

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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Institutional Biosafety Committee

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Welcome, Jatinder Gulani

Institutional Biosafety Committee (IBC)

In accordance with the National Institutes of Health (NIH) Guidelines, the National Cancer Institute-Frederick (NCI-F) has established an Institutional Biosafety Committee (IBC). The committee represents the interests of the surrounding community with respect to public health and protection of the environment, animal containment principles and biological safety.

The IBC is responsible for reviewing projects that involve, but are not limited to, rDNA/RNA, pathogen, oncogene, human material or other potentially infectious material, toxin, and genetically modified organism research conducted at or sponsored by NCI at Frederick.

IMPORTANT NOTE:
To ensure adequate and timely review, the IBC has implemented registration submission deadlines (see right).

THERE WILL BE NO EXCEPTIONS TO THIS REQUIREMENT.

NEW [Biosafety Level \(BSL\) Matrix](#)

NEW [IBC Amendments](#) available thru March 31, 2020.

NEW [IBC New Experimental, Renewal, Breeding and Storage Only](#)

IBC 2023 Meeting Dates	New/Renewal Registration Submission Deadlines
January 5	December 1
February 2	January 5
March 2	February 2
April 6	March 2
May 4	April 6
June 1	May 4
July 6	June 1
August 3	July 6
September 7	August 3
October 5	September 7
November 2	October 5
December 7	November 2

All meetings will be held in Building 549 Executive Board Room from 12:00 pm to 2:00 pm.

Current Topics in Biosafety

[NIH Guidelines](#)

[NIH Dual Use Research](#)

[Biosecurity News in Brief](#)

Strain database: To determine if a particular mouse strain is registered with the NCI-Frederick IBC, please contact [NCL-Frederick IBC](#), 301-846-1451.

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 IACSP 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 2.5×10^7 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)

Samples	CRE (ng/ μ l)				Mouse Genomic (ng/ μ l)			
	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 2.5×10^7 PFU Adeno-Cre Virus
 - Intranasal inoculation with 2.5×10^7 PFU Adeno-Cre Virus
 - Control – No Inoculation
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- 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.
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- 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

Samples	CRE (ng/ μ l)						Mouse Genomic (ng/ μ l)					
	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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