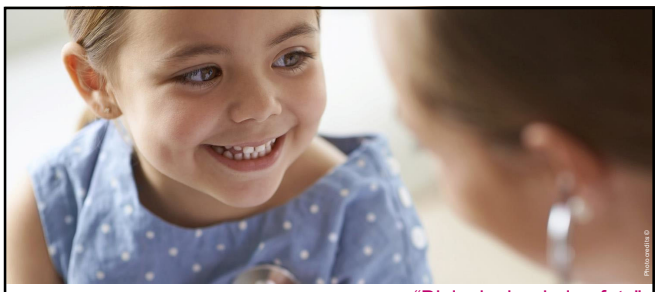


"Biologicals viral safety"

Stéphane Basmaciogullari
Cell Bank and Testing Development Head
Biosafety expert (Sanofi, Vitry sur Seine)

1

Organization 1/2

- **Introduction**
  - Bio production, Cell bank and Biosafety
- **I – What is a virus?**
  - I.1 – Discovery and definition
  - I.2 – Structure
  - I.3 – Replication
- **II – Pharmacopeia**
  - II.1 – Country specific pharmacopeia
  - II.2 – Harmonization
  - II.3 – Internal documentation (each pharmaceutical company)

2

2

Organization 2/2

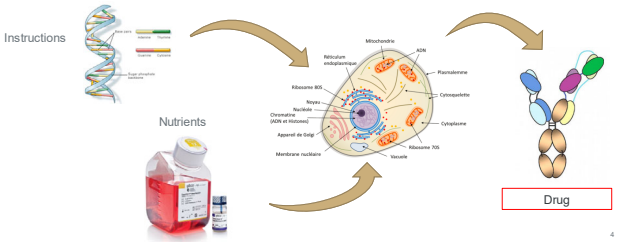
- **III – Viruses and industrial processes**
  - III.1 – Entry point
  - III.2 – Different kinds of consequences
  - III.3 – Strategy to ensure product quality / patient safety
- **IV – Viral testing**
  - IV.1 – How to detect
  - IV.2 – When to detect
  - IV.3 – Cost of testing

3

3

Introduction

Production of biopharmaceuticals :



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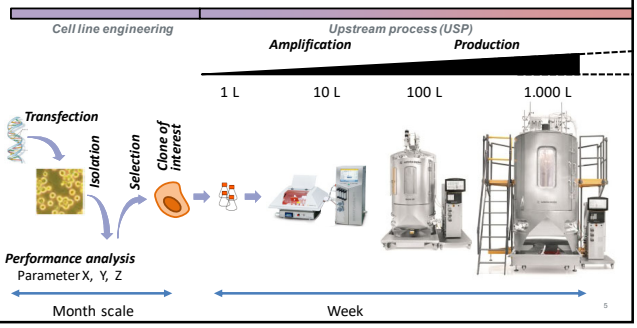
Introduction



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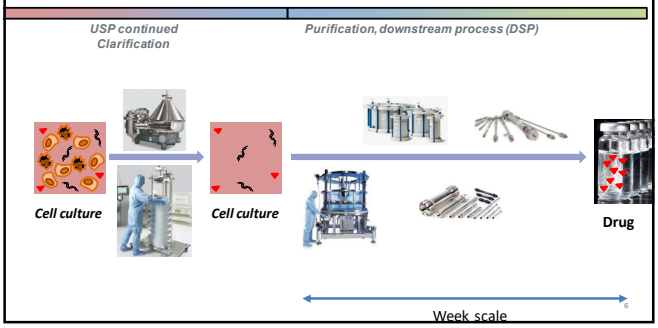

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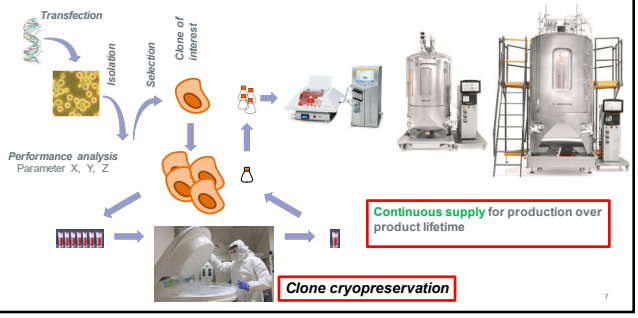
Introduction



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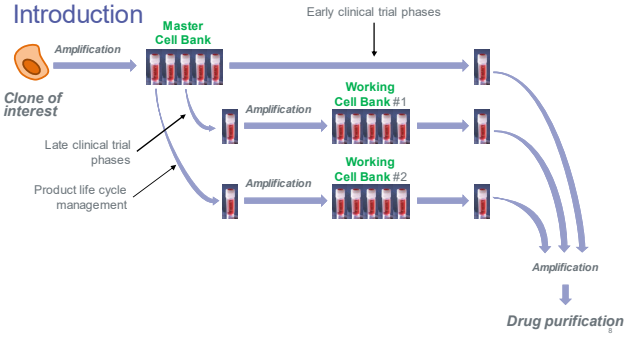
Introduction



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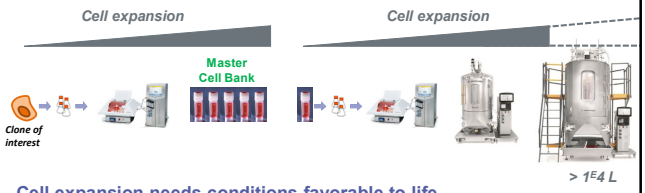
Introduction

Production of biopharmaceuticals :



Cell expansion needs conditions favorable to life …

… also the best conditions for the growth of adventitious agents

9

Introduction

Production of biopharmaceuticals : Cell banks

Continuous supply for production over product lifetime

The cryopreservation format is regulated (GMP, good manufacturing practices)
- MCB, WCB

- Clone must be **phenotypically and genetically characterized**
- Clone **stability** over time must documented
  - Gene copy number
  - Population doubling time
  - Productivity
- Bank **homogeneity** must be documented
- **Sample storage** is regulated
- **Biosafety** must be demonstrated
  - Sterility (bacteria, mycoplasma, funghi)
  - Adventitious viral agents
  - TSE/BSE risk

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Introduction

The best conditions for the growth of adventitious agents

Challenge:

Ensure that no contaminant enters / is present at any step of the manufacturing process : be able to

1/ Detect contaminants

2/ Avoid contaminant ingress during manufacturing

3/ Eliminate contaminants / Clearance capacity

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Introduction

The best conditions for the growth of adventitious agents

Challenge:

Ensure that no contaminant enters / is present at any step of the manufacturing process : be able to

1/ Detect contaminants

2/ Avoid contaminant ingress during manufacturing

3/ Eliminate contaminants / Clearance capacity

Focus:
Viral contaminants

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Part I:

What is a virus?

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I.1 – Discovery and definition

I.1.1 – Discovery: Tobacco mosaic virus

Adolf Meyer (1843-1942) :
Dmitri Ivanovski (1864-1920) :

-The ***agent*** can be transmitted and induce disease.
- The ***agent*** does not grow on medium like bactaria / fungi (1882).
-The ***agent*** goes through the Chamberland filter (1892).

An enzyme ? A toxin ? A small bacteria ?

A Meyer D Ivanovski

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I.1 – Discovery and definition

I.1.1 – Discovery: Tobacco mosaic virus

Martinus Willem Beijerinck (1851-1931) :

Independently from Meyer/Ivanovski:
- Similar conclusions + the ***agent*** only replicates when it is associated to some cellular metabolism (1898)
Neither a **bacteria, a fungus, a toxin**

contagium vivum fluidum, *virus*

MW Beijerinck

1. Crude extracts from diseased plants passing through porcelain filter candles do not show bacterial growth during three months of storage, but remain infective. Subsequent plant inoculation by injection readily leads to infection and reproduction of the characteristic symptoms.
2. Unlike bacteria, the infectious agent diffuses laterally into agar for at least 2 mm.
3. The agent multiplies in plants, as shown by serial transfers from plant to plant, and cannot be a toxin.
4. The agent multiplies only in actively growing tissues. It is not able to grow by itself but is carried away by the growth of dividing cells where multiplication in the living protoplasm is enormous.
5. Transport is 'through the phloem', upwards and downwards according to laws directing the movement of nutrients; in stems it is primarily vertical with little lateral spread.
6. The agent resembles living cells in that it is killed at 90 °C.
7. The agent may be dried in infected leaves (in a herbarium) and in filter paper soaked in infectious sap.
8. The agent may remain in dry soil during winter and infect plants from the soil; it can also be transferred in potting soil.
9. The agent retains infectivity after alcohol precipitation from sap and subsequent desiccation at 40 °C.

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I.1 – Discovery and definition

I.1.2 – Viruses in animals / bacteria

1898 foot-and-mouth disease (Bovine), Friedrich Loeffler (1852-1915), Paul Frosch (1860-1928)

1901 Yellow fever (Human), Carlos Finlay (1833-1915), Walter Reed (1860-1928) :
Reed demonstrates that the virus is transmistted through a mosquito vector: *Aedes aegypti*

1915 Bacteriophages (Bacteria), Félix d'Hérelle (1873-1949), Frederic Twort (1877-1950) :
Viruses capable of infecting bacteria ("phagotherapy" *Shigella sp. Rickettsia sp.*).

1931 Electron microscopy, Max Knoll (1887-1969), Ernst Ruska (1906-1988)

1938 First EM pictures of bacteria and viruses, Helmut Ruska (1908-1973)

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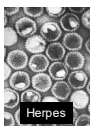
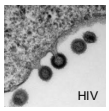
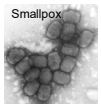
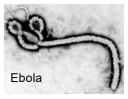
I.1 – Discovery and definition

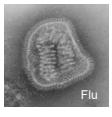
I.1.2 – Viruses in animals / bacteria

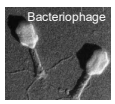












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I.1 – Discovery and definition

I.1.3 – Definition

Smaller than a bacteria*, induces diseases (transmitted)*
Cannot replicate on its own, a host and its metabolism is needed
Not a living thing

Must enter a cell and hijack its metabolism to replicate
Ends up killing the host cell*

* *Not always...*

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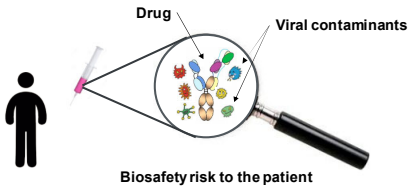
I.1 – Discovery and definition

I.1.4 – Challenge in the pharmaceutical industry

Viruses end up killing their host cells…



Biosafety risk to the patient

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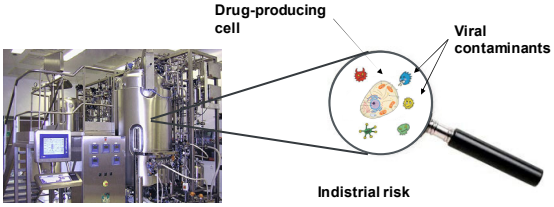
I.1 – Discovery and definition

I.1.4 – Challenge in the pharmaceutical industry

Viruses end up killing their host cells…



Indistrial risk

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I.2 – Structure

… and how it impacts:

1/ Detection

2/ Avoiding ingress during manufacturing

3/ Elimination/Clearance

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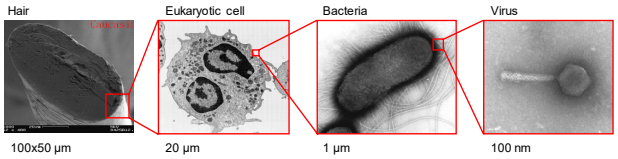
I.2 – Structure

I.2.1 – Size



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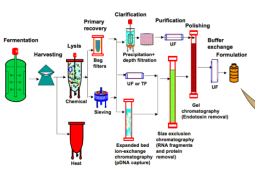
I.2 – Structure

I.2.1 – Size

Size matters !
-Invisible (light microscope)
-Not retained by filters used to capture cells

Raw material



Drug

23

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I.2 – Structure

I.2.1 – Size

Size matters !
-Invisible (light microscope)
-Not retained by filters used to capture cells

Raw material

Detection
Process clearance
capacity

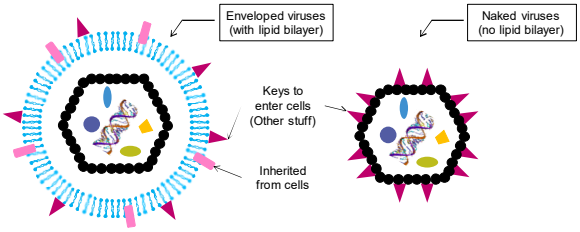
Drug

24

24

I.2 – Structure

I.2.2 – Envelope or not envelope



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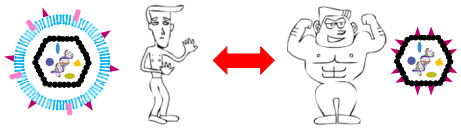
I.2 – Structure

I.2.2 – Envelope or not envelope



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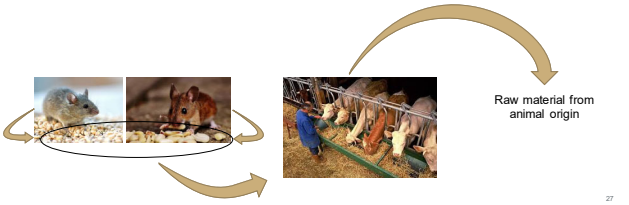
I.2 – Structure

I.2.2 – Envelope or not envelope

- Impacts resistance

Naked viruses can go through the digestive tract and retain infectivity

-Rota
-Noro (calici)
-Astro
-Adeno



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I.2 – Structure

I.2.2 – Envelope or not envelope

- Impacts resistance

Naked viruses can go through the digestive tract and retain infectivity

Transmission
Contamination routes

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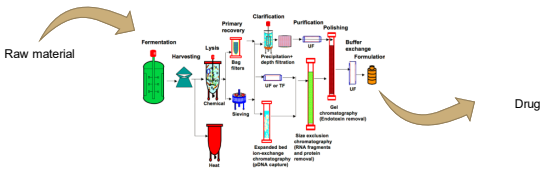
I.2 – Structure

I.2.2 – Envelope or not envelope

- Impacts resistance

Naked viruses resist better mechanical stress, extreme conditions (pH,high temperatures, dessication)



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I.2 – Structure

I.2.2 – Envelope or not envelope

- Impacts resistance

Naked viruses resist better mechanical stress, extreme conditions (pH,high temperatures, dessication)

Process clearance capacity

30

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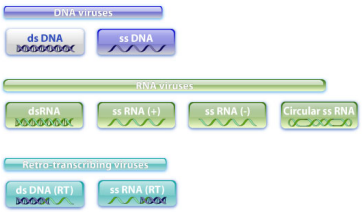
I.2 – Structure

I.2.3 – Genetic material

- DNA
- RNA
- Both



Always ends up in the cells
MAY end up integrated into the host's genome

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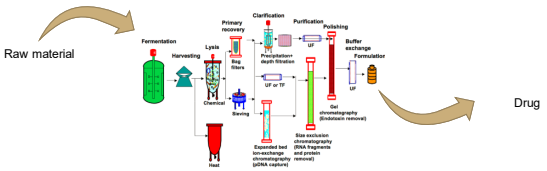
I.2 – Structure

I.2.3 – Genetic material

- Impacts detection

Looking for the viruse's genetic material



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I.2 – Structure

I.2.3 – Genetic material

- Impacts detection

Looking for the viruse's genetic material

Molecular biology tools to detect viruses

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I.3 – Replication

… and how it impacts:

1/ Detection

2/ Avoiding ingress during manufacturing

3/ Elimination/Clearance

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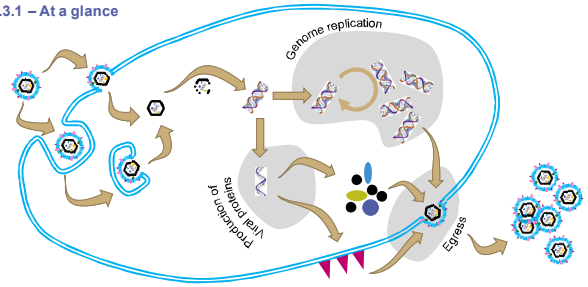
I.3 – Replication

I.3.1 – At a glance



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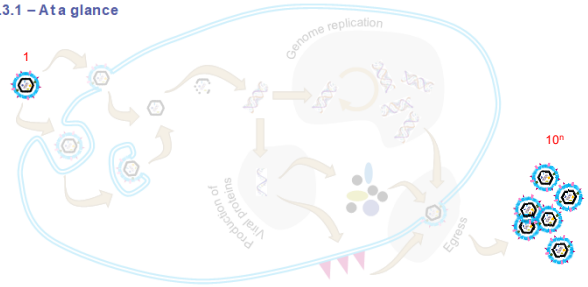
I.3 – Replication

I.3.1 – At a glance



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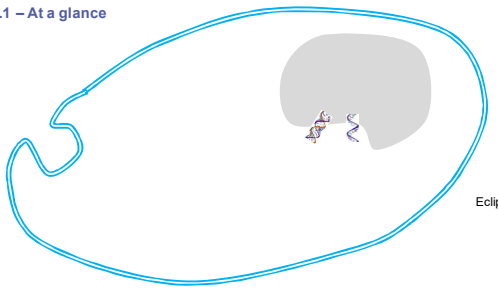
I.3 – Replication

I.3.1 – At a glance



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I.3 – Replication

I.3.1 – At a glance

Viral amplification in vitro helps detection

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I.3 – Replication

I.3.1 – At a glance

Elipse phase: no viral structures can be detected

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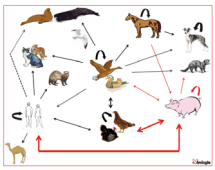
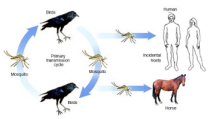
I.3 – Replication

I.3.2 – Host "specificity"

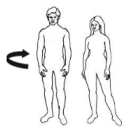
Flu



West Nile



Chickenpox



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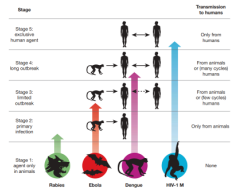
I.3 – Replication

I.3.2 – Host "specificity"



Origins of major human infectious diseases.
Wolfe ND, Dunavan CP, Diamond J.
Nature. 2007 May 17;447(7142):279-83. doi: 10.1038/nature05775.

Disease transmission from animals to human has happened many times in human kind history, long before politics even existed

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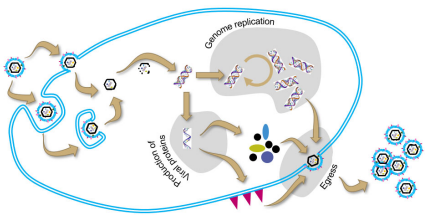
I.3 – Replication

I.3.2 – Host "specificity"



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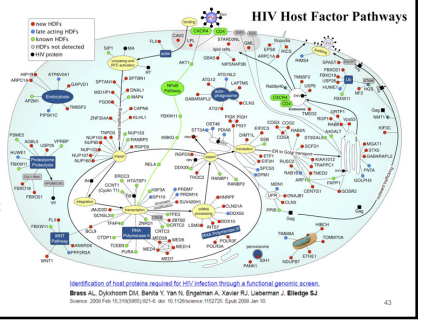
I.3 – Replication

I.3.2 – Host "specificity"

A virus can replicate in cells from two different types (tissue or species) if cofactors involved in its replication are present.

The requirement for specific cofactors participates to host specificity



Identification of host proteins required for HIV infection through a functional genomic screen.
Brass AL, Dykxhoorn DM, Benita Y, Yan N, Engelman A, Xavier RJ, Lieberman J, Elledge SJ.
Science. 2009 Feb 13;319(5865):921-6. doi: 10.1126/science.1152725. Epub 2009 Jan 10.

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I.3 – Replication

I.3.2 – Host "specificity"

Wisely chose the animal/cell model used to amplify (hence detect) viruses or the phenotype they induce

The requirement for specific cofactors participates to some host specificity

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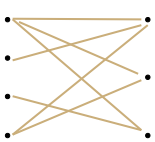
I.4 – Conclusion

Size

Genetic material

Structure (Resistance)

Species specificity



Detect

Prevent ingress

Clearance

50

50

I.4 – Conclusion

Basic research

Knowledge

Industry practices

If you know viruses, you know how to show they are not in your product. Or better said, you know how to show that despite the use of a sound approach you cannot detect them in your product.

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Part II :

Pharmacopeia

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II.1 – Country-specific pharmacopeia

II.1.1 – A set of rules to observe

Each country decides of a **set of rules (the pharmacopeia)** that drug manufacturers should comply with before a drug can be considered for clinical trial/marketing application.

The **marketing application** that is country specific **is reviewed by each country** and if it does not fulfil pharmacopeia expectations, the application is not accepted, the drug cannot be marketed.

Among many other things, the marketing application should demonstrate that **the drug is safe and free of adventitious agents**.

In Europe, the marketing application must be approved by Europe AND countries.

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II.1 – Country-specific pharmacopeia

II.1.2 – Examples

Agencies by countries

France : Agence nationale de sécurité du médicament et des produits de santé (ANSM)
UK: Medicines and Healthcare products Regulatory Agency (MHPRA)
Allemagne: Paul Ehrmich Institut (PEI) / The Federal Institute for Drugs and Medical Devices (Bundesinstitut für Arzneimittel und Medizinprodukte, BfArM)
Europe : European Medicine Agencies (EMA)
USA : Food and Drug Administration (FDA)
Japan : Pharmaceuticals and Medical Devices Agencies (PMDA)
Etc…

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II.2 – Harmonization

ICH harmonisation for better health

The **International Council for Harmonisation** of Technical Requirements for Pharmaceuticals for Human Use (ICH) is unique in **bringing together the regulatory authorities and pharmaceutical industry to discuss scientific and technical aspects of pharmaceuticals and develop ICH guidelines**. Since its inception in 1990, ICH has gradually evolved, to respond to increasingly global developments in the pharmaceutical sector and these **ICH guidelines are applied by a growing number of regulatory authorities**. ICH's mission is to achieve greater harmonisation worldwide to ensure that safe, effective and high quality medicines are developed, and registered and maintained in the most resource efficient manner whilst meeting high standards. Since its announcement of organisational changes in October 2015, ICH has grown as an organisation and now includes 19 Members and 35 Observers.

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II.2 – Harmonization

ICH harmonisation for better health

INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED TRIPARTITE GUIDELINE

VIRAL SAFETY EVALUATION OF BIOTECHNOLOGY PRODUCTS DERIVED FROM CELL LINES OF HUMAN OR ANIMAL ORIGIN
Q5A(R1)

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II.2 – Harmonization

The Pharma and ICH rules for biosafety are derived from what research came up with

Basic research
└Knowledge
└Industry practices

If you know viruses, you know how to show they are not in your product. Or better said, you know how to show that despite the use of a sound approach you cannot detect them in your product.

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II.3 – Internal documentation

In the pharma world (and industry generally speaking)

- **You must write down in procedures what you plan to do before you can start anything**
- **You must do what is written in procedures**
- **You must write down what you have actually done**

Inspections by agencies control all of that and any gap might delay production (something done that is not procedured, something procedured that is not done etc…)

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II.3 – Internal documentation

Regarding biosafety, each pharma has procedures that recapitulate expectations from international guidelines (country specific pharmacopeia, ICH etc…).

The procedure is the only document checked at the operational level.

It has to give clear instructions so that what is done is what agencies want to see in the clinical trials/marketing application.

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Part III :

Viruses and industrial processes

60

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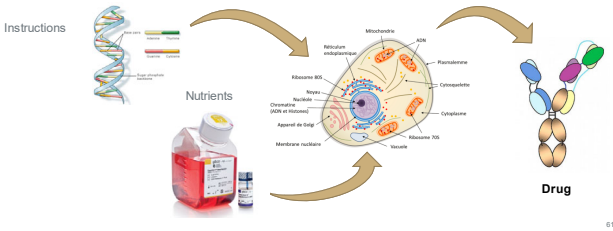
III.1 – Entry point



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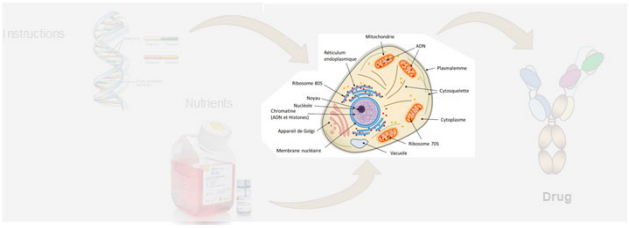
III.1 – Entry point

III.1.1 – The host cell



62

62

III.1 – Entry point

III.1.1 – The host cell

Characterisation of endogenous retrovirus in rodent cell lines used for production of biologicals.
Shepherd AJ, **Wilson** NJ, **Smith** KT.
Biologicals. 2003 Dec;31(4):251-60.

Table 1
Results of transmission electron microscopy (TEM), reverse transcriptase (RT) assay and infectious virus assays on 185 cell banks of rodent origin

	Cell type[1]	Total no. tested	TEM		RT	Infectivity assays	
			A/R type	C type		Mink S$^+$L$^-$	XC
Hybridomas	Souris NS0	40	35/35[2]	35/35	24/31	31/36	0/31
	Souris MH	76	63/63	63/63	38/65	31/68	2/71
	Souris-Humain H-MH	9	7/8	7/8	4/5	0/4	0/4
	Souris-Rat R-MH	3	2/2	2/2	2/2	1/3	0/3
	Souris-Mouton S-MH	1	1/1	1/1	1/1	0/1	0/1
	Rat BH	2	0/2	0/2	0/2	0/2	0/2
	Hamster CHO	44	12/34	31/34	9/34	0/39	0/8
	Hamster BHK	3	3/3	0/3	0/2	0/2	0/2

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III.1 – Entry point

III.1.2 – Raw material

64

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III.1 – Entry point

III.1.2 – Raw material

Massively parallel sequencing, a new method for detecting adventitious agents.
Onions D, **Kolman** J.
Biologicals. 2010 May;38(3):377-80. doi: 10.1016/j.biologicals.2010.01.003. Epub 2010 Mar 24.

Next Generation Sequencing : new **bovine parvovirus** identified in **bovine serum** used for cell culture

Viral nucleic acids in live-attenuated vaccines: detection of minority variants and an adventitious virus.
Victoria JG, Wang C, Jones MS, Jaing C, McLoughlin K, Gardner S, **Delwart** EL.
J Virol. 2010 Jun;84(12):6033-40. doi: 10.1128/JVI.02690-09. Epub 2010 Apr 7.

Next Generation Sequencing : detection of a **porcine parvovirus** in a **commercial vaccine** (conveyed by the use of porcine trypsin in cell culture)

65

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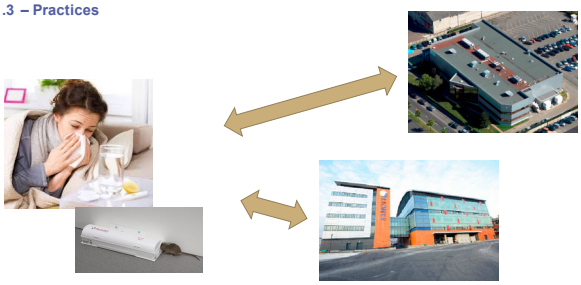
III.1 – Entry point

III.1.3 – Practices



66

III.2 – Different kinds of consequences

III.2.1 – Patient safety

PARALYTIC DISEASE ASSOCIATED WITH ORAL POLIO VACCINES.
HENDERSON DA, WITTE JJ, MORRIS L, **LANGMUIR** AD.
JAMA. 1964 Oct 5;190:41-8. No abstract available.

Vaccine against polio virus : **incomplete inactivation of some lots**

67

III.2 – Different kinds of consequences

III.2.1 – Patient safety

Viral nucleic acids in live-attenuated vaccines: detection of minority variants and an adventitious virus.
Victoria JG, Wang C, Jones MS, Jaing C, McLoughlin K, Gardner S, **Delwart** EL.
J Virol. 2010 Jun;84(12):6033-40. doi: 10.1128/JVI.02690-09. Epub 2010 Apr 7.

Identification and characterization of avian retroviruses in chicken embryo-derived yellow fever vaccines: investigation of transmission to vaccine recipients.
Hussain AI, Johnson JA, Da Silva Freire M, **Heneine W.**
J Virol. 2003 Jan;77(2):1105-11.

Porcine circovirus and avian retroviruses not pathogenic to humans
Breaching of the species barrier is possible

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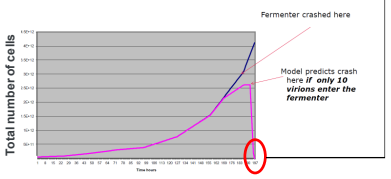
III.2 – Different kinds of consequences

III.2.2 – Industrial risk

David Onions, VSN conference talk, Gentilly, Sept 2017



No cells
⇨ No drug
⇨ No revenues

Delays to restart
⇨ Market share loss

Company fame

not always
All publicity is good publicity.

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III.2 – Different kinds of consequences

III.2.2 – Industrial risk

Identification and Quantitation of Vesivirus 2117 Particles in Bioreactor Fluids From Infected Chinese Hamster Ovary Cell Cultures

Yongchang Qiu,[1] Nathan Jones,[1] Michelle Busch,[1] Peng Pan,[1] Jesse Keegan,[1] Weichang Zhou,[1] Mark Plavsic,[2] Michael Hayes,[1] John M. McPherson,[1] Tim Edmunds,[1] Kate Zhang,[1] Robert J. Mattaliano[1]

[1]Biologics Development, Genzyme Corporation, 1 The Mountain Road, Framingham, Massachusetts 01701; telephone: 781-608-8206; fax: 978-263-9816; e-mail: qiuyongchang@yahoo.com
[2]Microbiological & Viral Safety, Genzyme Corporation, Framingham, Massachusetts

Identification and quantitation of Vesivirus 2117 particles in bioreactor fluids from infected Chinese hamster ovary cell cultures.
Qiu Y, **Jones N**, Busch M, Pan P, Keegan J, Zhou W, Plavsic M, Hayes M, McPherson JM, Edmunds T, Zhang K, Mattaliano RJ.
Biotechnol Bioeng. 2013 May;110(5):1342-53. doi: 10.1002/bit.24791. Epub 2012 Dec 25.

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III.2 – Different kinds of consequences

III.2.2 – Industrial risk

cell culture fluids and harvests or host cell lysates. In a recent viral contamination of bioreactors (Genzyme, 2009), the exact causative agent had remained unidentified after positive results in AAT assays were recorded. Since an initial random primer-based PCR approach failed to generate a clear lead hit, MS-based protein profiling was applied in an attempt to identify the potential viral proteins and hence the root cause. Here we report the successful identification and absolute quantitation of Vesivirus 2117 particles from the contaminated bioreactor fluid, and further discuss the advantages and pitfalls of MS-based protein sequencing, in comparison with other conventional tools.

Identification and quantitation of Vesivirus 2117 particles in bioreactor fluids from infected Chinese hamster ovary cell cultures.
Qiu Y, **Jones N**, Busch M, Pan P, Keegan J, Zhou W, Plavsic M, Hayes M, McPherson JM, Edmunds T, Zhang K, Mattaliano RJ.
Biotechnol Bioeng. 2013 May;110(5):1342-53. doi: 10.1002/bit.24791. Epub 2012 Dec 25.

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III.2 – Different kinds of consequences

III.2.2 – Industrial risk

Date	Open	High	Change	Change (%)	Low	High	Volume
Mar 06 2009	51.619998	53.170001	0.13	+0.25%	50.799999	53.170001	6,126,303
Mar 05 2009	53.049999	54.400001	1.82	+3.66%	52.479999	55.000003	6,870,412
Mar 04 2009	53.699999	52.479999	-4.04	-7.15%	50.050003	56.519996	14,659,041
Mar 02 2009	59.970001	56.519996	-4.41	-7.24%	56.109996	60.93	7,130,810
Feb 27 2009	63.584999	60.93	-3.7	-5.72%	60.519996	64.629997	5,637,515
Feb 26 2009	70	64.629997	-4.44	-6.43%	64.220001	70.470001	5,141,796
Feb 25 2009	70.059997	69.069999	-1.35	-1.92%	69.050003	70.459999	2,921,822
Feb 24 2009	69.410003	70.419998	1.01	+1.46%	69.410003	70.739997	3,045,269
Feb 23 2009	71.769996	69.410003	-2.16	-3%	69.240005	72.349998	3,631,269
Feb 20 2009	70.529998	71.559997	0.95	+1.35%	70.059997	72.010002	2,983,707
Feb 19 2009	71.279998	71.36	0.13	+0.18%	70.509994	71.769996	1,915,187

https://www.advfn.com/stock-market/NASDAQ/GENZ/stock-price

Genzyme Detects Virus Contamination of Bioreactor, Halts Production ...
www.biopharminternational.com/genzyme-detects-virus-contamin... ▾ Traduire cette page
17 juin 2009 - The virus strain, **Vesivirus** 2117, is known to interfere with the growth of Chinese hamster ovary (CHO) cells and is believed to have been introduced through a cell culture nutrient **Genzyme** confirmed that this virus was the cause of declines in cell productivity last year at its facilities in Allston and Geel, ...

Identification and quantitation of Vesivirus 2117 particles in ... - NCBI
https://www.ncbi.nlm.nih.gov/pubmed/23184768 ▾ Traduire cette page
de Y Qiu - 2013 - Cité 15 fois - Autres articles
25 déc. 2012 - (1)Biologics Development, **Genzyme** Corporation, 1 The Mountain Road, Framingham, MA 01701, USA. qiuyongchang@yahoo.com. The prevention of adventitious agent **contamination** is a top priority throughout the entire biopharmaceutical production process. For example, although viral **contamination** of ...

Genzyme Plant Shutdown Could Mean up to $300M in Lost Sales | GEN
https://www.genengnews.com/gen.../genzyme...up.../57506615 ▾ Traduire cette page
2 juil. 2009 - The company says that it decided to close the Allston facility following its discovery that a virus had **contaminated** a bioreactor. **Vesivirus** 2117 is not known to infect humans but slows the growth of cells that produce therapeutic proteins. The viral contaminant most likely came from a nutrient used in the ...

Virus stalls Genzyme plant : Article : Nature Biotechnology
https://www.nature.com/articles/nbt0809-681a ▾ Traduire cette page
de V Bethencourt - 2009 - Cité 32 fois - Autres articles
The company has announced that it will temporarily shut down the facility owing to a bioreactor **contamination** with **Vesivirus** 2117, which does not cause human infections, but impairs growth of the biologics-producing Chinese hamster ovary (CHO) cells. It reportedly originated from tainted nutrient medium and belongs to ...

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III.3 – Strategy to ensure product quality

III.3.1 – Selection of raw material

The absence of raw material from human / animal origin: low risk of introducing agents pathogenic to humans

- Serum from bovine origin
- Enzyme from porcine origin (Trypsin)

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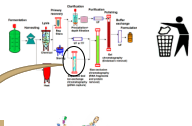
III.3 – Strategy to ensure product quality

III.3.2 – Demonstrate process clearance capacity

Should there be a virus, the system can remove/inactivate it and "none" is left in the drug

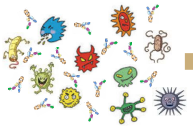


Input

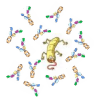
Step N-1 output

+

Virus added



Step N
All chemistry parameters determined



Output

Step N output

+

Residual virus

Reduction Factor = Log { Virus added / Residual virus }

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III.3 – Strategy to ensure product quality

III.3.2 – Demonstrate process clearance capacity

Virus quantification

qPCR => determination of viral genome copies

Molecular biology method: easy but no assurance that genomes detected belong to infectious viral particles

Coronavirus : des « traces infinitésimales » dans les eaux non potables de Paris

Des chercheurs du laboratoire attaché au service public de Paris ont détecté la présence de ce pathogène dans le réseau non potable de la ville grâce à l'amplification de son génome.

Par Martine Valo

Publié le 21 avril 2020 à 09h04 · Mis à jour le 21 avril 2020 à 12h41 · Lecture 3 min.

Le virus de la polio détecté dans les eaux usées à New York

Par Le Figaro avec AFP
Publié le 12/08/2022 à 19:04, mis à jour le 12/08/2022 à 19:56

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III.3 – Strategy to ensure product quality

III.3.2 – Demonstrate process clearance capacity

Virus quantification

Infectivity => determination of infectious viral particles

Cellular or animal mode required: more difficult, but detection confirms the presence of replication competent viruses

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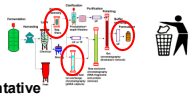
III.3 – Strategy to ensure product quality

III.3.2 – Demonstrate process clearance capacity

Several steps must be assessed



Steps are assessed at a small scale that must be representative

Affinity chromatography

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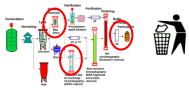
III.3 – Strategy to ensure product quality

III.3.2 – Demonstrate process clearance capacity



"Orthogonal" methods should be evaluated (methods that DO NOT rely on the same clearance mechanims

- Filtration/chromatography = removal
- pH/detergent treatment = inactivation

Several model viruses should be used with

- Big/small
- Enveloped /naked
- DNA/RNA genome

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III.3 – Strategy to ensure product quality

III.3.2 – Demonstrate process clearance capacity

Reduction factor documented for each selected step and each model virus

	Virus 1	Virus 2	Virus 3	…
Affinity chromatography	a1	a2	a3	…
Heat inactivation	b1	b2	b3	…
pH treetment	c1	c2	c3	…
Filtration	d1	d2	d3	…
	S1	S2	S3	…

Should there be X Virus 1 in the cell culture harvest
The amount of virus in the purified drug would be $X/10^{S1}$

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III.3 – Strategy to ensure product quality

III.3.2 – Demonstrate process clearance capacity

Adv Biochem Eng Biotechnol (2014) 139: 167–183
DOI: 10.1007/10_2013_213
© Springer-Verlag Berlin Heidelberg 2013
Published Online: 30 May 2013

Safety Assurance for Biologics Manufactured in Mammalian Cell Cultures: A Multitiered Strategy

Dayue Chen

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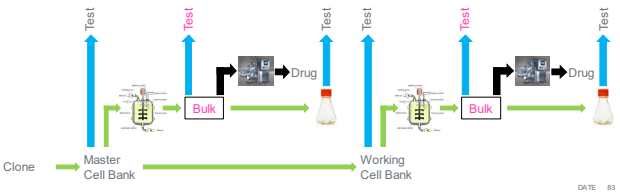
III.3 – Strategy to ensure product quality

III.3.3 – Document there is no virus (or show you cannot detect any)

Do testing at all steps of the manufacturing process (see part IV for details)



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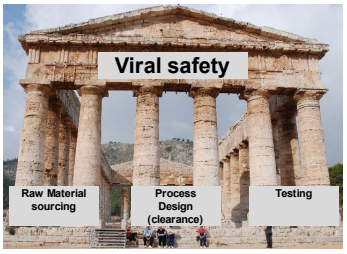
III.3 – Strategy to ensure product quality



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Part IV :

Viral testing

85

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IV – Viral testing

Challenge:

Ensure that no contaminant is present at any step of the manufacturing process : be able to

1/ Detect contaminants

2/ Avoid contaminant ingress during manufacturing

3/ Eliminate contaminants / Clearance capacity

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IV – Viral testing

Specific assay

Looking for a specific contaminant

The contaminant is detected AND identified

Less specific assay

Looking for a "familly" of contaminant through common characteristics

The kind of contaminant is detected but its identity is not knwon

Not specific

Looking for an effect of contaminants *in vitro* or *in vivo*

A contaminant is detected because of the outcome of the test, but we don't know what it is

Redundancy ensures nothing is missed (lowers the risk to miss a virus that would be present).

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IV.1 – How to detect

IV.1.1 - Specific

⇨ Detect the **genetic material of a virus by** PCR

-Lymphocytic Choriomeningitis Virus = murine virus
patient biosafety
-Minute Virus of Mice = murine virus
not a threat for humans, but can replicate in CHO cells
-Porcine Circovirus = porcine viruse
not a threat for humans, but found in Novartis virus

1/ Test article = cells to be tested
2/ DNA, RNAextraction (+/- RT)
3/ Readout = **qPCR**

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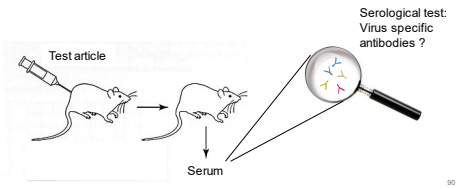
IV.1 – How to detect

IV.1.1 - Specific

⇨ Detect the **immunogenicity** of the test article



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IV.1 – How to detect

IV.1.1 - Specific

⇨ Detect the **immunogenicity** of the test article

-In mice
-In hamsters
-In rats

Mouse Antibody Production (MAP)
HAP
RAP

Ectromelia Virus; **Lymphocytic Choriomeningitis virus**, Hantaan Virus, Pneumonia Virus of Mice (PVM), Reovirus Type 3, Mouse Theilers Encephalomyelitis Virus, Lactic Dehydrogenase Virus, Sendai Virus, **Minute Virus of Mice (MVM)**, Mouse Adenovirus, Mouse Cytomegalovirus, Mouse Hepatitis Virus, Mouse Rotavirus, Polyoma Virus, Thymic Virus and K virus...

1/ Test article = lysed cells + medium
2a/ « per os » = maximizes the detection of enteric viruses
2b/ Nsal passage = maximizes the detection of respiratory viruses
2c/ Injection intraperitoneal = avoid epithelia
3/ Serum sampling at 28 days
4/ Readout = **ELISA** for specific viruses

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IV.1 – How to detect

IV.1.2 - Less specific

⇨ Detect **reverse transcription activity** > retroviridae (HIV, MLV)

Reverse transcriptase

ARN → ADN

RT assay

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IV.1 – How to detect

IV.1.2 - Less specific

1/ Test article = Cells and cell culture supernatant
2/ Inoculation on amplification cells (Mus dunni) + sample supernatant all along the incubation
3/ Ultracentrifugation, protein extraction : sample + matrice (ADN) + oligo + dNTPs
4/ Readout = **RT PCR**

93

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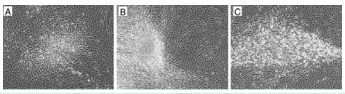
IV.1 – How to detect

IV.1.2 - Less specific

⇨ Detection of **infectious retroviruses** > retroviridae
Infectivity assay



1/ Test article = cells
2/ Inoculation of amplification cells (Mus dunni) + sample supernatant all along the incubation
3/ supernatant + indicator cells (shelter a transforming but defective virus)
4/ Readout = **presence of foci** (transforming virus complementation by the replication competent retrovirus = the transforming phenotype is visible)

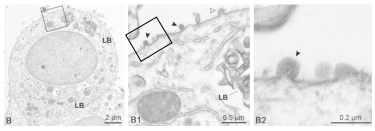
94

94

IV.1 – How to detect

IV.1.2 - Less specific

⇨ Detect **viral structures** by electron microsopy



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IV.1 – How to detect

IV.1.2 - Less specific

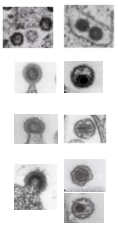
Retrovirus morphological classification

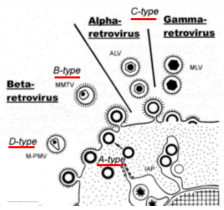
Type A. Round nucleocapsid, intracytoplasmic or vesicular = immature forms of B and T types

Type B. Round and complete nucleocapsid at the plasma membrane, not centered in mature viral particles outside of the cell

Type D. Round and complete nucleocapsid at the plasma membrane, centered, cylindrical/conical in mature viral particles outside of the cell

Type C. Partial nucleocapsid at the plasma membrane, centered, cylindrical/conical in mature viral particles outside of the cell





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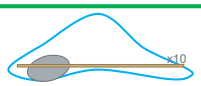
IV.1 – How to detect

IV.1.2 - Less specific

1/ Test article = cells
2/ Cells are pelleted and fixed
3/ Thin sections are made (~50nm)
4/ Readout = **TEM**, 200 cellules



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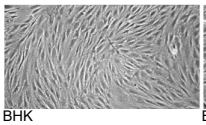
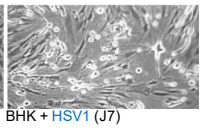
IV.1 – How to detect

IV.1.3 - Not specific

⇨ Detect **cytopathic effect** in vitro
in vitro adventitious agent detection


BHK


BHK + HSV1 (J7)

All tests on BHK ?

Can all viruses replicate in BHK ?

100

100

IV.1 – How to detect

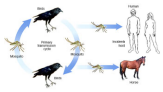
IV.1.3 - Not specific

⇨ Detect **cytopathic effect** in vitro

in vitro adventitious agent detection

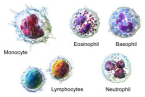
Influenza

West Nile



Chickenpox

HIV



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101

IV.1 – How to detect

IV.1.3 - Not specific

⇨ Detect **cytopathic effect** in vitro on **chosen indicator cells**

-Human cells
  Humans will receive the drug
-Non-human primate cells
  In case a human pathogen would not replicate in human cells
-Producer cells
  Maximize the chance to detect what was in there
-Other (case by case)
  Bovine, Porcine cells

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IV.1 – How to detect

IV.1.3 - Not specific

1/ Test article = cells
2/ Cells are lyzed and clarified by centrifugation
3/ The lysate is incubated with indicator cells + sample supernatant all along the incubation
4/ Readout = **cytopathic effect detection**

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IV.1 – How to detect

IV.1.3 - Not specific

⇨ Detect **pathogenic activity in vivo**

In vivo adventitious agent detection

Same as in vitro: the receiving organism must be selected based on how the product is made

-Eggs
-Mice (adult/suckling)
-Hamster
-Rabbits
-…

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IV.1 – How to detect

IV.1.3 - Not specific

1/ Test article = lyzed cells
2a/ per os injection
2b/ intracraniale injection
2c/ nasal route
2d/ intramuscle injection

(+ ctrl animals ~65 animaux/test)

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IV.1 – How to detect

IV.1.3 - Not specific

3/ animals are kept 14d – 21d
4/ Readout = **animal overal health**

« Pass » if 80% survive in good health

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IV.1 – How to detect

IV.1.3 - Not specific

IN VIVO ASAYS ARE UNETHICAL:

DUE TO THE ADVENT OF NEW TECHNOLOGIES, IN VIVO ASSAYS SHOUD BE ABANDONED AND REPLACED BY MORE SENSITIVE/SPECIFIC ASSAYS

MASS PARALLEL SEQUENCING IS ONE OPTION (already in place in the vaccine world, in the process of beeing accepted for biologiclas, ICH Q5A revision ongoing)

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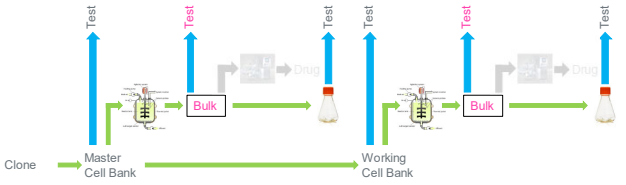
IV.2 – When to detect



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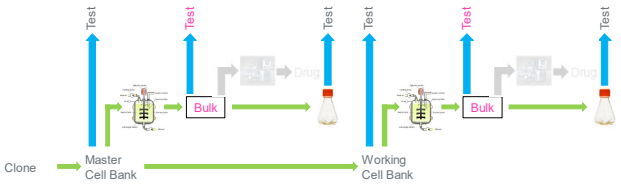
IV.3 – Cost of testing

n x 100,000 € per program



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Summary

Expectations in terms of biosafety are described in pharmacopeia

Methods are derived from what science is teaching us and testing should be sound (redundant, relevant to the product).

- Molecular biology / Immunological methods
- In vitro / in vivo testing

The rules are translated into procedures so that there is no ambiguity in what should be done at the operational level. It is expensive: do not do more, but do not do less than required either!

Biosafety is documented in the clinical trials/marketing application, and evaluated by agencies (each country). If it is not satisfactory, clinical trials cannot start/marketing is not authorized.

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