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Cell Culture

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Authentication – An Essential Part of Any Cell Culture Operation

Are You Confident You Are Working With The Cell Line You Think You Are?

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Authentication, the process of determining the true origin and identity of cell lines, should concern everyone using cell cultures. The consequences of working with a cell line that is misidentified or cross-contaminated with cells from a different origin can be devastating. Results invalidated, products devalued, years of research made irrelevant. Imagine discovering the hepatocytes you are using to study liver dysfunction are actually cervical carcinoma cells. Or your extensive work on a 'spontaneously transformed' human umbilical cord cell line is invalidated, as it is in fact a human bladder carcinoma epithelial cell line. This has been a bleak reality for some scientists.

Instances of cross-contamination of cell lines are more common than generally appreciated. Estimates suggest that up to 1 in 5 experiments in fields such as microbiology and cancer employ the wrong cells. In a study of 252 human cell lines18% were found to be cross-contaminants (Macleod *et al.*, 1999). Last year a study of over 500 human leukaemia-lymphoma cell lines showed 15% were not unique (Drexler *et al.*, 2003).

Can you afford not to take the precaution of authenticating your cell lines? A vivid demonstration of the importance of authentication has been provided through the progressive realisation, since the late 1960s, that a large number of human cell lines assigned unique identities and tissues of origin were, in fact, all cervical carcinoma HeLa cells. Table 1 lists cell lines that have been identified as HeLa contaminants. In addition, Table 2 (opposite page) shows examples of contaminated cell lines not involving HeLa.

Despite wide publicity of the HeLa contamination story, and the cell lines affected, there remains a lack of awareness of the dangers of cross-contamination of cell lines and the value of authentication. A search of literature published in 2003 to spring 2004 revealed researchers continuing to use cell lines, identified as HeLa contaminants, for purposes dictated by their original, incorrect designation. For example the KB cell line, an established HeLa contaminant, was referred to as being an oral carcinoma cell line and used for periodontal disease studies. Likewise, the WISH cell line, originally considered to be derived from human amnion cells but since shown to be a HeLa contaminant, has been used to study amnion pathophysiology.
Many cell lines are morphologically very similar, without use of recognised authentication techniques cross-contamination can remain undetected, potentially for years. Even where expression of certain markers suggests a cell line is unique this may not be the case.

Table 1. Cell lines determined to be HeLa contaminants listed in the ECACC catalogue.

Cell Line Name	ECACC Catalogue no.
AV3 (HeLa Derivative)	88102402
C16 (HeLa Derivative)	84121902
Chang Liver (HeLa Derivative)	88021102
Clone 1-5c-4 (HeLa deriative)	88021103
D98/AH2 Clone B (HeLa derivative)	85112701
FL (HeLa derivative)	90111910
GIRARDI HEART (HeLa derivative)	93120822
Hep-2C (HeLa derivative)	85020207
Hep2 (Clone 2B) (HeLa derivative)	85011412
Hep2 (HeLa derivative)	86030501
INT 407(HeLa derivative)	85051004
JIII (HeLa derivative)	93120824
KB (HeLa derivative)	94050408
L-41(HeLa derivative)	96121716
L132 (HeLa derivative)	89111004
WISH (HeLa derivative)	88102403
WKD (HeLa derivative)	93120839
WRL 68 (HeLa derivative)	89121403

For example, ED(67), originally considered a trophoblast-like cell line derived from human chorionic villus, appeared to be phenotypically distinct but has been shown to be genetically identical to the HeLa cell line (Kniss *et al.*, 2002). Suspicions were aroused when the cells were shown to lack properties of primary cells from the same origin i.e. primary villus cytotrophoblasts; secretion of human chorionic gonadotropin could not be detected. HeLa cells had never been grown in the laboratory; the origin of the HeLa contamination was traced to the WISH cell line.

Reduce the Risk

Every cell culture laboratory is at risk from cross-contamination and misidentification of cells. The common practice of exchanging cell lines between research laboratories, the use of several different cell lines in a single facility and the greater the number of individuals in contact with a cell line all contribute to the risk. As do a high throughput of short-term research staff, limited number of trained cell culturists and little in the way of defined cell culture disciplines and quality control.

Take measures to protect the quality and relevance of your work by authenticating the cell lines you use. ECACC advises adherence to three key practices to enhance confidence and reliability in the cell lines you work with:

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Table 2. Examples of contaminated cell lines not involving HeLa cell lines.

Cell Line	Originally referred to as:	Determined to be:
ECV304	'spontaneously transformed' human umbilical cord endothelial cells	genetically identical to T24 - a human bladder carcinoma epithelial cell line (MacLeod *et al.*, 1999)
TSU-Pr1	a unique human prostate cell line	a derivative of the bladder carcinoma cell line T24 (van Bokhoven *et al.*, 2001)
JCA-1	a unique human prostate cell line	a derivative of the bladder carcinoma cell line T24 (van Bokhoven *et al.*, 2001)
JROECL 47	a unique human oesophageal adenocarcinoma cell line	an admixture of the human colon adenocarcinoma cell line HCT 116 (Wijnhoven *et al.*, 2000)

(i) Start with Authenticated Cell Lines.

This can be achieved by obtaining cells lines from a well documented and quality controlled source such as a recognised culture collection. If a cell line is only available by transfer between research laboratories, request evidence of its providence. If records are not available authenticate the cell line prior to use.

(ii) Ensure Good Cell Culture Practice is in Place.

If high quality practical cell culture training is not readily available in-house, why not consider the cell culture training courses offered by ECACC?

(iii) Re-authenticate Cell Lines at Regular Intervals.

If you do not have the resources to authenticate cell lines at your laboratory use an established authentication service. If you are generating new cell lines it is advisable to define their DNA profiles at an early stage. Results from the study of 500 leukaemia-lymphoma cell lines, mentioned previously, implied that most contamination occurs during the initial establishment of a cell line (Drexler *et al.*, 2003). The EBV (Epstein-Barr virus) lymphocyte immortalisation and cell banking service at ECACC routinely stores blood samples on filter paper and compares DNA from the end lymphoblastoid cell lines generated with DNA from the original blood donors.

Methods of Authentication Used at ECACC

ECACC authenticates all cell lines deposited into its cell collection and offers a cell line authentication service. A combination of isoenzyme analysis, multilocus DNA fingerprinting and Short Tandem Repeat (STR) multiplex PCR analysis (also known as multiplex DNA fingerprinting) is used to authenticate cell lines.

Isoenzyme analysis utilises the property that isoenzymes have similar substrate specificity, but different molecular structures. This affects their electrophoretic mobility, thus producing specific mobility patterns for each species. It is a good tool for identifying different species. However, this technique will not distinguish between cell lines from the same species. To complement this multilocus DNA fingerprinting should be performed for non-human cell lines.

For multilocus DNA fingerprinting, DNA is extracted from a cell line, digested by restriction enzymes and subsequently separated by gel electrophoresis. Multilocus probes are applied to the separated DNA and visualised by chemiluminescence to produce the DNA fingerprint of the cell line. Multilocus DNA fingerprinting is used to differentiate cell lines both between species and within a species. However, multilocus DNA fingerprinting is prone to inter-run variation, so that to differentiate DNA from two different cell lines it is necessary to run them side by side on the same gel. This means that one gel cannot be compared with an archived banding pattern on another gel. Therefore DNA fingerprinting is primarily a confirmatory technique but still remains the only practicable method available for the routine authentication of non-human cell lines.

STR PCR profiling, the third authentication technique used at ECACC, is a powerful tool developed following advances in forensic techniques for DNA profiling. This technique generates multiple PCR amplicons, each incorporating a unique fluorescent marker. When these different amplicons are separated by chromatography, the relative distribution of emission peaks creates a unique profile or 'signature' that can be recorded digitally. This signature is reproducible and therefore can be compared with archived profiles. STR analysis enables ECACC to compare the profile of newly accessioned human cell lines with all the human cell lines already deposited in the collection. The existence of a large database of STR multiplex PCR profiles of human cell lines within the ECACC cell collection is an invaluable resource.

References

Drexler HG *et al.* False leukemia-lymphoma cell lines: an update on over 500 cell lines. Leukemia 2003, 17(2):416-426.

Kniss DA *et al.* ED(27) trophoblast-like cells isolated from first-trimester chorionic villi are genetically identical to HeLa cells yet exhibit a distinct phenotype. Placenta 2002, 23(1):32-43.

MacLeod RA *et al.* Widespread intraspecies cross-contamination of human tumour cell lines arising at source. Int J Cancer 1999, 83(4):555-563.

van Bokhoven A, *et al.* TSU-Pr1 and JCA-1 cells are derivatives of T24 bladder carcinoma cells and are not of prostatic origin. Cancer Res 2001, 61(17):6340-6344.

Wijnhoven BP *et al.* Human oesophageal adenocarcinoma cell lines JROECL 47 and JROECL 50 are admixtures of the human colon carcinoma cell line HCT 116. Br J Cancer 2000, 82(9):1510-1512

Is The Risk You Take Worth It?

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Introduction

Safety is a prerequisite in every work place. Cell culturists should be aware, not only of standard laboratory safety precautions but also the specific risks associated with cell culture. This includes the risks presented by the cell cultures themselves, the procedures specific to cell culture and the disposal of waste generated.

Risks Associated with Cell Lines

For animal cell culture the level of risk is dependent upon the cell line to be used and is based on whether the cell line is likely to cause harm to humans. A range of sources should be consulted to determine the risk presented by a cell line such as the supplier, the originator of the cell line and literature searches based on the identity of the cells. Factors to consider include their anatomical and species of origin which are directly related to the potential for infection by viruses or other agents pathogenic for humans. As shown in table 1, the cell cultures presenting the highest risk are likely to be those of human and primate origin especially those derived from peripheral blood, lymphoid cells and neural tissues. Where infection with an agent pathogenic for humans is known or suspected, the cell culture should be handled at a containment level appropriate to the agent concerned.

Table 1. Levels of risk presented by different types of cell cultures

Risk Level	Type of Cell Culture
Low Risk	• Non human/non primate continuous cell lines and some well characterised human or primate diploid lines of finite life span (e.g. the human foetal lung cell line, MRC-5)
Medium Risk	• Poorly characterised mammalian cell lines
High Risk	• Cell lines derived from human/primate tissue or blood. • Cell lines with endogenous pathogens (the precise categorisation is dependent upon the pathogen) – refer to the Advisory Committee on Dangerous Pathogens (ACDP) Guidelines, 1995, for details. • Cell lines used following experimental infection where the categorisation is dependent upon the infecting agent – refer to the ACDP guidelines, 1995, for details.

Although viruses are one of the most likely risks presented by cell cultures other potential hazards should be considered. These relate to components of the cell culture medium, other adventitious agents (e.g. contaminating mycoplasmas) and cell products, some of which may be biologically active molecules with pharmacological, immunomodulating or sensitising properties. In addition, the generation and use of modified cells, for example hybrids, transformed cells and cells containing recombinant DNA can be hazardous. These procedures could potentially result in the appearance of modified or reactivated viruses, novel fusion/hybrid proteins (especially in cross-species hybrids) and the expression of viral or cellular oncogenes.

Laboratory workers should never culture their own cells. *In vitro* transformation or genetic modification could result in malignant disease or expression of an unusual pharmacologically active protein if they were to be accidentally inoculated into the donor. Therefore, human cells should be obtained from individuals having no association with the experimental work.

The Risk Assessment Policy

Have the risks present in your cell culture laboratory been identified and evaluated? Are procedures in place to minimise or avoid the risks? Prior to commencing work a risk assessment must be performed consisting of two elements - (i) identification and evaluation of the risks (ii) definition of ways to minimise or avoid the risk. Risk assessments are a legal requirement under the Health and Safety at Work Act, UK (1974). They are designed to prevent injury, protect property and avoid harm to individuals and the environment. Once in place, staff must be aware of the potential risk, trained to minimise the risk and the risk assessment itself must be reviewed on a regular basis. There are also European Community directives covering Health and Safety at work. You can visit the European Agency for Safety and Health at Work website (www.europe.osha.eu.int) for information on legislation and standards, or you should contact your on-site Health and Safety representative.

Genetic Modification Safety Committee (GMSC)

All UK institutions that carry out work using and/or generating genetically modified organisms are required by law to have a GMSC. Prior to any work commencing, proposals for the intended work should go through the committee and be approved by the Health & Safety Executive (HSE) in the UK. In Europe, contact the National Competent Authority regarding the regulation of GM work.

Methods to Reduce Risk

The design and management of a tissue culture laboratory are important considerations with regard to safety. Ideally, work should be conducted in a single-use facility which, if at all possible, should be separated into an area reserved for handling newly received material (quarantine area) and an area for material which is known to be free of contaminants (main tissue culture

Safety in the Cell Culture Laboratory

facility). If this is not possible work should be separated by time with all manipulations on clean material being completed prior to manipulations involving the 'quarantine material'. Different incubators should also be designated. In addition, the work surfaces should be cleaned thoroughly between activities. The UK Advisory Committee on Dangerous Pathogens (ACDP) guidelines make recommendations regarding the laboratory environment. In addition, it is recommended that laboratories should have inward flow of air corridors to contain any risks within the laboratory. Conducting tissue culture in a shared facility requires considerable planning and it is essential that good technique is used throughout to minimise the risk of contamination. Staff training and the use of written standard operating procedures and risk assessments are keys ways to reduce the risks.

Procedures used in handling cell cultures present their own risks. Due to the repetitive nature of much cell culture work, repetitive strain injury (RSI) is a genuine risk. There are a number of ways to reduce this risk, such as task rotation, the use of automated pipettes and taking short breaks from repetitive tasks every 30 minutes. Sharps should not be used where there is a reasonable alternative. Comfort of the operator at the cell culture cabinet should not be overlooked. Using an unsuitable chair can cause injuries resulting from poor posture. With the acknowledgement of the importance of ergonomics at the tissue culture cabinet a wide variety of chairs are now available. Good designs include wheels for improved mobility and adjustable seats and backs.

Containment is the most obvious means of reducing risk from cell cultures. As with all work with infectious or potentially infectious material a microbiological safety cabinet should be used. For most cell lines the appropriate level of containment is Level 2 requiring a class II microbiological safety cabinet.

However, this may need to be increased to Level 3 depending upon the type of manipulations to be carried out and whether large culture volumes are envisaged. For cell lines potentially infected with HIV or Human T-Lymphotropic Virus (HTLV), Level 3 containment is required. It is crucial that microbiological safety cabinets are installed correctly and tested regularly e.g. every six months. All equipment and tools, such as tubes used for centrifugation, should be fit for purpose.

Other less obvious measures include restricting the movement of staff and equipment into and out of laboratories. Good laboratory practice and good bench techniques such as ensuring work areas are free from clutter and reagents are correctly labelled and stored, are also important for reducing risk and making the laboratory a safe environment in which to work. The risk of exposure to aerosols or splashes can be limited by avoiding rapid pipetting, scraping and pouring. In addition, it is recommended that people working in laboratories where human material is used are vaccinated against Hepatitis B.

Unexpected changes in cell phenotype such as cytopathic effect (CPE), indicate the need for re-assessment of the risks. In the event that cell culture products are allergenic, stringent containment and personal protection for the operators to prevent inhalation or contact with mucous membranes are required. When scaling up a procedure re-assess the risk as the potential for exposure may be increased.

Some individuals are allergic to penicillin. This sensitivity varies between individuals, but in in the worst case scenario a severe reaction could result in potentially fatal anaphylactic shock. Prior to starting work, prospective cell culture workers should be required by Occupational Health to complete a form stating any known sensitivity to penicillin. Individuals highly sensitive to this antibiotic should avoid working with the drug.

Disinfection and Waste Disposal

Routine cleaning of the laboratory should be undertaken involving the cleaning of all work surfaces both inside and outside of the microbiological safety cabinet, the floors and all other pieces of equipment e.g. centrifuges. Decontamination procedures must be capable of inactivating viruses and other contaminating agents in the presence of fluids that are often heavily loaded with organic material. Any employer has a 'duty of care' to dispose of all biological waste safely in accordance with national legislative requirements. Different forms of waste require different treatment.

One of the most important aspects of the management of all laboratory-generated waste is to dispose of waste regularly and not allow the amounts to build up. The best approach is 'little and often'.

Useful References:

The following publications are available from the Health and Safety Executive, www.hse.gov.uk:

Advisory Committee on Dangerous Pathogens (ACDP). Categorisation of biological agents according to hazard and categories of containment. Fourth Edition 1995. Now out of print – updated version due to be released in 2004.

ACDP Revised advice on laboratory containment with tissue samples in clinical cytogenetic laboratories.

Genetically Modified Organisms (Contained Use) Regulations 2000 (SI 2000/2831).

Control of Substances Hazardous to Health Regulations 2002

Safe working and the prevention of infection in clinical laboratories and similar facilities

Contact ECACC on ecacc.technical@hpa.org.uk for further information on:

- The different merits given to the major groups of disinfectants used in the cell culture laboratory
- The various examples of the ways in which tissue culture waste can be decontaminated and disposed of safety.

Improved *Ex Vivo* Expansion of Functional CD34⁺ Cells Using Stemline™ II Hematopoietic Stem Cell Expansion Medium

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Introduction

Hematopoietic stem cells (HSC) have the ability to repopulate the hematopoietic system by differentiating into all of the necessary erythroid, lymphoid, and myeloid lineages. Due to this rare ability, HSCs are used as therapeutic agents in the treatment of malignant and benign diseases of the blood forming and immune systems. There have been many advances in the area of clinical HSC research, but the availability of suitable cells for transplantation still remains a major limiting factor.[1,2]

HSCs can be isolated from three different sources: umbilical cord blood (CB), bone marrow, and mobilised peripheral blood. CB is currently the preferred source because it has been shown to have a lower risk of graft versus host disease (GVHD), presumably due to its immunological naiveté. However, because the volume of CB is limited, each umbilical cord generally has only enough cells to successfully transplant a small child. In order to transplant an adult, the HSCs from CB typically must be expanded *ex vivo*. It is critical that the expansion be performed in a manner to ensure that the HSCs not only differentiate along appropriate hematopoietic lineages, but also self-renew, leaving undifferentiated stem cells in the expanded culture. The differentiated cells will allow for short-term engraftment that will reduce the effects of neutropenia and thrombocytopenia in the patient. The undifferentiated cells will allow for long-term engraftment that will establish a new, permanent hematopoietic system for the patient. In order to expand these very specific cell types in the absence of potentially adventitious agents, which may be present in typical medium components, such as fetal bovine serum, an optimised serum-free medium is needed.

With these parameters in mind, Sigma-Aldrich has developed Stemline™ II Hematopoietic Stem Cell Expansion Medium (Code S0192). This formulation has lead to a significant increase in the number of cells expanded from cord blood CD34⁺ cells, as well as CD34⁺ cells from mobilised peripheral blood and bone marrow. Flow cytometry shows the surface antigen profiles to be consistent with previously reported profiles for *ex vivo* expanded cells. The expanded cells form all of the appropriate hematopoietic lineages in a colony-forming unit assay (CFU) and demonstrate long-term engraftment (primary and secondary recipient) in NOD/SCID mouse models. This serves as an indication that the expanded cells are representative of all of the proper lineages required for a successful transplant. Clinical studies are currently being designed to evaluate the engraftment potential of cells expanded in Stemline™ II Hematopoietic Stem Cell Expansion Medium.

Materials & Methods

All materials were supplied by Sigma-Aldrich Corporation (St. Louis, MO) unless otherwise stated.

Cell Preparation

For all experiments, cryopreserved, human CD34⁺cells were obtained from independent suppliers (Stemgenix, Amherst, NY; AllCells, LLC, Berkeley, CA) and were handled in a manner consistent with the manufacturers' instructions with regard to storage and reconstitution. Cells were counted using either a hemocytometer or Guava Personal Cytometer (Guava Technologies, Hayward, CA) to determine cell density and viability.

Serum-free Expansion Medium Preparation and Bench-Scale Expansion

Stemline™ Hematopoietic Stem Cell Expansion Media, IMDM, X-VIVO 15 (Cambrex, Walkersville, MD), HPGM (Cambrex, Walkersville, MD), QBSF-60 (Quality Biological, Gaithersburg, MD), StemPro-34 (Invitrogen, Carlsbad, CA), and StemSpan H3000 (StemCell Technologies, Vancouver, BC) were purchased fresh, aliquoted and stored according to the manufacturers' recommendations. For each experiment, a 10ml volume of each expansion medium was warmed to 25°C. One ml of each medium was pipetted in triplicate in 24-well culture plates (Corning/Costar, Corning, NY) to which thrombopoietin (TPO, Product Code T1568), stem cell factor (SCF, Product Code S7901), and granulocyte colony-stimulating factor (G-CSF, Product Code G0407) were added to a final concentration of 100ng/ml each. Sterile PBS was added to unused wells to maintain humidity. Plates were incubated at 37°C and 5% CO_2 for 15 minutes prior to the addition of the revived CD34⁺cells. Viable recovered CD34⁺ cells were added to each well at 1.0 x 10^4 cells/ml and allowed to proliferate in a humidified incubator at 37°C and 5% CO^2 for 10 days. Following the incubation period, the expanded total nucleated cells were counted.

Flow Cytometry

The direct determination of the absolute count of CD38⁺ and CD38⁻ cells was assessed utilizing the Immunotech Stem Kit CD34⁺ Hematopoietic Progenitor Cell (HPC) Enumeration Kit (Beckman-Coulter, Fullerton, CA), CD38-PE, CD34-ECD, and CD45-FITC Antibodies. The processed samples were identified and enumerated using Beckman-Coulter's flow cytometer (EPICS XL-MCL™).

Clinical-Scale Expansion

A 2-step, clinical-scale assay[3] using Teflon® culture bags (American Fluoroseal, Inc., Gaithersburg, MD) was set up for a comparison study between Stemline™ Medium (Product Code S0189) and Stemline™ II Medium. For clinical-scale studies, CD34⁺ cells were cultured for 7 days in 100ml Teflon culture bags containing 50ml of each culture medium plus cytokine concentrations as

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previously described. Cells were harvested from these bags and a 10ml aliquot was transferred to a second 100ml Teflon bag containing 90ml of each selected medium plus cytokines and cultured for an additional 7 day culture period. At the end of the culture protocol, cells were harvested, counted by hemocytometer, viability tested, and assayed for functional hematopoietic activity *in vivo* and *in vitro*.

NOD/SCID Studies

Immunodeficient NOD/SCID mice were used as recipients of transplanted human cord blood cells expanded at clinical-scale as previously described. NOD/SCID mice were lightly irradiated (3.0 Gy) 3-6 hours prior to infusion of human cells by injection intravenously into the retro-orbital plexus[4]. The mice, prior to and throughout the treatment period, were maintained under pathogen barrier conditions and their drinking water was supplemented with antibiotics. Depending on the experiment, between 500,000 and 6 million cells were transplanted. At various times after transplant, blood was obtained from each recipient by retro-orbital blood sampling. Human cells were distinguished by immunofluorescence and flow cytometry using an antibody to CD45 (Becton-Dickinson, Franklin Lakes, NJ). Presence of CD34 was used to distinguish progenitor cells and representation of human cells in the lymphocyte lineage was determined using CD19 and CD20; human myeloid cells were identified by CD15 and CD66b. The same panel of antibodies was used in the analysis of bone marrow at the termination of the experiment, or in preparation for injection into secondary recipients to verify the presence of self-renewing human stem cells in the primary recipients.

Results and Discussion

To test the ability of Stemline™ II Hematopoietic Stem Cell Expansion Medium to expand $CD34^+$ hematopoietic stem cells, we designed a bench-scale expansion assay. Cells were seeded into the wells of 24-well tissue culture plates. One milliliter of medium was added to each well with the appropriate cytokines to stimulate growth (100ng/ml each of TPO, SCF, and G-CSF). Each condition was performed in triplicate and seeded with 10,000 cells/ml in each well. This was the standard assay used to evaluate the expansion of $CD34^+$ cells from cord blood, bone marrow, or mobilised peripheral blood.

Due to the clinical importance and the donor-to-donor variability typical to the expansion of umbilical cord blood-derived cells, we elected to test 15 donors for expansion and surface antigen expression. The cells were counted on day 10 and the fold increase was determined by $cells_{final}/cells_{initial}$. In cord blood, Stemline™ and Stemline™ II outperformed the other serum-free HSC media (Figure 1A). While Stemline™ already performed better than or equal to the other HSC media, Stemline™ II exhibited a significant increase in expansion compared with the other products ($p< 0.00001$). Figure 1B represents the fold increase for each medium in all 15 donors. This also shows that Stemline™ II consistently provides the maximum number of total nucleated cells (TNC). The third graph in this series shows the percentage of $CD34^+$ cells/µl expanded from the initial $CD34^+$ cord blood cells, normalised to the number expanded in Stemline™ (Figure 1C). This represents an average ± S.E.M. for 3 donors. This data is further broken down into $CD38^-$ and $CD38^+$ progenitors as an indication of their degree of differentiation.

Using the same bench-scale assays, counted on day 14, for the expansion of bone marrow $CD34^+$ cells, Stemline™ performed as well as, or better than, the other competitors (Figure 2A). However, Stemline™ II was vastly superior to the other commercially available serum-free HSC media, giving approximately 5-fold more total nucleated cells ($n = 5$ donors; $p< 0.05$).

Figure 1.

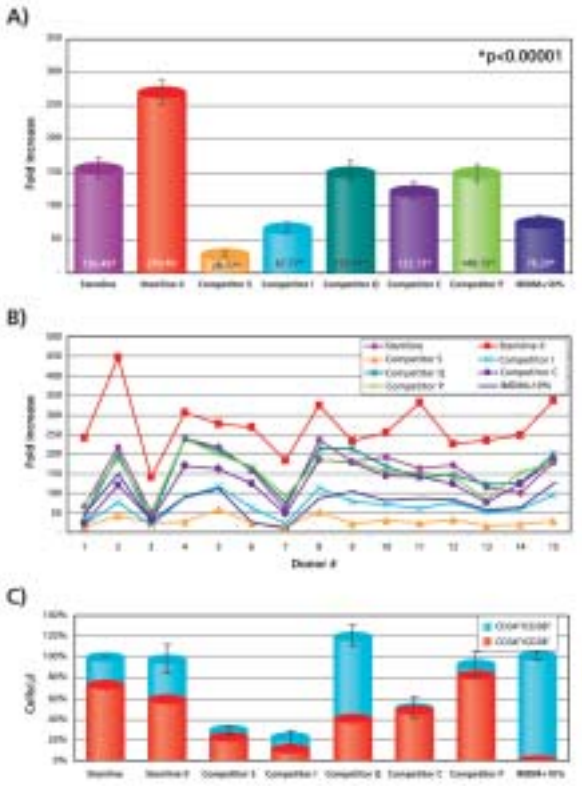

Figure 1. Expansion of $CD34^+$cells from cord blood in Stemline™ II compared to other commercially available, serum-free, HSC media. A. In cells purified from cord blood, Stemline™ II outperforms the other serum-free HSC media, exhibiting a significant increase in expansion ($p< 0.00001$). B. The second graph depicts the fold increase for each medium in all 15 donors. This also shows that Stemline™ II consistently provides the maximum number of TNC. C. The third graph in this series shows the percentage of $CD34^+$cells/ml expanded from the initial $CD34^+$cord blood cells, normalized to the number expanded in Stemline™. This represents an average ± S.E.M. for 3 donors. This data is further broken down into $CD38^-$ and $CD38^+$progenitors as an indication of their degree of differentiation.

Counted on day 14, cells derived from mobilised peripheral blood also consistently exhibited high levels of expansion of total nucleated cells when expanded in both Stemline™ products ($n = 7$; $p< 0.05$; Figure 3A). Flow cytometry on the expanded cells reveals that Stemline™ II also expanded a large number of $CD34^+$ stem cells (Figure 3B; both $CD38^+$ and $CD38^-$). Cells/µl

Expansion Medium for Hematopoietic Stem Cell

Figure 2.

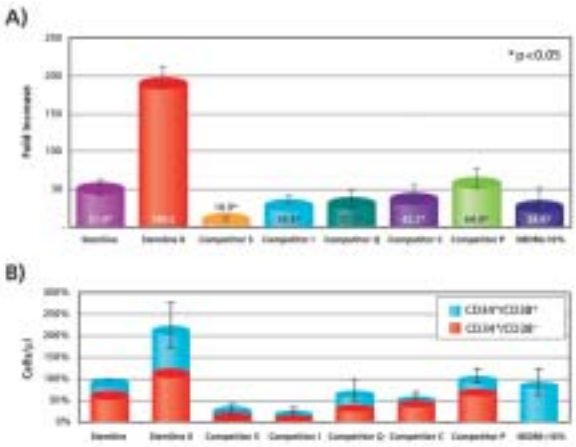

Figure 2. Stemline™II bench-scale expansion of CD34⁺cells from bone marrow compared to other HSC media. A. For the expansion of bone marrow CD34⁺cells, Stemline™II was vastly superior to the other commercially available serum-free HSC media (n = 5 donors; p< 0.05). B. Flow cytometry on the expanded cells reveals that Stemline™II also expanded a greater number of CD34⁺stem cells (both CD38⁺and CD38⁻). Cells/ml was normalized to the average number of cells/ ml in Stemline™ ± S.E.M. (n = 3 bone marrow donors).

were normalised to the average number of cells/µl in Stemline™ ± S.E.M. (n = 2 mobilised peripheral blood donors).

Overall, Stemline™ II Hematopoietic Stem Cell Expansion Medium provides for increased expansion of total nucleated cells from all three major cell sources. It also provides increased numbers of both early and late progenitors, as determined by the presence of CD38 surface antigens. These expansions also demonstrated another benefit of Stemline™ II Hematopoietic Stem Cell Expansion Medium. We often see donors, from all 3 sources, that do not generate enough cells to use for transplant. In these donors, Stemline™ II Hematopoietic Stem Cell Expansion Medium seems to rescue the proliferative capacity of the cells, in many cases allowing up to 10 times more expansion of TNC than any of the other commercially available products (data not shown).

Figure 3.

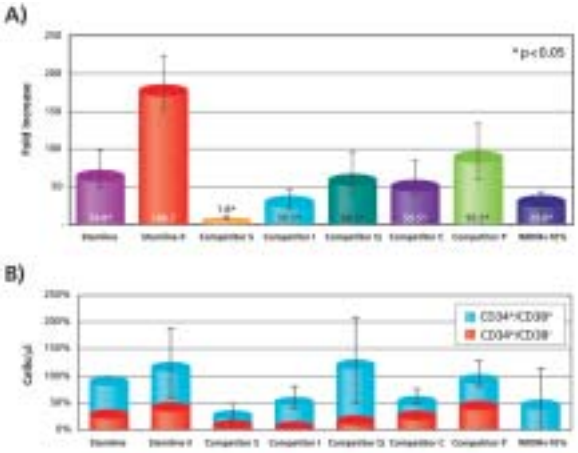

Figure 3. Bench-scale expansion of CD34⁺cells from mobilized peripheral blood in Stemline™II. A. In mobilized peripheral blood, both Stemline™ products consistently exhibit high levels of expansion of total nucleated cells (n = 7; p< 0.05). B. Flow cytometry on the expanded cells revealed that Stemline™II also expands a large number of CD34⁺stem cells (both CD38⁺and CD38⁻). Cells/ml were normalized to the average number of cells/ml in Stemline™ ± S.E.M. (n = 2 mobilized peripheral blood donors).

In order to prove that expansion of these cells was reproducible in a more clinically relevant format and that the cells retained their engraftment potential, we replicated a clinical-scale experiment to test both Stemline™ products. A two-step clinical-scale expansion was performed to compare cell growth in Stemline™ and Stemline™ II. Briefly, the cells were seeded into 100ml bags and incubated for 7 days. On day 7, a portion of the expanded cells was inoculated into a fresh 100ml bag for an additional 7 days. Both media demonstrated increased potential for expanding TNC from cord blood (Figure 4), supporting excellent growth and high viability (>80%). The expanded cells were analysed by flow cytometry for expression of CD34 and CD38. The majority of the CD34⁺ cells expanded in Stemline™ remained undifferentiated, early progenitors (CD34⁺/CD38⁻), while cells expanded in Stemline™ II contained both early (CD34⁺/CD38⁻) and late progenitor (CD34⁺/CD38⁺) phenotypes (Figure 5). Both media expand high levels of early progenitors, which is important for long-term engraftment. Stemline™ II also expands high levels of the late progenitors required for early engraftment and amelioration of the post-transplant nadir in mature myeloid cells.

After the two-step expansions were complete, a sample of cells from the Stemline™ and Stemline™ II media cultures were prepared for transplantation into NOD/SCID mice. Three different doses of cells were chosen for injection into the mice. A high percentage of the mice survived transplantation with cells expanded from both media (higher with Stemline™ II), all of which contained CD45⁺ human cells as proof of engraftment (a smaller number of which were also CD34⁺; Table 1). Both media expanded enough functional, early progenitors to achieve long-term engraftment. The higher survival rate in Stemline™ II may be explained by the higher levels of the late progenitors required for early engraftment and amelioration of the post-transplant nadir in mature myeloid cells. Further studies with secondary recipients are in process, with survival in excess of 9 weeks.

Figure 4.

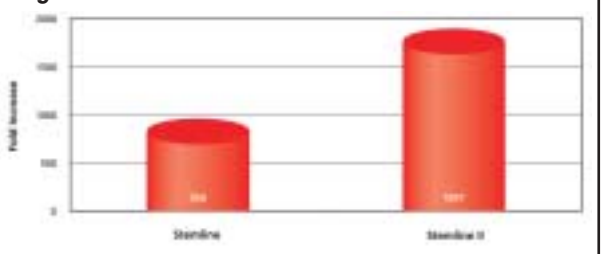

Figure 4. Comparison of Stemline™II to Stemline™in a clinical-scale expansion of CD34⁺cells from cord blood. A two-step clinical-scale expansion was performed to compare cell growth in Stemline™and Stemline™II. Both media demonstrate increased potential for expanding CD34⁺cells from cord blood, supporting excellent growth and high viability (> 80%).

Frequently asked questions - see page 13

Expansion Medium for Hematopoietic Stem Cell

Figure 5.

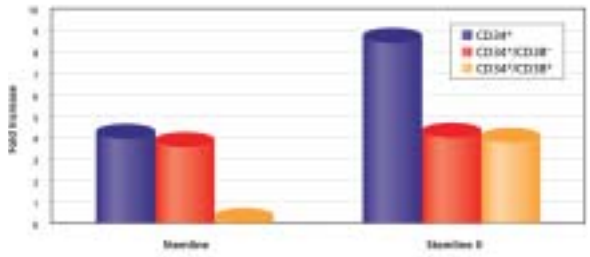

Figure 5. Flow cytometric analysis on CD34⁺cord blood cells from clinical-scale expansion. The majority of the CD34⁺cells expanded in Stemline™ remained undifferentiated, early progenitors (CD34⁺/38⁻), while cells expanded in Stemline™ II contained both early (CD34⁺/CD38⁻) and late progenitor (34⁺/38⁺) phenotypes.

References

1. Noort, W. A. and Falkenberg, J. H. F., Hematopoietic content of cord blood, in Cord blood characteristics: Role in Stem Cell Transplantation, Vol. 1, Cohen, S. B. A., *et al.*, (eds.) pp. 13- 37 (Martin- Dunitz, London, 2000).
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3. McNiece, I. K., *et al.*, Increased expansion and differentiation of cord blood products using a two- step expansion culture. Experimental Hematology, 28, 1181- 1186 (2000).
4. Szilvassy, S. J., *et al.*, Quantitation of Murine and Human Hematopoietic Stem Cells by Limiting- Dilution Analysis in Competitively Repopulated Hosts, in Hematopoietic Stem Cell Protocols, Vol. 1, Klug, C. A., and Jordan, C. T., (eds.) pp. 167- 187 (Humana Press, Totowa, New Jersey, 2002).

Table 1. Levels of risk presented by different types of cell cultures

	Stemline™			Stemline™ II		
Injected Cells	600,000	1,800,000	5,400,000	600,000	1,800,000	5,400,000
Survival Rate	5/10 50%	3/10 30%	3/7 43%	7/10 70%	6/10 60%	6/7 86%
Average % $CD45^+$	0.064 ± 0.061	0. 017 ± 0.006	0. 143 ± 0.081	0. 036 ± 0.013	0. 018 ± 0.019	0. 108 ± 0.162
Average % $CD45^+/CD34^+$	0. 000 ± 0.000	0. 003 ± 0.006	0. 007 ± 0.006	0. 011 ± 0.009	0. 002 ± 0.004	0. 010 ± 0.000
Both media expanded enough functional, early progenitors to achieve long- term engraftment. Further studies with secondary recipients are in process, with survival in excess of 9 weeks.						

Summary

In bench-scale expansions, Stemline™ and Stemline™ II media are capable of expanding $CD34^+$ cells from umbilical cord blood, adult bone marrow, and mobilised peripheral blood. Both Stemline™ media expand $CD34^+$ cells from all three sources better than other serum-free commercially available media. In clinical-scale expansions, both Stemline™ media were able to expand $CD34^+$ cells from cord blood. Flow cytometric analysis of the clinical-scale expansions revealed that Stemline™ and Stemline™ II expanded comparable numbers of early progenitor cells ($CD34^+/CD38^-$). Stemline™ II also demonstrated the additional benefit of a higher capacity for the expansion of the $CD34^+/CD38^+$ late progenitors required for short-term engraftment. Finally, cells expanded in both Stemline™ and Stemline™ II were capable of repopulating NOD/SCID mice using serial passage, demonstrating self-renewal of expanded cells, a critical functional test. Overall, the increased expansion along with the ability to produce the medium in a state-of-the-art cGMP facility makes Stemline™ II the best choice for clinicians seeking a serum-free product for their clinical hematopoietic stem cell applications.

Acknowledgements

We would like to thank the Saint Louis Cord Blood Bank, specifically Dr. Michael Creer, Mario Alonso, Donna Regan, Janet Schaeffler, and Cory Johnson for the flow cytometric analysis of the cells.

Ordering Information

Product	Description	Unit
S0192	Stemline™ II Hematopoietic Stem Cell Expansion Medium	500 ml 6 x 500 ml
S0189	Stemline™ Hematopoietic Stem Cell Expansion Medium	500 ml 6 x 500 ml
T1568	Thrombopoietin	5 µg
S7901	Stem Cell Factor	10µg
G0407	Granulocyte Colony-Stimulating Factor from Human	5µg

All products listed above are available from Sigma-Aldrich. For further information contact your local Sigma-Aldrich office or visit our website: **www.sigma-adlrich.com**

ECACC Cell lines Information

NS0 Serum-Free Cell Line

The NS0 mouse myeloma cell line is commonly used as a fusion partner for the generation of hybridomas. ECACC has successfully developed an NS0 serum-free adapted cell line, a variant of the parent. The NS0 serum-free cell line offers the opportunity to produce antibodies in the absence of animal-derived components. This is particularly relevant for the generation of antibodies for biopharmaceutical applications.

NS0 is a non-heavy and non-light chain synthesising myeloma therefore hybridomas generated express only the antibody of the parental primary B cell partner. The adapted cell line exhibits similar cell growth properties in comparison to the standard NS0 cell line grown in RPMI-1640 medium supplemented with 2mM L-glutamine and 10% foetal bovine serum (FBS).

The NS0 serum-free cell line is cultured in Hybridoma Medium supplemented with 8mM L-glutamine and 1% SyntheChol™ - a synthetic cholesterol supplement formulated by, and exclusively available from Sigma-Aldrich. The Hybridoma Medium is a propriety formulation containing inorganic salts, essential and non-essential amino acids, vitamins, recombinant human insulin, other organic compounds and trace elements. The absence of animal-derived materials eliminates the risk of contamination by adventitious agents associated with these components and reduces the performance variability in the medium. In addition, lower levels of contaminating proteins are present in the downstream production that could interfere with antibody purification.

Ordering Information

Product Name	Cat No.	Source
NS0 serum-free cell line	03061601	ECACC
Hybridoma Medium Cemically-defined Animal component-free	H4409	Sigma-Aldrich
SyntheChol™ NS0 Supplement, 500X (Aqueous solution cell *culture tested)	S5442	Sigma-Aldrich

New Cell Lines for the Collection

Rolf B1.T

A rat olfactory cell line that constitutively expresses glial fibrillary acidic protein, S100, the low affinity neurotrophin receptor p75 NTR, laminin, tenascin, and NCAM. These cells can support the re-growth of neurites from adult rat retinal ganglion cells. This cell line has potential value in research into mechanisms of glial support for axonal regeneration from adult mammalian central neurons and other aspects of differentiated glial cell biology. This cell line was deposited by Dr. Caroline Wigley (King's College, Guy's King's and St Thomas' School of Biomedical Sciences, London, UK).

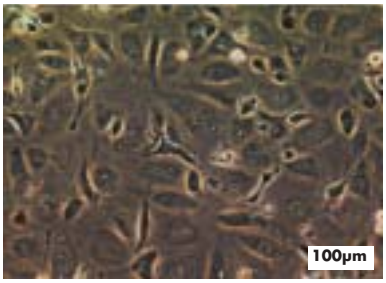
RCC4plusVHL & RCC4plusvectoralone

RCC4plusVHL is the renal cell carcinoma cell line RCC4 transfected with pcDNA3-VHL. The plasmid pcDNA3-VHL confers neomycin resistance and encodes the von-Hippel-Lindau (VHL) tumour suppressor gene product pVHL. The original renal cell carcinoma cell line RCC4 is VHL-deficient. The RCC4plusvectoralone, carrying the empty expression vector pcDNA3, can serve as a negative control cell line to study the effect of pVHL expression from pcDNA3-VHL. The cell lines were deposited by Professor Peter Ratcliffe, (Wellcome Trust Centre for Human Genetics, Oxford, UK).

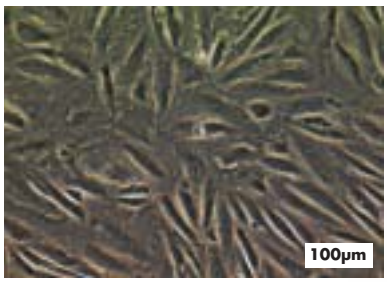
Ordering Information

Cell Line Name	ECACC Catalogue number
Rolf B1.T	03071601
RCC4plusVHL	03112703
RCC4plusvectoralone	03112702

RCC4plusvectoralone



RCC4plusVHL



ECACC Cell lines Information

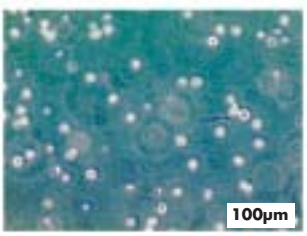
Protein-Free Cell Lines

ECACC offers a variety of cell lines that have been adapted to animal component and serum-free media. The absence of serum addresses a number of issues associated with animal-derived components in cell culture. A major regulatory concern is the presence of animal-derived components present in cell culture media used to generate biopharmaceutical products such as antibodies, recombinant proteins and vaccines. The absence of animal-derived components minimises the risk of contamination by adventitious agents associated with these components. Furthermore, lower levels of contaminating proteins are present in the downstream production that could interfere with the purification and performance of the final product. The absence of serum also reduces the performance variability between batches of medium. From a cell biology perspective, this promotes improved control and modulation of the cellular metabolism of the cell culture model under study. Both Sp2/0-Ag14-TurboDoma® and P3x63Ag8.653-TurboDoma® below have been adapted to grow in chemically defined protein- and peptide-free medium. For more information contact ECACC at **ecacc@hpa.org.uk** and ask for **information request 0401**.

Cell Line	Catalogue no.	Species	Tissue	Growth Mode
CHO (PROTEIN FREE)	00102307	Hamster	Ovary	Suspension/adherent depending on medium
Expression of recombinant proteins in the absence of animal derived products. Adapted to grow in suspension in CHO Animal Component-Free medium (Sigma C5467). Can be grown as an adherent cell line in CHO Animal Component-Free Attachment medium (Sigma C8730).				
MDCK-ProtCK-Free	02050101	Dog	Kidney	Adherent
Expression of recombinant proteins and replication of viruses for therapeutic applications. Adapted to MDCK Protein-Free Medium (Sigma M3678).				
Vero-Hektor™	03092503	Monkey	Kidney	Adherent
Expression of recombinant proteins and replication of viruses for therapeutic applications. Adapted to grow in chemically defined protein and peptide-free Hektor G medium.				
L-M	87032401	Mouse	Connective	Adherent
Derived from the NCTC clone 929 to grow in serum-free Medium 199 supplemented with 0.5% bactopeptone. Expression of recombinant proteins and replication of viruses.				
L929S	86032004	Mouse	Adipose	Suspension
Can be grown in serum-free DMEM supplemented with 0.5% bactopeptone.				
NS0 Serum-Free	03061601	Mouse	Myeloma	Suspension
Fusion partner for the generation of serum-free hybridomas. Adapted to grow in Hybridoma Medium (Sigma H4409) supplemented with SyntheChol™ (Sigma S5442) a synthetic cholesterol supplement.				
Sp2/0-Ag14-TurboDoma®	0603012501	Mouse	Hybridoma	Suspension
Fusion partner for the generation of serum-free hybridomas. Host for the expression of recombinant glycoproteins. Adapted to grow in the chemically defined protein and peptide-free TurboDoma® medium.				
P3X63Ag8.653-TurboDoma®	03092502	Mouse	Myeloma	Suspension
Fusion partner for the generation of serum-free hybridomas. Adapted to grow in chemically defined protein and peptide-free TurboDoma® medium.				

P3x63Ag8.653 - TurboDoma®

Sp2/0-Ag14-TurboDoma®



Vero-Hektor™

Information Request 0401

Depositing Cell Lines

New Head of General Collection at ECACC

Our new Head of the General Collection at ECACC is Dr. Peter Thraves. Peter obtained his honours degree in Biochemistry from the University of Liverpool, England. He went on to achieve a Ph.D. in Biochemistry in the Department of Biochemical Pharmacology at the Institute of Cancer Research, Sutton, Surrey. Following Peter's doctoral studies he obtained a Postdoctoral Fellowship in the Department of Biochemistry at Georgetown University Medical Center, in Washington, D.C. During his postdoctoral appointment Peter studied the role of poly-(ADP-ribose) polymerase on chromatin structure and its activation by carcinogens.

After his postdoctoral studies Peter was appointed Assistant Professor in the Department of Radiation Medicine at the Vincent T. Lombardi Cancer Research Centre, Georgetown University Medical Centre, Washington, D.C. During this appointment he continued his studies into ADP-ribose metabolism and developed additional areas of study involving the molecular and cellular mechanisms in human tumours exposed to ionising radiation. While still at Georgetown Peter developed additional areas of research interest into the development of human cell culture systems as *in vitro* models for carcinogenesis.

In 1998, Peter joined the biotechnology company Onyvax based at St. George's Hospital Medical School, London, creating human cells lines, both normal and cancerous, to serve as whole cell vaccines. In particular, to develop permanent cell lines from human tissues and optimise growth conditions for the production of a whole cell vaccine for prostate cancer. During his time at Georgetown and at Onyvax, Peter was responsible for generating many new permanent cell lines from several types of human tissue including prostate, breast, melanoma, colorectal tissues and kidney.

Following his appointment at ECACC, Peter will expand the General Collection to include cell lines of current and future use to the research and biomedical communities. The new cell lines will be brought into the collection with a view to creating groups of cell lines with common themes.

Contact Dr. Peter Thraves on peter.thraves@hpa.org.uk or + 44 (0)1980 612961, if you are interested in depositing a cell line into the collection or for further information.

Depositing Cell Lines in the Collections

The General and Hybridoma Cell Collections at ECACC have developed over the last 20 years. This development has followed requests from customers for particular cell types and donation of cultures by numerous investigators. Depositing cultures with ECACC promotes the distribution and use of cell lines throughout the scientific community and encourages the development of new cell-based technologies. Through donating cell lines to ECACC a depositor is internationally recognised as the originator and has their work secured for posterity.

ECACC is continuing to expand the diversity of its collections and is currently acquiring primary and permanent cell lines from epithelial, mesenchymal, endothelial, neuronal and embryonal tissues. In addition, ECACC is continuing to add to its human cell collection to include normal and disease states, various tumour cell types: breast, lung, colon, pancreas, skin, cervical and gonadal and cells from transgenic animals.

Depositing a cell line into the General Collection at ECACC is not only an easy process but is also **free of charge.** Depositors benefit from storing their cell lines in ECACC's state-of-the-art on-site storage facility that contains over 40 storage vessels. ECACC has one of the largest liquid nitrogen repositories for animal cells in Europe. It provides depositors with the guarantee of confidentiality, security, monitoring, annual reporting of stock status, stringent quality control testing and the dedication of ECACC's trained staff who are on site 24 hours a day.

All cell lines deposited in ECACC's General Collection are supplied strictly for research use only as specified in ECACC's Standard Terms and Conditions of Sale. ECACC does not own its cell lines and serves in the role of custodian. Consequently ECACC will observe any conditions or restrictions placed on the distribution of a cell line by the Depositor. In some cases the Depositors permission to release is obtained for each individual customer. In the event ECACC is approached with a request for commercial use of a cell line, such a request will always be referred to the Depositor.

Upon receipt of a cell line, ECACC will create a bank of the new cell line within its quarantine laboratories. Quality control measures are then in place to ensure viability of the bank, authentication and the absence of microbial contamination. On successful completion of all quality control checks the cell line is allocated a unique accession number and included in the catalogue with the approval of the depositor. The cell line is then made available to the scientific community in a tightly controlled manner. Advertisements within ECACC's Cell Line Catalogues, website and other promotional materials encourage worldwide distribution and applications.

For full details on 'How to deposit a cell line(s)' contact ECACC on + 44 (0)1980 612512 or ecacc@hpa.org.uk.

Media Expert™

The **MEDIA EXPERT™ (ME)** is the web's most comprehensive source for information on cell culture media formulation. Use the MEDIA EXPERT™ to learn about the major formula components found in cell culture media. ME explains why the component is used, what it does, and how it benefits or potentially causes problems in a cell culture system.

ME provides detailed information on each component, for example:

- component's primary function *in vivo* and *in vitro* with graphic representations and pathways
- chemical nature of component
- and much more!

The MEDIA EXPERT™ is intended as a handy reference. Each component discussion is essentially a mini-review article to help you better understand its role in cell culture.
The MEDIA EXPERT™ is also a problem solver. ME addresses a myriad of common symptoms seen in cell culture systems. It suggests possible causes and provides guidance for relieving the symptoms observed.

ME suggests paths of exploration to resolve common culture symptoms, such as:

- cell clumping
- poor cell attachment
- poor cell growth
- 10 other common symptoms

Stemline™ - Frequently Asked Questions

As a major Life Science supplier, Sigma-Aldrich has spent years helping customers with numerous application and technique inquiries.
By using some of the information gained from these customer contacts, we present some of our Frequently Asked Questions (FAQs) about our feature article. These FAQs will hopefully serve as a guide to help identify and resolve some of your concerns.

Are there animal-derived components in the medium?
No bovine or other animal-derived components are in Stemline™ II Medium (Product Code S0192). Human serum albumin is the only component of human origin. It has been found to be non-reactive (donor level) for Anti-HIV 1 & 2, Anti-HCV and HBsAg, but should be handled as if potentially infectious.

Are there growth factors or cytokines in the medium?
Other than insulin, Stemline™ II Medium is formulated without the addition of growth factors, to allow the end-user to supplement with a cytokine cocktail of choice.

Which growth factors or cytokines should be added to the medium?
The medium should work well with many of the commonly used cytokine cocktails. However, the medium was designed using 100 ng/ml each of Stem Cell Factor (SCF; Product Code S7901), Granulocyte Colony-Stimulating Factor (G-CSF; Product Code G0407) and Thrombopoietin (TPO, Product Code T1568). We recommend, that end-users try whichever cocktail they would normally use to expand $CD34^+$ cells, but use Stemline™ II as the base medium.

Is there a device master file (DMF) with the FDA for this medium?
A DMF is in process and should be filed with the FDA in the near future.

Should I use Stemline™ Medium (Product Code S0189) or Stemline™ II Medium (Product Code S0192) to expand cells in my application?
Both products work quite well for the expansion of total nucleated cells (TNC) from $CD34^+$ cells. Stemline™ II Medium significantly expands more TNC. However, the differentiation patterns in the two products are somewhat different. We recommend that both products should be tested in an application to determine which product generates the desired cell output.

Can the medium be used in clinical-scale applications in cell culture bags?
Stemline™ II Medium has been shown to be scalable into clinical-scale applications using cell culture bags, while still exhibiting high levels of expansion. The expanded cells have surface antigen profiles consistent with previously reported profiles for *ex vivo* expanded cells and demonstrate long-term engraftment (primary and secondary recipient) in NOD/SCID mouse models.

How stable is the product? Does the medium require supplementation with L-glutamine?
This medium is stable, when stored at 2-8°C and protected from light, until the date indicated on the label.
Supplementation with L-glutamine is not required prior to using this product.

For advice and information on all our products, contact your local Sigma-Aldrich office or visit our website www.sigma-aldrich.com

MegaCell™: The Next Generation of Classic Cell Culture Media

Authors:

Stacy Leugers, Terry Johnson, Anne Dennett, Amber Hoffelder, Heather Loke, and Laurie Donahue *Sigma-Aldrich Corporation, St. Louis, MO, USA*

Introduction

Historically, the majority of cell culture has been performed using classical media that were developed in the golden age of cell culture (1950's and 60's) such as Dulbecco's Modified Eagle Medium (DME), Minimum Essential Medium Eagle (MEM), and RPMI-1640. Depending on the exact medium formulation and cell type, 5-20% foetal bovine serum (FBS) is added to the medium to ensure proper cell growth. Today the trend is to use more defined media that have been developed to be less dependent on serum supplementation, but there are still numerous applications that use classical media supplemented with FBS. Due to increasing cost and decreasing availability of FBS, a new product line has been developed for those investigators interested in reducing their dependence on serum. The MegaCell™ product line is a set of five media based on classical media formulations that have been optimised to support cell growth with 3% serum supplementation comparable to growth observed with classical media containing 10% serum. Our understanding of cellular biochemistry and nutrition has increased greatly during the almost half century since these classical formulations were first reported in the literature. Utilising this knowledge, very specific components have been added to each individual medium to enrich them such that the required level of serum could be lowered. Various supplements, including lipids, buffers, amino acids, trace elements, and alternate energy sources, were added to each classical medium to create the MegaCell™ media product line. Sigma-Aldrich now offers five fortified MegaCell™ media products:
MegaCell™ MEM (Product Code M4067),
MegaCell™ DME (Product Code M3942),
MegaCell™ MEM: F12 (Product Code M4317),
MegaCell™ DME: F12 (Product Code M4192), and
MegaCell™ RPMI-1640 (Product Code M3817).

Customers who utilise the MegaCell™ media will be able to save money by reducing the levels of FBS needed without the need to wean cells.

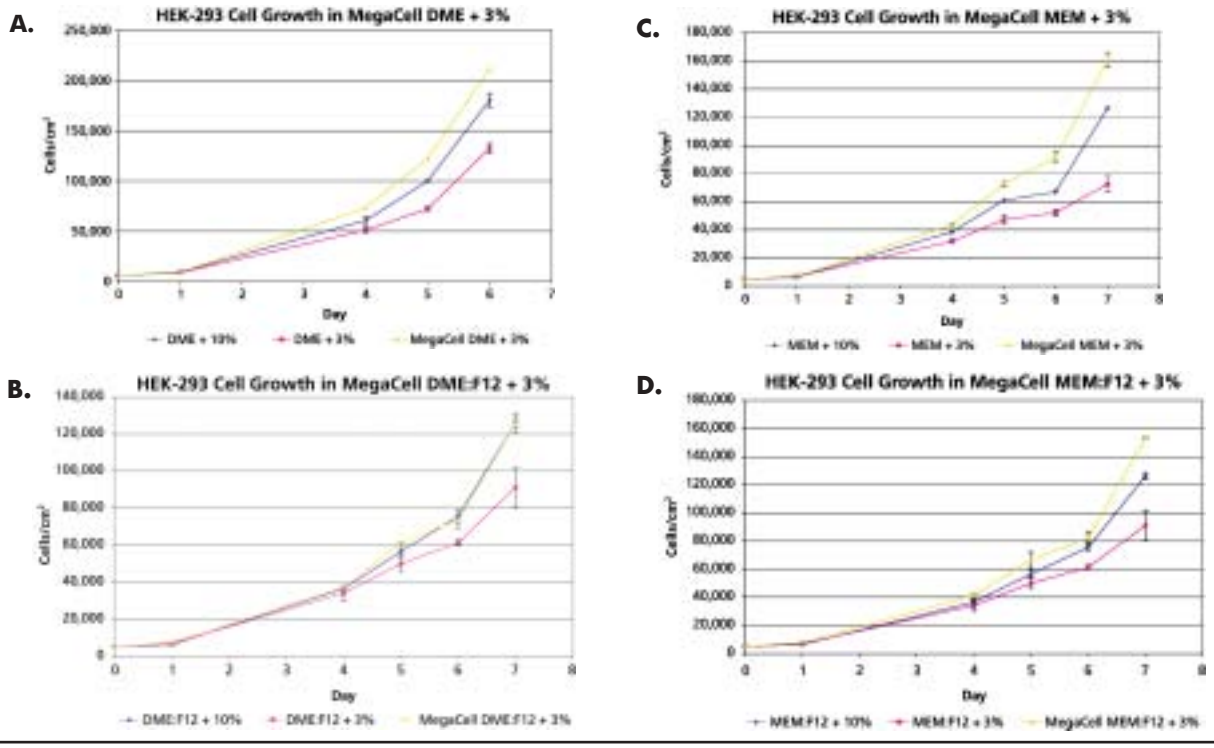
Materials and Methods

All materials were supplied by Sigma-Aldrich Corporation (St. Louis, MO) unless otherwise stated.

Media Preparation

All five of the MegaCell™ media were supplemented with 3% FBS and 4mM L-glutamine and warmed to 37°C before use. The competitor media tested were GIBCO's Advanced D-MEM (12491015), Advanced MEM (12492013), and Opti-MEM® I (31985070). All of these media are supplied by Invitrogen (Grand Island, NY) and were used according to the manufacturer's directions (supplemented with 3% FBS).

Figure 1. Attached cultures were seeded at 5,000 cells/cm² in 6-well plates in duplicate. *The assays were counted on days 1, 4, 5, and 6. When testing HEK- 293 cells all four media perform as well as, or better than, the 10% positive control. Figure (A) MegaCell DME (Product Code M3942); (B) MegaCell DME: F12 (Product Code M4192); (C) MegaCell MEM (Product Code M4067); (D) MegaCell MEM: F12 (Product Code M4317). Average of 2 wells for each experiment.*



MegaCell™: The Next Generation of Classic Cell Culture Media

Suspension Cell Culture

A hybridoma cell line, HFN 7.1, was used to evaluate the performance of MegaCell™ RPMI-1640 (Product Code M3817). The cell stocks were cultured in classical RPMI-1640 + 10% FBS in static T-flasks. The MegaCell™ RPMI-1640 development assays were performed in duplicate T-25 static flasks. The flasks were inoculated at 75,000 cells/ml with a final volume of 5ml/flask. The assays were counted using a hemacytometer and CASY® Counter (Scharfe Systems, Reutlingen, Germany) on days 1-4 and subcultured on day 3. The subculture was counted on days 1 and 4.

Attached Cell Culture

A human embryonic kidney cell line, HEK-293, was used to evaluate the performance of MegaCell™ DME (Product Code M3942), MegaCell™ MEM (Product Code M4067), MegaCell™ DME: F12 (Product Code M4192), and MegaCell™ MEM: F12 (Product Code M 4317). The cell stocks were cultured attached in T-225 flasks in classical DME + 10% FBS. For each development assay the cells were seeded in duplicate at 5,000 cells/cm^2 in 6- well plates containing 3ml of media in each well. The wells were counted on days 1, 4-6 and subcultured on day 5. The subculture was counted on days 1 and 6.

Results and Discussion

MegaCell™ media supplemented with 3% FBS are able to perform as well as, or superior to, the classical version of the same medium supplemented with 10% FBS. This is illustrated in Figures 1 and 2. Figure 1 (A-D) shows the results of the attached cultures and Figure 2 shows the results of the suspension cultures.

MegaCell™ Media Support the Growth of Multiple Attached and Suspension Cell Lines

Because of the diverse medium requirements of various cell lines and the variation in the composition of the MegaCell™ media products, some variability in the performance of different cell line and media combinations is expected. To better understand the characteristics of the MegaCell™ media products a survey was undertaken to evaluate the growth of some commonly used cell lines in the various MegaCell™ media. As expected, not all versions of the MegaCell™ media work for all cell types, but in general there was at least one version of the media that worked well with each cell line tested (Table 1).

MegaCell™ Media can be Supplemented with Calf or Adult Bovine Serum

Due to the increasing cost and decreasing availability of foetal bovine serum (FBS) the possibility of supplementing MegaCell™ media with less expensive and more readily accessible adult bovine serum (ABS) and/or calf serum (CS) was explored. ABS and CS are characteristically far less nutritionally rich than FBS; therefore it is not usually possible to use these sources of serum to achieve superior cell growth. Serum titrations were completed using both ABS and CS in the MegaCell™ media using the same assay design as employed in the previous studies. MegaCell™ media supplemented with either ABS or CS were able to perform as well as if supplemented with 3% FBS. The levels of the ABS and CS that were required to reach this performance level varied from 6-10% depending on the version of the media and the cell type tested. Figures 3 and 4 illustrate examples of the performance of the MegaCell™ media supplemented with ABS and CS.

MegaCell™ Outperforms the Competition

Media from the MegaCell™ product line were evaluated for their ability to support cell growth relative to other commercially available fortified media designed for use with reduced levels of serum supplementation (GIBCO's Advanced D-MEM, Advanced MEM and Opti-MEM® from Invitrogen). Attachment dependent and suspension cell

Figure 2. *The MegaCell™ RPMI (Product Code M3817) + 3% FBS outperforms both RPMI with 3% FBS and RPMI with 10% FBS. The MegaCell™ RPMI supplies more nutrients, allowing the cells to get to higher cell densities then ever seen before. For this assay HFN 7.1 cells were seeded at 75,000 cells/ ml in static T- 25 flasks and counted on days 1- 4. Average of 2 flasks.*

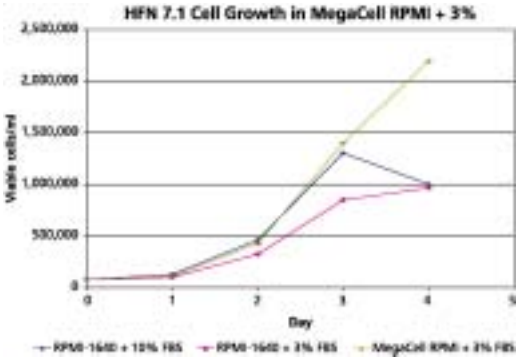
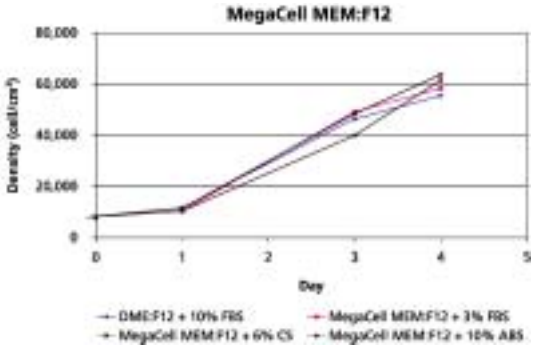


Figure 3. *MegaCell™ MEM: F12 was supplemented with 6% calf serum (CS) or 10% adult bovine serum (ABS) and growth was compared to DME: F12 + 10% FBS and MegaCell™ MEM: F12 + 3% FBS. Both the CS and the ABS- supplemented media performed as well as the MegaCell™ with 3% FBS and as well as the classical medium plus 10% FBS.*



MegaCell™: The Next Generation of Classic Cell Culture Media

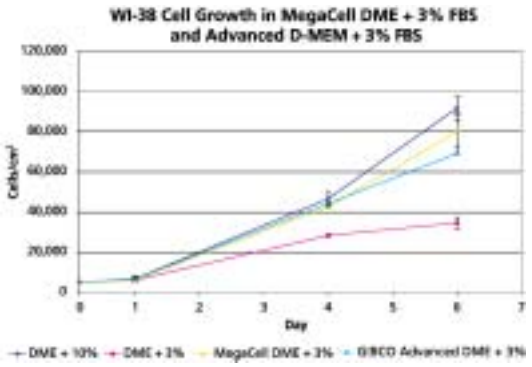
Table 1.

	Cell Line	Seeding Density	DME M3942	DME: F12 M4192	MEM M4067	MEM: F12 M4317	RPMI M3817
Attached	VERO	5000/cm²	–	+	+++	++	–
Attached	MDBK	5000/cm²	++++	+	+	+++	–
Attached	MRC-5	5000/cm²	++	+++	++	+++	–
Attached	WI-38	5000/cm²	+++	+++	+	+++	–
Suspension	SP20	75000/ml	–	–	–	–	++++
Suspension	HFN	75000/ml	–	–	–	–	++++

Note: Number of "+" indicates strength of application data results. Note: MegaCell™ is not recommended for 3T3 or MDCK cells.

lines were grown in MegaCell™ media and a comparable fortified medium with reduced levels of serum (3%) and a comparable non-fortified classical medium with 10% FBS. Example growth curves for attached (Figure 5) and suspension (Figure 6) cell lines are shown. In all cases, the MegaCell™ media outperformed the other media tested.

Figure 5. *When testing the Advanced D-MEM with attached WI-38 cells the MegaCell™ DME performs better than the Advanced D-MEM. Average of 2 wells.*



Summary

The MegaCell™ media product line is a group of fortified media based on classical media formulations that have been enriched to support cell growth under reduced serum conditions. When supplemented with 3% FBS these media have been shown to support cell growth comparable to non-fortified classical media supplemented with 10% serum without the necessity of weaning or adaptation. Additionally, the fortifications incorporated into the MegaCell™ media products have been found to allow the use of adult bovine and/or calf serum in place of more expensive and increasingly scarce fetal bovine serum. Recognising the diversity of cell lines in use, Sigma–Aldrich has developed a total product line of media with a broad range of formulations and nutritional components to better serve our customers.

Figure 4. *An example of the suspension data. The best serum substitute for FBS in MegaCell™ RPMI when growing HFN 7.1 cells was 6% calf serum (CS). The adult bovine serum (ABS) did not support growth as well as compared to the CS (data not shown).*

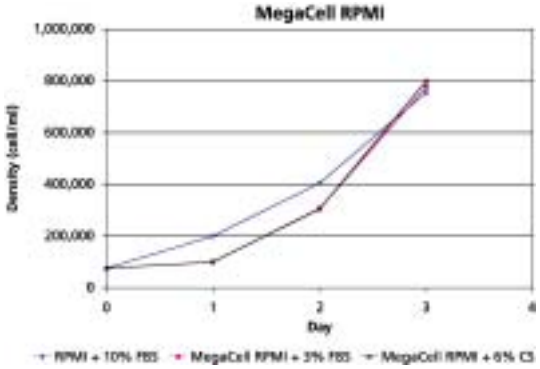
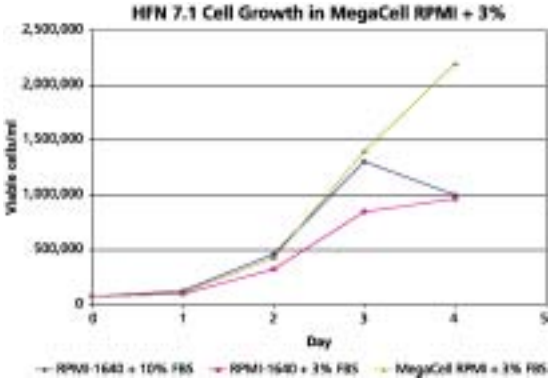


Figure 6. *MegaCell™ RPMI + 3% FBS outperforms GIBCO's Opti-MEM I + 3% FBS. The Opti-MEM I growth curve shows a large lag phase, while the MegaCell™ RPMI-1640 allows for exponential growth starting almost immediately after inoculation. Average of 2 wells.*



Prod No.	Description	Pack Size
M3942	MegaCell™ DME	500ml
M4067	MegaCell™ MEM	500ml
M4317	MegaCell™ MEM: F12 Ham	500ml
M3817	MegaCell™ RPMI-1640	500ml
M4192	MegaCell™ DME: F12 Ham	500ml

Sigma-Aldrich uses the MegaCell™ trademark pursuant to an agreement with Cortex Biochem, Inc.

Information Request 138

Ask ECACC.... Focus on Mycoplasma

In this issue ECACC has concentrated on answering the predominant queries relating to mycoplasma.

What are Mycoplasma?

Mycoplasma are the smallest (0.2-2µm in diameter) and most unusual of the prokaryotes. The microorganism's characteristics include:

- The absence of a rigid peptidoglycan cell wall and intracytoplasmic membrane
- The presence of small genome
- Reproduction by binary fission
- A requirement for cholesterol for membrane function and growth and the use of UGA codon for tryptophan

Mycoplasma contain the minimum macromolecular constituents required for self-replication in a cell-free medium, and so are intimately dependent upon the host for nutrients and protection. At least 10^8 colony-forming units of mycoplasma per millilitre of medium can be present within a cell culture (1,2,3).

There is No Visual Indication that my Cell Line is Contaminated with Mycoplasma. Do I Still Need to Check for Mycoplasma?

Yes, Mycoplasma can rapidly spread throughout the laboratory. All cells from normal or neoplastic tissues, primary or continuous, fibroblastic or epithelial and monolayer or suspension are susceptible to contamination. It is often difficult to detect mycoplasma because the contaminated culture may grow well and appear to be normal under a light microscope. Moreover, there is normally an absence of visual signs such as turbidity, cytopathic effect, and pH change.

What Changes in the Cell does Mycoplasma Contamination Cause?

Mycoplasma can cause alterations in the rate of cell growth (4), induction of morphological changes, cause chromosome aberrations (5), changes in amino acid and nucleic acid metabolism, induction of transformation, induction of apoptosis, induction of cytokines and oxidative radicals, macrophage activation, inhibition of antigen presentation, and induction of signal transduction (6). In addition, mycoplasma can also interfere with membrane receptor function and penetrate the host cells (7).

What are the Sources of Mycoplasma Contamination?

Exogenous mycoplasma arise from a variety of sources which include:

- Cross-contamination from already infected cell cultures from unknown sources. This is the most common cause of mycoplasma contamination
- Serum products
- Aerosols created from pipetting
- Multiple users of the laboratory who come into contact with contaminated cultures and spread it through usage of laboratory instrumentation, media and reagents
- General environment and products such as hand lotion dispensers and ethanol sprays

What Screening Methods can be used to Detect Mycoplasma?

Many different methods are employed to detect mycoplasma contamination in cultures, and each has advantages and disadvantages with respect to cost, time reliability, sensitivity and specificity. The methods used include: culture isolation (1), Figure1, indirect DNA stain (2), Figure 2, immunological methods (8), nucleic acid hybridisation (9), the use of MycoAlert™ to detect the activity of mycoplasmal enzymes, electron microscopy and PCR (10,11). Reliance on a single detection method is not advisable. If mycoplasma contamination is detected, it is important to find the source of the problem. ECACC conducts stringent quality control procedures for all its cell lines and can offer to test cultures for you using the two methods listed below:

Figure 1: Detection of Mycoplasma by Culture Isolation

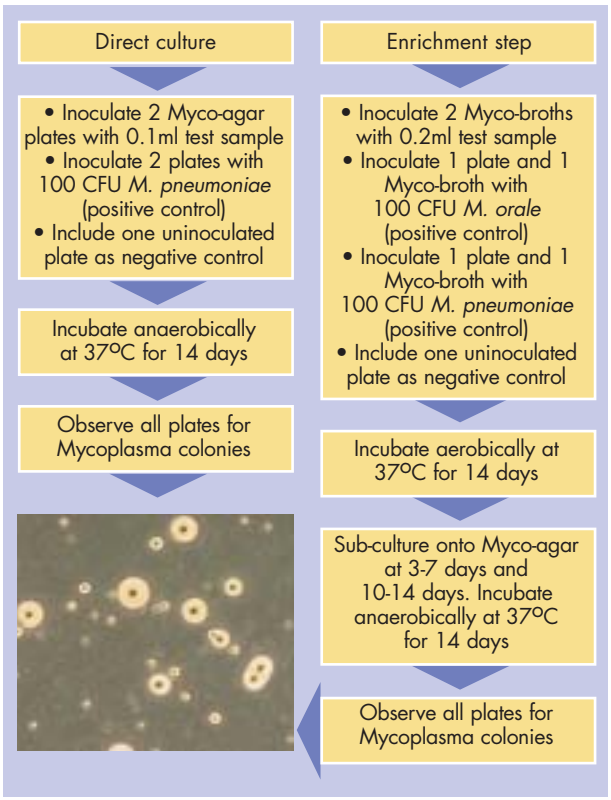
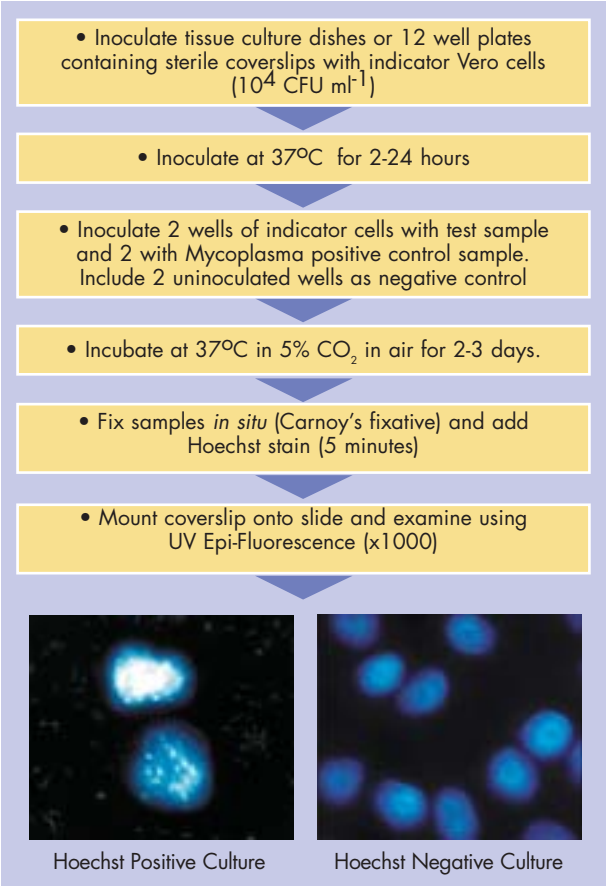


Figure 2: Detection of Mycoplasma by Indirect DNA Stain



ECACC also uses PCR as a rapid screening method for detection of mycoplasma using specific primers designed by ECACC. This technique allows large numbers of samples to be tested. It is recommended that PCR should be carried out in conjunction with culture isolation and indirect DNA stain.

	Performance Characteristics		
Method	**Sensitivity**	**Species Range**	**Speed**
PCR	Low/Medium	Uncertain	1 day
Indirect DNA Stain	Medium	All	2-3 days
Culture Isolation	High	Majority	3-4 weeks

What Do I Do if I Have Mycoplasma Contamination?

Mycoplasma are nicknamed the "crabgrass" of cell culture because their contamination is persistent and spreads rapidly to other cell lines and laboratories. The direct actions that need to be followed are:

- Discard the infected cell line(s)
- Discard any consumables, medium, constituents, and serum that have been in contact with the cell line.
- Fumigate laboratory including the micobiological safety cabinet.
- Inform all members of your institution (i.e. those that use cell lines) of the problem, so appropriate action can be taken by all.
- Obtain a fresh mycoplasma-free culture from either clean 'in-house' stock or from a reputable source.

How Can I Eradicate the Mycoplasma?

ECACC recommends that eradication should only be considered if a cell line is irreplaceable. This should be performed in an entirely separate facility to the general cell culture area to ensure that non-contaminated cell lines are not at risk. There is no guaranteed method to eradicate mycoplasma contamination from a cell line and manufacturers make many claims for products which should be viewed with caution. Antibiotic or chemical treatments are the most commonly used methods. ECACC has successfully employed fluoroquinolone based antibiotics (DNA gyrase inhibiting) include cycrofloxacin, MRA and Novobiocin. The more common antibiotics such as penicillin, kanamycin and gentamycin, although often reported otherwise, are ineffective against mycoplasma contamination.

A disadvantage in using antibiotic treatment is the risk of developing resistant strains of mycoplasma. Furthermore, the cytotoxic effects of the antibiotic can lead to cellular death and in some instances, loss of cellular characteristics is noticeable with antibiotic treatment. The entire procedure is time consuming and the eradication procedure may be ineffective in treating some mycoplasma species.

What Recommendations can ECACC Make to Prevent the Occurrence of Mycoplasma Contamination?

Do not purchase cell lines from unreliable sources. If this is not possible, quarantine all new cell lines in a facility that is separate from well established mycoplasma-free cell lines. Ideally, a separate designated quarantine laboratory would be preferable.

The use of the same containers of serum or medium by multiple users should be avoided. Both reagents should be tested frequently and discarded when mycoplasma has been detected. When a cell line has tested negative for mycoplasma it is good practice to freeze a number of ampoules as back-ups.

ECACC recommends that antibiotics should not routinely be used in cell culture. Use of antibiotics can mask poor aseptic technique. A more rapid detection method (i.e. Indirect DNA Stain and PCR) to test for mycoplasma contamination may be used for routine monitoring of active cell culture operations.

The risk of mycoplasma contamination is increased by staff who are poorly trained in core areas such as aseptic techniques and good laboratory practice.

ECACC recommends that a training regimen is in place at the institution before new staff handle the cell lines.

Users must be aware of the potential problems that may arise. Good laboratory management by the laboratory supervisor will contribute to the reduction/elimination of a risk of contamination.

Monitor for mycoplasma by;
- testing cultures once a month
- test all newly arrived cultures immediately
- test culture prior to freezing
- test when cultures are suspect

Otherwise contact ECACC at **www.ecacc.org.uk** and sign on the Level I Training Course which covers both practical and theoretical aspects of cell culture.

How Do I Submit a Sample for Mycoplasma Testing at ECACC?

A biohazard form and sample submission form (located on the ECACC website) must be completed and accepted by ECACC before any materials can be sent. ECACC will then contact you to confirm acceptance of the material and supply you with a reference number, which should be quoted in all further correspondence. Samples sent without a biohazard form will not be accepted for testing.

ECACC will accept cell cultures either growing or as frozen ampoules. If a frozen ampoule is sent then details of the media used must accompany it. This will be subjected to a handling fee to cover costs of establishing the cell lines in culture. Growing cultures must have been passaged twice in the absence of antibiotics and cryoprotectants.

Cultures should be growing for three days since the last medium change. Notification of the time of the last medium change must be made so that the minimum growth time can be completed at ECACC if necessary. The sample volume required is a 25cm^2 flask or frozen ampoule.

Flasks should be sent topped up to the neck with medium, in a non-vented flask, sealed to prevent leakage and packed in absorbent material for the event of spillage. Frozen ampoules should be sent with adequate dry ice to keep them frozen for the journey.

Packages should be clearly labelled with the storage temperature, sender, contact information, the quotation number provided by ECACC and should be addressed to the ECACC QC Department. Packaging and transportation should be in accordance with UN 602 regulation. Approved UN 602 packaging may be obtained from several suppliers, the details of which are available from any Royal Mail sales centre. If you use a courier service to deliver your samples, please contact the company involved for advice on their own approved packaging.

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1. Fundamental Techniques in Cell Culture...A Laboratory Handbook. 1st edition from ECACC and Sigma-Aldrich
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9. Non isotopic probing, plotting and sequencing. Kricka, L. J. (ed.), Academic Press, Inc. San Diego, CA.
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Ordering Information

Description	Price £
Indirect DNA Stain	110*
Culture Isolation	150*
PCR	60*
All three tests together	260

*A sample preparation fee of £90 per samples is charged if samples are received frozen or required passaging to remove antibiotics.

If you have any technical questions relating to ECACC products or services please contact ECACC on Tel: +44 (0)1980 612684 or by email: **ecacc.technical@hpa.org.uk**

Cell Culture Training Courses

ECACC Cell Culture training courses have attracted international scientists interested in improving their cell culture techniques and reducing microbial contamination. Depending upon the level you choose, the techniques covered by ECACC include:working aseptically, resuscitating, sub culturing, cryopreserving cells and lab management. You will also be provided with comprehensive instruction in fundamental areas of current significance by guest speakers who are specialists in their subjects.

Enhance your professional development and register. The course dates for 2004 are:

Level I 9th-12th November
Level II 5th-8th October

For a place on any of the courses, contact ECACC +44 (0)1980 612512 or email at ecacc@hpa.org.uk.

Sigma Capabilities in Cell Culture Media Development and Optimisation

Media Development and Optimsation

A proven process – for better results

Sigma Cell Culture R&D capabilities are built to meet your custom medium development needs, whatever they might be. Offering an integrated approach to medium selection, development, testing and validation, we'll deliver increased cell growth and productivity. That means faster results – and a greater competitive advantage for you.

Our Development Process

Our process consists of four phases, combining three different approaches that works to increase efficiencies, decrease time requirements and maximise overall medium performance against design goals.

Three Design Approaches

Analytical Approach – Our R&D staff selects the base media with the best performance characteristics (growth, productivity, etc.), speeding the selection process with a full range of analytical tools to perform raw material characterisation and spent medium analysis.

Molecular Approach – Microarray analysis helps to us identify new media components that might not be considered otherwise. This technique enables targeted testing for growth factors & receptors, hormones, attachment factors & receptors, plus signaling agents that affect cell growth or other cellular processes in your culture system leading to more optimal performance.

Statistical Approach – A multidimensional approach, based on Design-of-Experiment (DOE) principles, to optimising medium components. We use several methods to test multiple variables simultaneously, reducing the number of test conditions without a significant loss of information to speed the development process.

Creating Better Results Through a Better Process:

- Reduced experimental iteration resulting in better labor efficiency
- Early definition and recognition of interactions between components
- Better optimisation of interacting components
- More objectively defined endpoints
- Reduced development cycles of 3 to 9 months compared to linear methods
- Faster time to market and better return on investment

Defining the Measures of Success First:

- Cell growth rate
- Cell attachment efficiency
- Lack of cell aggregation (clumping)
- Product quality (e.g., glycosylation)
- Maximum cell density
- Resistance to agitation forces
- Product or virus yield
- Product or virus stability
- Maintenance of a differentiated or undifferentiated state

Selecting the Optimum Base Medium:

- Over 200 catalogue formulations and their additional variants
- Comprehenisve array of specialty media that include serum-free, protein-free, chemically-defined, and animal component-free formulations
- CHO family (over 35 formulae) media
- Hybridoma/NSO media
- Gene therapy (HEK 293, Per.C6) media
- Insect medium (S*f*9 and S*f*21)
- Stem cell media (hematopoietic, neural, mesenchymal, skin-derived)

Qualifying Key Raw Materials:

- Extensive testing program under cGMP specifications
- Complete traceability and full documentation with certificates of analysis, origin and suitability
- Multi-compendial (USP/EP) grade components
- Products for formulation include various lipid supplements, transferrin substitutes, plant hydrolysates, and recombinant growth factors and

Sigma Capabilities in Cell Culture Media Development and Optimisation

hormones that have been specifically designed and optimised for medium formulation

- Non-animal derived components include amino acids, lipids, vitamins, and transferrin substitutes

Verifying Performance in the Final System:

- Generation of application data under conditions that represent the final use of the medium in a production environment – e.g., stirred-tank, perfusion and hollow fiber bioreactors
- Validated scale up of final product in cGMP manufacturing processes further supported by method development, method validation and stability studies
- Ensured to meet the performance and regulatory demands of the biopharmaceutical industry

Supporting the Process through Quality Systems:

- Medium design controls under ISO guidelines
- Manufactured in FDA-registered and ISO 9001:2000 certified facility
- Process validation
- DMFs are on file and can be filed in support of your product
- Complete documentation available (CoAs, CoOs, batch records, etc)

Flexibility and Accountability in Collaboration:

- Flexible contract terms
- Defined deliverables
- Concise experimental plan (project format) with milestone evaluation points
- Timelines meeting and surpassing your expectations against objectives and costs
- Secure cell transfer and banking
- Scientist exchange at either Sigma-Aldrich R&D center or customer site
- Our internal analytical support or external testing services as needed
- Faster time to market and better return on investment

Analytical Assay Lab Capabilities

- Ion Chromatography
  - *Inorganic and organic ions*
  - *Biological buffers*
  - *Carbohydrates and oligosaccharides*
- HPLC with UV, PDA and FED
  - *Amino acid analysis (spent medium)*
  - *Oxidation state of transition metals*
  - *Peptide mapping (hydrolysates characterization)*
- Mass spectrometry (MALDI and ESI-TOF)
  - *Vitamin analysis (spent medium)*
  - *Fatty analysis*
- Enzymatic Analysis (YSI analyzer)
  - *Glucose*
  - *Lactate*
  - *Glutamine, glutamate*
- Capillary electrophoresis
  - *Charged molecules/ions*
- Particle size analysis

Unique Tools for Optimisation

- CHO Optimisation Kit 1
  - *6 animal component-free media including 2 chemically-defined formulas*
  - *Screen all 6 and select the best 3 performers for mixing experiments*
  - *Mixing experiments (using DOE methods) allow for further optimisation*
  - *Results can be analysed with Design Expert™ software or by sending results to Sigma-Aldrich R&D at no charge*
- New CHO bioreactor feeds
  - *20x vitamin and amino acid supplements*
  - *Allows you to test feed strategies*
- CHO Optimization Kit 2
  - *Consists of basal medium and 11 different concentrated supplements*
  - *Supplements include amino acids, vitamins, iron solutions, fatty acids, transferrin substitutes, and hydrolysates*
  - *Tremendous savings in labor and quality control in preparation of individuals components*
  - *Gives you vastly improved control and flexibility over your optimisation experiments*

ECACC and Sigma-Aldrich Partnership

Enhancing the ECACC and Sigma-Aldrich Distribution Partnership

For the last two years, Sigma-Aldrich has been successfully distributing ECACC cell lines within Europe.

Customers have benefited from:

- The ease of ordering ECACC's cell lines locally
- A reliable and speedy delivery service
- Purchasing the cell line using local currency
- The guarantee that the cell lines are authenticated and have undergone stringent quality control, including mycoplasma testing
- A local technical support service
- A free, 500ml bottle of the appropriate Classical Basal Medium with every cell line

ECACC and Sigma-Aldrich are pleased to announce that this service is now extended to the USA. Supply of the most popular cell lines, and those cell lines available exclusively from ECACC, will be supplied from Sigma-Aldrich inventory held in St. Louis within 48 hours. USA customers will soon be able to obtain the full ECACC range of cell lines from St. Louis. As the business develops, the number of ECACC cell lines held by Sigma-Aldrich as inventory will increase.

ECACC and Sigma-Aldrich believe that once again this emphasises the synergy between the two organisations and demonstrates how they can work together to serve the cell culture community in a progressive manner.

New Literature highlights

The New ECACC Cell Lines and Services Catalogue

This autumn ECACC will be releasing its new fully comprehensive 400+ page 2005 hardcopy catalogue. This catalogue will serve as a fundamental reference guide for the entire range of ECACC products and services with detailed specialist sections on:

- The General Cell Collection with full details on all cell lines including cross reference indexes for species and tissue origin
- International Histocompatibility Workshop Cell Collection including full HLA typing data
- A comprehensive guide to the human genetic cell line resource of over 27,000 cell lines representing 800 genetic disorders
- A listing of all available human pathogenic viruses from The National Collection of Pathogenic Viruses (NCPV)
- Description of ECACC's Human Genomic DNA Resources
- Full description of all the services ECACC has to offer along with detailed technical information and protocols.

Complete the attached reply card to ensure that you receive your own copy of ECACC's catalogue immediately upon publication.

ECACC's Human Genomic DNA Brochure - DNA from Cell Lines

Are you involved in genetic research?
ECACC can provide high quality, purified DNA from cell lines originating from:

- Apparently healthy, randomly selected human individuals
- A range of Primate Cell Lines
- The International Histocompatibility Workshop
- The Human Genetic Cell Line Collection
- The General Cell Line Collection

For a full comprehensive description of ECACC's DNA Resources, complete the reply card or contact ECACC on Tel: + 44 (0)1980 612512 or ecacc@hpa.org.uk

A New Cell Culture Product Guide for 2004/2005

Sigma-Aldrich is pleased to announce that a brand new Cell Culture Product Guide is in development. The expected release date is Autumn 2004. The product guide is designed to be a reference tool detailing the full range of Sigma Cell Culture products and services. It will include:

- A detailed listing of Sigma's unrivaled range of Cell Culture products, including media, balanced salt solutions, reagents and sera.
- Production capabilities and quality systems at our dual manufacturing facilities in St. Louis, USA, and Irvine, Scotland.
- Technical references and protocols including details of our web-based resources.
- Application data
- Media development and optimisation capabilities at our $55M Life Science Research Centre
- Analytical capabilities, for example spent media analysis
- Custom packaging opportunities
- FAQs

Complete the attached reply card to ensure that you receive your own copy of Sigma's Cell Culture Product Guide immediately upon publication.

To order direct from ECACC

Contact our customer service team

For direct sales, sales enquiries, delivery and pricing details contact ECACC between normal business hours, Monday – Friday, 8.45 – 17.00 (UK Time).

Orders can be placed by:

Fax: + 44 (0)1980 611315
Website: www.ecacc.org.uk
Email: ecacc@hpa.org.uk
Mail: European Collection of Cell Cultures, Health Protection Agency, Porton Down, Salisbury, Wiltshire, SP4 0JG

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