Supplementary material

**Rapid One-step Enzyme Immunoassay and Lateral Flow Immunochromatographic Assay for Colistin in Animal Feed and Food**

Jiayi Wang 1, Jinyu Zhou 1, Yiqiang Chen 1, \*, Xinpei Zhang 1, Yongpeng Jin 1, Xiaoqian Cui 1, Dongting He 1, Wenqing Lai 1, Lidong He 2

1*Beijing Advanced Innovation Center for Food Nutrition and Human Health, and State Key Laboratory of Animal Nutrition, College of Animal Science and Technology, China Agricultural University, Beijing, China*

2 *Department of Chemistry and Biochemistry, Florida State University, Tallahassee, FL, USA*

**\* Corresponding author:**

Yiqiang Chen, Ph. D, Associate Professor

Tel: (86-10)62733764; Fax: (86-10)62733588; E-mail: yqchen@cau.edu.cn



**Fig. S1** Schemes of one-step indirect ELISA, two-step indirect ELISA and one-step direct ELISA for colistin.



**Fig. S2** Ultraviolet-visible spectrum of four GNPs solutions synthesized by reducing chlorauric acid with 1.0, 1.6, 2.0 and 2.5 mL of citrate solution.

1. **A**
2. **B**
3. **C**
4. **D**

**Fig. S3** TEM images of four GNPs solutions synthesized by reducing chlorauric acid with 1.0 mL (A), 1.6 mL (B), 2.0 mL (C) and 2.5 mL (D) of citrate solution.

**Table S1** The IC50 values of all sera from mice immunized with two different dosages.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Group 1** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** |
| IC50 values (ng/mL) | 82 | 111 | 93 | 77 | 68 | 85 | 116 | 133 | 121 | 89 |
| **Group 2** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** |
| IC50 values (ng/mL) | 124 | 149 | 88 | 95 | 101 | 79 | 76 | 112 | 86 | 92 |

Note: Group 1: Low dosage group, 15 μg per immunization; Group 2: High dosage group, 60 μg per immunization.

**Table S2** Comparison of one step ci-ELISA, two step ci-ELISA and one step cd-ELISA regarding IC50 values and assay time (n=4).

|  |  |  |  |
| --- | --- | --- | --- |
|  | One step ci-ELISA | two step ci-ELISA | one step cd-ELISA |
| IC50 value (ng/mL) | 9.7±0.2 | 9.1±0.1 | 23.7±0.4 |
| Assay time | ~60 min | ~110 min | ~60 min |

**Table S3** Comparison of the developed ELISA method with that literature reported enzyme immunoassay.

|  |  |  |  |
| --- | --- | --- | --- |
| Limit of detection | Assay accuracy | Assay precision | Literature |
| 30 ng/mL in buffer  | --- | --- | Kitagawa et al., 1985 |
| 10.9 μg/kg in milk | 116-146% | 10.8-56.2% | Suhren and Knappstein, 2005 |
| 5.9 μg/kg in milk | 87.44-110.13%, | 8.91-10.78% | This study  |

**Table S4** Detection results of colistin in actual animal feed by ELISA, LFIA and HPLC method.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Sample No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Sample type | Swine feed | Swine feed | Swine feed | Swine feed | Swine feed | Swine feed | Chicken feed | Chicken feed | Chicken feed | Chicken feed |
| ELISA, mg/kg | 24.5 | － | 19.7 | － | 6.7 | － | － | 14.2 | － | － |
| LFIA, mg/kg  | 23.9 | － | 18.4 | － | 7.6 | － | － | 15.4` | － | － |
| LC-MS, mg/kg | 26.3 | － | 20.9 | － | 7.3 | － | － | 14.7 | － | － |
| Sample No. | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| Sample type | Chicken feed | Chicken feed | Milk | Milk | Milk | Milk | Meat | Meat | Meat | Meat |
| ELISA, mg/kg | 10.7 | － | － | － | － | － | － | 0.091 | － | － |
| LFIA, mg/kg | 9.9 | － | － | － | － | － | － | 0.087 | － | － |
| LC-MS, mg/kg | 10.2 | － | － | － | － | － | － | 0.096 | － | － |