Proficiency test for resorcylic acid lactones in bovine urine

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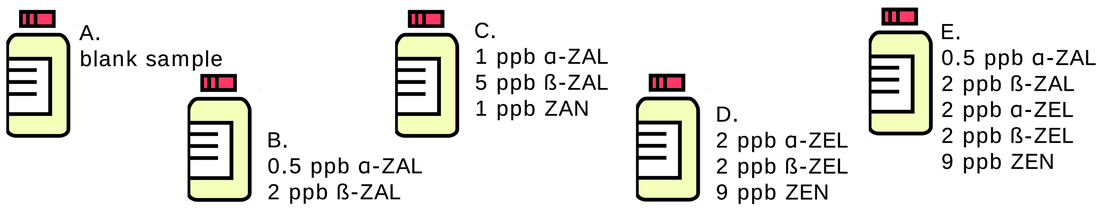
Introduction

Proficiency tests are a well known tool to evaluate and demonstrate the reliability of the data that are produced. In 2015 the European Union Reference Laboratory (EU-RL) organised a proficiency test for resorcylic acid lactones (RALs) in bovine urine.

When administered to animals, zeranol (α-zeralanol, α-ZAL) metabolizes to taleranol (ß-zeralanol, ß-ZAL) and zearalanone (ZAN). Next to the abuse of zeranol, RALs can also be present in animal tissues due to the metabolism of the *Fusarium* toxin zearalenone (ZEN) resulting in α-zearalenol (α-ZEL) and ß-zearelenol (ß-ZEL).

For this proficiency test five samples containing RALs originating from treatment with zeranol ranging from 0.5-10 µg/l were prepared. Additionally, two samples were enriched with ZEN and its metabolites.



Homogenous materials were prepared and sent to the participants.

In addition, the model designed by Launay et al. [11] was applied and evaluated to determine whether findings of RALs were due to abuse of zeranol or feed contamination with *Fusarium* toxins.

Results

Forty-three participants (26 National Reference Laboratories) subscribed for the proficiency test and 38 reported results. All labs included α-ZAL and ß-ZAL in their method, 31 included α-ZEL and ß-ZEL, 26 included ZAN and 31 included ZEN. A variety of purification methods/detection techniques/internal standards was applied.
An overview of the results are presented in Table 1 and Figure 1 .

Table 1. Results of RALs in materials B-E

Material	Compound	Consensus value (µg/l)	Range (µg/l)	Correct results (%)
B	α-ZAL	0.48	0.25-0.94	69
	ß-ZAL	1.9	0.4-5.36	69
	ß-ZEL	No statistical evaluation possible due to high uncertainy of consensus value		
	ZAN	No statistical evaluation possible		
	ZEN	No statistical evaluation possible		
C	α-ZAL	0.98	0.4-2.28	76
	ß-ZAL	4.5	0.8-12.8	62
	ß-ZEL	No statistical evaluation possible		
	ZAN	0.98	0.5-1.77	80
	ZEN	No statistical evaluation possible		
D	α-ZEL	1.8	0.81-3.8	67
	ß-ZEL	2.1	0.15-8.2	56
	ZEN	9.0	3.45-66.8	67
E	α-ZAL	0.45	0.25-1.06	69
	ß-ZAL	1.8	0.4-6.21	70
	α-ZEL	1.8	0.62-3.82	67
	ß-ZEL	2.1	0.1-11.4	59
	ZAN	No statistical evaluation possible		
	ZEN	9.1	2.83-70.5	73

In this test 7 false positive and 44 false negative results were reported. A result was assigned a FN if a compound was not detected, taken into account the reported scope of the participant, the consensus value and the reported CCα.

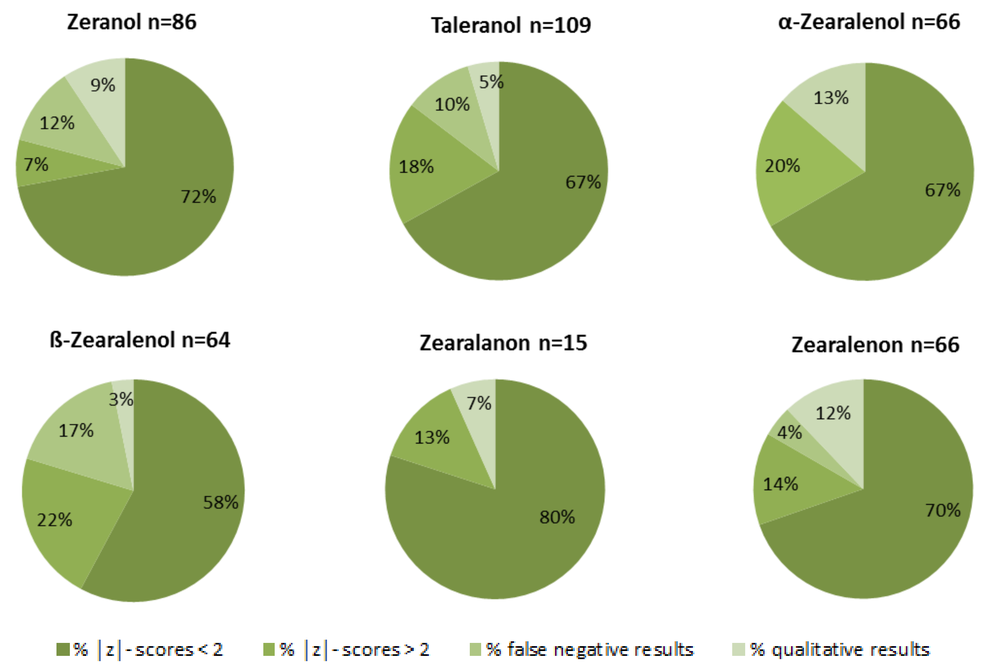


Figure1. Overview of |z|-scores <2 and >2, false negative results and qualitative results

Abuse or contamination?

Based on the research of Launay et al. [1] as also summarized in the EURL Reflection paper [2], material A, the blank sample, should be considered compliant in all cases. Materials B and C, in which zeranol and taleranol are the major compounds present, should be considered as samples for which there was proof of zeranol abuse. The majority of participants indeed classified these samples as non-compliant. Material D should be evaluated as compliant because of the relative high levels of *Fusarium* metabolites. Most of the laboratories classified this sample correctly. Evaluation of the results for E was the most challenging. Most of the laboratories classified this sample as non-compliant. However, due to the presence of *Fusarium* toxins, a significant number of laboratories classified this sample as compliant.

Conclusions

- Out of 43 labs, 8 labs showed optimal performance by correct quantification of the compounds and the absence of false positive and false negative results.
- Almost 10% of the participants was unable to report results.
- There is no specific method providing ‘the best’ results.
- The quantification and detection of RALs in bovine urine needs additional attention, due to many false negative results and many |z-scores| >2.

References

1] Launay, F.M., Prevalence of zeranol, taleranol and Fusarium spp. Toxins in urine: implications for the control of zeranol abuse in the European Union. 2004. Food Additives and Contaminants Vol. 21, pp 833-839.

[2] CRL Guidance Paper (7 December 2007)

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