

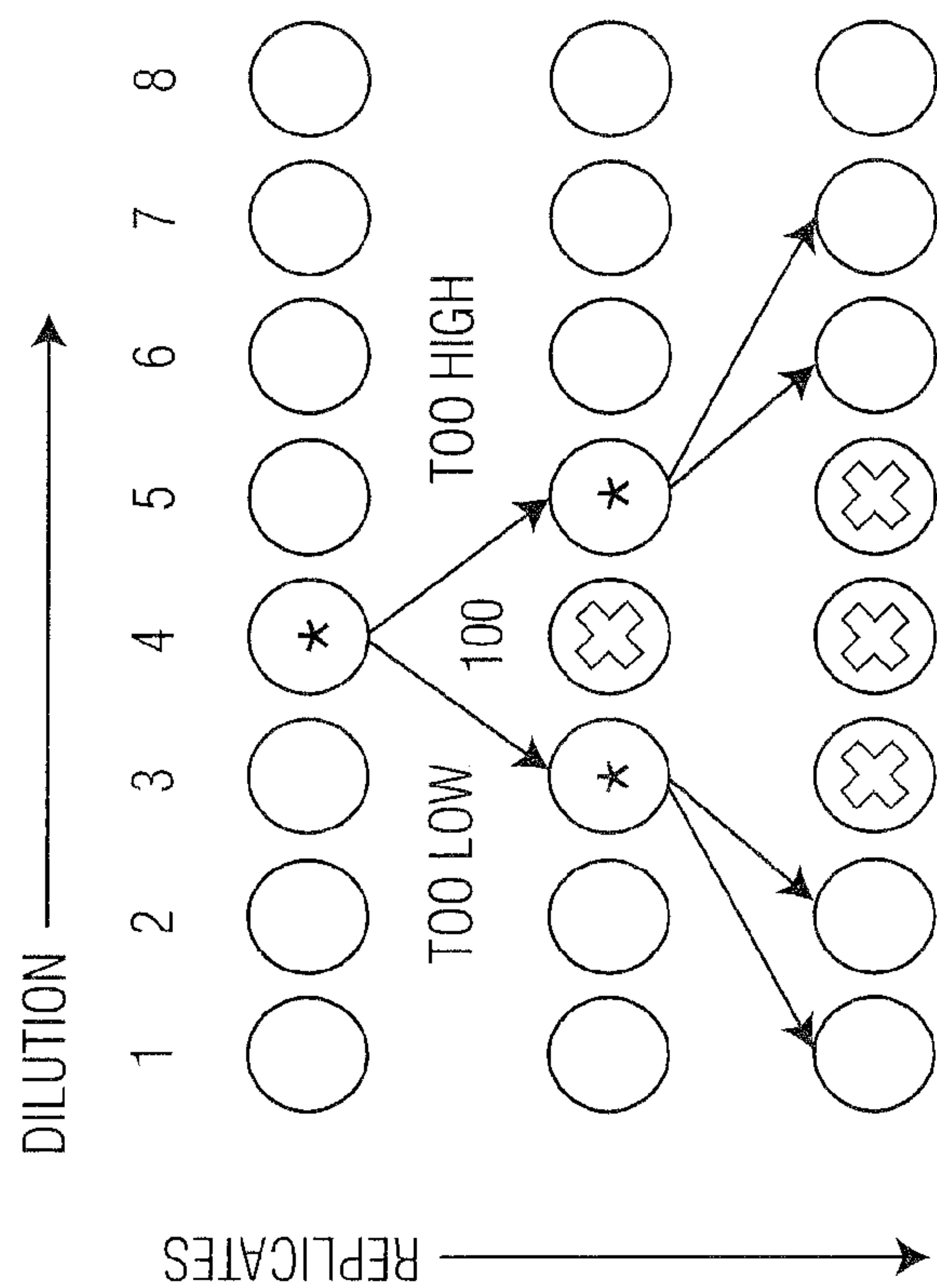


FIG. 1

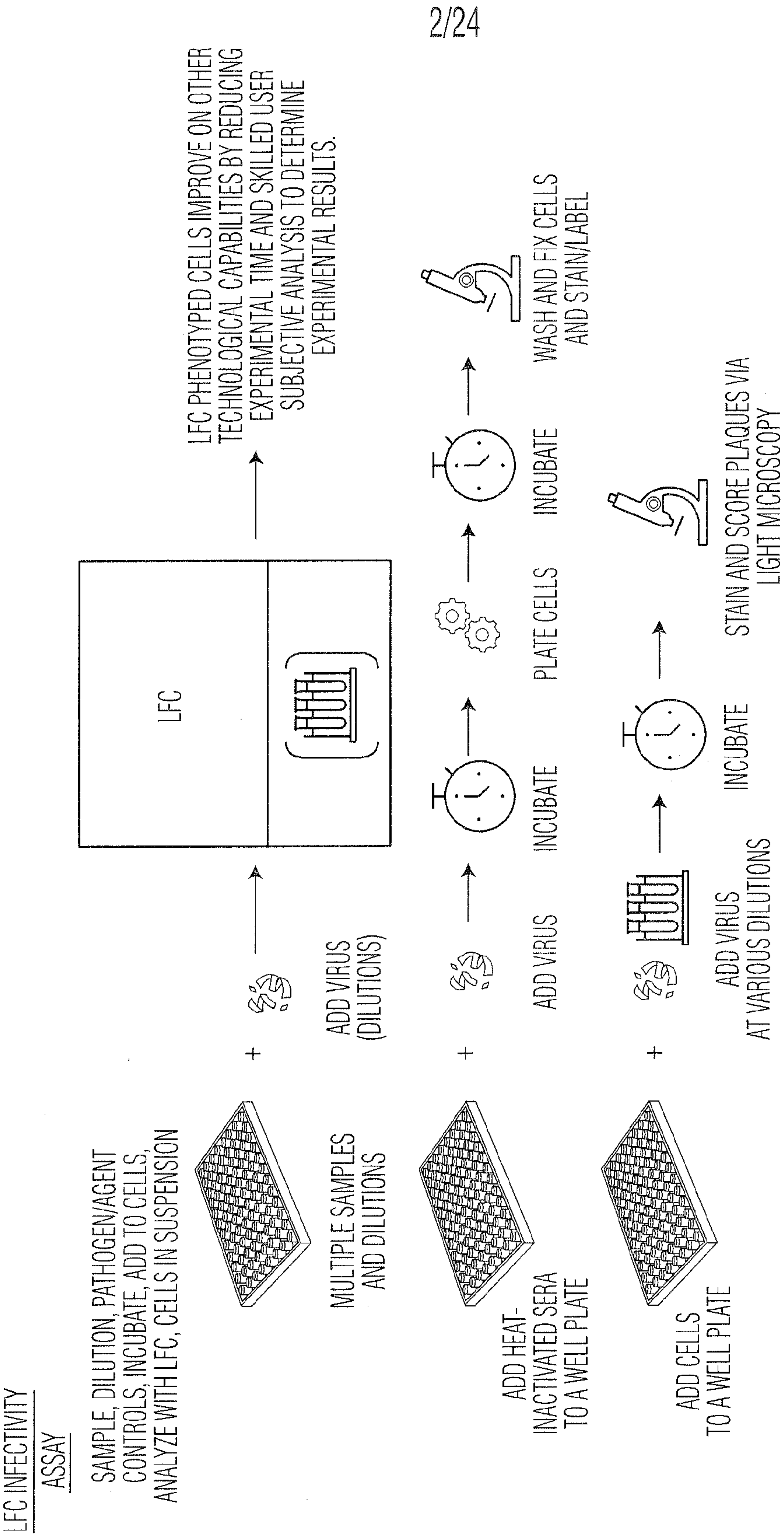


FIG. 2

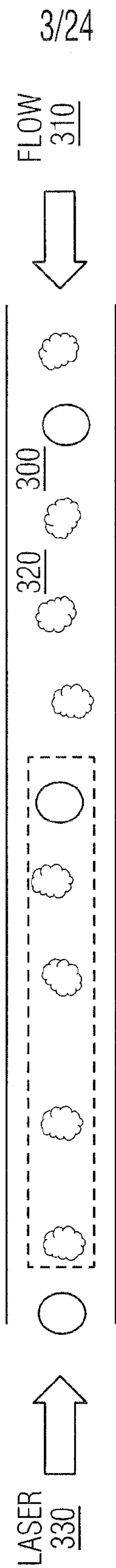
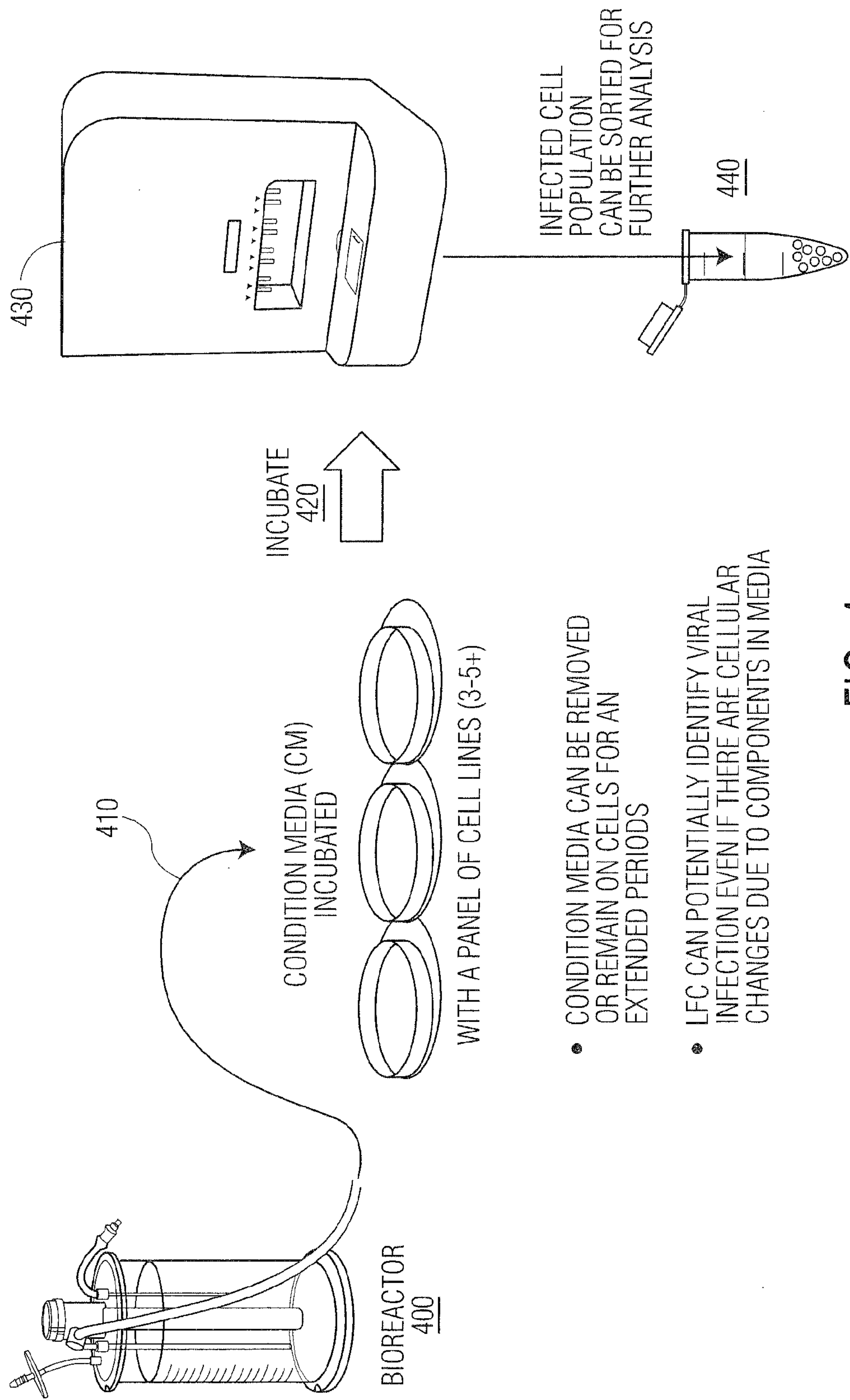


FIG. 3

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- CONDITION MEDIA CAN BE REMOVED OR REMAIN ON CELLS FOR AN EXTENDED PERIODS
- LFC CAN POTENTIALLY IDENTIFY VIRAL INFECTION EVEN IF THERE ARE CELLULAR CHANGES DUE TO COMPONENTS IN MEDIA

FIG. 4

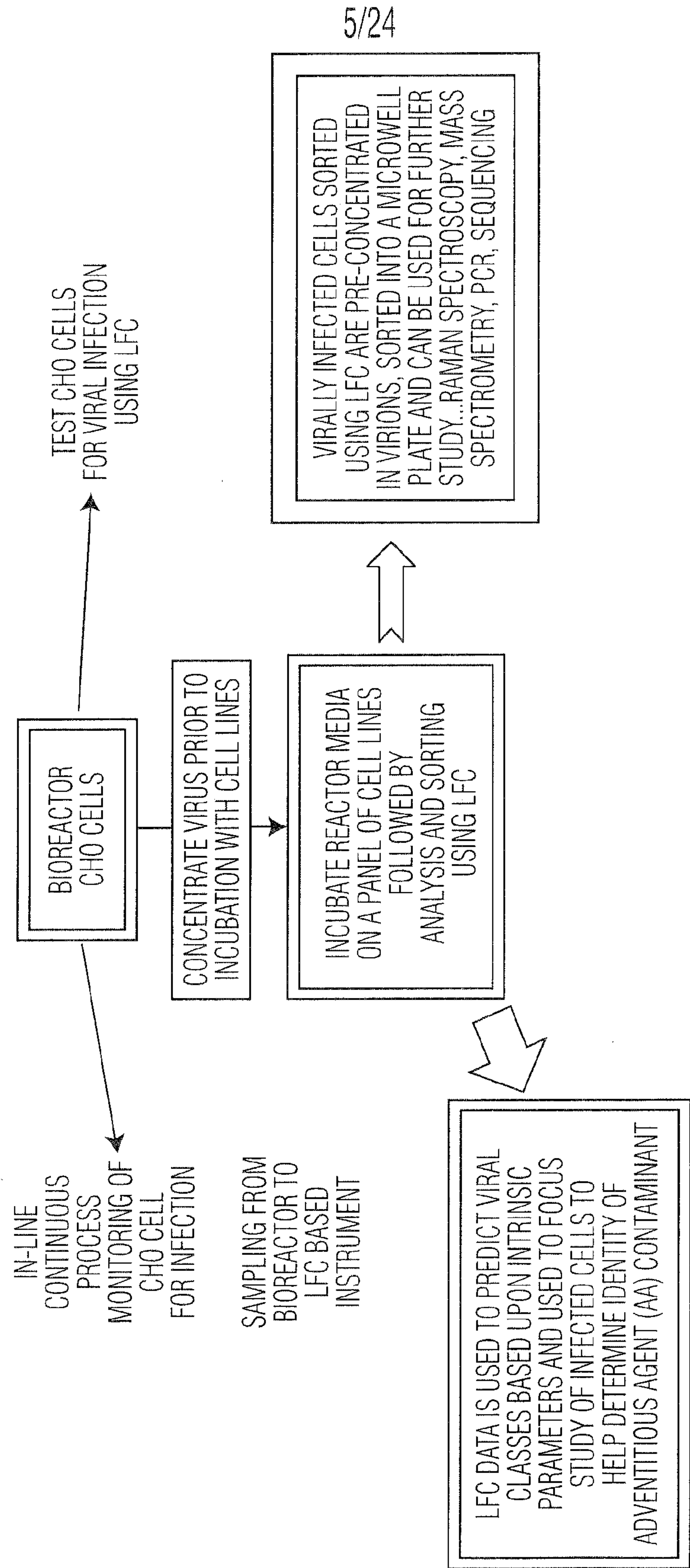


FIG. 5

CPE + REPLICATION/PERSISTS +	CPE + REPLICATION/PERSISTS –	CPE – REPLICATION/PERSISTS +	CPE – REPLICATION/PERSISTS –
ADENOVIRUS (hAdV) ENCEPHALOMYOCARDITIS (EMCV) MICE MINUTE VIRUS (MMV) REOVIRUS-3 (Reo-3)	ROSS RIVER VIRUS (RRV) FOWL PLAQUE VIRUS (FPV) CACHE VALLEY VIRUS (CVV) VESICULAR STOMATITIS VIRUS (VSV) VESIVIRUS 2117	PARAINFLUENZA (PIV-3) BUT... CPE SEEN IN THE FOLLOWING CELL LINES: HUMAN LUNG FIBROBLAST (MRC-5) HUMAN KIDNEY FIBROBLAST (324K) VERO	BOVINE VIRAL DIARRHEA VIRUS (BVDV) COXSACKIEVIRUS B3 (CVB3) MURINE HEPATITIS VIRUS (MHV) PSEUDORABIES VIRUS (PRV) PORCINE PAROVIRUS (PPV) BUT... COULD BE INFECTIOUS IN BABY HAMSTER KIDNEY CELL LINE (BHK)

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FIG. 6

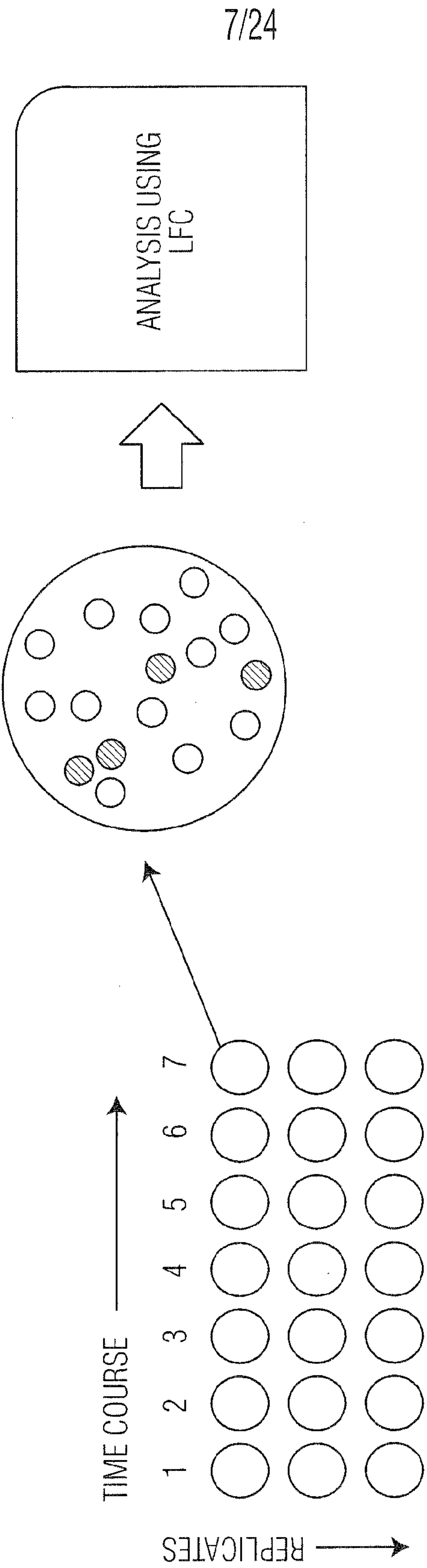


FIG. 7

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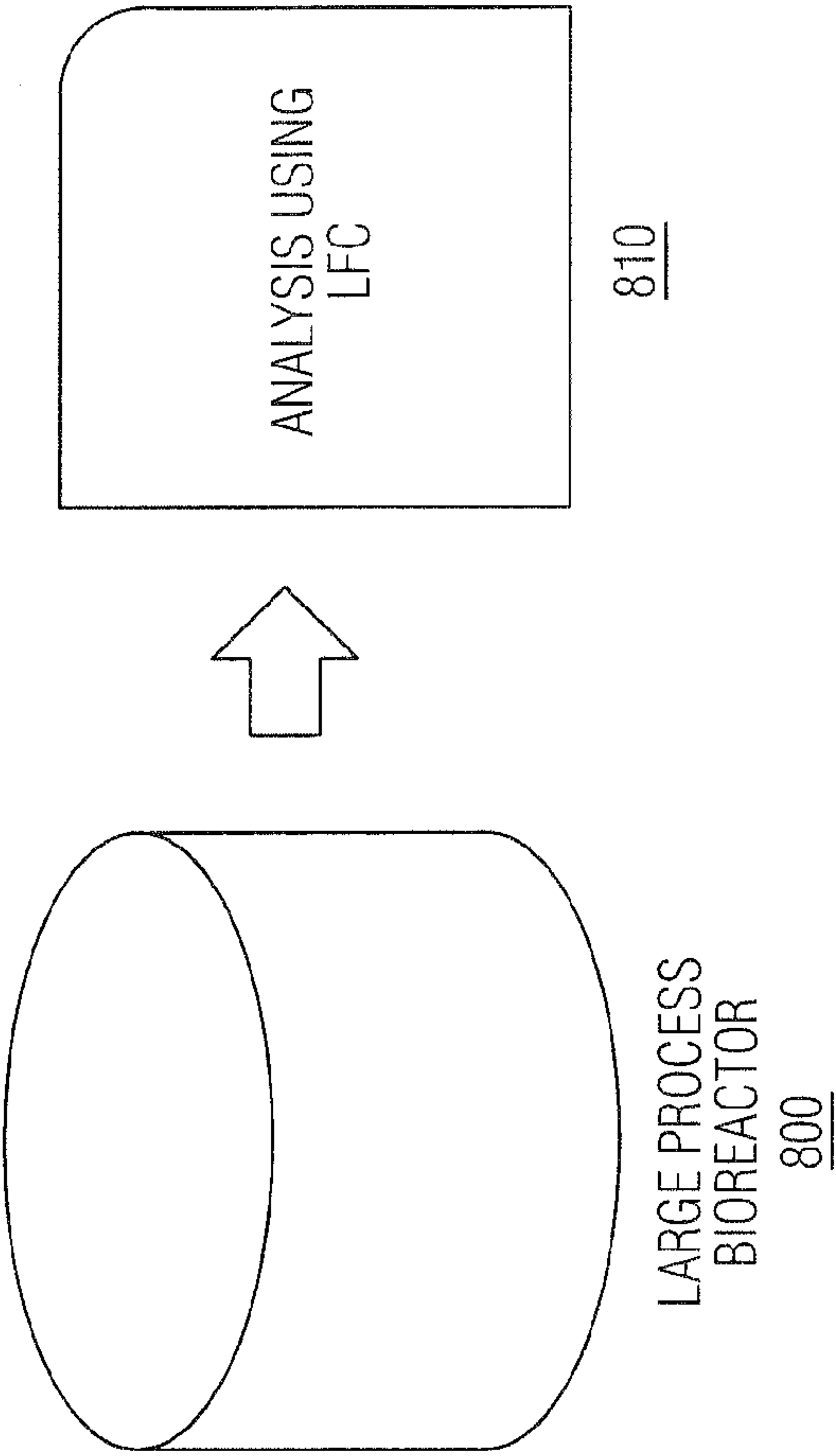


FIG. 8

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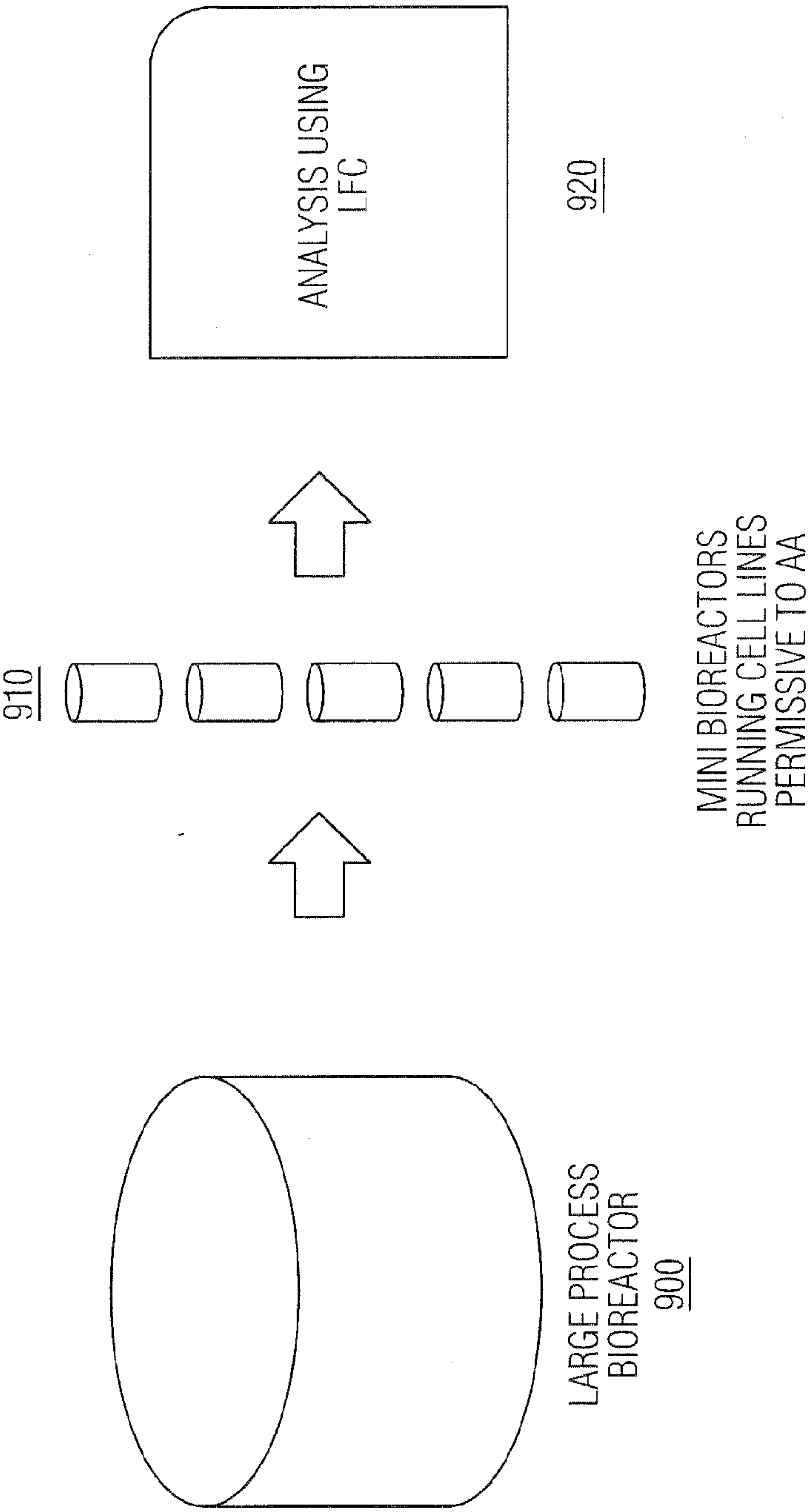


FIG. 9

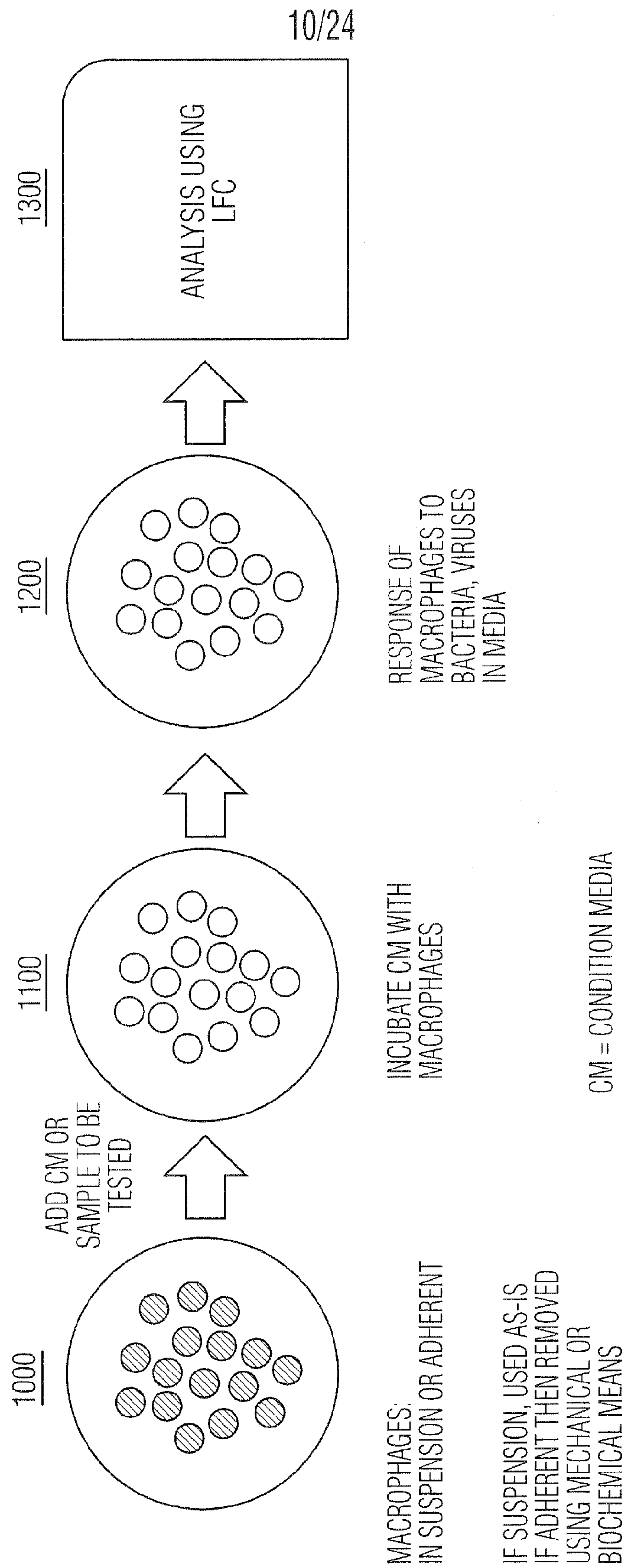


FIG. 10

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ANALYSIS OF UNKNOWN SAMPLES

ASSUMPTIONS:

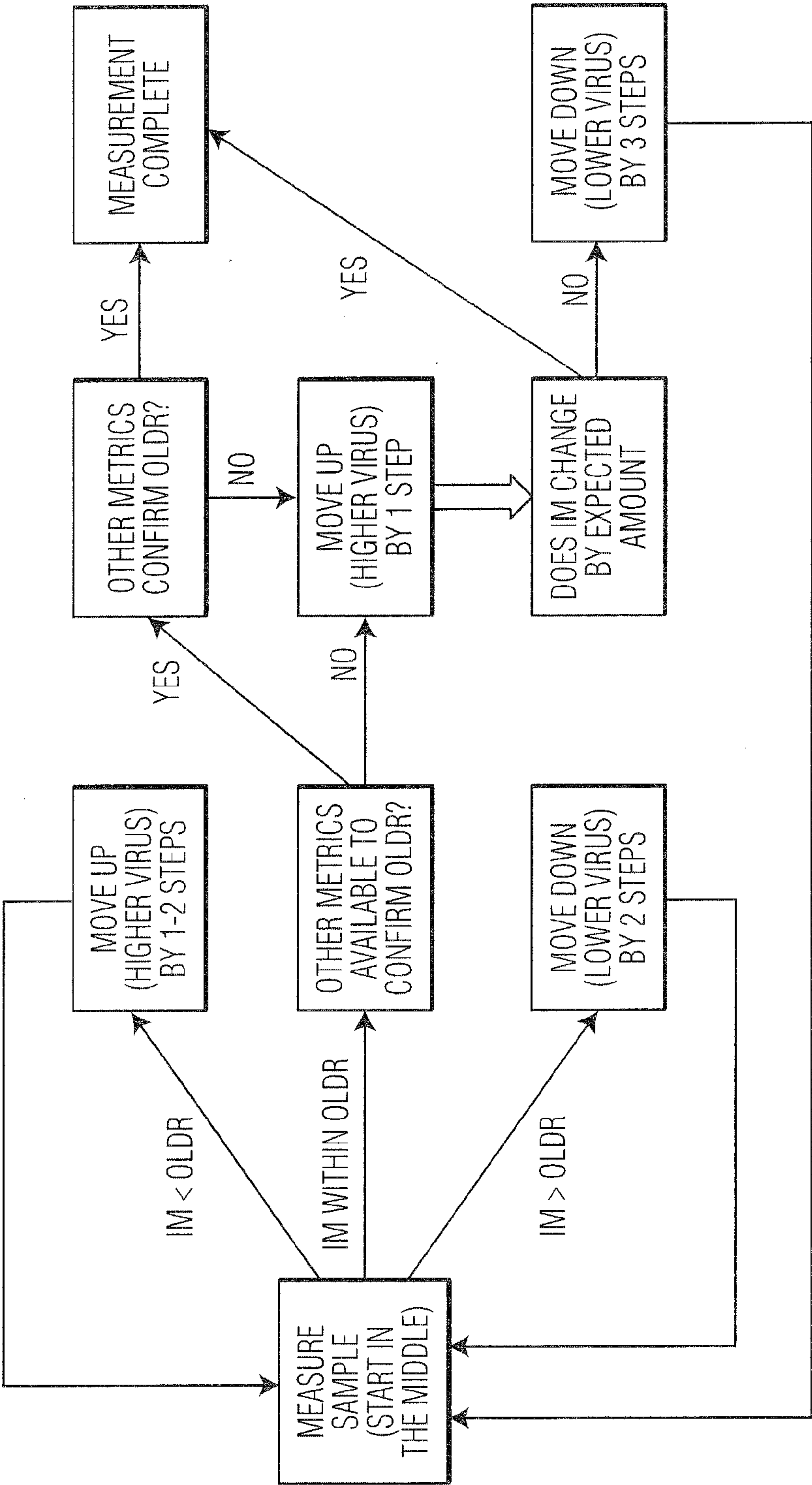
- AN INFECTION METRIC BASED ON RADIANCE MEASUREMENTS HAS BEEN IDENTIFIED
- CONTROL (UNINFECTED) AND MAXIMUM INFECTION METRIC ARE KNOWN FOR THE VIRUS/CELL COMBINATION
- THE TYPE OF FIT FOR THE CALIBRATION CURVE IS KNOWN (I.E., LINEAR VS LOGARITHMIC)

GOALS:

- OBTAIN THE OPTIMAL INFECTION METRIC (BEST ACCURACY, PRECISION, AND SIGNAL TO NOISE LEVEL – S/N) WITHIN THE LINEAR DYNAMIC RANGE OF THE CALIBRATION CURVE
- EITHER ONE MEASUREMENT AND DONE IF THAT DILUTION IS IN OPTIMAL LINEAR DYNAMIC RANGE (ODLR) OF THE CALIBRATION CURVE (FOR EXAMPLE 40-60% OF A MAXIMUM INFECTION METRIC) OR SEARCH FOR ONE ADDITIONAL INFECTION METRIC VALUE WITHIN THE ODLR AND THEN SELECT THE OPTIMAL DILUTION
- USE S/N TO DETERMINE SUITABILITY OF DATA POINT (CALCULATE N IN FLAT REGION OF DATA AND S AT PEAK METRIC SIGNAL) CHI²/10
- SEVERAL DIFFERENT DILUTIONS (VOLUMES OF VIRUS SAMPLE) CAN BE COMBINED AND AVERAGED TO CALCULATE THE TARGET VALUE IF THEY ARE WITHIN THE ODLR
- THE ODLR CAN BE ADJUSTED ON PER VIRUS/CELL LINE BASIS

FIG. 11

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IM = INFECTION METRIC
OLD R = OPTIMAL LINEAR DYNAMIC RANGE

FIG. 12

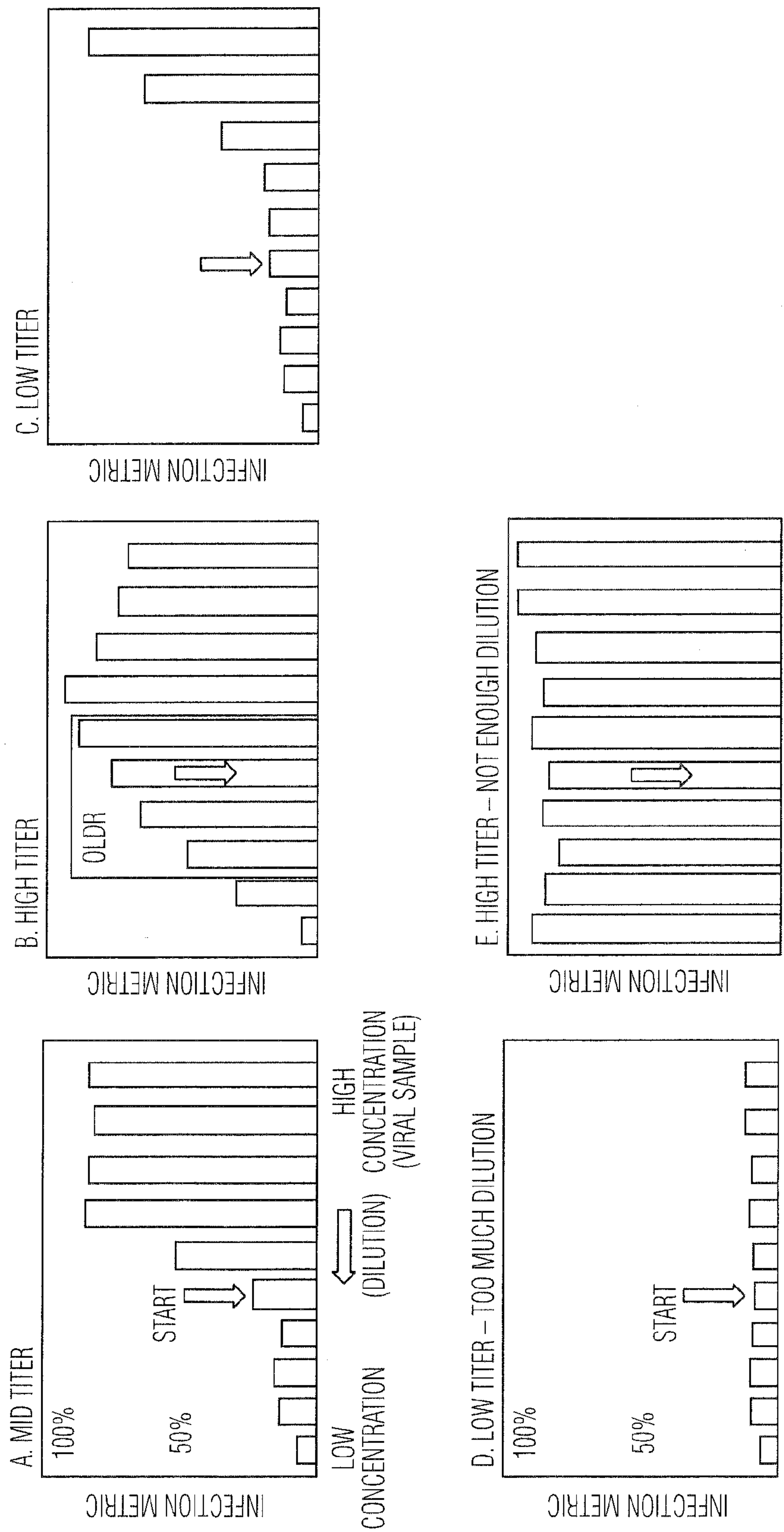


FIG. 13

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KNOWN: VIRUS, CELL, MEDIA, INCUBATION TIME, INFECTION METRIC
UNKNOWN: TITER

RESULTS: 1) TITER OF TESTED VIRAL SAMPLE AND 2) CALIBRATION CURVE

- 1. MAKE DILUTIONS OF VIRAL STOCK
- 2. ADD TO CELLS
- 3. INCUBATE FOR DESIGNATED TIME PERIOD
- 4. HARVEST CELLS AND ANALYZE DILUTIONS WITH REPLICATES ON RADIANCE INSTRUMENT
- 5. CALCULATE TITER BASED UPON ABSOLUTE TITER ALGORITHM
- 6. TITER USED TO CALCULATE DILUTION INFECTIVITY (TITERS)
- 7. CALIBRATION CURVE USED FOR FUTURE SAMPLES

FIG. 14

KNOWN: VIRUS, CELL, MEDIA
UNKNOWN: TITER, INCUBATION TIME, INFECTION METRIC

RESULTS: 1) TITER OF TESTED VIRAL SAMPLE AND 2) CALIBRATION CURVE

1. MAKE DILUTIONS OF VIRAL STOCK
2. ADD TO CELLS
3. INCUBATE FOR DESIGNATED TIME PERIOD
4. ANALYZE DILUTIONS WITH REPLICATES ON RADIANCE INSTRUMENT
5. DETERMINE BEST INFECTION METRIC AND TIME BASED ON TREND WITH DILUTION
 1. UNIVARIATE METRICS CAN SOMETIMES BE USED
 2. ENTIRE POPULATION HISTOGRAM OR SUBSET CAN BE USED AS PART OF THE INPUT VECTOR
 3. K-MEANS CLUSTERING CAN BE USED TO IDENTIFY ADDITIONAL METRICS
 4. COULD ALSO BE COMBINED WITH PLS TO CREATE A MULTIVARIATE METRIC
6. CALCULATE TITER BASED UPON ABSOLUTE TITER ALGORITHM
7. TITER USED TO CALCULATE DILUTION INFECTION METRIC (TITERS)
8. CALIBRATION CURVE USED FOR FUTURE SAMPLES

FIG. 15

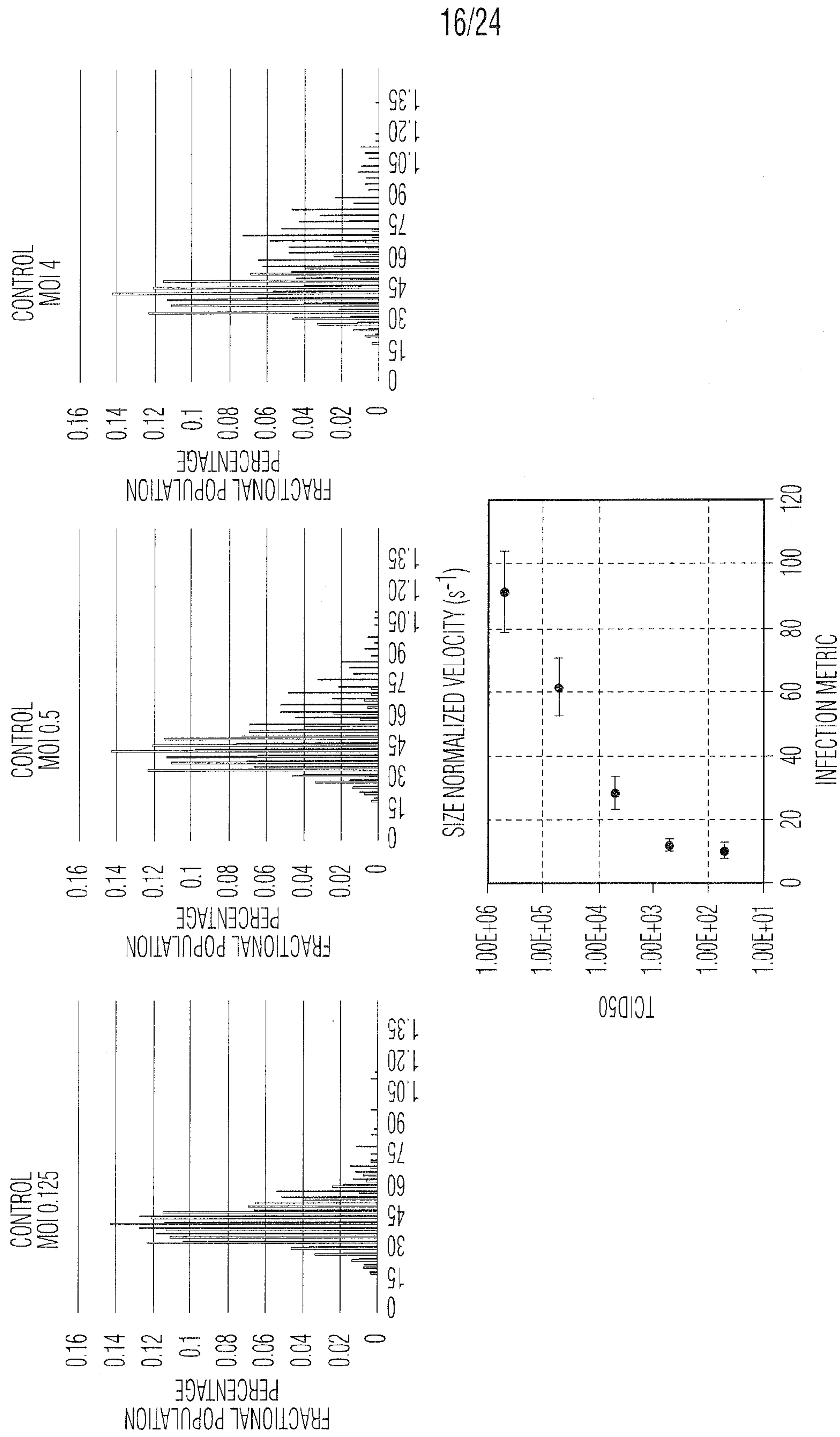


FIG. 16

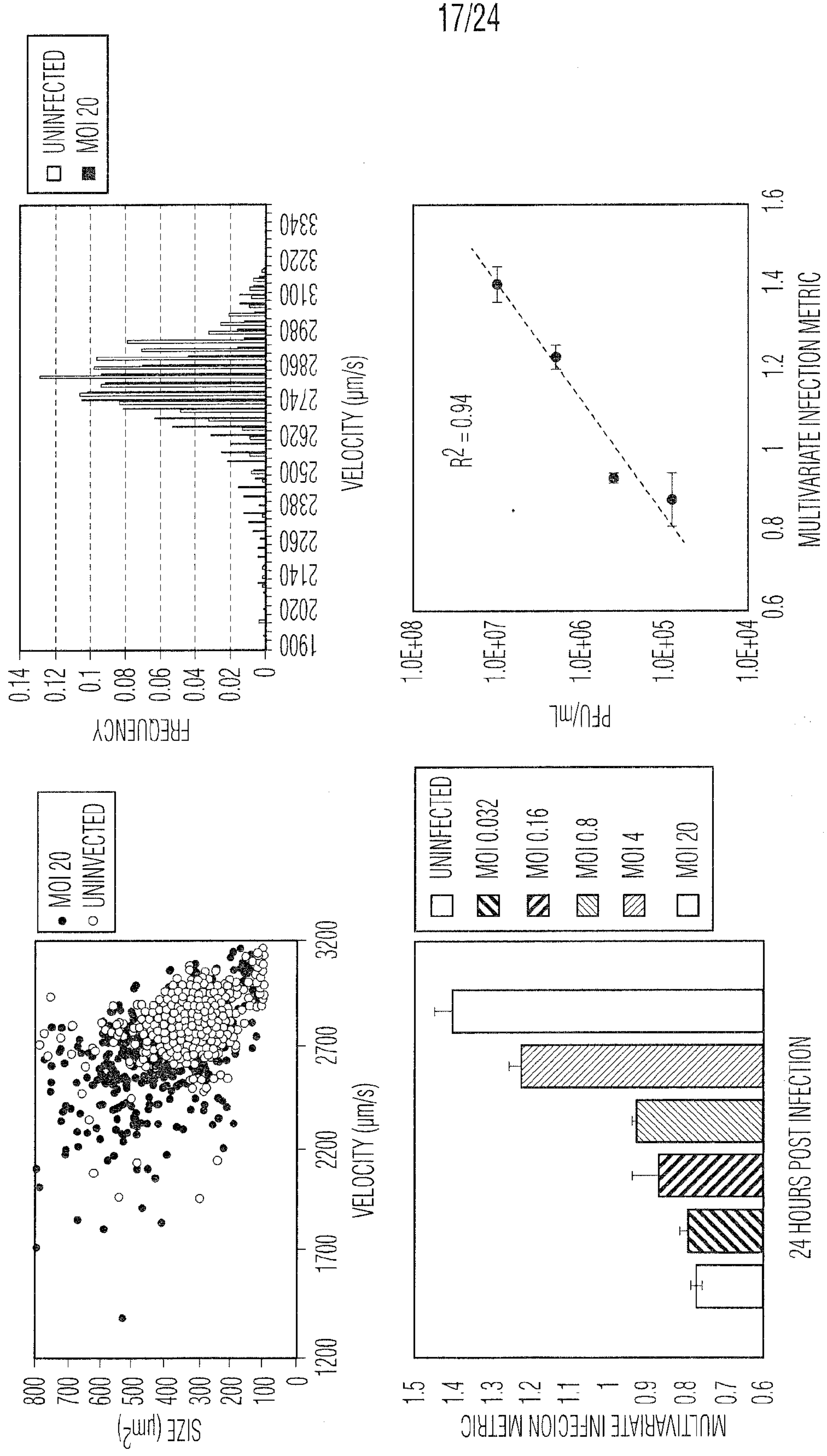


FIG. 17

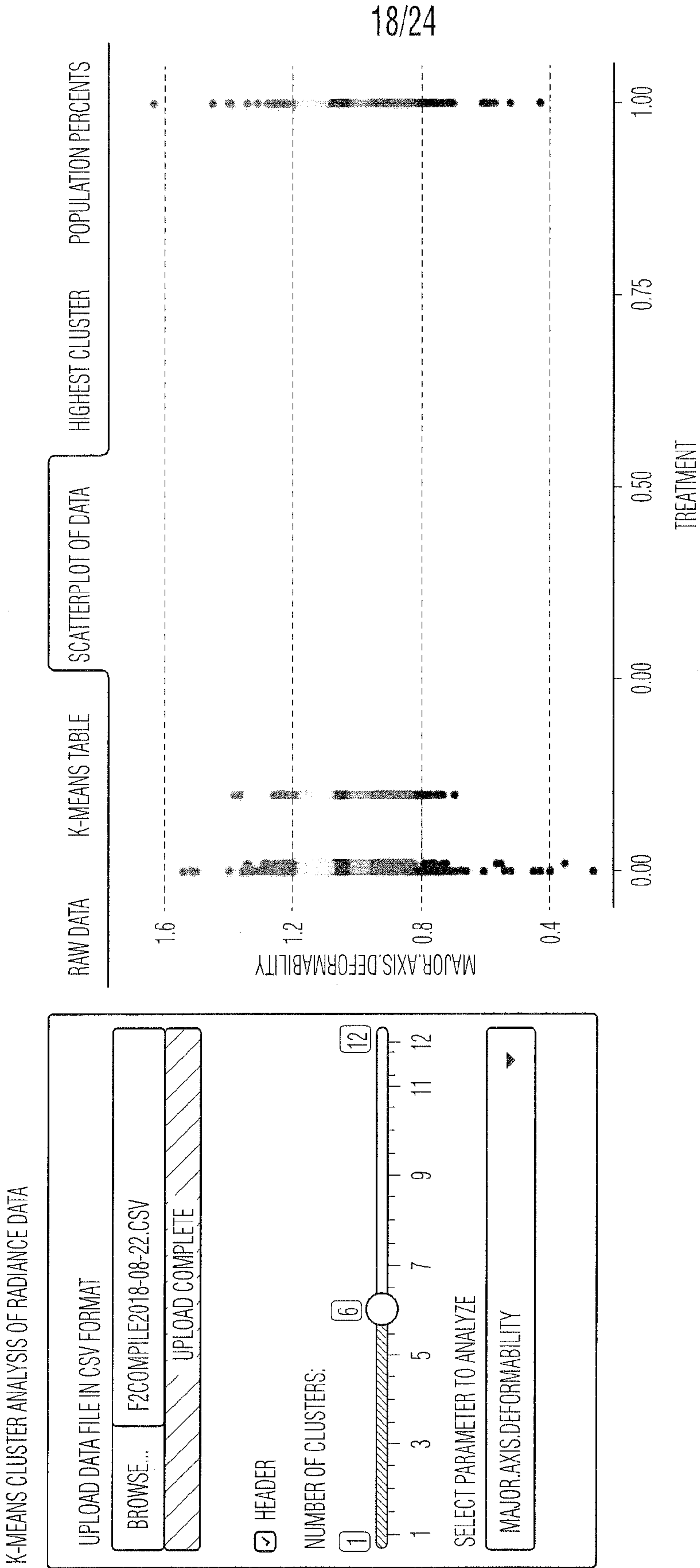
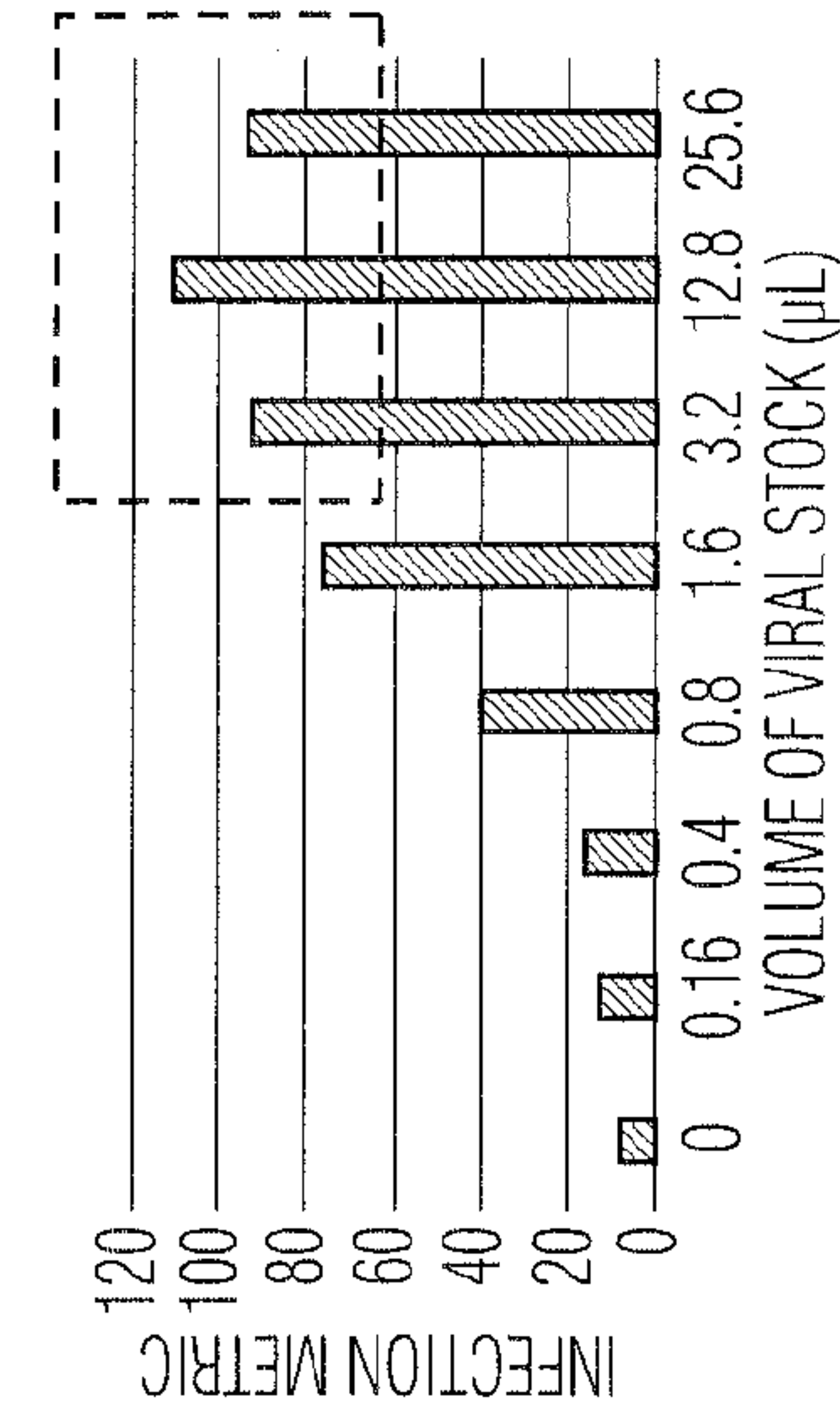


FIG. 18

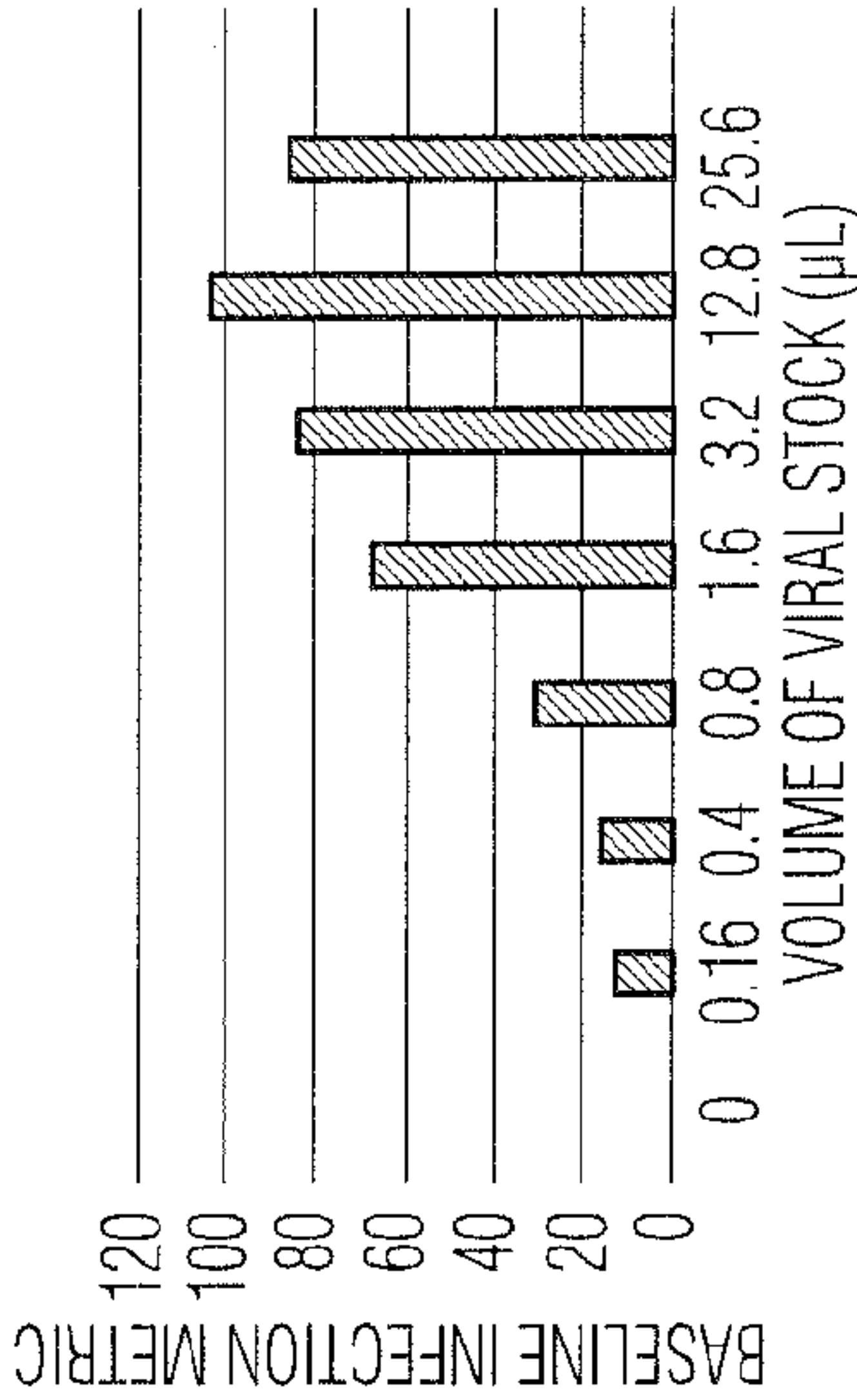
THIS ASSUMES AN INFECTION METRIC AND HARVEST TIME HAS ALREADY BEEN DETERMINED

1. ANALYZE SAMPLES AND IDENTIFY DILUTIONS THAT SPAN THE MAXIMUM INFECTION METRIC



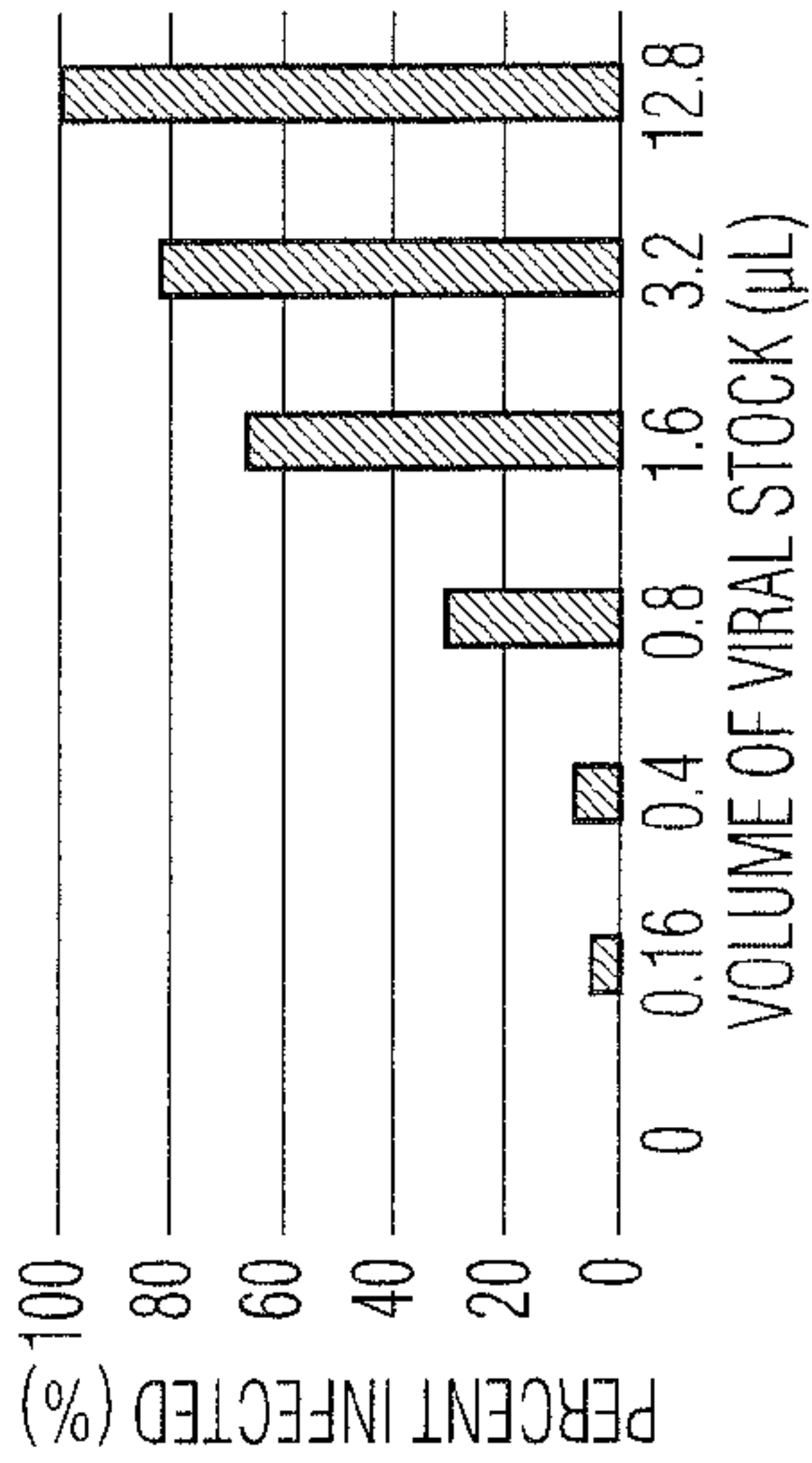
4. CALCULATE THE AMOUNT OF VIRUS ADDED (INFECTIOUS UNITS/mL) TO EACH DILUTION BASED ON THE NUMBER OF CELLS AT THE TIME OF INFECTION, THE PERCENTAGE OF UNINFECTED CELLS, THE POISSON DISTRIBUTION, AND THE VOLUME OF VIRUS ADDED AT THAT DILUTION.

2. SUBTRACT INFECTION METRIC OF UNINFECTED CELLS AS BASELINE



5. USING ONLY THE DILUTIONS THAT FALL WITHIN THE LINEAR RANGE (GENERALLY 0.5-40% INFECTED), CALCULATE THE OVERALL TITER BASED ON THE AVERAGE OF THE TITERS FOR THE DILUTIONS THAT FALL WITHIN THE LINEAR RANGE OF THE ASSAY.

3. ASSUME HIGHEST INFECTION METRIC IS 100% INFECTED, DISCARD DILUTIONS ABOVE 100%, CALCULATE PERCENT INFECTED FOR EACH DILUTION BASED ON SCALING



6.

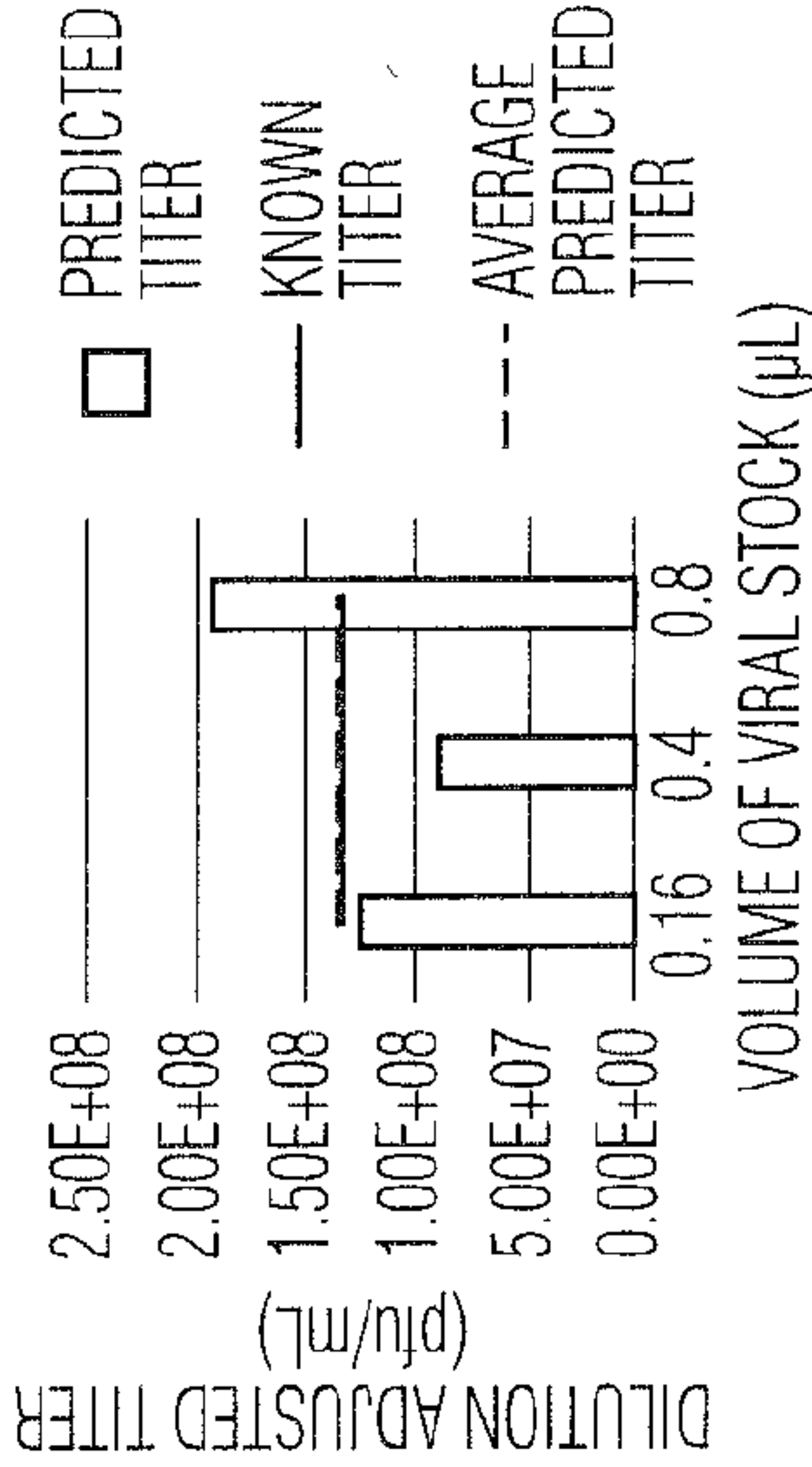


FIG. 19

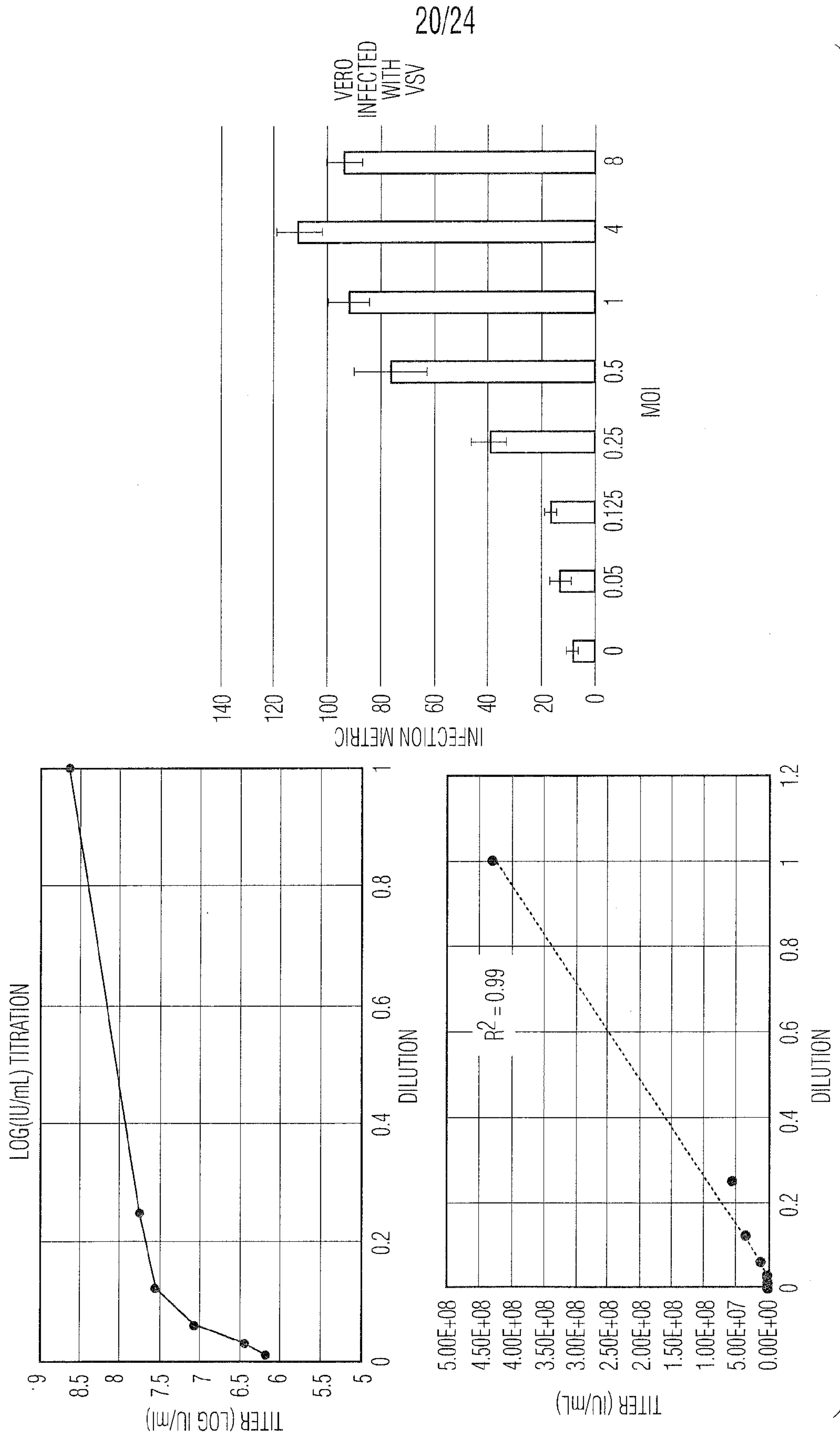


FIG. 20

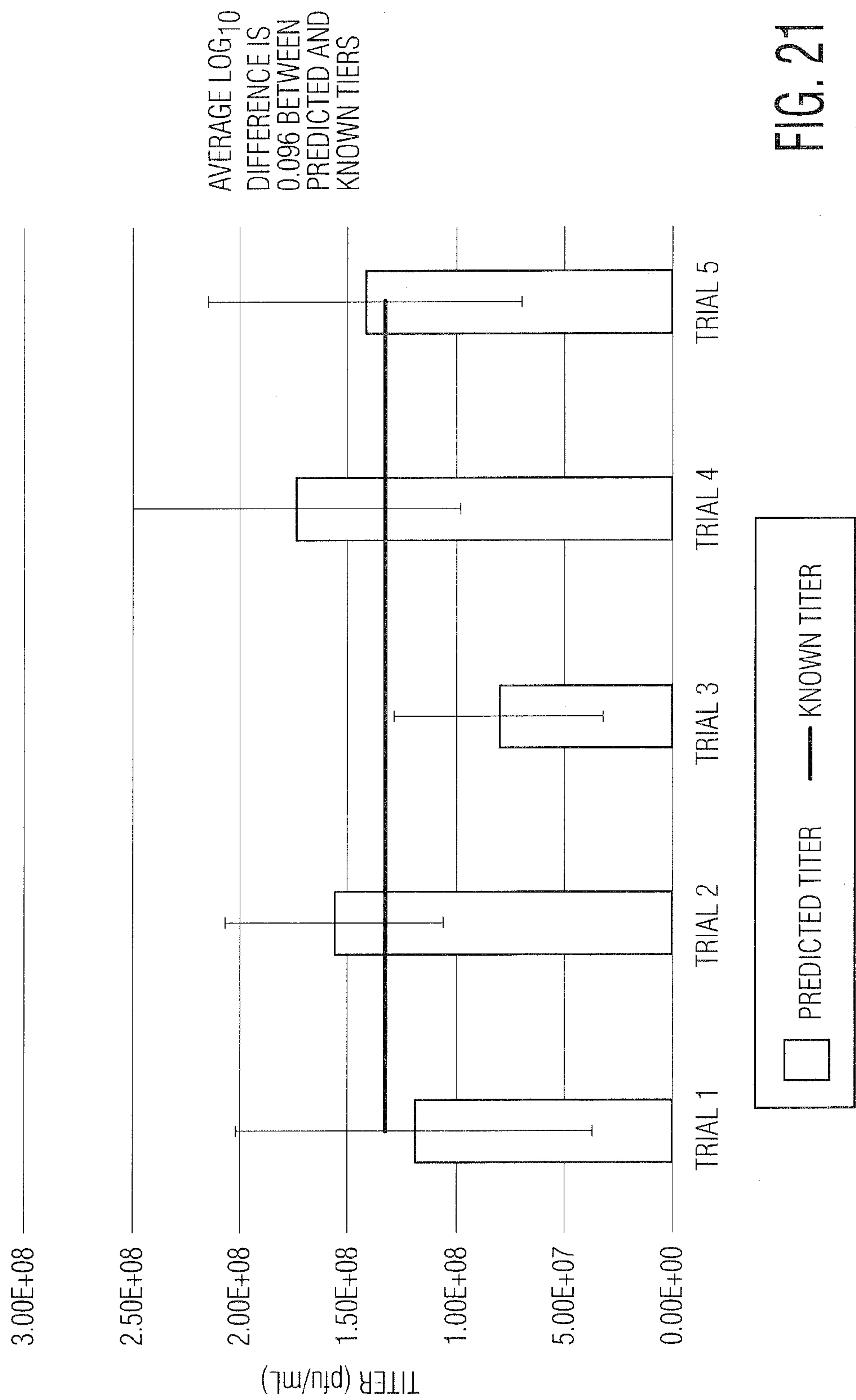


FIG. 21

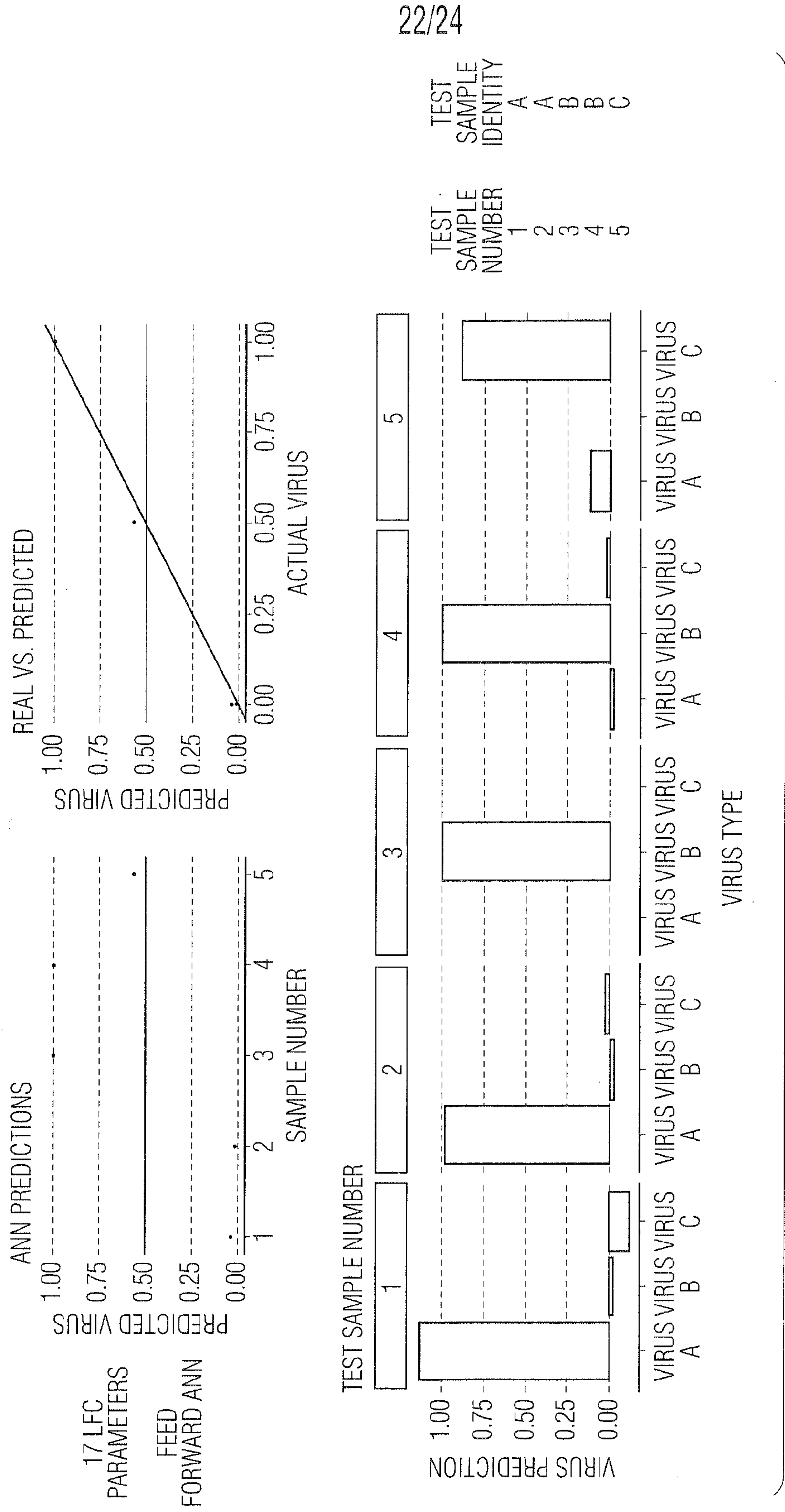
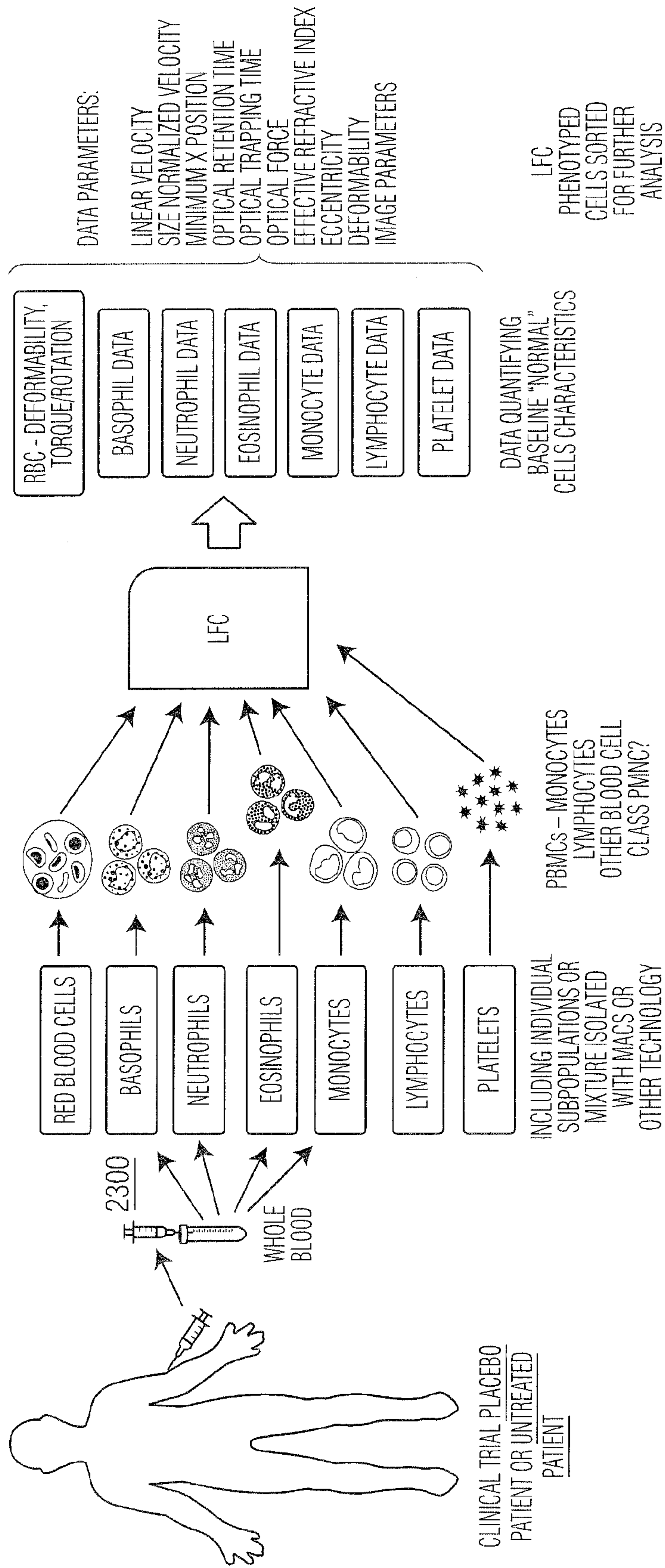
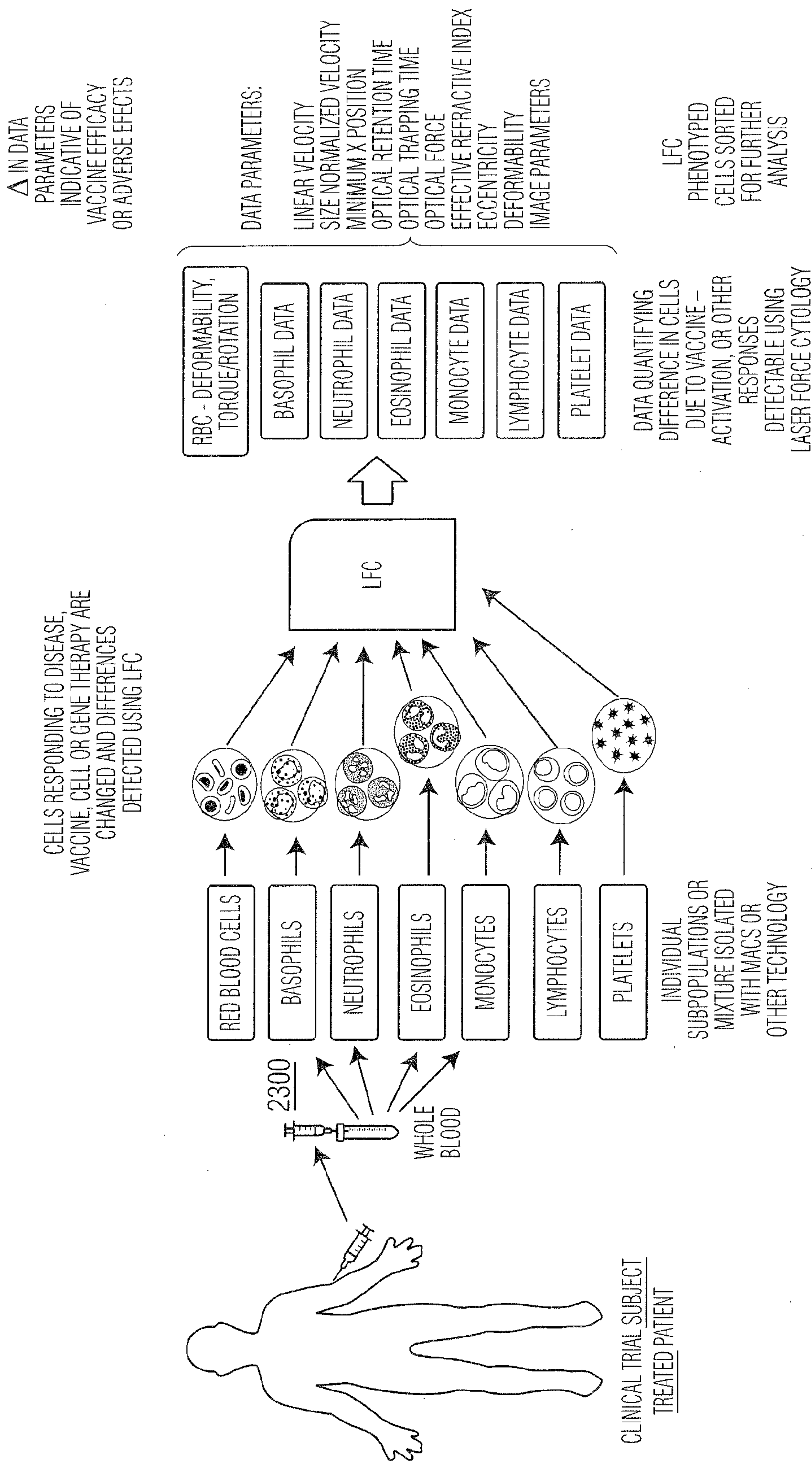


FIG. 22



2300 - PROCESS USING CENTRIFUGATION, OR RBC LYSIS, COMBINATION, OR OTHER METHOD

FIG. 23



2300 - PROCESS USING CENTRIFUGATION, OR RBC LYSIS, COMBINATION, OR OTHER METHOD

FIG. 24