



Prepare aquaria, stock fish, acclimate 10 days

T1.1 Prepare the 4 aquaria.

T1.2 Assess population distribution parameters: calculate mean for mass, size and associated standard deviations (if the population is gaussian).

T1.3 Stock 165 juveniles fish of at least 12-13 grams per aquarium.

T1.4 Record water parameter.

T1.5 Define feeding regime.

T1.6 Acclimate 10 days.

Monday, September 13

[Read more](#)

Recovery of the two strains *S.agalactiae* and *A.veronii* using TSA/TSB for 24h

T2.1 Recovery of the two strains

Wednesday, September 15

[Read more](#)

Cultivate bacteria 24h, draw growth-curves, count viable and dead cells, estimate biomass with spectrophotometer for $\lambda=600$ nm

T2.2 Stockage of bacteria for challenge test

T2.3 Production of vaccine Sa

T2.4 Production of vaccine Av

T2.5 Production of vaccine Sa + Av

T2.6 Production of vaccine Control

Thursday, September 16

[Read more](#)

Bacterial isolation for different organs in 5 randomly sampled juveniles using TSA/TSB or BHIA

T1.7 Bacterial isolation: Quality control step to prove that the fish population is free of diseases

Saturday, September 18

[Read more](#)



Mucus and blood swab for baseline week 0, end of acclimatation phase

T3.1 Weekly fish swabs (to collect biomarkers). Harvest head kidney and liver on 3 fish.

Monday, September 20

[Read more](#)



Immunization

T3.2 Immunization (Sa , Av , Sa + Av , Control)

Monday, September 20

[Read more](#)



Mucus and blood swab for week 1

T3.3 Weekly fish swabs (to collect biomarkers). Harvest head kidney and liver on 3 fish.

Monday, September 27

[Read more](#)



Mucus and blood swab for week 2

T3.4 Weekly fish swabs (to collect biomarkers). Harvest head kidney and liver on 3 fish.

Monday, October 4

[Read more](#)



Mucus and blood swab for week 3

T3.5 Weekly fish swabs (to collect biomarkers). Harvest head kidney and liver on 3 fish.

Monday, October 11

[Read more](#)



Immunization 2: booster

T3.6 Immunization second dose: booster(Sa , Av , Sa + Av , Control)

Monday, October 11

[Read more](#)



Mucus and blood swab for week 4

T3.7 Weekly fish swabs (to collect biomarkers). Harvest head kidney and liver on 3 fish.

Monday, October 18

[Read more](#)



Bacterial challenge test 1 (no booster)

T5.1 Expose to *Streptococcus agalactiae* the group vaccinated with Sa

T5.2 Expose to PBS the group vaccinated with Sa

T5.3 Expose to *Aeromonas veronii* the group vaccinated with Av

T5.4 Expose to PBS the group vaccinated with Av

T5.5 Expose to *Streptococcus agalactiae* the group vaccinated with Sa + Av

T5.6 Expose to *Aeromonas veronii* the group vaccinated with Sa + Av

T5.7 Expose to PBS the group vaccinated with Sa + Av

T5.8 Draw conclusions pt.1

Tuesday, October 19

[Read more](#)

Mucus and blood swab for week 5

T3.8 Weekly fish swabs (to collect biomarkers). Harvest head kidney and liver on 3 fish.

Monday, October 25

[Read more](#)

Mucus and blood swab for week 6

T3.9 Weekly fish swabs (to collect biomarkers). Harvest head kidney and liver on 3 fish.

Monday, November 1

[Read more](#)

Mucus and blood swab for week 7

T3.10 Weekly fish swabs (to collect biomarkers). Harvest head kidney and liver on 3 fish.

Monday, November 8

[Read more](#)

Mucus and blood swab for week 8

T3.11 Weekly fish swabs (to collect biomarkers). Harvest head kidney and liver on 3 fish.

Monday, November 15

[Read more](#)

Mucus and blood swab for week 9

T3.12 Weekly fish swabs (to collect biomarkers). Harvest head kidney and liver on 3 fish.

Monday, November 22

[Read more](#)



Mucus and blood swab for week 10

T3.13 Weekly fish swabs (to collect biomarkers). Harvest head kidney and liver on 3 fish.

Monday, November 29

[Read more](#)

Mucus and blood swab for week 11

T3.14 Weekly fish swabs (to collect biomarkers). Harvest head kidney and liver on 3 fish.

Monday, December 6

[Read more](#)

Lab assays to monitor immune response

T4.1 ELISA for each group of samples for IgM and IgT

T4.2 PCR of key immune genes

T4.3 Agglutination test

T4.4 Collect and analyze results

T4.5 Draw conclusions

Wednesday, December 8

[Read more](#)

Bacterial challenge test 2

T5.9 Expose to *Streptococcus agalactiae* the group vaccinated with Sa

T5.10 Expose to PBS the group vaccinated with Sa

T5.11 Expose to *Aeromonas veronii* the group vaccinated with Av

T5.12 Expose to PBS the group vaccinated with Av

T5.13 Expose to *Streptococcus agalactiae* the group vaccinated with Sa + Av

T5.14 Expose to *Aeromonas veronii* the group vaccinated with Sa + Av

T5.15 Expose to PBS the group vaccinated with Sa + Av

T5.16 Draw conclusions

Thursday, December 9

[Read more](#)

Final phase: draw conclusions

This is the content of the last section

Friday, December 10

[Read more](#)