Annual Report to Environmental Protection Authority

for

Activities under ERMA 200223

AgResearch Ltd

For the 12 months ending

30th June 2024

Submitted by Tim Hale
Delegated Facility Operator / Manager
AgResearch Ruakura
Animal Containment Facility

Contents

Summary of Activities – ERMA200223 Control 11 3

Outdoor Development Activities 3

Unforeseen adverse effects 3

ERMA200223 Liaison Group 3

Additional Supporting Information

Science Report 4

On Farm Management Summary 6

Ruakura Animal Ethics Committee Reports 10

MPI Verification Services Audit reports 48

Summary of Activities for the period 1st July 2023 to 30th June 2024

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 that were still alive at the end of the reporting period have now only been developed and maintained under the ERMA200223 approval.

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, calving of recipient cattle, lambing of recipient ewes and the transfer of embryos to recipient animals. Semen has been collected from Bulls for analysis / potential export or storage for future use.

Embryo Transfer activities this year have been in cattle only.

These transferred embryos fall within the approved organism description for the ERMA200223 approval and are for the study of gene function related to animals adapting to temperature changes.

All activities have been undertaken with the approval of the Ruakura Animal Ethics Committee.

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.

Unfortunately, due to circumstances mainly outside of AgResearch influence and despite further attempts, no progress has been made in resolving this Liaison group representation directly to date.

The Facility manager is in regular contact with Tainui Group Holdings (Land owner representative) on their development activities for Ruakura and impacts for the Animal Containment Facility.

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

- Following the capture and storage of the genetics in the form of cells, semen or embryos, the last remaining cattle engineered for modified milk composition were euthanised as an endpoint of these projects.
- Tissue, DNA and milk samples provide opportunities to continue and start new research collaborations based on the unique genetics of the animals.
- No safety concerns due to the genetic modifications have emerged from maintaining the animals.

Generating cattle genome edited for adaptation to warmer temperatures

- Semen from PMEL- and SLICK-edited bulls was collected and cryopreserved.
- Oocytes from PMEL- and SLICK-edited females were used to produce and cryopreserve embryos heterozygous for both edits.
- PMEL- and SLICK-edited females were artificially inseminated to induce a natural lactation and produce next generation calves that carry the mutation in a heterozygous state.
- Detailed monitoring of behavioural and physiological characteristics under warm and cold conditions was carried out. Preliminary results have shown reduced shade use, increased grazing and lower skin temperatures for PMEL-edited cattle under warm conditions. The edits had no impact in cold conditions.
- SLICK-edited cattle continued to be monitored for behavioural and physiological traits with evaluation of data ongoing.
- SLICK-edited cattle had shorter hair in spring and autumn with only the undercoat shorter in summer compared with unedited control cattle.
- Whole genome sequencing data is being evaluated to determine the potential for off-target mutagenesis events in any of the edited animals. This analysis is still in progress.
- We have produced seven (four female, 3 male) calves edited for the disruption of the NANOS2 gene. Once they reach sexual maturity, they will be tested for the impact of the NANOS2 disruption on female and male fertility.
- Activities and results were communicated to various stakeholders and presented at: International Embryo Technology Society, 50th Anniversary Meeting, Denver, January 2024 (invited) and American Dairy Science Association Dairy Digression Podcast, Episode Thirteen: Embryo-Mediated Gene Editing, Coat Color, and Herd Heat Absorption (https://www.youtube.com/watch?v=cg0whwfklGs), January 2024.

Overexpression of the histone demethylase KDM4B in transgenic cattle

- The project has been completed and no animals were retained and maintained.

Goats producing therapeutic proteins

- Goats were maintained to investigate longevity and potential long-term health effects.
- Several does were maintained as possible recipients for future embryo transfers.

Goats producing female-only offspring

- All remaining transgenic animals were culled.

Generating germline-complemented sheep and fertile founders for breeding sterile hosts

- One female NANOS2-/- and male NANOS2+/- cloned founder animal were bred using AI. One live F1 offspring was born, representing a heterozygous knockout genotype for future breeding and phenotype characterisation.

Generating immune-compatible sheep for xenotransplantation

- 4 female double knockout ewes (GGTA and CMAH) were used for AI, but no viable offspring were obtained.

On Farm Management Summary for year ending 30/06/2024

Animal Numbers 01/07/2023– 30/06/2024 (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 (1/07/23)	Births	Transfer In	Transfer Out	Aged In	Aged Out	Killed	Deaths	Closing (30/06/24)
Casein (ERMA200223)									
Total Casein	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
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 / hLZ KI - (ERMA200223)									
MA Cows	**13**				0		13		**0**
Total BLg -	**13**	**0**	**0**	**0**	**0**	**0**	**13**	**0**	**0**
Erbitux (ERMA200223)									
Total Erbitux	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Climate Smart (ERMA200223)									
R2yr Heifer	**0**				**12**				**12**
R1yr Heifer	**12**				**4**	**12**			**4**
Heifer Calves	**0**	4				**4**	**0**		**0**
R2yr Male	**0**				**12**				**12**
R1yr Male	**12**				**3**	**12**			**3**
Bull Calves	**0**	4				**3**	**1**	**0**	**0**
Total Climate Smart	**24**	**8**	**0**	**0**	**19**	**31**	**1**	**0**	**31**
KDM4B (ERMA200223)									
Total KDM4B	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Conventional Cattle									
MA Cows	**73**		0	19	0		8		**46**
Total Conventional	**73**	**0**	**0**	**19**	**0**	**0**	**8**	**0**	**46**
Cattle Total	**110**	**8**	**0**	**19**	**19**	**31**	**22**	**0**	**77**
Cattle alive developed under ERMA approvals (Tg and non Tg progeny)									**31**

[1] Aligns with normal livestock reconciliation aging practice.

Stock Class	Open (1/07/23)	Births	Transfer In	Transfer Out	Aged In	Aged Out	Killed	Deaths	Closing (30/06/24)
Goats									
Erbitux & Enbrel (ERMA200223)			[All Erbitux line]						
Ma Doe	**4**						4		**0**
R1yr Doe	**2**				0		2		**0**
R1yr Male +	**1**				0		1		**0**
Total Erbitux & Enbrel	**7**	**0**	**0**	**0**	**0**	**0**	**7**	**0**	**0**
non Med inherit (ERMA200223)									
Total TCR	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Conventional Goats									
MA Doe	**5**		29				8		**26**
R1yr Doe	**3**				0		3		**0**
Male R1yr +	**2**				0		2		**0**
Total Conventional	**10**	**0**	**29**	**0**	**0**	**0**	**13**	**0**	**26**
Goat Total	**17**	**0**	**29**	**0**	**0**	**0**	**20**	**0**	**26**
Goats alive developed under ERMA approvals (Tg and non Tg progeny)									**0**

Stock Class	Open (1/07/23)	Births	Transfer In	Transfer Out	Aged In	Aged Out	Killed	Deaths	Closing (30/06/24)
Sheep									
AI on Hooves (ERMA200223)									
MA Ewes	**9**				1		0	2	**8**
2th Ewes	**1**				9	1			**9**
Ewe Hgts	**9**				1	9			**1**
Ewe Lamb	**0**	1				1	0		**0**
MA Ram	**2**				1		2		**1**
R1yr Ram	**6**				0	1	0		**5**
Total	**27**	**1**	**0**	**0**	**12**	**12**	**2**	**2**	**24**
Conventional Sheep									
MA Ewes	**41**				5		22	2	**22**
2th Ewes	**5**				0	5			**0**
2th Ram	**0**				1		1		**0**
R1yr Ram	**1**				0	1	0		**0**
Total Conventional	**47**	**0**	**0**	**0**	**6**	**6**	**23**	**2**	**22**
Sheep Total	**74**	**1**	**0**	**0**	**18**	**18**	**25**	**4**	**46**
Sheep alive developed under ERMA approvals (Tg and non Tg progeny)									**24**

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

For cattle there has been one movement of conventional animals out of the facility during the period. 19 conventional cattle which had never held transferred embryos.

21 MA cows (13 GM) and 1 GM bull calf have been humanely killed, all have been disposed of in offal holes on-site, as surplus or following veterinary advice during this period.

For goats there has been one movement of 29 animals on to the facility during the period.

20 (7 GM) goats of varying ages have been humanely 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 no movement of animals on or from the facility during the period.

23 (2 GM) sheep of varying ages have been humanely killed and 4 (2 GM) sheep died during the period; these animals have also been disposed of in offal holes on-site, as surplus or unsuitable animals, or following veterinary advice.

41 cattle recipients have been used for ET (embryo transfer). All animals are regularly monitored for live weight and health status.

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.

Animals on the facility continue to be managed in a way which aligns with normal farming practice in New Zealand, grazing outdoors on pasture with some crops and supplementary feeding of hay, balage, silage or meal concentrates when required.

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

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

Surplus pasture is conserved when possible for use in periods of low growth, as balage, silage 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.

Nearly 3ha within the facility was undersown with a chicory and clover mix in anticipation of a dry summer.

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 was not applied this season.

Operationally we continued juggling animal movements and grazing for much of the season within the facility around construction activities to install water and waste water services for Tainui Group Holdings inland port development which is to the south east of the Animal Containment Facility.

Milk Production 23/24 season

No GM cows calved and no GM goats kidded specifically for seasonal milk production again this year. This has meant there was again no milk stored this year for surplus disposal by irrigation to pasture.

Ruakura Animal Ethics Committee Reports

The Ruakura Animal Ethics Committee (RAEC) removed the requirement for interim reporting on a quarterly basis as approvals are now normally only approved for a 12-month period with formal reporting required at the end of the approval period.
Regular updates on approved activities are provided verbally to the RAEC at scheduled fortnightly meetings during the year.

Below are the active approvals during the 12-month period of this report:

RAEC 2022-0381 – Maintenance of Cattle on the Animal Containment Facility

RAEC 2023-0685 - Climate smart cattle - production and characterisation

RAEC 2023-2024 - Maintenance breeding of different cloned sheep genotypes

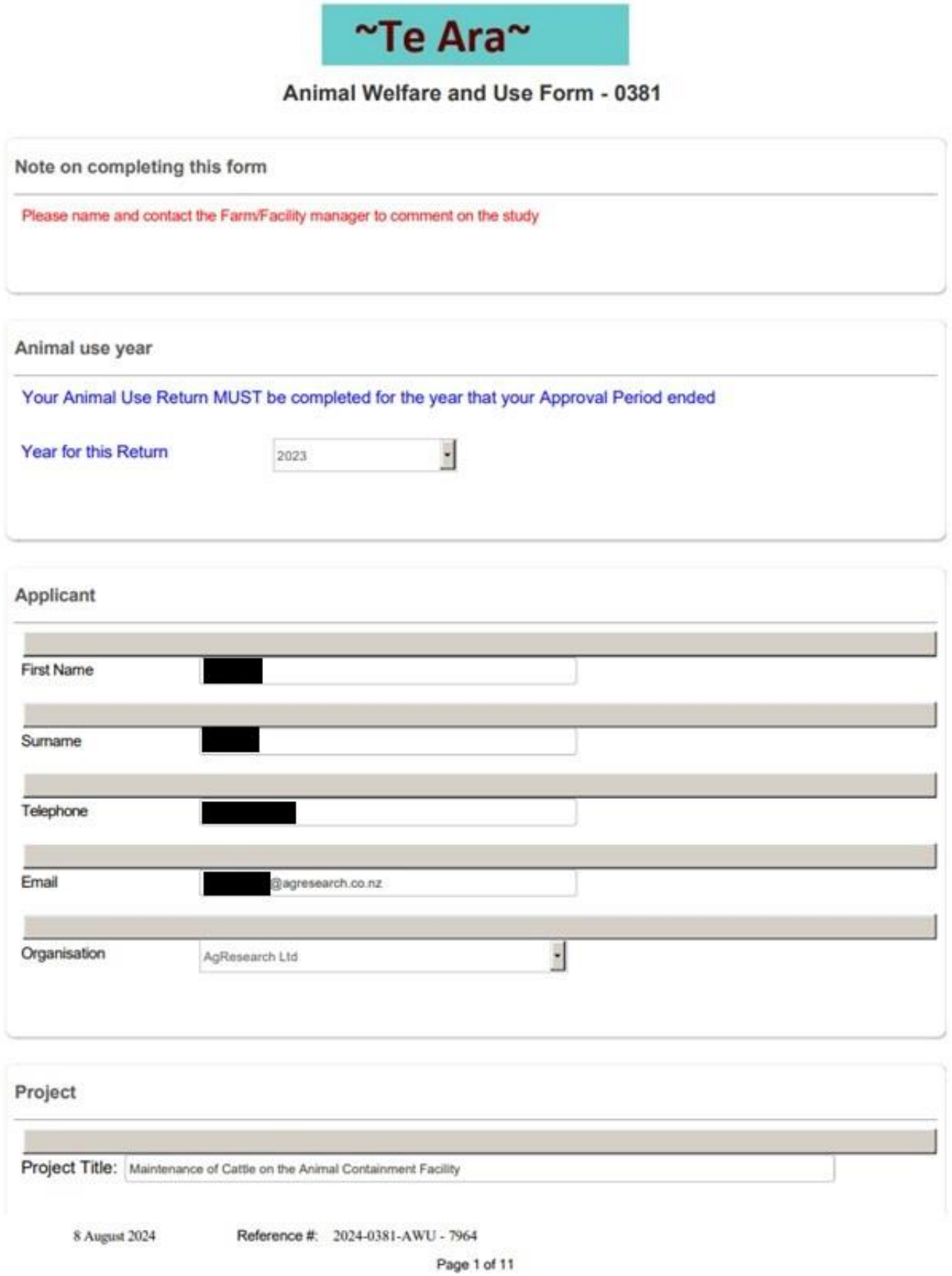
RAEC 2024-2288 - Characterisation of animals produced as part of the climate-smart cattle programme

RAEC 2024-2337 - Climate-Smart cattle embryo development

RAEC 2024-2385 - Collection of cloned transgenic goat fetuses for cell line rejuvenation

Reports Received during the period: *(These reports may contain information on activity in last years EPA reporting period.)*

RAEC 2022-0381 – Maintenance of Cattle on the Animal Containment Facility – Animal Welfare and Use report at end of trial approval period.



~Te Ara~

Animal Welfare and Use Form - 0381

Note on completing this form

Please name and contact the Farm/Facility manager to comment on the study

Animal use year

Your Animal Use Return MUST be completed for the year that your Approval Period ended

Year for this Return 2023

Applicant

First Name [redacted]

Surname [redacted]

Telephone [redacted]

Email [redacted]@agresearch.co.nz

Organisation AgResearch Ltd

Project

Project Title: Maintenance of Cattle on the Animal Containment Facility

8 August 2024 Reference #: 2024-0381-AWU - 7964

Page 1 of 11

Summary of manipulations

Briefly describe all the treatments/manipulations (type and frequency) each animal underwent during this application	standard husbandry treatments 1 cow was stimulated prior to ovum pick-up treatment to recover oocytes and production of IVF embryos Humane killing of animals no longer required or injured

Did the manipulations go according to plan — (•) Yes ( ) No

Were there any adverse events during the project — (•) Yes ( ) No

If any changes or issues outlined within this form, or adverse events reported, require a change in grading from that previously proposed and approved, ensure this is captured in your Animal Use Return below

Did the adverse event increase the Impact Grading of the animals affected — (•) Yes ( ) No

Were any animals withdrawn from the experiment or euthanised prematurely (if not already covered above) — ( ) Yes (•) No

Do you have documents to support your report — ( ) Yes (•) No

We are now required to report examples of the 3R's to MPI along with our animal use statistics.

Please briefly describe and comment on each of the approaches you outlined in your application to implement the 3R's

Did you implement them successfully?

Did additional opportunities to implement the 3R's arise during the study?

Replacement

Implementation

Presently, no alternatives exist that could replace the use of animals in this project.

Reduction

Implementation

Only a limited number of recipients were maintained in support of a publicly funded research programme into adapting dairy cattle to warming climatic conditions. A minimum number of animals for different transgenic lines were maintain to support accumulation of data on benefits and risks of the technology.

Refinement

Implementation

The animals are well cared for by experienced and competent staff with support from a veterinarian with extensive experience in caring for the unique animals under containment conditions.

Reflecting on the study

Provide a comment on the welfare of animals during the study - e.g. general health, nutrition, shade/shelter provisions, mental state etc

animals are cared for by dedicated staff, had good general health and were well fed

What went really well in the study

access to a pool of recipient animal to support another programme

Is there anything about the study you would do differently if you had to repeat or do a similar study

not having a separate application for maintaining recipients and instead include them into a programme were they are being used

Is there any advice you would like to give others carrying out a study in a similar area or using similar techniques

this only involved standard practices

Were there any staffing or resourcing issues that affected the study ○ Yes ◉ No

Were there any health and safety concerns or issues during the study ○ Yes ◉ No

Animal use details for each species

Complete the "USED" column only

Remember to include the Impact Grading for the animals affected by the Adverse Event(s)

ANIMAL USE RETURN

1. Animal Type Cattle

2. Source:

☐ Breeding unit	☐ Commercial	☑ Farm	☐ Born during project
☐ Captured	☐ Imported	☐ Public sources	

	Proposed	Used
Farm	94	34
Source total	100	34

3. Status:

☑ Normal/Conventional	☐ SPF/Germfree	☐ Diseased	☑ Transgenic/Chimera
☐ Protected wildlife	☐ Pregnant	☐ Unborn/Prehatched	☐ Other

	Proposed	Used
Normal/Conventional	79	19
Transgenic/Chimera	19	15
Status total	100	34

4. Purpose:

☐ Teaching	☐ Species conservation	☐ Environmental	☐ Animal husbandry
☐ Basic biological	☐ Medical research	☐ Veterinary research	☐ Testing
☐ Biological agents	☐ Compromised Offspring	☐ Alternatives	☑ Other

	Proposed	Used
Purpose other	100	34

Purpose total	100	34

Details of "other" purpose

Research into the application of transgenic and genome editing technology to genetically improve cattle for animal welfare, environmental and medical benefits.

5. Re-use of animals:

☑ No prior use ☑ Previously used

	Proposed	Used
No prior use	14	0
Previously used	86	34
Question 5 Total	100	34

Details of how these animals have been used previously in Research, Testing and Teaching

Conventional cows have been previously used as recipients for embryo transfer. GM cattle have been previously used for genotyping and phenotyping.

6. Grading of manipulations:

☑ A ☑ B ☑ C ☑ D ☐ E

	Proposed	Used
Grade A	78	19
Grade B	21	13
Grade C	1	1
Grade D	0	1
Grade total	100	34

7. Animals alive at end of project:

- ☐ Retained
- ☐ Returned
- ☐ Released
- ☐ Disposed of
- ☐ Rehomed
- ☑ None alive

8. Animals DEAD at end of the project:

- ☐ Killed for Research, Testing or Teaching on body or tissues
- ☑ Other dead:

	Proposed	Used
Other dead	0	34
Dead total	0	34

For the other dead numbers listed above, please confirm the breakdown of numbers in the following categories:

Note: When filling in **PROPOSED** numbers: ONLY complete the numbers for those animals planned to be **euthanised / killed as a planned end to the project**.

The other categories of animals that die during the project must be reported at the end of the project in the Animal Welfare and Use Form.

- ☑ Euthanised / killed as a planned end to project
- ☐ Died - related to manipulations
- ☐ Died - unrelated to manipulations
- ☑ Euthanised for welfare reasons

	Proposed	Used
Euthanised / killed	0	30
Euthanised for welfare reasons	0	4

Details for the animals killed/died above (for Animal Use form only)	TG cow had acquired a hip injury resulting in lameness / 2 Recips for Cancer eye / 1 TG cow poor (large) udder effecting movement. 30 animals were euthanised including maintained TG animals due to end of project and old recipients no longer fit foe purpose.
Where will animals be killed	Animal Containment Facility
Method of Killing the animals	Other

Please remember to list an SOP in Section 11 for your method of killing such as drug administration or decapitation

Details for "Other" method of killing	pentobarbitone via intravenous injection / Disposal via Firearm
How and where will the animal's bodies be disposed of	deep burial

9. Total number of animals:

	Proposed	Used
Total number of animals	100	34

10. Animals bred for research, testing and teaching but not used and killed:

Were animals bred to enable this project to take place but not used in the project and killed

○ Yes ◉ No

USE ADD ANOTHER FOR EACH ANIMAL TYPE/SPECIES

Changes from proposed

Were there any changes in animal numbers, Impact grade or details from those Proposed and Approved? ◉ Yes ○ No

Please explain why the changes occurred

No calves were born during the approval period.
Some of the recipients were no longer fit for purpose, investigation of the GM lines came to an end and overall animal numbers had to be reduced due to reduced footprint of usable area on the ACF.
One animal acquired a hip injury that changed its grading to D and was humanely killed.
One animal was humanely killed at the planned end of the investigation.

Roles on project

Roles on project:

Tick all the roles on the project

- [x] Program manager
- [ ] Lead Technician / Research Associate
- [x] Project staff
- [ ] Project staff without access to ~ Te Ara ~
- [x] Biometrician
- [x] Farm or Facility Manager
- [ ] Commercial Farmer
- [x] Veterinarian

Program manager

Name: Select Some Options

Program manager Comments:

Project staff

Project staff with access to ~ Te Ara ~

First Name: [redacted]

Surname: [redacted]

Email: [redacted]@agresearch.co.nz

Organisation: AgResearch Ltd

Project staff with access to ~ Te Ara ~

First Name: [redacted]

Surname: [redacted]

Email: [redacted]@agresearch.co.nz

Organisation: AgResearch Ltd

Project staff with access to ~ Te Ara ~

First Name: [redacted]

Surname [redacted]

Email [redacted]@agresearch.co.nz

Organisation AgResearch Ltd

Project staff with access to ~ Te Ara ~

First Name [redacted]

Surname [redacted]

Email [redacted]@agresearch.co.nz

Organisation AgResearch Ltd

Project staff with access to ~ Te Ara ~

First Name [redacted]

Surname [redacted]

Email [redacted]@agresearch.co.nz

Organisation AgResearch Ltd

Name Select Some Options

Project Staff comments

Biometrician

Biometrician

First Name [redacted]

Surname [redacted]

Email [redacted]@agresearch.co.nz

Organisation AgResearch Ltd

Name Select Some Options

Biometrician Comments

Farm or facility manager

First Name: Tim

Surname: Hale

Email: tim.hale@agresearch.co.nz

Organisation: AgResearch Ltd

AgResearch Farm/Facility: Ruakura Animal Containment Facility

Facility Manager comments:

Name and contact the farm / facility manager(s) to comment here.

(If there is more than one manager, multiple names and comments can be added here)

Name: Tim - Hale

Facility Manager Comments: This approval basically covers the alive recipients and GM cattle which are not part of a current specific AEC approved project.
Beyond specific animals identified within this report they have a well cared for environment and uneventful life within the ACF confines.
Staff are all experienced and competent in their responsibilities.

Commercial farmer

Name: Select Some Options

Veterinarian(s)

Name: Select Some Options

Veterinarian Comments:

Applicant sign off

Signed: This form was signed by AgResearch Ltd - [redacted]@agresearch.co.nz) on 22/02/2024 3:11 PM

If the form is complete (use the completeness checker) it will AUTO-SUBMIT once you have signed

RAEC 2023-0685 - Climate smart cattle - production and characterisation - Animal Welfare and Use report at end of trial approval period.

~Te Ara~

Animal Welfare and Use Form - 0685

Note on completing this form

Please name and contact the Farm/Facility manager to comment on the study

Animal use year

Your Animal Use Return MUST be completed for the year that your Approval Period ended

Year for this Return: 2023

Applicant

First Name: [redacted]

Surname: [redacted]

Telephone: [redacted]

Email: [redacted]@agresearch.co.nz

Organisation: AgResearch Ltd

Project

Project Title: Climate smart cattle - production and characterisation

Summary of manipulations

Briefly describe all the treatments/manipulations (type and frequency) each animal underwent during this application

standard husbandry treatments
hair plucking
shaving of small coat patch
fitted with IceQube, cow manger ear tags and implanted Star-oddi data loggers
received SmaXtech rumen boluses
scanning of the coat in the visible and near infrared spectrum
transfer of embryos, edited for NANOS2 knockout, chimaeric homozygous/heterozygous NANOS2 knockout, cloned with embryonic cells
Use of 57 recipients for ET, range of 1 -3 ETs,
rectal ultrasound scanning, twice following ET around day 35 and 45 of gestation, around day 35 (3x20, 1x37) and 45 (3x20, 1x37). Pregnant animals of different ET groups were rescanned around day 80 (6), day 100 (6) and 165/187 (8) of gestation
delivery of two calves by C-section with recipients humanely killed
era punch biopsy
blood samples
behavioral observations
semen collection

	26/09/2023		15/11/2023		17/11/2023	
	Method	straws	Method	straws	Method	straws
#6	AV x3	28	AV, Electro	0	Manual	204
#14	AV x3	0	AVx2	373		
#16	AV x3	0	AVx2	250		
#17	AV x3	0	AVx2	0		
#22			AVx2	0	AVx2	343
#24			AVx2	183		
#27			AVx2	0	AVx2	160

Synchronisation protocols

day of gestation treatment
-28 PG
-16 PG
-13 CIDr in & GNRH
-3 CIDr out & PG
0 Heats
7 ET

day of gestation treatment
-10 CIDr In
-3 CIDr Out & 3mL PG
0 Heats
7 Transfers

Did the manipulations go according to plan — (•) Yes ( ) No

Were there any adverse events during the project — ( ) Yes (•) No

Were any animals withdrawn from the experiment or euthanised prematurely (if not already covered above) — ( ) Yes (•) No

Do you have documents to support your report — ( ) Yes (•) No

We are now required to report examples of the 3R's to MPI along with our animal use statistics.

Please briefly describe and comment on each of the approaches you outlined in your application to implement the 3R's

Did you implement them successfully?

Did additional opportunities to implement the 3R's arise during the study?

Replacement

Implementation

Presently, no alternatives exist that could replace the use of animals in this project.

Reduction

Implementation

Only a limited number of recipients are used for production of limited numbers of edited animals still predicted to meet programme objectives. We have used recipient animals multiple times for embryo transfer to minimise the number of animals.

Refinement

Implementation

All manipulations are carried out according to SOP's or contracted out to commercial service providers as technology experts which aim to minimize any pain or noxiousness by use of minimally invasive techniques, sedation, pre-emptive pain relief and gold standard nursing and husbandry. SOPs and husbandry protocols are regularly updated to ensure the best possible welfare conditions for the experimental animals.

Reflecting on the study

Provide a comment on the welfare of animals during the study - e.g. general health, nutrition, shade/shelter provisions, mental state etc

animals are well cared for by dedicated staff, had good general health and were well fed

What went really well in the study

hyperspectral scanning of the coats

Is there anything about the study you would do differently if you had to repeat or do a similar study

greater focus on generating embryos and animals from OPU-derived oocytes
switch from embryo-mediated genome edition to using cloning of edited embryonic cells

Is there any advice you would like to give others carrying out a study in a similar area or using similar techniques

the most efficient production of genome edited claves with defined mutations is likely to be achieved by editing embryonic stem cells followed by embryonic cloning with such cells.

Were there any staffing or resourcing issues that affected the study ○ Yes ◉ No

Were there any health and safety concerns or issues during the study ○ Yes ◉ No

Animal use details for each species

Complete the "USED" column only

Remember to include the Impact Grading for the animals affected by the Adverse Event(s)

ANIMAL USE RETURN

1. Animal Type Cattle

2. Source:

☐ Breeding unit ☐ Commercial ☑ Farm ☑ Born during project
☐ Captured ☐ Imported ☐ Public sources

	Proposed	Used
Farm	103	81
Born during project	22	2
Source total	125	83

3. Status:

☑ Normal/Conventional	☐ SPF/Germfree	☐ Diseased	☑ Transgenic/Chimera
☐ Protected wildlife	☑ Pregnant	☐ Unborn/Prehatched	☐ Other

	Proposed	Used
Normal/Conventional	111	61
Transgenic/Chimera	14	16
Pregnant	0	6
Status total	125	83

4. Purpose:

☐ Teaching	☐ Species conservation	☐ Environmental	☐ Animal husbandry
☑ Basic biological	☐ Medical research	☐ Veterinary research	☐ Testing
☐ Biological agents	☐ Compromised Offspring	☐ Alternatives	☐ Other

	Proposed	Used
Basic biological research	125	83
Purpose total	125	83

5. Re-use of animals:

☑ No prior use ☑ Previously used

	Proposed	Used
No prior use	28	2
Previously used	97	81
Question 5 Total	125	83

Details of how these animals have been used previously in Research, Testing and Teaching

All recipients (57) were previously used for embryo transfers for the production of live edited and control calves
Slick (6) and control (5) calves were previously characterized for genotype, hair phenotype and behaviour under the previous AE approval.
PMEL (8) and control (5) calves were previously characterized for genotype and lighter coat colour under the previous AE approval.
NANOS2 knock calves (2) were born during the project

6. Grading of manipulations:

☐ A ☑ B ☑ C ☐ D ☐ E

	Proposed	Used
Grade B	113	81
Grade C	12	2
Grade total	125	83

7. Animals alive at end of project:

☑ Retained ☐ Returned ☐ Released ☐ Disposed of
☐ Rehomed ☐ None alive

	Proposed	Used
Retained	119	63
Alive total	119	63

Who will be RETAINING the animals and any specific care that will be required

All animals will be retained at the Animal Containment Facility.

8. Animals DEAD at end of the project:

☐ Killed for Research, Testing or Teaching on body or tissues ☑ Other dead:

	Proposed	Used
Other dead	6	20

Dead total	6	20

For the other dead numbers listed above, please confirm the breakdown of numbers in the following categories:

Note: When filling in **PROPOSED** numbers: ONLY complete the numbers for those animals planned to be **euthanised / killed as a planned end to the project**.

The other categories of animals that die during the project must be reported at the end of the project in the Animal Welfare and Use Form.

- [x] Euthanised / killed as a planned end to project
- [ ] Died - related to manipulations
- [ ] Died - unrelated to manipulations
- [x] Euthanised for welfare reasons

	Proposed	Used
Euthanised / killed	0	18
Euthanised for welfare reasons	6	2

Details for the animals killed/died above (for Animal Use form only)	recipients not kept alive following C-section births for Group 91 calving. Recipients used for ET and never established a pregnancy are deemed not fit for purpose and can be removed from the ACF (19 during period) and any animals that die or are euthanised on animal welfare grounds are disposed of by deep burial at the ACF as approved by Ngati Wairere and in ERMA200223 controls. Again cross over with numbers for 381 with timing of events.
Where will animals be killed	on ACF
Method of Killing the animals	Other

Please remember to list an SOP in Section 11 for your method of killing such as drug administration or decapitation

Details for "Other" method of killing	Drug admin or using Firearm
How and where will the animal's bodies be disposed of	ACF

9. Total number of animals:

	Proposed	Used

Total number of animals	125	83

10. Animals bred for research, testing and teaching but not used and killed:

Were animals bred to enable this project to take place but not used in the project and killed

○ Yes ◉ No

USE ADD ANOTHER FOR EACH ANIMAL TYPE/SPECIES

Changes from proposed

Were there any changes in animal numbers, Impact grade or details from those Proposed and Approved? ◉ Yes ○ No

Please explain why the changes occurred

Fewer recipients were used as not as many embryo transfers were conducted as originally planned.

Roles on project

Roles on project:

Tick all the roles on the project

- ☑ Program manager
- ☐ Lead Technician / Research Associate
- ☑ Project staff
- ☐ Project staff without access to ~ Te Ara ~
- ☑ Biometrician
- ☑ Farm or Facility Manager
- ☐ Commercial Farmer
- ☑ Veterinarian

Program manager

Name: Select Some Options

Program manager Comments:

Project staff

Project staff with access to ~ Te Ara ~

First Name [redacted]

Surname [redacted]

Email [redacted]@agresearch.co.nz

Organisation AgResearch Ltd

Project staff with access to ~ Te Ara ~

First Name [redacted]

Surname [redacted]

Email [redacted]@agresearch.co.nz

Organisation AgResearch Ltd

Project staff with access to ~ Te Ara ~

First Name [redacted]

Surname [redacted]

Email [redacted]@agresearch.co.nz

Organisation AgResearch Ltd

Project staff with access to ~ Te Ara ~

First Name [redacted]

Surname [redacted]

Email [redacted]@agresearch.co.nz

Organisation AgResearch Ltd

Project staff with access to ~ Te Ara ~

First Name [redacted]

Surname [redacted]

Email [redacted]@agresearch.co.nz

Organisation AgResearch Ltd

Project staff with access to ~ Te Ara ~

First Name [redacted]

Surname [redacted]

Email [redacted]@agresearch.co.nz

Organisation AgResearch Ltd

Project staff with access to ~ Te Ara ~

First Name [redacted]

Surname [redacted]

Email [redacted]@agresearch.co.nz

Organisation AgResearch Ltd

Project staff with access to ~ Te Ara ~

First Name [redacted]

Surname [redacted]

Email [redacted]@agresearch.co.nz

Organisation AgResearch Ltd

Project staff with access to ~ Te Ara ~

First Name [redacted]

Surname [redacted]

Email [redacted]@agresearch.co.nz

Organisation AgResearch Ltd

Project staff with access to ~ Te Ara ~

First Name [redacted]

Surname [redacted]

Email [redacted]@agresearch.co.nz

Organisation AgResearch Ltd

Project staff with access to ~ Te Ara ~

First Name [redacted]

Surname [redacted]

Email [redacted]@agresearch.co.nz

Organisation AgResearch Ltd

Project staff with access to ~ Te Ara ~

First Name [redacted]

Surname [redacted]

Email [redacted]@agresearch.co.nz

Organisation AgResearch Ltd

Name Select Some Options

Project Staff comments

Biometrician

Biometrician

First Name [redacted]

Surname [redacted]

Email [redacted]@agresearch.co.nz

Organisation AgResearch Ltd

Name Select Some Options

Biometrician Comments

Farm or facility manager

First Name Tim

Surname Hale

Email: tim.hale@agresearch.co.nz

Organisation: AgResearch Ltd

AgResearch Farm/Facility: Ruakura Animal Containment Facility

Facility Manager comments:

Name and contact the farm / facility manager(s) to comment here.

(If there is more than one manager, multiple names and comments can be added here)

Name: Tim - Hale ×

Facility Manager Comments: Cattle used for this project have been grazed as per expectation for New Zealand farming practice and well cared for by the experienced staff looking after them.
There is cross over in Recipient cattle numbers reporting with 381 because of concurrent approvals but numbers here reflect actually used in this approval.

Commercial farmer

Name: Select Some Options

Veterinarian(s)

Veterinarian

First Name: [redacted]

Surname: [redacted]

Telephone: [redacted]

Email: [redacted]

Organisation: External Contractor

Comment on application	
Veterinarian	
First Name	[redacted]
Surname	[redacted]
Telephone	[redacted]
Email	[redacted]
Organisation	[redacted]
Comment on application	
Name	Select Some Options
Veterinarian Comments	

Applicant sign off

Signed: This form was signed by AgResearch Ltd - [redacted]@agresearch.co.nz) on 22/02/2024 3:11 PM

If the form is complete (use the completeness checker) it will AUTO-SUBMIT once you have signed

~Te Ara~

Animal Welfare and Use Form - 2024

Note on completing this form

Please name and contact the Farm/Facility manager to comment on the study

Animal use year

Your Animal Use Return MUST be completed for the year that your Approval Period ended

Year for this Return: 2024

Applicant

First Name: [redacted]

Surname: [redacted]

Telephone: +64[redacted]

Email: [redacted]@agresearch.co.nz

Organisation: AgResearch Ltd

Project

Project Title: Maintenance breeding of different cloned sheep genotypes

16 September 2024 Reference #: 2024-2024-AWU - 9520

Page 1 of 10

Summary of manipulations

Briefly describe all the treatments/manipulations (type and frequency) each animal underwent during this application	Overview of maintenance breeding activities for different NANOS2 and CMAH/GGTA genotypes 2023: 1) Natural mating - 26/05/23 a cloned NANOS2+/- ram (Howie) was mated with a cloned NANOS2-/- ewe (#1927, Sandy) as well as with 4 other ewes ((Serena, Venus, Rhonda, Minnie) of the CMAH-/-/ GGTA-/- genotype. From these matings we obtained one lamb from 1927 on 30/10/23 (alive and well), but no pregnancies from the 4 other ewes. 2) #1927 – Died twisted gut 9/11/23. As for the lamb it was brought into the PC2 room at the ACF on sawdust and feed 3 times a day and monitored until it accepted the bottle and came for feeding then we let it back into a paddock with shelter with youngest ewes (2022 brn animals) until it was big enough to go in with more sheep and weaned at recommended weigh. One non-experimental ewe died: 3) #1935 – Found dead. No known cause 27/02/24 Several non-experimental rams, which are not covered by this application, were euthanised on welfare grounds as part of normal farm maintenance: 4) Ram – Euthed 1/08/23 5) Rams x2 – Tendon problems, euthanised (adverse event) 11/10/23. As stated above, these were not part of the study but covered here regardless. Several recipient ewes were culled as surplus: 6) 1x 1/07/23, 12x 4/09/23, 1x 17/08/23, 2x 14/12/23 These animals listed under 3)-6) are not listed under animal usage numbers since they were subject to normal farm practice and not experimentally manipulated. They are only mentioned here for completeness. 7) mated ewes were scanned around D45 for pregnancy 8) The born lamb was blood sampled for genotyping.
Did the manipulations go according to plan	○ Yes ◉ No
Describe what did not go as planned	The 4 'xeno' ewes did again not get pregnant, even though the ram was fertile with another ewe (and had been on multiple occasions before). This is the 3rd season in a row that these ewes did not get pregnant, either by AI or mating. Thi s is despite the fact that they all performed well in OPU/IVF experiments and produced normal numbers of eggs, as well as possessing an anatomically normal reproductive tract and organs. This is an intruiging observation, pointing at a function of the CMAH/GGTA genes during early pregnancy recognition or embryo development. It could be related to immune rejection. We are planning to follow up this observation in the future.
Were there any adverse events during the project	◉ Yes ○ No

If any changes or issues outlined within this form, or adverse events reported, require a change in grading from that previously proposed and approved, ensure this is captured in your Animal Use Return below

Did the adverse event increase the Impact Grading of the animals affected ○ Yes ◉ No

Were any animals withdrawn from the experiment or euthanised prematurely (if not already covered above) ◉ Yes ○ No

Please describe the circumstances

see above summary

We are now required to report examples of the 3R's to MPI along with our animal use statistics.

Please briefly describe and comment on each of the approaches you outlined in your application to implement the 3R's

Did you implement them successfully?

Did additional opportunities to implement the 3R's arise during the study?

Replacement

Implementation

Live animals were required as recipients since exo vivo gestation is not possible. The unexpected but reproducible 'non-pregnant' phenotype of the GGTA/CMAH ewes, is a good illustration why live animal studies are so valuable. This observation was not predictabel based on avaialble in vitro evidence and warrants further investigation.

Reduction

Implementation

We used the minimal numbers of animals required to get at least one female NANOS2-/- (1 animal). The ram is +/- and the ewe is -/-, so the chances of getting -/- offspring is 50% and getting female -/- is 25%, so the ideal outcome is not guranteed. However, we only have one female -/- available and even generation of +/- females will increase our chances of obtaining NANOS2-/- females in future breeding.
By using one ram to simultaneously generate animals with different edits we reduced animal use. We will also did not include any wild-type animals because the resulting genotypes will not be required and have to be culled.

Refinement

Implementation

Manipulations were carried out according to SOPs to minimize any pain or noxiousness

Reflecting on the study

Provide a comment on the welfare of animals during the study - e.g. general health, nutrition, shade/shelter provisions, mental state etc

Animals were well looked after throughout.

What went really well in the study

Study went to plan and a heterozygous NANOS2 female was produced, which is valuable for further breeding.

Is there anything about the study you would do differently if you had to repeat or do a similar study

no

Is there any advice you would like to give others carrying out a study in a similar area or using similar techniques

no

Were there any staffing or resourcing issues that affected the study ○ Yes ◉ No

Were there any health and safety concerns or issues during the study ○ Yes ◉ No

Animal use details for each species

Complete the "USED" column only

Remember to include the Impact Grading for the animals affected by the Adverse Event(s)

ANIMAL USE RETURN

1. Animal Type Sheep

2. Source:

☑ Breeding unit	☐ Commercial	☐ Farm	☑ Born during project
☐ Captured	☐ Imported	☐ Public sources	

	Proposed	Used
Breeding Unit	7	6
Born during project	10	1
Source total	17	7

3. Status:

☐ Normal/Conventional	☐ SPF/Germfree	☐ Diseased	☑ Transgenic/Chimera
☐ Protected wildlife	☐ Pregnant	☐ Unborn/Prehatched	☐ Other

	Proposed	Used
Transgenic/Chimera	17	7
Status total	17	7

4. Purpose:

☐ Teaching	☐ Species conservation	☐ Environmental	☐ Animal husbandry
☑ Basic biological	☐ Medical research	☐ Veterinary research	☐ Testing
☐ Biological agents	☐ Compromised Offspring	☐ Alternatives	☐ Other

	Proposed	Used
Basic biological research	17	7
Purpose total	17	7

5. Re-use of animals:

☑ No prior use ☑ Previously used

	Proposed	Used
No prior use	10	1

Previously used	7	6
Re-use of animals total	17	7

Details of how these animals have been used previously in Research, Testing and Teaching

The NANOS2+/- ram was used for successful mating and AI, the 5 ewes were used for OPU. The lambs wiill not have been used before.

6. Grading of manipulations:

☐ A ☑ B ☐ C ☐ D ☐ E

	Proposed	Used
Grade B	17	7
Grade total	17	7

7. Animals alive at end of project:

☑ Retained ☐ Returned ☐ Released ☐ Disposed of
☐ Rehomed ☐ None alive

	Proposed	Used
Retained	17	6
Alive total	17	6

Who will be RETAINING the animals and any specific care that will be required

Retained at ACF, born naturally

8. Animals DEAD at end of the project:

☐ Killed for Research, Testing or Teaching on body or tissues ☑ Other dead:

	Proposed	Used

Other dead	0	1
Dead total	0	1

For the other dead numbers listed above, please confirm the breakdown of numbers in the following categories:

Note: When filling in **PROPOSED** numbers: ONLY complete the numbers for those animals planned to be **euthanised / killed as a planned end to the project**.

The other categories of animals that die during the project must be reported at the end of the project in the Animal Welfare and Use Form.

- ☐ Euthanised / killed as a planned end to project
- ☐ Died - unrelated to manipulations
- ☑ Died - related to manipulations
- ☐ Euthanised for welfare reasons

	Proposed	Used
Died - related	0 - 999999	1

Details for the animals killed/died above

(for Animal Use form only)

#1935 – Found dead. No known cause 27/02/24 - was pink tagged!
#1927 - Died with Twisted gut post lambing

9. Total number of animals:

	Proposed	Used
Total number of animals	17	7

10. Animals bred for research, testing and teaching but not used and killed:

Were animals bred to enable this project to take place but not used in the project and killed

- ○ Yes
- ◉ No

USE ADD ANOTHER FOR EACH ANIMAL TYPE/SPECIES

Changes from proposed

Were there any changes in animal numbers, Impact grade or details from those Proposed and Approved?

- ◉ Yes
- ○ No

Please explain why the changes occurred

4 instead of 5 GGAT/CMAH ewes were mated because one was not up for it due to health reasons.

Roles on project

Roles on project:

Tick all the roles on the project

- [x] Program manager
- [ ] Lead Technician / Research Associate
- [x] Project staff
- [ ] Project staff without access to ~ Te Ara ~
- [ ] Biometrician
- [x] Farm or Facility Manager
- [ ] Commercial Farmer
- [ ] Veterinarian

Program manager

Name: Select Some Options

Program manager Comments:

Project staff

Project staff with access to ~ Te Ara ~

First Name: [redacted]

Surname: [redacted]

Email: [redacted]@agresearch.co.nz

Organisation: AgResearch Ltd

Project staff with access to ~ Te Ara ~

First Name: [redacted]

Surname [redacted]

Email [redacted]@agresearch.co.nz

Organisation AgResearch Ltd

Name Select Some Options

Project Staff comments

Biometrician

Name Select Some Options

Farm or facility manager

Farm or Facility Manager

First Name Tim

Surname Hale

Email tim.hale@agresearch.co.nz

Organisation AgResearch Ltd

AgResearch Farm/Facility Ruakura Animal Containment Facility

Comment on application

Name and contact the farm / facility manager to comment here

Name Tim - Hale ×

Facility Manager Comments Beyond separation for mating and lambing these sheep run with the recipient ewes for management purposes and receive same health treatments etc.
Feet management and condition require regular monitoring and frequent pedicures.

Commercial farmer

Name: Select Some Options

Veterinarian(s)

Name: Select Some Options

Applicant sign off

Signed: This form was signed by AgResearch Ltd - [redacted] ([redacted]@agresearch.co.nz) on 27/08/2024 8:38 AM

If the form is complete (use the completeness checker) it will AUTO-SUBMIT once you have signed

~Te Ara~

Interim Report - 2288

Project Title

Project Title: Characterisation of animals produced as part of the climate-smart cattle programme

Interim Report Title

Title
(include timeframe covered)

Title
(include timeframe covered): First interim report 2288, 23 January to 30 June 2024

Interim Report Number: #1

Interim Report Date: 30/06/2024

Report

Interim Report Details - *briefly summarise progress on the project so far, including reference to any adverse events and approved amendments*	Interim Report 2288 Nine edited and control heifers were artificially inseminated to get them in calf and into lactation. Seven heifers were pregnant after the first round of AI, with one pregnant from the second round of AI with due dates of 30 November and 20 December 2024. Only one heifer, a wild type control, failed to become pregnant. We have been working with ABS to gain approval for the collection and production of semen and embryos, respectively for export to Australia. Following an internal audit by ABS and an audit by AssureQuality we received the approval for collection, processing and storage of bovine semen to Australia. We gained approval from MPI to transfer semen to ABS for processing and their return to AgResearch for storage. The first export eligible semen collection on 22 May 2024 was observed by AssureQuality as mandatory control. The collection of export -approved semen from our bulls is now complete. In addition, we received the approval for collection, processing and storage of in vitro bovine embryos to Australia. The first export eligible oocyte collection and embryo processing will need to be observed by AssureQuality as mandatory control. We are in communication with our collaborators at CSIRO in Australia. The existing but expired heads of agreement between AgResearch and CSIRO is presently being renewed. Conversations with the Australian Department of Agriculture, Fisheries and Forestry and the Office of the Gene Technology Regulator about required approvals for the transfer of bovine semen and embryos to CSIRO have been initiated. This year we had a better summer with warm days than for the 2022/23 summer. Data collected from observations, loggers and rumen boluses, and exercise challenges under warm conditions are presently being evaluated. We have optimized culture of bovine embryonic stem cells and have established embryonic stem cells with a beta-casein transgene as genetic marker. In addition, we have derived primary cells from a NANOS2 -/- bull calf and verified these as suitable donor cells to produce cloned, sterile host embryos. Protocols for the injection of embryonic stem cells into early host embryos have been developed. Final testing of injection of the transgenic embryonic stem cells into cloned NANOS2 host embryos and characterisation of injection outcomes is underway. This has taken longer than initially expected. Once completed, we will produce chimaeric embryos for transfer and development to mid-term. Genotyping of the latest NANOS2 edited calves revealed production of two males with the intended precision homozygous NANOS2 knockout. The detailed genotype analysis using single cell clones derived from one additional male and two female NANOS2 edited calves showed that the male calf is a potential candidate for a heterozygous NANOS2 knockout that could be fertile and suitable to produce sterile NANOS2 -/- male host embryos. Although both females had complex on-site edits, one of the females was identified to have a homozygous NANOS2 KO genotype and is suitable for the production of sterile NANOS2 -/- male host embryos.

Do you have documents to upload ○ Yes ◉ No

Signature(s)

First Name	[redacted]
Surname	[redacted]
Email	[redacted]@agresearch.co.nz
Organisation	AgResearch Ltd

Signed: This form was signed by AgResearch Ltd - [redacted]@agresearch.co.nz) on 30/06/2024 12:27 PM

~Te Ara~

Interim Report - 2337

Project Title

Project Title: Climate-Smart cattle embryo development

Interim Report Title

Title

(include timeframe covered)

Title *(include timeframe covered)*: First interim report 2337, 29 March to 31 July 2024

Interim Report Number: #1

Interim Report Date: 31/07/2024

Report

Interim Report Details - *briefly summarise progress on the project so far, including reference to any adverse events and approved amendments*

Interim Report 2337

Following required health testing of oocyte donors, we gained approval from MPI for collection of oocytes, processing and storage of in vitro bovine embryos that can be exported to Australia. The first export eligible oocyte collection and embryo processing was undertaken on 5/6/24 including four PMEL-edited, one SLICK-edited and four wild type (WT) donors. Overall, oocytes were collected on three consecutive weeks and was then stopped with donors getting 4 months into their pregnancies. Embryos produced from two mosaic donors (one PMEL and one SLICK) were biopsied to determine the genotype of the embryos. We cryopreserved 13, 11 and 5 embryos derived from the three non-mosaic PMEL donors. These embryos are heterozygous for both the PMEL and SLICK edits. For the four WT donor cows, 24, 5, 5, and 5 embryos were cryopreserved.

Six embryos were produced from the mosaic PMEL donor, and five embryos were confirmed to possess the edited allele of the natural PMEL mutation. These embryos are heterozygous for both the PMEL and SLICK edits.

Five embryos were produced from the mosaic SLICK donor. One embryo was confirmed to possess the edited allele of the natural SLICK mutation. Two embryos have an alternative edit that also results in the SLICK phenotype. These embryos are heterozygous for both the PMEL and SLICK edits.

Collection of oocytes will resume, except for one WT donor which already reached the minimum target of 20 embryos, once heifers have their first heat after calving.

Do you have documents to upload	○ Yes	◉ No

Signature(s)

First Name	[redacted]
Surname	[redacted]
Email	[redacted]@agresearch.co.nz
Organisation	AgResearch Ltd

Signed: This form was signed by AgResearch Ltd - [redacted]@agresearch.co.nz) on 07/08/2024 10:54 AM

Ministry for Primary Industries
Manatū Ahu Matua

Verification Report[1]

Report ID:	PBV/2501/2023/03
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:	2023-05-03 to 2023-08-09
Verification Date:	2023-08-08
Published:	2023-08-18 14:38
Next Due Date:	2024-02-09
Level/Step:	**6.1** (started on 5.2 , and ceiling is 6)
Report Type:	Scheduled
Peer Reviewed By:	Nicki Sherratt

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, AgResearch 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

Glossary of terms :

TF	Transitional Facility
ACF	Animal Containment Farm
ACU	Animal Containment Unit
BACC	Biosecurity Authority Clearance Certificate
CAR	Corrective Action Request
CF	Containment Facility
CTO	Chief Technical Officer
CTOd	CTO decision/permission under Section 52/53 of the Biosecurity Act 1993
DFO	Delegated Facility Operator
EPA	Environmental Protection Authority
GH	Glasshouse
GM	Genetically Modified
HSNO	Hazardous Substances and New Organisms
MPI	Ministry for Primary Industries
NC	Non-Compliance
NZFS - VS	New Zealand Food Safety - Verification Services
PBV	Performance Based Verification
PC1	Physical Containment Level 1
PC2	Physical Containment Level 2
PP	Plant Protection
PPE	Personal Protective Equipment
R&M	Repairs and Maintenance
SAC	Small Animal Containment

2. Executive Summary

The objective of this PBV was to verify compliance with the facility manual, the Import Health Standards, the Standards identified in the "Biosecurity" section of this report, the HSNO Act 1996 and the facility and operator approvals as held under the Biosecurity Act 1993.

This was a scheduled and announced inspection of the AgResearch Limited transitional and containment facility at the Ruakura site in Hamilton. The outcome of the verification undertaken 8/08/2023 had an acceptable outcome. One NC has been issued for Laboratories. Nothing required follow up from the previous verification and AgResearch – Ruakura returns to a six-monthly verification frequency.

MPI is satisfied that AgResearch is operating in compliance with the requirements of the standards it is approved to. As such the facility and operator approvals will be continued.

3. Operator Summary

The entry and exit meetings along with the reality check of the facility was carried out by Crystal Lange (MPI) with [redacted](DFO) and Tim Hale (DFO). Animal records were reviewed in the SAC and ACF. Laboratory records were reviewed prior to the reality check.

The Inspectors' authority under the Biosecurity Act 1993 and HSNO Act 1996 was confirmed. Health and Safety is covered by a visitor register. No additional hazards were notified. Crystal was accompanied at all times.

The inspection process included a review of onsite records and a reality check. Records reviewed included: staff training, biological products register, new organisms' registers, animal registers and internal audits. The reality check included PC1 and PC2 laboratories in Plant Protection, Animal Physiology, animal containment in SAC and ACF and the Glasshouses.

4. Verification Completed (this period)

Biosecurity

The following elements were verified in this PBV period:

Biosecurity:Containment Facilities for Plants: 2007	Acceptable
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
Biosecurity:Transitional and Containment Facilities for Invertebrates	Acceptable

Subject: Transitional Facilities for Biological Products

Note List:

[Crystal Lange]

Management of biological products is closely aligned with that of products under the Microorganism standard.

Subject: Facilities for Microorganisms and Cell Cultures: 2007a

Note List:

[Crystal Lange]

Normal audit frequency of six monthly can now be resumed following an acceptable verification outcome. Approval of two tenant companies and independent containment facilities can be confirmed.

Updated or new CTO approvals are supplied to the verifier in a timely manner. Decontamination or structural repairs are advised as needed.

Subject: Containment Facilities for Plants: 2007

Note List:

[Crystal Lange]

PC2 rooms in use had the permissions (CTO or HSNO) noted on the door. All plants were clearly labelled. R&M, general maintenance and housekeeping was recorded in a number of logs, some entries were therefore duplicated. PC1 trial work was labelled and contained in trays to prevent loss following irrigation.

Subject: Transitional and Containment Facilities for Invertebrates

Note List:

[Crystal Lange]

Nematology work is ongoing. Nematodes are managed through multiple levels of containment.

Subject: Containment Standard for Field Testing of Farm Animals

Note List:

[Crystal Lange]

Day old lambs not yet in the site register were advised. Calves 32 and 33 were seen to be tagged in compliance with the Standard. Buck goats and sheep were

seen during the reality check and discussed further. Cows in a selected paddock were located on the farm map (phone App) and movement history confirmed.

A perimeter breach and theft of an electric fence unit was notified to MPI. The inner fence was not breached.

Subject: Containment Facilities for Vertebrate Laboratory Animals

Note List:

[Crystal Lange]

Containment and cage card labelling was accessed. The delegated operator was knowledgeable of all strains held and future uses of each cell line. Enrichment tools were available in each rodent cage.
Room 80 (SAC) was inspected and approved for use under dual standards (154.03.03, 154.03.02/154.02.17).

Quality Assurance

Site functions with regards to research and land holdings were discussed. Landmark dates were confirmed.

The site containment manual had been updated this verification period with changes to the facility footprint following the removal of some laboratories and the resignation of the Site Maintenance Engineer.

Internal audits had been completed for all areas. The PP audit was assessed as part of the previous PBV. Training was up to date with one induction, two contractor and one cleaner being trained since the last verification

The following elements were verified in this PBV period:

Quality Assurance:Biosecurity Contingency Plans	Acceptable
Quality Assurance:Chief Technical Officer (CTO) Permissions and Decisions	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:Training and Competency of Personnel	Acceptable

Documentation and Certification

Two imports had been received over the last three months. Transfer records were maintained to a high standard and the Delegated Operator was able to advise on the status of all approved transfers.

There have been no modifications to laboratories, just removal of some from the footprint due to changes in leased space.

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
Documentation and Certification:Site Plans, Specification and Modifications	Acceptable

Identification, Traceability & Management

Work underway and projects to be started were discussed. Imports and transfers were reconciled. Transfers EM2609 and EM2610 had yet to occur. Animal inventories were supplied and were up to date (7/08/2023) for the ACF and as at 26/07/2023 for SAC. Updates for the SAC were notified at the PBV. Records for PC1 plants (unwanted organisms) was not reviewed at this visit. PC2 GH register recorded work for the unwanted organism M.minor. Plant trial work is recorded in individual lab books.

The following elements were verified in this PBV period:

Identification, Traceability & Management:Inventory Control and Accuracy	Acceptable
Identification, Traceability & Management:Product and Organism Identification	Acceptable
Identification, Traceability & Management:Segregation	Acceptable
Identification, Traceability & Management:Transfer of Goods and Organisms	Acceptable

Hygiene & Sanitation

Notification had been received of cluster files entering laboratories through light fittings recessed into the ceiling space. Replacement lights are being installed that are fully sealed units. Excessive rainfall and long term ponding has raised the duck population in areas of standing water. Farm and building vermin control continue to be managed well.

PPE was available PC1, PC2, Planthouse and Small Vertebrate facilities. Autoclave function was confirmed using dual iButtons in July (PP-PC2) and August (PP-Glasshouse and SAC). Sufficient offal holes are present on the farm to manage Large Vertebrate waste.

The following elements were verified in this PBV period:

Hygiene & Sanitation:Cleaning and Disinfection	Acceptable

The following elements were verified in this PBV period:

Hygiene & Sanitation:Personnel Hygiene and Personal Protective Equipment (PPE)	Acceptable
Hygiene & Sanitation:Pest, Vermin and Weed Control	Acceptable
Hygiene & Sanitation:Waste Management	Acceptable

Design and Construction

All facilities inspected were well constructed. Minor repairs were noted for PP.

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:Physical Containment Level 2 (PC2)	Acceptable
Design and Construction:Plant Houses and Glasshouses	Acceptable
Design and Construction:Signage	Acceptable

Subject: Laboratories

Note List:

[Crystal Lange]

Glass flasks of water were seen to be carried into the PC2 Embryo suite in fabric bags. These bags were placed on the floor prior to being placed on the bench. Fabric bags are not impervious, they also pose a safety risk as they weaken with age.

The self closers on doors in the PC2 GH and PP-PC2 (lab) were not working properly and the doors would not close without a push. Paint work in the PP-PC2 needs to be repaired to maintain the impervious finish. A small crack in the vinyl flooring in PP-PC1 was noted along with a bench needing to be sealed.

NON-COMPLIANCE Rated: 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.

Corrective Action Request
1. Ensure walls, floors and work surfaces are sealed and impervious.
2. Remedy self closing doors.
3. Review use of fabric bags.

To Be Completed By: 29/09/2023

Subject: Open Field Testing Facilities

Note List:

[Crystal Lange]

Notification received of breach of outer perimeter fence. Double fence line used for unlawful entry to the General farm sheds. As soon as the hole in the fence was noted an animal count was undertaken and no animals were missing. The only item found missing was an electric fence unit.

Hazardous Substances and New Organisms (HSNO) Act

Compliance with recording requirements and containment controls was verified for; ARPN013 (GMD02023, APP203832), GMC03001 (GMC001197), GMD04112 (GMD003420), GMO04/ARR005 [GMD04112] (GMD003421) and 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)

Ministry for Primary Industries
Manatū Ahu Matua

Verification Report[1]

Report ID:	PBV/2501/2024/01
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:	2023-08-09 to 2024-02-09
Verification Date:	2024-02-13
Published:	2024-02-28 15:58
Next Due Date:	2024-08-09
Level/Step:	**6.1** (started on 6.1 , and ceiling is 6)
Report Type:	Scheduled
Peer Reviewed By:	Elizabeth Buckley

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, AgResearch 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

Glossary of terms :

TF	Transitional Facility
ACF	Animal Containment Farm
ACU	Animal Containment Unit
ASNZS	Australian New Zealand Standard 2243.3 Microbiological Safety and containment
B3	Better Border Biosecurity
BACC	Biosecurity Authority Clearance Certificate
CAR	Corrective Action Request
CF	Containment Facility
CTO	Chief Technical Officer
CTOd	CTO decision/permission under Section 52/53 of the Biosecurity Act 1993
DFO	Delegated Facility Operator
EPA	Environmental Protection Authority
GH	Glasshouse
GM	Genetically Modified
HSNO	Hazardous Substances and New Organisms
IHS	Import Health Standard
MPI	Ministry for Primary Industries
NC	Non-Compliance
NZFS - VS	New Zealand Food Safety - Verification Services
PBV	Performance Based Verification
PC1	Physical Containment Level 1
PC2	Physical Containment Level 2

PP	Plant Protection
PPE	Personal Protective Equipment
R&M	Repairs and Maintenance
SAC	Small Animal Containment

2. Executive Summary

This was a scheduled and announced inspection of the AgResearch Limited transitional and containment facility at the Ruakura site in Hamilton.

The objective of this PBV was to verify compliance with the facility manual, the Import Health Standards, the Standards identified in the "Biosecurity" section of this report, the HSNO Act 1996 and the facility and operator approvals as held under the Biosecurity Act 1993.

The outcome of the verification undertaken 13/02/2024 had an acceptable outcome. One minor NC was issued for Laboratory Hygiene. The minor NC s at the previous inspection t was closed 26/09/2023.

MPI is satisfied that AgResearch is operating in compliance with the requirements of the standards it is approved to. As such the facility and operator approvals will be continued.

3. Operator Summary

The entry and exit meetings along with the reality check of the facility was carried out by Crystal Lange (MPI) with [redacted] (DFO) and Tim Hale (DFO). The Inspectors' authority under the Biosecurity Act 1993 and HSNO Act 1996 was confirmed. Health and Safety is covered by a visitor register. No additional hazards were notified.

Two key support roles for AgResearch (not affecting the transitional facility) are not being filled. Tim has added Facilities Support to his role. There is no adverse impacts between Tims' roles at present.

The inspection process included a review of onsite records and a reality check. Records reviewed included information supplied prior to the PBV by both DFOs as well as on the day. The reality check included PC1 laboratories in the South Wing, PC2 laboratories in Animal Physiology, animal containment in SAC and ACF. [redacted] (Animal Technician) led the tour of SAC and was able to answer all questions presented.

4. Verification Completed (this period)

Biosecurity

The facility demonstrated substantial compliance with the 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

The facility manual was updated in December 2023 to Version 4.3. Ongoing updates were advised at the PBV. The internal audits identified minor maintenance issues. Refresher training was not yet due. Two cleaners and four summer students had been inducted along with nine contractors and two casual workers.

CTO expiry dates are regularly checked. Two approvals expire this year and the B3 project ceases June 2024. A copy of the current Velvet Leaf CTO was requested and supplied in a timely manner.

The following elements were verified in this PBV period:

Quality Assurance:Chief Technical Officer (CTO) Permissions and Decisions	Acceptable
Quality Assurance:Operator Control	Acceptable
Quality Assurance:Operator Internal Verification	Acceptable
Quality Assurance:Training and Competency of Personnel	Acceptable

Documentation and Certification

Transfer of samples for the B3 project (C2024/90933) did not occur. The BACC direction has since been rejected by MPI. Three other imports during the verification period were all released under an IHS. Registers of imports, transfers and animals were up to date.

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

The following elements were verified in this PBV period:

Documentation and Certification:Site Plans, Specification and Modifications	Acceptable

Identification, Traceability & Management

Transfers were reconciled and confirmed. Four outward movements under transfer EM2609 preceded the return (EM2160) which is yet to occur. Mice registers supplied prior to the PBV were updated on the day. Calves and cows were selected for traceability. One Mob was selected for location status. Inventory matched when deaths and yet to calve cows (held in a fence break) were added in.

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:Transfer of Goods and Organisms	Acceptable

Hygiene & Sanitation

Installing of sealed lights in the Animal Physiology complex has been completed and there have been no further incidences of cluster flies.

iButton records were sighted for the three waste autoclaves (SAC, PP and GH) with all hold times exceeding the minimums in the ASNZS.

The following elements were verified in this PBV period:

Hygiene & Sanitation:Cleaning and Disinfection	Acceptable
Hygiene & Sanitation:Personnel Hygiene and Personal Protective Equipment (PPE)	Acceptable
Hygiene & Sanitation:Waste Management	Acceptable

Subject: Cleaning and Disinfection

Note List:

[Crystal Lange]

During the South Wing reality check cardboard was noted stored under a bench in 101, cobwebs in 117, and water staining was noted on wooden clipboard in 111. A minor NC has been issued for hygiene.

Corrective Action Request

Confirmation of action take to be emailed to the Inspector.

Design and Construction

All South Wing laboratories (PC1) were visited for the reality check along the the PC2 Tissue Cultures laboratories in Animal Physiology. Identification of issues at internal audits and by other staff in between times is pleasing to see. A split floor seal in 112 was on the facility maintenance list.

The SPF room was empty with mice only held in one room of the SAC. Perimeter fencing was secure at the ACF along with internal paddock fencing.

The following elements were verified in this PBV period:

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:Physical Containment Level 2 (PC2)	Acceptable

Hazardous Substances and New Organisms (HSNO) Act

Compliance with recording requirements and containment controls was verified for; GMC03001 (GMC001197), GMD04112 (GMD003420), and 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)