

**VIROBATHE:**

Adenovirus infectivity:  
wishes & reality

Work Packages 1 & 4





# Work Package 1

## Provision of adenovirus & norovirus

- 5.1 & 5.2 - Known virus to use in concentration studies on recovery rate, evaluate adenovirus infectivity assays
- 6.2 - As positive virus to use during training
- 7 - As positive control virus
  
- Large volume of virus mixed and 1.3ml added to ampoules (storage vials). Stored at low temperature
- Quantity of virus in each pool of virus is checked by plaque assay or PCR on several ampoules

# Adenovirus 2



UK National Collection of Pathogenic Viruses

Strain #213

Isolated in 1977

Human adenovirus C

Grows well in cell culture and forms plaques in plaque assay



# Cell culture

## A549 cells

European Collection of Cell Culture (ECACC)

Human Caucasian lung carcinoma 1967

Robust cells: split ratio 1:10

Fast growing in a weekly cycle

Sensitive to many adenovirus serotypes

# Work Package 4

## adenovirus infectivity assay - 1

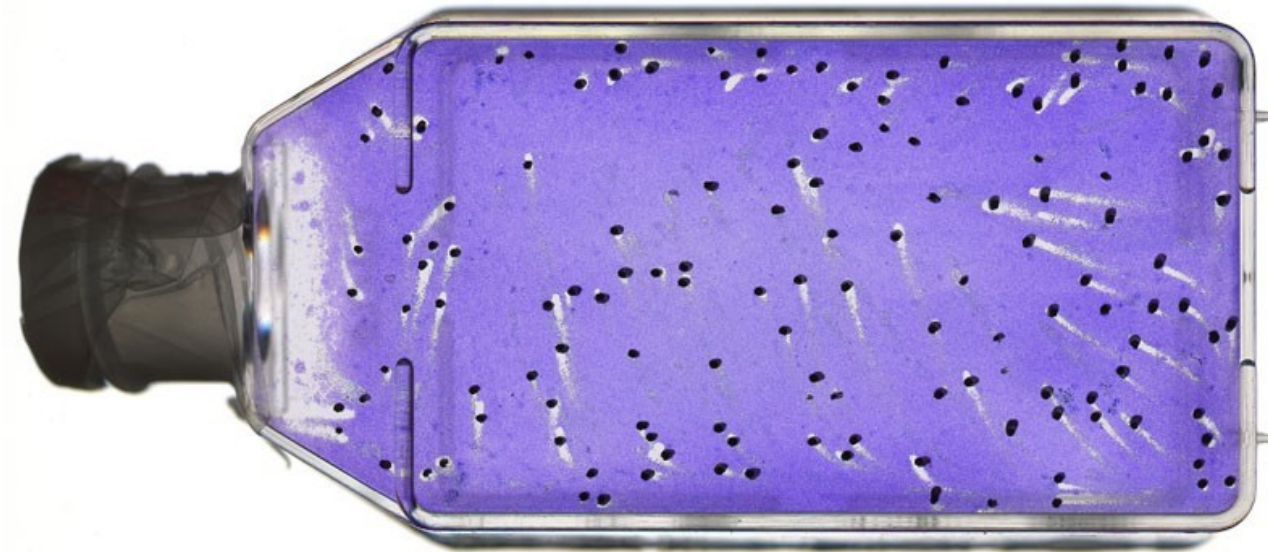


**Plaque assay** for 5.1 & 5.2 to count virus in concentrates from water processing and estimate the recovery rate

- 1ml virus added to 10L water sample
- Filtration by 1 of the 5 methods in 5.1 or 3 methods in 5.2
- 10ml concentrate
- 1ml per 25cm<sup>2</sup> flask with A549 cell monolayer
- CMC and stain with crystal violet after 8 days

Method not used in later Work Packages as not all serotypes produce plaques and growth would be too slow

# Adenovirus 2 plaque assay





# Work Package 4 adenovirus infectivity assay

For WP6.2 Training

For WP7 Surveillance

Need a liquid assay to detect infectious adenovirus of as many serotypes as possible

Ad 40/41 which was not expected to replicate in A549 cells

Most serotypes are slow growing so cpe not practical

5ml of sample concentrate

- Integrated cell culture & PCR (ICC-PCR)
- 5 days incubation, freeze & thaw, PCR on supernatant
- Inoculate control flask, freeze at Time 0 to check for residual virus



# ICC-PCR on Adenovirus Positive Water Samples

Sample Number	Positive No. of Flasks at T5	Comments on PCR gel band
12	1/2	Good
11	1/2	Faint
91	1/2	Faint
87	1/2	Good
83	1/2	Good
101	2/2 *	Good

\* To Barcelona

Seven other samples were positive by direct PCR and negative by ICC-PCR



# ICC-PCR results

- no residual virus found at T0
- Sequencing has not consistently identified adenovirus in material
- degeneration during transport?
- request information from each laboratory as to how many flasks inoculated & how many positive
- tomorrow - discuss your experiences using this technique

# And finally ...



- **adenovirus infectious assays are challenging**
- **plaque assay worked well**
- **ICC-PCR needs further analysis**