



Technical Specifications for the Release Analysis of "Master Cell Banks" and Retroviral Vectors

The Bambino Gesù Paediatric Hospital (OPBG) requires the activation of analytical services for the release of n=2 "Master Cell Banks" (MCB) and n=3 retroviral vectors to be used as "Starting Materials" in the production of CAR-T drugs. The analyses to be carried out are aimed at confirming identity, verifying virological and microbiological safety, and evaluating impurities in the materials subject to release. These analyses are complementary to additional tests carried out at the Officina Farmaceutica of the OPBG. For the release of retroviral vectors, both the vectors themselves and the collection of producer cells after the production of the vectors, known as "Post Production Cell Bank" (PPCB), are analyzed. Tables 1 and 2 respectively list the denominations of the required analyses for the two MCBs and the three retroviral vectors to be released.

Table 1. Required assays for MCB release (n=2).

	Assay	#
MCB (HEK Vec 293 RD114) identity	DNA Fingerprinting by STR Assay	x2
Microbiological evaluation	Sterility EP 2.6.1	x2
	Bacteriostasis (Sterility validation EP 2.6.1.)	x2
	LAL Validation and test	x2
	Detection of mycoplasma by culture and staining EP 2.6.7	x2
	Qualification of the test article for detection of mycoplasma (EP 2.6.7)	x2
Virological evaluation	Determination of viral contaminants (<i>in vitro</i>) using cell lines according to EMA/CHMP/ICH/804363/2022	x2
	Detection of porcine viruses using Vero and PPK cell lines according to 9CFR guideline and EP	x2
	Detection of bovine viruses using Vero and BT cell lines according to 9CFR guideline and EP	x2
	In vivo adventitious agent detection using embryonated hen eggs, suckling and adult mice	x2
	Quantitative PCR for HIV-1, HIV-2, HTLV 1&2, EBV, HCMV, HHV6, HHV7, HHV8, HAV, HBV, HCV, PCV1 (DNA), PCV2 or BCV(DNA), SV40	x2
	h. polyomavirus JC and BK sequences	x2
	h. papillomavirus	x2
	h. enterovirus	x2
	PCV1 (DNA), PCV2, BCV(DNA)	x2
Impurities evaluation	Assay for RD114 retrovirus Detection (Supernatant) (FDA-1999-D-0081) with GalV and RD114 as positive controls (with GalV and/or RD114 titration, if necessary)	x2
	Assay for RD114 retrovirus Detection (Cells) (FDA-1999-D-0081) with GalV and RD114 as positive controls (with GalV and/or RD114 titration, if necessary)	x2
	Detection of contaminating agents by TEM	x2

Table 2. Required assays for viral vector release (n=3).

PPCB assays		#
PPCB (HEK Vec 293 RD114) identity	DNA Fingerprinting by STR Assay	x3
Microbiological evaluation	Detection of mycoplasma by culture and staining EP 2.6.7	x3
	Qualification of the test article for detection of mycoplasma (EP 2.6.7)	x3
Virological evaluation	Determination of viral contaminants (<i>in vitro</i>) using cell lines according to EMA/CHMP/ICH/804363/2022	x3
	Detection of porcine viruses using Vero and PPK cell lines according to 9CFR guideline and EP	x3
	Detection of bovine viruses using Vero and BT cell lines according to 9CFR guideline and EP	x3
	In vivo adventitious agent detection using embryonated hen eggs, suckling and adult mice	x3
Impurity evaluation	Assay for RD114 retrovirus Detection (Supernatant) (FDA-1999-D-0081) with RD114 as positive control (with RD114 titration, if necessary)	x3
	Assay for RD114 retrovirus Detection (Cells) (FDA-1999-D-0081) with RD114 as positive control (with RD114 titration, if necessary)	x3
Viral vector assays		#
Microbiological evaluation	Detection mycoplasma by culture and staining (EP 2.6.7)	x3
	Qualification of the test article for detection of mycoplasma (EP 2.6.7)	x3
Virological evaluation	Determination of viral contaminants (<i>in vitro</i>) using cell lines according to EMA/CHMP/ICH/804363/2022	x3
	Detection of porcine viruses according to 9CFR guideline and EP	x3
	Detection of bovine viruses according to 9CFR guideline and EP	x3
	Assay for RD114 retrovirus Detection (Supernatant) (FDA-1999-D-0081)	x3
	In vivo adventitious agent detection using embryonated hen eggs, suckling mice and adult mice (ICH Q5a, PCT 1993)	x3
Impurity evaluation	Host cell DNA (E1B, E1A)	x3
	Osmolality	x3

Table 3. other assays to add as amendment

Viral vector assays		#
Preliminary assays	Evaluation to determine the cytotoxicity of the products under analysis prior to an <i>in vitro</i> test	x2
Impurity evaluation	Host Cell Proteins	x3
MCB and PPCB assays		#
Preliminary assays	Cell expansion and sample preparation	x4
Identity	Morphological evaluation	x3
Microbiological evaluation	Qualification of the method for sterility and test	x3
	Qualification of LAL method and test	x3

