

Lentivirus Vector Reference Standard Initiative

ISBioTech, Washington DC, March 7th 2017

Background/History

- › 2014 - Industrial partner bluebird bio seeking to form an LVV Working Group contact Keith Carson at ISBioTech
- › 2015 - ISBioTech Spring meeting – 1st LVV RSM meeting to gauge general interest in public discussions
- › 2016 - ISBioTech Spring meeting – Driving parties bluebird bio, Généthon and Lentigen held 2nd LVV RSM meeting to gauge general interest in public discussions
 - Among next steps it was decided that a survey would be conducted

Goals

- › The global availability of reference standard materials (RSMs) permits the **standardization of quantification techniques** across research and manufacturing organizations.
- › The LVV reference standard material is intended for **use in calibrating internal (product-specific) reference materials** used for quantitation assays, plus the qualification of assays.
 - Well-characterized reference standard preparations are important for the establishment of appropriate pre-clinical and clinical dosing, making data from different pre-clinical and clinical studies easier to compare.

Approach

- › Follow existing examples for the generation of other Viral Vector Reference Standards Materials available today through ATCC (Ad5, AAV2, AAV8, etc.)

Selected references for existing RSM

› Ad5 ATCC VR1516

1. Hutchins B Development of a reference material for characterizing adenovirus vectors Bioprocess. J. 125-28, 2002 Available from: BioProcessing Journal
2. Hutchins B, et al. Working toward an adenoviral vector testing standard. Mol. Ther. 2: 532-534, 2000. PubMed: 11124052
3. Summary of presentations can be found at <http://www.isbiotech.org/ReferenceMaterials/Adenovirus.html>

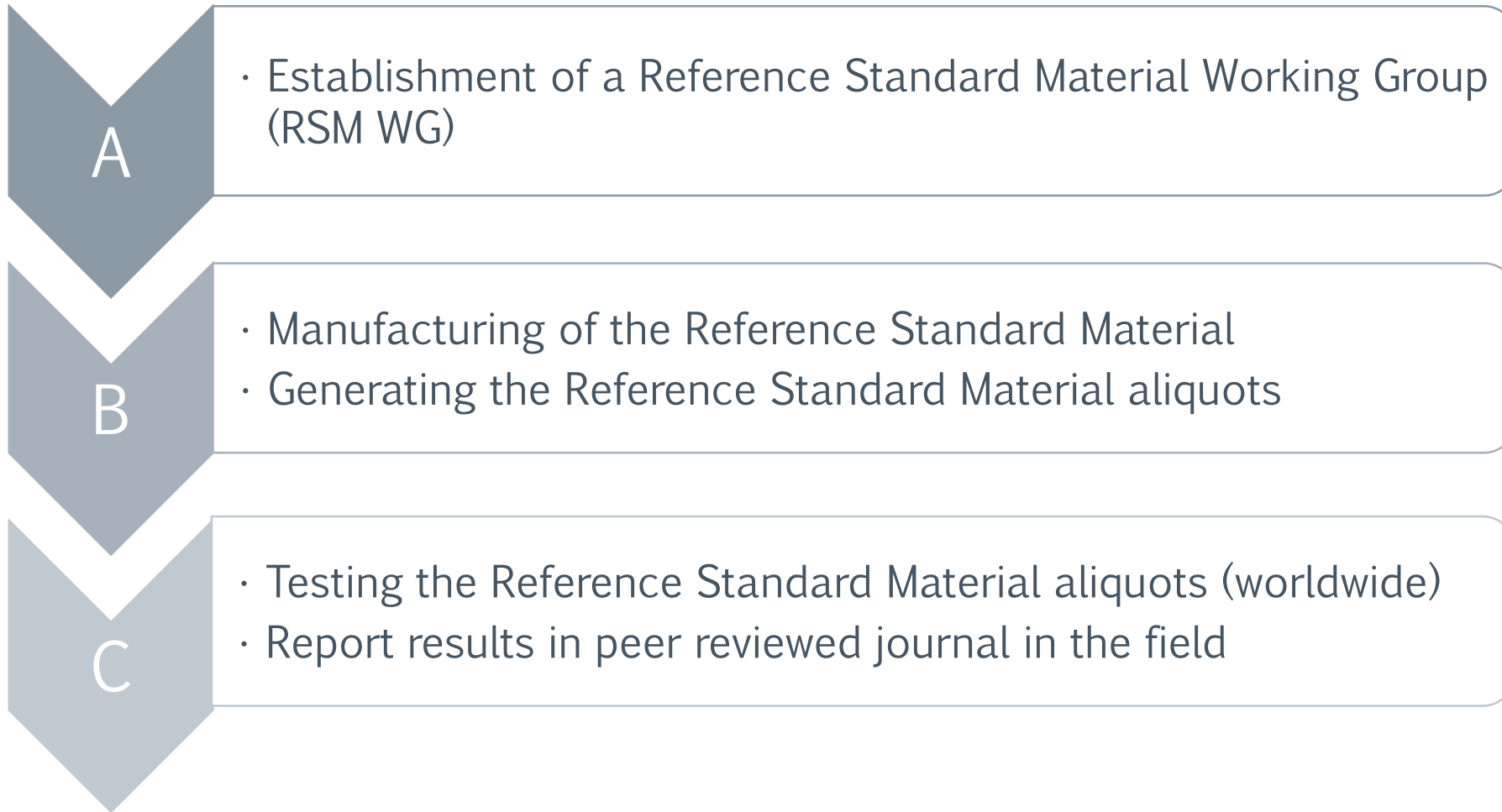
› AAV2 ATCC VR1616

1. Lock M, et al. Characterization of a recombinant adeno-associated virus type 2 Reference Standard Material. Hum. Gene Ther. 21(10): 1273-1285, 2010.

› AAV8 ATCC VR1816

1. Ayuso E., et al., 2014 Manufacturing and characterization of a recombinant adeno-associated virus type 8 reference standard material. Hum Gene Ther. 2014 Nov;25(11):977-87

Expected LVV RSM Workflow

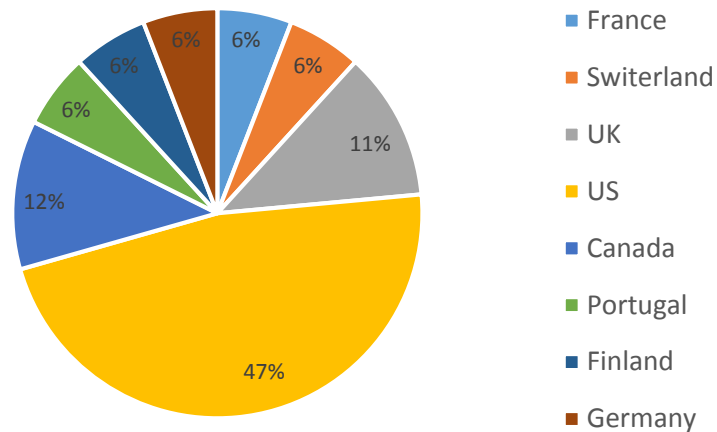


Lentivirus Vector Reference Standard Material

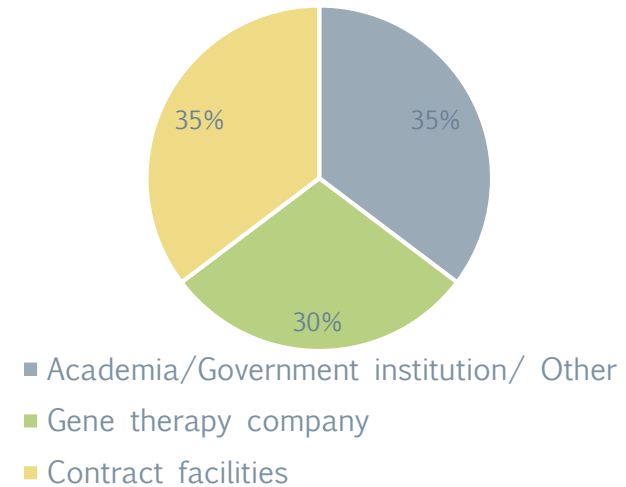
Survey Monkey results

Response analysis from 18 respondents

Survey participants per country

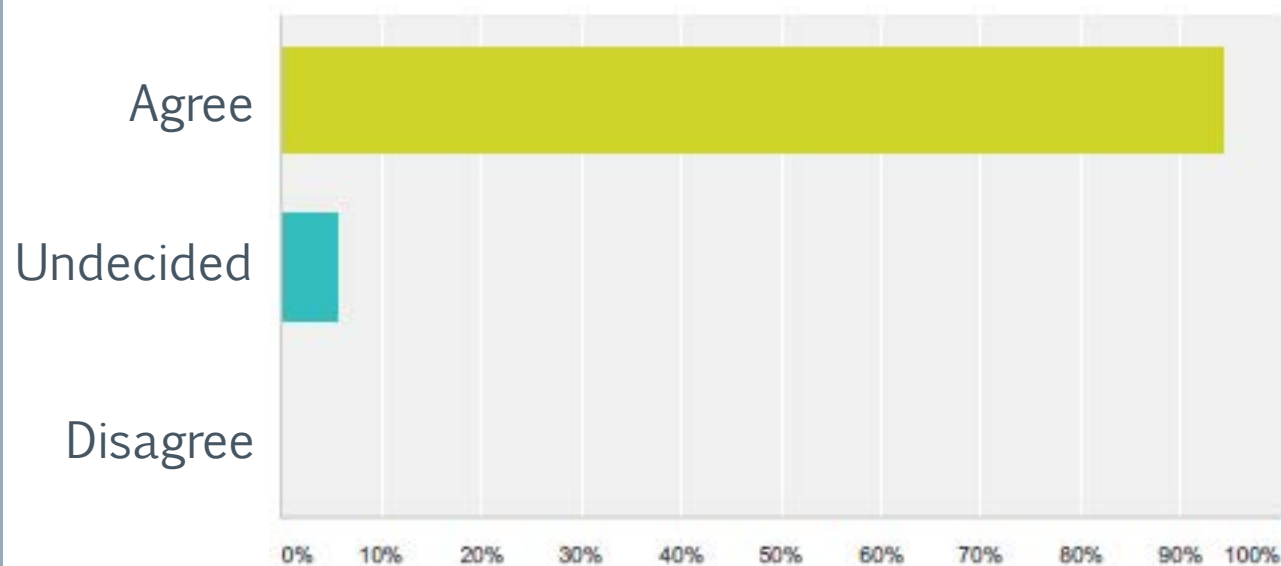


Survey participants per sector



Survey Results

- › Question 1: A 3rd generation HIV-1-based VSV-G pseudotyped LVV coding for a gene that is easily detected by FACS (GFP or equivalent) is proposed. Do you agree with this choice or would like to suggest an alternative?



Answer choices	Responses
Agree	94.44% (17)
Undecided	5.56% (1)
Disagree	0%
Total	18

Survey Results

- › Question 2: Please provide your estimate for 1) how many vials should be produced, 2) what the volume per vial should be and 3) what the vector concentration should be in each vial.

Participant estimates	#vials ^b	Volume (mL) ^b	Titer (TU/mL) ^b
# respondents ^a	11	14	8
Min	500	0.05	1.0E+06
Max	10000	100	1.0E+08
Mode	1000	1	5.0E+07
Mean	3045	8	4.7E+07

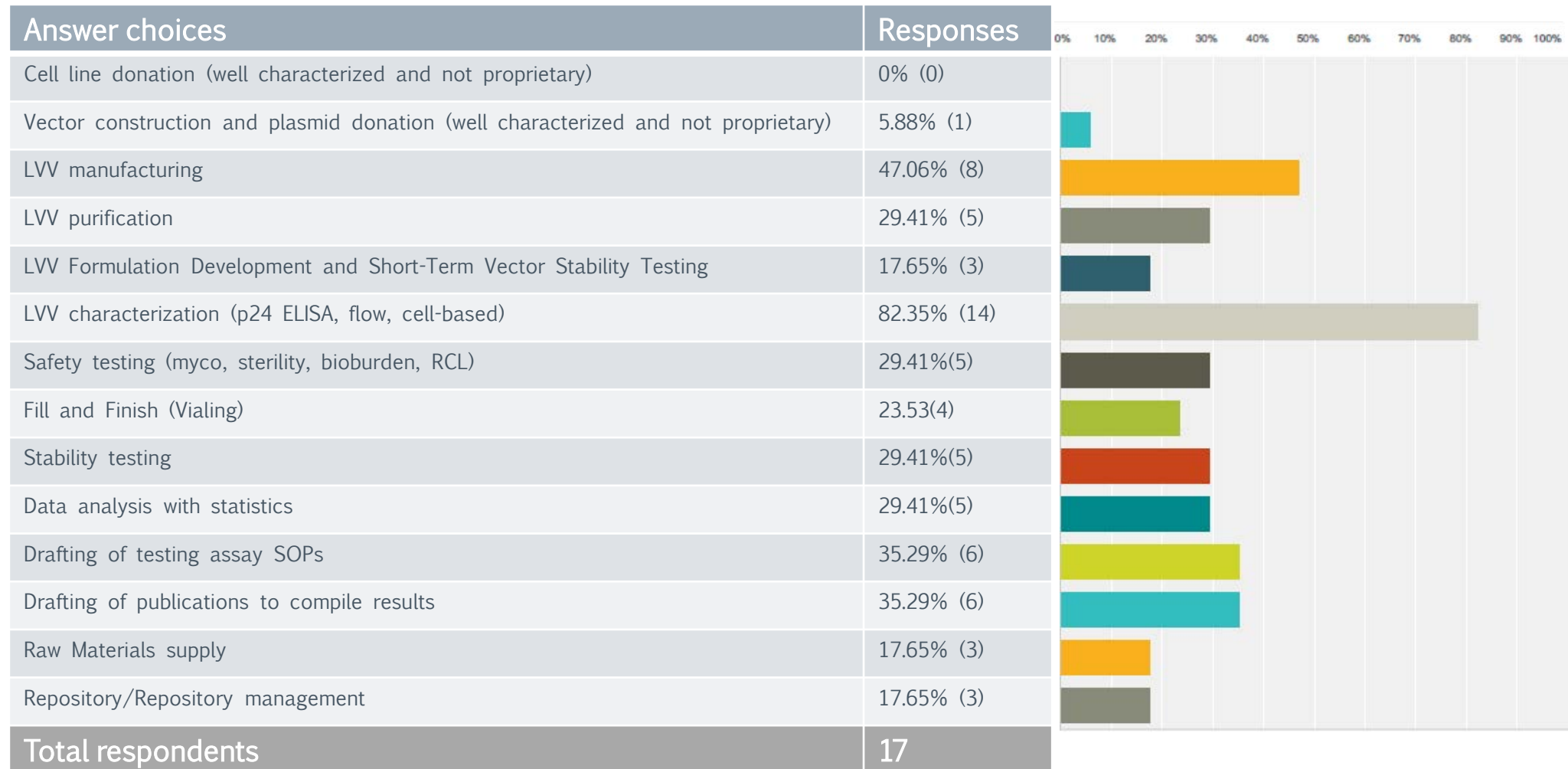
RECOMMENDED	5000	0.5	5.0E+7 to 1.0E+8
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^a Only responses providing numerical data included

^b Used average for ranges provided

Survey Results

- › Question 3: Please indicate the type of support your institution could potentially provide to support the LVV RSM initiative



Survey Results

Answer choices	Responses	Institute
Cell line donation (well characterized and not proprietary)	0% (0)	
Vector construction and plasmid donation (well characterized and not proprietary)	5.88% (1)	LentiGen (BD)
LVV manufacturing	47.06% (8)	Catapult (ES); LentiGen (BD); iBET (PA); Lonza H (RJ); bbb (MS); Finvector (HL); Wuxi App Tec (AM); NRC (SA)
LVV purification	29.41% (5)	LentiGen (BD); iBET (PA); bbb (MS); Finvector (HL); Wuxi App Tec (AM)
LVV Formulation Development and Short-Term Vector Stability Testing	17.65% (3)	LentiGen (BD), Bbb (GV); Finvector (HL)
LVV characterization (p24 ELISA, flow, cell-based)	82.35% (14)	Novartis (MJ); Catapult (ES); LentiGen (BD); McGill (AK); Mass Biologics (GS); iBET (PA); Lonza H (RJ); bbb (MS); bbb (GV); Finvector (HL); CEVEC (NF); Wuxi App Tec (AM); NRC (SA); NIBSC (YZ)
Safety testing (myco, sterility, bioburden, RCL)	29.41%(5)	LentiGen (BD), Bbb (GV); Lancaster (KB); Wuxi App Tec (AM); NIBSC (YZ)
Fill and Finish (Vialing)	23.53(4)	LentiGen (BD); Lonza H (RJ); Finvector (HL); NIBSC (YZ)
Stability testing	29.41%(5)	Mass Biologics (GS); NRC (SA); LentiGen (BD); Lonza H (RJ); Finvector (HL);
Data analysis with statistics	29.41%(5)	INSERM (NC); LentiGen (BD); bbb (MS); bbb (GV); NIBSC (YZ)
Drafting of testing assay SOPs	35.29% (6)	Novartis (MJ); Catapult (ES), INSERM (NC); LentiGen (BD); Mass Biologics (GS); bbb (MS);
Drafting of publications to compile results	35.29% (6)	Novartis (MJ); INSERM (NC), LentiGen (BD), McGill (AK); bbb (MS); Wuxi App Tec (AM)
Raw Materials supply	17.65% (3)	LentiGen (BD); bbb (GV); ISBioTech (KC);
Repository/Repository management	17.65% (3)	Catapult (ES); LentiGen (BD); NIBSC (YZ)

Points agreed and points
for discussion

Summary of points agreed so far...

- › Vector type: A 3rd generation HIV-1-based VSV-G pseudotyped LVV coding for a gene that is easily detected by FACS (GFP or equivalent)
- › Manufacturing of 5000 vials containing 0.5mL/vial of LVV at a concentration between 0.5E8 to 1E8 TU/mL
- › Process steps, raw materials and other manufacturing details required to understand the LVV RSM characteristics **need to be fully disclosed**

Summary of points agreed so far...

- › The testing would be performed by a number of industrial and academic partners in the Working Group under detailed SOPs that would be distributed along with LVV RSM samples. Data will be compiled and analyzed for publication purposes.
- › A well characterized cell bank and plasmids (or transformed bacteria) stocks should be kept at ATCC for reproducing another RSM
- › The LVV RSM will be made available from an unbiased repository like ATCC at minimal cost

Points for discussion

- › Lentiviral Vector Manufacturing
- › Lentiviral Vector Purification, Formulation and Fill Finish
- › Lentiviral Vector Characterization and Safety Testing
- › Drafting Requests for Donation
- › Stability studies: short and long-term
- › Distribution
- › Cost
- › Other points of discussion

Next Steps

- › Consolidate LVV RSM Working Group
 - Please respond to lvwg@isbiotech.org if you'd like to be included as a member of the **Working Group** for this initiative.
 - Please note that there is no limit to the number of individuals who can join from each organization, but there can only be one voting member from each organization.
- › Obtain alignment from the Working Group on points of discussion
- › We are working to identify a place and time for the next Working Group Meeting
- › Comments, please send to lvwg@isbiotech.org

Working Group so far..(through JoForm)

#	Name	Institute	Email	Job Title
1	Hugo Soares	iBET - Instituto De Biologia Experimental e Tecnologica	hsoares@ibet.pt	PhD Student
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5	Manjunatha	bub	manjuss168@gmail.com	RS
6	Martin Wisher	BioReliance	martin.wisher@sial.com	Head of Regulatory Affairs
7	Andre Sobczyk	Brain & Spine Institute, Paris	andre.sobczyk@icm-institute.org	Head of vectorology core facility
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21	Lexan Lhu	Charter Medical	llhu@chartermedical.com	Sales Engineer
22	Howard Kim	CCRM	howard.kim@ccrm.ca	Development manager

LVV RSM project ISBioTech 2015 and 2016 meeting minutes and list of attendees

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VIRAL REFERENCE MATERIALS



[Home Page](#) » Status: Completed and Available

Adenoviridae Image: Q-One BioTech / now part of [BioReliance](#)



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AAV2 Image: [Dr. Richard O. Snyder et. al., University of Florida,](#)
article published in [BioProcessing Journal](#) Vol. 7/No 2.



Status: [List of Participants](#)

PERV Image: Dr. Klaus Boller / [Science Photo Library](#)



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HIV Lentivirus Image: Q-One BioTech / now part of [BioReliance](#)



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Baculovirus Image: The Baculovirus Facility,
Department of Biochemistry, [University of Cambridge](#)



[Home Page](#) » Status: Project Ended

HERV Image: Russell Kightley / [Science Photo Library,](#)
as published in [NatureNews](#), 31 October 2006

Revised 5/16/2016

International Society for BioProcess Technology

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Questions and Information

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Working Group Executive Committee*

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*Placed in alphabetical order

Points of discussion for this meeting

Points for discussion this meeting

- › Lentiviral Vector Manufacturing
- › Lentiviral Vector Purification, Formulation and Fill Finish
- › Lentiviral Vector Characterization and Safety Testing
- › Drafting Requests for Donation

Lentiviral Vector Manufacturing

- › Vector constructs: Vector construction and plasmid donation (well characterized and not proprietary) was offered by 1 survey participant (Lentigen)
- › Cell line: no cell line donation identified

Lentiviral Vector Manufacturing

- › The method of manufacture of such LVV RSM
 - Adherent vs. suspension (FBS or serum free)
 - Transient transfection vs. producer cell line
- › Manufacturing scale – Consider a final volume 2500 mL of LVV at 0.5-1E8 TU/mL
- › Manufacturing site

Note

- › 8 Survey participants offered manufacturing capabilities

Lentiviral Vector Purification, Formulation and Fill Finish

- › Process steps and level of purity needed – Consider Purpose
- › Final formulation and short term stability
- › Purification and fill finish site

Note:

- 5 Survey participants offered DSP capabilities
- 3 Survey participants offered LVV Formulation Development and Short-Term Vector Stability Testing
- 4 Survey participants offered Fill and Finish (Vialing)

Lentiviral Vector Characterization Testing

- › p24 ELISA
- › Infectious titer by cell-based assay and PCR
- › Infectious titer by flow cytometry and GFP/equivalent expression
- › Other?

Note:

- 12 Survey participants offered testing capabilities

Lentiviral Vector Safety Testing

- › Mycoplasma
- › Sterility
- › Endotoxin
- › Bioburden
- › RCL
- › Other?

Note:

- 5 Survey participants offered Safety testing capabilities

Drafting of Requests for Donation (RFD):

› Need help drafting of RFD for:

- Cell Line
- Plasmid
- Production*
- Purification*
- Formulation and Short-Term Stability Testing
- Safety Testing
- Filling
- Characterization
- Long-Term Stability and Sterility Testing

* Include proposal for how the material, would be processed and why the people involved are qualified to do the work. The Working Group would then vote on the submissions and select one

Backup slides

Lentiviral Vector Stability Studies

- › Short-term field use & shipping configuration stability
- › Long-term stability at -80°C
- › Studies to be performed?

Note:

- 5 Survey participants offered Stability testing capabilities

Distribution

- › The LVV RSM will be made available from an unbiased repository like ATCC or other at minimal cost
- › The repository will restrict the number of vials that can be delivered to users and have all users sign a material transfer agreement (MTA) in which they agree to only use the material for the intended purposes, which are to validate internal reference materials and assays.

Cost

- › Costs to cover repository expenses and manufacturing expenses.

Note: There are multiple potential mechanisms (sponsors, granting mechanisms, vial cost, etc.) and it should be discussed collectively

Other points

- › Drafting of testing assay SOPs
- › Drafting of publications to compile results
- › Raw Materials supply
- › Repository/Repository management