Biosafety & Biosecurity

Dr. Nick Coleman

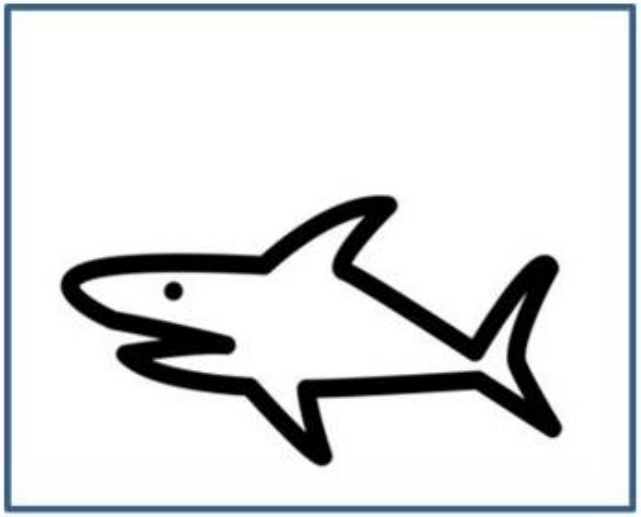
Learning objectives

Be able to :

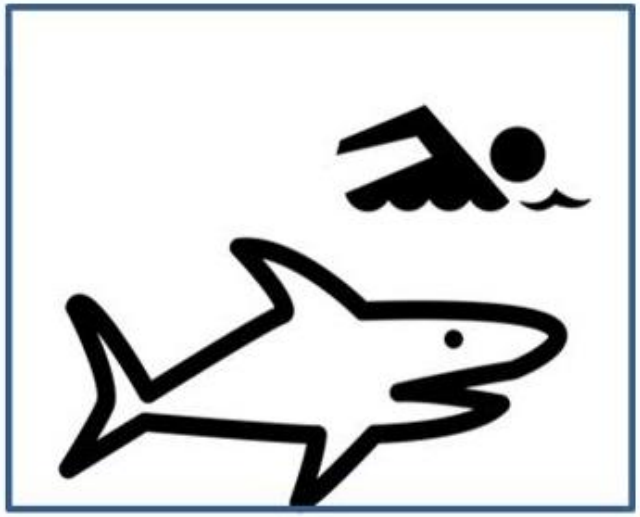
- Define ‘hazard’, ‘risk’, ‘biosafety’ and ‘biosecurity’
- Define the risk groups of microbes (GRAS, PC1, PC2, PC3, PC4, SSBA) and give an example of each group
- Define the safety features and behaviours appropriate for working in PC1 vs. PC2 labs
- Explain the ‘dual use’ problem as it applies to microbiology & discuss this problem using examples
- Explain how synthetic biology poses new risks for biosafety and biosecurity, and new dual-use dilemmas
- Define ‘gain of function’ experiments, and explain the possible risks and benefits of these, using examples

Risk assessment concepts

- **Hazard: intrinsic potential of something to cause harm**
- **Risk: *how likely* is the hazard to cause harm?**
  (context, exposure level / frequency, individual factors)
- **Risk assessment: "Do the benefits justify the risks?"**



HAZARD

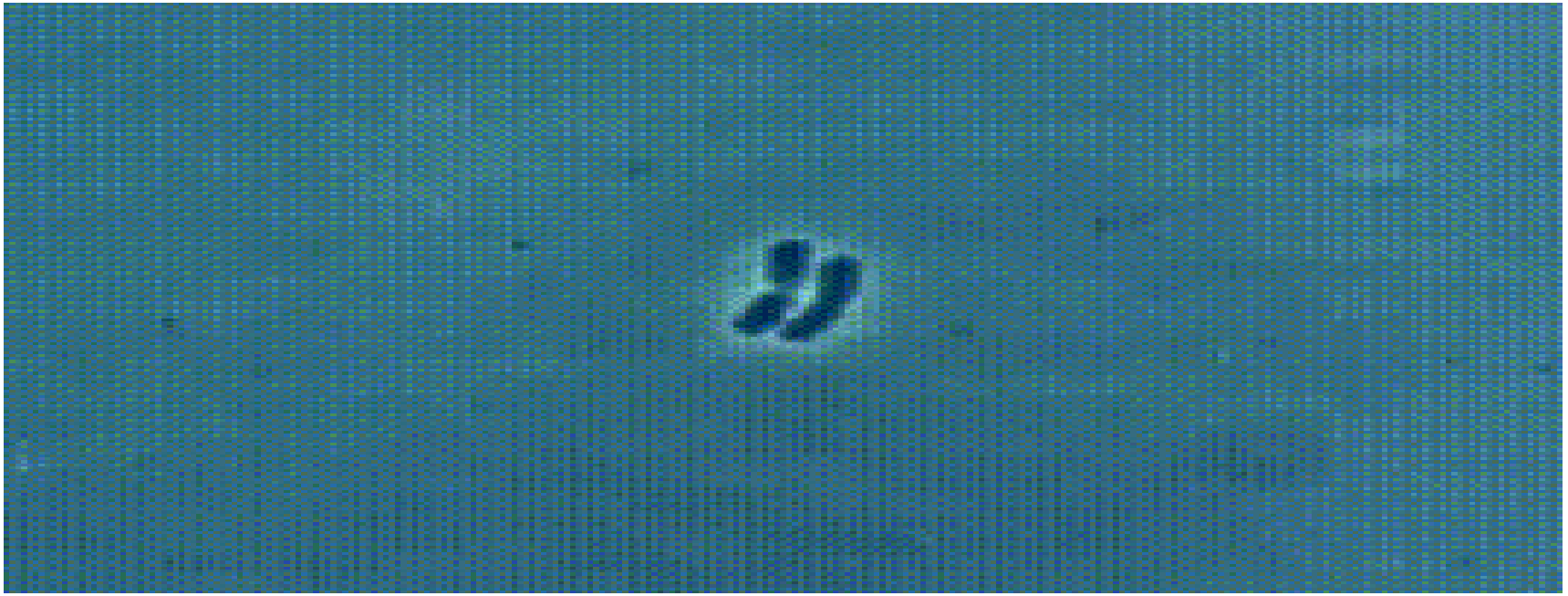


RISK

What makes microbes a unique hazard?

… compared to e.g. toxic chemicals or radiation

- **Ubiquitous:** hard to tell normal flora from threats
- **Potent:** tiny amounts can have huge impact
- **Detection** is tricky: e.g. ‘viable non-culturable’ cells
- **Self-replicating:** small numbers quickly become large



Biohazards and Biosafety

Biohazard: biological agent which can harm humans, animals, or plants; includes pathogens & toxins

Biosafety: materials & methods that prevent unintentional exposure to biohazards (pathogens / toxins)

Biohazards Symbol: Development of a Biological Hazards Warning Signal

SCIENCE, VOL. 158
7 July 1967

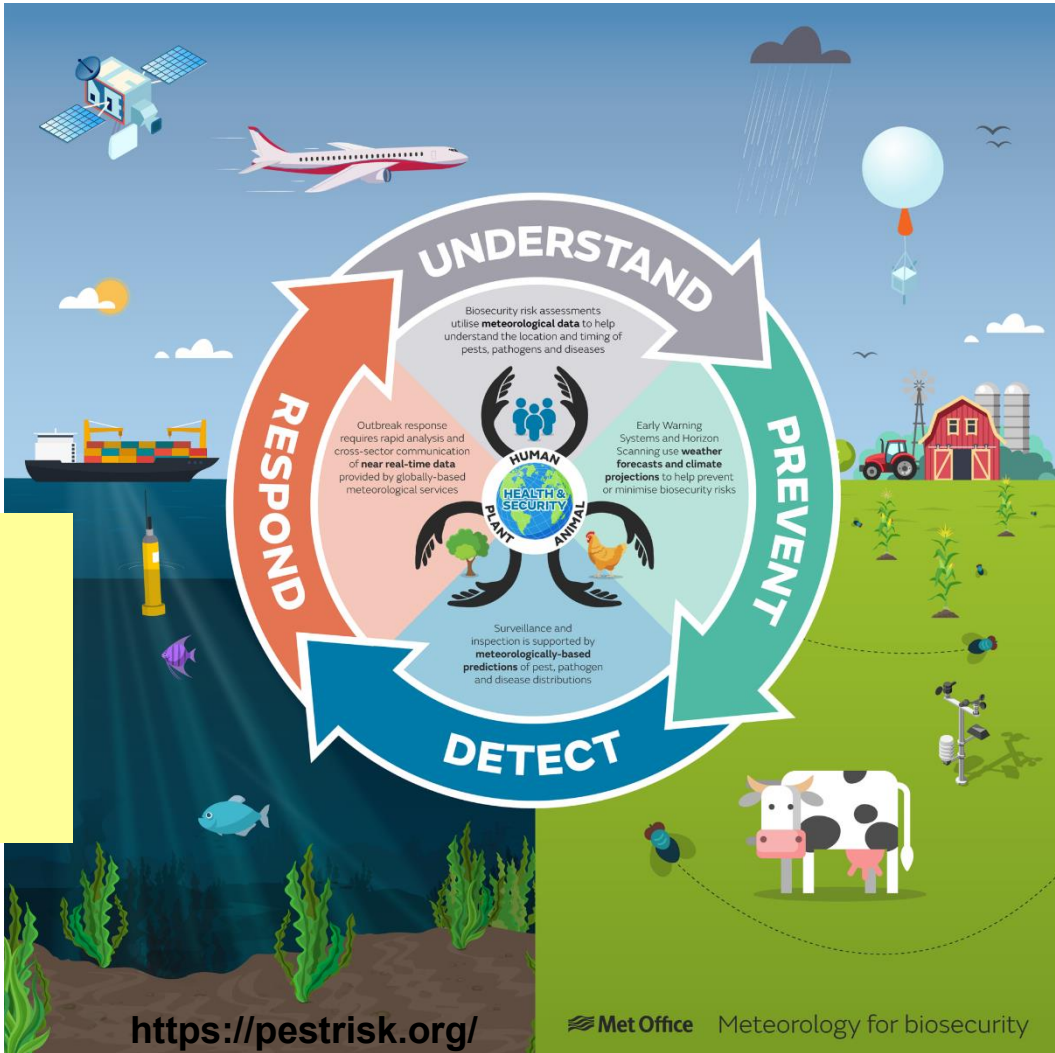
(i) striking in form in order to draw immediate attention; (ii) unique and unambiguous, in order not to be confused with symbols used for other purposes; (iii) quickly recognizable and easily recalled; (iv) easily stenciled; (v) symmetrical, in order to appear identical from all angles of approach; and (vi) acceptable to groups of varying ethnic backgrounds.

Biosecurity

…more difficult to define than ‘biosafety’, as it depends on context (human, agricultural, ecological etc.)

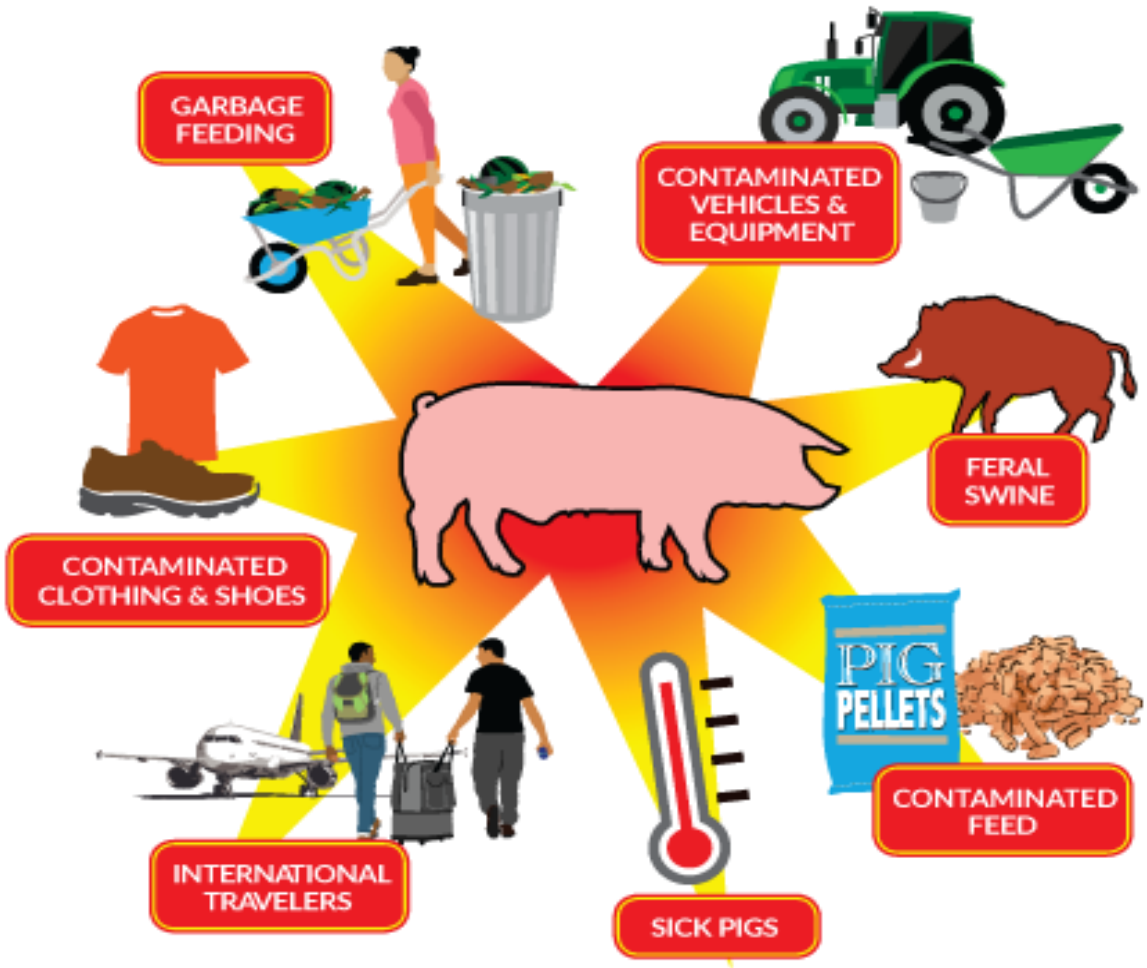
1. Precautions taken to protect humans from an introduced pathogen

Examples:
- SaRS-CoV2
- Tuberculosis
- Cholera



Biosecurity

2. Precautions taken to protect farms and food supply from accidental or deliberate contamination



Examples:
- **Foot & Mouth**
- **Rabies**
- **Hendra**

https://www.healthyagriculture.org/

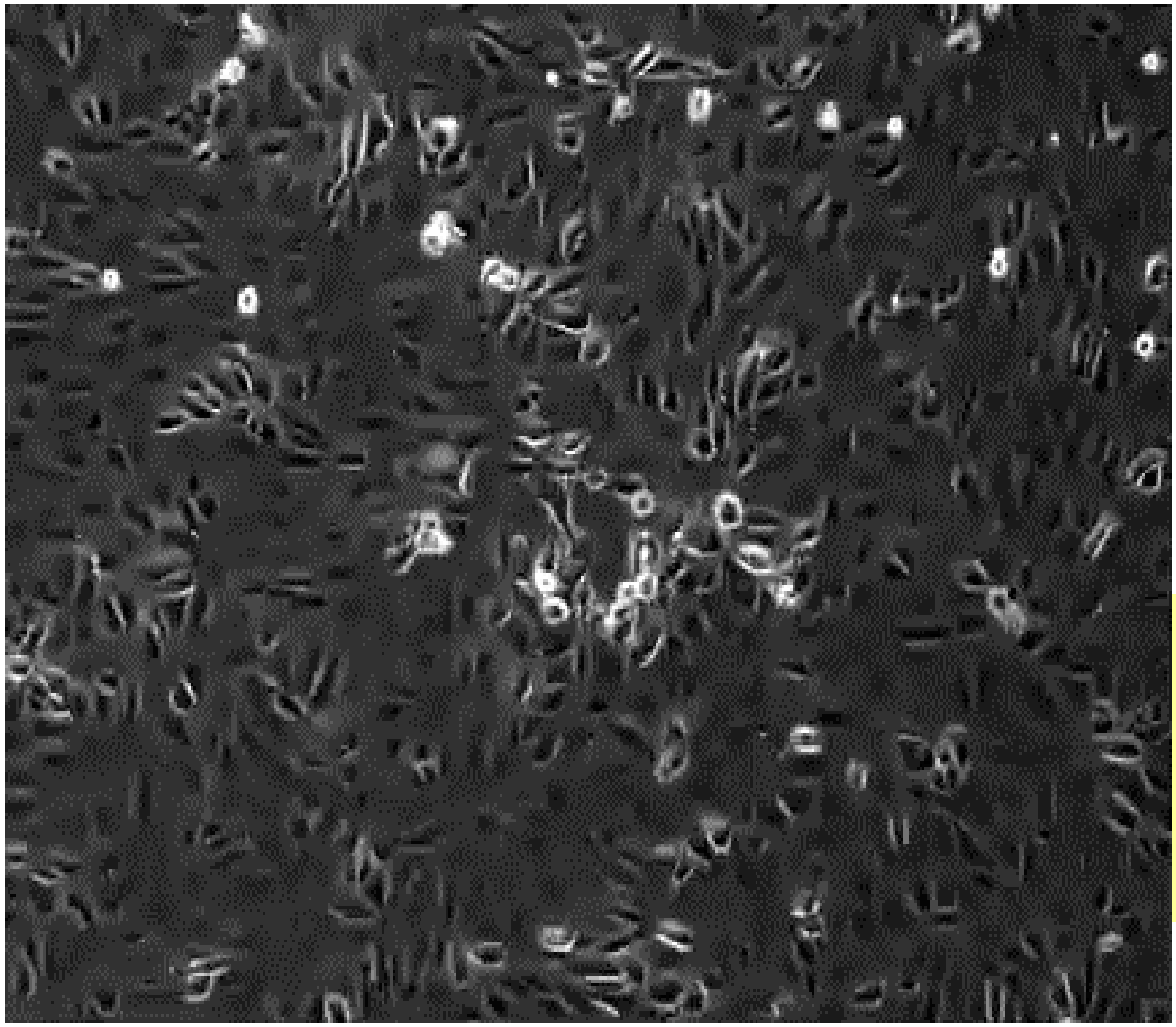
Biosecurity

3. Precautions taken to protect natural ecosystems from introduced biological threats

Examples:
- **Phytophthora**
- **Chytrid**
- **Myrtle rust**

Biosecurity

4. Precautions taken to prevent lab escape of pathogens



Examples:
- **Influenza 1918***
- **Influenza H5N1***
- **GMO's**

*** See later slides**

What do we mean by ‘precautions’?

- **Physical security / barriers**
  - Restricted access areas
  - Biosafety cabinet
  - PPE (lab coat, glasses, gloves)

- **Managing people**
  - Biosecurity laws
  - Careful recruitment
  - Appropriate training
  - Emergency response

What do we mean by ‘precautions’?

- **Managing animals, plants, microbes**
  - **Safe farm & food chain practices**
  - **Sterilisation / sanitisation procedures**

- **Information security**
  - **Inventories of biologicals**
  - **Documenting transfers / disposals**

strain	hazard level	date acquired	location
E.coli TOP10	low	5/02/1993	Freezer 1, box 2
E.coli BL21	low	7/07/1998	Freezer 1, box 2
P.aeruginosa PAO1	medium	15/06/2003	Freezer 1, box 3
S.aureus CCM 885	medium	14/04/2005	Freezer 1, box 3
B. anthracis 'Ames'	high	9/11/2001	secret (ssba)

Microorganism risk groups

Generally recognised as safe (GRAS)

- GRAS microbes are very safe. Will only cause disease in very rare cases (e.g. immunocompromised hosts)
- GRAS microbes can be handled outside the lab
- These are fermentation microbes e.g.
  - *Saccharomyces*
  - *Lactobacillus*
  - *Bacillus subtilis*

Microorganism risk groups

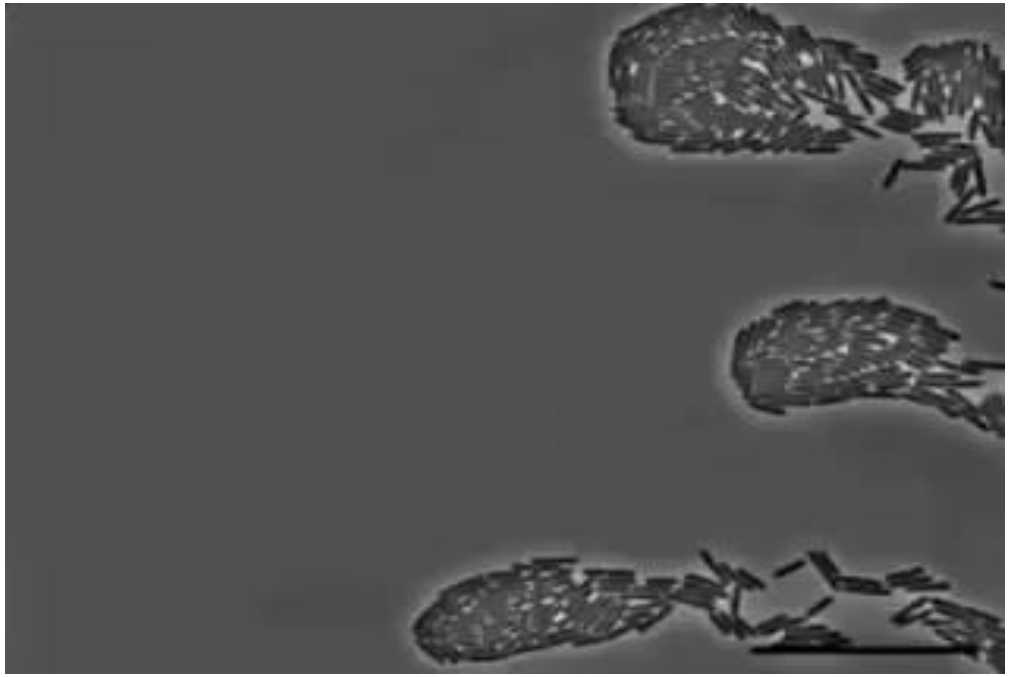
Risk group 1

- Risk group 1's will not cause disease in healthy humans
- Can be handled in a PC1 lab with minimal precautions
- Includes environmental microbes, normal flora, and lab strains used as model organisms or cloning hosts
  - Most *E.coli* strains
  - *Staph. epidermidis*
  - Non-toxic fungi e.g. *Penicillium*
  - Most protists e.g. *Paramecium*
  - All the Archaea !

Microorganism risk groups

Risk group 2

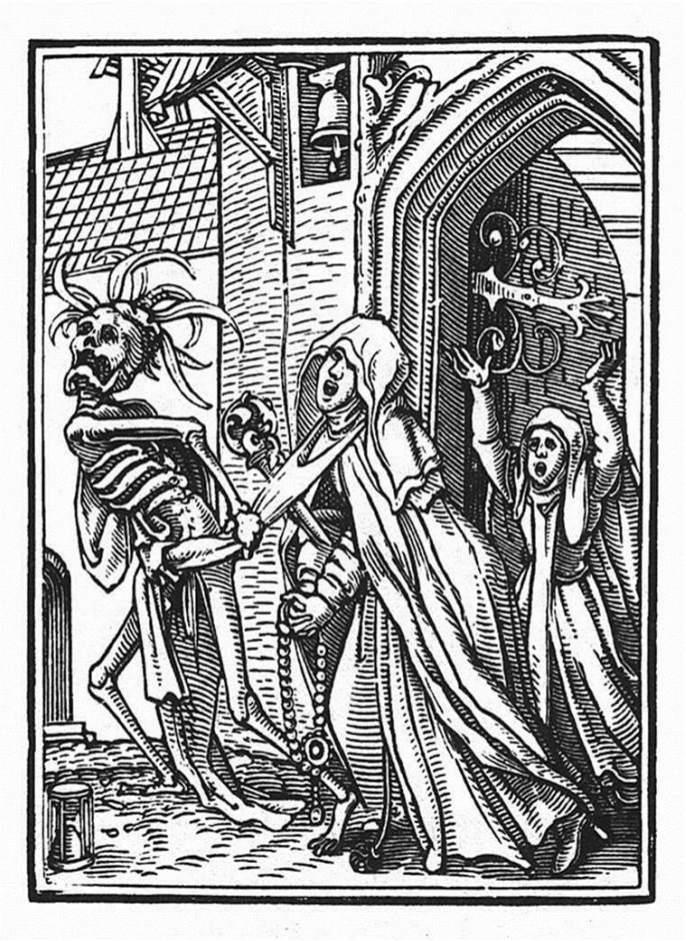
- **Risk group 2's can cause disease, but this is typically treatable, not life-threatening, not easily transmissible**
- **Can be handled in a PC2 lab**
- **Includes most common microbial pathogens**
  - **Pathogenic *E.coli***
  - ***Staph. aureus***
  - ***Ps. aeruginosa***
  - **Influenza virus**



Microorganism risk groups

Risk group 3

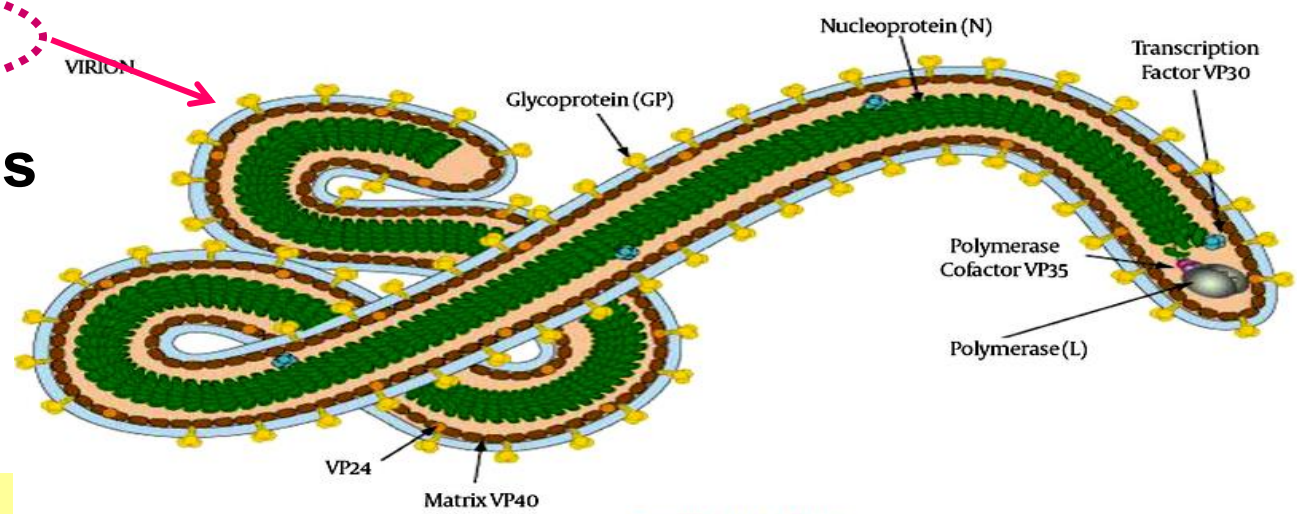
- Risk group 3's cause serious disease, which may be hard to treat and/or life-threatening
- Require handling in PC3 lab
- Very dangerous pathogens, e.g.
  - *Bacillus anthracis*
  - *Yersinia pestis* →
  - *Myco.tuberculosis*
  - HIV virus



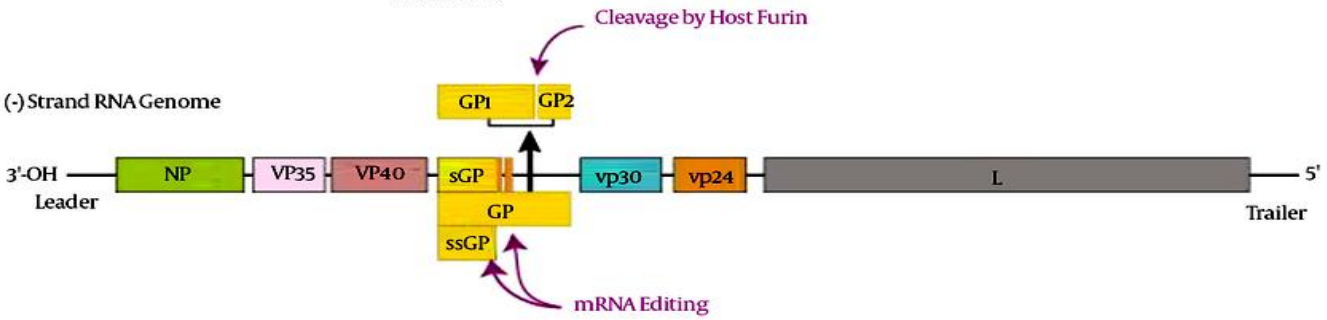
Microorganism risk groups

Risk group 4

- Risk group 4's cause deadly disease in humans, which is untreatable and also transmissible
- The scariest of all pathogens. Require PC4 lab.
- Ebola virus
- Marburg virus
- Lassa virus



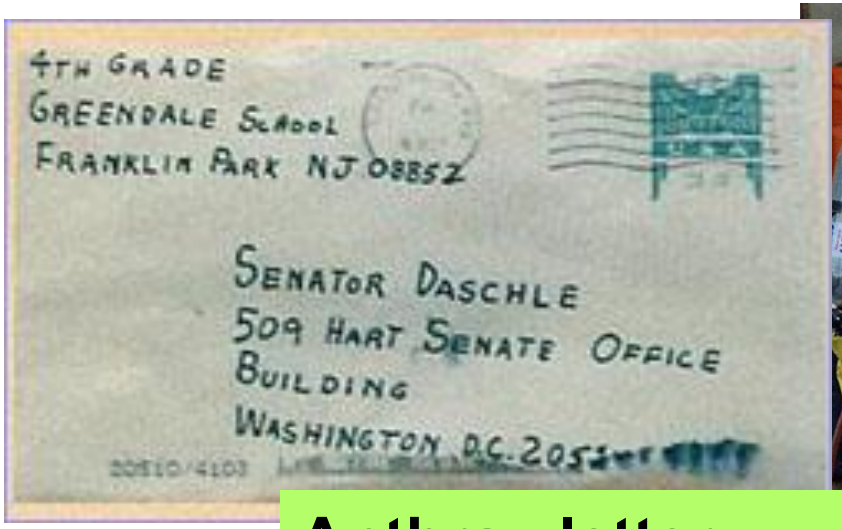
Ebola genome: just 19 kb and seven proteins



Microorganism risk groups

Security-sensitive biological agents (SSBA)

- Microbes and toxins that may be used for bioterrorism
- e.g. smallpox, *Ebolavirus*, *Bacillus anthracis* (anthrax)
- Holding SSBA's in a lab requires many extra layers of security compared to other pathogens



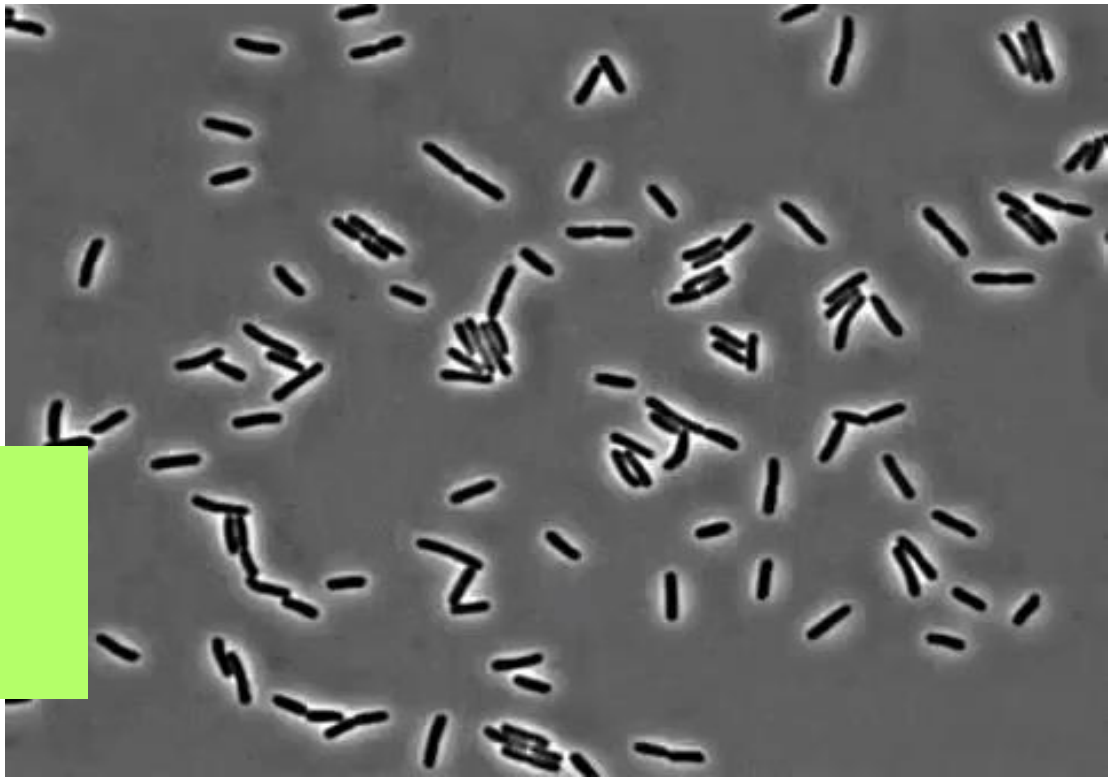
Anthrax letter sent to US Senate

Microbial GMO risk groups

Australian Government
Department of Health
Office of the Gene Technology Regulator

'Exempt dealings'

- **Lowest risk level – PC1 lab is OK**
- **Involve only standard host organisms e.g. *E.coli, B.subtilis, Saccharomyces***
- **Can add any genes except virulence factors or toxins**



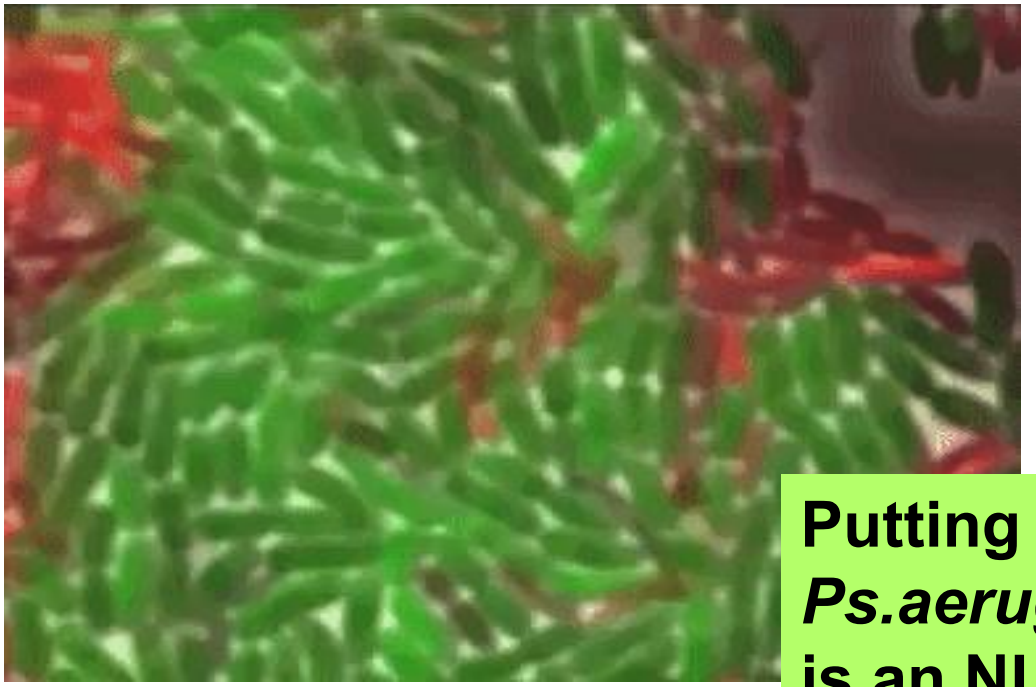
Adding GFP to *E.coli* is an exempt dealing

Microbial GMO risk groups

'Notifiable Low Risk Dealings'

Australian Government
Department of Health
Office of the Gene Technology Regulator

- **Medium risk level – PC2 lab**
- **May involve adding 'safe' genes to non-standard hosts**
- **OR adding virulence genes to standard hosts**



Putting GFP into *Ps.aeruginosa* is an NLRD

- **Must not involve: release of GMO to environment or creation of more dangerous pathogen***

***See later slides**

Physical containment levels: PC1 labs

https://www.ogtr.gov.au/resources

Building

- Fully enclosed space: walls, ceiling, lockable doors
- Surfaces must be easily decontaminated (non-porous)
- Hand-wash station (sink or sanitiser)
- Lab coat hooks / hangers

Behaviour

- Wear lab coat or gown
- Label all cultures
- Clean up spills
- Decontaminate wastes

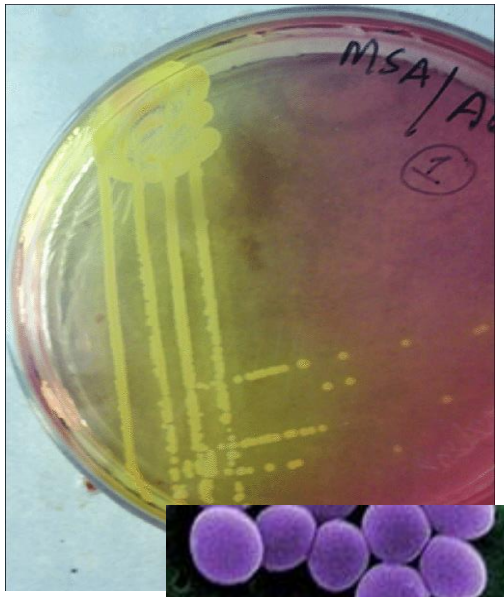
Commensal or ‘lab strains’ of *E.coli* can be handled in a PC1 lab

Physical containment levels: PC2 labs

https://www.ogtr.gov.au/resources

Building

- Same as PC1, plus the following:
- Eyewash station
- Biosafety cabinets
- Biohazard symbol on doors
- ‘Backflow prevention’ (water pipes)



Behaviour

- Same as PC1, plus the following:
- Wear gloves and safety glasses
- Fully enclosed footwear

Working with *Staph.aureus* requires a PC2 lab

The ‘Dual Use’ problem in microbiology

- **Any research on dangerous microbes could be used to help cure diseases OR used to make bioweapons e.g.**
  - **Reconstruction of 1918 flu** **LEGIT FEAR**
  - **Gain of function research on H5N1 flu** **LEGIT FEAR**
  - **Smallpox escaping deep freeze** **LEGIT FEAR**
  - **Engineering of SaRS-CoV2 ?** **FAKE NEWS**

Biological warfare

- Use of microbes as weapons was banned by the Geneva Protocol (1925) and the later Biological Weapons Convention (1972), which added bio-toxins

- Bioweapons are both cruel (do not kill quickly) and dangerous (spread to own troops / non-combatants)

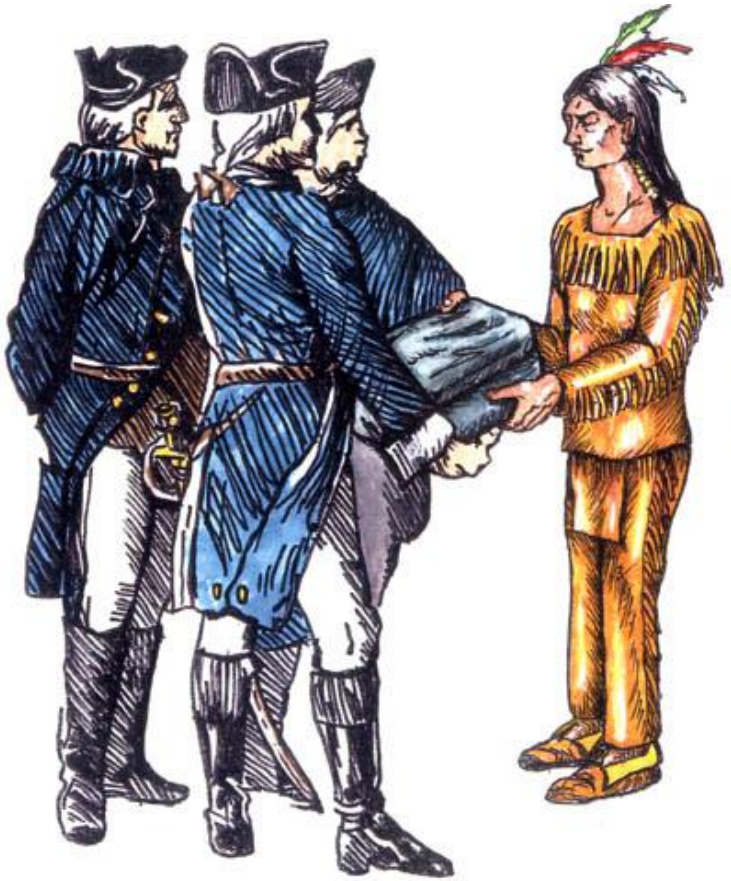
- …despite this, there are many horrible chapters in history where microbes were used as weapons

Biological warfare

- **Middle ages (500-1500 AD): Bodies of plague victims reportedly thrown into enemy cities via catapaults**

Biological warfare

- **1750-1800: Smallpox used against native Americans and native Australians by the British military & settlers**

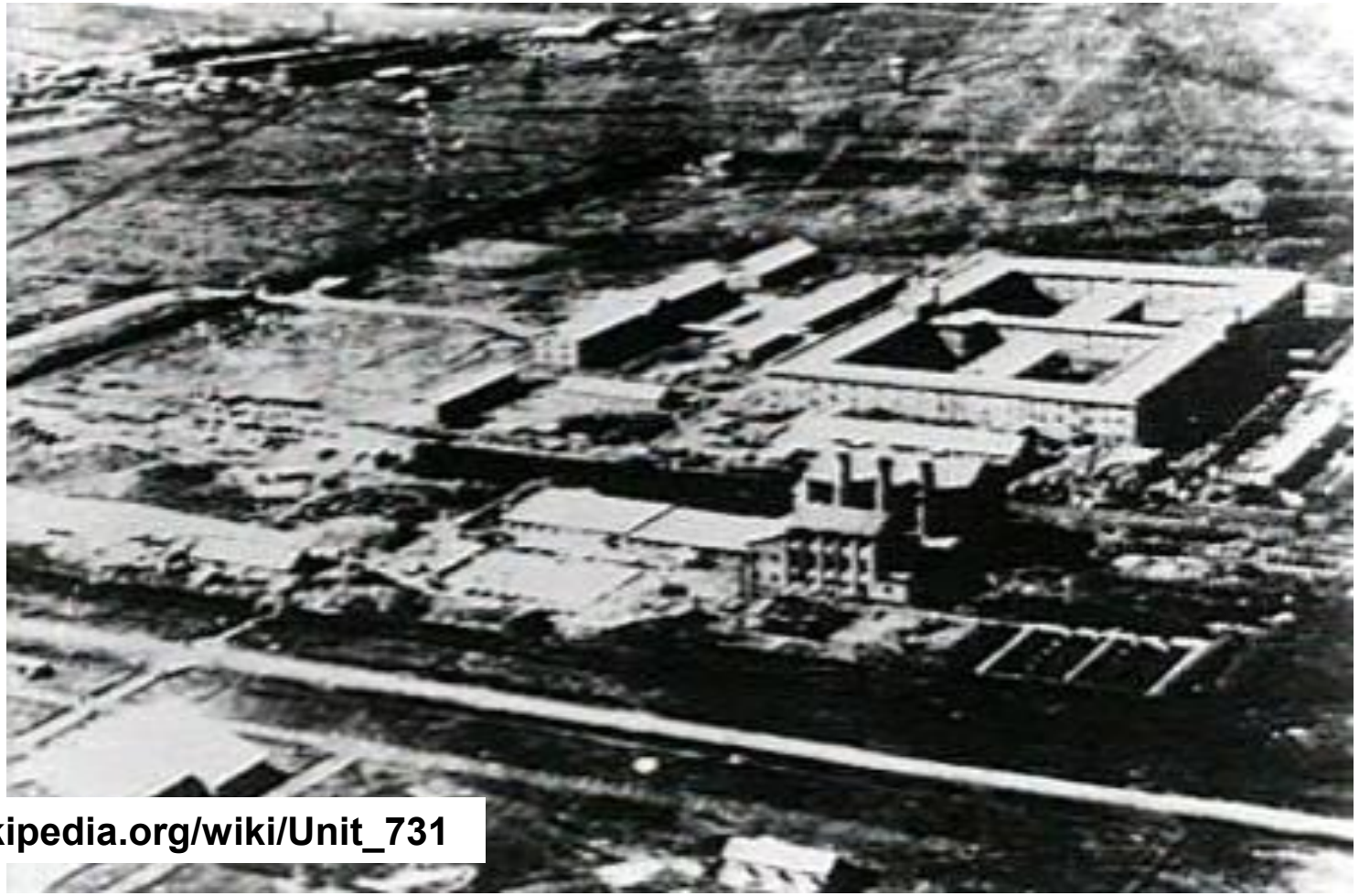


https://daily.jstor.org/how-commonly-was-smallpox-used-as-a-biological-weapon/

https://www.abc.net.au/radionational/programs/ockhamsrazor/was-sydneys-smallpox-outbreak-an-act-of-biological-warfare/5395050

Biological warfare

- **1936-1945: Japanese Army Unit 731 tested plague, cholera, typhoid, smallpox, botulism on prisoners**

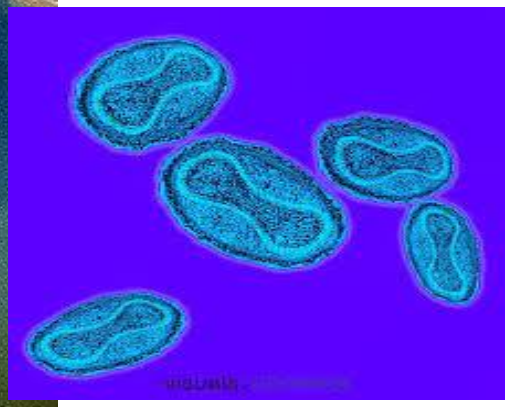


https://en.wikipedia.org/wiki/Unit_731

Biological warfare

- **1920's-1990's: Soviet Union weaponised, tested, and stockpiled several bacterial and viral agents. Many accidents and deaths. e.g. see below incident in 1971.**

"On Vozrozhdeniya Island in the Aral Sea, the strongest recipes of smallpox were tested… A research ship came to within 15 km of the island. The lab technician of this ship took samples of plankton from the top deck. The smallpox formulation (400 g. of which was exploded on the island) got her and she became infected. After returning home to Aralsk, she infected several people including children. All of them died"



Crit. Rev. Microbiol. 30 (4): 241–61

Smallpox: gone for good?

- Smallpox (variola virus) eradicated in the wild in 1977
- Global population now unvaccinated & susceptible
- Variola still stored in two PC4 labs in USA and Russia

→ Should we destroy it completely ?

→ Could it come back on its own? Or with help?

Synthetic biology and the 'Dual Use' problem

- **Advances in synthetic biology yield new ethical dilemmas**
- **We can now construct pathogens and toxins *in vitro* without access to the original source material**

→ DNA synthesis companies must routinely screen all orders for sequences of pathogens + toxins

But what happens when DNA synthesis becomes miniaturised and personalised?

DRUG DISCOVERY & DEVELOPMENT

GENOMICS/PROTEOMICS ONCOLOGY NEUROLOGICAL DISEASE INFECTIOUS DISE

DNA Script launches a benchtop DNA printer

By Sean Whooley | June 24, 2021

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the Syntax system, a
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integrated, automat
DNA synthesis (EDS) technology, t
parallel, comprised of up to 60 nuc

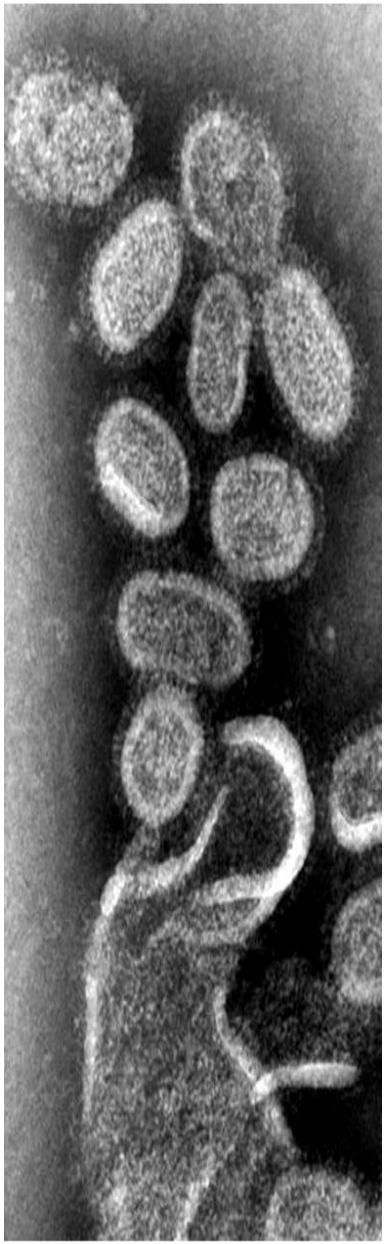
Synthetic biology and the 'Dual Use' problem

SCIENCE VOL 310 7 OCTOBER 2005

Characterization of the Reconstructed 1918 Spanish Influenza Pandemic Virus

Terrence M. Tumpey,[1]* Christopher F. Basler,[2] Patricia V. Aguilar,[2] Hui Zeng,[1] Alicia Solórzano,[2] David E. Swayne,[4] Nancy J. Cox,[1] Jacqueline M. Katz,[1] Jeffery K. Taubenberger,[3] Peter Palese,[2] Adolfo García-Sastre[2]

The pandemic influenza virus of 1918–1919 killed an estimated 20 to 50 million people worldwide. With the recent availability of the complete 1918 influenza virus coding sequence, we used reverse genetics to generate an influenza virus bearing all eight gene segments of the pandemic virus to study the properties associated with its extraordinary virulence. In stark contrast to contemporary human influenza H1N1 viruses, the 1918 pandemic virus had the ability to replicate in the absence of trypsin, caused death in mice and embryonated chicken eggs, and displayed a high-growth phenotype in human bronchial epithelial cells. Moreover, the coordinated expression of the 1918 virus genes most certainly confers the unique high-virulence phenotype observed with this pandemic virus.



Synthetic biology and the 'Dual Use' problem

Commentary on Tumpey et al paper *

"Tumpey et al. have constructed a virus that is perhaps the most effective bioweapon known"

"This would be extremely dangerous if it escaped, and there is a long history of things escaping"

"We are trying to understand what happened in nature and how to prevent a pandemic. In this case, nature is the bioterrorist"

"I didn't expect it to be as lethal as it was"

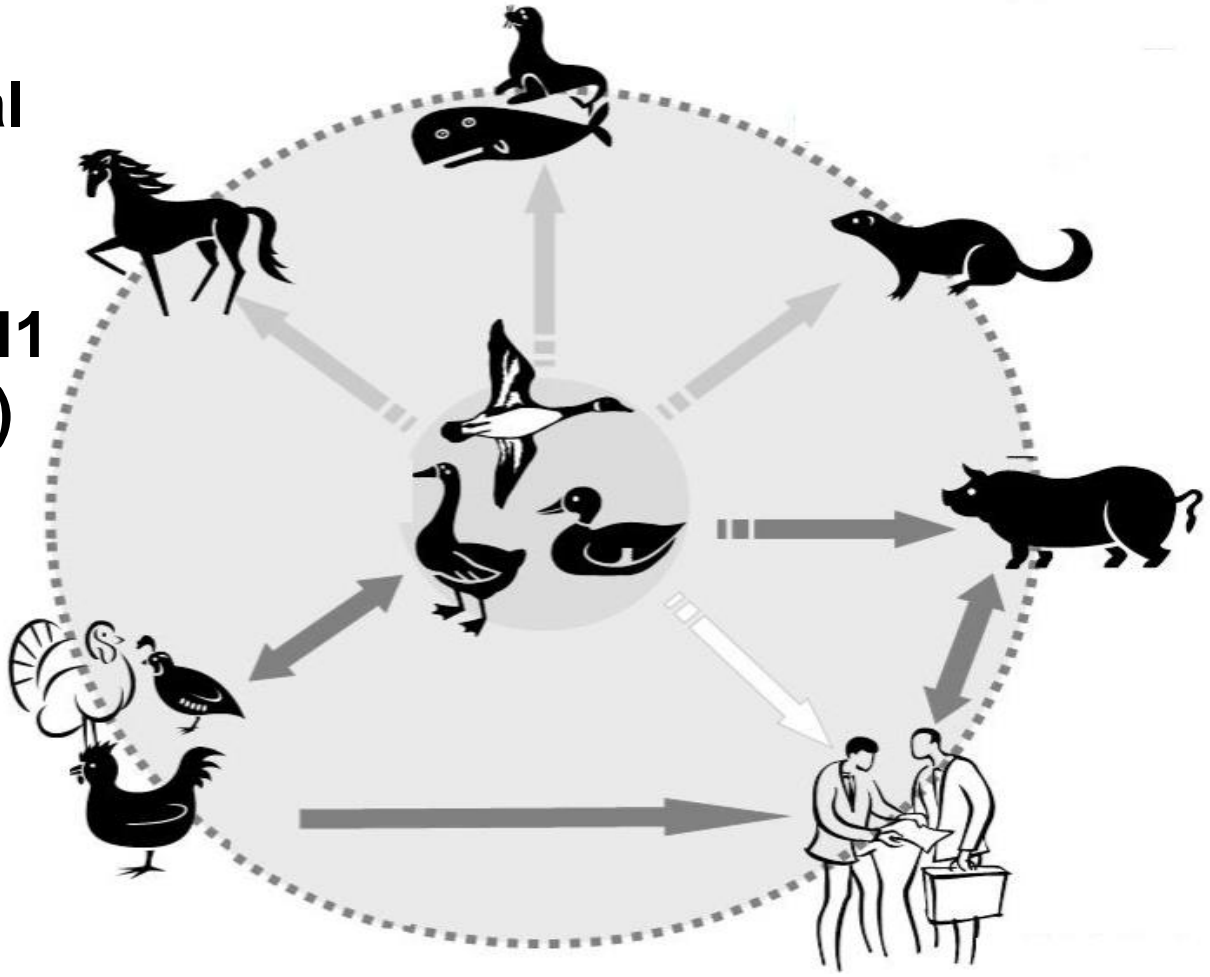
** Nature 437: 794-795*

More ethical dilemmas: 'Gain of function' research

- **Gain of function experiments aim to increase the virulence of a pathogen, in order to better understand it**

→ Another example of 'dual-use' research

- **Most controversial has been making an airborne-transmissible H5N1 influenza (bird-flu)**



Both wild and domestic birds are integral to the flu virus life cycle

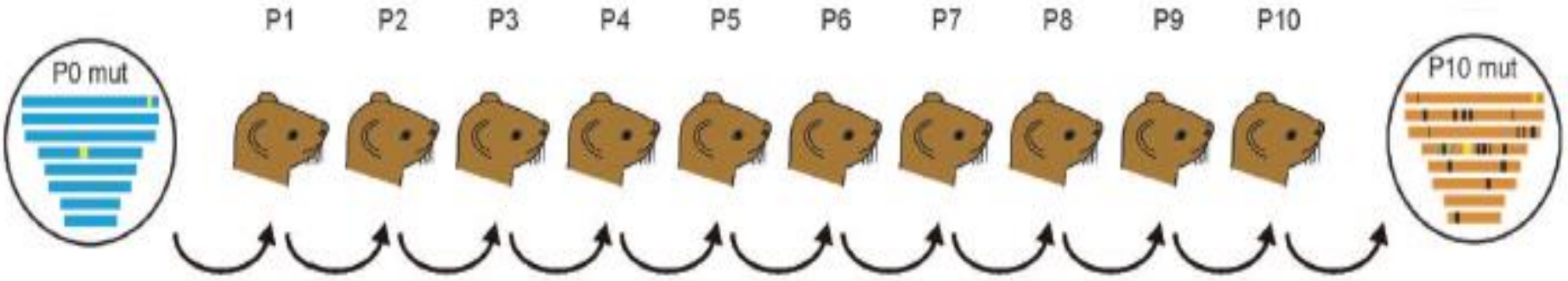
More ethical dilemmas: 'Gain of function' research

H5N1 22 JUNE 2012 VOL 336 SCIENCE

Airborne Transmission of Influenza A/H5N1 Virus Between Ferrets

Sander Herfst,[1] Eefje J. A. Schrauwen,[1] Martin Linster,[1] Salin Chutinimitkul,[1] Emmie de Wit,[1*] Vincent J. Munster,[1*] Erin M. Sorrell,[1] Theo M. Bestebroer,[1] David F. Burke,[2] Derek J. Smith,[1,2,3] Guus F. Rimmelzwaan,[1] Albert D. M. E. Osterhaus,[1] Ron A. M. Fouchier[1]†

Highly pathogenic avian influenza A/H5N1 virus can cause morbidity and mortality in humans but thus far has not acquired the ability to be transmitted by aerosol or respiratory droplet ("airborne transmission") between humans. To address the concern that the virus could acquire this ability under natural conditions, we genetically modified A/H5N1 virus by site-directed mutagenesis and subsequent serial passage in ferrets. The genetically modified A/H5N1 virus acquired mutations during passage in ferrets, ultimately becoming airborne transmissible in ferrets.



‘Gain of function’ research: self-policing

Pause on Avian Flu Transmission Research

“A perceived fear that the ferret-transmissible H5 HA viruses may escape from the laboratories has generated intense public debate on the benefits and potential harm of this type of research”

“We have agreed to a voluntary pause of 60 days on any research involving highly pathogenic avian influenza H5N1 viruses leading to the generation of viruses that are more transmissible in mammals”

→ **7 years !**

RON A. M. FOUCHIER,[1]* ADOLFO GARCÍA-SASTRE,[2] YOSHIHIRO KAWAOKA,[3] WENDY S. BARCLAY,[4] NICOLE M. BOUVIER,[5] IAN H. BROWN,[6] ILARIA CAPUA,[7] HUALAN CHEN,[8] RICHARD W. COMPANS,[9] ROBERT B. COUCH,[10] NANCY J. COX,[11] PETER C. DOHERTY,[12] RUBEN O. DONIS,[13] HEINZ FELDMANN,[14] YI GUAN,[15] JAQUELINE KATZ,[16] H. D. KLENK,[17] GARY KOBINGER,[18] JINHUA LIU,[19] XIUFAN LIU,[20] ANICE LOWEN,[21] THOMAS C. METTENLEITER,[22] ALBERT D. M. E. OSTERHAUS,[23] PETER PALESE,[24] J. S. MALIK PEIRIS,[25] DANIEL R. PEREZ,[26] JÜRGEN A. RICHT,[27] STACEY SCHULTZ-CHERRY,[28] JOHN STEEL,[29] KANTA SUBBARAO,[30] DAVID E. SWAYNE,[31] TORU TAKIMOTO,[32] MASATO TASHIRO,[33] JEFFERY K. TAUBENBERGER,[34] PAUL G. THOMAS,[35] RALPH A. TRIPP,[36] TERRENCE M. TUMPEY,[37] RICHARD J. WEBBY,[38] ROBERT G. WEBSTER[39]

27 JANUARY 2012 VOL 335 SCIENCE

‘Gain of function’ research: self-policing… for now

EXCLUSIVE: Controversial experiments that could make bird flu more risky poised to resume

Science

Two “gain of function” projects halted more than 4 years ago have passed new U.S. review process

8 FEB 2019 • BY JOCELYN KAISER

References

World Health Organisation (2006) Biorisk management. Laboratory biosecurity guidance. *https://www.who.int/csr/resources/publications/biosafety/WHO_CDS_EPR_2006_6.pdf*

Tumpey, T. et al (2005) Characterization of the reconstructed 1918 Spanish influenza pandemic virus. Science 310:77-80

Von Bubnoff (2005) The 1918 flu virus is resurrected. Nature 437:794–795

Herfst, S. et al (2012) Airborne Transmission of Influenza A/H5N1 Virus Between Ferrets. Science 336:1534-1541

Casadevall A, Shenk T. 2012. The H5N1 moratorium controversy and debate. mBio 3(5):e00379-12. doi:10.1128/mBio.00379-12.

Fouchier R. et al (2012) Pause on Avian Flu Transmission Research. Science 335:400