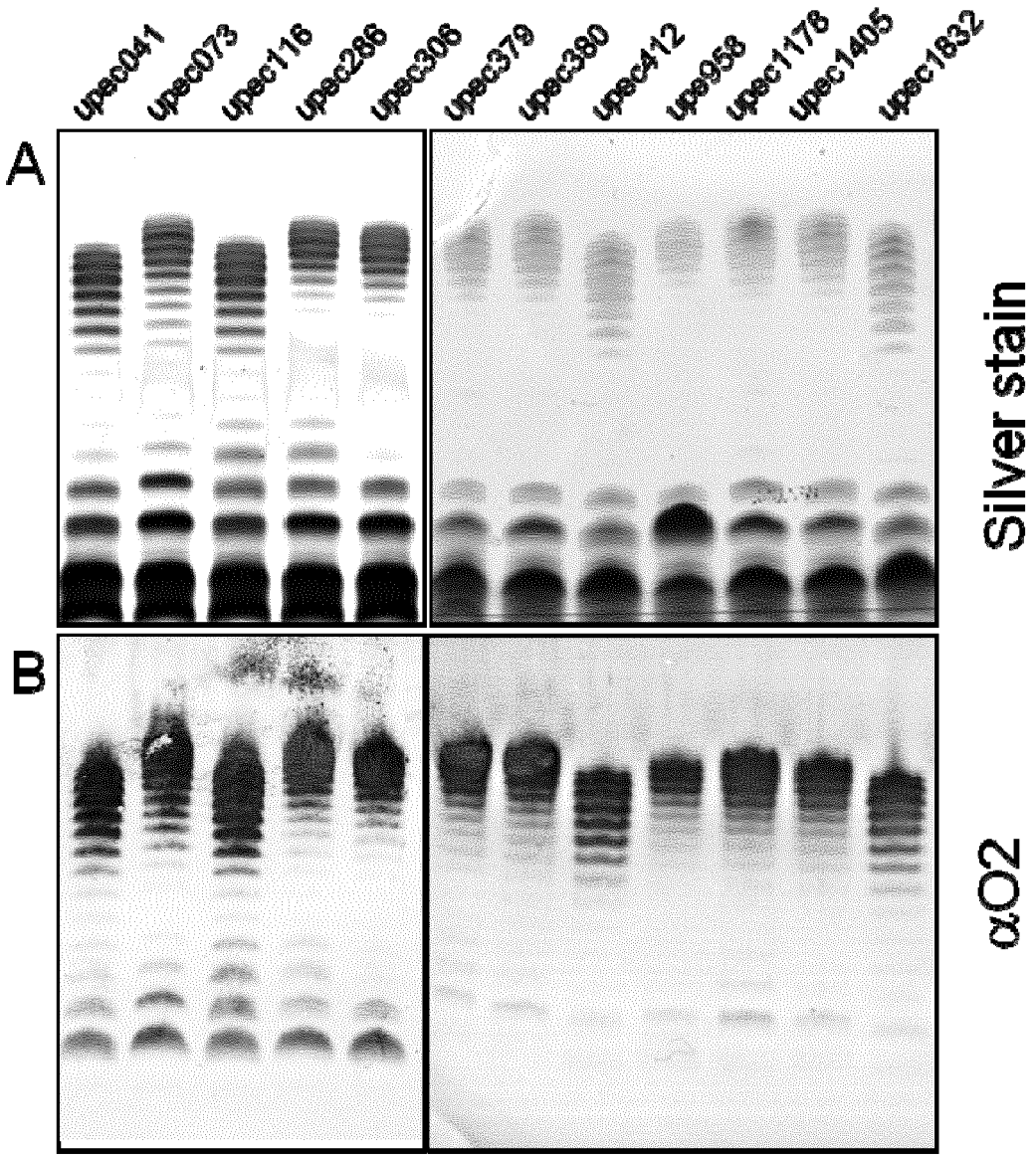

upec041
upec073
upec116
upec286
upec306
upec379
upec380
upec412
upe958
upec1178
upec1405
upec1832
A
B
Silver stain
αO2

FIG. 20 A-B

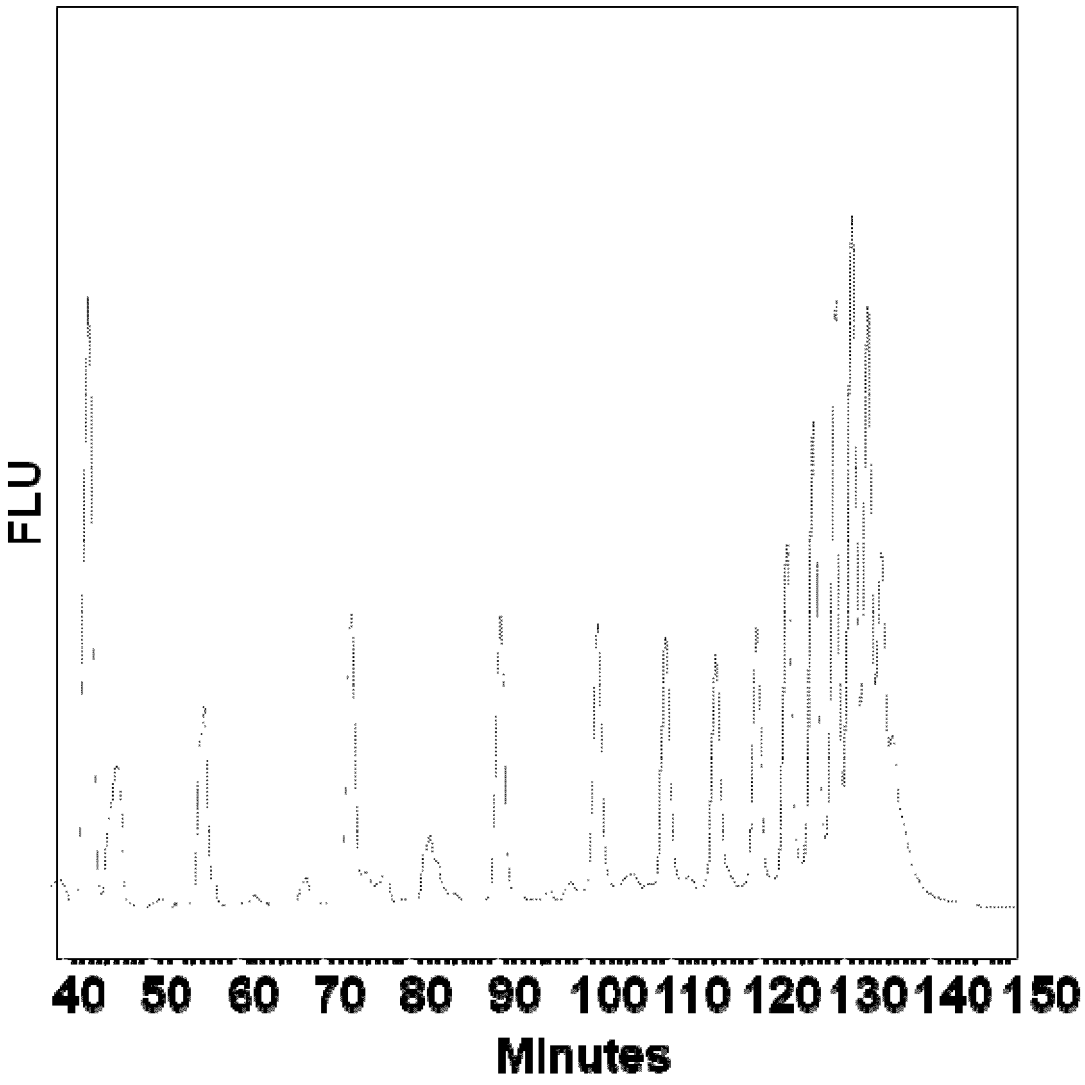


FIG. 21

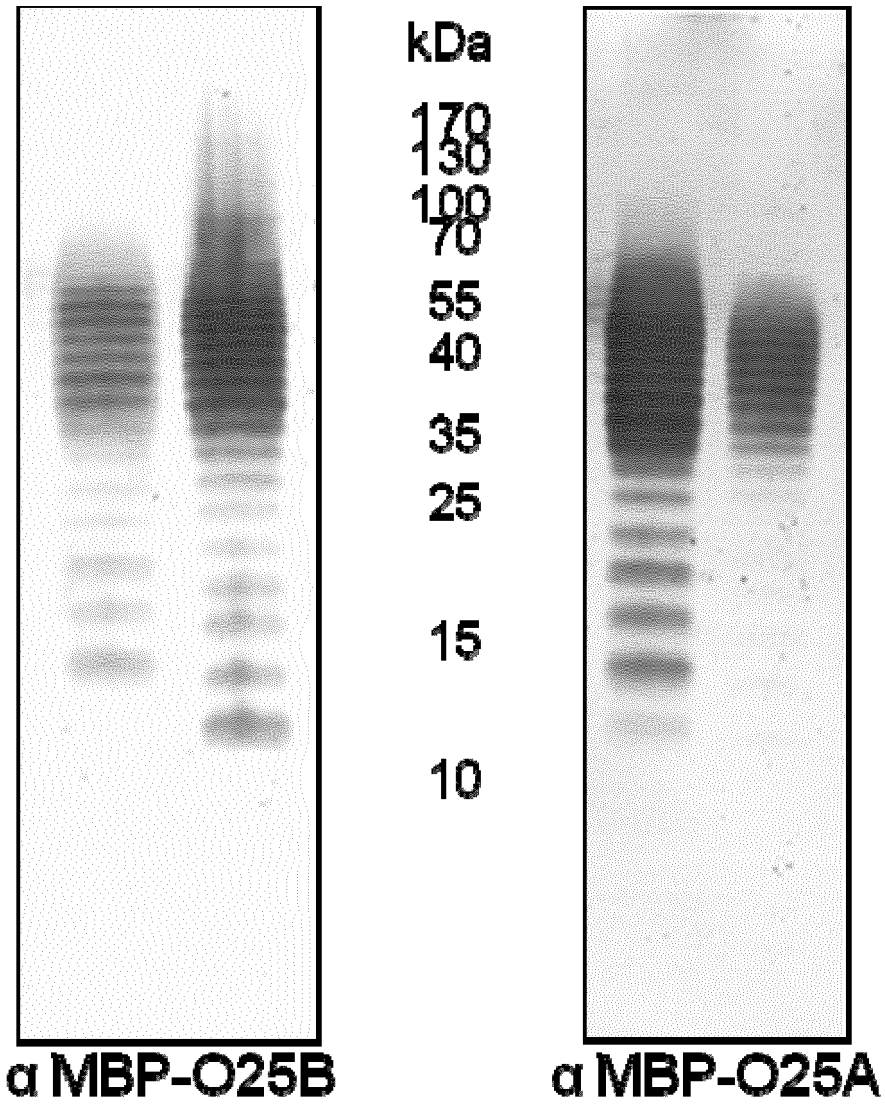


FIG. 22

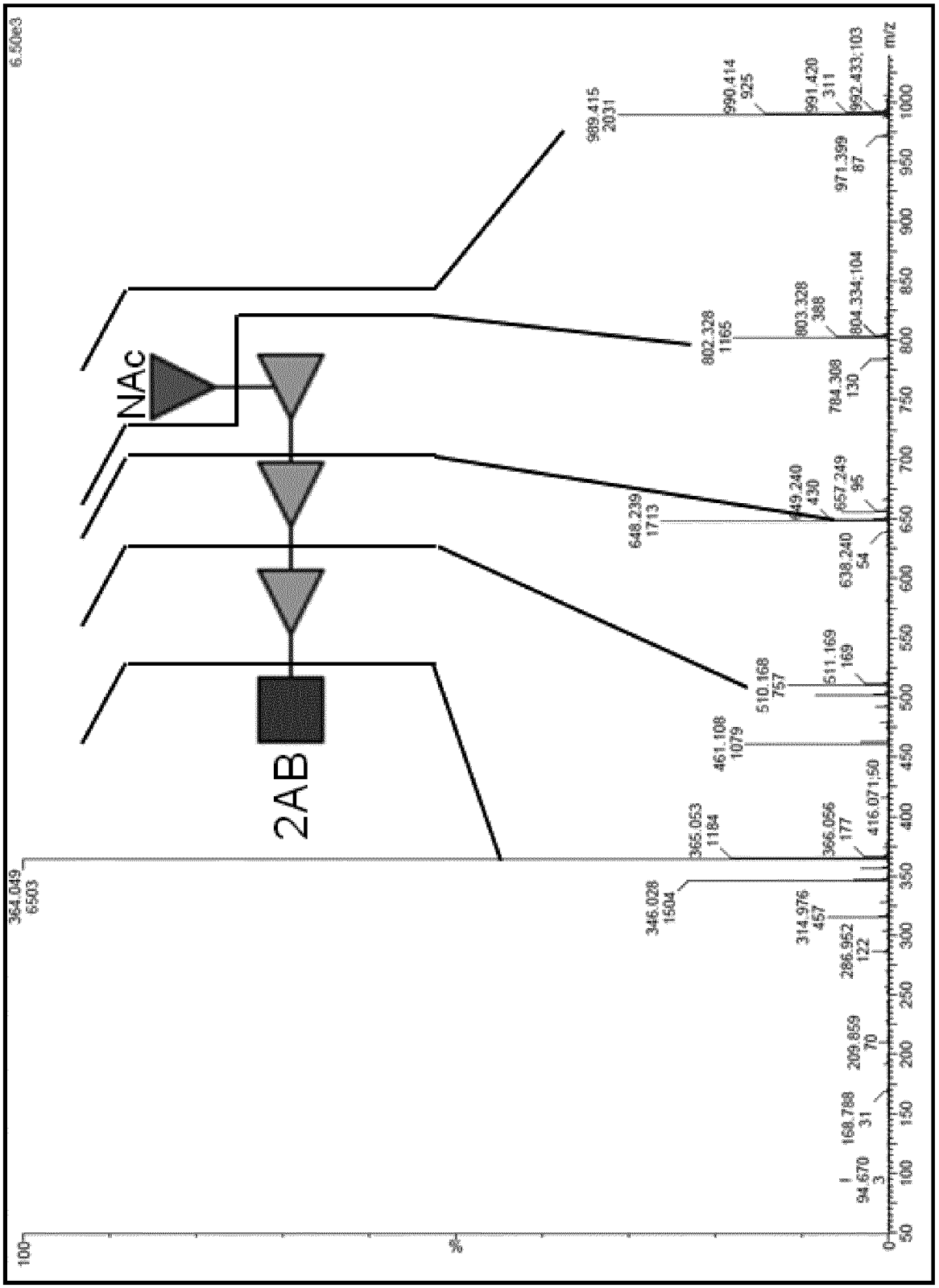

NAc
2AB
6.50e3
100
%
0
m/z
364.049
6503
346.028
1504
365.053
1184
366.056
177
416.071
150
461.108
1079
510.168
757
511.169
169
638.240
54
648.239
1713
649.240
430
657.249
95
784.368
130
802.328
1165
803.328
388
804.334
104
969.415
2031
990.414
925
991.420
311
992.433
103
971.399
87
314.976
457
286.952
122
209.859
70
168.788
31
94.670
3

FIG. 23

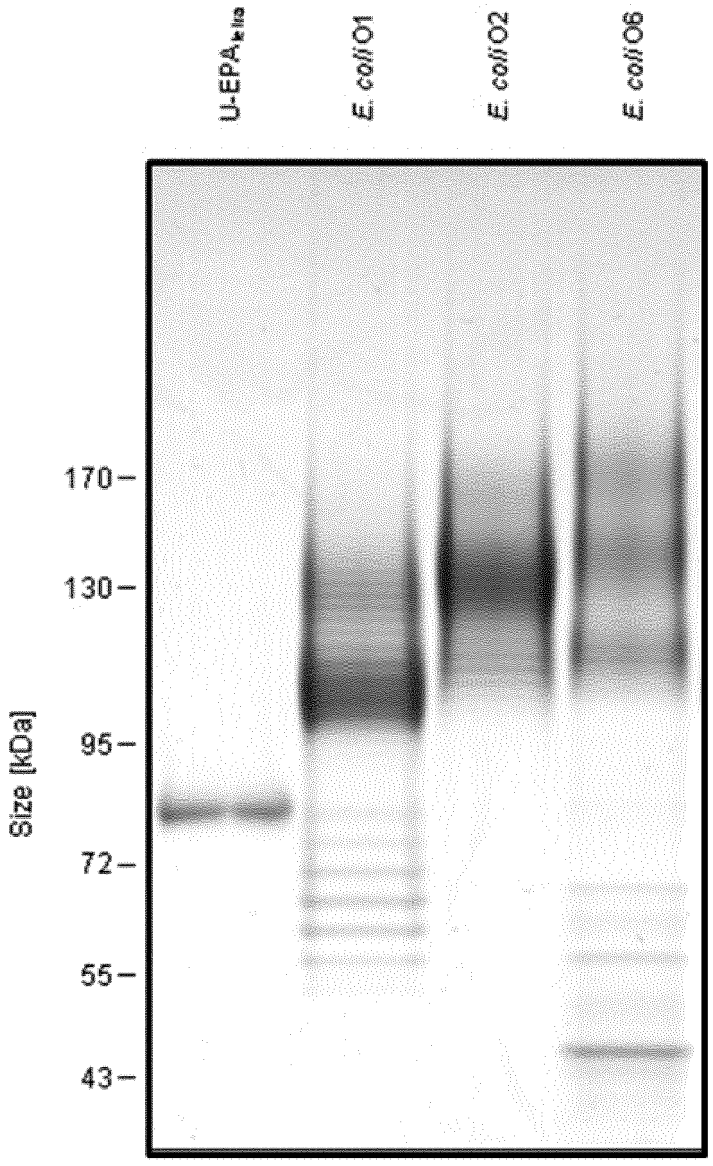


FIG. 24

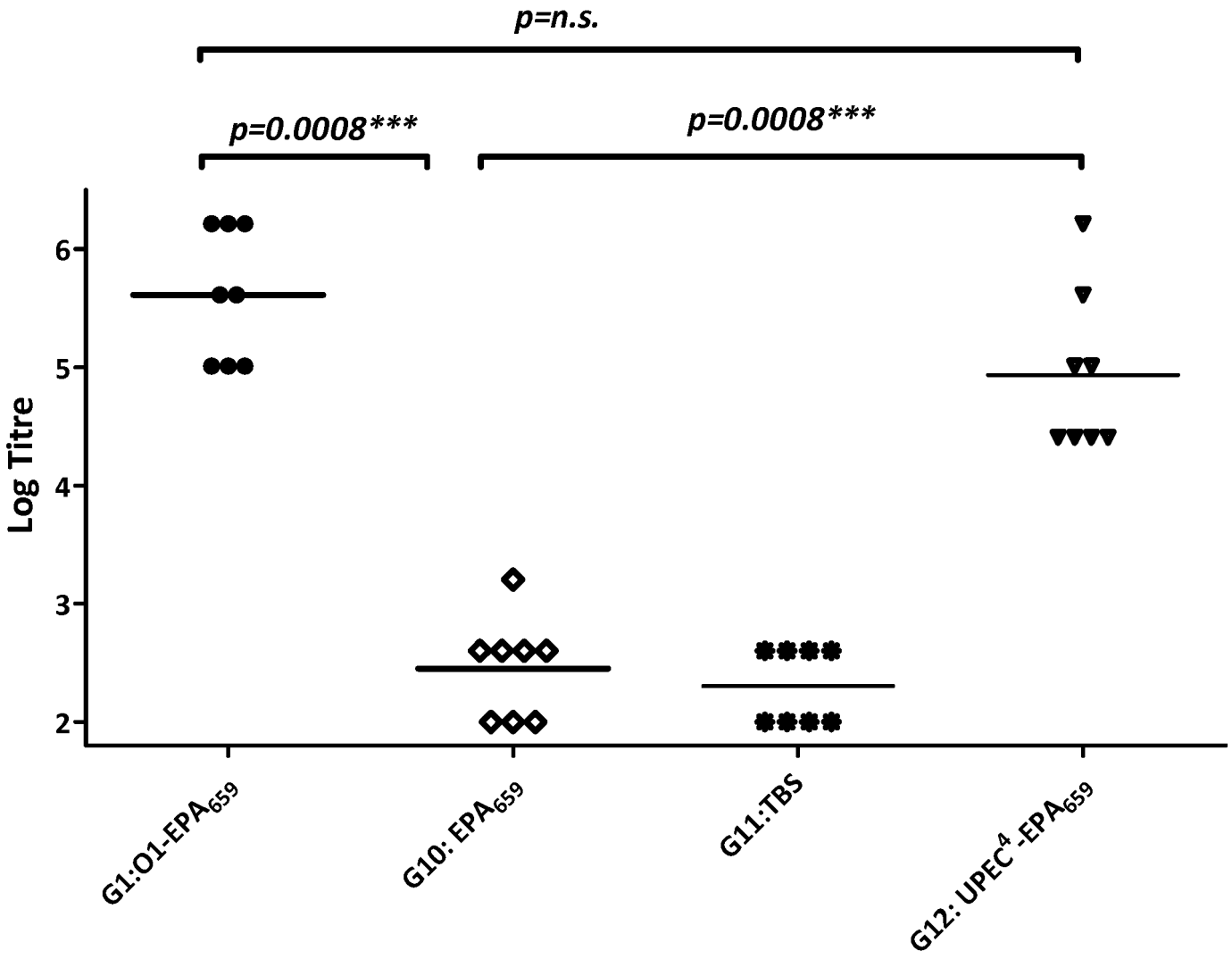

p=n.s.
p=0.0008***
p=0.0008***
Log Titre
6
5
4
3
2
G1:O1-EPA659
G10: EPA659
G11:TBS
G12: UPEC4 -EPA659

FIG. 25

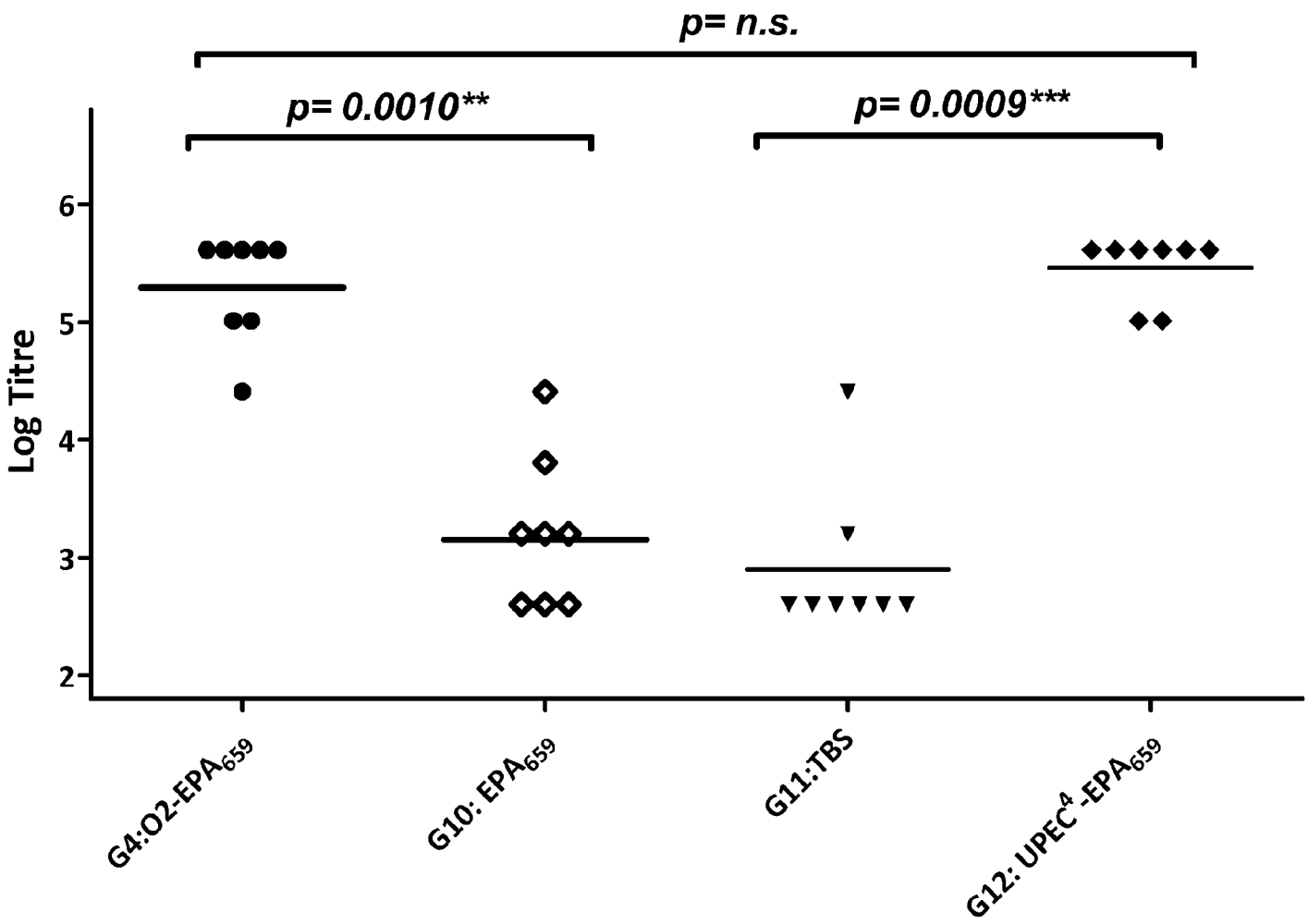

p= n.s.
p= 0.0010**
p= 0.0009***
Log Titre
6
5
4
3
2
G4:O2-EPA659
G10: EPA659
G11:TBS
G12: UPEC4 -EPA659

FIG. 26

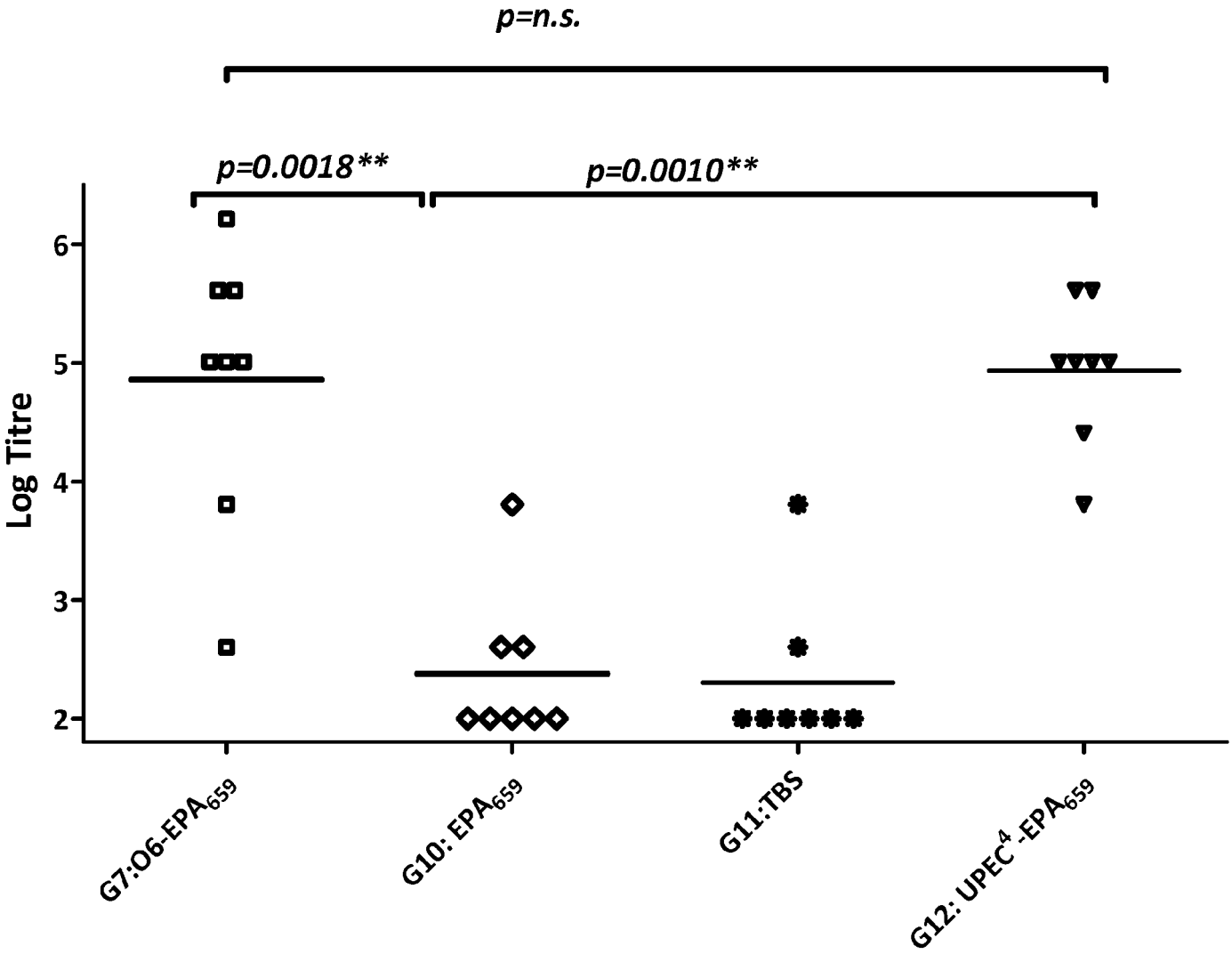

p=n.s.
p=0.0018**
p=0.0010**
Log Titre
6
5
4
3
2
G7:O6-EPA659
G10: EPA659
G11:TBS
G12: UPEC4 -EPA659

FIG. 27

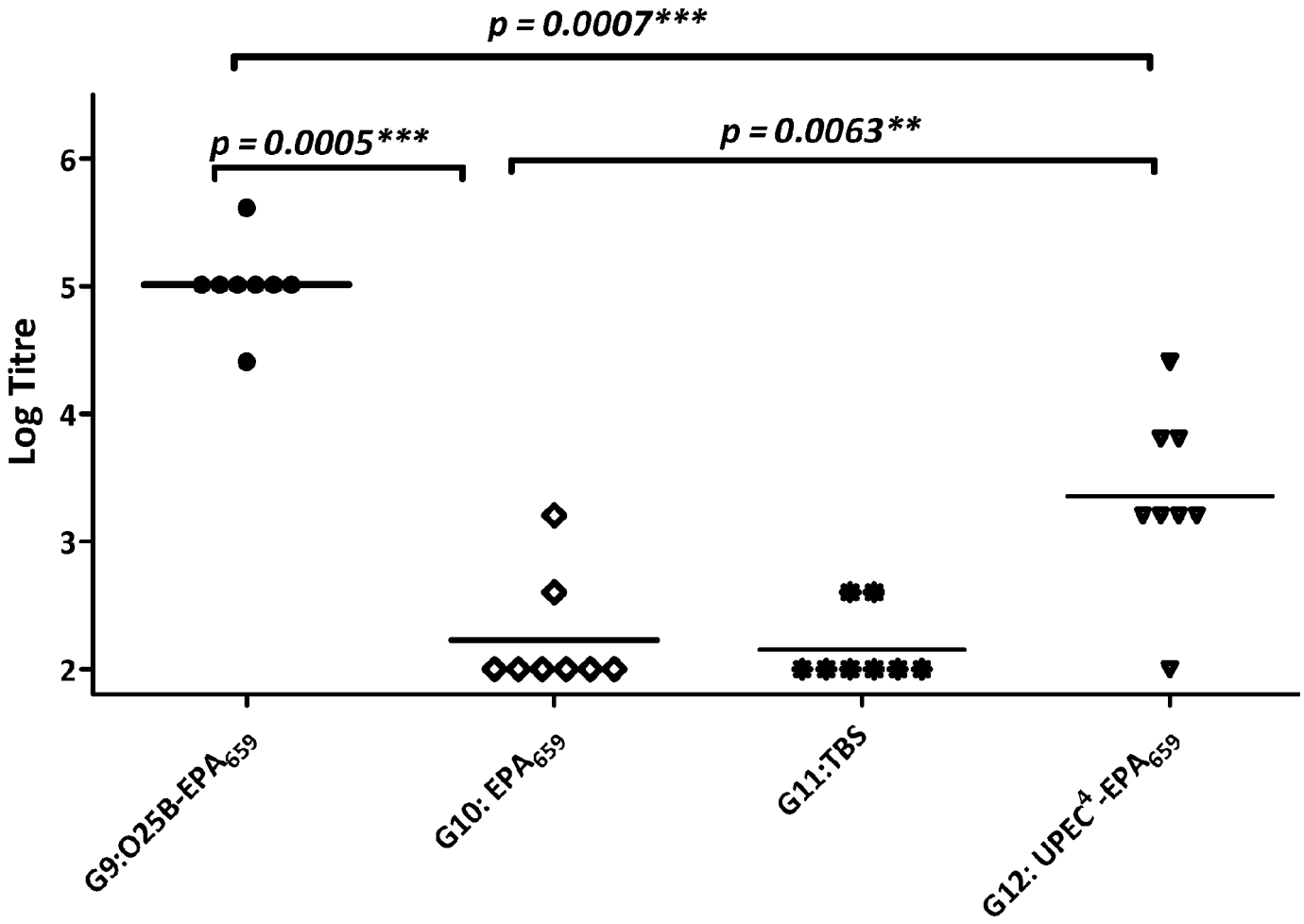

p = 0.0007***
p = 0.0005***
p = 0.0063**
Log Titre
6
5
4
3
2
G9:O25B-EPA659
G10: EPA659
G11:TBS
G12: UPEC4 -EPA659

FIG. 28

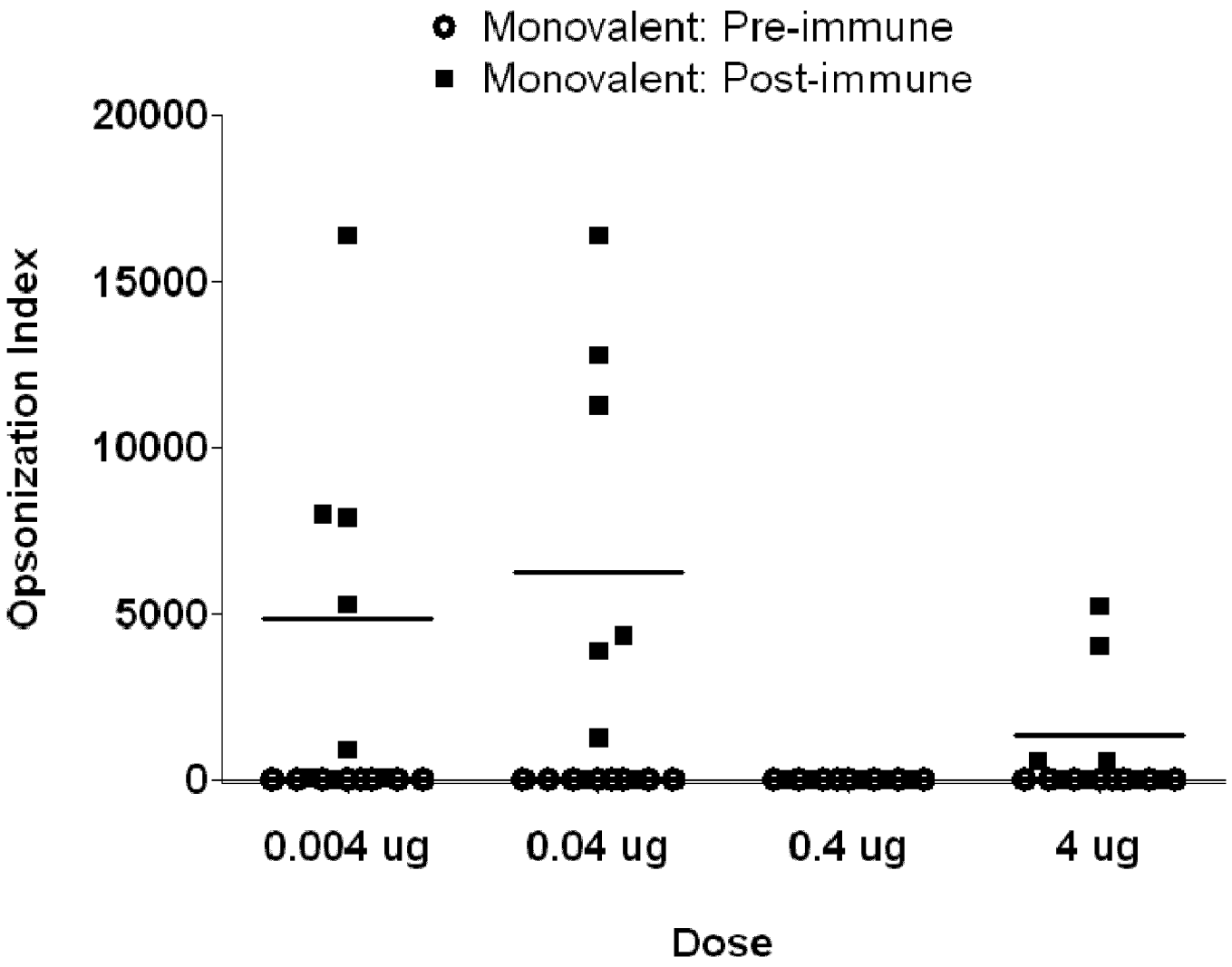

Monovalent: Pre-immune
Monovalent: Post-immune
Opsonization Index
20000
15000
10000
5000
0
0.004 ug
0.04 ug
0.4 ug
4 ug
Dose

FIG. 29A

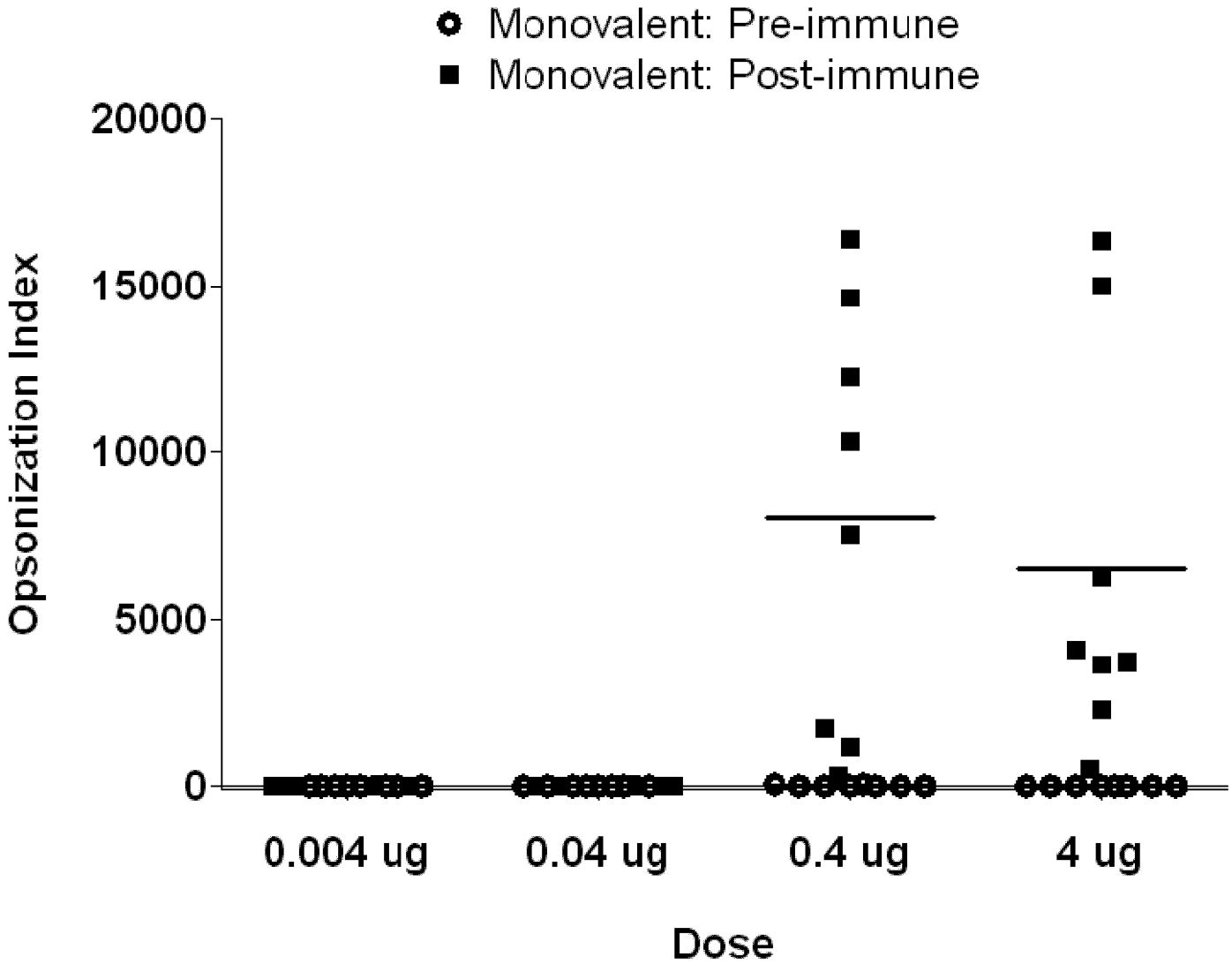

Monovalent: Pre-immune
Monovalent: Post-immune
Opsonization Index
20000
15000
10000
5000
0
0.004 ug
0.04 ug
0.4 ug
4 ug
Dose

FIG. 29B

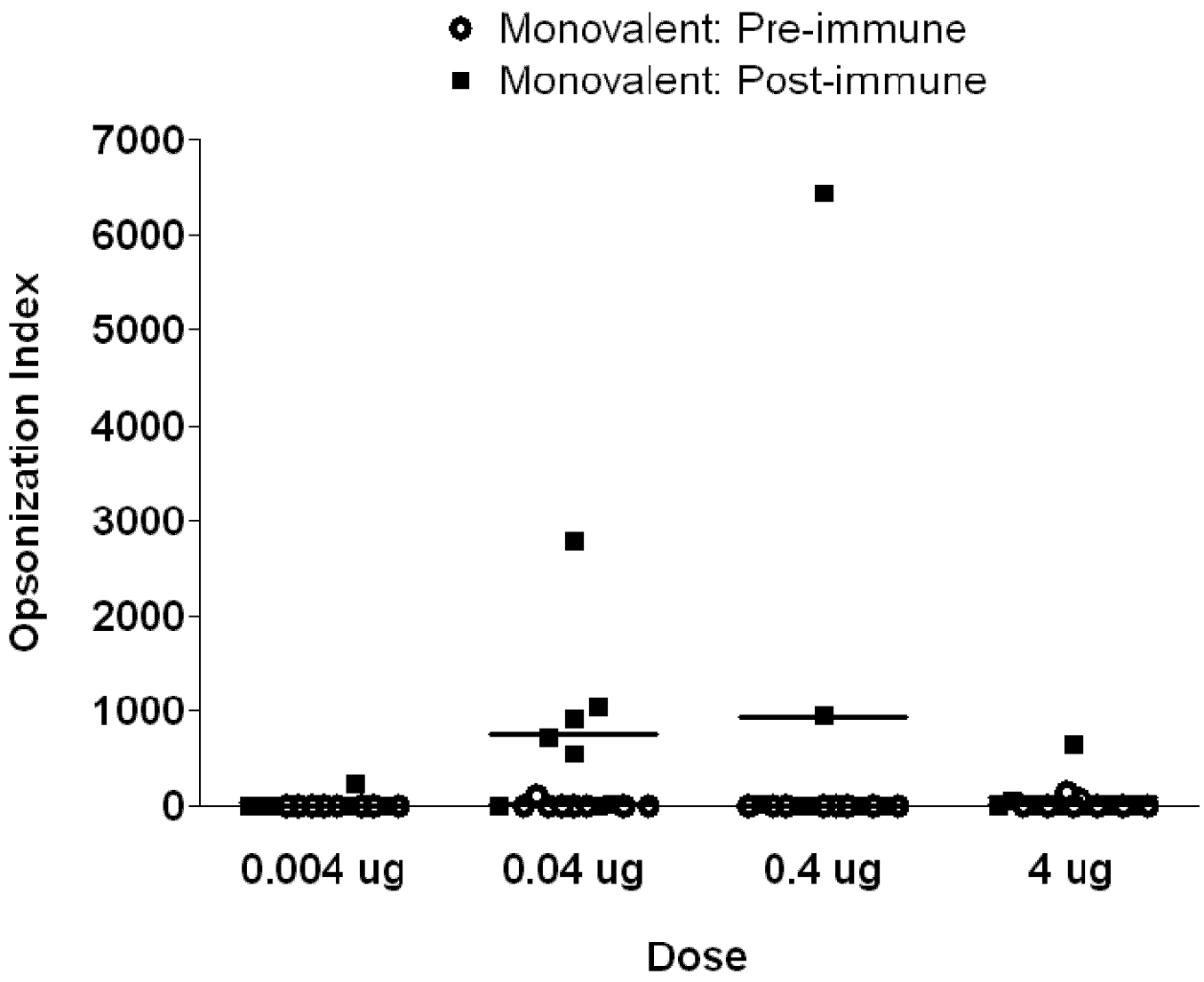

Monovalent: Pre-immune
Monovalent: Post-immune
Opsonization Index
7000
6000
5000
4000
3000
2000
1000
0
0.004 ug
0.04 ug
0.4 ug
4 ug
Dose

FIG. 29C

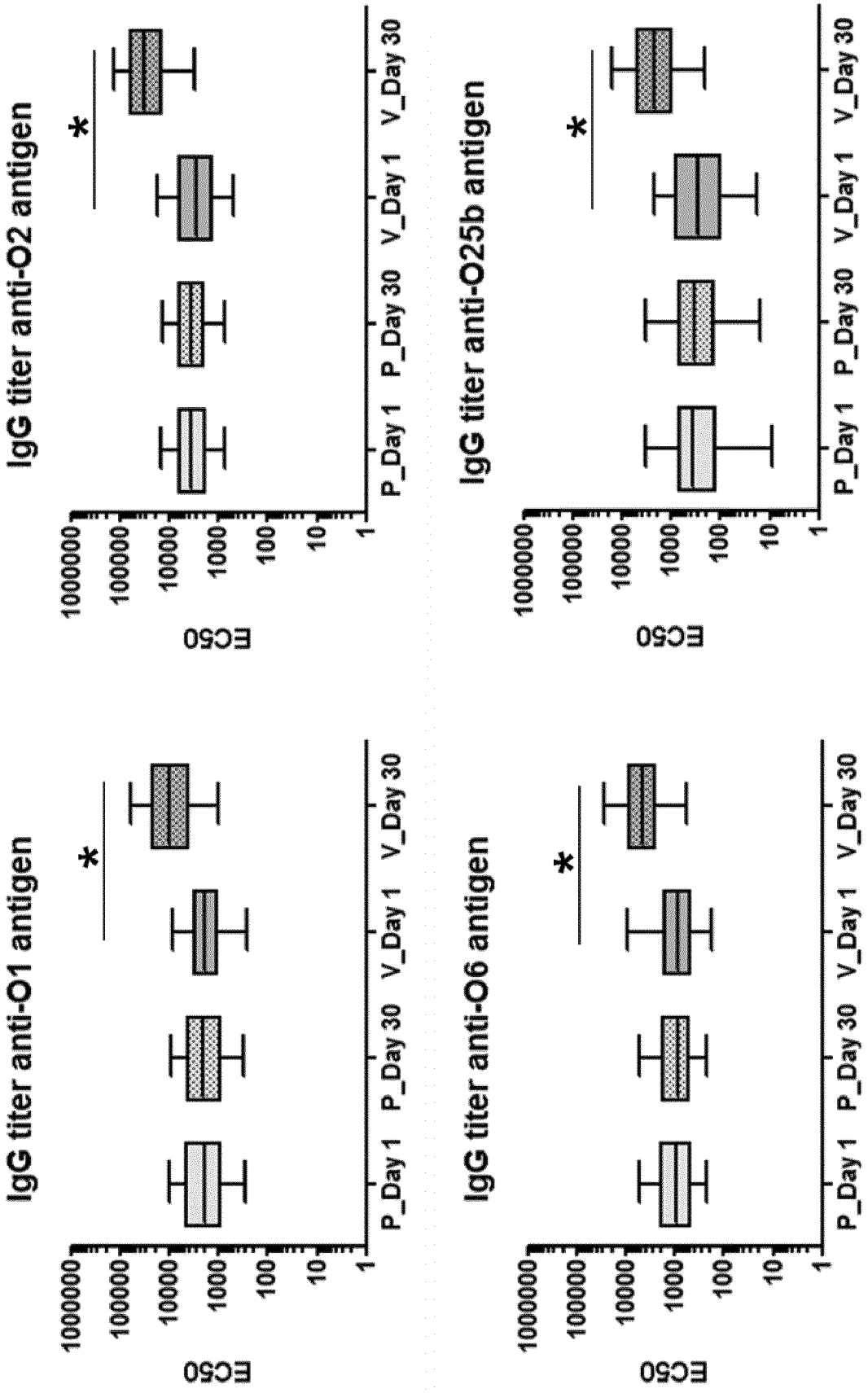

IgG titer anti-O1 antigen
IgG titer anti-O2 antigen
IgG titer anti-O25b antigen
IgG titer anti-O6 antigen
EC50
1000000
100000
10000
1000
100
10
1
*
P_Day 1
P_Day 30
V_Day 1
V_Day 30

FIG. 30

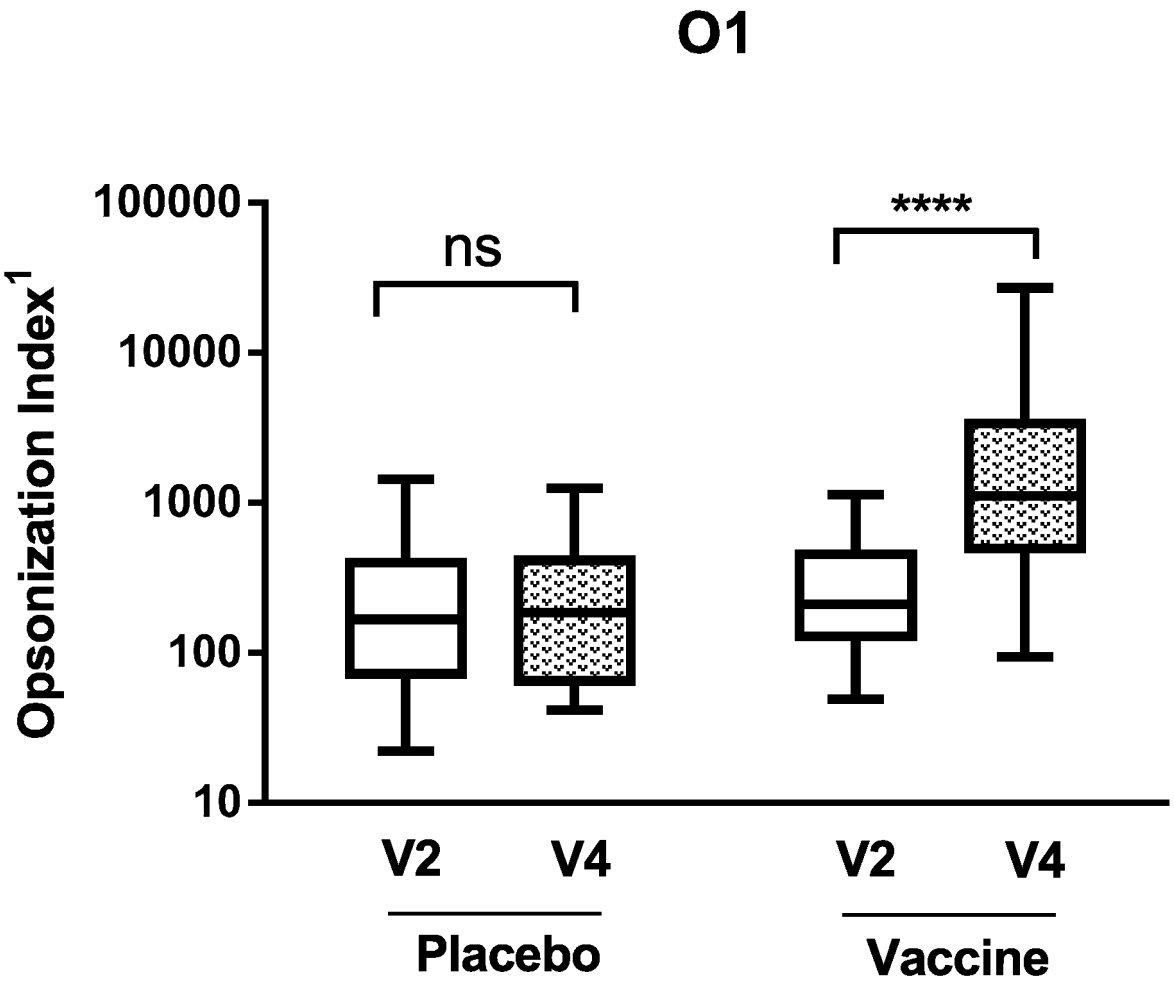

O1
Opsonization Index1
100000
10000
1000
100
10
ns
****
V2
V4
V2
V4
Placebo
Vaccine

FIG. 31A

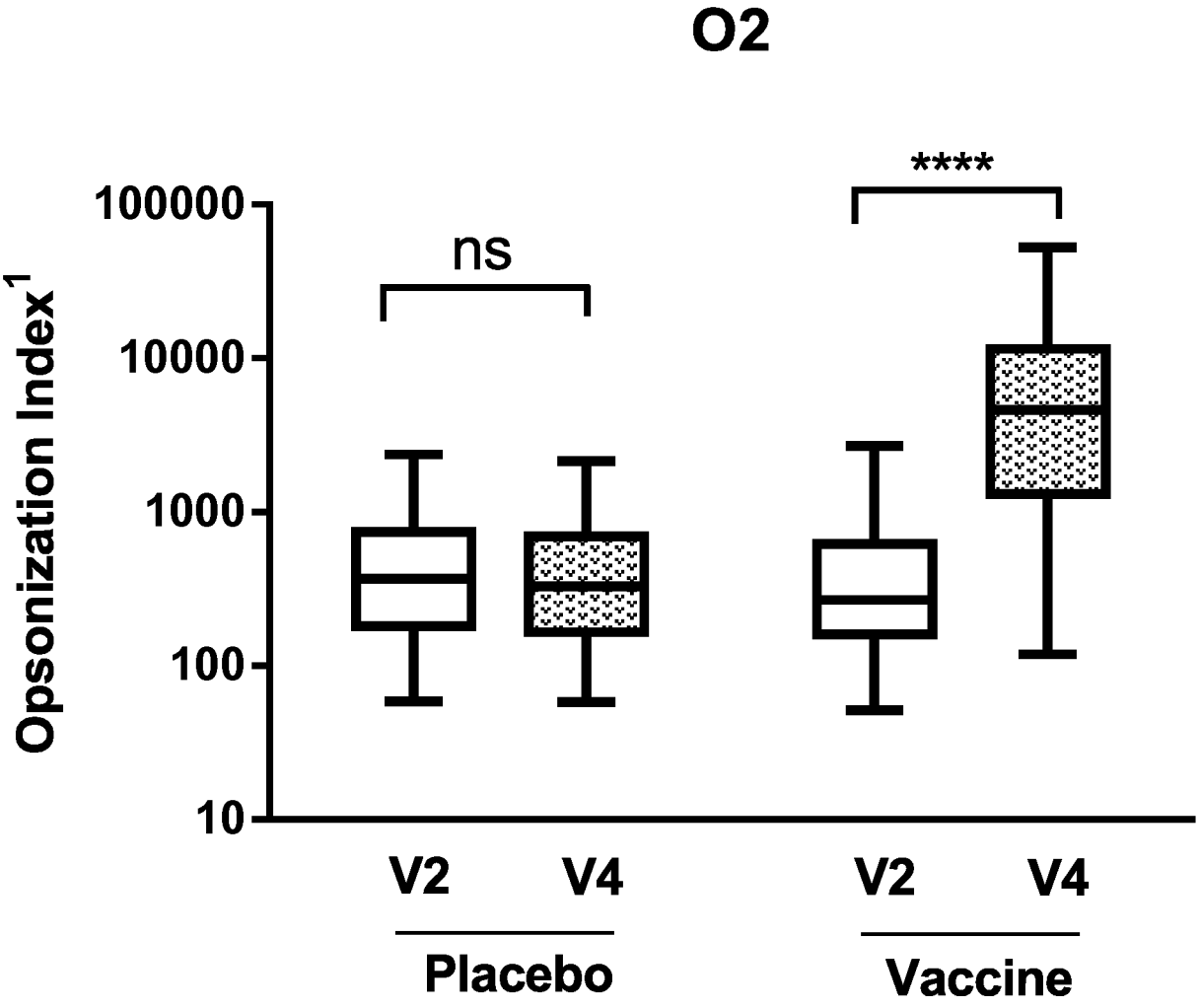

O2
****
ns
100000
10000
1000
100
10
Opsonization Index1
V2
V4
V2
V4
Placebo
Vaccine

FIG. 31B

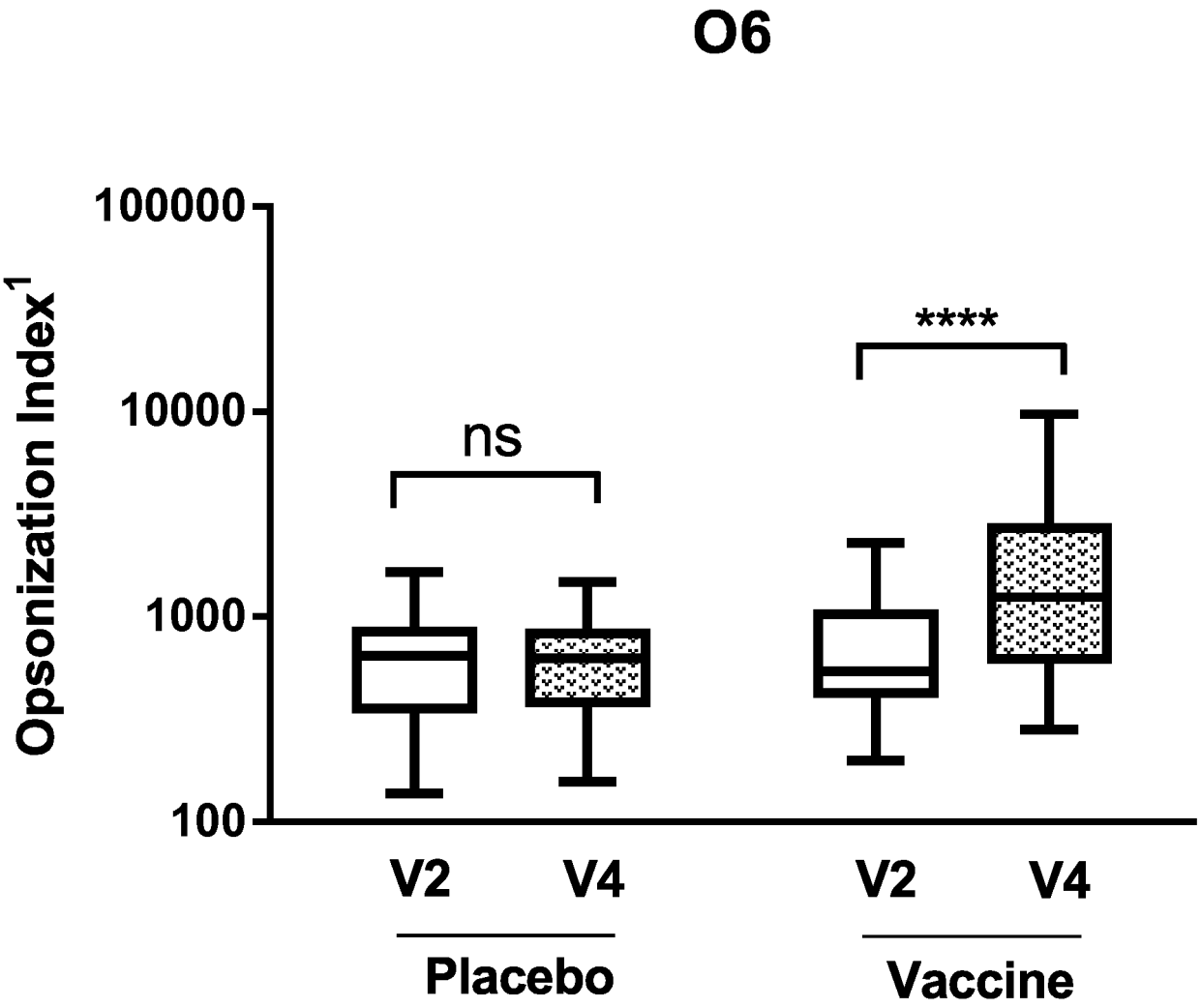

O6
Opsonization Index1
100000
10000
1000
100
ns
****
V2
V4
V2
V4
Placebo
Vaccine

FIG. 31C

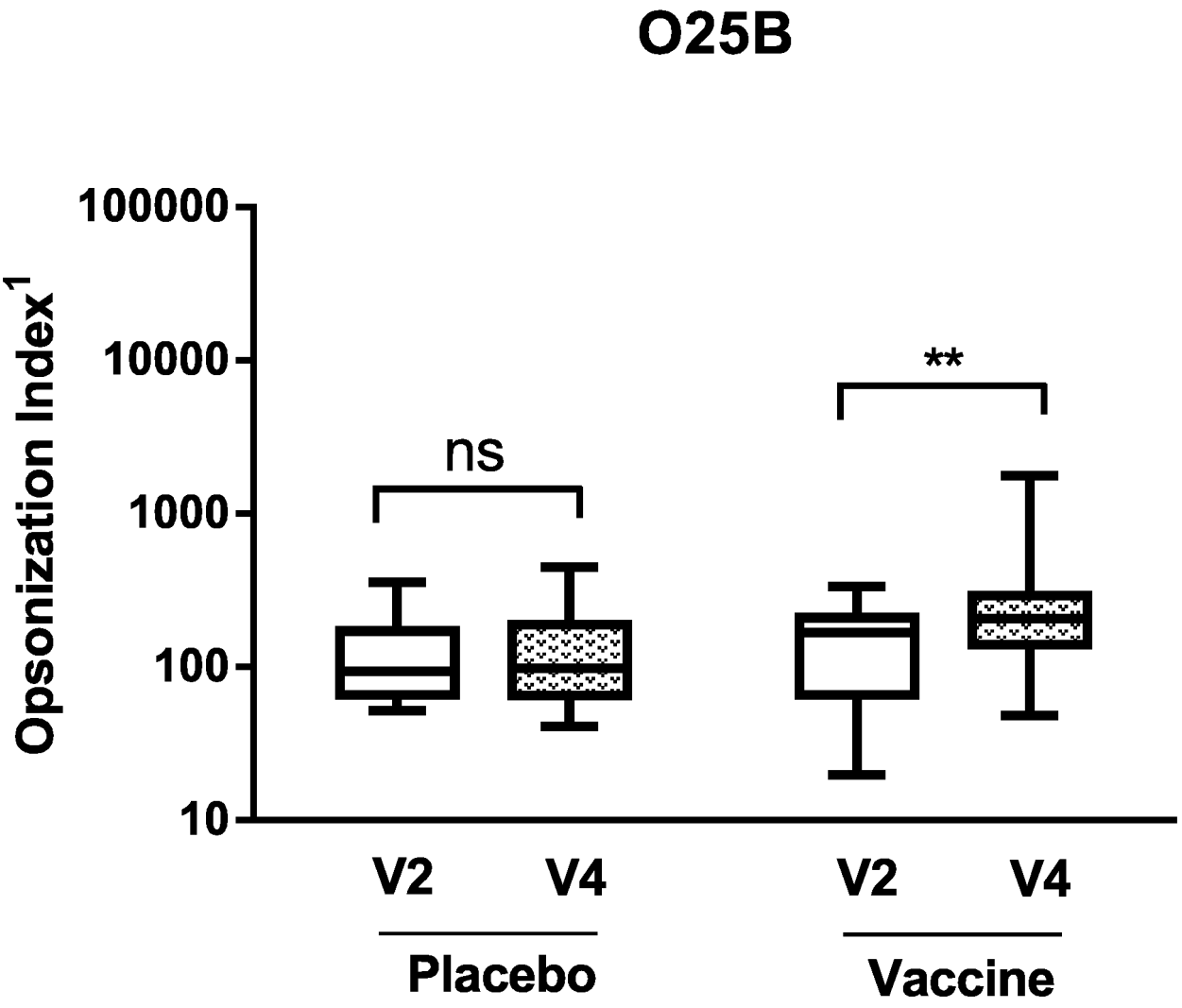

O25B
Opsonization Index[1]
100000
10000
1000
100
10
ns
**
V2
V4
V2
V4
Placebo
Vaccine

FIG. 31D

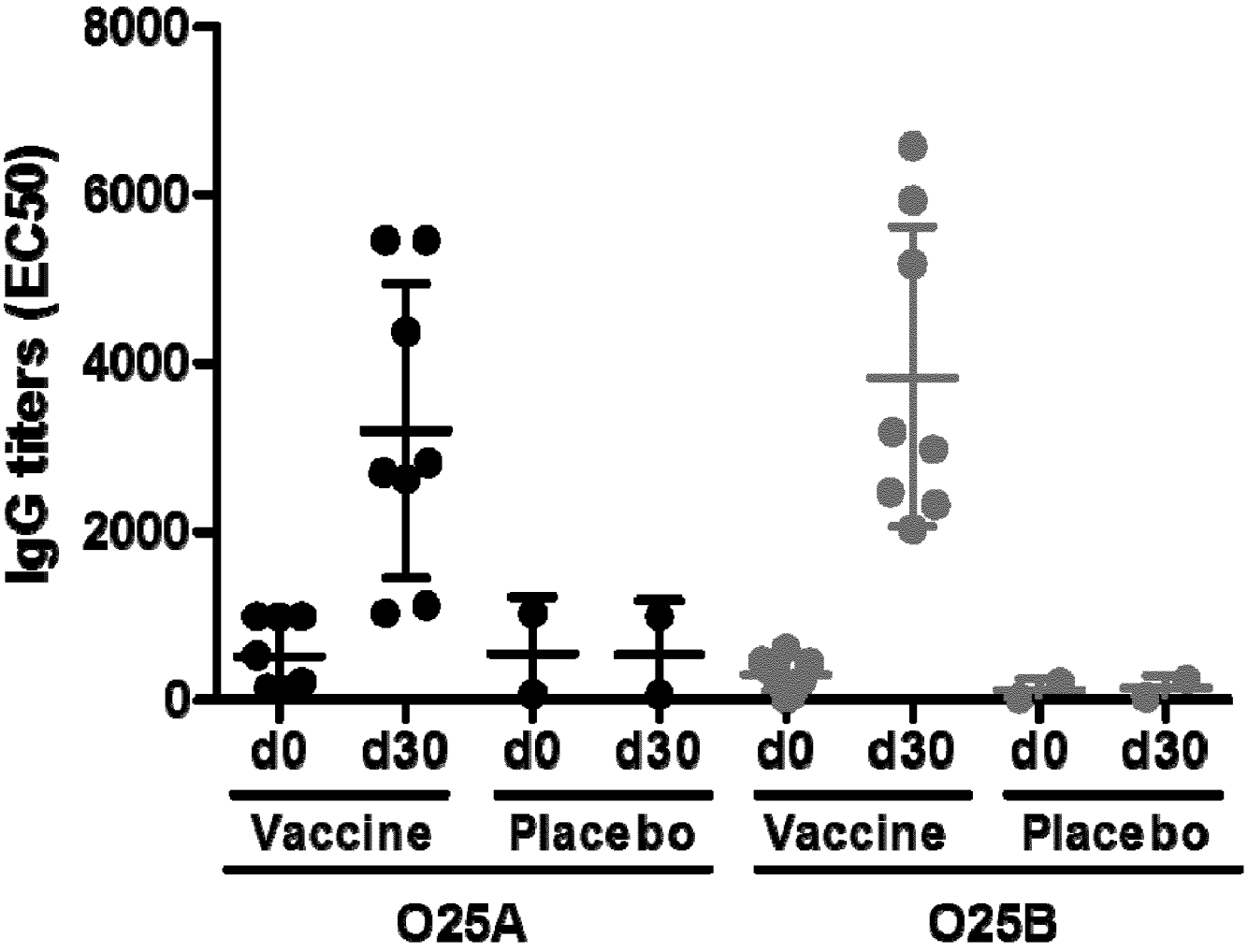

IgG titers (EC50)
8000
6000
4000
2000
0
d0
d30
d0
d30
d0
d30
d0
d30
Vaccine
Placebo
Vaccine
Placebo
O25A
O25B

FIG. 32

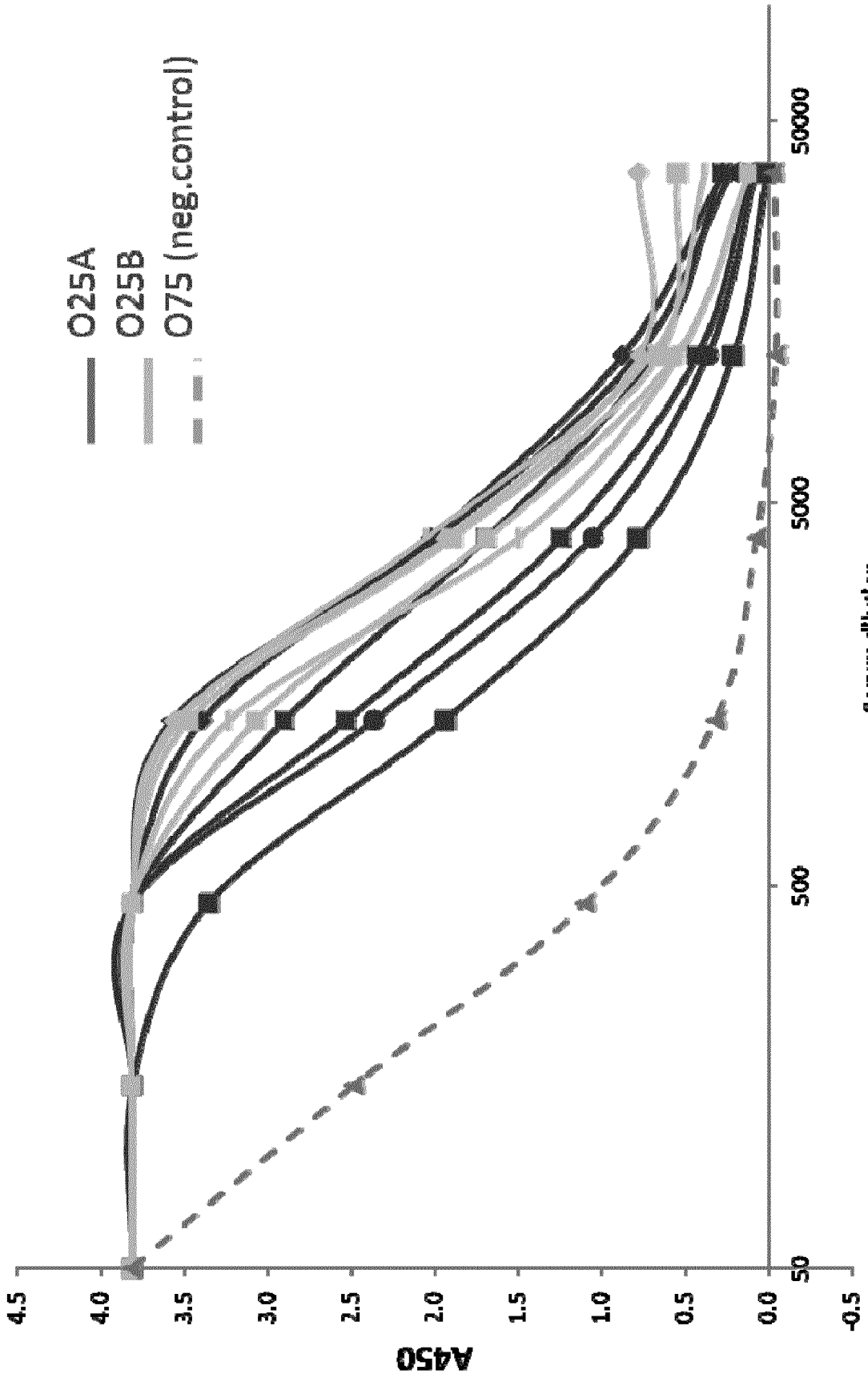

O25A
O25B
O75 (neg.control)
A450
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
50
500
5000
50000
Serum dilution

FIG. 33