final report

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Feed Test Validation

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ABSTRACT

The Ruminant Feed Ban is a cooperative initiative implemented by the Australian Government, the State and Territory Governments and the livestock and stockfeed industries to ensure that ruminant stockfeeds are not contaminated with Restricted Animal Materials. In support of this, a suitable testing regime is required to demonstrate freedom from contamination.

As there are currently no internationally recognised standards for the detection of RAM in ruminant stockfeeds, work is required to develop a testing capability to enable industry to demonstrate compliance with the Ruminant Feed Ban and to underpin regulation. This is the first of three projects aimed at developing this capability.

This project was able to validate a testing regimen consisting of a Lateral Flow Device (LFD) as a screening test and microscopy as a confirmatory test. The ability of two Polymerase Chain Reaction (PCR) tests to accurately identify the species from which RAM contaminant was derived was also validated.

BACKGROUND

This report presents relevant information relating to testing stockfeed for Restricted Animal Material (RAM) for consideration by State & Territory Government and industry stakeholders that are involved in the Ruminant Feed Ban. Specifically, the results presented in this report evaluate the suitability of feed tests for identifying RAM in ruminant feed stuffs.

There is currently no international standard for the testing for animal materials in feed stuff. Nor is there any agreement relating to the suitability or preferences of testing methods.

In February 2005 a Working Group of the Transmissible Spongiform Encephalopathies Freedom Assurance Program (TSEFAP) National Technical Committee (NTC) was convened to make recommendations to the TSEFAP National Advisory Committee (NAC) on technical aspects relating to the implementation of a stockfeed testing system to support the Ruminant Feed Ban in Australia. That report made 12 recommendations.

The Working Group concluded that a test system to detect RAM in domestically manufactured stockfeed should comprise a lateral flow device (LFD) for initial screening, with classical microscopy as the confirmatory test method. It was noted that Polymerase Chain Reaction (PCR) should continue to be used for import testing (if required) and such technology may eventually replace microscopy as a confirmatory test once further developed and evaluated.

The recommendations of most importance are listed below:

2. That a national laboratory capability be developed based on a test system using a lateral flow device (LFD) for screening and classical microscopy for confirmation and validated in a well designed study

6. That the value of PCR, in identifying the animal species in feeds positive for RAM by microscopy and for import testing for other types of RAM in fish meal, be assessed in properly designed blind studies.

8. That a domestic, risk-based random sampling protocol be developed as a second component once compliance testing is in place. Development of this system should be staged, beginning with a cost-benefit assessment with agreement regarding roles and responsibilities of the jurisdictions, the Commonwealth and industry, then followed by a pilot study of a number of selected manufacturers, resellers and end-users with a national random sample survey with design parameters derived from the pilot study.

9. Even though this is beyond the TOR of this project, the working group feels that the pilot study proposed in Recommendation 8 should be implemented in collaboration with the Stock Feed Manufacturers' Council of Australia (SFMCA) and also used to evaluate the efficacy of the existing equipment cleaning techniques for preventing cross-contamination with RAM as described in the FeedSafe QA program. This study be done before implementing the national random sample survey also proposed in Recommendation 8 above.

The purpose of this suite of projects is to ensure Australia has the ability to support the ruminant feed ban legislation requirements so that government and industry can

demonstrate compliance with the feed ban via official inspections[1] supported by a sampling and testing program capable of detecting RAM in manufactured ruminant feeds at all levels of production and use of stockfeed. Such a program would utilise a test or suite of tests that are "fit for purpose".

High screening sensitivity is required to ensure few or no true positives are missed. High specificity of the confirmatory procedure permits differentiation of true from false positive screening test results. At the confirmatory stage, tests able to identify the animal species from which the prohibited material came would be useful as they pose different levels of risk in terms of transmission of BSE.

This project is the first of three projects that address the recommendations of the *Feed Tests for RAM in Stockfeed Working Group Report* (Animal Health Australia, July 2005). This work addresses Recommendations 2 & 6 of the Report, which state:

2. *That a national laboratory capability be developed based on a test system using a lateral flow device (LFD) for screening and classical microscopy for confirmation and validated in a well designed study*

6. *That the value of PCR, in identifying the animal species in feeds positive for RAM by microscopy and for import testing for other types of RAM in fishmeal, be assessed in properly designed blind studies*

[1] Australian Ruminant Feed Ban Compliance Scheme – National uniform guidelines for Ensuring Compliance Through Inspection, Sampling and Testing Programs (http://www.animalhealthaustralia.com.au/tsefap/feedban_guidelines.pdf)

RECOMMENDATIONS

From the results of this project alone, it is recommended that:

1. A testing regimen (for quality assurance purposes) for the detection of RAM in ruminant stockfeeds be implemented that consists of the *FeedChek*® LFD at 1.0% Limit of Detection as a screening test and microscopy as a confirmatory test.

2. For regulatory purposes, microscopy could be implemented as a confirmatory test, either in conjunction with the LFD as a screening test or as a stand alone test.

3. That the AB PCR, NMI PCR and microscopy tests are suitable for use as confirmatory tests, if and when required.

4. That the *FeedChek*® LFD at 1.0% is suitable for use as a screening test for the purposes of QA for the production of stockfeed in single line mills producing feeds with and without RAM.

5. That the results be referred to the TSEFAP National Technical Committee for use in determining a suitable testing regime for underpinning the ruminant feed ban.

6. That AQIS review the test used for testing imported feed stuffs for RAM as it presently does not cover all species that constitute RAM.

7. This report is not to be made public unless SAFEMEAT Partners agrees to the release.

AIM & OBJECTIVES

The aim of the study is to contribute to the development of a suitable testing regimen for the detection of RAM in ruminant stockfeed, for inclusion in Australia's Ruminant Feed Ban.

The objectives of this study are to:

1. Assess in a blind study the value of PCR for identifying the species of RAM contamination found positive via classical microscopy.
2. Validate a testing system using a lateral flow device (LFD) for screening and classical microscopy for confirmation of all screened positive samples.

These objectives relate to delivering Recommendations 2 and 6 of the *Feed Tests for RAM in Stockfeed Working Group Report* (Animal Health Australia, July 2005), which identify:

> *2. That a national laboratory capability be developed based on a test system using a lateral flow device (LFD) for screening and classical microscopy for confirmation and validated in a well designed study*
>
> *6. That the value of PCR, in identifying the animal species in feeds positive for RAM by microscopy and for import testing for other types of RAM in fishmeal, be assessed in properly designed blind studies*

These objectives are to be achieved by dividing this project into two sub-projects, "Sub-Project A" and "Sub-Project B". The Materials and Methods and Results are addressed separately, below.

METHODS

Sub-Project A

Two PCR tests, one produced by the National Measurement Institute (NMI) (no name provided) the other produced by Agrigen Biotech (AB) (known as *Ruminant Screen*®) were used to test (blind) fish meal samples spiked with variable concentrations of bovine, ovine/hircine, and porcine material. The concentrations of the various RAM materials will be replicated in triplicate using the following concentrations:

- 0.00% (control)
- 0.15 %
- 0.30 %
- 0.50 %
- 1.00 %

The number of samples tested was 3 (triplicate) X 2 (tests) x 5 (concentrations) x 3 (species of RAM), resulting in a total of 90 samples.

The fish meal tested for this Sub-Project was tested negative for other types of RAM prior to use.

The Asia Pacific Laboratory Accreditation Cooperation (APLAC) (a subsidiary of NATA) conducted an international proficiency testing study for laboratories performing tests for the identification of RAM in feedstuffs (APLAC T047 Animal Materials in Feedstuff PT Program, July 2006). This study involved 22 laboratories from 15 countries and included two laboratories that used the NMI and AB PCR tests. As the methodology of that study was identical to this Sub-Project, the results of the APLAC trial have been applied to this work and no further testing was undertaken.

The final report for the APLAC T047 study can be found at Appendix 1, which contains full details of the materials and methods.

Sub-Project B

The two PCR tests (NMI and *Ruminant Screen*®), the LFD (*FeedChek*®) and classical microscopy were used to test (blind) commercially produced ruminant feed spiked with five different types of RAM (bovine, ovine, porcine, poultry and fish) at six different concentrations which were replicated three times. The various concentrations were:

- 0.00% (control)
- 0.05%
- 0.10 %
- 0.25 %
- 0.50 %
- 1.00 %

There were a total number of 288 samples tested as part of this project. Table 1 shows what samples were tested and the quantities tested for each test.

Table 1: Sample Testing Regime

	NMI PCR	**AB PCR** (***Ruminant Screen***®)	**LFD** (***FeedChek***®)	**Classical Microscopy**
Bovine	x 6 conc x triplicate	x 6 conc x triplicate	x 6 conc x triplicate	x 6 conc x triplicate
Ovine	x 6 conc x triplicate	x 6 conc x triplicate	x 6 conc x triplicate	x 6 conc x triplicate
Porcine	No testing[2]	x 6 conc x triplicate	x 6 conc x triplicate	x 6 conc x triplicate
Poultry	No testing[2]	x 6 conc x triplicate	x 6 conc x triplicate	x 6 conc x triplicate
Fish	No testing[2]	No testing[2]	x 6 conc x triplicate	x 6 conc x triplicate

The ruminant feed (supplied by a commercial feedlot) used for this sub-project was tested negative for RAM via classical microscopy prior to use.

Further details of the preparation and randomisation of samples can be found at Appendix 2.

[2] Testing was not undertaken as these tests cannot detect DNA from the species being used.

RESULTS

Sub-Project A

The full report of APLAC T047, at Appendix 1, identifies that samples were divided into three groups. These were Sample A (porcine-derived samples), Sample B (bovine-derived samples) and Sample C (ovine / hircine-derived samples).

Although each laboratory is represented by a code in Appendix 1, it can be revealed that the laboratory using the NMI test is represented by Code T047-009 and the laboratory using the AB test is represented by code T047-025.

Results from Appendix 1 show the following:

Table 2 Summary of Results for each PCR test in identifying contaminated and non-contaminated samples

	Sample A	**Sample B**	**Sample C**
NMI	**No result – not tested**	**Satisfactory**	**Satisfactory**
AB	**Satisfactory**	**Satisfactory**	**Satisfactory**

In this trial, neither test produced any false positive or false negative results. In fact, of the 21 laboratories reporting results:

- For Sample A, the laboratory using the AB test was one of five reporting no false positive or false negative results.
- For Sample B, the laboratories using the NMI and AB tests were two of ten reporting no false positive or false negative results.
- For Sample C, the laboratories using the NMI and AB tests were two of ten reporting no false positive or false negative results.

In addition to the laboratory using the AB test, the following laboratories achieved no false positives or false negatives for all three sample groups:

- T047-001
- T047-008
- T047-021

In addition to the laboratories using the NMI and AB tests, the following laboratories achieved no false positive or false negative results for sample groups B and C:

- T047-006
- T047-018
- T047-023
- T047-024

Sub-Project B

This project was to evaluate the performance of four tests that are designed to identify RAM in ruminant feeds. All results provided are qualitative in nature and therefore only show whether a test detected the presence or absence of RAM in the sample being tested. To assess the tests for their quantitative analysis capabilities was not within the Terms of Reference for this study.

Appendix 2 lists the raw results of the testing undertaken.

In order to measure the overall performance of the tests in identifying RAM, the following parameters were calculated:

- the number of true positives (TP)
- the number of true negatives (TN)
- the number of false positives (FP)
- the number of false negatives (FN)

In addition, the accuracy, sensitivity and specificity of each test were calculated as a basis of measuring the performance of the test and the laboratory undertaking the testing. These measurements are expressed as percentages by multiplying the results by 100.

Accuracy (AC)

This is the measure that identifies the capability of the laboratory to correctly identify both positive and negative samples. It is calculated by using the following equation:

$$AC = (TP + TN) / (TP + TN + FP + FN)$$

A score of 100% identifies that the laboratory correctly reported all positive and negative samples as such. A score below 100% identifies that samples were identified incorrectly. In order to show the rate of false positive or false negative results, sensitivity and specificity were calculated.

Sensitivity (SE)

Sensitivity is the measure of the ability of the test to classify a positive sample as positive.

$$SE = TP / (TP + FN)$$

Specificity (SP)

Specificity is the measure of the ability of the test to classify a negative sample as negative.

$$SP = TN / (FP + TN)$$

Table 3 summarises the results obtained by the different testing methodologies in detecting RAM in the samples provided to the laboratories.

Table 3 Summary of Results for Sub-Project 1B

Method	Test Results				No Results Provided	Number of Results	Sensitivity (%)	Specificity (%)	Accuracy (%)	Will identify					Cost per sample ($)[3]
	True Positive	False Positive	True Negative	False Negative						Avian	Bovine	Piscine	Ovine	Porcine	
Classical Microscopy	75	0	15	0	0	90	100	100	100	Y	Y	Y	Y	Y	130
LFD (0.1% LOD)	40	0	15	35	0	90	53.3	100	61.1	Y	Y	Y	Y	Y	35
LFD (1.0% LOD)	13	0	15	2	0	30	86.7	100	93.3	Y	Y	Y	Y	Y	35
Agrigen Biotech PCR	60	0	12	0	18	72	100	100	100	Y	Y	N	Y	Y	400
NMI PCR	29	0	6	1	55	35	96.7	100	97.2	N	Y	N	Y	N	550

[3] These costs are true for the testing of individual samples as at August 2007 and are to be used as a guide only. It is noted that savings may be made if samples are bulked together for testing. It is worth while discussing these costs with the relevant service providers.

DISCUSSION

The results clearly demonstrate that both the AB and NMI PCR tests are capable of detecting some types of RAM in contaminated stockfeed. The AB test demonstrated a sensitivity of 100% and specificity of 100% for those types of RAM tested whilst the NMI test showed a sensitivity of 96.7% and a specificity of 100%.

All tests were able to accurately identify the species from which RAM contaminant was derived, with the exception of piscine RAM by the AB PCR test and avian, piscine and porcine RAM by the NMI PCR test.

The development of a suitable testing regimen would ideally include a highly sensitive screening test and a highly specific confirmatory test. Screening tests would usually be inexpensive and confirmatory tests more expensive. The TSEFAP NTC Working Group has also previously recommended that the testing regimen consist of the LFD as a screening test, with microscopy or PCR as the confirmatory test. With this in mind, the LFD @ 0.1% LOD is unsuitable for use as a screening test, as it has a sensitivity of 53.3% and an accuracy of 61.1%.

However, the results of the LFD @1.0% LOD show that this test would be suitable as a screening test for quality assurance purposes as it has a sensitivity of 86.7%, an accuracy of 93.3% and is one quarter of the price of its nearest rival (microscopy).

For regulatory purposes, microscopy is clearly the most suitable test, either as a confirmatory test in conjunction with the LFD screening test, or as a stand alone test. This test is also the most cost effective and accurate test available for confirmatory purposes. If required, a PCR test could also be used as a confirmatory test, but these tests are three to four times more expensive than microscopy. It should also be noted that the results of this work indicate that the AB PCR is more sensitive and accurate than the NMI PCR.

In 2005 the ruminant feed ban working group agreed that it would be counterproductive to set a limit of detection (LOD) for RAM. This is due to the fact that legislation presently states that *no RAM is to be fed to ruminants* and that community and industry expectations would not be met. However, it was noted that in selecting a test it needed to support the state/territory legislation and would also act as a defacto LOD. It was also noted that the test/s would need to be used for upholding legislation and therefore would need to be sound enough to be used in a court of law for prosecutions.

Presently, the NMI PCR test is used for the testing of imported product. However, the test does not cover all species that can constitute RAM (as shown in Table 3). As such there is a need to review the test used for the import of feed stuffs into Australia. The AB PCR or microscopy may be more appropriate tests.

CONCLUSION

Subject to developing a methodology to nationally role out this testing regimen, a national capability to accurately detect RAM in ruminant stockfeed is readily achievable. This project has demonstrated that the application of the LFD as a screening test and microscopy as a confirmatory test is appropriate and valid, for quality assurance purposes.

From a regulatory perspective, the use of microscopy, with or without the use of LFD as a screening test, is also valid.

Both the AB and NMI PCR tests are suitable for use as an alternate confirmatory test with the AB PCR being slightly more sensitive and accurate than the NMI PCR test. The AB PCR test is preferred as it identifies a wider range of RAM than the NMI test.

APPENDIX 1 – APLAC T047 FINAL REPORT

APPENDIX 2 – SUB PROJECT B SAMPLE PREPARATION

APPENDIX 3 – SUB PROJECT B RESULTS

Feed Validation Test Sample	Microscopy			NMI			Agrigen Biotech			LFD		
	Test Results		Evaluation	Test Results		Evaluation	Test Results		Evaluation	Test Results		Evaluation
	Positive (+ve)	Negative (-ve)		Positive (+ve)	Negative (-ve)		Positive (+ve)	Negative (-ve)		Positive (+ve)	Negative (-ve)	
40 g Avian Negative Control (0.00 %)		-ve	Sat.					-ve	Sat.		-ve	Sat.
40 g Avian Negative Control (0.00 %)		-ve	Sat.					-ve	Sat.		-ve	Sat.
40 g Avian Negative Control (0.00 %)		-ve	Sat.					-ve	Sat.		-ve	Sat.
40 g Avian Positive RAM Spike (0.05 %)	+ve		Sat.				+ve		Sat.		-ve	Sat.
40 g Avian Positive RAM Spike (0.05 %)	+ve		Sat.				+ve		Sat.	+ve		Sat.
40 g Avian Positive RAM Spike (0.05 %)	+ve		Sat.				+ve		Sat.	+ve		Sat.
40 g Avian Positive RAM Spike (0.10 %)	+ve		Sat.				+ve		Sat.	+ve		Sat.
40 g Avian Positive RAM Spike (0.10 %)	+ve		Sat.				+ve		Sat.	+ve		Sat.
40 g Avian Positive RAM Spike (0.10 %)	+ve		Sat.				+ve		Sat.		-ve	**False Neg**
40 g Avian Positive RAM Spike (0.25 %)	+ve		Sat.				+ve		Sat.	+ve		Sat.
40 g Avian Positive RAM Spike (0.25 %)	+ve		Sat.				+ve		Sat.	+ve		Sat.
40 g Avian Positive RAM Spike (0.25 %)	+ve		Sat.				+ve		Sat.	+ve		Sat.
40 g Avian Positive RAM Spike (0.50 %)	+ve		Sat.				+ve		Sat.	+ve		Sat.
40 g Avian Positive RAM Spike (0.50 %)	+ve		Sat.				+ve		Sat.	+ve		Sat.
40 g Avian Positive RAM Spike (0.50 %)	+ve		Sat.				+ve		Sat.	+ve		Sat.
40 g Avian Positive RAM Spike (1.00 %)	+ve		Sat.				+ve		Sat.	+ve		Sat.
40 g Avian Positive RAM Spike (1.00 %)	+ve		Sat.				+ve		Sat.	+ve		Sat.

40 g Avian Positive RAM Spike (1.00 %)		+ve		Sat.					+ve		Sat.		+ve		Sat.
40 g Bovine Negative Control (0.00 %)			-ve	Sat.			-ve	Sat.			-ve	Sat.		-ve	Sat.
40 g Bovine Negative Control (0.00 %)			-ve	Sat.			-ve	Sat.			-ve	Sat.		-ve	Sat.
40 g Bovine Negative Control (0.00 %)			-ve	Sat.			-ve	Sat.			-ve	Sat.		-ve	Sat.
40 g Bovine Positive RAM Spike (0.05 %)		+ve		Sat.		+ve		Sat.		+ve		Sat.		-ve	Sat.
40 g Bovine Positive RAM Spike (0.05 %)		+ve		Sat.		+ve		Sat.		+ve		Sat.		-ve	Sat.
40 g Bovine Positive RAM Spike (0.05 %)		+ve		Sat.		+ve		Sat.		+ve		Sat.		-ve	Sat.
40 g Bovine Positive RAM Spike (0.10 %)		+ve		Sat.		+ve		Sat.		+ve		Sat.		-ve	**False Neg**
40 g Bovine Positive RAM Spike (0.10 %)		+ve		Sat.		+ve		Sat.		+ve		Sat.	+ve		Sat.
40 g Bovine Positive RAM Spike (0.10 %)		+ve		Sat.		+ve		Sat.		+ve		Sat.		-ve	**False Neg**
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40 g Bovine Positive RAM Spike (1.00 %)		+ve		Sat.		+ve		Sat.		+ve		Sat.	+ve		Sat.
40 g Bovine Positive RAM Spike (1.00 %)		+ve		Sat.		+ve		Sat.		+ve		Sat.	+ve		Sat.
40 g Bovine Positive RAM Spike (1.00 %)		+ve		Sat.		+ve		Sat.		+ve		Sat.	+ve		Sat.
40 g Fish Negative Control (0.00 %)			-ve	Sat.										-ve	Sat.
40 g Fish Negative Control (0.00 %)			-ve	Sat.										-ve	Sat.
40 g Fish Negative Control (0.00 %)			-ve	Sat.										-ve	Sat.
40 g Fish Positive RAM Spike (0.05 %)		+ve		Sat.										-ve	Sat.
40 g Fish Positive RAM Spike (0.05 %)		+ve		Sat.										-ve	Sat.
40 g Fish Positive RAM Spike (0.05 %)		+ve		Sat.										-ve	Sat.
40 g Fish Positive RAM Spike (0.10 %)		+ve		Sat.										-ve	**False Neg**
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40 g Fish Positive RAM Spike (0.50 %)		+ve		Sat.									-ve	**False Neg**
40 g Fish Positive RAM Spike (1.00 %)		+ve		Sat.								+ve		Sat.
40 g Fish Positive RAM Spike (1.00 %)		+ve		Sat.									-ve	**False Neg**
40 g Fish Positive RAM Spike (1.00 %)		+ve		Sat.									-ve	**False Neg**
40 g Ovine Negative Control (0.00 %)			-ve	Sat.		-ve	Sat.			-ve	Sat.		-ve	Sat.
40 g Ovine Negative Control (0.00 %)			-ve	Sat.		-ve	Sat.			-ve	Sat.		-ve	Sat.
40 g Ovine Negative Control (0.00 %)			-ve	Sat.		-ve	Sat.			-ve	Sat.		-ve	Sat.
40 g Ovine Positive RAM Spike (0.05 %)		+ve		Sat.	+ve		Sat.		+ve		Sat.		-ve	Sat.
40 g Ovine Positive RAM Spike (0.05 %)		+ve		Sat.	+ve		Sat.		+ve		Sat.	+ve		Sat.
40 g Ovine Positive RAM Spike (0.05 %)		+ve		Sat.		-ve	**False Neg**		+ve		Sat.		-ve	Sat.
40 g Ovine Positive RAM Spike (0.10 %)		+ve		Sat.	+ve		Sat.		+ve		Sat.	+ve		Sat.
40 g Ovine Positive RAM Spike (0.10 %)		+ve		Sat.	+ve		Sat.		+ve		Sat.	+ve		Sat.
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40 g Ovine Positive RAM Spike (0.25 %)		+ve		Sat.	+ve		Sat.		+ve		Sat.	+ve		Satisfactory
40 g Ovine Positive RAM Spike (0.25 %)		+ve		Sat.	+ve		Sat.		+ve		Sat.	+ve		Satisfactory
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40 g Ovine Positive RAM Spike (0.50 %)		+ve		Sat.	+ve		Sat.		+ve		Sat.	+ve		Satisfactory
40 g Ovine Positive RAM Spike (1.00 %)		+ve		Sat.	+ve		Sat.		+ve		Sat.	+ve		Satisfactory
40 g Ovine Positive RAM Spike (1.00 %)		+ve		Sat.	+ve		Sat.		+ve		Sat.	+ve		Satisfactory

40 g Ovine Positive RAM Spike (1.00 %)	+ve		Sat.	+ve		Sat.		+ve		Sat.	+ve		Satisfactory
40 g Porcine Negative Control (0.00 %)		-ve	Sat.						-ve	Sat.		-ve	Satisfactory
40 g Porcine Negative Control (0.00 %)		-ve	Sat.						-ve	Sat.		-ve	Satisfactory
40 g Porcine Negative Control (0.00 %)		-ve	Sat.						-ve	Sat.		-ve	Satisfactory
40 g Porcine Positive RAM Spike (0.05 %)	+ve		Sat.					+ve		Sat.		-ve	Satisfactory
40 g Porcine Positive RAM Spike (0.05 %)	+ve		Sat.					+ve		Sat.		-ve	Satisfactory
40 g Porcine Positive RAM Spike (0.05 %)	+ve		Sat.					+ve		Sat.		-ve	Satisfactory
40 g Porcine Positive RAM Spike (0.10 %)	+ve		Sat.					+ve		Sat.		-ve	**False Negative**
40 g Porcine Positive RAM Spike (0.10 %)	+ve		Sat.					+ve		Sat.		-ve	**False Negative**
40 g Porcine Positive RAM Spike (0.10 %)	+ve		Sat.					+ve		Sat.		-ve	**False Negative**
40 g Porcine Positive RAM Spike (0.25 %)	+ve		Sat.					+ve		Sat.		-ve	**False Negative**
40 g Porcine Positive RAM Spike (0.25 %)	+ve		Sat.					+ve		Sat.		-ve	**False Negative**
40 g Porcine Positive RAM Spike (0.25 %)	+ve		Sat.					+ve		Sat.		-ve	**False Negative**
40 g Porcine Positive RAM Spike (0.50 %)	+ve		Sat.					+ve		Sat.	+ve		Satisfactory
40 g Porcine Positive RAM Spike (0.50 %)	+ve		Sat.					+ve		Sat.		-ve	**False Negative**
40 g Porcine Positive RAM Spike (0.50 %)	+ve		Sat.					+ve		Sat.		-ve	**False Negative**
40 g Porcine Positive RAM Spike (1.00 %)	+ve		Sat.					+ve		Sat.	+ve		Satisfactory
40 g Porcine Positive RAM Spike (1.00 %)	+ve		Sat.					+ve		Sat.	+ve		Satisfactory
40 g Porcine Positive RAM Spike (1.00 %)	+ve		Sat.					+ve		Sat.	+ve		Satisfactory