6th Annual Cell Culture Technology Event
Recent advances, future prospects

Thursday, 07 March 2013
The Royal College of Pathologists, London, UK

Cell culture has matured to become *the* pivotal technology in biopharmaceutical research, development and production, and its use in this and other areas continues to expand rapidly. *In vitro* models are replacing animals in many tests and assays; its enormous potential in the fields of stem cell and regenerative medicine has hardly started to be realized; and its utility in research grows ever faster.

This conference will examine some of the latest applications of cell culture technology, some that are still "over the horizon", and some of the problems that must be solved before it can reach its full potential.

This event has CPD accreditation

Meeting chair: *Dr John Davis,* University of Hertfordshire; Vice-Chairman of ESACT-UK

9:00 – 9:45 **Registration**

9:45 – 10:00 **Introduction by the Chair**: *Dr John Davis,* University of Hertfordshire; Vice-Chairman of ESACT-UK

10:00 – 10:30 **Assessment of influenza pathogenicity using respiratory ex-vivo organ culture**
Bethany Nash, Animal Health and Veterinary Laboratories Agency, Surrey, UK
Pathogenicity studies are historically conducted in whole body systems requiring many animals for statistical significance. We have adapted ex vivo pig organ culture methods to investigate A(H1N1)pdm09 and Eurasian lineage H5N1 influenza virus pathogenesis in both pig and ferret systems as models of host and human influenza infection. This has enabled the number of animals used to be substantially reduced. Using real-time RT-PCR and immunohistochemistry we have investigated viral infection in both upper and lower respiratory tract infections to inform influenza pandemic preparedness.

10:30 – 11:00 **Cell Performance Monitoring On-Line**
Philip Matthius, Applikon Biotechnology UK
An innovative implementation of a new Quantitative microscopic imaging platform is used as a tool for on-line monitoring of viable cell density in bioreactors. This label-free technology can be used throughout the bioreactor cycle and will allow the user to track cell quantities, viability and morphology in real-time eliminating the need for sampling and reducing the risk of contamination. Tracking cell growth continuously allows for accurate prediction of optimum harvest times.

11:00 – 11:30 **Speakers' photo then mid-morning break/networking and trade show**
Please try to visit all the exhibition stands during your day at this event. Not only do our sponsors enable Euroscicon to keep the registration fees competitive, but they are also here specifically to talk to you

11:30 – 12:00 **Alternative cell sources for ocular surface stem cell therapy**
Dr Anna O'Callaghan, UCL Institute of Ophthalmology, UK
Limbal epithelial stem cells are responsible for the maintenance and repair of the corneal surface. Injury and disease can result in a deficiency of these stem cells affecting vision in these patients. Cultured limbal epithelial stem cell therapy can be used to repair the corneal surface. It is not always possible to use the patient's own corneal cells for this, and the use of alternative sources of stem cells from the patient such as from the mouth (oral mucosa) will be discussed.

12:00 – 12:30 **Investigating murine pluripotency using distinct culture conditions**
Dr Kirsten McEwen, MRC Clinical Sciences Centre, Hammersmith Hospital, UK
Murine embryonic stem cells provide a useful system to delineate the processes underlying naïve pluripotency. We have compared the transcriptional and epigenetic profiles of pluripotent cells cultured under two different culture conditions: traditional serum-containing and dual inhibition of Erk and Gsk3b, termed 2i. Distinct epigenetic properties are apparent, including altered abundance of DNA methylation. Our findings establish that culture in 2i instils a naïve pluripotent state with a distinctive epigenetic configuration that parallels several molecular features observed in the original in vivo cell population.

12:30 – 13:30 **Lunch/networking and trade show**

This is also a good time to fill out your feedback forms and any questionnaires

13:30 – 14:00 **Question and Answer Session**
Delegates will be asked to submit questions to a panel of experts. Questions can be submitted before the event or on the day

14:00-14:30 **Online Monitoring and Control of Glucose and Lactate in Small Volume Bioreactors**
Soenke Rosemann, Sartorius Stedim Biotech GmbH, Göttingen, D-37079, Germany
BioPAT®Trace is a dual-channel analyzer for the measurement of glucose and lactate. The online analyzing system BioPAT®Trace covers demands of long-term cell cultures and fast microbial processes in different scales such as small volume cultivations and large scale productions. The sterile sampling systems, based on filtration or dialysis, probes provide the perfect solution for reliable online sampling in bioreactors used in industrial and laboratory facilities. Special attention has been paid to ease of use in small scale cultivations. The dialysis mode of the BioPAT®Trace allows to collect data on glucose and lactate concentrations automatically and frequently without reducing the culture volume.

14:30 - 15:00 **Strategies for Increasing Protein Expression in Mammalian Transient Transfection Systems**
Dr. Jonathan Zmuda, Life Technologies, USA
Recent advances have allowed transient protein expression to become a fast, flexible and economical way to produce high quality recombinant proteins without the time and cost associated with generating stably transfected cell lines. The levels of protein generated via transient expression, however, have tended to be significantly lower than those of stably transfected cell lines, and when large amounts of protein are required, stable cell lines are often still preferred to transient systems. In order to further increase the utility of transient protein expression, the next key advances will need to approach, or equal, expression levels attained using stable expression systems without losing the speed and flexibility of transient systems. Here, we report on the development of a novel transient transfection system that uses high density HEK293F cell cultures to generate expression levels of greater than 1 g/L and the strategies utilized to achieve these results.

15:00 – 15:30 **Afternoon Tea/Coffee, networking and trade show**

15:30 – 16:00 **Recent advances in production of challenging proteins, using a proprietary Drosophila S2 cell-based platform: robustness, scalability, cGMP production**
Dr Wian de Jongh, CSO of Expres2ion Biotechnologies, Denmark
Drosophila S2 cells represent a efficient cGMP compatible eukaryotic platform for recombinant protein expression. Expres2ion has developed a proprietary and complete licensable production platform, to serve unmet needs in vaccine and research applications. The platform and relevant applications will be described.

16:00 – 16:30 **Industrially Generated Red Blood Cells for Transfusion**
Dr Nik Willoughby, Institute of Biological Chemistry, Biophysics and Bioengineering, Heriot-Watt University
Blood Transfusion has become a mainstay of modern medical practice. However problems persist both nationally and internationally in maintaining supply, managing the risk of transmission of infectious agents and ensuring immune compatibility between donor and recipient. Human embryonic stem cells (hES cells) offer a potentially limitless source from which to generate red blood cells (RBCs) for use in clinical transfusion. The work presented here will describe some of the biological and engineering challenges associated with scale-up of the cell culture and purification operations used to produce the large numbers of RBCs necessary for potential clinical supply.
Although this presentation describes development of a specific process, it is likely that the greatest scale-up hurdles will be specific to cellular therapies as a whole; therefore it is timely to consider process development now in order to prevent a major bottleneck occurring when allogeneic cellular therapies reach clinical trials.

16:30 – 17:00 **Next generation assays based on mechanistic toxicity for cell health monitoring**
Mr Craig Malcolm, Promega UK, UK
Investigating cytotoxicity in vitro often begins with measuring the number of viable cells remaining in cultures incubated with test compounds for 1-3 days. Alternatively, the number of dead cells can be measured by detecting biomarkers that leak from damaged cells into the culture medium. Currently available assay technologies will be described which go beyond providing simple “live or dead” information to indicate whether cells have died by apoptosis or necrosis. New methods which can detect involvement of various organelles and cellular pathways that may trigger events leading to cell death will also be discussed. The knowledge of which pathways are involved provides important information that can lead to identification of the molecular targets involved with initiating cytotoxic events. This talk will present an overview of methods for directly measuring cytotoxicity, detecting upstream pathways indirectly leading to cytotoxicity, and multiplexing assay combinations to more efficiently determine events leading to cell death. The growing importance of 3D cultures will also be discussed with data to describe developments in relation to optimised reagents for determining cell health status in the 3D culture paradigm.

17:00 **Chairman's summing up**

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About the Chair

John Davis is Visiting Lecturer in Biotechnology at the University of Hertfordshire, and Chairman of the UK Branch of the European Society for Animal Cell Technology (ESACT-UK). After a degree in Biochemistry at Sheffield, he moved in 1974 to Renato Dulbecco's laboratory where he was initiated into the art of cell culture. Following PhD studies in Leicester, he moved to Switzerland, working with both Norman Iscove and Georges Köhler, the latter starting him on his many years of research into the use of monoclonal antibodies, particularly in therapy. After a further postdoctoral position, at the University of Cambridge where he worked on the early stages of the development of Campath (Alemtuzumab; now also known as Lemtrada), he made the transition to industry, working first for PA Technology and then (for nearly 20 years) for the Bio-Products Laboratory. In 2007 he made the transition back to academia. In addition to undergraduate and postgraduate teaching, he now runs open courses on Basic Cell Culture and Intermediate/Advanced Cell Culture. He has served on both the UKCCCR subcommittee on the Use of Cell Lines in Cancer Research, and the EC task force on Good Cell Culture Practice. In addition he has edited a number of books on cell culture, including Basic Cell Culture: A Practical Approach, and (with Glyn Stacey) Medicines from Animal Cell Culture. His most recent book is Animal Cell Culture: Essential Methods, which was published in March 2011 by Wiley-Blackwell

About the speakers

After graduating from University College London in 1996 with a BEng in Biochemical Engineering, **Nik Willoughby** carried out a PhD within the Advanced Centre for Biochemical Engineering at UCL. Entitled "An Engineering Evaluation of Expanded Bed Adsorption for the Recovery of a Typical Bioproduct", the study looked at hydrodynamic and kinetic influences on the performance of this novel unit operation. After completing his PhD he worked for two years in purification development at Metris Therapeutics, before returning to UCL to help establish the Innovative Manufacturing Research Centre for Bioprocessing. This multi-disciplinary research group was funded by the EPSRC to facilitate rapid development and scale-up of bioprocesses. Nik moved to Heriot-Watt in April 2006, as part of the ScotChem initiative. His research group at Heriot-Watt is currently focused on scalable culture, purification and separation of stem cell-based cellular therapies. Under the BBSRC BRIC initiative they are investigating scalable separation of human adult and embryonic stem cells and have just started a major SFC-funded project looking at developing a manufactured hESC-derived replacement for donated blood. In addition Nik is heavily involved in sustainability strategies to reduce CO2 emissions from chemical and biological plant using novel techniques based around cyanobacterial photosynthetic fixation. In connection with this he is about to commence a Horizon-funded project developing techniques to produce added-value protein and bioenergy products within the brewing and distilling industry.

Craig Malcolm, PhD is currently Product Manager for Cell Analysis and Proteomics at Promega UK, based in Southampton. After receiving his PhD in Neurochemistry from St Andrews University he was in the pharmaceutical industry for seven years as a Team Leader in Molecular Pharmacology (Vernalis Ltd.), developing cell-based assays and supporting drug discovery screening projects. After several more years in product development as a Senior Cell Biologist (PerkinElmer), and various other roles in several other UK-based Life Sciences companies (Scientifica, Roche Applied Science), Dr Malcolm joined Promega UK last year and is responsible for cell-based assay reagents, proteomics products and related instrumentation in these areas.

Anna O'Callaghan is part of the Cells for Sight group headed by Professor Julie Daniels at the University College London Institute of Ophthalmology. Anna has been a Post-Doc there since 2009. Anna obtained her undergraduate degree in Biochemistry at the University of East Anglia, a Masters degree in Biochemical Engineering at University College London, and an Engineering Doctorate at University College London on limbal epithelial stem cells.

Bethany Nash has been a Senior Research Assistant within the Mammalian and Avian Influenza group at the Animal Health and Veterinary Laboratories Agency (AHVLA), Weybridge for 3 and a half years. Currently her work centres around inter-species transmission dynamics of influenza [e.g. H5N1 and A(H1N1)pdm09] and studying emerging influenza threats for pandemic preparedness planning. Previous to the AHVLA, Bethany spent time at the Centre of Infectious Diseases, Health Protection Agency, London researching correlates of immune response in human papillomavirus and cervical cancer.

Willem Adriaan (Wian) de Jongh, PhD, CSO and co-founder, ExpreS2ion Biotechnologies
Dr. de Jongh (South African) obtained a Bachelor degree followed by a M.Sc. (cum laude) in Chemical Engineering from the University of Stellenbosch, South Africa. Thereafter, he was awarded a doctorate in Biotechnology from DTU in 2006. During his PhD, Dr. de Jongh developed advanced cell line genetic engineering tools and applied metabolic engineering methodologies to cell lines with engineered improved production characteristics. Dr. de Jongh has six years' experience in the pharmaceutical industry in molecular biology; cell line development; project management; upstream process development; process scale-up; and process transfer to cGMP

manufacturing. Furthermore, Dr. de Jongh was instrumental in the development of the proprietary expression vector system ExpreS2ion Biotechnologies was founded on. Dr. de Jongh has also served on the steering committee and as project manager on several grant funded projects.

Nik Willoughby obtained his degree and PhD from UCL before spending time in industry with Metris Therapeutics and Lonza Biologics. In 2002, Nik returned to UCL to help establish the Innovative Manufacturing Research Centre for Bioprocessing. Nik moved to Heriot-Watt in 2006 to establish a cellular bioprocessing research group, with the group's focus divided between cellular therapies and sustainability research. Currently the cellular therapies team are focused on scalable culture, purification and separation of stem cell-based cellular therapies.

Kirsten McEwen completed a Biomedical Science Honours degree in New Zealand before undertaking a PhD in genomic imprinting at the University of Cambridge, UK with Anne Ferguson-Smith. Kirsten has continued her academic career as a post-doctoral fellow at the MRC Clinical Sciences Centre in London, working under Petra Hajkova in the Reprogramming and Chromatin group. Kirsten's focus is on basic reseach of epigenetic mechanisms in murine pluripotent stem cells.

Guy Matthews has worked at SAFC as a Technical Manager for the past 18 months, supporting Customers and SAFC's Business Development teams in biomanufacturing, this has been mostly focused on the development of upstream processes. Prior to joining SAFC Guy worked at Millipore in a business development role focused around the use of single use technology in bioproduction in both upstream and downstream. Prior to this Guy worked at a CMO in a quality function and has held a number of Business Development roles. The common factor in his career to date has been all the roles have been in the Biotechnology arena

Jonathan Zmuda, Ph.D., is an Associate Director of R&D leading the Cell Culture Essentials group at Life Technologies. Dr. Zmuda received his Ph.D. in Cell Biology from the University of Maryland, College Park, USA. Prominent among his roles, Dr. Zmuda works to discover, develop and commercialize technologies and products useful for cell biology applications, including cell-based assays, protein expression, cell culture media development, rare cell analysis and instrumentation while also focusing on the development, qualification and validation of methods used for QC testing.

Soenke Rosemann has a degree in business administration and in life sciences. Before starting to work for Sartorius Stedim Biotech he was with a venture capital company, focused on financing early stage companies in life sciences. Currently he is working in the Sartorius product management team for process analytical technology.

Philip Mathuis is founder and CEO of Ovizio Imaging Systems, a spin-off of the Université Libre de Bruxelles (ULB). After receiving his engineering degree he started his career in the telecommunications sector where he held management positions in several European countries. He obtained an MBA from ESCP Europe in Paris and is a seasoned entrepreneur with a passion for science and technology.

Keywords: cell, transfection, lasers, microscopy, Autophagy, Puncta, p62, Flux, cell, transfection, lasers, microscopy, dry powder, mammalian cell culture media, batch to batch consistency, human embryonic stem cells, clinical grade, cGMP, bioreactor, process development, large scale, upstream processes, recombinant protein production; cold-shock; CHO cells; cell engineering, cornea, eye, limbal epithelial stem cells, cell therapy,Influenza, organ culture, pigs, ferrets, respiratory tract,Soluble proteins, vaccines, cGMP,Drosophila , Red blood cells, human embryonic stem cells, scale-up, DNA methylation, genomic imprinting, pluripotency, Cell Line Selection, Media Development, cell, apoptosis, multiplexing, pathways, 3D culture, Online Monitoring,Glucose, Lactate

Event Web site: **www.regonline.co.uk/cellculture2013**

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FOR POSTER PRESENTATION

EFFECT OF DOXORRUBICIN IN CELL CULTURE OF CANINE BREAST TUMORS AND ITS CORRELATION WITH TGF-β1 EXPRESSION

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The canine mammary gland tumor is the most common malignant neoplasia in female dogs and its occurrence as spontaneous tumors in the dog provide a valid model tumor system available for testing cancer therapeutic agents or studying biology of cancer. Doxorubicin is an anthracycline drug used in chemotherapy protocol for patients with breast cancer in medicine and veterinary medicine. This medication has an excellent anti-tumor activity but its utility is limited due to acute and chronic toxicities (myelosuppression, immunosuppression and dose-cumulative cardiotoxicity). Transforming growth factor beta (TGF-β) has been shown to be overly produced during progression of various types of carcinomas, including breast cancer. The expression of TGF-β in breast tumors also has been positively correlated with disease and metastatic progression and lymph node involvement. Then, the aim of this study was to test the effectiveness of doxorubicin in different types of canine mammary cell cultures and to evaluate the TGF-β1 tumor expression in paraffin-embedded sections and its correlation with total number of cells obtained from cell culture. The neoplastic tissue samples were collected during the routine surgical procedures of the total mastectomies. This procedure was performed at the Veterinary Hospital of São Paulo State University (UNESP). A total of fifteen breast samples were collected, splitting them into three groups of five samples each one: Benign Mixed Tumor, Complex Carcinoma and Simple Carcinoma. A fragment was collected from each tumor and submitted by the same methodology of cultivation for everyone. The tumor specimen was washed in antibiotic medium (RPMI1640 with penicillin and streptomycin) and then was minced with scissors and transferred to culture flask containing now Dulbecco's Modified Eagle Medium (DMEM) and 10% Fetal Bovine Serum, maintained at 38,5°C in a humidified atmosphere of 95% air and 5% CO_2. At 4th passage cells were transferred into a six well plate until reach confluence and each well was used for a treatment with doxorubicin in different concentrations for 24 hours: Control - 0.25 - 0.50 - 0.75 - 1.0 - 2.0 μM. The cell viability was measured with the Trypan Blue 0,4% and hemocytometer (improved Neubauer). TGF-β1 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) expression was evaluated with imunnohistochemical staining in paraffin-embedded sections. In order to quantify the differences existing among the group treatments and number of total cells at 4th passage, the Kruskal-Wallis test was employed. For the comparison between TGF-β1 expression and tumor size (diameter) and number of total cells at 4th passage with TGF-β1 expression the Pearson and Spearman correlation test was chosen. The statistical program used was GraphPad InStat version 3.10. There were no significant differences among the groups and no correlation was observed between number of total cells and TGF-β1 expression. A positive correlation (p=0.0387) was found in TGF-β1 expression versus tumor size, demonstrating its involvement with tumor progression. Breast cancer cells viability was expressed similar among the groups of treatment. It was found that the higher concentration of chemotherapy, smaller cell viability was found. Despite of side effects originated from doxorubicin treatment, it is important to use this drug in *in vitro* therapeutic test with the purpose to search a new methodology of combination protocol with another highly effective novel non-toxic drug, which can lower the dose of doxorubicin because its excellent anti-tumor activity, mainly in metastatic breast cancer patients. In conclusion, the doxorubicin is effective on canine breast cancer cells with similar results in different types of mammary tumors and TGF-β1 expression was positive to correlate only with tumor size parameter.

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3D CULTURE PADRONIZATION OF BITCHES MAMMARY TUMOUS

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The general characteristic of cell culture is to allow maintenance of the cells in an independent environment from the original organism. The 3D cell culture has the main differential of provide to the cell a microenvironment like the one in original tissue. In this system, cells grow forming structures called multicellular spheroids with an internal cellular heterogeneity, microenvironment formation and better exposition to nutrients and oxygen. So, these differences normally create a greater similarity between the cultured cells and the living organism the cells are meant to represent, leading to more useful data and more relevant research mainly in chemotherapy researches. In breast cancer researches, the spheroids are being studied to understand the morphogenesis of the luminal space. The aim of this study was to standardize a 3D cell culture of canine breast cancer. So, the tumors were cultivated at 4th passage with Dulbecco's Modified Eagle Medium (DMEM) and Fetal Bovine Serum (FBS). Then, the medium was changed to HuMEC Ready Medium (Gibco®) that was specific for epithelial cells and cultivated at 2D cell culture up to the 20th passage and changed to 3D cell culture. The experiment used the AlgiMatrix® 3D Cell Culture System (Life Technologies®) with six well. The protocol was two milliliters of canine mammary cell suspension containing firming buffer to the AlgiMatrix® 3D cultures system. After 5 minutes, the culture medium of 5 ml was added to well, leaving one for the negative control with only medium and FBS. A fresh medium was added every day. The growth of tumor spheroids was assessed by observing formation of the spheroids in Algimatrix® well. Photographic registration was made at the 4th, 9th and 20th days with inverted microscope. It was observed that 3D cell culture presented a good development for canine mammary cells and can be used for experimental model and has an important role in researches for luminal morphogenesis and monitoring *in vitro* regulatory factors of this process. Furthermore, new chemotherapy protocols can be tested in this 3D cell cultures with more reliable results to those obtained in 2D cell culture.

Acknowledgements: Financial support by FAPESP (process: 2011/16331-0).

CORRELATION OF TRACE ELEMENTS IRON, COOPER AND ZINC OF CANINE MAMMARY TUMOR SAMPLES AND TOTAL NUMBER OF CELLS FROM ITS CELL CULTURE

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Trace elements play a very important role in various biological processes by activating or inhibiting enzymes. Selenium (Se), zinc (Zn), cooper (Cu) and iron (Fe) are very important structural and functional cofactors of several enzymes crucial for the biochemical cell activities. The involvement of some elements in the development of breast cancer seems to be connected with their functions as co-factors in enzymatic processes, specifically, Cu and Zn. They belong to the group of antioxidant metals, participating in the activity of antioxidant enzymes. Fe can also be involved because of its functions as regulating factor for angiogenesis. In cell culture, trace elements are important for the growth of cell. The use in serum free media may even reduce or eliminate the need for growth factors. Then, the aim of this study was to correlate trace elements Fe, Cu and Zn of canine breast cancer with its respective cell culture (total number of cells at fourth passage). Samples were collected during routine surgical procedures, mastectomies, performed at the Veterinary Hospital of São Paulo State University of Araçatuba, Brazil. A total of ten samples were collected. A fragment was kept in formalin 10% buffered for 24 hours and then liophilizated for 30 hours for analysis in Energy Dispersive X-Ray Fluorescence. The measurements were conducted using an EDX-720 model, Shimadzu Co, equipped with a Rhodio anode x-ray tube and irradiated area constant of five millimeters (performed at IPEN-USP, Sao Paulo State University). Another tumor specimen was collected for cell culture and washed in antibiotic medium (RPMI1640 with penicillin and streptomycin) and then was minced with scissors and transferred to culture flask and maintained at 37°C in a humidified atmosphere of 95% air and 5% CO_2 and 10% fetal serum bovine. At passage four, cells were transferred into a six well plate for cell counting with the hemocytometer (improved Neubauer). The statistical program used was GraphPad Instat version 3.10. The Pearson correlation for Fe, Cu, Zn and total number of cells was considered not significant. But the comparison between trace elements of canine breast cancer samples and canine mammary healthy tissues was significant performing the Unpaired t test for Fe (p=0,0002), Cu (p=0,0022) and Zn (p=0,0001). In conclusion, there is a high concentration of the Fe, Cu and Zn elements in malignant tumors comparing with normal breast tissues, but it was not possible to correlate with cell culture results.

Acknowledgements: Financial support by FAPESP (process: 2010/11232-1).

GRAM PER LITER EXPRESSION OF BIOLOGICALLY-ACTIVE AND PROPERLY GLYCOSYLATED PROTEINS USING A NOVEL MAMMALIAN TRANSIENT TRANSFECTION SYSTEM

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Recent advances have allowed transient protein expression to become a fast, flexible and economical way to produce high quality recombinant proteins without the time and cost associated with generating stably transfected cell lines. The levels of protein generated via transient expression, however, have tended to be significantly lower than those of stably transfected cell lines, and when large amounts of protein are required, stable cell lines are often still preferred to transient systems.

To further improve the utility of transient protein expression, the next key advances will need to approach, or equal, expression levels attained using stable expression systems without losing the speed and flexibility of transient systems. Here, we report the development of a novel transient transfection system that utilizes high density 293F cell cultures to generate expression levels of greater than 1 g/L of a human IgG and a non-IgG protein, respectively. To attain such high levels of protein expression, a novel cell culture media (Expi293™ expression medium) was developed to allow 293F cells to reach viable cell densities of 14 x10^6 cells/mL or greater while still maintaining high viability. These ultra-high density cultures enable transfection at higher cell densities than traditional protocols, significantly increasing the volumetric yield of protein. Additive increases in yield were further obtained through the generation of optimized 293F cells adapted for superior performance under high density culture conditions and selected for increased protein production. Finally, the transfection reaction itself was optimized through the development of a new transfection reagent (ExpiFectamine™ 293) that works in combination with proprietary transfection enhancers to increase overall protein yield.

When combined into a single expression system, these improvements increased protein levels significantly for both IgG and non-IgG recombinant proteins compared to the FreeStyle™ 293 expression system and expression levels of >1g/L were attained for multiple proteins. Analysis of N-linked carbohydrates on human IgG demonstrated the glycosylation patterns to be highly similar in both expression systems and a functional cell-based assay indicated that the biological activity of recombinant eryrthropoeitin was equivalent in the two systems. Together, these results indicate that significant increases in functional protein yields can be attained using a novel transient mammalian expression system that incorporates multiple advances in protein expression technology into a single, easy to use format that rivals protein expression levels of stably transfected cell lines.

DEVELOPMENT OF CELL CULTURE BASED LIVE VACCINE OF AAV-4 AND COMPARATIVE EVALUATION OF ITS EFFICACY WITH COMMERCIAL VACCINE

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The present study was designed to develop cell culture-based live vaccine for Avian Adeno Virus-4 (AAV-4), being the causative agent of hydropericardium syndrome in chickens. In this regard five field isolates of AAV-4 were propagated in primary chicken embryo liver cell culture upto 5th passage. These field isolates caused 70-80% mortality *via* subcut inoculation except one isolate (N-3317), which was non-pathogenic to birds at fifth passage. At this Passage level field isolate (N-3317) was used as live vaccine in 8 day old birds. This Live vaccine was subjected to safety and potency evaluation in comparison with Commercial HPS vaccine.
The antibody response to the used vaccines was evaluated by a self prepared ELISA. Groups of chicks injected *s/c* with 0.2 ml of commercial alum precipated and oil-base vaccines showed S/P ratios of 0.341 and 0.363 respectively. Live cell culture-based vaccine showed an S/P ratio of 1.169. All vaccinated groups survived the challenge dose of $10^{5.0}TCID_{50}$/ml of AAV-4. Cell culture-passaged live vaccine qualified the OIE standards of safety, sterility and potency.

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