

ANNEXURE-I

CELL LINE CHARACTERIZATION - TESTING REQUIREMENTS

S.No	Parameter	MCB	WCB	ECB	Tests to be done
1	Identity	yes	yes	No	Karyology
		yes	yes	No	Iso-enzyme analysis (Nucleoside Phosphorylase, Glucose -6- Phosphate Dehydrogenase, Lactate Dehydrogenase)
		yes	yes	No	DNA profiling using VNTR
2	Stability	yes	yes	No	Viability after revival
3	sterility	yes	yes	No	a) Bacterial and Fungal sterility-Atleast 1% of cryopreserved vials should be tested, both supernatant fluid and vial should be tested
		yes	yes	No	b) Mycoplasma-Both culture and indicator method should be used
		yes (MCB or WCB)		No	c) Mycobacteria
4	Viability	yes	yes	No	Post banking thaw viability should be more than 80%
5	Growth characteristics	yes	yes	No	Viability
		yes	yes	No	Morphology
		yes	yes	No	Cell doubling times
		yes	yes	No	Cloning/Plating efficiency
6	Homogeneity testing	yes	yes	No	Experiments to demonstrate Homogeneity and Growth characteristics may be combined, although the analysis should be carried out separately
7	Tumourigenicity test (in animals)	No	No	yes	Minimum of 10 animals should be used per sample with positive control (HeLa cells)
					Observation period for test in nude mice is 4 months (ATS treated new born mice can also be used)
8	Cytogenetics	yes	No	yes	Minimum of 100 cells from metaphase should be examined at approximately four equally spaced intervals during serial cultivation from the MCB through to the proposed in vitro cell age
9	Microbial agents	yes	yes	No	Test in adult mice: 0.5ml of sample should be injected intra peritoneally into atleast 10 adult mice (15-20gms) and observed for 4 weeks
		yes	yes	No	Test in suckling mice: 0.1ml of sample should be injected intra peritoneally into atleast 10 suckling mice (24 hrs old) and observed for 4 weeks
		yes	yes	No	Test in Guinea pigs: 5ml of sample should be injected intra peritoneally into atleast 5 guinea pigs (350-450gms) and observed for 42 days
		yes	yes	No	Test in Rabbits: 5ml of sample should be injected intra peritoneally into atleast 5 rabbits (1.5-2.5kgs) and observed for 4 weeks
10	Test in eggs	yes	yes	No	Test in embryonated chicken eggs: cells are injected into the allantoic cavity of each of at least ten embryonated hens' eggs, and into the yolk sac of each of at least another ten embryonated hens' eggs. The eggs are examined after not fewer than 5 days of incubation.
11	Tests in cell culture	yes	yes	No	cells should be inoculated with continuous simian cell line, human cell line and observed for 28 days
12	TEM	yes (MCB or WCB)		yes	At least 200 cells from the MCB or WCB and ECB or EOPC are examined by transmission electron microscopy (TEM) for evidence of contamination with microbial agents. Methods include negative staining and thin section.
13	Test for Retroviruses	yes	yes	yes	Culture supernatants need to be tested by a highly sensitive, quantitative, polymerase chain reaction (PCR) based RT assay or PERT assay
14	Adventitious agents	yes (MCB or WCB)		No	Detection of viral contaminants in bovine serum as per 9CFR (Bluetongue virus, Bovine virus diarrhoea virus, Reovirus, Rabies virus, Bovine adeno viruses, Bovine parvovirus, Bovine respiratory syncytial virus) and detection of Porcine circo virus, Hepatitis A virus, Hepatitis E virus , Infectious bovine rhinotracheitis virus, Bovine parainfluenza virus type 3, Bovine circo virus, Bovine polyoma virus, Bovine Herpes Virus, , Simian Immunodeficiency Virus and Simian Virus 40 - by PCR method. for the above mentioned tests "Massively Parallel Sequencing" may be quoted as option