

Supplementary materials for:

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Supplemental Appendix 1. Laboratory manual



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Antibiotic use was defined as patient reported intake of antibiotics (one dose or more) for at least 5 days in the first 10 days after the index GP consultation.

Data are presented as number (%), unless mentioned otherwise. SD = standard deviation.

* Pulmonary co morbidity = history of COPD, asthma or other lung disease. Cardiac co morbidity = history of heart failure, ischemic heart disease or other heart disease. DM = diabetes mellitus.

† Presented in days.

‡ Score for 14 patients' GP-recorded symptoms summed and scaled to range between 0 and 100 at day 1.

§ P value compared included versus excluded patients.

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INTRODUCTION

For WP 9, GRACE Contract no. LSHM-CT-2005-518226, the Central Lab has provided you with:

- This Investigator - Laboratory Manual
- An Investigator Requisition Form and a Local Lab Requisition Form
- Visit specific sampling kits for:

Observational study

- **Visit 1** (Pre-Treatment/Day 0)
- **Visit 2** (4 weeks after visit 1/Day 1)

Control group

- **Visit 0**

Sampling material supplied by the Central Lab (University of Antwerp, Belgium)

- Serum tube and serum transfer tube
- EDTA blood sampling tube
- Sputum container
- Nasopharyngeal swab with Virus Transport Medium
- Nasopharyngeal swab with Amies medium
- Microbanks with pearl medium (only for use in local labs)

Documents

The material is accompanied by two Requisition Forms: **An Investigator Requisition Form** (annex 1) and **A Local Lab Requisition Form** (annex 2).

For the investigator

- **The investigator** has to fill in the **Investigator Requisition Form** on the moment of the sampling. The **Investigator Requisition Form** will be kept by the investigator and a fax forwarded to the local National Network Facilitator (NNF) within 24 h after sampling. The **unfilled Local Lab Requisition Form** is accompanied by the samples, the unused sampling material and the unused labels, at the moment of the transport to the local lab

For the Local Lab

- The Local lab has to fill in the **Local Lab Requisition Form** on the moment of arrival of the samples. The **Local Lab Requisition Form** will be kept by the local lab and a fax forwarded to the local National Network Facilitator (NNF) within 24 h after arrival of the samples.

Please make sure that the Forms are filled in completely and legibly. If an error is made, cross out the mistake, write the correct information and initial and date the change.

STUDY PROCEDURES AND OBSERVATIONS

For all assessments to be performed per visit, please refer to the Sample Handling Procedures in chapter 4.

Overview of samples to be taken per visit

	Observational study		Control group
Sample	V1	V2	V1
Serum	S	S	
EDTA blood	S		S
Sputum	S		
Nasopharyngeal swab with virus transport medium	S	S	S
Nasopharyngeal swab with Amies medium	S	S	S
V1 = Day 0 (Pre-treatment) V2 = Day 1 (4 weeks after visit1)		S = Samples to be taken	

Overview of handlings to be performed by the local lab

Sample	Handling, Preparation or Test
Serum	Preparation of the serum and storage of the serum tube preferable at -70°C
EDTA blood	Store tube preferable at -70°C
Sputum	Direct microscopy
	Gram Stain
	Culture of <i>S. pneumoniae</i> and <i>Haemophilus spp.</i>
	Preparation of 2 Microbanks per <i>Streptococcus pneumoniae</i> and <i>Haemophilus species</i> isolate.
Nasopharyngeal swab with virus transport medium	Store the swab preferable at -70°C
Nasopharyngeal swab with Amies medium	Store the swab preferable at -70°C
V1 = Day 0 (Pre-treatment) V2 = Day 1 (4 weeks after visit1)	

Overview of analysis to be performed by the central lab or by the labs from the other Workpackages

Sample	Test	Observational study		Control group
		V1	V2	V1
Serum	Serology for <i>M. pneumoniae</i> , <i>C. pneumoniae</i> and <i>L. pneumophila</i>	T	T	
	C-reactive protein	T	T	
	Procalcitonin	T	T	
EDTA blood	Human Genetics	T		T
Sputum	Susceptibility testing on <i>S. pneumoniae</i> and <i>Haemophilus spp.</i>	T		
	Molecular analysis on <i>S. pneumoniae</i> and <i>Haemophilus spp.</i>	T		
Nasopharyngeal Swab in Viral transport medium	Viral culture	T		T
	Antigen tests	T		T
	Virus discovery	T	T	T
	PCR for <i>Chlamydia pneumoniae</i> , <i>Legionella pneumophila</i> , <i>Mycoplasma pneumoniae</i> , <i>Bordetella sp.</i> , <i>Streptococcus pneumoniae</i> and respiratory viruses	T	T	T
Nasopharyngeal swab in Amies medium	PCR for atypical bacteria	T	T	T
	Growth of <i>Streptococcus pneumoniae</i> and <i>Haemophilus spp.</i>	T		
V1 = Day 0 (Pre-treatment) V2 = Day 1 (4 weeks after visit1)		T = Perform test		

LABORATORY TEST ASSESSMENTS

To perform and/or to prepare the specimens for each test, please refer to the detailed description of each Laboratory Test in chapter 4.4 (Sampling Instructions)

Local Laboratory

- In your Local Laboratory the following tests are to be performed if the sample is present:

- Sputum : Direct microscopy, Gram stain and culture
-

If sputum cultures are positive for *S. pneumoniae* and/or *Haemophilus spp*



Preparation of Microbanks for the Central Lab

- In your Local Laboratory the following specimen have to be prepared for the Central Lab, if the sample is present:
 - A serum sample (in the 10 ml serum transfer tube)
 - Microbanks with sputum pathogens *S. pneumoniae* and/or *Haemophilus spp.* (see above)
- Additionally the following samples have to be stored until shipment to the Central Lab, if the sample is present:
 - An serum sample (see above)
 - An EDTA blood sample
 - Nasopharyngeal swab in virus transport medium
 - Nasopharyngeal swab in Amies medium
 - Microbanks (see above)

After preparation, put the samples, together with the unused labels, in a zip-lock bag. Store them in your freezer, preferably at -70°C until shipment to the Central Lab.

Only in case you do not have the facilities to freeze at -70°C you can store them at -20°C.

Central Laboratory (UA)

The Central Laboratory will perform the following tests on your collected samples:

- Serology for *M. pneumoniae*, *C. pneumoniae* and *L. pneumophila*
- CRP and procalcitonin in serum
- Transfer EDTA blood specimen to Workpackage 4 (WP4) for human genetics.
- Susceptibility testing on *S. pneumoniae* and *Haemophilus spp.*
- Transfer *S. pneumoniae* and *Haemophilus spp.* strains to WP6 and WP7, respectively, for molecular analysis
- PCR for *C. pneumoniae*, *L. pneumophila*., *M. pneumoniae*, *B. pertussis*, *S.pneumoniae* and respiratory viruses
- Aliquot PCR material and transfer to WP3 and WP5 for virus detection and virus discovery respectively
- Aliquot virus transport medium and transfer to WP3 for viral culture and antigen tests and to WP5 for virus discovery
- PCR of atypical bacteria using an nasopharyngeal swab in Amies transport medium
- Culturing *S. pneumoniae* and *Haemophilus spp.* using a nasopharyngeal swab in Amies transport medium

SAMPLE HANDLING PROCEDURES

Investigator Requisition Form

First complete the next information on the Investigator Requisition Form (Annex 1):

- Patient study number (**use same label as in CRF**, see 4.2 Material Preparation)
- Patient gender (F/M)
- Date of Birth (dd/mm/yyyy)
- Collection Date (dd/mm/yyyy)
- Collection Time (24 hours clock)
- Visit number (tick the right box)
- Specimens collected (tick the right box)

Material Preparation

Observational study

Visit 1 => take the observational study visit 1 sampling kit and open the big zip-lock bag.

Visit 2 => take the observational study visit 2 sampling kit and open the big zip-lock bag.

For Control group

Visit 0 => take the control group visit 1 sampling kit and open the big zip-lock bag.

Each tube has to be identified with a label. Labels are coded with a patient study number and a visit number.

Labels are to be stuck vertically on the tubes.

The same labels are used for the Patient Registration Form, Case Record Form (CRF), Investigator Requisition Form, Local Lab Requisition Form, ...