



VIROBATHE Final conference; March 20 & 21 2007

**Work Package 6.1 and 6.2:
Training workshop and multi-centre trial**

Work package leader: RIVM



rivm

Work package 6.1 & 6.2

WP 6.1: Training Workshop

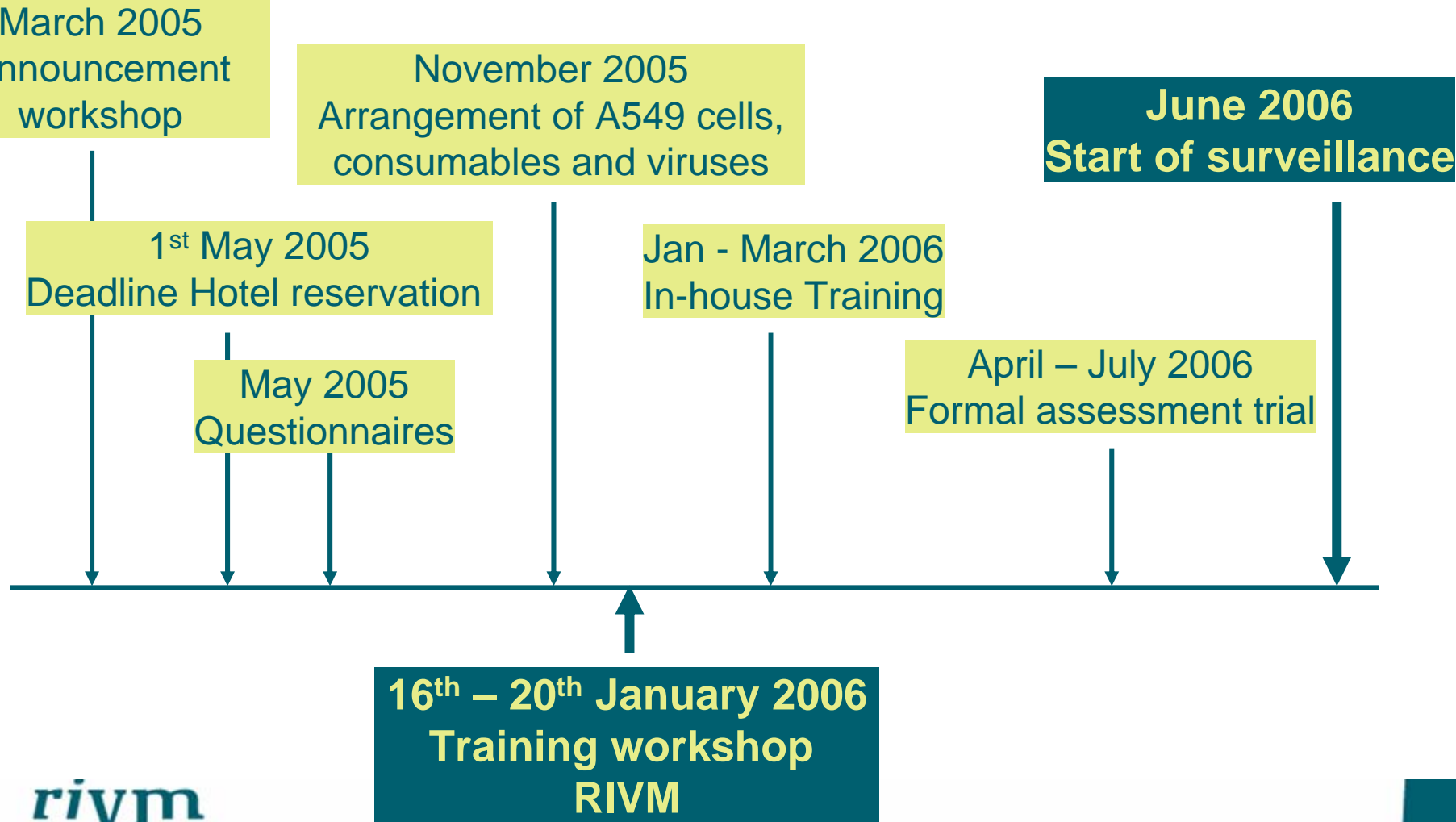
- 1) Receive Hands-on training in:
 - Virus concentration methods
 - (RT-)PCR
 - Cell culture

WP 6.2: Multi-centre trial

- 1) In-house training
- 2) Formal assessment trial



Time Schedule WP6.1 and 6.2



WP 6.1 Training workshop

- Questionnaire to participants to evaluate:
 - expertise
 - available techniques

Response	100%	15/15
Concentration	80%	12/15
PCR	87%	13/15
Cell culture	80%	12/15
Bacteria	67%	10/15
Phages	60%	9/15
Other	67%	10/15
Certification	40%	6/15



WP 6.1: Training Workshop

RIVM

Centre for Infectious Disease Control Netherlands

Laboratory for Zoonoses and Environmental Microbiology



rivm

16th-20th January 2006

WP 6.1: Training Workshop

1) Demonstration and

2) Hands-on training in:

- Virus concentration methods:

- Fresh waters: Glasswool filtration/beef extract elution

- Sea waters: Membrane filtration/ skimmed milk elution

- Molecular detection of viruses:

- Adenovirus: nested PCR

- Norovirus: nested RT-PCR

- Detection of infectious adenovirus

- Integrated cell culture-PCR



WP 6.1: Training Workshop

- 2) Presentation of progress from other work packages
- 3) Address safety aspects of handling potentially infectious virus
- 4) Planning audit arrangements and in-house training opportunities
- 5) Explanation of the statistical aspects of the project
- 6) Discuss choice of surveillance sites and sampling procedure



WP 6.1: Training Workshop

Review of the Training Workshop:

- Feedback questionnaire
- Overall score:
 - good to very good
- Useful remarks:
 - More product information was needed (producers, cost)
 - Participants should be able to contact each other (sharing address information)
 - More time could have been reserved to discuss other WP



WP 6.2: Multi-centre trial

Part 1: Training phase

- In house-training

- Practice virus concentration methods, (RT-)PCR for adeno- and noroviruses detection and the adenovirus infectivity assay
- Support by WP6 leader

Part 2: Formal assessment trial

- Assessment of detection methods

- (RT-)PCR for adeno- and noroviruses, adenovirus infectivity assay, concentration of natural samples

- Assessment of combined concentration/detection methods

- 12 tap waters/ artificial seawaters seeded with unknown concentrations of virus



WP6.2 Formal assessment trial

Criteria for the detection methods

Method	Criteria		Comment
	Number of positives	RNA/DNA dilution	
Adenovirus PCR	4 out of 6	1/10	
Norovirus RT-PCR	6 out of 6	1/1000	
ICC-PCR	3 out of 4	Undiluted; 1/10 (inhibition)	2 out of 2 negatives
Conc. natural sample (Noro- and adenovirus)	4 out of 5	Undiluted; 1/10 (inhibition)	

Passing criteria: continue to 2nd part of assessment trial

WP6.2 Formal assessment trial

Criteria of the combined concentration/detection methods

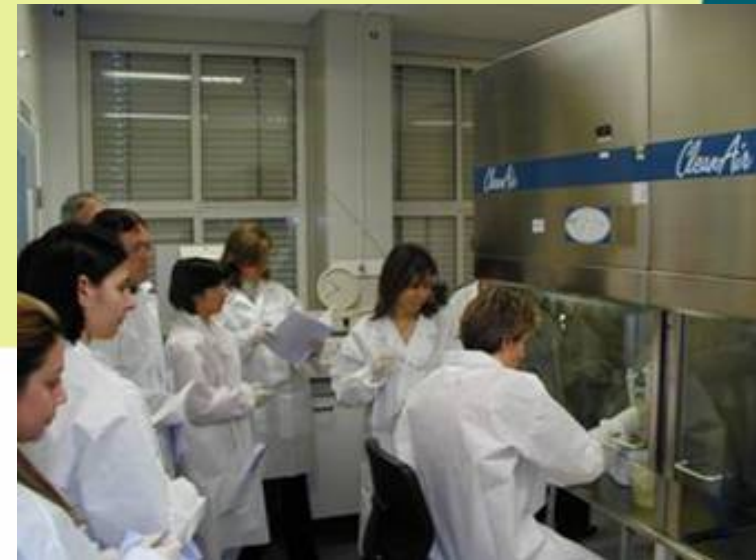
Method	Criteria		Comment
	Number of positives	RNA/DNA dilution	
Adenovirus PCR	6 out of 10	Undiluted	2 out of 2 negatives, duplo
Norovirus RT-PCR	6 out of 10	Undiluted	2 out of 2 negatives, duplo
ICC-PCR	6 out of 10	3x undiluted, 1x T0	2 out of 2 negatives, duplo

Passing criteria: continue to surveillance phase (WP7)

Results Formal assessment trial

Observed problems:

1. Too few positives
2. False positives
3. Absence of Internal Amplification Control (IAC)
4. Inhibition in natural samples
5. Very tight time schedule



Observed problems in WP6.2

1) Too few positives

- Concentration of IAC too high:
 - Competition with target RNA/DNA

Re-titrate IAC

- Incomplete inactivation of Uracil-N-Glycosylase (UNG)
 - Remaining UNG activity might degrade first round PCR product

Omit UNG

- Presence of inhibitors:
 - Originating from the water sample
 - Due to improper RNA/DNA extraction

Dilute the extracted RNA/DNA



Observed problems in WP6.2

2) False positives

- Contamination during concentration

- Insufficient decontamination of concentration equipment
- Contaminated pH-electrode
- Contamination with high titer spike suspensions (pos controls)

Use freshly prepared hypochlorite solutions

- Contamination in PCR

- Amplification products in PCR rooms

Use separate rooms for 1st and 2nd rounds

- Contamination in cell culture-PCR

- Cross contamination between cell culture flasks
- Positive T0 samples

Dilute T0 and T5 if contamination is excluded

Observed problems in WP6.2

3) Absence of Internal Amplification Control

- Concentration of IAC too low in original dilution
 - Prescribed dilution of IAC in SOP might not be sufficient

Re-titrate IAC

- IAC dilution is unstable
 - Dilutions were initially prepared in water
 - Freezing/thawing

Aliquot the diluted IAC in a buffered solution

- Presence of inhibitors in the sample

Observed problems in WP6.2

4) Presence of inhibitors in natural samples

- No IAC signal present in undiluted sample
 - If IAC signal is present in controls
 - More inhibition in marine than fresh waters

Dilute the sample (up to 1000 times)

Follow up:

- Smaller volume of concentrate in extraction
- Additional purification to eliminate inhibitors



Observed problems in WP6.2

5) Very tight time schedule

- At the time of the workshop

- Not decided yet on concentration methods

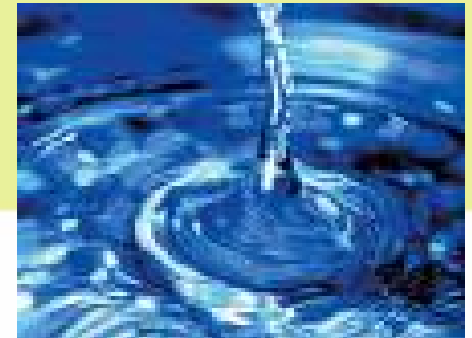
- Retesting during assessment

- Retitration of IACs; limited knowledge on norovirus GGI IAC concentration
- Get rid of contamination
- RNA/DNA extraction method not optimised for samples with high concentration inhibitors
- UNG re-activation

Further optimisation during surveillance phase

Future lessons

- 1) Plan more time for optimisation of new methods
- 2) More time is required for implementation
- 3) Positive controls should be available for all viruses
- 4) An interactive website will stimulate communication between laboratories
 - Will help to solve difficult results/problems more efficiently



WP6.2 Formal assessment trial



All participating laboratories
passed
the formal assessment



rivm