

# MRC-5 (Medical Research Council -5) Human Diploid Cell Culture using Tide Motion Bioreactors

## Background

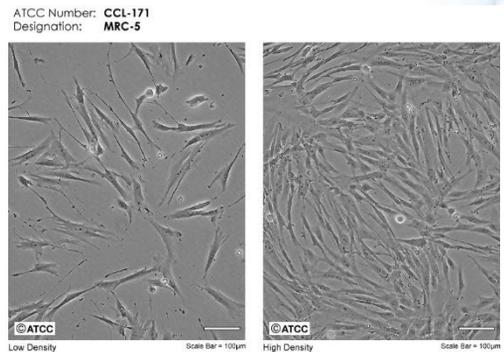
- Lung tissue obtained from 14-week fetus; Karyotype is 46,XY
- Fibroblast-like cell
- Population doubling to senescence is 42-48 passages (Jacobs,JP,1976)

## Applications

### Large scale vaccine/viral production:

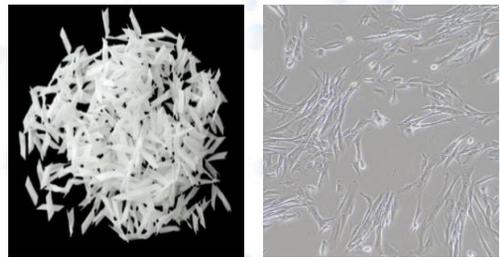
Adenovirus    Hep A/Hep B    Human Rabies  
 DTaP-IPV/Hib    MMR (MMR-II)    Varicella  
 Hep A            MMRV                    Zoster (shingles)  
 Hep B

## Gross morphology



## Culture of MRC-5 cells

MRC-5 diploid cells are an extremely challenging cell line to culture to high densities in solid 3-D matrices. Given their wide application in production of an array of human vaccines, (Jordan and Sandig, 2014) we have optimized the culture conditions using our Tide motion Bioreactors (CelCradle™ -500A) which use BioNOC™ II macrocarriers as the matrix. These cells have been cultured to high densities in these matrices that mimic a 3-D *in vivo* environment. Cells have been grown to approximately 19-fold the seeding densities.

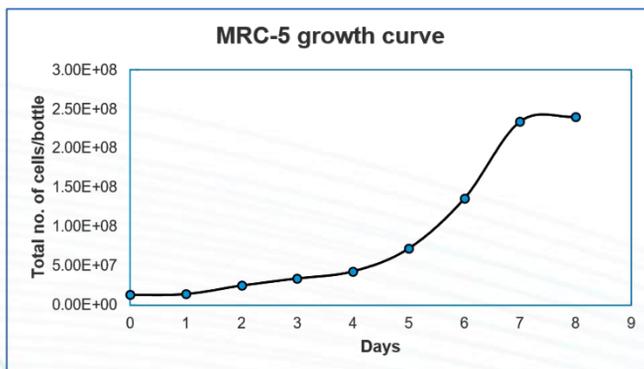


**MRC-5 cell growth supported by BioNOC™ II macrocarriers which provide a large surface area for growth**

(L)- BioNOC™ II macrocarriers  
 (R)- MRC-5 cells under 4x magnification

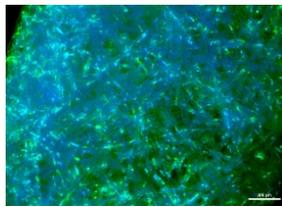
Cells are grown to typically high densities using a batch mode of culture in serum-containing medium as represented in (A) using these bench-top bioreactors of 500ml scale. Stained cells observed microscopically are represented in (B). The scalability and surface area for MRC-5 cell growth in different volume bioreactors is as represented in (C). The technology therefore, is very robust for linear scalability.

A



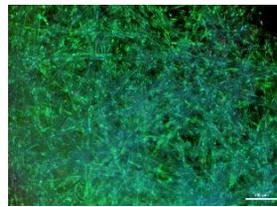
Media	DMEM + 15% FBS, 1% Penicillin-Streptomycin
Culture Period	Recommended Harvest at 7 <sup>th</sup> /8 <sup>th</sup> day
Seeding Density	15,000 cells per carrier (12.75 million cells per bottle)
Harvested	282,000 cells per carrier (240 million cells per bottle)
Fold change	19-fold

B



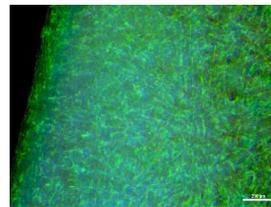
Day 1

40,000 cells/carrier



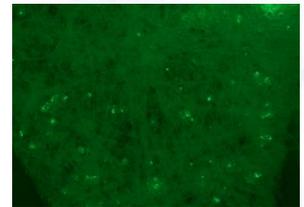
Day 2

85,000 cells/carrier



Day 3

282,000 cells/carrier



After harvest

Fluorescein diacetate (FDA) staining of live MRC-5 cells grown in DMEM containing 15% FBS and viewed under 4x magnification

Scalability

Surface Area for Cell Growth  
(in DMEM)

C

Model	Fixed Bed volume (L)	Cell numbers at harvest
CelCradle™	0.1	2.4x10 <sup>8</sup>
TideXcell™-002	2	4.8x10 <sup>9</sup>
TideXcell™-020	20	4.8x10 <sup>10</sup>
TideXcell™-100	100	2.4x10 <sup>11</sup>

Model	Fixed Bed volume (L)	Cell culture surface area in m <sup>2</sup>
CelCradle™	0.1	0.28
TideXcell™-002	2	5.6
TideXcell™-020	20	56
TideXcell™-100	100	280

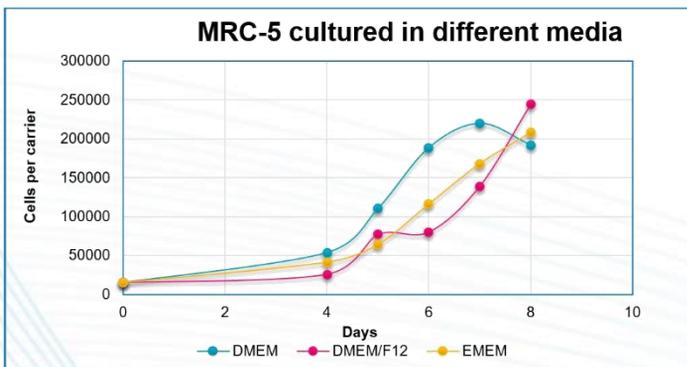
Optimum media for MRC-5 cell growth

MRC-5 cells have been reported to be cultured in EMEM, DMEM, DMEM: F12 media. Our results indicate a robust cell growth in DMEM supplemented with 15% FBS as reported above. This is a cost-effective solution as well, in terms of obtaining high cell densities with significantly less expensive media. The cell growth pattern of MRC-5 cells cultured on BioNOC™ II using different media is as shown in( D).

Recommendations for MRC-5 culture

- MRC-5 cells show optimum growth in DMEM medium supplemented with 15%FBS
- A low seeding cell density of 15,000 per carrier results in optimum growth and expansion of up to 19 fold
- The 7/8<sup>th</sup> day of culture is optimum for harvest in DMEM
- Culture of MRC-5 cells for virus production is optimum using passage 20-27 wherein 18-20 fold cell expansion is observed
- A simple DOE to test cell growth in 3D using different media such as DMEM, EMEM and DMEM:F12 supplemented with 15% FBS suggests that DMEM is optimum for cell expansion, recovery, better preservation of cell morphology and cost economy with almost comparable cell numbers.
- Application of these culture conditions in Tidemotion bioreactors demonstrates good cell expansion which can be used for production of cell banks/ viruses including Oncolytic Viruses and could form the basis for large-scale manufacturing.

D



## References

1. Jacobs, JP (1976). The Status of Human Diploid Cell Strain MRC-5 as an Approved substrate for the Production of Viral Vaccines. *Journal of Biological Standardization*. 4 (2): 97–99.
2. Jordan I and Sandig V (2014). Matrix and Backstage: Cellular Substrates for Viral Vaccines. **6(4): 1672–1700**