Additional Material 1

We used the Roche Cobas 8000's supporting reagent-CEA to detect the CEA concentration in the fecal supernatant, and compared it to the performance standard of serum CEA. The verified experimental protocol, data and conclusions are as follows:

1. Lower limits of detection

A quantitative fecal supernatant was added to the buffer solution (The buffer is a registered trademark and provided by the Guangzhou Forreal Biotechnology Co., Ltd.) in order to identify the CEA concentration. Therefore, we utilized the blank limit (LOB) to validate the measurement lower limit. LOB is estimated by measuring the duplication of a blank sample, and then calculating the average result and standard deviation (SD). We repeated the measurement 20 times with the buffer solution as the sample. The value of LOB is calculated according to calculation method in EP17 of CLSI (LOB = mean (blank) + 1.645SD $_{blank}$).

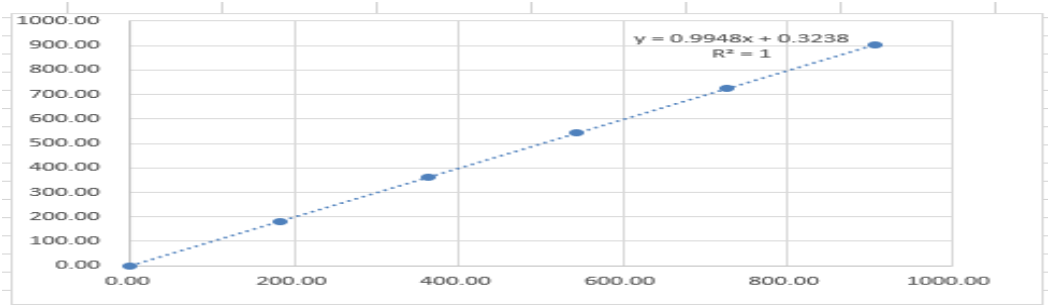
<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3
<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3

Conclusion: We concluded that the lower detection limit of the fecal supernatant was 0.30 ng/ml and verified the serum detection limit declared by the manufacturer.

2. Linearity

The linearity of the FCEA was validated according to CLSI guideline EP6-A. For each analyte, we generated five equally-spaced concentrations of samples. Next, three intermediate concentration pools were prepared by mixing high (H) and low (L) level patient fecal supernatant as follows, including 5L, 4L + 1H, 3L + 2H, 2L + 3H and 5H.The results of the linearity evaluation of the five concentrations of fecal supernatant prepared by mixing supernatant at high and low concentrations are shown in next Table.

dilution rate	theoretical value	Result 1	Result 2	mean value	bias %	Recovery %
Low	0.00	0.30	0.30	0.30	0.00%	100.30
4L:1H	181.40	180.00	180.00	180.00	-0.77%	98.60
3L:2H	362.80	354.00	366.00	360.00	-0.77%	97.20
2L:3H	544.20	543.00	546.00	544.50	0.06%	100.30
1L:4H	725.60	719.00	728.00	723.50	-0.29%	97.90
High	907.00	907.00	894.00	900.50	-0.72%	93.50



We utilized GraphPad for the statistics, took the theoretical concentration as the horizontal axis, and the measured mean value as the vertical axis in order to make the difference graph. Thus, we obtained the regression equation: $y = 0.9948x + 0.3238$, $R^2 = 1$. The 95% confidence intervals for slope and intercept are (0.9874-1.002) and (-3.729 to 4.377), respectively.

Evaluation criterion: R2 > 0.995, Recovery within ± 10 % of initial value (Provided by Roche).

Conclusion: R2 > 0.995, Recovery %< (± 10 %), the linear verification is passed. Linear range of verification: 0.3 - 907 ng/ml.

3. Clinically reportable range verification

We took a high-value stool sample, which was close to the maximum linear range, and serially diluted it with a buffer solution. The dilution factor includes a maximum recommended dilution factor of Roche's serum CEA. We repeated the measurement twice for each concentration. We statistically analyzed the linear relationship of each dilution concentration in order to determine the highest dilution factor.

experimental data and calculation							
dilution factor	measured result			theoretical value	deviation value	acceptable range	evaluation Result
	result 1	result 2	mean				
original value	975	970	972.5	966	0.67%	10.00%	pass
10	106	102.3	104.15	96.6	7.82%		pass
20	54.2	51.3	52.75	48.3	9.21%		pass
30	37	34	35.5	32.2	10.25%		no pass
40	28.8	27.6	28.2	24.15	16.77%		no pass
50	22.5	21	21.75	19.32	12.58%		no pass
Conclusion: The maximum dilution is 20 times.							
The clinical reportable range of this item is 0.3-20000 ng/ml.							

Furthermore, we checked the original data that was utilized in this study. There were no FCEA results diluted more than 10 times, which indicates that the FCEA results were all within the reportable range.

4. Precision evaluation

4.1 In-batch imprecision validation

The mixed fecal supernatant at both high and low levels was repeated 20 times in order to calculate the CV and SD values of in-batch precision.

Result evaluation: we took the 1/4 TEA that was required by CLIA'88 as target, and calculated it based on TEA of Roche's serum CEA as 25%. If the CV% of level 1 and level 2 were both less than 6.25%, then the verification would pass.

	Level 1 of FCEA				Level 2 of FCEA			
Run times	Run 1	Run 2	Run 3	Run 4	Run 1	Run 2	Run 3	Run 4
Rep 1	154.00	157.00	154.00	156.00	628.00	631.00	630.00	631.00
Rep 2	157.00	156.00	154.00	152.00	622.00	613.00	628.00	630.00

Rep 3	154.00	152.00	152.00	150.00	606.00	622.00	606.00	620.00
Rep 4	150.00	152.00	157.00	154.00	600.00	602.00	607.00	608.00
Rep 5	154.00	157.00	157.00	152.00	606.00	613.00	607.00	615.00
mean	154.05				616.25			
SD	2.35				10.79			
CV%	1.5%				1.8%			

Conclusion: The CV% of FCEA results of level 1 and level 2 were both less than 6.25%, and the verification passed.

4.2 Verification of imprecision between batches

The same individual used the same batch of CEA reagents on the same Roche instrument in order to determine the CEA results of the high and low value mixed fecal supernatants, thus reducing variability. For 20 consecutive days, a total of 40 test results were obtained. From them, the mean, standard deviation and coefficient of variation was calculated.

Result evaluation: we took the 1/3TEA required by CLIA’88 as the target, and calculated it based on the TEA of Roche's serum CEA as 25%. If the CV% of level 1 and level 2 were both less than 8.33%, then verification would pass.

	day1	day2	day3	day4	day5	day6	day7	day8	day9	day10
Level 1	154.00	157.00	154.00	150.00	154.00	157.00	156.00	152.00	152.00	159.00
Level 2	628.00	622.00	622.00	620.00	615.00	631.00	613.00	606.00	607.00	595.00
	day11	day12	day13	day14	day15	day16	day17	day18	day19	day20
Level 1	154.00	157.00	153.00	150.00	150.00	158.00	158.00	154.00	153.00	146.00
Level 2	628.00	612.00	620.00	615.00	620.00	630.00	620.00	606.00	592.00	598.00

	Level 1 of FCEA	Level 2 of FCEA
mean	153.90	615.00
SD	3.32	11.41
CV	2.2%	1.9%

Conclusion: The CV% of FCEA results of level 1 and level 2 were both less than 8.33%, and the verification passed.