Conference Reports

Improving *in vitro* methods by developing and using defined culture media

Copenhagen, 29th November 2009

In vitro methods are widely used to study activities at the cellular level. Furthermore, *in vitro* methods are powerful tools to replace or reduce animal experiments, either as stand-alone, or as part of a testing strategy.

Cells are maintained under well-established conditions, which typically involve incubation at 37°C with a humidified gas mixture of 5% CO₂. Furthermore, the medium to keep the cells alive is important. An often used basal medium is Dulbecco's Modified Eagles Minimal Essential Medium (DMEM). Dependent on the cell type this medium is supplemented with factors essential for proliferation, migration and differentiation of the cells. Usually, serum is used as supplement, often fetal bovine serum (FBS). The use of serum involves several problems: possible contamination, undefined (binding) factors, batch-to-batch variation, etc. The use of FBS also involves an ethical problem; blood collection may cause severe suffering to the animal (van der Valk et al., 2004). It was therefore concluded that the use of FBS should be strongly discouraged and chemically defined media should be preferred when using in vitro methods (Coecke et al., 2005; ESAC, 2008; van der Valk et al., 2004), Around 450 serum free media are now available (www.goodcellculture. com). Still, not for every cell type is a defined medium developed. In addition, the formulation of most commercially available media is not released, and these can thus not be regarded as strictly defined.

To discuss the development of culture media for specific cell types a workshop was organised by the Dutch-Belgian Society for In Vitro Methods (INVITROM), the European Society for Toxicology In Vitro (ESTIV) and the Danish In Vitro Toxicology Network, November 2009, in Copenhagen.

It was concluded that the development of serum-free media and cell adaptation processes is an ongoing process in several laboratories, often without knowledge of research processes, experiences or results of other laboratories regarding this topic. This information, particularly with regard to precise formulations, should be collected and made publicly available to facilitate the further development and use of defined cell and tissue culture media. Several databases already exist that collect this type of information (see below).

The participants supported the recent statement of the ECVAM Scientific Advisory Committee (ESAC) in which the use of serum and other animal components in cell and tissue culture was strongly discouraged and the development of defined media was required (ESAC, 2008). The use of defined media is also suggested in Good Cell Culture Practice (GCCP) (Coecke et al., 2005; Hartung et al., 2002). It was recommended to make GCCP part of Good Laboratory Practice and/or Good Manufacturing Practice to give it a legal basis.

During the workshop, the different components of a defined medium were discussed to facilitate the development of defined culture media. Furthermore, several approaches to adapt cells to serum-free media were discussed. In addition, the participants discussed how they developed a defined medium, also for specific cell types.

Details of the workshop and its recommendations will be published in a workshop report in Toxicology In Vitro (van der Valk et al., 2010).

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More information on already established media can be found on: http://www.focusonalternatives.org.uk/PDFs/FCS-free%20ta-ble%20May%2009.pdf; http://www.goodcellculture.com/

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ETHIOPIA: Alternatives in life science education at Ambo University

Ethiopia, 23rd December 2009

The Department of Biology at Ambo University in Ethiopia organised a one day seminar-workshop on alternatives to animal use in life science education. The event was held on 23rd December 2009 and organised in collaboration with Mahatma Gandhi-Doerenkamp Center (MGDC) for alternatives at Bharathidasan University in Tamil Nadu, India, the Doerenkamp-Zbinden Foundation (DZF), Switzerland, and I-CARE, Italy.

Ato Lakew Wondimu, Vice President of Ambo University, said in his welcoming address that this seminar was first of its kind in Ethiopia. He congratulated the MGDC, DZF and I-CARE for their noble work in protecting animals and propagating the mission of humane education.

P. Natarajan, Professor and Director of CATER, Ambo University, introduced the significance of alternatives in life science studies and research. He stated that the introduction of non-animal teaching aids has decreased animal experimentation in education by 30-50% in the last 15 to 20 years worldwide.

Mitiku Tesso, President of Ambo University, promised that Ambo University aims to spearhead a movement to bring humane education to Ethiopia and other parts of Africa. He predicted that the concept of humane education would gain momentum in Africa.

The Hon. **Adhana Haile**, Ethiopian Deputy State Minister for Education, emphasised the importance of developing new programs in biological research and following other countries in introducing humane education.

Mohammad A. Akbarsha, director and chair of the MGDC, fervently appealed to making learning biology lively and useful by using alternatives instead of dissection. He also addressed

options for toxicity and pharmacology testing with *in vitro* alternatives and computer-aided bio-informatics tools, i.e. *in silico* alternatives.

Subash Chand, Second Secretary to the Indian Embassy at Addis Ababa and Brook Lemma, Director of Research, Addis Ababa University, congratulated Ambo University on organising this unique seminar.

Shiranee Pereira, I-CARE, explained the need to bring the philosophy of "Ahimsa" into the teaching of Life Sciences. She spoke of animal sentience and pain and the need to adopt the 3Rs concept. Her talk covered details of painful animal experiments conducted in biomedical research and in the production of cosmetics and pharmaceuticals and contrasted these with the alternatives that have replaced eye and skin tests on animals.

M. C. Sathyanarayana, A.V.C. College, India, spoke on dissection alternatives and innovative digitalised alternatives to the use of animals in life science education. He explained that alternatives to dissection are clean, cheaper and safer and allow students to learn at their own pace. They are thus pedagogically, scientifically and ethically superior to conventional animal dissection. He urged Ethiopia to become a forerunner in Africa in starting the movement of humane education and protecting animals from being exploited for the purpose of education, research and testing.

The participants were given hands-on training on the use of tools and software in alternatives in animal science education. There were presented various simulators, mannequins and videos used as alternatives to animal use.

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