National Reference Centres and WHO Collaborating Centres

National Reference Centres (CNRs) play an important role in the Institut Pasteur's public health care initiatives. These research units are designated for four years as microbiology observatories for communicable diseases under a decree issued by the French Ministry of Health. World Health Organisation Collaborating Centres (WHOCCs) fulfil a similar role for the WHO within an international network of expert laboratories.

The reference and expertise activities of CNRs and WHOCCs are directly based on advances in research as regards molecular diagnosis and pathogenetic interpretation, made possible by the Institut Pasteur's multidisciplinary environment. This constantly developing research helps to improve rapid and reliable diagnosis and the detection of any unusual or new pathological phenomena.

Significant events in 2005

CNRs and WHOCCs for Arboviruses

Since spring 2005, serological diagnosis of infection due to the Chikungunya virus has been conducted on 436 samples among the 1,300 from Réunion. Serodiagnosis techniques have been transferred to Réunion's major private laboratories as well as to two hospital laboratories. This has enabled the CNR to provide diagnosis support at a regional level (Réunion, Seychelles, Mauritius, Comoros and Madagascar) as part of its WHOCC for Arboviruses activities. More than 20 strains have been isolated covering different clinical forms from the Réunion outbreak in April 2005. Sequencing has shown that the virus belongs to the East African genotype.

In response to requests from the WHO Global Outbreak Alert and Response Network (GOARN) in 2005, the WHOCC was actively involved in the field in the international response to yellow fever epidemics in Mali and South Kurdufan (Sudan) in November and December 2005.

The CNR has also sequenced the complete genome of the dengue viruses isolated on Réunion in 2004.

Influenzae Virus CNR (France-North) and Reference and Research WHOCC for Influenza

The year 2005 was marked by the spread of the avian influenza virus H5N1. In view of this, the Centre conducted research into the H5N1 virus in French patients returning from countries affected by the epizootic caused by the H5N1 virus. Moreover, as a WHO reference laboratory for H5 viruses, the Centre confirmed the detection of human cases of infection by the H5N1 virus in Cambodia and has helped to genetically analyse these viruses. Finally, the Centre has continued to assess and develop detection techniques for the H5N1 virus and has provided technical support to several countries throughout the world through the distribution of RT-PCR detection protocols.

In 2005, the **Arbovirus and *Influenzae* Virus CNR in the Antilles-Guiana region** detected the four dengue serotypes in French Guiana.

For the first time, cases of dengue have been confirmed in towns on the Maroni River which are normally free of the dengue vector *(Stegomyia aegypti)* and which have a high incidence of malaria.
After a 10 year absence in the French departments of the Americas, the DEN-4 serotype was detected in the Antilles and French Guiana. Its emergence provoked an epidemic in Guadeloupe and Martinique between July 2005 and January 2006. The CNR, working closely with the Antilles-Guiana Interregional Epidemiology Unit (CIRE), the teaching hospitals in Fort-de-France and Pointe-à-Pitre and the Health Monitoring Units in Martinique and Guadeloupe, played an active role in identifying the circulation of various serotypes in these two French overseas *départements*.

In 2005, the **Rabies CNR** processed 1,536 requests for rabies diagnosis (an increase compared with normal levels following a case of rabies in a dog imported from Morocco in August 2004). Among these 1,536 samples, three were diagnosed positive for rabies but from bats. Anti-Rabies Treatment Centre work continued at a steady pace in 2005, as in 2004. It seems that media coverage of incidents concerning animals introduced illegally into France has heightened public awareness of rabies, especially among travellers.

Viral Hemorrhagic Fevers CNR

In 2005, France experienced an epidemic of hemorrhagic fever with renal syndrome of unparalleled proportions since monitoring began on this infection: 253 cases were confirmed in the regions where the Puumala virus — whose reservoir is the bank vole — normally circulates. An early warning was given in April 2005. In June, the number of cases had exceeded those recorded in the whole of 2004, with 98 cases in the Ardennes *département* and 32 in the Jura. A similar situation occurred in Belgium and Germany, leading to a Europe-wide warning.

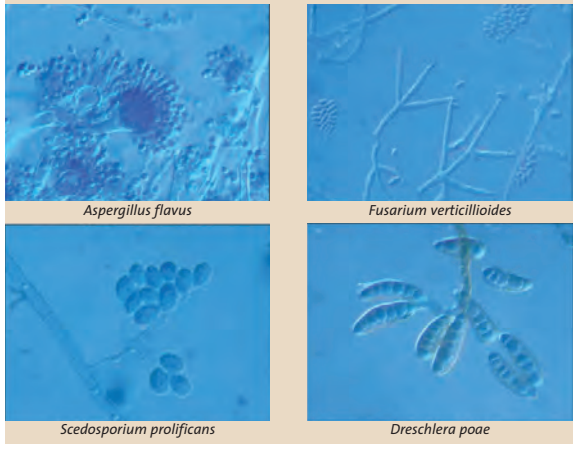
Reference and Research WHOCC for Enteroviruses and Viral Vaccines

In 2005, the WHOCC continued its research into the development and circulation of strains of the oral poliomyelitis vaccine (OPV) in partnership with laboratories of the Réseau International des Instituts Pasteur (RIIP) and other foreign laboratories (CDC, NIBSC). In particular, it carried out research into genetic drift (mutation and genetic recombination) in strains of the oral polio vaccine which were isolated in the various laboratories involved. This research thus led to the identification of highly modified, or even pathogenic, vaccine strains in Russia, Romania and Madagascar. More specifically, the WHOCC studied recombinant strains between vaccinal polioviruses and NPEVs circulating in Madagascar.

Mycology and Antifungals CNR

The RESOMYC server was introduced in early 2005 to facilitate disease notification and allow the CNR to improve its monitoring activities. As part of nosocomial infection monitoring, the Yeast Observatory was set up to analyse epidemiological and mycological data on yeast fungaemia occurring in all departments of 27 hospitals in the Paris area, including AP-HP hospitals dealing with severe pathologies. Monitoring of invasive aspergillosis has also begun in four regions, in partnership with the French National Institute for Health Monitoring (InVS).

Some species of fungus isolated in patients suffering from pulmonary or cutaneous lesions.



Aspergillus flavus — *Fusarium verticillioides*
Scedosporium prolificans — *Dreschlera poae*

The work carried out includes the development of a molecular identification technique for mucormycosis agents which should eventually aid diagnosis of these violent infections.

In 2005, the **Mycobacteria CNR** was able to collect various bacteria isolates responsible for pulmonary tuberculoses which are typical but for which the appearance of colonies in culture differs from that of the colonies of *M. tuberculosis*. Genetic analysis concerning molecular clocks that are different from those normally used has enabled the CNR to demonstrate that these isolates have an ancient source and evolution, considerably predating those of *M. tuberculosis*. While at a global level "homogenous" *M. tuberculosis* is considered to have evolved over a forty to fifty thousand year period, these isolates appear to have evolved over three million years. This opens up many new possibilities regarding the biology of mycobacteria and also the understanding of interactions between humankind, primates in general and tuberculosis bacilli.

Leptospira CNR and WHOCC for the Epidemiology of Leptospirosis

In terms of epidemiology, 2005 was characterised by a leptospira endemic in France that was similar to the one in 2004, i.e. the lowest incidence for 15 years. In the French Overseas Departments and Territories there was an unusual stability (apart from a fresh outbreak in Martinique) in relation to the previous year.
With regard to applied research, the CNR, in partnership with the Institut Pasteur in New Caledonia, published a real-time PCR method adapted to leptospirosis in 2005, which enables effective diagnosis with quantification and identification of the infecting species in a single day.

Escherichia coli/Shigella CNR

An epidemic of *E. coli* O157:H7 (haemolytic uraemic syndrome) occurred in South West France. All those affected had consumed frozen minced meat from the same batch. *E. coli* O157:H7 isolates carrying the genes coding for Shiga toxins 1 and 2, enterohemolysin and intimin were found in the stools of 18 out of 66 patients and in the meat. The epidemic stopped with the recall of the batches

Listeria CNR and WHOCC for Food-Borne Listeriosis

In 2005, the multiplex PCR typing method replaced conventional serotyping — due to its reproducibility, its easy implementation and low cost — having been tested by a multimember study coordinated by the CNR. This method was developed by the *Listeria* laboratory in partnership with the Genomics of Microbial Pathogens Unit, through the analysis of results obtained for more than 100 strains characterised by the DNA microarray technique.

National Reference Centres

Institut Pasteur in Paris

Anaerobic Bacteria and Botulism
Anaerobic Bacteria and Toxins Unit
Michel Robert Popoff, Jean-Philippe Carlier

Antibiotic Resistance
Antibacterial Agents Unit
Patrice Courvalin

Bordetella (Whooping Cough)
Bordetella Unit
Nicole Guiso-Maclouf, Valérie Caro

Borrelia
Molecular and Medical Bacteriology Unit
Danièle Postic, Elisabeth Ferquel
Associated laboratories: Institute of Bacteriology, Louis Pasteur University in Strasbourg

Anthrax
Toxins and Bacterial Pathogenesis Unit
Michèle Mock, Patricia Sylvestre
Associated laboratory: AFSSA/LERPAZ, Maisons-Alfort

Corynebacterium diphtheriae
Biodiversity of Emerging Pathogenic Bacteria Unit
Patrick Grimont, Anne Le Flèche

Escherichia coli and *Shigella*
Biodiversity of Emerging Pathogenic Bacteria Unit
Patrick Grimont, Francine Grimont
Associated laboratories: Microbiology Department, Robert Debré Hospital, AP-HP

Influenzae Virus (France-North)
Molecular Genetics of Respiratory Tract Viruses Unit
Sylvie van der Werf, Jean-Thierry Aubin

Leptospirosis
Spirochetes Laboratory
Guy Baranton, Danièle Postic

Listeria
Listeria Laboratory
Alban Le Monnier, Christine Jacquet

Meningococcus
Neisseria Unit
Jean-Michel Alonso, Muhamed-Kheir Taha

Mycobacteria
Mycobacteria Laboratory
Véronique Vincent, Gilles Marchal

Mycology and Antifungals
Molecular Mycology Unit
Françoise Dromer, Olivier Lortholary

Plague and other Yersinia Infections
Molecular and Medical Bacteriology Unit
Elisabeth Carniel, Françoise Guinet

Rabies
Lyssavirus Dynamics and Host Adaptation Research and Expertise Unit
Hervé Bourhy, Yolande Rotivel, Laurent Dacheux

Salmonella
Biodiversity of Emerging Pathogenic Bacteria Unit
Patrick Grimont, François-Xavier Weill

Vibrios and Cholera
Cholera and Vibrios Unit
Jean-Michel Fournier, Marie-Laure Quilici

Mérieux-Pasteur Research Centre in Lyon

Arboviruses
Biology of Viral Emerging Infections Unit
Hervé Zeller, Isabelle Shuffenecker
Associated laboratories: Institute of Tropical Medicine, Le Pharo-Marseille Armées, Army Health Service

Viral Hemorrhagic Fevers
Biology of Viral Emerging Infections Unit
Hervé Zeller, Marie-Claude Georges-Courbot

CNRs associated with the Institut Pasteur

Hepatitis B and C Viruses
Necker-Institut Pasteur Joint Liver Cancer Laboratory
Faculty of Medicine, Necker Enfants Malades Hospital
Valérie Thiers, Stanislas Pol
Christian Bréchot, Scientific Adviser

Associated CNR: Viral Hepatitis B and C CNR for the Monitoring of Blood Transfusions, National Blood Transfusion Institute
Associated laboratories: Bacteriology-Virology-Hygiene Laboratory, Avicenne Hospital, AP-HP

Institut Pasteur, French Guiana

Malaria Chemoresistance (Antilles-French Guiana region)
Parasitology Laboratory
Philippe Esterre, Eric Legrand

Arboviruses (Dengue, Yellow Fever) and *Influenzae* Viruses in the Antilles-French Guiana Region
Virology Laboratory
Jacques Morvan, Philippe Dussart

WHO Collaborating Centres

WHOCCs for Arboviruses and Viral Hemorrhagic Fevers
Biology of Viral Emerging Infections Unit
Hervé Zeller

Reference and Research WHOCCs for Influenza Viruses and other Respiratory Tract Viruses
Molecular Genetics of Respiratory Tract Viruses Unit
Sylvie van der Werf

FAO/WHO Collaborating Centre for Epidemiology of Leptospirosis
Spirochetes Laboratory
Guy Baranton

WHOCC for Food-Borne Listeriosis
Listeria Laboratory
Christine Jacquet

Reference and Research WHOCC for Enteroviruses and Viral Vaccines
Molecular Prevention
and Therapy of Human Diseases Unit
Francis Delpeyroux, Sophie Guillot

Reference and Research WHOCC for Rabies
Lyssavirus Dynamics and Host Adaptation Research and Expertise Unit
Hervé Bourhy

Reference and Research WHOCC for *Salmonella*
Biodiversity of Emerging Pathogenic Bacteria Unit
Patrick Grimont, François-Xavier Weill

Reference and Research WHOCC for *Yersinia*
Yersinia Unit
Elisabeth Carniel