Annual Report to Environmental Protection Authority

for

Activities under ERMA 200223

AgResearch Ltd

For the 12 months ending

30th June 2019

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Animal Containment Facility

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Summary of Activities for the period 1st July 2018 to 30th June 2019

This summary provides the information required by control 11 (Annual reporting) of the HSNO Act approval ERMA200223.

Outdoor Development Activities

All outdoor development activities being carried out within the Animal Containment Facility at Ruakura comply with the requirements of the ERMA200223 approval.

Cattle, still alive at the end of the reporting period have now only been developed and maintained under the ERMA200223 approval. During June the last of the cattle which were field tested or developed under the GMF98009 or GMD02028 approvals were euthanised and a Final report provided to the Environmental Protection Authority.

Goat development and maintenance activities now only involve animals developed under the ERMA200223 approval.

Cattle, Goat and Sheep activities, other than the maintenance or growing of animals, have been flushing eggs from fertile animals, kidding of recipient goats, milking of transgenic animals, lambing of recipient ewes and the transfer of embryos to recipient animals. Semen has been collected from Bucks and Bulls for storage for future use.

Embryo Transfer activities this year have only been in sheep.

These transferred embryos fall within the approved organism description for the ERMA200223 approval and are for either the production of human therapeutic proteins, or for the study of gene function.

All activities have been undertaken with the approval of the Ruakura Animal Ethics Committee and all animal approvals have been renewed during the year.

Further details on development activities are provided within the following Science, Management and Ethics reports.

Unforeseen adverse effects resulting from the genetic modifications

There have been no unforeseen adverse effects identified during this period.

Iwi liaison group relationship development and management activities

The ERMA200223 Liaison Group has still not officially met since December 2011.

As advised in previous annual reports, at the request of a group of Ngati - Wairere elders the Liaison meetings were put on hold, while representation and membership of the Liaison group was discussed within the Hapu.

Following some correspondence and individual contact, this group of Ngati - Wairere elders was invited and did visit Ruakura in October 2012 and a process to progress representation was discussed. Unfortunately due to circumstances outside of AgResearch influence and despite further attempts, no progress has been made in resolving this directly to date.

There has again been some informal contact with original monitoring group members and regular contact with Tainui Group Holdings on their development activities for Ruakura.

AgResearch's Portfolio Leader – Māori Agribusiness who has local affiliations, is still working to build a relationship with Ngati - Wairere for Liaison Group and other Ruakura initiatives of interest to Ngati - Wairere and Tainui purposes.

Additional Supporting Information

The following reports are supporting information provided to expand on the previous summary and provide evidence of wider compliance with ERMA200223 Controls and MAF/ERMA New Zealand Standard '*Containment Standard for Field Testing of Farm Animals'.*
This additional supporting information is also provided to enable equivalence to the previous annual reporting for the inactive GMF98009 approvals.

Science Report

Cattle modified for milk composition

- Cattle were maintained and milk analysed to characterise changes to the milk composition
- Semen was collected from one bull to preserve the engineered genetics

Generating cattle with lighter coat colour

- Primary cells for in vitro culture were derived from ear biopsy samples taken from three normal control calves and cryopreserved.
- Following cell banking, the control calves were euthanised according to EPA controls.
- Embryos were reconstructed by nuclear transfer with donor cells harbouring homozygous and heterozygous edits of the sequence variant for lighter coat colour and cryopreserved

Goats producing therapeutic proteins

- Assisted reproductive technologies were used to expand the number of transgenic goats, produce transgenic males, provide access to milk and confirm stability of genotype and phenotype
- Semen was collected from one buck to preserve the engineered genetics
- Goat-produced therapeutic antibody was purified from milk and functionally characterised

Generating germline-deficient sheep for embryo complementation (AI on hooves)

- *NANOS2* was knocked out by CRISPR-mediated genome editing in male ovine fetal fibroblasts (OFFs) of a composite breed, as well as female Poll-Dorset FFs (PDFFs).
- OFF heterozygous and homozygous, as well as PDFF homozygous female knockout (KO)-derived cloned blastocysts were obtained and transferred into recipient animals.
- For homozygous male KO embryos, 52 embryos were transferred into 23 ewes. A total of 6/52 (=12%) established viable pregnancies (around D50), At term, 6 lambs were born, 2 of which survived into adulthood. Resulting lambs will be analysed to confirm loss of germline.
- For heterozygous male KO embryos, 45 embryos were transferred into 14 ewes, but pregnancy rates have not yet been determined.
- For homozygous female KO embryos 55 embryos were transferred into 19 ewes. A total of 31±6% (17/55, n=3) established viable pregnancies (around D35).

- For embryo complementation with a reporter cell line, wildtype OFFs were modified to constitutively overexpress a red fluorescent protein (RFP).
- One clonal RFP cell line was used for aggregation with cloned *NANOS-/-* embryos for germline replacement.
- A total of 19 and 48 blastocysts were produced from *RFP* and chimaeric *NANOS2<>RFP* aggregate embryos, respectively, and transferred into recipient ewes.
- From these groups, 0 and 2 lambs, respectively, were born but none were viable. Another two lambs from the chimaera group were born prematurely at D114 and D133, both also were non-viable.

Generating immune-compatible sheep for xenotransplantation

- *GGTA* and *CMAH* genes were knocked out by CRISPR-mediated genome editing in male and female ovine fetal fibroblasts (OFFs) and used for cloning.
- OFF double knockout-derived cloned blastocysts were obtained from *GGTA*/*CMAH* null donor cells (double knockout or DKO).
- Following SCNT into abattoir-derived oocytes, blastocysts were transferred into surrogate recipients. At day 35 of development, ultrasonography scanning confirmed that 19% of in vitro derived male embryos (11/57, n=10) established a viable pregnancy. From these, 4 lambs were born but none survived. We repeated the experiments this breeding season, using both male and female DKO donor cells. Both had 44 embryos transferred into 17 ewes. Similar to last year's results, a total of 22.7% of both male and female embryos (10/44 after ET into 17 ewes for each group, n=4 runs) established viable pregnancies around D35, which are currently monitored at various stages of gestation.
- Resulting DKO lambs, if viable, will be analysed to confirm loss of α-Gal and Neu5Gc and evaluated as donors for xenotransplantation. Following puberty, the animals will be mated to establish a flock of potential donor animals.

Generating immune-compatible anephric sheep fetuses for kidney complementation

- *GGTA*/*CMAH* and *SALL1* genes were knocked out by CRISPR-mediated genome editing in male ovine fetal fibroblasts (OFFs) and used for cloning (=triple KO host).
- OFF double KO-derived cloned blastocysts were obtained from *GGTA*/*CMAH* null donor cells.
- Following SCNT into abattoir-derived oocytes, aggregated blastocysts from the following 4 groups were transferred into surrogate recipients:
  - Triple KO: 31 embryos into 10 recipients
  - Double KO: 37 embryos into 9 recipients
  - Triple<>double KO chimaeras: 60 embryos into 20 recipients
  - Wild-type controls: 6 embryos into 2 recipients

  No pregnancy scans are available for any of these groups yet.

Overexpression of the histone demethylase KDM4B in transgenic (TG) cattle

- One female animal that overexpresses the histone demethylase KDM4B is now approaching puberty. She will be used for breeding to preserve and expand this TG line. The line will aid future studies into improving somatic cell reprogramming.

On Farm Management Summary for year ending 30/06/2019

Animal Numbers 01/07/2018– 30/06/2019 (Births exclude still born or animals which die soon after birth reported in Animal Ethics Reports, Aged In and Out records changes in animal age[1])

Stock Class	Open (1/07/18)	Births	Transfer In	Transfer Out	Aged In	Aged Out	Killed	Deaths	Closing (30/06/19)
Casein (ERMA200223)									
MA Cows	**12**						10		**2**
Total Casein	**12**	**0**	**0**	**0**	**0**	**0**	**10**	**0**	**2**
MBP (ERMA200223)									
Total MPB	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
rhLF (ERMA200223)									
Total rhLF	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
BLg - (ERMA200223)									
MA Cows	**11**				5				**16**
R2yr Heifers	**5**				3	5			**3**
R1yr Heifers	**3**					3			**0**
Heifer Calves	**0**								**0**
R1yr + Bulls	**1**						1		**0**
Bull Calves	**0**								**0**
Total BLg -	**20**	**0**	**0**	**0**	**8**	**8**	**1**	**0**	**19**
Erbitux (ERMA200223)									
MA Cows	**1**						1		**0**
Total Erbitux	**1**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**
Coat Colour (ERMA200223)									
R1yr Bull	**0**				3		**3**		**0**
Bull Calves	**3**					3			**0**
Total Coat Colour	**3**	**0**	**0**	**0**	**3**	**3**	**3**	**0**	**0**
KDM4B (ERMA200223)									
R1yr Heifer	**0**				1				**1**
Heifer Calves	**1**					1			**0**
Total KDM4B	**1**	**0**	**0**	**0**	**1**	**1**	**0**	**0**	**1**
Conventional Cattle									
MA Cows	**36**				5		5		**36**
R2yr Heifers	**5**					5			**0**
Other classes	**0**		64						**64**
Total Conventional	**41**	**0**	**64**	**0**	**5**	**5**	**5**	**0**	**100**
Cattle Total	**78**	**0**	**64**	**0**	**17**	**17**	**20**	**0**	**122**
Cattle developed under ERMA approvals (Tg and non Tg progeny)									**22**

[1] Aligns with normal livestock reconciliation aging practice.

Stock Class	Open (1/07/18)	Births	Transfer In	Transfer Out	Aged In	Aged Out	Killed	Deaths	Closing (30/06/19)
Goats									
Erbitux & Enbrel (ERMA200223)									
Ma Doe	**16**		4		14		11		**23**
R2yr Doe	**14**				5	14			**5**
R1yr Doe	**5**				1	5			**1**
Doe Kid	**0**	1				1			**0**
Buck Kid	**0**	3				3			**0**
R1yr Male +	**4**				3		3		**4**
Total Erbitux & Enbrel	**39**	**4**	**4**	**0**	**23**	**23**	**14**	**0**	**33**
non Med inherit (ERMA200223)									
Total TCR	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Conventional Goats									
MA Doe	**20**			4	21		7		**30**
R2yr Doe	**21**					21			**0**
R1yr Doe	**0**								**0**
Male R1yr +	**0**								**0**
Kids	**0**								**0**
Total Conventional	**41**	**0**	**0**	**4**	**21**	**21**	**7**	**0**	**30**
Goat Total	**80**	**4**	**4**	**4**	**44**	**44**	**21**	**0**	**63**
Goats developed under ERMA approvals (Tg and non Tg progeny)									**33**

Stock Class	Open (1/07/18)	Births	Transfer In	Transfer Out	Aged In	Aged Out	Killed	Deaths	Closing (30/06/19)
Sheep									
AI on Hooves									
R1yr Ram	**0**				2				**2**
Ram Lamb	**0**	2				2			**0**
Total	**0**	**2**	**0**	**0**	**2**	**2**	**0**	**0**	**2**
Conventional Sheep									
MA Ewes	**3**				42		5		**40**
2th Ewes	**52**		119	1		42	9		**119**
Ewe Hgts	**0**								**0**
Total Conventional	**55**	**0**	**119**	**1**	**42**	**42**	**14**	**0**	**159**
Sheep Total	**55**	**2**	**119**	**1**	**44**	**44**	**14**	**0**	**161**
Sheep developed under ERMA approvals (Tg and non Tg progeny)									**2**

The preceding tables provide animal numbers by species over the reporting period in the development lines and are linked to the EPA approval. This includes transgenic and non-transgenic animals (progeny) and the conventional animals which are used to support the programmes.

For cattle there has been one movement of conventional animals into the facility during the period. 64 conventional beef animals, under 1 year of age and mixed sex were moved on to the facility for grass control purposes. There have been no movements of cattle from the facility.

20 cattle of varying ages have been euthanased (killed); these animals have been disposed of in offal holes on-site, having been identified as surplus or now unsuitable animals, or following veterinary advice during this period.

For goats there has been no movement of animals onto or from the facility (apart from approved exit and returns for surgery purposes) during the period. The transfer out and in of 4 females is some non Tg progeny of Tg animals which had been incorrectly counted as conventional animals previously.

21 goats of varying ages have been euthanased (killed) and no goats died during the period; these animals have also been disposed of in offal holes on-site, as now surplus or unsuitable animals, or following veterinary advice.

For sheep there has been one movement of 119 conventional animals into the facility and 1 conventional animal was moved off the facility as unsuitable for use as a recipient and the approved exit and returns for surgery purposes during the period.

14 sheep of varying ages have been euthanased (killed) and no sheep died during the period; these animals have also been disposed of in offal holes on-site, as unsuitable animals, or following veterinary advice.

For management purposes, as previously identified, the facility is treated as a separate small farm within the main Ruakura Farm. It is fully self-contained apart for some machinery requirements and specialist staffing.

The Ministry for Primary Industries Mycoplasma Bovis 'Notice of direction' on the Ruakura Farm, including the Animal Containment Facility was revoked in August 2018 following clear test results.

Animals on the facility continue to be managed in a way which is the normal farming practice in New Zealand, grazing outdoors on pasture.

This consists of daily shifts and restricted intakes depending on the age of the animal and its feed requirements. Examples are stage of pregnancy, lactating or rearing calf or kid, empty, young growing animals, etc.

No cattle, no goat and 162 sheep recipients have been used for ET (embryo transfer), with some others being mated with artificial insemination or bucks, on a rotational basis during the period. All animals are regularly monitored for live weight and health status.

All animals graze mainly on pasture, with some crops, supplementary feeding of hay, balage or concentrates when required.

Goats can at times receive a higher proportion of their daily intake as supplementary feed, as concentrates, to reduce their impact on pasture availability for cattle and often have access to covered shelter in inclement weather.

Surplus pasture is conserved when possible for use in periods of low growth, as balage or hay and there was only minimal purchasing of extra supplement (meal) required this season, mainly due to lower animal numbers which enabled maintenance of an adequate annual feed supply.

Regular pasture renewal is carried out with at least 10% of the facility receiving some form of renovation annually. Mineral supplementation is carried out using a mineral dispensing system through the water

troughs for assisting Facial Eczema control and other normal mineral deficiencies during identified periods of risk, as occurs on many farms.

Maintenance fertiliser applied this season contained no nitrogen and no selective additional Nitrogen (Urea) has been used on areas not used for milk/waste irrigation during the year.

Milk Production 18/19 season

No GM cows were calved at all or specifically for seasonal milk production again this year.

The milk from the GM goats which were milked was either used to feed kids, for science analysis or frozen on the facility.

This has meant there was again no milk stored this year for surplus disposal by irrigation to pasture.

Ruakura Animal Ethics Committee Reports

RAEC # 14510 - Maintenance of Cattle on the Animal Containment Facility

Ruakura Animal Ethics Committee Report: Third Quarter 2018

Transgenic Cattle

Summarised below are the animal numbers and animal health status of the various cattle groups for the reporting interval July to September 2018 according to the conditions for approval of Application 14510 Maintenance of cattle on the Animal Containment Facility.

A) Casein Plus cattle

A.1 Animal numbers
Initially 12
1 euthanised as no longer required

A.2 Health status
No health issues during reporting period

B) Beta-lactoglobulin (BLG) knockdown/knockout cattle

B.1 Animal numbers
20, no change

B.2 Health status
No health issues during reporting period

C) Coat Colour cattle

C.1. Animal numbers
3, no change

C.2 Health status
3 bull calves showed weight loss and diarrhoea, suspected cause of drinking each other's urine, as once separated they recovered

D) KDM4B cattle

D.1. Animal numbers
1, no change

D.2 Health status
No health issues during reporting period

E) Conventional recipient cattle

E.1. Animal numbers

41, no change

E.2. Health status
No health issues during reporting period

Ruakura Animal Ethics Committee Report: Fourth Quarter 2018

Transgenic Cattle

Summarised below are the animal numbers and animal health status of the various cattle groups for the reporting interval October to December 2018 according to the conditions for approval of Application 14510 Maintenance of cattle on the Animal Containment Facility.

A) Casein Plus cattle

A.1 Animal numbers
Initially 11
5 euthanised due to age related health issues

A.2 Health Status
Euthanasia because of cancer eye (6008, 8024, 9005), bad feet (8023), arthritis (9013).

B) Beta-lactoglobulin (BLG) knockdown/knockout cattle

B.1. Status of transgenic BLGkd cattle
Initially 20
1 male euthanised as no longer required

B.2 Health Status
No health issues during reporting period

C) Coat Colour cattle

C.1. Animal numbers
3, no change

C.2 Health status
No health issues during reporting period

D) KDM4B cattle

D.1. Animal numbers
1, no change

D.2 Health status
No health issues during reporting period

E) Conventional recipient cattle

E.1. Animal numbers

36, 5 euthanised due to eye related health issues.

E.2. Health status.
No health issues during reporting period

Ruakura Animal Ethics Committee Report: First Quarter 2019

Transgenic Cattle

Summarised below are the animal numbers and animal health status of the various cattle groups for the reporting interval January to March 2019 according to the conditions for approval of Application 14510 Maintenance of cattle on the Animal Containment Facility.

A) Casein Plus cattle

A.1 Animal numbers
6

A.2 Health status
Cow 7017 lameness, treated and recovered, has had some chronic mild stiffness in hindquarters- suspect arthritis

B) Beta-lactoglobulin (BLG) knockdown/knockout cattle

B.1 Animal numbers
19

B.2 Health status
No health issues during reporting period

C) Coat Colour cattle

C.1. Animal numbers
3, no change

C.2 Health status
No health issues during reporting period

D) KDM4B cattle

D.1. Animal numbers
1, no change

D.2 Health status
No health issues during reporting period

E) Conventional recipient cattle

E.1. Animal numbers
36, no change

E.2. Health status
No health issues during reporting period

Ruakura Animal Ethics Committee Report: Second Quarter 2019

Transgenic Cattle

Summarised below are the animal numbers and animal health status of the various cattle groups for the reporting interval April to June 2019 according to the conditions for approval of Application 14510 Maintenance of cattle on the Animal Containment Facility.

A) Casein Plus cattle

A.1 Animal numbers
Initially 6
4 euthanised due to old age issues

A.2 Health status
4 older cows age developed age related arthritis problems and/or had worn teeth. They were euthanised on animal welfare grounds.

B) Beta-lactoglobulin (BLG) knockdown/knockout cattle

B.1 Animal numbers
19

B.2 Health status
No health issues during reporting period

C) Coat Colour cattle

C.1. Animal numbers
Initially 3
3 euthanised as no longer required

C.2 Health status
No health issues during reporting period

D) KDM4B cattle

D.1. Animal numbers
1, no change

D.2 Health status
No health issues during reporting period

E) Conventional recipient cattle

E.1. Animal numbers

36, no change

E.2. Health status
No health issues during reporting period

RAEC #14511 - Maintenance, Breeding, Production and Characterisation of Transgenic Goats

Ruakura Animal Ethics Committee Report: Third Quarter 2018

Transgenic Goats

Summarised below is the status of the various goat groups and their offspring and any losses that have occurred during the reporting interval July to September 2018, in relation to the conditions for approval of Application 14511 " Maintenance, Breeding, Production and Characterisation of Transgenic Goats ".

Biosimilars

A) Erbitux Goats

A.1 Animal numbers
Initially 38 (34 females, 4 males)
1 female and 5 buck kids born on 28/08/2018
3 females euthanised because no longer required (old age etc.) and 1 due to bad feet causing welfare issues
2 buck kids euthanised as not GM positive

A.2 Treatments and activities during reporting interval
All have had routine animal health treatments and hoof care during the period

B) Enbrel Goats

B.1 Animal numbers
Initially (6 females, 0 males)

B.2 Treatments and activities during reporting interval
All have had routine animal health treatments and hoof care during the period

C. Recipients

C.1. Animal numbers
Initially (37 females, 0 males)
3 females euthanised due to age related issues on veterinarian advice.

C.2 Treatments and activities during reporting interval
All have had routine animal health treatments and hoof care during the period

Ruakura Animal Ethics Committee Report: Fourth Quarter 2018

Transgenic Goats

Summarised below is the status of the various goat groups and their offspring and any losses that have occurred during the reporting interval October to December 2018, in relation to the conditions for approval of Application 14511 " Maintenance, Breeding, Production and Characterisation of Transgenic Goats ".

Biosimilars

A) Erbitux Goats

A.1 Animal numbers
Initially 39 (32 females, 7 males)
6 females euthanised because no longer required (old age etc.) or due to bad feet causing welfare issues
3 Bucks euthanised following semen collection for storage of genetic material.

A.2 Treatments and activities during reporting interval
MOET was attempted for one goat but failed to ovulate
All have had routine animal health treatments and hoof care during the period

B) Enbrel Goats

B.1 Animal numbers
Initially (6 females, 0 males)
1 females euthanised due to bad feet causing welfare issues

B.2 Treatments and activities during reporting interval
MOET was attempted for three goats but all failed to ovulate
All have had routine animal health treatments and hoof care during the period

C. Recipients

C.1. Animal numbers
Initially (34 females, 0 males)
4 females euthanised because no longer required (old age etc.) or due to bad feet causing welfare issues

C.2 Treatments and activities during reporting interval
All have had routine animal health treatments and hoof care during the period

Ruakura Animal Ethics Committee Report: First Quarter 2019

Transgenic Goats

Summarised below is the status of the various goat groups and their offspring and any losses that have occurred during the reporting interval July to September 2018, in relation to the conditions for approval of Application 14511 " Maintenance, Breeding, Production and Characterisation of Transgenic Goats ".

Biosimilars

A) Erbitux Goats

A.1 Animal numbers
30 (26 females, 4 males)

A.2 Treatments and activities during reporting interval
All have had routine animal health treatments and hoof care during the period

B) Enbrel Goats

B.1 Animal numbers
5 females, 0 males
1 euthanised due to bad feet causing welfare issues

B.2 Treatments and activities during reporting interval
Natural mating of two goats
U/S scanning confirmed pregnancy for one goat
All have had routine animal health treatments and hoof care during the period

C. Recipients

C.1. Animal numbers
30 females, 0 males

C.2 Treatments and activities during reporting interval
All have had routine animal health treatments and hoof care during the period

Ruakura Animal Ethics Committee Report: Second Quarter 2019

Transgenic Goats

Summarised below is the status of the various goat groups and their offspring and any losses that have occurred during the reporting interval July to September 2018, in relation to the conditions for approval of Application 14511 " Maintenance, Breeding, Production and Characterisation of Transgenic Goats ".

Biosimilars

A) Erbitux Goats

A.1 Animal numbers
29 (25 females, 4 males)

A.2 Treatments and activities during reporting interval
All have had routine animal health treatments and hoof care during the period

B) Enbrel Goats

B.1 Animal numbers

4 females, 0 males

B.2 Treatments and activities during reporting interval
1 doe is maintaining a healthy pregnancy and due to kid early August 2019
All have had routine animal health treatments and hoof care during the period

C. Recipients

C.1. Animal numbers
30 females, 0 males

C.2 Treatments and activities during reporting interval
All have had routine animal health treatments and hoof care during the period

RAEC #14696 – AI-on-hooves: generating healthy NANOS2-deficient sheep for breeding sterile host embryos.

Ruakura Animal Ethics Committee Report: 2nd Quarter 2019

Transgenic Sheep

As of 30/06/2019, we report embryo transfer results from the following 2 experimental groups:
1) PDFF, female NANOS2 (-/-) cell clone #6
2) PDFF, female wild-type NANOS2 (+/+) control

Genotype	nET	Recipients	Pregnancy at D35 (%)	Fetus detected (%)
#6	12	4	3 (75)	3 (25)
WT	17	6	6 (100)	6 (35)

For both PDFF groups, pregnancy establishment and fetus detection was higher than expected (20%), which may indicate a beneficial effect of the PDFF-derived donor cell line. Pregnancies are being monitored to term with the aim of producing at least one viable lamb for breeding.

With respect to the two OFF3-derived NANOS2 rams, “Bunter” (*17/09/2018) and “Hammer” (*18/10/2018), both are alive and well. Their weight and scrotal circumference has been monitored in monthly intervals and they are showing linear growth rates up to now.

MPI Verification Services Audit reports

Ministry for Primary Industries
Manatū Ahu Matua

Ministry for Primary Industries
External Inspection Report:

Facility	AgResearch - Ruakura
Location	Ruakura Research Centre
	10 Bisley Rd, Hamilton
Facility Codes	TF02501 (364)
Operator	Tom Richardson
MPI Contact	[redacted] Tim Hale,
Primary Verifier	Crystal Lange
Inspection conducted By	Crystal Lange
Inspection Date	August 1 2018
Inspection report Date	August 6 2018

New Zealand Government

Growing and Protecting New Zealand

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Executive Summary

The external inspection of AgResearch's Ruakura campus transitional and containment facility in Hamilton was carried out by the Ministry for Primary Industries (MPI) August 1 2018 for the period April - September 2018.

No issues were noted with records or structural compliance for either of the animal containment units although goat identification should be reviewed to align with the 154.03.06 standard.

There were some minor structural issues noted in a laboratory and the glasshouse that may require closer scrutiny, the repair to the PC1 Endophyte Laboratory is not quite complete so that non-compliance issued following the last inspection will remain open.

The depth of internal auditing has improved and sample tracking at a surface level has been implemented. MPI will review this at the next scheduled inspection.

The Operating Manager has taken ownership of finding support and using devolved management tasks as a step toward succession planning.

Overall the audit outcome was satisfactory, two recommendation were made.

Sincerely

Crystal Lange
Technical Supervisor
Verification Services

Inspection Scope

The AgResearch site at the Ruakura is a transitional and containment facility, approved as such under the Biosecurity Act 1993 because it holds or processes goods that are risk goods as defined by the Biosecurity Act 1993 or may contain new organisms as defined by Hazardous Substances and New Organisms (HSNO) Act 1996.

The facility is approved to Environmental Protection Authority (EPA) Standards 154.03.02: *Containment Facilities for Microorganisms 2007a,* 154.03.06: *Containment Standard for the Field Testing of Farm Animals,* Standard 154.03.03: *Containment Facilities for Vertebrate Laboratory Animals,* 155.04.09 *Containment Facilities for Plants 2007, 154.02.08 Transitional & Containment Facilities for Invertebrates* and MAFBNZ Standard 154.02.17 *Transitional Facilities for Biological Products.* These standards specify the structural and operational requirements for transitional and containment facilities receiving and holding risk goods or new organisms.

The purpose of this inspection was to determine if the facility and operator approvals held by AgResearch and the HSNO Act approvals are being complied with. Consequently, the scope of the inspection includes the standards and HSNO approvals.

This report is written so that each Facility Manager can extract the parts that are specifically relevant to their own area.

References:
MAFBNZ Standard: *Transitional Facilities for Biological Products*
EPA Standard: *Facilities for Microorganisms and Cell Cultures: 2007a*
EPA Standard: *Containment Standard for the Field Testing of Farm Animals*
EPA Standard: *Containment Facilities for Vertebrate Laboratory Animals*
EPA Standard: *Transitional and Containment Facilities for Invertebrates*
EPA Standard: *Containment Facilities for Plants 2007*
Australian Standard & New Zealand Standard (AS/NZS) 2243.3: 2002 - *Safety in Laboratories: Microbiological aspects and containment facilities*
Facility Manual: version 2 June 2017
Approvals granted under the HSNO Act
CTO Approvals *Phytophthora agathidicida* (exp Dec 2018)
MPI permits to import and associated Import Health Standards (IHS's)

Inspection Process

This scheduled external inspection was conducted by Crystal Lange (MPI), with [redacted] (delegated Facility Operator and Operating Manager, [redacted] and Tim Hale (delegated Facility Operators). The inspection process included a review of onsite records where applicable, including staff training and internal audit records, registers, and physical inspection of the containment areas.

Inspection Findings

Animal Containment Facility

ERMA200223

Compliance with the controls of ERMA200223 was assessed. No issues were noted.
N.B. Controls 8, 11, 12, 14 and 15 are not applicable to this review.

Operational Requirements

Overall good operator control was demonstrated. Sampled records were up to date for animal treatments, transfer approvals, animal counts and training.

Control 1

AgResearch is meeting the requirements of all applicable controls.

Control 2

Three of the permissible species are in use for research purposes.

Control 3

Practices comply with EPA, Animal Ethics and MPI requirements.

Control 4

The Requirements of the Standard are being met:

Training	No induction or refresher training has been required.
Access	There have been no untoward security issues, perimeter checks are occurring and external security responses are triggered at random.
Internal Audit Programme	The internal audit was conducted June 2018. There were no areas of concern arising from this review.
Records	Registers were up to date, this inspection focus was on goat records.
Physical Requirements	Perimeter fencing was secure, internal fencing, gates and races were well maintained. Entry gates are monitored during daily activities.

Control 5

Sheep continue to be used to graze the perimeter. Stock rotation is managed with the AgResearch general farm to ensure co-grazing does not occur.

Control 6

There have been no reported breaches of containment or any suspicious activity.

Control 7

Animal records are maintained electronically. The full stock count was supplied at the inspection.

Control 9

Primary goat tagging is by ID chip. Where possible secondary systems are used however the nature of the goat makes this a repetitive process. Recommendation 2

Control 10

No pregnant animal slaughter has occurred.

Control 13
Contact is being maintained with Iwi as and when possible.

Non-Compliances & Corrective Actions

Non-Compliance: A failure to comply with requirements. MPI rates non-compliances as follows:

Critical: A **critical failure in an operation or system** that caused or could have caused **a serious (or significant) risk** to biosecurity.

Major: A **major failure in an operation or system** that **caused or could have caused** a risk to biosecurity.

Minor: A situation or incident that may not be a major failure but results in a **decrease in confidence** in the management of the facility and **may or may not** immediately **cause or lead** to a risk to biosecurity.

Corrective Action Request (CAR): A documented instruction issued by an inspector/enforcement officer, warranted as such under the Biosecurity Act, specifying a remedial action to be taken within a specific time frame in response to a non-compliance.

NOTES:

1. A specific non-compliance may have more than one CAR associated with it.
2. Unless otherwise directed, work will be permitted to continue while the CAR(s) are being actioned.

Non-Compliance #1 Rating: **Minor**

Laboratories not maintained to meet the requirements of section 4.7 of AS/NZS 2243.3:2002 as required by the Micro2007a and Biological Products standards

CAR(s)

1. Identify which units in the South Wing basement may hold risk goods
2. Ensure wall/floor joins (PP Room 10) are impervious (continued)

Date to be completed: September 28 2018

Recommendations

Recommendation: Non-binding advice provided to assist and facilitate the enhancement and improvement of structures, systems, processes and methodology to maintain compliance with the requirements of the standard(s) and minimize the likelihood of non-compliances occurring. Recommendations are generally based on observations made by the auditor during the course of the audit.

1. Check ceiling in SW117 for leaks, monitor ceiling
2. Review animal identification in line with the Farm Animal Standard (154.03.06)

MPI contact details

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Ministry for Primary Industries
Manatū Ahu Matua

Verification Report[1]

Report ID:	PBV/2501/2019/01
Outcome:	**Acceptable**
Issued to:	AgResearch - Ruakura Campus
Operator ID(s):	2501
Issued by:	Crystal Lange **Phone:** 079578319 **Email:** crystal.lange@mpi.govt.nz
Verification Period:	2018-09-10 to 2019-03-09
Verification Date:	2019-02-13
Published:	2019-02-14 08:39
Next Due Date:	2019-09-09
Level/Step:	**6.2** (started on 6.2 , and ceiling is 6)
Report Type:	Scheduled
Peer Reviewed By:	Davide Zazzaro

1 A Verification Report is a formal report issued when sufficient evidence has been assessed to arrive at an outcome for a verification period. This report may contain Technical Reviews and external audit findings completed during the period. Inadequate and/or untimely responses to deficiencies identified in this report, poor/unacceptable performance, or failure to pass subsequent audits may result in the escalating imposition of sanctions and/or interventions provided by law.

1. Premises Profile

AgResearch - Ruakura Campus is, under section 39 of the Biosecurity Act 1993, approved as a Transitional and Containment Facility in accordance with the requirements of the MPI/EPA standard(s) identified. Under section 40 of the Biosecurity Act, Tom Richardson is approved as an operator of that facility and is primarily responsible for the facility, compliance with facility approvals and all activities involving risk goods.

The standards that the facility is approved to specify the structural and operating requirements for containment and/or transitional facilities holding regulated organisms and risk goods that are, or may contain:

- Agricultural Compounds
- Animals
- Animal Products
- Biologicals
- Miscellaneous
- Non-risk Goods
- Plant Products

Physical Address :
10 Ruakura Campus Bisley Road, Ruakura, Hamilton

2. Executive Summary

This was a scheduled inspection of four of the six standards AgResearch's Ruakura site is approved to. The inspection process included a review of onsite records where applicable, including staff training and internal audit records, registers, and physical inspection of the containment areas. The outcome was acceptable with one non-compliance issued for laboratory design and construction.

Identification of goats had been reviewed and changes implemented since the last inspection. The sealing of the PC1 Endophyte Laboratory was completed and that non compliance closed October 30 2018. Inventory tracking needs to be completed this week to avoid an additional non-compliance being issued.

3. Operator Summary

AgResearch had three sectors being inspected this visit. Crystal Lange (MPI) met with the following Delegated Facility Operators [redacted] (laboratories), [redacted] (vertebrates) and Tim Hale (animal containment). [redacted] accompanied [redacted] as familiarisation for his biosecurity role as part of his succession planning.

MPI was advised that research under ERMA200223 (Cattle and Sheep) had been placed on temporary hold by the Ruakura Ethics committee as they review research results.
There have been no incidents or breaches that MPI should have been advised of.

4. Verification Completed (this period)

Biosecurity

Substantial compliance was demonstrated with the animal standards. Good compliance was demonstrated with the laboratory based standards.

The following elements were verified in this PBV period:

Biosecurity:Containment Facilities for Vertebrate Laboratory Animals	Acceptable
Biosecurity:Containment Standard for Field Testing of Farm Animals	Acceptable
Biosecurity:Facilities for Microorganisms and Cell Cultures: 2007a	Acceptable
Biosecurity:Transitional Facilities for Biological Products	Acceptable

Quality Assurance

Contingency planning has been well described for physical events. AgResearch is lacking in leadership for support for key roles in the management of the containment facility, staff are initiating succession planning on their own.

The 2018 annual report for EPA Approval ERMA200223 was submitted and accepted by the EPA. An updated facility manual was received and approved January 8 2019.

The following elements were verified in this PBV period:

Quality Assurance:Biosecurity Contingency Plans	Acceptable
Quality Assurance:Notifications to MPI/EPA	Acceptable
Quality Assurance:Operating Procedures	Acceptable
Quality Assurance:Operator Control	Acceptable
Quality Assurance:Operator Internal Verification	Acceptable
Quality Assurance:Organizational Structure and Management	Acceptable

Documentation and Certification

Release BACC's were on file for non GM mice that had completed quarantine.
Three imports (BACCs) chosen for traceability during the internal audit had not been confirmed during the internal audit or since. They were not able to be located during the MPI inspection as key staff were not available. This has been left with the Delegated Facility Operator (DFO) to follow up.

The following elements were verified in this PBV period:

Documentation and Certification:Biosecurity Authority Clearance Certificates (BACCs)	Acceptable
Documentation and Certification:Documentation and Record Keeping	Acceptable

Identification, Traceability & Management

All staff and tenants have a good knowledge of transfer protocol. A summary register of movements is maintained by the DFO and each business unit maintains its own register. Selected items chosen at the internal audit for traceability have yet to looked for. This needs to be completed by February 18 to avoid a non-compliance being issued.

Animal inventories for the Small Animal Containment and Animal Containment Facility were up to date. Cage cards or animal identification was present.

The following elements were verified in this PBV period:

Identification, Traceability & Management:Authorised Signatories	Acceptable
Identification, Traceability & Management:Inventory Control and Accuracy	Acceptable
Identification, Traceability & Management:Storage Areas	Acceptable

Hygiene & Sanitation

Personal Protective Equipment was available and in use. Disinfectant solutions were available in all laboratories. Lack of use of laboratories contributed to the accumulated dust and midges on the windowsills and benches. This has been included in the Non-compliance raised for Design and Construction.

Waste is chemically treated or autoclaved onsite or collected by a third party provider. i-button records were available for both the vertebrate and Plant Protection autoclaves.

The following elements were verified in this PBV period:

Hygiene & Sanitation:Cleaning and Disinfection	Acceptable
Hygiene & Sanitation:Personnel Hygiene and Personal Protective Gear	Acceptable
Hygiene & Sanitation:Pest and Vermin Control	Acceptable
Hygiene & Sanitation:Waste Management	Acceptable

Design and Construction

The animal containment areas continue to be suitable to hold the approved species. Temporary fencing measures were approved by MPI during installation of a water main.

All entry points had adequate signage. There have been no security events.

A number of laboratories had dusty windowsills, midges were on sills and benches indicating extended periods of disuse or poor hygiene. Window sills were peeling in South Wing 101, rust stains and rusty tweezers were noted on the sink bench in 114. Dairy Science Rm1 had absorbents in the floor already showing signs of water staining, which the DFO offered to disinfect and dispose of. The wall paper was peeling off of all wall areas, screw holes and cobwebs were noted. A non-compliance has been issued below.

The following elements were verified in this PBV period:

Design and Construction:Access and Security	Acceptable
Design and Construction:Animal Enclosures and Facilities (inc. invertebrates)	Acceptable
Design and Construction:Laboratories	Acceptable
Design and Construction:Open Field Testing Facilities	Acceptable
Design and Construction:Physical Containment Level 1 (PC1)	Acceptable
Design and Construction:Signage	Acceptable

Subject: Physical Containment Level 1 (PC1)

Note List:

[Crystal Lange]

Laboratories were not compliant with the ASNZS 2243.3 Safety in Laboratories. General hygiene was not up to standard in many of the laboratories, South Wing 101 and Dairy Science Rm 1 were dilapidated.

Hazardous Substances and New Organisms (HSNO) Act

No new research work has been initiated. Substantial compliance was observed with the controls of ERMA200223.

The following elements were verified in this PBV period:

Hazardous Substances and New Organisms (HSNO) Act:HSNO Act Approvals for Development of New Organisms	Acceptable
Hazardous Substances and New Organisms (HSNO) Act:HSNO Act Approvals for New Organisms for Containment	Acceptable

Mandatory Tasks

5. Definitions

Acceptable Where the Animal Products Officer (or Biosecurity Inspector) is satisfied that the operator is substantially complying with requirements; and where there have been any departures from

regulatory requirements, that the operator's corrective actions have been, or are being, applied appropriately and effectively.

	Departures from regulatory requirements, identified by the Animal Products Officer (or Biosecurity Inspector), are to be transferred to the operator's issue management system for resolution. (Key Topic / Non-compliance)
Unacceptable	Where the Animal Products Officer (or Biosecurity Inspector) has determined that the operator is not in substantial compliance with regulatory requirements; evidenced by inadequate operator controls. (Key Issue / Non-compliance)