

# Changzhou Live Products Rapid Detection Realized Online Detection through Informatization Means

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### Case Report

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#### Abstract

This paper introduces how to trace the sampling process through mobile phone terminal and use enzyme marker to carry out clenbuterol hydrochloride rapid detection in pig urine and upload the test results in real time based on Changzhou agricultural product supervision geographic information platform.

**Keywords:** Clenbuterol Hydrochloride; Rapid Detection; Operating Procedures

#### **Case Report**

Changzhou agriculture committee has established Changzhou agricultural product supervision geographic information platform through innovative supervision methods. At present, the platform has been put into use in the whole city, which has been widely praised. In order to regulate the quality and safety of livestock products in the city and standardize the sample test procedures, the platform develops operating procedures based on clenbuterol rapid test [1,2].

#### Sampling

Samples of live pigs should be selected to represent the level of the whole batch of products and not to select special individuals. Information related to sampling shall be recorded timely and accurately during sampling. Hence the sample should have been identified by the unit or individual [3-7].

**General Principles of Sampling:** Detailed sampling specifications refer to NY/ t5344.1-2006 sampling specifications for pollution-free food -- part 1: general provisions, NY/T 5344.6-2006 specifications for

pollution-free food products -- part 6: livestock and poultry products, NY/T 763-2004 sampling methods for pork, pork liver, pig urine, etc.

**Sample Collection by Handheld Terminals:** Registration and installation. Download "Changzhou agricultural product quality safety mobile supervision platform" app installation package, and install the app according to the instructions.

- Log in. Use the registered user name above to log in.
- Sample information entry. Enter into" product testing "module, click " product testing "on the screen at the bottom right, fill out sampling agencies, sampling personnel (at least two), sampling area, the tested unit, task source, test category, product category, inspection method, test standard and sample information according to displayed information.
- Sample photographing. Click "sampling photographing" to take photos on site and click "save".
- Signature of examinees. Conduct on-site electronic signature in the "signature of the examinee" column, and then click "OK".
- Uploading and modification of sample information. Click "to be uploaded", three operation buttons of "delete", "edit" and "upload" will appear under the

sample information bar. Click "delete" to delete this sample information, or click "edit" to modify this sample information, or click "upload" to upload this sample information.

#### Detection

Sample Detection: Detection principle. The basis for determination is the antigen antibody response. Monoclonal antibodies can bind to either clenbuterol or enzyme markers. When the sample contained clenbuterol hydrochloride, the binding site on the binding monoclonal was competitive with the enzyme marker, and the enzyme marker that did not bind to monoclonal was washed away after the washing procedure. After adding the matrix and hair colorant, the combined enzyme marker converted the colorless hair colorant into the blue product. The color depth was related to the concentration of clenbuterol in the sample. When the termination solution is added, the blue color changes to yellow, and then the absorbance is measured with an enzyme marker, which is inversely proportional to the concentration of clenbuterol in the sample.

#### **Test Instruments and Reagents**

- Instruments. Enzyme marker, homogenizer, oscillator, centrifuge, and micropipette.
- Reagents.
- Clenbuterol kit (R-biopharm Co, Germany).
- The enzyme marker and sample buffer provided are 4 times the concentrate. Before use, dilute the enzyme marker with 1:4 (1+3) deionized water and sample buffer (such as 25ml concentrate +75ml deionized water). The diluted buffer can be stored at 2-8 degrees for 4 weeks.
- The washing buffer provided is 20 times the concentrate. Dilute the concentrated washing buffer with 1:20 (1+19) with deionized water before use (e.g. 2ml of concentrate +38ml of deionized water, enough for 4 microholes).
- The clenbuterol ligands provided were 100 times concentrated. Due to the poor stability of the dilute enzyme ligands, only the actual amount of the enzyme ligands was diluted. Centrifuge (1 min /1000g) the concentrate before it is absorbed. Dilute concentrated enzyme markers with dilute buffer of enzyme markers at 1:100.

#### **Operation Steps**

- Sample pretreatment. If the urine sample is clear, it can be measured without treatment. If the urine is cloudy, filter until it is clear.
- Sample detection.

- Remove the kit from the refrigerator and place it at room temperature (20-25 °C).
- Insert the hole with sufficient quantity of standard and sample into the micro-hole frame, and make two parallel experiments on standard and sample to record the position of standard and sample.
- Add the standard or treated samples of 25mm L to their micro-pores, and add the solution of enzyme marker diluted by 75mm L to each pore. Gently blending, incubate for 30 minutes in the dark at room temperature (20 to 25 °C).
- Pour out the liquid in the hole and turn the micro hole rack upside down on the absorbent paper to pat (3 times per round) to ensure the complete removal of the liquid in the hole. Flush the hole with 250 mm L washing buffer, pour out the liquid in the micro hole again and repeat the operation twice.

#### 129/5000

- Join 100 mm L substrate/hair color reagent to each of the hole. Gently blending, incubate in the dark ion for 30 minutes at room temperature (20 to 25°C).
- Add 100 mm L reaction termination liquid to the micro-pore. The mixture measures absorbance at 450nm. The air is blank. The absorbance value must be read within 30 minutes after the stop solution is added.
- Read and calculation.
- Read the absorbance value of the sample at the wavelength of 450nm.
- Draw the standard curve by dividing the average value of the standard solution and sample absorbance value obtained by the ratio of blank solution and multiplying by 100. The calculated standard values plot a semi-log coordinate system curve corresponding to clenbuterol concentration, and the correction curve should become linear in the range of 200-2000ng/kg.
- Calculation. The concentration of each sample can be read from the correction curve.
- Judgment. The clenbuterol concentration in the urine sample was positive once the concentration was over 1mmg/kg.

#### **Matters Needing Attention**

- Do not use reagents with different batches. All reagents should be returned to room temperature before using, after use shall be immediately saved back 2-8°C. Since the stability of enzyme markers and antibodies is not well, only the actual dosage can be diluted when used.
- In the process of sample adding and washing, be careful not to let the pipette tip of the pipette touch the mixture in the hole to avoid cross contamination; keep the pores wet during the entire operation.
- During the incubation process at constant temperature,

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the micro orifice plate should be covered to avoid light exposure.

#### **Data Transmission**

After sample testing, login "Changzhou agricultural product quality safety mobile platform" and click to enter product testing - mobile terminal sampling. Click the uploaded sampling record in "the sampling list". Click on the "test", check the "checked unit information", "information detection unit" and "other information", according to test results to perfect the "inspection record", and then click "submit". The "success message" will be displayed after uploading.

#### Conclusion

This experiment ensured the accuracy of the data and made better use of the platform to supervise the quality and safety of livestock products in the city by online sampling of information means and real-time detection of clenbuterol hydrochloride in pig urine.

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