

Use of cell culture technology to minimize the need for animal trials in development and production of fish vaccines

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Background- Fish Diseases

Viral-

viral hemorrhagic
septicemia virus (VHSV)



Parasitic-
Sea lice

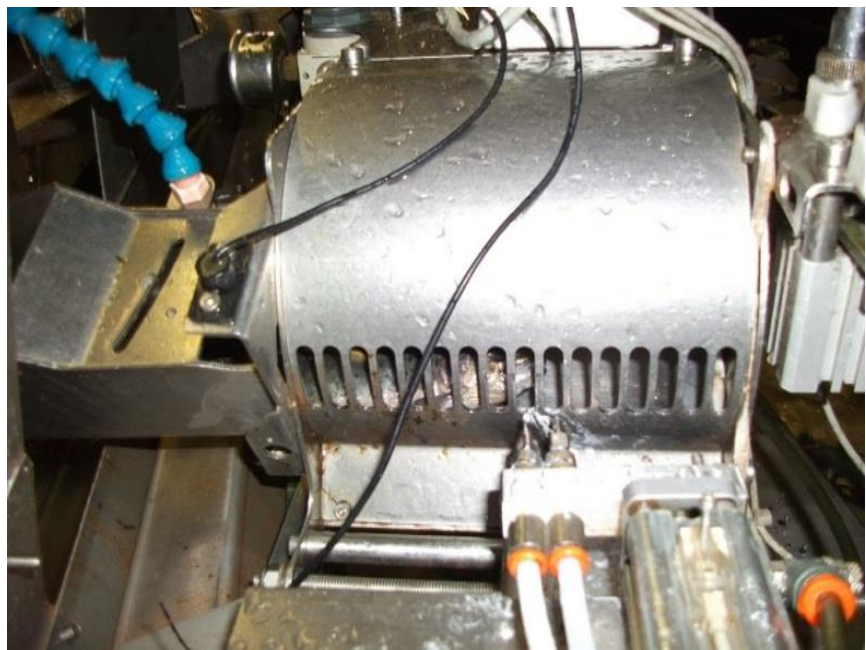
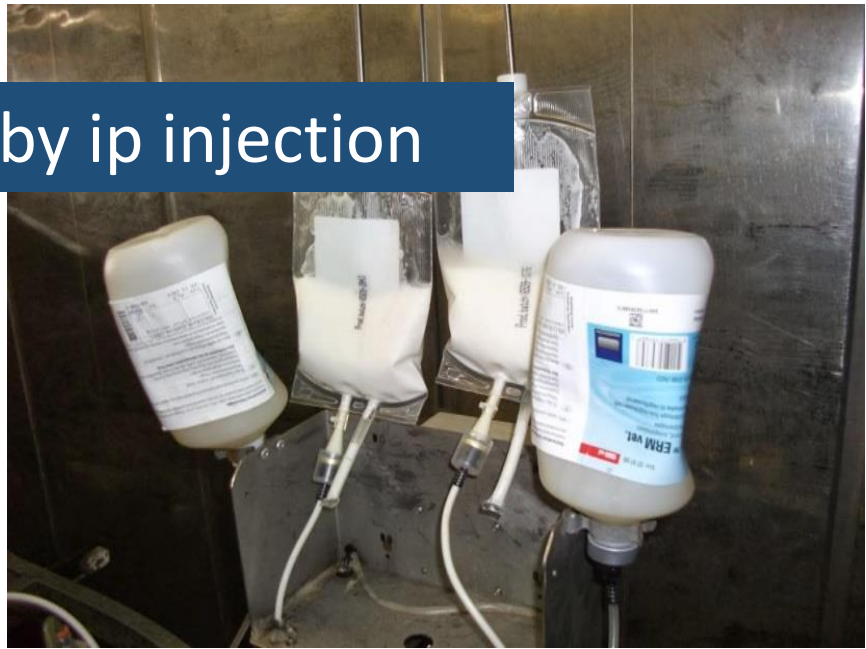
Bacterial-

Aeromonas salmonicida



Fungal-
*Branchiomyces
demigrans*

Fish vaccination by ip injection

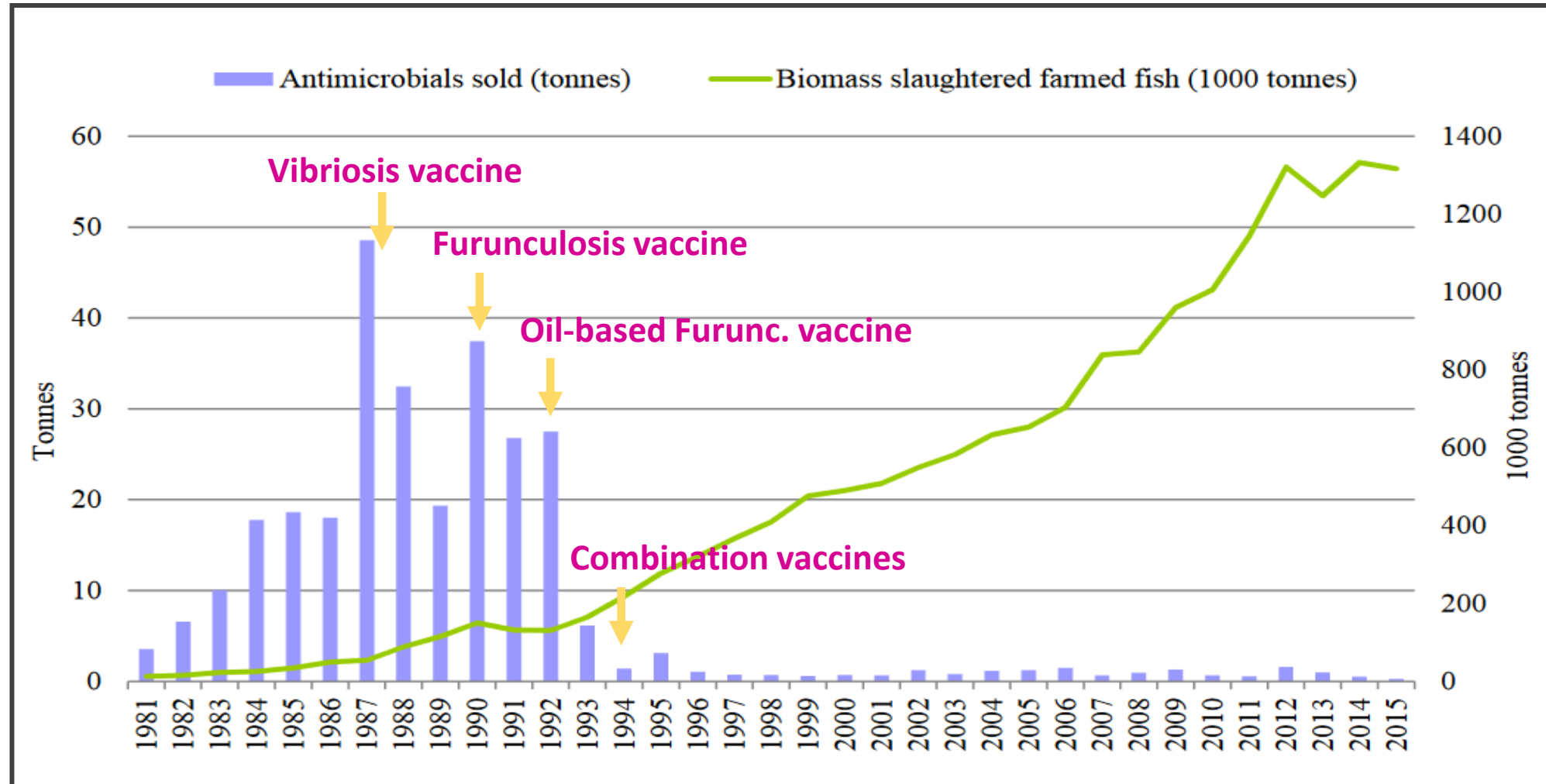


Dip-vaccination



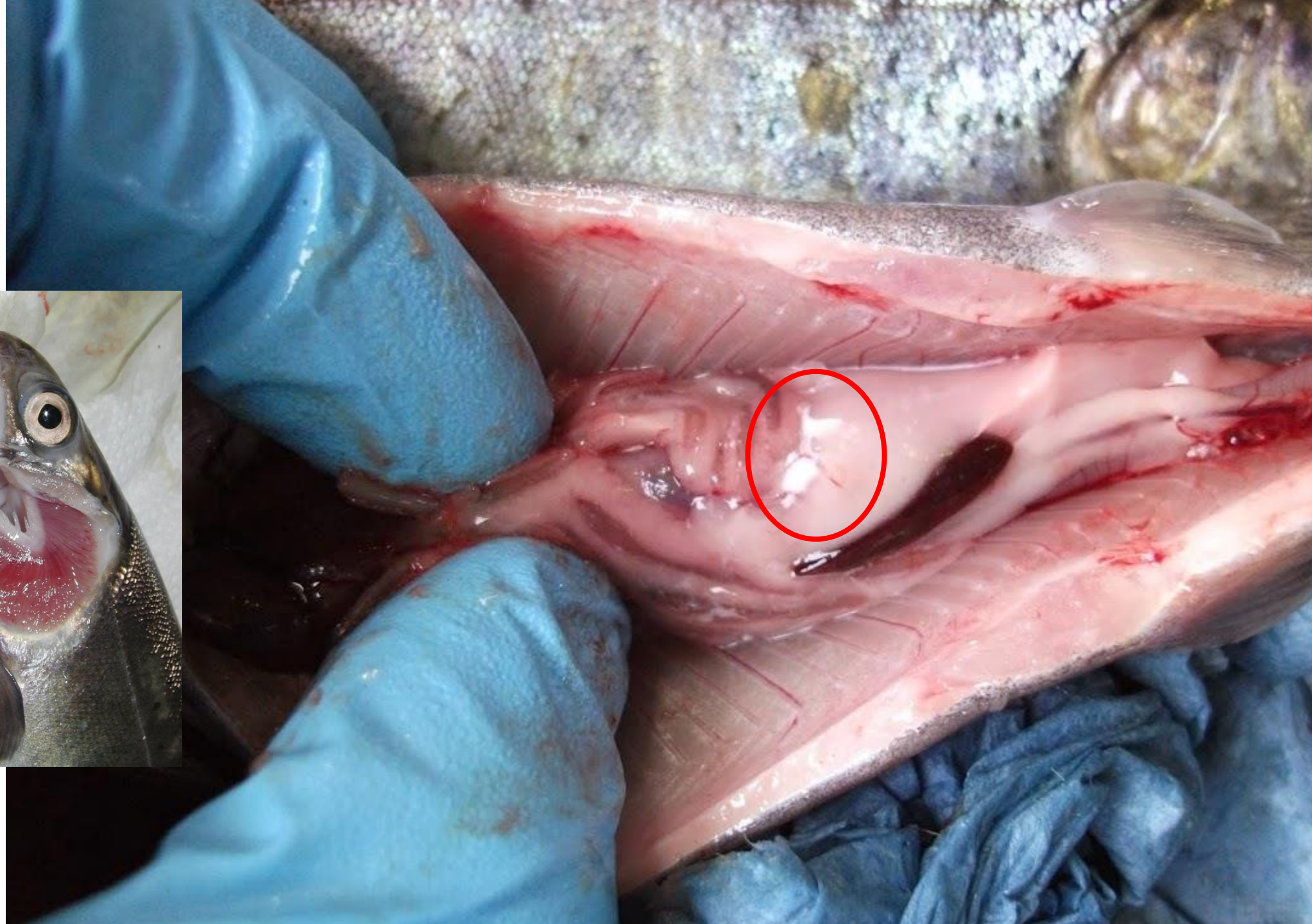
Background- Impact of vaccine in Norway

The use of antimicrobial has declined by about 99% (1987-2014)



Ref.1 Usage of antimicrobial agents and occurrence of antimicrobial resistance in Norway. 2014. Oslo and Tromsø

Ref.2 Fish Vaccination – A brief overview. Dr Marian McLoughlin



Aims

- 1. Replace the use of live animals with cell cultures for the initial screening of vaccine components.**
- 2. Gain a better understanding of the fish immune system to improve vaccine efficacy.**

Egtved disease (Viral haemorrhagic septicaemia, VHS)

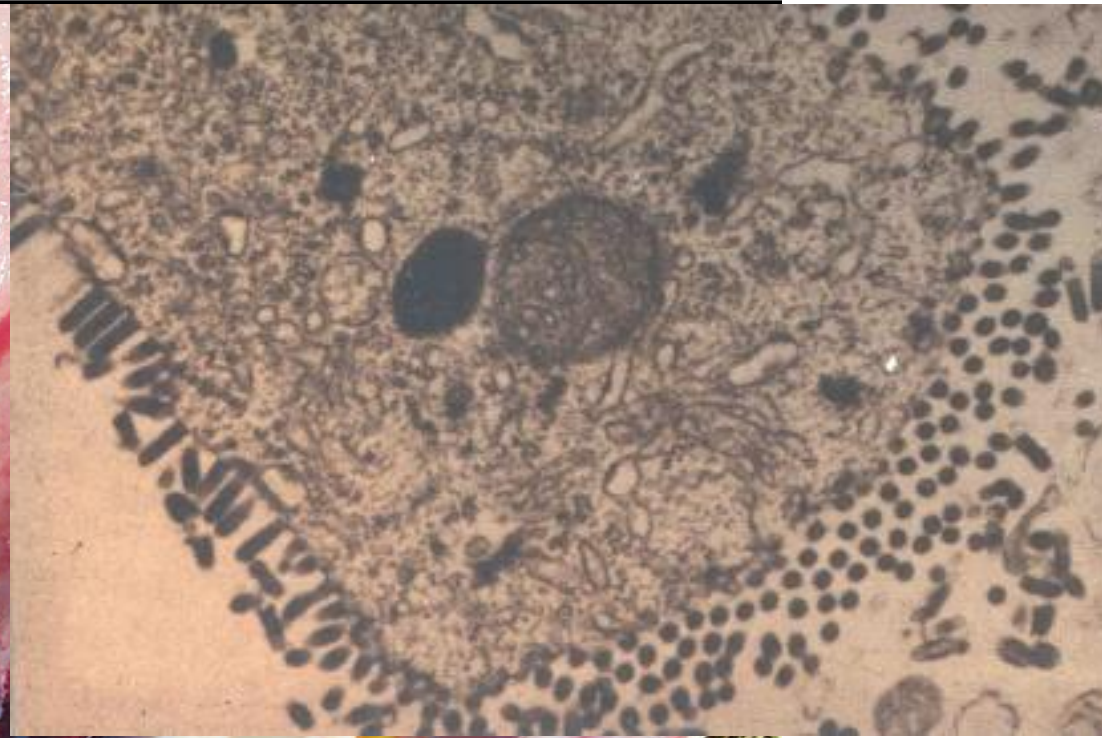
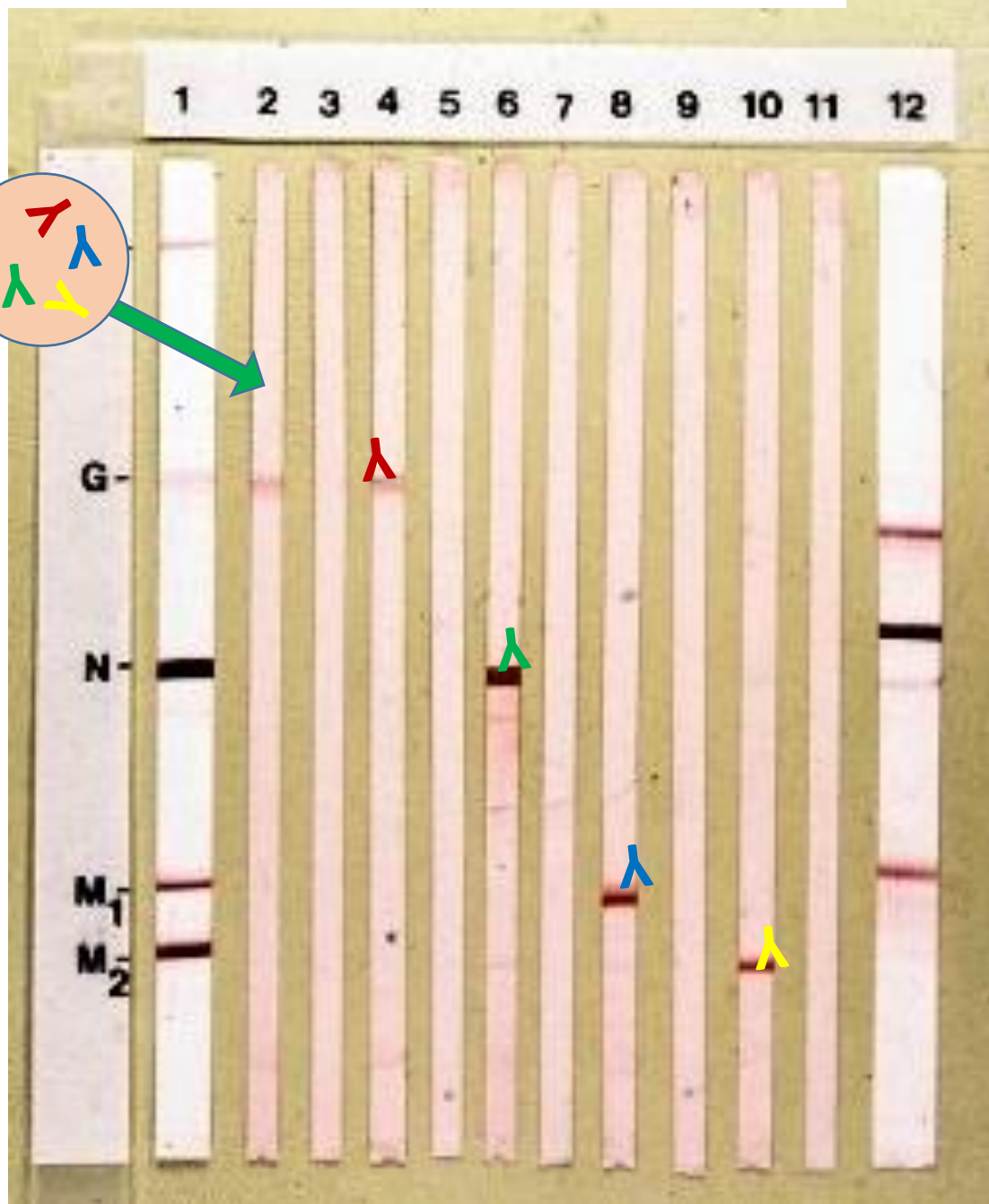
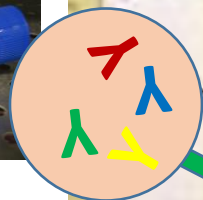
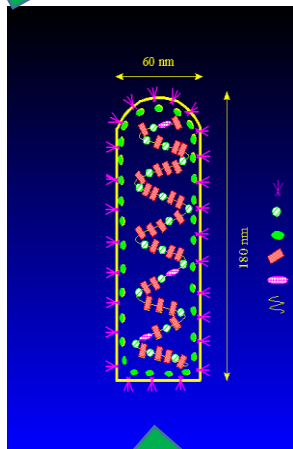
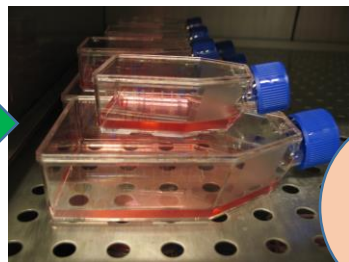


Photo by N.H.Henriksen, Danish Aquaculture

Monoklonale muse-antistoffer mod VHS virus -proteiner



Passive immunization against VHSV



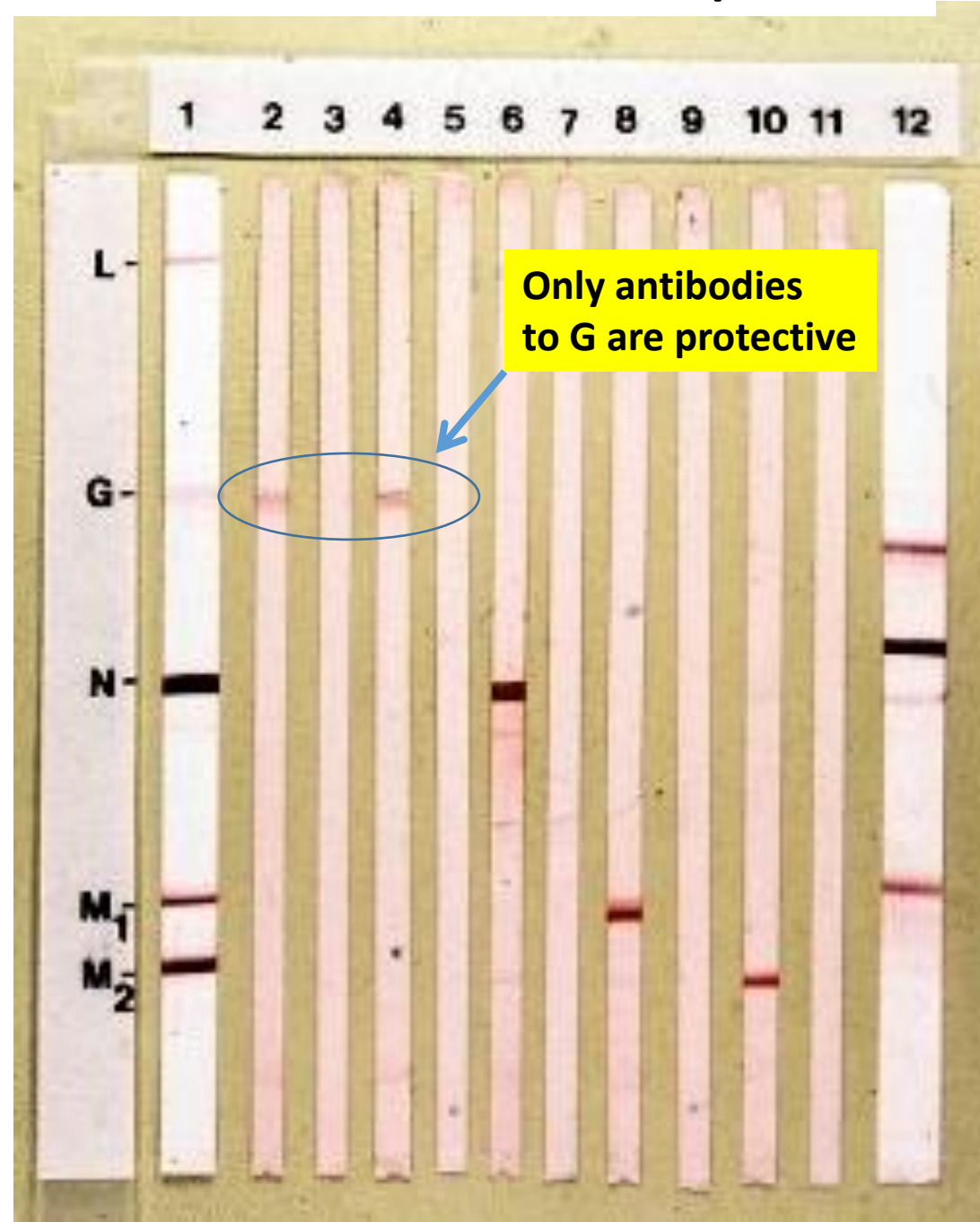
Y

Antibody
to VHSV

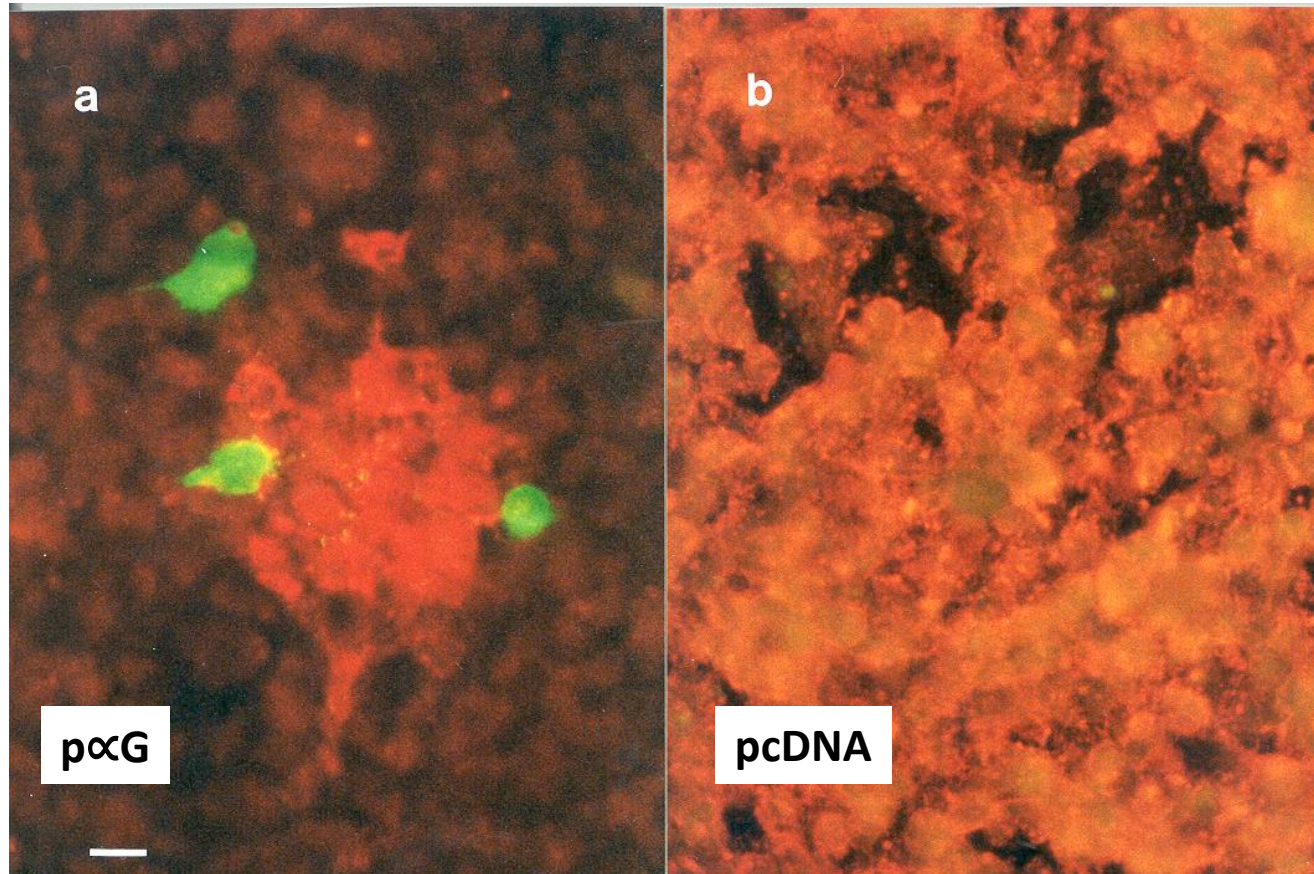
Virus challenge



Monoclonal antibodies to VHSV-proteins



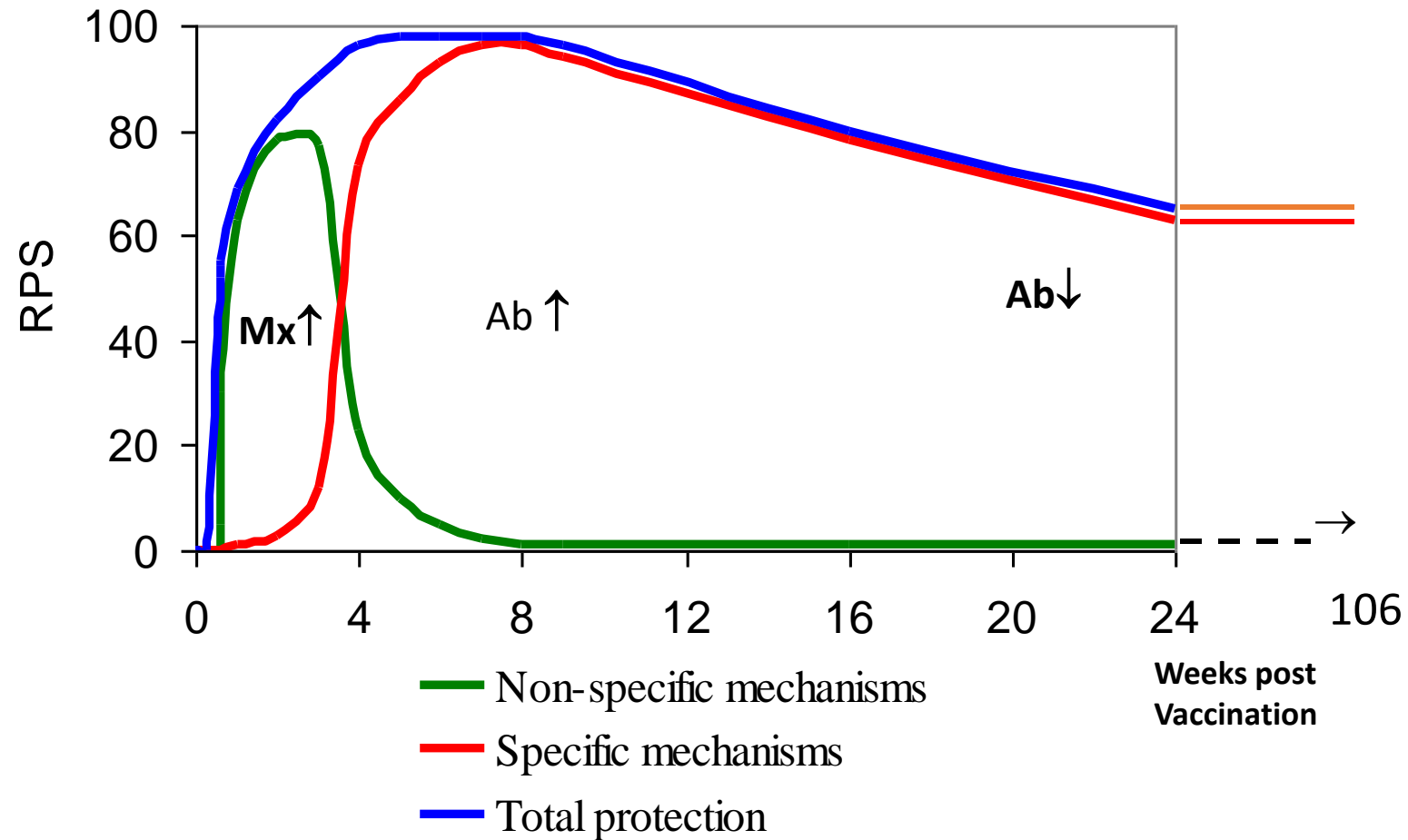
Mimicking antibody mediated protection against virus in transfected cells



Cell cultures were inoculated with virus 3 days after transfection with a plasmid encoding a virus-neutralizing antibody (pαG) or a control (pcDNA3).

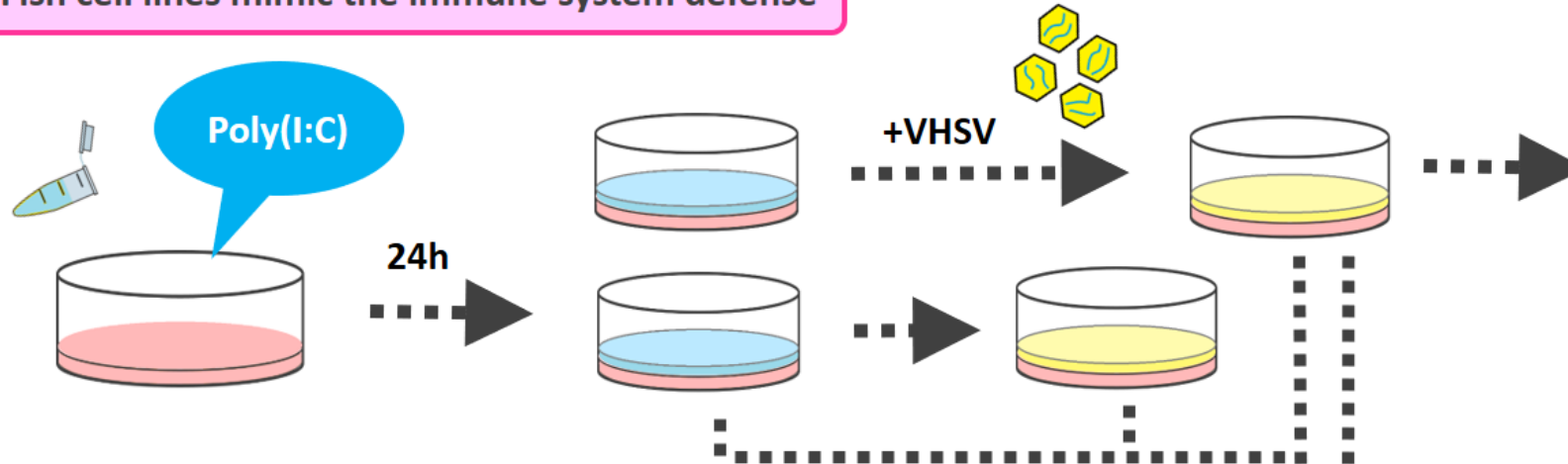
The antibodies produced by the pαG-cells protected the cell culture against the **cytopathogenic virus**

Time-course of protection following DNA vaccination against VHSV and IHNV



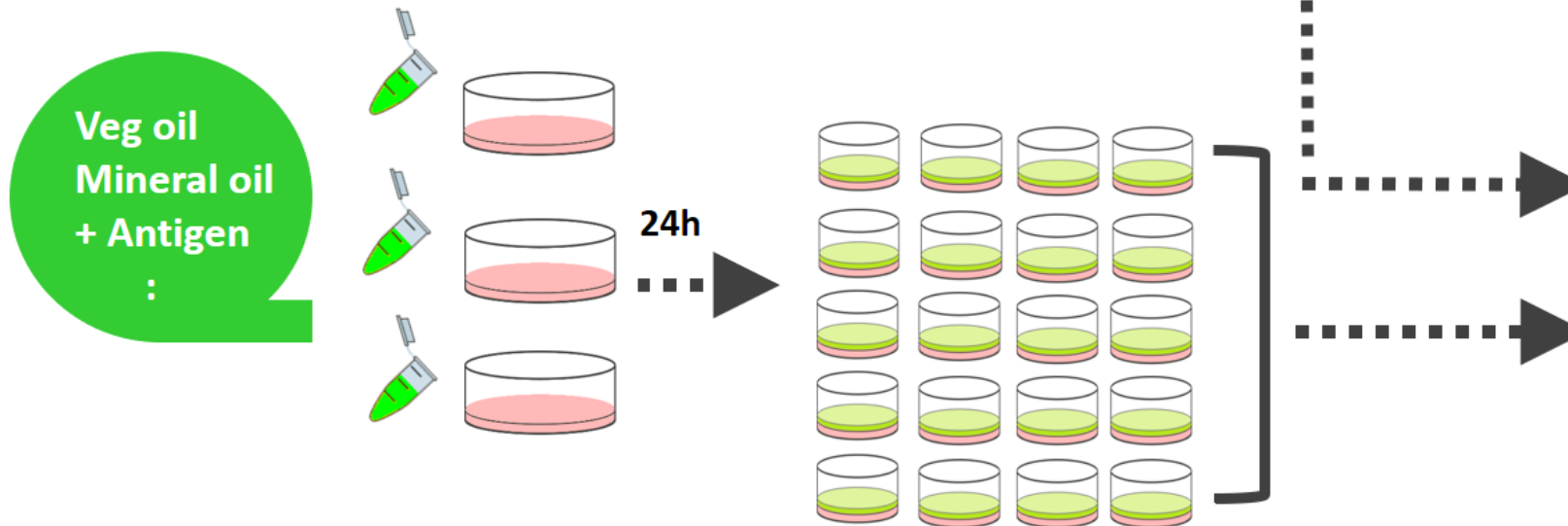
Workflow

Fish cell lines mimic the immune system defense



Confocal microscopy

In vitro platforms for evaluating adjuvants

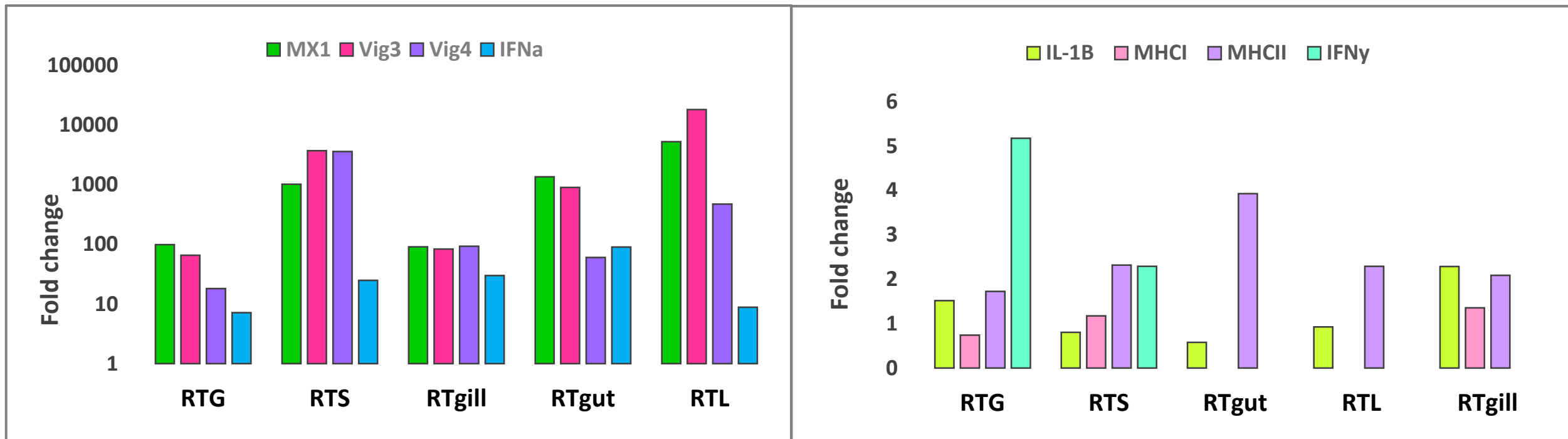


ELISA

QPCR analysis

Result - Fish cell lines mimic the immune response

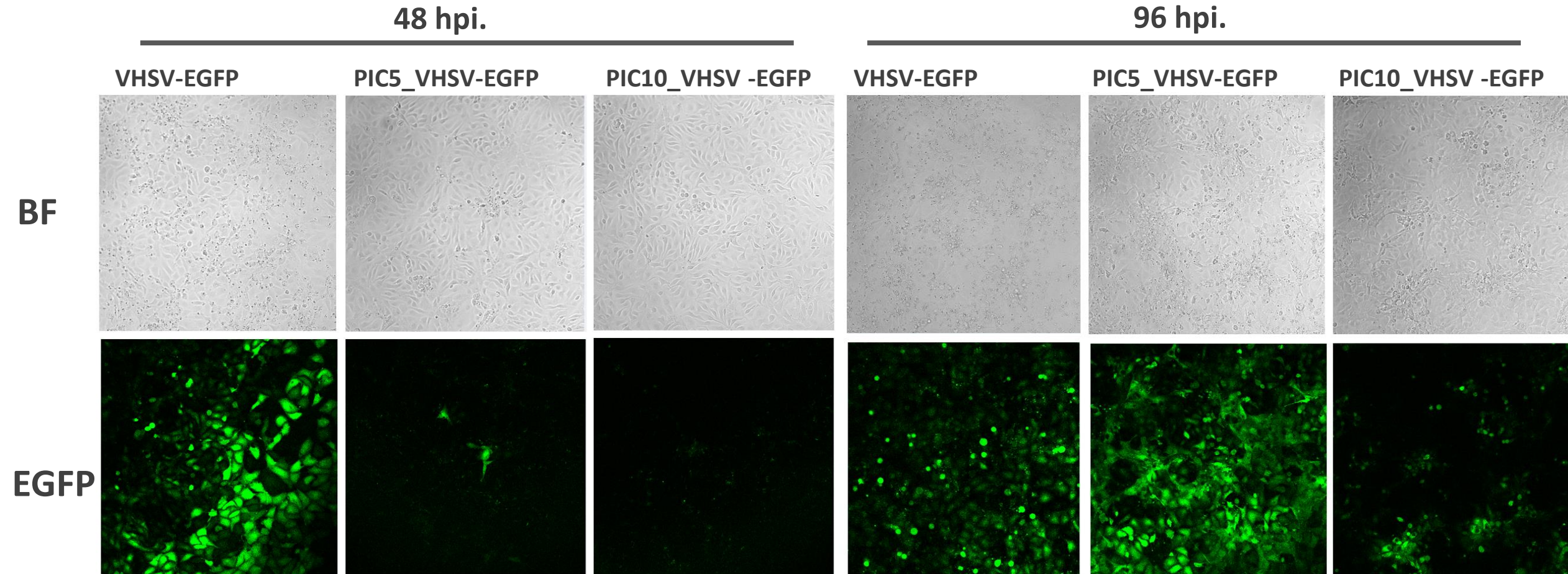
Immune gene expression analysis of fish cells treated with poly(I:C) for 24 hours
Poly(I:C)= synthetic dsRNA (potential vaccine adjuvant)



RTL (rainbow trout liver), RTgut (rainbow trout gut), RTgill (rainbow trout gill), RTG (rainbow trout gonad) and RTS (rainbow trout spleen)

Result - Fish cell lines mimic the immune system defense

Monitoring VHSV replication by confocal microscopy

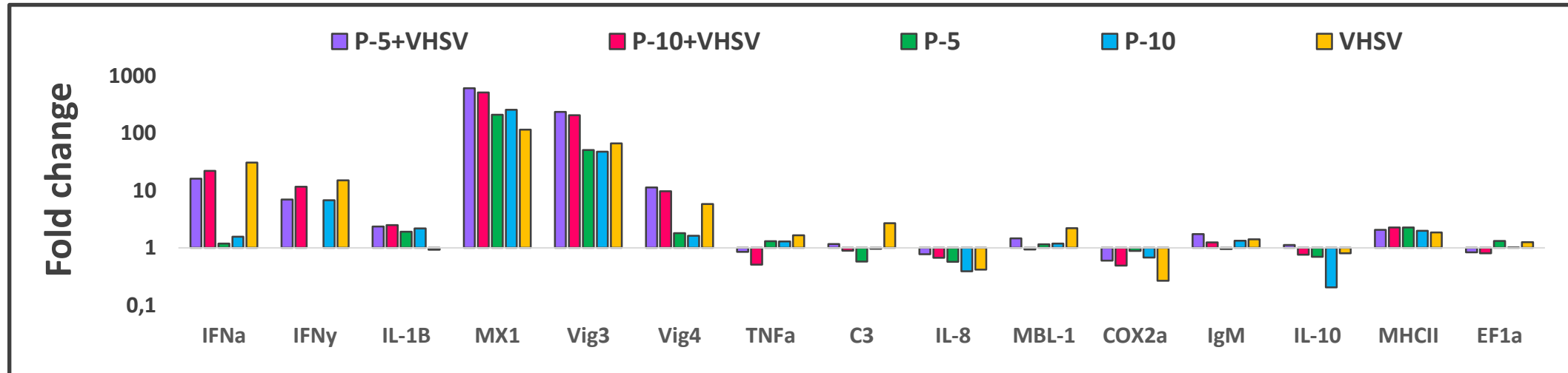


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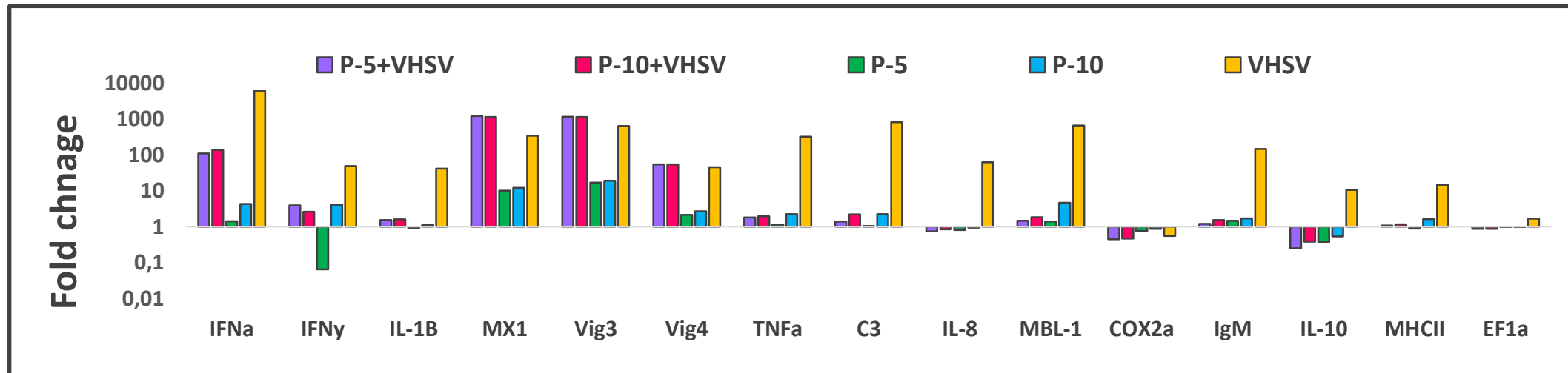
Result - Fish cell lines mimic the immune system defense

Quantitative analysis of the time-course of poly(I:C)-mediated antiviral assay

48h post-treatment



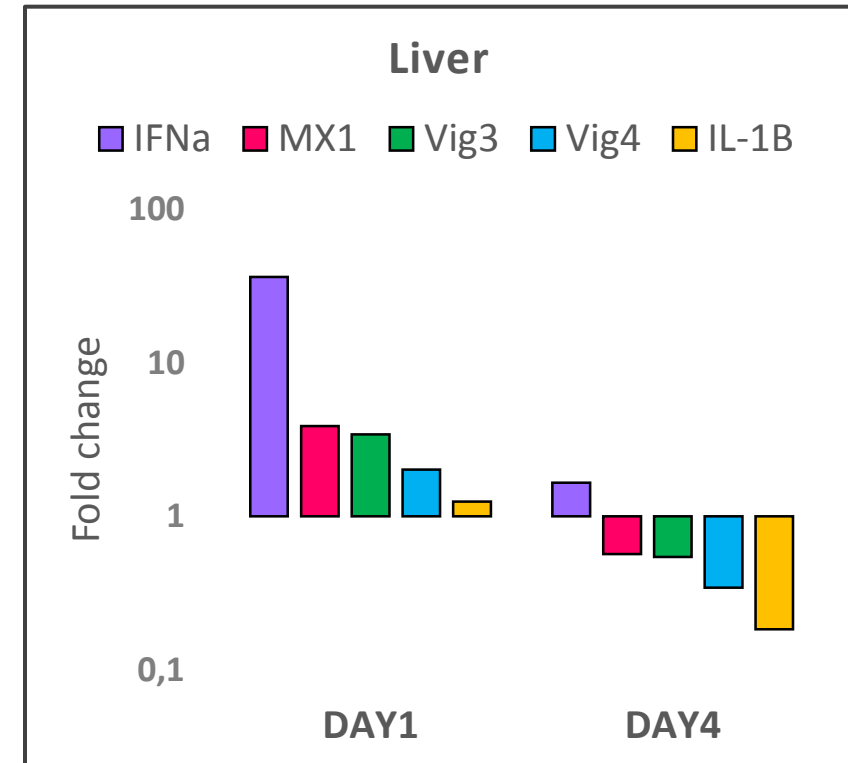
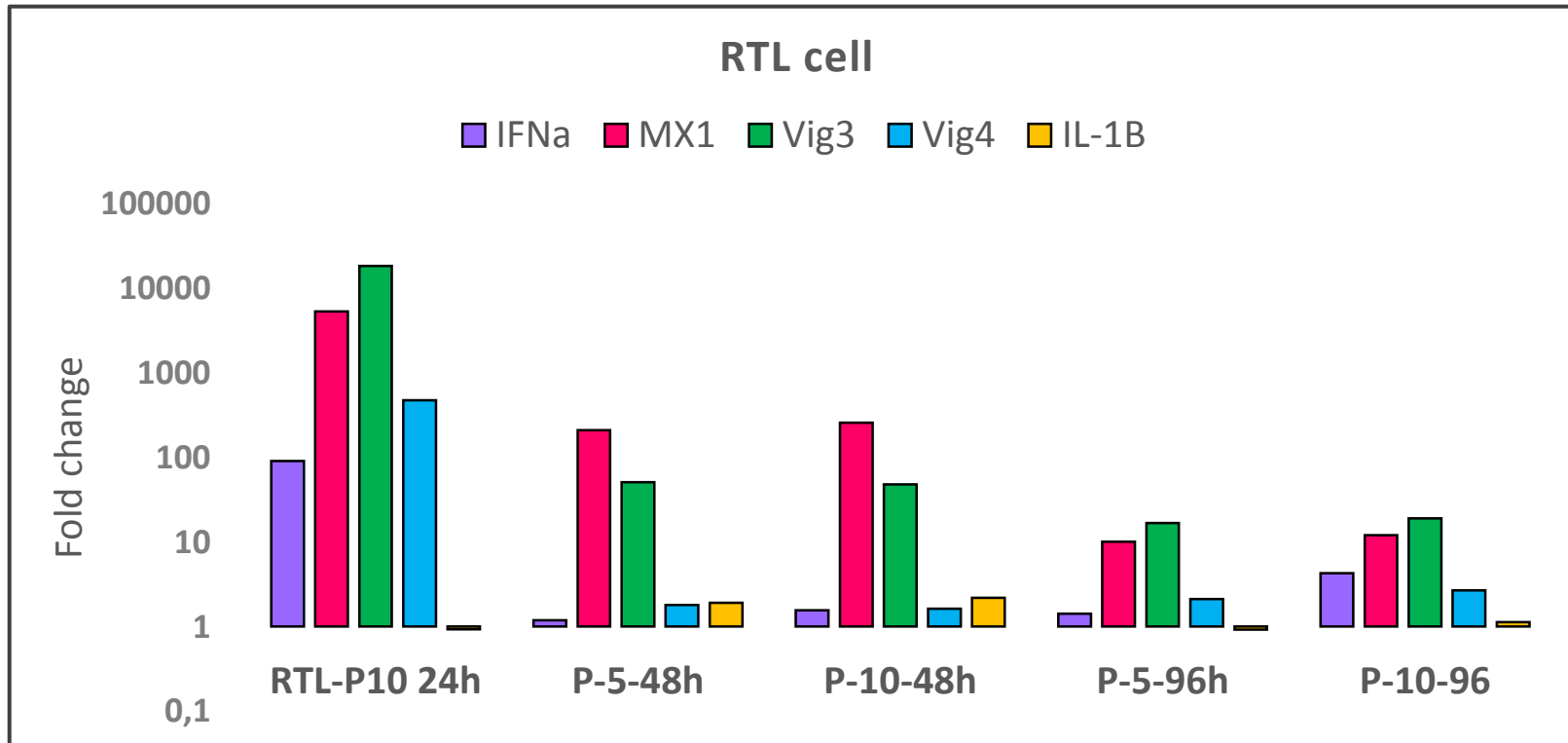
96h post-treatment



P-5: 5 μ g/ml of poly(I:C); P-10: 10 μ g/ml of poly(I:C)

Result - Fish cell lines mimic the immune system defense

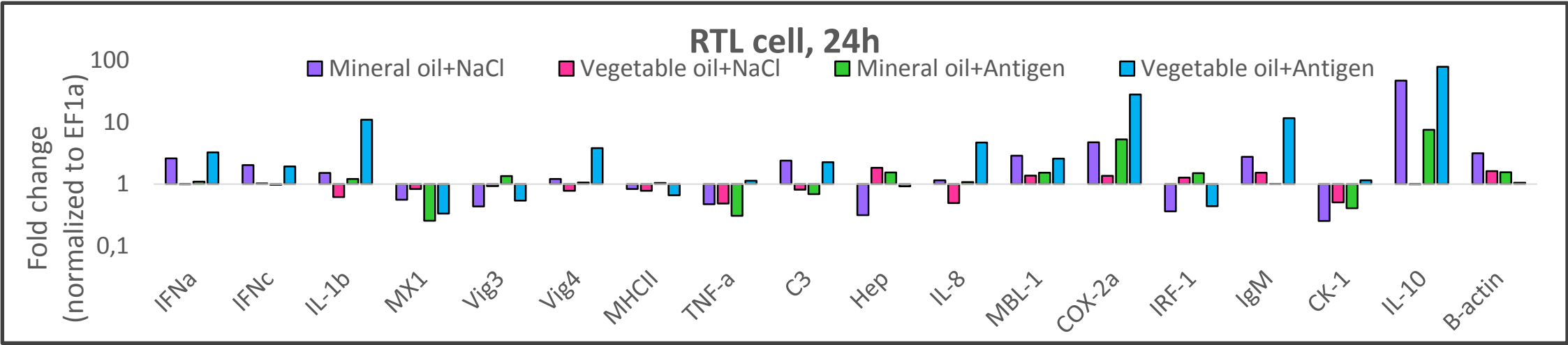
Quantitative analysis of the time-course of poly(I:C) induced gene expression



Oil-adjuvanted bacterial vaccines can induce adherances following ip vaccination



**Gene expression profiles of RTL cells stimulated with mineral or vegetable oil adjuvant
-correlation with vaccine induced side effects (?)**



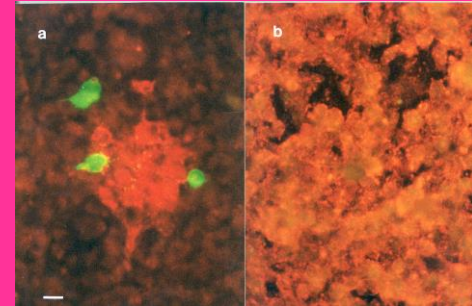
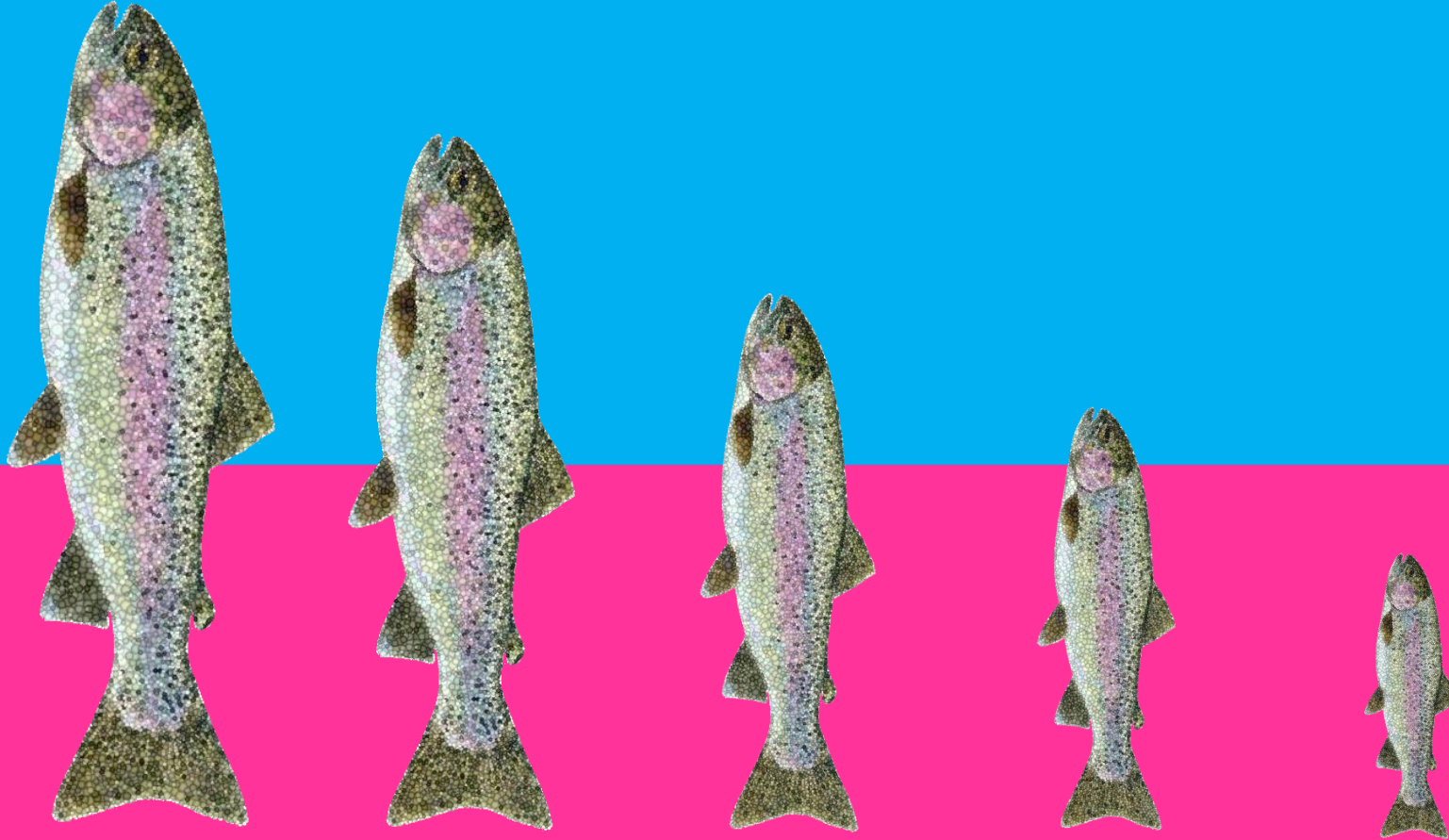
Conclusions

1. Vaccine-immunostimulant could protect the fish cell cultures against virus infection in a dose and time dependent manner, mimicking the innate immune response in the animal (fish) host.
2. Established cell lines can be used to address aspects of vaccine induced adaptive immunity, but panels of different cell lines are required to address the complexity of the immune system.
3. Cell culture technology has potential to partly substitute use of live fish in vaccine development.

Thanks **for** your attention!

Refinement

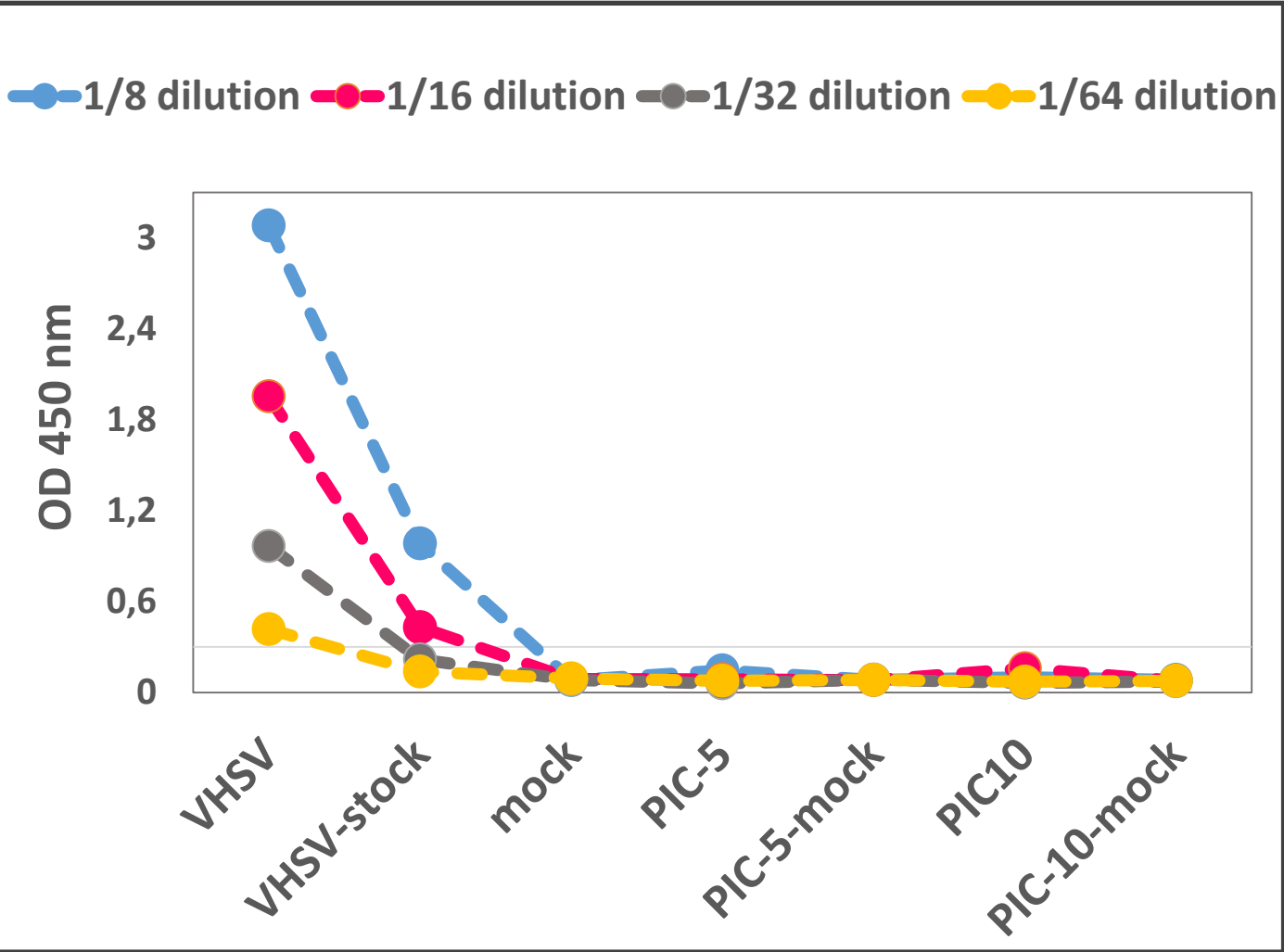
Reduction



Replacement

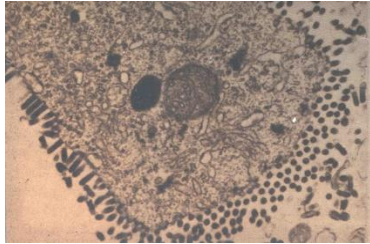
Result - Fish cell lines mimic the immune system defense

Detection of viral hemorrhagic septicemia virus (VHSV) using ELISA (enzyme-linked immunosorbent assay) - 48 hpi.

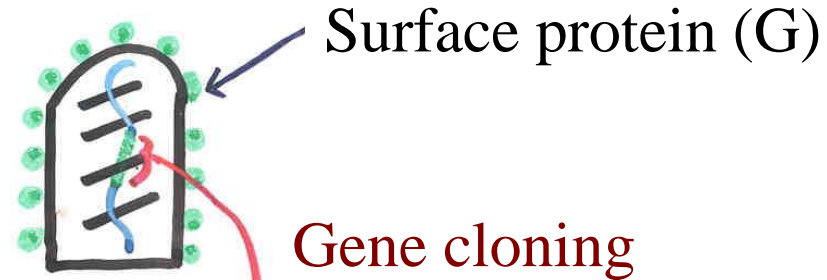


	VHSV	Poly(I:C)
VHSV	10 ^{4.4} TCID50/ml	-
VHSV-stock	10 ^{7.4} TCID50/ml	-
mock	-	-
PIC-5	10 ^{4.4} TCID50/ml	5 ug/ml
PIC-5-mock	-	5 ug/ml
PIC-10	10 ^{4.4} TCID50/ml	10 ug/ml
PIC-10-mock	-	10 ug/ml

DNA vaccination: the concept



Virus particle



Surface protein (G)

Vaccine plasmid
pcDNA3-vhsG

Fermentation +
Purification

Vaccination by
Intramuscular
injection

