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**SECOND REPLACEMENT SEED STOCK FOR MRC-5 CELLS**

**PROPOSAL FOR WHO REFERENCE CELL BANK STATUS**

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This document has been prepared for the purpose of inviting comments and suggestions on the proposals contained therein, which will then be considered by the Expert Committee on Biological Standardization (ECBS). Comments **MUST** be received by **8 October 2018** and should be addressed to the World Health Organization, 1211 Geneva 27, Switzerland, attention: Technologies, Standards and Norms (TSN). Comments may also be submitted electronically to the Responsible Officer: **Dr Hye na Kang** at email: [kangh@who.int](mailto:kangh@who.int).

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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 60 population doublings, when they become senescent and cease to replicate *i.e.* they are a ‘finite cell line’. The need to supply new vaccine manufacturers, particularly in the far east and the need to periodically replace stocks at manufacturers who previously received MRC-5 cells 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. A proposal for a replacement stock was submitted to the ECBS in 2006 and approved. The resulting replacement PDL12 bank (bank number: 660902-M01) was endorsed by the ECBS in 2007. Due to concerns about inadequate cell numbers per vial and the veracity of the population doubling level, the ‘M01’ bank has now been replaced with a new seed bank at a PDL13 (660902-M02). This second replacement MRC-5 seed bank consists of 189 vials at  $\sim 4.3 \times 10^6$  cells/vial at PDL13 (bank number: 660902-M02) and is proposed for endorsement by ECBS as a WHO Reference Cell Bank.

## Introduction and Background

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 60 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 compromising sterility and risking explosion of the glass ampoules. 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. 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.

In 2005/2006, with the support of the WHO ECBS and in liaison with manufacturers, NIBSC produced a replacement bank of MRC-5 cells derived from the PDL7 cells at the slightly higher passage level of PDL12. The PDL12 bank of cells (660902-M01) underwent a range of quality control tests and the results were deemed satisfactory. The ECBS approved the bank as a WHO Reference Cell Bank in 2007 as a direct replacement for the original MRC-5 PDL 7 stock. However, between 2008 and 2010, recipients commented that the number of

cells per vial was less than expected: In 2008 and 2009 two recipients requested a further vial of cells citing low cell counts as the reason. In 2010 two recipients commented on low cell counts, and one requested an investigation. An internal study at NIBSC verified this, but could not resolve the discrepancy with the cell bank documentation. It was also determined that the true PDL of the 'PDL12' bank was actually  $\geq$ PDL 13. It was therefore decided to create a second reference cell bank and to withdraw the 'PDL12' bank from further distribution.

A second replacement MRC-5 seed bank of 189 vials at  $\sim 4.3 \times 10^6$  cells/vial was produced in 2014 at PDL 13 (bank number: 660902-M02). The 'M02' bank is now proposed for endorsement by ECBS as a WHO Reference Cell Bank.

It should be noted that since this preparation as 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 13 seed stock (660902-M02) will be part of the vaccine manufacturer's validation process and tests results reported for the seed stock are primarily intended for information only.

## Source Material

MRC-5 cells were established at NIBSC in September 1966 and assigned an NIBSC accession number of 660902. The original seed stock was cryopreserved in 1966 at a population doubling level (PDL) 7. It was frozen in 481 glass ampoules in 54 pools, with each pool containing 7 to 10 ampoules (Jacobs *et al.*, 1970). The new seed bank presented here (cell bank number 660902-M02) was derived from six MRC-5 PDL7 ampoules, with two ampoules taken from each of 3 pools. The 6 ampoules from the 3 pools were cultured separately until cell identity from each ampoule was confirmed to be correct (*i.e.* authenticated as MRC-5) by STR DNA profiling, following which the cells from the 6 thawed ampoules were pooled together.

## Cell Culture

Production of the 'M02' bank was carried out in a cleanroom within the UK Stem Cell Bank's suite of cGMP-consistent, EUTCD-compliant Cleanrooms. All culturing processing was carried out in a Class II microbiological safety cabinet ('grade A' clean air environment) within a cleanroom background of 'grade B' air. Cells were cultured to 24x 300cm<sup>2</sup> flasks and cryopreserved at PDL13 as  $\sim 9.45 \times 10^8$  cells at 99% cell viability in 1ml volumes at  $\sim 4.3 \times 10^6$  cells/ml in 189 screw-capped polypropylene 1.8ml vials. Vials are stored in real-time temperature-monitored liquid nitrogen vessels in vapour-phase below -140°C.

## Cell Bank Testing

Five cryovials were thawed for testing. On thawing, the mean cell count was  $4.45 \times 10^6$ /vial and coefficient of variation (CV) - 5.36%. Mean cell viability was 97%, CV - 1.57%. DNA profile was confirmed as MRC-5 using an ISO-17025 accredited test. Mycoplasma was tested by a QPCR method conforming to EU pharmacopeia as negative ( $<2.5$  copies/ml;  $<10$ cfu/ml of culture supernatant). Cell cultures were tested for sterility in-house using an assay conforming to ISO 9001. Cryovial homogeneity was tested using the IncuCyte live cell imaging system which showed insignificant variation of cell growth kinetics between cryovials: CV - 1.67%. Cells were tested for the following viruses: CMV; HTLV1; HIV1; HBV; HCV; EBV, by an independent CPA accredited laboratory.

## Vials on Hand

Of the 189 vials produced, 5 were used for testing; 14 were used to derive a new PDL15 distribution bank; 22 have been stored off-site for disaster recovery; 22 have already been distributed to vaccine manufacturers. There are therefore 126 vials on hand at NIBSC, with a further 22 in reserve in off-site storage. There is thus a total remaining stock of 148 vials. 140 of the remaining 148 vials would be available for distribution. The remaining 8 would be held back for potential future side-by-side performance testing of a replacement bank. The 22 vials assigned for disaster recovery are intended for distribution in the ~2 year period following the disaster, until a replacement bank becomes available. The replacement bank would not be derived from the M02 (PDL 13) bank but from the original 1966 PDL 7 cells. There are 83 ampoules of the PDL 7 cells held at NIBSC and another 20 ampoules held off-site. The current rate of distribution is ~9 vials per year. Therefore, at this rate the M02 bank can be expected to last 16 years. Taking into account the 103 original PDL 7 ampoules, if the glass seal of all ampoules remained intact, there would be the potential to maintain MRC-5 supplies for further ~350 years. In reality, it is likely that supplies can be maintained for at least 50 years.

The PDL13 bank has so far been distributed to the following:

# vials	Supplied to
1	Chengdu Kanghua Biopharma, China
5	HLL Biotech, India
2	AB&B Bio-Tech, China
1	Tianjin Cansino Biotech, China
1	TomegaVax, USA
1	Chengdu Institute of Biological Products
1	Chengdu Kanghua Biopharma, China
1	Shan Xi Peisen Biological Products, China
1	Zhejiang Pukang Biotechnology, China
1	Cadila Healthcare, India
1	Shanghai Institute of Biological Products, China
2	Taizhou Scivac, China
1	Univ of Electronic Science & Technology, China
1	Batavia Biosciences, Netherlands
2	Taizhou Scivac Biotech, China

## Proposed Distribution Control

If the cell bank is endorsed by the ECBS, it is proposed to establish procedure for review and approval of the requests in a manner similar to how requests for the WHO Vero 10-87 cells are handled. In this way some scrutiny will be applied to ensure all recipients are *bone-fide* vaccine manufacturers. It would be possible for NIBSC to do the assessment of the requests based on agreed criteria, but it would be preferable if there could be some NIFDC involvement in the Chinese requests. At the moment it is difficult to judge from NIBSC which Chinese requestors are likely to produce approved vaccines.

## 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 PCR records
- Morphology records (light microscopy)
- Homogeneity testing records (viability and growth rates)
- DNA profile records
- Virus screening records
- Cryopreservation storage records

## Discussion and Conclusions

The CLMF is held at NIBSC and a copy can be made available for the WHO. It should be noted that the PDL 13 (660902-M02) MRC-5 cell bank is a seed stock or ‘reference cell bank’ and that recipients/manufacturers of these cells 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 13 bank are now being distributed to manufacturers.

It is requested that the WHO endorse the new MRC-5 PDL 13 cell bank (660902-M02) as a replacement for the PDL12 (660902-M01) seed stock for viral vaccine production, diagnostic and surveillance purposes.

## References

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