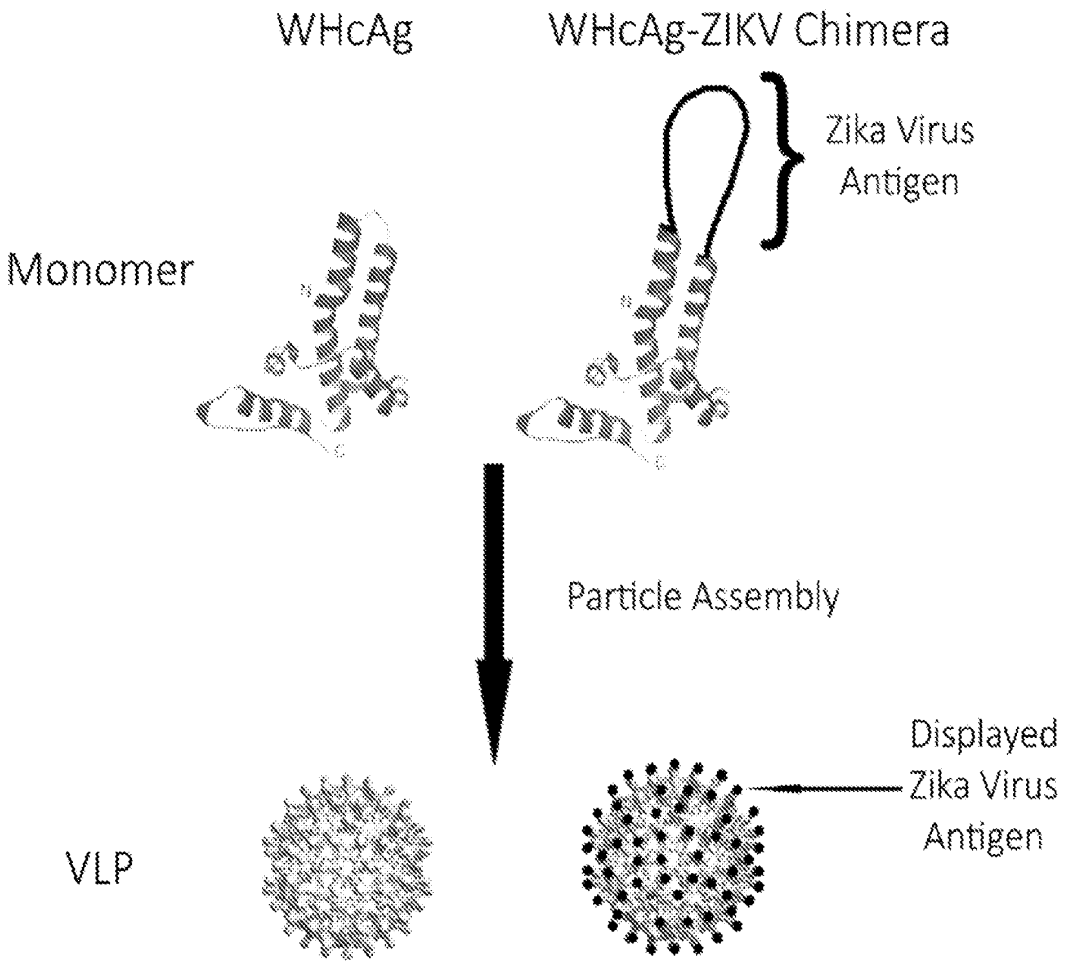

WHcAg
WHcAg-ZIKV Chimera
Zika Virus Antigen
Monomer
Particle Assembly
Displayed Zika Virus Antigen
VLP

FIG. 1

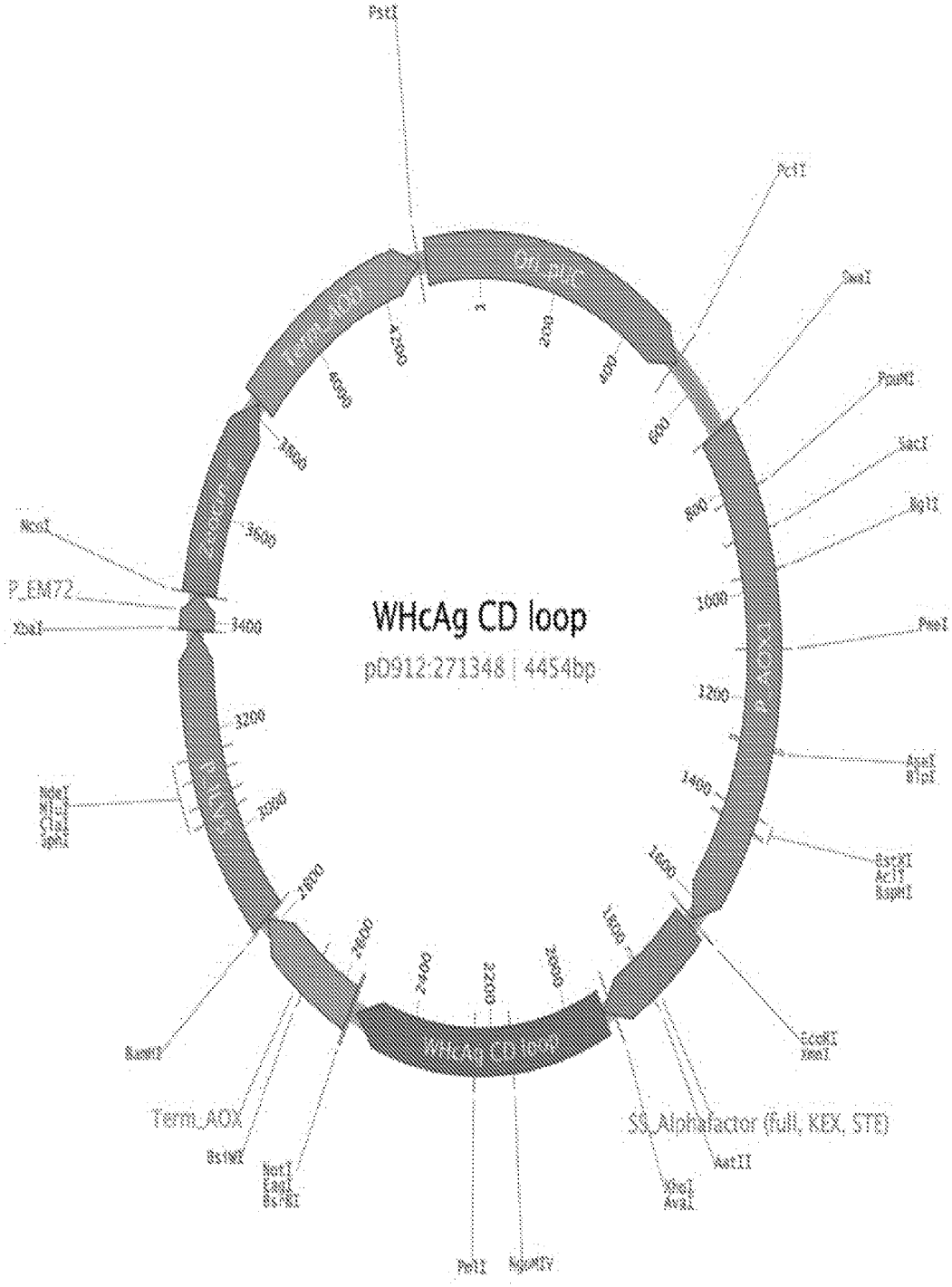
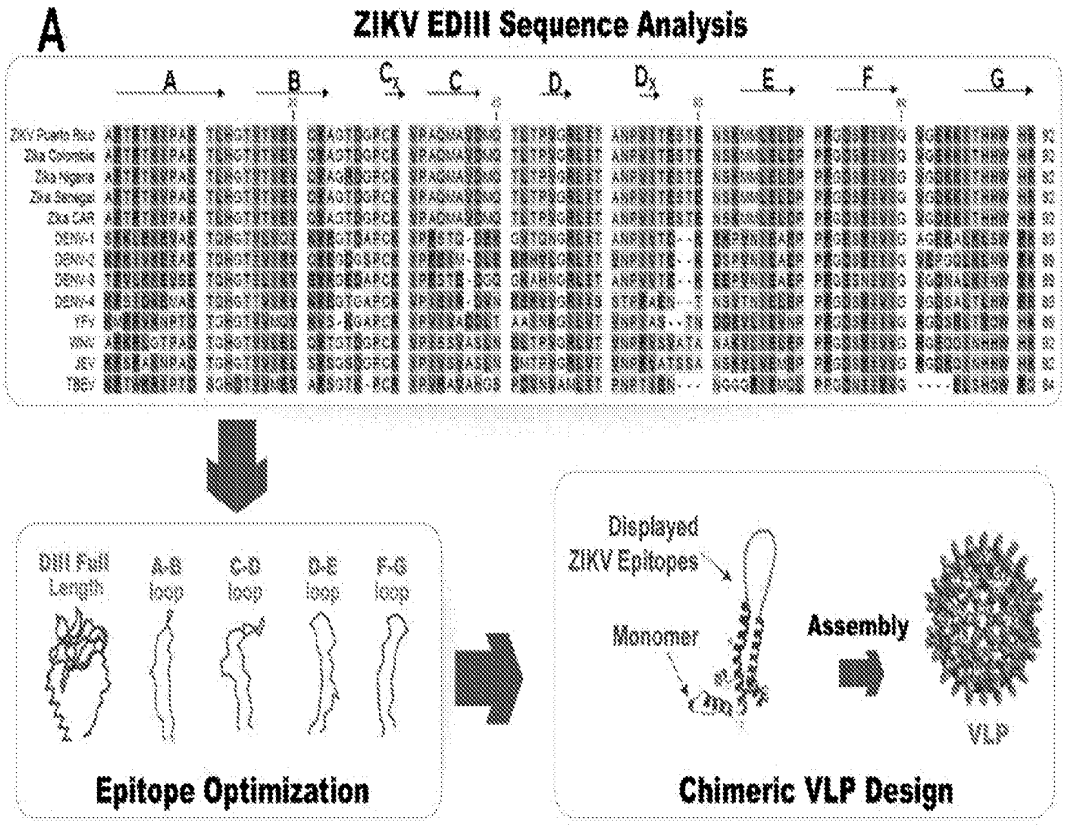

WHcAg CD loop
pD912:271348 | 4454bp
P_EM72
Term_AOX
SS_Alphafactor (full, KEX, STE)

FIG. 2


A
ZIKV EDIII Sequence Analysis
A
B
Cx
C
D
Dx
E
F
G
ZIKV Puerto Rico
Zika Colombia
Zika Nigeria
Zika Senegal
Zika CAR
DENV-1
DENV-2
DENV-3
DENV-4
YFV
WNV
JEV
TBEV
DIII Full Length
A-B loop
C-D loop
D-E loop
F-G loop
Epitope Optimization
Displayed ZIKV Epitopes
Monomer
Assembly
VLP
Chimeric VLP Design

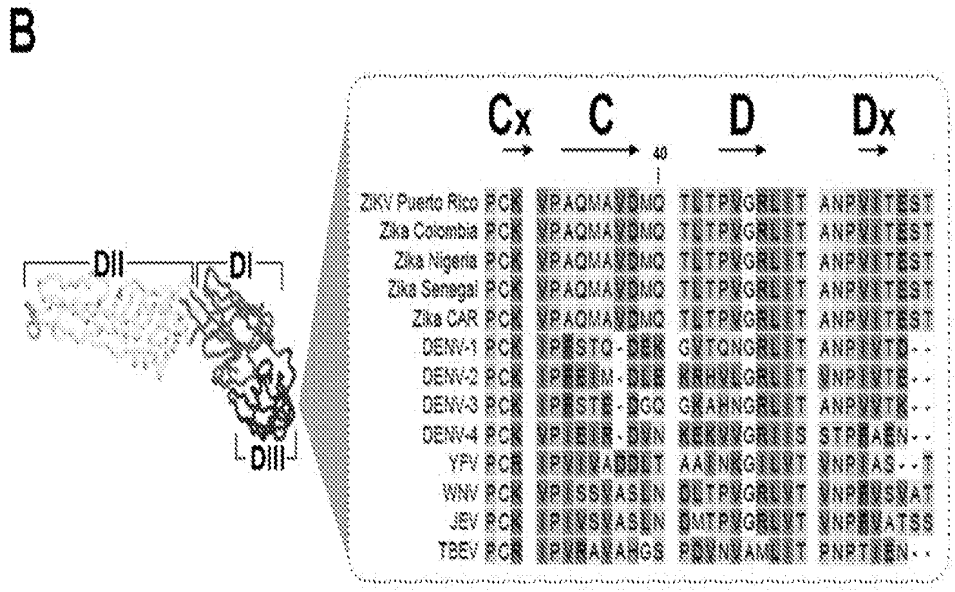

B
DII
DI
DIII
Cx
C
D
Dx
40
ZIKV Puerto Rico
Zika Colombia
Zika Nigeria
Zika Senegal
Zika CAR
DENV-1
DENV-2
DENV-3
DENV-4
YFV
WNV
JEV
TBEV

FIG. 3

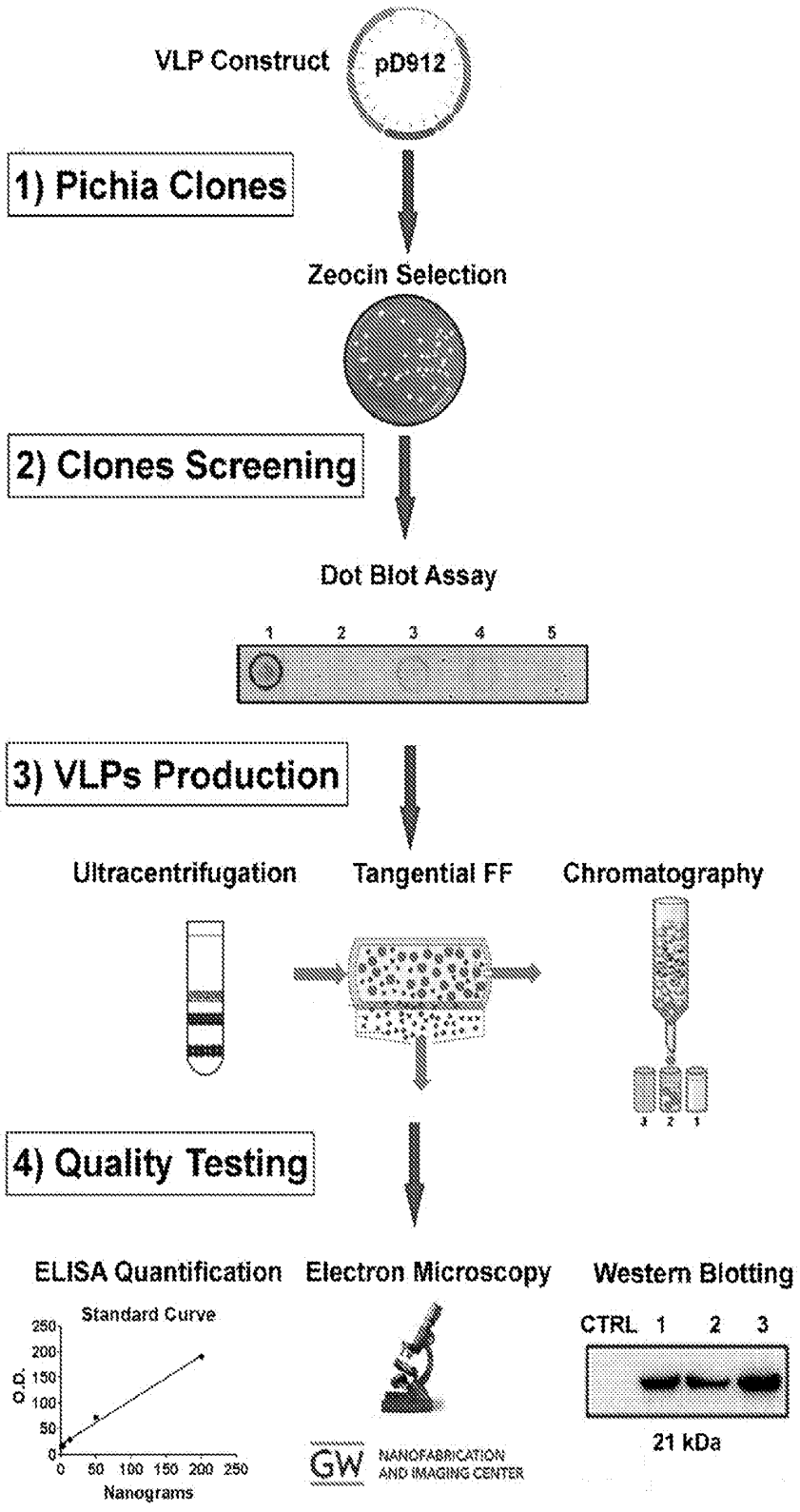

VLP Construct
pD912
1) Pichia Clones
Zeocin Selection
2) Clones Screening
Dot Blot Assay
1 2 3 4 5
3) VLPs Production
Ultracentrifugation
Tangential FF
Chromatography
4) Quality Testing
ELISA Quantification
Standard Curve
O.D.
250
200
150
100
50
0
0 50 100 150 200 250
Nanograms
Electron Microscopy
GW NANOFABRICATION AND IMAGING CENTER
Western Blotting
CTRL 1 2 3
21 kDa

FIG. 4

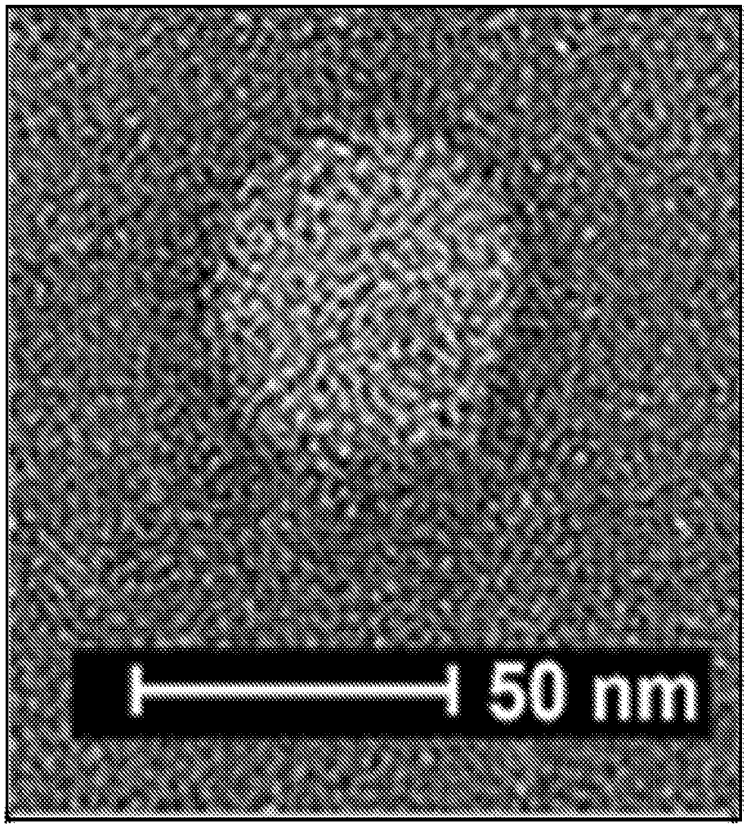


FIG. 5

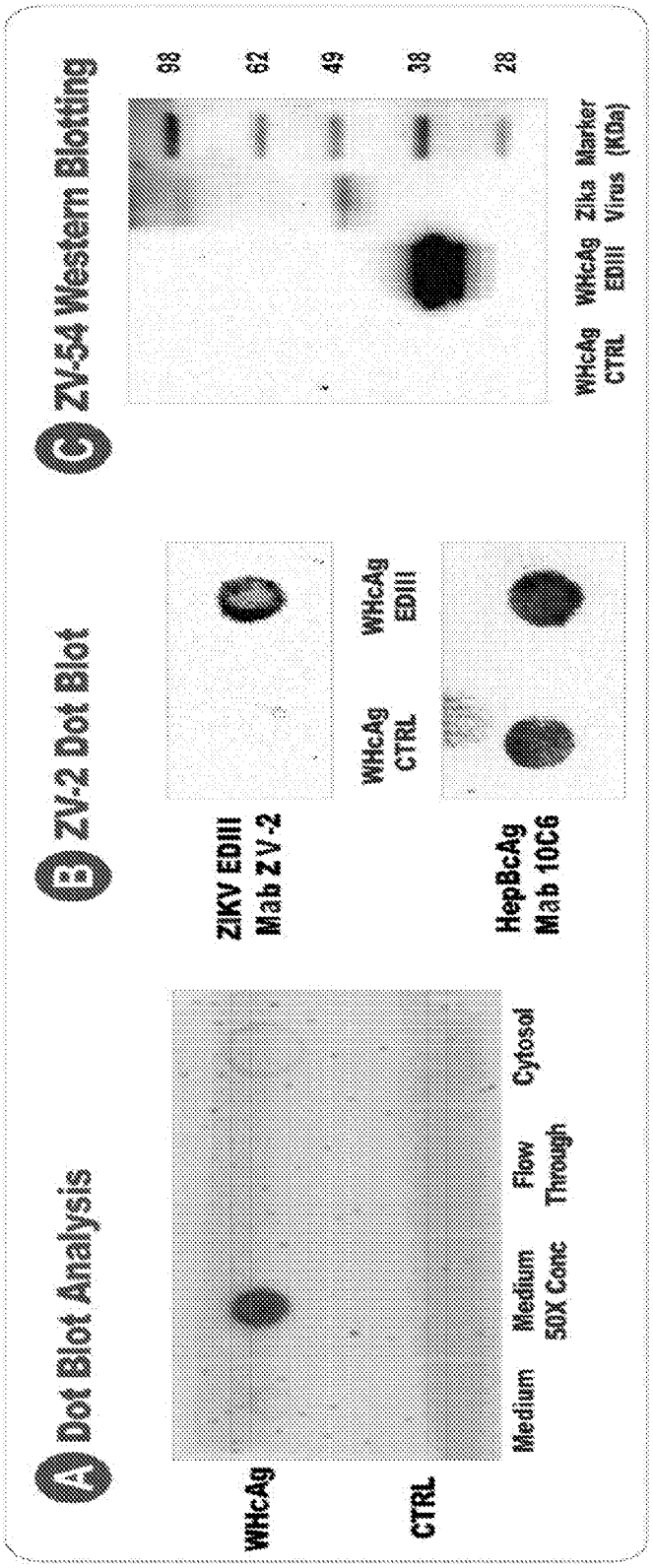

A Dot Blot Analysis
WHcAg
CTRL
Medium
Medium 50X Conc
Flow Through
Cytosol
B ZV-2 Dot Blot
ZIKV EDIII Mab ZV-2
WHcAg CTRL
WHcAg EDIII
HepBcAg Mab 10C6
C ZV-54 Western Blotting
98
62
49
38
28
WHcAg CTRL
WHcAg EDIII
Zika Virus
Marker (KDa)

FIG. 6

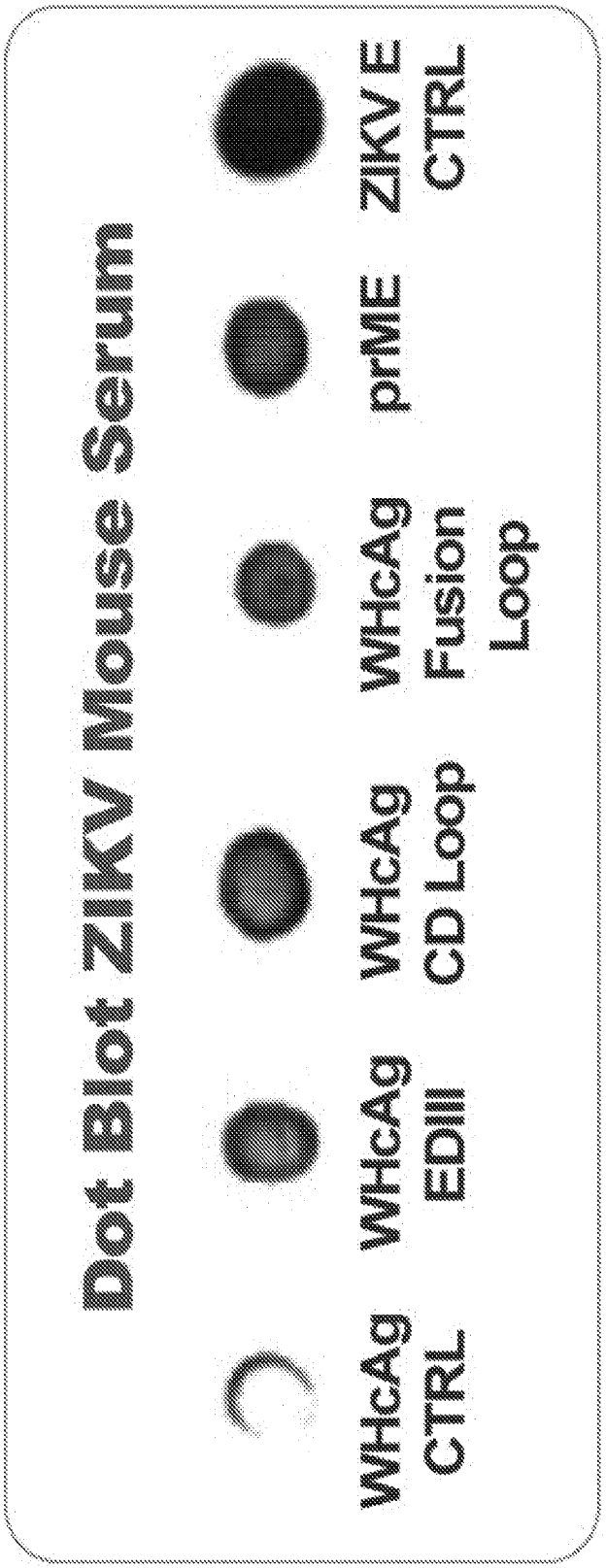

Dot Blot ZIKV Mouse Serum
WHcAg CTRL
WHcAg EDIII
WHcAg CD Loop
WHcAg Fusion Loop
prME
ZIKV E CTRL

FIG. 7

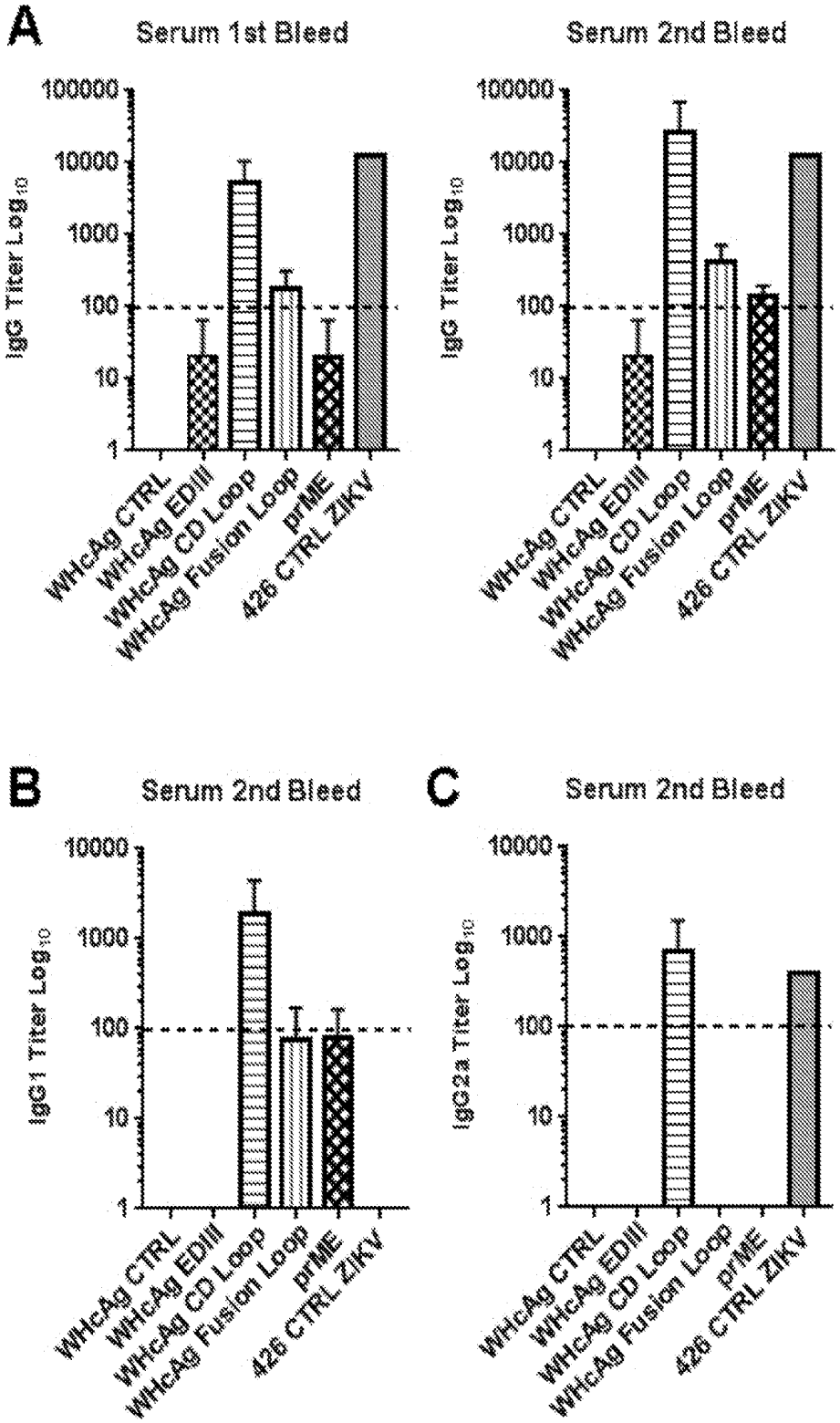

A
Serum 1st Bleed
Serum 2nd Bleed
IgG Titer Log10
100000
10000
1000
100
10
1
WHcAg CTRL
WHcAg EDIII
WHcAg CD Loop
WHcAg Fusion Loop
prME
426 CTRL ZIKV
B
Serum 2nd Bleed
IgG1 Titer Log10
C
Serum 2nd Bleed
IgG2a Titer Log10

FIG. 8

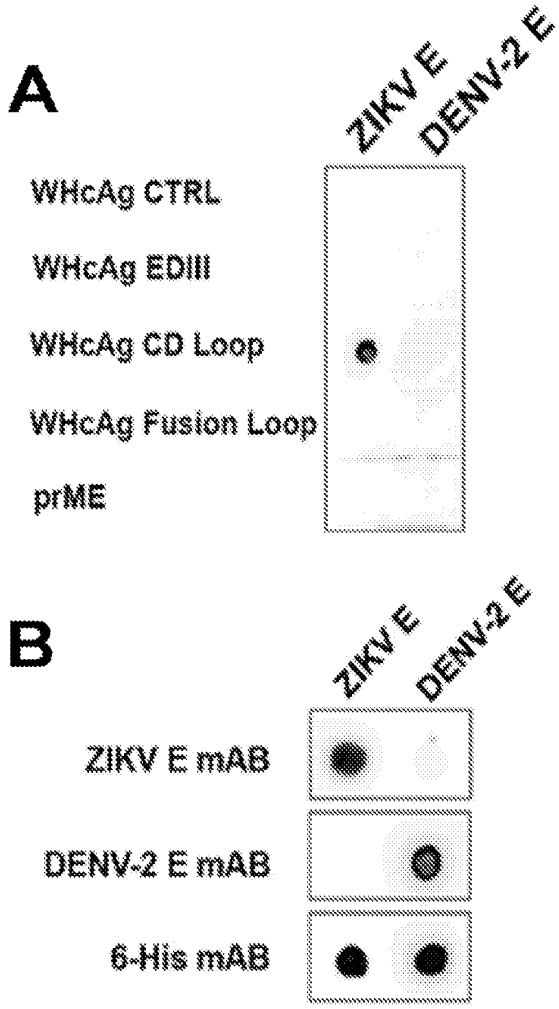

A
ZIKV E
DENV-2 E
WHcAg CTRL
WHcAg EDIII
WHcAg CD Loop
WHcAg Fusion Loop
prME
B
ZIKV E
DENV-2 E
ZIKV E mAB
DENV-2 E mAB
6-His mAB

FIG. 9

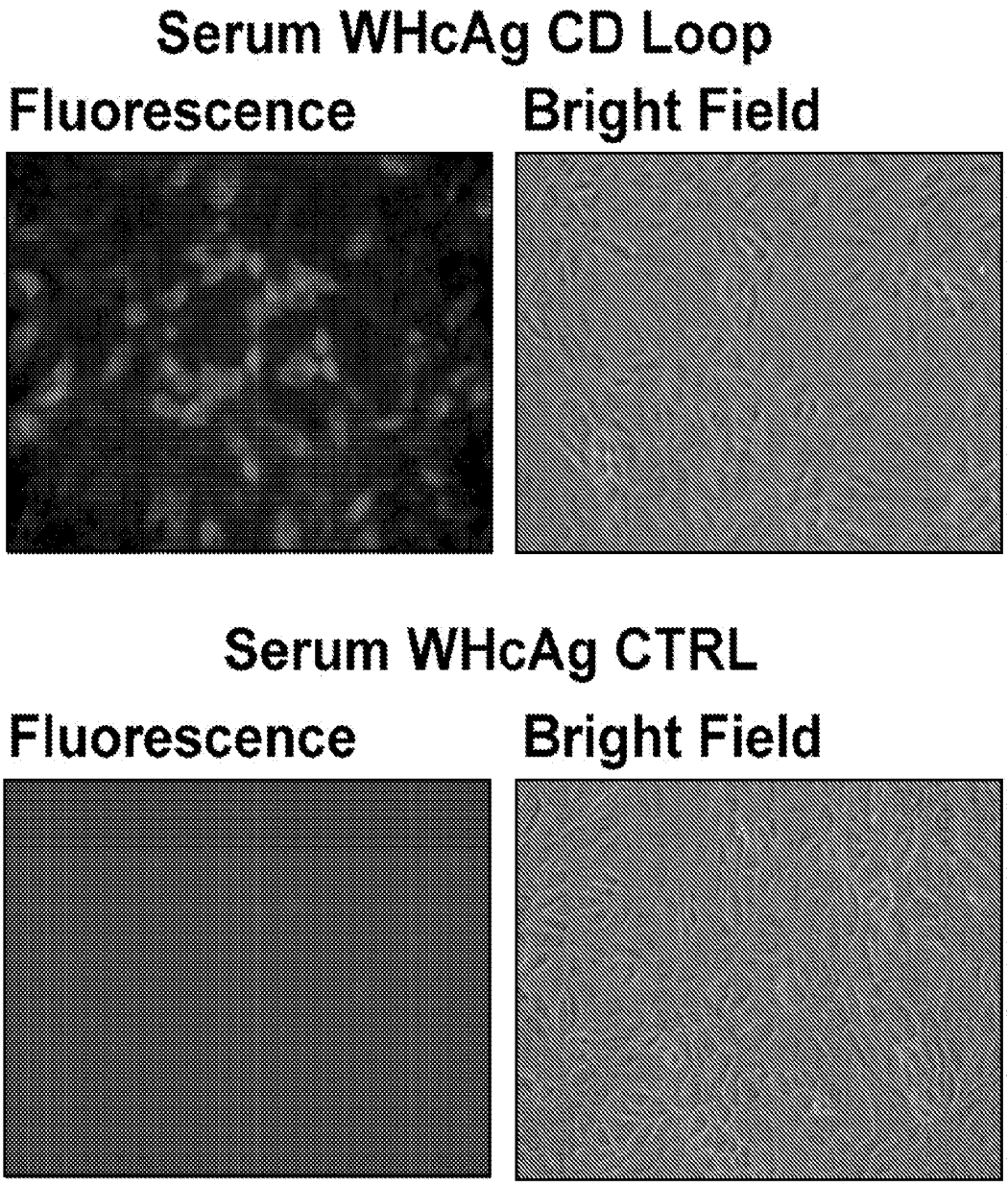

Serum WHcAg CD Loop
Fluorescence
Bright Field
Serum WHcAg CTRL
Fluorescence
Bright Field

FIG. 10

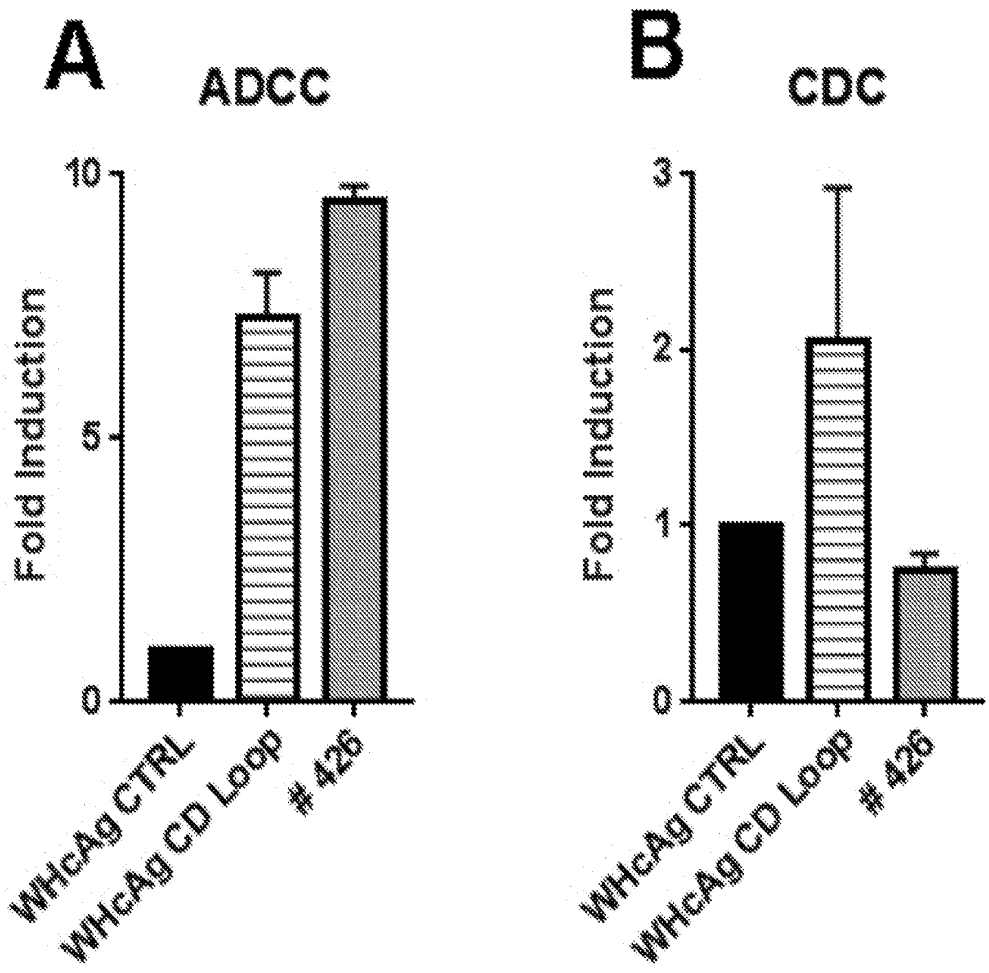

A
ADCC
Fold Induction
10
5
0
WHcAg CTRL
WHcAg CD Loop
426
B
CDC
Fold Induction
3
2
1
0
WHcAg CTRL
WHcAg CD Loop
426

FIG. 11

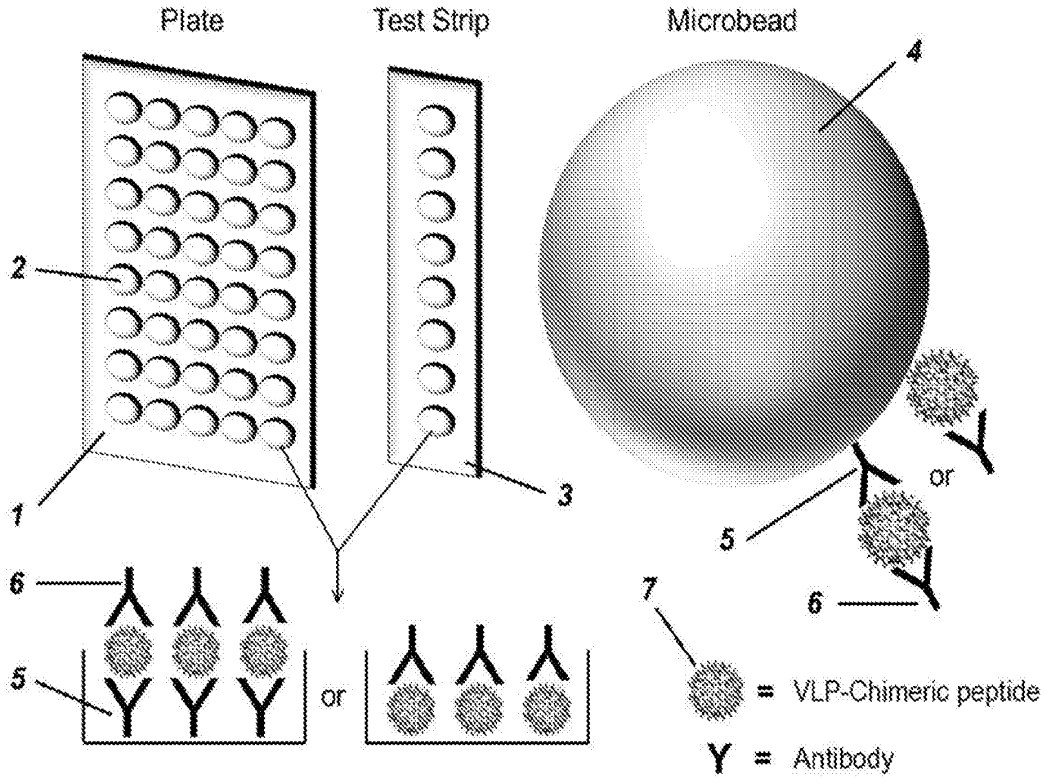


Plate
Test Strip
Microbead
1
2
3
4
5
6
7
or
or
= VLP-Chimeric peptide
Y = Antibody

FIG. 12

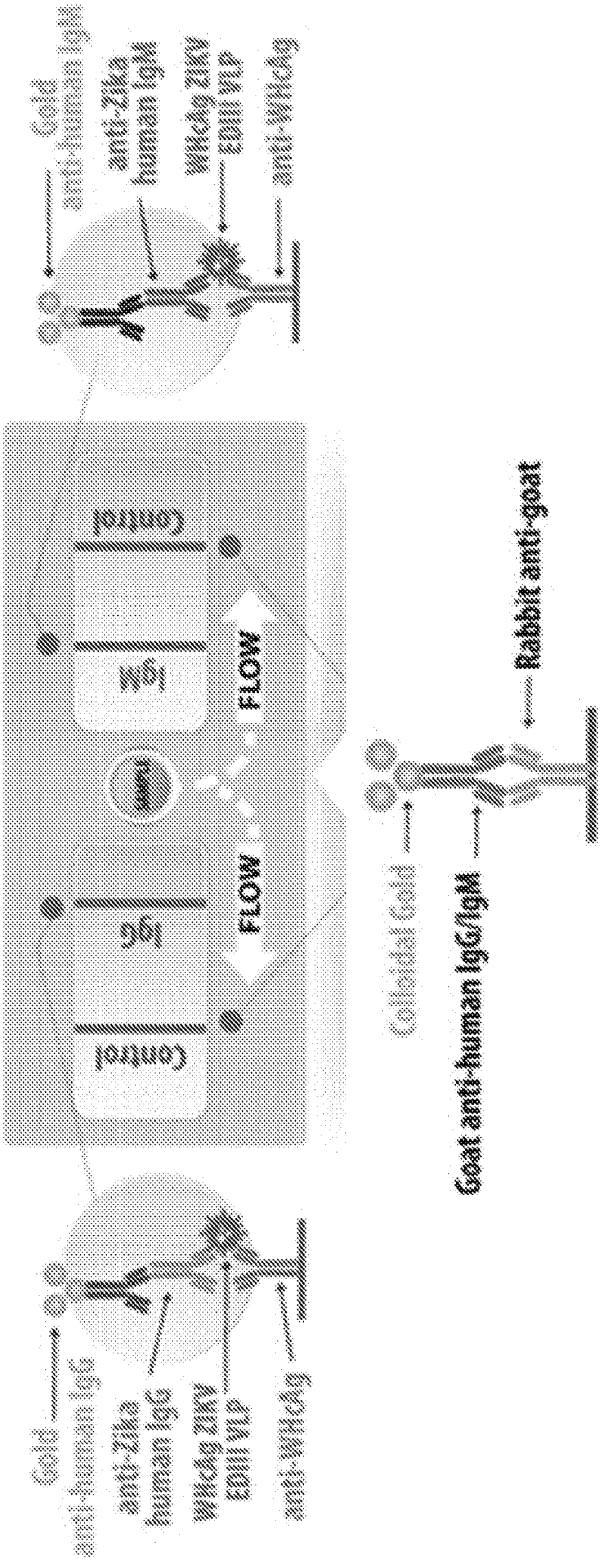

Gold anti-human IgM
anti-Zika human IgM
WhcAg ZIKV consl VLP
anti-WhcAg
Control
IgM
FLOW
Control
IgG
FLOW
Colloidal Gold
Goat anti-human IgG/IgM
Rabbit anti-goat
Gold anti-human IgG
anti-Zika human IgG
WhcAg ZIKV consl VLP
anti-WhcAg

FIG. 13

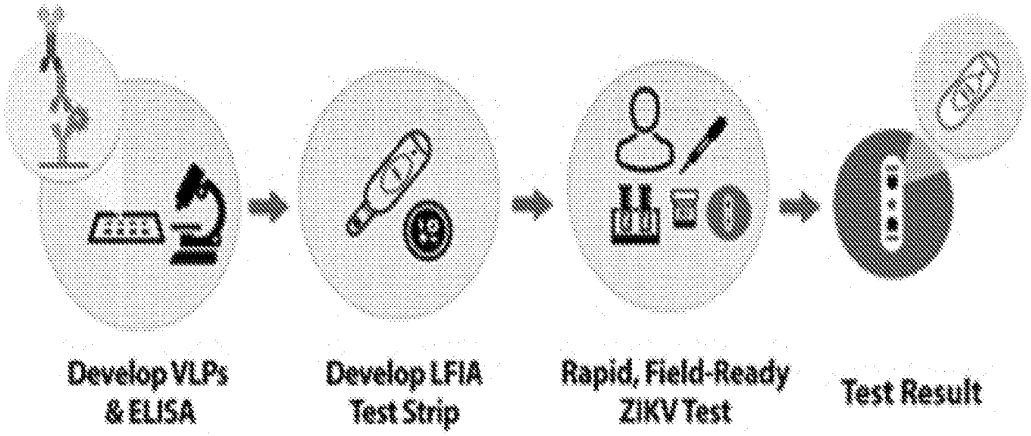

Develop VLPs & ELISA
Develop LFIA Test Strip
Rapid, Field-Ready ZIKV Test
Test Result

FIG. 14

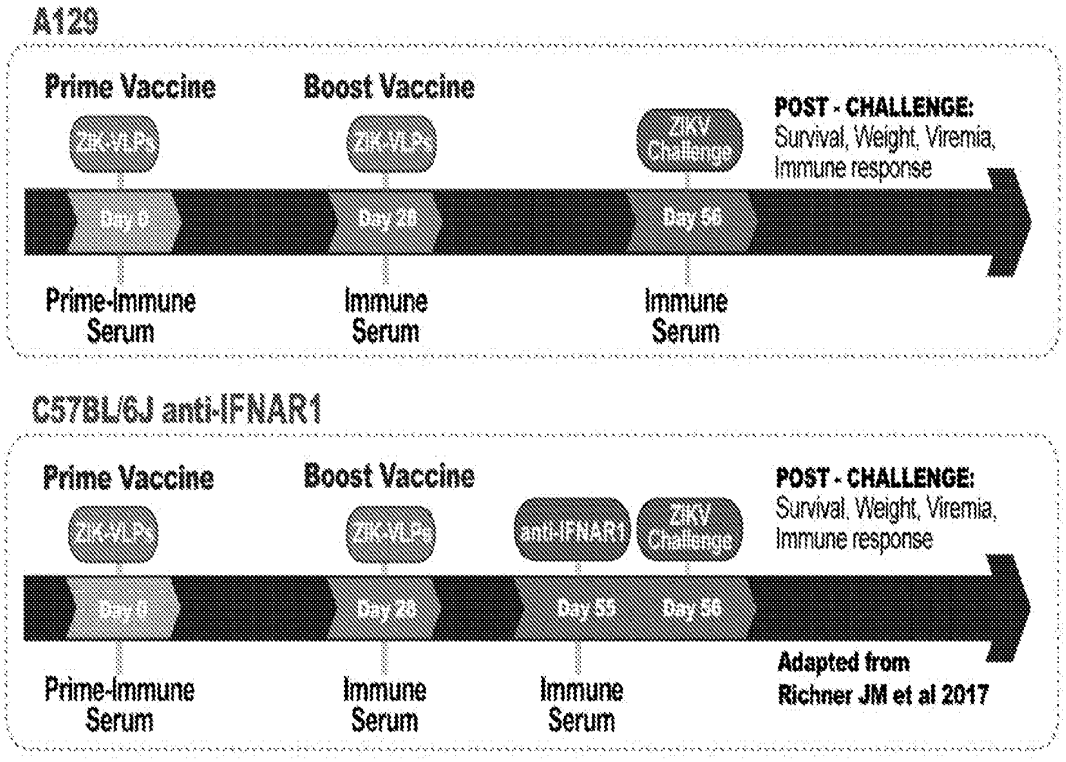

A129
Prime Vaccine
Boost Vaccine
ZIKV Challenge
POST - CHALLENGE:
Survival, Weight, Viremia,
Immune response
Day 0
Day 28
Day 56
Prime-Immune Serum
Immune Serum
Immune Serum
C57BL/6J anti-IFNAR1
Prime Vaccine
Boost Vaccine
anti-IFNAR1
ZIKV Challenge
POST - CHALLENGE:
Survival, Weight, Viremia,
Immune response
Day 0
Day 28
Day 55
Day 56
Prime-Immune Serum
Immune Serum
Immune Serum
Adapted from
Richner JM et al 2017

FIG. 15

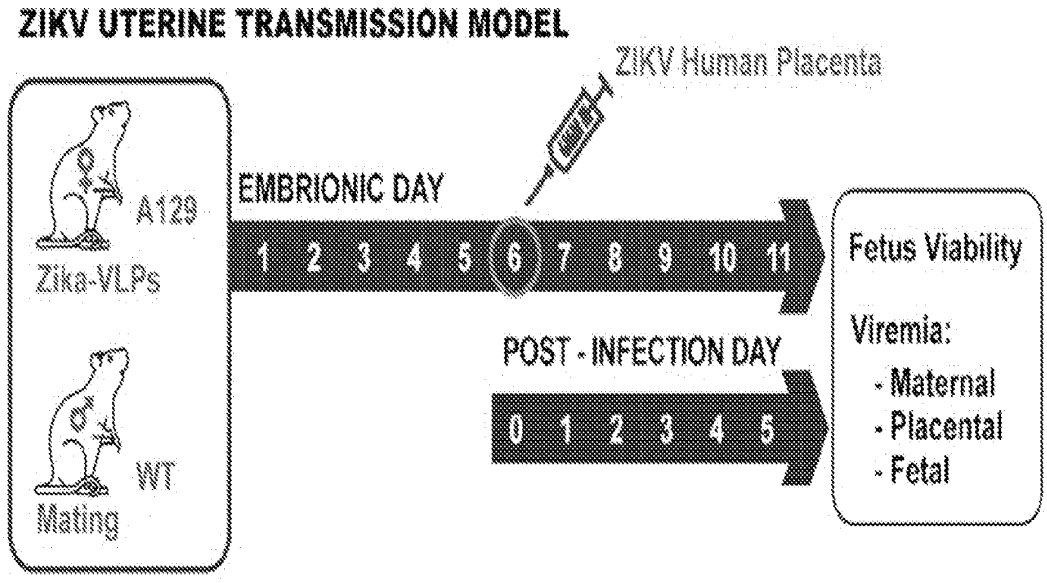

ZIKV UTERINE TRANSMISSION MODEL
ZIKV Human Placenta
A129
Zika-VLPs
WT
Mating
EMBRIONIC DAY
1 2 3 4 5 6 7 8 9 10 11
POST - INFECTION DAY
0 1 2 3 4 5
Fetus Viability
Viremia:
- Maternal
- Placental
- Fetal

FIG. 16

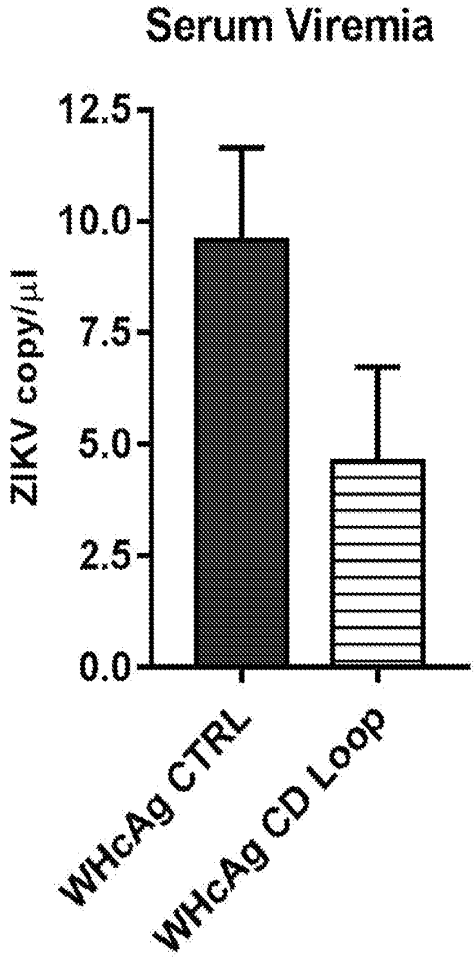

Serum Viremia
ZIKV copy/μl
12.5
10.0
7.5
5.0
2.5
0.0
WHcAg CTRL
WHcAg CD Loop

FIG. 17