Annual Report to Environmental Protection Authority

for

Activities under ERMA 200223

AgResearch Ltd

For the 12 months ending

30th June 2022

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, kidding of goats, 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 disrupted planned follow up interactions with Te Haa o te Whenua O Kirikiriroa which have not been rescheduled at this time.

Members of the AgResearch Animal Science team presented on the current cattle project at the Hui: Māori, Genetics, and Genomics - Wānanga Tuatoru, in Cambridge, July 2021.

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
- Milk from different transgenic lines is functionally analysed as part of international collaborations

Generating cattle genome edited for adaptation to warmer temperatures

- Eight calves edited for the slick mutation and five non-edited control calves were born in February. Three of the 'slick' calves were genotyped as non-mosaic, 100% edited for the precise mutation while the others had various degrees of the precise mutations plus other small on-target sequence changes.
- Detailed analyses of the genotypes and coat and behavioural characteristics under warm and cold conditions are in progress.
- We have transferred high breeding worth embryos that were edited for lighter coat colour (PMEL) embryos. The embryos were produced by IVF with oocytes from a pool of 14 oocyte donors and the same sire. One PMEL calf and five non-edited control calves were born in June 2022 with additional calves expected to be born in September 2022.
- Genotyping of the PMEL calf has identified the presence of two different alleles. The intended 3 bp deletion and a target site specific but unintended 6 bp deletion. Both mutations delete amino acids in the signal peptide of the protein.
- DNA samples from the PMEL and control calves were prepared and sent for parentage testing by SNP-chip.
- Activities and results were communicated to various stakeholders, and presented at the Hui: Māori, Genetics, and Genomics - Wānanga Tuatoru, Cambridge, July 2021; Transgenic Animal Research Conference XIII, virtual event, August 2021 and 6th International Livestock Biotechnology Symposium, virtual event, February 2022
- New knowledge generated was published in two scientific articles (Laible et al., *BMC Genomics* 22, 856, 2021; Wei et al., *Frontiers in Genetics*, accepted, 2022)

Goats producing therapeutic proteins

- Goats were maintained to investigate longevity and potential long-term health effects
- Some of the goats were used as embryo transfer recipients for the production of new lines of transgenic offspring
-

Goats producing female-only offspring

- One cloned transgenic goat and one (out of two) AI controls remained in good health into adulthood and were phenotypically characterised for sperm morphology, transgene presence and transmission ratio distortion in vitro.

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

- Cloned, gene-edited animals were maintained into adulthood from four different *NANOS2* genotypes, namely: male homozygous knockout (Group 1), male heterozygous knockout (Group 2), female homozygous knockout and female wild-type cell lines (Group 4). These animals are presently being maintained and/or bred.
- Female *NANOS2*$^{-/-}$ and male *NANOS2*$^{+/-}$ cloned lambs were used for breeding using ovum-pickup and in vitro fertilisation (IVF) or AI, respectively. Both genotypes displayed normal fertility.

Generating immune-compatible sheep for xenotransplantation

- 5 adult cloned ewes, carrying deletions in the *GGTA* and *CMAH* genes, were used for OPU-IVF to generate gene-edited offspring of both sexes.

Overexpression of the histone demethylase KDM4B in transgenic cattle

- One cloned female animal overexpresses the histone demethylase KDM4B fused to a GFP reporter transgene. This animal (#1801) was used for repeated ovum pick-up, followed by IVF with wild-type sperm to generate transgenic offspring of both sexes.
-

On Farm Management Summary for year ending 30/06/2022

Animal Numbers 01/07/2021– 30/06/2022 (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 (1/07/21)	Births	Transfer In	Transfer Out	Aged In	Aged Out	Killed	Deaths	Closing (30/06/22)
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	**15**				0		1		**14**
Total BLg -	**15**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**14**
Erbitux (ERMA200223)									
Total Erbitux	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Climate Smart (ERMA200223)									
Heifer Calves	**0**	7							**7**
Bull Calves	**0**	10							**10**
Total Climate Smart	**0**	**17**	**0**	**0**	**0**	**0**	**0**	**0**	**17**
KDM4B (ERMA200223)									
MA Cows	**1**				0				**1**
Total KDM4B	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**
Conventional Cattle									
MA Cows	**50**		0	4	31		0		**77**
R2yr Heifers	**31**		0		0	31			**0**
Other classes	**22**	0	0	22	0	0	0	0	**0**
Total Conventional	**103**	**0**	**0**	**26**	**31**	**31**	**0**	**0**	**77**
Cattle Total	**119**	**17**	**0**	**26**	**31**	**31**	**1**	**0**	**109**
Cattle developed under ERMA approvals (Tg and non Tg progeny)									**32**

[1] Aligns with normal livestock reconciliation aging practice.

Stock Class	Open (1/07/21)	Births	Transfer In	Transfer Out	Aged In	Aged Out	Killed	Deaths	Closing (30/06/22)
Goats									
Erbitux & Enbrel (ERMA200223)									
Ma Doe	**19**						7		**12**
R2yr Doe	**0**								**0**
R1yr Doe	**0**								**0**
Doe Kid	**0**	2							**2**
Buck Kid	**1**	1				1	1		**0**
R1yr Male +	**0**				1				**1**
Total Erbitux & Enbrel	**20**	**3**	**0**	**0**	**1**	**1**	**8**	**0**	**15**
non Med inherit (ERMA200223)									
Total TCR	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Conventional Goats									
MA Doe	**22**						3		**19**
R2yr Doe	**0**								**0**
R1yr Doe	**0**								**0**
Male R1yr +	**0**				2				**2**
Kids	**2**	3				2			**3**
Total Conventional	**24**	**3**	**0**	**0**	**2**	**2**	**3**	**0**	**24**
Goat Total	**44**	**6**	**0**	**0**	**3**	**3**	**11**	**0**	**39**
Goats developed under ERMA approvals (Tg and non Tg progeny)									**15**

Stock Class	Open (1/07/21)	Births	Transfer In	Transfer Out	Aged In	Aged Out	Killed	Deaths	Closing (30/06/22)
Sheep									
AI on Hooves									
MA Ewes	**0**				12			2	**10**
2th Ewes	**12**				3	12			**3**
Ewe Hgts	**0**				4	3			**1**
Ewe Lamb	**3**	2				4	0		**1**
MA Ram	**1**				1		1		**1**
R2yr Ram	**1**				0	1	0	0	**0**
R1yr Ram	**0**				2	0	1		**1**
Ram Lamb	**2**	1				2			**1**
Total	**19**	**3**	**0**	**0**	**22**	**22**	**2**	**2**	**18**
Conventional Sheep									
MA Ewes	**50**				4		3		**51**
2th Ewes	**4**				0	4			**0**
Ewe Hgts	**0**				5	0			**5**
Ewe Lamb	**0**	5				5			**0**
2th Ram	**7**				0		7		**0**
R1yr Ram	**0**					0			**0**
Ram Lamb	**0**	1							**1**
Total Conventional	**61**	**6**	**0**	**0**	**9**	**9**	**10**	**0**	**57**
Sheep Total	**80**	**9**	**0**	**0**	**31**	**31**	**12**	**2**	**75**
Sheep developed under ERMA approvals (Tg and non Tg progeny)									**18**

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 2 movements of conventional animals out of the facility during the period. This was 22 mixed sex conventional beef animals, under 2 years of age on the facility for grass control purposes and 4 Ma cows which failed to perform for ET breeding and complied with the approved removal criteria. There has been no movements of cattle into the facility.

1 Ma GM cow has been humanely killed and has 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.

11 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.

12 sheep of varying ages have been humanely killed and 2 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.

73 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 often have access to covered shelter in inclement weather.

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

Dry 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.

No maintenance fertiliser was applied this season, but the majority of the grazing area within the facility received lime at 1000kg/ha.

Operationally we have also been 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 21/22 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 # 15088 - Generation of climate-smart cattle from edited embryos

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

RAEC # 15082 - Somatic cell transfer cloning to induce female-only offspring in goats

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

RAEC # 15051 - Maintenance of cloned sheep for breeding and phenotype evaluation.

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

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

AE ReportA 15051 ~ (Status=ACCEPTED)(Applicant=[redacted]) (AE APPLICATION 15051) Maintenance of cloned sheep for breeding and phenotype evaluation

Group	Line	Question	Answer
		0. ADMINISTRATIVE DETAILS	
0	1	Title	(AE APPLICATION 15051) Maintenance of cloned sheep for breeding 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 Containment Facility
0	7	Start Date (dd/mm/yyyy)	05/02/2021
0	8	Finish Date (dd/mm/yyyy)	06/02/2022

0	9	Number of animals used ~ Species used	35 ~ 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.	We did not transfer any vitrified embryos, as originally planned, which reduced the number of animals (no ET recipients, no offspring).
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.	Semen collection from the two different NANOS2 genotypes has finished with two successful collections from Bunter and Howie. Also on this application are 3 companion rams, two which are offspring from a clone but these have maintained as standard ACF practice. There are also 3 ewes that are offspring from clones and therefore are pink tagged and are maintained as recipients. Bunter and 75 are both Johnes positive but are not clinically unwell. 2 x OPU (May and June) was performed on 7 and 6 (after one death) poll dorset cloned females, after Howie's semen had been successfully tested for IVF. The majority of ewes had a laparotomy rather than laparoscopy. It resulted in a shorter procedure which was seen as an improved benefit to the sheep even if it meant the surgical wound was larger. Given that the animals had a laparotomy rather than a laparoscopic procedure, they needed a 6-week recovery time from surgery. Oocyte recovery was an average 10 oocytes/ewe on both occasions. Animals stimulated well out of season using our current stimulation protocol. 4 cloned 'xenogirl' females (not Bertha) were naturally bred to a ram for 2 cycles along with 3 controls as per modification #2969. None of the clones became pregnant but the reasons for this is unclear. All 3 controls became pregnant and lambed without incident. Twice we carried out mating with Howie, once to 3 ewes (77, 78, 79), then to 5 ewes for December 2021 mating. 7 lambs were born from the first mating (one male killed at birth), another 3/5 ewes are still pregnant.
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	On the first OPU date, 2 out of 7 sheep had poorer oxygenation and required extra care during and post op. This was likely due to chronic pneumonia and lung consolidation from having pneumonia as lambs. The following day, the sheep were all doing well. One ewe 1930 had a swelling at the laparotomy site, which was well covered with antibiotics and pain relief. On the 2nd OPU date, 1 out of 6 sheep had trouble with intubating as she had a very long epiglottis and had to recover to be anaesthetised again. When she woke up again, she had to be on oxygen and given planipart as she had laboured breathing.

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	see above, 2 ewes had temporarily poorer oxygenation on the day of anesthesia, 1 had trouble intubating and 1 had swelling at laparatomy site.
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.	- Rachel (NANOS2-/-) died on 1st July as reported (adverse event 246) - One wild-type clone (Martha) died spontaneously and for no obvious reason (as per post-mortem) on 3/12/2021.
1	9	If Yes please detail and state whether or not this affected the outcome of the project	The death of Rachel has have made the surviving animals even more precious and will slow down progress because there now only 2, not 3 NANOS2-/- females available.
		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	A lot less animals were used as we had no offspring born apart from some control lambs
2	4	Refinement	We ended up naturally mating the xenogirls rather than doing AI due to unavailability of sexed semen. While it was unfortunate they didn't get pregnant from NM, it is likely they wouldn't get pregnant from AI either so overall it saved them at least one AI surgery. OPU surgery and anaesthesia was undertaken by experienced veterinarians with multimodal pain relief
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)	no comments
		98. NOTES ~ Read only	
98	1	Status Change	[redacted] 23/03/2022) SUBMIT

98	2	Committee Decision	(04/04/2022 RESUBMIT[redacted]) Please add Tim Hale to this report and gain signatures prior to submission.
98	3	Status Change	([redacted] 03/05/2022) SUBMIT
98	4	Committee Decision	(12/05/2022 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	[redacted] ~ approved ~ 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 ~ approved ~ Job (Research Farm Manager, Ruakura) Location (Ruakura; Manager-Animal Containment Facility,Yard; First Aid)	FOM Ruakura / Facility Operator
99	99	[redacted] ~ approved ~ Job (Senior Scientist) Location (Ruakura; An Phys. First Aid)	Principal investigator, general oversight

AE ReportA 15082 ~ (Status=ACCEPTED)(Applicant=[redacted]) (AE APPLICATION 15082) Somatic cell transfer cloning to induce female-only offspring in goats (modified from 14710)

Group	Line	Question	Answer
		0. ADMINISTRATIVE DETAILS	
0	1	Title	(AE APPLICATION 15082) Somatic cell transfer cloning to induce female-only offspring in goats (modified from 14710)
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)	03/09/2020
0	8	Finish Date (dd/mm/yyyy)	24/12/2021
0	9	Number of animals used ~ Species used	48 ~ 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 got 3 instead of 20 clones and AI offspring, hence the reduced number.
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)	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 have obtained one cloned buck from a C-section, who appears to have moderate, rather than high impact on his animal welfare status
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.	In Sept/Oct 2020, we synchronised 26 does for ET of cloned embryos and 6 does for AI. Of these, 23 does had undergone ET (with 3 undergoing surgery but no transfer due to not ovulating), which resulted in 1 pregnant animal that held to D60 and beyond. At term, we obtained one live cloned buck by C-section. In parallel, only one of the six AI'ed animals got pregnant, resulting in twin bucks. In March 2021, we synchronized 42 does and transferred into 23 of them, including 3 recipients for the parental cell line as positive controls. 33 does had undergone surgery but ET had not occurred for various reasons (lack of synchrony response, adhesions). Some of these animals were also synchronised in Sept/Oct 2020, so they had undergone the programme twice over a 6-month period. Embryos were transferred at the 1-2 cell stage, and looked good at transfer, using the same set of does as were used in the September/October cloning runs. At the ~35-day pregnancy check there were zero viable pregnancies in any group. Total over the 6 month period were 45 does used, 38 had undergone surgery (ET or AI) with 27 undergoing surgery (and synchrony) twice. No animal was rejected from surgery twice. This December 2021, we attempted semen collection from Brownie and two AI controls, using 5 does on natural heats, over 2 AV session. Both were unsuccessful with the bucks showing modest interest but not riding. This was probably due to the bucks being inexperienced and still out-of-season.
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	Pregnancy rate per embryo and term survival was about 5-fold lower than expected for SCT goat clones. We suspect that there may be an underlying problem with the recipient herd as AI success was also considerably lower than expected.
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?	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	
2	3	Reduction	
2	4	Refinement	
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)	We did not have the options of testing and selecting better surrogate recipients but it became apparent during the trial that a number of does did not perform well even after being AI'ed. In the future, we will endeavour to identify poor recipients earlier, before they get to several years of age, perhaps by using regular AI to confirm their suitability.
		98. NOTES ~ Read only	
98	1	Status Change	([redacted] 18/01/2022) SUBMIT
98	2	Committee Decision	(28/01/2022 RESUBMIT[redacted]) Please include information about the semen collection that was approved in modification 3053. Additionally, the numbers in 1.2 don't match what is written in 0.9, this could be because some of the animals were synchronised multiple times but this is not clear currently. If animals were used multiple times please include the total number of times animals underwent synchrony and surgery. In 1.2 there is indication that 42 does were synchronised, suggesting that the

			minimum number of animals used over this project was 42 + 3 offspring= 45 animals (not 35). Please gain 2 missing signatures. If the stats need to be altered then please contact the animal ethics office and it can be put into RESUBMIT for editing.
98	3	Status Change	([redacted] 15/02/2022) SUBMIT
98	4	Committee Decision	(03/03/2022 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	[redacted] ~ approved ~ 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 ~ Job (Research Farm Manager, Ruakura) Location (Ruakura; Manager-Animal Containment Facility,Yard; First Aid)	FOM Ruakura / Facility Operator
99	99	[redacted] ~ approved ~ Job (Senior Scientist) Location (Ruakura; An Phys. First Aid)	Principal investigator, general oversight

AE ReportA 15088 ~ (Status=ACCEPTED)(Applicant=[redacted]) (AE APPLICATION 15088) Generation of climate-smart cattle from edited embryos

Group	Line	Question	Answer
		0. ADMINISTRATIVE DETAILS	
0	1	Title	(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
0	7	Start Date (dd/mm/yyyy)	09/09/2020
0	8	Finish Date (dd/mm/yyyy)	09/09/2021
0	9	Number of animals used ~ Species used	74 ~ 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.	18 recipient cows were used in two different rounds of synchronisation and/or embryo transfers. In the application we had estimated that 10 calves will be produced during the approval period from November 2020 transfers. However, no pregnancies from edited embryos were established and hence control pregnancies aborted. 14 instead of 15 cows were used for ovum pick up.
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	
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.	Modification 2798 Synchronisation of 28 recipients and transfer of 15 edited and 10 control embryos. Ultrasound pregnancy scanning and abortion of control embryos at around day 45 of gestation. Modification 2860 Synchronisation of 20 recipients and transfer of 16 edited embryos. Ultrasound pregnancy scanning and abortion of pregnancies at around day 45 of gestation. Modification 2911 14 high breeding worth cows had 6 rounds of weekly ovum pick up (OPU) and a last OPU session after a 3 week interval. Modification 2914 Synchronisation of 30 recipients and transfer of 15 edited and 10 control embryos. Ultrasound pregnancy scanning at day 35, 49 and 83 of gestation. Some recipients were re-used over the different ET rounds
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	Embryo transfers under modification 2798 did not establish pregnancies from edited embryos. This was later shown to have been caused by a toxic culture component and was resolved for subsequent embryo transfers. OPU cows were vet checked soon after arrival and it was recommended to give the cows a 6 week rest period prior to starting any OPU which was unexpected but didn't impact the trial. Animals over that time recovered from any uterine infections and gained body condition (remnant issues from previous farm) and OPU all went to plan from there.
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?	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.	Recipients pregnant with control embryos (Mod 2798) were aborted (prior to half gestation) in the absence of pregnancies with edited embryos.
1	9	If Yes please detail and state whether or not this affected the outcome of the project	The issue has been resolved and embryo transfers have/are going to be repeated.
		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	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.
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 gold 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)	Regular review and update of husbandry protocols aids our aim to achieve gold standard nursing and husbandry. Recipients need a regular turnover to maintain a recipient herd that keeps fit for purpose.

		98. NOTES ~ Read only	
98	1	Status Change	([redacted] 28/09/2021) SUBMIT
98	2	Committee Decision	(14/10/2021 ACCEPTED[redacted])
		99. PERSONNEL SIGNATURES	
99	1	Committee	RUAKURA
99	1	Programme leader and Facility manager 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	[redacted] ~ approved ~ 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, U/S, ET
99	99	[redacted] ~ Job (Associate Research Director - Delivery) Location (Invermay; Administrator: Megan Struthers +6434899072)	Associate Research Director
99	99	[redacted] ~ approved ~ 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] ~ approved ~ 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] ~ approved ~ Job (Farm Senior - Farm Technical) Location	Farm Senior

		(Ruakura; Containment Unit; First Aid)	
99	99	[redacted] ~ Job (Senior Scientist) Location (Ruakura; An Phys. First Aid)	Senior Scientist
99	99	[redacted] ~ approved ~ Job (Science Team Leader - Animal Biotechnology) Location (Ruakura; Repro-An Phys, Fire Warden)	Principal Scientist, Science Team Leader

MPI Verification Services Audit reports

Ministry for Primary Industries
Manatū Ahu Matua

Verification Report[1]

Report ID:	PBV/2501/2021/02
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:	2021-03-09 to 2021-08-27
Verification Date:	2021-08-06
Published:	2021-08-17 16:25
Next Due Date:	2022-02-27
Level/Step:	**6.2** (started on 6.1 , 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 Required
CAR	Corrective Action Request
COVID-19	Coronavirus Disease of 2019
CTO	Chief Technical Officer
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
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
VS	Verification Services

2. Executive Summary

The objective of this visit was to verify compliance with the facility manual, the Import Health Standard(s), the standards identified in the "Biosecurity" section of

this report and the facility and operator approvals as held under the Biosecurity Act 1993.

This was a scheduled and announced inspection of the AgResearch Limited TF and CF at Ruakura, Hamilton on 6 August 2021. The outcome of this verification was acceptable with one NC issued for PC1 laboratories.

Registers were reviewed, animals, enclosures and laboratories reviewed. 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] (delegated Operator) and Tim Hale (delegated Operator ACF) of AgResearch. The reality check included ACF, SAC including the Pig unit (Goat Shed), PC1 laboratories in Dairy Science, South Wing, Plant Protection and the PC1 and PC2 Plant Protection glasshouses.

The Inspectors' authority under the Biosecurity Act 1993 and Hazardous Substances and New Organisms Act 1996 was confirmed. Health and Safety is covered by a visitor register. No additional hazards were notified.

Operator Control continues to be reviewed and strengthened. Staff in the Pig unit demonstrated good understanding of risk assessment although dissemination of knowledge could be improved.

4. Verification Completed (this period)

Biosecurity

No issues were identified. Specifies for each standard are noted below.

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]

Only two imports had occurred under this standard during the past six months. Research work is winding down in general with a number of laboratories having little or no recent use.

Subject: Facilities for Microorganisms and Cell Cultures: 2007a

Note List:

[Crystal Lange]

Registers were reviewed, recent transfers and training events were confirmed. PC1 laboratories excluding those in the APC were visited. The PC2 laboratories in Plant Protection was also excluded at the last minute.

Notification of transactions to the delegated Operator are continuing.

Subject: Containment Facilities for Plants: 2007

Note List:

[Crystal Lange]

Controls were reviewed for CTO permissions for velvet leaf, M.minor and black-grass. AgResearch was found to be compliant with the conditions of the relevant approvals. HSNO Approval GMD02023 was in use.

Labelling of pots and seed storage was acceptable. The glasshouse manger was knowledgeable of all work under way.

A replacement autoclave was due to be installed that week.

Subject: Transitional and Containment Facilities for Invertebrates

Note List:

[Crystal Lange]

The PC2 glasshouse was confirmed to comply with PC2 holding requirements. Appropriate signage was present.

Subject: Containment Standard for Field Testing of Farm Animals

Note List:

[Crystal Lange]

The register was supplied and animal transactions discussed. Deaths and animal removals were explained. There were no health issues that required notification to MPI. Training was up to date for all staff and the facility manual (V4) was issued in June 2021. Selected animals from the register were confirmed as present in containment. Sections of the perimeter fence were viewed when entering and leaving site and while travelling to the Pig unit.

Compliance with the 154.03.06 Standard and HSNO Approval ERMA200223 was assessed and confirmed as being compliant.

Subject: Containment Facilities for Vertebrate Laboratory Animals

Note List:

[Crystal Lange]

Quarantine of 46 animals had occurred with only one death. Completion of quarantine was notified to MPI and approval given to transfer the animals to another Containment Facility. Recording of daily quarantine checks was identified as deficient by the facility.

Animal rooms were well maintained. The animal register was checked against cage cards for HD K/O and JMJ3.

The Pig unit is approved under this standard but managed by the Animal Containment Farm staff. The facility was clean, tidy and secure. Pigs were alert and active. Work was underway for HSNO Approval GMD102650.

Quality Assurance

A number of CTO permissions are held. It was confirmed the PTA approval was not renewed after expiry in 2018. Copies of Velvet Leaf, M.minor and black-grass had been

supplied to MPI. A copy of the Chilean mayten permission was requested. Compliance with controls for black-grass and velvet leaf were discussed and confirmed as being compliant.

Internal audits for the site were completed 27/05-23/6 2021. Key points were the continued failure of the Plant Protection autoclave and a number of hygiene and structural issues for laboratories.

Training had been completed for staff and cleaners. Training for Laboratory Managers was underway.

Excellent (and timely) notification has been received for incidents that have occurred during the past six months (security events and broken panel in the PC2 glasshouse)

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

Subject: Operating Procedures

Note List:

[Crystal Lange]

During the Piggery visit the delegated Operator was advised that anyone accessing the Pig unit does not access the PC2 laboratory without showering first.

It was agreed clarity needs to be gained to cover visitors not working with the pigs and access to other laboratories in the Animal Physiology building (e.g. PC1) as these rooms/equipment/laboratory gowns are used by staff accessing PC2 laboratories as well.

A written protocol should then be supplied to relevant staff and placed on the Pig unit door.

Documentation and Certification

All imports were recorded. Records maintained during vertebrate quarantine (C2021/505534) were discussed. The facility was aware of a deviation in recording of daily checks from historic to present imports with the change in supervisory staff. This will be redressed at the earliest opportunity.

Registers for the three animal facilities were maintained and supplied in hard copy, internal audit records and the laboratory/small vertebrate/plant house pre-audit report were supplied in time for part of the external audit preparation.

The following elements were verified in this PBV period:

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

Identification, Traceability & Management

Inventory control for M.minor was discussed as numbers can not be confirmed pre-trial without destroying the soil structure and pupation burrows. Grass pots were well labelled, animals tagged, clipped or notched as required.

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

Hygiene & Sanitation

Overall the hygiene of working areas was maintained to a good level except for Dairy Science, particularly in the storage areas. PPE was available and appeared clean.

Autoclave validation records were supplied for Plant Protection and Small Animal Colony. Chemical treatment was not able to be viewed due to hygiene protocols for the Pig unit that had not been formalised. Pig unit waste (non GM) is collected in wheelie bins and disposed of on farm.

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:Quarantine Isolation	Acceptable
Hygiene & Sanitation:Waste Management	Acceptable

Design and Construction

Issues with the electronic security system and extended periods where doors are required to be open for maintenance work have been notified to MPI.

All animal cages, pens enclosures and fencing had been maintained to an acceptable level. Issues with laboratory based facilities are noting in the NC below.

The following elements were verified in this PBV period:

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

Cracks and separating joins in the vinyl flooring of the Nematology laboratory (PP 14) need sealing.

Dust, cobwebs and midges were present in Dairy Science. The storage area with the -80 freezer was especially dirty. Wall/bench seals need replacing and a hole on the sink bench (12A) must be sealed. A bench in 26 had a chip and sections needed to be joined together with new impervious sealing.
Silicon sealant was not present around number of door frames which allows spills and cleaning fluids to seep into the joinery. While a number of doors have been done, the initiative has not been taken to roll this out across the site.

A bench in SW114 was sagging.

Collectively these issues form the PC1 NC. Completion of repairs should be confirmed with MPI by 10 September 2021.

Hazardous Substances and New Organisms (HSNO) Act

ERMA200223, GMD02013, GMD02023, APP203820, APP203942 and GMC03001 were among the approvals in use. It was confirmed GMC100216 was not yet 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)

Ministry for Primary Industries
Manatū Ahu Matua

Verification Report[1]

Report ID:	PBV/2501/2022/01
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:	2021-08-07 to 2022-02-27
Verification Date:	2022-02-18
Published:	2022-03-18 15:34
Next Due Date:	2022-08-27
Level/Step:	**6.2** (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 Required
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
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 18/02/2022 was acceptable with one NC issued for PC1 laboratories. Issues raised at the last PBV were confirmed as closed during the verification period. CAR 2501-2021-01 was issued for a breach of controls imposed by a CTO permission. MPI does not yet enforce compliance with CTO permission controls unless the NC also is a NC with a MPI transitional or containment standard. As the controls of the decision were immediately modified, the critical NC was nullified so only the requirement for MPI approval for transfer was outstanding. This major NC has been adequately addressed in the CAR response.

Security events were notified to MPI as they occurred, one was outside MPI oversight, one contravened AgResearch access policy (technical NC) and one was due to a faulty device. None of these required intervention or further action from MPI.

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). Tim Hale (DFO) was present for the ACF and Pig Unit assessment. The Inspectors' authority under the Biosecurity Act 1993 and HSNO Act 1996 was confirmed.

Health and Safety is covered by a visitor register. Access requirements under the current Traffic Light level had been emailed to MPI. Under COVID-19 Traffic Light Red, where onsite visits are deemed necessary, time spent on site by MPI staff must be minimised. As such the reality check only included ACF, SAC (including the Pig unit (Goat Shed)), PC1 laboratories in Dairy Science and South Wing.

Since the last PBV [redacted] as been appointed the Science Facility Manager and retained her MPI portfolio. [redacted] (Associate Research Director) had been appointed the Operator Representative for AgResearch. Crystal and [redacted] met with [redacted] during the verification.

4. Verification Completed (this period)

Biosecurity

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]

The biological products register showed two of the last three imports had been disposed of. Of the recent imports only C2021/1278625 was still in use.

Subject: Facilities for Microorganisms and Cell Cultures: 2007a

Note List:

[Crystal Lange]

A PC2 laboratory was accessed without the knowledge of the Laboratory Supervisor in violation of the site Contractor Access protocols. One of the contractors had been inducted, the other was not.

Laboratory Supervisor made contact with with the Contractors prior to their departure and sanitised the sink and flushed the drain where they had been working.

The AgResearch DFO was notified immediately as was MPI. Immediate actions taken were confirmed to be acceptable and a Root Cause Analysis undertaken. As a result all PC2 access for contractor access cards has been removed. Additionally, Transitional and Containment training will be part of the site induction as opposed to specific training 'when access is required'.

Subject: Containment Facilities for Plants: 2007

Note List:

[Crystal Lange]

1. CAR 2501-2021-01 was issued following a breach in the controls of the CTO Permission for black grass (Alopecurus myosuroides). As the Permission stipulated the Ruakura facility then containment controls are applied as per the EPA/MPI Standard.

Product was transferred without a valid movement authority (this standard) and without destruction (CTOd). The amendment of the CTO permission to allow transfer of material for a specific purpose changes the risk rating of the NC in relation to the CTO and only leaves the unauthorised movement. As such the CAR was downgraded from a Critical to Major non compliance.

There was good dialogue between all parties and a resolution was quickly achieved.

2. A non-urgent security incident was notified where a lock failure meant the emergency exit release needed to be used so staff could exit the facility.

Subject: Containment Standard for Field Testing of Farm Animals

Note List:

[Crystal Lange]

Farm staff reviewed the manual as part of the internal audit. Findings and suggestions were all recorded and reported to the Designated Operator and MPI.

Health records were reviewed for selected animals. The electronic mob tally had not been updated with an additional animal (1801) as the Farm Senior had yet to return to the office for data entry. This was confirmed verbally by phone call.

Compliance with the controls of ERMA200223 was confirmed.

Subject: Containment Facilities for Vertebrate Laboratory Animals

Note List:

[Crystal Lange]

A summary register was email to MPI and strains selected for the review. Cage cards were check and numbers in cages reconciled. Cards for culled animals are kept in each room until collected by the DFO to update the register.

Compliance with controls GMC03001 and GMD04112 was confirmed.

Quality Assurance

Positive COVID detection scenarios have been used to develop onsite procedures for removing the COVID-19 risk from the working environment. These include non use (72 hours), deep clean of laboratories or an essential worker wearing full bio-hazard PPE during the subsequent 72 hrs following a staff member reporting positive.

Version 4 of the facility manual is in place (approved 29/06/2021). Changes to the site footprint have since been made by email/photo and were approved by MPI.

Site wide training refreshers are scheduled for February/March 2022 for staff and contractors. Cleaner training was current and four staff had been inducted.

Internal audits were completed November through December. No issues were raised for the ACF/SAC or Piggery. R&M and hygiene issues were noted across a significant

number of laboratories and reproductive structures were found to be inadequately contained in the PP Glasshouse.

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:Operating Procedures	Acceptable
Quality Assurance:Operator Control	Acceptable
Quality Assurance:Operator Internal Verification	Acceptable
Quality Assurance:Training and Competency of Personnel	Acceptable

Corrective Action Requests (CARs)

CAR ID:	2501/2021/01	**Status:**	COMPLETE
Date issued:	21 December 2021	**Issued by:**	Crystal Lange
Subject:	Quality Assurance:Chief Technical Officer (CTO) Permissions and Decisions		
Date completed:	07 January 2022		
Reason:	Advice from TF2501 that plant tissues samples were sent from the TF with out destruction, in contravention to control 4 of CTO permission for Alopecurus myosuroides (black grass).		

Documentation and Certification

Only two imports have been received this PBV period. BACCs were on file. Record keeping was to a high standard. Biological products, small and large animal records were acceptable.

Additions to the laboratory footprint were made during the period. Approval was granted remotely from photographs supplied. These rooms were inspected during the reality check and confirmed to be acceptable.

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

Three authorised signatories are in place. Registers were up to date and transfers reconciled without issue.

The following elements were verified in this PBV period:

Identification, Traceability & Management:Authorised Signatories	Acceptable

The following elements were verified in this PBV period:

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

Hygiene & Sanitation

Validation was confirmed for the new PP Glasshouse autoclave. iButton reports for PP PC2 and SAC confirmed treatment parameters have been maintained. Waste from the Piggery is disposed of within the ACF.

Cleaning is discussed under Design and Construction: Laboratories.

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

Issued noted in the last PBV were addressed or added to ongoing routine maintenance. The NC was closed 9/09/2021. See notes under the Laboratories heading for issues noted this verification.

MPI was notified of a break-in at the Glasshouses. The affected area was not part of the containment facility. MPI was notified as there was the potential to access the PC1 area although this did not occur. PC2 remained secure.

The glasshouse was visited to check the wet wall replacement and autoclave installation. New rooms in Dairy Science (11, 17) were inspected as well as 36-42. South Wing First floor laboratories were visited. 101 had been approved to Plants PC1 remotely. As it was already approved to Biologicals (154.02.17) nothing additional was required.

The following elements were verified in this PBV period:

Design and Construction:Access and Security	Acceptable

All animal cages, pens enclosures and fencing had been maintained to an acceptable level. Issues with laboratory based facilities are noting in the NC below.

The following elements were verified in this PBV period:

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

Cracks and separating joins in the vinyl flooring of the Nematology laboratory (PP 14) need sealing.

Dust, cobwebs and midges were present in Dairy Science. The storage area with the -80 freezer was especially dirty. Wall/bench seals need replacing and a hole on the sink bench (12A) must be sealed. A bench in 26 had a chip and sections needed to be joined together with new impervious sealing.
Silicon sealant was not present around number of door frames which allows spills and cleaning fluids to seep into the joinery. While a number of doors have been done, the initiative has not been taken to roll this out across the site.

A bench in SW114 was sagging.

Collectively these issues form the PC1 NC. Completion of repairs should be confirmed with MPI by 10 September 2021.

Hazardous Substances and New Organisms (HSNO) Act

ERMA200223, GMD02013, GMD02023, APP203820, APP203942 and GMC03001 were among the approvals in use. It was confirmed GMC100216 was not yet 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

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)