

EPI SOUTH PLUS - WORK PACKAGE 4

Mediterranean Regional Laboratory Network

WP4 TRAINING

WEST NILE AND BIOSAFETY II IN THE LABORATORY

24 - 28 June 2013, Instituto de Salud "Carlos III", Madrid (SPAIN)

REPORT



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International Division, Institut Pasteur (France)



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Department of Microbiology Reference Laboratories,
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Introduction

The second EpiSouth laboratory training session took place at the Instituto de Salud “Carlos III”, Madrid (Spain) from 24 to 28 June 2013. It has been organised by the WP4 team with the Laboratory of Arboviruses and imported viral diseases of the ISCIII and Philippe Dubois, consultant on Biosafety, previously WHO trainer.

1.1. OBJECTIVES OF THE TRAINING

The objectives of this training were to improve the diagnosis and understanding of West Nile and the level of biosafety in the laboratories of the EpiSouth MRLN and to enhance the networking among participating laboratories. The biosafety modules were complementary to the modules discussed on the first training, but could be attended by new trainees.

For the Agenda, see Annex 1.

1.2. PARTICIPANTS

The WP4 requested the heads of laboratories that were represented during the first training to nominate the same trainee if possible. For the new participating laboratories, the criteria were the same than for the first training: permanent staff of the laboratory, active person working at the bench, English and/or French speaker, able to provide training to other laboratory members at the return in her/his laboratory.

The twenty laboratories (7 EU and 13 non-EU) that participated were:

Albania	Italy (ass. lab*)	Palestine
Algeria	Jordan	Romania
Bosnia & Herzegovina	Kosovo	Serbia
Bulgaria	Lebanon	Spain
Cyprus	Libya	Tunisia
FYROM	Malta	Turkey
Greece	Montenegro	

** The Italian laboratory member of the core group could not propose a trainee for the training, so one from the associated laboratory “Lazzaro Spallanzani” has been proposed to participate with the agreement of the EpiSouth coordination and the Italian lab part of the core group.*

In the frame of the good collaboration and the optimisation of already existing expertise on West Nile, international experts, especially from the EuroWestNile project that is coordinated by the host laboratory, were involved to give lectures. The EpiSouth WP5 was also invited.

For the list of participants see Annex 2.

2. The training

As for the first training on Dengue and Biosafety, the training combined theoretical and practical courses related to the laboratory diagnosis of West Nile and Biosafety.¹

Likewise, trainees worked in pairs during theoretical and practical courses and compared results from different available commercial serological diagnostic kits for West Nile diagnosis in order to address limits and differences between the techniques. The aim of this comparison analysis is to identify strengths and weaknesses of the different available kits and be able to choose (performance or price) the most adapted for the home situation.

The training is finalised by a case study session.

Theoretical part

The **global context** of West Nile has been set by different experts; lectures on epidemiology, clinic, ecology, veterinary and laboratory-related lectures have been given.

A **round table** with international experts of the Region has been organised on the first day of the training so as to share experience and introduce the complex issue of the emergence of West Nile in the Mediterranean region. The aim of this round table was to discuss about **how a country with no case of West Nile faces the first suspected case and how this country should get prepared to face the possible next outbreaks by organising a surveillance of West Nile**. There was a focus on the laboratory point of view but the discussion was also about the other partners that the laboratory staff should include in case of a WN outbreak (role of the government/ officials/ Public Health institutions/etc. in the different countries, place of the veterinarians, of the entomologists, etc.).

The discussion was structured around 5 axes that have been introduced by different intervening experts:

First situation, a country with no experience in West Nile detection faces a possible first case

1. What happens when the apparition of the 1st suspected case of West Nile appears in the country? (*Intervener: Anna Papa-Konidari, Aristotile University of Thessaloniki, Greece*)

¹ Note: The presentations of the training are available on the private area of the EpiSouth website in the ENWA WP4 section: https://nwa.episouthnetwork.org/index.php?tab=3&id_area=2066&topic=Training

2. What kind of investigation/outbreak team should be set-up for a case of a suspected WN outbreak? (*Intervener: Mari Paz Sanchez Seco, Instituto de Salud Carlos III, Spain*)

3. In a country that never faced an outbreak before, what is the set of minimum tools that a laboratory should have to support the diagnosis of WN? (*Intervener: Gülay Korukluoğlu, Turkish Public Health Institution, Turkey*)

Now, the country is becoming endemic and it is decided to set-up a surveillance system.

4. What experts should be part of a surveillance team? (*Intervener: Aykut Özkul, Ankara University*)

5. What are the new tools that a (future) reference laboratory should acquire? Technology updates? (*Intervener: Maria Rosaria Capobianchi, Istituto Nazionale per le Malattie Infettive (INMI) "L. Spallanzani", Italy*)

A module on **case studies** has been organised at the end of the training to summarize and put into perspective what has been learnt throughout the week. This module has been more developed (4 hours instead of 2 hours and use of an interactive tool) during this second training upon request of the trainees at the end of the first training.

The **biosafety modules** were organised in two parts: a biorisk assessment module, including exercises on a computer-based tool and an interactive lecture (with video images) on facility design (BSL3) and maintenance.

2.1. PRACTICAL PART

The objective of the practical course was to learn how to perform or to improve the West Nile laboratory diagnosis (molecular biology and serology).

The used laboratory protocols for PCR methods have been developed by the Spanish partners (ISCIH and CISA-INIA) and for serology kits were used.

Real-time RT-PCR

It enables the detection of West Nile virus DNA in acute phase samples and is the preferred molecular tool to detect West Nile virus early in the course of West Nile infection. Identification with this technique can distinguish the different serotypes. An “in-house” protocol and primers have been used for the multiplex PCR method used by the ISCIH laboratory and shared with the trainees.

IgG and IgM ELISA

As most of the laboratories used commercial kits to perform the serology of WN. WP4 asked the participating laboratories, before the training, what commercial kits were accessible in their country, in order to use these kits for the training. Except 2 laboratories that each used different kits difficult to access in all other participating countries, the laboratories were using the same 2 kits. Therefore, it has been decided to use these 2 kits and compare them for their sensitivity and specificity during the course. This to discuss the limits and strengths of these 2 kits, using a panel of well characterised sera from patients. The used panel has kindly been provided by the Greek laboratory of the MRLN (Anna Papa-Konidari, Aristotle University of Thessaloniki), also identified as a supportive laboratory for the MRLN.

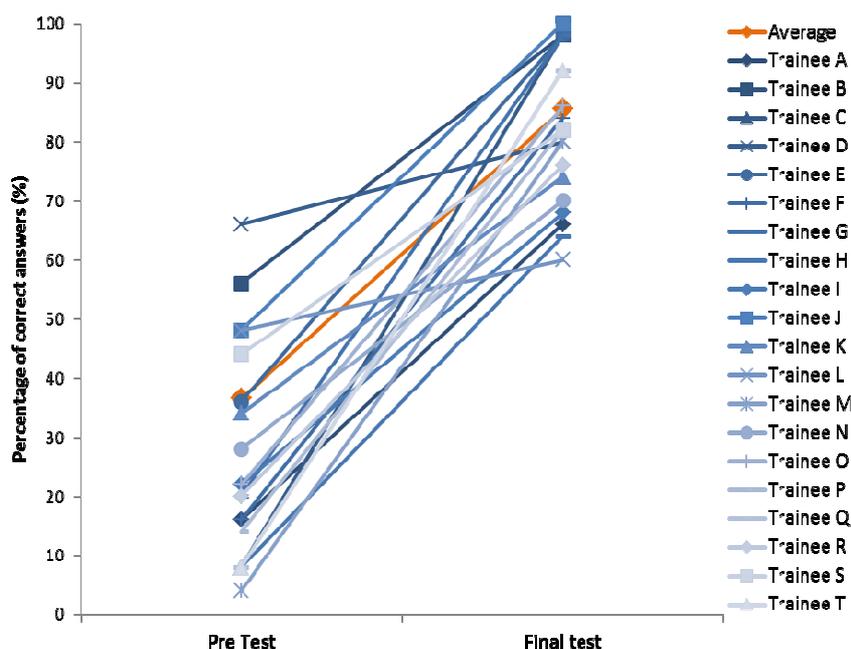
Five sera were analysed by ten pairs of trainees. Each serum was tested twice by 2 different pairs of trainees (each pair using a different kit for IgG and IgM). The results obtained from the different kits and trainees have been discussed and analysed collectively. It appeared that the most expensive kit was also the most performant one.

3. Evaluation

3.1. EVALUATION OF THE TRAINEES

The efficiency of the training has been evaluated by a short test on biosafety/biorisk knowledge. As for the first training, the same test was done by the trainees on the first morning and on the last afternoon of the training week (*See Annex 3*).

Graph 1 describes the level of knowledge of the trainees on biosafety and biorisk before and after the training. It shows that the level of the trainees was heterogeneous before and after the training but the level of all the trainees clearly improved: from 4 to 66% of good answers at the pre-test and from 60 to 100% of good answers (12 trainees over 17 had 100% of correct answers).



Graph 1: Improvement of the level of knowledge of the trainees on biosafety and biorisk before and after the training.

3.2. EVALUATION OF THE TRAINING

On the last afternoon of the training, an evaluation survey (see *Annex 4*) has been addressed to the trainees to evaluate the different modules of the training (see *Graphs 2 to 5*) and to assess their expectations for the future of the MRLN and EpiSouth network (see *Table 1*). A discussion completed this assessment.

All the trainees were very satisfied by the training (overall score of the quality: 4.5/5) and 100% of the trainees declared that the training met their expectations.

Regarding the sequencing module, during the discussion, there was also a great demand to have more time to focus on. This topic has been explored during this training, but as it is a vast topic and trainees had heterogeneous skills on the subject, it has been difficult to totally reach the expectations. At least, this has raised awareness towards this discipline and underlined the necessity of sequencing surveys to control the spread of the disease.

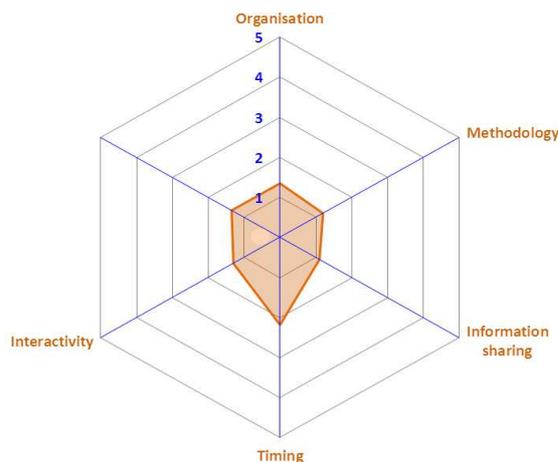
The Table 1 describes the perspectives and expectations of the trainees after the training and after the end of the project.

The trainees are expecting the continuation and sustainability of the MRLN after the project ends. Their top 3 expectations are respectively to improve capacity (mostly with training), to

exchange and share information and to extend the topics to other pathogens (emerging plus respiratory diseases have been mentioned).

Concerning their expectations for the possible next trainings, they would like more practice, an extended time or a reorganisation of the schedule to have an agenda including the next steps in WN diagnosis (IFA, seroneutralisation, culture). The seroneutralisation and cell culture remained a request. However due to i) the important number of participants ii) their heterogeneous background and level iii) the few having access to BSL3 facilities in their home situation, the possibility of organising such a training and its impact would remain very limited. Nevertheless, the IFA technique could be considered as a technique to be taught in a future training on WN.

Further requests were expressed concerning further training on the use of sequences and sequence analysis for molecular epidemiology of WN.



Graph 2: Evaluation of the lectures of the external experts.
(1: very good → 5: very bad)

Graph 3: Evaluation of the Biosafety modules.
(1: very good → 5: very bad)

Figure 1: Average representation of the results of the evaluation of the course (1= very good, 5= very bad)

Overall students were satisfied with the course a point of improvement seemed to be the timing of the sessions (too less time for sequence analysis and too less time for practical work)

Table 1: Perspectives and expectations of the 20 trainees after the training, written comments overview.

Items of the questionnaire	n
How would you apply what you have learnt within the next 6 months?	
Introduce / implement methods in the lab - Foster collaboration	14

Change / adapt methods already in place in the lab	6
Apply what have been learnt during the training	5
Share / transfer knowledge to lab colleagues	4
Do you have any idea to improve such training session?	
More practice	5
Extend time period / Reorganise the schedule	5
Reallocate the timing	3
Other new techniques	3
More sequencing	2
Using more the experience of the trainees	2
What do you expect for a possible continuation of the Mediterranean Regional Laboratory Network and of the whole EpiSouth network?	
Improve capacity	9
Exchange and share information	8
Extend to other pathogens	6
Sustain the Network	5
Organisation of EQA	3
Identification of supporting laboratory	2
Other *	5

* *Other:*

- Develop multi-centric project in this field ;
- The concientisation of the European commission about the problem of: 1/ The emergence of some viral zoonosis due to Global climate change; 2/ The threat represented for the A. Albopictus colonization of the Mediterranean basin in relation to autochthonous transmission of Dengue fever, YFV ;
- Project about epidemiology, management data, share our knowledge and experience of cases ;
- Audiences will be very important to follow-up the application and the progress regarding to the topics that was submitted during this meeting ;
- The laboratory part has to find a new formula to work together (example: Create a website - interactive!) to put our questions, protocols, etc.

Conclusion and wrap-up

This second EpiSouth laboratory training has consolidated the trust and the networking among the laboratory staff from the whole Mediterranean and Balkans region that had participated to the first training and fostered the collaboration with the new members. The WP4 has succeeded in improving laboratory capacity by training staff on Biosafety and West Nile diagnosis. As a result of the evaluation of the first training, the number of practical modules and of interactive and group exercises has been increased. However, trainees persist to ask for more practice. But we feel that it is very important for the lab staff to be aware of the global context.

All the trainees wish to have a continuation of the network and its activities. In particular, they expect to have the occasion to continue to exchange information (such as protocols or experience) and to improve the capacities of their laboratory through training.

Annexes

ANNEX 1: Agenda of the training



Agenda of the EpiSouth plus WP4 training on "West Nile and Biosafety II in the laboratory" 24 - 28 June 2013 - Instituto de Salud Carlos III, Madrid



Legend:

- General presentation and Evaluation
- Lectures
- Laboratory courses
- Biosafety modules
- Computer-based exercises

	MONDAY 24	TUESDAY 25	WEDNESDAY 26	THURSDAY 27	FRIDAY 28
08:30-09:00	Welcome	Debriefing	Debriefing	Debriefing	Debriefing
09:00-09:15	Presentation training			Available sequencing techniques	Case studies
09:15-09:30	Presentation participants	PCR	Biorisk assessment II	A. Zaballos	
09:30-09:45	Evaluation of participants	Interpretation	Feedback	Sequence editing	
09:45-10:00	WP4	J. Fernandez	Discussion	J. Fernandez	
10:00-10:15	Break	Break	P. Dubois	Sequence assembly	
10:15-10:30	Introduction to West-Nile (WN)	Break	Break	Break	
10:30-10:45	M.A. Jimenez	WN in mosquitoes and birds	Biosafety II		
11:00-11:15	Global surveillance and epidemiology of WN - focus on Mediterranean	R. Soriguer	Facility design and maintenance		
11:15-11:30	M.A. Jimenez	Veterinary infections, laboratory diagnosis and surveillance	Other concerns		
11:30-11:45	Clinical aspects of WNV infection	A. Özkul	C. Batejat, P. Dubois		L. Franco
11:45-12:00	M.P. Sanchez-Seco				
12:00-12:15	Presenting EuroWestNile project				
12:15-12:30	C. Curia Martinez				
12:30-12:45					
12:45-13:00					
13:00-13:15	Lunch	Lunch	Lunch	Lunch	Lunch
13:15-13:30					
13:30-13:45					
13:45-14:00					
14:00-14:15	Round table for sharing experiences				
14:15-14:30	Greece (A. Papa)				
14:30-14:45	Italy (M.R. Capobianchi)				
14:45-15:00	Spain (M.P Sanchez-Seco)				
15:00-15:15	Turkey (S. Korukluglu, A. Özkul)				
15:15-15:30					
15:30-15:45	Break				
15:45-16:00	PCR				
16:00-16:15	Launching				
16:15-16:30	with Lecture				
16:30-16:45	"Multiplex amplification for differential diagnosis of neurotropic flaviviruses"				
16:45-17:00	J. Fernandez, A. Vazquez				
17:00-17:15					
17:15-17:30					
17:30-17:45					
17:45-18:00					
18:00-18:15					
18:15-18:30					
18:30-18:45					
18:45-19:00					
				Locating and discharging related sequences	Discussion
				J. Fernandez	WP4 and ISCIII
				Break	Evaluation of participants
				Sequence alignment	WP4
				A. Vazquez	Break
				Distance methods	General Conclusion
				J. Fernandez	WP4, ISCIII
				Phylogeny	
				A. Vazquez	

ANNEX 2: List of participants

**Organisation: Kathleen Victoir and Sabah Boufkhed (International Division, Institut Pasteur)
Gülay Korukluoglu (Microbiology Department, Turkish Public Health Institution)**

with: Antonio Tenorio and the team of the Laboratory "Arbovirus and imported viruses" (ISCIII, Madrid)

and: Philippe Dubois and Ingegerd Kallings (Biosafety expert)

TRAINEES			
COUNTRY	Participants		Name of the Institute, Name of the Laboratory
	First name	Name	
1 ALBANIA	Majlinda	KOTA	Institute of Public Health, Laboratory of Virology
2 ALGERIA	Aissam	HACHID	Institut Pasteur d'Algérie - Sidi-Fredj Annex, Virology Department
3 BOSNIA-HERZEGOVINA	Almedina	HADZIHASANOVIC-MORO	Clinical Center University of Sarajevo, Institute for Clinical Microbiology
4 BULGARIA	Iva	CHRISTOVA	National Center of Infectious and Parasitic Diseases, NRL on tick-borne infections
5 CYPRUS	Panayiota	HADJIKYPRIANOU	Nicosia General Hospital, Immunology Laboratory
6 FYROM	Golubinka	BOSHEVSKA	Institute of Public Health of R.Macedonia, Laboratory for Virology and molecular diagnostics
7 GREECE	Elpida	GAVANA	Aristotle University of Thessaloniki, Medical School, Department of Microbiology
8 ITALY	Monia	PACENTI	Azienda Ospedaliera di Padova (Padova University Hospital), U.O.C. Microbiologia e Virologia
9 JORDAN	Mahmoud	SHAFEI	Central Public Health Laboratory
10 KOSOVO	Xhevat	JAKUPI	National Institute of Public Health of Kosovo, Department of Microbiology
11 LEBANON	Sylvia	KARAM	Rafik Hariri University Hospital, Department of laboratory medicine
12 LIBYA	Adem	ELJERBI	National Centre for Diseases Control, Diseases Control Reference Laboratory
13 MALTA	Clive	MUSCAT	Mater Dei Hospital, Pathology Department, Virology Laboratory
14 MONTENEGRO	Zeljka	ZEKOVIC	Institute of Public Health, Centre for Medical Microbiology, Department for Virology and Serology
15 PALESTINE	Issa	ISHTIAH	Central Laboratory of Ministry of Health
16 ROMANIA	Ani Ioana	COTAR	National institute for research & development in microbio& immuno "Cantacuzino", Laboratory for vector-borne infections
17 SERBIA	Slavica	RAKIC-ADROVIC	Institute of Virology Vaccines and sera "Torlak", National Reference Laboratory for Arboviruses
18 SPAIN	Marcello	SALVATORE ROSSI	Institute of Health "Carlos III" (ISCIII), Laboratory of Arbovirus and imported viruses
19 TUNISIA	Nahed	HOGGA	Institut Pasteur de Tunis, Laboratory of Clinical Virology
20 TURKEY	Ihsan	DURMAZ	Turkish Public Health Institution, Department of Microbiology Reference Laboratories, Virology Laboratory

TRAINERS			
Lecturers			
1 FRANCE	Christophe	BATEJAT	Institut Pasteur
2 FRANCE	Philippe	DUBOIS	Consultant on Quality and Biorisk Management
3 GREECE	Anna	PAPA-KONIDARI	Aristotle University of Thessaloniki, Medical School, Department of Microbiology
4 ITALY	Maria Rosaria	CAPOBIANCHI	Istituto Nazionale per le Malattie Infettive (INMI) "L. Spallanzani"
5 TURKEY	Aykut	ÖZKUL	Department of Virology - Faculty of Veterinary Medicine - Ankara University

Spanish team and experts			
1 SPAIN - ISCIII	Antonio	TENORIO	ISCIII, Centro Nacional de Microbiología, Laboratory of Arbovirus and imported viruses
2 SPAIN - ISCIII	Carlos	CURIA MARTINEZ	ISCIII, Centro Nacional de Microbiología, Laboratory of Arbovirus and imported viruses
3 SPAIN - ISCIII	Fernando	DE ORY	ISCIII, Centro Nacional de Microbiología, Laboratory of Serology
4 SPAIN - ISCIII	Leticia	FRANCO	ISCIII, Centro Nacional de Microbiología, Laboratory of Arbovirus and imported viruses
5 SPAIN - ISCIII	Mari Paz	SANCHEZ-SECO	ISCIII, Centro Nacional de Microbiología, Laboratory of Arbovirus and imported viruses
6 SPAIN - ISCIII	Ana	VAZQUEZ	ISCIII, Centro Nacional de Microbiología, Laboratory of Arbovirus and imported viruses
7 SPAIN - ISCIII	Angel	ZABALLOS	ISCIII, Centro Nacional de Microbiología, Laboratory of Arbovirus and imported viruses
8 SPAIN - CISA-INIA	Jovita	FERNANDEZ	CISA-INIA (Instituto Nacional de Investigaciones y Experiencias Agronómicas y Forestales, Centro de Investigación en Sanidad Animal), Emerging and transboundary diseases
9 SPAIN - CISA-INIA	Miguel Ángel	JIMENEZ	CISA-INIA (Instituto Nacional de Investigaciones y Experiencias Agronómicas y Forestales, Centro de Investigación en Sanidad Animal), Emerging and transboundary diseases
10 SPAIN - EBD-CSIC	Ramón	SORIGUER	EBD-CSIC (Estación Biológica de Doñana - Spanish Council for Scientific Research)

EPISOUTH PLUS WP4 TEAM			
1 FRANCE - IP	Kathleen	VICTOIR	Institut Pasteur, International Division
2 FRANCE - IP	Sabah	BOU FKHED	Institut Pasteur, International Division
3 TURKEY	Gülay	KORUKLUOGLU	Turkish Public Health Institution, Department of Microbiology

ANNEX 4: Pre-test (and Final test) to assess the progress made during the training

Biorisk Management

Biorisk Management	
Pre-Course Assessment	
Your name:	
Your country:	
Your email:	
Date:	
Pre-course assessment score:	
1.	The term "laboratory biorisks" integrates two principal ideas. What are they? Please also try to define each of these two concepts.
2.	Which of the following organizations has published the international standard for Laboratory Biorisk Management? a. European Committee for Standardization (CEN) b. Food and Agriculture Organization (FAO) c. International Standards Organization (ISO) d. World Health Organization (WHO) e. World Organisation for Animal Health (OIE)

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3. Describe the purpose of the international standard for Laboratory Biorisk Management. Why do you think it is important?

4. To implement a Laboratory Biorisk Management system, a laboratory should address which three specific processes?

- a. Risk Groups, Safety Levels, Equipment Maintenance
- b. Assessment, Mitigation, Performance
- c. Hazard identification, Security Guards, Waste Management
- d. Personal Protective Equipment, Biosafety Cabinets, Decontamination

5. Define **risk** in words.

6. Which mathematical definition of **risk** is most correct?

- a. Risk = function (likelihood, consequences)
- b. Risk = hazard x threat x vulnerability
- c. Risk = probability x consequences x vulnerability
- d. Risk = function (consequences, vulnerability)

7. What is the difference between a **hazard** and a **threat**? Define these concepts in words.

Hazard:

Threat:

8. What are the four categories of mitigation control measures?

- a. Risk assessment, engineering controls, practices and procedures, administrative controls
- b. Engineering controls, practices and procedures, administrative controls, personal protective equipment
- c. Personal protective equipment, engineering controls, administrative controls, decontamination methodologies
- d. Risk groups, biosafety levels, biosafety cabinets, waste management

9. In general, what is the first method to consider to reduce laboratory biorisks? Choose only one.

- a. Biosafety cabinets
- b. Personal protective equipment
- c. Elimination or substitution
- d. Decontamination methods
- e. Good laboratory practices

<p>10. Describe what performance means in relation to biorisk management?</p>
<p>11. What are the three main components of performance?</p> <ul style="list-style-type: none">a. Organization, staffing, management oversightb. Facilities, equipment, proceduresc. Standard operating procedures, good laboratory practices, management oversightd. Control, assurance, improvemente. Responsibility, staffing, financial resources
<p>12. What does "PDCA" stand for in a quality management system?</p> <ul style="list-style-type: none">a. Plan, decontamination, clean-up, assessmentb. Preliminary demonstration of comprehensive activityc. Pre-assessment, decontaminate, call for assistance, post-assessmentd. Precise decision criteria analysise. Plan, do, check, act
<p>13. Why do you think PDCA is important?</p>

ANNEX 5: Questionnaire for the trainees to assess the training



EpiSouth

Network for the Control of Public Health Threats
in the Mediterranean Region and South East Europe

Training on West Nile and Biosafety II in the Lab

24-28 June 2013, Instituto de Salud "Carlos III" (Spain)

Organisation team:

Kathlen Victoir and Sabah Boufkhed

Gulay Korukluoglu

Antonio Tenorio and his team

Philippe Dubois

(WP4) International Division, Institut Pasteur

(WP4) Microbiology Department, Turkish Public Health Institution

(Lab) Unit of Arboviruses and imported viral diseases

(Biosafety) Consultant

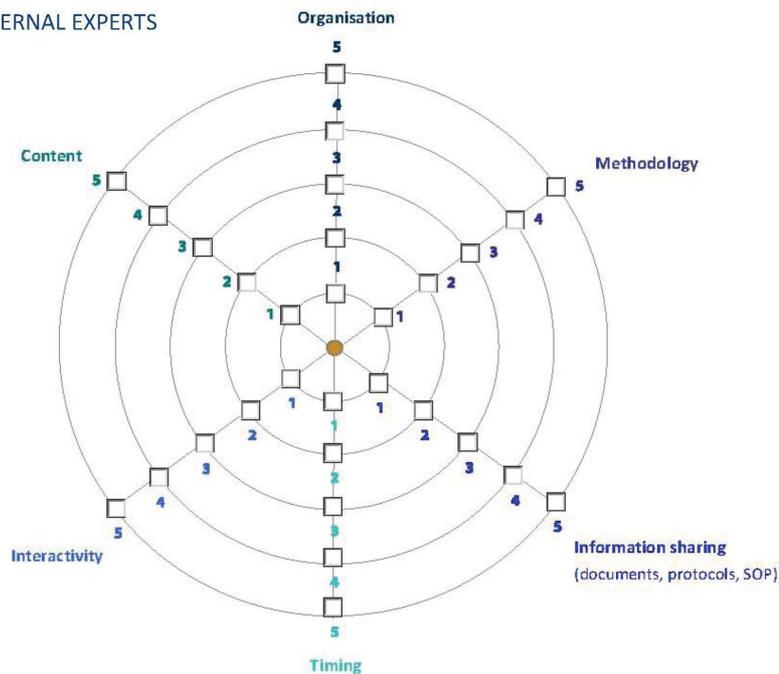
EVALUATION OF THE TRAINING

A. To evaluate the quality of this training, we kindly ask you to rate the following items of the training

1 → **VERY GOOD**

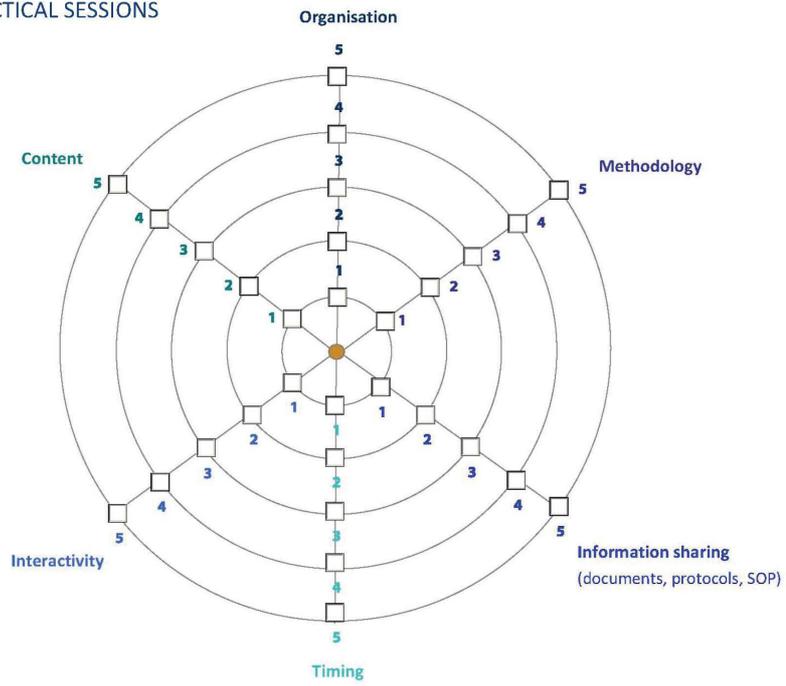
5 → **VERY BAD**

LECTURES OF EXTERNAL EXPERTS



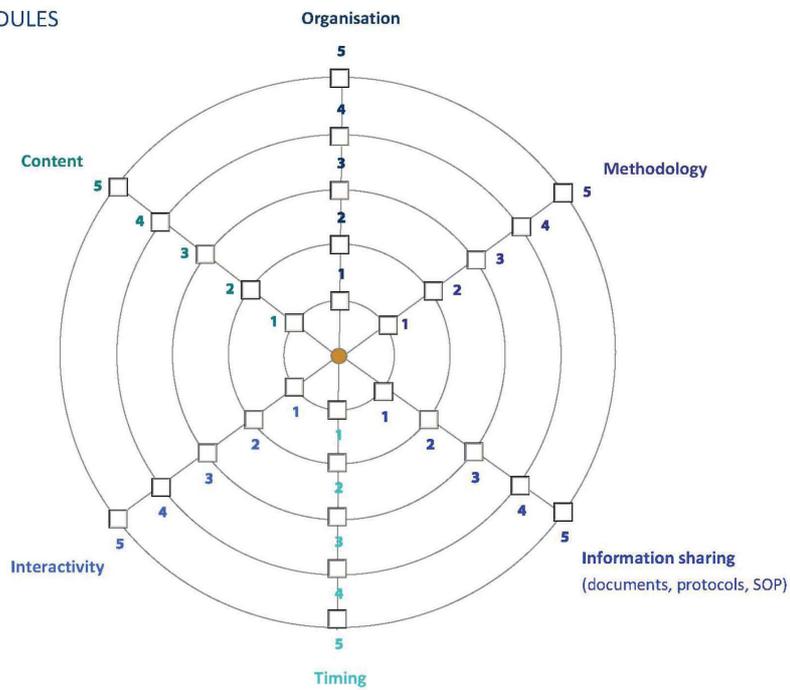
Additional comments or advises for the lectures of external experts:

LABORATORY PRACTICAL SESSIONS



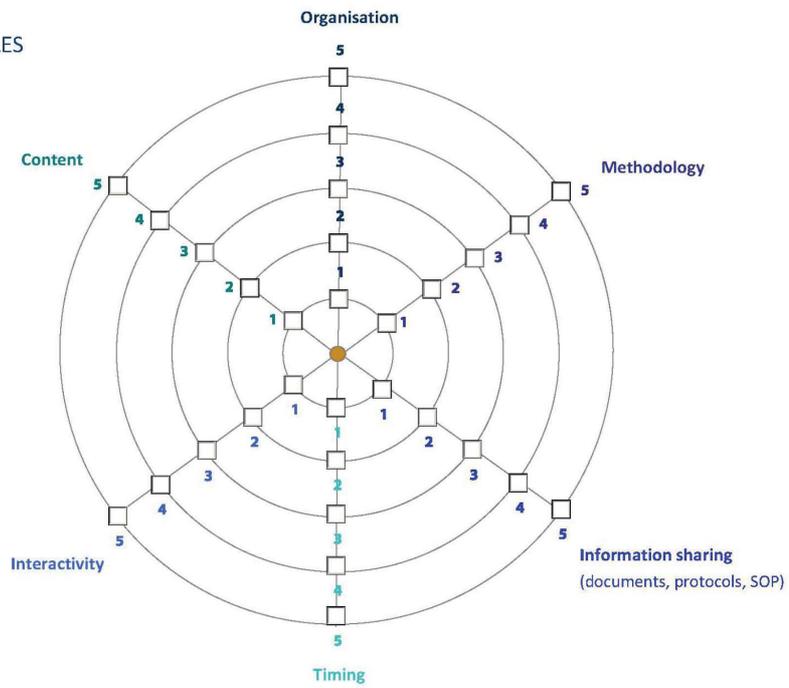
Additional comments or advises for the laboratory practical sessions:

BIOSAFETY MODULES



Additional comments or advises for the biosafety modules:

SEQUENCING MODULES



Additional comments or advises for the sequencing modules:

B. Did the training met your expectations? (circle your answer) Yes No Don't know

If the training did not met your expectations: what could have been improved? What was missing?

C. How would you apply what you have learnt within the next 6 months?

D. Do you have any idea to improve such training session?

E. What do you expect for a possible continuation of the Mediterranean Laboratory network?

F. What do you expect for an possible continuation of the whole EpiSouth network?

Any comment or remark?