# POSTER PRESENTATIONS

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# Improving welfare of cattle housed in a high-containment facility using behavioural analysis

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## Introduction

Animal behaviour is an observable measure of the effects the external environment has on an animal. The relatively non-invasive nature of behavioural analysis minimises the influence of human presence. Appropriate acclimatisation to a novel experience or environment is an important refinement technique adopted by animal research facilities to improve welfare.

Miguel-Pacheco *et al* (2014) used CCTV to investigate the impact of cattle lameness on eating and milking behaviour.[1] They found lame cattle fed less and volunteered themselves less frequently for milking compared to their able-bodied conspecifics suggesting that lameness impacts behaviour and willingness to be milked. Using human observation, Grandin (2010) demonstrated habituating naive antelope and bison to restraint crates and regulated procedures (e.g. blood sampling) improved handling and reduced cortisol levels as the animals were overall calmer and less flighty.[2] Through habituation, Grandin was able to improve the animal's welfare during regulated procedures by reducing the overall stress caused by the restraining process.

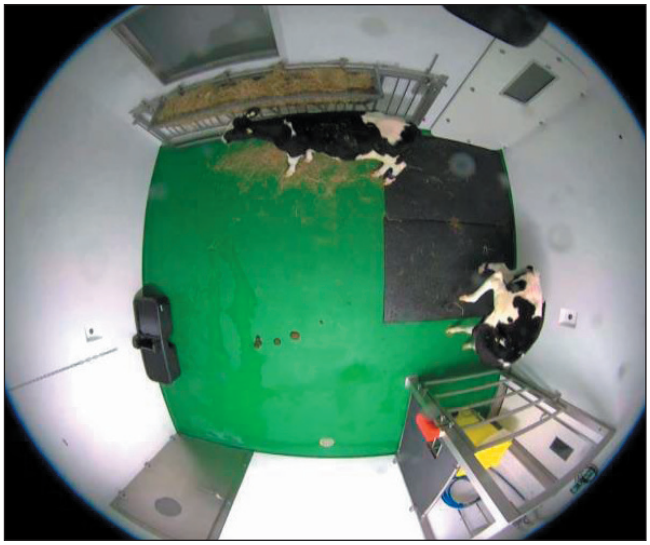

**Figure 1.** The CCTV camera angle used for video footage.

The Pirbright Institute (TPI) has been trialling the use of behavioural analysis through CCTV usage and human observation to measure cattle acclimatisation to a high-containment SAPO4 facility.

## Phase 1: Observation

Objective: To determine the length of time needed for acclimatisation by analysing the voluntary entry rate of cattle into yokes recorded through human observation.

- During phase one, 17 cattle were yoked (see figure 2) in the morning over 12 days as part of the feeding routine, 7 of those were additionally yoked in the afternoon.
- Feed buckets were placed out, the yokes opened and the staff members then waited out of sight for the cattle to enter.
- The cattle were initially allowed six minutes to enter the yokes by themselves. It was then decided this was too long and the time then reduced to three minutes.
- After the time allowed, if they had not entered, coaxing them in with the feed bucket was permitted.
- The number of cattle entering voluntarily, being coaxed or refusing to yoke were recorded on the data collection sheet. A comments box was available for any additional information e.g. a full health check, blood sampling, vaccination.

**Figure 2.** Cattle in the yokes during feeding time.

## Phase 1 Results

Between days 4 and 6 the number of cattle voluntarily yoking plateaued with a minor decrease seen on day 5 (Figure 3) which is as yet unexplained.

- A decrease in voluntary yoking was seen on day 3 possibly as a result of being health checked and having their feet inspected whilst in the yokes the previous day. They may have not been fully habituated to the yokes and environment at this point.

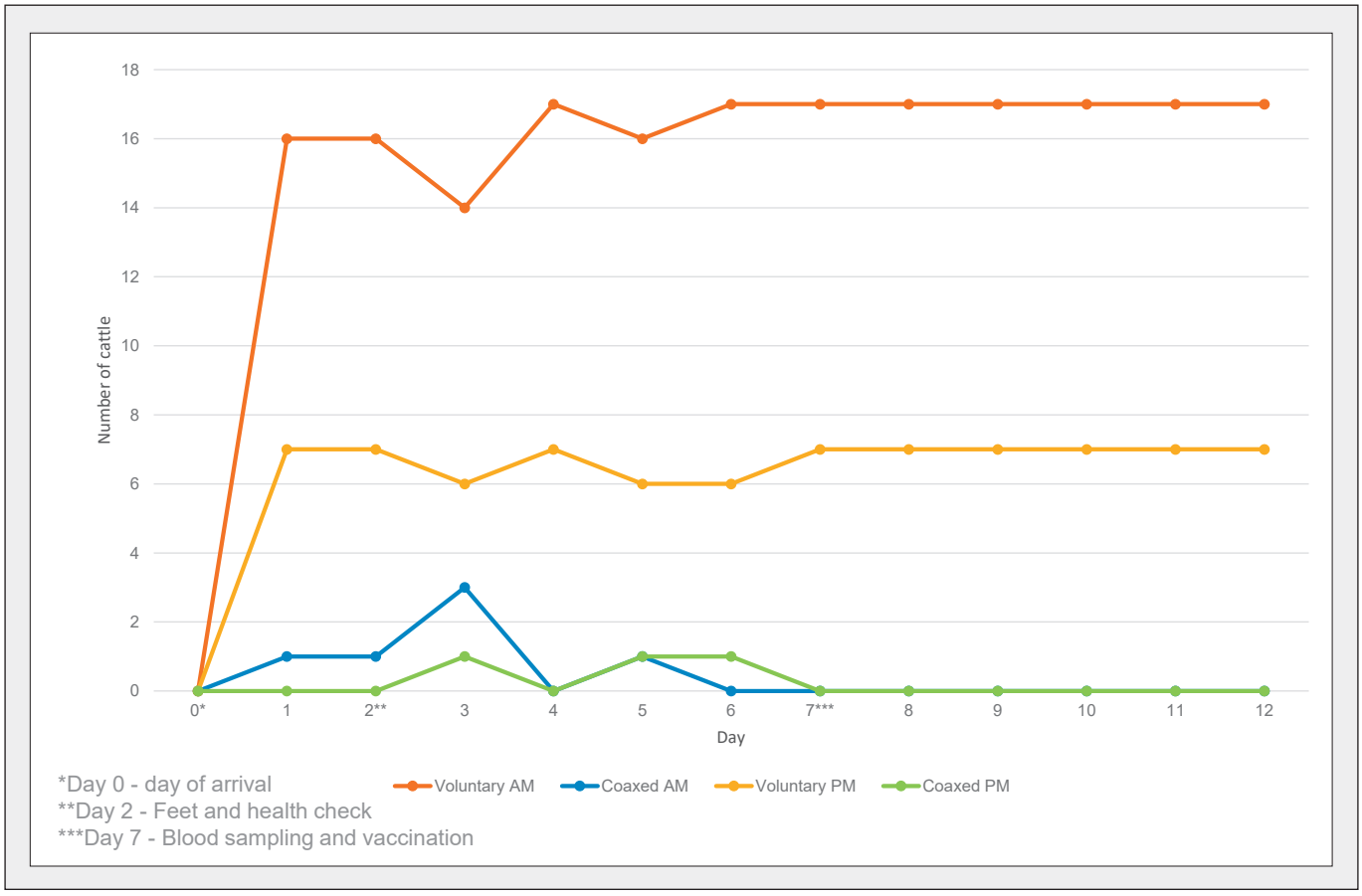


**Figure 3. Phase 1:** Yoking frequencies of 17 cattle at TPI during the combined AM(N=17) and PM(N=7) yoke over the acclimatisation period from 28/10/2019-09/11/2019.

– No decrease in the number of cattle entering the yokes was observed the day after their first regulated procedure (day 8) suggesting full acclimation.

– Being yoked PM as well as AM did not seem to have a noticeable positive or negative effect on the cattle entering the yokes.

## Phase 2: Time Trial

**Objective:** To determine the length of time needed for acclimatisation by analysing the voluntary entry rate of cattle into yokes recorded through cattle yoking timings.

– During phase 2, 44 cattle were yoked (see Figure 4) in the morning over 7 days as part of the feeding routine.

– Feed buckets were placed out; the yokes were opened and the staff members then waited out of sight for the cattle to enter.

– As a result of phase one, the cattle were allowed three minutes to enter the yokes by themselves.

– After the time allowed, if they had not entered, coaxing them in with the feed bucket was permitted.

– The number of cattle entering voluntarily being coaxed or refusing to yoke were recorded on the aforementioned data collection sheet.

– The CCTV cameras were then consulted and, using Waveview, the amount of time it took each animal from three study groups (study one N=5, study two N=2, study three N=4) to enter the yokes was determined. This was then analysed.

## Phase 2 Results

– Through human observation, the cattle voluntarily yoking plateaus on day 4 (see Figure 4). A reduction in numbers can be seen from day 6 onwards where two were euthanised due to the study ending.

– When analysing the timings, the cattle voluntarily self-yoke the quickest on day 6 with a large reduction in time seen between days 4 and 6 for both groups one and two (see figure 5).

– The amount of time reduces from day 4 and continues to do so suggesting around this time they are more comfortable in a novel environment and being yoked.

– These pilot studies indicate complete acclimatisation occurs around days four to six yet further study is

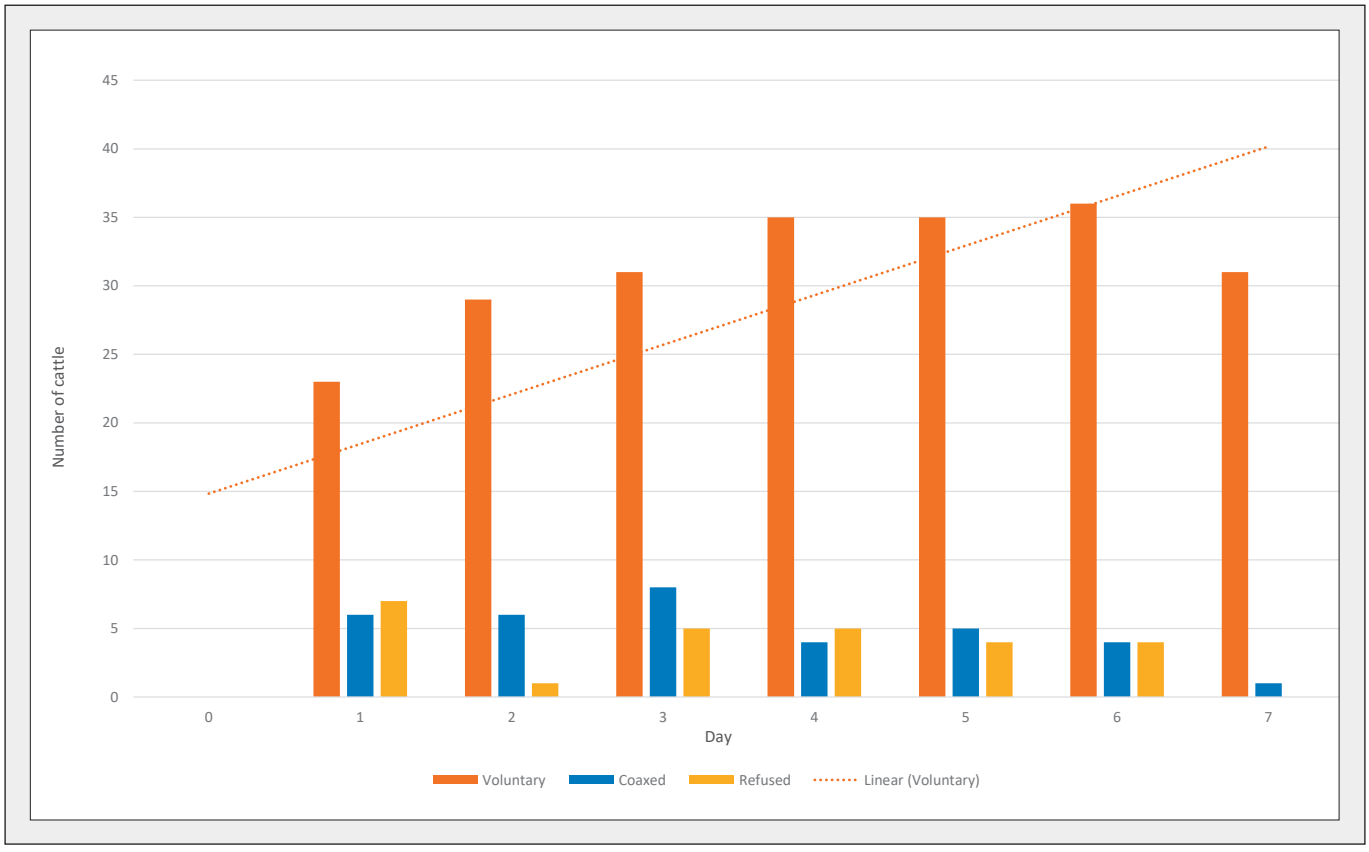


**Figure 4. Phase 2:** Total number of cattle (N=44) observed entering the yokes during the AM yoke of the acclimatisation period collated from three studies (17/02/2020-18/07/2020).

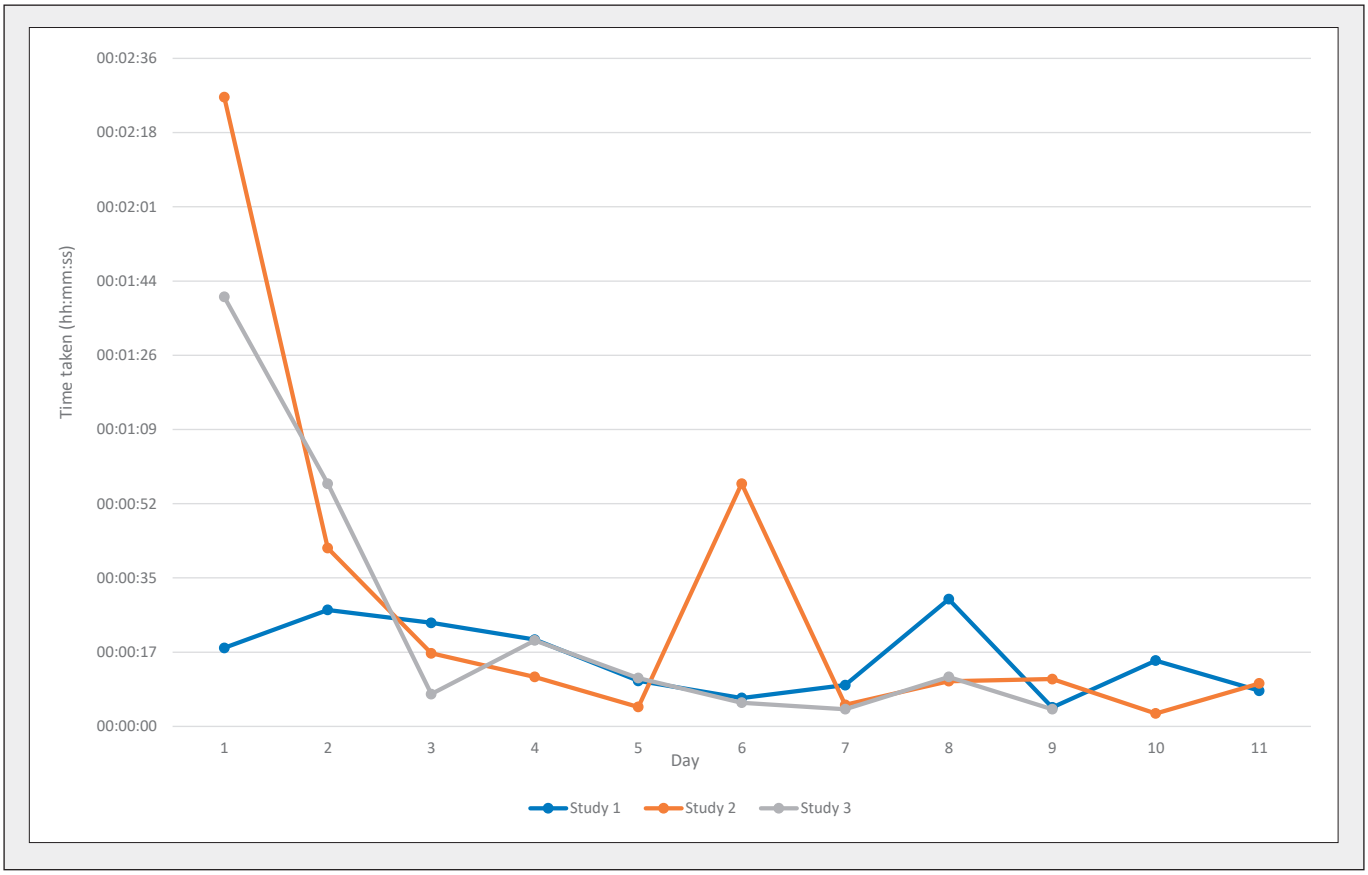


**Figure 5. Phase 2:** The average amount of time taken per day for cattle (N=11) to self-yoke across three separate studies (17/02/2020 –18/07/2020).

needed to confirm this. From the data generated, behavioural analysis and CCTV use appear to be very useful in helping determine and further improve the appropriate acclimatisation period for cattle in a high-containment environment. This is essential in aiding refinement to improve both animal welfare and scientific integrity.

## Future studies

Data collected from IceRobotics motion tracking bracelets and additional Noldous collar tracking software will help expand the current locomotion data. Ongoing studies into the use of animal mounted animal accelerometers and location tracking systems are also being conducted. The present yoking study will also be including more CCTV footage and timings. This will be used to determine how long it takes the cattle to enter the yokes using the premise that stress cattle will enter slower than those that are fully habituated.

**Ethical statement:** all legal and ethical aspects were considered when conducting this study including the use of animals. Those participating in this study consented to take part and were aware of any image recording that took place.

## References

1 **Miguel-Pacheco, G.G., Kaler, J., Remnant, J., Cheyne, L., Abbott, C., French, A.P., Pridmore, T.P. and Huxley, J.N.** (2014). Behavioural changes in dairy cows with lameness In an automatic milking system, *Applied Animal Behaviour Science,* Vol 150, pp 1-8.

2 **Grandin, T.** (2010). Habituating Antelope and Bison to Cooperate With Veterinary Procedures, *Journal of Applied Animal Welfare Science,* vol 3 (3), pages pp 253-261.

# Efficacy of Medola's Blue Stain for the assessment of *Syphacia muris* egg viability

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## Introduction

*Syphacia muris* (Nematoda: Oxyuridae); commonly known as the rat pinworm, is a parasite of rodents. This ubiquitous nematode commonly infects laboratory rodents, primarily rats, via direct and indirect transmission routes. Daily shedding of pinworm ova in the host results in ingestion of the ova and contamination of water, food and bedding. This provides a continual inoculum that results in frequent re-exposure of the host to the parasite making the control of pinworms difficult (Meade & Watson. 2014).[1]

The assessment of *S. muris* ova viability following the disinfection of an infested facility with an effective ovicidal agent can be costly and time consuming. Both viable and non-viable ova will persist following treatment. It is not possible to visually assess the viability of ova using standard microscopy. Therefore to test the viability of ova following treatment with an ovicidal agent traditionally ova are subjected to hatching analysis using a suitable hatching media.

Most hatching media have a short shelf life and to ensure that unhatched pinworm ova are truly non-viable, it is advisable to test the hatching media on viable pinworm ova before analysing treated ova. Further to this, using hatching media for the visual assessment of hatched ova is not ideal as juvenile nematodes are digested in the hatching media within 30 minutes of hatching. Therefore, assessment is purely based on ova being correctly orientated on a Sellotape slide to allow the break in the ova cell wall that the juvenile nematode has vacated to be visible.

Medola's blue stain has been used in the viability assessment of plant-parasitic nematode ova for many years. We propose that this stain is a useful tool in determining the viability of *S. muris* ova in environmental and rodent samples when determining treatment/ decontamination efficacy in rodent facilities.

## Method

- A total of 20 Homozygous Scottish (HS) rats of known health status with an established *S. muris* infection were sampled. The animals were group housed 3-4 rats with husbandry procedures carried out in accordance with ASPA (1986).[2]

- Ova were collected by Sellotape impressions of the anal area. Samples were taken in the afternoon to optimise collection as demonstrated by Van de Gulden (1967).[3] The Sellotape impressions were dissected at 40x magnification into sections containing 25 viable ova and placed sticky side up and fixed to glass slides using 10 mm acid free craft dots. Non-viable ova were identified by the presence of degradation of the lipids inside the infective juveniles, this indicated that the nematodes within the ova were unable to hatch. These ova were omitted from the tapes.

- Viable control: 10 replicates of 25 viable ova were immersed in a 0.05% solution of Medola's blue stain in an 0.85% saline solution for 30 minutes at room temperature before being de-stained by immersion in distilled water for 30 minutes at room temperature and hatched following the hatching procedure.

- Non-viable control: 10 replicates of 25 viable ova were heat killed by immersing the sellotape slides in distilled water at 65°C for 5 minutes. Once air dried the 10 replicates of 25 viable ova were immersed in a 0.05% solution of Medola's blue stain in an 0.85% saline solution for 30 minutes at room temperature before being de-stained by immersion in distilled water for 30 minutes at room temperature and hatched following the hatching procedure.

- Chemical control: 10 replicates of 25 viable ova were chemically killed by immersing the Sellotape slides in 2% Neopredisan solution for 2 hours. Once

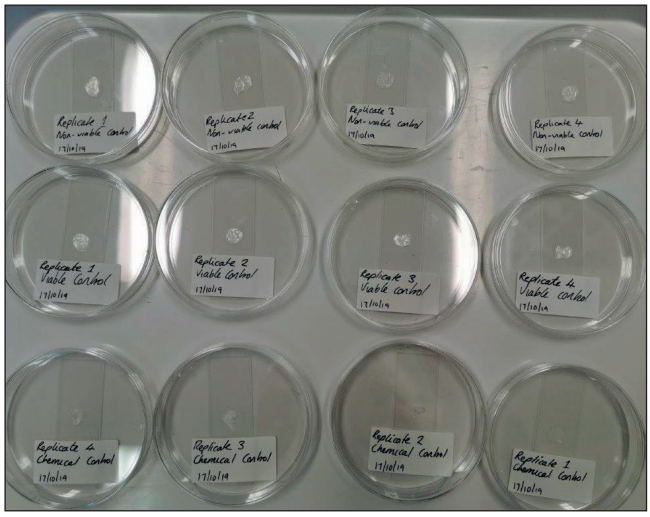

**Figure 1.** Anal tapes mounted on glue spot slides before staining. Non-viable control is heat control.

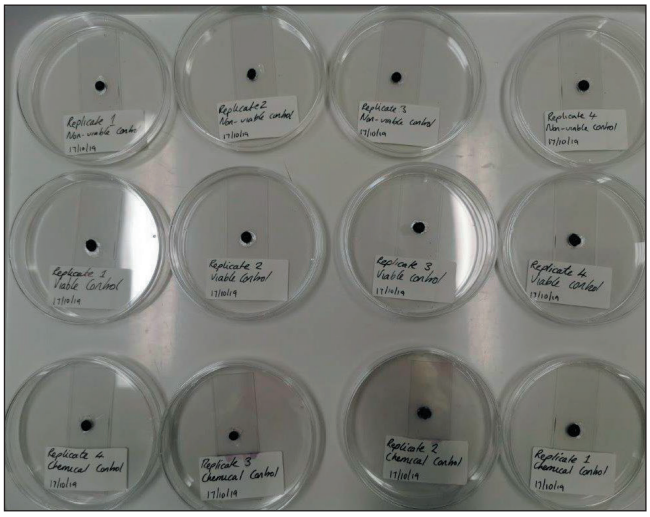

**Figure 2.** Anal tapes mounted on glue spot slides with Medola's blue stain. Non-viable control is heat control.

air dried the 10 replicates of 25 viable ova were immersed in a 0.05% solution of Medola's blue stain in an 0.85% saline solution for 30 minutes at room temperature before being de-stained by immersion in distilled water for 30 minutes at room temperature and hatched following the hatching procedure.

– Hatching: The hatching medium used was prepared according to the method previously reported by Dix *et al*: 2004.[4] Viable, non-viable and chemical control ova were covered in hatching medium and incubated in ambient air at 37°C overnight. Slides were scanned x60 magnification and the number of hatched and non-hatched ova recorded. Ova were considered nonviable if the operculum was intact or the ova contained larva. An ova without larva or those with an open operculum were considered viable. Data was recorded for each replicate.

## Results

The results can be found in table 1.

All heat killed ova and all Neopredisan killed ova took up the Medola's blue stain. There was a minor difference in the intensity of staining observed between the heat killed and Neopredisan killed ova. Heat killed ova presented with a blue colour whereas Neopredisan killed ova presented as a blue/purple colour. None of the 250 untreated ova took up the Medola's blue stain.

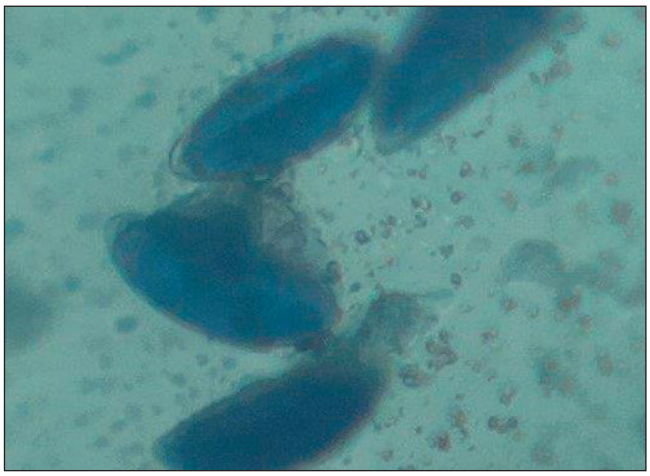

**Figure 3.** A *Syphacia muris* non-viable ova stained with Medola's blue after Neopredisan treatment.

| **Chemical Control 2% Neopredisan for 2 hours.** | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Replicate number | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Average | Total |
| Number of *S. muris* ova stained with 0.05% Medola's blue | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 250 |
| Number of *S. muris* ova stained after destaining for 30 mins | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 250 |
| Number of *S. muris* ova hatched in hatching media | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | | | | | | | | | | |
| **Heat Control 65°C for 5 mins in a water bath.** | | | | | | | | | | | | |
| Replicate number | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Average | Total |
| Number of *S. muris* ova stained with 0.05% Medola's blue | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 250 |
| Number of *S. muris* ova stained after destaining for 30 mins | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 250 |
| Number of *S. muris* ova hatched in hatching media | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | | | | | | | | | | |
| **Untreated ova** | | | | | | | | | | | | |
| Replicate number | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Average | Total |
| Number of *S. muris* ova stained with 0.05% Medola's blue | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Number of *S. muris* ova stained after destaining for 30 mins | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Number of *S. muris* ova hatched in hatching media | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 250 |

**Table 1.** *Syphacia muris* Medola's blue assessment results

The heat treated and Neopredisan treated ova could not be successfully hatched demonstrating that the stained ova were non-viable as expected. All untreated ova were successfully hatched demonstrating that the unstained ova remained viable. The Medola's blue stain did not have any effect in the ability of viable ova to hatch.

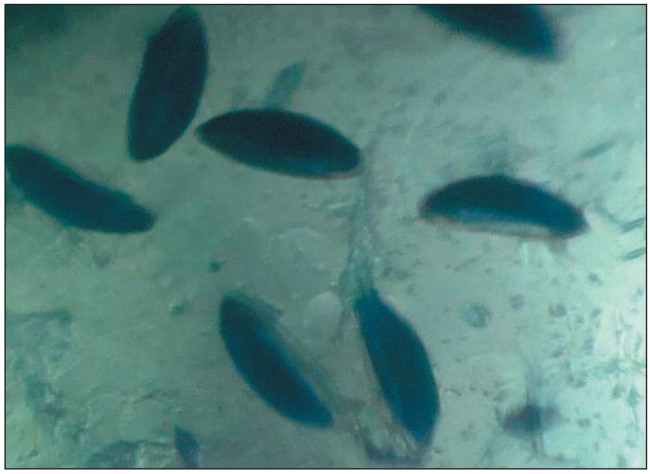

**Figure 4.** *Syphacia muris* non-viable ova stained with Medola's blue after heat treatment.

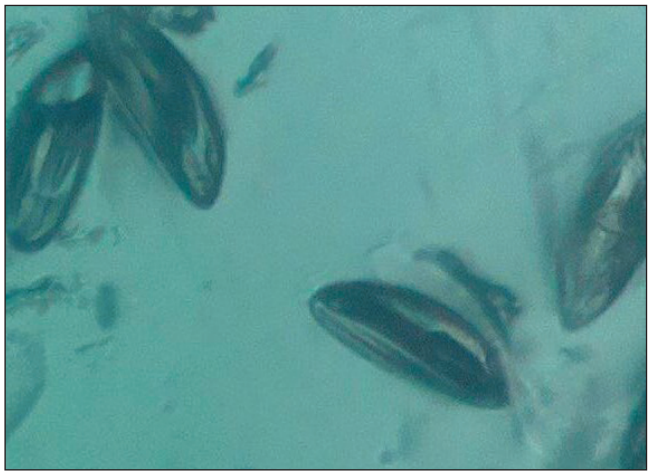

**Figure 5.** *Syphacia muris* viable ova, stained with Medola's blue.

## Conclusion

The exact mechanism of uptake of this stain is not known for this organism. However, we observed that viable nematode ova with an intact operculum did not allow the stain to pass the outer cuticle of the infective juvenile. In nonviable ova or those without an intact operculum, the stain was readily observed in the juveniles. It is possible that upon death, the permeability of the infective juvenile nematode cuticle may have changed allowing the stain to enter and permanently bind with the dead nematodes.

Irrespective of the mechanism of action, this study confirms that Medola's blue stain is a useful tool in determining the viability of *Syphacia muris* ova which has historically been impossible without the use of hatching media. This is a more cost-effective, technically simplified and less time-consuming method of determining ova viability which could be adopted by animal facility personnel. This method could be used in facilities to confirm effective decontamination where ova may still be present in the environment after chemical treatment. This stain could also provide a useful tool to determine the efficacy of disinfectants for use in animal facilities.

## References


1. **Meade, T.M., Watson, J.** (2014). Characterisation of rat pinworm (Syphacia muris) epidemiology as a means to increase detection and elimination. *Journal of American Association of Laboratory Animal Science. Nov; 53(6):661-7.*
2. **ASPA. 1986. Guidance on the operation of the Animals (Scientific Procedures) Act 1986**. Home Office 01.12.2017.
3. **Van der Gulden WJ.** (1967). Diurnal rhythm in egg production by Syphacia muris. Experimental Parasitology 21:344–347.
4. **Dix, J., Astill, J., Whelan, G.** (2004). Assessment of methods of destruction of Syphacia muris ova. Laboratory Animals 38:11-16.

# Cage side determination of post-mortem interval in mice

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## Introduction

Unexpected death of animals within a research facility can indicate underlying welfare issues, cause the loss of scientific data and potentially represents a compliance breach under the Animals (Scientific Procedures) Act 1986 (ASPA).

Currently there is no validated scoring system to estimate the *post-mortem* interval in mice beyond *rigor mortis.*[1]

The aims of this preliminary study were to:

1. Document internal and external macroscopic post-mortem changes in wild-type mice between 0-48h.
2. Develop a simple visual cage-side scoring system to help technicians estimate the time of death.
3. Inform and improve Home Office reporting of unexpected mouse deaths.

## Materials and methods

30 male mice (C57BL/6$^{c-/c-}$; 15-18 weeks old) were used in this prospective, randomised and 'blind' study.

- The mice were euthanised in their home cage ($CO_2$, 20% cage volume.min$^{-1}$) and were randomly allocated to 1 of 5 groups: examination at 0, 3, 16, 24 and 48hr post-euthanasia (n=6 per group).
- The cadavers were maintained in ventral recumbency at stable temperature (22 ± 2°C) and humidity (53 ± 5%), singly housed on aspen chips bedding within Tecniplast GM500 cages until *post-mortem* examination.
- Rectal temperature was measured at the time of dissection.
- Post-mortem examinations and dissections were performed as previously described by an operator unaware of the time of death of the animal.[2] The operator scored the qualities of internal and external organs against a composite 3-points scoring system (Green, Amber, Red) developed during a pilot study.
- Organs scored included: eyes (EE); skin and extremities (SE); abdominal cavity (AC); liver and gallbladder (LG); spleen (SP); intestines (IN).
- Data was analysed using Fisher's Exact test for categorical data with pairwise comparisons (for organs) and one-way ANOVA with Tukey's multiple comparison (rectal temperatures) to give results for statistical significance between all the time-points.
- All analysis was performed using R version 3.6.0[3] with package R companion version 2.3.25[4].

## Results

The appearance of the abdominal cavity, liver/gallbladder, spleen and intestines changed significantly between 0 and 48 hour *post-mortem.* The changes observed on the skin, extremities and eyes were qualitatively identifiable but not statistically different by scoring.

**Changes between 0-3hr:** No significant change was identified in any of the organs scored. However the rectal temperature decreased to 21.9 +/- 0.8°C by 3 hour, in equilibrium with the room temperature.

**Changes between 0-16hr:** the appearance of the abdominal cavity was the only statistically significant difference (p,0.5). Discolouration, the presence of free fluid, change in organ's shape and odour were the 1$^{st}$ signs noted.

**Changes between 0-24hr:** In addition to the abdominal cavity (p,0.05), the appearance of the intestines changed (p,0.05) with gaseous distension and occasional free fluid bathing the abdominal cavity.

**Changes between 0-48hr:** In addition to changes to the abdominal cavity and the intestines, the appearance of the liver/gallbladder (p,0.01) and the spleen (p,0.01) changed with gravitational blood pooling (*livor mortis),* swollen appearance and crumbly texture. The gallbladder was autolysed or broken.

## Proposed visual *post-mortem* scoring system

Based on the experimental results outlined above, the proposed scoring tools aim to support technicians in their estimation of the *post-mortem* interval in mice. The tool also includes additional macroscopic trends that were not statistically significant but may assist determination of timing.

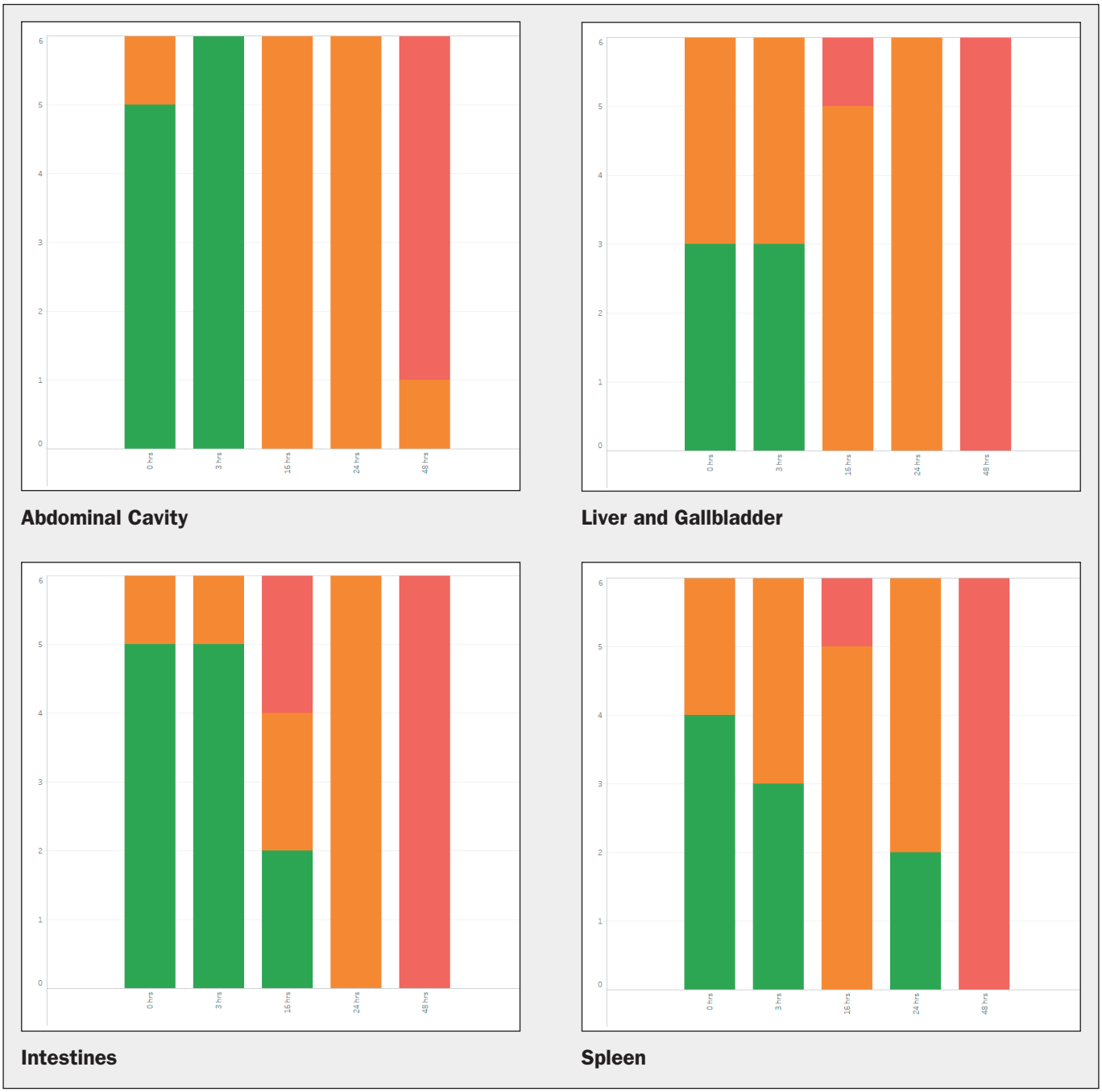
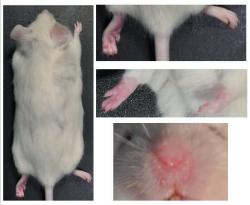
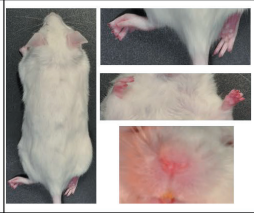
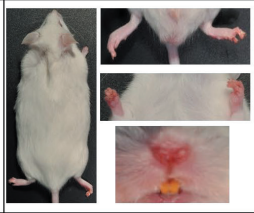
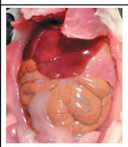
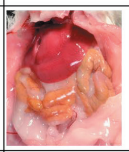
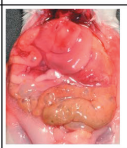
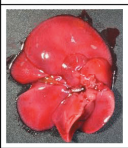
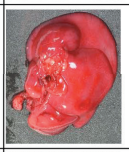
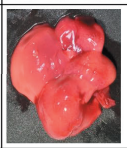
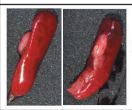
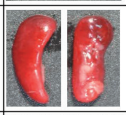
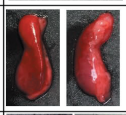
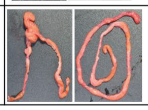
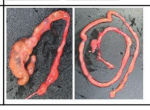
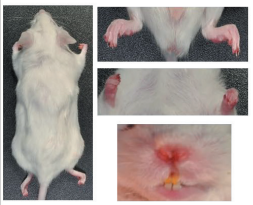
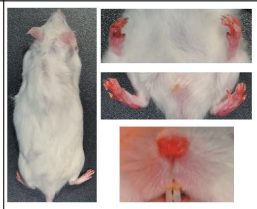
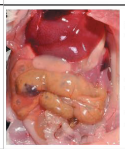
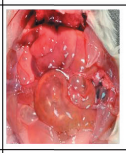
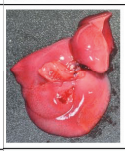
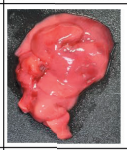
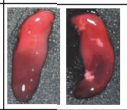
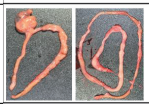
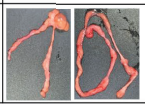



**Figure 1**: Number of mice scoring Green, Amber or Red at each time point (0, 3, 16, 24 & 48hr) for the major organs: abdominal cavity; intestines; liver and gallbladder; spleen. * p<0.05, ** p<0.01. Due to the number of comparisons, significant comparisons are only shown for;

- 0hr and the earliest significant following time point
- 48hr and the closest significant time point

| | 0hr | 3hr | 16hr |
|---|---|---|---|
| SE |  ➢ Good skin elasticity. ➢ Pale paws and digits. ➢ No presence of blood under nail bed. ➢ Pale nose. |  ➢ Good skin elasticity. ➢ Pale paws and digits. ➢ Some accumulation of blood under nail bed. ➢ Pale nose. |  ➢ Reduced skin elasticity. ➢ Base of paws and digits darker in colour. ➢ Accumulation of blood under nail bed. ➢ Nose starting to show signs of blood pooling. |
| AC |  ➢ Organs: shape, colour and position normal. ➢ Free fluid and odour: None. ➢ Inner abdominal wall normal. |  ➢ Organs: shape, colour and position normal. ➢ Free fluid and odour: None. ➢ Inner abdominal wall normal. |  ➢ Organs: abnormal shape, mild discolouration, easily blanched. ➢ Odour: present. ➢ Inner abdominal wall normal. |
| LG |  ➢ Liver: Shiny, plump, normal colour size and texture. ➢ Gall bladder present and normal. |  ➢ Liver: Shiny, plump, normal size, texture. Mild colour gradient from blood pooling. ➢ Gall bladder: present but empty. |  ➢ Liver: Shiny, plump, abnormal shape, texture. Increased colour gradient from blood pooling. ➢ Gall bladder: autolysed or broken. |
| Sp |  ➢ Shiny, plump, normal colour size and texture. |  ➢ Plump, normal colour size and texture. Surface dull. |  ➢ Abnormal shape, colour gradient from blood pooling. Surface dull. |
| In |  ➢ Normal colour and shape. Easily manipulated without breakage. Mesenteric intact (in situ). No discolouration. |  ➢ Normal colour and shape. Easily manipulated without breakage. Mesenteric intact (in situ). No discolouration. |  ➢ Mild discolouration. Presence of gas bubbles. Difficult to manipulated without breakage. |

| | 24hr | 48hr |
|---|---|---|
| SE |  ➢ Marked reduction of skin elasticity ➢ Base of paws and digits show blood pooling ➢ Marked accumulation of blood under nail bed ➢ Clear signs of blood pooling in nose |  ➢ No skin elasticity ➢ Paws and digits show marked blood pooling and dehydration ➢ Marked blood pooling in nose |
| AC |  ➢ Organs: distended, discolouration, easily blanched. ➢ Free fluid and odour: present. ➢ Inner abdominal wall thick and fragile. |  ➢ Organs: distended, discolouration, easily blanched. ➢ Free fluid and odour: present. ➢ Inner abdominal wall: marked discolouration. |
| LG |  ➢ Liver: Shiny, plump, abnormal shape, texture. Increased colour gradient from blood pooling. ➢ Gall bladder: autolysed or broken. |  ➢ Liver: Shiny, distended, marked abnormal shape, texture friable. Increased colour gradient from blood pooling. ➢ Gall bladder: autolysed or broken. |
| Sp |  ➢ Distended, colour gradient from blood pooling. Surface dull. |  ➢ Distended and abnormal shape. Marked colour gradient from blood pooling. Surface dull. |
| In |  ➢ Mild discolouration and distention. Presence of gas bubbles. Easily broken when manipulated. Fluid filled. |  ➢ Marked distention and presence of gas bubbles. Loss of definition. Easily broken when manipulated. Fluid filled. |

## Discussion and conclusion

- This poster provides the foundation for a macroscopic scoring system for the determination of *post-mortem* interval in male mice.
- Key tissues for examination include the abdominal cavity, intestines, liver, gallbladder and spleen.
- However, this method was not sensitive enough to detect early changes (0-3h *post-mortem*).
- External macroscopic changes of the skin, paws and extremities may be helpful indicators of the time of death but would require further investigation.
- Microscopic changes of the skin, paws and extremities may be helpful indicators of the time of death but would require further investigation.
- To increase generalisability this study can be expanded upon by varying the sex and strain of the mouse, the method of euthanasia and the animal's *post-mortem* positioning.

## Acknowledgements

Chris Lelliott, Claire Rogerson, Hannah Spendlove, Gary Stephens.

## References

1 **Capas-Peneda, C., Goncalves-Monterio, S., Oliveira, B., Duarte-Arajo, M.,** (2016). 'How do you tell how long a mouse has been dead? Rigor mortis as a tool to estimate mice time of death in animal house facilities.' FELASA Confrence 2016. FELASA2016_SC_SM_MDA_BO_v2.pdf

2 **Scudamore, C.L., Busk, N. and Vowell, K.,** (2014). A simplified necropsy technique for mice *Laboratory Animals 2014; 48: 342-344*.

3 **R Core Team, 2019 'R:** A language and environment for statistical computing' https://www.scirp.org/reference/referencespapers.aspx?referenceid=2631126

4 **Salvatore, Mangiafico,** 2020. rcompanion: Functions to support extension program evaluation' https://CRAN.R-project.org/package=rcompanion

# Maximising efficacy of your health monitoring programme

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## Abstract

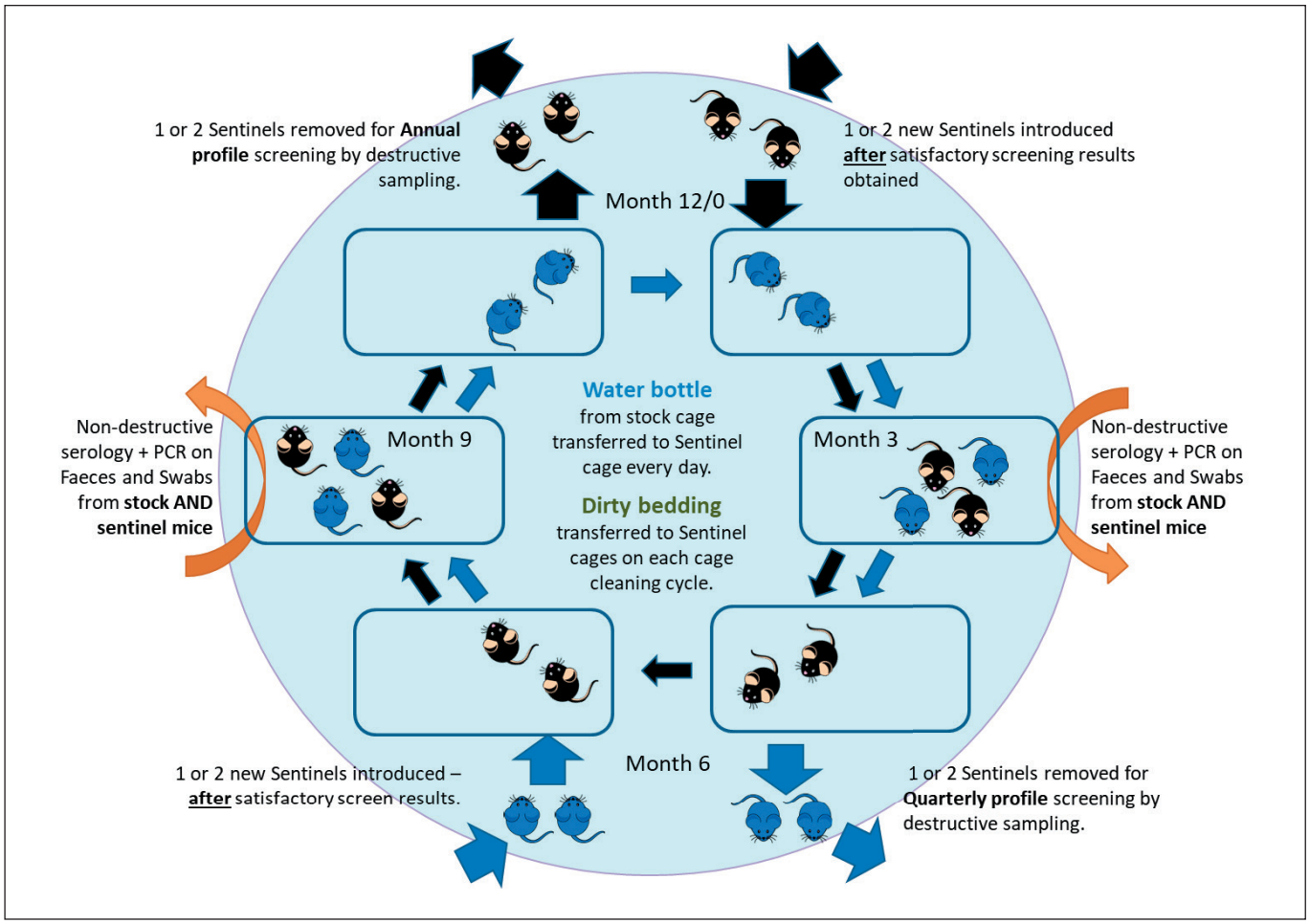
There are a multitude of health monitoring techniques/methods available. It can be difficult to know which of these to use and when to use them. This is an attempt to simplify, maximise the efficacy of and ultimately **reduce** the number of animals used in Health Monitoring (HM) programmes. Whilst this is not exhaustive and may not suit all situations it may provide some information that allows you to optimise your current methods and give greater confidence in HM results.

## Rolling sentinel programme

This is where 2 to 4 sentinels are housed in a sentinel cage on each rack. For each screen interval (usually every quarter) 1 or 2 are sampled by necropsy. If results of the screen are clear, then 1 or 2 new sentinels are introduced to the sentinel cage. On the next screen, the older sentinels are used.

*Advantages…*

Using only 1 or 2 sentinels where there would have originally been 3 used in each screen.



The sentinels left in the cage can be re-sampled and tested if there are any unexpected results, confirming or discounting the original result.

By using non-destructive sampling (tail bleeds + PCR (polymerase chain reaction) for 2 of the quarterly screens at 3 and 9 months, alternating with the destructive screens, the numbers of sentinels used can be reduced by 50%. This method also increases the sentinel exposure time therefore increasing the likelihood of any infections being detected.

Identifying 'new' and 'old' sentinels is essential for this to work.

## Appropriate sampling

Wherever possible the samples taken for screening should be appropriate to the agents being screened for; faeces for Gastrointestinal infections (GI), throat swabs for respiratory infections and so on. PCR allows samples to be pooled (up to 10 individuals) so decreasing the cost of screening.

Pooling large numbers of samples is tempting but can also be a problem. Faecal samples can vary in the amount of positive material present, so a weak signal can be diluted out in pools of large numbers, leading to false negative results.

Conversely, a positive result from a large pool will require further tests to pinpoint the originator(s) of the positive material.

## Exhaust air dust screening

A recent development in the application of PCR as a screening method is the use of exhaust air dust from the exhaust plenum filter of each rack/AHU. The main problem being that you cannot control for any background levels of nucleic acid from infectious agents originating from diet (from wild mice during manufacturing process or during storage) so its use as a primary method of screening is potentially problematic.

## Soiled bedding transfer protocol

There are some basic changes that can be made here in order to maximise the transfer of any infectious agents. During each change, only 1 row on the IVC should be transferred to the sentinel cage e.g. Week 1 – Row 1 and so on until all rows have been transferred at least once.

Only occupied cages should have their bedding transferred and the volume of bedding transferred should be adjusted to ensure maximum levels of exposure occurs.

Once all rows on a rack have been transferred, there should be a period of 4 weeks where the sentinels have clean bedding to allow any sero-conversion to take place prior to screening.

**The transfer of dirty cage lids and dirty water bottles** from stock cages to the sentinel cage on each soiled bedding transfer can help to increase the chance of transmission of any infectious agents to the sentinels, particularly those agents which do not transmit easily by soiled bedding alone. Any enrichment can also be transferred to the sentinel cage.

## Non-destructive sampling

It is now possible to sample and screen animals effectively by using non-destructive methods:

- Tail bleed for viral screening by serological testing.
- Faecal, throat and fur-swab PCR for bacteria and parasites.

By combining these methods with a rolling sentinel programme, we can reduce the numbers of animals used by up to 50% without compromising the robustness of your screening. There may even be an increase in the effectiveness of sentinels by increasing their overall exposure time to any infections.

## Limits of techniques used

It is always worth bearing in mind that each technique used in health monitoring has its own limits. Such as, serology being indirect detection of viruses and PCR indicating only the presence/absence of nucleic acid of infectious agents with no information on infectivity. By combining these techniques with direct observation methods such as microbiological culture, parasitology by microscopy and gross morphology we increase the likelihood of zeroing in on genuine infections.

## Ask your screening provider for advice

Health Monitoring is a complex process and it can be hard to know which techniques or which tests to use. Also the interpretation of results and the significance of any findings can be confusing. The laboratory that carries out your screening for you, should be able to provide any support and advice needed, free of charge.

## Discussion

It is worth highlighting that all health monitoring assays currently available to us are not 100% perfect. That is to say that each method on its own will have certain limitations on the knowledge that can be gained from its

results e.g., Serology will only tell you if an infection has occurred at some point in the past, rather than an active infection. PCR can only tell you if nucleic acid from a specific agent is present or absent, with no information on whether the agent is dead or alive or indeed a product of an active infection. The more direct methods available in health monitoring of microbiological culture and parasitology by microscopy can only be carried out effectively by necropsy, but these methods do demonstrate that an active/viable infection is happening at the time of sampling, along with providing opportunity to gain an overall picture of the animals general health.

With these points in mind, the logical way forward, if we want to reduce the numbers of animals used in health monitoring programmes without compromising confidence in the results, it is recommended that a programme which uses a combination of the methods available to us is used.

Relying on one or two methods for health monitoring potentially raises the likelihood of missing infections or the occurrence of false positives which may be acted upon without the possibility of checking results by another method. It could be said that the 'gold standard' in health monitoring should be to detect an infectious agent in the animals themselves by more than one method. The combined screening approach allows this to happen, as well as reduce the overall numbers of animals used. This approach also goes some way to fulfilling the recently proposed 5Rs (Reduce, Refine, Reuse, Robustness and Repeatability).

## Acknowledgements

The author would like to thank Owen Hazelby, Samantha Lupton, James Salmons, Peter Scobie-Trumper, Mark Staplehurst, Alistair Thompson and Frank Ward for their support in the production of this work.

## References

1 **FELASA** (2014). Recomendations for the health monitoring of mouse, rat, hamster, guinea pig and rabbit colonies in breeding and experimental units. *Laboratory Animals 2014. Vol 48(3). 178-192.*

2 **Dickinson, A., *et al*** (2016). Caveats of PCR. An overview of the caveats of PCR as a Primary Method of Laboratory Animal Health Monitoring. Dickinson. A, et al. *LASA Forum, Summer 2016.*

3 **Leblanc, M. *et al*** (2014). False Positive Results after Environmental Pinworm PCR Testing due to Rhabditid Nematodes in Corncob Bedding.. *J Am Assoc Lab Anim Sci. 2014 Nov; 53(6): 717-724.*

4 **Thompson, A.** (2012). If PCR is always the answer, then perhaps you are asking the wrong questions. Thompson A. Guest Editorial, *Lab Animals Europe Vol 12(8) Aug 2012.*

5 **Dickinson, A. *et al*** (2015). Comparison of faecal PCR with traditional methods in the detection of Syphacia obvelata and Pasteurella pneumotropica. (Poster*). Animal Technology and Welfare 2015 Dec: 14(3): 221-222.*

6 **Dickinson, A., *et al*** (2016). Contaminating DNA can give false positives in "Sentinel Free" health monitoring by PCR on IVC exhaust air dust samples. (Poster) *Laboratory Animal Science Association Annual Conference. November 2016.*

7 **Henderson, K.S. *et al*** (2013). Efficacy of Direct Detection of Pathogens in Naturally Infected Mice by Using a High-Density PCR Array. *JAALAS Nov 2013 Vol 52(6) p763-772.*

8 **Dickinson, A.** (2017). Combined screening strategy to reduce the numbers of sentinel animals used, whilst maintaining confidence in results (Poster) *Animal Technology and Welfare*. Vol 16.3. pp 229-231.

# An electronic based experimental protocol has 'ARRIVEd'

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## Background and Objectives

Historically Study Protocols are paper based. At the Institute of Cancer Research each research group generated their own protocol template making documents difficult to follow for persons conducting out of hours support as well as the Home Office Inspector. Other additional disadvantages experienced with the paper systems such as potentially slow approval processes where study proposals are passed back and forth between submitter and approver, the lack of version control to approve documents or even the unnecessary flow of potentially 'dirty' documents into the animal facility.

In an attempt to revolutionise and harmonise our Study Protocol design we have developed an online protocol and submission process. This has streamlined the process and eradicates many of 'copy and paste' errors associated with previous versions.

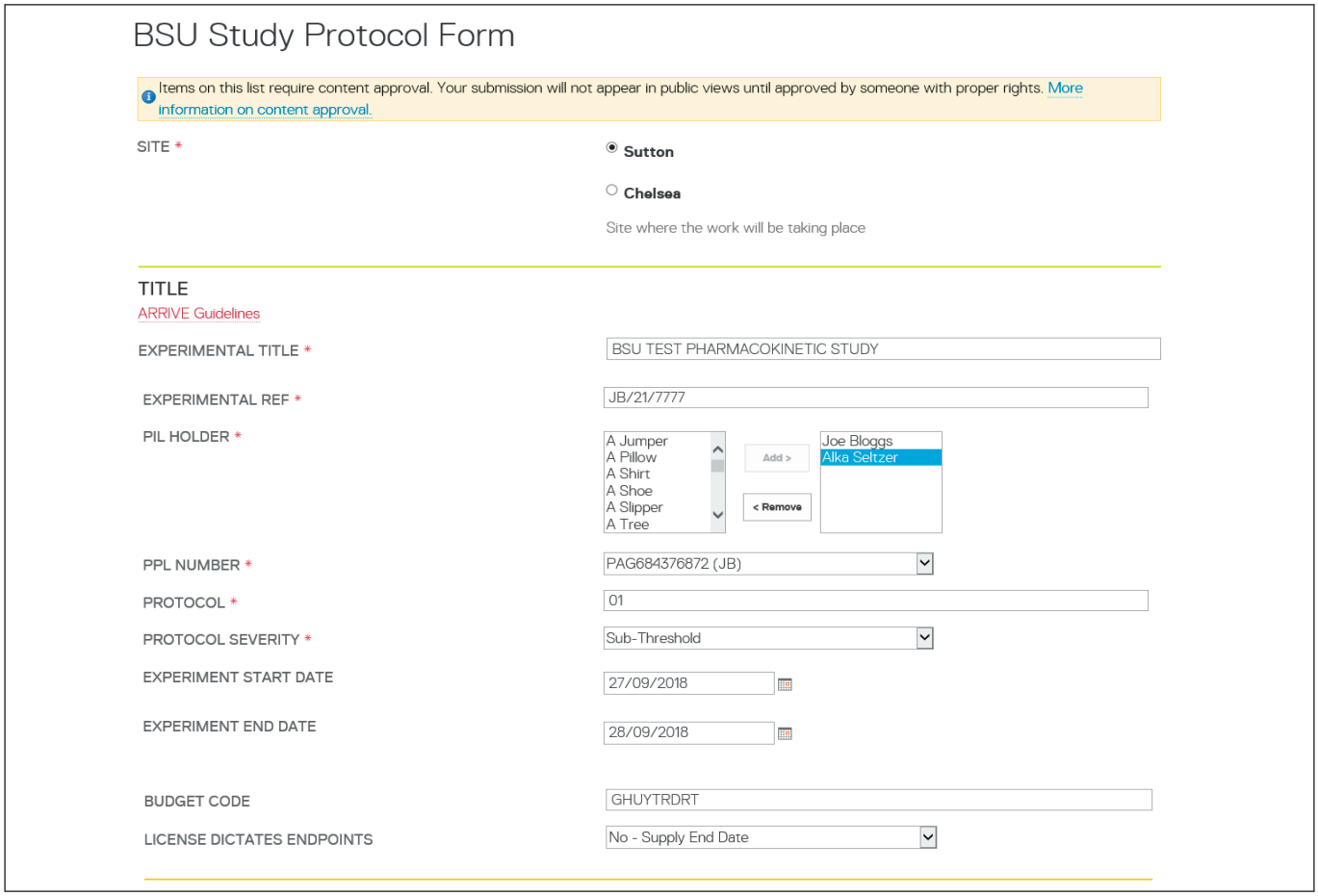



**Figure 1.** BSU Study Protocol form.

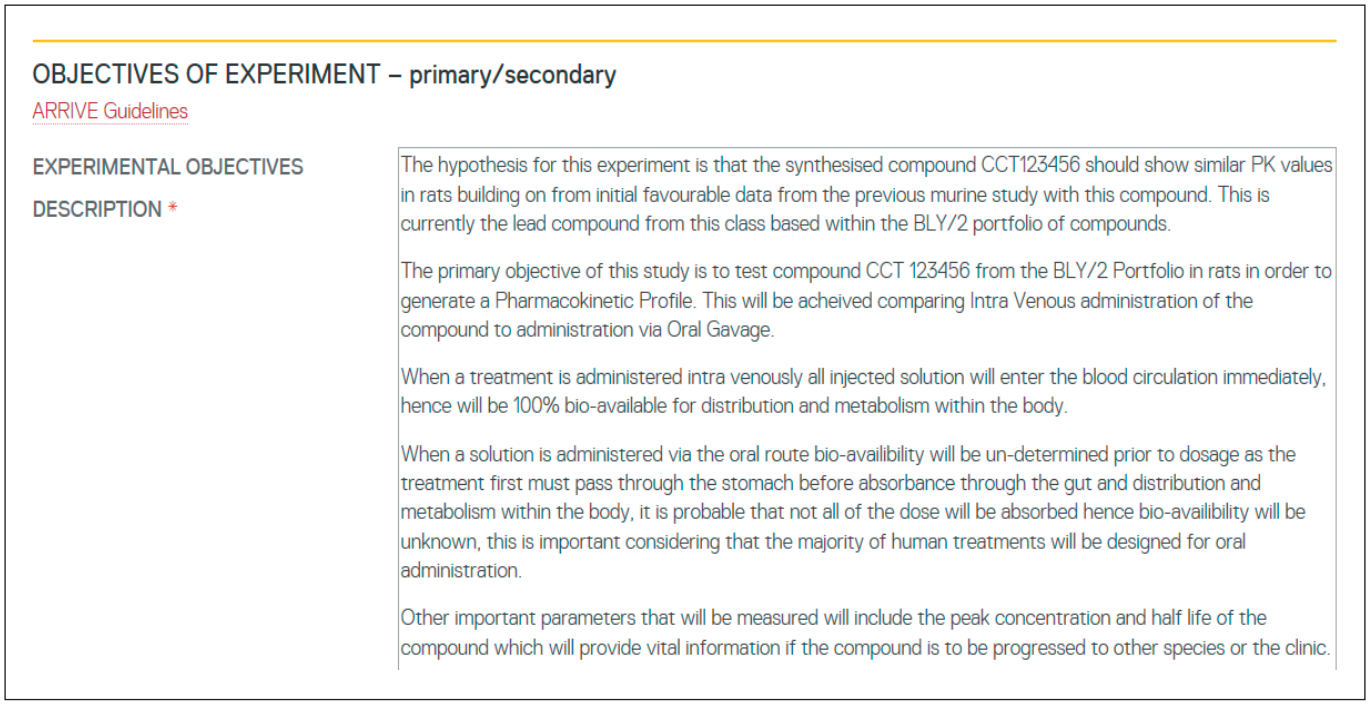



**Figure 2.** Screen shot of the Objectives of Experiment section.

## Creating a document

Protocols are initiated via the internal main intranet. It is possible for any member of staff to submit a protocol. Submitters are only permitted to view their own documents and each study must be assigned to one or more active Personal Licence (PIL) holders. This is made possible by the large volume of drop-down fields embedded within the document. To minimise data input errors Project and personal licence numbers along with PIL contact details have been uploaded to a separate database that links with the protocol and will fill in details automatically.

## Content

The submitter is encouraged to be as detailed as possible when explaining their proposal. The protocols should be detailed enough to permit repeatability of study conditions as well as ensuring that other PIL holders involved with the study can follow.

Particular attention is drawn to the objectives or hypotheses for the experiment to be clearly defined. A number of pre-populate drop down boxes are provide to ensure accuracy.

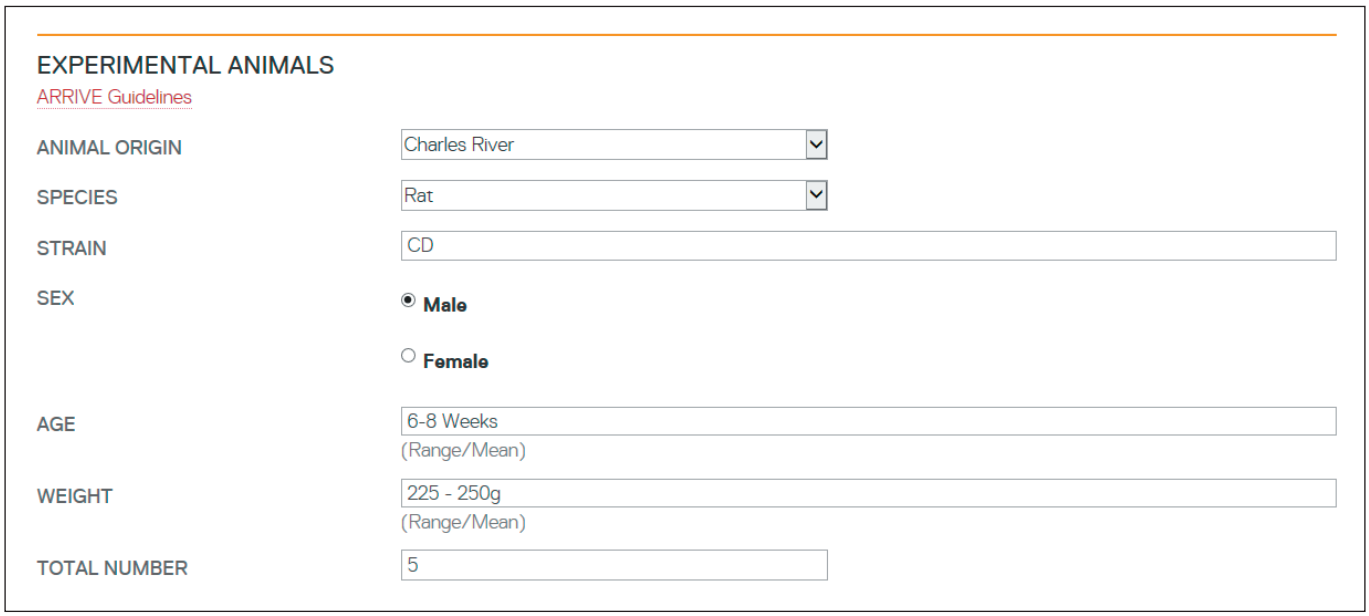



**Figure 3.** Screen shot of the experimental animals section.

## The ARRIVE Guidelines

**A**nimal **R**esearch: **R**eporting of ***In Vivo*** **E**xperiments guidelines are intended to improve the reporting of research using animals – maximising information published and minimising unnecessary studies. The electronic protocol has been developed with full consideration to the ARRIVE guidelines, each title section has a direct link to the guidelines enabling the submitter to fully understand the requirements for each section. This places Animal Welfare at the core.

The protocol template has been designed to capture the spirit of the 3Rs through the questions it poses the submitter, aiding the publishing of work or repeating experiments. The protocol is primarily a working document, so a balance was found as to the areas of ARRIVE to include in the study design and areas to include in the study design and areas to omit until the write up phase of the experiment.

### SAMPLE SIZE CALCULATION

ARRIVE Guidelines

NO. OF EXPERIMENTAL ANIMALS * 3

NO. OF CONTROL ANIMALS * 2

EXPERIMENTAL UNIT * Animal

### STUDY DESIGN

ARRIVE Guidelines

DESCRIBE ALLOCATION METHOD

Prior to ordering animals from the external supplier it is essential to ensure that sufficient resources are available to support the study for example compound availability and space to house the animals and perform the study. A ready supply of consumables should always be available and must be ordered in advance.

A completed study protocol will be sent electronically in advance of the study to the Project License Holder (PPL) and Named Animal Care and Welfare Officer (NACWO) to authorize the study start.

Animals will be recieved from an external supplier at the Biological Services Unit (BSU). Upon receipt in to the building animals will be randomly allocated in to experimental groups, all animals will be housed in individually ventilated cages and fed an irradiated diet water will be provided ad-libitum, unfortunately diet supplements such as "forage mix" will not be supplied due to potential interferance with experimental data.

When animals have been allocated and housed they will be transported to a holding room within the BSU and allowed to acclimatize, this will allow factors such as transport stress or settling the hierachy within the group to settle or form, Acclimatization will be for a minimum of 7 days prior to enrolment on a study.

During acclimatization animals will be routinely handled by BSU staff during cage maintenance or health checking activities. 24 hours prior to the start of the study all animals will have their tails carefully washed with lukewarm water and hibiscrub, this helps to acclimatize the animal with the handler, additionaly a clean tail is easier to cannulate and will reduce the potential risk of infections.

All procedures will be performed in a Microbiological Safety Cabinet (MSC) therefore prior to the start of the

Cage 1 - Controls

Vehicle Treated Control - 1 Animal (Identified as No.1)

Non-Treated Animal for Control Blood - 1 Animal (Identified with No Mark)

Cage 2 - Compound CCT 123456

Drug Treated - 3 Animals (Identified as Animal Nos. 2, 3,and 4)

The study will proceed as follows -

Collect Control Blood from the designated animal - this will allow the lab analysts to optimize their methodology and produce accurate base line curves for analizing the main study, this animal will be housed with the control vehicle animal for the remainder of the study to provide companionship reducing variability within the study.

27/09/2018 - IV Phase (Please see Experimental Procedures for Specific Details).

28/09/2018 - IV Phase concludes after 24hr timepoint. Cannulas removed, animals allowed to rest and "washout"

04/10/2018 - PO Phase - Animals re-canulated (Please see Experimental Procedures for Specific Details).

05/10/2018 - PO Phase concludes after 24hr timepoint. Animals culled by Schedule 1 method.

05/10/2018 - Samples transfered to bio-analylitical laboratory for analysis.

## Electronic submission and version control

Electronic submission and approval means that documents only require printing when approved, this eliminates the requirement to transfer paper items from outside of the facility reducing the risk of pathogen transferal and eradicates the risk of an unapproved version being used as the 'official' version.

Version Control means that changes are more easily tracked and that the approved version is easily identified.

EXPERIMENTAL PROCEDURES

ARRIVE Guidelines

DESCRIBE PROCEDURE(S)

**27/09/2018 - IV PHASE**

Animals will be allocated and identified as described in the study design section of this protocol.

All animals will be assessed for good health and weighed to calculate dosage volumes on the day of the study, weight information will be noted in the attached form to this protocol..

Animals will be gently warmed prior to cannulation for 5 minutes at 37 degrees C, to promote vasodilation.

Animals will be cannulated in one of the lateral tail veins using 24g BD Angiocaths (cannulas), angiocaths will be secured using two short lengths of Tensoplast and flushed with 0.2ml of pre-prepared Heparin/ Saline solution to maintain patency, the cannula will be secured so that it is not overly tight to the tail or subversly not overly loose, either scenario is likely to lead to the cannula being chewed out.

Control blood will be collected from the control blood animal prior to the study start - 1000ul of blood will be collectected via the cannula on to heparin and transported on wet ice to the bio-analytical lab for the team to optimize their methods and create accurate baseline curves for the further analysis of the study.

Drug formulation will be prepared as per the instructions laid out below in Drug Formulations.

When the formulation is in solution, gently warm animals for 5 minutes at 37 degrees C, to promote vasodilation and prepare injection syringes. Needles (27g Butterfly) and Syringes (1ml) will be prepared with the test and vehicle substances (one per animal) IV substances must be in solution with no blemishes and at room temperature with a neutral pH at the time of dosing.

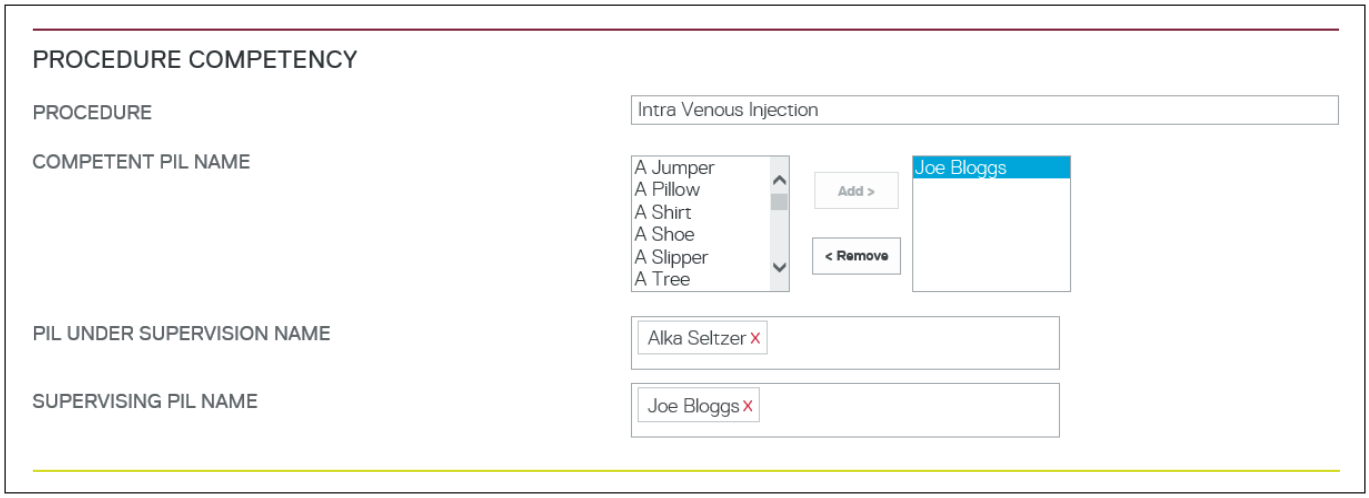
Dose animals Intra Venously in opposite lateral tail vein, to that of the cannula, stem bleeding and place back in warming box, animals will be injected at 1 minute intervals.

| | |
|---|---|
| TUMOUR TYPE | BAL456 |
| INDUCTION METHOD | Sub Cutaneous |
| INDUCTION SITE | Right Flank |
| TUMOUR SIZE LIMIT | 12mm |
| MEASUREMENT REGIME | Twice Weekly |
| WEIGHING REGIME | Twice Weekly |
| CELL BATCH | 123456 |
| DATE SCREENED | 27/09/2018 |
| RESULT ATTACHED | (•) Yes ( ) No |

| | |
|---|---|
| SURGERY | ☐ **Yes**<br>☑ **No**<br>☐ **To be Determined** |
| ANAESTHESIA | ☐ **Yes**<br>☑ **No**<br>☐ **To be Determined** |
| ANAESTHETIC METHOD and AGENT | N/A |
| ANALGESIA | ○ **Yes**<br>◉ **No** |
| ANALGESIC ADMINISTRATION ROUTE | N/A |
| ANALGESIC REGIME | N/A |

## EXPERIMENTAL PROCEDURES - THERAPY DETAILS

| | |
|---|---|
| AGENT(S) | CCT 123456<br>Agent 1 |
| BATCH REF | 1<br>Batch Ref1 |
| DRUG FORMULATION, DOSAGE (mg/kg, mg/ml, ml/kg), and TREATMENT REGIME | **IV Formulation**<br>Compound will be dissolved in DMSO (10%) before adding Tween 20 (5%) vial will be sonicated for 10 minutes before transfer to the BSU.<br>10 minutes prior to dosing Saline (85%) will be added and the formulation vortexed for 1 minute.<br>The formulation will be made up at 1mg/kg (1mg/ml) and must be a clear solution (See Formulation Sheet).<br>A single intravenous dose at 1ml/kg of the formulation will be administered<br>DRUG FORMULATION, DOSAGE (mg/kg, mg/ml, ml/kg), and TREATMENT REGIME 1 |
| AGENT 2 | CCT 123456 |
| BATCH REF 2 | 1 |
| DRUG FORMULATION, DOSAGE (mg/kg, mg/ml, ml/kg), and TREATMENT REGIME 2 | **PO Formulation**<br>Compound will be dissolved in DMSO (10%) before adding Tween 20 (5%) vial will be sonicated for 10 minutes before transfer to the BSU.<br>10 minutes prior to dosing Saline (85%) will be added and the formulation vortexed for 1 minute.<br>The formulation will be made at 1mg/kg (0.2mg/ml) and must be a smooth suspension (See Formulation Sheet).<br>A single gavage dose at 5ml/kg will be administered |



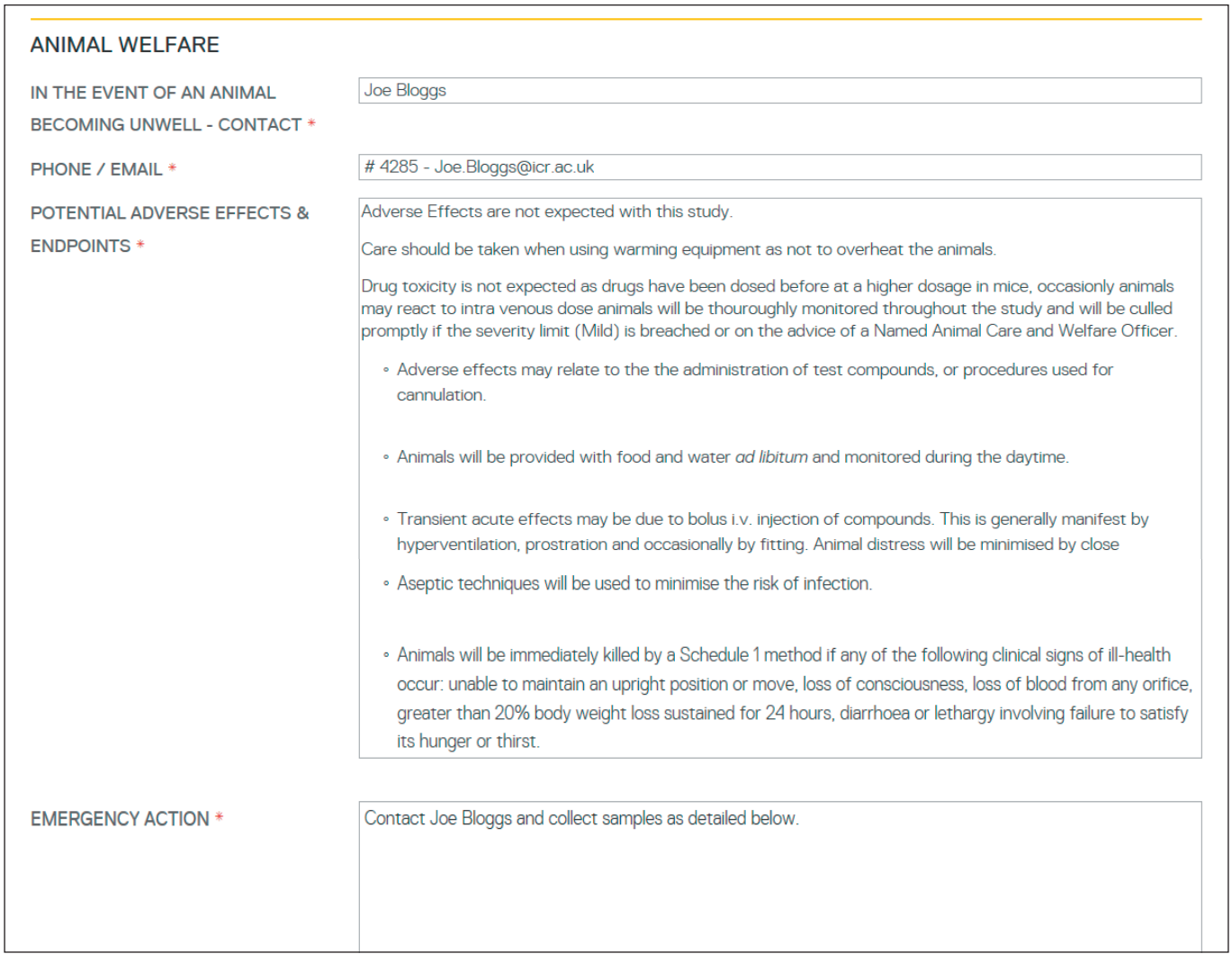

## Procedural Competency

Study Proposers are required to declare all regulated Schedule 1 techniques to be performed within the study proposal in the procedural competency section of the submission.

This will detail PIL holders whom are signed off within their Personal Training Record and those whom are working under supervision. In such cases a supervising PIL holder from the identified list must be indicated.

## Auditing

Competency Declarations are spot audited to Personal Training Records by the Named Training and Competency Officer (NTCO) and inaccuracies treated as a non-compliance.



## Submission Process

At each stage of the submission process both submitter and approver will receive email notification of all status changes to the document.

An approver will check the study proposal and either approve or reject it, when approved the document will be printed, signed and dated. Rejected submissions will be electronically redirected to the submitter with an explanation for editing and resubmission.

Approvers will be Named Animal Care and Welfare Officers (NACWO) or NACWO trained.

The primary advantage of electronic approval is the ease and speed of ensuring the documents reaches the correct person, this allows the reviewer to check the study proposal at a time when they can give it full attention.

## Features and Benefits

- Faster approval process.
- Drop-down boxes employed within the template to aid accuracy of information.
- Author can save document as draft to re-edit prior to submitting.
- Submitters must declare full Project License authority for their study.
- Training and Competency auditing may commence prior to the study commencing.
- Version History allows both submitters and reviewers to track document changes and gives Quality Control as to the 'Approved Version'.