Supporting Information

Raman Sensor Assisted by Cascade Signal Amplification for Simultaneous Detection of Two Extracellular Antibiotic Resistance Genes

Xinli Shi[a], Qi Wang[c], Yan Zhang[a], Chunping Ge[a], Yujun Sun[a], Yuqi Zhang[a], Mengmeng Li[a], Xiao Gong[d], Xinyue Song[*, b], Shusheng Zhang[*,b,e]

[a]College of Chemistry and Chemical Engineering, Linyi University, Linyi 276005, P. R. China.

[b]Shandong Provincial Key Laboratory of Tumor Imaging Equipment Development and Tumor Diagnosis & Treatment Integration Technology, School of Medicine, Linyi University, Linyi 276005, Shandong, People's Republic of China

[c]College of Chemistry and Chemical Engineering, Shandong University, Jinan 250100, P. R. China.

[d]Senior Veterinarian, Livestock Development Promotion Center of Pingyi County

[e]State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, China.

[*]Corresponding Author Email: *pygongxiao@163.com (X. Gong); songxinyue428@163.com (X. Y. Song) ; Shushzhang@126.com (S. S Zhang)*

Contents

1. Apparatus

The general morphology of the products was analyzed using transmission electron microscopy (TEM, JEOL JEM-2100). Ultraviolet-visible (UV-vis) absorption spectra were obtained with a spectrophotometer (Thermo Nanodrop 2000).

Table S1: Sequences of the used DNA in this experiment.

Name	Sequences (5'-3')
DNA1	Bio-TCAACCAAGTCAT-P
DNA2	Bio-GGCCAGATCACCG-P
H1	TCTGAGAATAGTGACCAGCTGAGGTTCCCCCCATGTGTAGA TGGGGGAACC
H2	CGATATCGTTGGTCTGAGCTGAGGTTCCCCCCATGTGTAGA TGGGGGAACC
bla-TEM	CACTATTCTCAGAATGACTTGGTTGA-P
bla-CTX-M-1	ACCAACGATATCGCGGTGATCTGGCC-P
H3	Bio-T8-TCTGAGAATAGTGACCAGCTGAGGGTCCTGGTCACTAGGG GGCCGC
H4	CGATATCGTTGGTCTGAGCTGAGGACTCTCAGACCAAGCG GCCCCC
Capture DNA3	SH-T8-TAGTGACCAGG
Capture DNA4	SH-T8-TTGGTCTGAGAG
ROX-DNA5	ROX-TTTTTTCCTAGCGAC-SH
Cy3-DNA6	Cy3-TTTTTTCCTAGCGAC-SH
1-mis (vs. *bla-TEM*)	CACTATTCTGAGAATGACTTGGTTGA
3-mis (vs. *bla-TEM*)	ACCAACGATTAGGCGGTGATCTGGCC
w-mis (vs. *bla-TEM*)	ACAGCTGGGAGACGAAACCTGCGTAA
bla-TEM forward primer	TTGCTCACCCAGAAACGC
bla-TEM reverse primer	CGACCGAGTTGCTCTTGC
1-mis (vs. *bla-CTX-M-1*)	ACCAACGATTTCGCGGTGATCTGGCC
3-mis (vs. *bla-CTX-M-1*)	ACCAACGATTAGGCGGTGATCTGGCC
w-mis (vs. *bla-CTX-M-1*)	ACAGCTGGGAGACGAAACCTGCGTAA
bla-CTX-M-1 forward primer	AGGAAGACTGGGTGTGGCA
bla-CTX-M-1 reverse primer	CAGATTCGGTTCGCTTTCAC

2. Materials and Chemicals

$HAuCl_4 \cdot 4H_2O$, trisodium citrate dihydrate, and sodium acetate were sourced

from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The streptavidin-modified magnetic beads (MBs) were acquired from Beyotime (Shanghai, China). To identify typical penicillin resistance genes, *bla*$_{-TEM}$ and *bla*$_{-CTX-M-1}$ sequences were retrieved from the National Center for Biotechnology Information (NCBI) database using the Basic Local Alignment Search Tool (BLAST) and subsequently synthesized by Sangon Biotechnology Ltd (Shanghai, China). Oligonucleotides were also ordered from Sangon Biotechnology Ltd (Shanghai, China), and their sequences of oligonucleotides are presented in Table S1. Klenow Fragment (3'→5' exo-), Nt.BbvCI and deoxynucleotide solution mix (dNTPs) were produced from New England Biolabs. All reagents were of analytical grade and did not require further purification. All solutions were prepared using deionized water (>18.25 MΩ).

Table S2: Information for environmental samples.

Sample number	Matrix	Location		Total DNA (ng/μL extract)	*bla*$_{-TEM}$ copies (copies/μL extract)	*bla*$_{-CTX-M-1}$ copies (copies/μL extract)
		N	E			
S1	Water from Sewage plant inlet	34.94	118.37	29.05	1.4×10^{5}	2.1×10^{4}
S2	Water from Sewage plant outlet	34.94	118.37	69.95	6.5×10^{2}	2.4×10^{4}
S3	Water from a pond in Sewage plant	34.94	118.37	29.7	5.6×10^{2}	1.8×10^{4}
S4	Water from Yi River	35.07	118.22	48.85	7.1×10^{1}	2.1×10^{4}
S5	Untreated water from hospital	35.11	118.40	109	1.9×10^{5}	1.4×10^{5}
S6	Treated water from hospital	35.11	118.40	68.25	1.2×10^{5}	8.3×10^{4}
S7	Water from a small pond in Linyi University	35.06	118.16	5.6	4.0×10^{3}	1.9×10^{4}

3. Polyacrylamide gel-electrophoresis (PAGE) analysis

In this work, we employed polyacrylamide gel electrophoresis to validate the DNA cascade cycling process. For the gel-electrophoresis analysis, a mixture containing 10 μL of the sample solution and 2 μL of loading buffer (6×) was loaded into 10% of polyacrylamide gel and run in 1× TBE buffer at 100 V for 120 min. After staining with the Super Red at 37°C for 30 minutes, the gel was imaged using a Bio-Rad imaging system. The lanes in the PAGE analysis are as follows in Figure S1a. Lane 1: trigger DNA1 (2 μM); Lane 2: H3 (1 μM); lane 3: Capture DNA3 (2 μM); lane 4: trigger DNA1 (1 μM) + H3 (1 μM); lane 5: H3 (1 μM) + Capture DNA3 (1 μM) ; lane 6: trigger DNA1 (1 μM) + H3 (1 μM)+ Capture DNA3 (1 μM) ; lane 7: trigger DNA1 (1 μM) + H3 (1 μM)+ Capture DNA3 (1 μM)+ trigger DNA2 (1 μM) + H4 (1 μM)+ Capture DNA4 (1 μM); lane 8: trigger DNA1 (1 μM) + H3 (1 μM)+ Capture DNA3 (1 μM)+ trigger DNA2 (1 μM) + H4 (1 μM)+ Capture DNA4 (1 μM) + Kf+Nt. The lanes in the PAGE analysis (Figure S1b) were as follows. Lane 1: trigger DNA2 (2 μM); Lane 2: H4 (1 μM); lane 3: Capture DNA4 (2 μM); lane 4: trigger DNA2 (1 μM) + H4 (1 μM); lane 5: H4 (1 μM) + Capture DNA4 (1 μM); lane 6: trigger DNA2 (1 μM) + H4 (1 μM)+ Capture DNA4 (1 μM); lane 7: trigger DNA1 (1 μM) + H3 (1 μM)+ Capture DNA3 (1 μM)+ trigger DNA2 (1 μM) + H4 (1 μM)+ Capture DNA4 (1 μM); lane 8: trigger DNA1 (1 μM) + H3 (1 μM)+ Capture DNA3 (1 μM)+ trigger DNA2 (1 μM) + H4 (1 μM)+ Capture DNA4 (1 μM) + Kf+Nt.

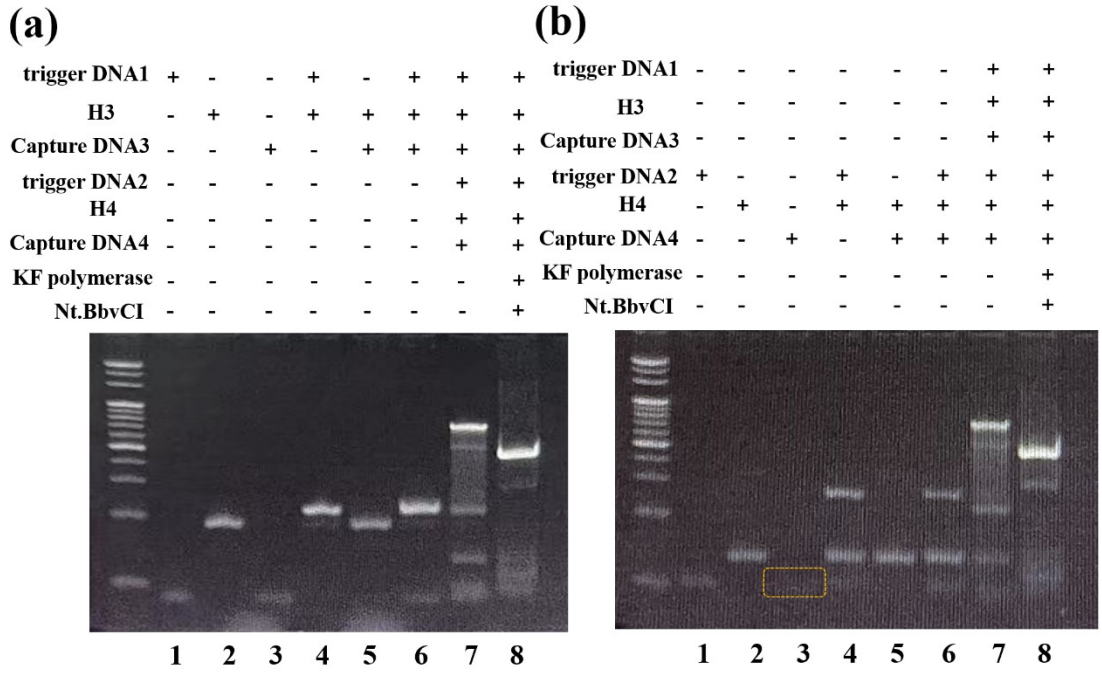

Figure S1: (a) PAGE analysis for cycle 3; (b) PAGE analysis for cycle 4.

4. Grain-size diagram and optimization of the incubation time

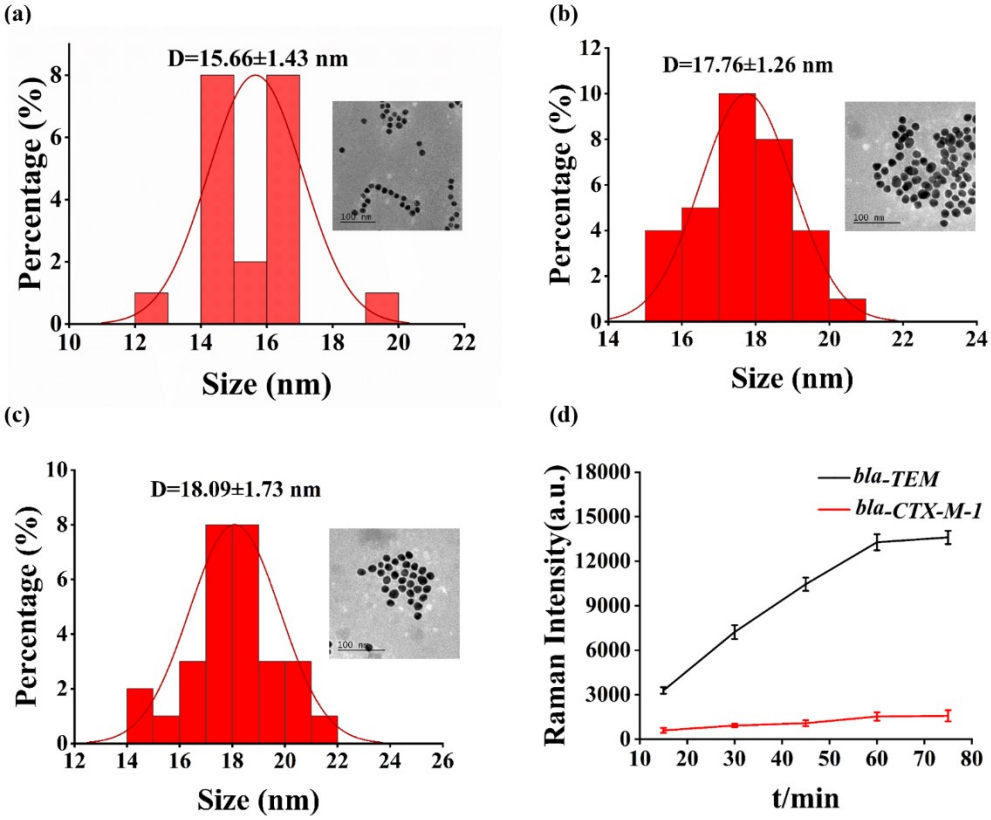

Figure S2: (a) particle size distribution of the synthesized AuNPs (inset: TEM image of the synthesized AuNPs); (b) particle size distribution of SERS tag-1 (inset: TEM image of the obtained SERS tag-1); (c) particle size distribution of SERS tag-2 (inset: TEM image of the obtained SERS tag-2); (d) effects of incubation time on the SERS intensity response to 0.5 aM *bla*$_{-TEM}$ and *bla*$_{-CTX-M-1}$.

5. Accuracy

Table S3: Spiked recoveries of *bla*$_{-TEM.}$

	Normalized Raman Intensity	Concentration	Recovery/%	Average Recovery/%	RSD/%
2 zM	3357.25	1.93 zM	96.50	106.88	7.37
	3503.25	2.10 zM	105.00		
	3628.25	2.26 zM	113.00		
	3631.25	2.26 zM	113.00		
0.2 aM	11490.25	0.20 aM	100.00	106.25	4.51
	11606.25	0.21 aM	105.00		
	11678.25	0.22 aM	110.00		
	11684.25	0.22 aM	110.00		

20 aM	19605.25	20.06 aM	100.30	103.12	6.00
	19530.25	19.22 aM	96.11		
	19699.25	21.16 aM	105.81		
	19771.25	22.05 aM	110.24		

Table S4: Spiked recoveries of *bla*$_{CTX-M-1}$.

	Normalized Raman Intensity	Concentration	Recovery/%	Average Recovery/%	RSD/%
2 zM	622.75	1.88 zM	94.00	104.38	8.76
	631.75	2.00 zM	100.00		
	650.75	2.29 zM	114.50		
	643.75	2.18 zM	109.00		
0.2 aM	1288.75	0.22 aM	110.00	103.75	9.94
	1253.75	0.19 aM	95.00		
	1266.75	0.19 aM	95.00		
	1292.75	0.23 aM	115.00		
20 aM	1937.75	23.44 aM	117.19	106.38	9.81
	1915.75	20.02 aM	100.09		
	1908.75	19.04 aM	95.18		
	1942.75	22.61 aM	113.07		

6. Linear relationship diagram for *bla*$_{CTX-M-1}$

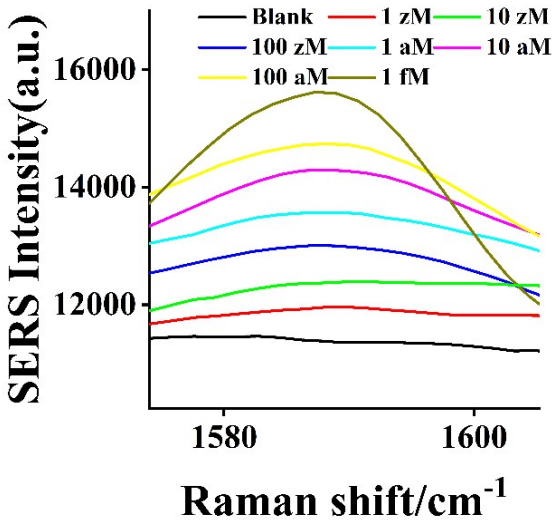

Figure S3: Localized enlargement at 1588 cm^{-1} in Figure 3b.

7. Interference Study

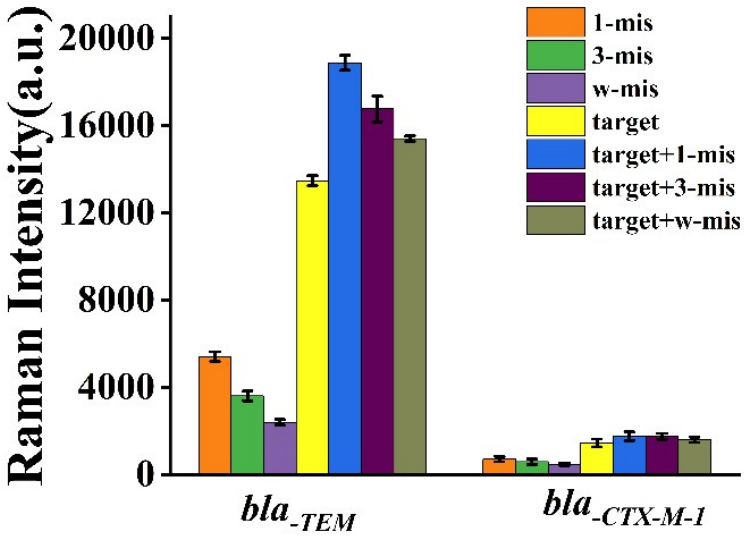

Figure S4: SERS sensor specific Raman intensities for the detection of the target substances (*bla-TEM* and *bla-CTX-M-1)* and similar compounds.

8. The *bla-TEM* and *bla-CTX-M-1* sequences alignments of the environmental samples

Table S5: The *bla-TEM* sequences alignments of the environmental samples.

The partial sequence of *bla-TEM* from the NCBI database:
CGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGG**C**GCG
GTATTATCCCGT**G**TTGACGCCGGGCAAGAGCAACTCGGTCG
S1：
CGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGTGCGG
TATTATCCCGTGTTGACGCCGGGCAAGAGCAACTCGGTCG
S2:
CGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCG
GTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCG
S3:
CGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCG
GTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCG
S4:
CGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCG
GTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCG

S5:

CGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGTGCGG

TATTATCCCGTGTTGACGCCGGGCAAGAGCAACTCGGTCG

S6:

CGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGTGCGG

TATTATCCCGTGTTGACGCCGGGCAAGAGCAACTCGGTCG

S7:

CGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCG

GTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCG

Table S6: The *bla*$_{-CTX-M-1}$ sequences alignments of the environmental samples.

The partial sequence of *bla*$_{-CTX-M-1}$ from the NCBI database:

GTTGTGGGGGATAAAACCGGCAGCGGTGACTATGGCACCACCAACGATATCGCGGTG

ATCTGGCCAAAAGATCGTGCGCCGCTGATTCTGGTCACTTA

S1：

GTTGTGGGGGATAAAACCGGCAGCGGTGACTATGGCACCACCAACGATATCGCGGTG

ATCTGGCCAAAGATCGTGCGCCGCTGATTCTGGTCACTTA

S2:

GTTGTGGGGGATAAAACCGGCAGCGGTGACTATGGCACCACCAACGATATCGCGGTG

ATCTGGCCAAAAGATCGTGCGCCGCTGATTCTGGTCACTTA

S3:

GTTGTGGGGGATAAAACCGGCAGCGGTGACTATGGCACCACCAACGATATCGCGGTG

ATCTGGCCAAAAGATCGTGCGCCGCTGATTCTGGTCACTTA

S4:

GTTGTGGGGGATAAAACCGGCAGCGGTGACTATGGCACCACCAACGATATCGCGGTG

ATCTGGCCAAAAGATCGTGCGCCGCTGATTCTGGTCACTTA

S5:

GTTGTGGGGGATAAAACCGGCAGCGGTGGCTATGGCACCACCAACGATATCGCGGTG

ATCTGGCCAAAAGATCGTGCGCCGCTGATTCTGGTCACTTA

S6:

GTTGTGGGGGATAAAACCGGCAGCGGTGGCTATGGCACCACCAACGATATCGCGGTG

ATCTGGCCAAAAGATCGTGCGCCGCTGATTCTGGTCACTTA

S7:

GTTGTGGGGGATAAAACCGGCAGCGGTGACTATGGCACCACCAACGATATCGCGGTG

ATCTGGCCAAAAGATCGTGCGCCGCTGATTCTGGTCACTTA

9. PCR analysis for the extracted *bla*$_{-TEM}$ and *bla*$_{-CTX-M-1}$

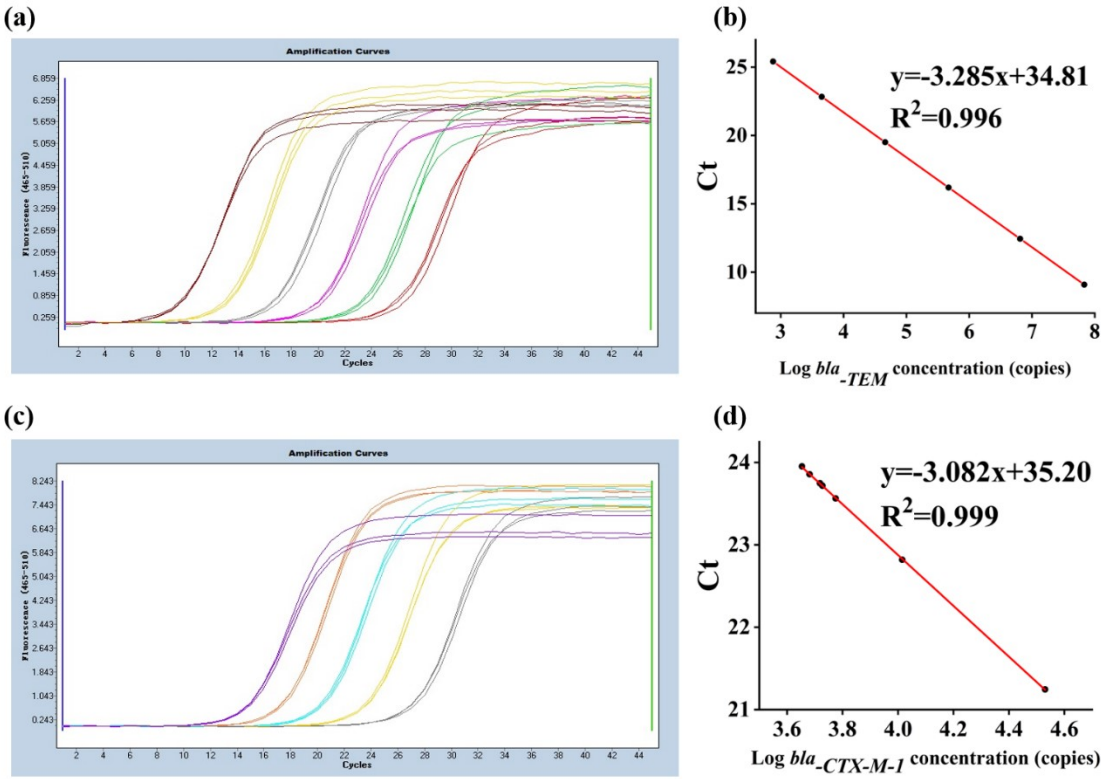

Figure S5: Amplification curve of *bla*$_{-TEM}$ (a), *bla*$_{-CTX-M-1}$ (b) in qRT-PCR; Standard curve of *bla*$_{-TEM}$ (c), *bla*$_{-CTX-M-1}$ (d) obtained by qRT-PCR.

10. Calculation of the limit of detection (LOD)

The LOD was calculated based on the below equation [1]:

$$x_{LOD} = \frac{s_b \times t_{a,n-1} - b}{m} \quad (1)$$

$$\text{LOD} = 10^{xLOD} \quad (2)$$

Where s_b is the standard deviation of the blank sample, $t_{a,n-1}$ is the α-quantile of Student's t-function with n-1 degrees of the freedom (n represents for the number of

measurement), *b* represents for the obtained intercept and *m* represents for the slope of the calibration curve.

References

[1] a) S. Lu, J. Du, Z. Sun, C. Jing, *Anal. Chem.* **2020**, 92, 16229-16235; b) G. L. a. J. D. W. Long, *Anal. Chem.* **1983**, 55, 993-792; c) H.-P. Loock, P. D. Wentzell, *Sens. Actuators B Chem.* **2012**, 173, 157-163.