

Abstract examples:

**Novel hapten design, antibody recognition mechanism study and a highly sensitive immunoassay for diethylstilbestrol in shrimp**

**新合剂设计、抗体识别机制研究以及对虾中二乙基己烯雌酚的高灵敏度免疫分析方法**

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**Abstract**

Over the past few years, there has been a lack of progress in the quality of diethylstilbestrol (DES) antibodies used in immunoassay. In this study, a new immunizing hapten was designed for remarkably sensitive and specific antibody generation against diethylstilbestrol. By introducing a benzene ring instead of the traditional linear chain alkane as the hapten spacer, a more specific immune reaction was induced in antibody production. The developed polyclonal antibodies were characterized using an indirect competitive enzyme-linked immunosorbent assay (icELISA). Under optimized conditions, the half maximal inhibitory concentration ( $IC_{50}$ ) of the best polyclonal antibody was 0.14 ng/mL and it displayed low cross-reactions (CRs) with the structural analogs such as hexestrol (HEX) and dienestrol (DI). The molecular modeling and quantum chemical computation revealed that the lowest CR of the DES antibody to DI was mainly due to the huge three-dimensional conformational difference between DES and DI. Finally, a highly sensitive icELISA method based on the polyclonal antibody was developed for the determination of DES in shrimp tissue. The limit of detection (LOD) was as low as 0.2  $\mu\text{g}/\text{kg}$  in shrimp and the recovery rates from spiked samples ranged from 83.4% to 90.8% with a coefficient of variation of less than 13.8%. These results indicated that the use of an aromatic ring as the immunizing hapten spacer arm could be a potential strategy for the enhancement of anti-DES antibody sensitivity, and the established icELISA was applicable to DES detection in shrimp.

## **Evaluation of online somatic cell count estimation in automatic milking systems**

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### **Abstract**

Somatic cell count (SCC) is one of the most important and widely used mastitis diagnostics. For detecting subclinical mastitis, online SCC related measurements are more and more used in automatic milking systems (AMS). Sensors such as an automated online California Mastitis Test (O-CMT) allow for high frequency screening of high SCC cows within a herd, which makes it potentially powerful to identify episodes of mastitis. However, the performance of O-CMT measurements, as compared to SCC determined in the laboratory (L-SCC), has only scarcely been described. The aims of this study were to (1) assess the agreement between the O-CMT measurement averaged over different time windows and the corresponding L-SCC measurements; (2) determine the optimal time window for averaging O-CMT as compared to L-SCC; (3) explore the added value of time-series of frequent O-CMT measurements in individual cow udder health monitoring compared to L-SCC measurements. Data were collected from 50 farms in 6 different European countries that were equipped with AMS using O-CMT measurements and also performed regular L-SCC testing. Using Bayesian latent class analysis, we estimated the sensitivity and specificity in detecting high SCC of both measurements, assuming no gold standard. We found that the overall concordance correlation coefficient (CCC) between O-CMT and L-SCC was 0.53 but differed substantially between farms. The CCC between O-CMT and L-SCC improved when averaging O-CMT over multiple milkings, with an optimal time-window of 24 h. Surprisingly, sensitivity and specificity of O-CMT<sub>24h</sub> were both higher than test characteristics of L-SCC in classifying high SCC events. Exploration of time series of daily O-CMT recordings show that this is an effective screening tool to capture episodes of high SCC. Altogether, we conclude that while O-CMT agrees moderately with L-SCC, because of its high measurement frequency, it is a promising on-farm tool for udder health monitoring.

## 四环素类广谱单克隆抗体的制备及其间接竞争 ELISA 检测方法的建立 Preparation of broad-spectrum Anti-Tetracycline monoclonal antibodies and development of an indirect competitive ELISA to detect residues of Tetracyclines

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### 目的

四环素类(Tetracyclines, TCs)药物是畜牧养殖业广泛使用的广谱抗生素之一, 批准用于动物的有四环素(TC)、土霉素(OTC)、金霉素(CTC)和强力霉素(DC)。在食品动物和环境中如果超过残留限量, 不仅损害人体健康, 还会造成耐药菌的传播, 对其进行灵敏、快速检测是控制其不合理使用的重要手段。本研究的目的是基于四环素类公共结构, 设计合成米诺环素(MNC)全抗原, 制备高亲和力、广谱性四环素类单克隆抗体, 并建立可用于检测四环素残留的间接竞争 ELISA 检测方法。

### 方法

以 9 氨基-米诺环素为半抗原, 通过戊二醛法将半抗原分别与钥孔血蓝蛋白(KLH)、牛血清白蛋白(BSA)和鸡卵白蛋白(OVA)进行偶联, 将人工抗原MNC-KLH/BSA免疫雌性Balb/c小鼠, 检测血清效价和抑制, 使用杂交瘤细胞筛选技术获得细胞株, 通过小鼠体内诱生法制备单抗。分别以TC/OTC/CTC/DC/MNC-BSA为包被原, 优化ELISA条件, 建立间接竞争ELISA检测方法。

### 结果

全抗原MNC-BSA经MALDI-TOF鉴定合成成功, MNC-KLH/OVA全抗原经抗体检测鉴定合成成功。小鼠经免疫并筛选, 获得单克隆抗体9F2, 经鉴定该抗体为IgG1( $\kappa$ )型。ELISA优化结果显示, 最佳包被原为DC-BSA, 最佳包被浓度为25 ng/ml, 单克隆抗体最佳工作浓度为30 ng/ml, 最适PH为7.5, 最佳反应缓冲液为10 mM PB, 且可耐受40%甲醇, 抗体对TC、OTC、CTC、DC、MNC、替加环素、山环素、甲氧环素、罗列环素、美他环素、赖甲环素的半数抑制浓度(IC<sub>50</sub>) 在0.16-30.1  $\mu$ g/L之间。

### 结论

成功合成了MNC全抗原, 制备出了高亲和力、广谱性单克隆抗体。建立了快速、灵敏的间接竞争ELISA检测方法, 为检测动物源性食品中四环素类残留奠定了基础, 具有一定的研究价值和应用价值。

**关键词:** 四环素类药物; 广谱识别性; 单克隆抗体; 酶联免疫吸附实验

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