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Replacement Seed Stock for MRC-5 cells

Glyn Stacey¹, Ross Hawkins¹, Penny Carter², Charles Hunt,¹ and Lesley Young¹

¹Division of Cell Biology and Imaging and UK Stem Cell Bank and ²Quality Assurance, National Institute for Biological Standards and Control, South Mimms, Hertfordshire, EN6 3QG, UK

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SUMMARY

The original seed stock of MRC-5 cells ("PDL7") was established in the 1960s at the laboratories later known as the National Institute for Biological Standards and Control in the UK. These original stocks have been released by NIBSC for the development of vaccines over several decades. MRC-5 cells are a human diploid fibroblast culture which can be passaged in vitro for approximately 50 population doublings, when they become senescent and cease to replicate i.e. they are a 'finite cell line'. Manufacturers' stocks of these cells clearly need periodic replacement from NIBSC and this has meant that NIBSC stocks of these cells have become depleted. This fact, combined with an observed deterioration of the original glass vials in which the cells were originally frozen resulted in the need to replace the stocks supplied to manufacturers to ensure continuity of supply of these cells for vaccine development.

NIBSC, with the support of the WHO ECBS and in liaison with manufacturers, has produced a replacement bank of MRC-5 cells at a slightly higher passage level than the original stock. This new bank of cells has undergone a range of quality control tests and the results from these tests have been deemed satisfactory.

This replacement stock of MRC-5 cells is now offered for adoption by ECBS as a 'reference cell bank' (as defined by the 'WHO Cell bank Monitoring Group (WHO, 2003)), as a direct replacement for the original MRC-5 "PDL 7" stock. The cell line data sheet and 'Information For Use' (IFU) that will be released with the material are attached as appendices.

INTRODUCTION

The original seed stock of the MRC-5 culture was frozen in 1966 at population doubling level (PDL) 7.

It was frozen in 481 glass ampoules in 54 pools, each containing 7-10 ampoules per pool (Jacobs et al., 1970). MRC-5 cells were found to support the growth of a range of human viruses and were established as a cell type that could be used for vaccine manufacture. The original stock of "PDL 7" cells has been released by NIBSC for vaccine development for a number of decades (Wood and Minor, 1991). MRC-5 cells can be maintained in vitro for approximately 50 population doublings, at which point they become senescent and cease to replicate i.e. they are a 'finite cell line'. As a result, manufacturer's periodically request replacement stocks of cells and after 40 years of use, the "PDL 7" seed stock was becoming diminished. In addition to this the integrity of the glass ampoules appeared to be deteriorating. It was agreed that there was a need to replace the original seed stock of cells but any new stock would inevitably require passage of the original cells and thus an increase in population doubling level. A proposal for this replacement stock was submitted to WHO in 2005 and this was endorsed by the ECBS .

After consulting with manufacturers, it was decided to produce a new seed stock of MRC-5 cells at an approximate population doubling level of PDL 12, which would, according to users of the cells, still provide sufficient in vitro passage capacity for preparation of manufacturers cell banks and production including allowance for the likely passages required to adapt cells to new serum-free culture conditions.

It should be noted that since this preparation is a seed stock ('reference cell bank') for vaccine development, any manufacturers master and working banks derived from it will require full qualification and evaluation as described under the WHO requirements for cell substrates (WHO, 1998; WHO, 2003). It is also important to recognise that the MRC-5 seed stock is very different to a reference material and cannot be directly evaluated under the usual criteria for an international reference material submitted to ECBS. The following sections describe the preparation and quality control of the new MRC-5 seed stock which support ongoing supply of these cells for vaccine development based primarily on the history of safe use of the original seed stock. As for the original 'PDL7' seed stock; fitness for purpose of the new PDL 12 seed stock will be part of the vaccine manufacturer's validation process and tests results reported for the seed stock are primarily intended for information only.

ASSAY METHODS AND STUDY DESIGN

The new seed stock was derived from six 'PDL 7' ampoules i.e. 2 ampoules from each of 3 pools. The 3 cell pools were cultured separately under conditions consistent with cGMP and in a Grade A clean air environment. Sterility testing was carried out in-house (40 vials) and externally (20 vials) according to EU Pharmacopeia and Identity confirmed by DNA profiling.

The cells were pooled 1 passage prior to cryopreservation and four hundred and fifty vials were cryopreserved using a rate controlled freezer. A total of 64 vials were used for QC testing and further cell bank production. The seed stock was homogeneity tested by subjecting 40 vials to viability testing on thawing and growth rate analysis for 5 subcultures. The seed bank was screened for the presence of contaminating bacteria, fungi, mycoplasma and for the following viruses: CMV; HTLV1; HIV1; HBV; HCV; EBV. Sterility testing was performed according to EU Pharmacopeia by an independent laboratory accredited under MHRA. Viral testing was performed by an independent CPA accredited laboratory.

RESULTS OF TESTING ON THE PDL 12 SEED STOCK

Cell identity tests showed identical profiles by DNA profiling (15 hypervariable alleles by 'STR' methodology) for all cell 'pools' used to prepare the final PDL 12 stock

Viability and homogeneity testing: Vials 011-020, 211-220, 236-245, 436-445, from the sequentially numbered vials of the bank, were submitted for viability testing by trypan blue dye exclusion test. Percentage viabilities exceeded 80% in all cases and showed no trend from early to late aliquotted vials.

Karyological studies: 4 studies, each of 20 metaphase spreads consistently showed a normal human male karyotype, 46,XY.

In all cases of sterility, mycoplasma and viral testing no microbiological contaminants were detected.

OTHER SUPPORTING DOCUMENTATION: 'CELL LINE MASTER FILE'

A 'Cell Line Master File' (CLMF) has been produced which provides the following information:

- History and production of the original (PDL 7) bank, including electron micrographs, karyology data, morphology and culture conditions.
- Media records (batch numbers, volumes, expiry dates, serum TSE certificate)
- Thawing records (viability counts, seeding densities)
- Subculture records
- Pooling records
- Cryopreservation records (with rate controlled freezer cooling data)
- Mycoplasma culture records
- Mycoplasma PCR records
- Morphology records (light microscopy)
- Homogeneity testing records (viability and growth rates)
- DNA profile records
- Karyology records
- Virus screening records
- Cryopreservation storage records

The cell line data sheet and 'Information For Use' (IFU) document that will be released with the material are attached as appendices.

CONCLUSIONS

The CLMF is held at NIBSC and a copy can be made available for the WHO. It should be noted that the PDL 12 MRC-5 cell bank is a seed stock or 'reference cell bank' and that recipients/manufacturers of these cell should establish their own Manufacturers Master Cell Banks for thorough re-qualification, as recommended by the World Health Organisation for cell substrates (Requirements for the use of animal cells for in vitro substrates for the production of biologicals (WHO, 2003). Vials from the PDL 12 bank are now being distributed to manufacturers.

PROPOSAL

It is requested that the WHO endorse the new MRC-5 PDL 12 cell bank as a replacement for the PDL 7 seed stock for viral vaccine production, diagnostic and surveillance purposes.

ACKNOWLEDGEMENTS

All staff from the Division of cell Biology and Imaging who contributed to the work of this project are thanked for their time and invaluable expert contribution, as this study would not have been possible without their input.

REFERENCES

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APPENDIX 1: MRC-5 SAFETY DATA SHEET

Cell Line:	MRC-5
NIBSC Accession No:	660902
Description of cells:	Human foetal lung, diploid fibroblast
Cell growth medium:	MEM + 10% FCS
Subculture routine:	Subculture at a ratio of 1:4 every 3 to 4 days

Handling of cell lines received from NIBSC – Safety Information.

Frozen ampoules

Cells are provided as frozen cultures in plastic cryovials. Each cryovial contains cells cryopreserved in media containing foetal bovine serum and cryoprotectant (DMSO).

- The ampoules are packed in dry ice (solid CO₂) pellets, which may cause frost-bite on contact with skin.
- Care should be taken when thawing the ampoule, as residual liquid nitrogen may be present in the ampoule and may present an explosive hazard.

Resuscitate the thawed cells into cell growth medium in one 25 cm² culture flask.

Cell Culture Hazards

The cells do not present a known infectious or toxic hazard, however it is recommended that the cells are handled at containment level 2.

APPENDIX 2: MRC-5 'IFU' CONTENT

MRC-5 – HUMAN DIPLOID FIBROBLASTS

NIBSC ACCESSION NUMBER: 660902

Version Date: 26 July 2007

“This material is not qualified under the In Vitro Diagnostics Directive of the European Union”

1. INTRODUCTION

Human diploid fibroblast cell line: MRC-5.

This product is intended for use as a ‘Reference Cell Bank’ and any manufacturer’s master and working cell banks derived from it will require full qualification and evaluation, as described under the WHO requirements for cell substrates (WHO, 1998).

References

- WHO (1998) World Health Organisation Expert Committee on Biological Standardization (47th Report) Requirements for the use of animal cells as *in vitro* substrates for the production of biologicals. WHO technical report series 878, World Health Organisation, Geneva.
- Jacobs JP, et al. Characteristics of a human diploid cell designated MRC-5. Nature 227: 168-170, 1970.

2. UNITAGE

2ml plastic cryovials

3. CONTENTS

Human diploid fibroblast MRC-5 cells provided as frozen cultures in 2ml plastic cryovials. Each vial contains cells cryopreserved in medium containing foetal bovine serum and cryoprotectant (DMSO).

4. CAUTION

THIS PREPARATION IS NOT FOR ADMINISTRATION TO HUMANS.

The preparation contains material of human origin, which has been tested and found negative for:

CMV by PCR	Hep C by PCR
HTLV1 by PCR	
HIV 1 by PCR	Hep B PCR
EBV by PCR	
Mycoplasma/Ureaplasma by PCR	

As with all materials of biological origin, this preparation should be regarded as potentially hazardous to health. It should be used and discarded according to your

own laboratory's safety procedures. Such safety procedures probably will include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening ampoules or vials, to avoid cuts.

5. DIRECTIONS FOR OPENING THE SCREW CAP VIALS.

Vials have a screw cap. The cap should be removed by turning anti-clockwise. Care should be taken on removal of cap to prevent the contents escaping.

6. STABILITY

It is the policy of NIBSC not to assign an expiry date to their cell banks. These materials are held at NIBSC within assured, temperature-controlled storage facilities. They should be stored on receipt as indicated on the relevant datasheet. Users who have data supporting any deterioration in the characteristics of any cell bank are encouraged to contact NIBSC.

7. CITATION

In any circumstance where the recipient publishes a reference to NIBSC materials, it is important that the title of the preparation and any NIBSC code number, and the name and address of NIBSC are cited correctly.

8. LIABILITY AND LOSS

Information provided by the Institute is given after the exercise of all reasonable care and skill in its compilation, preparation and issue, but it is provided without liability to the Recipient in its application and use.

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If any of the Goods supplied by the Institute should prove not to meet their specification when stored and used correctly (and provided that the Recipient has returned the Goods to the Institute together with written notification of such alleged defect within seven days of the time when the Recipient discovers or ought to have discovered the defect), the Institute shall either replace the Goods or, at its sole option, refund the handling charge provided that performance of either one of the above options shall constitute an entire discharge of the Institute's liability under this Condition.

9. MATERIAL SAFETY SHEET

Physical properties (at room temperature)			
Physical appearance	Liquid (pink or yellow)		
Fire hazard	None		
Chemical properties			
Stable	Yes	Corrosive:	No
Hygroscopic	No	Oxidising:	No
Flammable	No	Irritant:	No
Other (specify)	<i>Contains material of human origin</i>		
Handling:	Handle according to Biosafety Level 2 guidelines		
Toxicological properties			
Effects of inhalation:	Not established, avoid inhalation		
Effects of ingestion:	Not established, avoid ingestion		
Effects of skin absorption:	Not established, avoid contact with skin		
Suggested First Aid			
Inhalation	Seek medical advice		
Ingestion	Seek medical advice		
Contact with eyes	Wash with copious amounts of water. Seek medical advice.		
Contact with skin	Wash thoroughly with water.		
Action on Spillage and Method of Disposal			
Spillage of vial contents should be taken up with absorbent material wetted with an appropriate disinfectant. Rinse area with appropriate disinfectant followed by water.			
Absorbent materials used to treat spillage should be treated as biological waste.			

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