Importance of Clenbuterol Determination

Clenbuterol is a beta-adrenergic agonist used in a variety of ways, mainly as a bronchodilator for those affected with breathing disorders, and as a feed additive to promote leanness in animals used for meat production. When consumed by pigs, clenbuterol enhances protein synthesis resulting in increased muscle fiber size, along with a decrease in the build up of fatty tissue.

The potential risk for human health posed by the presence of β-agonists is high, due to the severity of the possible adverse effects. The β-agonist clenbuterol has been implicated in many poisoning cases in European and Asian countries. Although meat is the most frequently analyzed sample matrix, other matrices, such as water, milk and feed are also routinely analyzed.

While regulations for clenbuterol vary by country, the FAO of the United Nations and the World Heath Organization reconfirmed that the recommended residual limit in meat samples as 10 ppb.

Performance Data

Sensitivity:

Sample	Limit of Detection
Feed	6 ppb
Urine	3 ppb
Meat	3 ppb

General Limited Warranty: Abraxis, Inc. warrants the products manufactured by the Company, against defects and workmanship when used in accordance with the applicable instructions for a period not to extend beyond the product's printed expiration date. **Abraxis makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose.**

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Clenbuterol Strip Test

A Screening Test for Rapid Detection of Clenbuterol in Feed, Urine, and Formula Samples

Product No. 522530

1. General Description

The Abraxis Clenbuterol Strip Test is designed solely for the preliminary screening of clenbuterol in contaminated samples (please contact Abraxis for food matrix of interest). This test is suitable for the qualitative screening of Clenbuterol in feed at 6 ppb, and in urine and meat at 3 ppb (see Sample Preparation, Section D). The Clenbuterol Strip Test provides preliminary qualitative test results only. Other conventional methods, such as ELISA, HPLC, or GC/MS, should be used to obtain quantitative results or to confirm positive samples.

2. Safety Instructions

Discard samples according to local, state and federal regulations.

3. Storage and Stability

The Clenbuterol Strip Test components and reagents should be stored at room temperature (20-30°C). Protect from light and moisture. Reagents may be used until the expiration date on the box.

4. Test Principle

The Clenbuterol Test Strip consists of a membrane strip containing a Clenbuterol conjugate. A Control Line, produced by a different antibody-antigen reaction, is also present on the membrane strip. Clenbuterol, if present in a sample solution, will bind with gold-labeled antibodies in the sample pad, forming an antibody-antigen complex. The solution wicks up causing the fluid to pass over an area containing the immoblized Clenbuterol conjugate specific to the gold-labeled antibodies on the nitrocellulose membrane (test line). The excess unbound gold-labeled antibodies will continue to migrate up the strip and bind to different immobilized antibodies specific to the gold-labeled antibody, producing a second visible line (control line). In the absence of Clenbuterol in the sample or extract, the colloidal gold labeled antibody contacts the immobilized Clenbuterol conjugate on the strip. An antibody-antigen reaction occurs forming a visible line. The Control Line is not influenced by the presence or absence of Clenbuterol sample or extract, and therefore, should be present in all reactions. The formation of two visible lines indicates a negative result (below the detection limit or cut-off). If Clenbuterol is present in the sample or extract, it competes with the immobilized Clenbuterol conjugate in the test area for binding sites on the colloidal gold labeled antibody. If a sufficient amount of Clenbuterol is present in the sample or extract, it will fill all of the available binding sites, thus preventing attachment of the labeled antibody to the immobilized Clenbuterol conjugate and, therefore no line will develop. If a colored line is not visible in the Test Line region, or if the Test Line is significantly lighter than the Control Line, the sample or extract is positive (above the detection limit or cut-off).

5. Limitations of the Clenbuterol Strip Test

The Clenbuterol Strip Test is designed to screen contaminated samples. Samples must be prepared as instructed in the Sample Preparation Section (Section D).

Mistakes in handling the test can cause errors. Possible sources for such errors include: inadequate storage conditions of the test strip or reagents, inaccurate volumes of sample, extract or reagents, too long or too short incubation times, and extreme temperatures (lower than 10°C or higher than 30°C) during the test performance.

The Abraxis Clenbuterol Strip Test provides preliminary qualitative screening results. Reasonable judgment should be applied to any test results, particularly when preliminary positive results are obtained. Other conventional methods, such as ELISA, HPLC, or GC/MS should be used to obtain quantitative results or to confirm positive samples.

A. Warnings and Precautions

1. Prior to use ensure that the product has not expired by verifying that the date of use is prior to the expiration date on the label.
2. Unused microtiter wells should be resealed in the aluminum pouch with desiccant and stored protected from light and moisture.
3. One dropper is used for each sample or extract for the entire strip test procedure. Do not discard the dropper until the test is complete. Use only the appropriate labeled dropper for the appropriate sample or extract as the use of droppers from other samples or extracts will result in contamination and produce inaccurate results.
4. Use only the dropper provided in the pouch with the strip test cassette. Substitution of other droppers could result in inaccurate sample or extract volumes.
5. Use reasonable judgment when interpreting results.
6. As the sample or extract runs through the test strip, the membrane may become tinted pink. This does not invalidate the test or cause inaccurate results.

B. Reagents and Materials Provided

1. Strip test cassette in a sealed pouch
2. Dropper in a sealed pouch
3. Phosphate Buffered Saline (PBS)

C. Additional Reagents and Materials (not provided)

1. Timer
2. Clean, dry collection container
3. Sample Grinder

D. Sample Preparation

1. *Feed* – Grind sample to the particle size of well ground coffee. Proceed to Sample Extraction, Section G.
2. *Urine* – Adjust to room temperature. Proceed to Strip Test Procedure, Section G.
3. *Meat* – Weigh 4g of sample into a centrifugal tube, place into a boiling water bath for 10 minutes. Allow the extract to cool to room temperature, then proceed to Sample Extraction, Section G.

E. Additional Reagents and Materials (for Sample Extraction and Concentration, not provided)

1. Deionized or distilled water
2. Sodium Chloride
3. Methanol
4. 15 mL or 50 mL conical vials
5. 1.5 mL or 2.0 mL microcentrifuge vials
6. Serological pipettes, 5 mL or 10 mL
7. Balance with 0.01 g accuracy
8. Centrifuge capable of 3000 rpm (1700 x g), optional
9. Micropipette (200 µL,1000 µL) with disposable tips

F. Sample Extraction (for Feed and Meat)

For Feed

1. Prepare 1000 mL of 60% methanol by adding 600 mL methanol to 400 mL deionized or distilled water.
2. Weigh 1 g ground sample (section D) and 0.2 g Sodium Chloride in 15 mL conical vial.
3. Add 5 mL of 60% methanol/water to samples.
4. Vortex or shake for 1 minute. Allow sediments to settle for at least 2 minutes.
5. *Dilute extract 4-fold by removing 60 µL extract to 180 µL PBS in 1.5 mL or 2.0 mL microcentrifuge vial.
6. Proceed to Strip Test Procedure, Section G.

*Note: Diluted extract must contain 20% or less methanol concentration in sample.

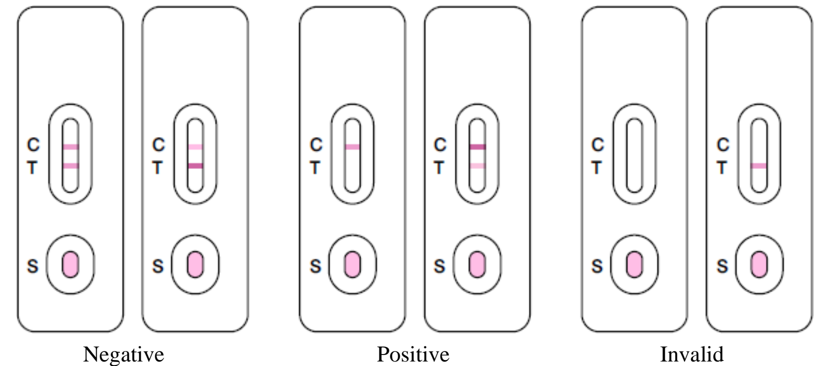
For Meat

1. Remove 3-5 drops of the boiling extractions into a 1.5 mL or 2.0 mL centrifugal tube.
2. Allow 5-10 minutes for meat/solids to settle to the bottom of the centrifuge tube, or centrifuge at 2500 rpm for 5 minutes. Collect the supernatant as the sample for detection.

G. Strip Test Procedure

1. Prepare sample according to Sample Preparation instructions (Section D), for matrices requiring sample extraction, refer to Sample Extraction Instructions (Section F).
2. Remove the strip test cassette and sample dropper from the sealed pouch. Place the cassette on a leveled surface.
3. Collect the sample using the dropper, and add 2-3 drops (about 80-100 µL) of sample without air bubbles into the sample well on the test cassette.
4. Allow the sample to enter the test window, interpret the results of your data within 5-10 minutes according to the Interpretation of Results Criteria (Section H).

H. Interpretation of Results



1. Negative (below the detection limit or cut-off): T (Test) line is equal to or darker than C (Control) line.
2. Positive (above the detection limit or cut-off): T line is not present or significantly lighter than C line.
3. Invalid Test: C line is not present. Retest using another aliquot of sample or extract with new microtiter well and test strip.

Note: Illustration is for demonstration of test line intensity range only, since overall intensity may vary slightly with different lots of reagents, samples, extracts, etc. Results should be determined ***within 5 minutes*** after completion of the strip test procedure. Interpretation of the results beyond the 5 minutes may produce inaccurate results.