Comparative study of the value of flotates from TCE and PE / TCE sedimentations for the detection of constituents of animal origin
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A. ANSELMO & P. VEYS

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1. Introduction

Since 2022, Commission Implementing Regulation (EU) 2022/893 amending Annex VI to Regulation (EC) No 152/2009[1] has introduced a double sedimentation protocol for the detection of terrestrial invertebrate constituents. This double sedimentation is based on a complementary second settling step on flotates within a mixture of 30% petroleum ether / 70% tetrachloroethylene. As defined by the EURL-AP in the Standard Operating Procedure (SOP) *"Operational protocol for the combination of light microscopy and PCR"*[2], the double sedimentation protocol must only be performed following the single sedimentation protocol and applied only on feed or feed materials intended for ruminant when terrestrial vertebrates' remains couldn't be disclose from a first single tetrachloroethylene sedimentation.

This situation requires two separate protocols to be applied to the same feedstuff rather than a single procedure, which would otherwise reduce the use of toxic reagents and save time. During several workshops of the EURL-AP network, the possibility of streamlining the sedimentation process by ultimately using a single protocol was therefore proposed by some national reference laboratories and discussed.

To assess whether the use of a single protocol could be envisaged, the EURL-AP, on agreement of DG Santé, conducted the present internal study comparing the effectiveness of flotates of the two sedimentation protocols. The objective of this study was to determine whether the double sedimentation protocol could provide results as reliable and conclusive as those obtained with the single sedimentation protocol for the flotates.

2. Material and method

Sample set

For this study, the EURL-AP selected several samples from past EURL-AP proficiency tests[3–7] for which mandatory homogeneity studies had already delivered results. To best represent the diversity of particles that can be observed by light microscopy, seven samples with different compositions were chosen:

	Matrix	Adulterant	Reference
A	Aquafeed	Porcine PAP 1%	2019-sample 8 [3]
B	Pig feed	Porcine haemoglobin 1%	2021-sample 4 [4]
C	Poultry feed	Ruminant PAP 0.1 % Porcine PAP 0.1 % Poultry PAP 0.1 %	2022-sample 1 [5]
D	Pig feed	Milk powder 2 %	2022-sample 2 [5]

E	Sheep feed	Porcine blood meal 0.5 % Bovine hairs 0.1 %	2023-sample 7 [6]
F	Pig feed	Feather meal 0.1 % Egg powder 1 %	2024-sample 1 [7]
G	Poultry feed	*Tenebrio molitor* meal 0.5 %	2024-sample 3 [7]

Sample preparation and observations

To compare the results obtained from the two sedimentation protocols, 10 entities of each sample were subjected to 10 single TCE sedimentations and 10 double PE / TCE sedimentations, producing each ten flotates.

The 10 flotates with densities < 1.62 (specific gravity) obtained from the single TCE sedimentation and the 10 flotates < 1.26 (specific gravity) from the double PE / TCE sedimentation (also refer as 'final flotate'[1]) were examined. These fractions only were used to assess the effectiveness of the two protocols. All flotates were sieved using a 250 μm square mesh sieve before slide mounting and only the < 250 μm fractions were used.

Based on these flotate fractions, 4 microscope slides were prepared for each repetition using different mounting media: two slides mounted in glycerol without staining, one slide stained with Fehling's solution, and one slide stained with cystine or TMB / H_2O_2, depending on the adulterant.

In total, 80 slides were examined for each sample – 40 per type of flotate.

Qualitative results sorting

Results of the microscopic observations were established in accordance with the EURL-AP SOP "*Observation flowchart by light microscopy*"[8] and Annex VI of Commission Regulation (EC) No 152/2009[1].

Therefore, based on the decision limit set at a minimum of 5 particles, considering the 4 slides observed per repetition, the flotate fractions could be declared positive, negative or < LOD for the detection of constituents of animal origin.

Data treatment and statistics

All data obtained were compiled and are presented in **Annex 1**. Statistical analyses were performed using Spyder 6 with Python 3.13.9.

To compare the results obtained with the single sedimentation TCE protocol and the double sedimentation PE / TCE protocol, a McNemar test[9] for significance of change was

applied. This non-parametric test is used to compare two proportions from paired samples. In this study, samples are considered paired because several observations were made on the same sample. The McNemar test does not consider cases in which both methods yield either negative or positive results; it is based exclusively on discordant pairs, where the two methods produce different outcomes. The test statistic, with continuity correction, was calculated as:

$$X^2 = \frac{(|b - c| - 1)^2}{b + c}$$

where *b* and *c* represent the numbers of discordant pairs. A pair is considered discordant when the results of the two measurements are different. In our case, *b* will represent the number of positive results obtained exclusively via single TCE sedimentation and *c* the number of positive results obtained exclusively via double PE / TCE sedimentation. As the test cannot accommodate results reported as < LOD, these values were deliberately classified as negative.

To complement the results obtained with the McNemar test, an odds ratio[10] was used. The odds ratio is a statistical measure that quantifies the strength and direction of the association between two methods. In simple terms, it indicates how much more likely one method is to yield one result compared with the other. For paired data, the odds ratio was calculated as:

$$OR = \frac{b}{c}$$

where *b* and *c* correspond to the numbers of positive results obtained exclusively via the single TCE sedimentation and via the double PE / TCE sedimentation, respectively. If the odds ratio value is greater than 1, then method A (TCE sedimentation) is considered more effective than method B (PE / TCE sedimentation), and vice versa if the value is less than 1. To be interpreted correctly, the odds ratio must be associated with a 95% confidence interval (CI). If this interval does not contain 1, we can conclude that there is a significant difference between the two methods. On the other hand, if the CI contains 1, there is no significant difference between the two methods but simply a trend.

3. Results and discussion

Among the seven samples analysed, samples A, B, and E were not conclusive in determining which of the two sedimentation protocols was more effective [**Annex 1**]. Effectively, for these three samples, all flotates were declared positive for the animal constituents present independently of the sedimentation mode. No qualitative

difference in the observations could be noticed: muscle fibres, blood globules were observed in sample A and B. For sample E (containing hairs), although the overall systematic positive result outcome obtained by each sedimentation method, it could nevertheless be noted that the double PE / TCE sedimentation had a more concentrating impact on the hair presence in the final flotate, resulting in a higher frequency of hair fragments on the slides. Although this small qualitative influence on sample E, in general for these samples no impact of the sedimentation protocol could be determined for the results expression.

In contrast, different outcomes were observed for samples C, D, F, and G which are detailed hereafter. Given the limited number of observations per sample, data augmentation was performed to allow statistical analysis of these samples. For each sample, new combinations of the observed slides were generated to produce additional results. This approach was based on combinations of three slides rather than four, to reflect the minimum number of observations required under current legislation. This combination of observed slides allowed us to obtain 120 new results for each sample. The resulting outcomes were binary: a result was considered positive when ≥ 5 particles were observed across the three slides, and negative when < 5 particles were observed.

Sample C: Poultry feed + 0.1 % ruminant PAPs + 0.1 % porcine PAPs + 0.1 % poultry PAPs

Basic observations for this sample revealed that muscle detection, at levels above the limit of decision, in the flotates was more readily achieved using the double sedimentation protocol (9/10) than the single sedimentation protocol (5/10) (**Table 1**). This difference can be explained by the ability of the double PE / TCE sedimentation protocol to concentrate particles of interest and, thus, reduce the proportion of inconclusive particles in the flotate fraction < 1.26. The lowered number of non-relevant particles improves the visibility of the particles of interest.

Table 1. Basic results of the 10 microscopic observations carried out based on the four slides from the flotates < 1.62 and < 1.26 for sample C.

Flotate number	Conclusion for single sedimentation TCE	Conclusion for double sedimentation PE/TCE
1	< LOD	+
2	+	+
3	+	+
4	+	+
5	+	+
6	+	-
7	< LOD	+
8	< LOD	+
9	< LOD	+

10	< LOD	+

Following the data augmentation for this sample, the number of positive and negative results for each type of sedimentation was compiled in **Table 2**.

Table 2. Number of positive and negative results obtained with data augmentation for single TCE sedimentation and double PE / TCE sedimentation for sample C (discordant pairs are in italics).

	+ by PE / TCE	- by PE / TCE	Totals
+ by TCE	12	*4*	16
- by TCE	*91*	13	104
Totals	103	17	240

Based on these observations, the McNemar test was applied, yielding a *p-value* < 0.001 (***). The odds ratio calculated following the test indicates a value of 0.04 (95%CI [0.016 ; 0.120], suggesting that double sedimentation has a better ability to detect the presence of particles for this type of sample. These results demonstrate a significant difference between the two sedimentations, with the double PE / TCE sedimentation allowing a better detection of muscles particles

Sample D: Pig feed + 2% of milk

In the case of dairy product detection, and more specifically lactose crystals, the single TCE sedimentation protocol yielded more positive results (7/10) than the double PE / TCE sedimentation protocol (0/10) (**Table 3**). The density of lactose crystals (1.53) explains the difficulty in detecting milk in flotates with densities < 1.26 since many crystals settled down and concentrated in the intermediate flotate.

Table 3. Basic results of the 10 microscopic observations carried out based on the four slides from the flotates < 1.62 and < 1.26 for sample D.

Flotate number	Conclusion for single sedimentation TCE	Conclusion for double sedimentation PE/TCE
1	+	-
2	+	-
3	+	< LOD
4	+	< LOD
5	< LOD	< LOD
6	< LOD	< LOD
7	< LOD	< LOD
8	+	< LOD
9	+	-
10	+	-

For this sample, following the data augmentation, the number of positive and negative observations for each type of sedimentation was compiled in **Table 4**. The McNemar test was applied on these data, yielding a *p-value* < 0.001 (***). For this sample, given absence of positive observation results for the double PE / TCE sedimentation, the odds ratio could not be calculated. These results demonstrate again a significant difference between the two sedimentations, with this time the single TCE sedimentation tending to allow better detection of milk particles.

Table 4. Number of positive and negative results obtained with data augmentation for single TCE sedimentation and double PE / TCE sedimentation for sample D (discordant pairs are in italics).

	+ by PE / TCE	- by PE / TCE	Totals
+ by TCE	3	*113*	116
- by TCE	*0*	4	4
Totals	3	117	240

Sample F: Pig feed + 0.1 % feather meal + 1 % egg powder

For this sample, the double PE / TCE sedimentation protocol produced more positive results for feather detection (9/10) than the single TCE sedimentation protocol (0/10) (**Table 5**). As observed for muscle detection, this difference can be attributed to the concentration of relevant particles and the reduction of the proportion of inconclusive particles in the flotate fraction < 1.26, leading to improved feather visibility.

Table 5. Basic results of the 10 microscopic observations carried out based on the four slides from the flotates < 1.62 and < 1.26 for sample F.

Flotate number	Conclusion for single sedimentation TCE	Conclusion for double sedimentation PE/TCE
1	< LOD	+
2	< LOD	+
3	-	+
4	-	+
5	-	+
6	-	+
7	-	-
8	-	+
9	-	+
10	-	+

Following the data augmentation, the number of positive and negative observations for each type of sedimentation was compiled in **Table 6**. The McNemar test was applied yielding a *p-value* < 0.001 (***). For this sample, also, given the total absence of positive observation results for the single TCE sedimentation, the odds ratio cannot be calculated. These results suggest a significant difference between the two

sedimentations, with the double PE / TCE sedimentation tending to allow better detection of feather particles.

Table 6. Number of positive and negative results obtained with data augmentation for single TCE sedimentation and double PE / TCE sedimentation for sample F (discordant pairs are in italics).

	+ by PE / TCE	- by PE / TCE	Totals
+ by TCE	0	*0*	0
- by TCE	*120*	0	120
Totals	120	0	240

Sample G: Poultry feed + 0.5 % *Tenebrio molitor* larvae meal

As the double PE / TCE sedimentation protocol was initially developed to facilitate the detection of insect-derived particles, it was expected that this would facilitate the observation of insect particles. Based on the 10 basic microscopic observation results, the difference between the two sedimentation was nevertheless not evident (**Table 7**).

Table 7. Basic results of the 10 microscopic observations carried out based on the four slides from the flotates < 1.62 and < 1.26 for sample G.

Flotate number	Conclusion for single sedimentation TCE	Conclusion for double sedimentation PE/TCE
1	+	+
2	+	< LOD
3	+	+
4	< LOD	< LOD
5	< LOD	+
6	+	+
7	+	+
8	< LOD	+
9	< LOD	< LOD
10	+	+

However, following the results obtained with data augmentation (**Table 8**), the application of the McNemar test yielded a *p-value* < 0.001 (***), combined with an odds ratio of 0.05 (95%CI [0.012 ; 0.197]). This result suggests that double PE / TCE sedimentation is more effective than single TCE sedimentation for detecting insect particles.

Table 8. Number of positive and negative results obtained with data augmentation for single TCE sedimentation and double PE / TCE sedimentation for sample G (discordant pairs are in italics).

	+ by PE / TCE	- by PE / TCE	Totals
+ by TCE	74	*2*	76

- by TCE	*42*	2	44
Totals	116	4	240

Effectiveness: single against double sedimentation

To assess which of the two sedimentation protocols was more effective for an overall animal particle detection in flotates, the analysis focused on compilation of samples showing divergent results between the two methods (samples C, D, F, and G).

All observation results obtained through data augmentation and used for the McNemar test are shown in **Table 9**. Following the test, a *p-value* < 0.001 (***) was obtained. For the whole dataset, an odds ratio of 0.47 (95%CI [0.378 ; 0.585]) was achieved. These results seem to indicate that, for the detection of animal particles in flotates, there is a significant difference between the two sedimentation protocols. This difference tends towards the double PE / TCE sedimentation, which appears to be at large more effective than the single TCE sedimentation

Table 9. Number of positive and negative results obtained with data augmentation for single TCE sedimentation and double PE / TCE sedimentation for sample C, D, F and G (discordant pairs are in italics).

	+ by PE / TCE	- by PE / TCE	Totals
+ by TCE	89	*119*	208
- by TCE	*253*	19	272
Totals	342	138	960

However, this varies depending on the type of sample. For example, the results obtained for sample D show that single TCE sedimentation is significantly more effective for detecting milk than double PE / TCE sedimentation.

4. Conclusions

The study conducted to evaluate the effectiveness of the single TCE sedimentation compared with the double PE / TCE sedimentation revealed an overall advantage in favour of the double PE / TCE sedimentation protocol for improving microscopic observations on the floating fractions. However, as demonstrated in detail, the relative performance of each protocol is matrix-dependent. In certain cases, particularly for milk detection, the single TCE sedimentation proved more suitable. Nevertheless, in this case, it has to be noted that milk powder is an authorised ingredient in animal feed, including feed intended for ruminants[2]. Although its presence must currently be reported, when detected, milk powder is not considered as a PAP and, therefore, it does not influence the TSE control policy.

At present, the use of the double PE / TCE sedimentation protocol is not systematic since it depends on the outcome of microscopic observations obtained following a first single TCE sedimentation. Furthermore, the double PE/TCE sedimentation is only limited to feed or feed materials intended to ruminants. Adopting the double PE / TCE sedimentation as a sole applicable protocol, regardless of the context – understanding the type or material or its intended use – , would inevitably increase sample handling time (longer sedimentation time). Nevertheless, this option could also ultimately reduce overall observation time by limiting matrix interference in the flotate fraction < 1.26, thereby facilitating particle identification.

Acknowledgement

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Annex 1

Sample	Flotate	Single sedimentation TCE - flotate <1.62						Double sedimentation PE/TCE - flotate <1.26					
		Slide 1 Glycerol	Slide 2 Glycerol	Slide 3 Fehling	Slide 4 Cystine	Slide 5 TMB/H2O2	Conclusion	Slide 1 Glycerol	Slide 2 Glycerol	Slide 3 Fehling	Slide 4 Cystine	Slide 5 TMB/H2O2	Conclusion
Aquafeed + 1 % porcine PAP	1	>5 muscles, 1 red globule	>5 muscles, 1 red globule	>5 muscles, 2 red globules		bubble release + green staining	+	>5 Muscles, >5 red globules	4 Muscles, >5 red globules	> 5 muscles, > 5 red globules		bubble release + green staining	+
	2	>5 muscles, >5 red globules	>5 muscles, 3 Particules rouge	>5 muscles, 2 red globules		bubble release + green staining	+	>5 Muscles, >5 red globules	>5 Muscles, >5 red globules	> 5 Muscles, > 5 red globules		bubble release + green staining	+
	3	>5 muscles,2 red globules	>5 muscles, 3 Particules rouge	>5 muscles, 2 red globules		bubble release + green staining	+	>5 Muscles, >5 red globules	>5 Muscles, >5 red globules	>5 Muscles, >5 red globules		bubble release + green staining	+
	4	>5 muscles,4 red globules	>5 muscles, 3 Particules rouge	>5 muscles, 2 red globules		bubble release + green staining	+	>5 Muscles, 3 red globules	>5 Muscles, 1 red globule	>5 Muscles, 4 red globules		bubble release + green staining	+
	5	>5 muscles,5 red globules	>5 muscles, 2 Particules rouge	>5 muscles, >5 red globules		bubble release + green staining	+	>5 Muscles, 3 red globules	>5 Muscles, 2 red globules	>5 Muscles, >5 red globules		bubble release + green staining	+
	6	>5 muscles, >5 red globules	>5 muscles, >5 red globules	>5 muscles, 2 red globules		bubble release + green staining	+	>5 Muscles, >5 red globules	>5 Muscles, >5 red globules	>5 Muscles, >5 red globules		bubble release + green staining	+
	7	>5 muscles, 3 red globules	>5 muscles, >5 red globules	>5 muscles, >5 red globules		bubble release + green staining	+	>5 Muscles, 3 red globules	>5 Muscles,5 red globules	>5 Muscles, >5 red globules		bubble release + green staining	+
	8	>5 muscles, 4 red globules	>5 muscles, >5 red globules	>5 muscles, >5 red globules		bubble release + green staining	+	>5 Muscles, >5 red globules	>5 Muscles, >5 red globules	>5 Muscles, >5 red globules		bubble release + green staining	+
	9	>5 muscles,2 red globules	>5 muscles, 4 Particules rouge	>5 muscles, 3 red globules		bubble release + green staining	+	>5 Muscles, >5 red globules	>5 Muscles, >5 red globules	>5 Muscles, >5 red globules		bubble release + green staining	+
	10	>5 muscles,2 red globules	>5 muscles, 3 Particules rouge	>5 muscles, 2 red globules		bubble release + green staining	+	>5 Muscles, >5 red globules	>5 Muscles, >5 red globules	>5 Muscles, >5 red globules		bubble release + green staining	+
Pig feed + 1 % porcine haemoglobin	1	red globules >5	red globules >5	red globules >5		bubble release + green staining	+	red globules >5	red globules >5	red globules >5		bubble release + green staining	+
	2	red globules >5	red globules >5	red globules >5		bubble release + green staining	+	red globules >5	red globules >5	red globules >5		bubble release + green staining	+
	3	red globules >5	red globules >5	red globules >5		bubble release + green staining	+	red globules >5	red globules >5	red globules >5		bubble release + green staining	+

Group	#												
	4	red globules >5	red globules >5	red globules >5		bubble release + green staining	+	red globules >5	red globules >5	red globules >5		bubble release + green staining	+
	5	red globules >5	red globules >5	red globules >5		bubble release + green staining	+	red globules >5	red globules >5	red globules >5		bubble release + green staining	+
	6	red globules >5	red globules >5	red globules >5		bubble release + green staining	+	red globules >5	red globules >5	red globules >5		bubble release + green staining	+
	7	red globules >5	red globules >5	red globules >5		bubble release + green staining	+	red globules >5	red globules >5	red globules >5		bubble release + green staining	+
	8	red globules >5	red globules >5	red globules >5		bubble release + green staining	+	red globules >5	red globules >5	red globules >5		bubble release + green staining	+
	9	red globules >5	red globules >5	red globules >5		bubble release + green staining	+	red globules >5	red globules >5	red globules >5		bubble release + green staining	+
	10	red globules >5	red globules >5	red globules >5		bubble release + green staining	+	red globules >5	red globules >5	red globules >5		bubble release + green staining	+
Poultry feed + 0.1 % ruminant PAP + 0.1 % porcine PAP + 0.1 % poultry PAP	1	Blank	Blank	Blank	3 muscles		< LOD	Blank	2 muscles	>10 muscles	7 muscles		+
	2	Blank	Blank	3 muscles	7 muscles		+	Blank	Blank	2 muscles	4 muscles		+
	3	Blank	3 muscles	4 muscles	5 muscles		+	Blank	1 muscle	8 muscles	4 muscles		+
	4	Blank	1 muscle	1 muscle	5 muscles		+	2 muscles	1 muscle	7 muscles	8 muscles		+
	5	Blank	1 muscle	2 muscles	4 muscles		+	4 muscles	4 muscles	>10 muscles	>10 muscles		+
	6	Blank	2 muscles	2 muscles	4 muscles		+	Blank	Blank	Blank	Blank		-
	7	Blank	2 muscles	3 muscles	1 muscle		< LOD	Blank	1 muscle	1 muscle	10 muscles		+
	8	Blank	Blank	Blank	3 muscles		< LOD	>10 muscles	4 muscles	>10 muscles	3 muscles		+
	9	Blank	Blank	1 muscle	1 muscle		< LOD	Blank	4 muscles	10 muscles	1 muscle		+
	10	Blank	1 muscle	Blank	2 muscles		< LOD	2 muscles	8 muscles	5 muscles	>10 muscles		+
Pig feed + 2 % milk	1	6 lactose crystals	4 lactose crystals	Blank	Blank		+	Blank	Blank	Blank	Blank		-
	2	2 lactose crystals	5 lactose crystals	Blank	Blank		+	Blank	Blank	Blank	Blank		-
	3	5 lactose crystals	4 lactose crystals	4 lactose crystals	3 lactose crystals		+	Blank	4 lactose crystals	Blank	Blank		< LOD
	4	4 lactose crystals	3 lactose crystals	Blank	Blank		+	1 lactose crystal	Blank	Blank	Blank		< LOD
	5	3 lactose crystals	1 cristal de lactose	Blank	Blank		< LOD	1 lactose crystal	Blank	Blank	Blank		< LOD
	6	1 cristal de lactose	3 lactose crystals	Blank	Blank		< LOD	1 lactose crystal	Blank	Blank	Blank		< LOD

	#												
	7	3 lactose crystals	2 lactose crystals	Blank	Blank		< LOD	Blank	1 lactose crystal	Blank	Blank		< LOD
	8	9 lactose crystals	8 lactose crystals, 1 feather	Blank	Blank		+	Blank	3 lactose crystals	Blank	Blank		< LOD
	9	4 lactose crystals	3 lactose crystals	Blank	Blank		+	Blank	Blank	Blank	Blank		-
	10	9 lactose crystals	8 lactose crystals, 1 feather	Blank	Blank		+	Blank	Blank	Blank	Blank		-
Sheep feed + 0.5 % porcine blood meal + 0.1 % cow hairs	1	5 hairs	5 hairs	4 hairs		red globules + bubble release + staining	+	>10 hairs, > 10 red globules	>10 hairs, > 10 red globules	>10 hairs, > 10 red globules		>10 hairs, red globules (>10) + bubble release + staining	+
	2	2 hairs	7 hairs	2 hairs		red globules + bubble release + staining (5)	+	>10 hairs, > 10 red globules	>10 hairs, > 10 red globules	>10 hairs, > 10 red globules		>10 hairs, red globules (>10) + bubble release + staining	+
	3	2 hairs	4 hairs	2 hairs		red globules + bubble release + staining (>10)	+	>10 hairs, > 10 red globules	>10 hairs, > 10 red globules	>10 hairs, > 10 red globules		>10 hairs, red globules (>10) + bubble release + staining	+
	4	5 hairs	7 hairs	3 hairs		hairs, red globules + bubble release + staining (5)	+	>10 hairs, > 10 red globules	>10 hairs, > 10 red globules	>10 hairs, > 10 red globules		>10 hairs, red globules (>10) + bubble release + staining	+
	5	>10 hairs	6 hairs	7 hairs		hairs, red globules + bubble release + staining (>10)	+	>10 hairs, > 10 red globules	>10 hairs, > 10 red globules	>10 hairs, > 10 red globules		>10 hairs, red globules (>10) + bubble release + staining	+
	6	6 hairs + red globules	10 hairs + red globules	8 hairs + red globules		red globules (6) + bubble release + staining verte	+	>10 hairs, > 10 red globules	>10 hairs, > 10 red globules	>10 hairs, > 10 red globules		>10 hairs, red globules (>10) + bubble release + staining	+
	7	5 hairs + red globules	>10 hairs + red globules	5 hairs		red globules (4) + bubble release + staining verte	+	>10 hairs, > 10 red globules	>10 hairs, > 10 red globules	>10 hairs, > 10 red globules		>10 hairs, red globules (>10) + bubble release + staining	+
	8	10 hairs + red globules	7 hairs + red globules	7 hairs + red globules		red globules (>10) + bubble release + staining verte	+	>10 hairs, > 10 red globules	>10 hairs, > 10 red globules	>10 hairs, > 10 red globules		>10 hairs, red globules (>10) + bubble release + staining	+

Group	#						Result						Result
	9	4 hairs + red globules	7 hairs + red globules	7 hairs + red globules		red globules (7) + bubble release + staining verte	+	>10 hairs, > 10 red globules	>10 hairs, > 10 red globules	>10 hairs, > 10 red globules		>10 hairs, red globules (>10) + bubble release + staining	+
	10	6 hairs + red globules	7 hairs + red globules	5 hairs + red globules		red globules (10) + bubble release + staining verte	+	>10 hairs, > 10 red globules	>10 hairs, > 10 red globules	>10 hairs, > 10 red globules		>10 hairs, red globules (>10) + bubble release + staining	+
Pig feed + 0.1 % feather meal + 1 % egg powder	1	Blank	Blank	2 muscles	1 plume		< LOD	5 feathers	>5 feathers	>5 feathers	>5 feathers		+
	2	Blank	Blank	1 plume, 1 acarien	Blank		< LOD	>5 feathers	>5 feathers	>5 feathers	>5 feathers		+
	3	Blank	Blank	Blank	1 plume		-	>5 feathers	>5 feathers	>5 feathers	>5 feathers		+
	4	Blank	Blank	Blank	Blank		-	>5 feathers	>5 feathers	>5 feathers	>5 feathers		+
	5	Blank	Blank	Blank	Blank		-	>5 feathers	>5 feathers	3 feathers	>5 feathers		+
	6	Blank	Blank	Blank	Blank		-	>5 feathers	>5 feathers	1 feathers	>5 feathers		+
	7	Blank	Blank	>5 muscles ?	Blank		-	>5 feathers	>5 feathers	3 feathers	>5 feathers		+
	8	Blank	Blank	Blank	Blank		-	1 feather	Blank	Blank	Blank		-
	9	Blank	Blank	Blank	Blank		-	>5 feathers	>5 feathers	>5 feathers	>5 feathers		+
	10	Blank	Blank	Blank	Blank		-	2 feathers	2 feathers	4 feathers	3 feathers		+
Poultry feed + 0.5 % *Tenebrio molitor* larvae meal	1	3 insect particles	1 insect particle	4 insect particles + 1 muscle	3 insect particles		+	>5 insect particles	>5 insect particles	3 insect particles + 1 muscle	6 insect particles		+
	2	Blank	5 insect particles	2 insect particles + 3 muscles	1 insect particle		+	1 insect particles	3 insect particles	Blank	1 insect particles		< LOD
	3	Blank	3 insect particles	1 insect particle + 1 muscle	3 insect particles + 1 muscle		+	>5 insect particles	>5 insect particles	>5 insect particles	>5 insect particles		+
	4	Blank	Blank	1 insect particle + 1 muscle	2 insect particles		< LOD	3 insect particles	Blank	1 insect particles	Blank		< LOD
	5	Blank	Blank	2 muscles	Blank		< LOD	>5 insect particles	>5 insect particles	>5 insect particles	>5 insect particles		+
	6	1 insect particle	2 insect particles	2 insect particles + 2 muscles	Blank		+	>5 insect particles	>5 insect particles	>5 insect particles	>5 insect particles		+

7	1 insect particle	2 insect particle	2 insect particles + 2 muscles	Blank		+	2 insect particles	4 insect particles	Blank	Blank		+
8	Blank	1 insect particle	1 insect particle	Blank		< LOD	2 insect particles	4 insect particles	1 insect particles	1 insect particles		+
9	1 insect particle + 1 antenne	Blank	Blank	1 muscle		< LOD	3 insect particles	2 insect particles	Blank	Blank		< LOD
10	Blank	1 insect particle	1 insect particle + 2 muscles	2 insect particles		+	>5 insect particles	>5 insect particles	>5 insect particles	>5 insect particles		+