CONffIDENCE: Contaminants in food and feed: Inexpensive detection for control of exposure

IMPROVEMENT TO THE EXISTING TETRASENSOR AND EXTENSION OF SCOPE TO FEED, URINE AND THERMALLY PROCESSED MEAT MATRICES

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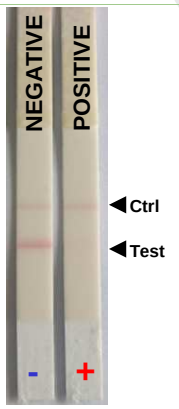
I. INTRODUCTION

Tetrasensor is a competitive receptor-based lateral flow dipstick assay developed by Unisensor and detecting many Tetracycline compounds at least at Maximum Residue Limit (MRL) values in different matrices such as milk, honey and raw animal tissues. Within WP2b (T2b4 – D2b3) of Conffidence FP7 Eu-Project, detection of tetracycline family residues with Tetrasensor was improved and extended to 3 additional matrices : urine, feed and heat-processed meat.
In order to fit with these matrices, new sample processing was developed and reagents were adapted to improve the test line signal.
This dipstick-based assay allows the detection of all Tetracycline compounds at low levels of detection in each matrices (≤ 25 µg/kg for Tissues, ≤ 50 ng/ml for Urine and ≤ 200 µg/kg for Feed) in less than 20 minutes. Performance of this Tetracycline screening assay was evaluated by an external laboratory (Fera, Sand Hutton, York, UK) for four tetracycline compounds in a range of matrices.

II. PRINCIPLE OF THE TEST

This diagnostic test exploits the activity of a receptor for the recognition of Tetracycline molecules present in the sample. The test requires the use of two elements provided in the kit. The first element is a freeze-dried reagent mix containing a certain amount of labeled receptor and the second is a dipstick consisting of a set of membrane with two capture lines (***figure A***). After sample processing (see protocol in paragraph III), when a liquid format of your sample is obtained, the supernatant is added together with the reagent receptor and the dipstick. While starting to run vertically on the strip, the liquid passes through the capture lines. The first line (TEST LINE) captures the remaining active receptor. The second upper line (CTRL LINE) serves as a control line for result interpretation. In case of positive sample, the contaminant will prevent the binding of the colored receptor to the test line giving a test line intensity lower than those of the control line (ratio Test / Ctrl ≤ 1 with optical reader). Results are interpreted by visual observation of the line intensities or with the help of an optical reader "Readsensor" (***figure B***).

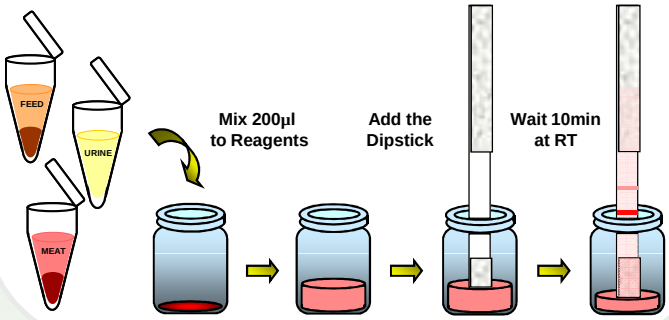
A



B

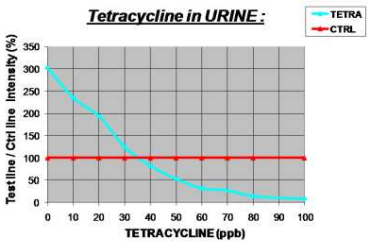
III. PROTOCOLS

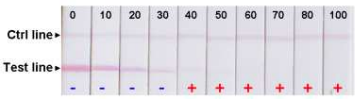
MATRIX	 FEED	 URINE	 (COOKED) MEAT
DILUTION	**3gr** + 27ml Buffer1 GRIND **2min** Take **1ml** of liquid sample CENTRIFUGE **1min**	**200µl** + 1,8ml Buffer2	**10gr** + 30ml Buffer1 GRIND **2 min** Take **1ml** of liquid sample CENTRIFUGE **1min**
TEST	Mix **200µl** to 200µl Buffer2 Use **200µl** for the test	Use **200µl** for the test	**If RAW :** Use **200µl** for the test **If COOKED :** Mix **400µl** to 200µl Buffer2 Use **200µl** for the test



IV. EXAMPLE OF RESULTS

Representative experiment of Tetrasensor analyses of pig Urine samples spiked to reach concentrations of Tetracycline ranging from 10 ng/ml (ppb) to 100 ng/ml (ppb).
The test is considered as positive when the test line intensity is lower than the control line.
The limit of detection for Tetracycline obtained in this experiment is between 30-40 ng/ml (ppb).





V. SENSITIVITY

Compound	Detection capability (µg/kg - ppb)*		
	Muscle	**Feed**	**Urine**
Chlortetracycline	≤20	~100	≤12.5
Oxytetracycline	~25	≤200	≤50
Tetracycline	~25	≤200	~50
Doxycline	≤20	≤100	≤12.5

**Other compounds at least detected in animal Muscle (≤ 25 µg/kg - ppb) :*
Minocycline, Demeclocycline, Sancycline, Amicycline, Meclocycline, Methacycline.

Note : *The detection capability is the concentration level at which ≤5% false compliant rate (β error) will occur (n=21). The Tetrasensor assay performance was found to be consistent across a variety of known blank samples (more than 20 different / matrix type) of porcine muscle (raw/cooked), porcine and bovine urine and porcine feedingstuffs.*

VI. CONCLUSION

In conclusion, we have improved our generic Tetracycline-dipstick assay. This updated format allows detection of most of the Tetracyclines in a large range of matrices including raw animal tissues, processed meat, feed and urine. This format of Tetrasensor is commercialized at Unisensor under product code TM630.

The research leading to these results has received funding from the European Community's Seventh Framework Programme (*FP7/2007-2013)* under *grant agreement* n° 211326.