Considerations for risk group assessment of recombinant organisms

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Recombinants

- Recombinants: microorganisms that contain recombinant DNA
- Recombinant DNA: DNA constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell (NIH definition)
- Require special consideration with respect to risk factors used in risk assessment
- Viral gene transfer vector, bacterial strain harboring recombinant plasmid, etc.

Biological Agent – Risk Group Classification

- Microorganisms are classified into 4 Risk Groups
- Risk Groups 1 to 4 represent organisms that are increasingly hazardous

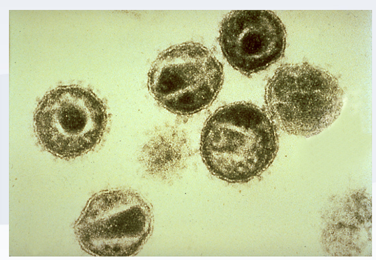
RG 1

S. cerevisiae

RG 2

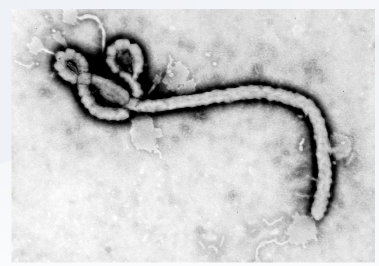
Salmonella spp.

RG 3



HIV

RG 4

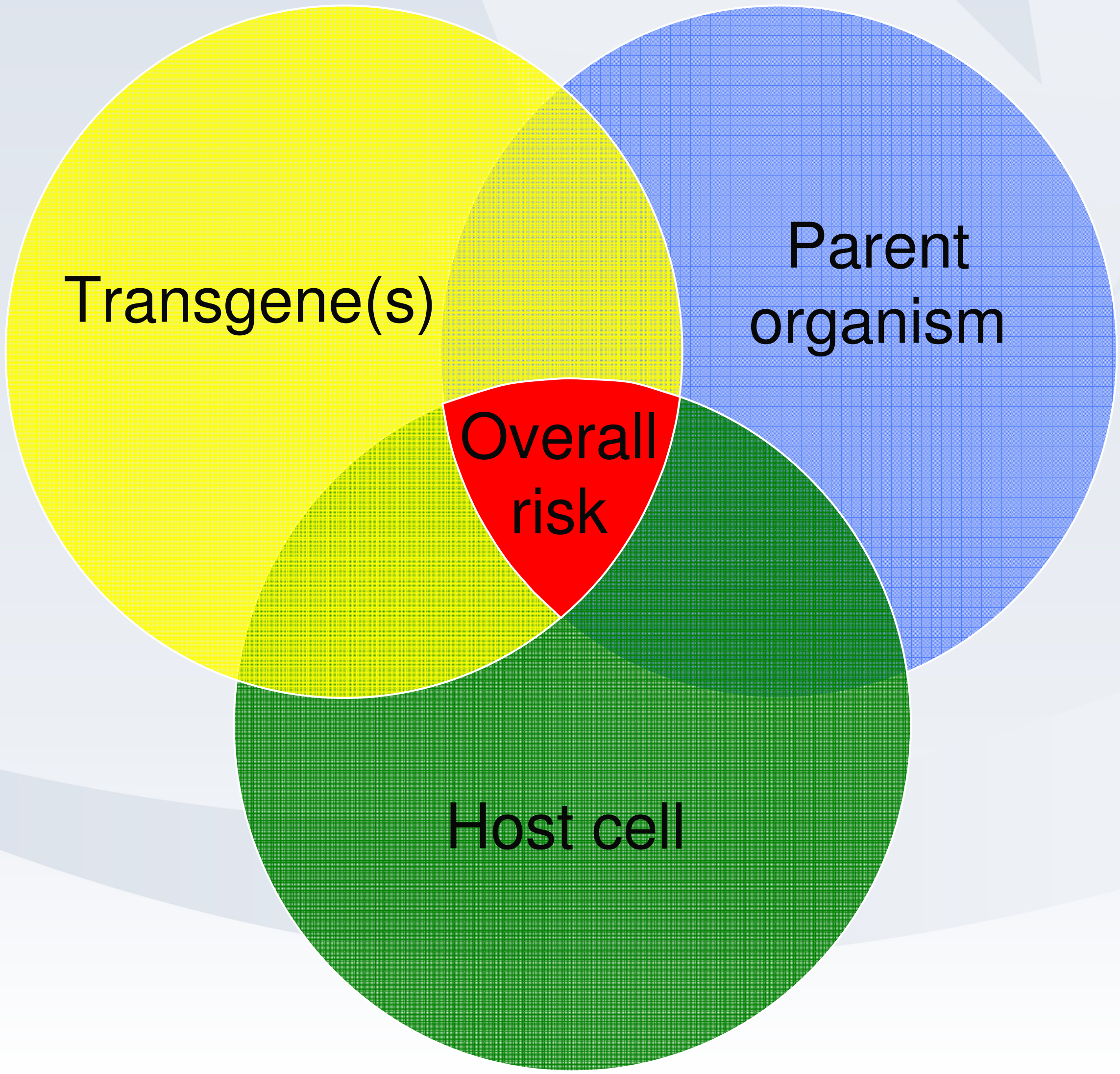


Ebola virus

Risk factors for Risk Group Assessment

- Pathogenicity and virulence
- Infectious dose
- Mode of transmission
- Ability to spread / communicability
- Survival in the environment
- Host range
- Endemicity
- Economic and public health impacts
- Availability of prophylaxis and therapeutics
- Vectors (vector-borne diseases)

Recombinants Risk Assessment



Transgene considerations

- Encodes a known or suspected virulence factor?
  - Toxin, capsule, etc.
- Potential to alter the host range or cell tropism of the virus?
  - VSV-G pesudotyped lentiviral vectors
- Potential to increase or decrease the replication capacity of the virus?

Transgene considerations

- Encodes a known oncogene?
- Potential for altering the cell cycle?
- If the modification has resulted in a form of attenuation, how extensively has this strain been utilized without incident and/or has the attenuation been proven in animal models?
- Does the modification have an effect of increasing or decreasing the efficacy of available treatment or prophylaxis?
  - Antibiotic resistance gene

Parent Organism considerations

- What is the risk group of the parent organism?
- Does the viral DNA integrate into the host genome?
- Essential genes have been deleted?
- What is the probability of generating replication-competent viruses (RCV)?
  - Number of recombination events necessary to generate RCV?
  - How easy to detect RCV? Any test available?

Host consideration

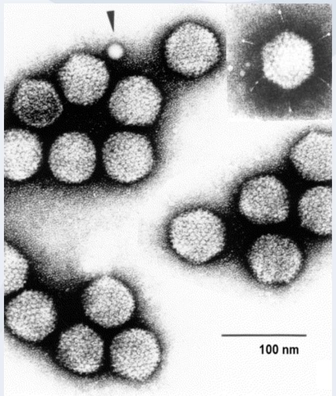
- What is the probability of generating replication-competent viruses?
  - Presence of cryptic retroviruses?
  - Probability of creating replication competent viruses through homologous recombination?
- Is the host permissive to recombinant replication?
  - If host is a non-permissive transgenic animal, does it have cells that could support recombinant replication?

Viral vectors

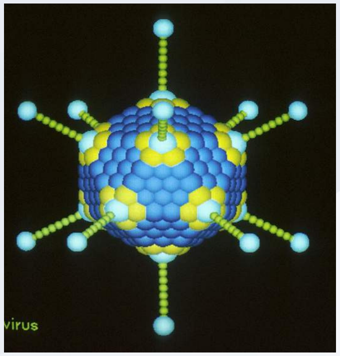
- Modified viruses engineered to express a foreign gene

- Many different types of viral vectors available depending on application
  - Adenovirus, AAV, retrovirus, lentivirus, poxvirus, herpesvirus, baculovirus, etc.

Adenovirus vectors



- Widely used for gene therapy applications
- Can infect wide range of cells including non-dividing cells
- Do not usually integrate into host genome



- Replication-deficient viruses are produced from two components: viral DNA vector and packaging cell line (e.g. 293 cells)
- Virus can be spread through droplets and fecal-oral route
- May persists for days to weeks on environmental surfaces

Adeno-Associated virus (AAV) vectors

- Wild-type AAV is not associated with any human disease
- Unable to replicate on its own. Requires helper virus (adenovirus or herpesvirus) co-infection to replicate
- Integrates into chromosome 19 of host genome
- Respiratory and gastrointestinal route of infection

Retrovirus/Lentivirus vectors

- Can infect a broad range of cells
- Specificity can be altered by replacing *env* gene (e.g. VSV-g)
- Provirus DNA integrates into host genome
- Some retroviruses can only infect dividing cells. Lentivirus can infect both dividing and non-dividing cells
- Can supply *gag*, *pol* and *env* in trans (e.g. from a packaging cell line or plasmid) to obtain replication defective viruses
- Can be spread by parenteral route. Use of needles and sharps should be minimized
- Enveloped virus. Not likely to survive for long period on environmental surfaces

Retrovirus/Lentivirus vectors

Features to improve safety:

- Deletion of viral pathogenicity genes (*vpr, vpu, vif, nef, tat,* etc.)
- Transient production systems
- Deletion of LTR’s and packaging site

Retrovirus/Lentivirus vectors

Concerns:

- Replication competent viruses
- Insertional mutagenesis
- Rescue by other human pathogenic viruses (e.g. reactivation of cryptic virus in host)

Poxvirus vectors

- Replicates in cytoplasm
- Can obtain high level of transgene expression
- Not designed to be replication defective
- Vaccination may be recommended for personnel working with recombinant poxviruses
- Quite stable at room temperature on environmental surfaces

Herpesvirus vectors

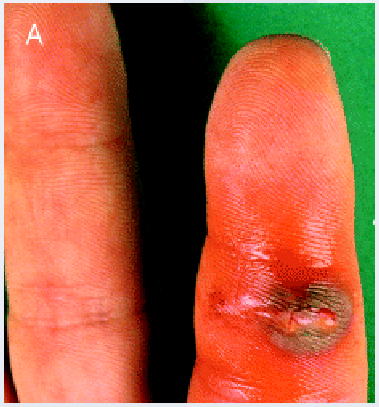
- Replicates in the nucleus and can establish latent infection but does not integrate in the host genome
- Can be used for transgene expression in neuronal cells
- Can be spread through direct contact
- Latency poorly understood. Exposure to recombinant could reactivate latent infection in host
- Enveloped virus. Not likely to survive for extended period on environmental surfaces

Baculovirus vectors

- Lytic viruses pathogenic to arthropods only
- Unable to replicate in mammalian cells
- Can however deliver genes to mammalian cells (requires high M.O.I.)

Increased virulence of recombinants: some real life examples

- Modified Vaccinia virus infection in a vaccinated lab worker. Mempel *et al.*, *J. Invest. Dermatol.* 2003
- Change of 11 a.a. in a complement-inactivating protein of vaccinia creates the smallpox protein sequence. Rosengard *et al.*, *PNAS* 2002
- Expression of IL-4 by a recombinant mousepox results in fatal infection in normally resistant mice. Jackson *et al., J. virol.* 2001
- Disruption of one operon creates hypervirulent *M. tuberculosis* that rapidly kills mice. Shimono *et al., PNAS* 2003



Mempel et al., J. Invest Dermatol 2003

Challenges

- Risk assessment often incomplete because information may be unavailable for newly engineered agents and their transgene
  - Chimeric viruses
  - Transgene of unknown function
- Probability of generating replication competent viruses may be difficult to estimate

Resources

- **Office of Laboratory Security**

  www.phac-aspc.gc.ca/ols-bsl/index.html

  Phone: (613) 957-1779
- **Office of Biohazard Containment and Safety**

  www.inspection.gc.ca/english/sci/bio/bioe.shtml
- Biological Safety Principles and Practices, 4th Edition. 2006 ASM Press