Annual Report to Environmental Protection Authority

for

Activities under ERMA 200223

AgResearch Ltd

For the 12 months ending

30th June 2023

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

Contents

Summary of Activities for the period 1st July 2021 to 30th June 2022

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.

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 Rams for analysis or storage for future use.

Embryo Transfer activities this year have been in cattle and 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.

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.

Frustratingly, 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.

AgResearch's Manager Māori - Strategy and Engagement who has local affiliations, and his team are working diligently to build a relationship with Ngati - Wairere for Liaison Group and other Ruakura initiatives of interest to Ngati -Wairere and wider Tainui. COVID restrictions previously disrupted planned follow up interactions with Te Haa o te Whenua O Kirikiriroa which still have not been rescheduled for this purpose at this time.

The Facility manager is in regular contact with Tainui Group Holdings 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

- Cattle were maintained to investigate longevity and potential long-term health effects
- The genetic engineered cattle show the same age-related health issues known from conventional cattle with increasing age.
- Oocytes were retrieved by ovum pick up from one cow with a disruption of the gene encoding the milk protein beta-lactoglobulin. The oocytes were used to produce and cryopreserve IVF embryos with a disruption of the gene for beta-lactoglobulin and are intended for the future production of knockout calves.
- Milk from different transgenic lines is functionally analysed as part of international collaborations.

Generating cattle genome edited for adaptation to warmer temperatures

- Seven calves edited for the slick mutation and five non-edited control claves were monitored for behavioural, physiological and hair characteristics.
- Eight additional calves edited for the PMEL coat colour dilution mutations and 5 non-edited control calves were produced.
- Six of the *PMEL*-edited calves were no-mosaic for the precise mutation. One calf was mosaic and the other carried alleles that were not precisely edited.
- The PMEL calf that was born in the previous reporting year was diagnosed as being blind and was euthanised. The eyes of the calves were histologically evaluated and by MRI scanning.
- Absorbance of thermal energy was measured in the lighter coloured edited cattle and non-edited control cattle. The edited cattle absorbed approximately 40% less thermal energy than the genetically matched controls.
- Detailed monitoring of behavioural and physiological characteristics under warm and cold conditions are in progress but were impacted by a very wet summer and warm winter.
- Whole genome sequencing was undertaken and the sequence data our now used to determine whether the calves experienced any potential off-target mutagenesis.
- We have generated edited embryos with a disrupted *NANOS2* gene to confirm the impact of this gene on male fertility. Edited embryos were transferred, and ultrasound scanning identified multiple pregnancies.
- We generated chimaeric embryos by aggregation of in vitro fertilised male *NANOS2* KO embryos with cloned male embryos, where only one copy of the *NANOS2* gene was disrupted. The chimaeric embryos were transferred into recipients for development to term.
- Activities and results were communicated to various stakeholders and presented at: Queenstown Research week, August 2022; Online workshop, May 2023; School class, Solway College, June 2023

Overexpression of the histone demethylase KDM4B in transgenic cattle

- Oocytes were retrieved by ovum pick up from one cloned cow that overexpresses the histone demethylase KDM4B fused to a GFP reporter transgene. The oocytes were used to produce and cryopreserve transgenic IVF embryos of both sexes and are intended for the future production of transgenic calves.

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

- One cloned transgenic buck and two non-transgenic AI control bucks, as well as several recipient does, were maintained into adulthood.

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

- Female *NANOS2*$^{-/-}$ and male *NANOS2*$^{+/-}$ cloned founder animals were bred using ovum-pickup and in vitro fertilisation (IVF) and AI. Both genotypes displayed normal fertility and 7 live F1 offspring were born, representing both homozygous and heterozygous knockout genotypes for future breeding and phenotype characterisation.

Generating immune-compatible sheep for xenotransplantation

- 5 female double knockout ewes (*GGTA* and *CMAH*) were used for ovum-pick and IVF, but no viable offspring were obtained. The same animals were also used for AI to generate gene edited offspring of both sexes.

On Farm Management Summary for year ending 30/06/2023

Animal Numbers 01/07/2022– 30/06/2023 (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 (1/07/22)	Births	Transfer In	Transfer Out	Aged In	Aged Out	Killed	Deaths	Closing (30/06/23)
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 - (ERMA200223)									
MA Cows	**14**				0		1		**13**
Total BLg -	**14**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**13**
Erbitux (ERMA200223)									
Total Erbitux	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Climate Smart (ERMA200223)									
R1yr Heifer					**12**				**12**
Heifer Calves	**7**	6				**12**	**1**		**0**
R1yr Male					**12**				**12**
Bull Calves	**10**	3				**12**		**1**	**0**
Total Climate Smart	**17**	**9**	**0**	**0**	**24**	**24**	**1**	**1**	**24**
KDM4B (ERMA200223)									
MA Cows	**1**				0		1		**0**
Total KDM4B	**1**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**
Conventional Cattle									
MA Cows	**77**		0	0	0		4		**73**
R2yr Heifers	**0**		0		0	0			**0**
Other classes	**0**	0	0	0	0	0	0	0	**0**
Total Conventional	**77**	**0**	**0**	**0**	**0**	**0**	**4**	**0**	**73**
Cattle Total	**109**	**9**	**0**	**0**	**24**	**24**	**7**	**1**	**110**
Cattle alive developed under ERMA approvals (Tg and non Tg progeny)									**37**

[1] Aligns with normal livestock reconciliation aging practice.

Stock Class	Open (1/07/22)	Births	Transfer In	Transfer Out	Aged In	Aged Out	Killed	Deaths	Closing (30/06/23)
Goats									
Erbitux & Enbrel (ERMA200223)									
Ma Doe	**12**						8		**4**
R2yr Doe	**0**								**0**
R1yr Doe	**0**				2				**2**
Doe Kid	**2**	0				2			**0**
Buck Kid	**0**	0				0	0		**0**
R1yr Male +	**1**				0				**1**
Total Erbitux & Enbrel	**15**	**0**	**0**	**0**	**2**	**2**	**8**	**0**	**7**
non Med inherit (ERMA200223)									
Total TCR	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Conventional Goats									
MA Doe	**19**						14		**5**
R2yr Doe	**0**								**0**
R1yr Doe	**0**				3				**3**
Male R1yr +	**2**				0				**2**
Kids	**3**	0				3			**0**
Total Conventional	**24**	**0**	**0**	**0**	**3**	**3**	**14**	**0**	**10**
Goat Total	**39**	**0**	**0**	**0**	**5**	**5**	**22**	**0**	**17**
Goats alive developed under ERMA approvals (Tg and non Tg progeny)									**7**

Stock Class	Open (1/07/22)	Births	Transfer In	Transfer Out	Aged In	Aged Out	Killed	Deaths	Closing (30/06/23)
Sheep									
AI on Hooves (ERMA200223)									
MA Ewes	**10**				3		4	0	**9**
2th Ewes	**3**				1	3			**1**
Ewe Hgts	**1**				9	1			**9**
Ewe Lamb	**1**	9				9	1		**0**
MA Ram	**1**				1		0		**2**
R1yr Ram	**1**				6	1	0		**6**
Ram Lamb	**1**	6				6	0	1	**0**
Total	**18**	**15**	**0**	**0**	**20**	**20**	**5**	**1**	**27**
Conventional Sheep									
MA Ewes	**51**				0		9	1	**41**
2th Ewes	**0**				5	0			**5**
Ewe Hgts	**5**				0	5			**0**
Ewe Lamb	**0**					0			**0**
2th Ram	**0**				0		0		**0**
R1yr Ram	**0**				1	0			**1**
Ram Lamb	**1**					1			**0**
Total Conventional	**57**	**0**	**0**	**0**	**6**	**6**	**9**	**1**	**47**
Sheep Total	**75**	**15**	**0**	**0**	**26**	**26**	**14**	**2**	**74**
Sheep alive developed under ERMA approvals (Tg and non Tg progeny)									**27**

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 no movements of conventional animals in or out of the facility during the period.

6 Ma cows (2 GM) and 1 GM heifer calf have been humanely killed and 1 GM bull calf died, all have been disposed of in a offal hole on-site, 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.

22 (8 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 onto or from the facility (apart from approved exit and returns for surgery purposes) during the period.

14 (5 GM) sheep of varying ages have been humanely killed and 2 (1 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.

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.

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.

57 cattle recipients have been used for ET (embryo transfer). 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, silage or meal 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 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.

Wet summer / autumn conditions meant nearly 10ha within the facility was undersown with new grass seed to boost pasture recovery. 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 applied this season.

Operationally we continue to be juggling animal movements and grazing 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 22/23 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 # 15407 - Maintenance of Cattle on the Animal Containment Facility

RAEC # 15409 - Maintenance of Goats on the Animal Containment Facility

RAEC # 15467 - Generation of climate-smart cattle from edited embryos

RAEC # 15523 - Phenotyping goats for transmission ratio distortion and generation of female-only offspring

RAEC # 15567 - Breeding cloned sheep for generating absolute transmitters and phenotype evaluation

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

RAEC 2022-0423 – Maintenance of Goats on the Animal Containment Facility

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

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

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

AE ReportA 15407 ~ (Status=ACCEPTED)(Applicant=[redacted]) (AE APPLICATION 15407) Maintenance of Cattle on the Animal Containment Facility

Group	Line	Question	Answer
		0. ADMINISTRATIVE DETAILS	
0	1	**Title**	(AE APPLICATION 15407) (AE APPLICATION 15081) Maintenance of Cattle on the Animal Containment Facility
0	2	**Applicant**	[redacted]
0	3	**Project proposer (If not the person named above)**	
0	5	**Institution**	AgResearch Limited
0	6	**Location**	AGR Ruakura

0	7	**Start Date (dd/mm/yyyy)**	01/07/2021
0	8	**Finish Date (dd/mm/yyyy)**	01/08/2022
0	9	**Number of animals used ~ Species used**	96 ~ Cattle
0	10	**Number of animals used ~ Species used**	
0	11	**Number of animals used ~ Species used**	
0	12	**Number of animals used ~ Species used**	
0	13	**If the number of animals used is not the same as the approved number of animals proposed for use in your application please explain why there is a difference.**	Originally proposed were 140, including 15 calves to be born during the project. No calves were born and maybe there was an error on existing animal numbers in the application as only 96 cattle were maintained.
0	15	**AgResearch Staff - please ensure the person responsible for entry of animal use data in to Animal Use database.is named on this form**	
0	17	**Animal Manipulation Grades - please include the grading change for any animals affected by Adverse Event(s)**	
0	18	**The grades must reflect the summed impacts of both the initial state of the animal and the induced effect of the experimental procedure, not the induced effect alone**	
0	19	**What was the maximum animal manipulation grading approved in your proposal? (It is recorded in ANIMAL USE justification line 2 on your application)**	B (LITTLE IMPACT)
0	20	**Was the maximum grading of manipulations for some or all of the animals indicated in your proposal appropriate? (YES or NO)**	yes
0	21	**If, now that you have completed the manipulations, you think that the maximum grading was different from your proposal please explain why.**	
0	22	**What should the maximum grading now be?**	
0	23	**If you have changed the grading for some or all of the manipulations please remember to use the appropriate grading on the AEStats form**	
		1. MANIPULATIONS	
1	1	**Please note that an answer is required for points 3, 5 and 7.**	

		Even a No answer must be included	
1	2	**Briefly outline the manipulations carried out (including any approved modifications). Please include treatments, numbers of animals etc.**	Cattle were maintained according to normal farm practices. Two cows (BLG KO cow and KDM4B cow) underwent four rounds of Ovum pick up. The resulting oocytes were fertilised in vitro with WT semen to produce embryos. The embryos were biopsied for genotyping and cryopreserved for future transfer.
1	3	**Did the manipulations go according to plan Yes or No?**	yes
1	4	**If the manipulations did not go according to plan please state what happened**	
1	5	**Were any adverse effects on animal welfare noted. (Bruising, swelling at injection sites, failure to adapt to changed conditions etc) Yes or No?**	no
1	6	**If Yes please detail any adverse effects on animal welfare**	
1	7	**Were any animals withdrawn from the experiment or euthansed prematurely Yes or No?**	One cow was euthanised, 1 GM Cow (15015) and one recipient died with no conclusive cause of death identified..
1	8	**If Yes please state why this was necessary, state whether or not it was as a result of the manipulations and if it was a result of the manipulations please detail why it was necessary.**	15015 was humanely killed following veterinarian investigation showing that the animal was healthy except for presenting lameness. Further investigation was not deemed necessary at the time by [redacted] and [redacted] Hence no PM was conducted. One recipient that was not being used at the time was found dead. No conclusive reason was identified with PM not deemed necessary.
1	9	**If Yes please detail and state whether or not this affected the outcome of the project**	This has not affected the outcome of the project.
		2. COMMENTS from STAFF	
2	1	**Please comment on your approaches you described in your application to address the 3R's. Were they successful?**	
2	2	**Replacement**	There are no tissue culture or other alternative models available to reliably predict the full impact of specific genetic modifications on the phenotype, the stability of the phenotype, long term health effects or data on the ability to safely contain and maintain cattle in outdoor containment.
2	3	**Reduction**	Only a minimum number of animals were maintained to ensure programme objectives can be met.
2	4	**Refinement**	Any pain or noxiousness was minimized by sedation, pre-emptive pain relief and high standard nursing and husbandry.
2	5	**Based on your experience of this and other experiments, do you have any comments that may assist those carrying out similar work in future and which might improve the welfare of animals in a similar trial and /or improve the efficiency of animal handling, staff safety, etc. (i.e. If**	Regular review and update of husbandry protocols aids our aim to achieve high standard nursing and husbandry. No changes to protocols were made in this reporting period.

		you had to do this again what would you do differently)	
		98. NOTES ~ Read only	
98	1	**Status Change**	[redacted]19/10/2022) SUBMIT
98	2	**Committee Decision**	(02/11/2022 RESUBMITaec_agr_[redacted]) Was a Post mortem completed on the cow that was found dead, and/or 15015? Please attach if so. All people named on the original application must be listed for viewing. If specific protocols have been updated, please briefly outline this as this information may be applicable to other applications/SOPs and would be beneficial for the committee to understand.
98	3	**Status Change**	[redacted]11/11/2022) SUBMIT
98	4	**Committee Decision**	(16/11/2022 PREVIEWED[redacted]
98	5	**Committee Decision**	(24/11/2022 ACCEPTED[redacted])
		99. PERSONNEL SIGNATURES	
99	1	**Committee**	RUAKURA
99	1	**Programme leader, Facility manager & Lead Technician must sign. All other personnel that were involved in this project must be named so that they can view and add to this report but they do not need to sign it.**	
99	99	**AEC_ABS_[redacted] ~ Job () Location (;)**	commercial veterinarian, OPU, ET
99	99	**AEC_AHC_[redacted]~ Job () Location (;)**	commercial veterinarian, calving
99	99	**[redacted]~ Job (Veterinarian and Animal Welfare Officer) Location (Lincoln Science Centre;)**	Animal Welfare Officer

AE ReportA 15409 ~ (Status=ACCEPTED)(Applicant=[redacted]) (AE APPLICATION 15409) Maintenance of Goats on Animal Containment Facility

Group	Line	Question	Answer
		0. ADMINISTRATIVE DETAILS	
0	1	**Title**	(AE APPLICATION 15409) (AE APPLICATION 15080) Maintenance of Goats on Animal Containment Facility
0	2	**Applicant**	[redacted]
0	3	**Project proposer (If not the person named above)**	
0	5	**Institution**	AgResearch Limited
0	6	**Location**	AGR Ruakura
0	7	**Start Date (dd/mm/yyyy)**	18/07/2021
0	8	**Finish Date (dd/mm/yyyy)**	18/09/2022
0	9	**Number of animals used ~ Species used**	44 ~ Goats

0	10	**Number of animals used ~ Species used**	
0	11	**Number of animals used ~ Species used**	
0	12	**Number of animals used ~ Species used**	
0	13	**If the number of animals used is not the same as the approved number of animals proposed for use in your application please explain why there is a difference.**	The total number of receipients available for AI was 28, not 41 as originally indicated, because a number of recipients were already transferred into in other trials with cloned embryos in March 2021.
0	15	**AgResearch Staff - please ensure the person responsible for entry of animal use data in to Animal Use database.is named on this form**	
0	17	**Animal Manipulation Grades - please include the grading change for any animals affected by Adverse Event(s)**	
0	18	**The grades must reflect the summed impacts of both the initial state of the animal and the induced effect of the experimental procedure, not the induced effect alone**	
0	19	**What was the maximum animal manipulation grading approved in your proposal? (It is recorded in ANIMAL USE justification line 2 on your application)**	B (LITTLE IMPACT)
0	20	**Was the maximum grading of manipulations for some or all of the animals indicated in your proposal appropriate? (YES or NO)**	No
0	21	**If, now that you have completed the manipulations, you think that the maximum grading was different from your proposal please explain why.**	High rate of dystocia, with does not progressing into second stage of labour without assistance due to lack of pelvic/vaginal expansion. One doe subsequently needed to be euthanased post-partum due to complications from a difficult birth.
0	22	**What should the maximum grading now be?**	D (HIGH IMPACT)
0	23	**If you have changed the grading for some or all of the manipulations please remember to use the appropriate grading on the AEStats form**	
		1. MANIPULATIONS	
1	1	**Please note that an answer is required for points 3, 5 and 7. Even a No answer must be included**	

1	2	**Briefly outline the manipulations carried out (including any approved modifications). Please include treatments, numbers of animals etc.**	We undertook one run of AI. In this run, 11/28 does got pregnant (5 females and 11 males = 16 born, kept 5 females and 1 male). Manipulations included abdominal ultrasound to monitor pregnancy, management of parturition and blood sampling. Some of the recipients were GM.
1	3	**Did the manipulations go according to plan Yes or No?**	No
1	4	**If the manipulations did not go according to plan please state what happened**	With the overall AI pregnancy rate being 12/34=35% across two AI runs (one the previous season), we are sitting below the expected commercial pregnancy rate (67%) using proven semen. Out of the 2016-2017 born animals, 8 does have had at least 3 opportunities to get pregnant but have never been found pregnant at any stage (including AI attempts). This suggests poor fertility in the does, perhaps combined with bad luck using low numbers. Following kidding in the 2nd AI run, we have lost 4 recipients. All but one male kid have been humanely killed.
1	5	**Were any adverse effects on animal welfare noted. (Bruising, swelling at injection sites, failure to adapt to changed conditions etc) Yes or No?**	yes
1	6	**If Yes please detail any adverse effects on animal welfare**	High rate of dystocia, with does not progressing into second stage of labour without assistance due to lack of pelvic/vaginal expansion. One doe subsequently needed to be euthanased post-partum due to complications from a difficult birth. This emphasises concerns with the welfare of the does in regards to ability to birth kids.
1	7	**Were any animals withdrawn from the experiment or euthansed prematurely Yes or No?**	no
1	8	**If Yes please state why this was necessary, state whether or not it was as a result of the manipulations and if it was a result of the manipulations please detail why it was necessary.**	
1	9	**If Yes please detail and state whether or not this affected the outcome of the project**	
		2. COMMENTS from STAFF	
2	1	**Please comment on your approaches you described in your application to address the 3R's. Were they successful?**	
2	2	**Replacement**	n/a
2	3	**Reduction**	Recipient does were re-used several times for producing and gestating embryos. The conventional wild type animals and their offspring have also been utilised in other projects, eg. 13210, Adrenal Responsiveness in Dairy Goat Kids; 13209, Evaluate the efficacy of a non-penetrating captive bolt to humanely euthanize Goat Kids and 14642, Collection of transgenic cloned fetuses for cell line rejuvenation in goats. GM as well as conventional wild type goats have also been used in another GM goat project (15082) aimed at enabling production of female-only offspring.

2	4	**Refinement**	All manipulations were carried out according to SOPs which aim to minimize any pain or noxiousness by use of minimally invasive techniques, sedation and anaesthesia, pre-emptive pain relief and high standard nursing and husbandry.
2	5	**Based on your experience of this and other experiments, do you have any comments that may assist those carrying out similar work in future and which might improve the welfare of animals in a similar trial and /or improve the efficiency of animal handling, staff safety, etc. (i.e. If you had to do this again what would you do differently)**	Need to keep better track of recipient age and lactation status, making sure they are not being left empty. This should improve recipient fertility. There were 3 Adverse Events associated with application 15409: Adverse Event 273, 289 and 346.
		98. NOTES ~ Read only	
98	1	**Status Change**	[redacted] 22/12/2022) SUBMIT
98	2	**Committee Decision**	(12/01/2023 RESUBMIT[redacted] Thank you for submitting your report. Preview comments are: Numbers listed as used is not what was predicted and in original application it indicated that there were 41 goats available with up to 56 born. Given 16 goats were born, this suggested that number of resident goats was considerably less than indicated and it would be good to indicate why this occurred. This question is asked on both Report A (0-13) and Stats (1.8) 0-20 this should be No, and the resultant questions answered. More manipulations were carried out than listed in 1.2. This included abdominal ultrasound to monitor pregnancy, management of parturition, rearing of offspring and blood sampling. 2-4 gold standard should use high standard instead. Several adverse events occurred, and all should be covered in the report A, it is useful to refer to the adverse event number to link for easy reference. All people listed on application need to be listed on Report A for viewing purposes (they all do not need to sign). Stats- Adverse event regraded some of these to D and C, so a separate stats form will be needed for those regraded D, and C grading indicated for those regraded C. The application indicated that some animals were genetically modified so that will need to be indicated please. If you need support with any of this please email me. Thanks [redacted]
98	3	**Status Change**	[redacted] 31/01/2023) SUBMIT
98	4	**Committee Decision**	(08/02/2023 RESUBMIT[redacted] Thank you for submitting your ReportA, please make the following changes: Please clarify that all goats covered by this application were normal/conventional (not genetically modified) with the purpose of breeding being maintaining of conventional animals for use in other experiments such as recipients for embryo transfer or normal controls. Otherwise, please contact us if your stats form needs to be unlocked so you can update normal vs genetically modified.
98	5	**Status Change**	[redacted] 14/02/2023) SUBMIT
98	6	**Committee Decision**	(21/02/2023 PREVIEWED[redacted])
98	7	**Committee Decision**	(24/02/2023 ACCEPTED[redacted])

		99. PERSONNEL SIGNATURES	
99	1	**Committee**	RUAKURA
99	1	**Programme leader, Facility manager & Lead Technician must sign. All other personnel that were involved in this project must be named so that they can view and add to this report but they do not need to sign it.**	
99	99	**AEC_ABS_[redacted] ~ Job () Location (;)**	external vet
99	99	**AEC_AHC_[redacted]~ Job () Location (;)**	external vet
99	99	**[redacted] ~ Job (Veterinarian and Animal Welfare Officer) Location (Lincoln Science Centre;)**	AWO
99	99	**[redacted]~ approved ~ Job (Animal Technician) Location (Ruakura; Animal Phys Yard, First Aid)**	Animal technician
99	99	**HALET ~ approved ~ Job (Research Farm Manager, Ruakura) Location (Ruakura; Manager-Animal Containment Facility,Yard; First Aid)**	FOM Ruakura / Facility Operator

AE ReportA 15467 ~ (Status=ACCEPTED)(Applicant=[redacted]) (AE APPLICATION 15467) Generation of climate-smart cattle from edited embryos

Group	Line	Question	Answer
		0. ADMINISTRATIVE DETAILS	
0	1	**Title**	(AE APPLICATION 15467) (AE APPLICATION 15088) Generation of climate-smart cattle from edited embryos
0	2	**Applicant**	[redacted]
0	3	**Project proposer (If not the person named above)**	
0	5	**Institution**	AgResearch Limited
0	6	**Location**	AGR Ruakura Containment Facility
0	7	**Start Date (dd/mm/yyyy)**	06/09/2021
0	8	**Finish Date (dd/mm/yyyy)**	20/11/2022
0	9	**Number of animals used ~ Species used**	100 ~ Cattle
0	10	**Number of animals used ~ Species used**	
0	11	**Number of animals used ~ Species used**	
0	12	**Number of animals used ~ Species used**	

0	13	**If the number of animals used is not the same as the approved number of animals proposed for use in your application please explain why there is a difference.**	less animals were born during the approval period and not all recipients were used
0	15	**AgResearch Staff - please ensure the person responsible for entry of animal use data in to Animal Use database.is named on this form**	
0	17	**Animal Manipulation Grades - please include the grading change for any animals affected by Adverse Event(s)**	
0	18	**The grades must reflect the summed impacts of both the initial state of the animal and the induced effect of the experimental procedure, not the induced effect alone**	
0	19	**What was the maximum animal manipulation grading approved in your proposal? (It is recorded in ANIMAL USE justification line 2 on your application)**	C (MODERATE IMPACT)
0	20	**Was the maximum grading of manipulations for some or all of the animals indicated in your proposal appropriate? (YES or NO)**	yes
0	21	**If, now that you have completed the manipulations, you think that the maximum grading was different from your proposal please explain why.**	
0	22	**What should the maximum grading now be?**	C (MODERATE IMPACT)
0	23	**If you have changed the grading for some or all of the manipulations please remember to use the appropriate grading on the AEStats form**	
		1. MANIPULATIONS	
1	1	**Please note that an answer is required for points 3, 5 and 7. Even a No answer must be included**	
1	2	**Briefly outline the manipulations carried out (including any approved modifications). Please include treatments, numbers of animals etc.**	initially we had 13 pregnant cows that calved in Nov 2022 73 recipients were synchronised (some more than once; total of 119 synchronisations) and 61 cows were ETed (some more than once; total of 95 ETs). 27 live calves were born during the approval period (15PMEL and control calves; 12 slick and control calves) 12 slick edited and control calves were implanted with a data logger and were fitted with sensors to record behavioral information,

			complemented by on site observations under restricted shade access. The calves were exercised via short walk under warm conditions, and initially small patches of coat were clipped for hair sampling, later replaced by plucking some hair. 3 slick and 2 control bull calves were castrated. Slick/control calves were also observed during cold mornings, IRT images taken, I-buttons underneath the tail fitted for temperature measurements, plus blood and fecal sampling. Measurements of skin thickness and sweating rate were planed but not done due to shifts in priority and delays in resourcing the specialised device, respectively.
1	3	**Did the manipulations go according to plan Yes or No?**	no
1	4	**If the manipulations did not go according to plan please state what happened**	Not all of the various manipulations did go to plan. The pregnancy rates following ET were highly variable with no correlation to the type of IVP embryo. Some ETs had to be repeated. 3 of the slick edited calves lost their temperature loggers. [redacted] checked the remaining calves and were happy with how they looked, so we left them in. Four adverse events were reported, one death due to misadventure (AE 309), one about unintended blindness (AE 336), one unexplained death (AE 340), one small calve that was euthanised on welfare grounds (AE 356).
1	5	**Were any adverse effects on animal welfare noted. (Bruising, swelling at injection sites, failure to adapt to changed conditions etc) Yes or No?**	yes
1	6	**If Yes please detail any adverse effects on animal welfare**	One calf edited in the PMEL gene for a coat colour mutation was born blind. Genotyping revealed the presence of an unintended PMEL allele with a 6bp deletion in addition to the intended 3bp deletion. The unintended allele might be linked to the blindness.
1	7	**Were any animals withdrawn from the experiment or euthansed prematurely Yes or No?**	yes
1	8	**If Yes please state why this was necessary, state whether or not it was as a result of the manipulations and if it was a result of the manipulations please detail why it was necessary.**	The blindness made the care of the animal diffcult with existing resources at the ACF. We hypothesise that the blindnessis was caused by the uninteded allele. The unintended allele was generated by an inaccurate editing event at the target site which was not detected in the embryo biopsy. The editing was done in IVP embryos which provides little control over when and to what extent editing is happening. Alternatively, the editing can be done in cultured cells which allows to determine the exact editing genotype before animals are generated. However, generating edited animals from the edited cells by cloning has a low efficiency.
1	9	**If Yes please detail and state whether or not this affected the outcome of the project**	One out of nine calves was affected in this way. All others showed the expected coat colour dilution phneotype, with most being fully edited for the intended PMEL edit.
		2. COMMENTS from STAFF	
2	1	**Please comment on your approaches you described in your application to address the 3R's. Were they successful?**	

2	2	**Replacement**	There are no tissue culture or other alternative models available to reliably predict the full impact of specific genetic modifications on the phenotype including animal welfare, climate adaptation and sustainability.
2	3	**Reduction**	We continually strive to reduce the number of animals we use for these projects. Embryos are biopsied and screened for intended genotype and only validated embryos will be transferred for development to term. Only a minimum number of animals for each line of genetically modified cattle will be generated that ensures programme objectives will be met. The biopsy screening was not as accurate as expected resulting in an enrichment for but not limiting live animals to the intended genotypes. Due to the high variability of pregnancy rates following transfers, some transfers had to be repeated with additional embryos.
2	4	**Refinement**	All manipulations are carried out according to SOP's or contracted out to ABS which aim to minimize any pain or noxiousness by use of minimally invasive techniques, sedation, pre-emptive pain relief and high standard nursing and husbandry.
2	5	**Based on your experience of this and other experiments, do you have any comments that may assist those carrying out similar work in future and which might improve the welfare of animals in a similar trial and /or improve the efficiency of animal handling, staff safety, etc. (i.e. If you had to do this again what would you do differently)**	To regularly replace recipient animals to avoid accumulation of old not fit for purpose animals and be able to always include some heifers with the highest fertility in embryo transfer runs.
		98. NOTES ~ Read only	
98	1	**Status Change**	([redacted] 26/01/2023) SUBMIT
98	2	**Committee Decision**	(08/02/2023 RESUBMIT[redacted]) Thank you for submitting your ReportA, please make the following changes: Section 1.2. Please summarise other manipulations (other than implantation with data logger) that occurred to calves as per modification 3076 and 3156 – and if they did not occur, please outline why this did not happen. Please elaborate on the calves born – 12 were slick edited that survived until getting a data logger, what were the others? (this affects the statistics report – Q2). Section 1.4, Please reference AE 309,336 & 340 within this section. As section 6.11 of the statistics refers to an additional dead animal, please also explain within the Report A who that is and what happened to it (or create an adverse event if it fits that criteria). Section 2.4. As gold standard is a protocol that is not easily defined and changes over time, we would prefer if the terminology used was 'high standard' rather 'gold standard'.
98	3	**Status Change**	([redacted] 10/02/2023) SUBMIT
98	4	**Committee Decision**	(23/02/2023 PREVIEWED[redacted])
98	5	**Committee Decision**	(24/02/2023 ACCEPTED[redacted])
		99. PERSONNEL SIGNATURES	
99	1	**Committee**	RUAKURA

99	1	**Programme leader, Facility manager & Lead Technician must sign. All other personnel that were involved in this project must be named so that they can view and add to this report but they do not need to sign it.**	
99	99	**AEC_ABS_[redacted] ~ Job () Location (;)**	Commercial Veterinarian
99	99	**AEC_AHC_[redacted] ~ Job () Location (;)**	commercial Veterinarian
99	99	**[redacted] ~ Job (Veterinarian and Animal Welfare Officer) Location (Lincoln Science Centre;)**	Veterinarian, Animal Welfare Officer
99	99	**[redacted] ~ approved ~ Job (Animal Technician) Location (Ruakura; Animal Phys Yard, First Aid)**	Animal Technician, ET, U/S, Blood sampling, Disbudding
99	99	**[redacted] ~ Job (Farm Senior) Location (Ruakura; Farm. First Aid)**	Farm Senior
99	99	**HALET ~ approved ~ Job (Research Farm Manager, Ruakura) Location (Ruakura; Manager-Animal Containment Facility,Yard; First Aid)**	Farm Operations Manager / Facility Manager
99	99	**[redacted] ~ Job () Location (;)**	or viewing purposes as added via Mod 3076
99	99	**[redacted] ~ Job (AW-Technician (AWR3RUA)) Location (Ruakura (RUA);)**	or viewing purposes as added via Mod 3076
99	99	**[redacted] ~ Job (Senior Statistician) Location (Ruakura; North Wing, Ground floor)**	Statistician
99	99	**[redacted] ~ approved ~ Job (Principal Scientist) Location (Ruakura; Dairy Science Building)**	Programme Leader
99	99	**[redacted] ~ Job (Farm Senior - Farm Technical) Location (Ruakura; Containment Unit; First Aid)**	Farm Senior
99	99	**[redacted] ~ approved ~ Job (Senior Scientist) Location (Ruakura; An Phys. First Aid)**	Scientist
99	99	**[redacted] ~ approved ~ Job (Senior Scientist) Location (Ruakura; Kahikatea building, Ngahere complex Room 6)**	Scientist, data collection, methods and animal handling, competent with animals - added per MOD 3076

AE ReportA 15523 ~ (Status=ACCEPTED)(Applicant=[redacted]) (AE APPLICATION 15523) Phenotyping goats for transmission ratio distortion and generation of female-only offspring

Group	Line	Question	Answer
		0. ADMINISTRATIVE DETAILS	
0	1	**Title**	(AE APPLICATION 15523) Phenotyping goats for transmission ratio distortion and generation of female-only offspring (14710, 15082)
0	2	**Applicant**	[redacted]
0	3	**Project proposer (If not the person named above)**	
0	5	**Institution**	AgResearch Limited
0	6	**Location**	AGR Ruakura Containment Facility
0	7	**Start Date (dd/mm/yyyy)**	31/01/2022
0	8	**Finish Date (dd/mm/yyyy)**	31/12/2022
0	9	**Number of animals used ~ Species used**	13 ~ Goats
0	10	**Number of animals used ~ Species used**	
0	11	**Number of animals used ~ Species used**	
0	12	**Number of animals used ~ Species used**	
0	13	**If the number of animals used is not the same as the approved number of animals proposed for use in your application please explain why there is a difference.**	We only attempted sperm collection by AV to morphologically and molecularly evaluate the sperm in functional assays in vitro, using 2 bucks and 11 cycling does. One control buck was not used because the other one was a better match, both age- and weight-wise, to the treatment buck. We had also planned to conduct an in vivo assay with the semen. This included AI of does and subsequent PCR-based sexing and evaluation of transgene presence in flushed embryos. However, the treatment buck (Brownie) has only given sperm once in the six collections since February 2022, while the control (Blackie) has given normal numbers in all six collections. Thus, Brownie did not produce sufficient high-quality semen to undertake this assay. Given his poorer sperm quantity and quality, it was too risky to perform oocyte superovulation and embryo flushing, which are invasive animal assays. There is a

			high risk that the assay would fail due to low production and recovery of embryos. In that case, we would have used up all sperm, precluding generation of other transgenic bucks of this genotype in the future. We therefore focused our efforts on more in-depth in vitro characterization of the sperm, as summarized above.
0	15	**AgResearch Staff - please ensure the person responsible for entry of animal use data in to Animal Use database.is named on this form**	
0	17	**Animal Manipulation Grades - please include the grading change for any animals affected by Adverse Event(s)**	
0	18	**The grades must reflect the summed impacts of both the initial state of the animal and the induced effect of the experimental procedure, not the induced effect alone**	
0	19	**What was the maximum animal manipulation grading approved in your proposal? (It is recorded in ANIMAL USE justification line 2 on your application)**	C (MODERATE IMPACT)
0	20	**Was the maximum grading of manipulations for some or all of the animals indicated in your proposal appropriate? (YES or NO)**	no
0	21	**If, now that you have completed the manipulations, you think that the maximum grading was different from your proposal please explain why.**	We did not use any in vivo assays, only collection by artificial vagina (AV), so grade B would have been sufficient.
0	22	**What should the maximum grading now be?**	B (LITTLE IMPACT)
0	23	**If you have changed the grading for some or all of the**	

		manipulations please remember to use the appropriate grading on the AEStats form	
		1. MANIPULATIONS	
1	1	**Please note that an answer is required for points 3, 5 and 7. Even a No answer must be included**	
1	2	**Briefly outline the manipulations carried out (including any approved modifications). Please include treatments, numbers of animals etc.**	Collections by AV attempts in February 2022 saw the two bucks being reluctant and quite nervous, but we did get a small amount of semen, both from Brownie and one control (Blackie), which was frozen. Overall, semen quality was poor, in terms of motility, viability (fresh and post-thaw) and yield. We continued collections into April (8 collections in total), using both naturally cycling does and a cohort of 5 PG-injected does, Which were part of the 11 does. One control buck was not used because the other one was a better match, both age- and weight-wise, to the treatment buck.
1	3	**Did the manipulations go according to plan Yes or No?**	No
1	4	**If the manipulations did not go according to plan please state what happened**	Altogether, we carried out ten AV collections between December 2021 and April 2022. The first four collections did not work for either buck, as they were either too early in the season or the bucks were too immature. From collection 5 onwards, Blackie delivered sperm every time and we had sufficient straws after 2 collections. At the same time, Brownie delivered once in the past 6 collections. Apart from the one time that he gave a full ejaculate, he only gave small volumes at the first attempt and almost nothing at the second. So, there were clear differences in sperm quantity between the two bucks. Physically (=weight, body condition), both bucks are very similar. Brownie was showing normal behaviours (pawing at doe, grunting and mounting). The doe does not need to be on heat for him to show these behaviours and he was not distracted. With no obvious libido issue, a plausible explanation for his lower sperm count was that the dominant-negative transgene was expressed and has compromised overall sperm quantity. Overall, 98% and 96% of Blackie's and Brownie's sperm, respectively, were morphologically normal.
1	5	**Were any adverse effects on animal welfare noted.**	no

		(Bruising, swelling at injection sites, failure to adapt to changed conditions etc) Yes or No?	
1	6	**If Yes please detail any adverse effects on animal welfare**	
1	7	**Were any animals withdrawn from the experiment or euthansed prematurely Yes or No?**	no
1	8	**If Yes please state why this was necessary, state whether or not it was as a result of the manipulations and if it was a result of the manipulations please detail why it was necessary.**	
1	9	**If Yes please detail and state whether or not this affected the outcome of the project**	
		2. COMMENTS from STAFF	
2	1	**Please comment on your approaches you described in your application to address the 3R's. Were they successful?**	
2	2	**Replacement**	It is not possible to replace semen and animal production with non-animal alternatives (e.g. in vitro or computer models).
2	3	**Reduction**	We use as few animals as necessary (see biometric evaluation) to detect TRD with various assays.
2	4	**Refinement**	The way experiments are carried out are refined to reduce pain or suffering as much as possible.
2	5	**Based on your experience of this and other experiments, do you have any comments that may assist those carrying out similar work in future and which might improve the welfare of animals in a similar trial and**	n/a

		/or improve the efficiency of animal handling, staff safety, etc. (i.e. If you had to do this again what would you do differently)	
		98. NOTES ~ Read only	
98	1	**Status Change**	[redacted]02/02/2023) SUBMIT
98	2	**Committee Decision**	(08/02/2023 RESUBMIT[redacted]) Thank you for submitting your ReportA, please make the following changes: Section 1.2; Please provide more details regarding the animals used as it is currently unclear which two have been classified as Grade A on the statistics report. The behaviour described for the bucks during manipulation would be consistent with a Grade B impact. Was the third buck used (as per application), if not please provide a brief explanation why not. If used, please outline his contribution/manipulations.
98	3	**Status Change**	[redacted] 13/02/2023) SUBMIT
98	4	**Committee Decision**	(14/02/2023 PREVIEWED[redacted])
98	5	**Committee Decision**	(16/02/2023 ACCEPTED[redacted])
		99. PERSONNEL SIGNATURES	
99	1	**Committee**	RUAKURA
99	1	**Programme leader, Facility manager & Lead Technician must sign. All other personnel that were involved in this project must be named so that they can view and add to this report but they do not need to sign it.**	
99	99	**AEC_ABS_[redacted] ~ Job () Location (;)**	external vet
99	99	**AEC_AHC_[redacted] ~ Job () Location (;)**	external vet
99	99	**[redacted] ~ Job (Veterinarian and Animal Welfare Officer) Location (Lincoln Science Centre;)**	awo

99	99	[redacted] **~ Job (Animal Technician) Location (Ruakura; Animal Phys Yard, First Aid)**	Animal technician
99	99	**HALET ~ Job (Research Farm Manager, Ruakura) Location (Ruakura; Manager-Animal Containment Facility,Yard; First Aid)**	FOM Ruakura / Facility Operator
99	99	[redacted] **~ Job (Senior Statistician) Location (Ruakura; North Wing, Ground floor)**	statistical oversight
99	99	[redacted] **~ approved ~ Job (Farm Senior - Farm Technical) Location (Ruakura; Containment Unit; First Aid)**	animal tech
99	99	[redacted] **~ approved ~ Job (Senior Scientist) Location (Ruakura; An Phys. First Aid)**	Principal investigator, general oversight
99	99	[redacted] **~ approved ~ Job (Science Team Leader - Animal Biotechnology) Location (Ruakura; Repro-An Phys, Fire Warden)**	team leader

AE ReportA 15567 ~ (Status=ACCEPTED)(Applicant=[redacted]) (AE APPLICATION 15567) Breeding cloned sheep for generating absolute transmitters and phenotype evaluation

Group	Line	Question	Answer
		0. ADMINISTRATIVE DETAILS	
0	1	**Title**	(AE APPLICATION 15567) Breeding cloned sheep for generating absolute transmitters and phenotype evaluation
0	2	**Applicant**	[redacted]
0	3	**Project proposer (If not the person named above)**	
0	5	**Institution**	AgResearch Limited
0	6	**Location**	AGR Ruakura
0	7	**Start Date (dd/mm/yyyy)**	04/03/2022
0	8	**Finish Date (dd/mm/yyyy)**	31/12/2022

0	9	**Number of animals used ~ Species used**	56 ~ Sheep
0	10	**Number of animals used ~ Species used**	
0	11	**Number of animals used ~ Species used**	
0	12	**Number of animals used ~ Species used**	
0	13	**If the number of animals used is not the same as the approved number of animals proposed for use in your application please explain why there is a difference.**	The proposed number was 95 animals. We used less because we i) did not generate enough embryos to fill 45 recipients (only 25), ii) used only 10 OPU donors (not 12), iii) had less offspring than expected (21, not 35) and lost some pink tagged Howie/Hoss offspring (#75, #76, AI control #61) due to Johne's. We also lost some ewes and lambs at term and beyond. One modification suggested that 5 ewes would be added, however these were not 5 new ewes just 5 ewes that had an additional sample taken from them.
0	15	**AgResearch Staff - please ensure the person responsible for entry of animal use data in to Animal Use database.is named on this form**	
0	17	**Animal Manipulation Grades - please include the grading change for any animals affected by Adverse Event(s)**	
0	18	**The grades must reflect the summed impacts of both the initial state of the animal and the induced effect of the experimental procedure, not the induced effect alone**	
0	19	**What was the maximum animal manipulation grading approved in your proposal? (It is recorded in ANIMAL USE justification line 2 on your application)**	D (HIGH IMPACT)
0	20	**Was the maximum grading of manipulations for some or all of the animals indicated in your proposal appropriate? (YES or NO)**	yes
0	21	**If, now that you have completed the manipulations, you think that the maximum grading was different from your proposal please explain why.**	
0	22	**What should the maximum grading now be?**	
0	23	**If you have changed the grading for some or all of the manipulations please remember to use the**	

		appropriate grading on the AEStats form	
		1. MANIPULATIONS	
1	1	**Please note that an answer is required for points 3, 5 and 7. Even a No answer must be included**	
1	2	**Briefly outline the manipulations carried out (including any approved modifications). Please include treatments, numbers of animals etc.**	We conducted 2 OPU sessions from a cohort of 10 cloned gene-edited stimulated sheep in April/May 2022. The resulting IVP embryos were vitrified and used for 2 embryo transfer sessions in July 2022. In those 2 ET sessions, a total of 48 embryos were transferred into 19 sychronised recipients (25 receips synchronised), includinig 3 putative chimaeric embryos. An additional 5 AI controls were included in the trial. All pregnancies were monitored through to term by ultrasonography. At term, we induced parturition with short-acting Dex and obtained 7 live offspring from the AI group (all alive) and 7 live offspring (plus 7 dead) from the IVP group. All 5 ewes for AI were live but only 2 from the 10 IVP receips survived, including several that required c-section or slaughter recovery, instead of vaginal lambing. A cohort of 5 IVP lambs was bottle-fed until weaning, one lamb was euthansed prematurely due to perceived inability to cope with heat stress (respiratory difficulties). Bloods were collected once on 26/05/22 from the 5 GGTA/CMAH (=xeno) sheep but not from the control Bunter was maintained but no further semen collections were undertaken as he was infertile and positive for Johne?s disease. He was losing weight so was euthanised on veterinarian?s advice.
1	3	**Did the manipulations go according to plan Yes or No?**	No
1	4	**If the manipulations did not go according to plan please state what happened**	The proportion of dead lambs and recipient ewes was higher than expected for IVP embryos. The euthanasis of ewes was due to slaughter Caesarian of dams. All 7 dead lambs were post mortemed, they included: 1 twin born dead, long bottom jaw 3 euthanased with short bottom jaw (parrot mouth) 1 euthanased oversized, limb deformities and renal deformities 1 euthanased, below 2kg and not trying to breathe 1 euthanased postnatally, reduced lamb capacity (PM report yet to come) Among this group was one putative IVP<>cloned chimaera. The genetic analysis of this animal is not completed because additional tissues (brain, kidney, liver) need to be analysed for chimaerism. However, early analysis of blood inidicate that the animal was most likely not chimaeric because no evidence of the gene-edited sterile IVP host could be detected. Thus, it appears that this animal may have been a clone, which is consistent with the observed phenotype. By contrast, all AI control lambs (7) wer born and suckling dams, even though this group also included two assists, both larger single lambs. They were born to older maiden ewes who are not as stretchy in vaginal area.

1	5	**Were any adverse effects on animal welfare noted. (Bruising, swelling at injection sites, failure to adapt to changed conditions etc) Yes or No?**	Yes
1	6	**If Yes please detail any adverse effects on animal welfare**	Several adverse effect on ewes and lambs were noted and are summarised in the follwing adverse event reports: 320, 349, 350, 352, 353, 354.
1	7	**Were any animals withdrawn from the experiment or euthansed prematurely Yes or No?**	No
1	8	**If Yes please state why this was necessary, state whether or not it was as a result of the manipulations and if it was a result of the manipulations please detail why it was necessary.**	
1	9	**If Yes please detail and state whether or not this affected the outcome of the project**	
		2. COMMENTS from STAFF	
2	1	**Please comment on your approaches you described in your application to address the 3R's. Were they successful?**	
2	2	**Replacement**	The goal of our research is to produce and fully characterise livestock with novel genotypes and new phenotypic traits. These goals cannot be achieved without animals as research subjects. Replacement (e.g. with in vitro models or simulations) is not possible and not appropriate.
2	3	**Reduction**	We continually strive to reduce the number of animals we use for these projects. Animals from each line of gene-edited sheep will be genotyped to make sure that only those of the desirable genotyped will be maintained for future experiments.
2	4	**Refinement**	Manipulations were carried out according to SOPs which aim to minimize any pain or noxiousness by use of minimally invasive techniques, sedation and anaesthesia, pre-emptive pain relief and high standard nursing and husbandry. Specifically, clones may be higher risk and we were extra careful with anaesthetic doses, anaesthesia depth, length and recovery during OPU.
2	5	**Based on your experience of this and other experiments, do you have any comments that may assist those carrying out similar work in future and which might improve the welfare of animals in a similar trial and /or improve the efficiency of animal handling, staff safety, etc. (i.e. If you had to do this again what would you do differently)**	More experimental repeats with putative IVF<>SCT embryos need to be conducted, since n=1 only provides anecdotcal evidence. We have so far only observed the phenotype of one animal, which appears to be non-chimaeric. The same applies to the in vivo survival from IVP embryos, which showed run-to-run variation, even though the runs were conducted within just 4 days and used all the same reagents (survival of embryos per transferred recipient was 0/7=0% in run 1, 7/7=100% in run 2). But we need to be prepared for poorer potential outcomes and reduced survival with frozen/thawed/biopsied IVP embryos.

		98. NOTES ~ Read only	
98	1	**Status Change**	[redacted] 03/02/2023) SUBMIT
98	2	**Committee Decision**	(10/03/2023 PREVIEWED[redacted])
98	3	**Committee Decision**	(16/03/2023 ACCEPTED[redacted])
		99. PERSONNEL SIGNATURES	
99	1	**Committee**	RUAKURA
99	1	**Programme leader, Facility manager & Lead Technician must sign. All other personnel that were involved in this project must be named so that they can view and add to this report but they do not need to sign it.**	
99	99	**AEC_ABS_[redacted] ~ Job () Location (;)**	external vet
99	99	**AEC_AHC_[redacted] ~ Job () Location (;)**	external vet
99	99	**[redacted] ~ Job (Veterinarian and Animal Welfare Officer) Location (Lincoln Science Centre;)**	AWO
99	99	**[redacted] ~ Job (Animal Technician) Location (Ruakura; Animal Phys Yard, First Aid)**	Animal technician
99	99	**[redacted] ~ Job (Post Doctoral Scientist) Location (Ruakura)**	help with surgery
99	99	**HALET ~ Job (Research Farm Manager, Ruakura) Location (Ruakura; Manager-Animal Containment Facility,Yard; First Aid)**	FOM Ruakura / Facility Operator
99	99	**[redacted] ~ Job (Senior Statistician) Location (Ruakura; North Wing, Ground floor)**	statistical oversight
99	99	**[redacted] ~ Job (Farm Senior - Farm Technical) Location (Ruakura; Containment Unit; First Aid)**	animal tech
99	99	**[redacted] ~ approved ~ Job (Senior Scientist) Location (Ruakura; An Phys. First Aid)**	Principal investigator, general oversight
99	99	**[redacted] ~ approved ~ Job (Science Team Leader - Animal Biotechnology) Location (Ruakura; Repro-An Phys, Fire Warden)**	team leader

Ministry for Primary Industries
Manatū Ahu Matua

Verification Report[1]

Report ID:	PBV/2501/2022/02
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:	2022-02-19 to 2022-08-27
Verification Date:	2022-08-23
Published:	2022-09-06 17:49
Next Due Date:	2023-02-27
Level/Step:	**6.2** (started on 6.2 , and ceiling is 6)
Report Type:	Scheduled
Peer Reviewed By:	Rana Fathizargaran

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
COVID-19	Coronavirus Disease of 2019
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
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 23/8/2022 and 26/08/2022 was acceptable with one NC issued for PC1 laboratories. A NC was also identified while preparing for the PBV. This is noted in the report; however it was predominantly resolved prior to the verification. Follow-up from this is being undertaken by the recipient facility.

Issues raised at the last PBV were confirmed as closed during the verification period.

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) 23/08/2022 and Tim Hale (DFO) 26/08/2022. The Inspectors' authority under the Biosecurity Act 1993 and HSNO Act 1996 was confirmed. Health and Safety is covered by a visitor register. Crystal was accompanied at all times.

The reality check included PC1 laboratories in Dairy Science and South Wing, the PP Glasshouses, SAC, ACF and Piggery[redacted]was present to supply relevant pig records and discuss welfare including heating and cooling.

A new site Maintenance Engineer had been appointed. This appointee has an electrical background and replaces the previous incumbent who had a building background. A 10 year basic maintenance plan has been outlined. There is no firm outcome after this as the long term plan for this site has yet to be decided by AgResearch.

4. Verification Completed (this period)

Biosecurity

Acceptable compliance to all standards was observed. Transfer CM5930 highlights some of the risks posed to AgResearch by allowing tenants to use the umbrella of the 2501 facility approvals.

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 and Containment Facilities for Invertebrates

Note List:

[Crystal Lange]

All four annexes of the PC2 glasshouse were assessed for compliance with the invertebrate standard. The frames for the mesh screens of the inner doors were replaced as part of 2021/2022 maintenance. Isolated air-conditioning units were installed for cooling control. Small concrete bunds were present in each annex.

Drainage holes were noted on the lower window frames which may drain the the outside, it was agreed all internal holes will be sealed as a preventative measure.

Sealing was confirmed 20/07/2022.

Subject: Containment Standard for Field Testing of Farm Animals

Note List:

[Crystal Lange]

Earthworks for the municipal water supply and land port access road has changed drainage patterns. Significant standing water was present at the time of the verification. As contractors are entering the site, extra protocols have been put in place to allow easy of access but maintain containment integrity.

The annual report to the EPA had been drafted and was planned to be submitted on time.

Overseas cases of Foot and Mouth Disease has led to a revision of the M.bovis plan to ensure preparedness.

Quality Assurance

All active CTO approvals were current. An application is underway for as recently reclassified weed (Golden dodder (Cuscuta campestris)).

Amendments to the facility manual were summarised in the pre-audit report. Internal audits were completed in a timely manner for laboratories, glasshouses, small (SAC) and large vertebrates (Piggery) and animal containment (ACF). Training had been completed for nine new laboratory staff, Glasshouse training is due and the PP DFO is aware of this.

The following elements were verified in this PBV period:

Quality Assurance:Chief Technical Officer (CTO) Permissions and Decisions	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

All BACC's were held on file, two had been tracked as part of the internal audit. All requested documents were available to view. The Glasshouse log book and autoclave records were held within the Glasshouse facility.

There have been no structural changes to the site, however tenanted spaces have changed.

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

Reconciliation of multiple transfers CM581 and JBiC056 was undertaken. Transfer JBiC263 (Bovine Serum Albumin) was tracked to the -20° freezer in PP. Registers were maintained in detail for the Glasshouses (PC1 and PC2). Cage cards for mice in the SAC matched the register. Embryo transfer records were reviewed for the piggery and records sampled for lambs, ewes and cows. A stock count was undertaken for selected paddocks of sheep and goats.

The following elements were verified in this PBV period:

	Identification, Traceability & Management:Inventory Control and Accuracy	Acceptable
⊛	Identification, Traceability & Management:Transfer of Goods and Organisms	Acceptable

The following elements were verified in this PBV period:

Identification, Traceability & Management:Transport of Risk Goods and Organisms	Acceptable

Subject: Transfer of Goods and Organisms

Note List:

[Crystal Lange]

This issue is now closed.

Transfer CM5930 was raised for two strains imported on a HSNO approval. These were held in containment (Micro2007a) by a tenant and directed to a transitional facility (Biological products). The recipient facility did not hold appropriate approval to receive or hold these cell lines. It appears there was a loss of knowledge of the status of those cell lines over time. Transfer CM5930 was reviewed by 4 people (internal and external parties) and the errors were not noted by anyone.

The recipient facility has destroyed the GM strain and is seeking clarification with the EPA/Animal Imports around the status of the non GM line.

This non-compliance can now be considered closed.

Hygiene & Sanitation

ibutton records for PP and SAC were reviewed. ibutton records for the new autoclave in the Glasshouse resulted in an extension of the waste cycle ('soil') to 30 minutes to ensure an adequate time/temperature parameter was reached due to the thickness of substrates treated.
Piggery waste is transferred to the ACF for onsite disposal.

Pest control is monitored by Ecolab. Moderate activity has been reported. Brush strips were installed on doors in Animal Physiology following the finding of a dead mouse.

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:Pest, Vermin and Weed Control	Acceptable
Hygiene & Sanitation:Waste Management	Acceptable

Design and Construction

Laboratories in Dairy Science and South Wing were inspected along with the Glasshouses and controlled growth rooms. A minor NC was issued for PC1 Laboratories.

Some mice cages had activity wheels. The DFO was knowledgeable as to which strains and sexes were better matched to utilise this enrichment. Health investigations at the ACF were discussed.

Repairs to wallpaper, paint and air-conditioning units had been completed in Dairy Science. The new wash-up build for the the Embryo Lab (PC2) was underway.

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]

Site laminar flow hoods are tested every two years inline with a risk assessment approved by MPI. Testing is due in 2023.

Subject: Physical Containment Level 1 (PC1)

Note List:

[Crystal Lange]

Unlabelled reagent bottles were seen in Dairy Science, shelves were dusty and cobwebs were abundant in elevated ceiling spaces. Floors around fridge edges were grimey. There was a hole in the floor behind the ice machine and a patch of vinyl in a doorway was seen to be cracking.

Corrective Action Request
1. Ensure reagent bottles are labelled and dated as applicable.
2. Address hygiene issues
3. Fix flooring issues

To Be Completed By: 7 October 2022

Hazardous Substances and New Organisms (HSNO) Act

A register was maintained of AgResearch HSNO Approvals and those of tenants.

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

The following elements were verified in this PBV period:

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

Subject: HSNO Act Approvals for Development of New Organisms

Note List:

[Crystal Lange]

Compliance with the controls (1, 2, 5-8, 14, 15, 19) of EPA decision APP203820 was confirmed 18/07/2022.

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/2501/2023/01
Outcome:	**Unacceptable**
Issued to:	AgResearch - Ruakura Campus
Operator ID(s):	2501
Issued by:	Crystal Lange **Phone:** 079578319 **Email:** crystal.lange@mpi.govt.nz
Verification Period:	2022-08-24 to 2023-02-27
Verification Date:	2023-02-09
Published:	2023-02-13 12:13
Next Due Date:	2023-05-09
Level/Step:	**5.1** (started on 6.2 , and ceiling is 6)
Report Type:	Scheduled
Peer Reviewed By:	Els Maas

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
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 9/02/2023 was UNACCEPTABLE due to CAR:2501/2022/01 being issued during the verification period for an unapproved transfer. Two minor NCs were issued following the the reality check of the verification, one for laboratory hygiene, the other for perimeter fencing.

The CAR was closed promptly however it was the second transfer NC within the past two verification periods. It identified a lack of understanding of the types of risk goods held and the controls required to move these goods. It was confirmed that transfers and transfer documentation will be a focus area of the upcoming refresher training. The NC issued for perimeter fencing was addressed prior to the completion of this report. Photographic evidence was supplied, and this NC is now closed. The NC issued at the previous PBV had been closed by the due date.

MPI is satisfied that the DFOs are knowledgeable of the requirements of the standards and are proactive in addressing knowledge gaps of staff. In the main, AgResearch can be seen to be operating in compliance with the requirements of the standards it is approved to. As such the facility and operator approvals will be continued although increased verification frequency will be occurring for the laboratories operating under the Biological and Microorganisms Standards.

3. Operator Summary

The entry meetings along with the reality check of the facility was carried out by Crystal Lange (MPI) with [redacted] (DFO) and Tim Hale (DFO). Farm records were reviewed while in the ACF. Laboratory records were reviewed with [redacted] followed by the exit meeting (Tim was not present).

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 and the Animal Physiology Complex, and animal containment in SAC and ACF.

Discussions were held over decontamination of equipment prior to relocation within the facility or external to it, along with the future plans for the site including

the ACF in relation to the land port and the annexing of tenant organisations from the AgResearch facility.

4. Verification Completed (this period)

Biosecurity

Summary notes have been presented for each standard verified.

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

Subject: Transitional Facilities for Biological Products

Note List:

[Crystal Lange]

A summary of activity for the past six months was supplied prior to the inspection. Registers were viewed on site and a reality check of TF/CF laboratories undertaken. Hygiene was noted as an issue in PP and a non-compliance was issued. Additionally a CAR had been issued during the PBV period for an unapproved transfer to a non-TF. This along with a major NC for transfer issued during the previous PBV period indicated a lack of understanding of Biosecurity requirements. The laboratory side (Biological and Micro) of the Ruakura facility is now on increased audit frequency.

Subject: Facilities for Microorganisms and Cell Cultures: 2007a

Note List:

[Crystal Lange]

A summary of activity for the past six months was supplied prior to the inspection. Registers were reviewed on site and the HSNO Approval code of a new transfer was advised. Dust and sealing of surfaces was identified as part of the non-compliance issued following the reality check.

Subject: Containment Standard for Field Testing of Farm Animals

Note List:

[Crystal Lange]

Internal audits for the Piggery (Vertebrate Standard) and ACF were supplied prior to the verification. The facility has been subjected to ongoing and repetitive flooding as a result of landowner activity. Some areas were still inundated at the time of the PBV. The double fenced perimeter was not being grazed. Sheep, goats and cattle were adequately contained. Ear tag numbers were used for traceability. The changes to the register were discussed and the new calves were seen in the cattle yards. The supplied register noted a high number of deaths of ewes and does. This was discussed. All culls were confirmed to have been for animal welfare reasons.

A minor non-compliance was issued for gates in the perimeter fenced not being secured as required by the standard.

Subject: Containment Facilities for Vertebrate Laboratory Animals

Note List:

[Crystal Lange]

A summary of activity of the past six months was supplied prior to the inspection. Mouse lines are being reviewed and valued genetics are being moved into cryo-preservation. Three rooms in the SAC were in use. The Piggery was not in use and the Gene Transfer Unit had been removed from the facility prior to refurbishment.

Records were reviewed as part of the Ruakura site PBV and a satisfactory reality check was completed.

Quality Assurance

MPI has been notified in a timely manner of any issues that have been identified on site. Advice is sought as required where clarification is required. Two new CTO permission applications have been submitted. Expiry dates are regularly reviewed and the CTO permissions expiring in 2023 had been noted. Internal audits have been completed in a timely manner with no significant findings noted. Training was up to date with ten inductions having been completed during the verification period. Site refresher training is not yet due. The induction training process for new staff was described.

The following elements were verified in this PBV period:

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

Subject: Operator Internal Verification

Note List:

[Crystal Lange]

With the next verification (high frequency) co-coinciding with the next internal audit round it was agreed that only the internal audit for PP needs to be completed prior to MPIs visit.

Documentation and Certification

All requested records were available to be viewed. Registers were sighted for imported and transferred goods. The new PC1 laboratory under construction in Animal

Physiology was visited as was the completed Aspiration Laboratory. It was agreed that while the Aspiration Laboratory meets PC1 requirements, it will not be included into the TF at this time. MPI was also advised that the site footprint would be amended once the tenant separation is complete.

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

Registers for the TF/CF laboratories, SAC and ACF were up to date. Storage locations of biological products were recorded, mouse cage card labelling matched the supplied register and number of occupants, calf tags were used to trace back to sire and dam. Health records for ACF animals were also sighted.

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	Unacceptable

Corrective Action Requests (CARs)

CAR ID:	2501/2022/01	**Status:**	COMPLETE
Date issued:	29 November 2022	**Issued by:**	Crystal Lange
Subject:	Identification, Traceability & Management:Transfer of Goods and Organisms		
Date completed:	13 December 2022		
Reason:	Email for facility advising of unapproved transfer to a non TF.		

Hygiene & Sanitation

Dust was identified between units in the PP Freezer Room, on power-point covers and the phone shelf in the Media/autoclave room, on high surfaces in SBL and power-point covers along a work bench in Nematology. Coved strips added to the bench tops in SBL require the sealing to be checked and replaced as needed, the coved strips in the Micro

Laboratory require sealing to the bench to prevent any seepage down the wall. A strip of bench edge in the Endophyte lab requires sealing. A patch of wall in Animal Physiology (17d) requires the patched plaster to be painted in order to seal the wall.

PPE was seen to be being worn by staff. PPE for visitors was available at all locations. All risk goods waste is autoclaved. iButton verification for the three autoclaves was reviewed and found to be acceptable.

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:Pest, Vermin and Weed Control	Acceptable
	Hygiene & Sanitation:Waste Management	Acceptable

Subject: Cleaning and Disinfection

Note List:

[Crystal Lange]

⊛ Dust was noted on surfaces in a number of laboratories reflection poor cleaning practices and restrictive observation. Sealing of benches is required to ensure all surfaces are impervious. As a consequence a minor NC has been issued.

CORRECTIVE ACTION REQUEST
1. Address hygiene (dust) issues
2. Ensure surfaces are sealed and impervious.

To Be Completed By: 3/03/2023

Design and Construction

Doors in the PP suite were not closing properly despite having self-closers. The alignment of doors, latches and frames should be reviewed.

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

Subject: Animal Enclosures and Facilities (inc. invertebrates)

Note List:

[Crystal Lange]

This issue is now closed.

The spine road access gates were chained closed with the inner gate overlaid by monitored electric fencing. The inner gate is chained shut but offset from the electric fence Either set of gates could be removed as all gudgeons face up and are unpinned.

MAF Reg Standard 154.03.06: Containment standard for field testing of farm animals
4.2.1 Perimeter fences
Where swinging or sliding gates are incorporated into either the inner or outer perimeter fence (such as at the vehicle entrance-way and stock loading race), the gudgeons or rollers shall be of such type or so placed, as to prevent the gates being lifted from them.

This is a minor non-compliance and must be addressed by 17/02/2023.
This was actioned and closed 10/02/32023 while the report was in the process of being written.

Hazardous Substances and New Organisms (HSNO) Act

APP203192s67A, APP203239, ERMA200223 and GMC03001 were among the approvals in use.

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)