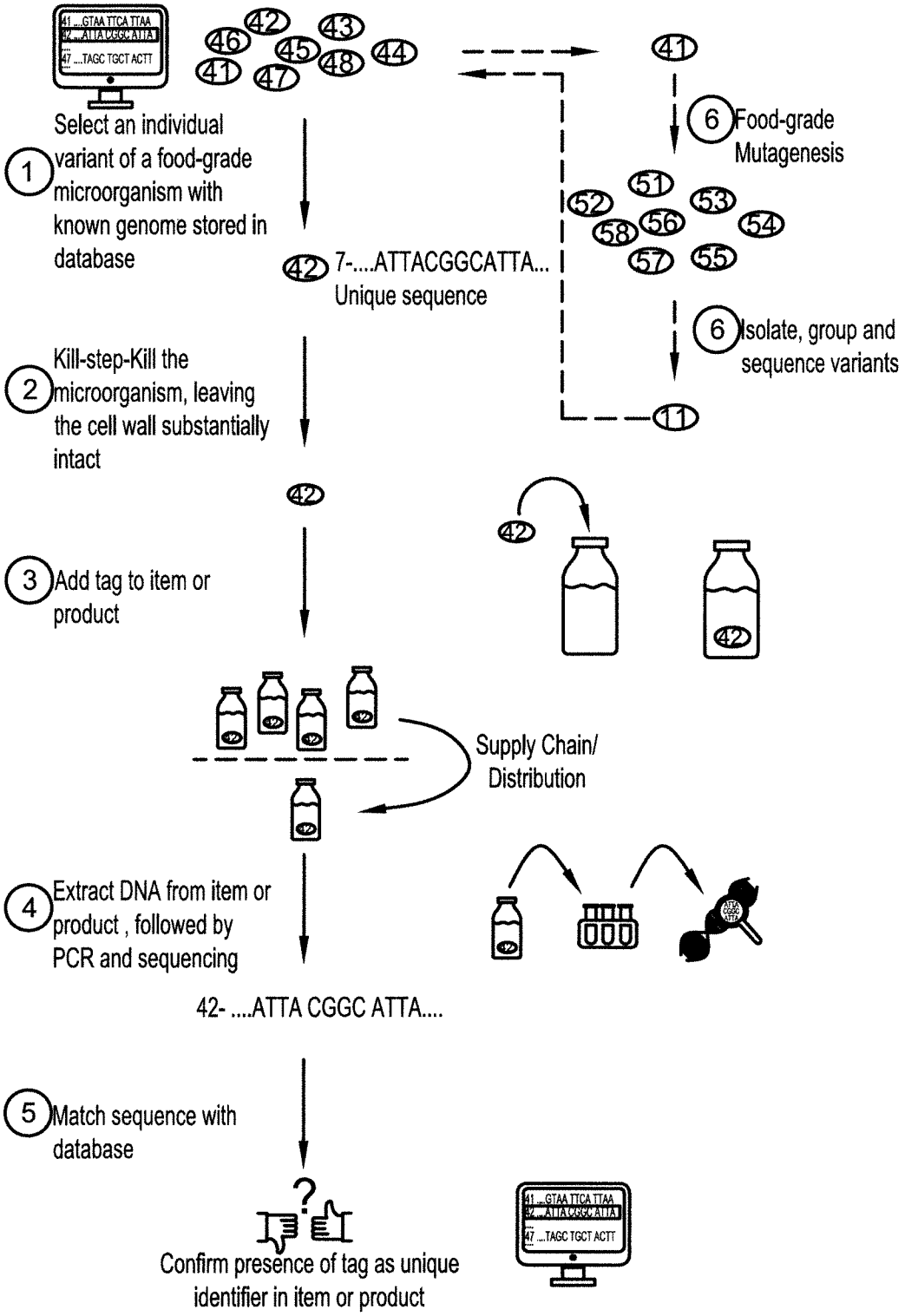

41 ...GTAA TTCA TTAA
42 ...ATTA CGGC ATTA
47 ...TAGC TGCT ACTT
Select an individual variant of a food-grade microorganism with known genome stored in database
1
42 7-....ATTACGGCATTA...
Unique sequence
Kill-step-Kill the microorganism, leaving the cell wall substantially intact
2
Add tag to item or product
3
Supply Chain/ Distribution
Extract DNA from item or product , followed by PCR and sequencing
4
42- ....ATTA CGGC ATTA....
Match sequence with database
5
Confirm presence of tag as unique identifier in item or product
6 Food-grade Mutagenesis
6 Isolate, group and sequence variants
11

FIG. 1

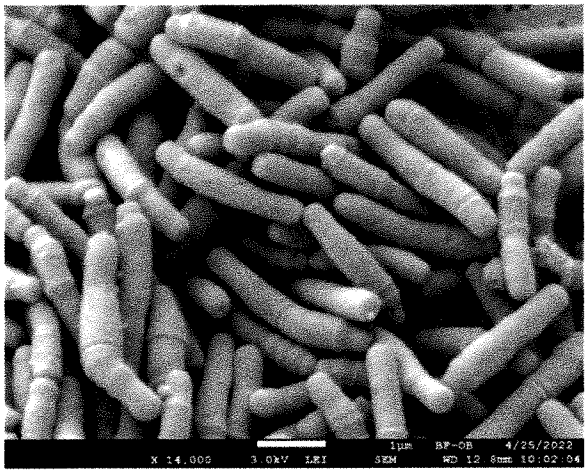


FIG. 2A

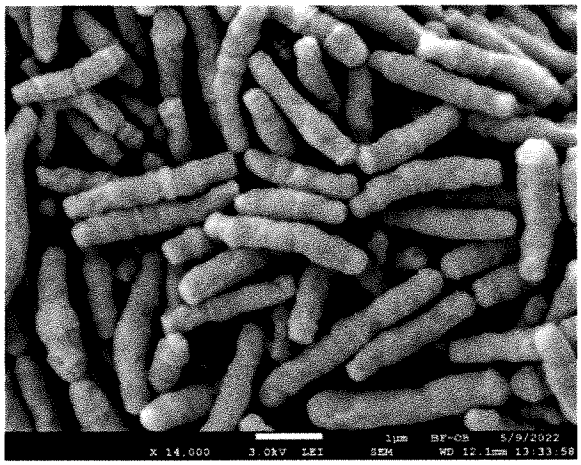


FIG. 2B

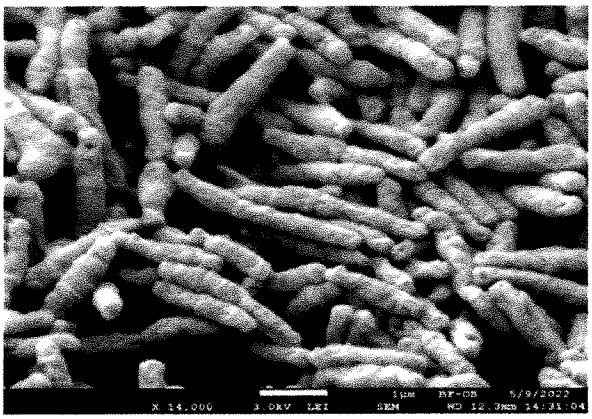


FIG. 2C

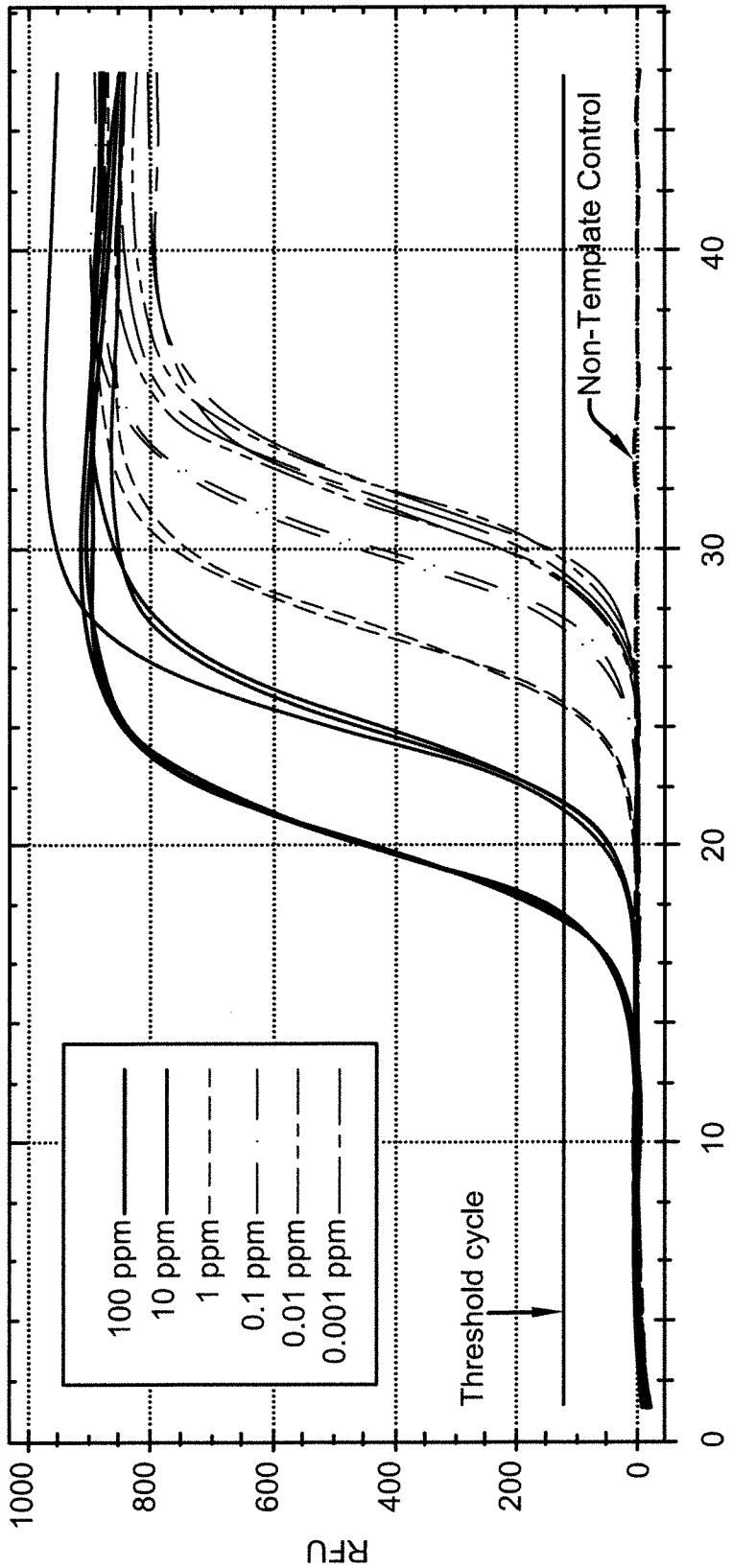

RFU
1000
800
600
400
200
0
0
10
20
30
40
Cycles
100 ppm
10 ppm
1 ppm
0.1 ppm
0.01 ppm
0.001 ppm
Threshold cycle
Non-Template Control

FIG. 3

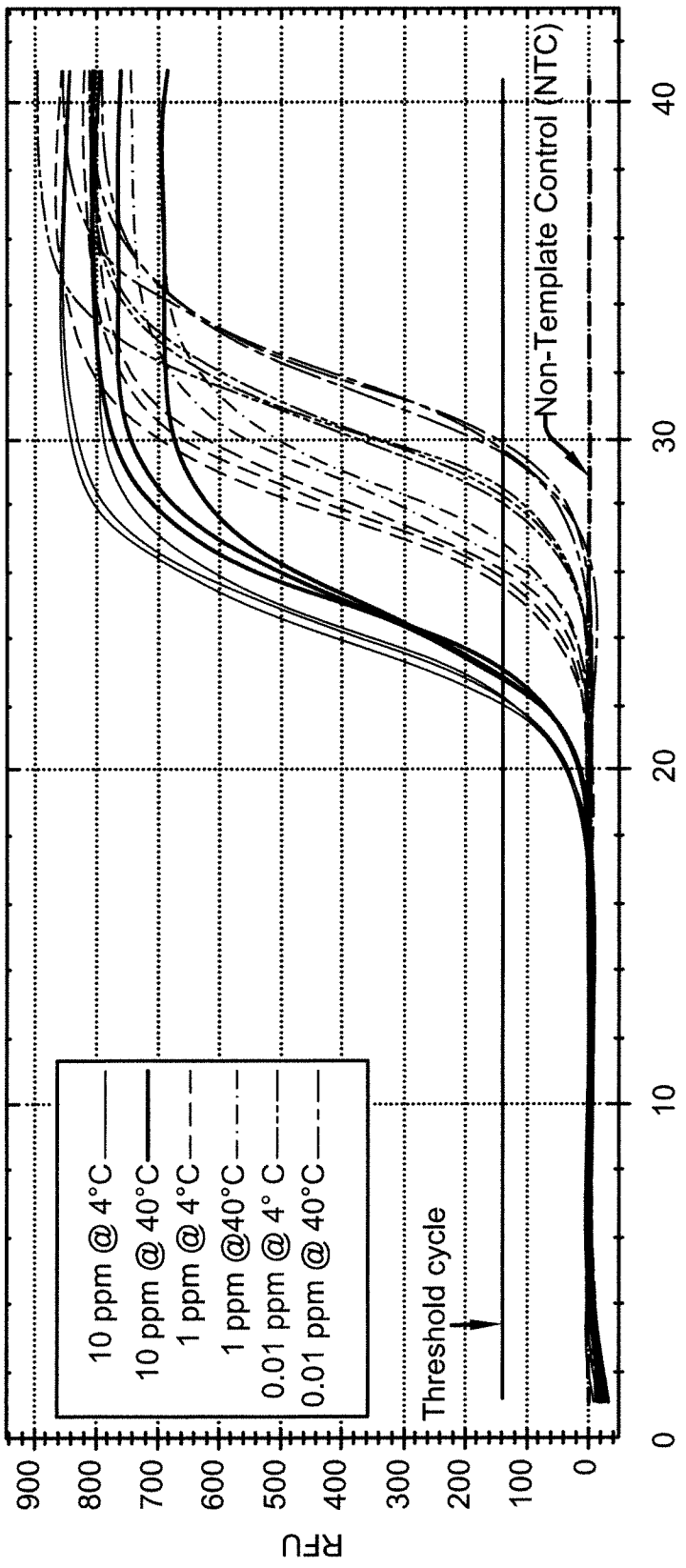

RFU
900
800
700
600
500
400
300
200
100
0
0
10
20
30
40
Cycles
10 ppm @ 4°C
10 ppm @ 40°C
1 ppm @ 4°C
1 ppm @40°C
0.01 ppm @ 4° C
0.01 ppm @ 40°C
Threshold cycle
Non-Template Control (NTC)

FIG. 4

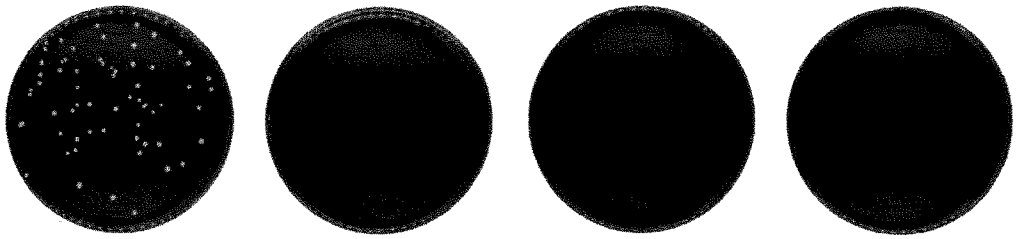

Control
Untreated
90°C; 10 min
80°C; 20 min
121°C; 15 min

FIG. 5

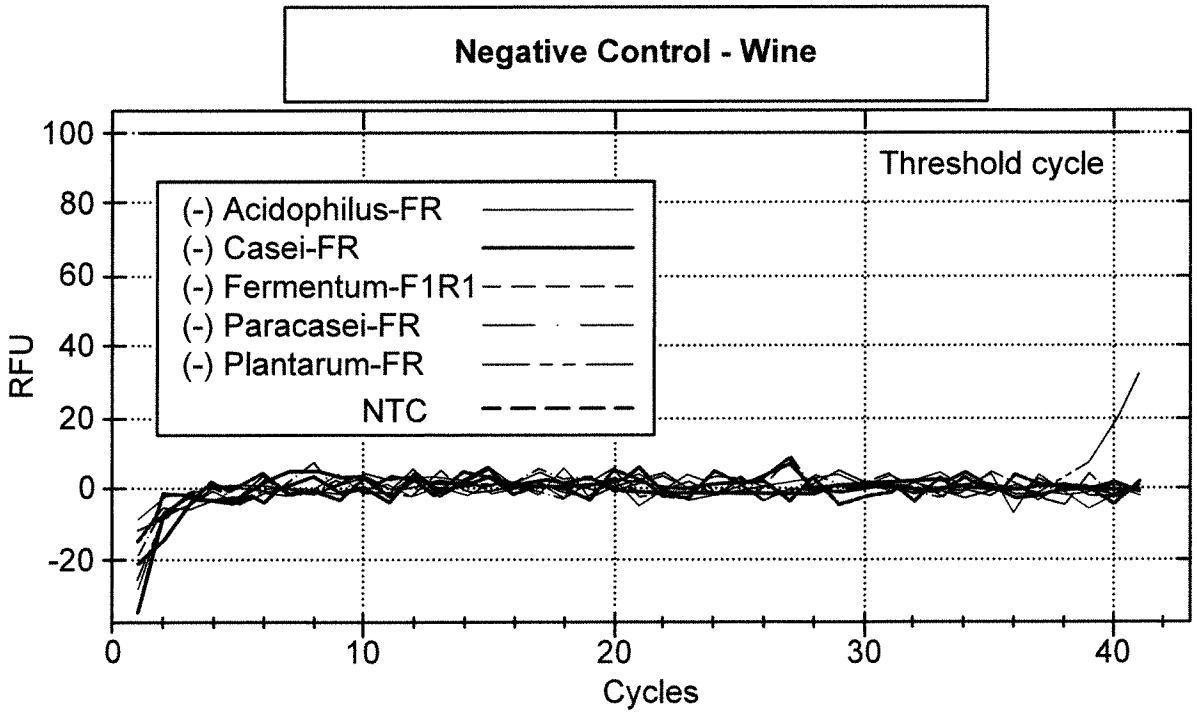

Negative Control - Wine
Threshold cycle
(-) Acidophilus-FR
(-) Casei-FR
(-) Fermentum-F1R1
(-) Paracasei-FR
(-) Plantarum-FR
NTC
RFU
100
80
60
40
20
0
-20
0
10
20
30
40
Cycles

FIG. 6A

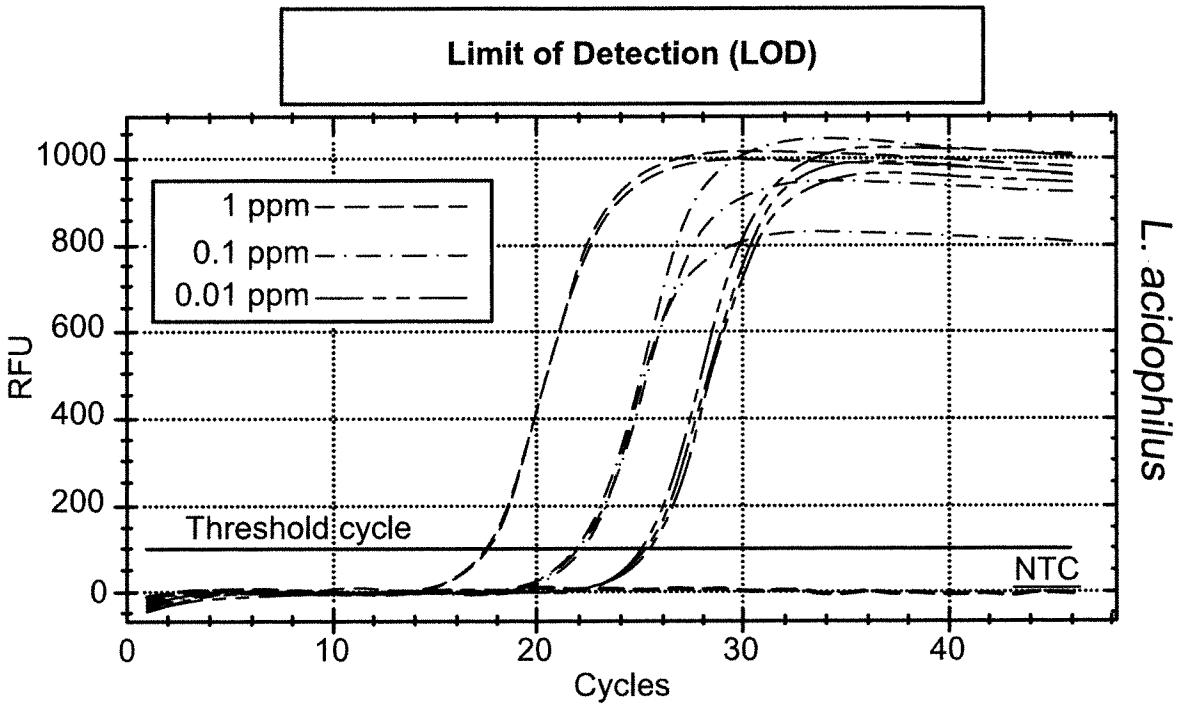

Limit of Detection (LOD)
1 ppm
0.1 ppm
0.01 ppm
Threshold cycle
NTC
RFU
Cycles
L. acidophilus

FIG. 6B

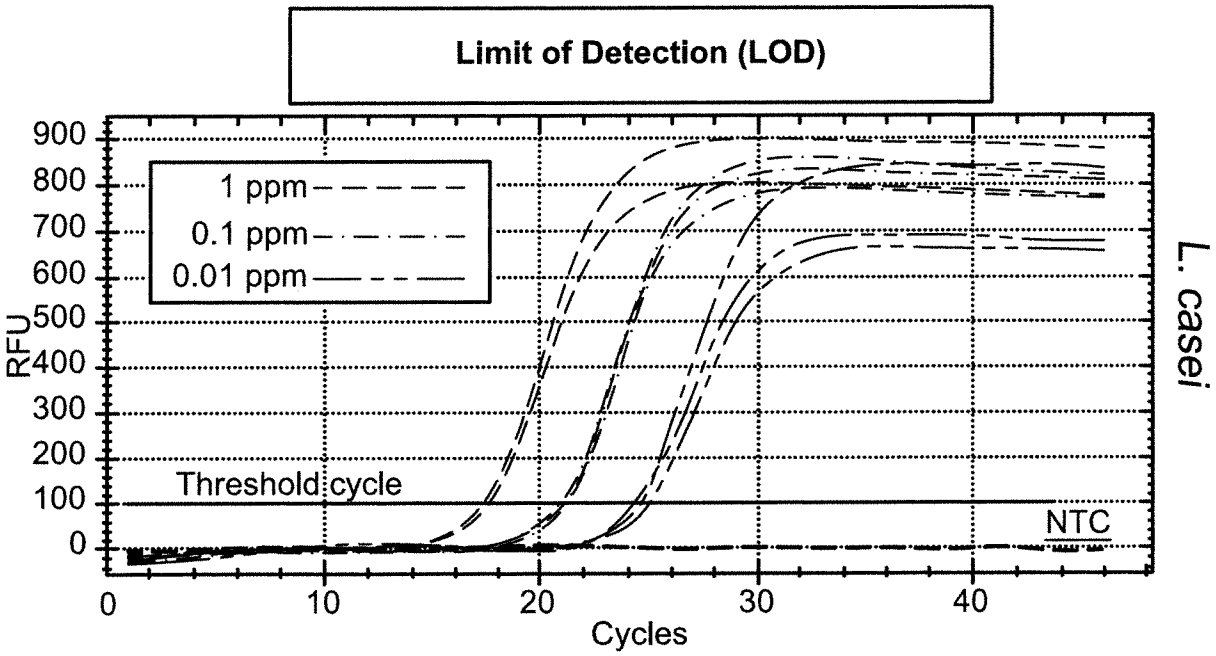

Limit of Detection (LOD)
1 ppm
0.1 ppm
0.01 ppm
Threshold cycle
NTC
RFU
Cycles
L. casei

FIG. 6C

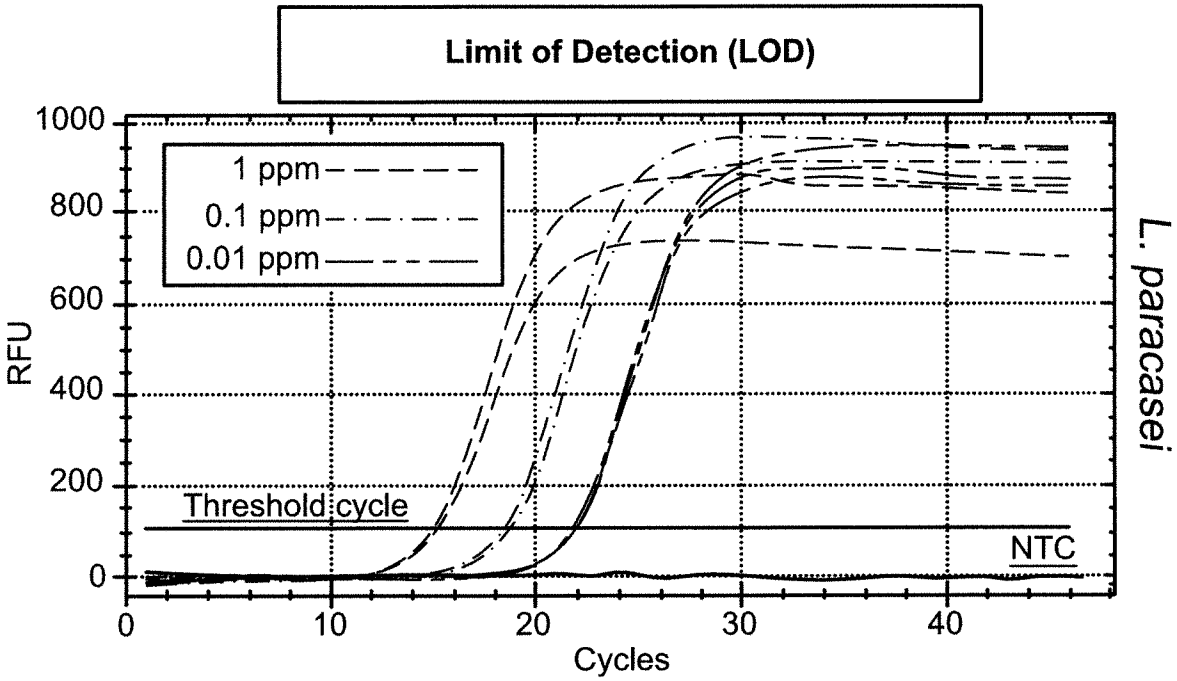

Limit of Detection (LOD)
1 ppm
0.1 ppm
0.01 ppm
Threshold cycle
NTC
RFU
Cycles
L. paracasei

FIG. 6D

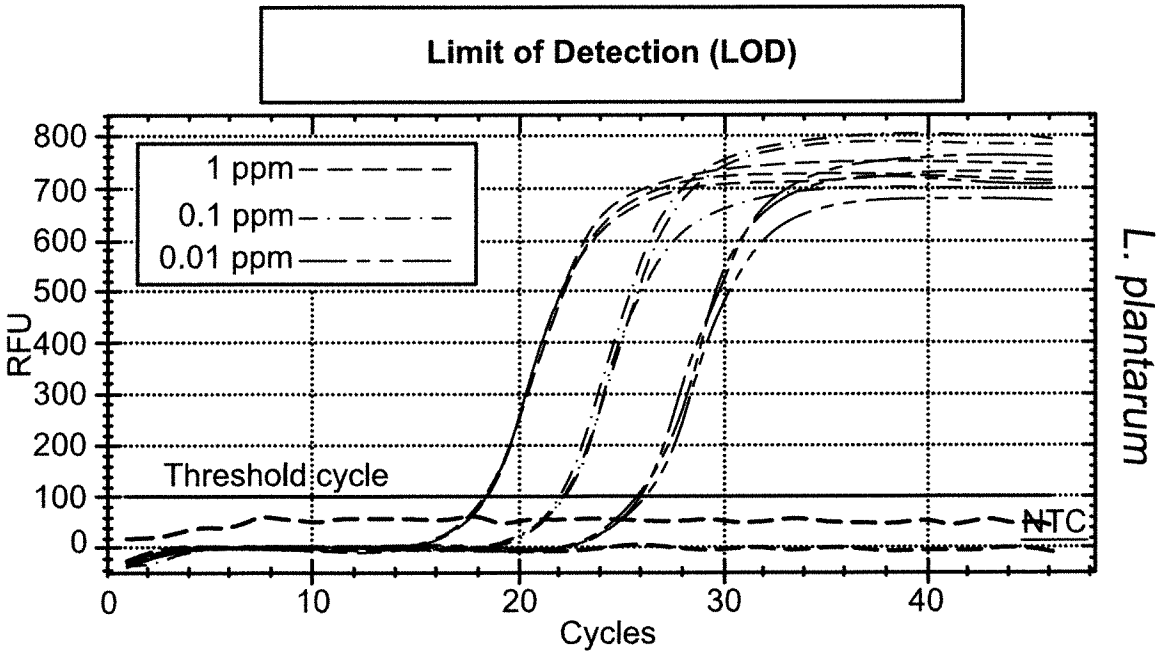

Limit of Detection (LOD)
1 ppm
0.1 ppm
0.01 ppm
Threshold cycle
NTC
RFU
Cycles
L. plantarum

FIG. 6E

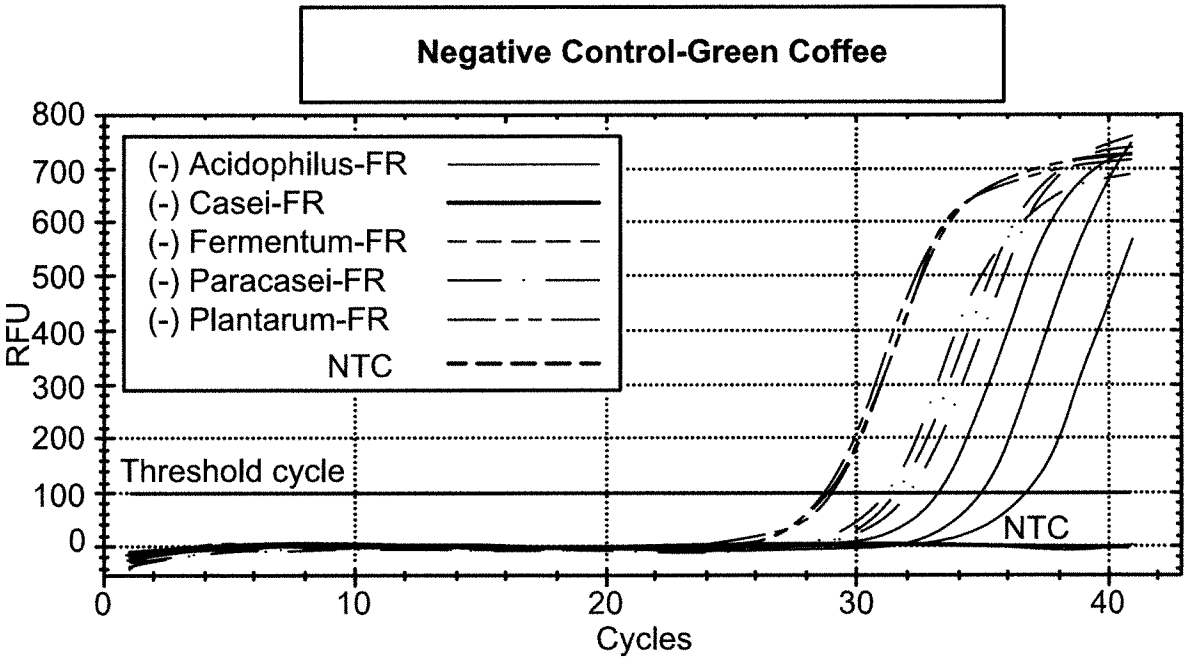

Negative Control-Green Coffee
(-) Acidophilus-FR
(-) Casei-FR
(-) Fermentum-FR
(-) Paracasei-FR
(-) Plantarum-FR
NTC
Threshold cycle
NTC
RFU
Cycles
0
100
200
300
400
500
600
700
800
0
10
20
30
40

FIG. 7A

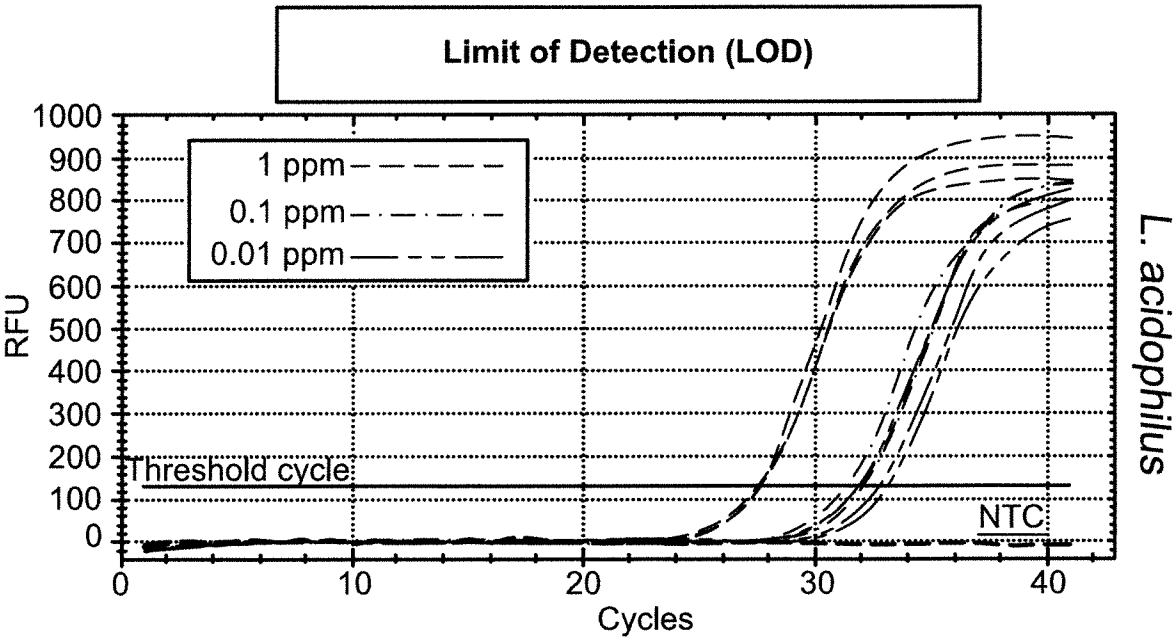

Limit of Detection (LOD)
1 ppm
0.1 ppm
0.01 ppm
Threshold cycle
NTC
RFU
Cycles
L. acidophilus
0
100
200
300
400
500
600
700
800
900
1000
0
10
20
30
40

FIG. 7B

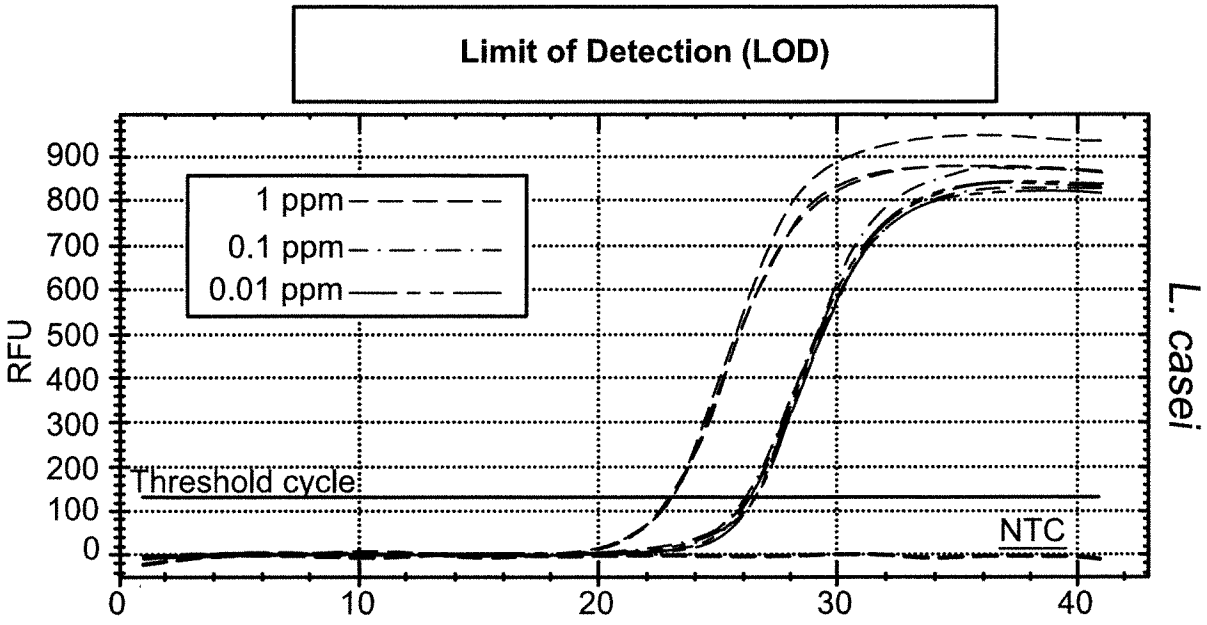

Limit of Detection (LOD)
1 ppm
0.1 ppm
0.01 ppm
Threshold cycle
NTC
RFU
900
800
700
600
500
400
300
200
100
0
0
10
20
30
40
L. casei

FIG. 7C

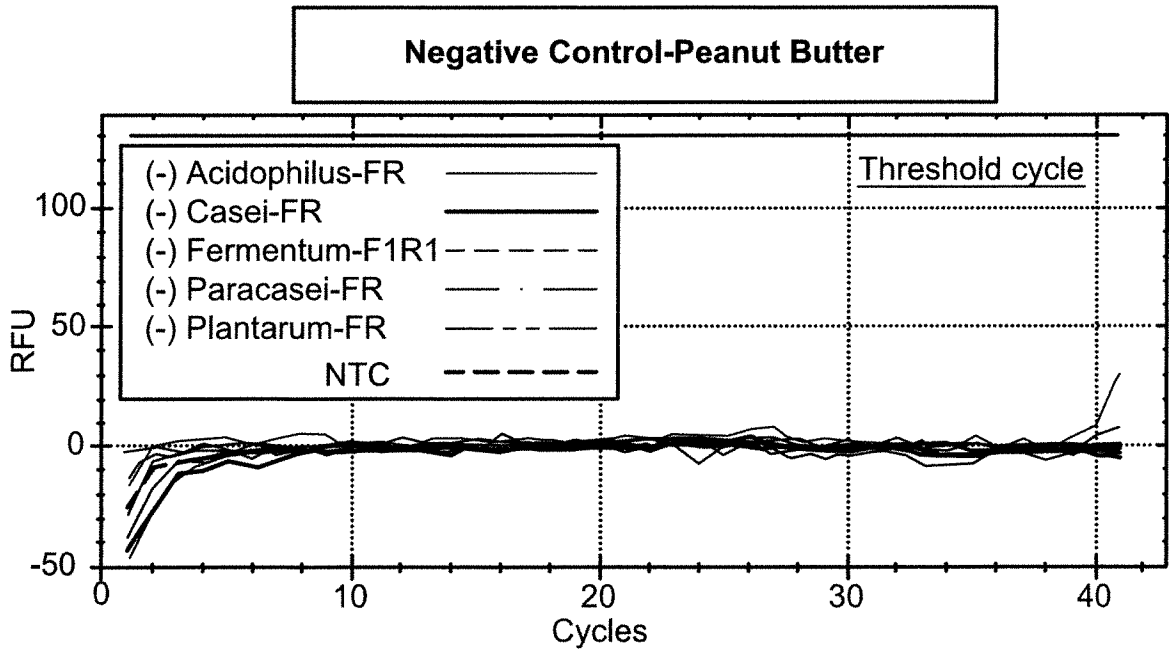

Negative Control-Peanut Butter
(-) Acidophilus-FR
(-) Casei-FR
(-) Fermentum-F1R1
(-) Paracasei-FR
(-) Plantarum-FR
NTC
Threshold cycle
RFU
100
50
0
-50
0
10
20
30
40
Cycles

FIG. 8A

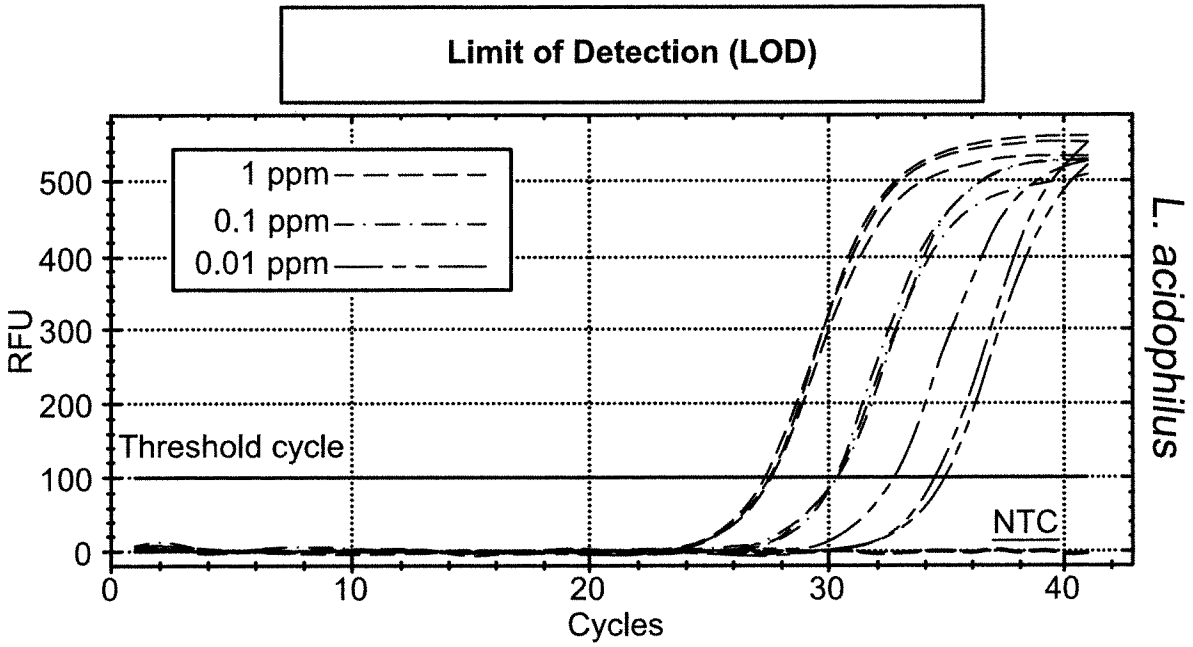

Limit of Detection (LOD)
1 ppm
0.1 ppm
0.01 ppm
RFU
500
400
300
200
100
0
Threshold cycle
NTC
0
10
20
30
40
Cycles
L. acidophilus

FIG. 8B

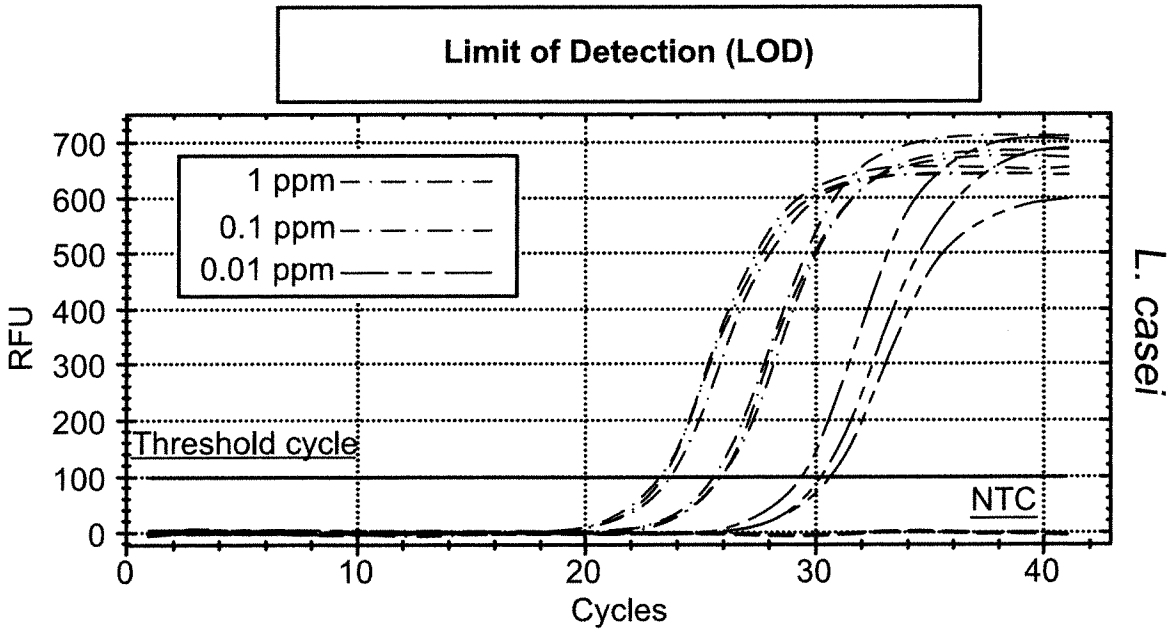

Limit of Detection (LOD)
1 ppm
0.1 ppm
0.01 ppm
RFU
700
600
500
400
300
200
100
0
Threshold cycle
NTC
0
10
20
30
40
Cycles
L. casei

FIG. 8C

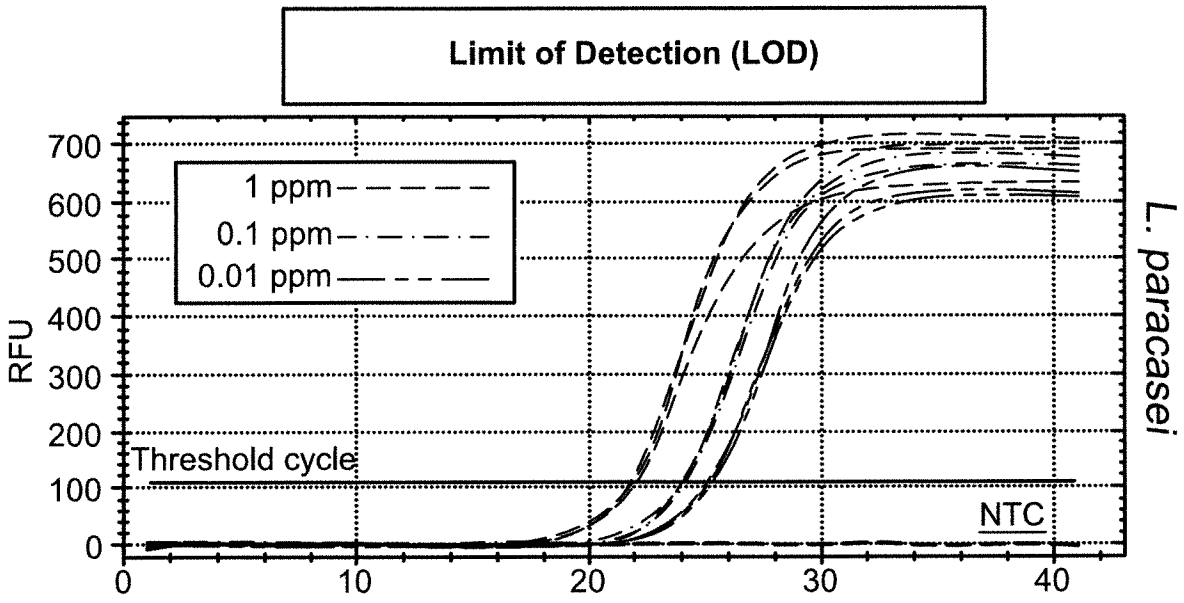

Limit of Detection (LOD)
1 ppm
0.1 ppm
0.01 ppm
RFU
Threshold cycle
NTC
L. paracasei
0
100
200
300
400
500
600
700
0
10
20
30
40

FIG. 8D

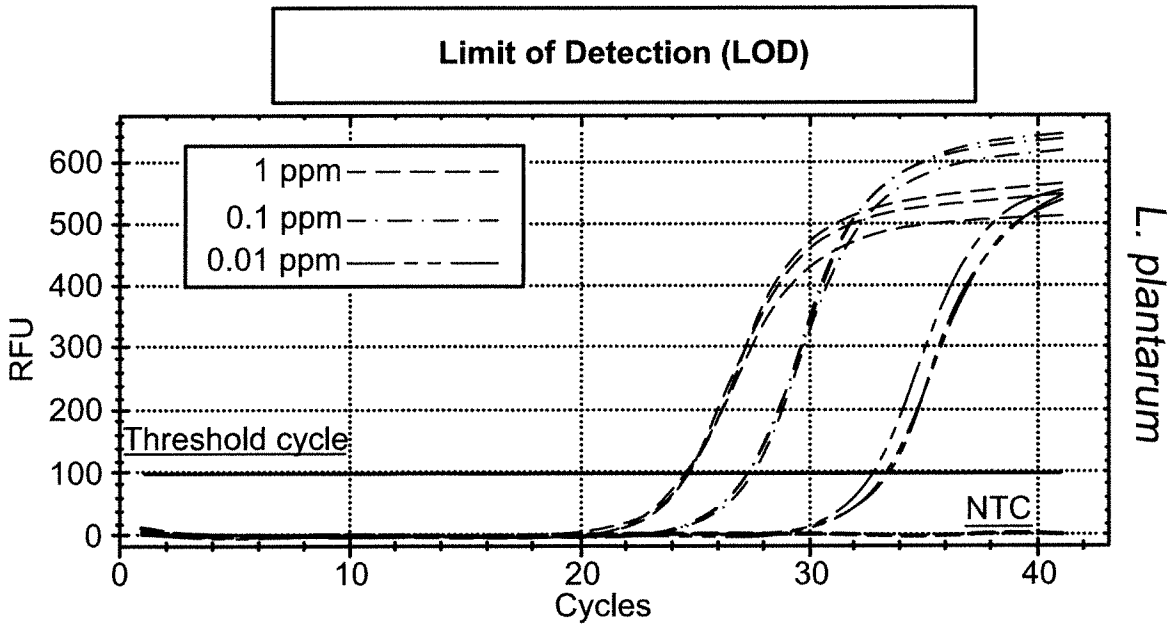

Limit of Detection (LOD)
1 ppm
0.1 ppm
0.01 ppm
RFU
Threshold cycle
NTC
L. plantarum
0
100
200
300
400
500
600
0
10
20
30
40
Cycles

FIG. 8E

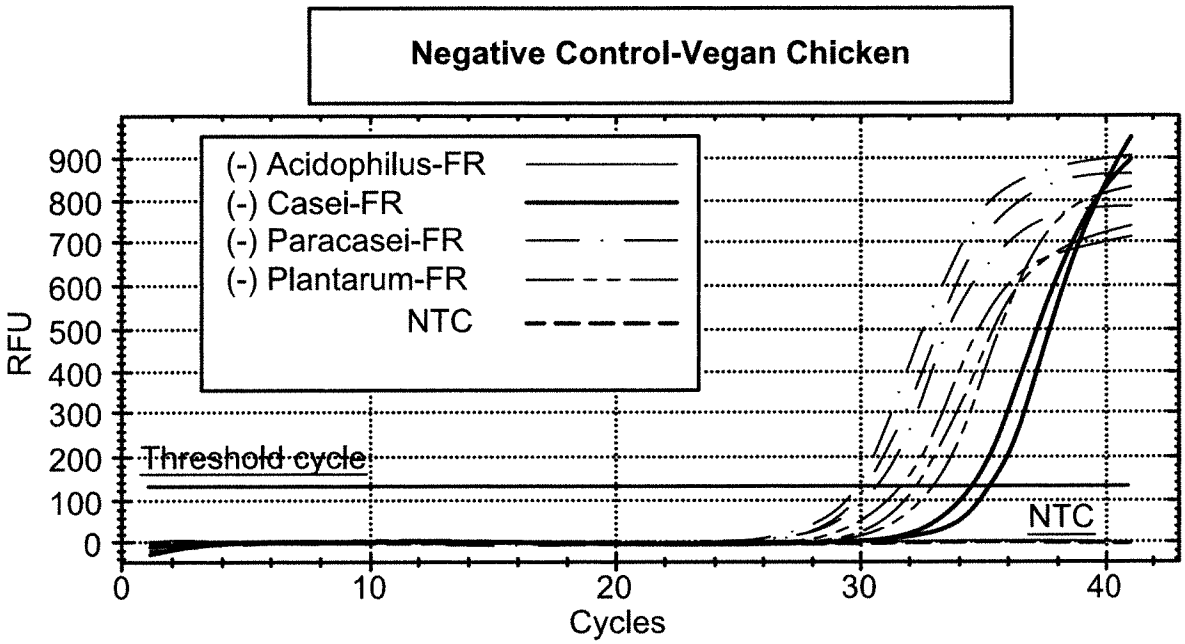

Negative Control-Vegan Chicken
(-) Acidophilus-FR
(-) Casei-FR
(-) Paracasei-FR
(-) Plantarum-FR
NTC
RFU
900
800
700
600
500
400
300
200
100
0
Threshold cycle
NTC
0
10
20
30
40
Cycles

FIG. 9A

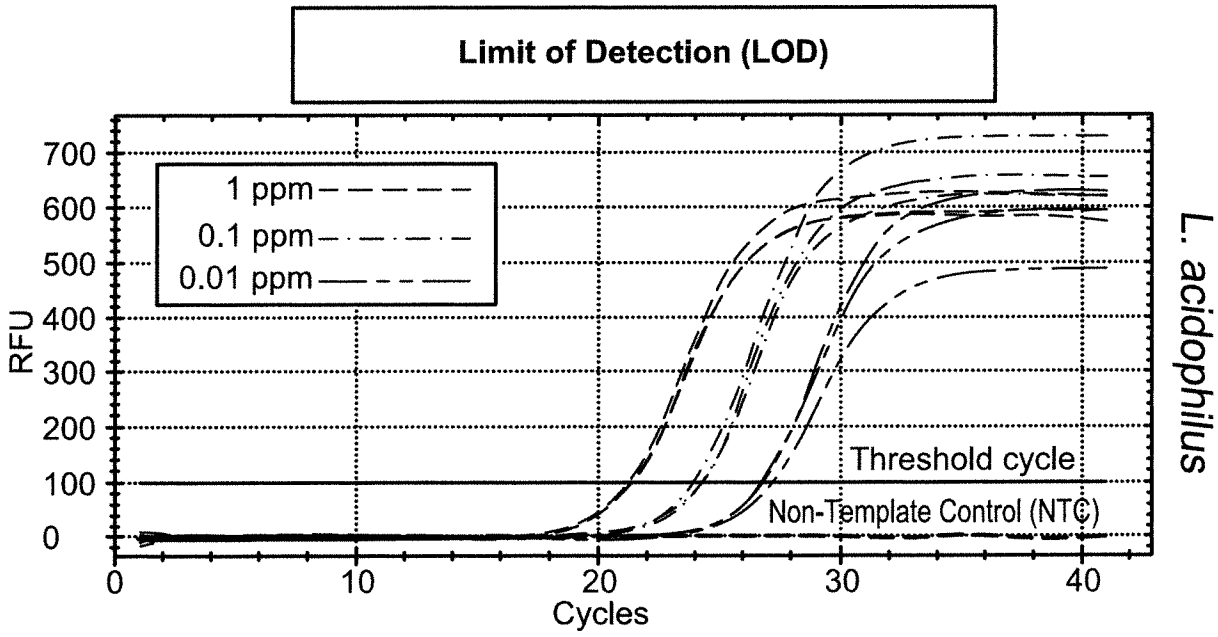

Limit of Detection (LOD)
1 ppm
0.1 ppm
0.01 ppm
RFU
700
600
500
400
300
200
100
0
Threshold cycle
Non-Template Control (NTC)
0
10
20
30
40
Cycles
L. acidophilus

FIG. 9B

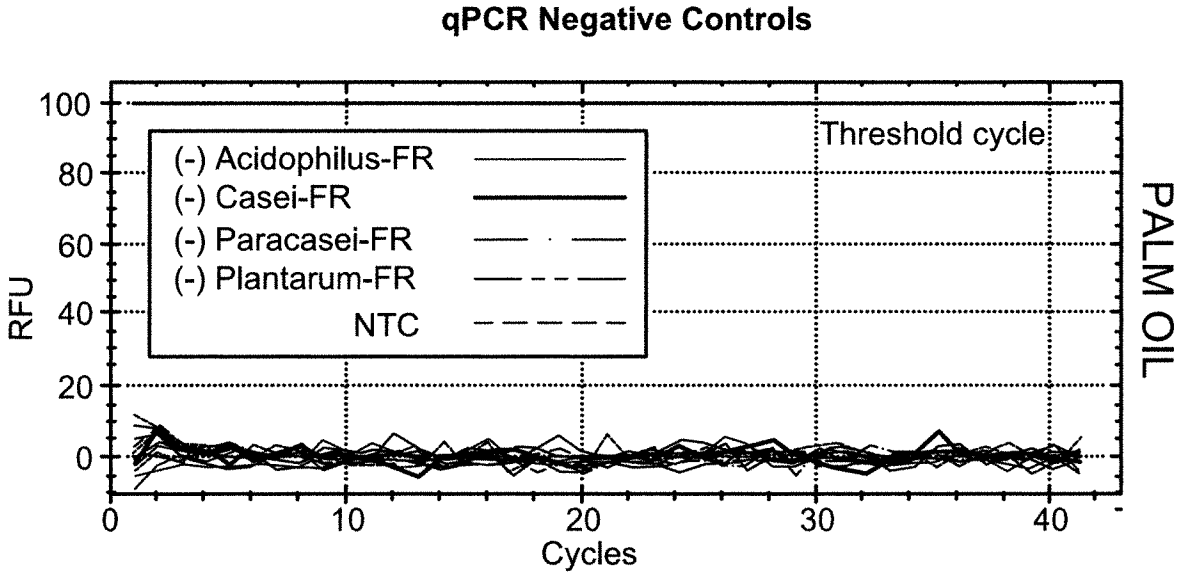


FIG. 10A

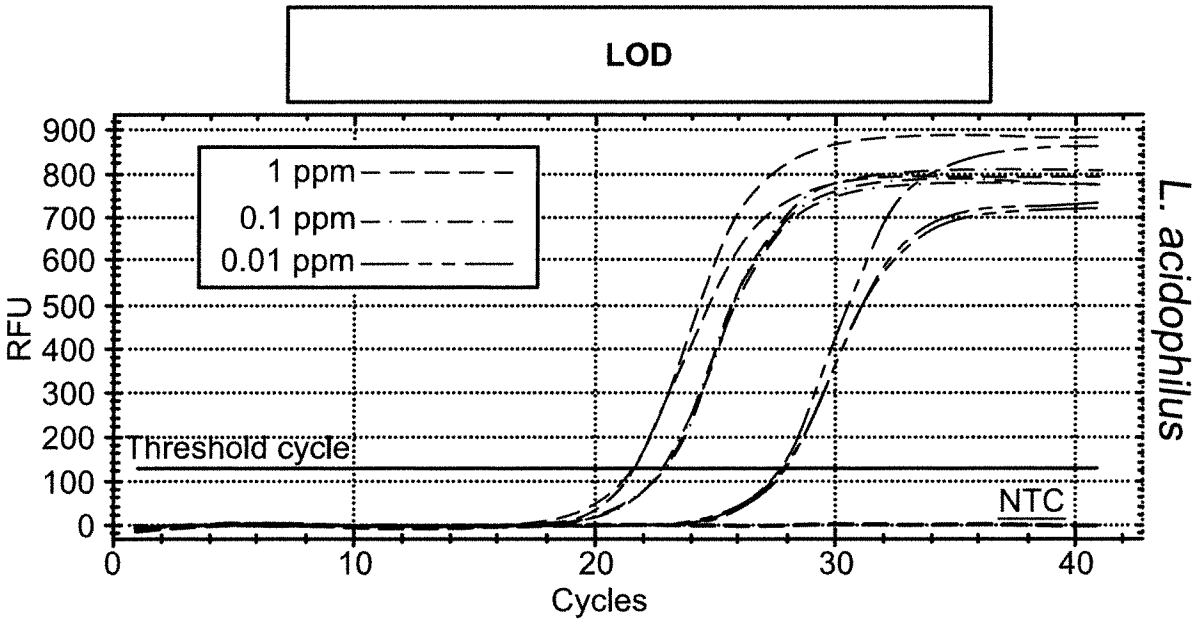


FIG. 10B

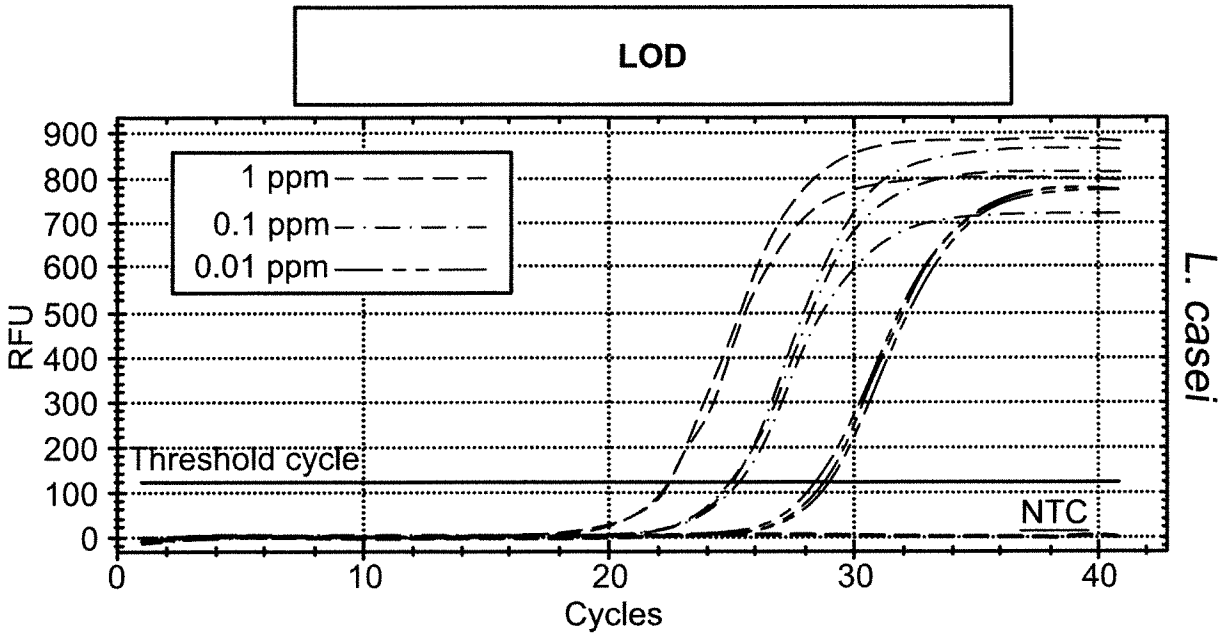


FIG. 10C

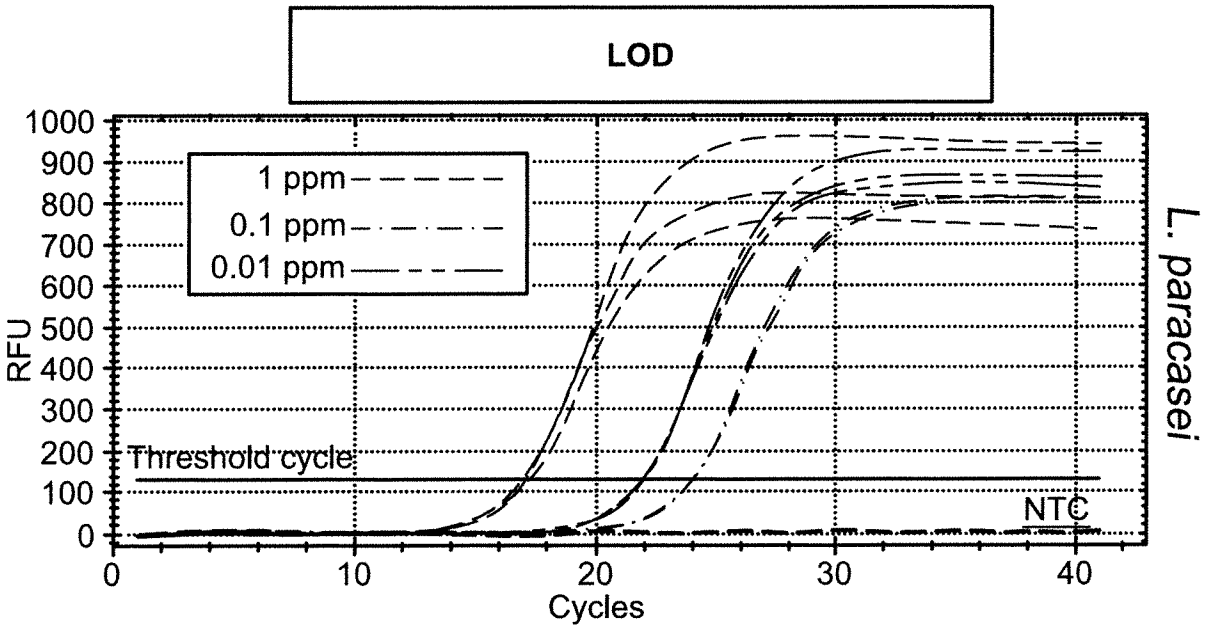


FIG. 10D

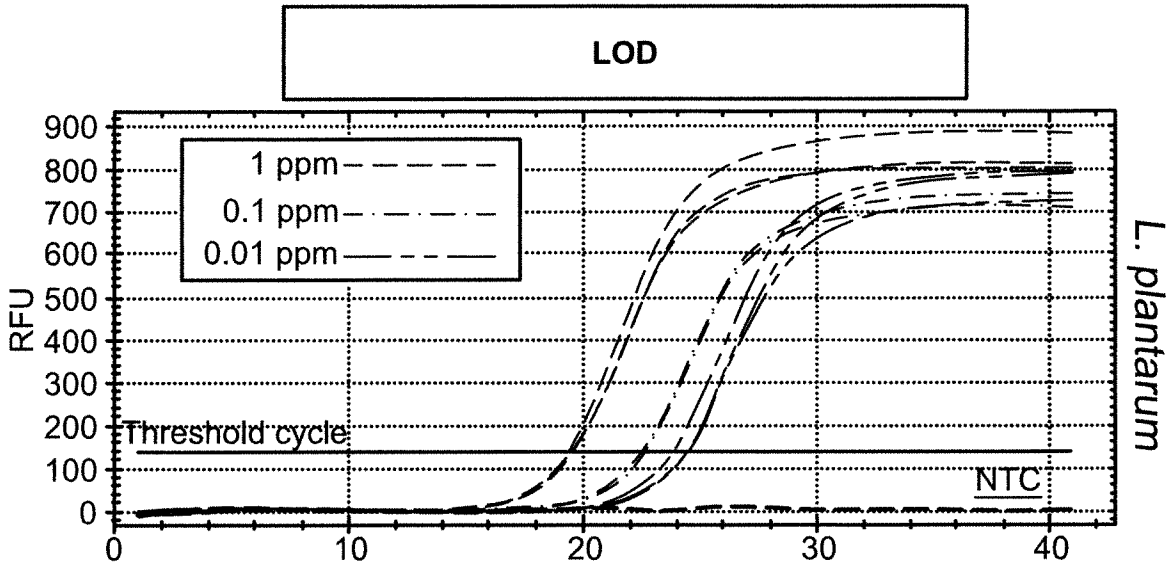


FIG. 10E

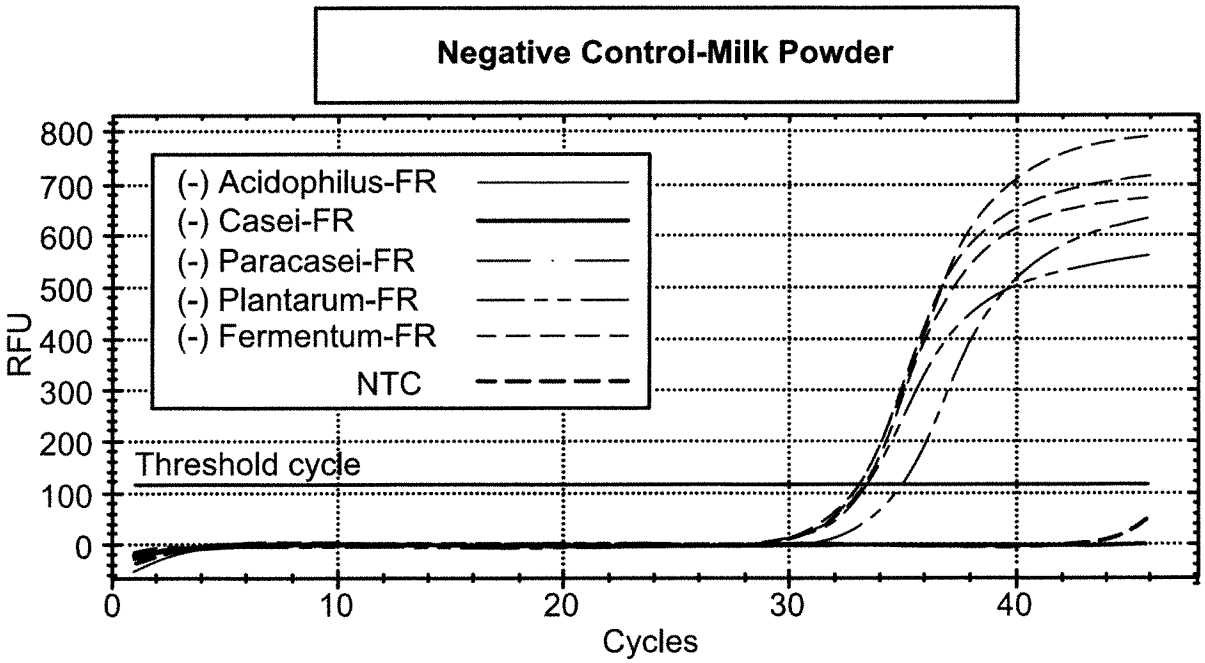


FIG. 11A

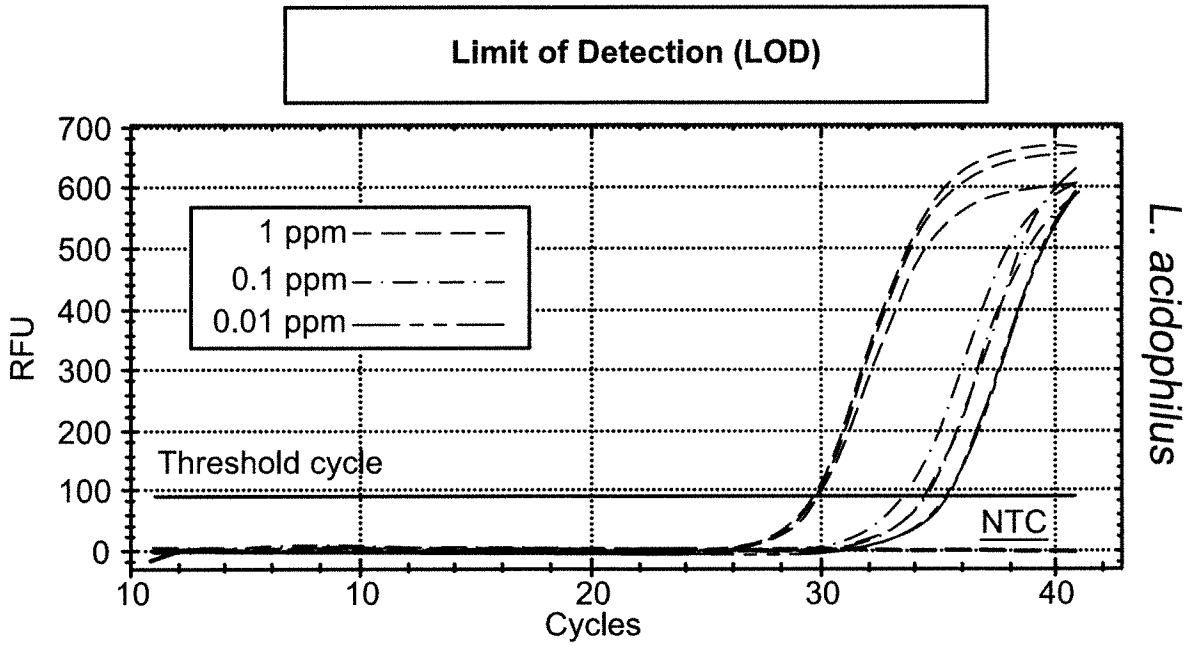


FIG. 11B

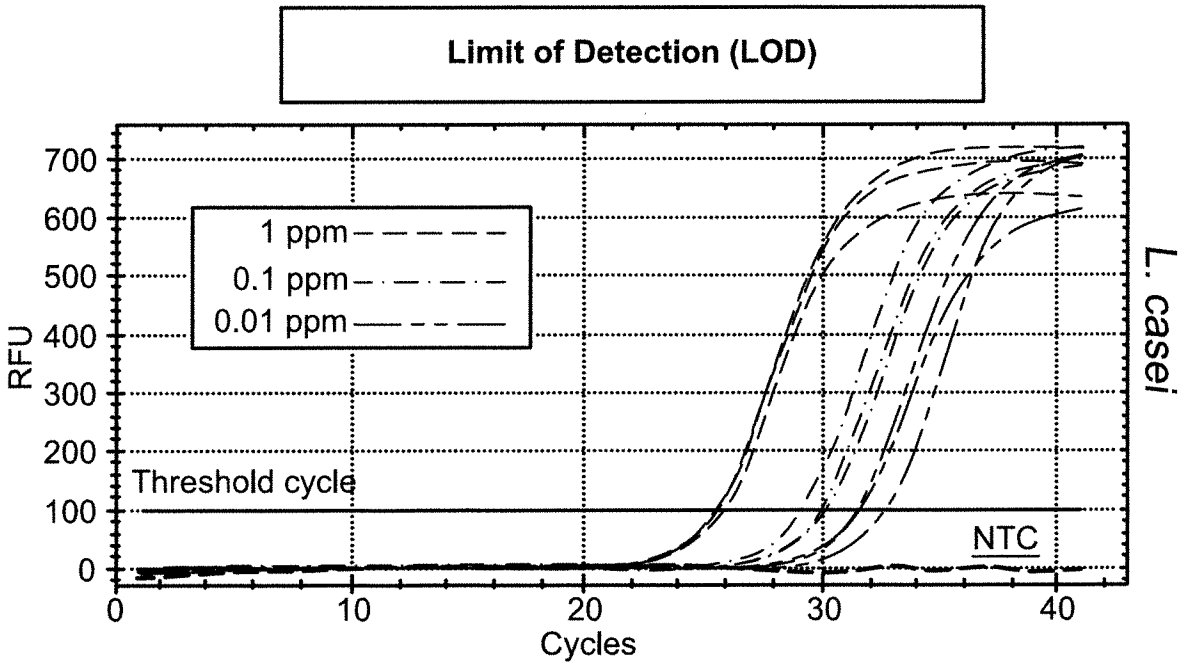


FIG. 11C

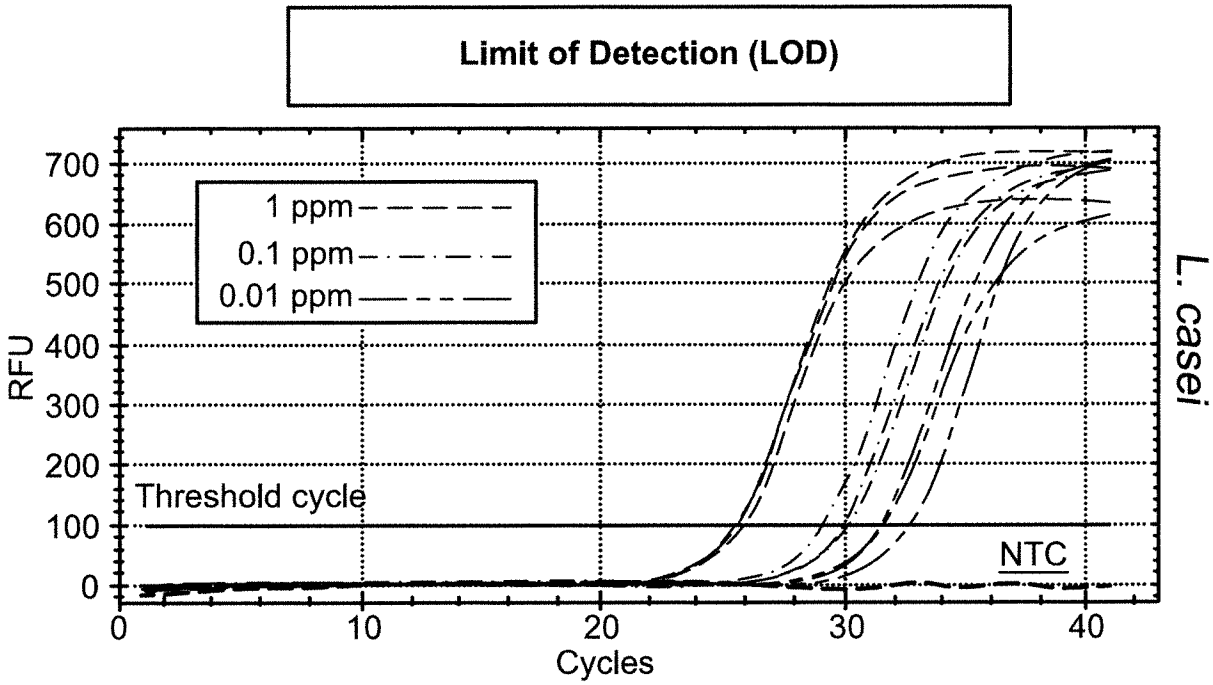


FIG. 11D

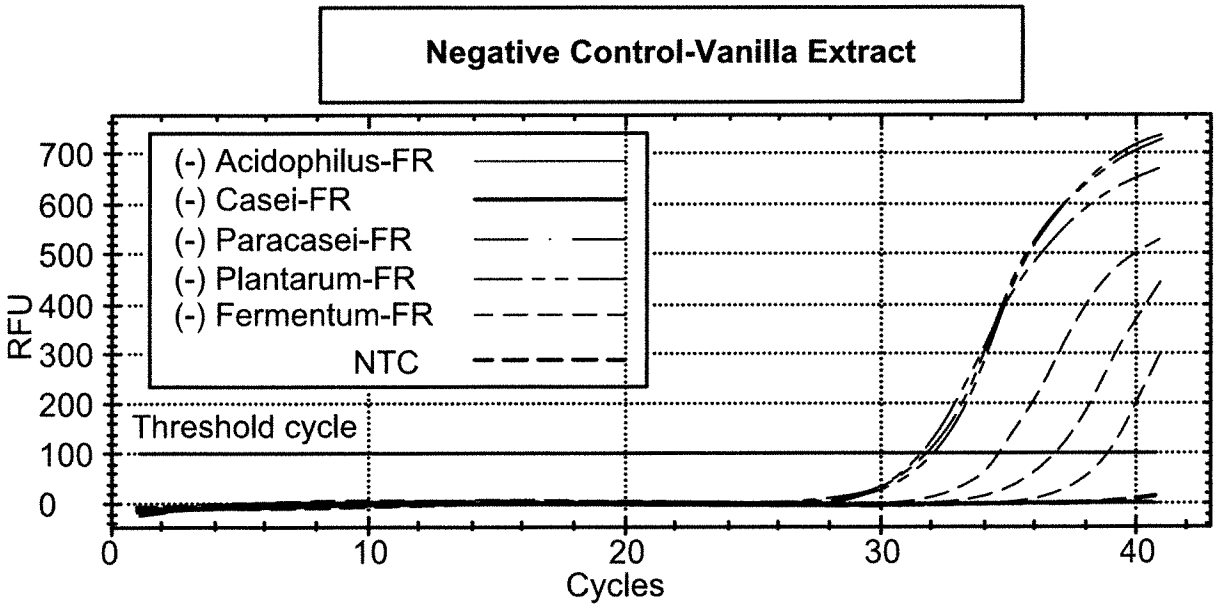


FIG. 12A

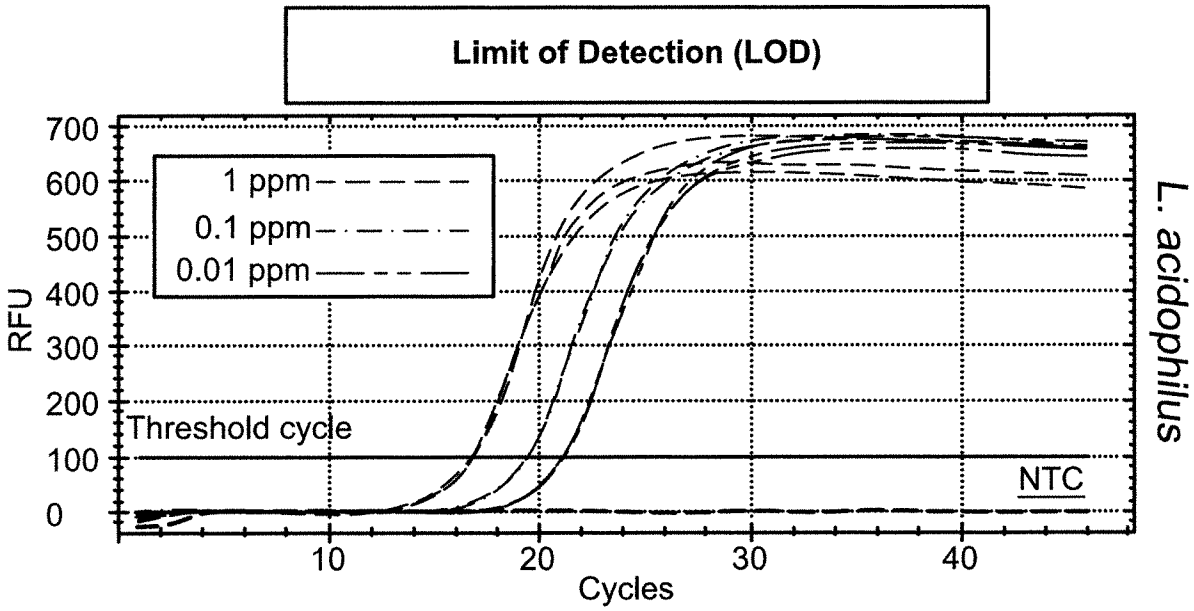


FIG. 12B

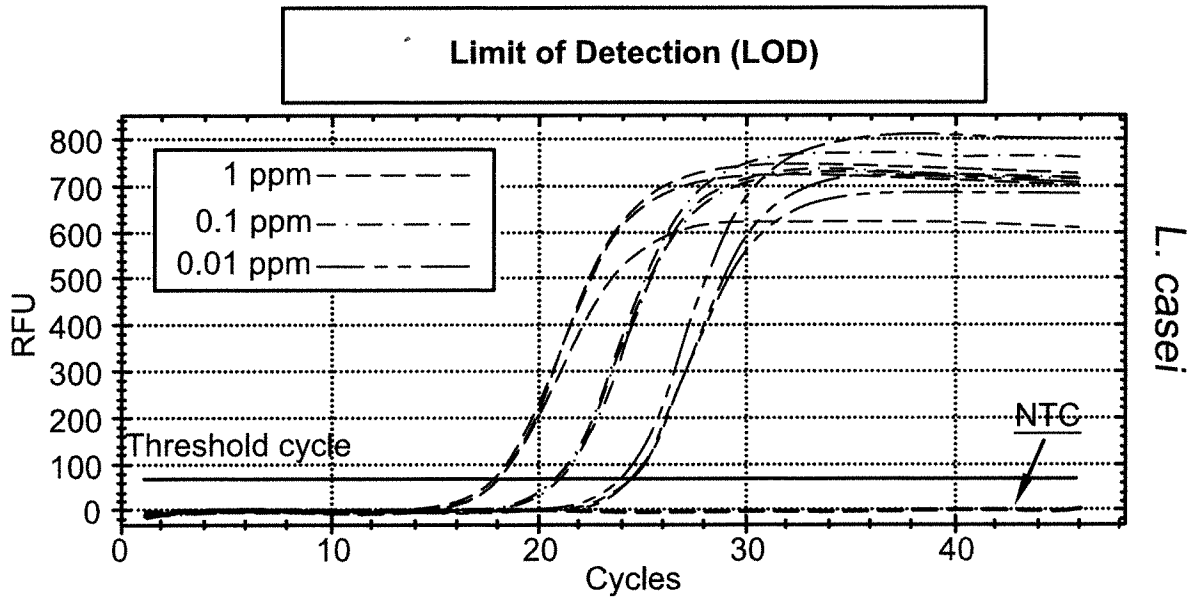


FIG. 12C

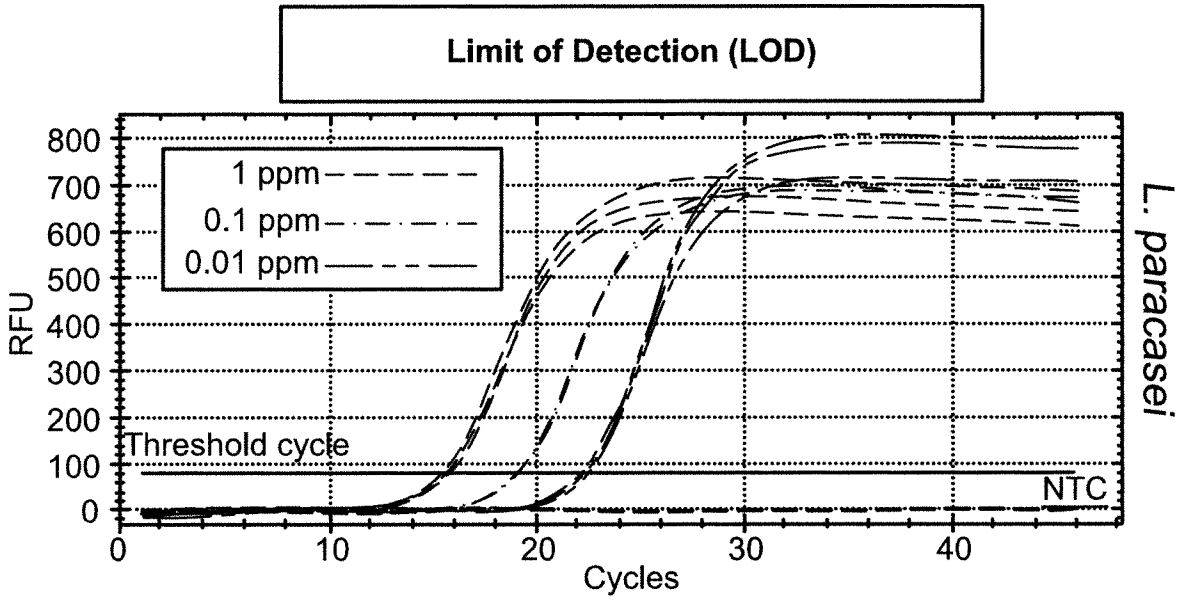


FIG. 12D

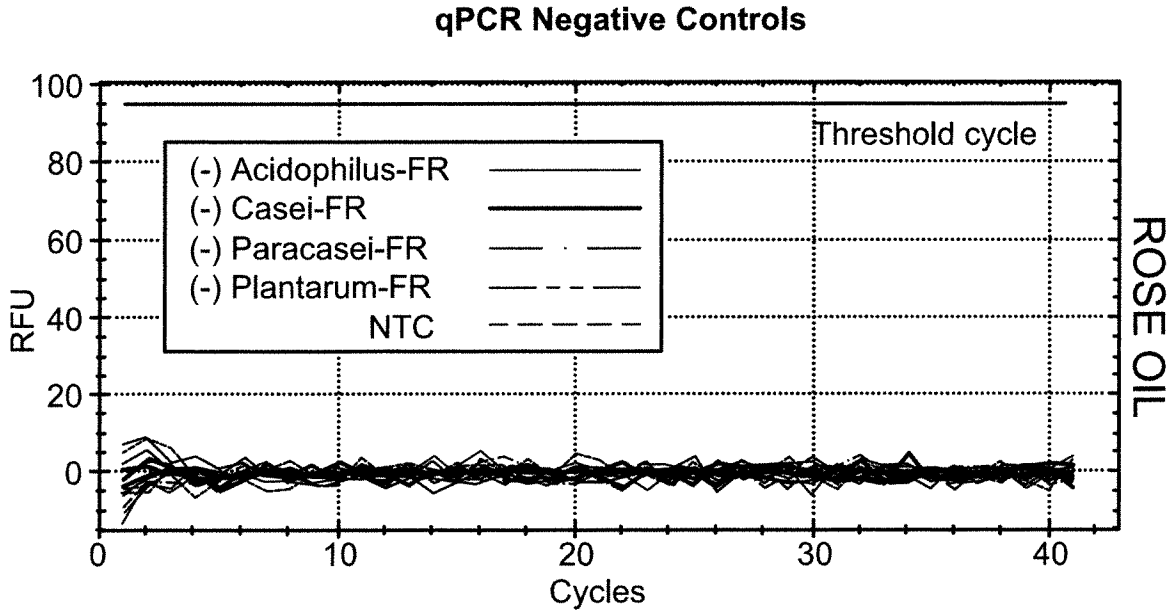


FIG. 13A

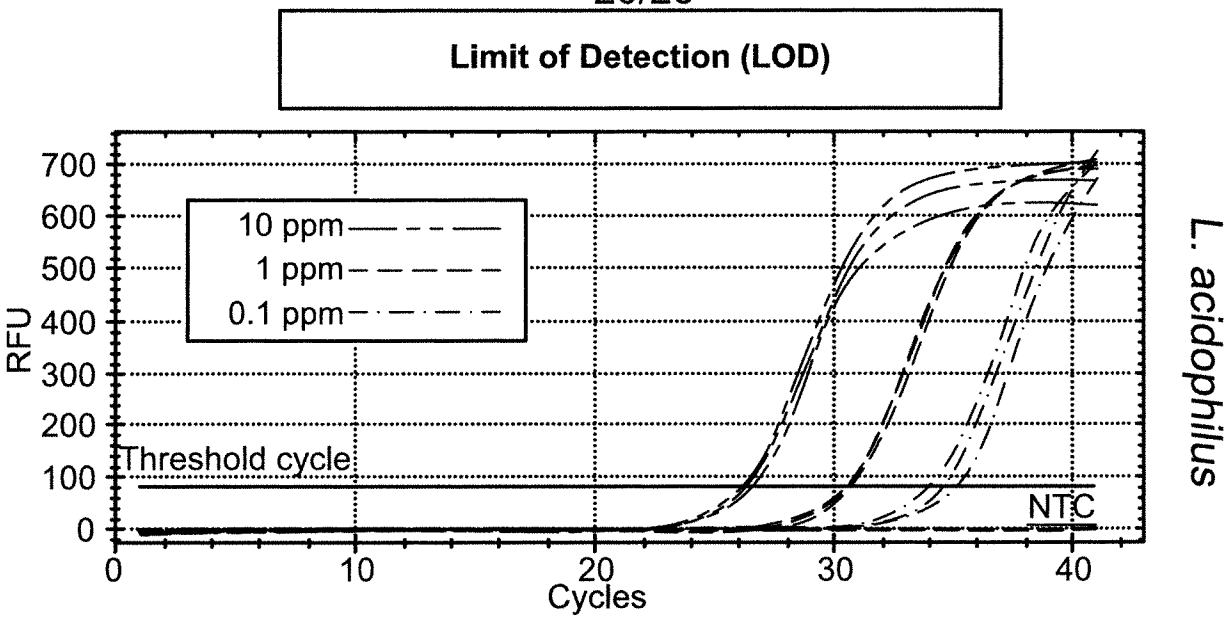


FIG. 13B

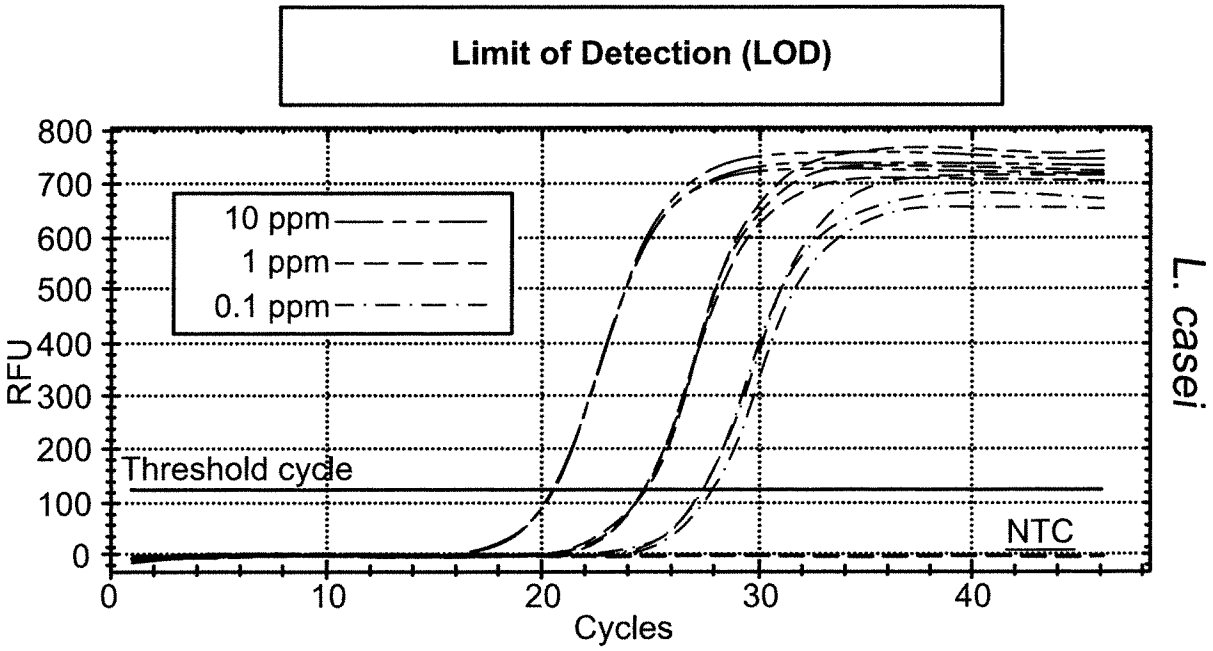


FIG. 13C

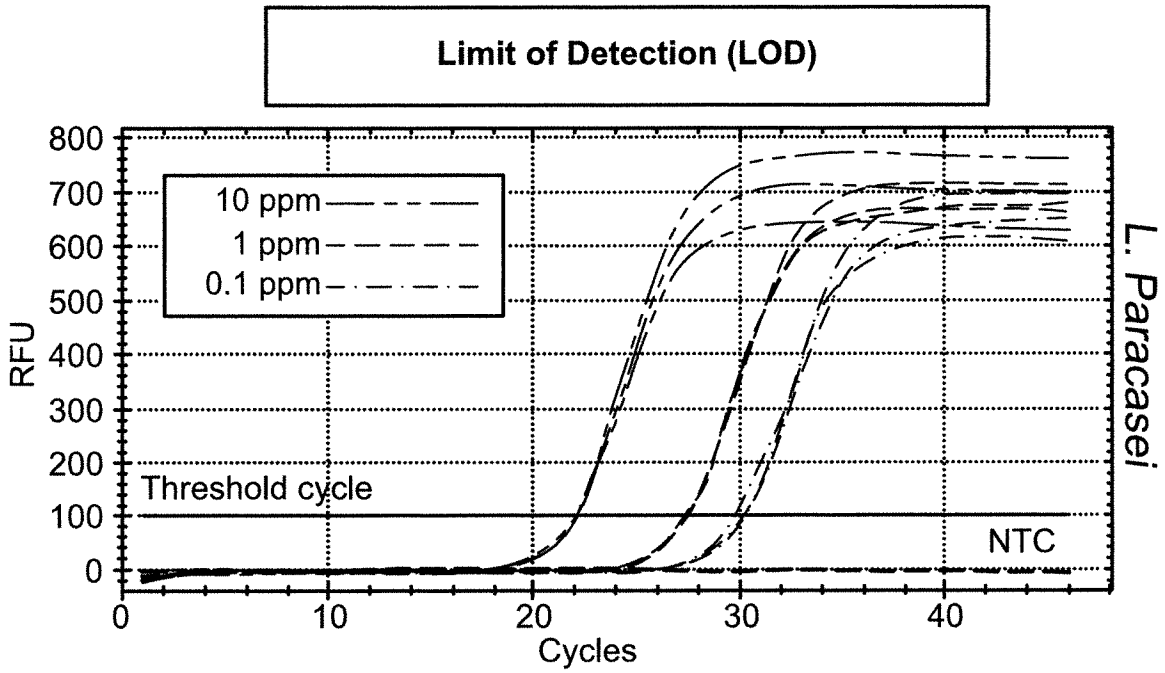


FIG. 13D

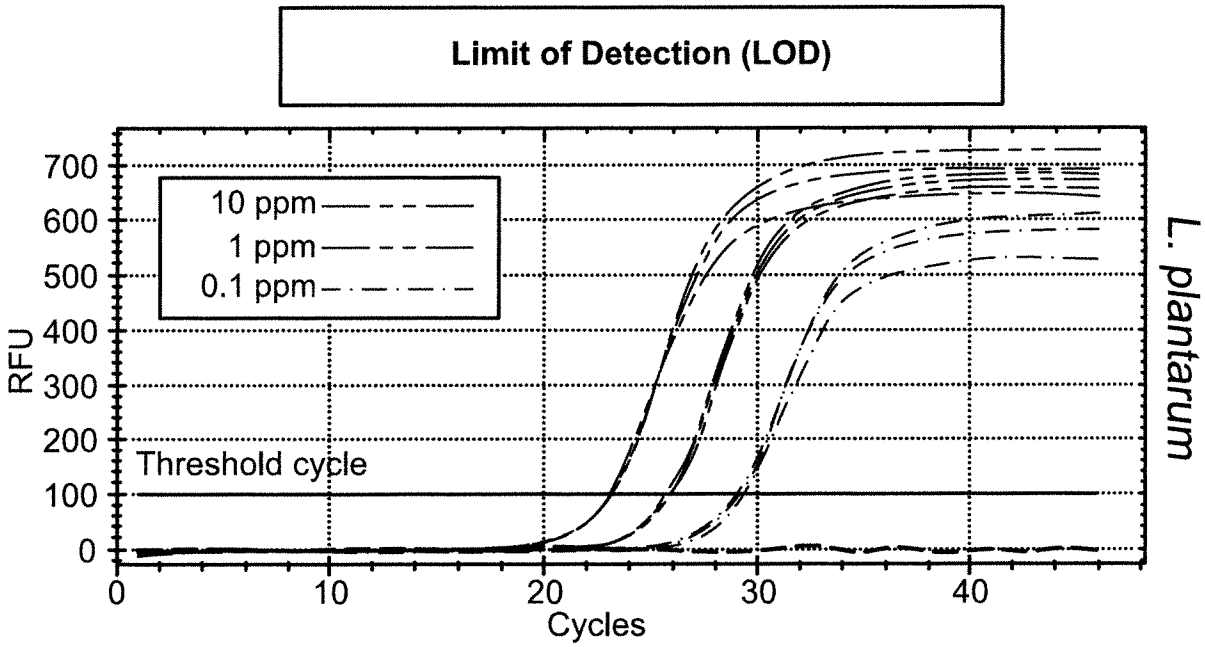


FIG. 13E

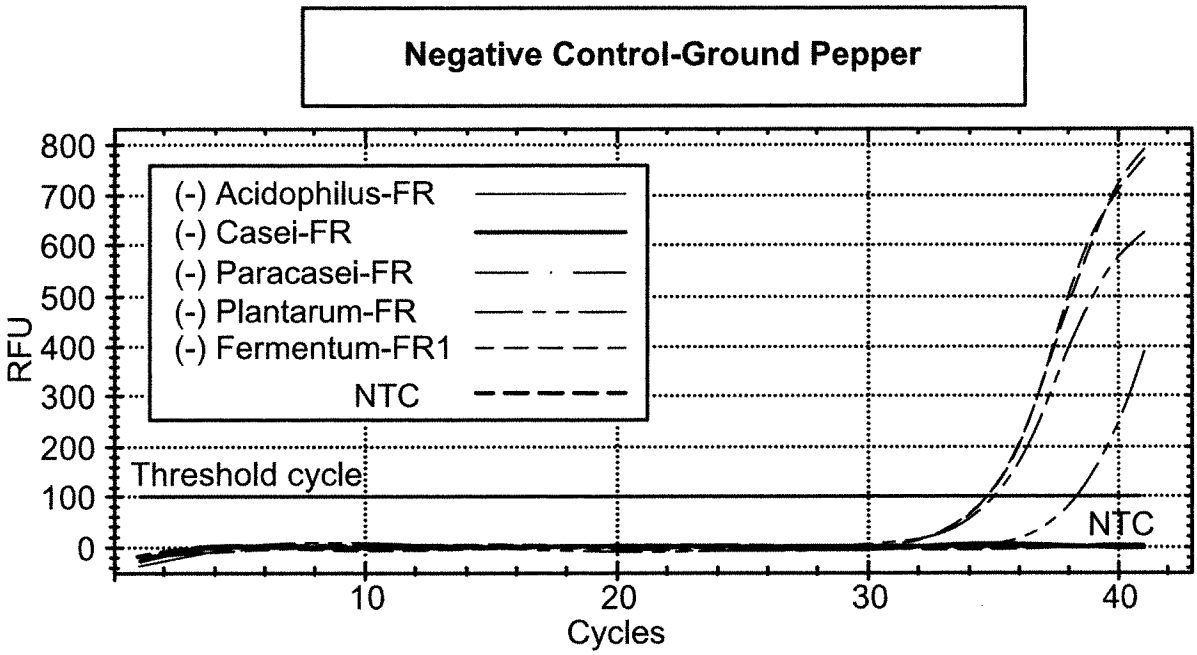


FIG. 14A

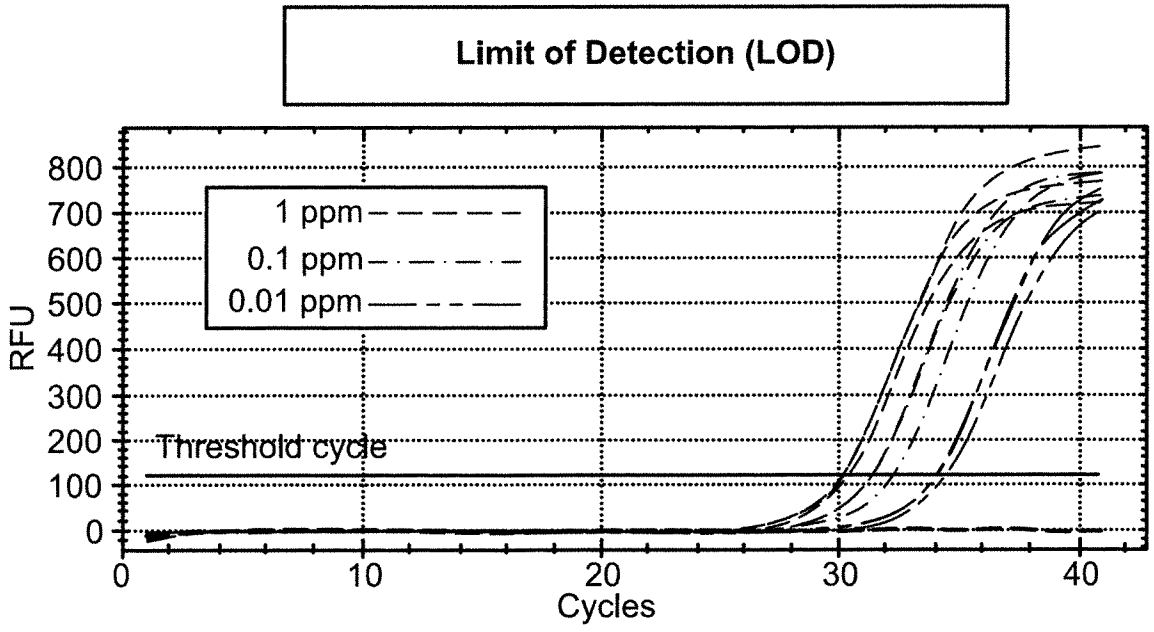


FIG. 14B

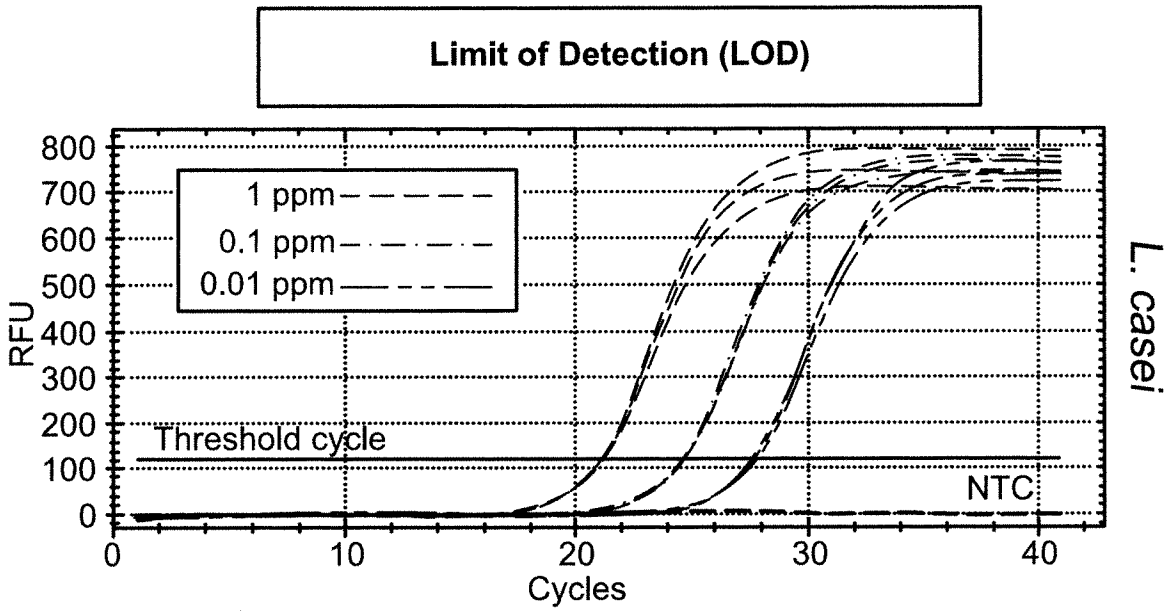


FIG.14C

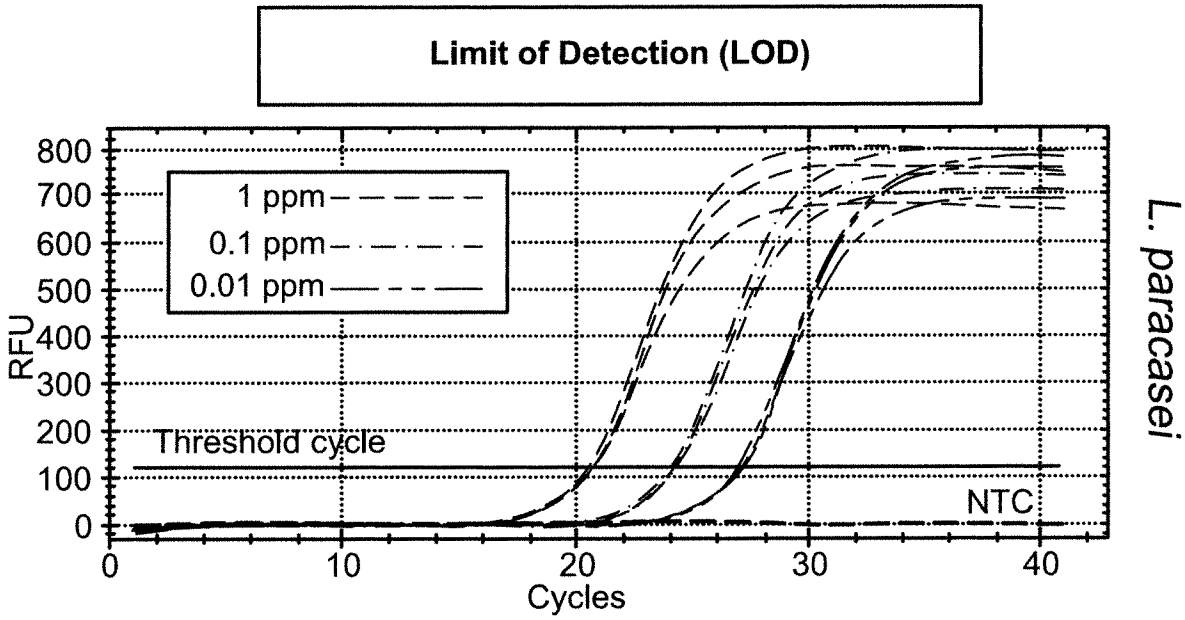


FIG. 14D

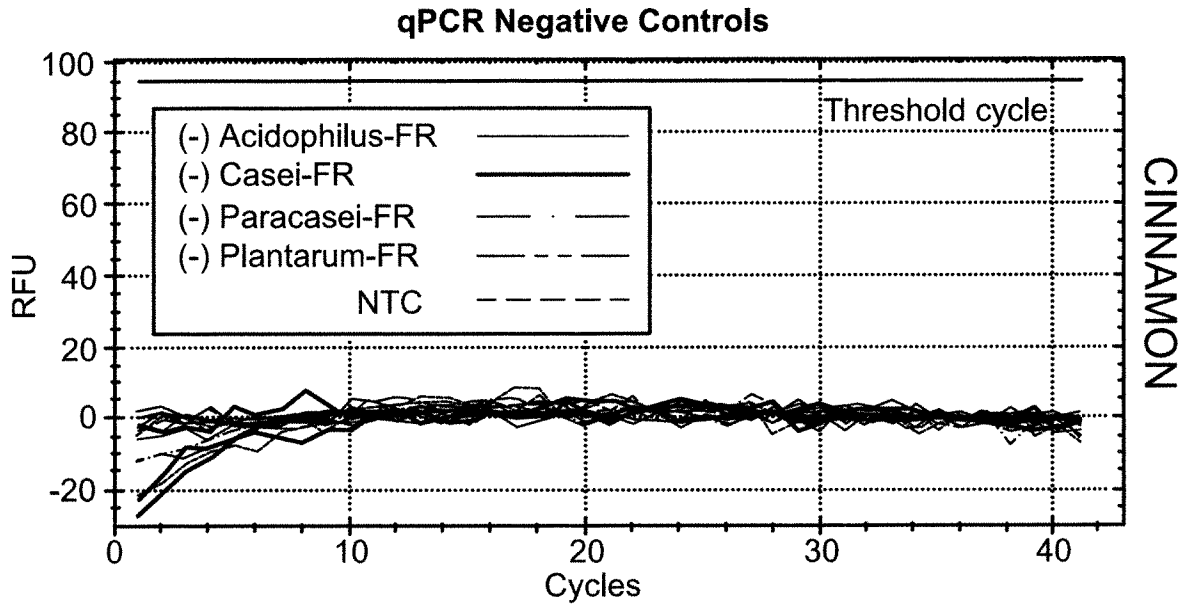


FIG. 15A

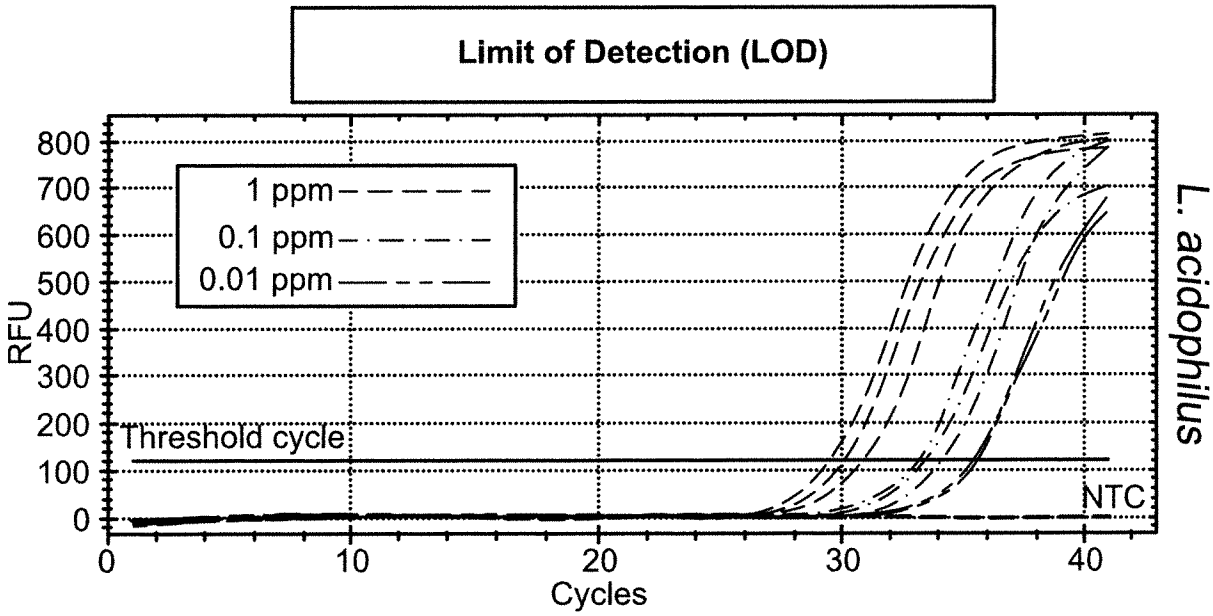


FIG. 15B

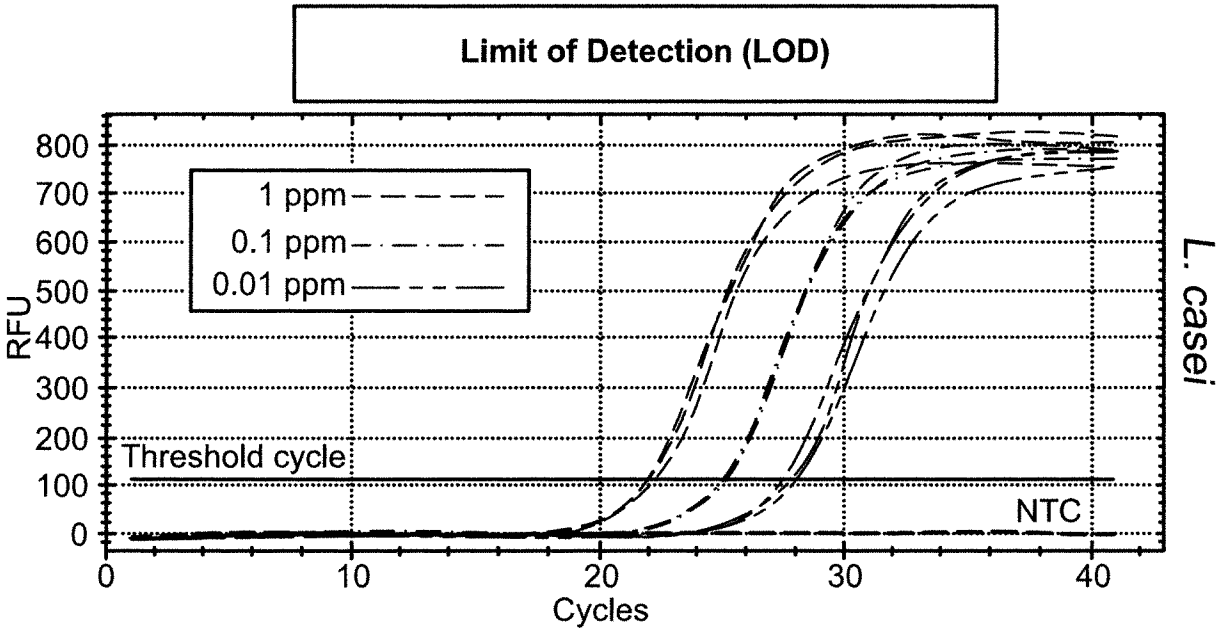


FIG. 15C

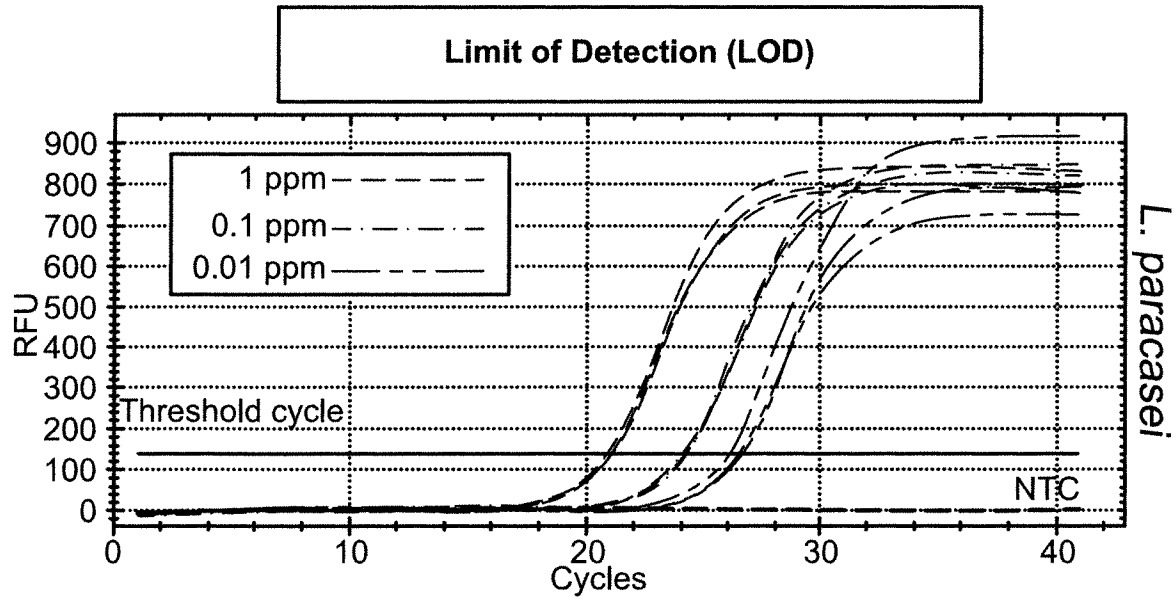


FIG. 15D

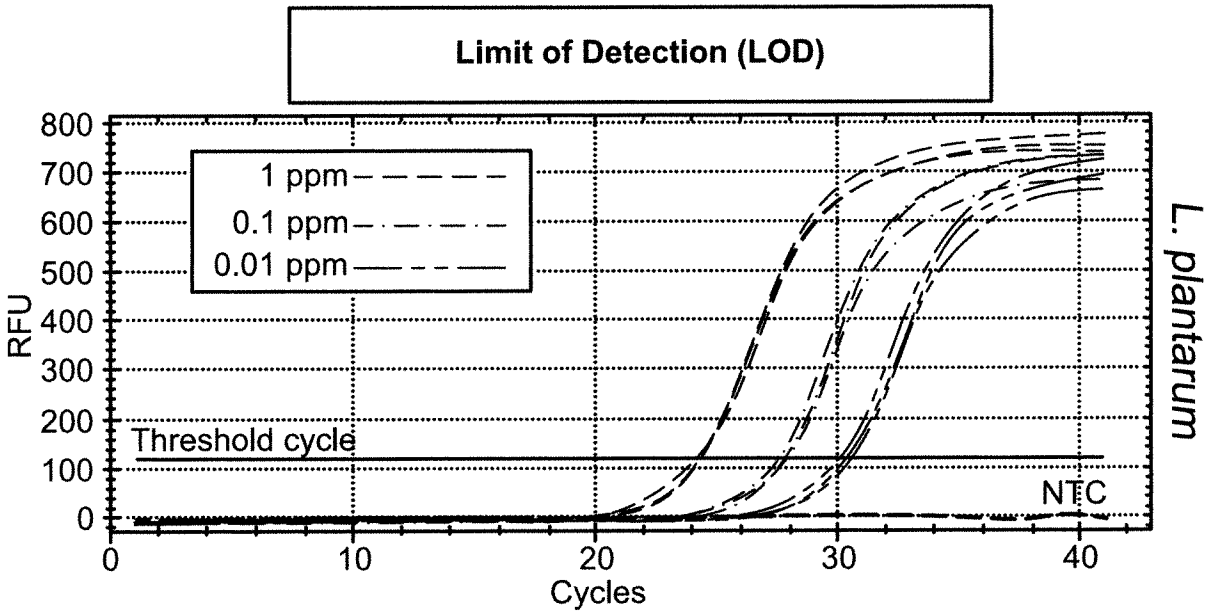

Limit of Detection (LOD)
800
700
600
500
400
300
200
100
0
RFU
1 ppm
0.1 ppm
0.01 ppm
Threshold cycle
NTC
0
10
20
30
40
Cycles
L. plantarum

FIG. 15E