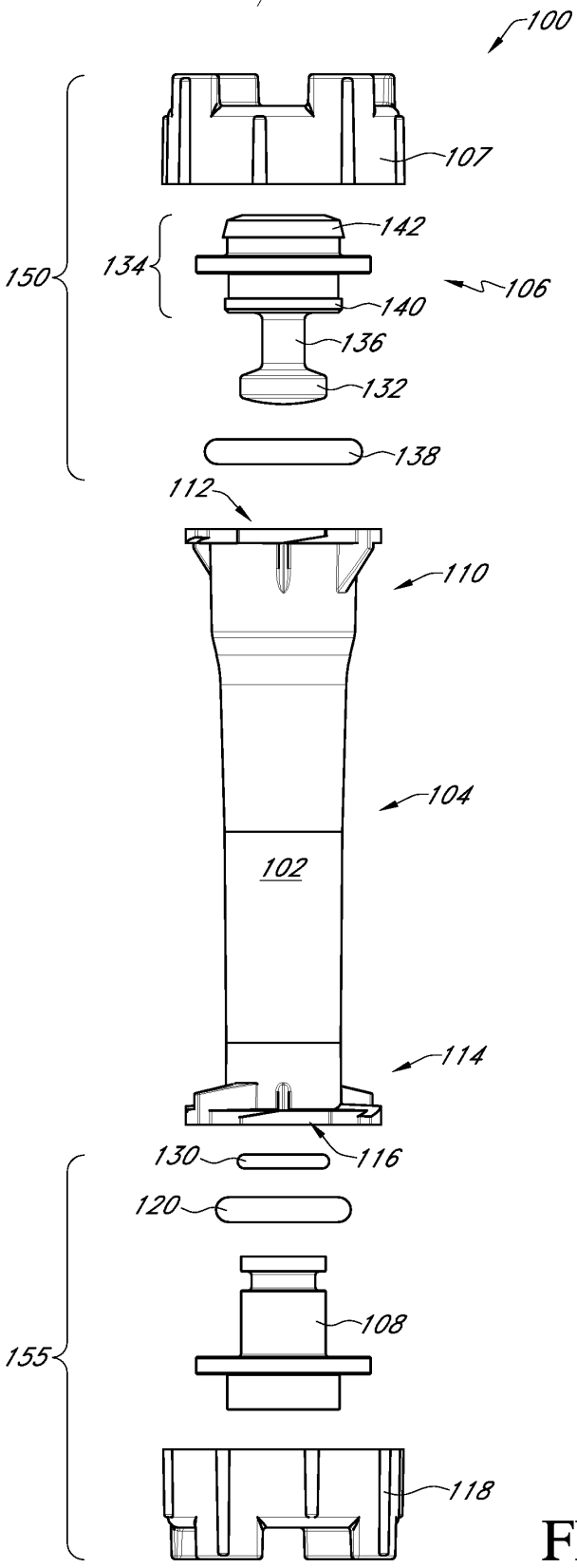

100
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155
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FIG. 1A

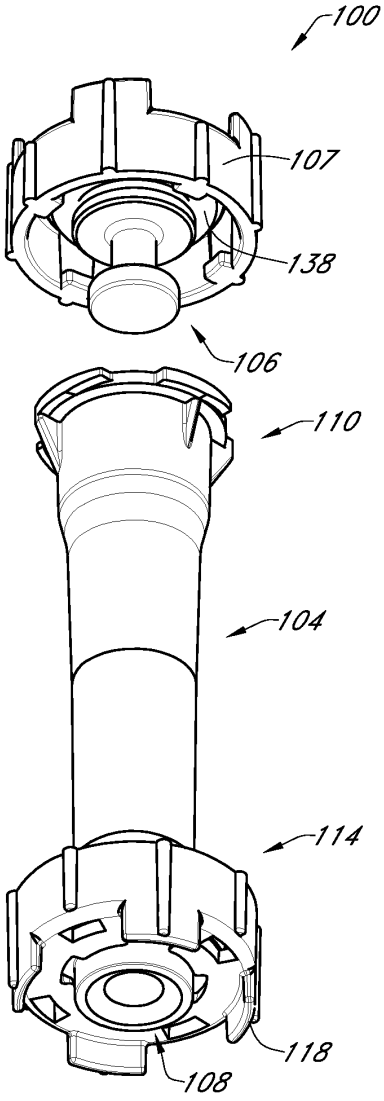


FIG. 1B

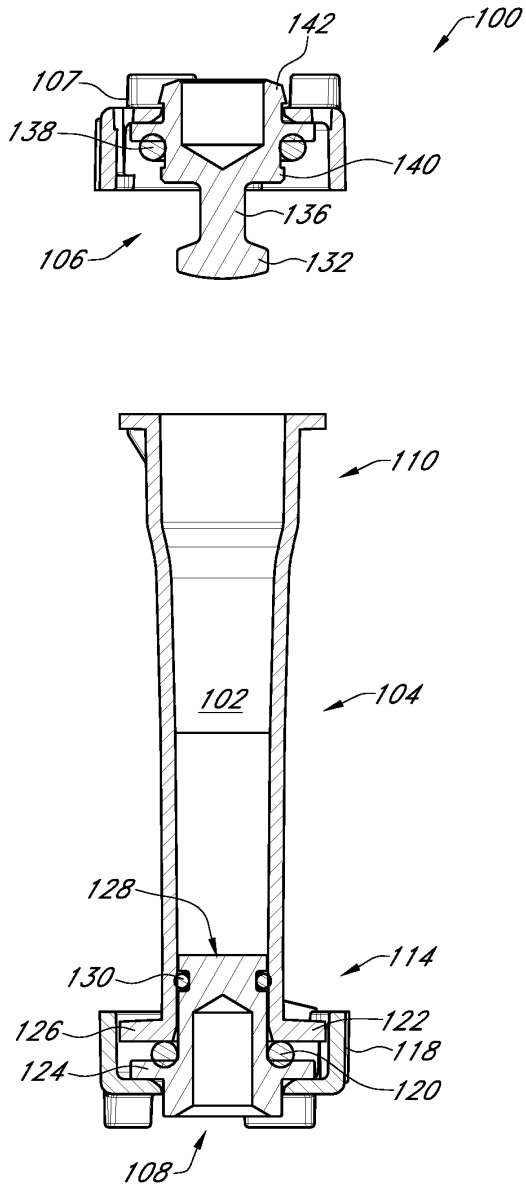

142
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FIG. 1C

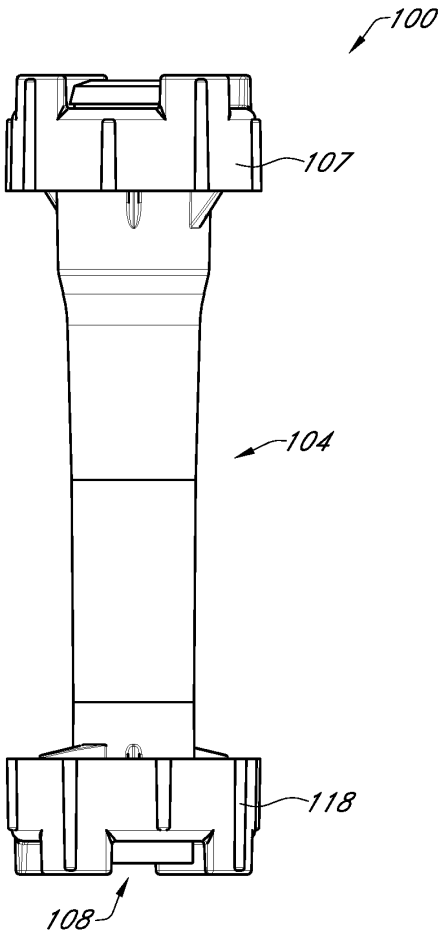


FIG. 1D

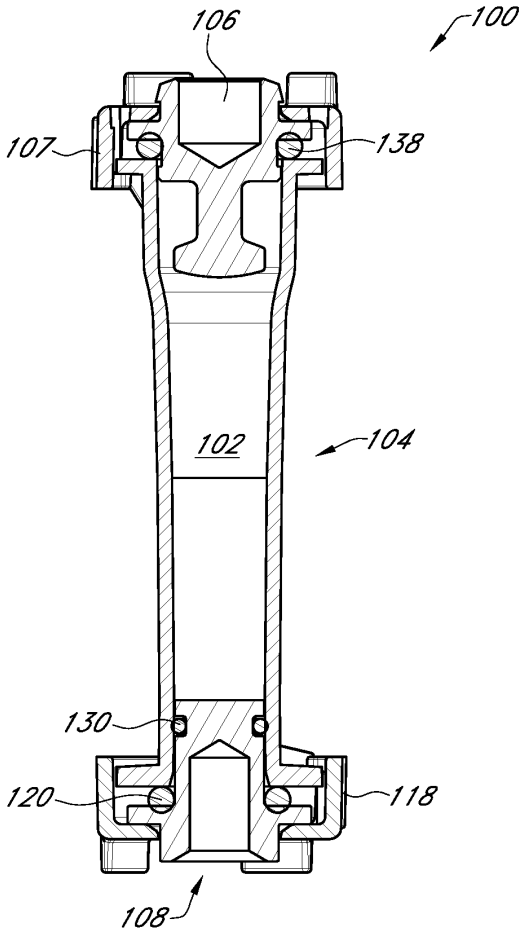


FIG. 1E

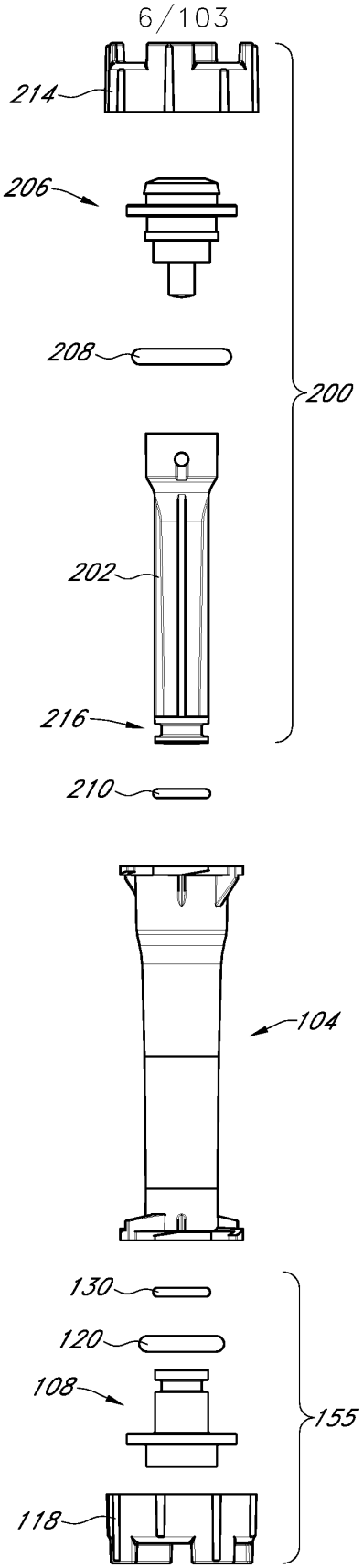


FIG. 2A

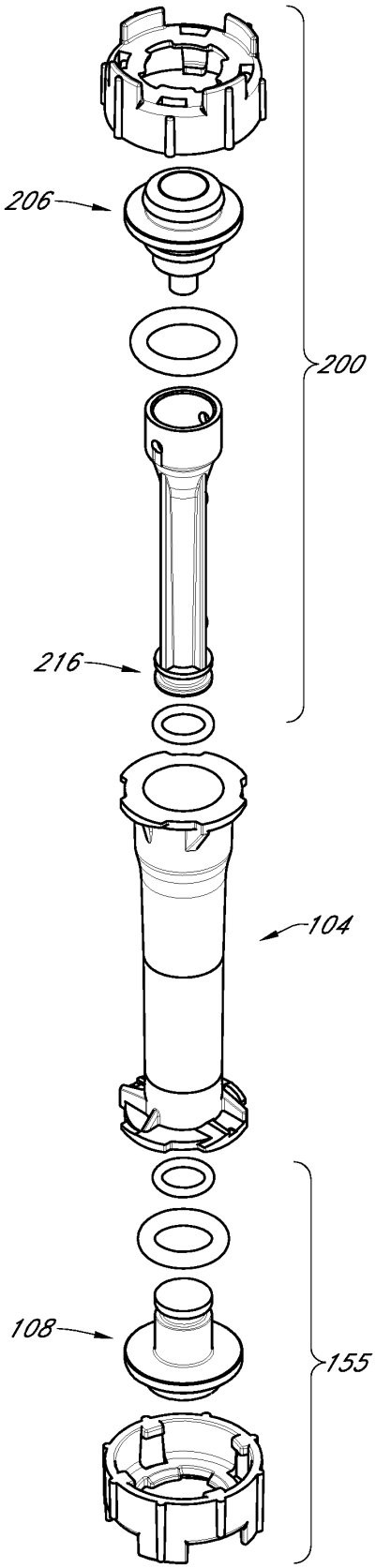


FIG. 2B

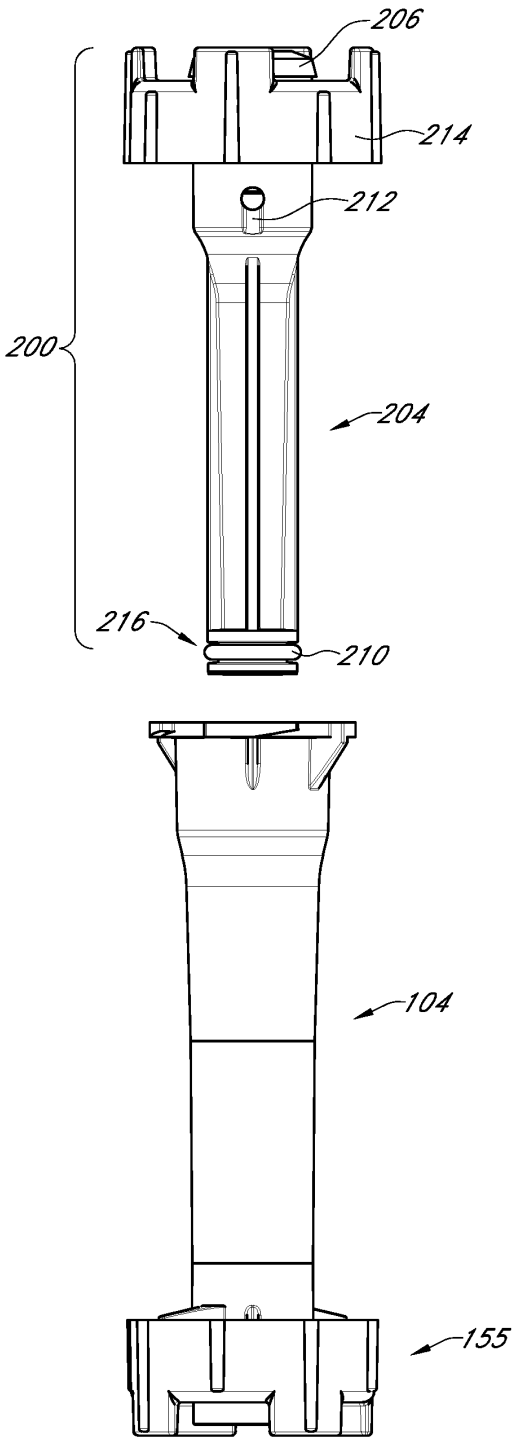


FIG. 2C

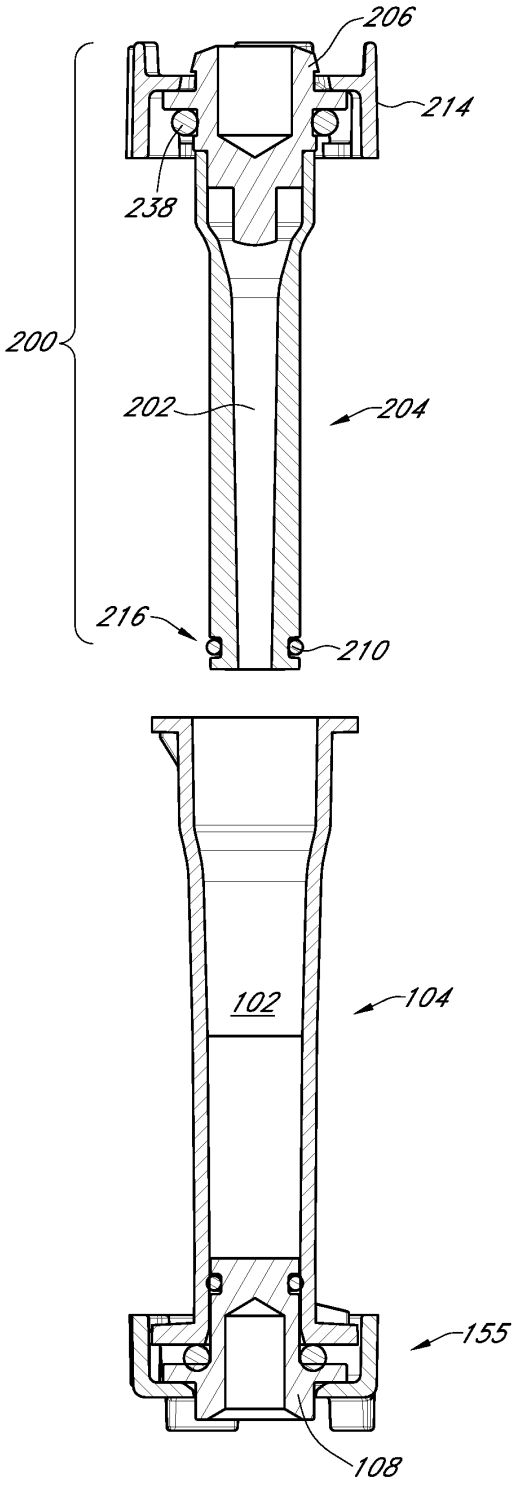


FIG. 2D

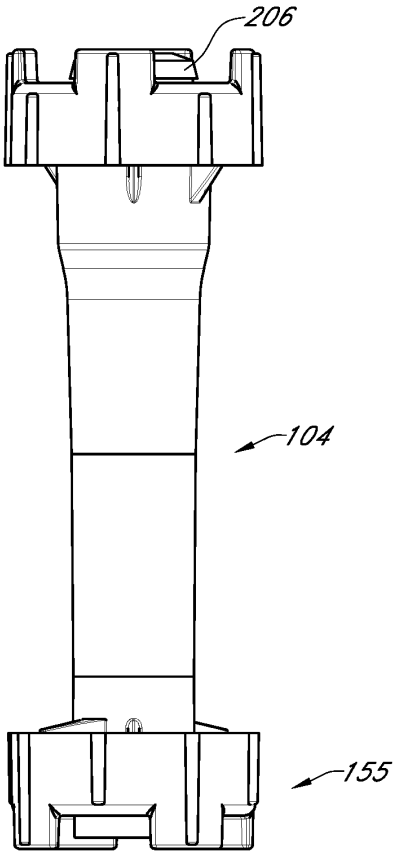


FIG. 2E

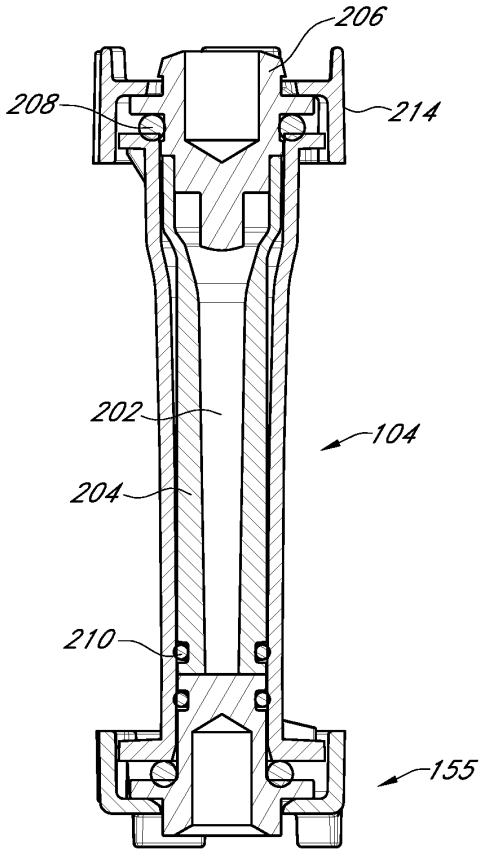


FIG. 2F

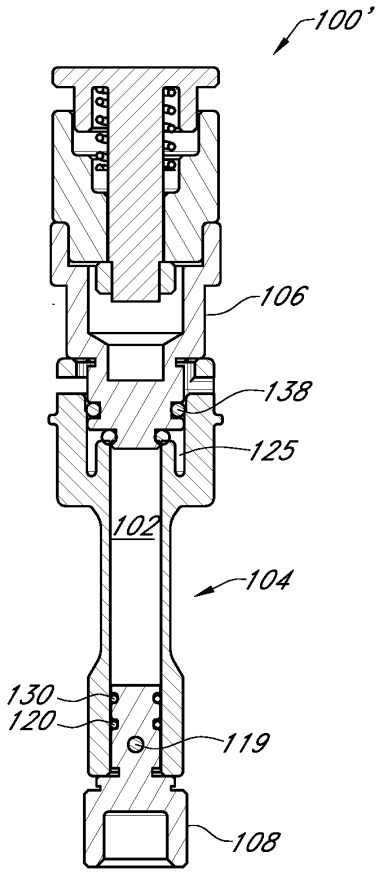


FIG. 3

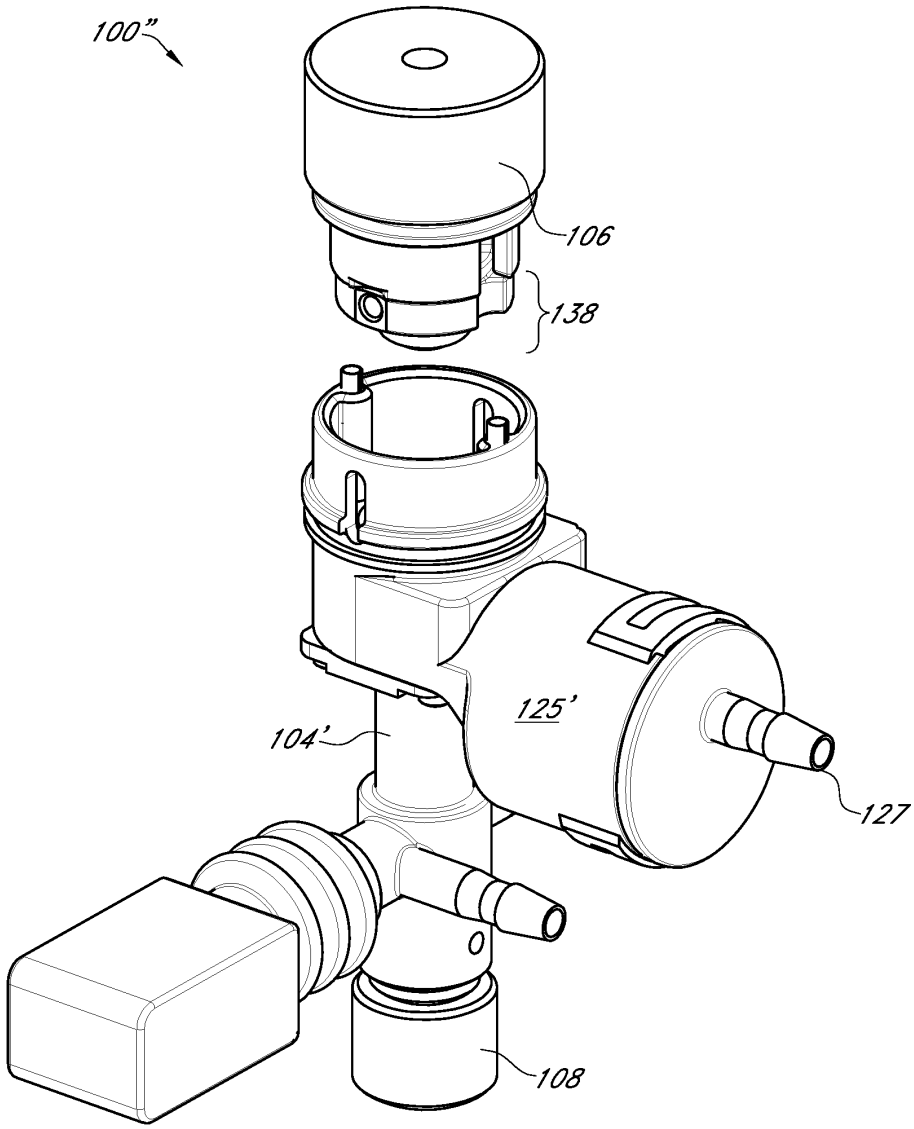


FIG. 4

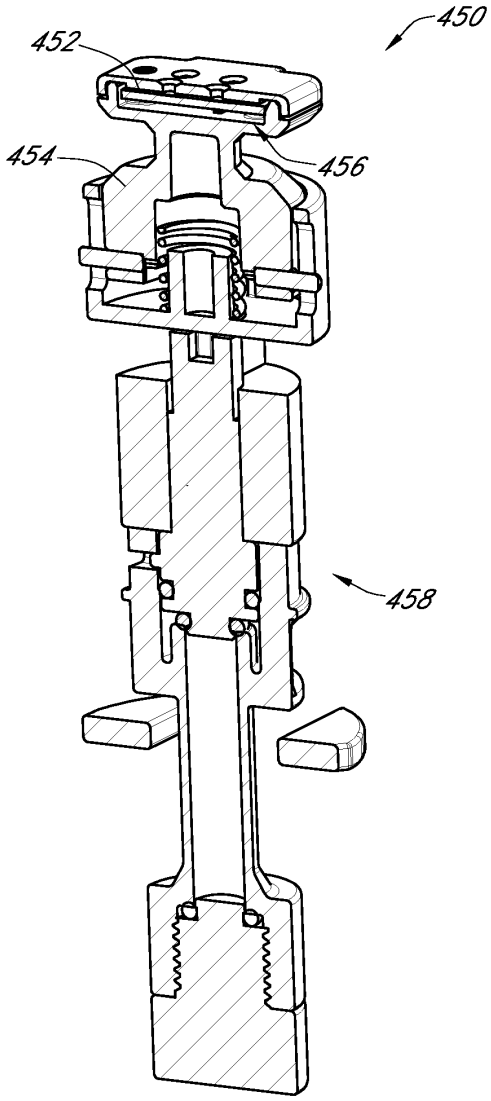


FIG. 5

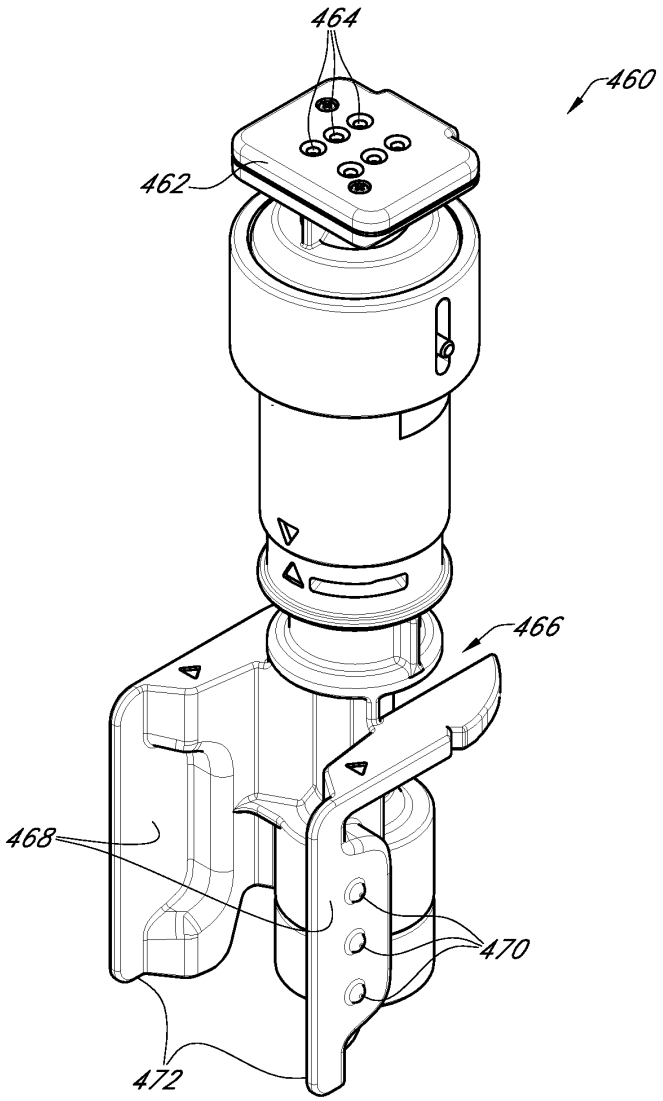


FIG. 6A

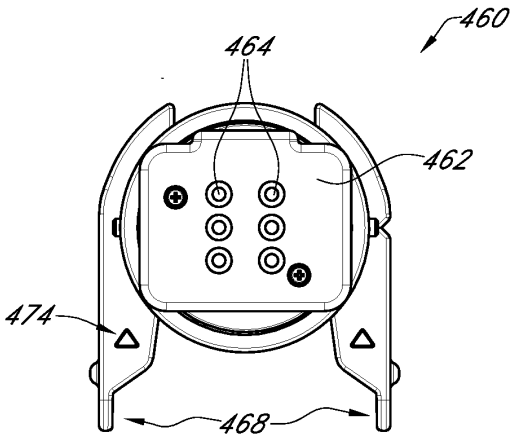


FIG. 6B

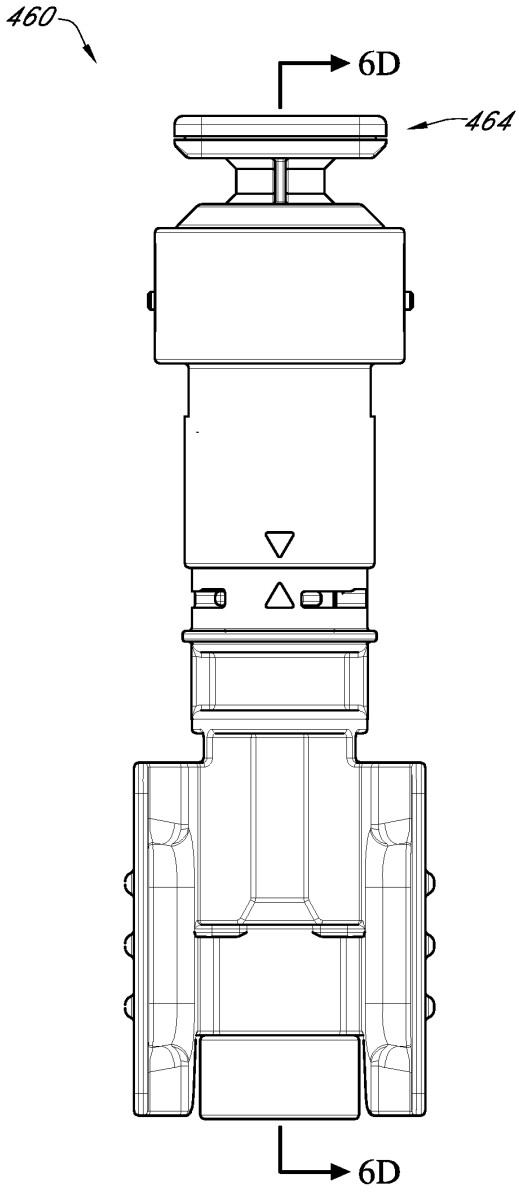


FIG. 6C

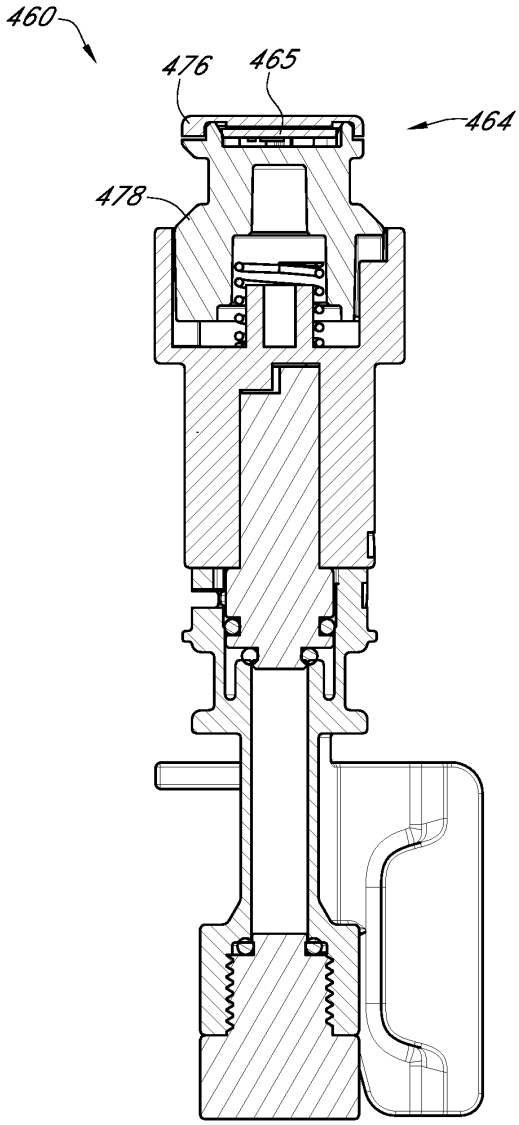


FIG. 6D

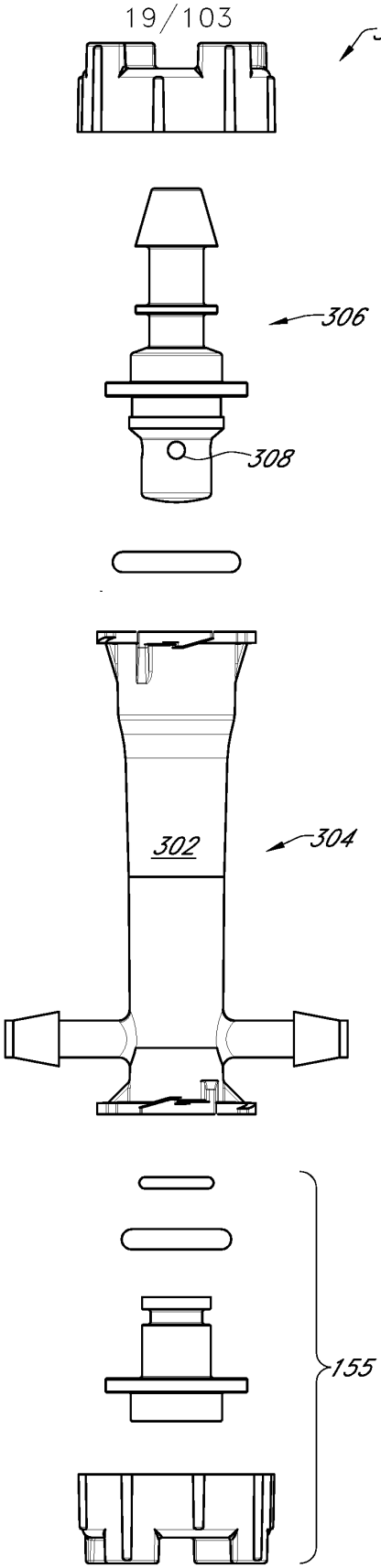


FIG. 7A

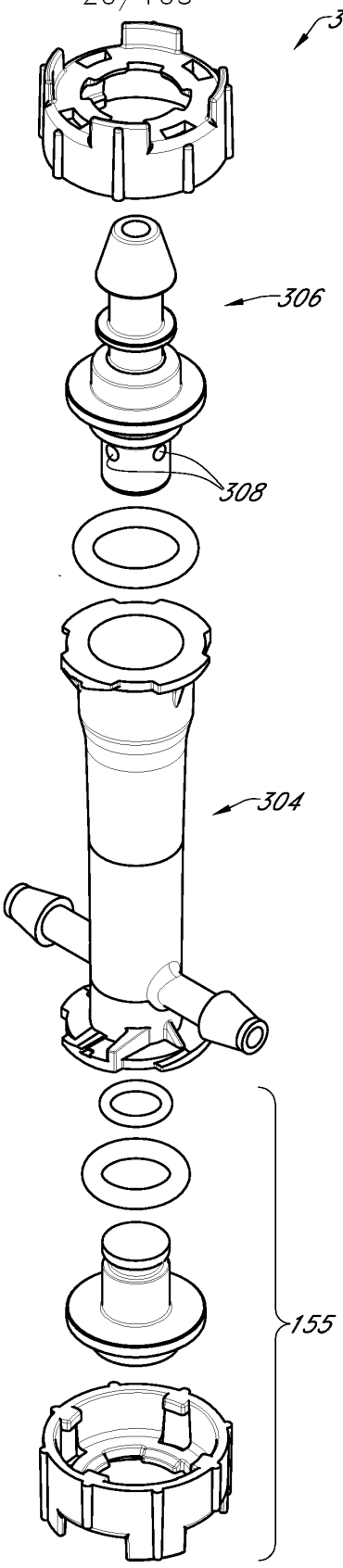


FIG. 7B

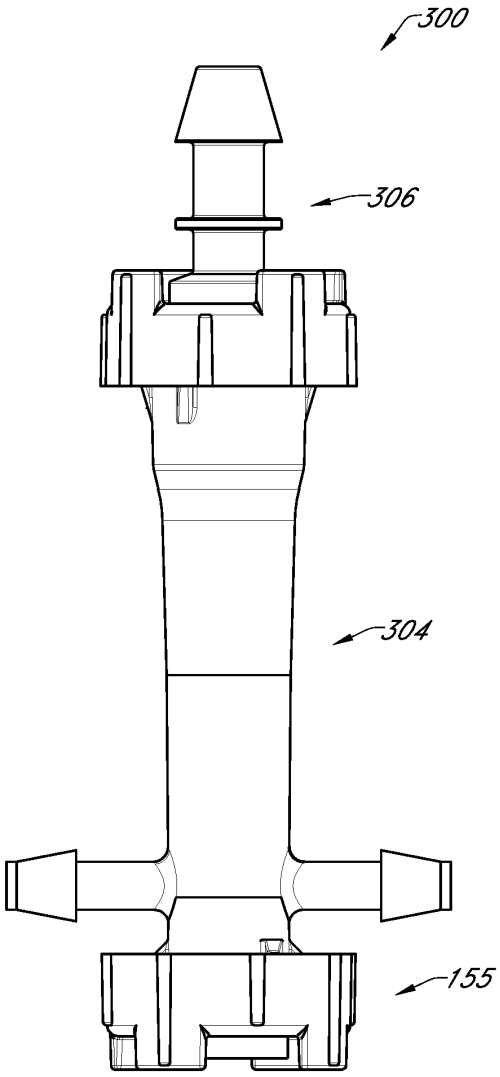


FIG. 7C

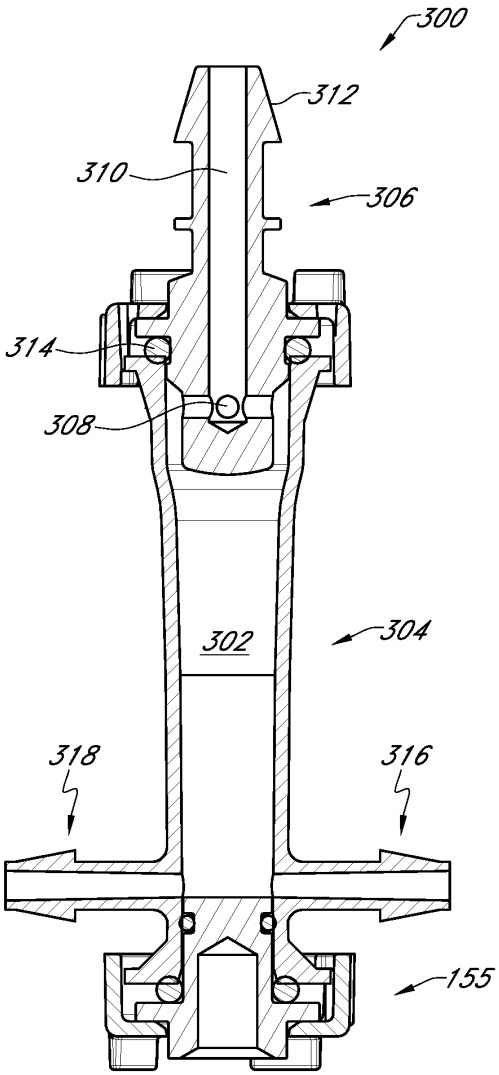


FIG. 7D

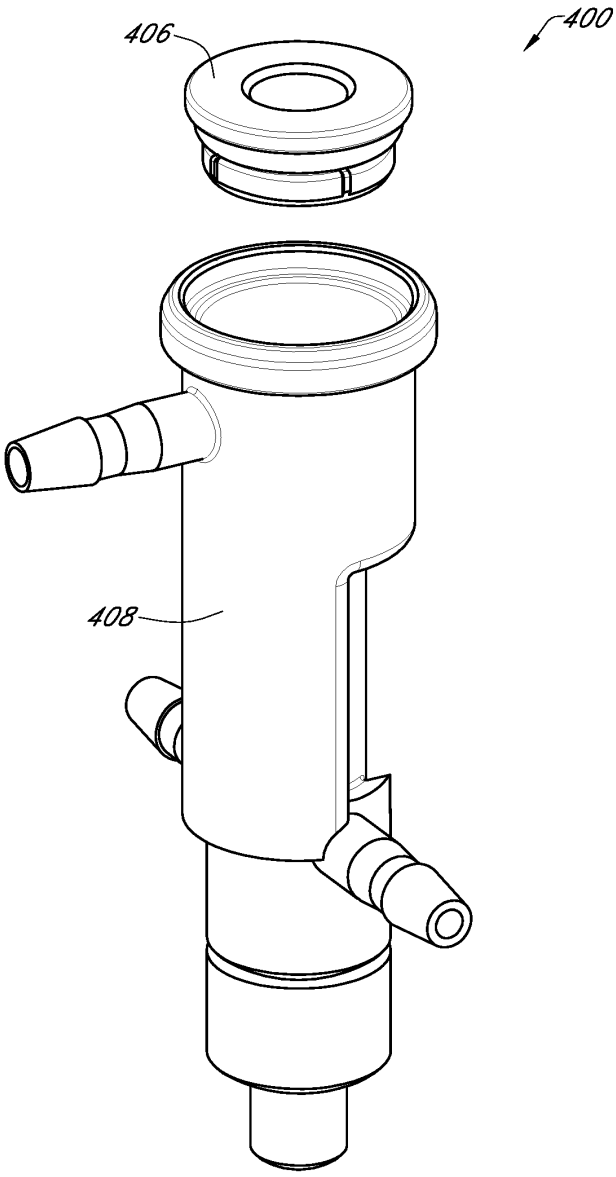


FIG. 8A

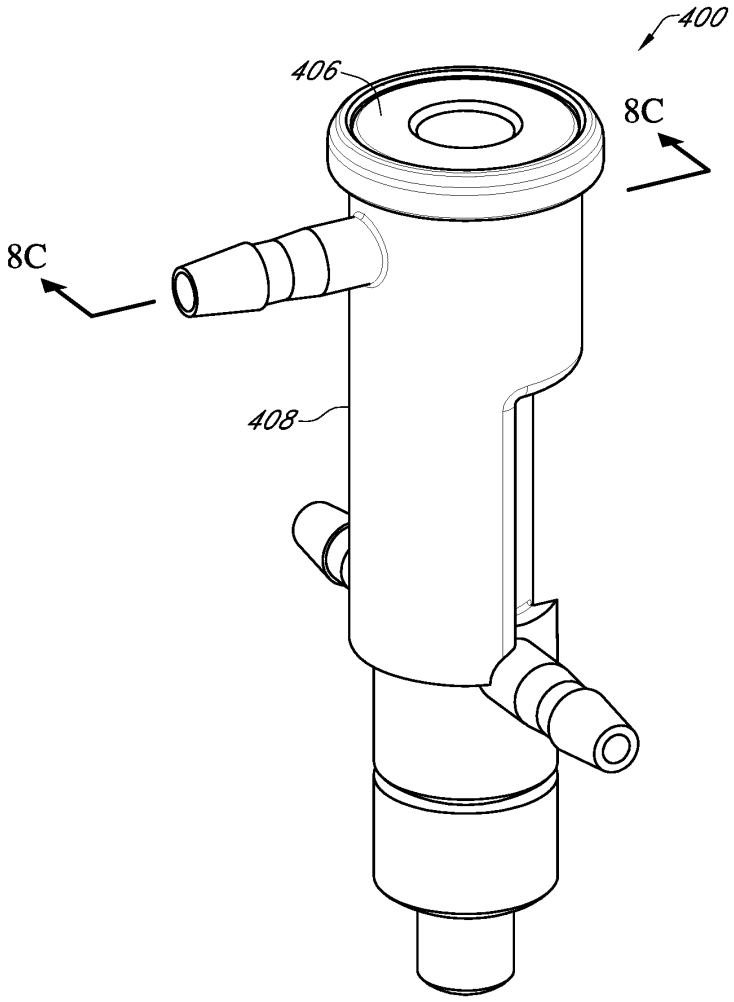


FIG. 8B

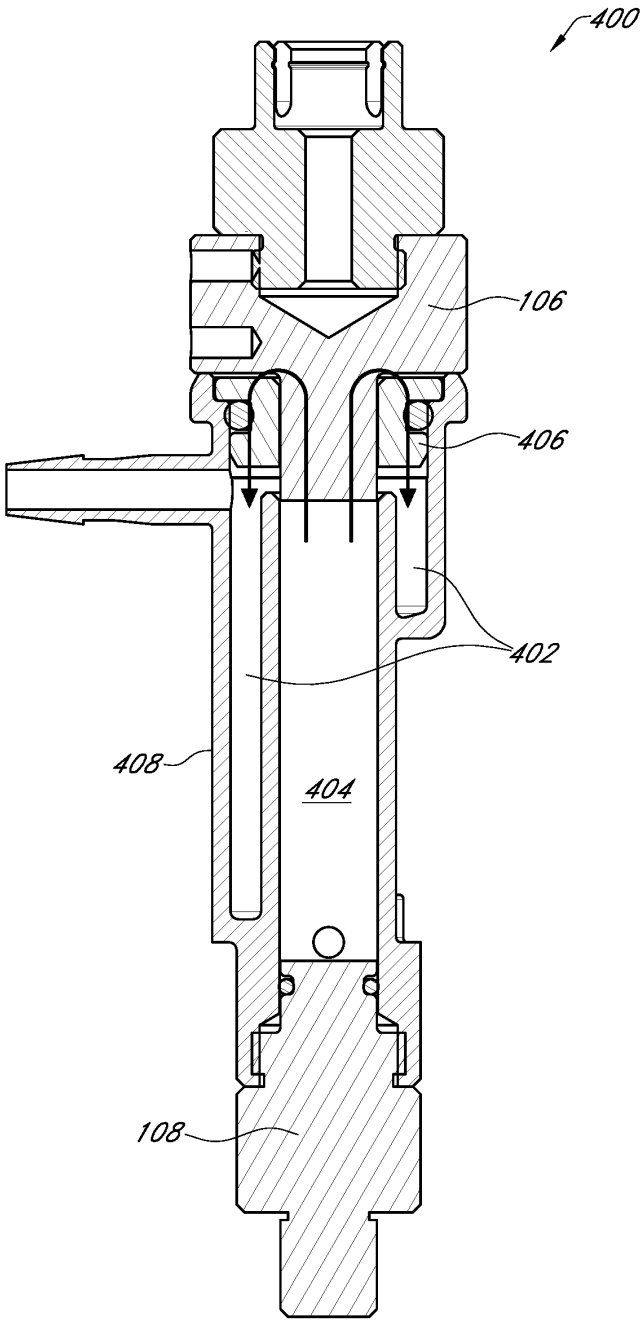


FIG. 8C

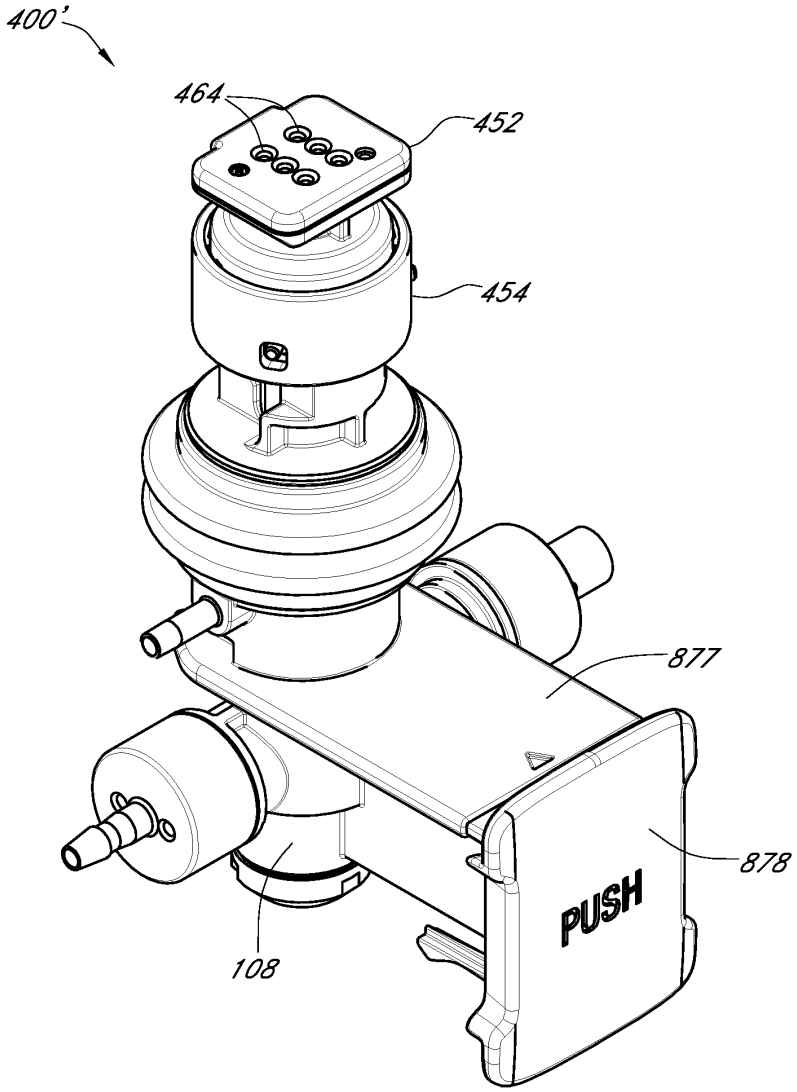


FIG. 9

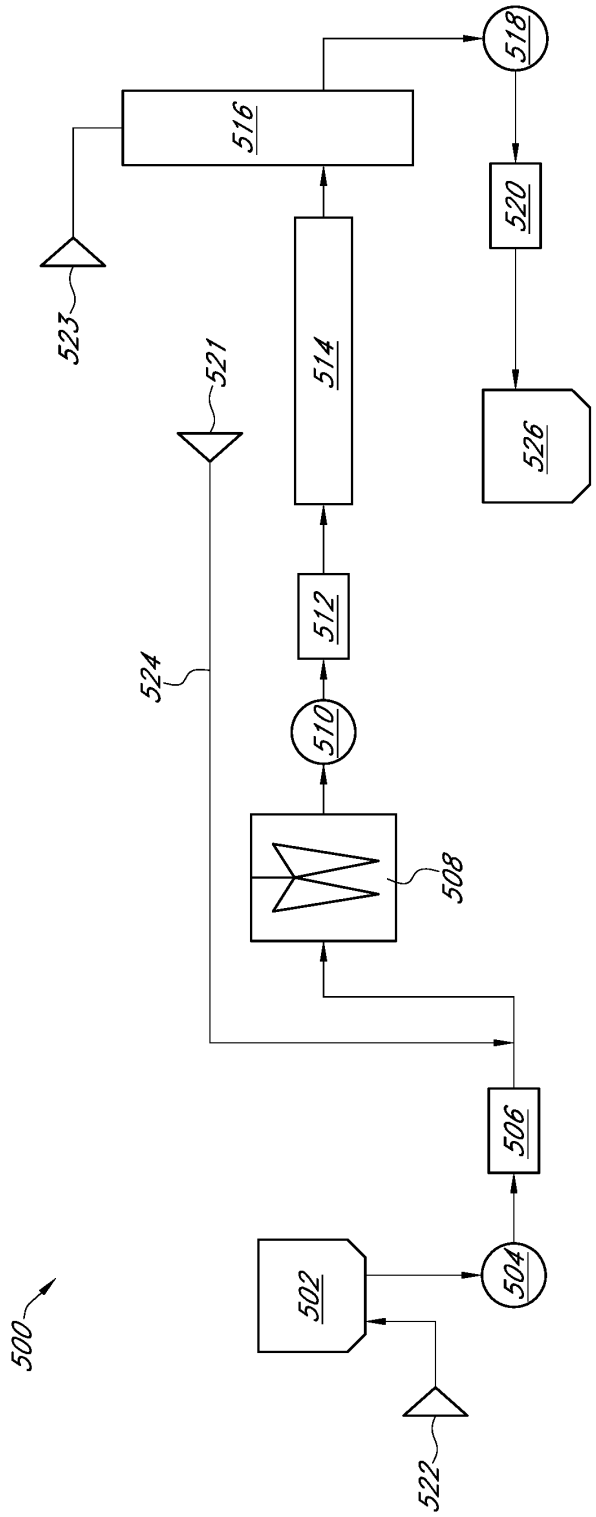


FIG. 10A

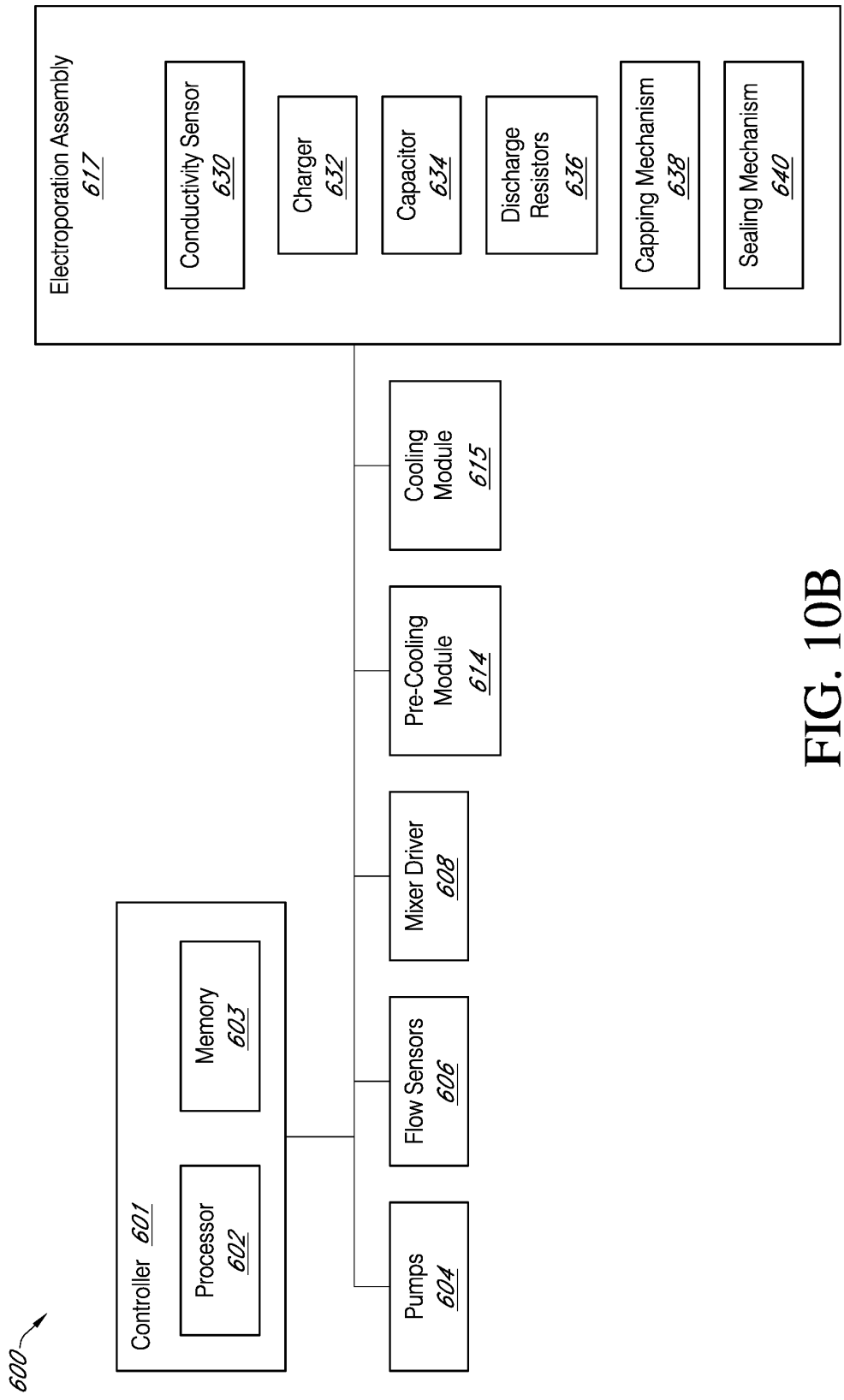

600
Controller 601
Processor 602
Memory 603
Pumps 604
Flow Sensors 606
Mixer Driver 608
Pre-Cooling Module 614
Cooling Module 615
Electroporation Assembly 617
Conductivity Sensor 630
Charger 632
Capacitor 634
Discharge Resistors 636
Capping Mechanism 638
Sealing Mechanism 640

FIG. 10B

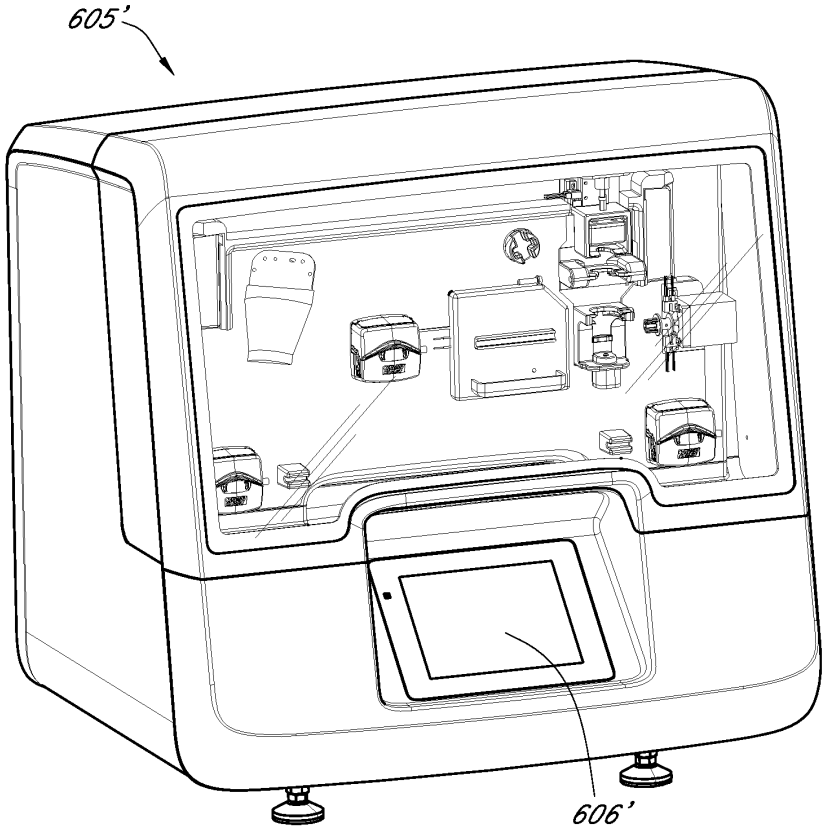


FIG. 11A

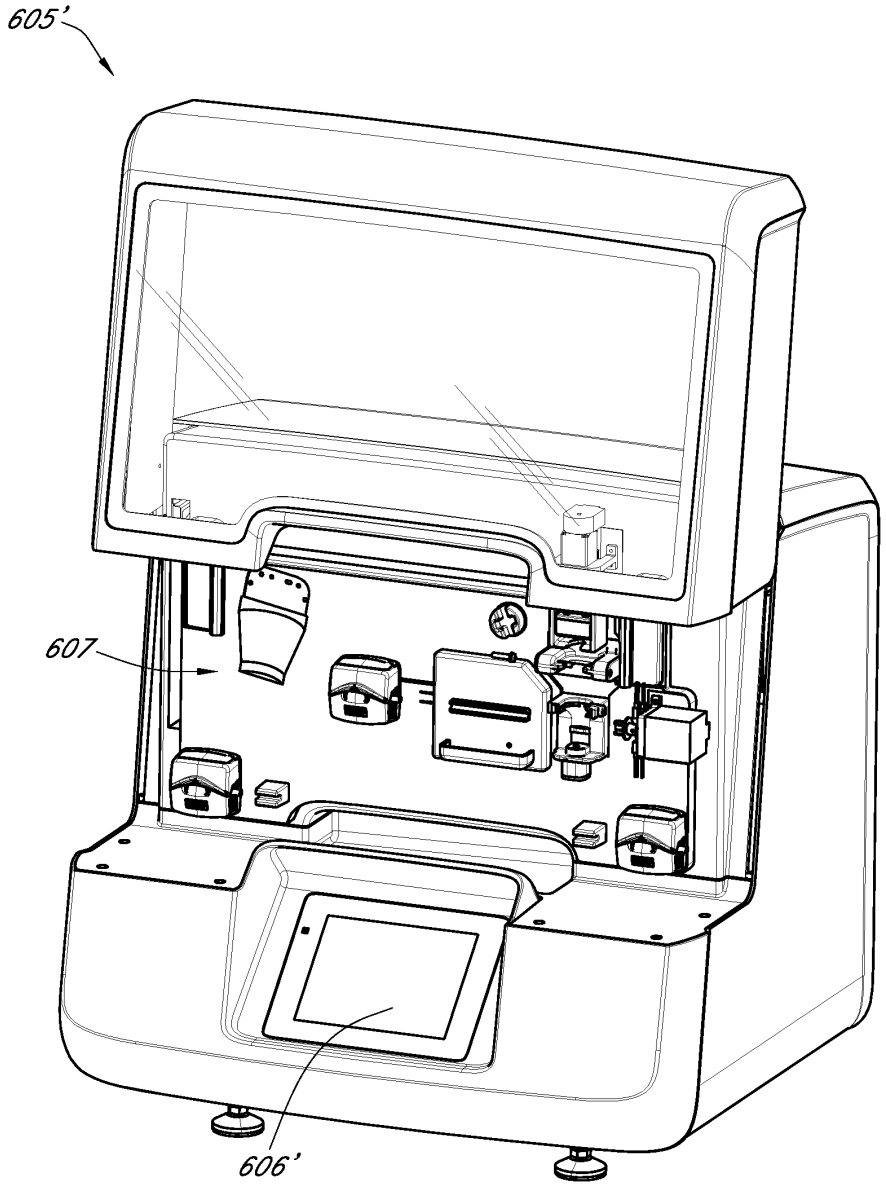


FIG. 11B

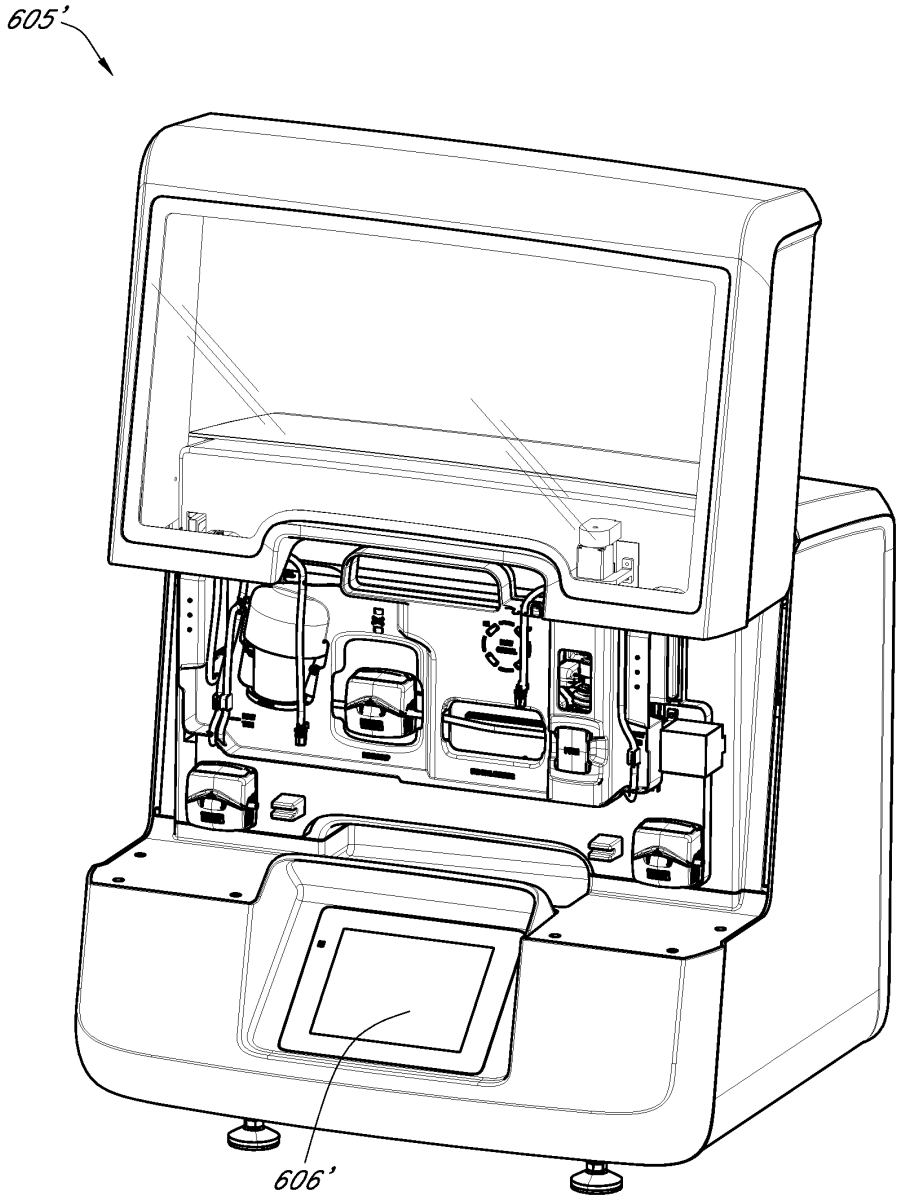


FIG. 11C

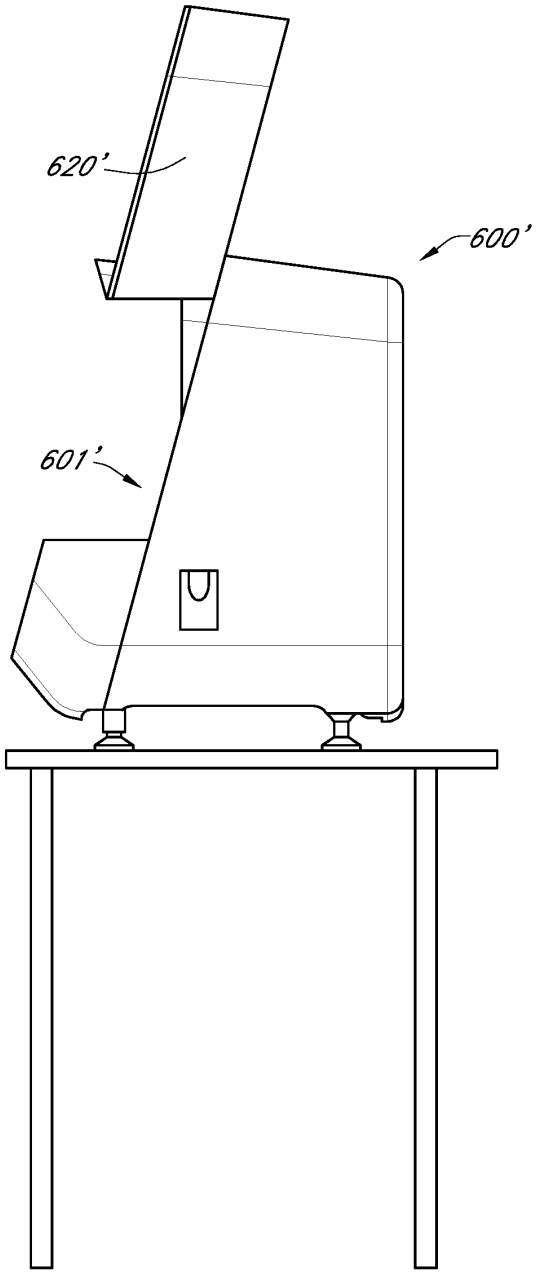


FIG. 11D

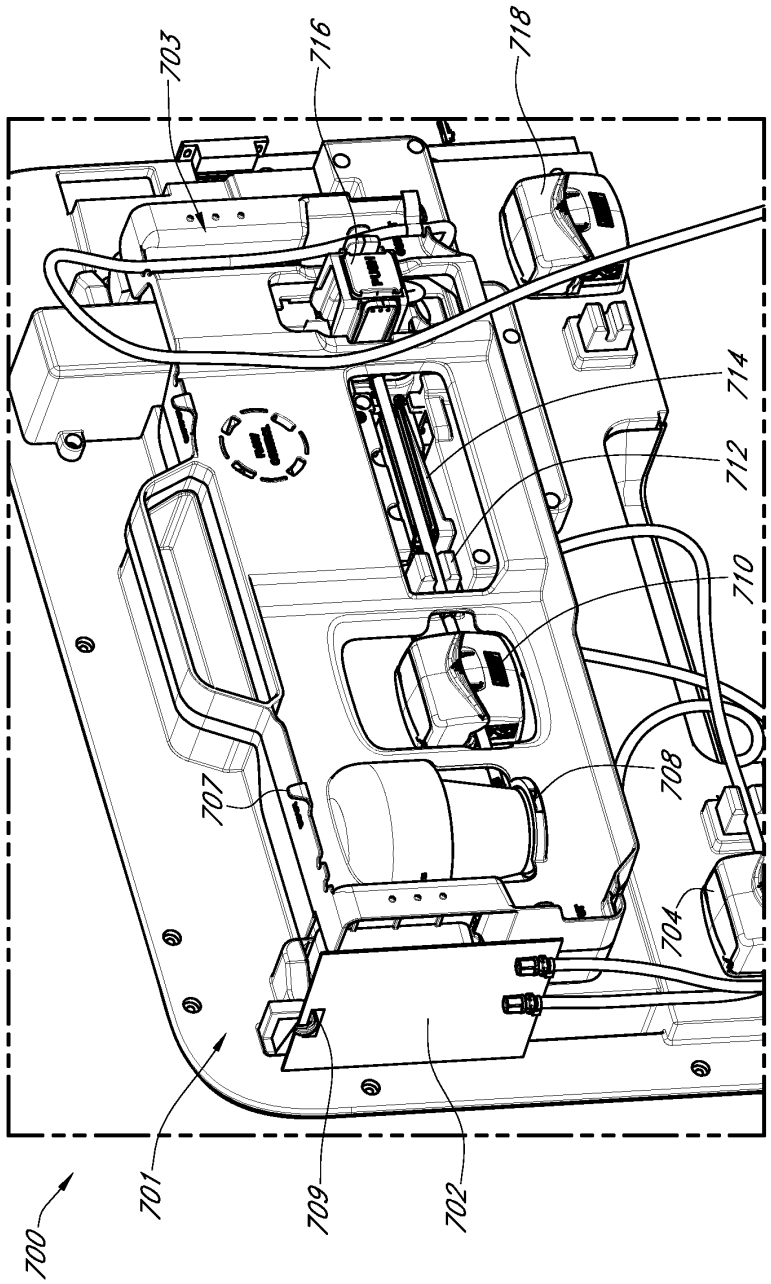


FIG. 12A

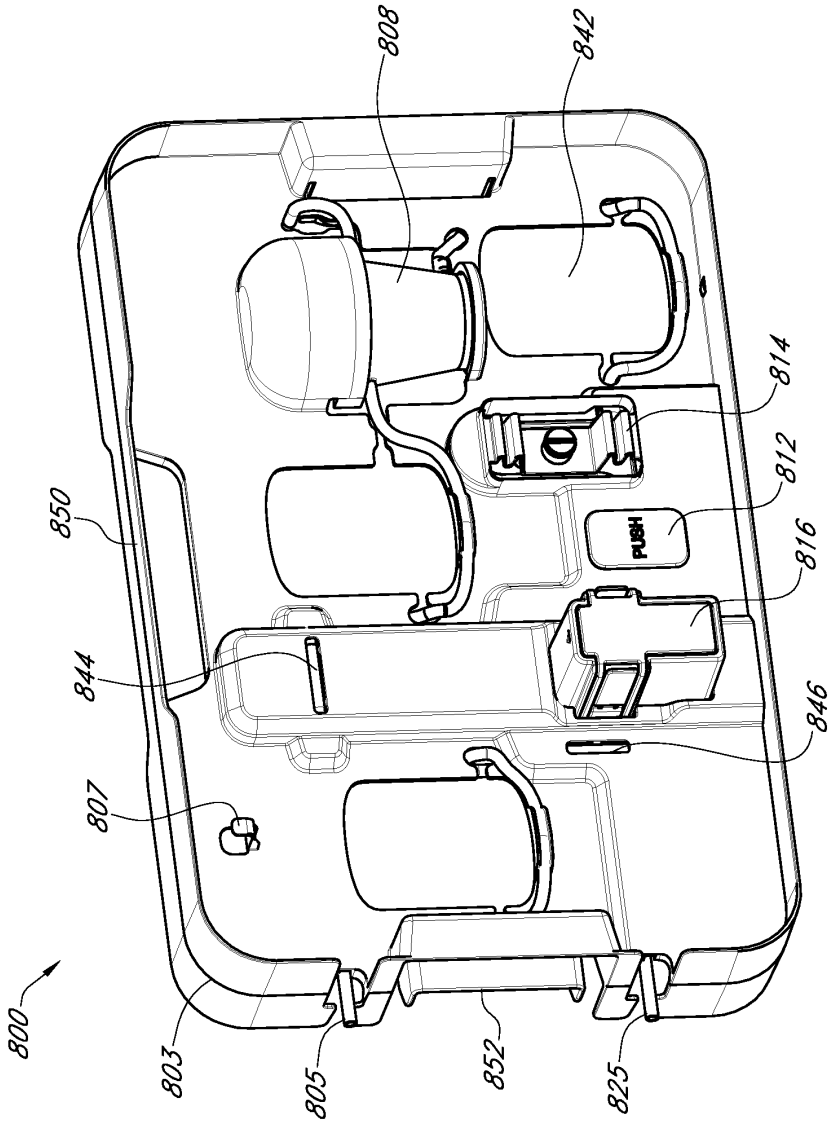


FIG. 12B

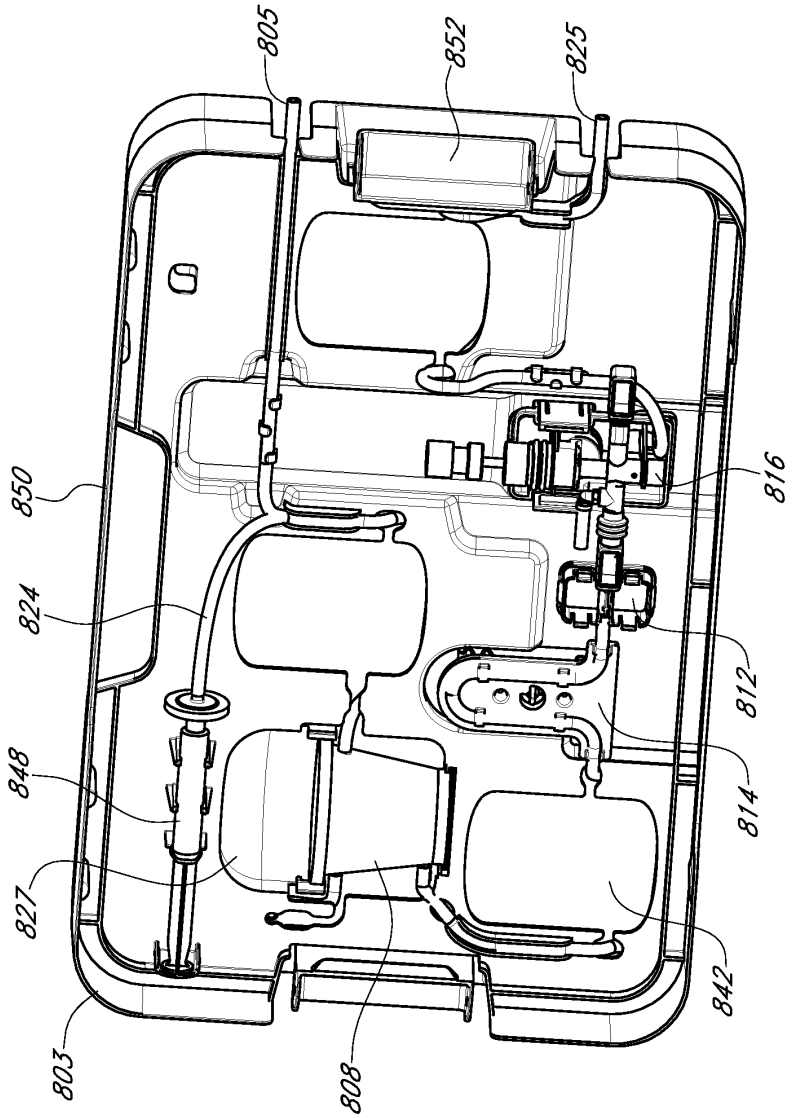


FIG. 12C

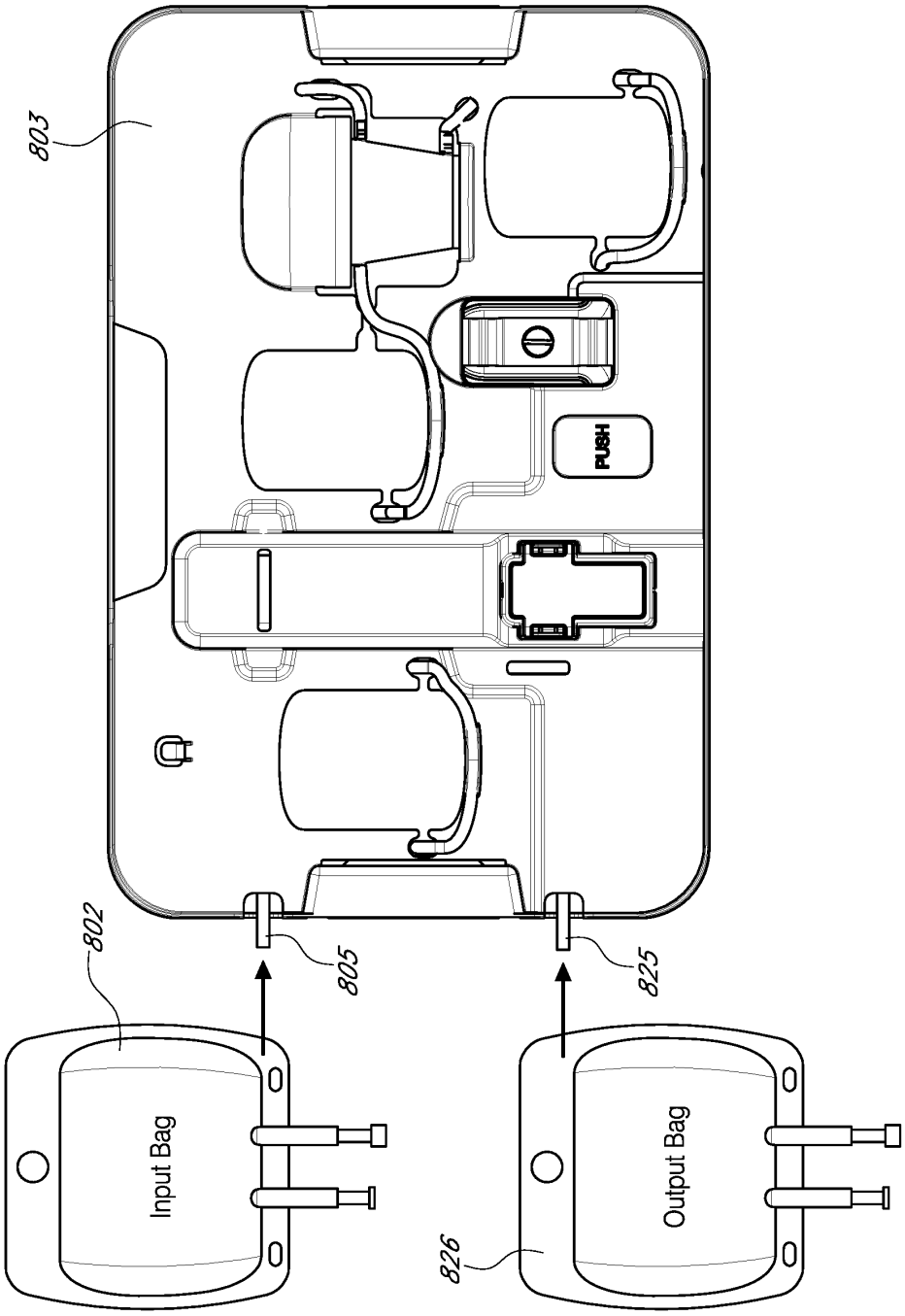


FIG. 12D

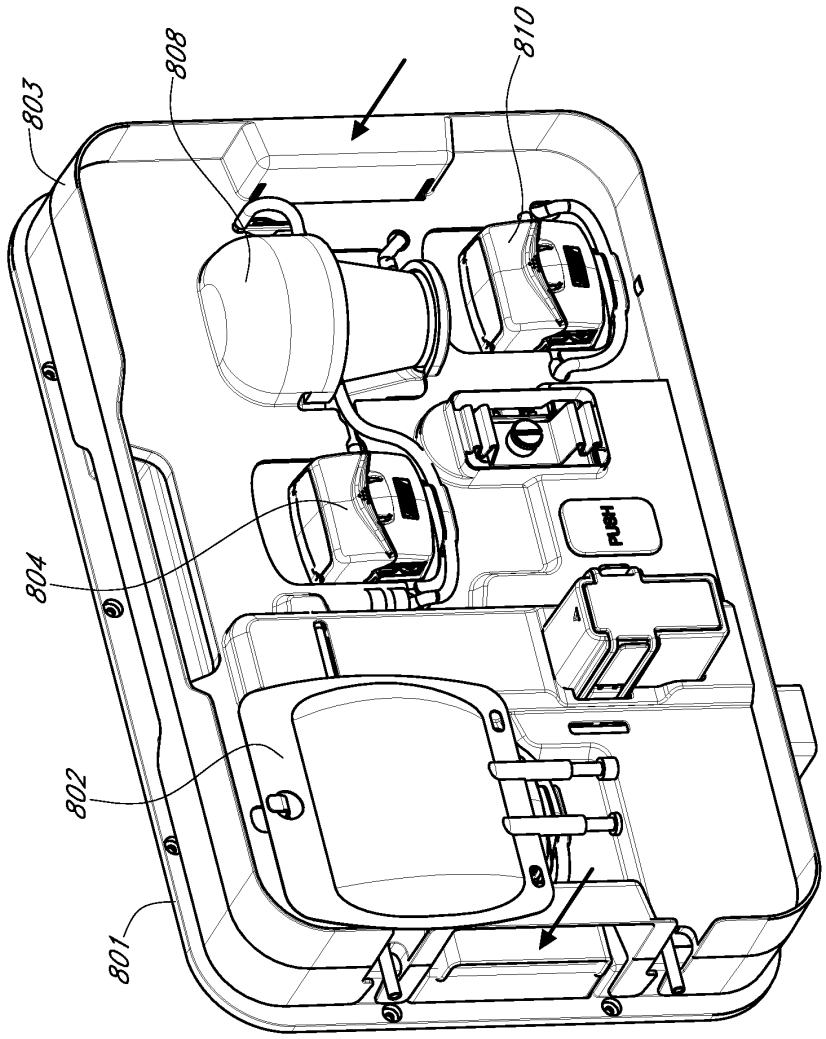


FIG. 12E

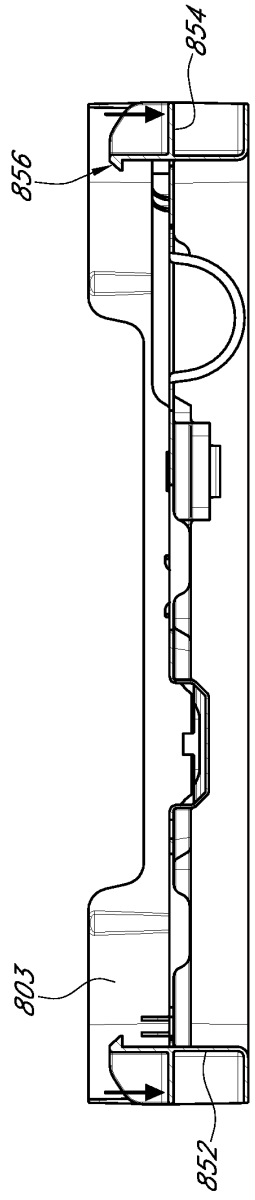


FIG. 12F

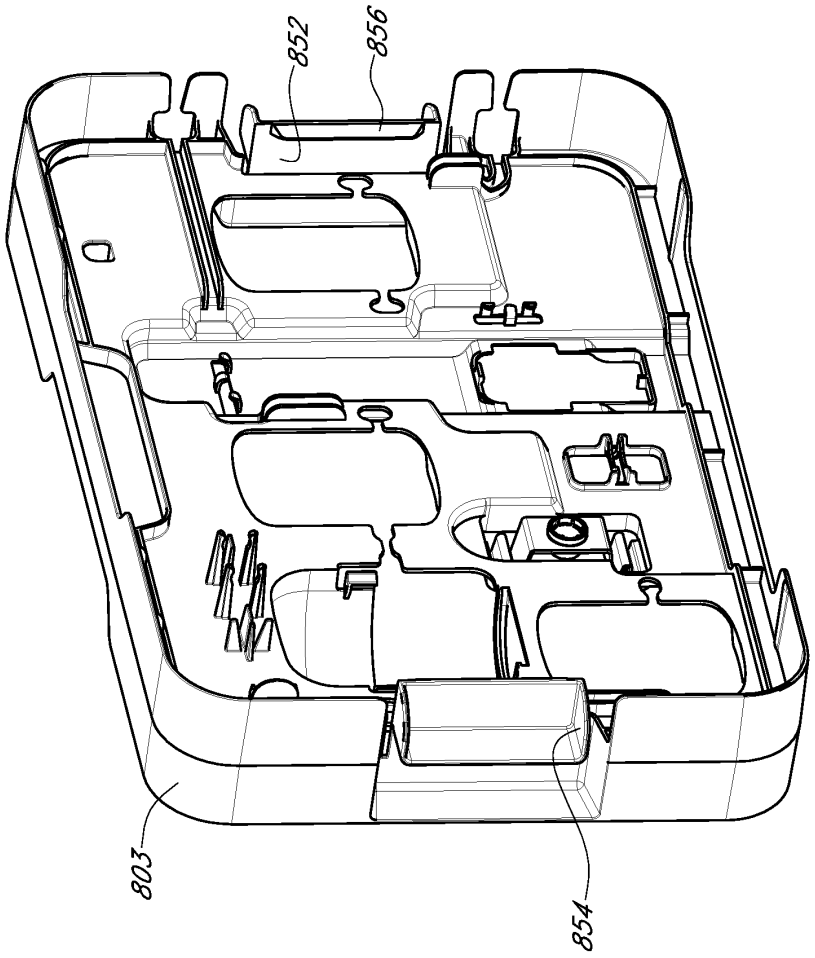


FIG. 12G

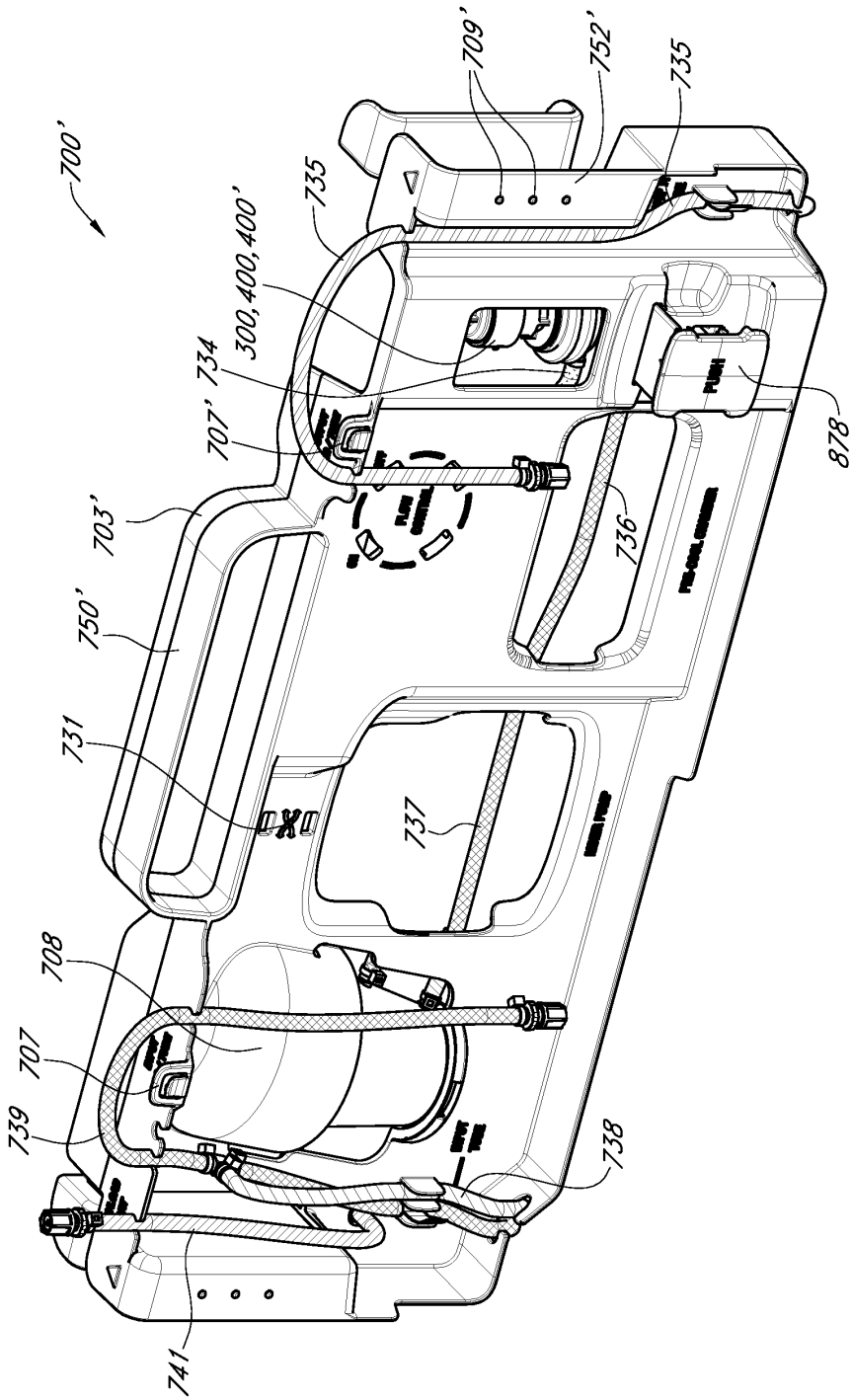

700'
709'
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300,400,400'
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707'
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FIG. 13A

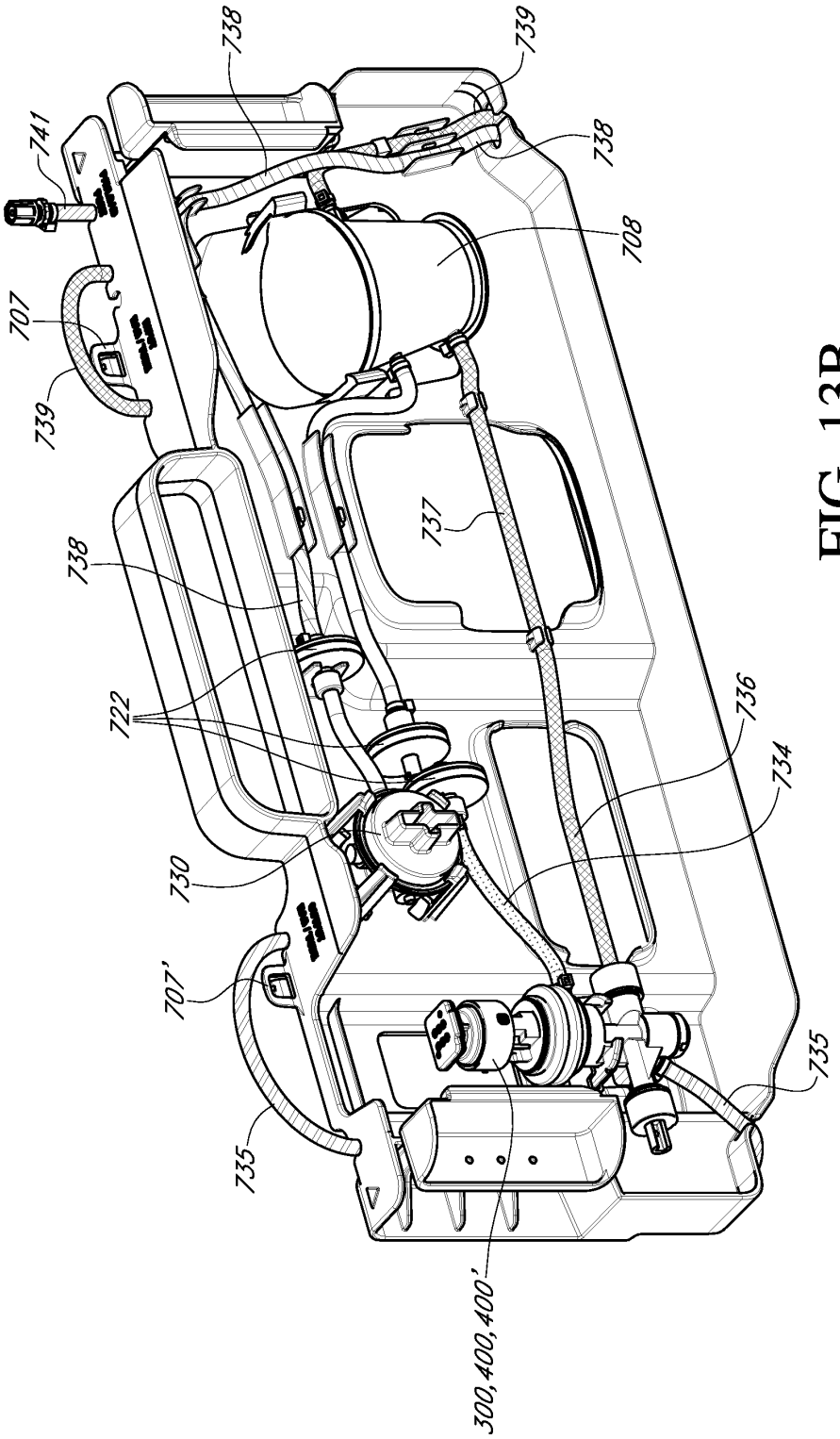

738
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730
707'
735
735
300, 400, 400'

FIG. 13B

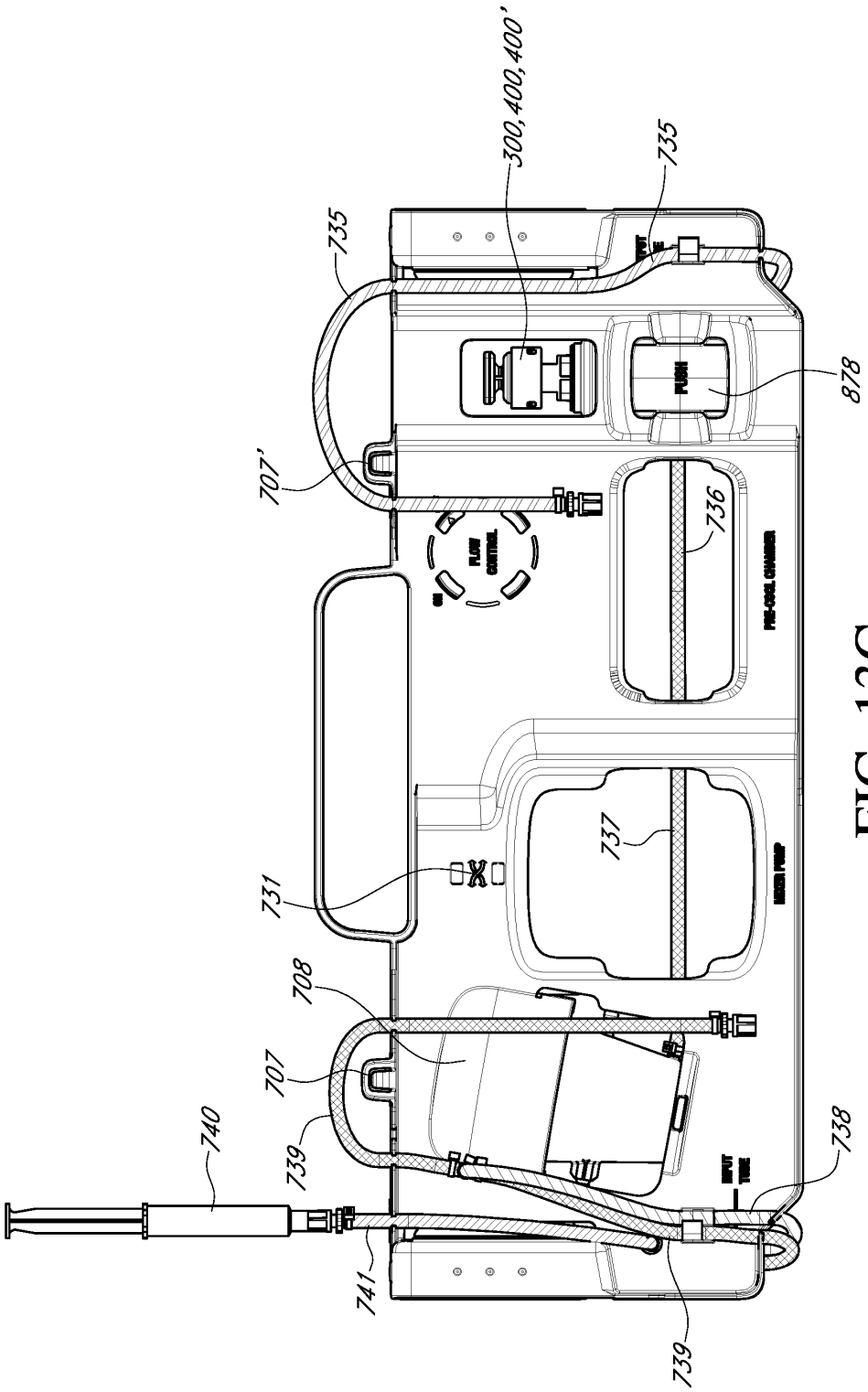

300, 400, 400'
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878
707'
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PUSH
FLOW CONTROL
PRE-COOL CHAMBER
MIXER PUMP
VENT TUBE

FIG. 13C

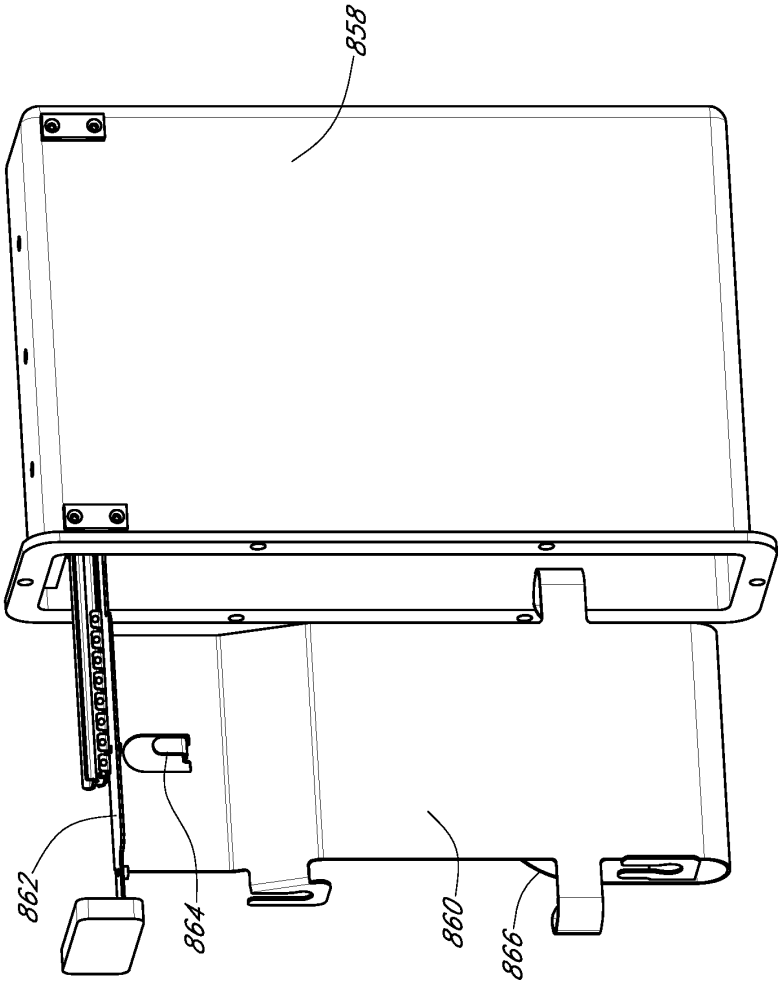


FIG. 14A

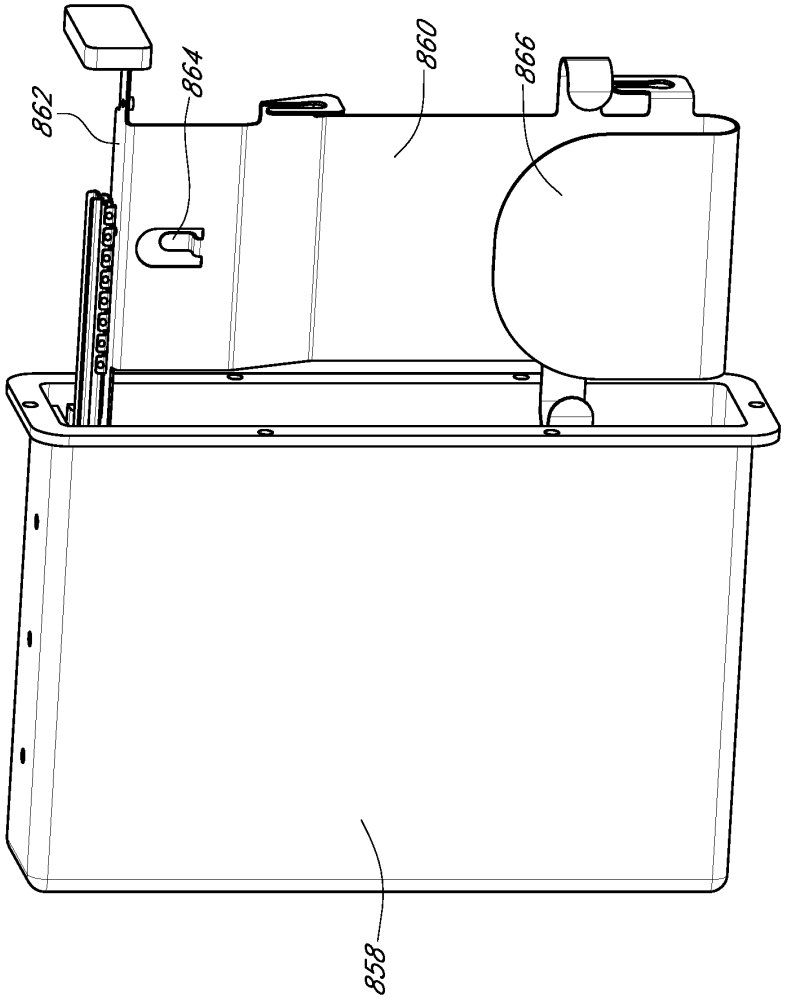


FIG. 14B

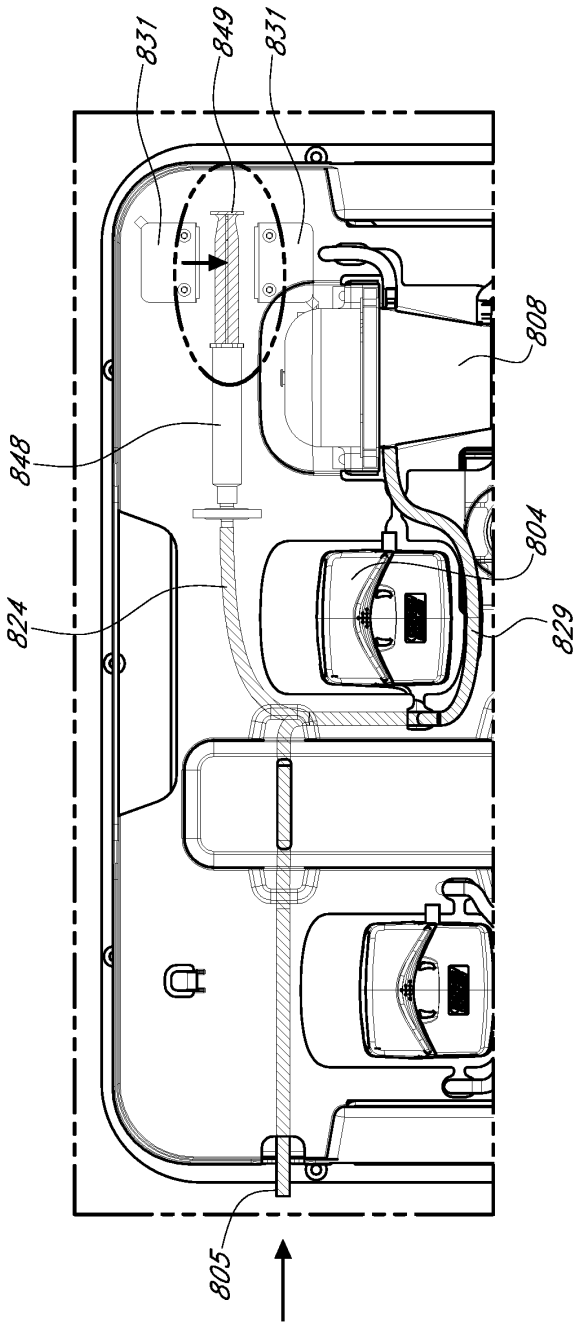


FIG. 15A

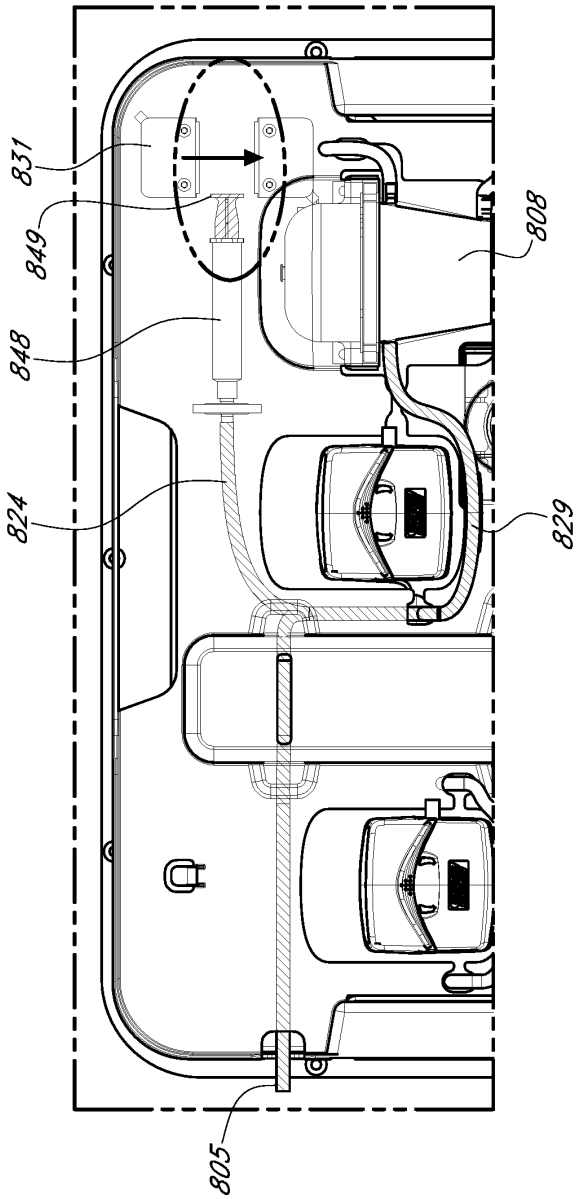


FIG. 15B

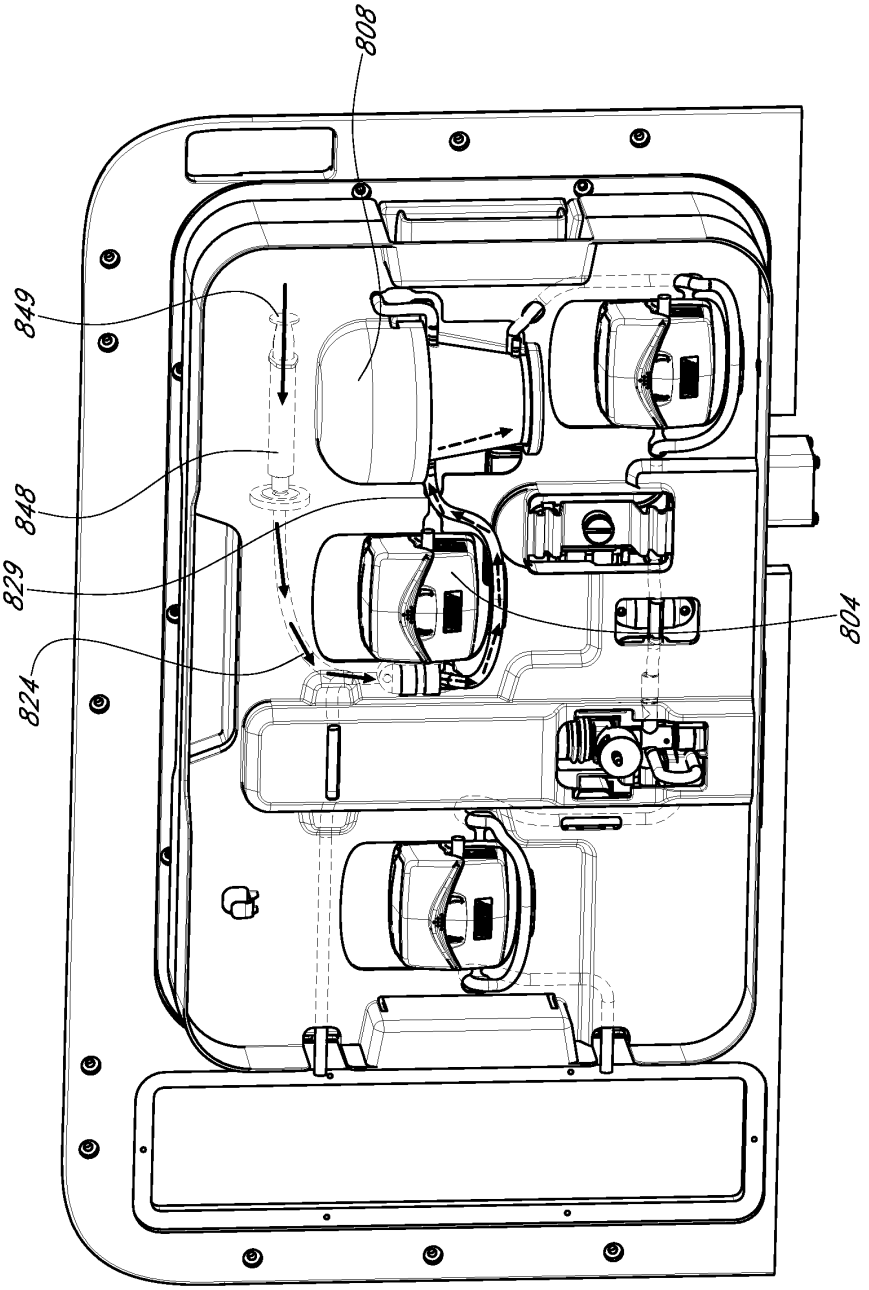


FIG. 15C

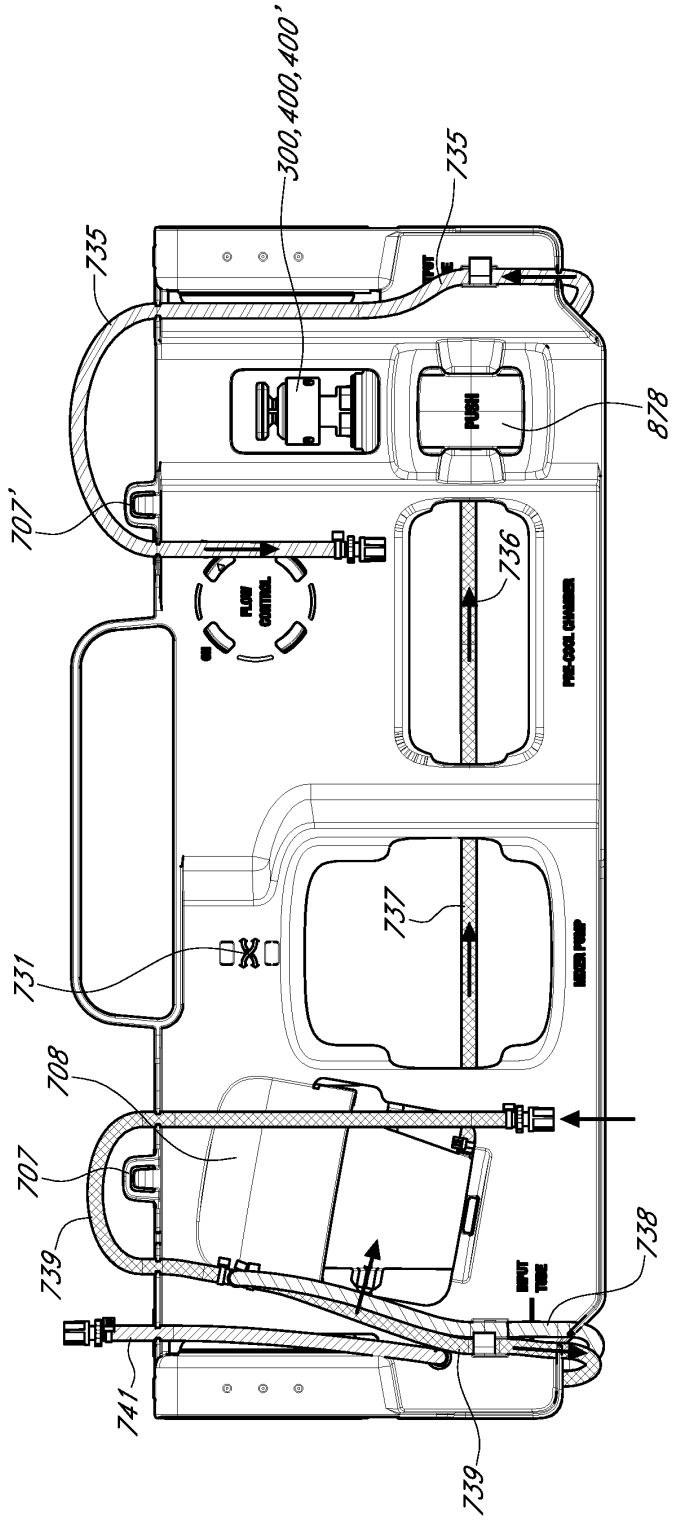

300, 400, 400'
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PUSH
FLOW CONTROL
PRE-COOL CHAMBER
MIXER PUMP
INPUT TUBE

FIG. 15D

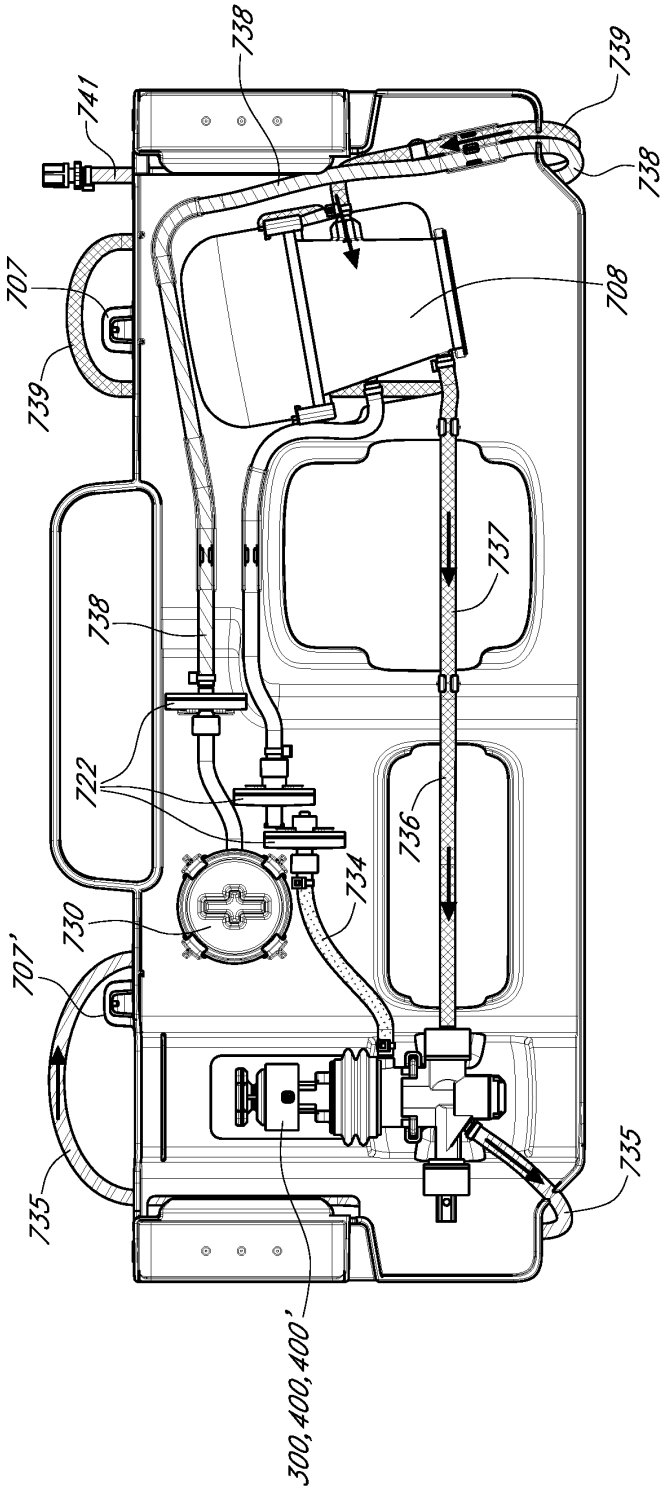

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300, 400, 400'

FIG. 15E

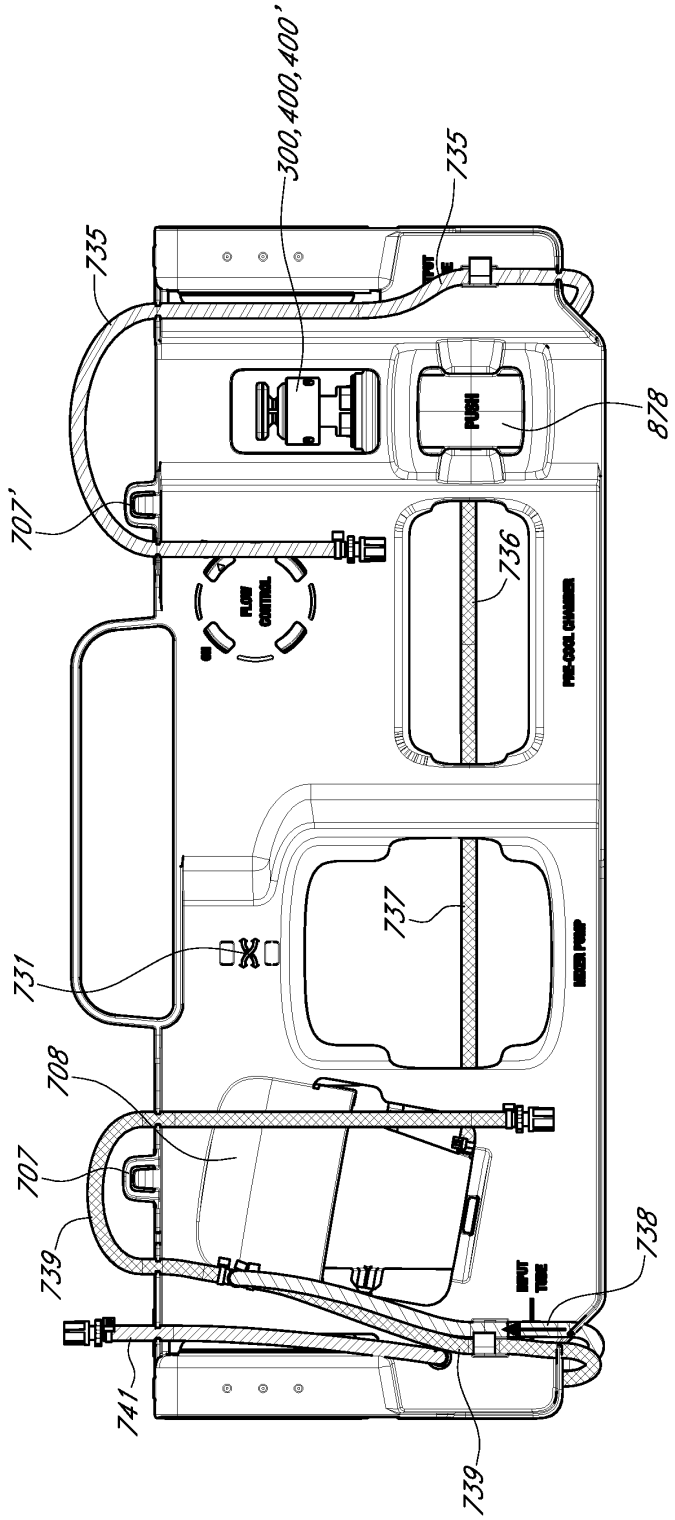

300, 400, 400'
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PUSH
FLOW CONTROL
PRE-COOL CHAMBER
MIXER PUMP
INPUT TUBE

FIG. 15F

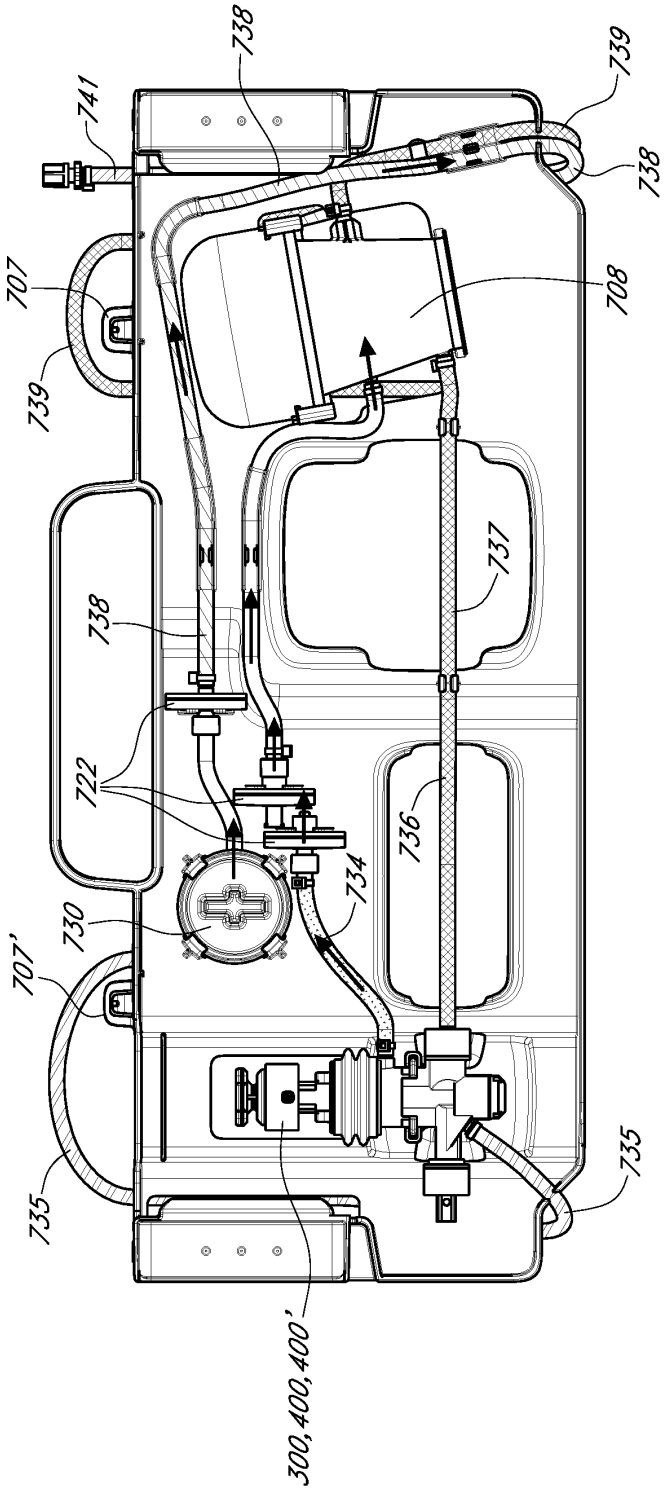

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707'
735
735
300, 400, 400'

FIG. 15G

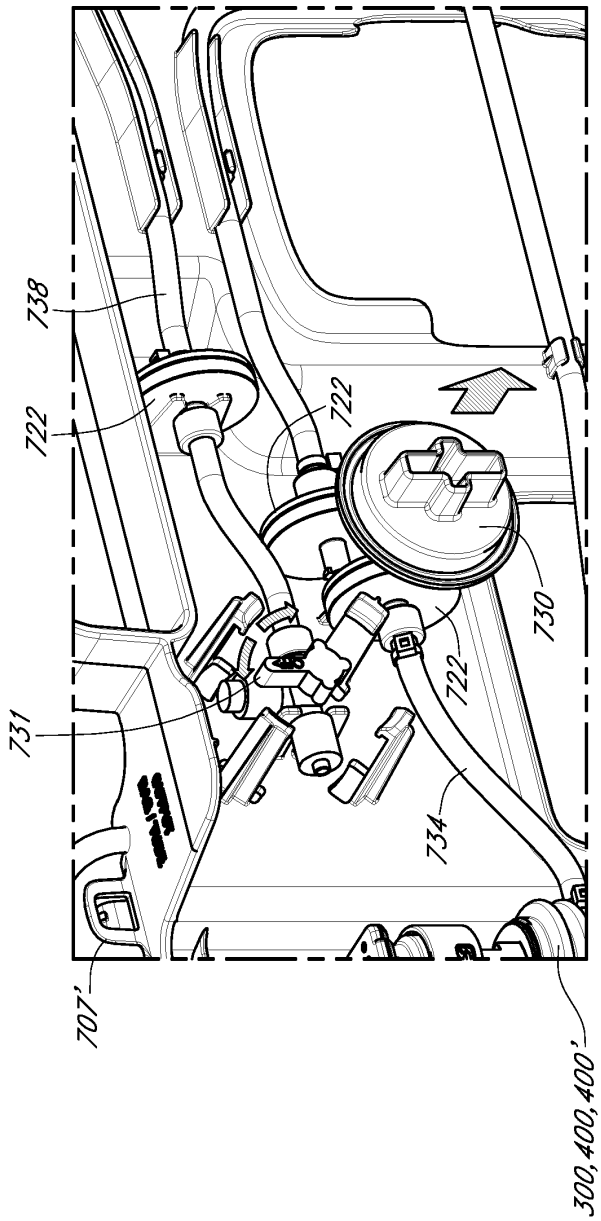


FIG. 15H

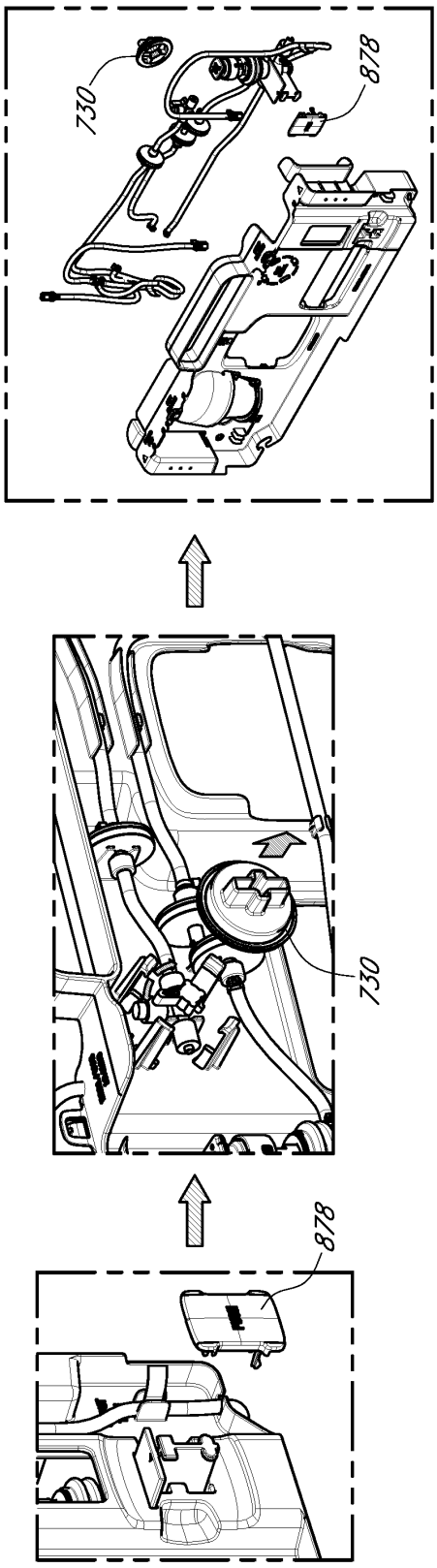


FIG. 15I

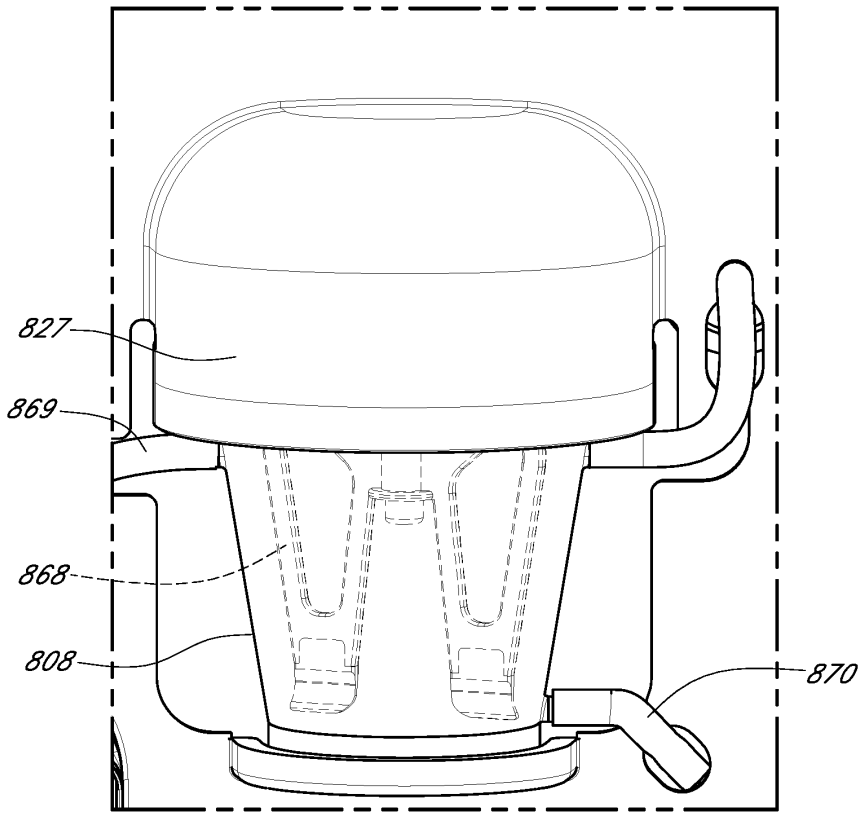


FIG. 16A

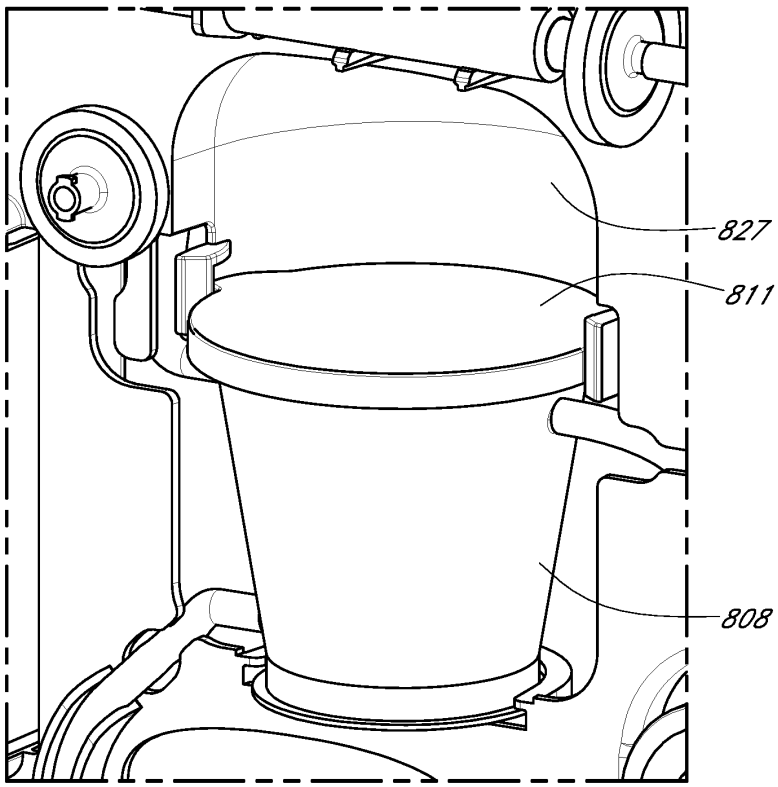


FIG. 16B

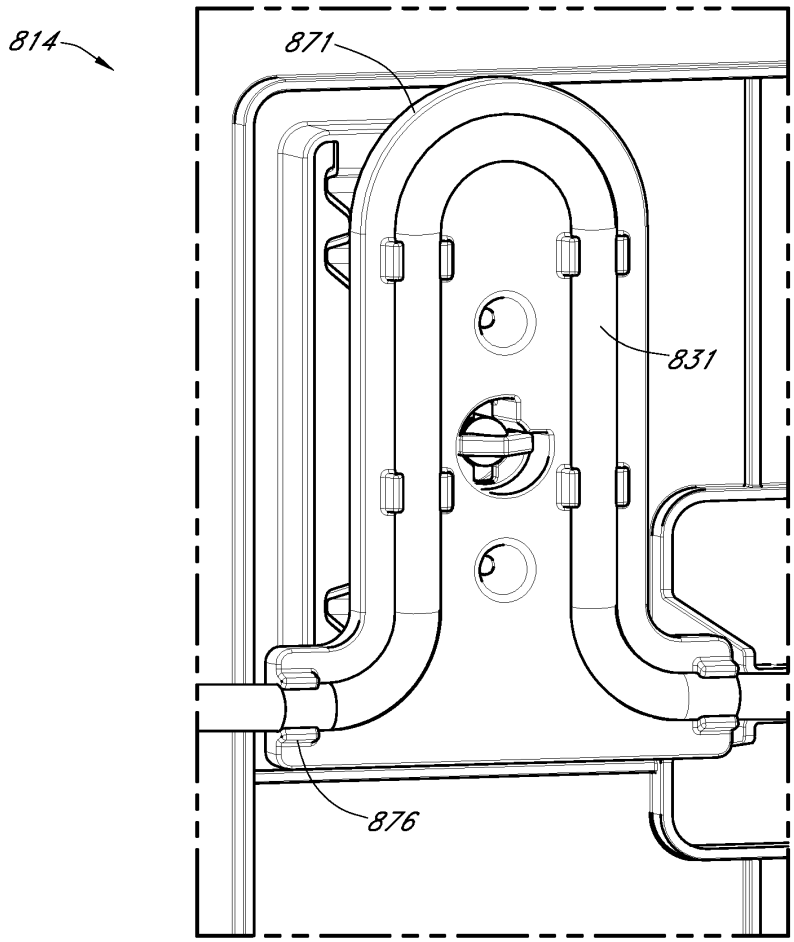


FIG. 17A

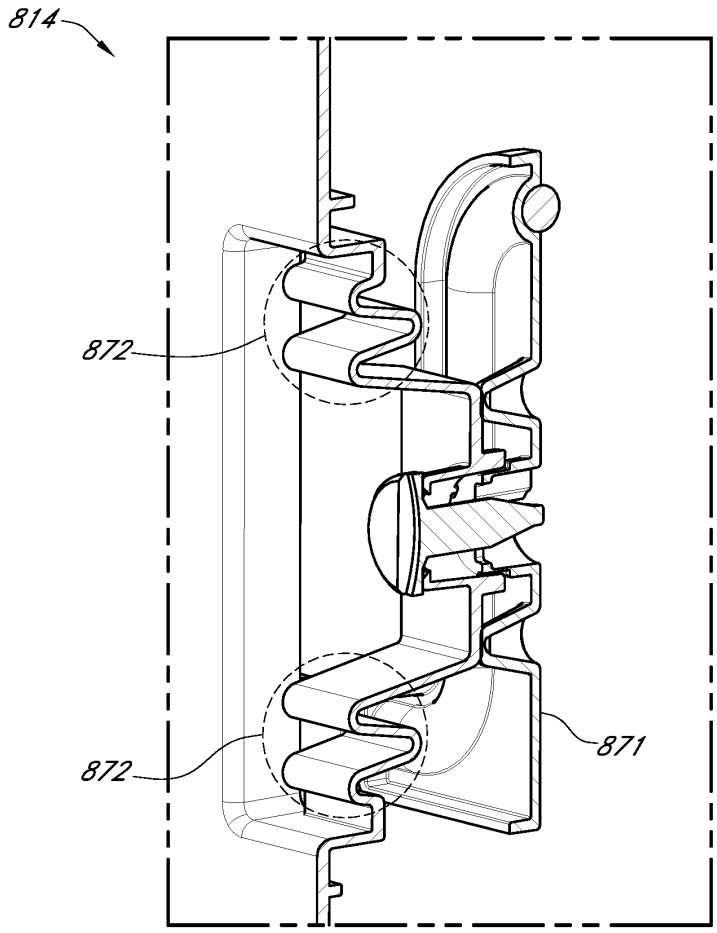


FIG. 17B

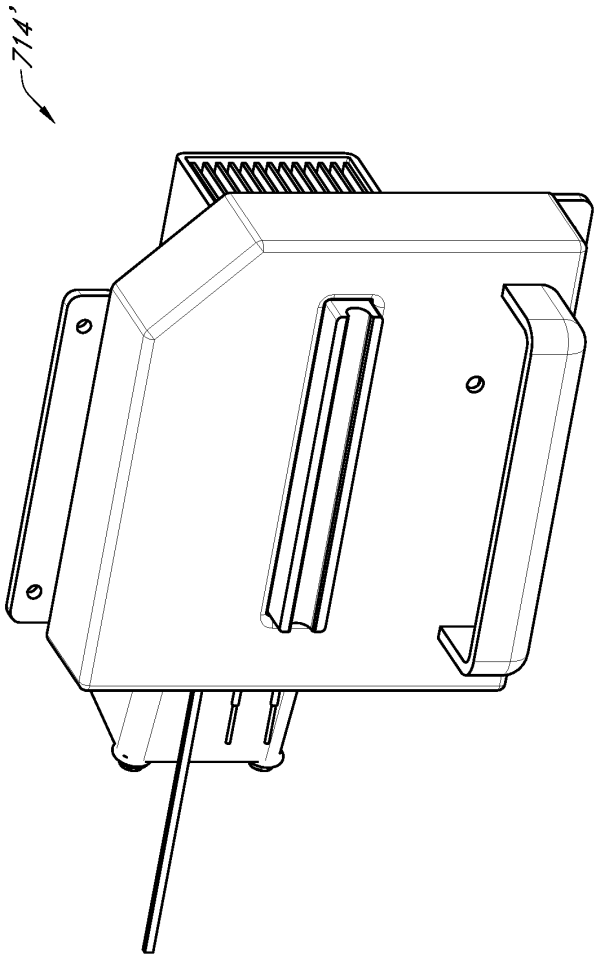


FIG. 17C

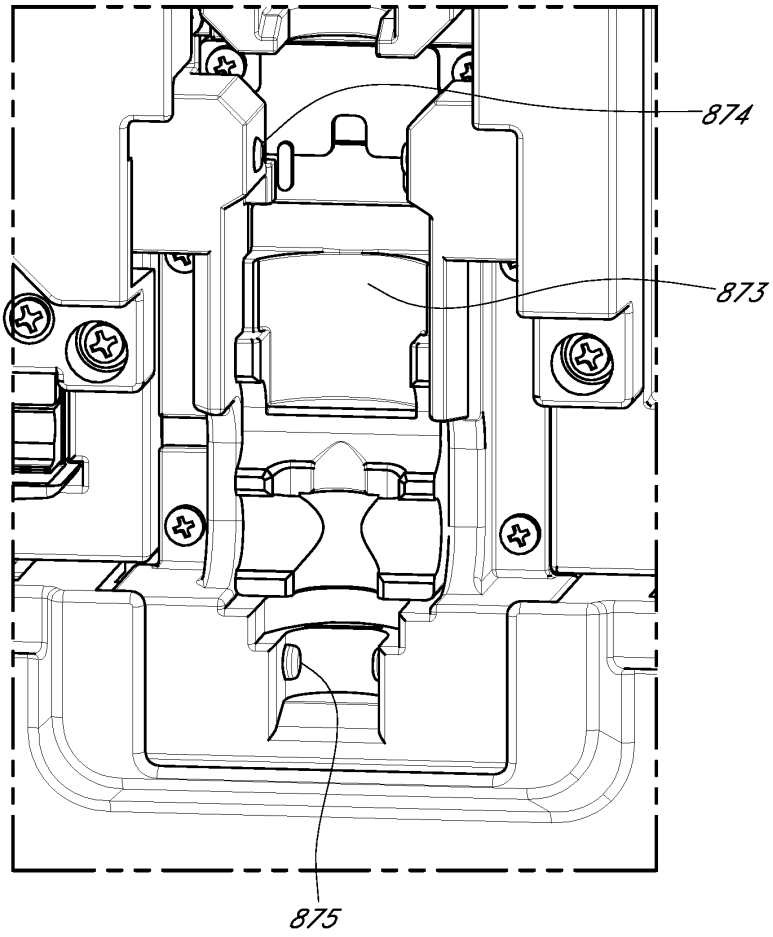


FIG. 17D

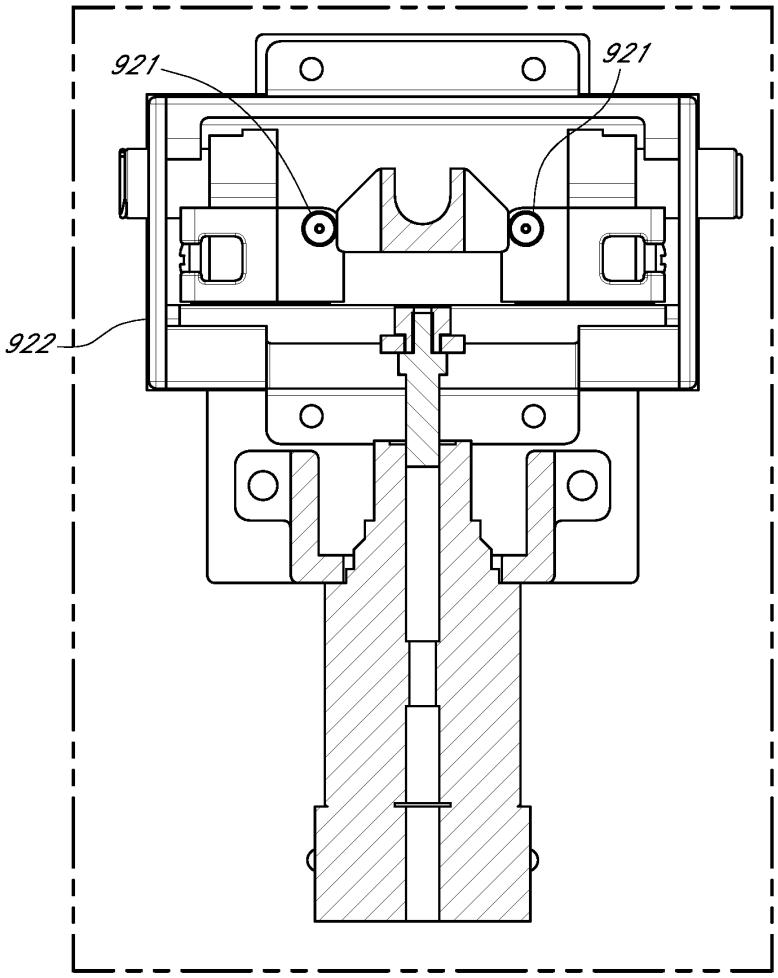


FIG. 18A

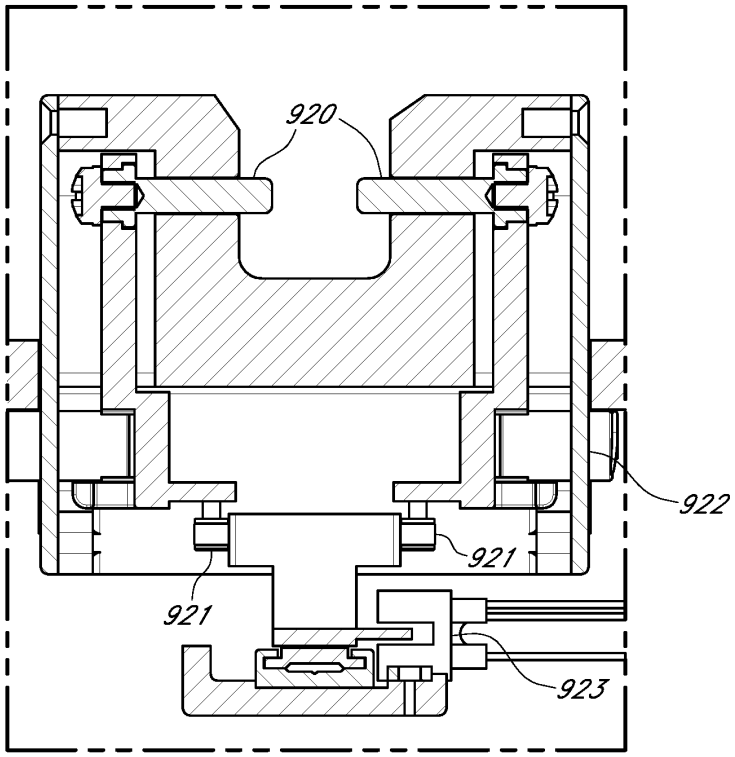


FIG. 18B

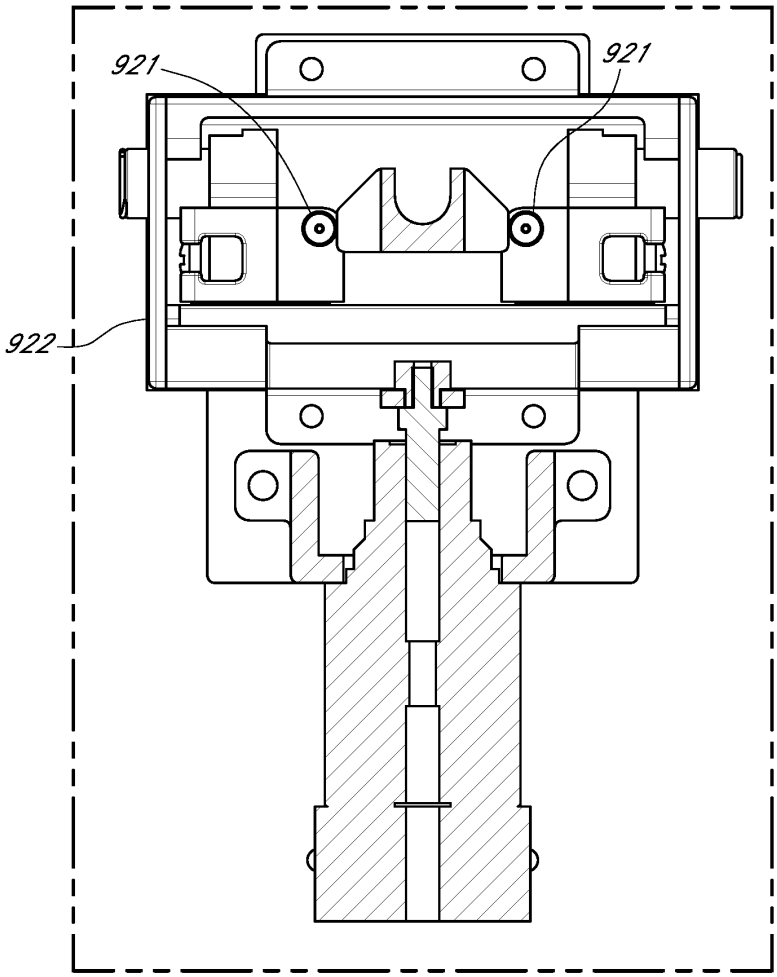


FIG. 18C

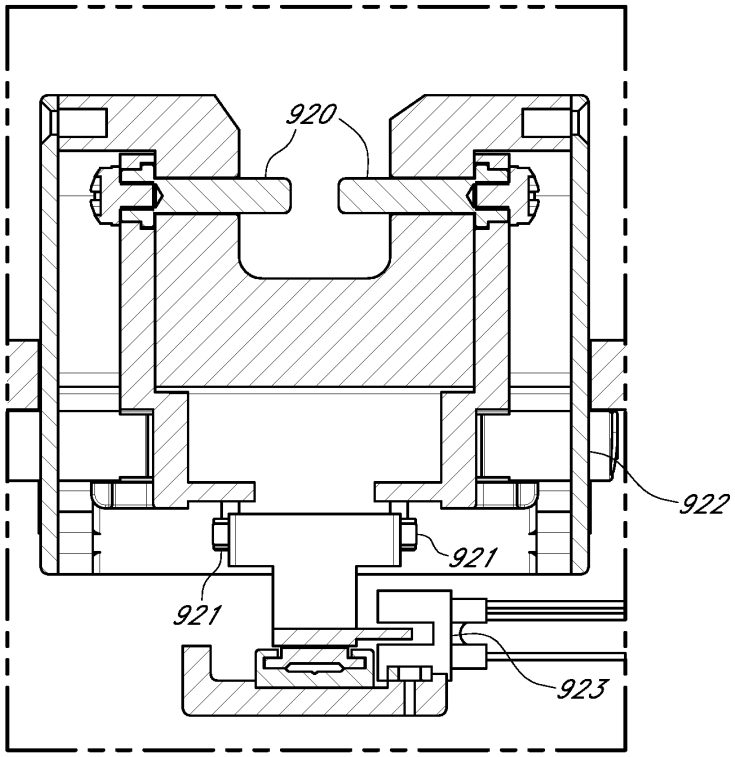


FIG. 18D

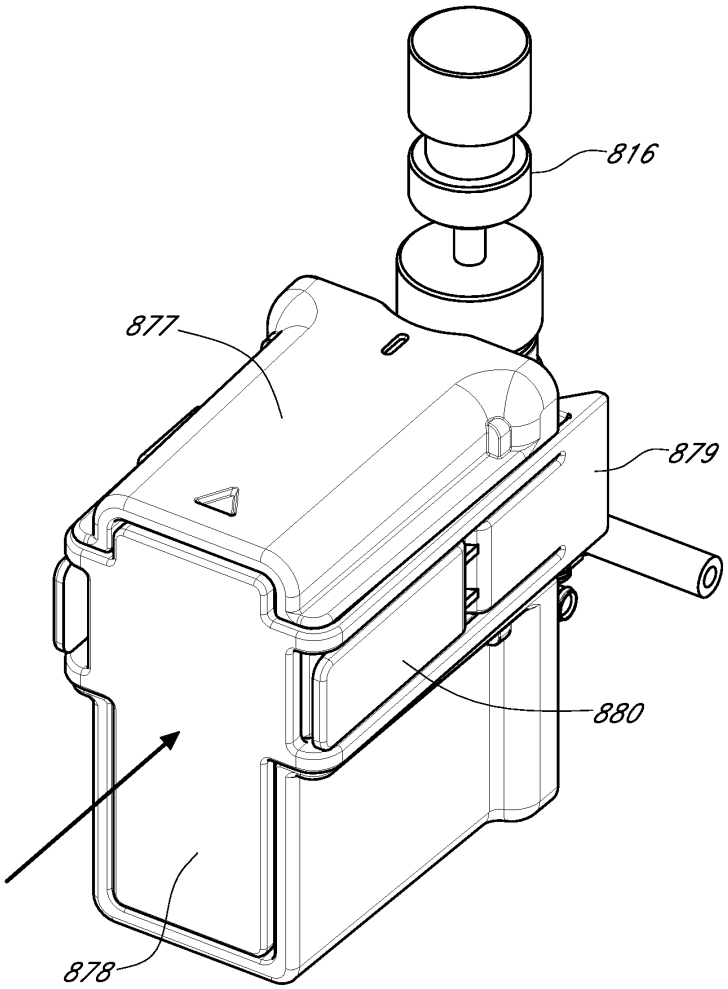


FIG. 19A

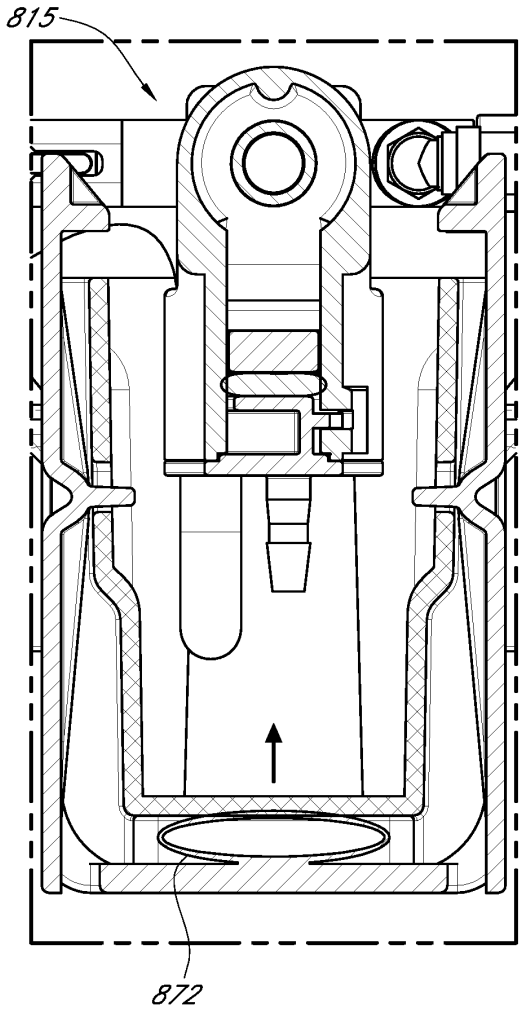


FIG. 19B

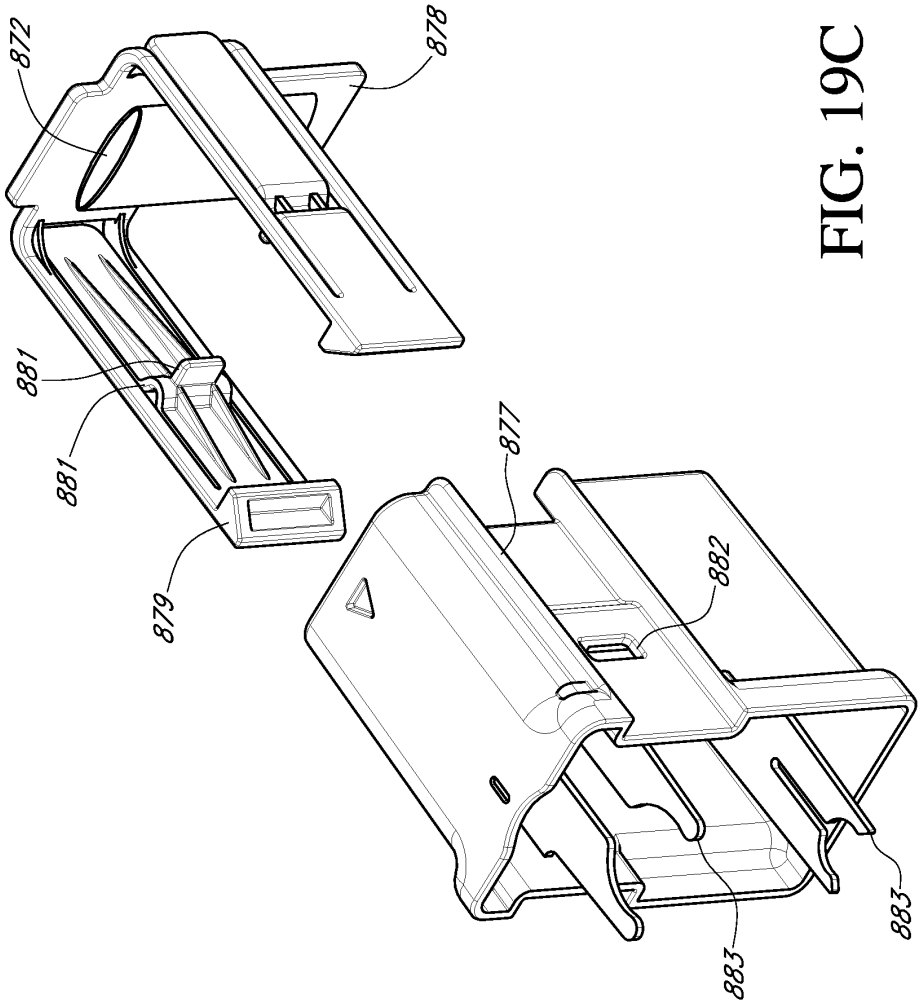


FIG. 19C

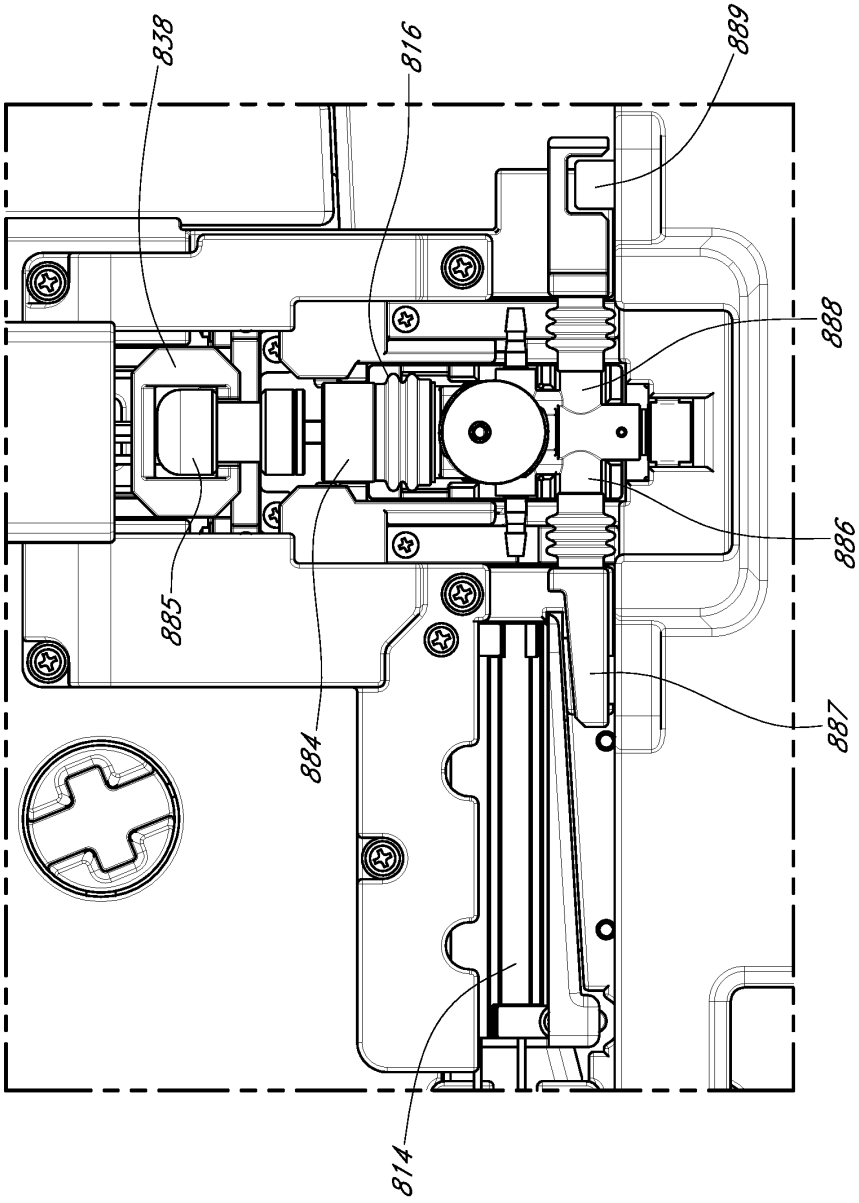


FIG. 20A

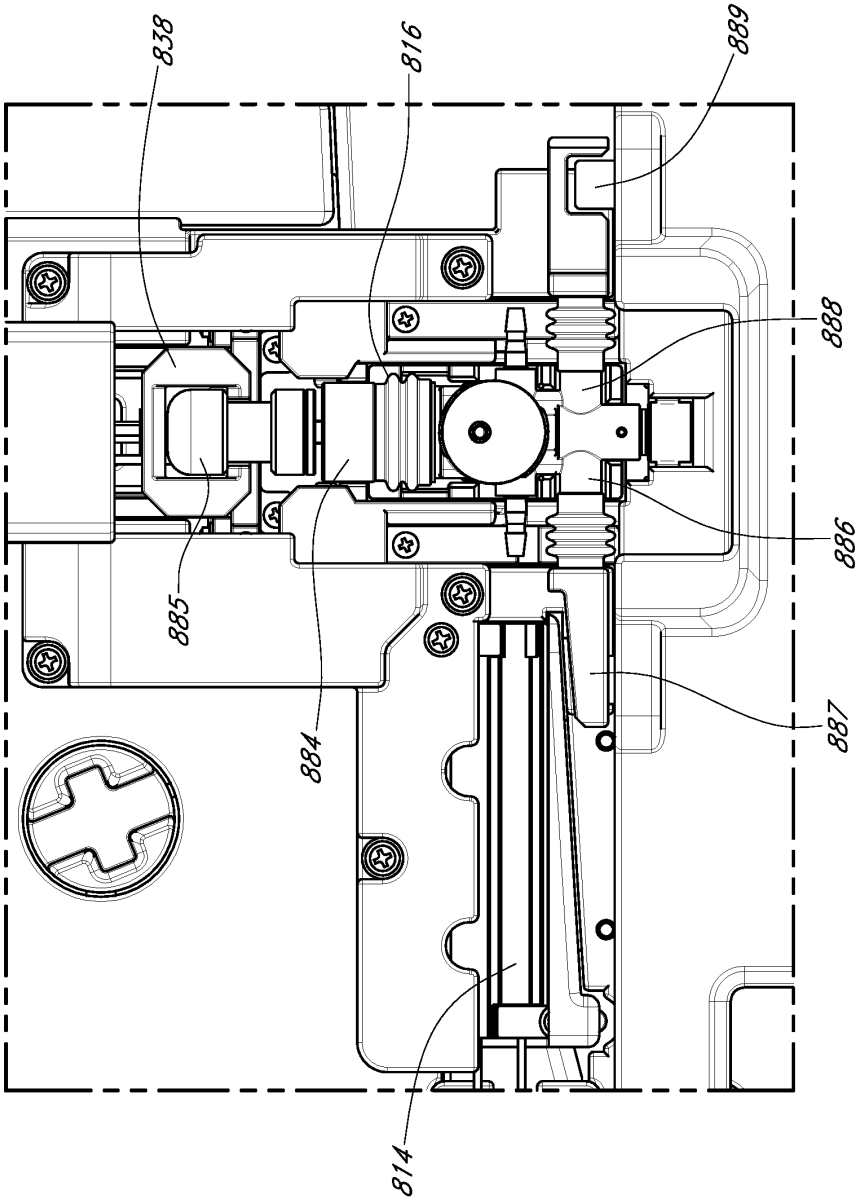


FIG. 20B

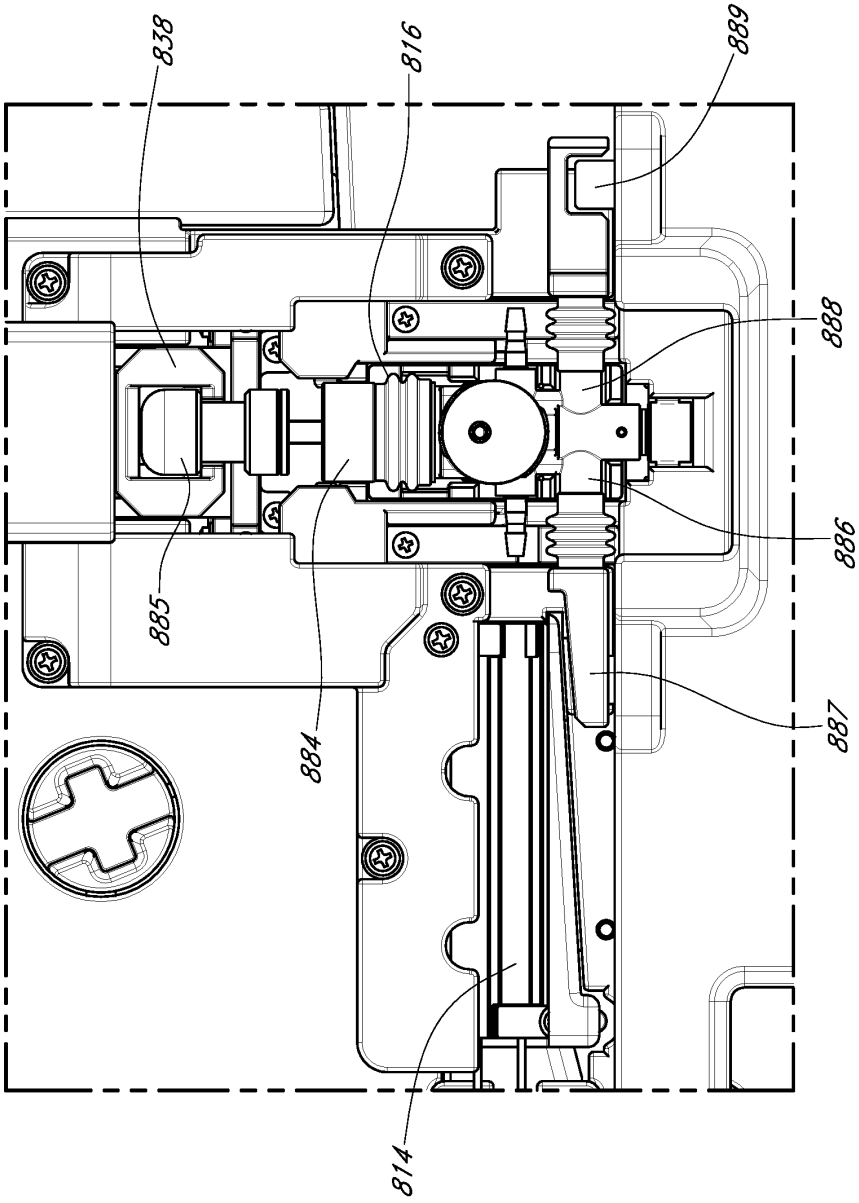


FIG. 20C

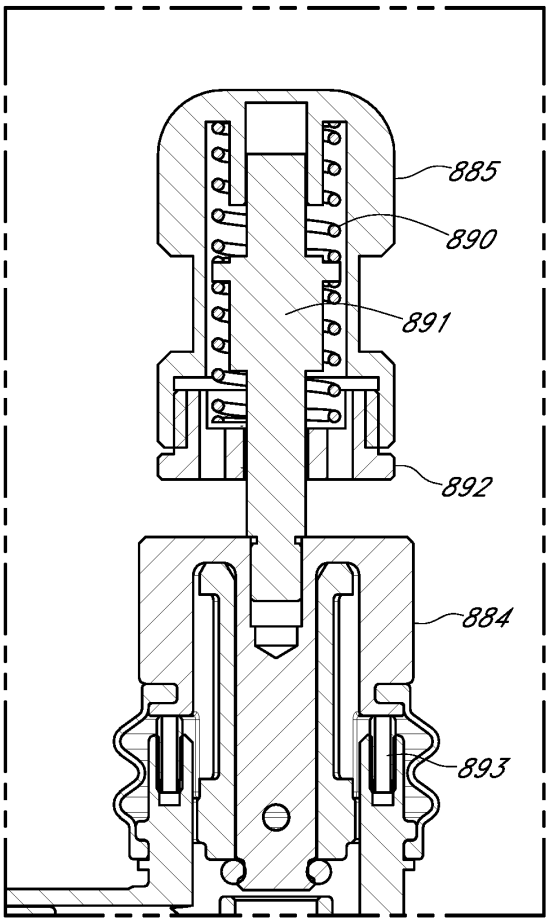


FIG. 21

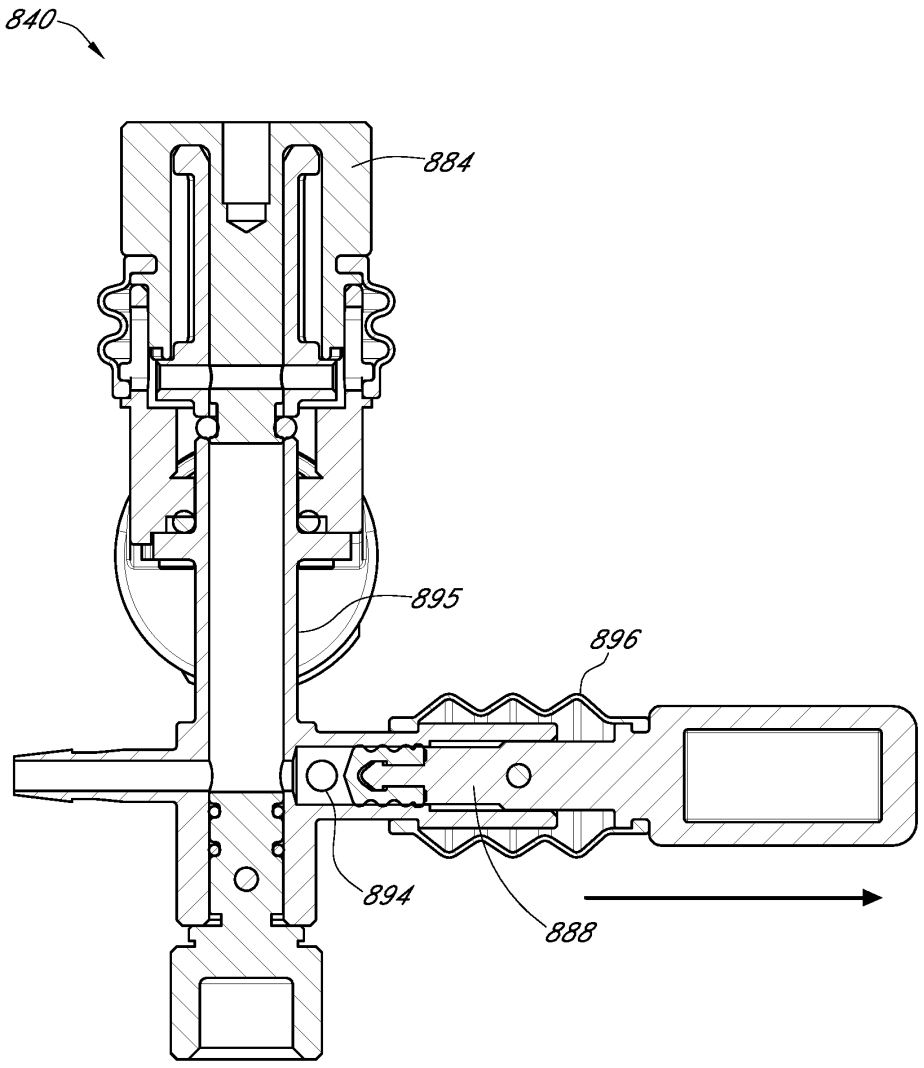


FIG. 22A

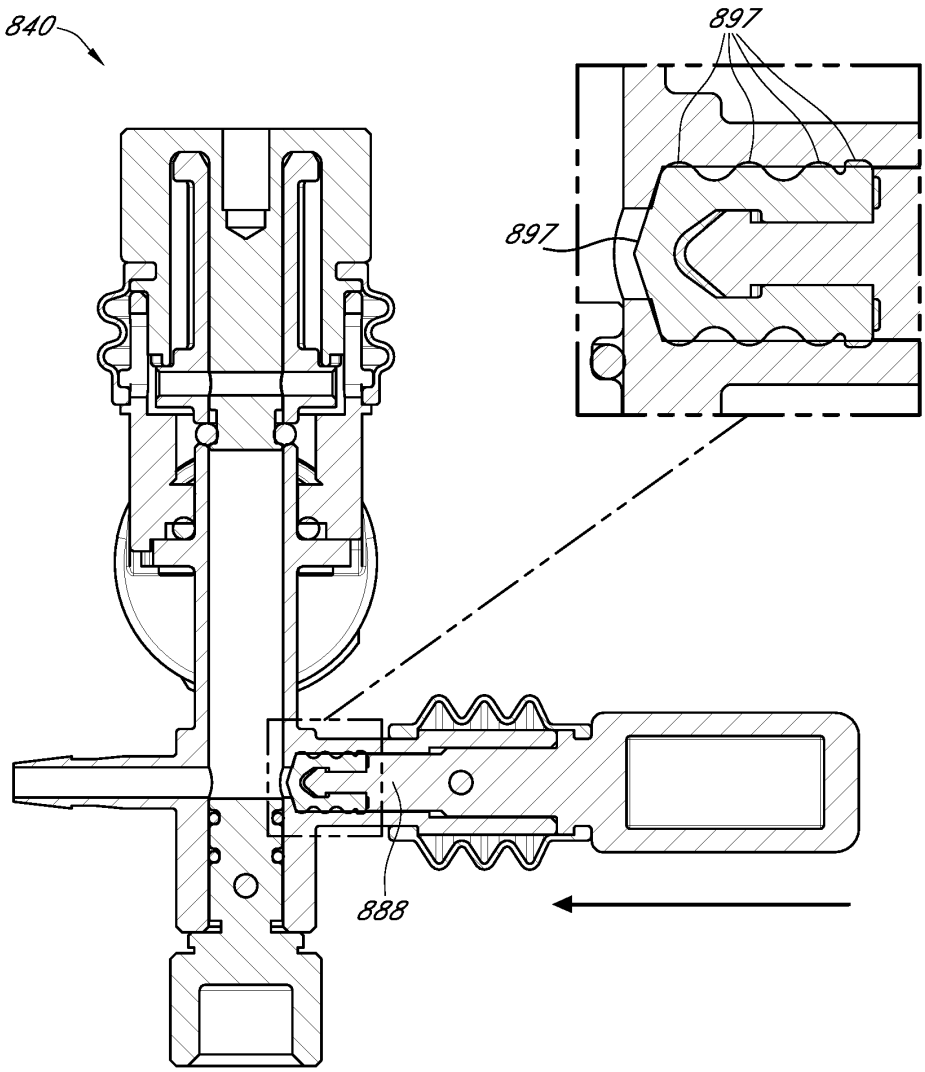


FIG. 22B

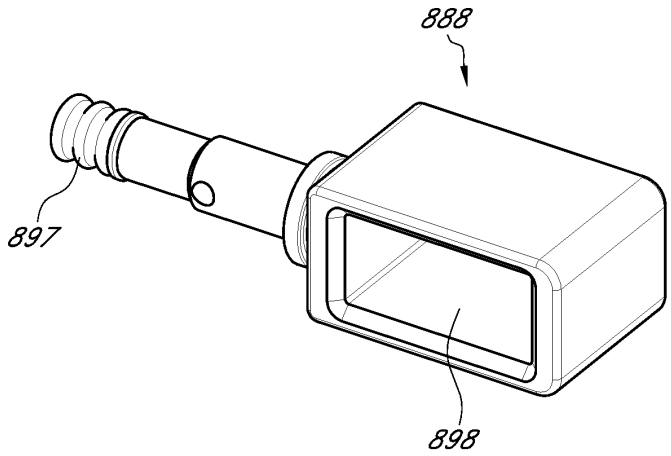


FIG. 22C

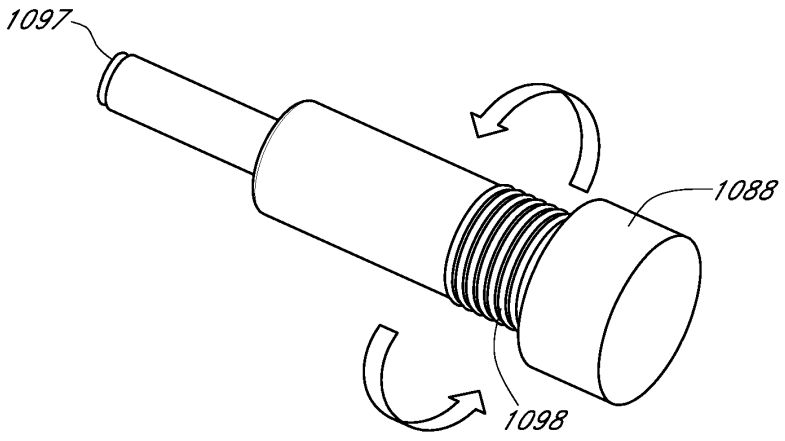


FIG. 22D

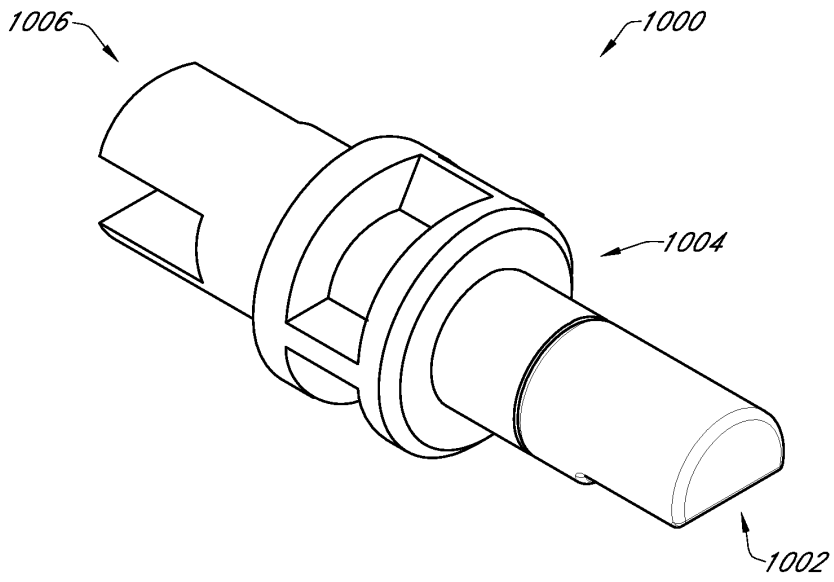


FIG. 22E

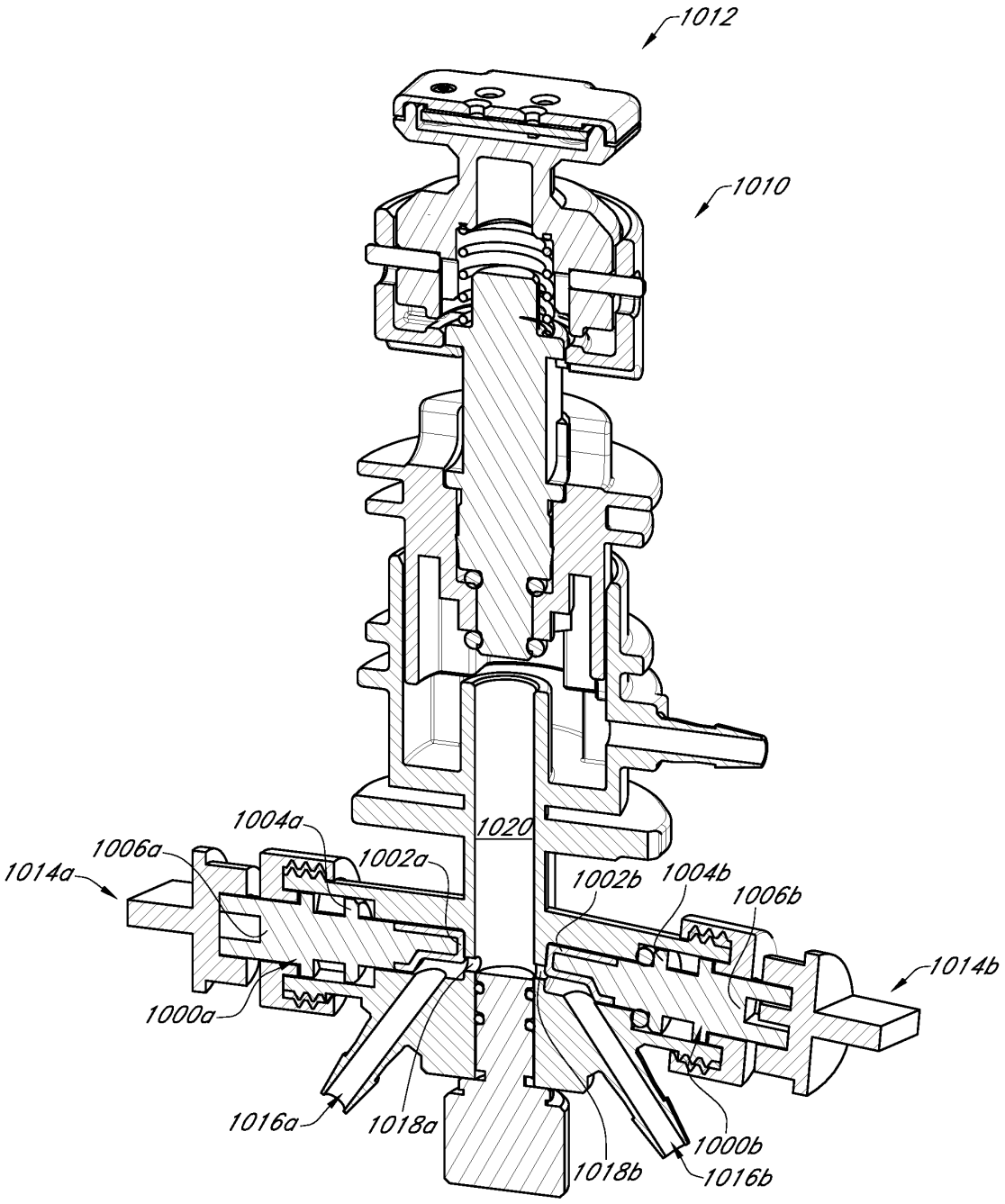

1012
1010
1004a
1006a
1014a
1002a
1020
1002b
1004b
1006b
1014b
1000a
1016a
1018a
1018b
1000b
1016b

FIG. 22F

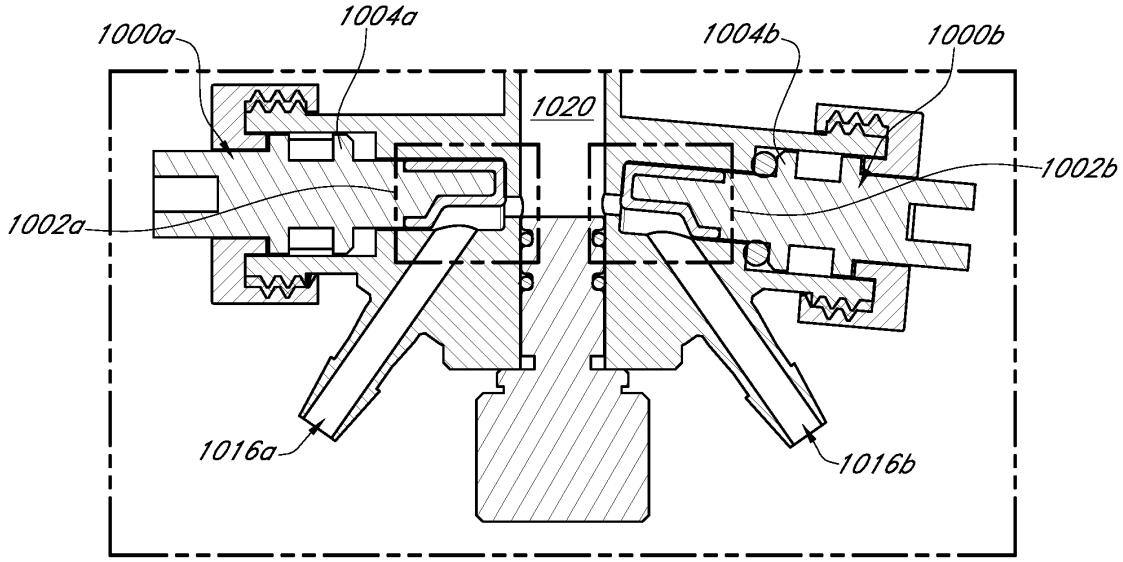


FIG. 22G

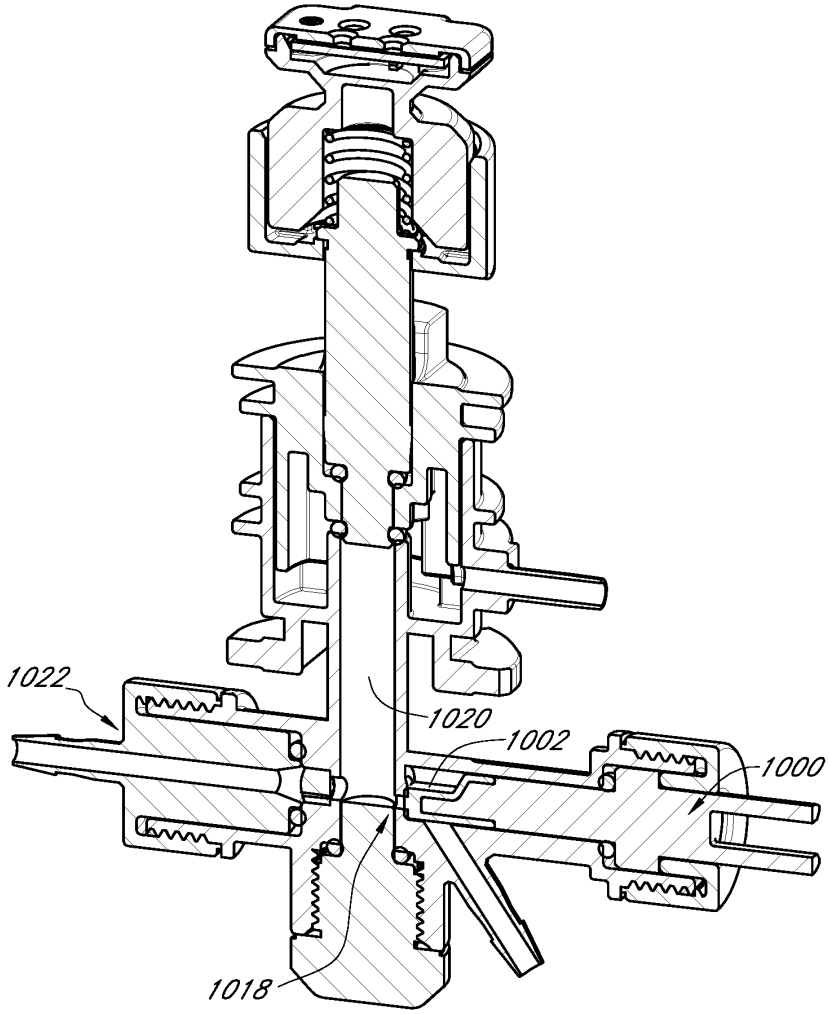


FIG. 22H

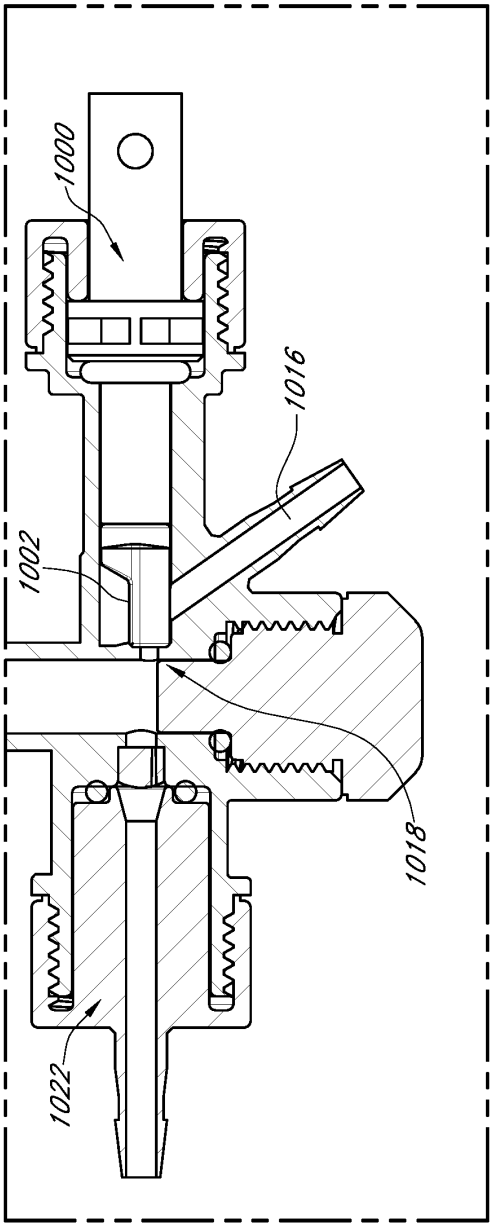


FIG. 22I

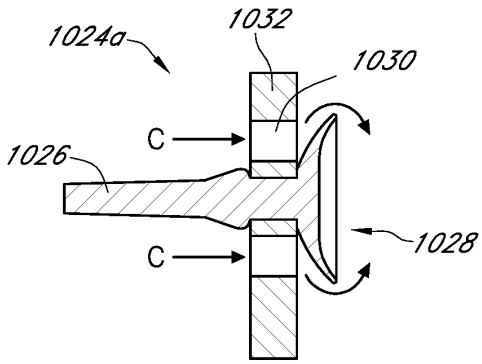


FIG. 22J

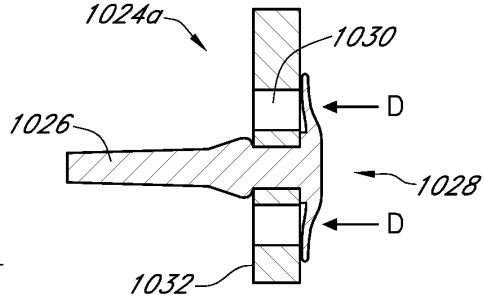


FIG. 22K

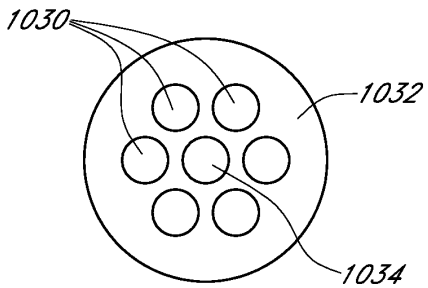


FIG. 22L

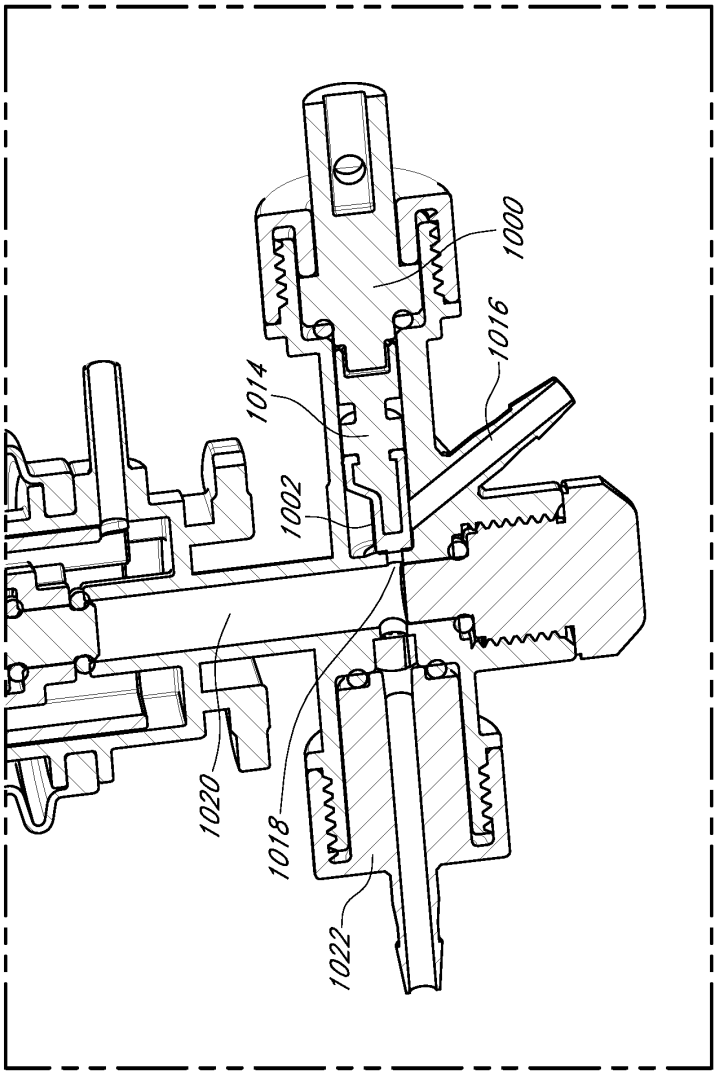


FIG. 22M

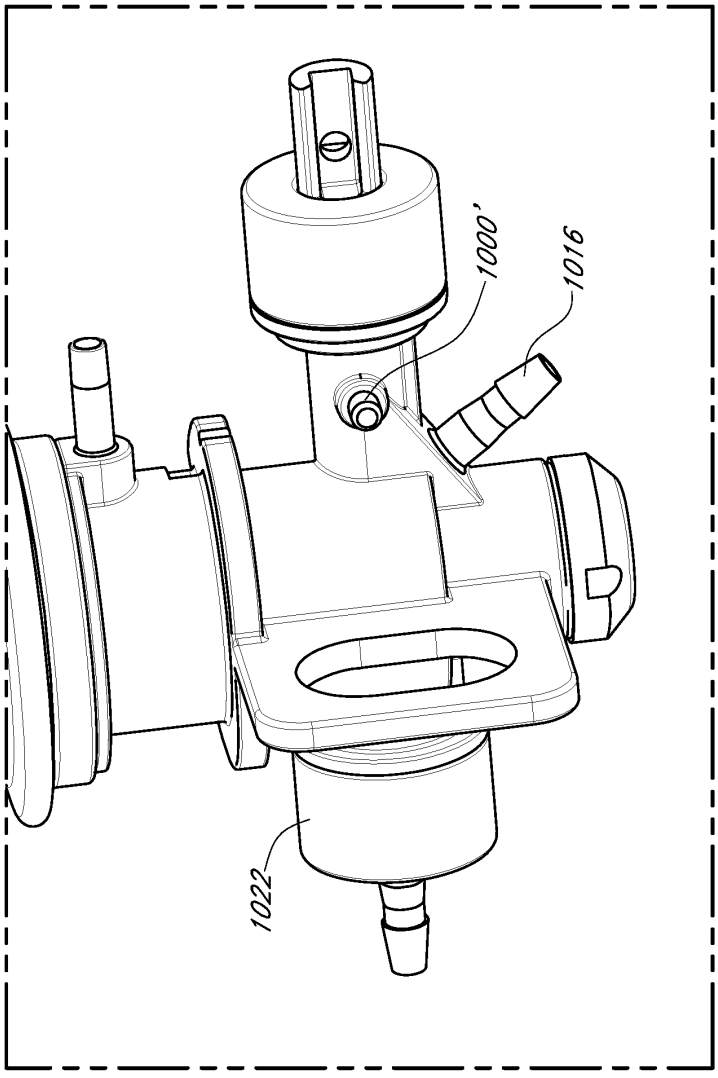


FIG. 22N

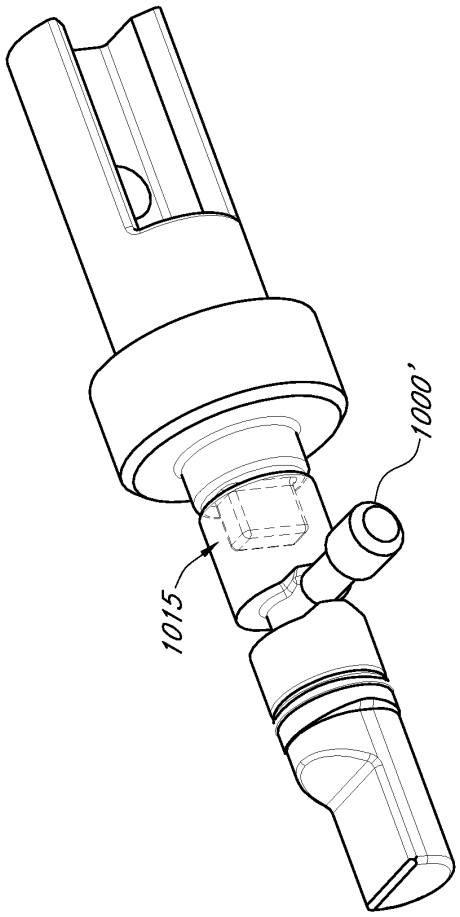


FIG. 22O

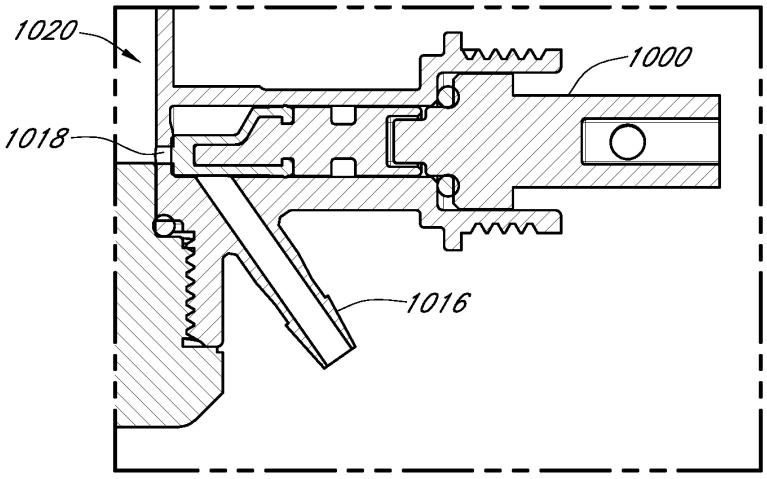


FIG. 22P

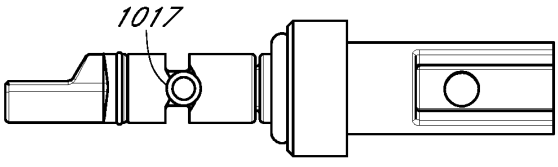


FIG. 22Q

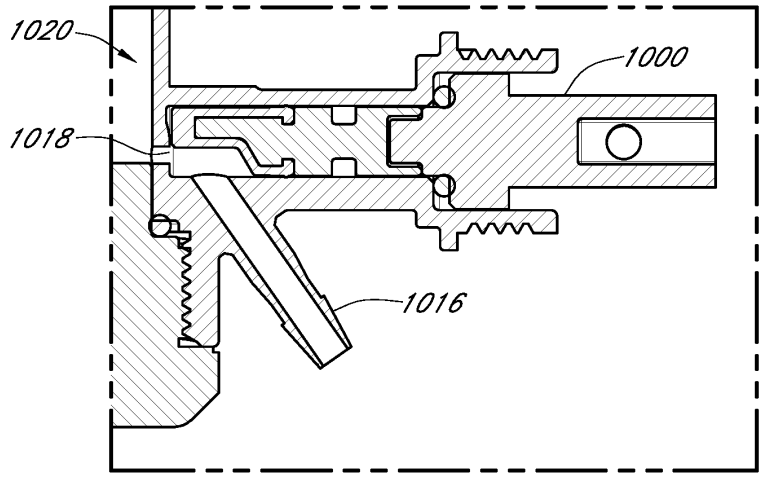


FIG. 22R

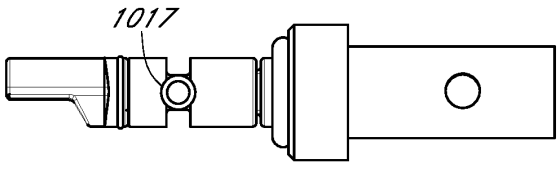


FIG. 22S

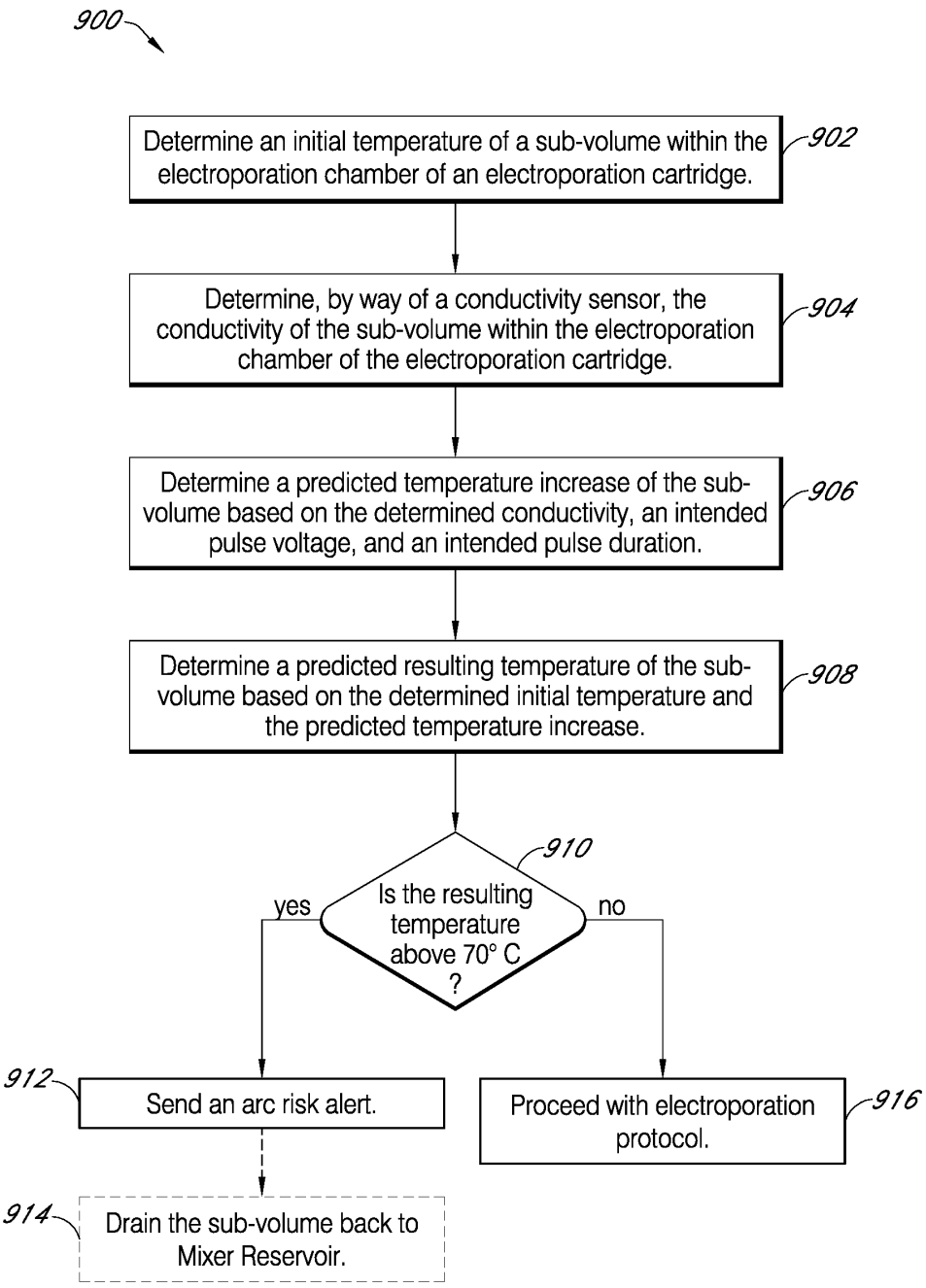

900
Determine an initial temperature of a sub-volume within the electroporation chamber of an electroporation cartridge.
902
Determine, by way of a conductivity sensor, the conductivity of the sub-volume within the electroporation chamber of the electroporation cartridge.
904
Determine a predicted temperature increase of the sub-volume based on the determined conductivity, an intended pulse voltage, and an intended pulse duration.
906
Determine a predicted resulting temperature of the sub-volume based on the determined initial temperature and the predicted temperature increase.
908
910
Is the resulting temperature above 70° C ?
yes
no
912
Send an arc risk alert.
914
Drain the sub-volume back to Mixer Reservoir.
Proceed with electroporation protocol.
916

FIG. 23

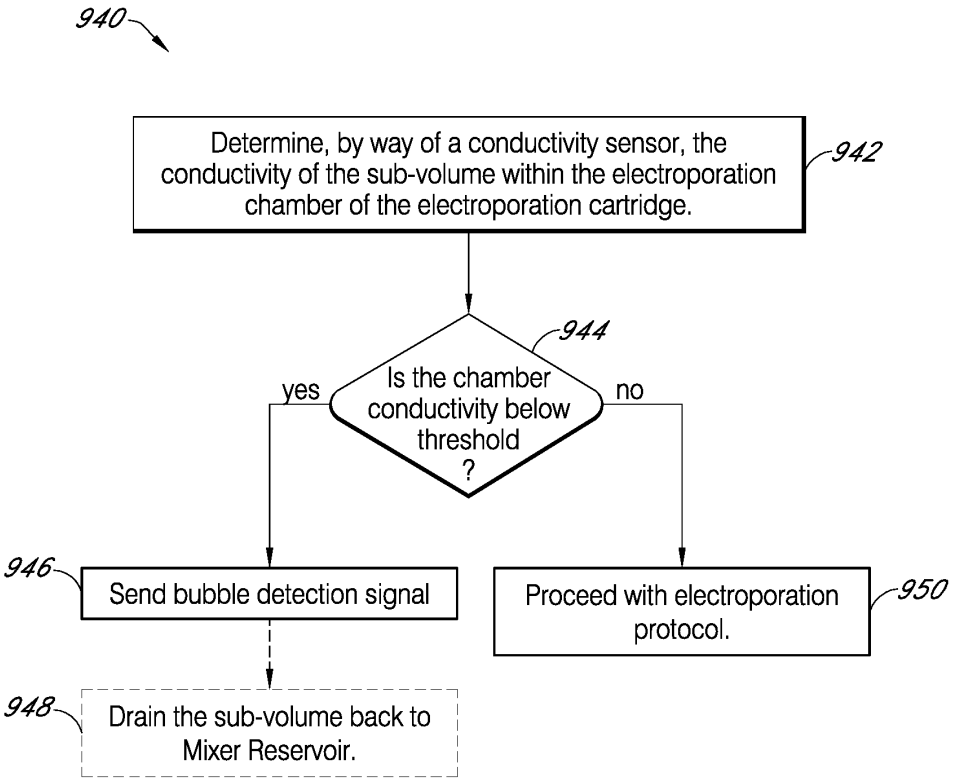

940
Determine, by way of a conductivity sensor, the conductivity of the sub-volume within the electroporation chamber of the electroporation cartridge.
942
944
Is the chamber conductivity below threshold ?
yes
no
946
Send bubble detection signal
948
Drain the sub-volume back to Mixer Reservoir.
Proceed with electroporation protocol.
950

FIG. 24

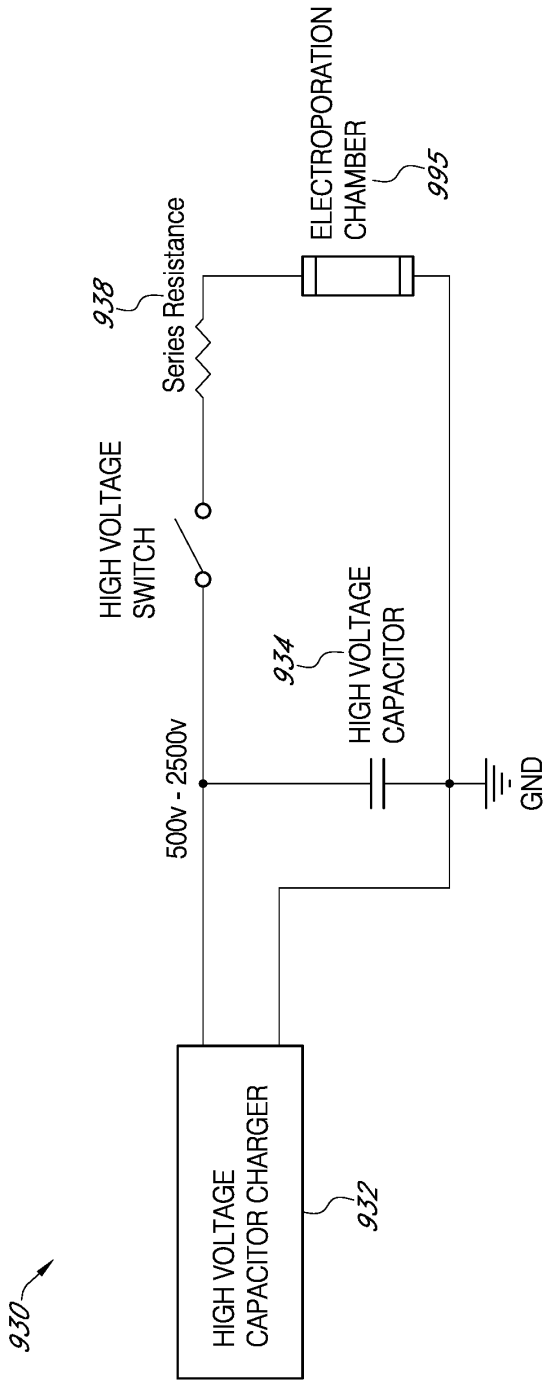

930
HIGH VOLTAGE CAPACITOR CHARGER
932
500v - 2500v
HIGH VOLTAGE SWITCH
938
Series Resistance
934
HIGH VOLTAGE CAPACITOR
GND
ELECTROPORATION CHAMBER
995

FIG. 25

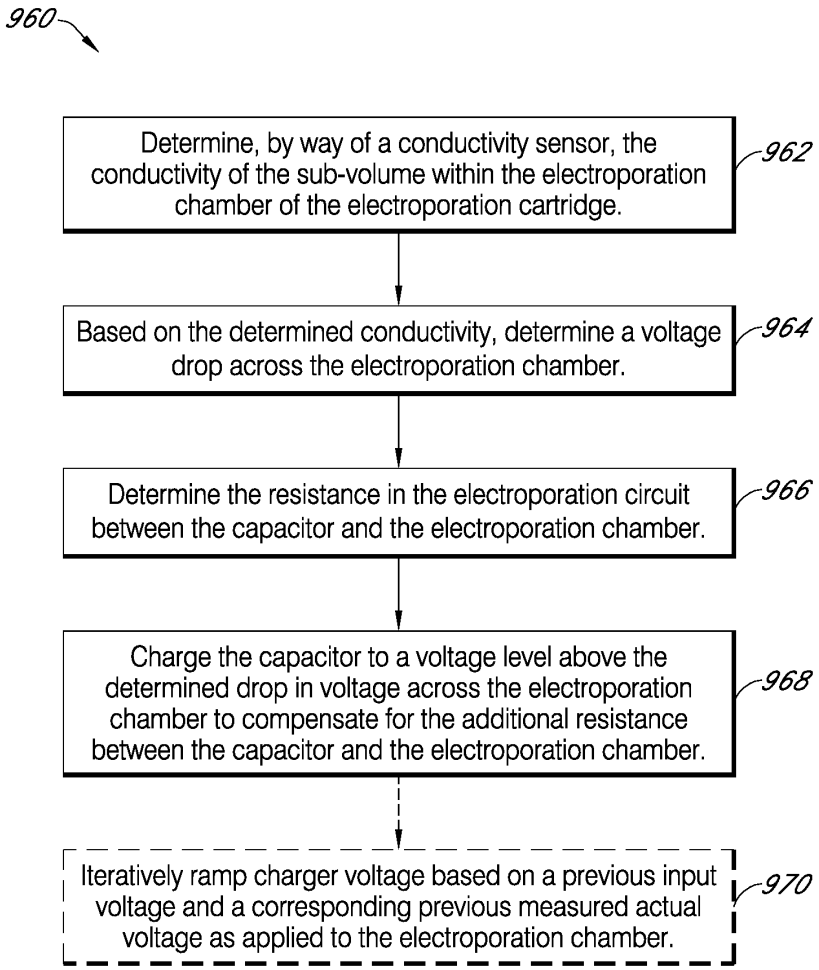

960
Determine, by way of a conductivity sensor, the conductivity of the sub-volume within the electroporation chamber of the electroporation cartridge.
962
Based on the determined conductivity, determine a voltage drop across the electroporation chamber.
964
Determine the resistance in the electroporation circuit between the capacitor and the electroporation chamber.
966
Charge the capacitor to a voltage level above the determined drop in voltage across the electroporation chamber to compensate for the additional resistance between the capacitor and the electroporation chamber.
968
Iteratively ramp charger voltage based on a previous input voltage and a corresponding previous measured actual voltage as applied to the electroporation chamber.
970

FIG. 26

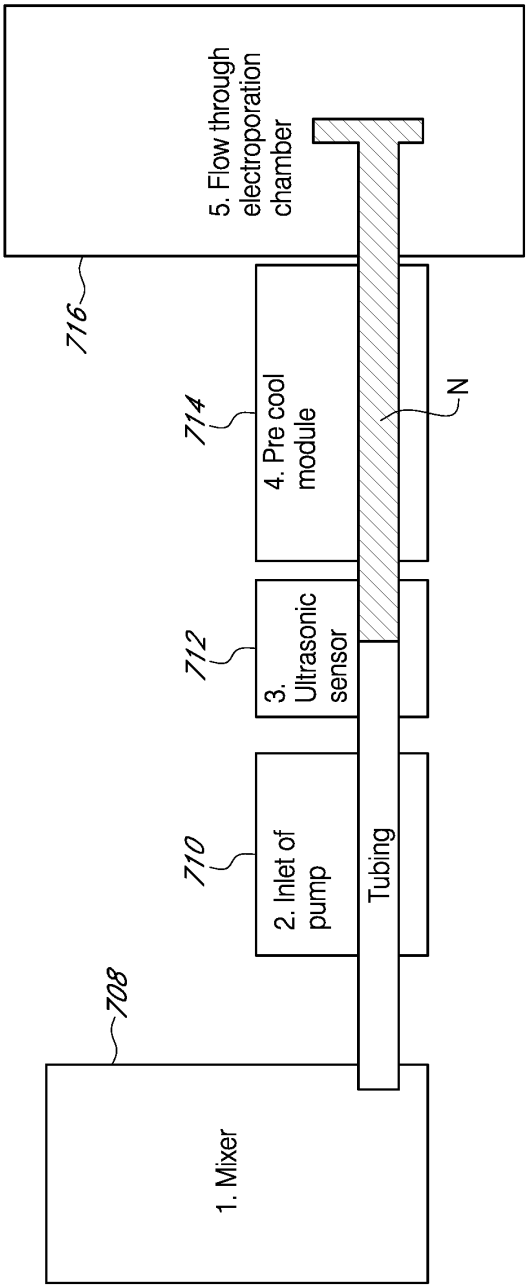

1. Mixer
2. Inlet of pump
3. Ultrasonic sensor
4. Pre cool module
5. Flow through electroporation chamber
Tubing
N
708
710
712
714
716

FIG. 27A

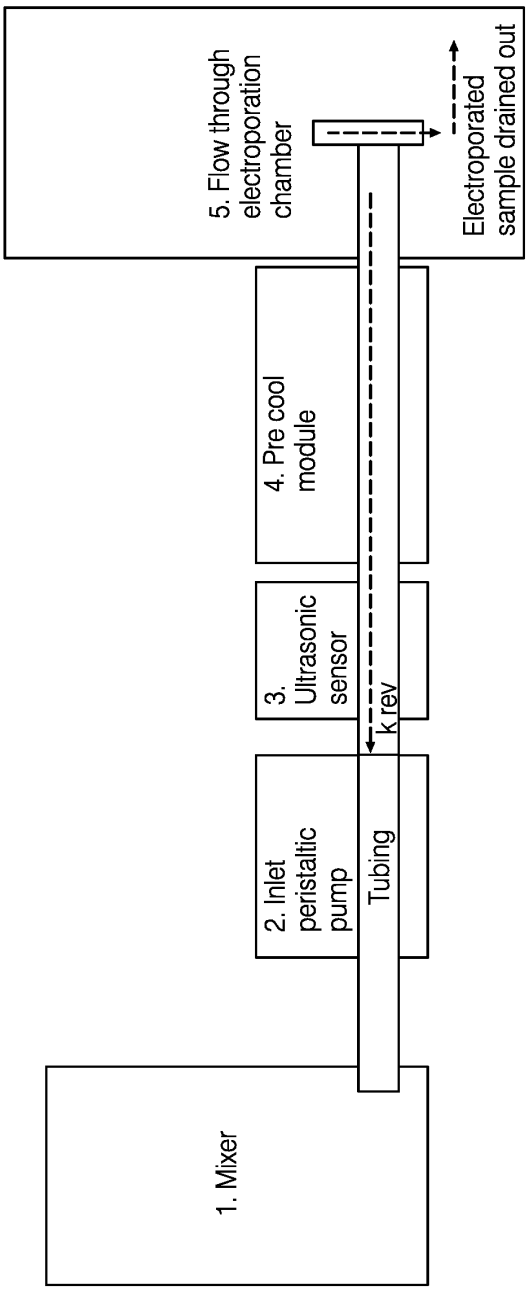

1. Mixer
2. Inlet peristaltic pump
Tubing
3. Ultrasonic sensor
k rev
4. Pre cool module
5. Flow through electroporation chamber
Electroporated sample drained out

FIG. 27B

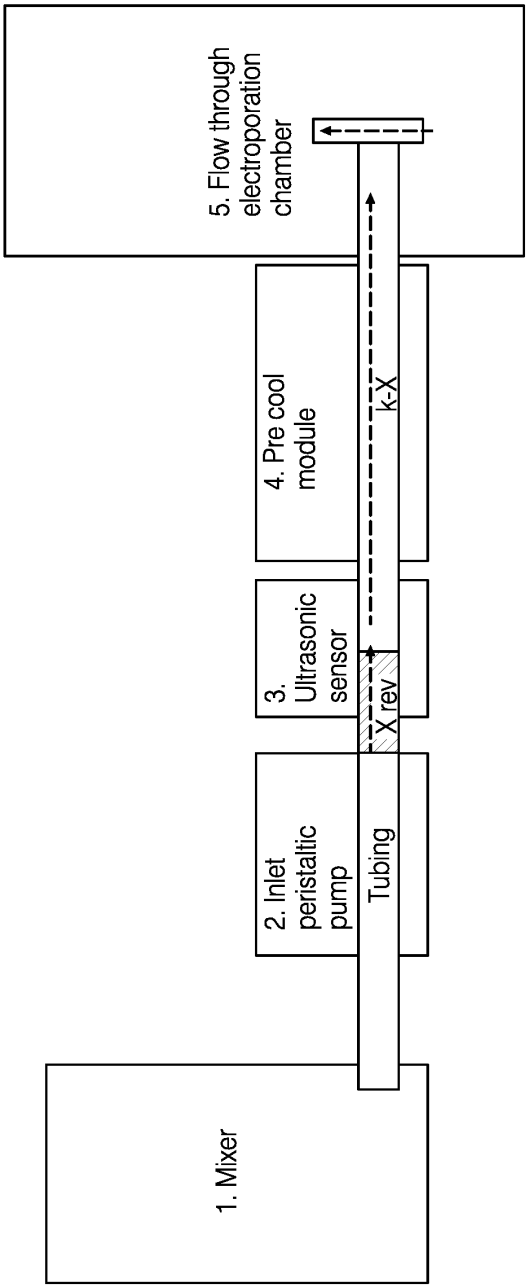

5. Flow through electroporation chamber
4. Pre cool module
k-X
3. Ultrasonic sensor
X rev
2. Inlet peristaltic pump
Tubing
1. Mixer

FIG. 27C

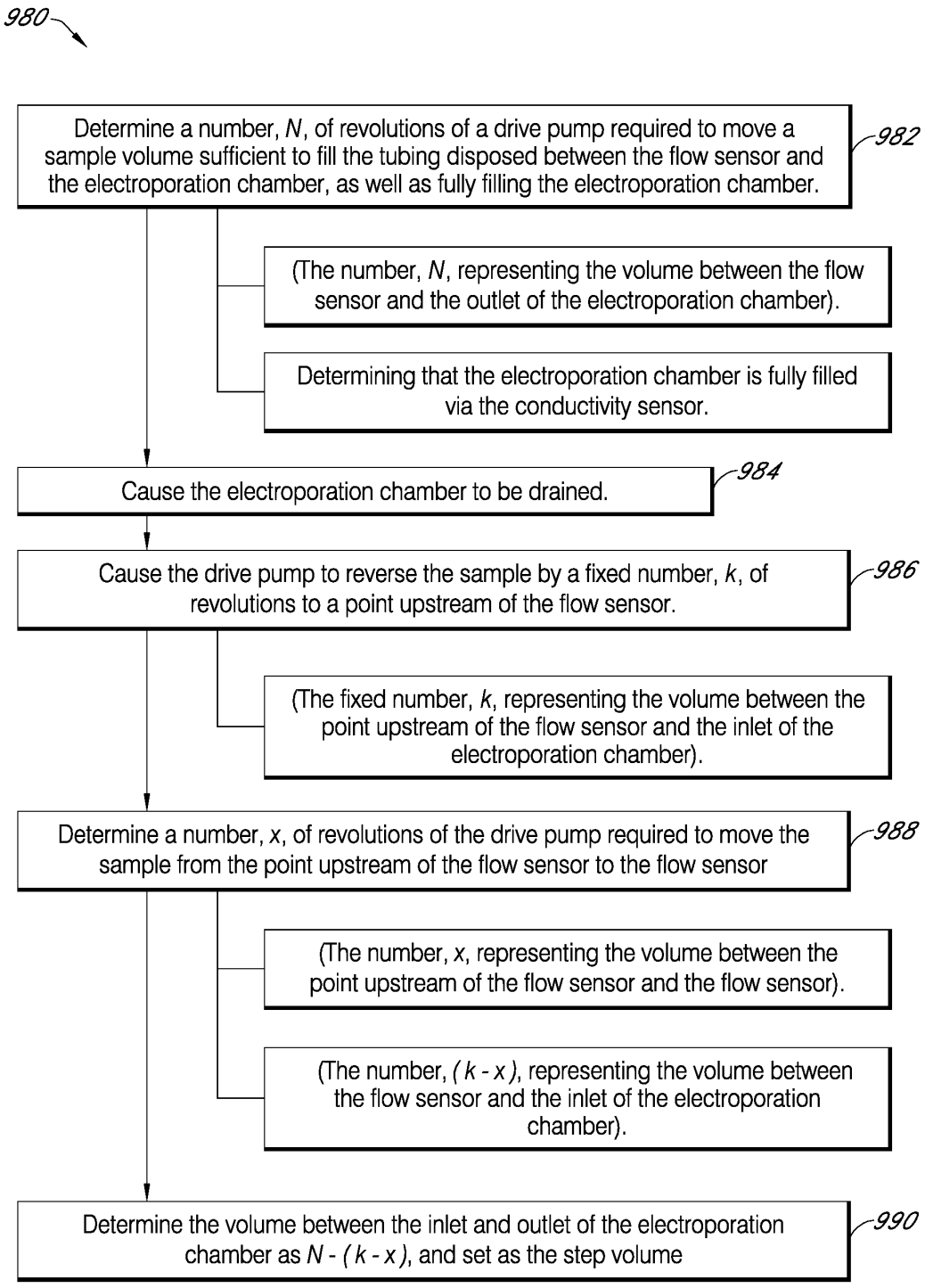

980
Determine a number, N, of revolutions of a drive pump required to move a sample volume sufficient to fill the tubing disposed between the flow sensor and the electroporation chamber, as well as fully filling the electroporation chamber.
982
(The number, N, representing the volume between the flow sensor and the outlet of the electroporation chamber).
Determining that the electroporation chamber is fully filled via the conductivity sensor.
Cause the electroporation chamber to be drained.
984
Cause the drive pump to reverse the sample by a fixed number, k, of revolutions to a point upstream of the flow sensor.
986
(The fixed number, k, representing the volume between the point upstream of the flow sensor and the inlet of the electroporation chamber).
Determine a number, x, of revolutions of the drive pump required to move the sample from the point upstream of the flow sensor to the flow sensor
988
(The number, x, representing the volume between the point upstream of the flow sensor and the flow sensor).
(The number, (k - x), representing the volume between the flow sensor and the inlet of the electroporation chamber).
Determine the volume between the inlet and outlet of the electroporation chamber as N - (k - x), and set as the step volume
990

FIG. 27D

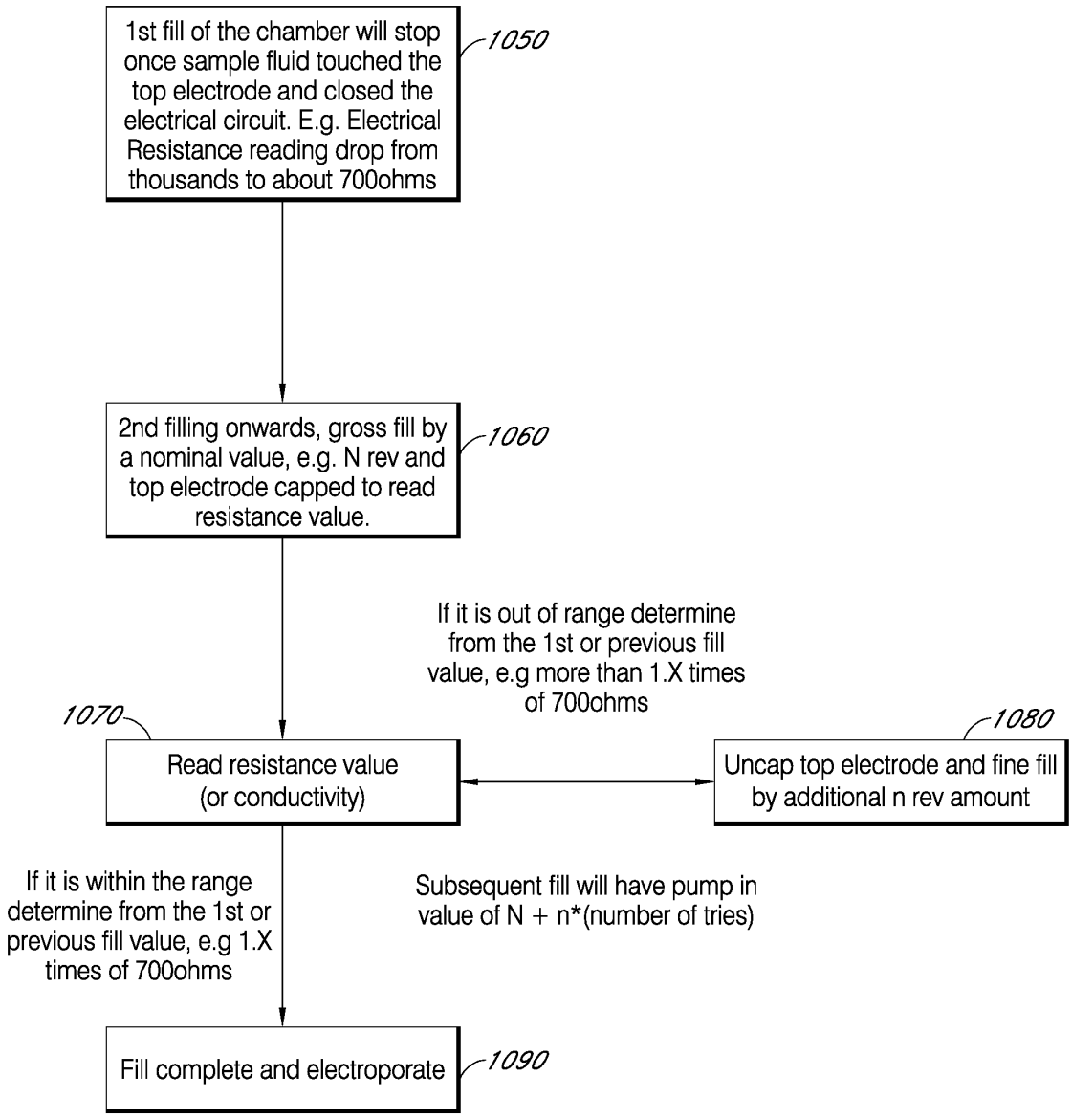

1st fill of the chamber will stop once sample fluid touched the top electrode and closed the electrical circuit. E.g. Electrical Resistance reading drop from thousands to about 700ohms
1050
2nd filling onwards, gross fill by a nominal value, e.g. N rev and top electrode capped to read resistance value.
1060
If it is out of range determine from the 1st or previous fill value, e.g more than 1.X times of 700ohms
1070
Read resistance value (or conductivity)
1080
Uncap top electrode and fine fill by additional n rev amount
If it is within the range determine from the 1st or previous fill value, e.g 1.X times of 700ohms
Subsequent fill will have pump in value of N + n*(number of tries)
Fill complete and electroporate
1090

FIG. 27E

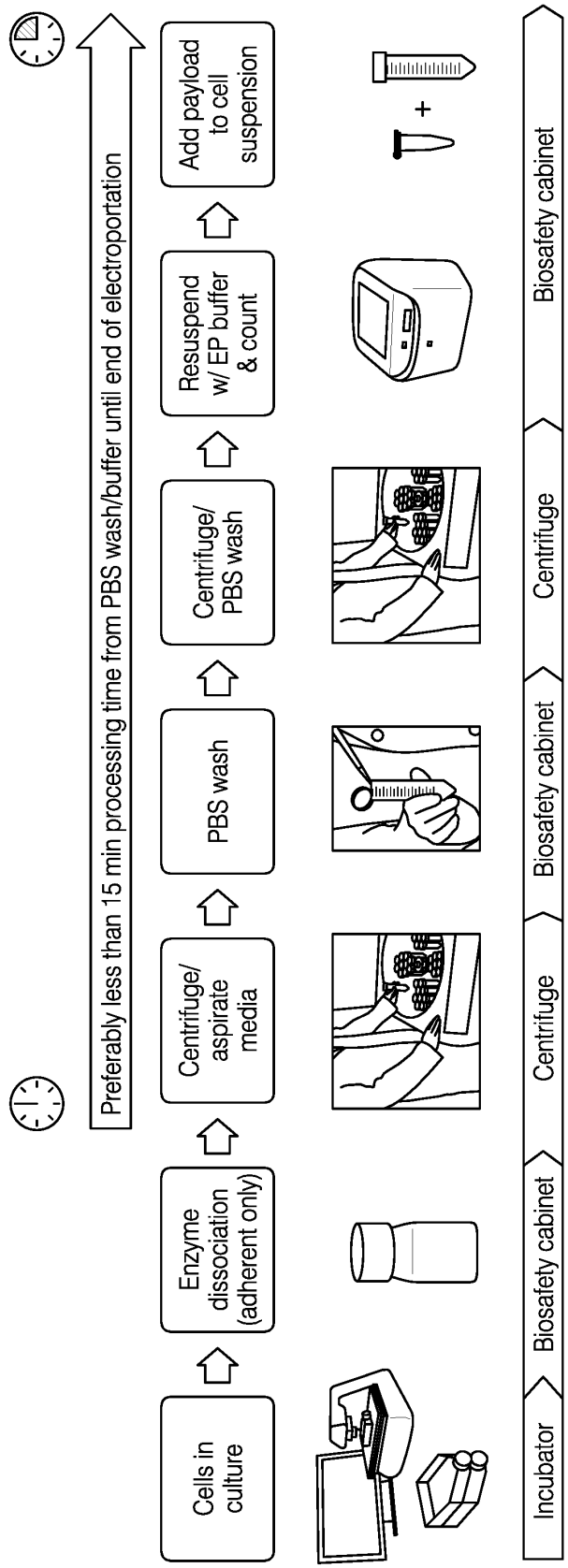

Preferably less than 15 min processing time from PBS wash/buffer until end of electroportation
Cells in culture
Enzyme dissociation (adherent only)
Centrifuge/ aspirate media
PBS wash
Centrifuge/ PBS wash
Resuspend w/ EP buffer & count
Add payload to cell suspension
Incubator
Biosafety cabinet
Centrifuge
Biosafety cabinet
Centrifuge
Biosafety cabinet
+

FIG. 28

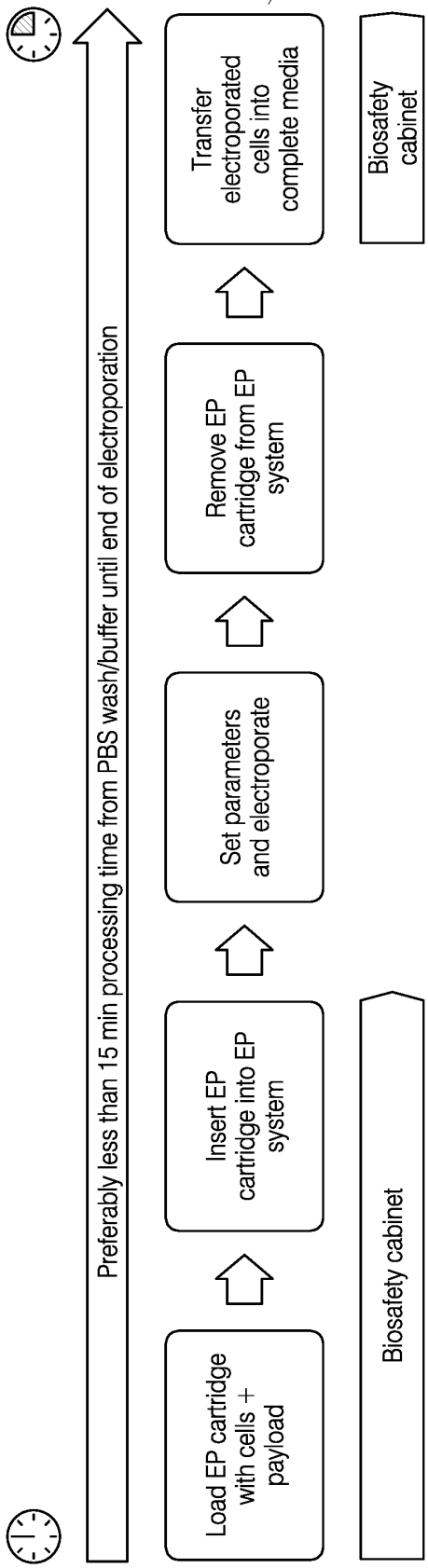

Preferably less than 15 min processing time from PBS wash/buffer until end of electroporation
Load EP cartridge with cells + payload
Insert EP cartridge into EP system
Set parameters and electroporate
Remove EP cartridge from EP system
Transfer electroporated cells into complete media
Biosafety cabinet
Biosafety cabinet

FIG. 29A

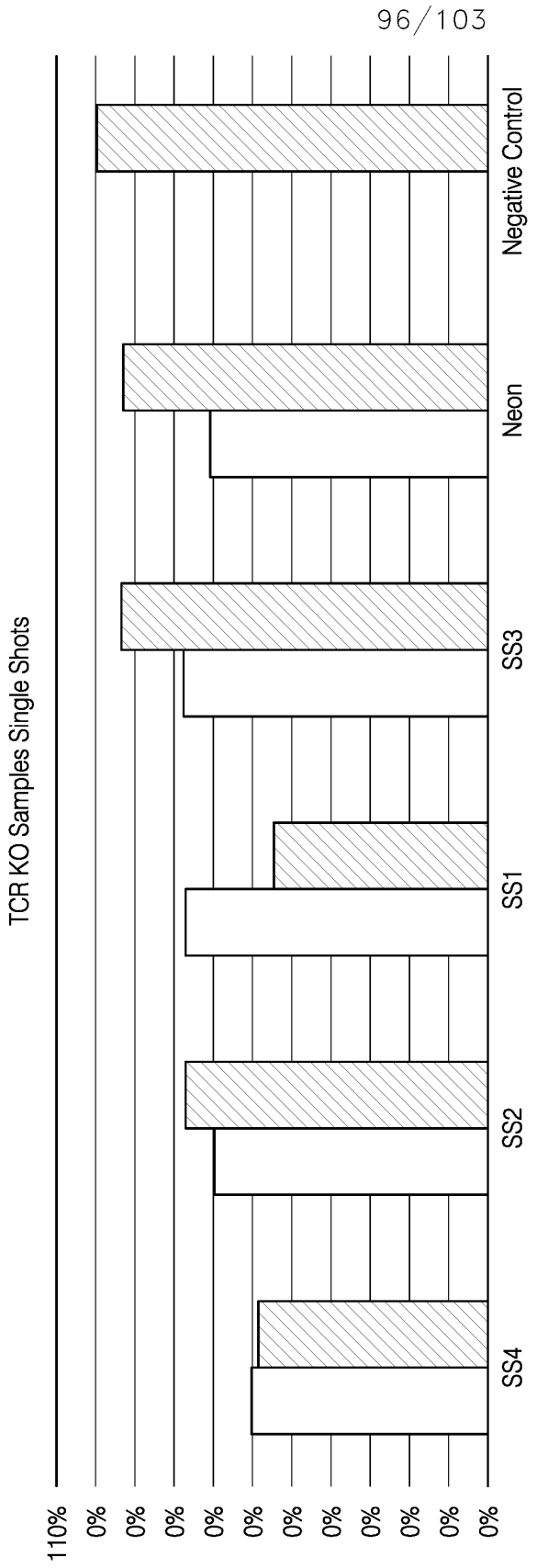

TCR KO Samples Single Shots
110%
0%
0%
0%
0%
0%
0%
0%
0%
0%
0%
0%
SS4
SS2
SS1
SS3
Neon
Negative Control
TCR KO %
Viability %
Day4, activated T-cells: PB13834
EP program: 2300V/3ms/4-pulses
Buffer: R buffer
Cas9+gRNA: TRAC-1
Cells: 50x10^6/mL

FIG. 29B

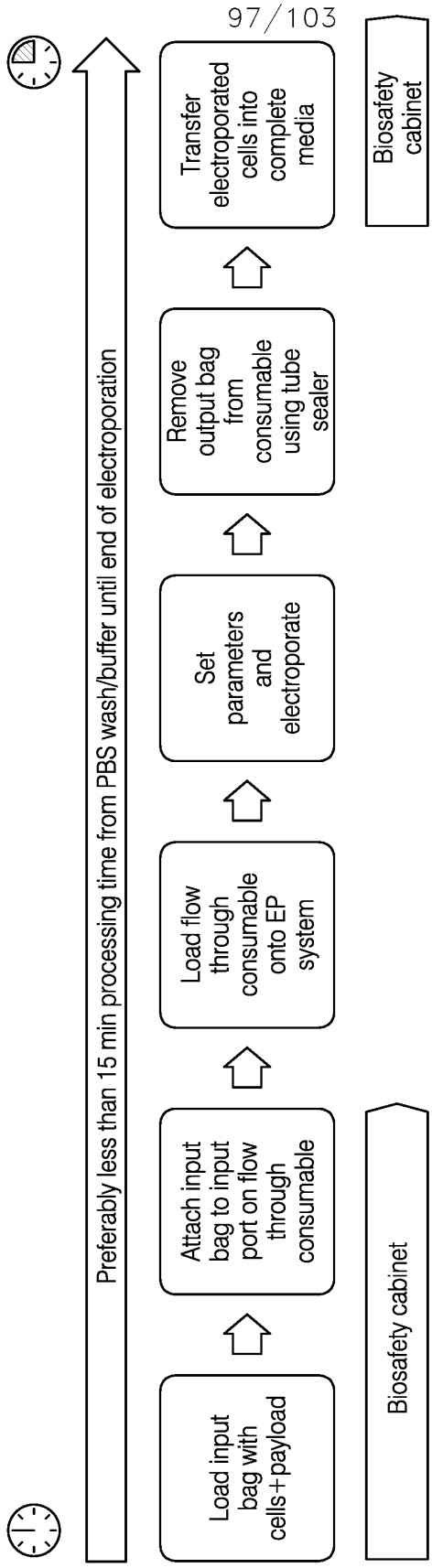

Preferably less than 15 min processing time from PBS wash/buffer until end of electroporation
Load input bag with cells+payload
Attach input bag to input port on flow through consumable
Load flow through consumable onto EP system
Set parameters and electroporate
Remove output bag from consumable using tube sealer
Transfer electroporated cells into complete media
Biosafety cabinet
Biosafety cabinet

FIG. 30A

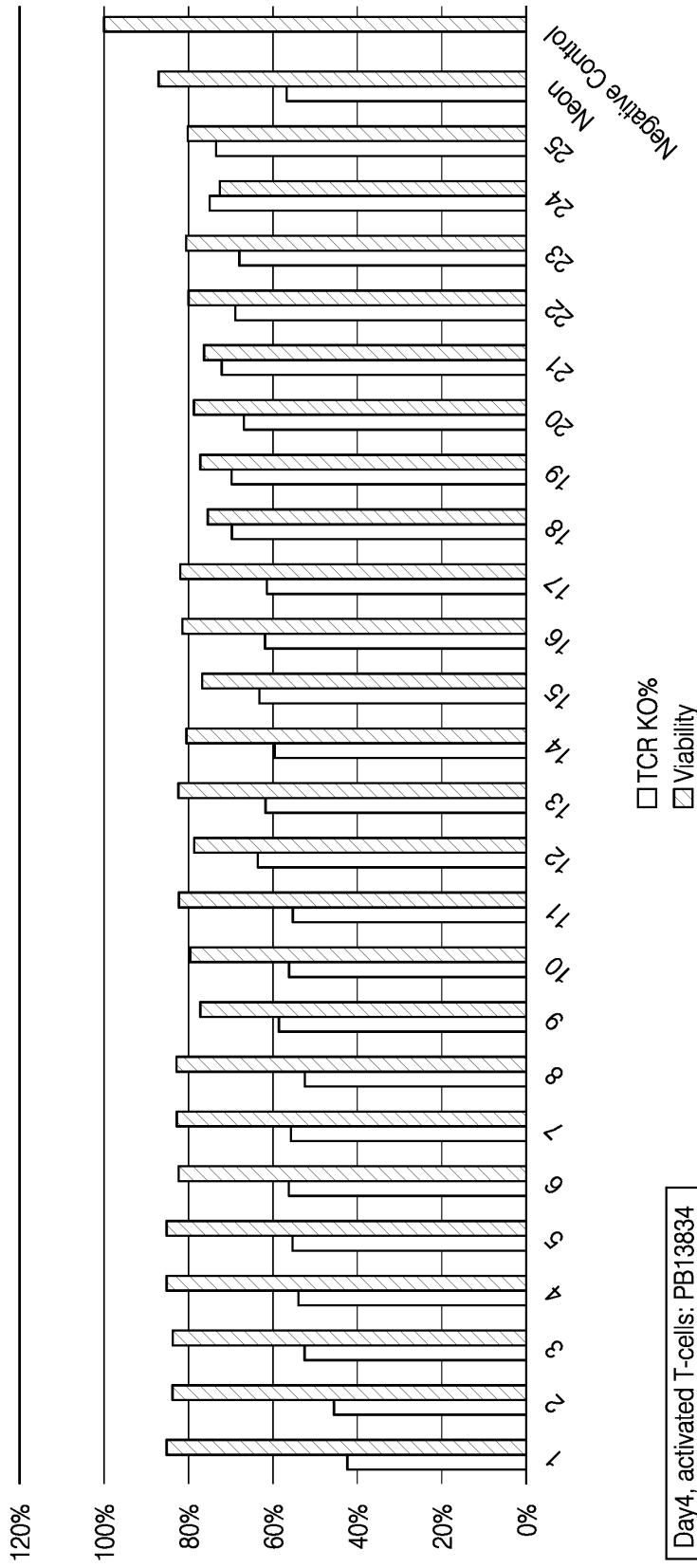

TCR KO Samples Flow through #2
120%
100%
80%
60%
40%
20%
0%
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
Neon
Negative Control
TCR KO%
Viability
Day4, activated T-cells: PB13834
EP program: 2300V/3ms/4-pulses
Buffer: R buffer
Cas9+gRNA: TRAC-1
Cells: 50x10^6/mL

FIG. 30B

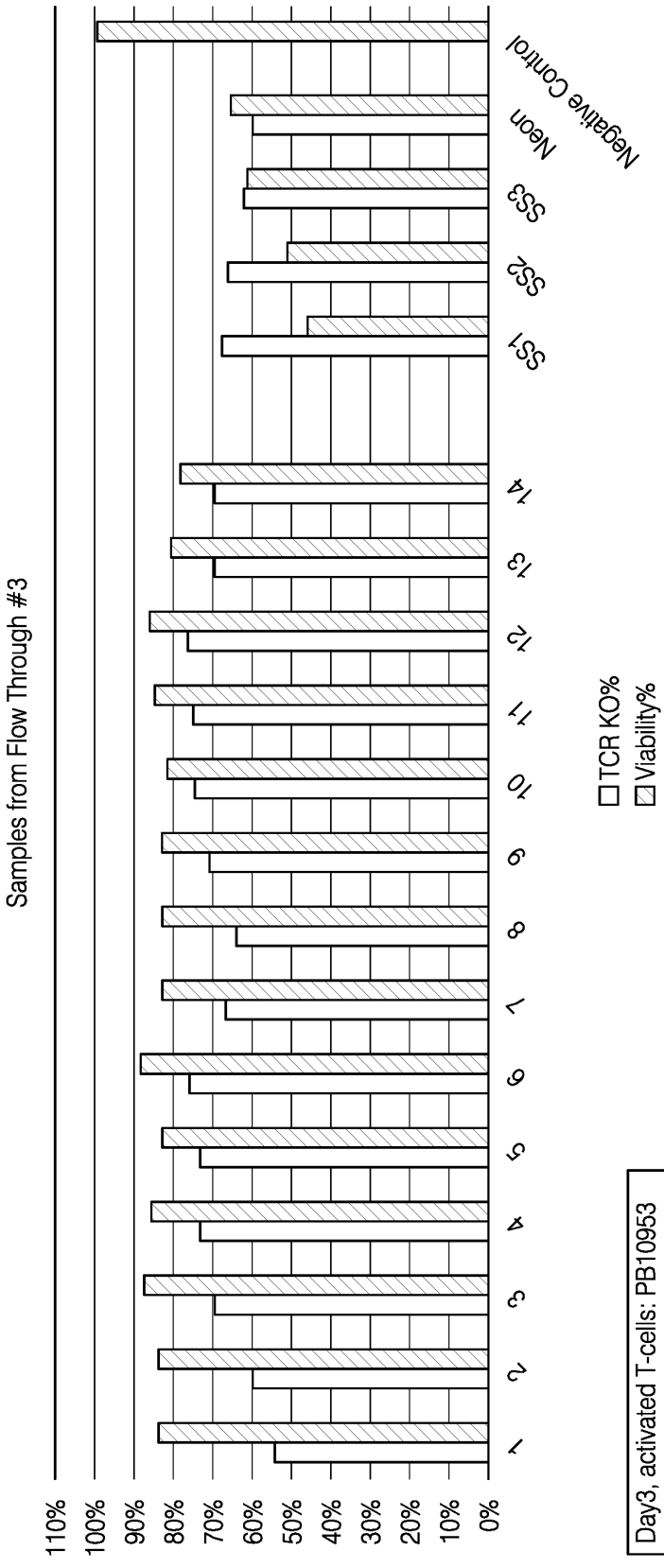

Samples from Flow Through #3
110%
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1
2
3
4
5
6
7
8
9
10
11
12
13
14
SS1
SS2
SS3
Neon
Negative Control
TCR KO%
Viability%
Day3, activated T-cells: PB10953
EP program: 1600V/10ms/3-pulses
Buffer: R buffer
Cas9+gRNA: TRAC-1
Cells: 50x10^6/mL

FIG. 31

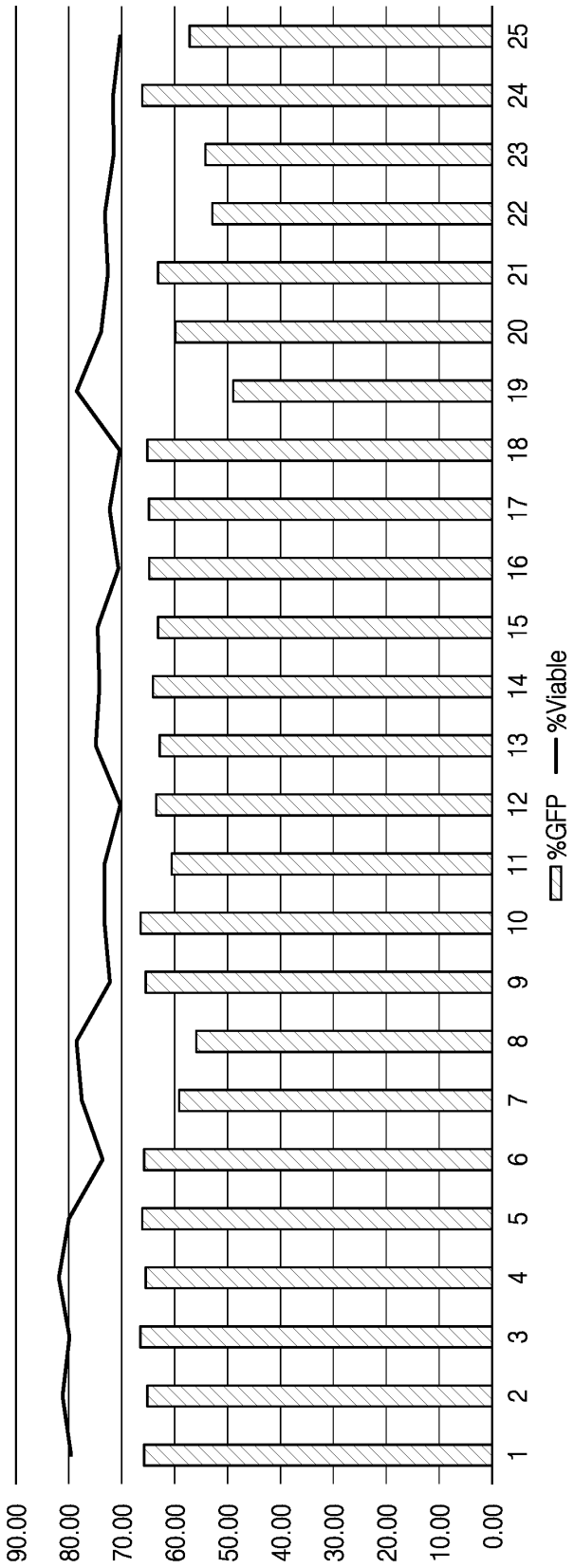

90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
%GFP
%Viable
Jurkat Cells, 50x10^6/mL
EP program: 1700V/20ms/1-pulse
Buffer: R buffer
GFP Plasmid, 4.6kB

FIG. 32

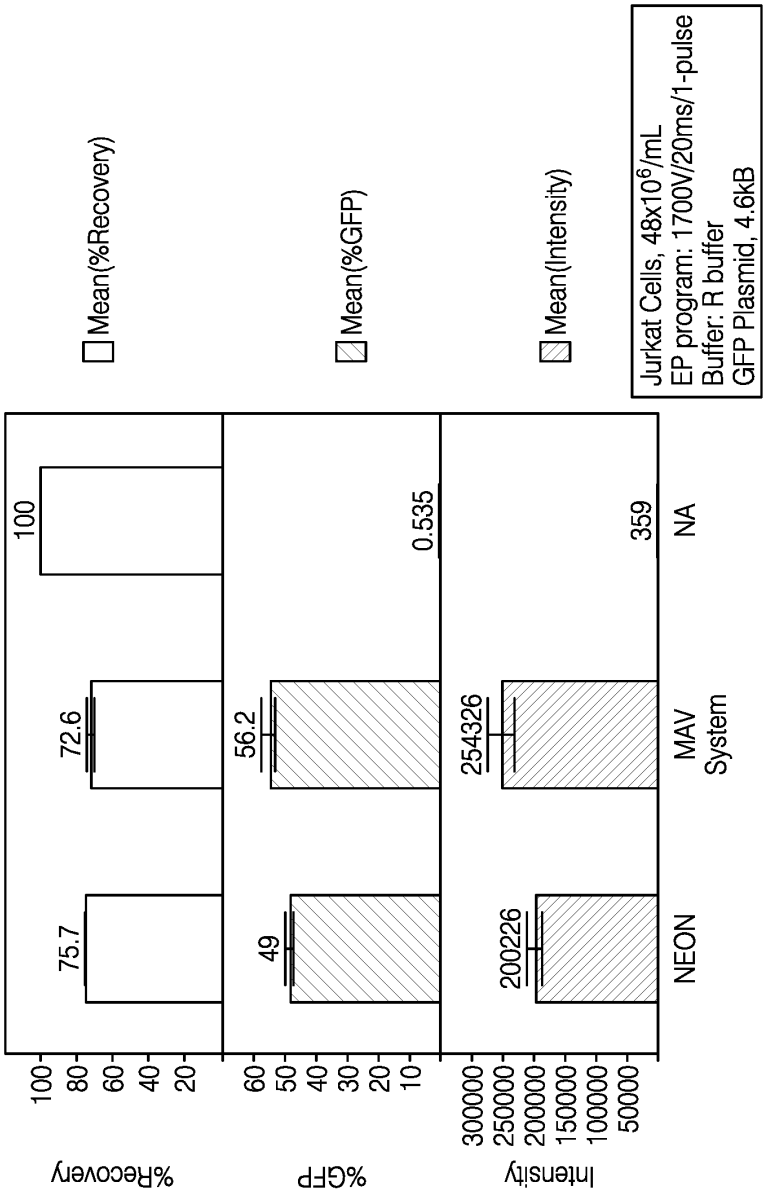

Mean(%Recovery)
Mean(%GFP)
Mean(Intensity)
Jurkat Cells, 48x10^6/mL
EP program: 1700V/20ms/1-pulse
Buffer: R buffer
GFP Plasmid, 4.6kB
%Recovery
%GFP
Intensity
75.7
72.6
100
49
56.2
0.535
200226
254326
359
NEON
MAV System
NA

FIG. 33

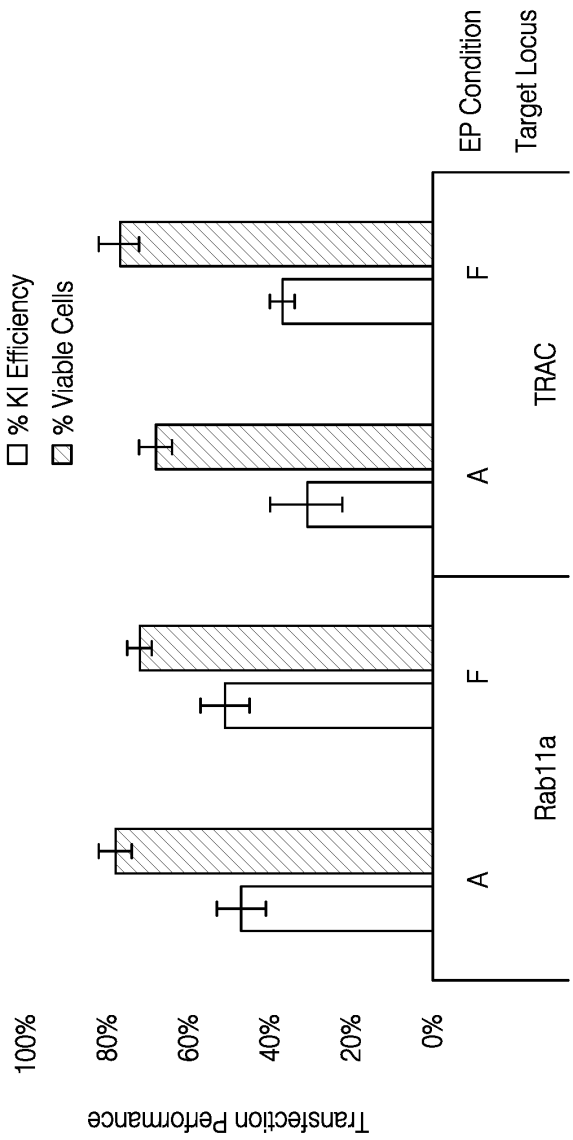

% KI Efficiency
% Viable Cells
Transfection Performance
100%
80%
60%
40%
20%
0%
EP Condition
Target Locus
A
F
A
F
Rab11a
TRAC

FIG. 34

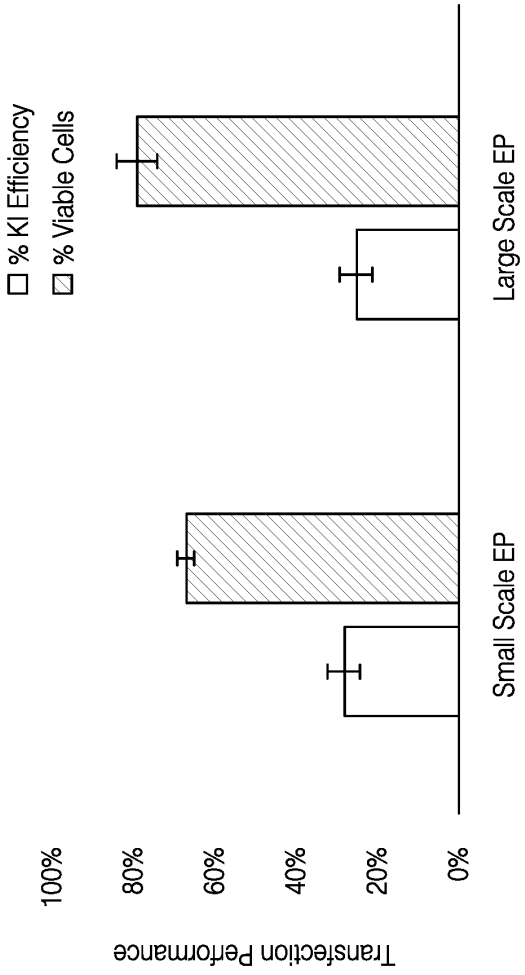

% KI Efficiency
% Viable Cells
Transfection Performance
100%
80%
60%
40%
20%
0%
Small Scale EP
Large Scale EP

FIG. 35