Interlaboratory testing on screening tests for tetracyclin and sulfathiazole residues on honey matrix

International Honey Commision

World Network of Honey Science
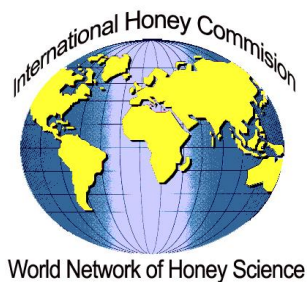
Famille Michaud Apiculteurs depuis 1920

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1. INTRODUCTION

The aim of this interlaboratory study was first to identify the screening tests able to detect the veterinary drugs on honey, and then to test these ones on several samples.

The first round was to evaluate the situation on tetracyclin and sulfathiazol.

A questionnaire had been sent to the participants, in order to list the screening tests available, used in our application, and appropriated with the European requirements for residues limits.

For tetracyclin, three different kits have been tested:
-Antimicrobial Array II from Randox
- Ridascreen Tetracyclin from R-Biopharm
-Tetrasensor from Unisensor

For sulfathiazole, one kit has been tested:
-Antimicrobial Array I from Randox

The materials were dispatched in March 2013 to 15 participants .Each of them received 5 honey test materials to be analyzed for sulfathiazol and/ or for tetracyclin.
Participants were from 11 countries (Belgium, France, Germany, Italy, Switzerland, Portugal, United Kingdoms, India, Argentina, Poland and Serbia).

5 different test materials were offered:
-N° 1 Incurred honey at 10ppb Tetracyclin
-N° 2 Blank honey
-N° 3 Incurred honey at 10ppb Sulfathiazol
-N° 4 Spiked honey with 8ppb Tetracyclin and 5ppb Sulfathiazol
-N° 5 Spiked honey with 15ppb Tetracyclin and 15ppb Sulfathiazol

Each participant received 5 samples in a proper contener, refrigerated with ice block.
The instructions were sent by e mail to each participant.

On 15 participants, one laboratory had a problem with the custom, 2 others participants sent the results after closing date.

The results presented in this report concern 12 laboratories.

For each analyte, two approaches have been done, the first one is based on the calculation of the standard deviation for proficiency, the assigned value and the bias, the second one on the evaluation of the screening capability.

1.1 Summarized results

1.1.1 For tetracyclin run by Randox AM II

Sample	Assigned value µg/kg	Standard uncertainty (ux)	Number of score 2*sigma ≤x-X≤2*sigma	Total number of score	% 2*sigma≤x-X≤2*sigma
1	16.064	0.891	6	7	85.7
2	Nd		7	7	100
4	5.813	0.367	5	6	83.3
5	16.004	1.69	5	7	71.42

1.1.2 For tetracyclin run by Ridascreen Elisa

Sample	Assigned value µg/kg	Standard uncertainty (u$_x$)	Number of score 2*sigma ≤x-X≤2*sigma	Total number of score	% 2*sigma ≤x-X≤2*sigma
1	18.083	2.989	2	6	33.33
2	Nd		6	6	100
4	10.183	2.66	2	6	33.33
5	16.882	3.323	3	6	50

1.1.3 For sulfonamides run by Randox AM I

Sample	Assigned value µg/kg	Standard uncertainty (u$_x$)	Number of score 2*sigma ≤x-X≤2*sigma	Total number of score	% 2*sigma ≤x-X≤2*sigma
2	Nd		7	7	100
3	13.038	1.202	5	7	71.42
4	5.389	1.124	4	6	66.6
5	15.750	1.530	5	7	71.42

2. PREPARATION

The samples preparations were carried out by Famille Michaud Apiculteurs in February 2013.
The matrix was prepared by blending one by one honey blank sample, honey incurred sample and honey spiked sample.
The spiking solution was prepared with tetracyclin and sulfathiazole.
Five different samples were prepared, each of them were homogenized and divided in samples serials.
Samples were stored at -20°C until dispatch.

The reference standards used for spiking are the following ones:

Molecule	Supplier	Reference	Batch number
Tetracyclin	Sigma Aldrich	87128	BCBF8374V
Sulfathiazol	Sigma Aldrich	S9876	SLBB2307V

3. HOMOGENEITY CHECK OF THE SAMPLES

The homogeneity was performed on a screening method by Famille Michaud Apiculteurs on Randox system and also on a confirmatory method by Intertek on HPLC MSMS in February 2013.
The principle is the quantification of the heterogeneity between 10 packaged samples and analyzed in a random order: by calculation of an interlaboratory standard deviation (two analyses of a measurand according to annex B of the standard ISO 13528).

The test is homogeneous if the standard deviation between the different results do not exceed 0.3 σ, where σ is equal to 10%.

So all the materials were proved to be homogenous in this trial.

4. STABILITY CHECK OF THE SAMPLES

The stability was performed on a screening method by Famille Michaud Apiculteurs on Randox system and also on a confirmatory method by Intertek on HPLC MSMS in the beginning of June 2013.
The principle is the quantification of the deviation between the value obtained during the homogeneity check and the value obtained for three packaged samples and analyzed in a random order, at the end of the period given for laboratories to send their results of analyses. It is performed by analyzing twice a measurand according to annex B of the standard ISO 13528.

The test is stable if the standard deviation between the different results do not exceed 0.3 σ, where σ is equal to 10%.

So all the materials were proved to be stable in this trial.

The average results obtained for the test materials on HPLC MS MS :
-N° 1 Incurred honey at 12.4 ppb Tetracyclin
-N° 2 Blank honey
-N° 3 Incurred honey at 8 ppb Sulfathiazol
-N° 4 Spiked honey with 7.6 ppb Tetracyclin and 4.7 ppb Sulfathiazol
-N° 5 Spiked honey with 13.3 ppb Tetracyclin and 12.3 ppb Sulfathiazol

5. STATISTICAL EVALUATION

The participant results were analyzed according to the NF ISO 13528; statistical methods usally followed in proficiency testing by interlaboratory comparison.
The standard deviation for proficiency, the assigned value and a bias were calculated for each sample, each molecule and each protocol according to the requirements of § 7.1 and 7.4 of the NF ISO 13528.

5.1 Assigned value calculation

Assigned value X represents the median of the concentration values determined by the participants.

Standard uncertainty of the assigned value Ux is calculated as follow:
$Ux=1.25*s/\sqrt{p}$ (where s is the standard deviation of the robust mean and p the number of participant)

5.2 Target standard deviation for proficiency

The target standard deviation σ reflects best practice for the protocols in question.
Usually, the target standard deviation is calculated with the Horwitz equation according to the paragraph 6.4 of the NF ISO 13528.

In this proficiency test, we choose to define the target standard deviation as a value which reflects best practice for screening assays according to the paragraph 6.3 of the NF ISO 13528.

The value chosen is 10%.

5.3 Bias calculation

Bias is calculated as following:

x-X
X is the Assigned value
And x the participant report result mean

The laboratory performance evaluation was established taking into account the following criteria:
Satisfactory : when -2*sigma<(x-X)<2*sigma
Unsatisfactory : when x-X is out of this interval

6. SCREENING CAPABILITY

The screening capability has been evaluated regarding the limit of detection provided by the supplier of each kit and the level of contamination of each sample.

7. RESULTS

7.1. Qualitative analysis: Interpretation on screening capability

7.1.1 For tetracyclin run by Randox AM II

All the results are complying regarding the LOD of 8.65 ppb given by the supplier.

7.1.2 For tetracyclin run by Ridascreen

All the results are complying regarding the LOD of 4ppb given by the supplier.

7.1.3 For tetracyclin run by Tetrasensor

Regarding the LOD given by the supplier of 8ppb
For the sample 1, 12.5% of false negative results
For the sample 4, 37.5% of false positive results
For the sample 5, 25% of false negative results
Comments: all the interpretations have been done visually without the Readsensor except for the lab 1, it is interesting to note that the results of this laboratory are all complying.

7.1.4 For sulfathiazol run by Randox AM I

The LOD given by the supplier is 5 ppb for sulfathiazole.
For samples 2, 3 and 5, the results are complying regarding this LOD.
For the sample 4, the homogeneity test result gave an average level of 5 ppb, and the stability test an average level of 4.7 ppb, we could consider that this sample was contaminated at the limit of detection announced by the supplier.
On this sample, 42.8% of false negative results were found.

7.2. Quantitative analysis

7.2.1 Tetracyclin

7.2.1.1 Tetracyclin run by Randox AM II

Percentages of recovery were calculated and reported by the participants.

- **Sample 1**

Assigned value = 16.06 ppb.
Ux = 0.891.

Laboratory number	Result	% recovery	x-X	2*sigma	moins 2*sigma
4	14.845		-1,215	3,212	-3,212
5	11.77		-4,29	3,212	-3,212
6	15.975		-0,085	3,212	-3,212
7	17.565		1,505	3,212	-3,212
9	16.215		0,155	3,212	-3,212
10	18.24	122	2,18	3,212	-3,212
11	16.375		0,315	3,212	-3,212
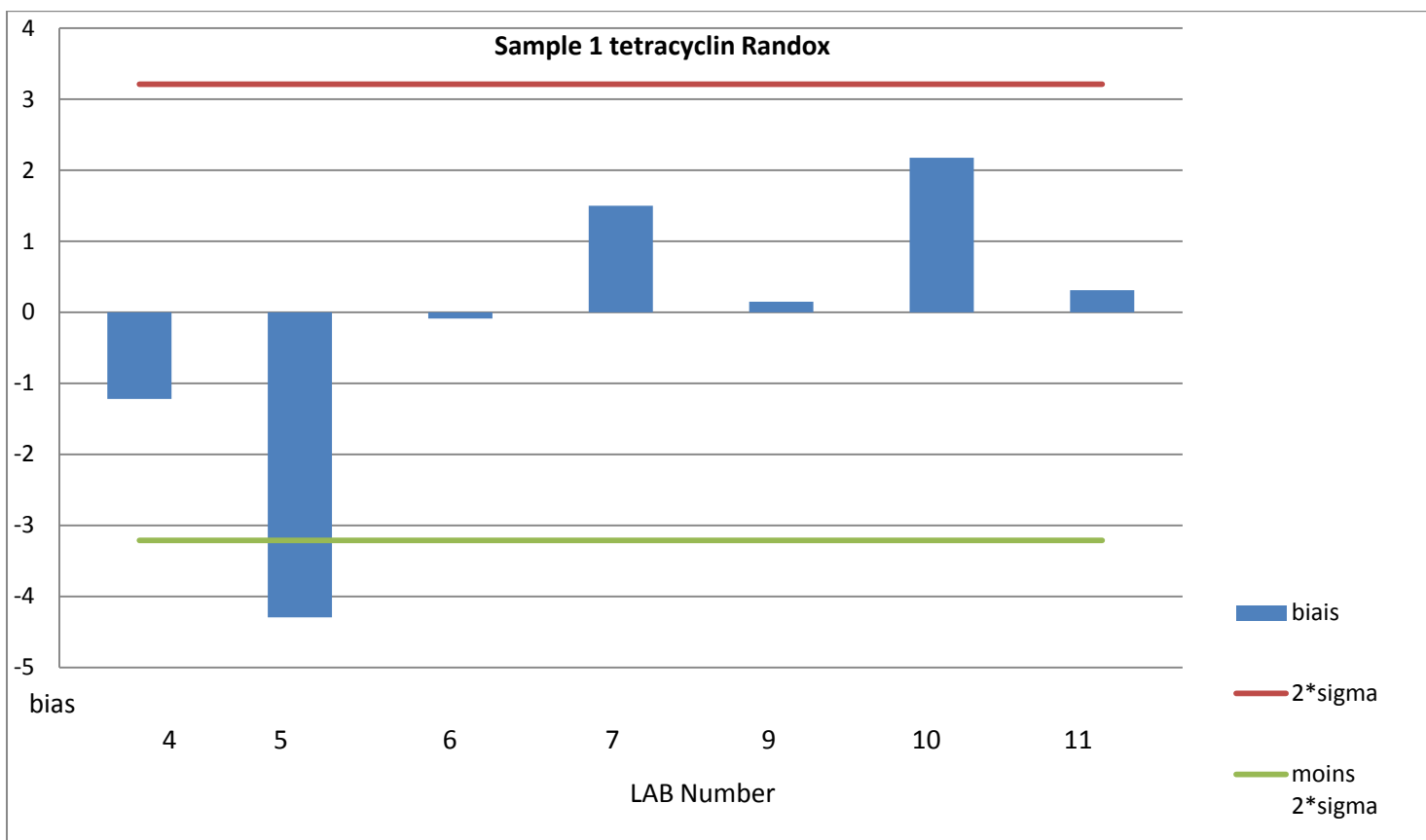
- **Sample 2**

Laboratory number	Result
4	Not Detected
5	Not Detected
6	Not Detected
7	Not Detected
9	Not Detected
10	Not Detected
11	Not Detected

- **Sample 4**
  Assigned value = 5.81 ppb.
  Ux = 0.367.

Laboratory number	Result	% recovery	x-X	2*sigma	moins 2*sigma
4	7.035		1,225	1,162	-1,162
5	5.385		-0,425	1,162	-1,162
6	Not detected		#VALEUR!	1,162	-1,162
7	5.905		0,095	1,162	-1,162
9	5.31		-0,5	1,162	-1,162
10	6.125	122	0,315	1,162	-1,162
11	5.26		-0,55	1,162	-1,162
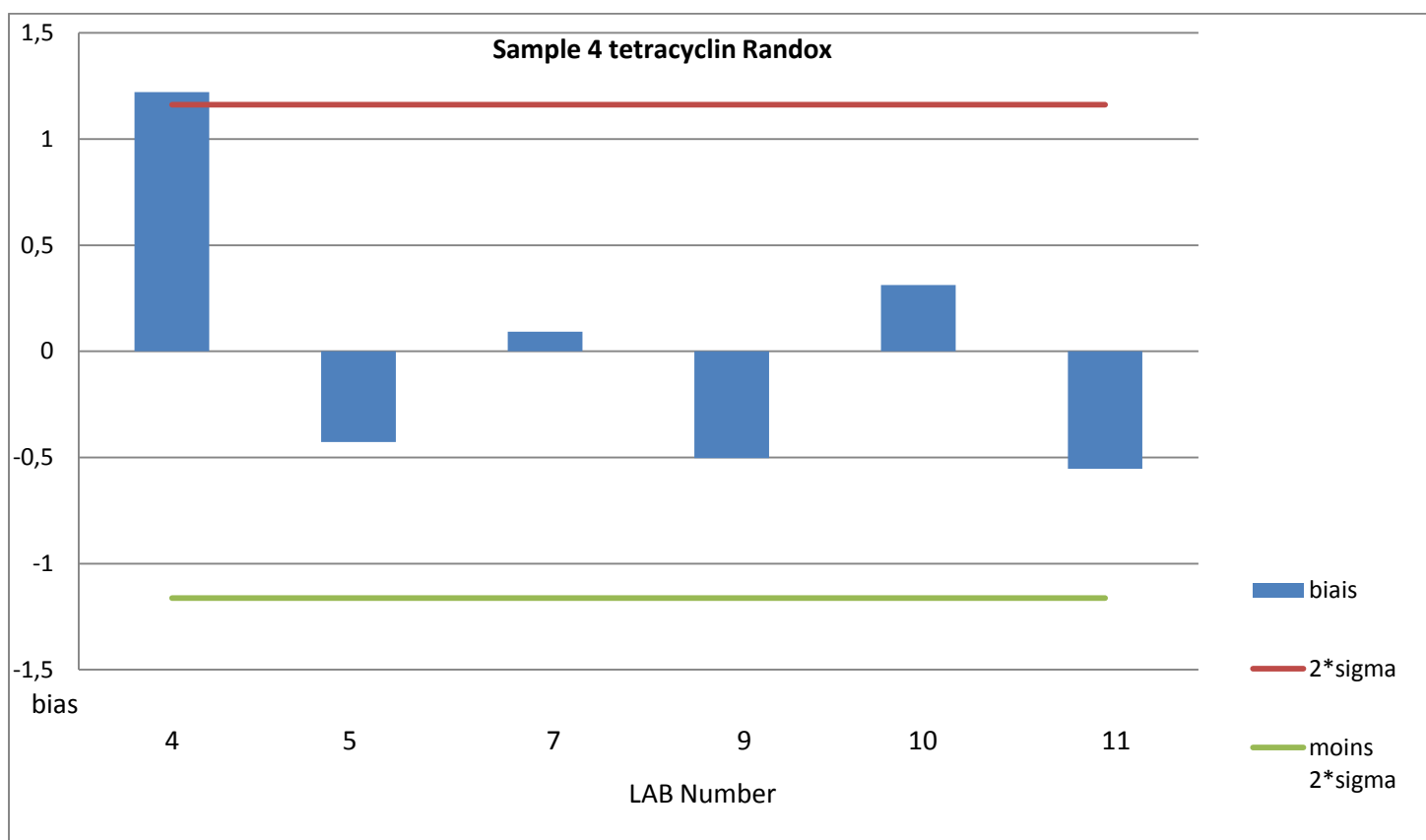
- **Sample 5**
  Assigned value = 16.004 ppb.
  Ux = 1.69.

Laboratory number	Result	% recovery	x-X	2*sigma	moins 2*sigma
4	16.19		0,186	3,2008	-3,2008
5	10.105		-5,899	3,2008	-3,2008
6	13.49		-2,514	3,2008	-3,2008
7	18.95		2,946	3,2008	-3,2008
9	15.535		-0,469	3,2008	-3,2008
10	19.625	122	3,621	3,2008	-3,2008
11	17.6		1,596	3,2008	-3,2008
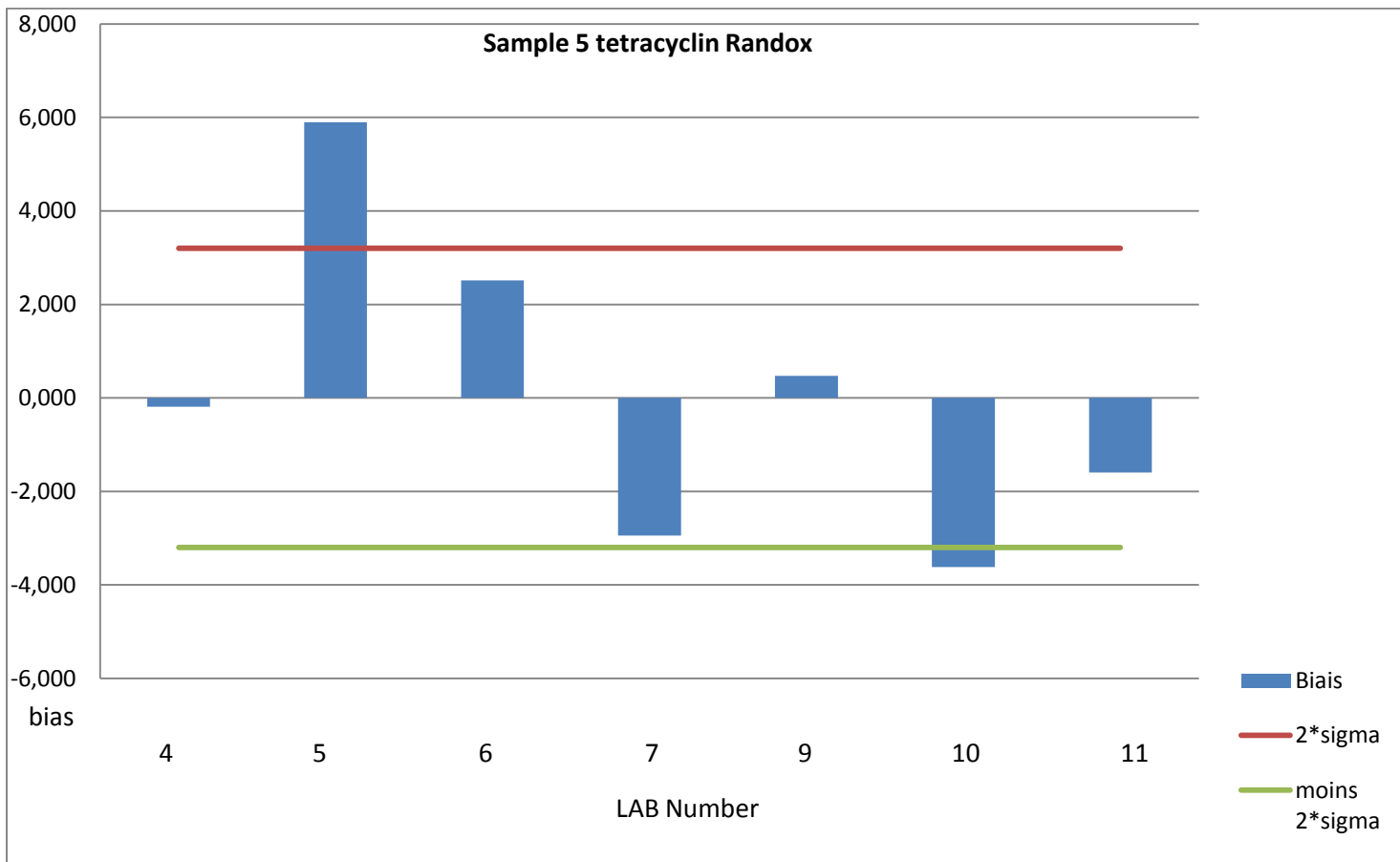
On the sample 4, one laboratory obtained a negative result, but the concentration of tetracyclin in this sample (5.813 ppb) is under the LOD of the test (8.65 ppb), the global results for this sample could be consider as correct.
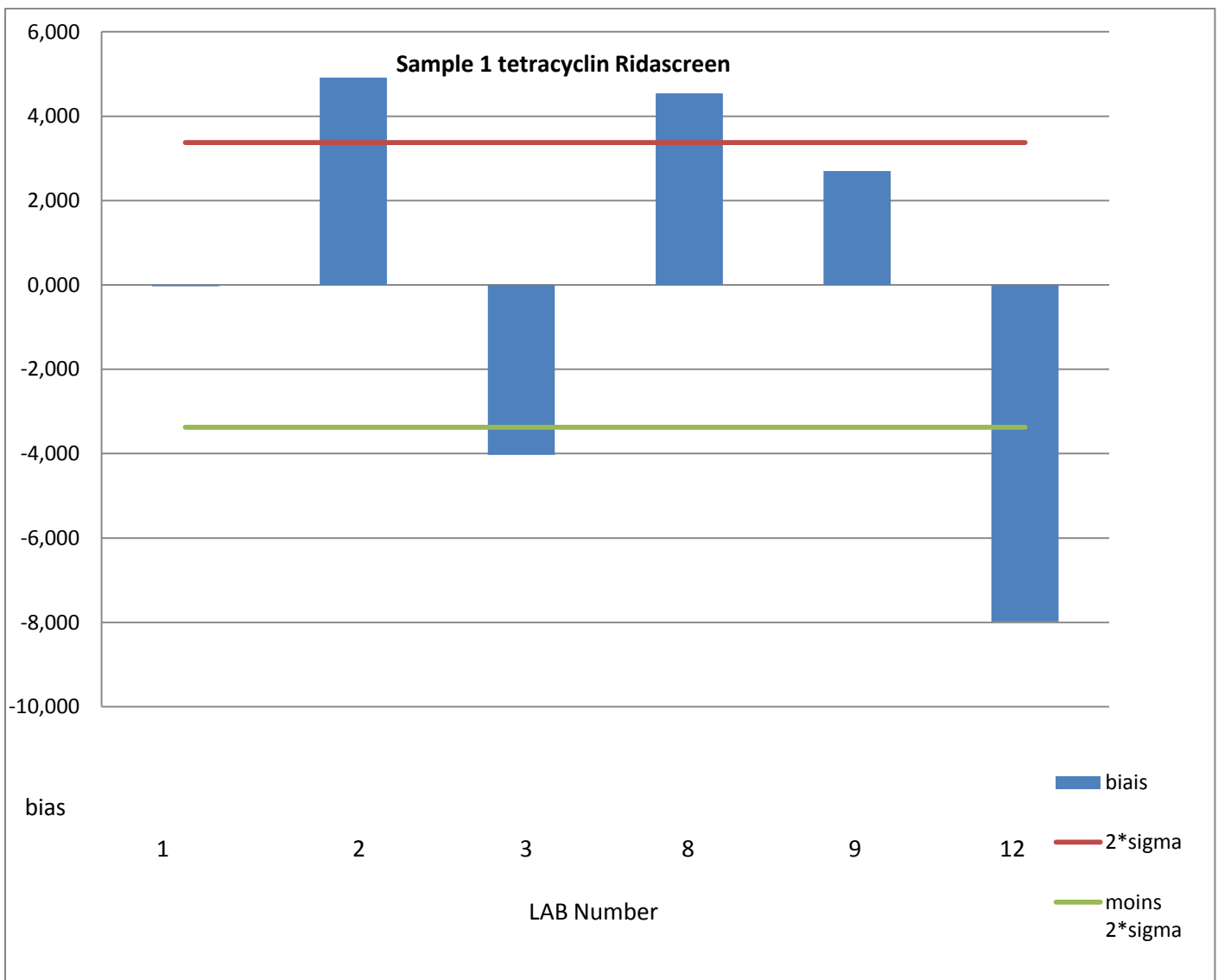
7.2.1.2 Tetracyclin run by Ridascreen Elisa

- **Sample 1**
  Assigned value = 18.083 ppb.
  Ux = 2.989.

Laboratory number	Result	% recovery	x-X	2*sigma	moins 2*sigma
1	18.05	172	-0,033	3,6166	-3,6166
2	23		4,917	3,6166	-3,6166
3	14.05		-4,033	3,6166	-3,6166
8	22.625		4,542	3,6166	-3,6166
9	20.77		2,687	3,6166	-3,6166
12	10.1		-7,983	3,6166	-3,6166

- **Sample 2**

Laboratory number	Result
1	Not detected
2	Not detected
3	Not detected
8	Not detected
9	Not detected
12	Not detected

- **Sample 4**
  Assigned value = 10.183 ppb.
  Ux = 2.66.

Laboratory number	Result	% recovery	x-X	2*sigma	moins 2*sigma
1	9.05	172	-1,133	2,0366	-2,0366
2	12		1,817	2,0366	-2,0366
3	5.3		-4,883	2,0366	-2,0366
8	16.4		6,217	2,0366	-2,0366
9	13.45		3,267	2,0366	-2,0366
12	4.9		-5,283	2,0366	-2,0366
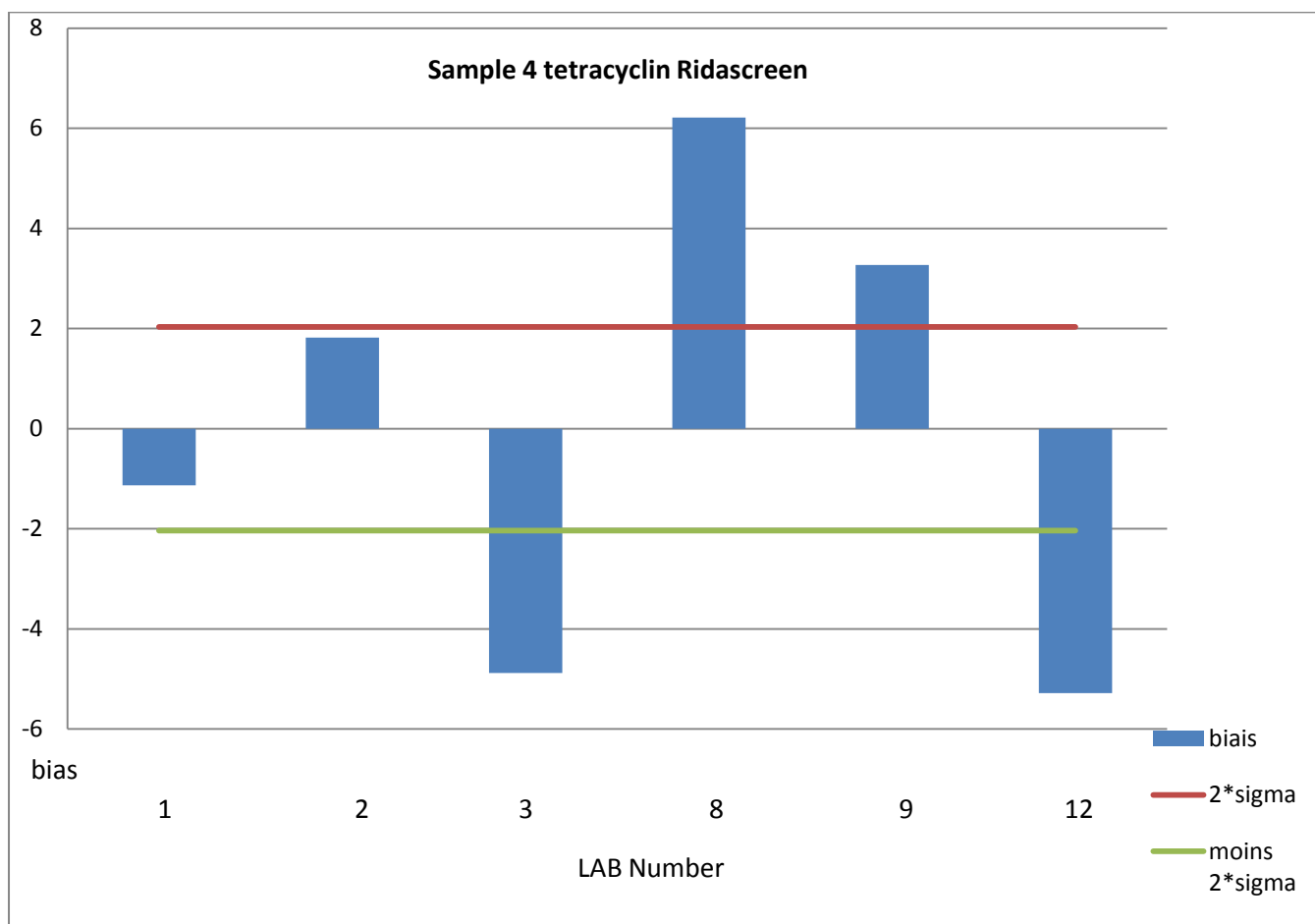
- **Sample 5**
  Assigned value = 16.882 ppb.
  Ux = 3.323.

Laboratory number	Result	% recovery	x-X	2*sigma	moins 2*sigma
1	17.15	172	0,268	3,3764	-3,3764
2	19.5		2,618	3,3764	-3,3764
3	11.65		-5,232	3,3764	-3,3764
8	24.84		7,958	3,3764	-3,3764
9	19.12		2,238	3,3764	-3,3764
12	9		-7,882	3,3764	-3,3764

Sample 5 tetracyclin Ridascreen
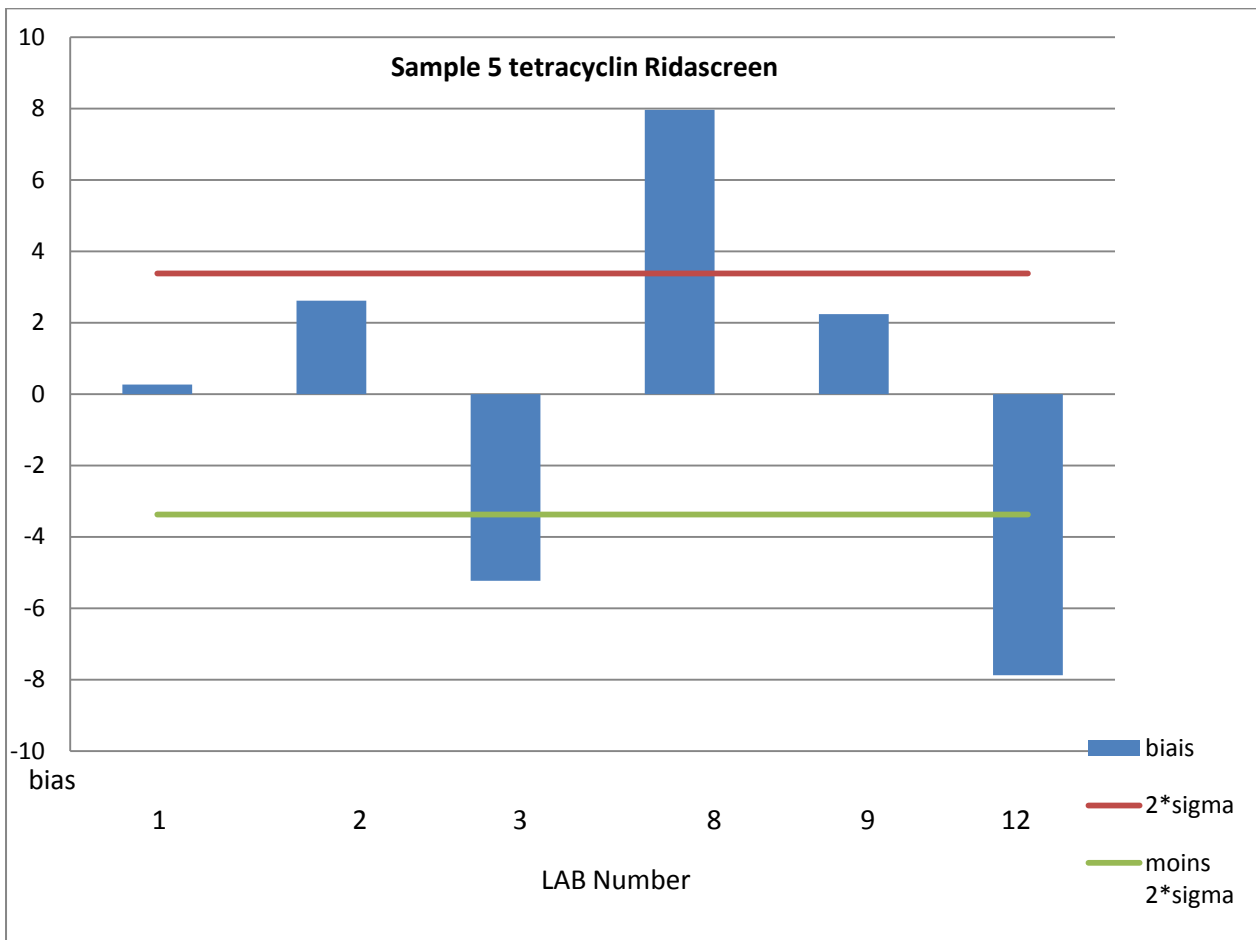
7.2.2 Sulfonamides by Randox AM I

- **Sample 2**

Laboratory number	Result
4	Not Detected
5	Not Detected
6	Not Detected
7	Not Detected
9	Not Detected
10	Not Detected
11	Not Detected

- **Sample 3**
  Assigned value = 13.038 ppb.
  Ux =1.202.

Laboratory number	Result	% recovery	x-X	2*sigma	moins 2*sigma
4	14.325		1,287	2,6076	-2,6076
5	12.34		-0,698	2,6076	-2,6076
6	12.84		-0,198	2,6076	-2,6076
7	14.8		1,762	2,6076	-2,6076
9	7.62		-5,418	2,6076	-2,6076
10	15.98	96	2,942	2,6076	-2,6076
11	11.75		-1,288	2,6076	-2,6076
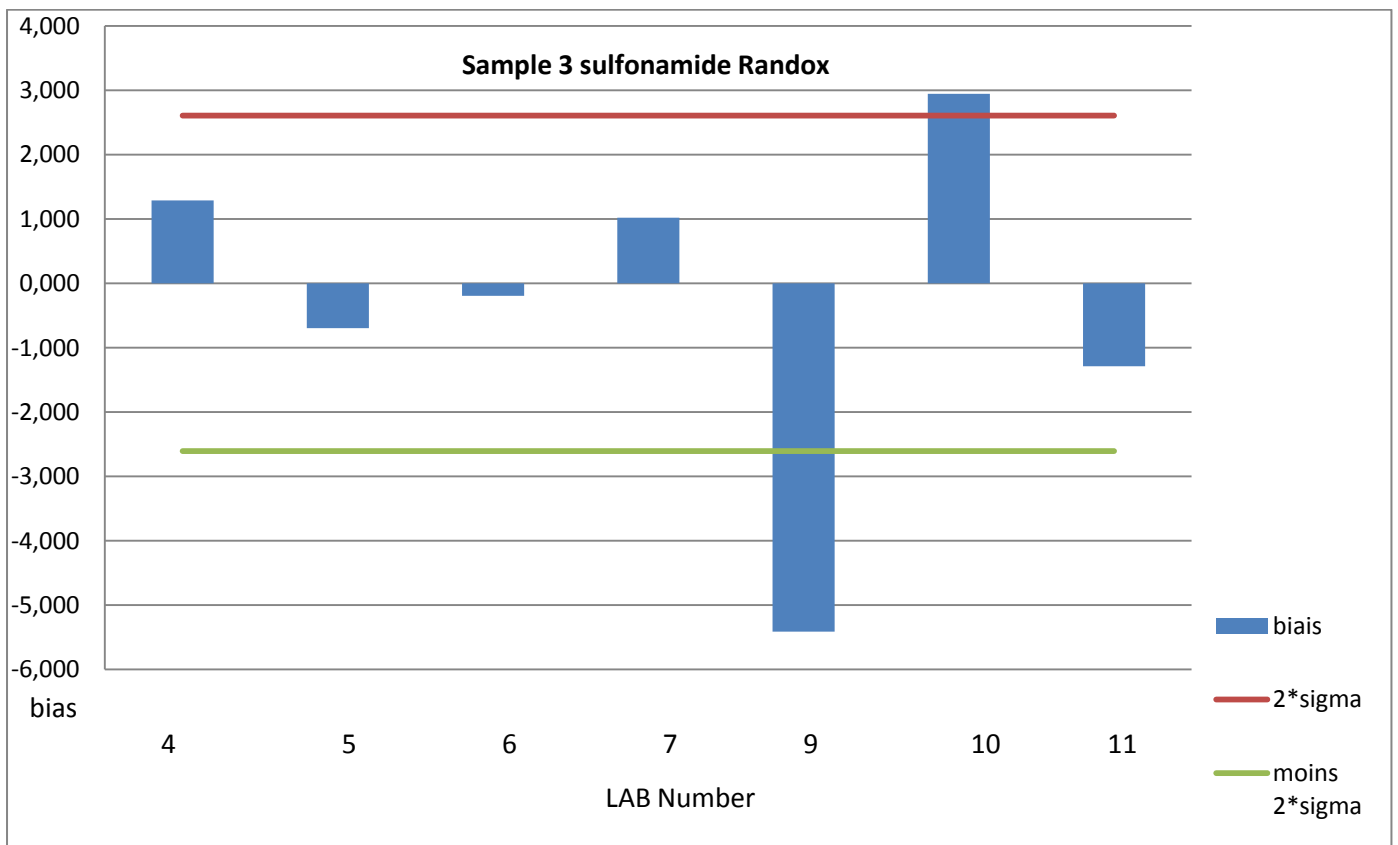
- **Sample 4**
  Assigned value = 5.389 ppb.
  Ux =1.124.

Laboratory number	Result	% recovery	x-X	2*sigma	moins 2*sigma
4	7.81		2,421	1,0778	-1,0778
5	4.45		-0,939	1,0778	-1,0778
6	Not detected		#VALEUR!	1,0778	-1,0778
7	5.88		0,491	1,0778	-1,0778
9	2.02		-3,369	1,0778	-1,0778
10	5.83		0,441	1,0778	-1,0778
11	6.27		0,881	1,0778	-1,0778
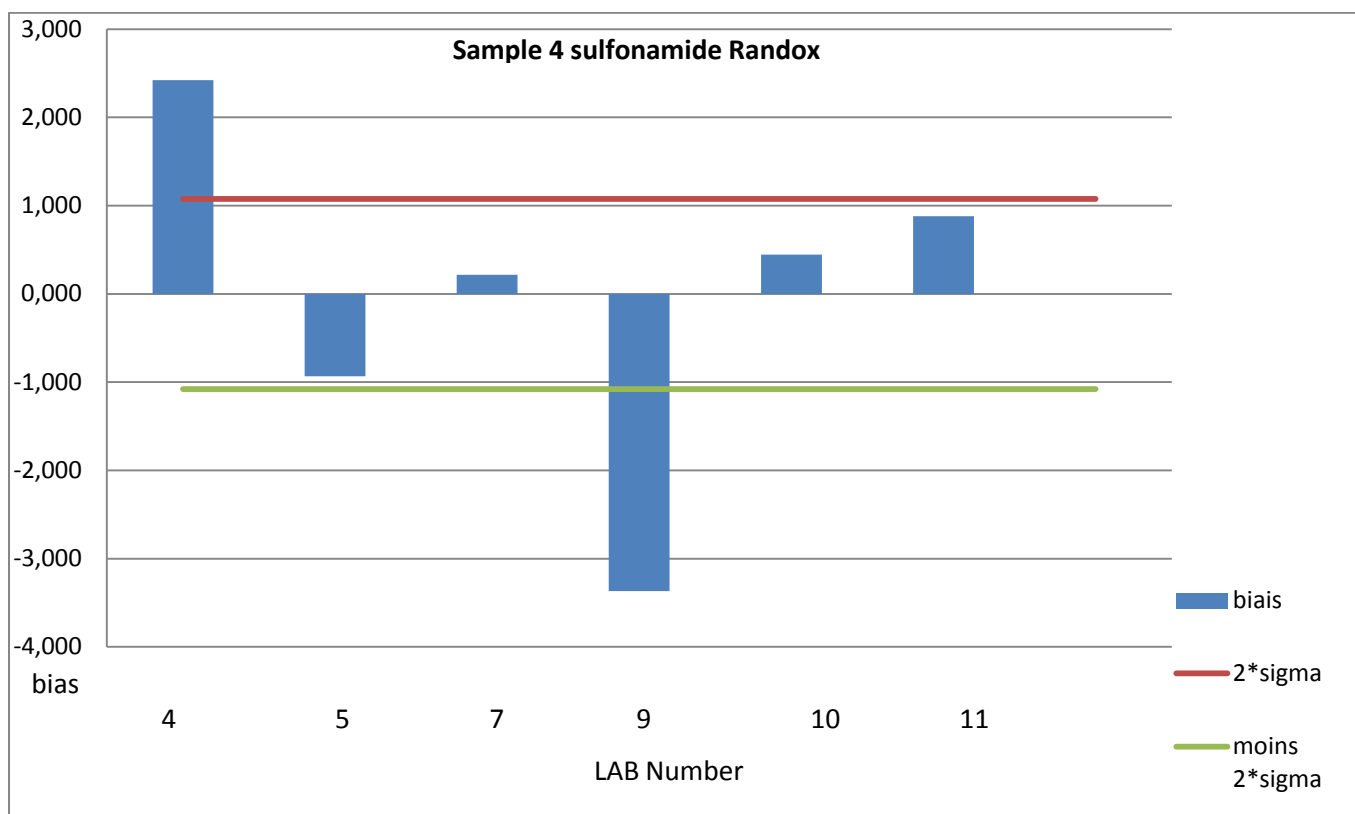
- **Sample 5**
  Assigned value = 15.75 ppb.
  Ux =1.530.

Laboratory number	Result	% recovery	x-X	2*sigma	moins 2*sigma
4	17.48		1,73	3,15	-3,15
5	15.21		-0,54	3,15	-3,15
6	13.48		-2,27	3,15	-3,15
7	17.39		1,64	3,15	-3,15
9	8.32		-7,43	3,15	-3,15
10	19.48		3,73	3,15	-3,15
11	16.30		0,55	3,15	-3,15
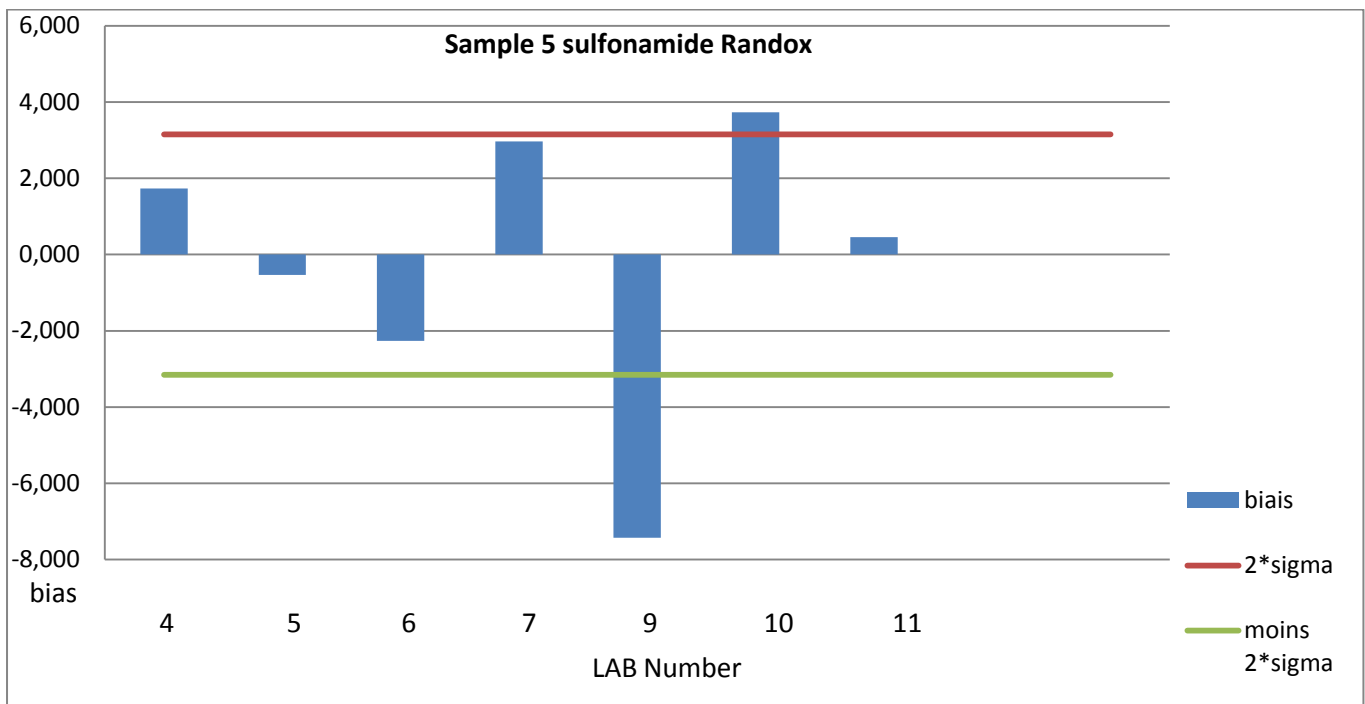
Three laboratories obtained a negative result for the sample 4, whereas this sample have been spiked at 5 ppb of sulfathiazole corresponding to the LOD of the test, so 42% of false negative results.

One laboratory found positive results for sulfamethoxazole on samples 2, 3, 5.

7.3.Tetracyclin by Tetrasensor

- **Sample 1**

Laboratory number	Result
1	POS
2	NEG
3	POS
4	POS
6	POS
7	POS
8	POS
9	POS

- **Sample 2**

Laboratory number	Result
1	NEG
2	NEG
3	NEG
4	NEG
6	NEG
7	NEG
8	NEG
9	NEG

- **Sample 4**

Laboratory number	Result
1	NEG
2	NEG
3	LOW POS
4	LOW POS
6	NEG
7	NEG
8	LOW POS
9	NEG

- **Sample 5**

Laboratory number	Result
1	POS
2	NEG
3	POS
4	POS
6	POS
7	NEG
8	POS
9	LOW POS

8. CONCLUSION

It is important to mention that these tests are screening tests and not quantitative tests, that's why we could conclude that for tetracycline, Randox and Ridascreen tests gave satisfactory results.

For Tetrasensor, it seems that the Readsensor is necessary to obtain reliable results, an additional test might confirm this point.

For the sulfonamides, the LOD of the supplier has to be re-evaluate.

However, the statistical evaluation was interesting to manage, the interpretation has to be done carefully due to the fact that this study is based on qualitative results and not quantitative.