

# Speed Poster Presentation (15.45-16.15)



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6. Karen Ekkelund Petersen (Scanbur)
7. Anders Fick Thomsen (Aarhus University)
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Critical Incident Reporting System in Laboratory Animal Science (CIRS-LAS)



**UNIVERSITÄTS  
KLINIKUM  
Jena**

**It's time to rethink! - CIRS-LAS**  
**Critical Incident Reporting System in Laboratory Animal Science**  
**Learn from negative results in animal based research**

Sponsored by the  
Federal Ministry  
of Education  
and Research

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Why do we need CIRS-LAS?

- Every year a high number of publications based on animal experiments are published
- Negative results are often not published and get lost
- We need all results – positive and negative – to learn from it!

→ CIRS-LAS provides a database to collect critical incidents and negative results

→ Used by researchers, students, technicians, animal keepers to report and investigate

What to achieve with CIRS-LAS?

Anonymous reports of

- Critical incidents
- Complications
- Negative experiences



Exchanging experience

- Discussing via blog function
- Evaluation by expert panel
- Transparent failure management

Support the 3R principles

- Refinement, Reduction

**We need your support for animal welfare!**

Our aims

- ✓ Reduction of animals used in experimental projects
- ✓ Refinement of animal welfare
- ✓ No repetition of failed experiments
- ✓ Increased transparency
- ✓ More than 150 registered members

www.CIRS-LAS.de





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Conclusion:

- Open-minded and constructive failure management
- Exchange of experiences to improve animal welfare
- Provides warranty for anonymity and impunity
- Enables more reliability in animal experiments
- Supports 2 of the 3R-principles – Reduce and Refine
- **Learn from critical incidents to avoid them and to prevent repeating!**
- **Start with your support in animal welfare!**



Inhaled pharmaceuticals: Correlation between in vitro and in vivo lung effects

**Bile salt enhancers for inhalation: correlation between *in vitro* and *in vivo* lung effects**

AstraZeneca

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**Background**

Systemic drug delivery of macromolecules via the lungs requires crossing of the air-blood barrier, however large molecules do not cross easily. Absorption enhancers, such as bile salts, can improve systemic availability, but may be harmful to the lungs due to their mechanisms of action, e.g. by increasing the permeability of the air blood barrier.

We tested the potential effects of bile salts on the lung function in two *in vitro* methods and compared the results to *in vivo* data.

**Conclusion**

Lung surfactant function inhibition *in vitro* ranks substances in the same order as induction of rapid shallow breathing *in vivo*.

Measurements of *in vitro* inhibition of LS function may predict potential for adverse respiratory functional response to enhancers. This can assist in early decision-making by use of an *in vitro* model, thereby reducing the need for animal testing.

***In vitro* LS function**

Lung surfactant (LS) function was assessed in an "artificial alveolus", the Constrained Drop Surfactometer (CDS).

A cycling drop of LS was continuously exposed to the bile salts, mimicking the physiological conditions in the lungs. The LS function was continuously monitored at increasing exposure concentrations.

**3D human airway *in vitro* model**

TEER and cell viability were measured by 2 consecutive exposures to up to 100 µM bile salts in the MucilAir<sup>TM</sup> cell model.

***In vivo* acute toxicity**

The breathing patterns of mice exposed to bile salts show acute effects indicative of respiratory irritation expressed as induction of rapid shallow breathing (RSB).

**Results**


There were no effects on barrier integrity (TEER change in the MucilAir<sup>TM</sup> model) for any of the bile salts tested, which may support that the respiratory effect observed is a result of interaction with surfactant, rather than a direct epithelial toxicity.

Substance and structure	NaTCA	NaGCA	NaTDCA	NaDC	Lactose
<i>In vitro</i> inhibition	220±45 µg/mg LS	360±55 µg/mg LS	360±55 µg/mg LS	1100±200 µg/mg LS	No inhibition
Breathing patterns	Normal		Rapid shallow breathing		
LOAED, <i>in vivo</i> induction of RSB defined as a 10% change from baseline, Effect on tidal volume at each dose	0.88 µg/kg BW	3.65 µg/kg BW	6.07 µg/kg BW	9.34 µg/kg BW	Not tested

## Better welfare through positive reinforcement training and VAB catheter

### Better welfare through positive reinforcement training and VAB catheter

Animal facility and Vet team



**Background**

**Procedure**

**Benefits**

**Background**

We have been working with pigs, in the animal facility at Lundbeck, for many years. But it has always involved a lot of screaming, involuntarily cooperation with the pigs and heavy lifts for the animal technicians. The blood samples was collected from the jugular vein, while the pig was fixed on its back. This is the most common way, to take a blood sample from a minipig.


We were sure, that the procedures, some how, could be optimized so that the pigs would have a better and less stressful time at Lundbeck.

Tick if	Method of
<input checked="" type="checkbox"/>	<b>Reduction</b> (Reduction of the numbers of animals used)
<input type="checkbox"/>	<b>Replacement</b> (Replacing the use of animals with In vitro/In silico methods)
<input checked="" type="checkbox"/>	<b>Refinement</b> (Refinement of procedures leading to less severe procedure (improved instrumental setup, improved housing and /or improved humane endpoints)

**Initiative**

We are now having the pigs surgically prepared by the provider, so we receive them with a permanent catheter. The catheter gives us access to the vein, without going tough the skin.


We have implemented that the pigs are being trained, to the different procedures they are going through, at their time at Lundbeck.



Utensils for positive reinforcement training:  
Treats and treat bag, clicker, bowl for training, and target stick

**Procedure**


First step to better welfare in the pig facility, was to implement the Vascular Access Button (VAB) catheters. The catheter is placed behind the pigs ear. It leads to the vein, by two tubes. One tube for dosing, and one for blood collection. The catheter is protected by a cap, outside the pig. (See picture)



The catheter lets us take blood samples, without penetrating the skin. That makes training much easier, because we don't brake the trust with the pig, by hurting it.


The training starts, the day the pigs arrive. Getting to know us, habituation to the treats and getting used to the new facility. Next step is to teach the pig, that every time it hears the click, it will get a treat. When this basic classical conditioning is established the pig can learn almost anything.

The studies often includes IV dosing in the morning, and several blood samples through out the day. Based on this, we have made a training plan for the pigs.



The pigs is first taught, to follow a target stick. This is very useful. It lets us move the pig from one place to an other, in a fun and voluntarily way. We use the target stick to move the pigs to the scale, climb a ramp up on a table and move the pigs from stable to stable.

When the pig is comfortable on the elevated table, we teach it to hold its head still. We use a feeding bowl with a knob in the middle, where the pig place its snout and stands still. Slowly we begin to touch the pig. With small steps, the pig begins to accepts being touched. Now we can exam the pigs body and work with the catheter, while the pig is calm and voluntarily interact.



Pictures 1-4:  
1. The pig follows a target stick in the hallway, down the ramp, staying calm on the elevated table and standing still by the bowl while drawing blood or dosing.

**Benefits**

**On time**  
Both with the VAB catheter and voluntarily participation, it allows us to take the blood samples exactly on the requested minutes.

**Less stress**  
Because there is no assaults when the blood samples are being taking, we can visible see that the plasma samples are more clear. As the animals participates voluntarily, the animals and the animal care takers are less stressed.

**Less noise**  
We never need to protect our ears, when we take blood samples anymore, because the pigs voluntarily participate and therefor don't scream.

**More playful pigs**  
We can see a significant different in the pigs behavior after we began to train them. They are more curious on us, they offer a lot of behavior, they enjoy running around and play in the stable and we see less undesirable behavior due to too little stimulation. E.g. aggression to each other and tail biting.

**Time used**  
The pigs always arrive 10-14 days prior to study start for acclimatization. This period is enough to have them trained and ready to voluntarily participate in the PK studies.

**Conclusion**

The new initiatives, has made an overall better atmosphere, in the pig facility.

The pigs are enjoying their time at Lundbeck, their voluntarily participate in the studies, and they are less afraid of us.

The animal technicians has a better work environment, both physical and mentally. There is less heavy lifting, much lower risk of accidents, and now it is a lot more fun to work with the pigs.

Tick if	Proedure is implemented
<input checked="" type="checkbox"/>	Proedure is implemented

Presented at Knowledge Sharing Day - 2019

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Ultra Micro blood reduces the severity of the procedure and the number of animals used

Poster #222

## Ultra Micro blood reduces the severity of the procedure and the number of animals used

Frederik Rode, Elin Eneberg, Silas Anselm Rasmussen, Dorthe Belling, Tina Brønnum Pedersen and Animal Facilities  
*Translational DMPK, NCSR & Animal Facilities*

### Background

**New method:** To reduce the number of animals, improve animal welfare, and increase the quality of the scientific output, we have implemented an "Ultra micro-sampling" procedures in our PK studies with mice.

**Traditionally,** in a mouse pharmacokinetic (PK) study, blood is sampled from the tongue or, the cheek (~ 60-120 µL). In contrast, a micro-sample is <50 µL (UK 3R Centre), which is 5% of the total blood volume in a mouse (~1 ml). In this initiative, we aim to reduce the blood volume taken to <20 µL (Ultra Micro-sampling). Due to the blood loss induced by cheek sampling, sparse sampling is necessary to obtain a 'full PK time profile, i.e. several mice are needed to complete one PK profile. For the bioanalysis of PK samples, less than 5 µL plasma is required by high sensitive analyses (e.g., UPLC-MS/MS). Therefore the 60-120 µL blood is excessive.

Tick if	Method of
<input checked="" type="checkbox"/>	<b>Reduction</b> (Reduction of the numbers of animals used)
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### Initiative

### Procedures

**Traditional sampling from the chin:** A vein is punctured in the chin of the animal. At the same time, the animal is held firmly by the scruff of the neck. This fixation works like a stasis, allowing the blood to flow freely down the chin down into the collecting tube. 60 µL blood is needed, although our analysis (Data) shows that approximately 100 µL is removed from the animals, per sample. This relatively large volume for a mouse limits the number of blood samples to two per animal pre-ethanasia.

Traditional Cheek blood sampling 60-120 µl

→

New Method Micro-sampling 10-20µl

Micro-sampling of tail blood makes it possible to get a full PK profile per mouse (5-6 samples weekly). The method involves no anaesthesia and only mild restraint of the mouse and a small point puncture of a lateral tail vein (micro-sampling from the tail). A blood droplet is sucked into a capillary tube (Minivette POCT) and the blood is subsequently pipetted into a small centrifuge tube allowing for plasma separation.

100µl blood vs. 10µl blood droplets for reference.

Cheek blood sampling 3 animals, 2 x 100 µl blood per animal

Sparse sampling (left) vs. full profile sampling (right). Six blood samples are needed for a full-time concentration profile (middle). From either three cheek-sampled ● animals or one micro-sampled \* animal.

### Data

Uncorrected values

Values corrected for blood loss

Blood was sampled from mice receiving an IV dose of a human antibody. One group of mice (Red) was sampled using cheek blood (~100 µl) and the second group (Blue) was sampled using micro-sampling (20 µl). Initial results (left graph) displayed a 66% higher peripheral volume of distribution for the compound in the animals sampled via cheek blood compared to animals samples sampled via the tail. However, when adjusting the results of the cheek blood sampling for an expected blood loss of 100 µL per blood sample, the two profiles were not different (right graph). Each time point contains 3 plasma samples, and this can be obtained with 3 mice using micro-sampling or 9 mice using the traditional sampling.

### Conclusion

Tick if	Procedure is implemented
<input checked="" type="checkbox"/>	Procedure is implemented

When fully implemented internally and at CROs, this method will reduce the number of mice used for PK to between 33% and 50% of the current use in PK studies. Blood sampling is less invasive and stressful for all animals undergoing blood sampling. Additionally, the results from micro-sampled animals are more accurate and give better estimations of PK, TK, and PD. The disadvantage of full profile micro-sampling can be that sampling times are less flexible compared to Sparse sampling designs. A combination of using Sparse sampling times with micro samples is to be used for some experiments.

This method is implemented for PK and PD exposure in mice in Antibody projects. In the future, this initiative can be used for both mice and rats (all strains) replacing more invasive blood sampling in all rodent studies, including external experiments.

### Benefits of micro-sampling


The main aim of this Initiative is to reduce and refine any mouse and rat experiment that uses blood sampling

Furthermore, fewer animals reduce the cost of animal housing

Our results demonstrate improved accuracy of measurements on blood from small volumes compared to large blood volumes due to unwanted removal of compound with repeated blood sampling.

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Investigating the proliferative and serum-reducing effect (s) of various protein hydrolysates on "Vero cells" as an ATMP bioproduction cells




La science pour la santé  
From science to health

### Investigating the proliferative and serum-reducing effect (s) of various protein hydrolysates on "Vero cells" as an ATMP bioproduction cells

Chloé LEZIN<sup>1,2</sup>; Georges UZAN<sup>2</sup>; Mohamed Essameldin ABDELGAWAD<sup>1,3</sup>

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#### Abstract

Fetal bovine serum is widely used in cell culture as an additive to media. Serum is a provider of different elements such as nutrients, growth factors, hormones, binding/transport proteins, or even attachment and spreading factors<sup>1</sup>. In the present work, we investigated whether peptones can be used as an alternative to fetal bovine serum in an expansion media for VERO cells.

Data indicates that the raw material and the process used to manufacture peptones have an impact on the proliferation of VERO. Moreover, whether cell culture medium will be combined with casein, wheat or soy-based peptones, we can reduce the serum by 50% and still obtain the same performance regarding the proliferation using 5% of serum plus 0.5 g/L of peptones.


The best peptones were combined and tested with different media: DMEM 10%FBS (control media) versus a novel optimized Vero media: BMBD145 Av

Our results illustrate how satisfactory is the batch to batch consistency of casein-based peptones. Furthermore, one batch of wheat-based peptone, was given different post process treatment. It appeared that gamma irradiation and ultra filtration have a positive impact on proliferation. For casein-based peptones, it turned out to be the opposite.

Further, peptones give a better proliferation results when used with an optimized cell culture media rather than with a conventional/general one.

#### Methods and Materials

- **MATERIAL**
  - Cell line: VERO CCL-81 (ATCC<sup>®</sup>, batch 70005907)
  - Media: DMEM, high glucose, L-glutamine (Gibco, batch 20078) / BMBD145 (in house media)
  - FBS (Eurobio, ref: CVFSVF00-01, batch 566505-5256)
  - BrdU kit (Ozyme, 5813S)
  - Peptones, all supplied by OrganoTechnie
    - TN1, batches: 174603X / 182108
    - T4, batches: 173908 / 173908G
    - W106, batch: 180903UG
    - W206, batches: 180903 / 181105X / B51801M
    - S203, batches: 182801 / 182203X
    - S204, batches: 175103X / 175103X
- **METHODS**
  - **Media preparation**  
All peptones were fully solubilized in DMEM. A 20ml syringe and a 0.22 um filter were used for a sterilizing filtration. Aliquots (20ml) were kept at +4°C before utilization.  
Control media: 10%, 5%, 2%, 1% FBS  
Test media: 5%, 2%, 1% FBS + 0.5g/L peptones or peptones mixtures
  - **Cell culture**  
Cells (division 5) were cultivated in 96 well-plates from FALCON. The seeding was 2500 cells/cm<sup>2</sup>. It was a 4 days culture with a re-nourishment at day 2. Each assay was done in triplicate.
  - **Proliferation assay**  
It was done with a BrdU kit. BrdU is a pyrimidine analogue that incorporate into newly synthesized DNA of proliferating cells in place of thymidine. It is a colorimetric detection whereas the intensity at OD<sub>490</sub> can be used directly as an indicator of cells proliferation.
  - **Statistic analysis**  
It was performed using the method of one-way analysis of variance (ANOVA)



#### Results

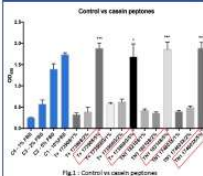


Fig.1: Control vs casein peptones

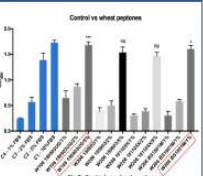


Fig.2: Control vs wheat peptones

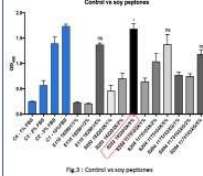


Fig.3: Control vs soy peptones

#### Discussion

All peptones were compared to the 5% FBS control (C2), we can observe that:

- T+ that did not go under gamma irradiation treatment (#173908) performs better than the one that did (#173908G)
- Good batch to batch consistency for TN1
- Best wheat peptone (W106, #180903U) went under ultra filtration and gamma irradiation.
- BrdU is not adapted when the proliferation is so strong that cell multilayers are formed.
- In conclusion, we could decrease the amount of FBS used by 50% and have the same proliferation (and even better) when adding 0.5g/L of peptones.
- Peptones are more efficient when used with an optimized media rather than a conventional/commercial one.

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#### References

1. Biossion G, Fardet L, et al (2010) Serum-free Cell Culture: The Serum-Free Media Interactive Online Database. *Aliment*, 27, 53-62
2. Bredius JG, van der Wal ACW, van der Wal ACW, et al (2011) Tissue-specific modulation of stem cells for cell therapy manufacturing. *Regenerative Medicine* 7:7-11. doi:10.1016/j.rme.2011.01.001
3. Biossion G, van der Wal ACW, van der Wal ACW, et al (2011) The use of fetal bovine serum: what is the scientific problem? *Food and Agriculture, Org*, 20, 219-27
4. Hiras G, Singha LA, Chaney M (1975) Tissue bovine serum: a multistep standard. *Proceeding of the Society for Experimental Biology and Medicine* 149, 344-347
5. Knepper RL, Maxwell CG, Sussman W, et al (1988) The presence of transcription factors in fetal bovine sera. *In Vitro Cell Dev Biol-Animal* 24(170-175). doi:10.1007/BF02880010
6. Inoué S, Gohda H, Wajima T (2014) Evaluation of human placental serum fetal bovine serum for culture of transgenic animal cells. *Cell Culture* 10:170-180. doi:10.1007/s11434-014-0514-0
7. Ghanem C, Abdou M, Ibrahim A, El-Sayed M, et al (2008) The presence of transcription factors in fetal bovine sera. *In Vitro Cell Dev Biol-Animal* 24(170-175). doi:10.1007/BF02880010
8. Ghanem C, Abdou M, Ibrahim A, El-Sayed M, et al (2008) The presence of transcription factors in fetal bovine sera. *In Vitro Cell Dev Biol-Animal* 24(170-175). doi:10.1007/BF02880010
9. Inoué S, Gohda H, Wajima T (2014) Evaluation of human placental serum fetal bovine serum for culture of transgenic animal cells. *Cell Culture* 10:170-180. doi:10.1007/s11434-014-0514-0
10. Inoué S, Gohda H, Wajima T (2014) Evaluation of human placental serum fetal bovine serum for culture of transgenic animal cells. *Cell Culture* 10:170-180. doi:10.1007/s11434-014-0514-0

Tighter control of relative humidity improves murine breeding performance – a retrospective analysis

## Tighter control of relative humidity improves murine breeding performance – a retrospective analysis

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### Aim of the study

In the current study we aimed to investigate the effect of relative humidity (RH) on breeding performance of mice. The breeding of mice housed with RH controlled steadily at cage level was compared to breeding of mice housed in cages with RH controlled less steadily at room level. The comparison was done on data for eight months (January to August) and pups per litter, preweaning mortality and total litter loss was compared between the groups. Data were collected on mice of various backgrounds. The rationale for the study was to evaluate potential effects of RH on breeding performance. An improvement of breeding performance could potentially reduce the number of breeding mice being used for research.

### Study design

For group 1 an air handling unit capable of controlling RH (ScanClim®) was used to control RH at minimum 55%. In group 2 a setup with an air handling unit not controlling RH was used, and the animals in this system were subject to RH controlled at room level. The statistical analyses used were t-test comparing the groups after checking the data for normal distribution and binomial testing was used when appropriate (GraphPad Prism 7). The data collection was performed as part of routine data collection on breeding from the facility. Welfare assessments were performed daily and the housing of animals complied to regulations set by the Home Office UK.

### Results

RH was kept at 55% and above in group 1, where RH was controlled at cage level, whereas group 2 was subjected to RH varying from 34 to 76%. Over 2000 litters were born in the period of data collection.

- The number of pups per litter was significantly higher for group 1 ( $p < 0.001$ ). The same was found when only investigating mice C57BL/6 strains ( $p < 0.001$ ) (figure 1-2).
- Total litter loss registered at weaning was significantly higher in group 2 ( $p < 0.01$ ) (figure 3).
- No significant difference was found in preweaning mortality (graph not shown).
- To investigate how the variations in RH affected breeding performance standard deviations for the RH in gestational period and preweaning period postpartum were compared to the pups per litter and total litter loss. No connections between the variation in RH and pups/litter could be found using this method. However, low variation in RH the first week after birth was associated with the lowest total litter loss observed (figure 4).

#### Standard deviation of relative humidity first week after birth

Figure 4 Standard deviation of relative humidity in the first week postpartum compared to the total litter loss preweaning. The expected value is the average of all values of total litter loss. \*\*\*  $P < 0.001$

#### Pups per litter

Figure 1 Pups per litter in group 1 and 2. Graph is shown with mean & standard error of the mean. \*\*\*  $P < 0.001$

#### Pups per litter (all C57BL/6 strains)

Figure 2 Pups per litter of all C57BL/6 strains in group 1 and 2. Graph is shown with mean & standard error of the mean. \*\*\*  $P < 0.001$

#### Total litter loss

Figure 3 Total litter loss in group 1 and 2. Graph is shown with mean & standard error of the mean. \*\*  $P < 0.01$

### Limitations

The unit used for adjustment of humidity can only humidify, thus the RH is set to minimum 55% but could go above during periods with high humidity. The animals were housed in different rooms and the breeding data is collected on various strains. The data should be seen as a initial result and we aim to investigate this in further detail. Together with other data we strongly believe that RH affects breeding of mice.

### Conclusions

Our results strongly suggest that RH can affect murine breeding performance significantly. When controlling RH to not drop below 55% the mice had more pups per litter and fewer total litters were lost preweaning, when compared to the animals housed under less steadily room controlled RH. Further investigation is warranted on how different levels of stable RH versus variation in humidity affect the breeding performance.

Innovative Life Sciences



## Pig training – a refinement, research and welfare initiative

The screenshot shows a video player interface with a dark blue background. At the top left is the Aarhus University logo and text: 'AARHUS UNIVERSITET INSTITUT FOR KLINISK MEDICIN'. At the top center is the title 'Pig training – a refinement, research and welfare initiative' and authors 'Thomsen AF, Jakobsen K, Kousholt BS'. At the top right is the Danish 3R-Center logo. The main video area shows a woman smiling next to a pig. Below the video is a timeline labeled 'DAYS: 0 7 14' with a play button icon. A tooltip says 'Click on the icon to see a specific video'. Below the timeline is a bar labeled 'Socialization'. To the right is a list of activities with pig icons: 'Target stick' (2 icons), 'Novel objects' (2 icons), 'Habituation' (3 icons), 'Hoof trimming and Weighing' (4 icons), and 'Sampling and control' (5 icons). At the bottom are buttons for 'HOME', 'ABSTRACT', and 'ACKNOWLEDGEMENT'.

AARHUS UNIVERSITET  
INSTITUT FOR KLINISK MEDICIN

### Pig training – a refinement, research and welfare initiative

Thomsen AF, Jakobsen K, Kousholt BS

DAYS: 0 7 14

Click on the icon to see a specific video

Socialization

- Target stick
- Novel objects
- Habituation
- Hoof trimming and Weighing
- Sampling and control

HOME ABSTRACT ACKNOWLEDGEMENT



# Mouse Kidney Parvovirus: A Newly Characterized Parvoviral Pathogen of Research Mice

**IDEXX BioAnalytics**

## Mouse Kidney Parvovirus: A Newly Characterized Parvoviral Pathogen of Research Mice

Marcia Hart, Cynthia Besch-Wilford, Marcus Grim, Robert Livingston, IDEXX BioAnalytics, Columbia, MO

### Introduction

- Anecdotal reports of inclusion body nephropathy (IBN) due to an unknown etiology for decades.
- Histopathologic lesions (epithelial intranuclear inclusions in renal tubules) observed in immunodeficient and immunocompetent mice.
- Recently, two independent research groups have attributed observed lesions to a new parvovirus of the Chapparrinovirus genus.
- Mouse Kidney Parvovirus (MKPV) or Murine Chapparrinovirus (MuCPV) are taxonomically the same species.
- Virus identified in both wild and laboratory mice.
- Virus is genetically divergent from other known mouse parvoviruses such as MVM and MPV.

**Example of observed histologic lesions**

MKPV: virus inclusions in the nucleus  
normal uninfected nucleus

### Developing a PCR Diagnostic Test

**Assay Design**

- The IDEXX BioAnalytics fluorescent MKPV real-time PCR assay was designed to a target that is 100% conserved among the Murine chapparrinovirus sequences obtained from both wild and research mice in the NCBI database.

**MKPV PCR Assay Validation Process**

Analytical validation with serial dilutions of known positive controls included:

- Assay amplification efficiency of 95-105% (Figure A)
- Linearity over 5 points, calculated coefficient of variation (CV) of crossing points (Cp) < 3% with CV calculated with absolute values < 20%, (2 value = 0.999) (Figure B)
- Amplification of <math>10^7</math> template molecules per PCR reaction
- Clinical validation involved testing serial dilutions of clinical samples, and target confirmation by sequence analysis.
- Clinical correlation study resulted in 100% positive real-time PCR results obtained for all histopathology samples tested.

A. Amplification Curves

B. Linearity Plot

**Sequence Analysis**

MKPV PCR assay amplified virus in the feces and tissues from wild mice, pet store mice, immunocompetent and immunocompromised research mice. Sequence analysis was performed on virus from wild mice and research mice from 10 institutions in Canada, Europe, the Middle East, and the United States, and revealed multiple MKPV virus strains.

The MKPV PCR assay detects genetically divergent strains of MKPV and serves as a useful tool in screening samples for MKPV.

### Number of positive fecal samples as detected by real-time PCR

Strain Type	Positive Samples
Pet Store	17/19
Competent Strain/Strains	13/12
Immunocompromised	21/18
Strain Unspecified	10/10

### Number of positive non-fecal samples as detected by real-time PCR

Material Type	Positive Samples
Biological Materials	15/16
Environmental	22/49
Other mouse samples	8/10

**Materials evaluated for this survey include:**

- Biological materials - cell lines, tumors, tumor fragments, and Matrigel
- Environmental - rack filters and cage waste
- Other mouse materials - kidneys, urine, unspecified mouse tissue, and pet waste

### Detection by Dirty Bedding Sentinels

**Colony Mice**

- 3 cages naive female nude mice (4 mice per cage)
- Mice housed in cages containing dirty bedding from a known positive colony for 2 weeks.
- MKPV was detected in the feces by real-time PCR for up to 12 weeks post exposure.

**Sentinel Mice**

- 3 cages of naive ICR female mice (4 per cage)
- Mice housed on a separate rack from colony mice
- Sentinel mice exposed to 30 cc dirty bedding from colony cage every 2 weeks for 4 weeks.
- MKPV was detected in the feces by real-time PCR for up to 6 weeks post exposure.

### Number of MKPV positive cages as detected by real-time PCR of feces

Mice	Time of Testing (Weeks)						
	0	2	4	6	8	10	12
Nude	0/3	ND	3/3	ND	3/3	ND	3/3
Sentinel	0/3	3/3	3/3	3/3	3/3	*	*

**Table Legend**

1. ND = Time points not performed. 2. \* = One point that was pending testing.

### Summary

- MKPV infection has been detected in a wide range of mouse stocks and strains.
- MKPV can be detected in a variety of sample types including feces, urine, mouse tissues, rack filters, cage waste and biological material such as cell lines and tumors.
- MKPV infection can be detected in soiled bedding sentinel mice as early as 2 weeks after exposure to dirty bedding.
- MKPV shedding was detected for up to 6 weeks in sentinel mice and 12 weeks in nude mice.

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[www.idexxbioanalytics.com/mkpv](http://www.idexxbioanalytics.com/mkpv)

PREPARE guidelines for better Science (film)

**TOO LATE!!**

**HOW CAN WE IMPROVE ANIMAL STUDIES?**

**WE ARRIVED BECAUSE WE WERE PREPARED**  
 VISIT: [NORECOPA.NO/PREPARE](http://NORECOPA.NO/PREPARE)

**PRECISION**  
**REPLICABILITY**  
**HEALTH AND SAFETY**  
**TRANSLATABILITY**

**PILOTS**  
**CABIN CREW**  
**GROUND STAFF**  
**AIR TRAFFIC CONTROLLERS**

**RECIPE**  
**NORECOPA.NO/PREPARE**

**SUGGESTIONS FOR**

- GUIDELINES
- DATABASES
- INFORMATION CENTRES
- JOURNALS, DISCUSSION LISTS, ETC.

**SUGGESTIONS FOR**

- A MASTER PLAN
- A CONTINGENCY PLAN
- A CONTRACT (ANIMAL FACILITY & RESEARCH GROUP)

**COLLABORATION**

- ANIMAL CARERS AND TECHNICIANS
- VETERINARIANS
- FACILITY MANAGERS
- SCIENTISTS AND THEIR STAFF
- ANIMAL WELFARE AND ETHICS COMMITTEES
- REGULATORS

**THIS WAY WE CAN**

- REDUCE THE RISK OF ANIMALS SUFFERING
- IMPROVE OUR METHODS - DO BETTER SCIENCE!
- WRITE BETTER APPLICATIONS
- IMPROVE MANUSCRIPT QUALITY

**norecopa**