Autumn 2003

Cell Culture

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Feature Articles

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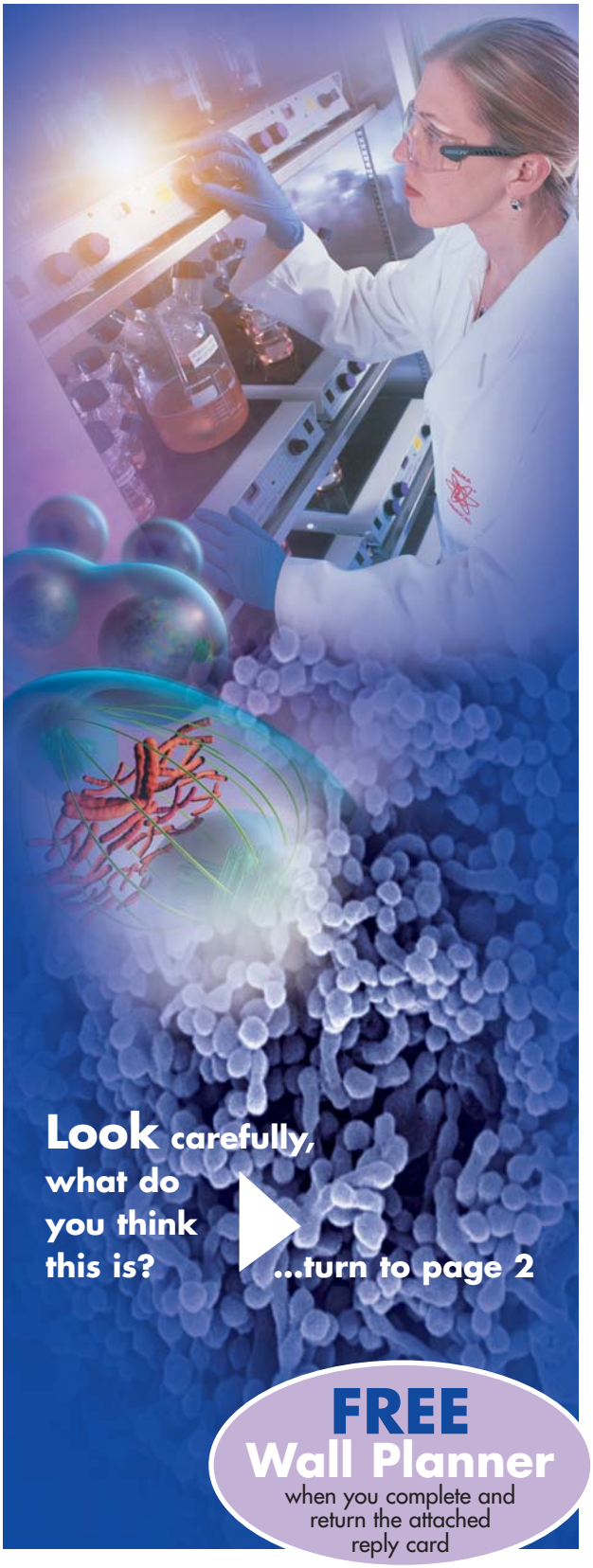


Mycoplasma – A Cells Worst Enemy

Pippa Bracegirdle, Clare Wilson and Bryan Bolton *European Collection of Cell Cultures, Health Protection Agency, Porton Down, Wiltshire, UK*

Answer from front page

A scanning electron micrograph of an infected cell, showing the cell membrane completely covered with Mycoplasma.

Are your cell lines free from Mycoplasma contamination?

Consider the effects Mycoplasma contamination has on the properties and functions of a cell line and how this might affect your research:

- Affect uptake across cell membranes
- Interfere with membrane receptor function
- Cause morphological change
- Influence amino acid and nucleic acid metabolism
- Induce cell transformation

In addition to the effects mycoplasmas might have on an individual cell line, the introduction of a contaminated culture can devastate a cell culture facility due to it's ability to spread rapidly through all cell cultures, causing an outbreak situation.

The solution

The best way to avoid the introduction of Mycoplasma is to obtain your cell lines from a recognised culture collection such as ECACC. However, it is still necessary to carry out regular testing of cell lines in routine culture and at the time of cell banking, so that any contamination can be quickly identified and removed from the facility. ECACC provides a Mycoplasma testing service, which is used by many of our customers. Three Mycoplasma detection methods are currently routinely employed at ECACC, each having particular strengths and weaknesses (see Table 1). Reliance on a single detection method for anything other than screening purposes is not advisable, and if a cell line has not been tested for some time ECACC recommends testing by all three methods.

Method	Sensitivity	Species range
PCR	Low/Medium	Uncertain
Indirect DNA Stain	Medium	All
Culture Isolation	High	Majority

Method	Speed	US FDA Approval
PCR	1 day	No
Indirect DNA Stain	2-3 days	Yes
Culture Isolation	3-4 weeks	Yes

Table 1: Mycoplasma detection methods.

Testing for Mycoplasma

The protocols outlined opposite are routinely used by ECACC for testing all manufactured cell banks. A more detailed version of these protocols is provided in the popular ECACC and Sigma-Aldrich joint publication ''Fundamental Techniques in Cell Cultures – A laboratory handbook'. For a free copy of this publication complete the reply card or visit **www.ecacc.org.uk.**

Detection of Mycoplasma by Indirect DNA Stain (Hoechst 33258)

DNA staining methods such as Hoechst stain, are suitable for the detection of Mycoplasma in both cell cultures and cell culture reagents and can give results within 24 hours (Figure 1). However, direct staining is relatively insensitive, with a detection limit of 10^6 colony forming units (CFU) ml^{-1}. Co-culturing the test sample with an indicator cell line such as Vero (ECACC Product No. 84113001-1v1) can improve the sensitivity to 10^4 CFU ml^{-1} by increasing the available surface area upon which mycoplasmas can adhere.

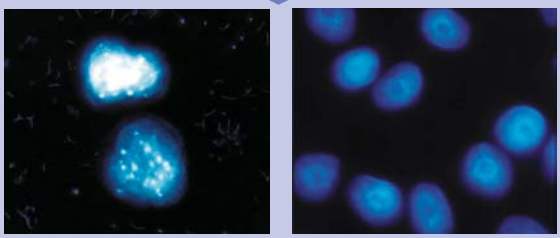
Detection of Mycoplasma by Culture

Detection of mycoplasmas using both direct culture and an enrichment step, is regarded as the reference method, with a theoretical detection level of 10 CFU ml-1 (Figure 2). This method is suitable for the detection of mycoplasmas in both cell cultures and reagents, with results available within four weeks. However, it is worth noting that certain strains of *Mycoplasma hyorhinis* can not be cultured *in vitro*. *Mycoplasma sp.* Colonies have a characteristic "fried egg" appearance.

ECACC Service

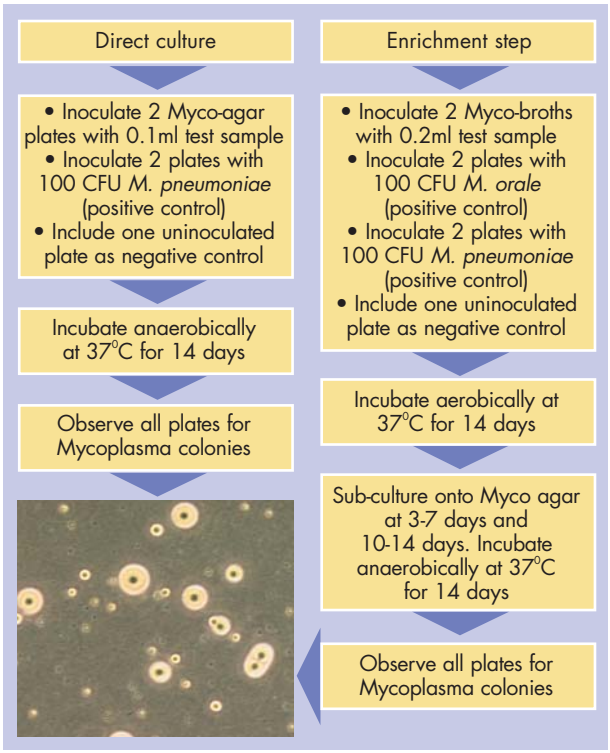
Figure 1: Detection of Mycoplasma by Indirect DNA Stain

- Inoculate tissue culture dishes or 12 well plates containing sterile coverslips with indicator cells (10^4 CFU ml^{-1})
- Inoculate at 37°C for 2-24 hours
- Inoculate 2 wells of indicator cells with test sample and 2 with Mycoplasma positive control sample. Include 2 uninoculated wells as negative control
- Incubate at 37°C in 5% CO_2 in air for 3-5 days. Discard any dishes that are contaminated with bacteria or fungi
- Fix samples *in situ* (Carnoy's fixative) and add Hoechst stain (5 minutes)
- Mount coverslip onto slide and examine using UV Epi-Fluorescence (x1000)



Hoechst Positive Culture | Hoechst Negative Culture

Figure 2: Detection of Mycoplasma by Cell Culture Isolation



Important Notes for both Methods

1. These test procedures should be carried out in a microbiological laboratory away from the cell culture laboratory.
2. *M. pneumoniae* is a potential pathogen and must be handled in class II microbiological safety cabinet operating to ACDP Category 2 Conditions.
3. Hoechst stain is toxic and should be handled and discarded with care.

To make use of this service visit the ECACC website for details on how to send samples for testing or contact our technical support co-ordinator on **+44 1980 612684**

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 sample is charged if samples are received frozen or require passaging without antibiotics.

Information Request 201

Cell Culture Training Courses

Learn how to maintain your cell lines free from contamination by attending ECACC's long established training courses. Call today to book your place and gain valuable in depth practical and theoretical knowledge from ECACC's cell culture experts. The ECACC Cell Culture training courses are designed to accommodate the newcomer to cell culture (Level 1) through to the experienced cell biologist (Level 2).

Level I (2004)
13 - 15 January
11 - 13 May
9 - 11 November

Level II (2004)
2 - 5 March
5 - 8 October

Contact our Business Administrator and Training Coordinator:
lisa.reynolds@hpa.org.uk

The ECACC Cryostorage Facility: Development of a cGMP Safe Depository

David Lewis and James Biggins *European Collection of Cell Cultures, Health Protection Agency, Porton Down, Wiltshire, UK*

The Need for Second Site cGMP Storage

Cultured mammalian cells, often following genetic manipulation, are increasingly associated in some way with the manufacture and/or testing of a therapeutic substance that is manufactured according to cGMP. The current direction of EU regulations is towards extending the requirements of cGMP further "upstream" of the manufacturing process, to include clinical trial materials and the early provenance of cell lines used in a cGMP operation. Consequently any cell line that is likely to be used in a cGMP process should be banked as a Master Cell Bank (MCB), and validated early in the research and development phases of a project.

Subsequently, all the work conducted using cells from this MCB represents a cumulative investment, which eventually can assume a very high value. In order to secure such an investment the owners of the MCB are advised to arrange a second site, back-up storage for either a part of the original MCB or a direct derivative. Cells recovered from back-up storage must be fit for use in the same cGMP operation as the Primary stock so the second site storage conditions need to be cGMP compliant.

For many years ECACC has provided second site storage for valuable third party cell lines. In recognition of the increasing need for a Safe Deposit facility that is able to support the needs of cGMP operations, ECACC has set up, and is now validating a dedicated cGMP Safe Deposit facility.

The ECACC Safe Deposit Facility for cGMP Cell Banks

The cGMP Safe Deposit vessel is being established within ECACC's new, state-of-the-art cryostorage facility located on the Health Protection Agency Porton Down site. This facility is serviced by two 10,000L liquid nitrogen tanks providing >100% reserve capacity. The Inventory storage vessels are configured for automatic replenishment and each vessel is monitored by an electronic telemetry alarm system. This facility benefits from the latest safety advances including oxygen sensors linked to powerful air ventilation. A 24 hour security service operates at the Porton Down site.

The cGMP Safe Deposit vessel is a Custom Biogenic Systems V3000 Isothermal model (Figure 1) designed so

Figure 1. Validation of cGMP Safe Deposit vessel.

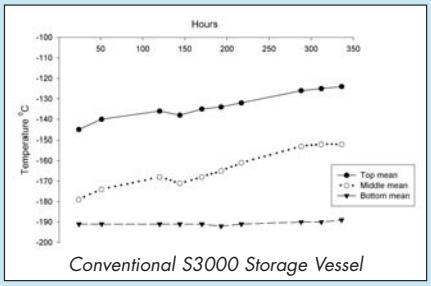
that the liquid nitrogen is contained in a "jacket" which surrounds the storage compartment. This design allows vapour phase storage, which minimises the opportunity for cross contamination between vials through the liquid nitrogen medium. In addition the temperature distribution within an Isothermal vessel covers a narrower range, and can be more closely controlled when compared to a conventional vapour phase vessel.

The "conventional" design of a liquid nitrogen storage vessel requires that the liquid nitrogen is delivered into the actual storage compartment of the vessel. The inventory is then stored either submerged in a large volume of liquid nitrogen (liquid phase storage), or otherwise in the gaseous space above the surface of a smaller volume of liquid nitrogen (vapour phase storage). Liquid phase storage guarantees a constant storage temperature of –196^{0}C, but presents a number of operational and biological risks the most outstanding of which is the possibility that contaminants may pass between vials through the liquid nitrogen medium. Such a mode of contamination was demonstrated when hepatitis B virus contaminated units of human bone marrow stored in liquid nitrogen at a UK blood laboratory. Consequently vapour phase storage is considered more suitable for a cGMP Safe Depository that will contain multiple cell lines.

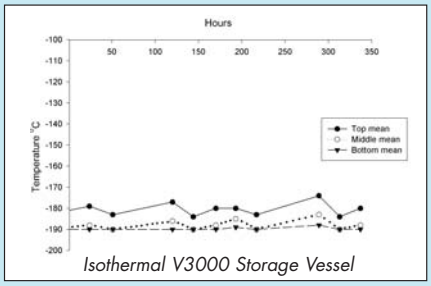
In a conventional liquid nitrogen storage vessel, vapour phase storage has the disadvantage that a temperature gradient inevitably extends from the surface of the liquid nitrogen to the upper regions of the compartment just beneath the lid. This temperature gradient expands and contracts as the liquid nitrogen fill level fluctuates. Provided the temperature does not exceed -135^{0}C, such fluctuations are unlikely to affect the cell stocks. This value represents the glass transition temperature of water below which molecular movement ceases and all biological activity is suspended. Nevertheless broad, fluctuating temperature gradients make it more difficult to control this critical threshold and therefore should be

The ECACC Cryostorage Facility: Development of a cGMP Safe Depository

(i) Temperature Profile at Different storage levels within the vessels

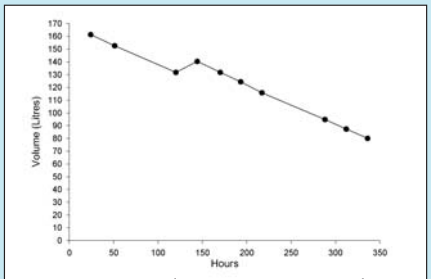


Conventional S3000 Storage Vessel

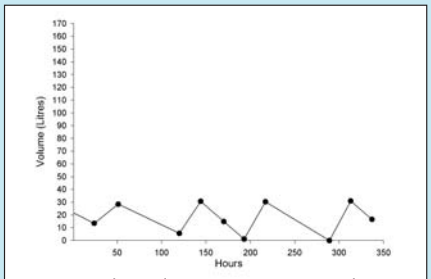


Isothermal V3000 Storage Vessel

(ii) Decrease in Liquid Nitrogen Volume over time



Conventional S3000 Storage Vessel



Isothermal V3000 Storage Vessel

Figure 2. *A comparison between the Conventional S3000 and Isothermal V3000 Storage Vessel. At the start of the experiment the temperature at the top of the Conventional S3000 storage vessel is approximately 40°C warmer than the bottom of the vessel compared to 10°C for the Isothermal V3000 storage vessel (i). As the liquid nitrogen volume decreases (ii) the corresponding temperatures at the top and middle of both vessels increase. This increase is significantly lower for the isothermal V3000 vessel in that the temperature remains below –170°C at all times. However, the temperature in the conventional S3000 vessel continues to decrease to below –130°C for the top of the tank which is higher than the critical glass transition temperature of –135°C. Therefore the isothermal V3000 tank provides a more stable storage environment than the conventional S3000, which is highly dependent on the volume of nitrogen in the tank, to achieve the required temperatures particularly towards the top of the tank.*

minimised. Isothermal inventory storage vessels have been identified as a means of achieving this.

Validation of the cGMP Safe Deposit Isothermal Vessel

The cGMP Isothermal storage vessel has been temperature mapped to determine the temperature at different locations in the storage compartment. These determinations have been repeated at different points in the liquid nitrogen fill cycle. The effects of opening the lid and the subsequent recovery times have also been determined. Similar mapping has been applied to a conventional vessel for the purpose of comparison. Results are summarised in Figures 2.

Operation of the ECACC cGMP Safe Depository

Only cell stocks that have been tested and shown to be free of microbial contaminants will be stored in the cGMP Safe Depository. In particular candidate Safe Deposits must be thoroughly tested for mycoplasma contamination. It is more difficult, perhaps impracticable to eliminate the possibility of any virus contaminant, which is why vapour phase storage is so important.

Features of the ECACC cGMP Safe Depository:

- The operational procedures for Isothermal V3000 Storage Vessel have been validated
- Vapour phase storage to minimise the opportunity of cross contamination
- Security and restricted access
- Confidentiality
- Staff on site 24 hours each day
- Maintenance and supervision by specialist, trained staff
- Eurotherm Chessel telemetry alarm system
- Continuous temperature logging, both electronic and manual
- Regular resuscitation of standard control "Monitor" cell lines that are stored in the Safe Deposit vessels
- Event logging and reporting
- Annual reporting of stock status, stock movements, the maintenance of storage conditions and the results from monitor cell lines

Conclusion

ECACC has been storing cell banks for almost 20 years and has one of the largest liquid nitrogen repositories for animal cells, in Europe. Recent initiatives will enable ECACC to make this expertise available to those who wish to secure cGMP cell stocks. For further details, including prices visit the ECACC website on **ecacc.org.uk**

Reference

Tedder, M. Zukerman, A. Goldstone, A. Hawkins, A. Fielding, E. Briggs, D. Irwin, S. Blair, A. Gorman, K. Patterson, D. Linch, J. Heptonstall and N. Brink. 1995. Hepatitis B transmission from contaminated Cryopreservation tank. Lancet 346:137-140

Information Request 204

An Efficient Approach to Cell Culture Medium Optimisation for Chinese Hamster Ovary (CHO) Cells

Justin Gifford, Ken Kao, Scott Ross, and Matthew Caple *Sigma-Aldrich Corporation, St. Louis, MO, USA*

Application Notes

- Six unique animal component-free media for maximum recombinant protein production
- Convenient format and rapid screening of multiple media formulations
- Detailed instructions for media mixing, experimental design, and data analysis
- Simple yet powerful mixing experiments using DOE methodology

Introduction

One of the most challenging aspects of culturing recombinant CHO cell clones is providing for the diverse nutritional requirements that are unique to every transfected cell line. In order to minimise the amount of time required for medium development, Sigma has recently developed a medium optimisation kit, CHO Kit 1 (Product Code CH0001). This kit consists of six diverse animal component-free CHO media formulations to provide for a wide range of nutritional requirements. These formulations serve not only as a quick and easy screen, but also function as a platform for statistical medium optimisation by using a three-point mixing design. By selecting the top three performing media from this initial screening, it is possible to further increase growth and productivity by following a statistical approach to media mixing provided by Design Expert® computer software. Taken together, our data strongly suggests that using CHO Kit 1 with a combination of media screening and a statistical approach to media mixing can facilitate the development of an optimised medium for any recombinant CHO clone.

Flexible format, rapid screening and media mixing

The six diverse animal component-free media, included in the kit, are designed to maximise cell growth and recombinant protein production in a wide variety of CHO cell clones. Two of these six media are chemically defined and all differ in amounts of amino acids, vitamins, salts, trace elements, recombinant human insulin, and other organic compounds. The format of the kit allows the user to rapidly screen all six of the media for cell growth, recombinant protein production or whatever the criteria may be. If this initial screen yields satisfactory results, the researcher may decide that no further optimisation is required. However, if the initial screening does not satisfy the specified criteria, a series of media-mixing assays can be completed.

Media mixing is the most efficient way to meet the diverse nutritional requirements of a particular cell line. As a result, the media selected for these mixing assays are absolutely critical. In order to select the best candidates to be mixed, it is important to examine all of the data from the initial screen and determine which criteria (i.e. growth kinetics, productivity) are most important. The format of this kit will use the three best performing media selected from the initial screen to perform the blending assays. Figure 1 depicts the diagram and mixing table used for the blending of three media selected (media A, B, and C).

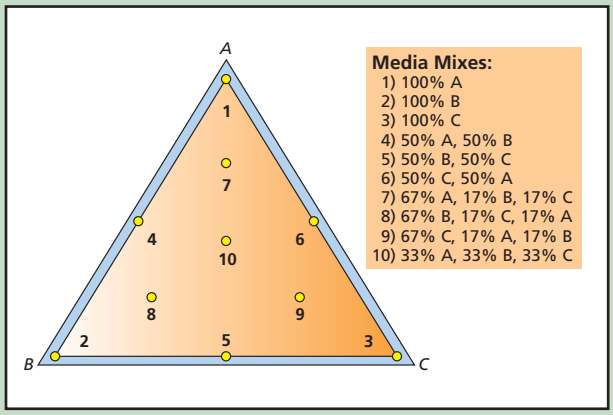
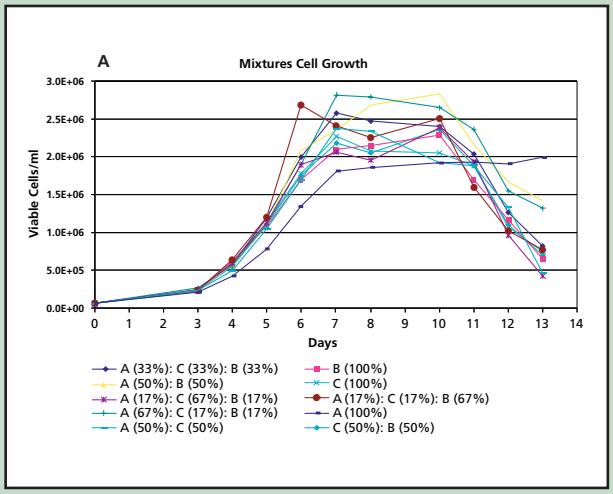


Figure 1. Representation of the three-point mixture triangle with the associate media mixes. *Each medium is set to 100% at vertices of the triangle (points 1, 2 and 3). Mixing begins at 50% between two of the media along the sides (points 4, 5 and 6). This is followed by a 67%, 17% and 17% mix of all three media within the interior of the triangle (points 7, 8 and 9). The final mixture will be 33% of all three media as seen at the axis of the triangle (point 10).*

Once the media-mixing assay has been completed, there are two options for data analysis. The first option is to visually analyse the data for each criterion, as can be done by examining viable cell growth (Figure 2A). At the same time, maximum cell growth data can be normalised and plotted on the mixture triangle to see where the best mixes might be located (Figure 2B). The same method can be applied to analyse the productivity of recombinant protein and any other criteria.

Product Information



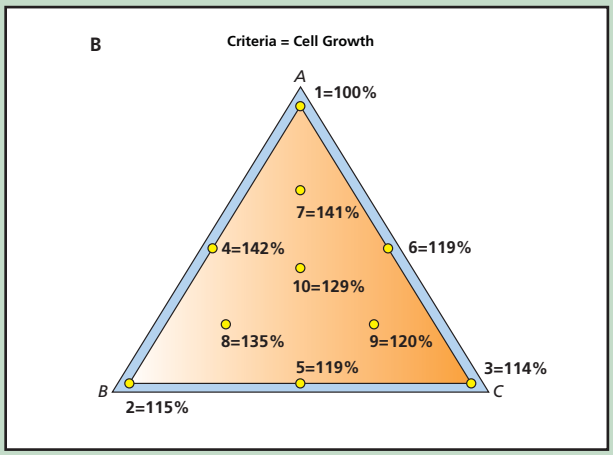


Figure 2. Representative comparison of cell growth for media mixes performed. *Media mixes above were formulated and tested based on results of the initial screen of all six media in the kit. A). Ten cell growth curves were obtained from the three-point medium-mixing assay as indicated in the three-point mixture triangle. B). The maximum cell densities from the different media mixes were normalised by using the number obtained from medium A as 100%. This data was plotted on the mixture triangle in order to estimate where the best mixes might be located.*

The second method of data analysis is more in-depth, in which a design-of-experiment (DOE) software package, such as Design-Expert, can be used. The software analyses the media-mixing data and allows the researcher to assign importance values to each criterion. Based on these inputs, mathematical models are used to predict the outcome of an infinite number of combinations of the three media, and their desirability based on which criteria are most important. The final outcome is one or several best-fit media designed specifically to meet the nutritional requirements of a particular cell line. This is illustrated in Figure 3 by a contour plot generated by the software to depict where on the mixture triangle the most desirable media mixes are located.

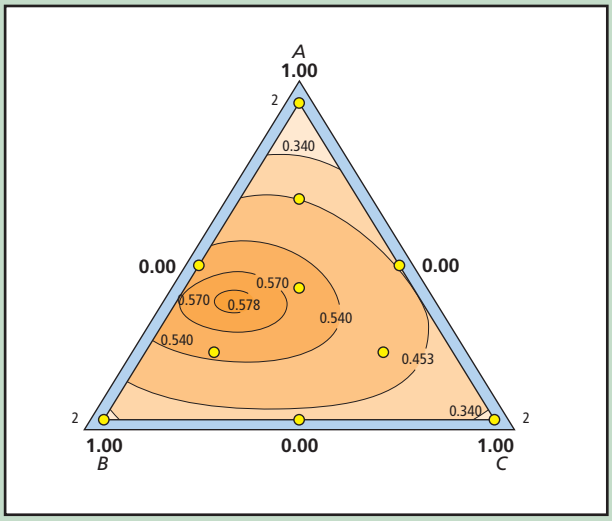


Figure 3. Contour plot indicating where the most desirable media mixes exist. *After inputting all the data collected from the mixture assay, importance values were assigned to each criterion. Design Expert then analysed the data and generated a contour plot. Values indicated on the graph are based on 1.000 being the most desirable. Any point on the graph can be selected and identified by the exact ratio of the three media.*

As more and more recombinant CHO clones have been developed, it has become increasingly important to streamline the medium optimisation process. To meet this need, CHO Kit 1 has been developed to provide researchers with a format for medium development that is efficient yet powerful. The convenient format allows for a rapid screening of multiple diverse CHO formulations designed for maximum recombinant protein production. In addition, the powerful mixture experiments coupled with the DOE software provide an invaluable tool for boosting cell growth and productivity. In either application, it is obvious that CHO Kit 1 will meet the needs of the majority of medium optimisation projects.

Ordering Information

Sigma Prod. No.	Description	Pack Size
CH0001	CHO Kit 1, Animal Component-Free	1 kit

Further Information

For further details on all our cell culture products visit **sigma-aldrich.com/cellculture**

Information Request 203

Cryopreservation and Storage of Cell Lines

Elizabeth Fashola-Stone, Marc Jones and Bryan Bolton *European Collection of Cell Cultures, Health Protection Agency, Porton Down, Wiltshire, UK*

Benefits that Cryopreservation Offers

Cryopreservation is invaluable when dealing with cells of limited life span. It allows cells to be stored at ultra-low temperature for future use without having to resort to the continuous cultivation of cell lines. Other main advantages include:

- Reduced risk of microbial contamination
- Reduced risk of cross contamination with other cell lines
- Reduced risk of genetic drift and morphological changes
- Work conducted using cells at a consistent passage number
- Reduced costs

The Requirements for Successful Cryopreservation

A large amount of development work has been undertaken to ensure successful cryopreservation and resuscitation of a wide variety of cell lines of different cell types. The basic principal of cryopreservation is to slow freeze and quick thaw. The most reliable and reproducible way to achieve a slow freeze at a rate of -1°C to -3°C per minute is with the use of a programmable rate controlled freezer. The cost in acquiring such equipment is often beyond the budget for the majority of research laboratories. An alternative approach is to freeze passively by keeping the ampoules for 24 hours in a Nalgene Mr Frosty (Sigma Product No. C1562) filled with isopropyl alcohol at –80°C.

Cryopreservation also depends upon the use of a high concentration of serum/protein (>20% should be used and in many cases serum is used at 90%) and cryoprotectants such as dimethyl sulphoxide (DSMO, Sigma Product No. D2650) or glycerol (Sigma Product No. G2025). Both cryoprotectants help to prevent the cells from rupturing due to the formation of ice crystals. DMSO is the most common cryoprotectant used at a final concentration of 10%, however, this is not always appropriate because DMSO induces differentiation in some cell lines (e.g. HL60, ECACC Product No. 98070106-1v1). In such cases, glycerol is often used as the alternative (refer to ECACC data sheet for details of the correct cryoprotectant for a particular cell line). It is essential that immediately prior to cryopreservation cultures should be healthy with a viability of >90% and in the log phase of growth. The latter parameter can be achieved by using pre-confluent cultures (i.e. cultures that are below their maximum cell density).

Ultra-Low Temperature Storage of Cell Lines

Following controlled rate freezing, cells can be cryopreserved in a suspended state for an indefinite period provided a temperature of less than –135°C is maintained. ECACC strongly discourages the idea of long term storage at -80°C. Such ultra-low temperatures can only be attained by specialised electric freezers or more usually by immersion in liquid or vapour phase nitrogen. The advantages and disadvantages of each are summarised below:

Table 1. Comparison of ultra-low temperature storage methods for cell lines.

Methods	Advantages	Disadvantages
Electric (-135°C) Freezer	• Ease of maintenance • Steady temperature • Low running costs	• Requires liquid nitrogen back-up • Mechanically complex • Storage temperatures high relative to liquid nitrogen
Liquid Phase Nitrogen	• Steady ultra-low (-196°C) temperature • Simplicity and mechanical reliability	• Requires regular supply of liquid nitrogen • High running costs • Risk of cross-contamination via the liquid nitrogen
Vapour Phase Nitrogen	• No risk of cross-contamination from liquid nitrogen • Low temperatures achieved • Simplicity and reliability	• Requires regular supply of liquid nitrogen • High running costs • Temperature fluctuations between - 135°C and - 190°C

Ask ECACC.... Focus On Cryopreservation

ECACC receives a diverse range of technical enquires and in this issue we have focused on the freezing of cell lines which account for a large proportion of the queries we deal with.

How should I store my frozen cells on arrival..?

On arrival, Frozen vials should be transferred immediately to vapour phase LN_2 or Liquid phase LN_2 if vapour phase not available. Do not use a –80°C freezer as an alternative as this can reduce the viability of the cells.

Why is Vapour phase LN_2 preferred to liquid phase LN_2..?

If ampoules are immersed in Liquid Phase LN_2 it increases the risk of LN_2 seeping into the vial, this would cause potential problems of cross-contamination and increased risk of the ampoule exploding when thawed.

What Safety precautions should I take when thawing ampoules..?

It is important to first check the Material Safety Data Sheet (MSDS) that is supplied with the cells to ensure that the correct level of containment is observed for the cell lines you are using. When handling the ampoules it is important to wear insulated safety gloves and full-face visor in addition to a lab coat.

When should I freeze down a bank of cells..?

Once in culture it is advisable to freeze down a token bank of 3-5 ampoules as soon as possible. This will provide a source for Master banks from which you can create working stocks to use. This tiered banking system will minimise risks associated with maintaining cells in permanent culture such as microbial contamination, genetic drift, loss of characteristics of interest or senescence of finite life span cell lines.

How can I get the correct freezing rate..?

Cells should be frozen slowly at a rate of -1 to -3°C per minute to prevent cell damage. To achieve this you can either use a rate programmable freezer or an Isopropanol bath, such as a "Mr. Frosty" (Sigma Product No. C1562). Ampoules should then be transferred directly to LN_2 storage.

What Quality Control testing should I perform on frozen stocks..?

After freezing down a bank it is important to thaw 2-3 ampoules to check for cell count / viability and freedom from Bacteria, Fungi and Mycoplasma. This testing should be done on all master and working banks produced prior to use. It is also important to confirm that Master and Working banks are genetically identical by DNA profiling. ECACC performs this same level of QC testing on all available cell lines together with any additional testing as required (such as BVDV testing for Bovine cell lines).

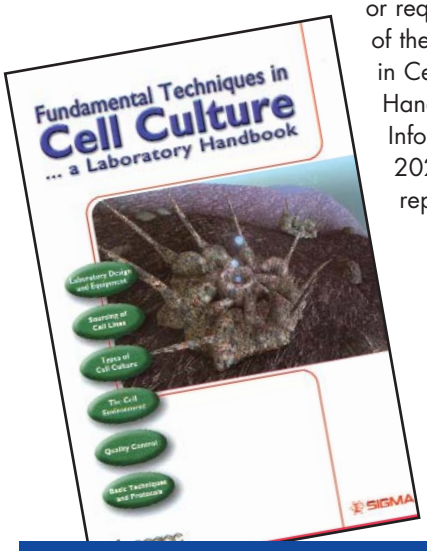
Ordering Information

Prod. No.	Description	Pack Size
ECACC 98070106-1vl	HL60 cell line	Growing Culture Frozen
Sigma C6164	Cell Culture Freezing Medium-DMSO, cell culture tested	50ml 5x50ml
Sigma C1562	Freezer Container, Nalgene Mr Frosty	1 each

If you have any technical questions relating to ECACC products or services please feel free to call me on +44 (0)1980 612684 or email me on **ecacc.technical@hpa.org.uk**
Marc D F Jones,
Technical Support Co-ordinator, ECACC.

Further Information

For further information and protocols on cell culture methods visit the ECACC and Sigma-Aldrich websites or request a **FREE** copy of the Fundamentals in Cell Culture Handbook using Information Request 202 on the enclosed reply card.



Information Request 202

New

CHO DHFR⁻Medium, Animal Component-Free

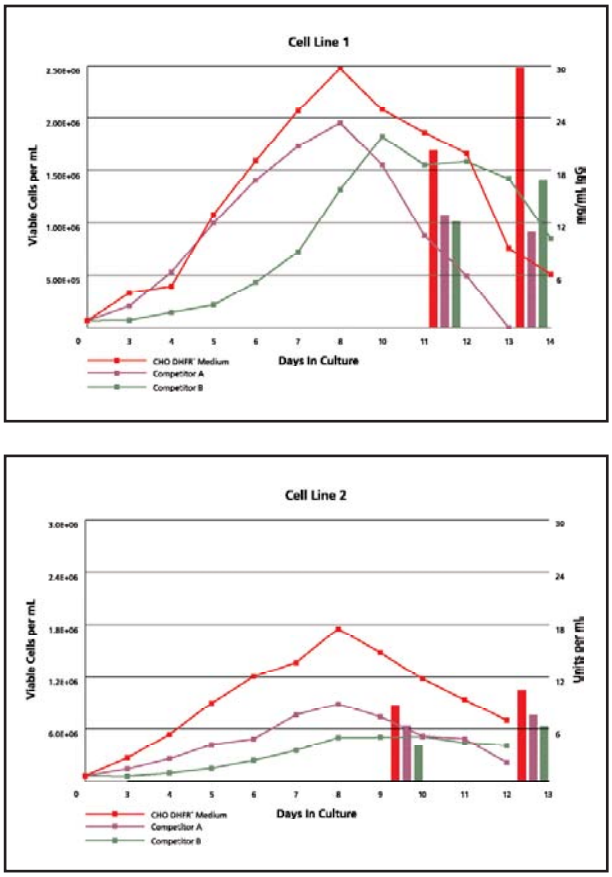
This animal component-free CHO media has been designed to provide outstanding cell growth and recombinant protein productivity in Chinese Hamster Ovary (CHO) cells that are engineered to contain the dihydrofolate reductase (DHFR⁻) amplification system.

- Animal Component-free — contains no transferrin or other products of animal origin
- Designed for DHFR⁻ CHO clones — no hypoxanthine or thymidine
- Outperforms the competition in terms of cell growth and productivity
- Supports corrected glycosylation of recombinant proteins
- Exceptional performance in other selection systems.

Products

Sigma Prod. No.	Description	Pack Size
C8862	CHO DHFR⁻ Medium, Animal component-free.	1L 6x1L

Information Request 205



Figures 1 & 2: *Cell growth and recombinant protein productivity in two CHO DFHR⁻ clones were compared after culture in C8862 and in animal component-free media from two competitors. Samples were taken daily to analyse cell growth and recombinant protein productivity. The data indicates that C8862 consistently supports the highest cell densities and recombinant protein productivity.*

Reduce Serum Supplementation with MegaCell™ Media

- Reduce FBS supplementation to 3% or less and increase productivity at the same time!
- Enriched with growth factors, amino acids, and specially engineered supplements
- Five formulations that support a wide variety of cellular applications
- Enhanced buffer capacity
- Save time and money with MegaCell™

To request a free sample of MegaCell™ please quote promotion code V20 or email your full details to **eurpromotions@sial.com**. Only 1 sample per person. Offer valid until 30th November 2003.

Products

Sigma Prod. No.	Description	Introductory Pack Size
M4067	MegaCell™ Minimum Essential Medium Eagle	500 ml
M4317	MegaCell™ Minimum Essential Medium/Nutrient Mixture F-12 Ham	500 ml
M3942	MegaCell™ Dulbecco's Modified Eagle's Medium	500 ml
M4192	MegaCell™ Dulbecco's Modified Eagle's Medium/ Nutrient Mixture F-12 Ham	500 ml
M3817	MegaCell™ RPMI-1640 Medium	500 ml

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TrypZean™ Solution from Sigma-Aldrich

New

TrypZean™ Solution, (1x) is the first animal component-free trypsin solution optimised for cell dissociation. It is formulated with TrypZean™ a recombinant bovine trypsin expressed in corn and manufactured by Sigma-Aldrich utilising ProdiGene's proprietary transgenic plant protein expression system.

- **Animal component-free –** TrypZean™ eliminates the risk of viruses, BSE and other potential adventitious agents
- **TrypZean is recombinant trypsin –** Using the same enzyme (with the same kinetics) for cell detachment means minimal protocol changes for you
- **Enzyme inhibition –** Soybean trypsin inhibitor and other inhibitors work the same with TrypZean™ as they do with native trypsins (on a weight-to-weight basis)
- **High purity –** TrypZean™ provides increased specificity and eliminates contaminating activities found in lower purity enzymes
- **Convenience –** TrypZean™ Solution is formulated at the optimal concentration to dissociate adherent cells

Products

Sigma Prod. No.	Description	Pack Size
T3449	TrypZean™ Solution, 1x	100 ml
		500 ml

Information Request 207

Frequently Purchased Cell Lines and Appropriate Relevant Media

Cell line Name	Cell Line Description	Cell Line ECACProd. No.	Media Sigma Prod. No.	Media description
L929 3T3 L1 VERO	Mouse Adipose Fibroblast Mouse Embryo Fibroblast Monkey African Green Kidney	85011425 86052701 84113001	D 6546	Dulbecco's Modified Eagle's Medium high glucose, without L-glutamine, with 110mg/L sodium pyruvate and sodium bicarbonate.
CHO/DHFR⁻	Chinese Hamster Ovary	94060607	C8864	CHO/DHFR⁻ medium animal component free
PE/CA/PJ15 KG-1	Human Tongue Epithelial Human Bone Marrow Myeloblast	96121230 86111306	I3390	Iscove's Modified Dulbecco's Medium without L-glutamine, with sodium bicarbonate.
293 (HEK) HELA MRC-5	Human Embryo Kidney Human Cervix Carcinoma Human Lung Fibroblast	85120602 93021013 84101801	M 2279	Minimum Essential Medium Eagle with Earle's salts and sodium bicarbonate, without L-glutamine.
HL60 SP2/0-Ag14 THP-1	Human Lymphoblast Mouse Suspension Human Monocyte Leukaemia	98070106 85072401 88081201	R 0883	RPMI 1640 without L-glutamine, with sodium bicarbonate.
CHO-K1 SH-SY5Y LT	Chinese Hamster Ovary Human Neuroblast Human Lung Fibroblast	85051005 94030304 96061947	N 4888	Nutrient Mixture F-12 HAM without L-glutamine, with sodium bicarbonate.
CHO (PROTEIN FREE)	Chinese Hamster Ovary (Protein Free)	0.0102307	C 5467	CHO Medium animal component-free.

Information Request 208

New Cell Lines

ECACC Expands its Hybridoma Collection

ECACC has over 450 cell lines in its Hybridoma Collection. The specificity of the secreted antibodies cover a wide range of antigens including bacterial, viral, plant, immunoglobulin, major histocompatibility complex and oncogenes.

One of ECACC's largest depositors of hybridomas during the last 18 years has been the Molecular Immunology Group at the world renowned Sir William Dunn School of Pathology, University of Oxford. Their main area of research is the study of the immune response particularly the molecular interaction of leukocytes and their surface proteins.

This research has led to a series of over 120 monoclonal producing hybridomas, the OX series, 55 of which are currently available from ECACC. The latest five OX cell lines are listed below (Table 1) with the most frequently requested hybridomas from Oxford, UK and other sources. ECACC is also able to supply the myelomas that include NS0, SP2/0-Ag14 and P3X63Ag8.653.

Full details of all the cell lines listed above can be obtained by requesting our CD-ROM or from our website **www.ecacc.org.uk**

Table 1. Details of ECCAC's Hybridomas expansion.

	Hybridoma Name	ECACC Product Number	Antibody Reactivity	Antibody Subclass	Depositor	Reference
NEW	OX-89	03062501	Anti-mouse CD134	IgG1	Professor A.N. Barclay, Molecular Immunology Group, Oxford	Immunol 2001 166:6972
NEW	OX-90	03062502	Anti-mouse CD200	IgG2a	Professor A.N. Barclay, Molecular Immunology Group, Oxford	Science 2000 290:1768
NEW	OX-101	03062503	Anti- rat CD47	IgG1	Professor A.N. Barclay, Molecular Immunology Group, Oxford	Eur.J. Immunol 2000 30:2130
NEW	OX-102	03062504	Anti-rat CD200 Receptor	IgG1	Professor A.N. Barclay, Molecular Immunology Group, Oxford	Immunity 2000 13:233
NEW	OX-104	03062505	Anti-human CD200	IgG1	Professor A.N. Barclay, Molecular Immunology Group, Oxford	Immunol 2001 102:173
	OX-7	84112008	Anti Thy-1.1	IgG1	Prof. A Williams, MRC Cellular Immunology Unit, Oxford	Biochem J 1980;187:1
	OX-86	96110601	Anti mouse CD134	Rat IgG1	Dr D W Mason, MRC Cellular Immunology Unit, Univer	Eur J Immunol 1996;26:1695
	OX-26	84112014	Anti rat transferrin receptor, CD71	IgG2a	Prof. A Williams, MRC Cellular Immunology Unit, Oxford	Nature 1984; 312:162
	OX-19	84112012	Anti rat CD5	IgG1	Prof. A Williams, MRC Cellular Immunology Unit, Oxford	Eur J Immunol 1982;12:511
	OX-38	88051303	Anti rat CD4	IgG2a	Prof. A Williams, MRC Cellular Immunology Unit, Oxford	J Exp Med 1985;162:117
	OX-8	84112009	Anti rat CD8	IgG1	Prof. A Williams, MRC Cellular Immunology Unit, Oxford	Eur J Immunol 1980;10:609
	OX-33	86100902	Anti rat CD45	IgG1	Prof. A Williams, MRC Cellular Immunology Unit, Oxford	Eur J Immunol 1985;15:168
	Mycl-9E10	85102202	Anti human c-myc gene product	IgG1, kappa	Dr G Evan, Ludwig Institute for Cancer Research	Mol Cell Biol 1985;5:3610
	OKT3	86022706	Anti human CD3	IgG2a	Obtained from ATCC	Proc Nat Acad Sci, USA 1980; 77:4914
	W6/32	84112003	Anti HLA-A,B,C	IgG2a	Prof. A Williams, MRC Cellular Immunology Unit, Oxford	Cell 1978;14:9; Nat.1979;279:243
	OKT4	87012602	Anti human CD4	IgG2b	Obtained from ATCC	Proc Nat Acad Sci, USA 1980;77:4914
	ED1	89040701	Anti rat macrophage	IgG1	Dr C D Dijkstra, Cell Biology and Immunology Dept	Immunol 1985;54:589
	OKTP	86022707	Anti human CD71	IgG1	Obtained from ATCC	US Patent 4,364,934
	W3/25	84112002	Anti rat CD4	IgG1	Prof. A Williams, MRC Cellular Immunology Unit, Oxford	Cell 1977;12:663; Immunol Rev 1983;74:57
	YTS154.7.7.10	87072285	Anti rat Thy-1	Rat IgG2b	Prof. H Waldmann/ Dr S P Cobbold, Dept of Path.	Nature 1984;312(6):548
	2-179-E11	92030602	Anti human TNF alpha	IgG1	Dr A Meager, Div of Immunobiology, National Instit.	Hybridoma 1987;6:305
	7D4	88111402	Anti murine IL-2 receptors	Rat IgM kappa	Obtained from ATCC	Proc Nat Acad Sci, USA 1983;80:5694
	7R2/A4	92030601	Anti human interferon gamma	IgG1	Dr A Meager, Div of Immunobiology, National Instit.	Meager A (1987) In:Lymphokines & Interferon:A Practical Approach, p105

The Cytotoxic and Protective Activity of *Clostridium Botulinum* Exoenzyme C3 in Mammalian Cells

Dr. Joanne Ayriss

Joanne Ayriss[1], Hazel Evans[2], Elizabeth Fashola-Stone[1], Oliver Chow-Worn[1], J. Mark Sutton[1] and Cliff Shone[1]. Health Protection Agency, Porton Down, Salisbury, UK[1], University of Bath, Bath, UK[2]

The *Clostridium Botulinum* C3 exoenzyme (C3 bot) belongs to the family of ADP-ribosyltransferases that specifically inactivate Rho GTPases in mammalian cells. C3 ADP-ribosylation of Rho GTPases causes biological inactivation and inhibition of Rho-dependant processes including cell signalling, cell transformation and regulation of the actin cytoskeleton. C3 exoenzymes have become established tools for studying the role of these target proteins. This short communication describes the opposing properties of C3 bot on neuronal and non-neuronal cells.

C3 bot was introduced into NIH3T3 fibroblasts or NG108 neuroblastoma cells (both purchased from ECACC) by osmotic treatment (Okada and Rechsteiner, 1982). Following treatment, the fibroblasts rounded (Panel Aii) and actin filaments became disaggregated (Panel Bii) in comparison to untreated cells (Panels Ai and Bi). No such effects were observed following C3 treatment of NG108 cells (Panel Cii) indicating a fundamental difference in Rho signalling on neuronal and non-neuronal cells. Treatment of NG108 cells with lysophosphatidic acid (LPA) induced neurite retraction (Panel Di) that was inhibited in the presence of C3 (Panel Dii). The bioacivity of the phospholipid messenger LPA occurs through stimulation of signalling pathways via GTP binding protein-coupled receptors (GPCR's) in many cell types including neuronal cells (Fukushima et al., 2002). Thus, it is predicted that LPA signalling of neurite retraction is inhibited by C3 Rho modification in neuronal cells.

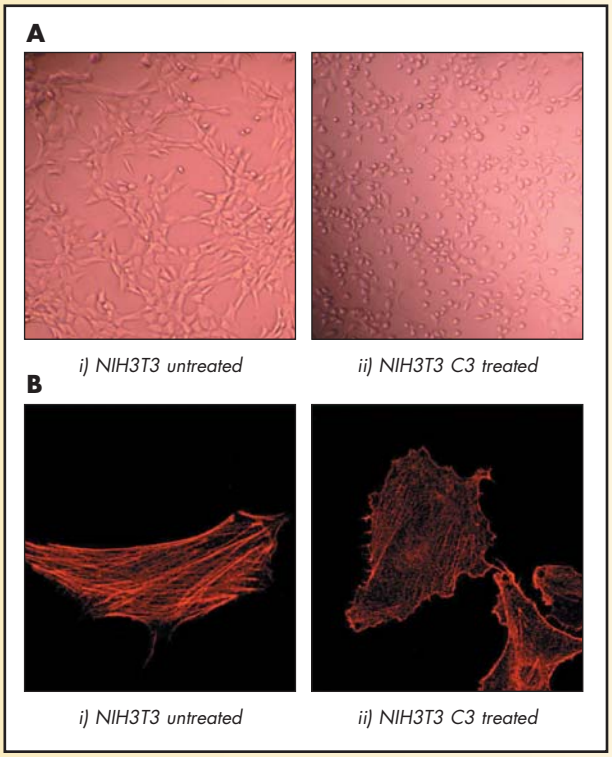


Figure 1. *NIH3T3 cells (2 x 10^5/ml) were treated with 15µg/ml of C3 bot in uptake media (Dulbecco's Modified Eagle Medium, 0.5M sucrose, 10% polyethylene glycol and 0.1mg/ml ovalbumin) for 10 min at 37°C. Actin staining: cells were fixed with 4% paraformaldehyde and treated with Rhodamine-Phalloidin (0.2µM final concentration) for 30 minutes at room temperature.*

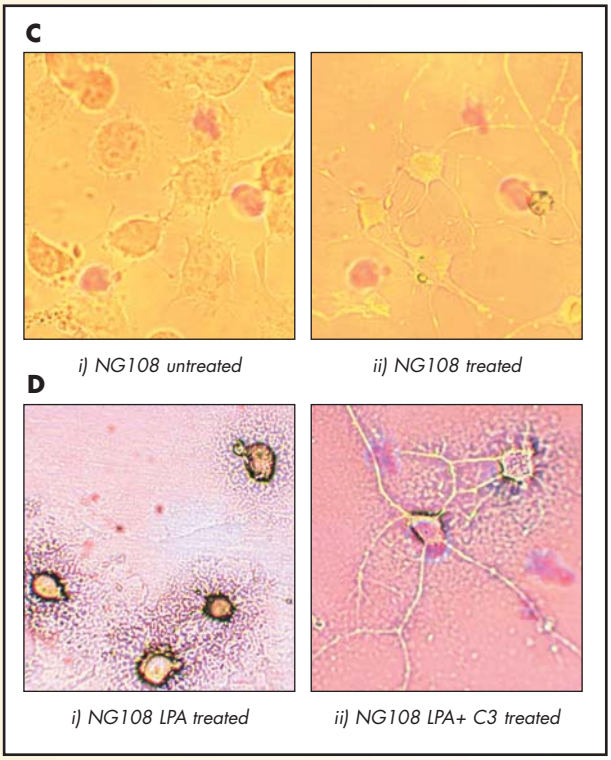


Figure 2. *NG108 cells (2 x 10^5/ml) were grown to 40% confluency in supplemented Dulbecco's Modified Eagle Medium. C3 bot (30µg/ml) was added for 3h at 37°C prior to LPA (1µM) treatment for 5 minutes at 37°C.*

Reference

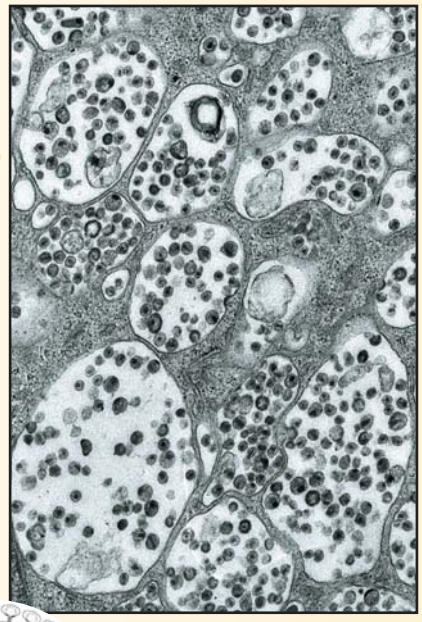
- Fukushima *et al.*, 2002. Molecular and Cellular Neuroscience. 20:271-282.
- Okada and Rechsteiner, 1982. Cell. 29:33-41.

National Collection of Pathogenic Viruses

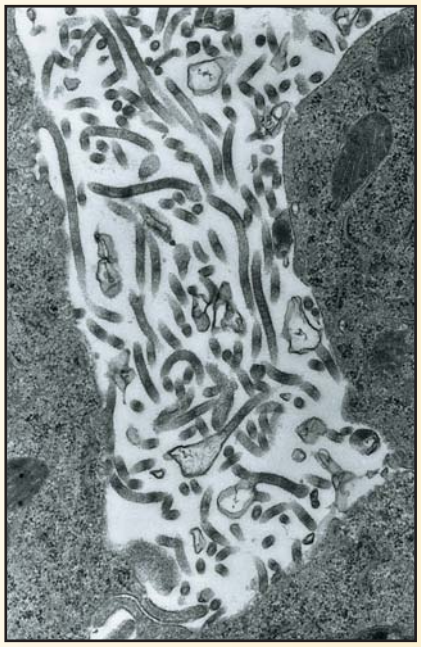
Mark Outlaw, Health Protection Agency, Porton Down, Salisbury, UK

The National Collection of Pathogenic Viruses (NCPV) preserves well-characterised, authenticated human pathogenic viruses in a secure facility, and is able to supply the agents or nucleic acids derived from them, to the scientific community according to national and international guidelines. The strategy and policy of NCPV is overseen by an expert virology advisory panel composed of distinguished scientists and clinicians from universities, medical schools, government agencies and industry. The collection is primarily comprised of human pathogenic viruses requiring handling at biosafety levels 3 or 4, but has expanded to encompass hazard group 2 pathogens. Most of the material is in the form of cell-cultured virus stocks, but where desirable or necessary, provision has also been made for selected clinical material and uncultivable viruses to be archived as serum, tissue or other biological samples, or in the form of cloned material.

The collection contains materials, particularly in the area of emerging virus diseases, which are not readily available to the wider scientific community. We expect the collection to be of benefit in the future development and testing of vaccines and antiviral compounds, in the development and validation of diagnostic test systems, and in the conservation of biodiversity. Appropriate banking and quality control procedures have been put in place for proper archival maintenance of titred infectious virus stocks and their provision to users. Authenticity of stocks has been primarily determined by direct sequencing of PCR products amplified from appropriate genomic regions, and also through use of monoclonal antibodies. In parallel, an incremental programme to make available non-infectious virus-derived materials such as DNA or RNA from purified virus or from infected cells, individual virus genes (PCR products, cloned DNA), and viral proteins has been developed.



HIV virus in an ECACC cell line.



Ebola virus in an ECACC cell line.

Images above are courtesy of Barry Dowsett, Health Protection Agency, Porton Down.

The wide range of cell lines used by NCPV are all supplied by ECACC, which is located on the same site as NCPV. It is vital for the NCPV that viruses we supply have been propagated on high quality, fully authenticated cells. All medium, serum and other additives are traceable and pass necessary quality standards. Relevant procedures for cell banking with ECACC are already available and applicable to NCPV, and many existing protocols are modified for banking viruses. Also, ECACC acts as a model in the development of a proactive accessioning policy, a database of possible customers and their requirements. In addition, NCPV holds the patent virus deposits on behalf of ECACC and carries out any virological investigations ECACC may require. For more information about NCPV at the Health Protection Agency, Porton Down, contact Dr Mark Outlaw on *ncpv@hpa.org.uk*.

An editorial enhances scientific awareness? Have you ever thought about having an editorial in our Newsletter?

ECACC Distributes over 6,000 cell culture each year to laboratories world-wide for various applications in cell science. We thought it might be both useful and interesting to share some of these applications with our customers. The two applications presented in this issue are from group within the Health Protection Agency – Porton Down site (formerly CAMR).

To benefit from further exposure of your research ECACC and Sigma-Aldrich welcomes submissions from scientists for inclusion in future newsletters. If you would like an editorial contact ***elizabeth.fashola-stone@hpa.org.uk.***

All you want to know about Sigma-Aldrich

as a manufacturer but were afraid to ask

As a leading supplier of cell culture products, Sigma-Aldrich is frequently contacted by its customers with a variety of application and technical inquiries.

By using some of the information gained from these customer contacts, we are pleased to present some of our **Frequently Asked Questions** (FAQs). These FAQs will hopefully serve as a guide to help identify and answer some of your potential enquiries.

Does Sigma manufacture their cell culture products?

Yes. In the past 20 years Sigma-Aldrich has invested over $50 million in facilities dedicated to support both the research and industrial cell culture communities.

Where are your manufacturing facilities?

One in St.Louis, USA, whilst our European facility is in Irvine, UK.

We have a technology transfer programme to enable the interchangeable manufacture of products between these two sites.

Do your facilities operate to a quality standard?

Yes. Sigma-Aldrich operates according to the cGMP quality systems at both the St.Louis and Irvine cell culture facilities. Both are registered with the Food & Drug Administration for the manufacture of class 1 medical devices in accordance with 21CFR#820. Whilst both facilities operate to ISO 9001:2000 and the UK facility is also registered to ISO 13488:1996 and conforms to ISO 14001:1996.

What are Sigma-Aldrich's cell culture manufacturing capabilities?

We can manufacture liquids in batch sizes up to 10,000L and dry powders in batch sizes up to 4,000 Kg.

Can you provide custom products and packaging?

Yes. We are happy to manufacture customer-specific products supported by confidentiality agreements, as necessary. We offer a wide range of liquid packaging options from 5ml to 500L, and are also able to custom package dry powders and manufacture tablets.

Can we visit the cell culture manufacturing facilities?

Yes. We are justifiably proud of our facilities. We actively encourage our customers to visit them for a full audit or a more informal tour.

Do you have R&D capability and support?

Yes. Our new $55 million Life Science and High Technology center is open to promote future new product development and is evidence of our continued investment in technology to serve our customers. Our dedicated cell culture R&D is not only involved in the development of Sigma-Aldrich catalogue products, but also supports the development, optimisation and manufacture of custom media formulations.

Is Sigma-Aldrich able to offer serum-free and animal component-free media?

Yes. As part of our Value Added Materials Programme we are sourcing and developing alternatives to animal-derived components, e.g., amino acids, lipids. With these, we have formulated serum-free and animal component-free media for commercially important cell lines, e.g., CHO, NS0, HEK-293, Per,C6®, MDBK/MDCK and hybridomas.

OK, but aren't you really just a chemical company dipping your toe into another marketplace?

Absolutely not. Cell culture is one of our major corporate initiatives. However, due to our background and overall manufacturing capabilities, it does mean that we are the supplier of many of our competitors' raw materials!

Does your background offer any other advantages?

Oh yes! We are able to call upon unparalleled analytical capabilities to assist in the development of customer specific media. Also, we are able to develop and manufacture unique products such as synthetic, cholesterol substitute, SyntheChol™.

Further Information

For further information on Sigma-Aldrich's cell culture capabilities visit the website **sigma-aldrich.com/cellculture** where you can visit our virtual tour of the production facilities. For details on the bulk supply of Synthecol™ please email **eurpromotions@sial.com**

Information Request 208

To order direct from ECACC - European Collection of Cell Cultures

Health Protection Agency, Porton Down, Salisbury, Wiltshire, SP4 0JG

Contact our friendly customer service team

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