



« Molecular diagnosis for the early detection of avian Influenza A and Newcastle disease viruses »

6–10 June 2011
1 week

In charge scientist
Emmanuel Albina

Introduction

Newcastle disease (ND) virus and Influenza A virus have been responsible for serious losses in poultry industry. The Highly Pathogenic Avian Influenza (HPAI), commonly known as “bird flu” caused by virus subtype H5N1 from Asian origin is an economic and public health threat for many countries in Asia, Africa, Middle East and Europe and present serious risks to human health.

Early detection of HPAI or ND is essential for a rapid control of these diseases. Various methods can be used for this detection, however molecular diagnosis by the reverse transcriptase polymerase chain reaction (RT-PCR) is the most rapid, sensitive and specific method.

RT-PCR is a method that can amplify specific sequences of viral nucleic acids to make accurate diagnosis. Two different methods can be used; the conventional procedure which requires several steps and the new technology called real-time RT-PCR (rRT-PCR) which gives results in a shorter time.

Sequencing of the cleavage site included in the amplified region of the hemagglutinin gene is advised for the identification of pathogenic strains and for epidemiological tracing of the isolated viruses.

Those molecular assays require specialized staff, procedures and equipment to be properly performed.

Course objectives

The general objective of the course is to provide practical training in molecular diagnosis of avian Influenza and Newcastle Disease and to provide knowledge of different protocols and procedures to be used both for conventional and real time PCR. This will include advised or harmonized protocols and procedures, equipment and diagnosis guidelines.

Specific objectives

- Training in molecular diagnosis of HPAI and ND
- Knowledge on different protocols procedures of RT-PCR
- Knowledge on different procedures of rRT-PCR
- Information about molecular typing and phylogenetic analysis of HPAI and ND

At the end of the training participants should be able to establish diagnostic methods, and provide training as well as continuous support to laboratory staff.

Organization

The course will be organized and coordinated by the research unit: « Emerging and Exotic Animal Diseases Control » of CIRAD (TA-15/G, campus International de Baillarguet, 34398 Montpellier, France)

The course will be open to a maximum of eight participants. The programme will alternate theoretical presentations and practical training. The first part will be focused on the conventional PCR whereas the second part will deal with real time PCR.

Participants qualifications

Participants must be actively involved in animal diseases diagnosis and have experience in molecular biology techniques. A minimum of theoretical knowledge in PCR is required.

The training course will be conducted in English or in French according to the origin of the participants.

Training costs:

Pedagogic : **1 300 €**
Travel towards Montpellier : to be determined by participant
Housing expenses (about) : allow a minimum of 80 € a day

If necessary, a customized estimate can be established upon request.

Application procedure

Participants should submit the attached assessment form together with a Curriculum Vitae, a motivation letter and eventually the name of the institution delivering the fellowship at least two months prior to the beginning of the training (hence by April 10, 2011 the latest) and send it by letter or e-mail to the following address:

**CIRAD Enseignement en Elevage et Médecine Vétérinaire Tropicale
TA A-15/B
Campus international de Baillarguet
34398 MONTPELLIER Cedex 5
France**

Tel : 33 (0) 4.67.59.39.02

Fax : 33 (0) 4.67.59.37.97

E-mail : marie-caroline.estienne@cirad.fr

Enseignement en Elevage et Médecine Vétérinaire Tropicale

http://www.cirad.fr/ur/formation_elevage_en

INFLUENZA TRAINING: PRACTICAL ASSESSMENT QUESTIONNAIRE

1 - Have you any technical skill in Molecular Biology?

- YES
- NO

If YES, months of experience?

2 - Which PCR equipment are you using in your laboratory?

- None
- Brand and type of machine:

3 - Do you perform molecular diagnosis in routine?

- YES
- NO

If YES:

- On which diseases?
- Which genes are you amplifying?

4 - Give one main drawback and one advantage of conventional PCR

Drawback:

Advantage:

5 - Paul has received to his laboratory 4 samples with a suspicion of *Mycoplasma pneumoniae*. He prepared a mix PCR and the PCR reaction according to the following table:

<u>MIX PCR for one reaction</u>		<u>Thermal cycler conditions</u>		
PCR Buffer 10X:	5µl	94°C	2 min	1 cycle
dNTP Mix (containing 10mM of each dNTP):	0.5µl	94°C	30 sec	30 cycles
Specific forward primer 20µM	1µl	55°C	30sec	
Specific reverse primer 20µM	1µl	72°C	30sec	
Taq DNA polymerase	0.5µl			
Template DNA	2µl	72°C	7 min	
Water	40µl	4°C	overnight	

After migration of 10µl of the PCR reactions, all results were negative including the positive control. Instead of Paul, what would be your first hypothesis to explain this unexpected result?

.....

.....

.....

.....

.....