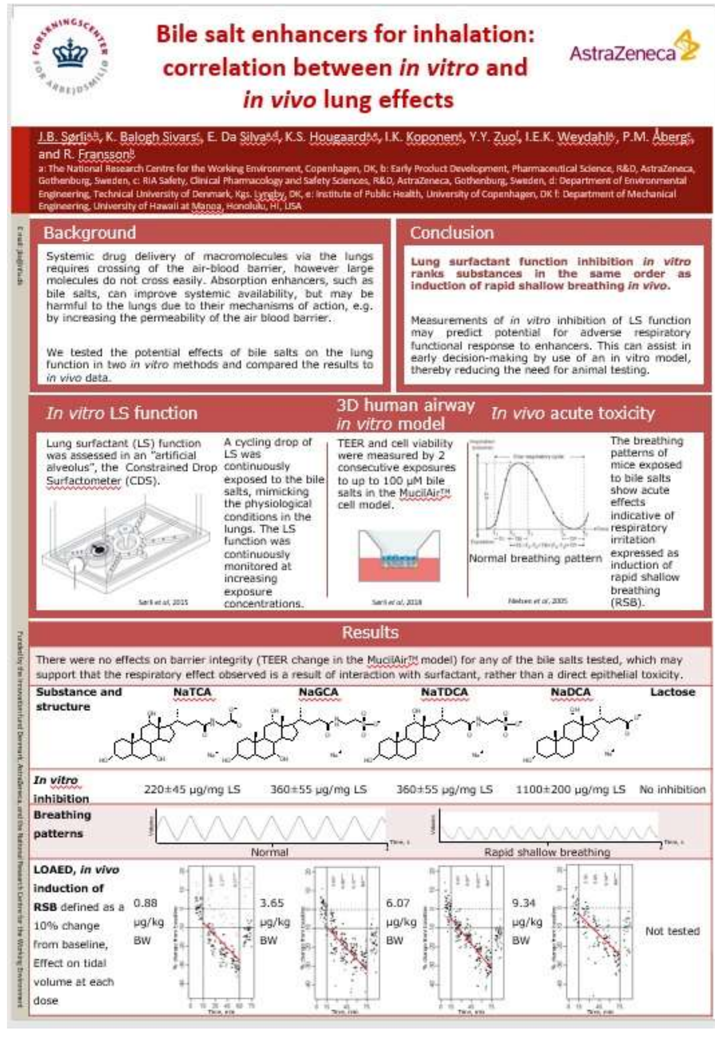
Speed Poster Presentation (15.45-16.15)

1. **Sabine Bischoff** (Universitätsklinikum Jena)
2. **Jorid Birkelund Sørli** (The National Research Center for the Working Environment)
3. **Louise Maria Grønbech Nielsen** (Lundbeck)
4. **Tina Brønnum Pedersen** (Lundbeck)
5. **Mohamed Essameldin Abdelgawad** (Cairo University)
6. **Karen Ekkelund Petersen** (Scanbur)
7. **Anders Fick Thomsen** (Aarhus University)
8. **David Mayo** (IDEXX BioAnalytics EMEA)
9. **Adrian Smith** (Norecopa/The Danish 3R-Center)

Critical Incident Reporting System in Laboratory Animal Science (CIRS-LAS)

Jorid Birkelund Sørli (The National Research Center for the Working Environment)

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



Louise Maria Grønbech Nielsen (Lundbeck)

Better welfare through positive reinforcement training and VAB catheter

Better welfare through positive reinforcement training and VAB catheter

Animal facility and Vet team

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
★	**Reduction** (Reduction of the numbers of animals used)
	Replacement (Replacing the use of animals with In vitro/In silico methods)
★	**Refinement** (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	
★	Procedure is implemented

Presented at Knowledge Sharing Day - 2019

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Tina Brønnum Pedersen (Lundbeck)

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

Lundbeck

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.

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Initiative

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.

Resources
Reduce warming
Quicker
Reduced housing
Reduced animals
Animal welfare
Reduce restraint
Less material
Less invasive
Other samp-ling routes
Scientific
Multiple endpoints
Better PK profiles

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-euthanasia.

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.

100 vs 10µl blood

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

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

Concentration
Time

Micro blood sampling 1 animal, 6 x 10 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
Plasma Conc ug/mL
Time (h)
Cheek-sampling
Micro-sampling

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

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.

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

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

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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[1]. 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.

Introduction

One of the most famous cell culture media additive is fetal bovine serum (FBS). Its annual production is around 600 000 liters a year[2]. This raised both ethical and scientific issues. Methods used to harvest it are often criticized because of the harm it can cause to the animal well-being[3].

Moreover, the use of FBS implies several major drawbacks such as a lack of batch to batch consistency[4,5], a potential source of zoonoses[6], downstream processing challenges[7,8], and high cost[9].

Peptones, on the other hand are defined as being any water-soluble protein hydrolysates containing amino acids, peptides, inorganic salts, but also vitamins, sugars and lipids[10]. They are used as an additive to media in order to increase cell proliferation, biomolecules production.

VERO are widely used in the pharmaceutical industry, in clinical or research laboratories. They have been utilized to produce several human vaccines. The classic percentage of FBS used for their culture is 10%.

In this study, the objective is to investigate if peptones could be used as a safe and vegetal (xeno-free) alternative to FBS as a media supplement in an expansion media for VERO.

Methods and Materials

- **MATERIAL**
  - Cell line: VERO CCL-81 (ATCC®, batch 70005907)
  - Media: DMEM, high glucose, L-glutamine (Gibco, batch 20078) / BMBD145 (in house media)
  - FBS (Eurobio, ref: CVFSVF00-01, batch S66505-5256)
  - BrdU Kit (Ozyme, 6813S)
  - Peptones, all supplied by Organotechnie
    - TN1, batches: 174603X / 182108
    - T+, batches: 173908 / 173908G
    - W106, batch: 180903UG
    - W206, batches: 180903 / 181105X / BS1801M
    - S203, batches: 182601 / 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/cm². 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 synthetized DNA of proliferating cells in place of thymidine. It is a colorimetric detection whereas the intensity at OD_{450} 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)

Media preparation → Seeding (Day zero) → Change media (Day 2) → BrdU kit → ANOVA

Results

Control vs casein peptones

Fig.1 : Control vs casein peptones

Control vs wheat peptones

Fig.2: Control vs wheat peptones

Control vs soy 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.**

Contact

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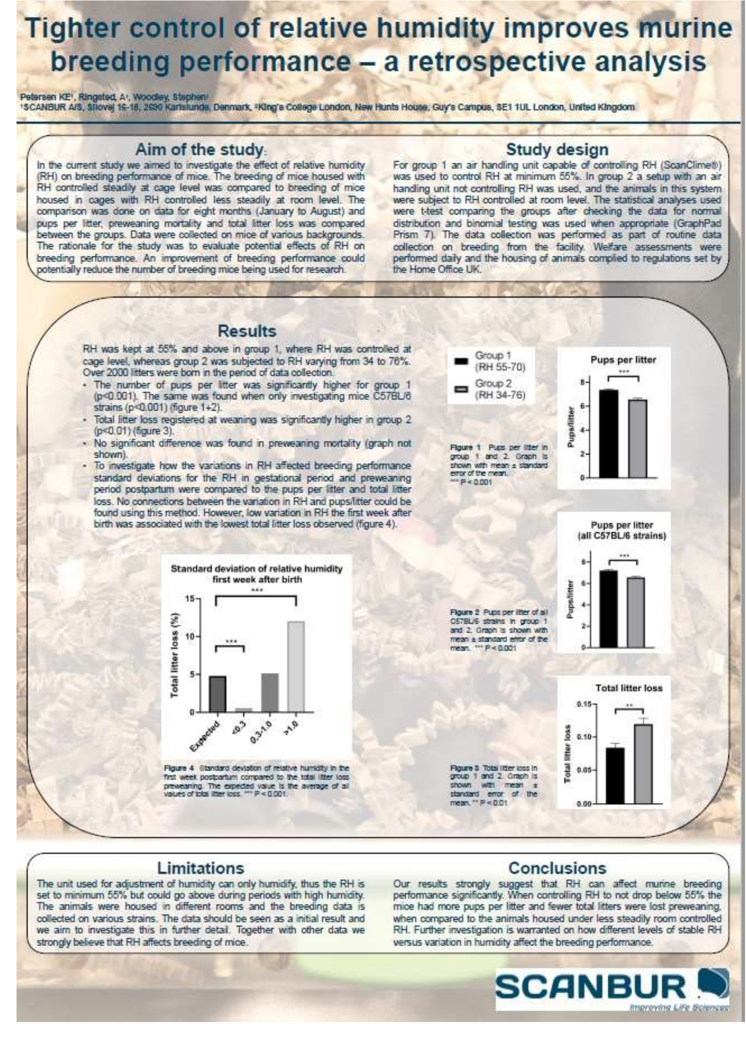
Karen Ekkelund Petersen (Scanbur)

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



Pig training – a refinement, research and welfare iniative

AARHUS UNIVERSITET
INSTITUT FOR KLINISK MEDICIN

Pig training – a refinement, research and welfare initiative

Thomsen AF, Jakobsen K, Kousholt BS

Click on the icon to see a specific video

DAYS: 0 7 14

Socialization

Target stick

Novel objects

Habituation

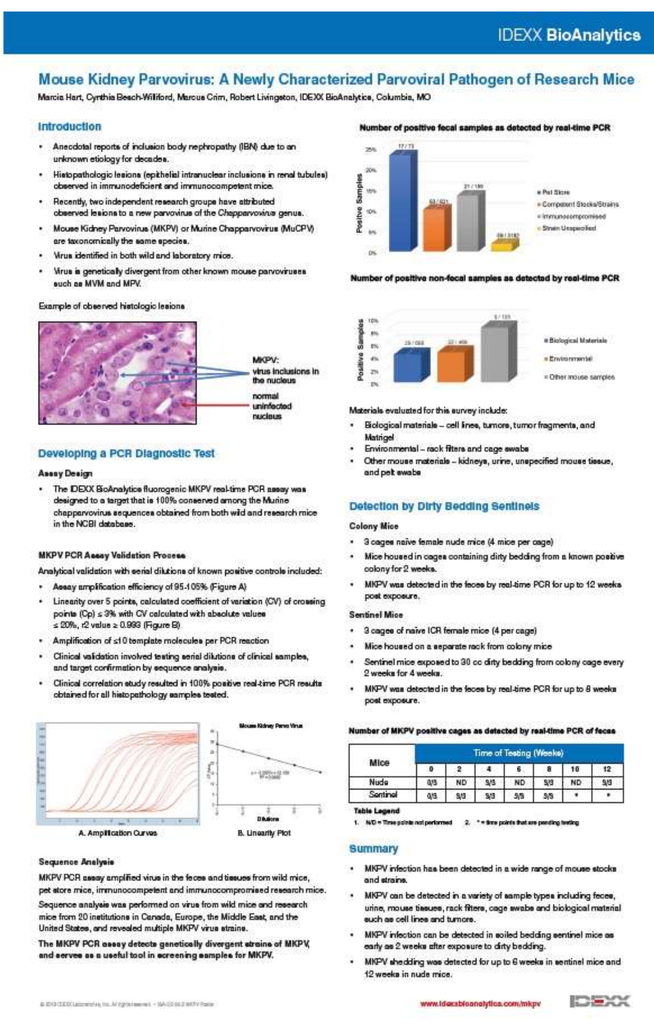
Hoof trimming and Weighing

Sampling and control

HOME ABSTRACT ACKNOWLEDGEMENT

David Mayo (IDEXX BioAnalytics EMEA)

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-Williford, Marcus Crim, 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 *Chapparvovirus* genus.
- Mouse Kidney Parvovirus (MKPV) or Murine Chapparvovirus (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 fluorogenic MKPV real-time PCR assay was designed to a target that is 100% conserved among the Murine chapparvovirus 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%, r2 value ≥ 0.993 (Figure B)
- Amplification of ≤10 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.

Mouse Kidney Parvo Virus

Dilutions

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 20 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

Positive Samples

Pet Store

Competent Stocks/Strains

Immunocompromised

Strain Unspecified

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

Positive Samples

Biological Materials

Environmental

Other mouse samples

Materials evaluated for this survey include:

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

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 8 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. N/D = Time points not performed
2. * = time points that are 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 swabs 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.

www.idexxbioanalytics.com/mkpv

IDEXX

Adrian Smith (Norecopa/The Danish 3R-Center)

PREPARE guidelines for better Science (film)

TOO LATE !!

RECIPE

NORECOPA.NO/PREPARE

HOW CAN WE IMPROVE ANIMAL STUDIES ?

PILOTS
CABIN CREW
GROUND STAFF
AIR TRAFFIC CONTROLLERS

- PRECISION
- REPLICABILITY
- HEALTH AND SAFETY
- TRANSLATABILITY

- 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

WE ARRIVED BECAUSE WE WERE PREPARED
VISIT: NORECOPA.NO/PREPARE

norecopa

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

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