



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

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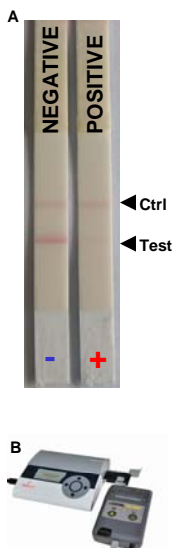
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## I. ABSTRACT

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 Confidence 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 Tetracycline compounds at very low levels of detection (< 50 ppb - µg/kg) in each matrices in less than 20 minutes. For those who need less sensitive results, it is possible to further dilute the sample preparation to proportionally turn up the limit of detection. Accurate performances of this Tetracycline screening assay will soon be confirmed by in lab validation studies (within D.2b.7 deliverable of Confidence project) by FERA-CSL partner.

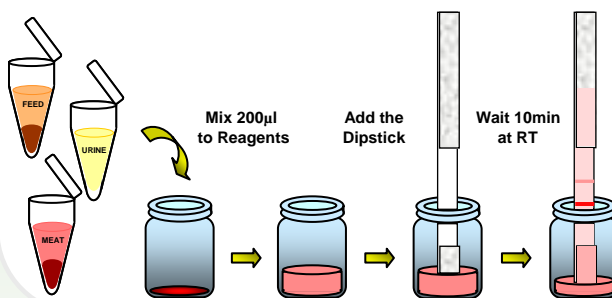
## II. PRINCIPLE OF THE TEST

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 there is a liquid format of your sample, 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 line intensity lower than those of the control line (or 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).



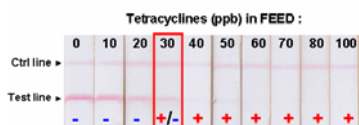
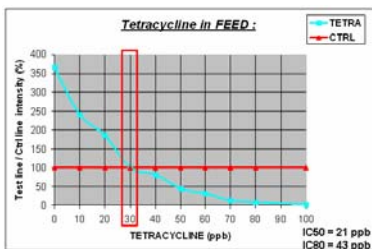
## III. PROTOCOLS

MATRIX	FEED	URINE	(COOKED) MEAT
DILUTION / EXTRACTION	3gr + 27ml Buffer1 GRIND 2min Take 1ml of liquid sample CENTRIFUGATION 1min	200µl + 1,8ml Buffer2	10gr + 30ml Buffer1 GRIND 2 min Take 1ml of liquid sample CENTRIFUGATION 1min
SUPERNATANT / (2 <sup>nd</sup> DILUTION)	Use 200µl for the test	Use 200µl for the test	<b>IF RAW:</b> Use 200µl for the test <b>IF COOKED:</b> Mix 400µl to 200µl Buffer2 Use 200µl for the test



## IV. EXAMPLE OF RESULTS

Representative experiment of Tetrasensor analyses of Feed samples spiked to reach concentrations of Tetracycline ranging from 10 ppb to 100 ppb. The test is considered as positive when the test line intensity becomes ≤ to that of control line. The limit of detection for Tetracycline obtained in this experiment is 30 ppb.



## V. SENSITIVITY

MATRICES : (Included dilution)	Crude Tissue (4X)	Cooked Meat (6X)	Feeds (10X)	Urine (10X)
Spiked - Incurred				
TETRA*	20ppb	ND	25ppb	30ppb
OXYTETRA*	10ppb	ND	20ppb	-
CHLORTETRA*	<5ppb	ND	<10ppb	6ppb
DOXY*	<5ppb	<15ppb	<5ppb	3ppb

\* Detects also all the main other members of Tetracycline family (LOD to be determined).

## 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, urine, honey, egg, water and seafood.