EVALUATION OF RESIDUAL QUANTITY OF ADENO-CRE IN THE MOUSE CAGES OF INDIVIDUALLY VENTILATED RACK AFTER INOCULATION OF MICE WITH ADENO-CRE VIRUS.

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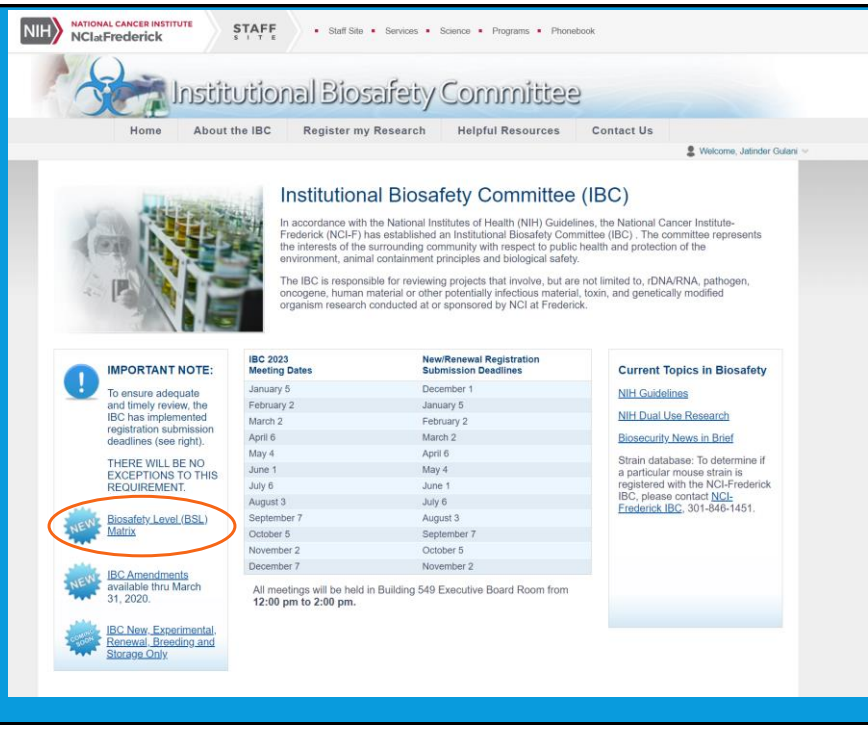

Biosafety Level Matrix

Biological Materials for Direct Inoculation into Animals	Containment Level Requirements		Necropsy
	Practices/Procedures/Manipulations	Animal Housing	
Adenovirus/Adeno-Cre; Adenoviral Vectors	BSL 2	ABSL 2 for 2 weeks, then ABSL 1 with required cage change prior to transition	BSL 2
Known Human Pathogens/Microorganisms/Bacteria (Refer to IBC since specific strains of bacteria and other infectious materials in Risk Group 1 may not require this containment level).	BSL 2	ABSL 2 for duration of the study	Follow LASP SOP 3.049F for ABSL 2 practices
Known Mouse Pathogens (with potential zoonotic effects)	BSL 2	ABSL 2 for duration of the study	BSL 2
Vaccinia	BSL 2	ABSL 2 for duration of the study	BSL 2

Biological Materials of Murine Origin	Containment Level Requirements		Necropsy
	Practices/Procedures/Manipulations	Animal Housing	
Unmodified Murine Cells	BSL1	ABSL1	BSL 1
Murine Cells Transfected with Non-Viral Elements	BSL 1	ABSL1	BSL 1
Modified Murine Cells – Transduced with MLV-Based Vector with an Insert that was Designated as Benign (i.e. GFP) by the IBC*	BSL 1	ABSL 1	BSL 1
Modified Murine Cells – Transduced with MLV-Based Vector with an Insert that was Designated as Non-Benign (e.g. Oncogene) by the IBC*	BSL2	ABSL 2 for the duration of the study	BSL 2
Modified Murine Cells – Transduced with Lentiviral Vector **	BSL 2	ABSL 2 for 10 days, then ABSL 1**	BSL 2
Modified Murine Cells-Transduced with Adenoviral Vector ***	BSL 2	ABSL 2 for 2 weeks, then ABSL 1***	BSL 2

Biological Materials of Human Origin	Containment Level Requirements		Necropsy
	Practices/Procedures/Manipulations	Animal Housing	
Known Human Pathogens/Microorganisms/Bacteria (Refer to IBC since specific strains of bacteria and other infectious materials in Risk Group 1 may not require this containment level).	Biosafety Level to be determined by IBC review	N/A	N/A
Unmodified Human Cell Lines	BSL 2	ABSL 1	BSL 2
Modified Human Cells-Transfected with Non-Viral Elements	BSL 2	ABSL 1	BSL 2
Modified Human Cells-Transduced with MLV-Based Vectors with An Insert that was Designated as Benign (i.e. GFP) by the IBC*	BSL 2	ABSL 1	BSL 2
Modified Human Cells-Transduced with MLV-Based Vectors with An Insert that was Designated as Non-Benign (i.e. Oncogene) by the IBC*	BSL 2	ABSL 2 for the duration of the study	BSL 2
Modified Human Cells – Transduced with Lentiviral Vector **	BSL 2	ABSL 2 for 10 days, then ABSL 1**	BSL 2
Modified Human Cells-Transduced with Adenoviral Vector ***	BSL 2	ABSL 2 for 2 weeks, then ABSL 1***	BSL 2

CHALLENGES WITH ABSL-2 HOUSING

- Providing routine husbandry care is labor intensive.
- Operations are costly.
- Limited availability of animal housing space, which meets ABSL-2 standards.
- Difficulty in procuring PPE during COVID times.

FIRST EXPERIMENT

8–10-week-old female C57BL/6J mice

- **Intratracheal inoculation** with 2.5 x 10^7 PFU **Adeno-Cre Virus**
- **Intratracheal inoculation** with **Saline**

- Sample type: **Dry Swab** of the mouse body, mouse cage, biosafety cabinet and floor of the animal holding room
- Sampling time points: **day 10, 14, 21 and 28 post-inoculation.**

- PCR detection of Cre gene (215 bp) which is part of the Adeno-Cre was utilized to assess the presence of Adeno-Cre virus
- PCR detection of mouse genomic DNA (IL2 exon3 – 300 bp) was used as the positive control.

Adeno-Cre: *Replicative defective human adenovirus serotype 5 with Cre-recombinase*

MULTIPLEX PCR

The LabChip® GX Touch™ nucleic acid analyzer:

- Microfluidic technology generates high-resolution data
- Optimal for DNA and RNA quantitation and sizing which can be done in seconds using automated capillary electrophoresis separation.

ABSL-2 HOUSING

Lab Product Individually Ventilated Rack

Biosafety Cabinet

Personal Protective Equipment (PPE)

	CRE (ng/µl)				Mouse Genomic (ng/µl)			
Samples	**D10***	**D14***	**D21***	**D28***	**D10**	**D14**	**D21**	**D28**
Adeno-Cre Cage #1 Mouse Body Swab	-**	-	-	-	-	-	5.5	-
Adeno-Cre Cage #2 Mouse Body Swab	-	-	-	-	2.9	-	-	-
Adeno-Cre Cage #3 Mouse Body Swab	-	-	-	-	-	-	5.6	-
Adeno-Cre Cage #4 Mouse Body Swab	-	-	-	-	-	-	-	1.3
Adeno-Cre Cage #5 Mouse Body Swab	-	-	-	-	-	6.7	-	-
Adeno-Cre Cage #1 Cage Swab	-	-	-	-	-	-	-	-
Adeno-Cre Cage #2 Cage Swab	-	-	-	-	-	-	-	-
Adeno-Cre Cage #3 Cage Swab	-	-	-	-	1.3	-	-	0.8
Adeno-Cre Cage #4 Cage Swab	-	-	-	-	1.5	2	1.4	-
Adeno-Cre Cage #5 Cage Swab	-	-	-	-	3.4	-	-	-
Saline Cage #1 Mouse Body Swab	-	-	-	-	-	1.1	-	-
Saline Cage #2 Mouse Body Swab	-	-	-	-	-	-	-	-
Saline Cage #3 Mouse Body Swab	-	-	-	-	4.2	1.4	11.9	-
Saline Cage #4 Mouse Body Swab	-	-	-	-	-	-	-	1.4
Saline Cage #5 Mouse Body Swab	-	-	-	-	-	-	0.4	-
Saline Cage #1 Cage Swab	-	-	-	-	-	-	-	-
Saline Cage #2 Cage Swab	-	-	-	-	-	-	-	1.4
Saline Cage #3 Cage Swab	-	-	-	-	-	-	3.9	-
Saline Cage #4 Cage Swab	-	-	-	-	3.5	-	6.2	-
Saline Cage #5 Cage Swab	-	-	-	-	2.5	-	2.1	-
Biosafety Cabinet	-				-			
Animal Holding Room Floor	-				-			
Cre (+)	20.6			9.3				10.7

The PCR assay sensitivity was minimum of 50 copies for the Cre gene.

* Time post Adeno-cre inoculation.

** No amplification observed.

SUMMARY OF FIRST EXPERIMENT

- **Adenoviral DNA was not detectable on the animal body and in cage environment after 10 days of intratracheal Adeno-Cre viral inoculation in mice.**

SECOND EXPERIMENT

8–10-week-old female C57BL/6J mice

- **Intratracheal inoculation** with 2.5 x 10^7 PFU **Adeno-Cre Virus**
- **Intranasal inoculation** with 2.5 x 10^7 PFU **Adeno-Cre Virus**
- **Control – No Inoculation**

- Sample type: **Wet Swab** of the mouse body and mouse cage
- Sampling time points: **4 hours, 24 hours, 3 days, 7 days, 10 days and 14 days** post-inoculation.

- PCR detection of Cre gene (215 bp) which is part of the Adeno-Cre was utilized to assess the presence of Adeno-Cre virus
- PCR detection of mouse genomic DNA (IL2 exon3 – 300 bp) was used as the positive control.

Adeno-Cre: Replicative defective human adenovirus serotype 5 with Cre-recombinase

	CRE (ng/µl)						Mouse Genomic (ng/µl)					
Samples	4 hr*	24 hr	3 day	7 day	10 day	14 day	4 hr	24 hr	3 day	7 day	10 day	14 day
Intratracheal mouse # 1 Body Swab	-**	-	-	-	-	-	2.9	-	-	1.8	6.9	6.4
Intratracheal mouse # 2 Body Swab	-	-	-	-	-	-	1.2	-	-	-	6.3	7.2
Intratracheal mouse # 3 Body Swab	-	-	-	-	-	-	3	-	-	14	-	1.9
Intratracheal mouse # 4 Body Swab	-	-	-	-	-	-	-	0.6	-	31.3	3.3	3.1
Intratracheal Cage Sample	-	-	-	-	-	-	-		-	3.4	3.3	4.3
Intranasal mouse # 1 Body Swab	-	-	-	-	-	-	2.3	1.4	-	6.4	3.7	1.7
Intranasal mouse # 2 Body Swab	9.1	-	-	-	-	-	2.2	2.7	-	8.5	6.3	4.4
Intranasal mouse # 3 Body Swab	-	6.9	-	-	-	-	3.3	-	-	1.6	4.4	-
Intranasal mouse # 4 Body Swab	-	-	-	-	-	-	-	0.8	3	12.8	3.2	20.9
Intranasal Cage Sample	7.5	-	-	-	4.5	-	-	-	-	-	6.8	1.8
Control mouse # 1 Body Swab	-	-	-	-	-	-	-	-	3.4	5.8	15.7	-
Control mouse # 2 Body Swab	-	-	-	-	-	-	3.3	1.1	-	8.1	-	-
Control mouse # 3 Body Swab	-	-	-	-	-	-	1.3	-	-	3.8	5.9	-
Control mouse # 4 Body Swab	-	-	-	-	-	-	8.6	-	8.5	-	2.8	22.3
Control Cage Sample	-	-	-	-	-	-	-	-	-	0.7	3.3	-
Cre (+)	20.6					9.3						10.7

The PCR assay sensitivity was minimum of 50 copies for the Cre gene.
* Time post adeno-cre inoculation

** No amplification observed.

SUMMARY OF SECOND EXPERIMENT

- Adenoviral DNA was not detectable on the animal body and in cage environment:
  - After 4 hours of intratracheal Adeno-Cre viral inoculation in mice, even with the wet swab.
  - After 10 days of intranasal Adeno-Cre viral inoculation in mice.

CONCLUSIONS

- This data demonstrates that adenoviral DNA dissipates quickly after Adeno-Cre inoculation in mice, which helped institutional biosafety committee at NCI Frederick in determining the length of ABSL-2 housing of mice (biocontainment) post Adeno-Cre virus inoculation.
- Two weeks (14 days) of ABSL-2 housing provides adequate safety controls for studies involving the use of Adenoviral vectors in mice.

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