Minutes of the Lentivirus Reference Material Meeting at the ISBiotech 9th Spring Meeting, Norfolk, VA, March 4-6, 2019 (12h00-13h15).

Anja Rodenbrock from CNRC replaced Sven Ansorge and presented an update of the programme and the plan of the preliminary tests and the manufacturing of the lentivirus reference material. The details are provided in the joint PPT-presentation.

Slide Presentation at Spring 19 LV Referenc

Discussions: addition of sucrose for better preserving the viral vector during clarification (filtration). P. Gagnon reminded that an increased sucrose (or trehalose) concentration leads to increased viscosity and thus shear during filtration. However, O.-W. Merten indicated that the addition of 5% sucrose led to a considerable protection of GaLV-TR pseudotyped lentiviral vector when added before filtration. Thus, the addition of sucrose seems to be a good choice to reduce vector inactivation during clarification.
The manufacturing protocol will not be directly translated to a 200L scale, but will be assessed in beforehand at a 10L scale. The 10L scale will serve to lock-in the process conditions, to decide on the purification options (choice of filters from two different vendors), and to send purified material to ATCC in order to de-risk sterile-filtration.

Timing: 3L reactor run(s) – terminated by April 2019
10L reactor run(s) – achieved by September 2019 (sterile filtration will be performed in 2020)
200L reactor run by December 2019 with a back-up run (if necessary) by February 2020.

Characterisation of the lentiviral reference material (LVV RM Characterization, slide 20):

There should be a distinction between the safety tests (endotoxin, sterility, mycoplasma, adventitious agents (in vitro method), RCL) which should be performed by one company, such as BioReliance, Eurofins, or another company) and the other tests. These tests should be performed by many contributors which should be provide protocols or SOPs for testing and indicate which of the proposed tests they would do. In this context, the most important tests are the tests for infectious titer, the particle titer (p24) and purity and SOPs will be required.
Concerning statistical evaluation, J. Reiser has identified a statistician at FDA.

Table 1.0

Attribute	Recommended Methods	Testing performed by	Report
Identity	SDS-PAGE and Western Blot for viral gag proteins	1 or 2 Organizations (Company, academia or CTO)	Confirm identity
	Vector sequence	1 Organization	Confirm identity

O.-W. Merten, Boston, 9th of March 2019.

Purity	Host cell protein	1 or 2 Organizations (Company, academia or CTO)	Results
	Host cell DNA	1 or 2 Organizations (Company, academia or CTO)	Results
	Total protein	1 or 2 Organizations (Company, academia or CTO)	Results
	Residual Benzonase	1 or 2 Organizations (Company, academia or CTO)	Results
Safety testing[1]	Endotoxin	1 CTO	EU/mL
	Sterility	1 CTO	Pass
	Mycoplasma	1 CTO	Negative
	Adventitious agents (in vitro methods)	1 CTO	Negative
Infectious titer	Either ddPCR or qPCR via classical TaqMan of genomic DNA from transduced target cells such as HCT116 or HEK293	As many as possible organizations with WG SOP. Statistical analysis to be performed.	ig/mL
Particle concentration	Commercial p24 ELISA	As many as possible organizations with WG SOP. Statistical analysis to be performed.	Nanograms p24 per LVV RM
RNA genome copies	RT qPCR	As many as possible organizations with WG SOP. Statistical analysis to be performed.	Number of copies
Replication-competent lentivirus (RCL)[1]	qPCR Assay for VSV-G[2]	1 CTO	Negative

Material list:

The material list for the 200L run(s) is available, but the list for the 10L reactor is still to be established (the materials required for the 3L runs will be provided by the CNRC). As soon as both list are available, the vendors (=potential donators) have to be contacted and it is proposed that not only Keith but also other members of the group contact vendors. It will be important to dispatch this specific activity and ideally, those who have already (good) contacts to the vendors should contact them.

O.-W. Merten, Boston, 9th of March 2019.

Keith indicated that the price for the USP material is about 41000-42000 US$. The costs for the DSP material is slightly higher.

O.-W. Merten, Boston, 9th of March 2019.