TRAINING SCHOOL IN EXPERIMENTAL DESIGN & STATISTICAL ANALYSIS OF BIOMEDICAL EXPERIMENTS REPORT

Supporters:

February 1-3 2016

Voss, Norway

FRAME delivers regular training schools in experimental design and statistics to increase awareness among scientists about the need to reduce animal numbers in experiments and to refine procedures. Participants gain a better understanding of how to properly design and effectively analyse their experimental programmes so that they can go on to produce higher quality science, which has made the most efficient use of a minimum number of animals.

Training School in experimental design & statistical analysis of biomedical experiments report

VOSS, NORWAY

INTRODUCTION

Russell and Burch (1959)[1] identified that the way in which reduction in laboratory animal use can most effectively be achieved is by rigorous experimental design and appropriate statistical analysis of any results. An effective experimental design must incorporate the aims of the work to be carried out, the choice of experimental animals and techniques, the parameters to be measured and the methods to be used for analysis of the results. However, there is a general lack of knowledge on the part of biologists about these issues, and an urgent need for statistical experts to appreciate the issues and problems that biologists face. A Training School involving these individuals was an ideal opportunity to facilitate dialogue and enhance the application of experimental design and statistical analysis to animal experimentation to improve: a) animal welfare; b) the amount of information from a given number of animals involved; and c) the quality of biomedical research and testing.

The tenth Training School was co-organised with Norecopa and the University of Bergen and was held in Voss, Norway. It received CPD accreditation from the Laboratory Animal Science Association (LASA), UK. In addition to the *ecopa* travel bursary detailed below this event was supported by Laboratory Animals Ltd and the Norwegian Society for Protection of Animals, NSPA (Dyrebeskyttelsen Norge) whose generous contributions allowed the registration fee to be subsidised.

OBJECTIVES

The key objectives of the Training School were:

- to provide researchers from across Europe with an understanding of basic design concepts that they do not seem to be gaining from other sources,

- to give them the ability to use more efficient designs for their experiments, and

- to stimulate engagement with the Three Rs and useful discussion between animal users in industry and academia on both refinement and reduction.

STRUCTURE

The programme (appendix 1) was taught by 6 expert tutors:

- Dr Michael Festing

- Dr Derek Fry

- Dr Michelle Hudson-Shore [also acted as Training School coordinator]

- Dr David Lovell

- Professor Adrian Smith [also acting as local coordinator]

- Aurora Brønstad

1. Russell, W.M.S. & Burch, R.L. (1959). The Principles of Humane Experimental Technique, 238pp. London, UK: Methuen and Co.

The format included lectures, group discussions, and individual exercises. The programme was structured to lead the participants from simple experimental design and statistical ideas, through more complex methods and analysis to effective presentation of findings. Participants were also able to discuss their own research problems/experiences with the Training School tutors. This interactive approach strengthened and supplemented the information given in the more traditional lectures. The residential nature of the School also fostered networking and dissemination of information between participants.

PARTICIPANTS

On this occasion as the hosting country the majority of participants were from Norway but the School also attracted participants from nine other countries (table 1) and a variety of research backgrounds. The majority of participants were postgraduates and postdoctoral researchers, but several other roles were also represented including senior members of staff, such as a professor and head of department (table 2).

Table 1: Origin of participants who attended the Training School, February 2016

Country	Number of Participants
Norway	26
UK	8
Denmark	7
Netherlands	2
Canada	2
Switzerland	1
Germany	1
Singapore	1
Sweden	1
France	1
Total	**50**
No. on waiting list	**11**

Table 2: Scientific roles represented among participants, February 2016

Scientific Position/Role
PhD Student
Post Doc Research
Scientist/Scientific Officer
Masters Student
Researcher/Research Assistant
Biostatistician
Head of Department
Project Leader
Professor
Research Technician
Innovation Coordinator
Technical Manager
Senior Advisor
Veterinarian
Senior Lecturer
Associate/Assistant Professor
Customer Service Manager
Section Leader
Chief/Principle Engineer
Residency in Laboratory Animal Science

OUTCOMES

Participant Feedback

The Training School was very well received with participant's providing very positive feedback. The majority of participants who provided feedback said that they would recommend the course to colleagues, that the instructors provided helpful assistance and agreed that the course had exposed them to new knowledge and practices (see appendix 2 for a full summary of responses). Many participants made additional comments, such as:

> **The content of the course was excellent. I have learnt a lot and got a good sense of the topics I want to study further.**
>
> **I feel very lucky to have participated in this School, because I came across with the basic principles of protocol design, updated my knowledge about methods of statistics, different programs and met experts of this field, who did their best in order to pass their knowledge and share their expertise with us. After this experience I feel more confident to consult researchers, I am more efficient during discussions and I can prove and explain more accurately my ideas to them.**

Knowledge Acquisition

To gain insight in to how successful training of this nature is the Training School tutors designed a set of questions which were given to the participants at the start and end of the school. The answers to these questions were collated and analysed to determine the participants existing knowledge of the subject and then to establish if and what they have learned as a consequence of the training. There was an increase in understanding of all the areas tested (figure 1) and the individual overall scores also increased after the training (figure 2).

Figure 1: Comparison of the overall understanding by area of experimental design, before and after training by participants in the Voss Training School 2016.
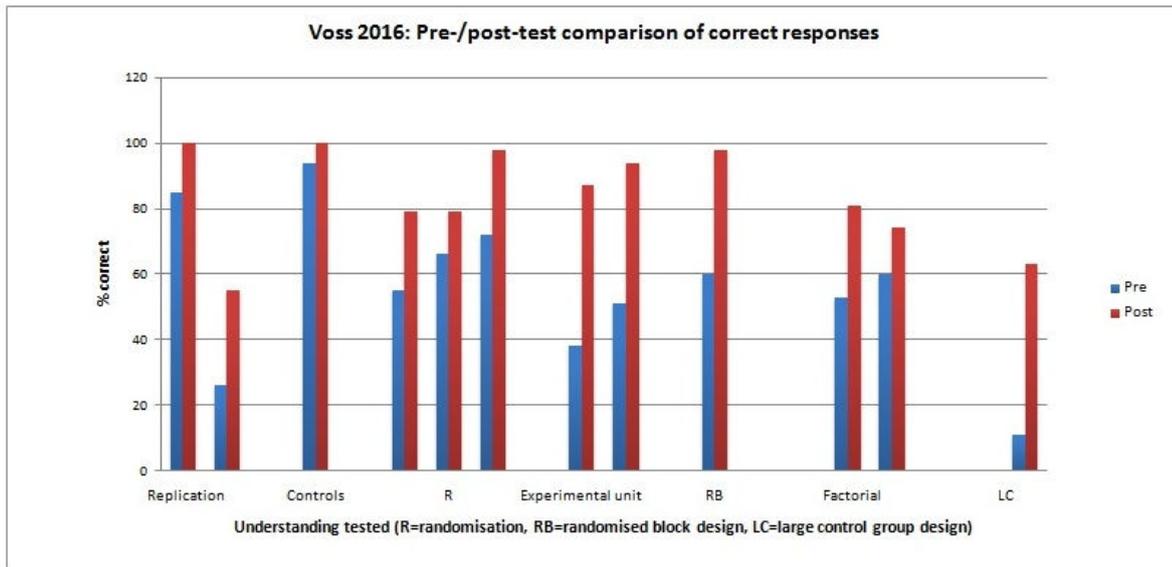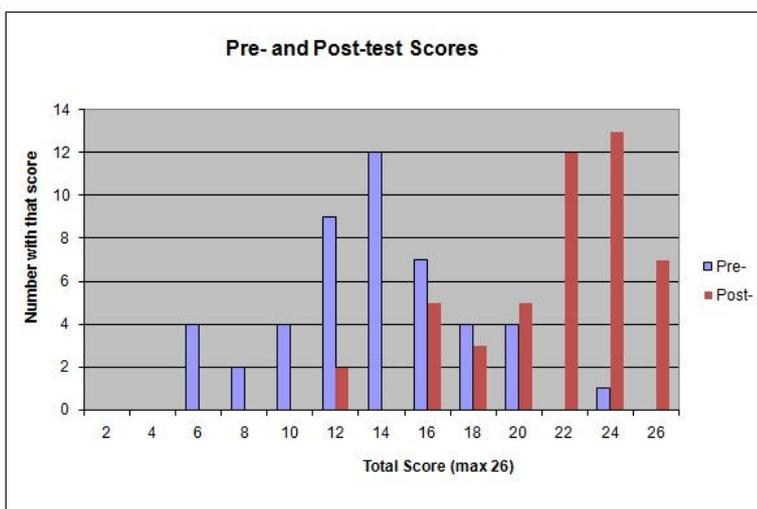

Figure 2: Comparison of participants overall test scores before and after the Voss Training School, 2016.

TRAVEL BURSARY

Thanks to the European consensus-platform for alternatives (*ecopa*) we were able to offer four travel bursaries to help young scientists to attend the event. This could be used towards travelling to attend and the application criteria were:

1.   Be engaged in laboratory animal science
2.   Be able to attend the whole course
3.   Not attended the Training School before
4.   Applicants must send an overview of their expected travel expenses
5.   Priority will be given to young scientists

One of the conditions of accepting the bursary was that the recipient provided a short report on the impact the training would have on their own research and more broadly in their institution/country, including how they intend to disseminate the knowledge they acquired within their network (appendix 3). The four recipients were: Jassia Pang, Singapore; Alexandra Durrant, UK; Alexandros Zervas, Greece and Susannah Williams, France.

The availability of this bursary demonstrated the demand for training of this nature. Due to the timing of the award from *ecopa* it was only possible to provide a week long opportunity to apply. Despite this short time scale, 33 applications were received from 14 different countries including, Ethiopia, USA, Australia and Singapore.

Appendix 1	Training School Programme, Voss 2016

Session Number	Session Title and Content
Sunday	**Informal Discussion and Introductions:** *Hotel Bar*
Monday	**Basic Principles**
	Registration – Outside conference facilities on lower ground floor
1	*Introduction to course: The Three Rs, legal and ethical aspects.* *Quiz 1.*
2	*Principles of experimental design. Need for better design. Types of experiment (pilot, exploratory, confirmatory), objectives, controls, experimental units, replication, randomization, blinding.*
	BREAK
3	*Common failings: unclear objectives, bias, lack of power, failure to randomize/blind, pseudoreplication. Costs of poor design.*
4	**Group Exercise 1:** *Objectives, controls, experimental units.*
	LUNCH
5	*Revision of basic statistical inference. Null and alternative hypotheses, SD vs. SE, outliers, type I & type II errors, variables affecting significance, summary statistics.*
6	*Sources of variability in animal studies and how they may be controlled.*
	BREAK
7	**Computer session**: *Simulating experiments and the importance of controlling variability. Randomisation, sampling, Type 1 and Type 2 errors*
	DINNER – Hotel Restaurant
Tuesday	**Experimental Designs and Statistical Analysis**
8	*The analysis of variance, interactions, post-hoc tests, assumptions, data transformations.*
9	**Group Exercise 2:** *Finding design faults.*
	BREAK
10	*Completely randomised, randomised block and latin square designs. Power calculations, resource equation.*
11	*Qualitative data, contingency tables, non-parametric tests.*
	LUNCH
12	*Factorial "designs".*
13	**Group Exercise 3:** *Choosing the right design & over-night exercise.*
	BREAK
	SOCIAL EVENT – Visit local waterfall
14	*Experiments to test relationship: correlation, regression.*

15	*Computer session*: ANOVA, power analysis and software.
	DINNER – Hotel Restaurant
Wednesday	**Applied Experimental Design and Presentation for Publication**
16	*Discussion of overnight exercise: Planning an experimental programme.*
17	*Presentation of results: The ARRIVE guidelines, summary statistics, graphs, diagrams, tables.*
	BREAK
18	***Quiz 2 and discussion***
19	***Parallel Sessions 1:*** ***Group Exercise 4:*** *Write an experimental protocol in your field that would meet the ARRIVE Guidelines* ***Discussion of participants problems***
	LUNCH
20	***Parallel Sessions 2:*** ***Group Exercise 4:*** *Continued* ***Discussion of participants problems***
21	*Literature searches and systematic reviews*
22	*Databases and other 3R-resources*
23	*Answers to Quiz 2 & take home messages.*
	CLOSE

Appendix 2	Training School Feedback Summary, Voss 2016

The Design of the Course

The objectives of the course were clear to you	Agree **42**	No opinion **1**	Disagree **3**	
The course contents met with your expectations	Agree **41**	No opinion **1**	Disagree **4**	
The lecture sequence was well planned	Agree **39**	No opinion **6**	Disagree **1**	
The contents were illustrated with adequate examples	Too few **10**	Enough **34**	Too many **2**	
The academic level was appropriate	Too low **1**	Correct **39**	Too high **6**	
The course exposed you to new knowledge and practices	Agree **45**	No opinion **0**	Disagree **1**	
You would recommend this course to your colleagues	Agree **43**	No opinion **2**	Disagree **1**	

The Conduct of the Course

The lectures were clear and easy to understand	Agree **38**	No opinion **6**	Disagree **2**	
The course material handed out was adequate	Agree **31**	No opinion **7**	Disagree **8**	
The computer lab sessions were clear and easy to understand	Agree **29**	No opinion **10**	Disagree **7**	
The instructors provided helpful assistance	Agree **38**	No opinion **6**	Disagree **2**	

Background Information

How did you hear about the School?	Internal email **12**	Colleague **25**	FRAME website **3**	Some ticked >1
Other, please specify: **4** (Inc. advisor, Norecopa and VOLE)	Flyer **0**	Direct email **6**	Facebook, Twitter **0**	

Future Training

I would be interested in further training to be able to teach the topics covered in this course	Definitely **19**	Maybe **17**	Not at all **10**	

Total Forms 47/50 (92%)

Appendix 3	Bursary Recipient Reports, Voss 2016

NAME: Alexandra Durrant
AWARDED: 257.54€

REPORT: During my PhD I am going to investigate maturational differences in pain pathways and immune systems, and how these two systems interact. In order to do this I would need to collect tissue from a range of rodents at different ages, and so I want to ensure that my experimental designs, and practical techniques, are of the highest quality and standard before beginning any animal work. Developmental studies also require careful planning from a practical and statistical point of view, and as a first year doctoral student, this course will prove to be invaluable across these areas from the start to the end of my project as I now have increased knowledge and understanding of how to properly design experiments, which will strengthen my data, and more importantly reduce the numbers of animals I will need to use to collect the data.

I am an active member of the PhD community at my university with over 50 students in my year, us being the fourth cohort of students to undertake this Life Sciences PhD programme as part of a BBSRC funded doctoral training programme. I am therefore able to communicate with numerous students in all stages of their PhD, many of whom work with animals. As well as being able to pass the information and training I received from the FRAME school to PhD students, I am involved in helping final year undergraduate Neuroscience students, and so will share information with students who may be considering a career in research. My supervisor and his colleagues lead large groups of many research scientists and they actively encourage dissemination of best practice to other colleagues either in the form of informal seminars or more formally through research lectures. I will soon be giving a presentation to my research colleagues regarding the information I received from FRAME, especially the information concerning the experimental unit, as I found this to be the most interesting and valuable thing I learnt. Being able to relay information on how to correctly determine the experimental unit has the potential to prevent pseudoreplication in almost all of my colleagues' studies, and thus will reduce the need for further research and use of animals to properly replicate the results. I have made my notes from the school available to my research group so they can access this information at any time, and have introduced posters and booklets on good search practice on animal alternatives into our laboratory. My colleagues and I are committed to the 3Rs and I am certain that new information and practices I have been exposed to on this course will be actively disseminated to our immediate research groups and more widely to the entire research community at The University of Nottingham.

I have also been given the opportunity to present at a poster at the International Association for the Study of Pain (IASP) conference in Japan, which means that I will be able to share information and practices I learnt at the FRAME training school with potentially hundreds of animal researchers from across the world.

I would like to thank FRAME and the University of Bergen for organising the event, and I would like to extend my appreciation to eco*pa* for helping to provide me with funds to be able to attend the school.

NAME: Susannah Williams
AWARDED: 252.16€

REPORT: Prior to attending the course I had worked on projects involving animal models to test new therapies for neurodegenerative brain diseases and tested the therapies with behavioural cognitive and motor tests, but these projects had been finalised before I began working on them. It was becoming more and more apparent as new projects were coming up that I needed to better understand the stats behind planning an experiment to get enough power in the result instead of using an arbitrary number of animals which may be more or less that needed (either way is not good).

The training course was extremely well set out, with a mix of lecture style and interactive slots that meant the day did not became monotonous and made everyone an active participant. Which when I was later talking with some colleagues about the course I would frame certain points firstly with a question on what did they know about it, making them think first and leading to a discussion which included what both parties had learnt from different sources rather than a one person monologue of a fact where you can never be sure if the listener is really taking it in. For example over a work lunch with a mix of post docs and a stagiaire (french apprentice placement students) we argued what the n number should be in two different situations - the stagiaire got it incorrect which shows how stats are not necessarily fully understood post BSc degree level and the importance of either attending extra courses or actively discussing science and disseminating knowledge when you find gaps in each other's info is really important for basic understanding and building on ideas.

So on a social work level information has been spread and the course has already enhanced group discussion both directly of stats and when talking about hypothetical experiments and how you would need to set it up.

Secondly, in a more direct way relating to projects in existence, I have been working on some behavioural tests with rats looking for the best way to test for a deficit in this animal model, and with the pilot data I now have the course has equipped me with the right thinking of what tests to do next, from the simple steps of finding my significant difference to do the power equations to

how i will set up the proper experiment and the treatments each animal will receive - not all animals can be tested on the same day, make sure I either keep these groups the same or randomise. But also the next macaque studies we will do blinded results analysis to try and minimise user bias.

Thirdly, and which has yet to happen, I will have a meeting with some of the people on one of the rat projects to discuss what was learnt on the course in the context of the next experiment and how and why we should run in the way I recommend - of course no doubt this will be a two way exchange of info and the result will be based on a mixture of their experiences - there is an approx. 5% loss of animals before the experiment will be finished. But in this set up the knowledge of what has been gleaned from the course will be given with most impact and in a way easily transferable to their future studies. I have also summarised a lot of the power points in the the slides I think most important for easy reference to flick back through and to demonstrate points easily to others.

As for future courses, I would recommend this course to researchers I speak to who are coming into animal experimentation and project planning as a way to they can find easily understandable and approachable. And for myself I think I will now look at attending a short stats course that covers how to do the general tests on particular software where I hope I can stick to the same software for a few years.

NAME: Alexandros Zervas
AWARDED: 473.63€

REPORT: My name is Alexandros Zervas and I received a bursary to attend the FRAME Training School in Experimental Design and Statistics, which took place in Voss, Norway in February 2016. I am a veterinary surgeon highly interested in Laboratory Animal Medicine and a PhD student at LRMS of Medical School, University of Athens.

The subject of my thesis is "Evaluation of the protective effect of plant extracts in the rat model of osteoporosis" under the supervision of Professor Ismene Dontas. During my 3, 5-year Lab Animal Medicine practice, I always have to be prepared to provide researchers information about difficulties may come up. I try to be well informed and I usually ask for help from experts in different fields (i.e. the statistician). I feel very lucky to have participated in this School, because I came across with the basic principles of Protocol Design, updated my knowledge about methods of Statistics, different programs and met experts of this field, who did their best in order to pass their knowledge and share their expertise with us. After this experience I feel more confident to consult researchers, I am more efficient during discussions and I can prove and explain more accurately my ideas to them. For example, every time a scientist comes to me for assistance in designing a protocol, now I spend some time to explain aspects of Protocol Design which are vital for the analysis later. Researchers and I always try to predict possible difficulties that may come up and find better ways of design to avoid them. Our mutual target is to retrieve the best possible results with as less as possible animals used, the promotion of 3Rs, animal welfare and ethics taken in first account. Everyone involved in research has to feel responsible and to make the correct decision every time in order to reach this target.

This knowledge is also to be transmitted to my colleagues back in London and in Athens. I am going to present the titles of the lectures I attended with my comment at the regular week meeting at both places (RVC, LRMS) to give a glimpse to my colleagues as food for thought and literature research. I have already been asked for my opinion by colleagues, staff and researchers and I really like to contribute with fresh ideas in order to help them to find answers of ambiguous questions, the best possible solution and try to prevent problems that may occur later.

For all these reasons, I have to thank the FRAME and *ecopa* Scientific Committees for this opportunity to attend such an important meeting.

NAME: Jassia Pang
AWARDED: 555€

REPORT: As a research veterinarian, I not only head and collaborate on research projects; I spend a lot of time consulting and reviewing protocols for other biomedical investigators. Having gone through veterinary school and a postdoctoral fellowship in laboratory animal medicine consisting of several biostatistics and basic statistics courses, I thought I had a pretty good albeit basic grasp of designing and analysing animal experiments when I started my new position in Singapore. However I soon realised how much more I did not know as it pertained to animal work specifically, nor were there many others whom I could consult locally and learn from. I then realised the lack of knowledge and thus training in such topics in our community. As part of the system that is to safeguard the responsible and appropriate use of animals in research, I believe it is essential that not only I, but my colleagues as well, need to ensure that we are able to provide comprehensive advice and review of any projects requiring animals. Participating in the FRAME Training School provided the opportunity from which I was able to gather knowledge and also identify resource to help address this issue.

Through the training received, I was able to better understand what an experimental unit is, and thus determine the size of groups required. This is especially crucial for me as I was in the midst of designing a new experiment and applying for a grant. All this while I had based my calculations on previous experience, mentor suggestions and the literature but was never really quite sure if I was right, however now I am better able to justify my numbers using the techniques learnt and I hope this will refine and define the total number of animals I require from here on out. In addition, I am more critical of papers I read having

obtained a more solid understanding of the various ways results are presented graphically. This helps me tease out and have greater appreciation for the findings, as well as hone my skills in reviewing the claims made.

As part of the ethics committee, this training will also greatly impact the way I review protocols. I had previously found myself getting muddled by the different terminologies and not really knowing whether the analyses chosen were appropriate, but now I feel that I will be able to discuss this more confidently both with other committee members and the investigators themselves. At the institutional level, I hold a joint appointment at 2 institutions, and both of which have requested that I share the knowledge obtained from the FRAME Training School. I will be running at least 2 sessions in the coming months, explaining and providing an overview of what we covered. In this way the knowledge spreads, and I am confident this will improve the standard of animal experimentation in our community. I also hope to be able to offer my services to any investigator who needs assistance designing animal based projects, especially those who are transitioning to in vivo modelling.

As part of the scientific committee for an upcoming international conference, I have also raised this as an area of training that should be offered. As a result, we will be running a 3Rs track, including several sessions on animal experimental design. Personally, I intend to continue this learning path, extending my understanding on designing experiments and including non-animal alternatives so that I can provide better advice to the investigators I work with and refine the projects I head. Ultimately, I strongly feel that we as laboratory animal veterinarians strive to have a world where no animal will need to be used as models. But until we get to that stage, it is our responsibility to ensure that the privilege of utilising animals is held to the highest measure. By being able to design and run well-thought animal experiments when required, I believe this is our contribution to steps in the right direction.