

Session II

New Quality Challenges for Cell Therapy Products

13:45-16:00

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Summary

- QC of autologous cell therapy products
- QC of allogeneic cell therapy products
- Testing of bovine derived products
 - risk from BVDV and BPyV
- Quantitative PCR assay for mycoplasma detection



Routes for Potential Contamination

- Starting materials
 - Cells
 - Donor
 - Manipulation prior to banking
- Media components and reagents
 - FCS - bovine viruses, TSE
 - Glucose, amino acids, salts - MVM contamination?
 - Trypsin - porcine viruses
 - Growth factors - various sources
- Cell manipulation and processing
 - Operators



Ensuring the safety of biological products

- Screening of raw materials for adventitious/endogenous contaminants
 - cell banks, vector banks, media/reagents
- Screening of process intermediates
 - bulk harvests, purified vector lots
- Incorporate virus removal/inactivation processes in downstream purification



Ensuring the safety of cell therapy products

- Screening of raw materials for adventitious/endogenous contaminants
 - cell banks, media/reagents
- Screening of process intermediates
 - manipulated and expanded cells
- Validation of cell processing steps and cleaning between production runs
- Lot release tests



QC of Autologous Cell Therapies

- Donor screening
 - HIV 1 & 2, HTLV I & II, HBV, HCV
- In process testing
 - sterility
 - mycoplasma
 - adventitious viruses: *in vitro* and *in vivo* assays
 - bovine, porcine or human viruses for media components
 - cell identification : cell markers, isoenzymes
 - cell number, viability, morphology
 - cell function



QC of Autologous Cell Therapies (2)

- Lot release testing
 - sterility
 - LAL
 - mycoplasma
 - adventitious viruses: *in vitro* assay
 - cell identification : cell markers, isoenzymes
 - cell number, viability, morphology
 - cell function



QC of Autologous Cell Therapies (3)

- Process validation
 - Cell manipulation steps validated for:
 - microbiological control
 - cell viability, growth and/or differentiation
 - cell identity and purity
 - cell activity or function
 - Cleaning validation for inactivation of bacteria, fungi and viruses



QC of Allogeneic Cell Therapies

- Donor screening
 - human retroviruses (HIV 1 & 2, HTLV I & II)
 - hepatitis B
 - hepatitis C
 - hepatitis A
 - cytomegalovirus
 - Epstein Barr virus

 - CJD and vCJD risk factors



QC of Allogeneic Cell Therapies (2)



QC of Allogeneic Cell Therapies (3)

- Lot release tests
 - tests for the absence of microbial and viral contaminants
 - sterility (14 days)
 - adventitious viruses, *in vitro* assay (14 days)
 - mycoplasma (28-35 days)
 - LAL assay
 - determination of cell viability, number and morphology
 - determination of potency



Bovine derived products

- CPMP Note for Guidance on use of Bovine Serum in the Manufacture of Human Biological Products. CPMP/BWP/1793/02 Draft 2002
- Testing for bovine viruses
 - bovine viral diarrhoea virus (BVDV)
 - antibodies to BVDV
 - bovine polyoma virus



Bovine viral diarrhoea virus

- Frequently present in foetal calf serum
- Inactivated by gamma-irradiation
- Vero, CHO and CEF cells are permissive for BVDV
- Human MRC-5 and WI-38 cells are not permissive for BVDV



Bovine Polyoma Virus

- Stable, non-enveloped, ds-circular DNA, oncogenic. Related to human pathogen, SV40
- No evidence of disease association
- Bovine sera: 40-60% Ab +ve
- Human sera (veterinarians): 70% Ab +ve
- FCS: PCR +ve, 3 studies: 60%, 70%, 90%
- PCR correlates with virus isolation (1 study)
- Concern for cell therapy products?
- Only use irradiated FCS.



Sample Tested	Number positive/number
Foetal bovine serum	64/73 (88%)
New-born calf serum	2/3
Bovine calf serum	0/3
Adult bovine serum	0/2
Horse serum	0/1
Cell lines	6/11



Replication of BPyV

- Westcott et al, 1987
 - Primary kidney cells from 2 week old calves cultured in HS, lamb serum and gamma-irradiated FBS: 18/64 were positive.
- Schuurman et al, 1991
 - Inoculated onto *Macaca* kidney cells
 - Observed CPE after 4-7 weeks culture in 5/5 sera +ve by PCR
- Growth in human cells?
- Is BPyV a safety concern for cell therapy products?



Mycoplasma Testing

ASSAY	% POSITIVE
Mycoplasma	
– Research	
• Cells (421/4412)	9.5
• Products (6/416)	1.4
– Regulatory	
• Cells (53/1483)	3.5
• Products (42/5921)	0.7



Regulations for *Mycoplasma* Detection

- European Pharmacopeia requirement is most stringent
 - “...use not more than 100 CFU per 60 mm plate containing 9 mL of solid medium and not more than 40 CFU per 100 mL container of the corresponding liquid medium:...”
- Direct culture
 - inoculation of broth and agar with sample
 - incubate replicate samples aerobically and anaerobically
 - examined macroscopically
 - total assay time 35 days
- Hoechst DNA staining
 - incubate with indicator cell line (Vero)
 - stain and look for *mycoplasma* DNA in cytoplasm



Mycoplasma Q-PCR assay

- Target the consensus 16S and 23S rRNA spacer region from 60 species of *Mycoplasma*
- Multiple primer and probes: 9 primers and 2 probes
- Single tube PCR using TaqMan® technology
- Why PCR?
 - Fast turnaround time
 - Long culture assay not suitable for products with short shelf life

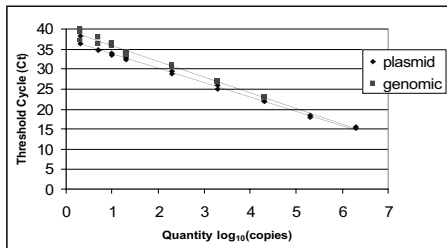


Mycoplasma Q-PCR Control Plasmids

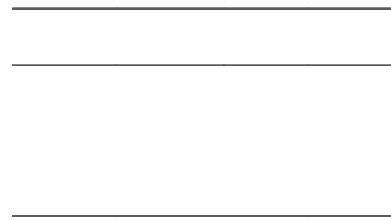
- Control plasmids from 10 species of *Mycoplasma* used are:
 - *A. laidlawii*
 - *M. fermentans*
 - *M. hyorhinis*
 - *M. pirum*
 - *M. salivarium*
 - M. arginini*
 - M. gallisepticum*
 - M. orale*
 - M. pneumoniae*
 - M. synoviae*



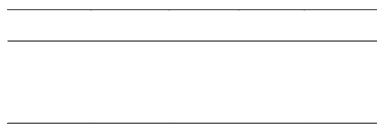
Standard Curve for *M. pneumoniae* - plasmid DNA Vs. genomic DNA



Mycoplasma Q-PCR Specificity



Comparison of *in vitro* assay and Q-PCR



*This titer is based on the presence of *mycoplasma* in Vero cells.



Conclusions

- What is the risk of contamination of human cell therapy products with BVDV and BPyV?
- Q-PCR assays for mycoplasma detection has broad specificity and is a sensitive and rapid method.
- Q-PCR assay can be used as a screening tool for samples that are not suitable for long term culture methods - such as cell therapy products
- Formal comparison of Q-PCR method with Pharmacopoeial methods to be completed.



Acknowledgements

- Wang-Ting Hsieh
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- Andrew Chadder

Gene therapy - general safety tests and vector specific safety issues

O.-W. Merten
Généthon, Evry/F



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Agenda:

- Introduction
- Cell bank
- RCV (RCR, LV-RCR, RCA, rcAAV)
- Quality control of clinical vector lots
- Supplementary material:
 - Raw materials
 - Bacterial cell bank
 - Plasmid stock
 - Virus seed stock/vector bank (e.g. AdV)

Starting materials - contaminants

- Cells (cell banks)
- Plasmids (bacteria - cell banks)
- Viruses/vectors
- Raw materials (medium, medium additives (e.g. serum), trypsin)
- Fungi, yeasts
- Bacteria
- Mycoplasma
- Adventitious viruses
- Replication competent viruses
- Prions
- Others (DNA, proteins, endotoxins, ...)



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Viral vectors - adventitious agents testing

- No **reliance on purification processes** - imperative to ensure freedom of starting materials from adventitious agents...
- Cell banks, master vector banks, biological additives,... have to be tested/screened:
 - mycoplasma, bacteria, fungi
 - broad screens to detect broad range of viruses
 - specific assays for viruses of particular concern

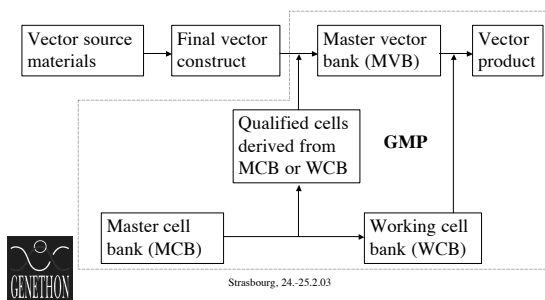
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Which starting materials are used for which vector production:

Vector	MLV	MLV, LV	AAV	AdV
Production system	Stable producer cell lines	Transient, transfection	Transient, transfection ± infection	Transient, infection
Other raw materials	+	+	+	+
Producer cell	+	+	+	+
Bacterial cell bank		+	+	
Plasmid		+	+	
Helper/deficient virus			(+)	+

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Seed lot system - lytic vector



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Cells - cell banks

Use of the cell bank concept:

- The master cell bank (MCB) is a collection of cells of uniform composition derived from a single tissue or cell
- The working cell bank (WCB) is derived from one or more ampoules from the MCB
- One of both (normally the MCB) has to be:
 - characterized as accurately as possible in terms of biological structure and functional activities
 - and certified to be free of endogenous and exogenous contaminating agents

Regulatory requirements for the characterization of cell lines (1):

1. Documentation of the history and genealogy of the cell line (tracability!)
2. Details of production and storage procedures used for the MCB and WCB
3. Data which establishes the identity of the cell line:
 - morphology
 - (karyology), constant chromosomal markers
 - isozyme analysis
 - DNA finger printing
 - analysis of immunological markers



Regulatory requirements for the characterization of cell lines (2):

4. Characteristics of the inserted gene:
 - complete nucleotide sequence of the gene of interest
 - detailed restriction endonuclease map, including coding sequence
 - copy number
5. Data which establishes the stability of the cell under the conditions used (i.e. product expression rates)
6. Growth characteristics of the cell - details of the culture system to be used

Regulatory requirements for the characterization of cell lines (3):

7. Testing for viruses:
 - Classical virology (in vitro, in vivo)
 - Tests for reverse transcriptase
 - Electron microscopic analysis
 - MAP, RAP, or HAP tests if appropriate
8. Absence of bacteria, fungi, and mycoplasma
- (9. Tumorigenicity testing)
- (10. Tests for the expression of oncogenes)
11. Specific tests concerning cells used for gene therapy purposes (e.g. RCRs)

Type	Name	Method	MCB	WCB	End production cells
Microbial	Sterility	Culture methods	+	+	+
	Mycoplasma	Cell culture only, indicate DNA detection test	+	+	+
	Mycobacterium spp.	Culture method	+		
Broad virus testing	In vitro assay for adventitious viruses	Co-culture with detector cells (MRC-5: Vero, a cell line of the same species/strain as the cell line to be tested)	+	-	+
	In vitro assay for adventitious viruses	Inoculation assay and linking mice, and embryonated eggs	+	-	+
	Electron microscopy		+	-	+
	Mouse antibody production test - 16 mouse viruses (*)	In vivo	+	-	-
Virus specific testing	Bovine virus test	Different tests	+	-	+ (if PCS is used)
	PPV test	In vitro assay using primary porcine kidney cells	+	-	+ (if syngeneic used)
	MVM	PCR	-	-	+
Retrovirus	RCRs	Felton 8-11 focus assay after amplification	+	-	+
	RCRs	Co-cultivation with cells, amplification, PCR cell envelope	+	+	+
Identity		Isotype analysis or DNA fingerprinting	+	+	+
Tumorigenicity		In Vitro	-	-	+

Safety testing of cell banks of a rec. MLV producer cell line (murine):

Remark, the need for testing for potential virus contaminants depends essentially on risk assessment.

(*) Mouse viruses detected in the MAP-test:
 Ectromelia v., Hantann v., K v., Lactic Dehydrogenase v., Lymphocytic Choriomeningitis v., MVM, mouse adenovirus, mouse cytomegalovirus, mouse encephalomyelitis v., mouse hepatitis v., mouse Rotavirus, Pneumonia v. of mice, Polyoma v., Reovirus 3, Sendai v., Thymic V.

Quality control of the producer cells of human origin (e.g. HEK293, Per.C6, used for AdV production):

- Additional tests in comparison to murine RVV producer cells:
 - Retrovirus test: F-PERT (Fluorescence-Product Enhanced Reverse Transcriptase) assay
 - Specific virus tests for viruses infecting human cells (test of MCB by PCR-methods):
HIV 1 & 2, HTLV 1 & 2, HBV, HCV, HHV-6, HHV-7, HHV-8, EBV, CMV, AAV, SIV, HPV, SV40, human and simian spuma virus
(It is not necessary to test for mouse specific viruses)

Cell banks - references to presentations of the EDQM Meeting

- M. Wisher - QC of cell banks, starting materials, final product and state of art testing methods - cell therapy

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Replication competent viruses (RCV):

- Generation by (overcoming of the defective functions):
 - **Recombination** (with related viruses (e.g. AdV), viral sequences harboured in the genome of the cell (e.g. RV))
 - **Complementation** (of any defective function inherent in the vector either by cellular or viral genes mobilisation of the vector, e.g. AdV, superinfection by wild type AdV, complementation of $\Delta E1a$ -AdV by the lymphotropic herpesvirus EBV)

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Replication competent retrovirus (RCR):

- MCB and WCB (if developed):
 - A) cells - 10^8 cells or 1% of cell bank (whichever is smaller)
 - B) supernatant - 5%
- Production cells:
 - cells - 10^8 cells or 1% of the viable cell mass
- Clinical grade vector:
 - supernatant - 5%

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Envelope of the potential RCRs? - choice of the amplification cell line.

- Envelope of the retroviral packaging vector to be produced potential RCRs have the same envelope
- In the case that the producer cells were produced via transduction using a retroviral vector containing an distinct envelope from the packaging vector RCRs containing this envelope might be produced

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Envelope of the potential RCRs? - choice of the amplification cell line.

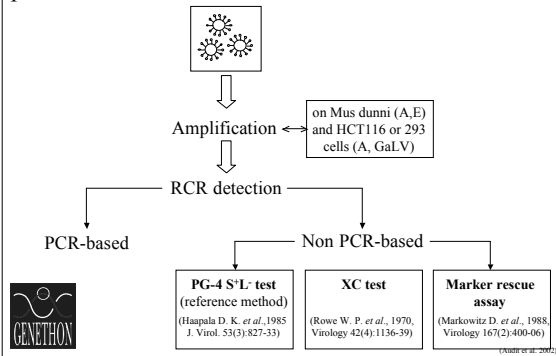
☐ **the MCB (only the MCB!) has to be tested for two different RCRs have to be performed.**

- Choice of the amplification cell line:
 - Mus dunni: (amphotropic), ecotropic RCRs
 - HEK293, HCT116: amphotropic, VSV-G, GaLV enveloped RCRs

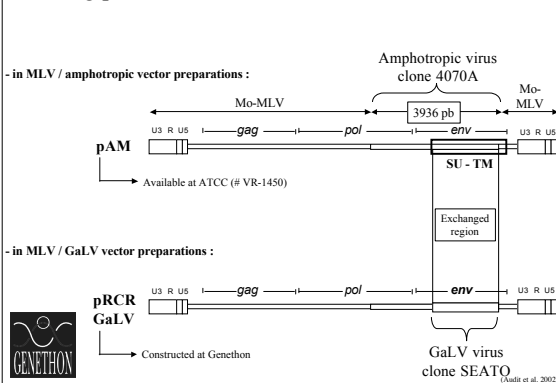


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RCR detection in MLV vector preparations/MLV producer cell lines:

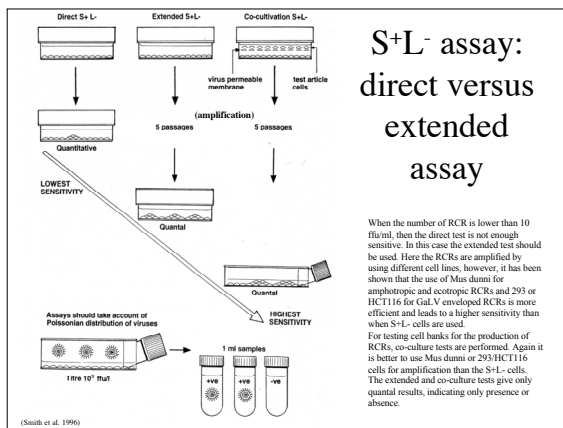


Existing positive controls for RCR detection



S⁺L⁻ assay:

- Choice of the amplification cell line:
 - Mus dunnii: (amphotropic), ecotropic RCRs
 - HEK293, HCT116: amphotropic, VSV-G, GaLV enveloped RCRs
- Detection cell lines (S⁺L⁻, non-transformed phenotype, harbouring a sarcoma virus genome, leukemia negative):
 - PG4 (feline): amphotropic, GaLV enveloped RCRs
 - SC-1/XC, D56 (murine): ecotropic RCRs

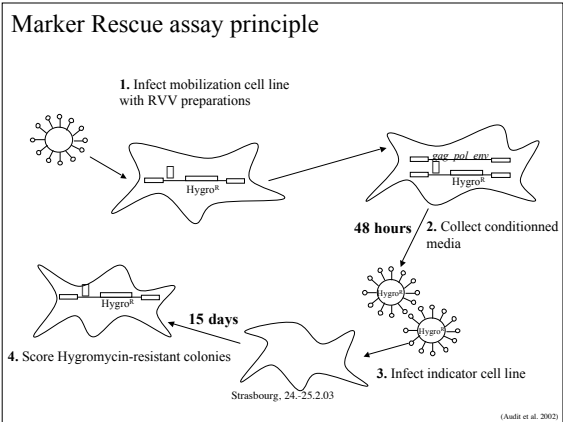


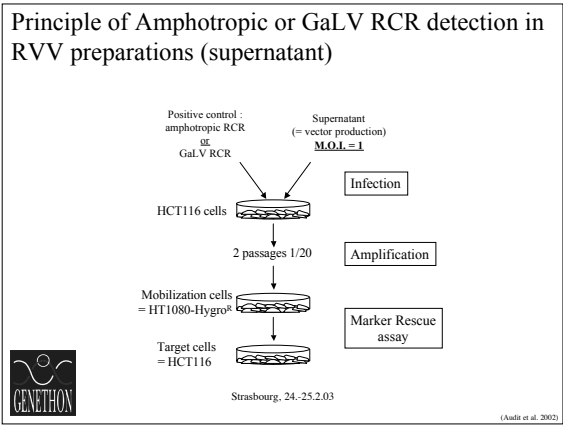
S⁺L⁻ assay:

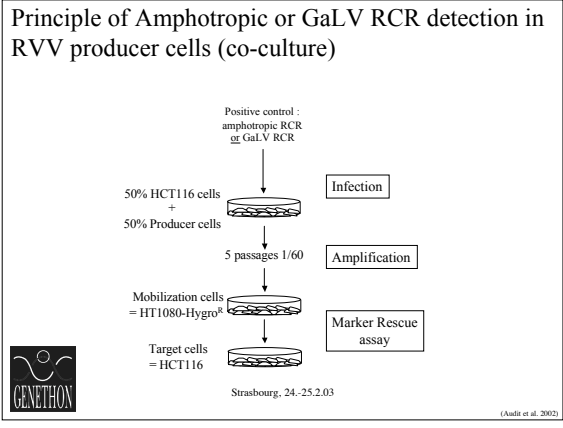
- Detection limits:
 - supernatant in an extended assay after amplification:
 - 1 RCR after an dilution of 10⁻⁶
 - producer cells in a co-culture experiment:
 - 1 RCR infected cell in 10⁶ un-infected cells (amplification on 293 cells)

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(GaLV-RCR, Chen et al. 2001)







Detection limits - comparison amphotropic - GaLV (RCR input):

Test article: supernatant

		Positive control	
		Amphotropic RCR	GaLV RCR
HCT116 cells number	5.8.10 ⁶ cells (6 wells plate)	1	1
	1.5.10 ⁶ cells (T25)	≤ 5	≤ 5
	3.8.10 ⁶ cells (T75)	≤ 5	≤ 5

Test article: producer cell line

		Positive control	
		Amphotropic RCR	GaLV RCR
Cells number	1.5.10 ⁶ HCT116 cells + 1.5.10 ⁶ producer cells (6 wells plate)	1	1
	3.8.10 ⁶ HCT116 cells + 3.8.10 ⁶ producer cells (T25)	≤ 5	≤ 5
	11.2.10 ⁶ HCT116 cells + 11.2.10 ⁶ producer cells (T75)	≤ 5	≤ 5

(Avallé et al., 2002)

Comparison Marker Rescue versus S⁺L⁻ assay:

- Marker Rescue Assay is about 100 times more sensitive than the S⁺L⁻ assay (Kim et al. 1998 for amphotropic RCRs)
- Marker Rescue Assay is faster and much easier to read than the S⁺L⁻ assay


Comparison of the S⁺L⁻ and the Marker rescue assay (Kim et al. 1998)

Dilution	Marker rescue (p24) assay	S ⁺ L ⁻ assay after 24h amplification
10 ⁷	+	+
10 ⁶	+	+
10 ⁵	+	+
10 ⁴	+	+
10 ³	+	+
10 ²	+	+
10 ¹	+	-
10 ⁰	-	-

(Note: Supernatant from a high titer RV PA317 based producer cell line)

RCR-testing:

- Products to be tested:
 - MCB, WCB
 - Clinical lot intermediates
 - Purified vector lots



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Methods for the detection of recombinants in retroviral vector (MLV) preparations:

Recombinant		Indicator (mobilisation) cell line *	Detection cell line *	Sensitivity **
RCR	Replication competent retrovirus	Vector +	Vector -, follow-up of transgene expression	1/10 ⁷
Replication defective recombinant	Env recombinant (ER)	Gag-pol +, vector +	Vector -, follow-up of transgene expression	100/10 ⁷
	Gag-pol recombinant (GPR)	Env +, vector +	Vector -, follow-up of transgene expression	100/10 ⁷

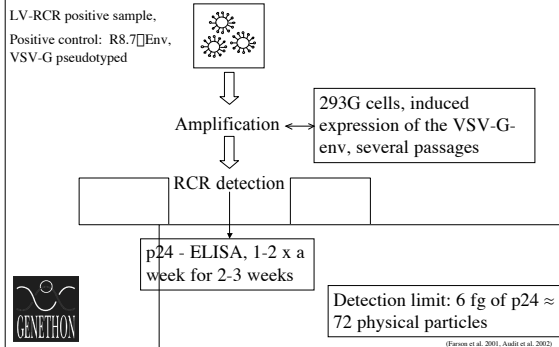
* has to be infectible by the env protein to be expected or used
 ** Cosset et al. J. Virol. 69 (1995), 7430-7436.

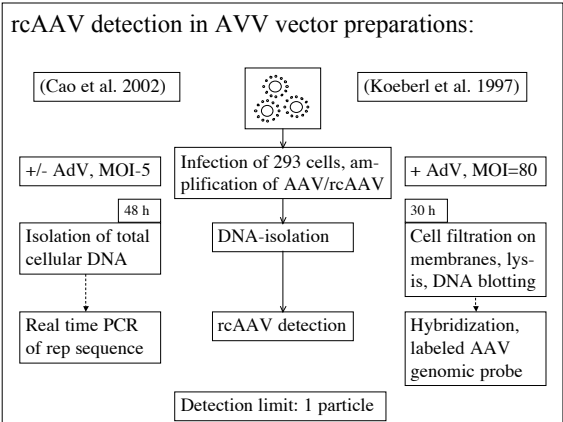
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Other RCV-tests

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Detection of replication-defective recombinants in LV vector preparations:





Summary: other RCV-tests:

RCV	Amplification cell line	Test principle	Sensitivity	Reference
LV-RCR	293G, induction of the expression of the VSV-G env protein	p24 - ELISA (absence of RCR: decrease of the p24 levels over several passages)	6 fg of p24 (= 72 pp)	Farson et al. 2001, Audit et al. 2002
RCA	A549, HeLa S3	Presence of RCA - appearance of a CPE	1-2 RCA/10 ¹² pp or 0.33 PFU/ml	Smith et al. 1996, Ma et al. 2002
rcAAV	293 +/- wildtype adenovirus	Real time PCR for rep, or DNA-hybridization using a labeled AAV genomic probe	1 rcAAV particle	Cao et al. 2002, Koeberl et al. 1997

RCV testing - references to presentations of the EDQM Meeting

- K. Mitrophanous - Need for reference materials for retroviruses and lentiviruses

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Quality control of a clinical lot:

- | | |
|---|---|
| <ul style="list-style-type: none"> - Sterility - Absence of mycoplasma - Absence of adventitious agents (in vitro, EM) - Endotoxin - Viral titration | <ul style="list-style-type: none"> - Identity (restriction, PCR) - Function (transgene expression) - Degradation of cellular DNA - Cellular Protein - pH - Particulates - Fill volume - Stability |
|---|---|



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Test	Bulk harvest	Clinical lot	Ex vivo transduced lot
Sterility	+ and/or	+	
Mycoplasma cultivation and fluorochrome	+		
Bioburden	+		
In vitro assay for adventitious viruses	+		
In vitro assay for adventitious viruses	(+)		
MVM PCR	+		
Vector titer	+		
Co-cultivation S'L with PG4 (ampho.)			+
Infection S'L with PG4 (ampho.)	+ OR	+	+
Co-cultivation cell line with 293 (if Gal.V)			+
Infection of 293 cells (if Gal.V)	+ OR	+	+
LAL test		+	
General safety		+	
Co-cultivation S'L with FC10			+

Overview of retroviral GMP product safety testing:

Residual protein!
Residual DNA!

Vector specific quality control tests for clinical lots:

- | | |
|--|--|
| MLV: | AdV: |
| - RCR-detection (e.g. S ⁺ /L ⁻ , mobilisation assay) | - RCA detection |
| | - absence of AAV |
| LV: | - Particles versus infectious units (reason potential toxicity of AdV-particles: 100:1, for phase 1 trials*) |
| - LV-RCR-detection | |
| AAV: | - absence of empty capsides |
| - Detection of AdV | |
| - rcAAV detection | |



(* in discussion to be reduced to 30:1)
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Quality control testing of rAdV:

	Test	Specification
Cell Lysate	Bacterial culture	Absence
	Fungal culture	Absence
	Mycoplasma culture	Absence
	Mycoplasma PCR	Absence
	Adventitious viruses in vitro	Absence (except AdV)
Purified Vector	Bacterial culture	Absence
	Fungal culture	Absence
	Endotoxin	< 5 EU/ml
	Identity	Positive
	Function	Positive
	Titer (iu)	> 10 ⁸ iu/ml
	Titer (vp)	> 10 ¹⁰ vp/ml
	Particle to iu ratio	< 30
	RCA	< 1 vp/5x10 ¹⁰ vp



Vector testing - references to presentations of the EDQM Meeting

- G. Sharpe - Manufacture & characterisation of adenoviral vectors for gene therapy clinical trials.
- D. Malarme - Stability of viral vectors for gene therapy
- B. Hutchins - Characterization & use of an adenovirus reference material

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Conclusions:

- To provide confidence in the safety of potential gene therapy protocols it is essential that all starting materials, cell banks, seed stocks as well as final products have to fulfill the quality requirements (GLP, GMP) with respect to
 - characterisation,
 - absence of contaminants
 - stability, etc.

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- Smith K.T. et al. (1996) Gene delivery systems for use in gene therapy: an overview of quality assurance and safety issues. Gene Ther. 3, 190-200.
- Stadler J. et al. (2003) Raise free purification process for supercoiled plasmid DNA to be used in gene therapy. J. Gene Med. Submitted.

Supplementary Material:

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Agenda:

- Introduction
- Cell bank
- RCV (RCR, LV-RCR, RCA, rcAAV)
- Quality control of clinical vector lots
- Supplementary material:
 - Raw materials
 - Bacterial cell bank
 - Plasmid stock
 - Virus seed stock/vector bank (e.g. AdV)

Raw material quality attributes:

Raw materials = materials which are directly or indirectly used in the process for the production of a biopharmaceutical.

Attributes	Tools to provide assurance
Identity	Testing, traceability, labels
Purity	Testing, Inspection of item(s), Vendor certificate of analysis
Suitability for intended use	Process validation, Vendor audit programme, Performance testing if needed
Traceability	Vendor audit programme, Vendor certification, Certificate of analysis, Contractual obligations under change control, Labelling, control



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Raw materials:

- ICH Topic Q 5 A: Quality of biotechnological products: viral safety evaluation of biotechnology products derived from cell lines of human or animal origin (4 March 1997)
- Lubiniecki A.S. and Shadle P.J. (1997) Raw material considerations. Dev. Biol. Standard 91, 65-72.
- CPMP. Note for guidance on the use of bovine serum in the manufacture of human biological medicinal products. (25 April 2002)
- Merten O.-W. (2002) Development of serum-free media for cell growth and production of viruses/viral vaccines - Safety issues of animal products used in serum-free media. Dev. Biol. 111, 233-257.
- Merten O.-W. (2002) Virus contaminations of cell cultures - a biotechnological view. Cytotechnology, in press.



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Raw materials - references to presentations of the EDQM Meeting

- E. Balbirnie - Cell Drugs™ - Quality challenges
- M. Wisher - QC of cell banks, starting materials, final product and state of art testing methods - cell therapy
- D. Galbraith - Virological and microbiological safety aspects of novel cell therapies and tissue engineered products.

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Bacterial seed stocks (1):

- Requirements: cell stock has to be checked for identity, safety, and quality:
- Purity: 1. Absence of bacterial and fungal contaminants
2. Absence of bacteriophages (for E. coli)
- Identity: 1. API identification system (use of miniaturised biochemical tests)
2. Random Amplified Polymorphic DNA - fingerprinting analysis..., PCR analysis

Bacterial seed stocks (2):

- Genetic stability:

1. Restriction enzyme digestion (detection of eventual rearrangements)
2. Gene copy number
3. Plasmid retention (number and percentage of bacteria retaining the antibiotic resistance gene)
4. Nucleotide sequencing of the plasmid
5. Determination of the molecular weight of the expressed gene (SDS-gel electrophoresis, Western blot)

- Viability testing of the bacterial cell banks

Bacterial seed stocks - references to presentations of the EDQM Meeting

- D. Faucher - Quality control of NV1FGF plasmid DNA for PAD gene therapy
- M. Schleef - Improvements in GMP plasmid-DNA production: vector topology and product safety

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Plasmids

- Plasmids possess DNA sequences necessary for
 - selection and replication in bacteria
 - eukaryotic promoters and enhancers
 - transcription termination/polyadenylation addition sequences for gene expression.



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Criteria for lot release of plasmids for phase 1 clinical studies:

- Potency
- General safety
- Sterility
- Purity
- Quantity
- Identity (DNA sequence of the inserted gene, restriction enzyme analysis)

(CBER. Points to consider on plasmid DNA vaccines for preventive infectious disease indications. December 1996)

GMP plasmid seed lot:

Endotoxin	LAL test <100 EU/mg
DNA homogeneity	>95% ccc, agarose gel
RNA+ssDNA contamination	Undetectable by agarose gel/HPLC
E.coli genomic DNA	<10 μ g/mg of plasmid DNA
Protein	<10 μ g/mg of plasmid DNA
Sterility	No colonies after 21 days of tryptose broth culture
Identity	Restriction digestion, coding sequence
Purity	Spectrophotometric scans
A _{260/280}	1.75 – 1.80
Potency	Transfection experiment



(J. Oac; Stadler et al. 2003)

(GMP) plasmid lots - references to presentations of the EDQM Meeting

- D. Faucher - Quality control of NV1FGF plasmid DNA for PAD gene therapy
- D.R. Thatcher - Stability of plasmid DNA vectors in gene therapy
- M. Schleef - Improvements in GMP plasmid-DNA production: vector topology and product safety

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Virus seed stock/vector bank (1):

- Details of establishment, production and storage procedures used for the seed stock
- The identity (integrity, stability) of the vector/virus has to be proven, by using:
 - Immunological markers (e.g. molecular analysis of capsid proteins or nucleic acids)
 - Phenotypic characterization (e.g. host range)
 - Sequence analysis, restriction enzyme mapping, or PCR



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Virus seed stock/vector bank (2):

- Testing for sterility, mycoplasma, and adventitious viruses, as for the vector producer cell line (e.g. HEK293)
 - Sterility
 - Absence of mycoplasma
 - Absence of adventitious viruses
- In addition for replication defective vectors:
 - Absence of replication competent viruses



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