

B. PAST, PRESENT AND FUTURE OF BACTERIA AND VIRUS FIGHTERS

Milestones of 150 years bacteria and virus fighting

1851 – 1900

- 1851 - Charles Chamberland (France) developed filters for bacteria, which lead to the **discovery of viruses**.
- 1853 - Pierre Roux (France) discovered that diphtheria was caused by **the toxin**, rather than by the bacterium itself.
- 1855 - Bunsen burner, invented by Michael Faraday.
- 1856 - Louis Pasteur (France) discovered that **fermentation** is the result of the activity of micro-organisms.
- 1859 - British biologist Charles Darwin published his book “On the origin of species by means of natural selection”. It will change the face of biology and society forever.
- 1866 - Mendel published his work on **heredity**.
- 1871 - Charles Darwin published his book “The descent of man and selection in relation to sex”, in which he looked for the evolution of humans and sexual evolution.
- 1873 - Hansen discovered *Mycobacterium leprae*.
- 1876 - The term “enzyme” is used for the first time after **trypsin** is discovered in pancreatic juice. Louis Pasteur discovered **anaerobic organisms**.
- 1879 - Louis Pasteur discovered accidentally that weakened cholera bacteria do not cause cholera in chickens. Start of **attenuate life vaccines**.
- 1880 - Louis Pasteur developed his germ theory of diseases. Robert Koch (Germany) started using solid cultures – gelatine-to-grow bacteria.
- 1882 - Robert Koch discovered the **bacterium that caused tuberculosis (TB)**.
- 1885 - Louis Pasteur developed a **vaccine against rabies**.
- 1889 - Ducrey discovered the bacterium *Haemophilus ducreyi*, which causes chancre.
- 1892 - **Viruses** were hypothesized as the cause of the tobacco mosaic disease in plants.
- 1898 - Tobacco mosaic virus identified by Martinus Beijerinck (The Netherlands) “*Contagium vivum fluidum*”. Friedrich Loeffler and Paul Frosch passed the filterable animal virus of foot and mouth disease from calf to calf.

1901 – 1925

- 1900 - Walter Reed & James Carroll (USA) showed that **yellow fever virus** was caused by the first human filterable virus and that it is transmitted by mosquitoes.
- 1901 - Hugo De Vries’ book “The mutation theory” suggested that changes in species are the result of mutations.
- 1905 - Donovan discovered the causative organism *D. granulomatis* of granuloma venerea, an ulcerative sexual transmitted disease.
- 1906 - Wassermann developed a serologic test to detect antibodies against *T. pallidum* the etiologic agent of syphilis.
- 1909 - The terms **gene, genotype, phenotype** were used for the first time.
- 1910 - H.T. Ricketts (USA) showed that Mexican typhus was transmitted by the human body louse.
- 1916 - Frederick W. Twort discovered the filterable agent responsible for bacterial lyses and called it “glassy transformation”.
- 1917 - Felix d’Herelle named Twort’s discovery **bacteriophage**.
- 1918 - K. Landsteiner and E. Popper successfully **passaged poliomyelitis** from a filtrate of spinal cord material from a fatal case in a nine-year old boy to two old world (rhesus) monkeys.

1926 – 1950

- 1926 - Theodor Svedberg & Robin Fahraeus devised the **ultracentrifuge**.

- 1932 - E.W. Goodpasture, G.J. Buddingh & L. Richardson grew vaccinia in the developing **chick embryo**.
- 1933 - Ernest Runska built the **first electron microscope**, able to enlarge objects 12 000 times.
- 1939 - E. Runska presented the first electron micrograph of any virus: tobacco mosaic virus.
- 1940 - F.M. Burnet isolated influenza virus through amniotic sac of the chick embryo.
- 1942 - YF vaccine caused Hepatitis B in US soldiers.
First electron micrograph of **polio** virus.
- 1944 - Oswald Avery, Colin McLeod, Maclyn McCarthy showed that **DNA** is the hereditary material for most living organisms.
- 1948 - J. Enders, T. Weller and F. Robbins (USA) learned how to grow mumps and polioviruses in chick embryo tissues without bacterial contamination, opening the way for more efficient vaccine production.

1951 – 1975

- 1952 - Jonas Salk developed a **polio vaccine**.
- 1953 - James Watson (USA) & Francis Crick (UK) developed the **double helix model of DNA**. This ignited the beginning of the molecular revolution and our initial understanding of gene function.
Frederick Sanger (UK) determined **protein structure** of insulin.
- 1954 - J.S. Younger: preparation of trypsin dispersed monkey kidney cells.
- 1955-1959 - Period in which a lot of new arboviruses (virus transmitted by arthropods (mosquitoes)- have been discovered such as: Chikungunya (1955); Crimean Congo hemorrhagic fever (1956); Sindbis virus (1956); o’Nyong-Yong (1959), ...
- 1959 - Japanese scientists discovered that resistance to antibodies in *Shigella dysenteriae* was passed from one bacterium to another by small circles of DNA known as “plasmids”; Milestone in the development of genetic engineering.
- 1964 - C. Yanofsky and S. Brenner proved that the **order of bases in DNA coincides with the order of amino acids in proteins**.
- 1965 - A **vaccine against measles** became available.
- 1967 - Marburg virus: first discovery of a Filovirus, etiologic agent of Marburg hemorrhagic fever.
- 1968 - W. Arber (Switzerland) discovered “**restriction enzymes**” able to cut at specific sites DNA. Together with D. Nathane (US) and H. Smith they shared in 1978 the Nobel prize for this finding. Milestone in genetic engineering.
- 1970 - Lassa virus: first discovery of an Arenavirus, causing Lassa hemorrhagic fever.
Temin discovers **reverse transcriptase enzyme** : RNA → DNA.
- 1973 - S. Cohen and H. Boyer (US) showed that DNA could be cut with one type of enzyme, joined together again with another type and reproduced by inserting them into bacteria *E. Coli*. Start of genetic engineering.
International Committee on Nomenclature of Viruses (INCV) was erected (4400 viruses: 1200 animal; 2200 bacteriophages; 1000 plant).
- 1975 - G. Kohler and C. Milstein produced specific antibody secreting cells in continuous cell culture (**monoclonal antibody**: MAAb), later to be applied extensively in viral diagnosis.

1976-2000

- 1976 - The last recorded **small pox case** was reported in Somalia.
Two outbreaks of severe hemorrhagic fever occurred simultaneously between June and November 1976 around Maridi in Western Equatoria, Southern region of the Democratic Republic of the Sudan and in the Equateur Region in the Northwest of the Republic of Zaire (now Democratic Republic of the Congo), in an area close to the Yambuku Catholic mission. This was exactly **hundred** years after the Belgian King Leopold II organized in Brussels an International Geographic Conference in his palace in Laken, to gather the most famous explorers of that time, such as M. Stanley, to help him to select his private Belgian colony.
The outbreaks at a distance of less than 400km’s were shown to be caused by two new members of the deadly filoviruses. “**Ebola_{Sudan}**” and “**Ebola_{Zaire}**”. They differed antigenetically from each other as well as from the Marburg virus (see 1967). **PLITM was involved in the discovery of Ebola_{Zaire}**.
H.W. Lee discovered the Hantaan virus, etiologic agent of Haemorrhagic fever with renal syndrome.

- 1977 - Frederick Sanger developed DNA sequencing, allowing the unequivocal characterization of the genetic information in each living organism.
- 1980 - The World Health Organization General Assembly officially declared on 8th May that small pox has been eradicated. **This was the first global eradication of a human virus.** Bernard Poiesz and Bob Gallo isolated the human T-lymphotropic virus (HTLV-1) from lymphocyte T cultures of a patient with a T-cell cutaneous lymphoma. In 1981 and 1982, respectively, Hiruma and Yoshida isolated the same retrovirus from T-cell lines derived from patients with adult T-cell leukaemia¹.
- 1981 - First clinical description of new emerging disease at the West coast of the United States, at that time still called “Gay related immune disease (GRID)” or the “four H’s: homosexual, heroin addicts, hemophiliacs and Haitians”, now Acquired Immune Deficiency Syndrome (**AIDS**).
- 1982 - The first human insulin, made by bacteria as a result of genetic engineering was marketed.
- 1983 - First isolation of a new retrovirus human immunodeficiency virus type-1 by Luc Montagnier (France) and his team.
Kary Mullis developed the **polymerase chain reaction (PCR)**. PCR made it possible to elucidate the genetic composition of viruses and bacteria, without the need to culture them. PCR made the impossible, possible. For example: Hepatitis C virus could never be isolated, but was identified and characterized with the help of PCR (see F. Ryan Book p85).
- 1984 - June 1984 was the official start of the Zairian-Belgian research program “Projet SIDA”, an international research project based in Kinshasa, Zaire, which provided many early epidemiological insights into AIDS.
- 1985 - A commercial enzyme-linked immuno-absorbent assay (ELISA) for the detection of antibodies against HIV-1 became available (based on the “in-house” ELISA test developed by R. Gallo at the National Cancer Institute – US). Of crucial importance to screen rapidly large groups of people, especially in blood transfusion centres: in addition it is the only way to find out if someone is infected with HIV, since at average the first 10 years after infection, no specific clinical sign of symptoms can be observed.
- 1986 - Genetically engineered tobacco plants were grown outside in the field trials for the first time in the USA.
- 1987 - Transmission studies in chimpanzees helped to establish that the main agent of parenterally acquired non-A, non-B hepatitis was likely to be an enveloped virus. These studies made available a pool of plasma known to contain a relative high titre of the agent. With the help of molecular biology techniques, the genome could be cloned. Clones covering the entire viral genome were assembled and the complete nucleotide sequence determined. **Hepatitis C** was identified, first virus characterized without being able to grow the virus in cell culture. (p182 – Book Principles and Practice of Clinical Virology by A.J. Zuckermann & J.E. Banatvala & J.R. Pattison).
- 1990 - Start of ambitious human genome project.
World Wide Web invented by T.B. Lee & R. Caillieau.
- 1995 - The bacterium *Haemophilus influenzae* was the **first living organism** in the world to have its **entire genome sequenced**.
- 1996 - Start of the high active antiretroviral treatment (HAART) of HIV infection and AIDS?

2002-2006

- 2001 - Craig Venter and colleagues published the **complete genetic code** of the **laboratory mouse**.
- 2003 - **Stem cells** extracted from the bone marrow were used to **treat patients** with **heart failure** for the first time in Brazil by Emerson C. Perin and Hans F.R. Dohmann. For the **first time** ever the **efficacy of two AIDS vaccines in humans**, one in North America, the other in Thailand was **monitored**. Unfortunately, with a **negative result**.
- 2004 - Breakthrough of nanotechnology in medicine. Ehud Shapiro and his team at the Weizmann Institute of Science in Israel made a molecular DNA computer which was able to detect the presence of diagnostic markers for cancer – and then release treatment molecules in the right place. Although this has only been tested in test-tubes, body trials are expected soon².

1. Jaap Goudsmit, author of the book "Viral sex. The nature of AIDS". page 148.
2. Berenson et al. An autonomous molecular computer for logical control of gene expression. Nature May 27, 2004;429(6990):423-429
www.timelinescience.org/index.php
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How the hundredth anniversary of the Prince Leopold Institute of Tropical Medicine (ITM) fits into 150 years fight against bacteria and viruses?

It was remarkable to observe that bacteriology and virology are relative young scientific disciplines, which started only 150 years ago. As often happens, progress in scientific knowledge was many times related to the design of new innovative techniques, which allowed to discover a totally new unexpected world. The discovery of the "unusual" is only done by those who are gifted to be alert for it, and to do something with it, instead of being disappointed and to stop the experiments. One of them was the discovery of filters in 1851 which allowed filtering out bacteria from solutions. The filtrate (the solution which passed through the filter) was supposed to be free from bacteria and not be able to cause disease when injected into animals and/or plants. Total surprise when, in 1898, Martinus Beyerinck described the filtrate of a plant extract, when injected into plants caused a plant disease. Something smaller than bacteria was able to provoke disease. A new infectious agent was discovered: *Tobacco mosaic virus*. In the same year, F. Loeffler and P. Frosch passed the filterable animal virus of foot and mouth disease from calf to calf.

Mycobacterium leprae and *Mycobacterium tuberculosis*, the etiologic agents of leprosy and tuberculosis were already discovered in 1873 and 1882, less than a quarter of a century before the "School for Tropical Medicine in Brussels" was erected in Brussels. These two diseases remained one of the main targets of fight against bacteria in the history of ITM.

The bacteria and virus fight of ITM was strongly affected by the existence of the Congo Freestate. It helped to focus the attention on tropical diseases. Leopold II was impressed by the number of diseased and deaths due to tropical diseases. He insisted to fight these tropical diseases¹.

On 30 June 1960, the foundation of the Democratic Republic of Congo, was of crucial importance for the bacteria and virus fight of the Institute. It initiated the move of the "Out of Africa group"². A group of active Belgian bacteria, virus and parasite fighters with extensive field experience returned to Belgium. Many joined the ITM, others went to Janssen Pharmaceutica and the Belgian Universities. Although the diplomatic relations between Belgium and the Democratic Republic Congo in 1960 was interrupted, not all of the physicians and paramedici returned. A private fund was erected in April 1961 called "Fonds Médical Tropical" (FOMETRO) (Fund of Tropical Medicine). This fund was supported by all the medical faculties of the Belgian universities, as well as by ITM. This fund allowed the coordination of many medical activities.

1. Contribution of PLITM in the fight against bacteria and viruses in the period 1960 – 2005

The staff members

The “Out of Africa group”² could be considered experts in infectious tropical diseases most common in the third world. They have seen and studied all these diseases in their real context. They brought home the “field experience”, to me synonym for: “knowing to do a lot with a minimum of tools and infrastructure”. One of them was Prof. Dr. S.R. Pattyn.

S.R. Pattyn obtained his medical doctor (MD) degree at the University Gent, Belgium (1953) as well as his tropical medicine diploma (1953) at ITM, Belgium. After a training in virology at the University of Leiden, The Netherlands in 1954 and mycology training (1958) at the Centers for Disease Control, Atlanta, USA, S.R. Pattyn left for the Belgian Congo. From 1954 up to 1959 he was appointed as head of the section pathology in Elizabethville (now Lubumbashi) where he erected a new virology section to study entero- and adenoviruses. In the period 1959-1960 he was appointed as Professor Bacteriology at the University of Congo and Ruanda-Burundi. By his return to Belgium, S.R. Pattyn was appointed as Professor bacteriology-virology at ITM. He erected three units: virology, mycobacteriology and bacteriology. The objectives of the virology unit at that time period were to develop the necessary protocols to isolate and quantify viruses using cell culture, plaque assays mice as well as chick embryo's; to isolate and characterize enteroviruses, coxsackie and echoviruses collected in the Democratic Republic Congo; to isolate and quantify arboviruses and to study their replication in mouse organ cultures, as well as the effect of antiviral drugs.

In the Mycobacteriology Unit the objectives were to isolate, characterize and to study the clinical significance of mycobacteria different from *Mycobacterium leprae* (etiologic agent of leprosy) and *M. tuberculosis* (etiologic agent of Tuberculosis). An impressive collection of different mycobacteria from different geographical origin was established. Of a gold value for future retrospective molecular epidemiological studies to study the drug resistance of *M. tuberculosis*. S.R. Pattyn deserved a lot of credit for his enormous contribution in the fight against leprosy. Since *M. leprae* did not grow in culture, S.R. Pattyn optimized the mouse footpad technique to grow *M. leprae*. This allowed him to evaluate the efficacy of drugs to inhibit the growth of *M. leprae*, as well as to monitor drug resistance of *M. leprae*. During his long career, S.R. Pattyn was heavily involved in the evaluation of controlled therapeutical trials in pauci and multibacillary leprosy treatment with different regimens of drugs and of different duration.

In 1972 the energetic S.R. Pattyn combined a part-time professorship at ITM with a new full professorship in microbiology at the University of Antwerp where he became head of the Department of Medical Microbiology.

Prof. Dr. Françoise Portaels joined ITM in 1968. She started as a research assistant to work overseas at the National University of Zaire, Kinshasa from 1970 up to 1974. She isolated and identified a lot of new mycobacteria species different from *M. leprae* and *M. tuberculosis* originating from Zaire. Subsequently she studied the drug susceptibility of *M. leprae* and *M. tuberculosis*, the etiologic agent of the devastating disease “*Ulcus Buruli*”, as well as the treatment of this disease, including drug susceptibility testing. She reinforced the capacity of the mycobacteria unit

considerably. On an annual base, about 5000 specimens and strains from Belgium, Europe and overseas were isolated, identified and tested for drug susceptibility.

She became internationally acknowledged as a World Health Organization collaboration center for the diagnosis and surveillance of *Mycobacterium ulcerans*; worldwide coordinator of the WHO/IUATLD (International Union Against Tuberculosis and Lung Disease) supranational reference laboratory network for tuberculosis drug resistance surveillance; national reference laboratory for tuberculosis and mycobacteria. She was also actively involved in improving the treatment of tuberculosis, especially multi-drug resistant tuberculosis as member of the World Health Working Group on direct observed treatment of tuberculosis (DOTS-PLUS). She became also member of the World Health Organization (WHO) task force on Buruli Ulcer. She generated a unique mycobacterial collection: more than 10 000 well documented isolates (human, animal and environmental) from various geographic origins. This unique collection was used for many retrospective studies (epidemiology for non-tuberculous leprosy disease, molecular epidemiology of tuberculosis) and isolates have been frequently requested by international colleagues.

S.R. Pattyn and F. Portaels deserved a lot of credit to have focused so well on tuberculosis, at a time the world thought that tuberculosis was under control.

How the battle against tuberculosis was won and lost

In March 1993, Jorgen Lehmann asked the pharmaceutical company, Ferrosan, in Malmö, Sweden to synthesize p-amino-salicylic acid (PAS). His rationale was: if acetylsalicylic acid (aspirin) was able to stimulate growth of *M. tuberculosis*, it must be possible to make a derivative with an antagonistic activity.

In 1948, the British Medical Council compared the results of treatment in three groups of tuberculosis patients: one given PAS alone, one given streptomycin alone, and a third given the two drugs simultaneously. The trial was demonstrated that the **combination** of the two drugs reduced considerably the risk of development of streptomycin resistant strains of tubercle bacilli during the six months following the start of treatment. **Combination therapy** was born and considered to be an important breakthrough in the fight against tuberculosis. However, this combined therapy was only effective in eighty percent of the tuberculosis patients treated. PAS caused regularly some side effects and by lowering its concentration, streptomycin resistance increased. New drugs were needed.

The battle was won?

In 1952, by an extraordinary coincidence, three pharmaceutical companies, Bayer in Germany and Squibb and Hoffman La Roche in USA, had simultaneously discovered the same anti-tuberculosis wonder drug: isoniazide.

A perfect drug does not exist and what was expected happened. In April 1952, no less than six separate studies had shown that *M. tuberculosis*, readily became resistant to isoniazide. Neither PAS, streptomycin and isoniazide as single drug was the miracle cure for tuberculosis.

In 1960, John Crofton, a tuberculosis expert working at the university of Edinburgh, together with his colleagues has shown the world that by treating patients with all three drugs at the same time, tuberculosis was completely curable.

In 1963, the pharmaceutical giant CIBA, developed a new drug of remarkable potency against tuberculosis; rifampicin. Less toxic than streptomycin and as potent as isoniazid, it could also be taken in tablet form. In 1967, ethambutol was discovered by Lederle laboratories in the USA.

Rifampicin replaced streptomycin and ethambutol replaced PAS. Together with isoniazid they became the optimal combination therapy in the fight against tuberculosis.

In the developed world, sanatoria were closed. The success of the fight against tuberculosis resulted in a progressive and massive reduction in both the infection rates and death rates from tuberculosis in every country throughout the developed world. This revolution came slowly, steadily over two decades, from the mid sixties to the mid seventies.

Was the terror really over?

Ironically, it was the very success of combination-drug therapy that had allowed the switch of emphasis from hospital in patient treatment of tuberculosis to the **poorly monitored** community based therapy. The key issue was: what was really happening to patients after they had been discharged back into the community? No less than 89 percent of patients disappeared from community follow-up and never completed their treatment. In the home environment patients did not adhere enough to the efficacious combined drug therapy.

The worst had still to come.

The congruence of tuberculosis with the new disease AIDS for the first time described in 1981, made the world facing the greatest public health disaster since the bubonic plague.

Frank Ryan, M.D. *The forgotten plague*. 1992. Boston.

G. van der Groen joined as an assistant the bacteria and virus fighters team of S.R. Pattyn in September 1973. As a biochemist, with only very little practical experience in bacteriology, no experience at all in virology, he had to start from zero. In 1973-1974, he was involved in the research on the differentiation and the growth of mycobacteria. The best thing he did that year was to stop smoking. Since mycobacteria were growing too slowly and his “research genes” which code for “patience” were not yet sufficiently activated, he switched to virology and focused on the study of the multiplication of different arboviruses in mouse organ cultures, and in mouse peritoneal macrophages. G. van der Groen learned a lot of the basic virologic laboratory skills from L. De Vleeschouwer and J. Peel, under the critical supervision of S.R. Pattyn. In close collaboration with Dr. Van den Berghe, University of Antwerp they studied the antiviral effect of different drugs on polio, coxsackie and arboviruses.

Prof. Dr. Peter Piot, MD, executive director Joint United National Programme on HIV/AIDS and under-secretary general of the United Nations joined as an assistant the Department of Microbiology in 1974. P. Piot was intrigued by the study of sexual transmitted disease and their impact on the health of mother and child. He first learned in the lab how to isolate and to grow the bacteria causing sexual transmitted infections and diseases under the practical guidance of Eddy Van Dyck, an extremely well trained and experienced bacteriologist. The first three of an extreme long list of P. Piot’s publications were dealing with *ureaplasma urealyticum*, causing urethritis, and *Neisseria gonorrhoeae*, etiologic agent of gonorrhoeae.

1976 Emergence of the Ebola virus

On Monday, October 4, 1976, a blue thermos flask arrived at our laboratory. When the thermos was opened we saw two broken glass tubes, floating in ice water. In this, a letter was floating, written by Dr. Courteille with the following content: “I have enclosed liver biopsies and blood samples from the autopsy of Sister Myriam, born Louise Ecran, aged forty-two years, who just died here in the Ngaliema Hospital. She comes from the Mission Hospital of Yambuku. We have received reports of hundreds of cases in the Yambuku area and in the Bumba zone generally. Apparently, there are no survivors”. This looked serious!

We inoculated the sample into newborn mice, weanling mice and a continuous cell line of Vero cells. Newborn and weanling mice died respectively on the 5th and 7th day, and a cytopathic effect in Vero cells was observed 11 days post inoculation. All these manipulations had been done on a bench with no more protection than a lab coat and a pair of gloves in a laboratory with free access. This allowed the brand

new director of ITM, Prof. Dr. L. Eyckmans to be present in the laboratory, at the moment these manipulations were ongoing. A few days later we were not allowed by the World Health Organization and the Belgian authorities to continue to work on these samples because our laboratory was lacking the necessary safety infrastructure to work with dangerous pathogens. We had to send the samples to Ernie Bowen and David Simpson at Porton Down, United Kingdom and to Karl M. Johnson and Patricia Webb at the Centers for Disease Control (CDC), Atlanta, USA. Both teams possessed high security laboratory level four, equipped with glove-port cabinets and secure animal rooms, kept under negative pressure. In such laboratories there was always a rigid barrier between the virus and the manipulator. The negative pressure prevented the escape of the pathogen out of the glove-port cabinet or the laboratory.

We had not sent all the samples, because we were too curious to reconfirm the cytopathic effect in the Vero cells.

On 14 October 1976, ITM, Porton Down-UK and CDC Atlanta, USA reported simultaneously that they isolated a virus morphologically similar to the Marburg virus (see frame article entitled: "Ebola and Marburg filovirus outbreaks") but immunologically different. Later on this virus was named Ebola_{Zaire}. This virus was genetically different from Ebola_{Sudan}, which was isolated from patients with or similar clinical haemorrhagic fever in Southern Sudan (see framework article entitled: "Ebola and Marburg filovirus outbreaks"), where a similar epidemic occurred between June and November 1976.

After the virus was isolated, S.R. Pattyn, P. Piot and G. van der Groen were asked to travel to Zaire as members of an international medical commission to help to control the Ebola haemorrhagic fever outbreak. For P. Piot and me it was our first trip ever to Sub-Saharan Africa. Our family was told we left for two weeks, but we stayed away for almost three months. Working three months under very primitive field conditions focussed G. van der Groen's interest on the development of very simple methods for the laboratory diagnosis of viral haemorrhagic fever viruses, as well as it encouraged him to do the maximum with the minimum of tools available. This trip made him also aware of the tremendous public health problems in developing countries, especially Zaire. P. Piot discovered the power of epidemiology in the study of a new infectious disease, since he was intensively involved in the epidemiological investigations. These included possible modes of transmission, the incubation period, secondary attack rates and related risk factors and distribution of clinical defined cases in time, geography and amongst persons.

"Officier de l'Ordre National du Léopard, Zaire 1977"

During this expedition, P. Piot escaped from a helicopter crash. He had to fly back from the Yambuku missionary hospital where the Ebola outbreak started. He refused to get on board of an helicopter, due to a threatening storm which was coming up. The pilots were laughing with this refusal and took off. Less than one hour after departure, they crashed in the tropical forest. P. Piot together with local people went a few days later to the place where the helicopter crashed and recovered the dead swollen bodies. Coffins had to be made in the heart of the forest. He organised their reshipment to Kinshasa. At the end of the expedition, he received from President Mobutu the award "Officier de l'Ordre National du Léopard Zaire" for his brave humanitarian action. It was the first award in the long list of awards he would receive during his impressive career.

Expansion of the study of viral haemorrhagic fever viruses as well as sexual transmitted infections 1977-1981

G. van der Groen was invited by Dr. K.M. Johnson to work at the Centers for Disease Control, Atlanta, USA for a three month period to get acquainted to work with very dangerous pathogens in a high security laboratory. With this experience, he was capable to design a high security laboratory at the Institute of Tropical Medicine, Antwerp, which was unique in that it was the smallest high security laboratory on earth using for the first time flexible film type of isolator to contain class 4 pathogens. This prototype of isolator became commercialized and was used in the United States, Japan, Australia, the USSR and The Netherlands. This experience brought him to visit most of the high security laboratories in the world. He had a chance to continue his training on viral haemorrhagic fevers by working two more times at CDC, Atlanta, as well as in the US Army Medical Research Laboratories, Frederick Maryland, as well as at the Institute of Poliomyelitis in Moscow, USSR. That's the reason he was for sure on the list of the KGB as well as CIA! In the meantime, he had participated at two more expeditions, one in Zaire (1979), the other in Cameroon (1981), in order to find Ebola virus in nature. Until now, we don't know where Ebola virus hides in nature, despite extensive research efforts. This work was partially combined with the search for monkeypox viruses. Monkeypox viruses caused a disease which clinically was difficult to be differentiated from small pox. Consequently, each monkeypox outbreak in Sub-Saharan Africa could be considered as a revival of the smallpox virus which was declared eradicated in 1980. Since 1979, he started to work regularly as consultant for the WHO in the field of viral haemorrhagic fever diagnosis and laboratory safety. Since 1981, the laboratory became acknowledged as a WHO collaborating center for viral haemorrhagic fever virus reference and research.

After the Ebola adventure, P. Piot together with E. Van Dyck continued and intensified the study of sexual transmitted infections on different pathogens such as *Neisseria gonorrhoeae*, *Haemophilus vaginalis* and *Chlamydia trachomatis*. They expanded their studies to African countries such as Swaziland, Southern Africa and Kenya.

In 1980, P. Piot became an associate professor and head of the Division of Microbiology.

Ebola and Marburg filovirus outbreaks

Ebola and Marburg filovirus outbreaks

In August 1976, 318 cases of Ebola haemorrhagic fever occurred in 55 villages around the catholic mission of Yambuku, north central Democratic Republic of Congo (DRC). 83% of the cases were fatal. Both sexes and all age groups were affected. The mission hospital was the epicentre of the outbreak. Injections with unclear needles and syringes at the mission hospital was the only plausible risk factor in 26%, person-to-person transmission was likely in 47%, and both transmission modes were possible in 11%. The threat to health workers became apparent when 13 of 17 Yambuku hospital staff were infected and 11 died.



Ebola outbreak 1976, Yambuku, DRC

Four syringes and needles were issued to the nursing staff each morning of the catholic mission in Yambuku. These limited number of syringes & needles were sometimes rinsed between patients in a pan of warm water. More than 300 patients a day were treated with this limited unsterile set. At the end of the day, they were boiled. 26% of the Ebola infections in Yambuku at least were due to injections with unsterile needles & syringes

Foto 5

Blood samples of a Belgian missionary sister (Ecran Louise, on the picture at the middle of the bottom part of the composite picture) who died, were sent to the Institute of Tropical Medicine (ITM) Antwerpen, Belgium.



The samples arrived on Monday, 4 October 1976, and were processed by Prof. Dr. S.R. Pattyn and his young collaborator Dr. Sc. Guido van der Groen. All samples and first passage material were shared between ITM, Porton Down, UK (Dr. E. Bowen) and the Centers for Disease Control, Atlanta, USA (Dr. K.M. Johnson; Dr. P. Webb). On 14 October 1976, all three laboratories reported they had isolated a virus which was morphologically similar to the Marburg virus, but immunologically different. The new virus was named Ebola and became the second species of the *filovirus* (threadlike shaped viruses) genus.

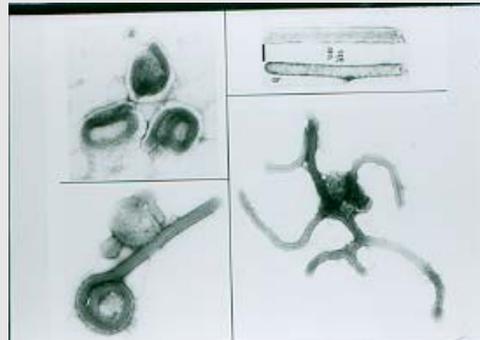


Foto 6

In June 1977, Dr. D. Heymann (CDC, USA), described at the catholic mission of Tandala, north-western DRC, a nine year old female, with clinical symptoms of Ebola haemorrhagic fever. Ebola virus was isolated from a post mortem blood specimen.

Foto 7

The next major Ebola outbreak in DRC occurred in 1995 in Kikwit (315 cases; case fatality rate (CFR) 81%). This outbreak took place in an unprecedented atmosphere of legitimate news reporting and tabloid exploitation.

The Marburg (MBG) outbreak in DRC occurred in Durba, 1998./1999. 75 cases had been identified (CFR 83%). This was worldwide the first MBG outbreak of that size. Media attention was limited as the area of the outbreak was difficult to access because of the ongoing armed conflict. Cumulative total of filovirus cases in DRC since 1976 is estimated 709 (CFR 86%). So far, the 709 filovirus

haemorrhagic fever (HF) cases in DRC represent 32% of the total filovirus HF cases reported worldwide (2160: CFR 71.8%). DRC being so far the country with the largest number of filovirus outbreaks. Filovirus outbreaks occurred mainly in central Africa (DRC, Southern Sudan, Gabon, Ivory Coast, Uganda, and Republic of Congo). Between 13 October 2004 – 27 June 2005, 436 MBG cases including 362 deaths (CRF: 84%) have recently been reported in Angola. So far the largest MBG outbreak reported ever! Sporadic filovirus infections have been reported in Germany, Yugoslavia, South Africa, Kenya, Zimbabwe, Russia, United Kingdom, USA, Italy, Philippines.

Despite considerable efforts, the reservoir (living organism in which filoviruses hide away) host remained unknown.

The damage on the local level of some of the filovirus outbreaks has been devastating at times, but was marginal on the international level, despite the considerable media attention these outbreaks received. Since 1967, approx. 2160 cases (CFR 71.8%) due to Marburg and Ebola, have been diagnosed, compared with 40 million cases (CFR 100%) due to HIV-1 since 1981!

The filovirus outbreaks in Central Africa could be kept under control by the consequent application of the following straightforward strategy:

- to prevent transmission by early case detection and isolation, barrier nursing, safe burials and to improve the basic hygiene in hospital settings
- to establish a field laboratory for filovirus diagnosis
- to treat patients by preventing and treatment of dehydration
- to inform, guide and influence the local public

ITM, Antwerp has built up during the years, a remarkable expertise in fighting filovirus infections in the field. ITM collaborated with local authorities to help control each of the filovirus outbreaks in DRC as well as in Uganda. ITM, Antwerp participated also in expeditions in DRC (Tandala, June and July 1979) and Cameroon (Mouloundou (1989) in order to find the Ebola reservoir*.

Combine the high case fatality rate (71.8%) of filovirus infections in sub-Saharan Africa with possible airborne transmission of Ebola_{Reston} in a quarantine facility for monkeys in Reston, near Washington DC, USA in 1989, and you get the kind of horror scenario that fuels the interest of media, public and bio-terrorists.

According to Ken Alibek**, Marburg and Ebola viruses have been weaponized by the Russians during the cold war period between them and the USA.

We should remain vigilant since it is not excluded that new filoviruses still hide in nature and/or in laboratories, which combines the pathogenicity of Ebola_{Zaire} with the aerogenic spread of Ebola_{Reston}. Filovirus X?

* Borchert M et al. Viewpoint: Filovirus haemorrhagic fever outbreaks: much ado about nothing? Tropical Medicine and International Health 2000;5:318-324.

** Alibek K. Biohazard, New York 1999.

1981 – 1985 *Start at ITM of a multidisciplinary fight against the new health time bomb “HIV/AIDS”*

Kaposi's sarcoma (KS) was a rare form of relatively benign cancer that tended to occur in older people and had been described in the past in Africa. But by March 1981, at least eight cases of a more aggressive form of KS had occurred amongst young gay men in New York. At the same time, there was an increase, in both California and New York, in the number of cases of a rare lung infection Pneumocystis carinii pneumonia (PCP). In June, the Centers for Disease Control (CDC) in Atlanta, published a report about the occurrence, without identifiable cause, of PCP in five men in Los Angeles.

A few days later, following these reports of PCP and other life-threatening opportunistic infection (infectious which occur due to a weakened immune defence), the CDC formed a Task Force on Kaposi's sarcoma and opportunistic infections (KSOI)³. In December 1981, it was clear that the disease affected other population groups, when the first PCP cases were reported in injecting drug users. At the same time the first case of AIDS was documented in the UK.

The disease still did not have a name. Names like: lymphadenopathy, gay-related immune deficiency (GRID) and community-acquired immune dysfunction were circulating. In June 1982, a report of a group of cases amongst gay men in Southern California suggested that the disease might be caused by an infection agent that was sexually transmitted.

By the beginning of July 1982, a total of 152 cases from 23 states, had been reported to CDC. Later that month, the first reports appeared that the disease was occurring in Haitians, as well as haemophiliacs. Therefore, the disease was labelled a disease of "the four H's: homosexuals, heroin addicts, haemophiliacs and Haitians". By August 1982, the disease was referred to by its new name of Acquired Immune Deficiency Syndrome (AIDS).

1982 French and Belgian scientists hypothesized that AIDS was also circulating in Africa

Jacques Liebowitch, a physician and immunologist, was intrigued by the occasional African and Haitian immunodeficient individuals that he and other European doctors had recently seen⁴. In 1982, Liebowitch put forward the hypothesis that AIDS was a viral disease of African origin that caused illness and death by, as he put it, "completely burning out the immune system" (immune deficiency).

In Belgium, P. Piot was also in favour of this hypothesis, when he and some clinicians (like Henry Taelman) at the clinic of ITM, Antwerp, observed patients from Zaire with clinical signs and symptoms compatible with the AIDS cases described in the United States. In support of P. Piot's thinking, in 1982-1983 Dr. Nathan Clumeck at Brussels St Pierre University Hospital, observed AIDS-like cases among Zairian people who either lived in Belgium or had come to Belgium for treatment of their profound immunodeficiencies. Clearly, these Zairian cases differed strongly from the American AIDS cases in that: none were gay, used injected drugs, or had visited Haiti. Most striking was that more than a third were women.

1983 Discovery of the virus causing AIDS

In February 1983, the virus causing AIDS was isolated from the lymph glands of a French AIDS patient by the team of Luc Montagnier in Paris, France. They named their virus "lymphadenopathy-associated virus (LAV)". They began to isolate this type of virus from other AIDS patients. Today, this virus is known as the human immunodeficiency virus type 1 or HIV-1.

First isolation in France of a new retrovirus from patient Bru suspected to be an AIDS patient.

On January 3, 1983, Rozenbaum told Françoise Brun-Vézinet he was about to take a lymph gland biopsy from a homosexual with generalized lymph gland swelling. Brun-Vézinet arrived at the Pitié-Salpêtrière on the day of the biopsy, where Rozenbaum allowed her half of the removed lymph gland, a piece of tissue about half a cubic centimetre. She rushed the biopsy across Paris, to the Pasteur Institute where she was working. She put it into the fridge of Luc Montagnier's lab. She informed Luc Montagnier and in the evening he went to the fridge where he found the bottle with a note attached: "Lymph node biopsy from Mr. "Bru". This man has lymphadenopathy. He is homosexual. He may go on to develop AIDS".

Donning surgical gown and gloves, he started to work in the laminar flow cabinet and prepared half of the sample for tissue culture. The other half was kept frozen. Every day after that, he would go to inspect the cultures, using an invert microscope to observe the growing cells through the transport bottoms of the flasks. This microscope magnifies the cells by a factor of about four hundred.

Every three days, he would remove a sample of the supernatant and pass it to Françoise Sinoussi, who would screen it for the presence of reverse transcriptase enzyme. If the enzyme was present, it would confirm that Bru's lymphocytes were infected with a retrovirus.

At about the fifteenth day, a strange cytopathic effect did occur, as well as Françoise Sinoussi was obtaining some small activity in the reverse transcriptase testing. Six days later, on January 23, sufficient amount of reverse transcriptase enzyme was found. So, they believed they had isolated a retrovirus. Which one? A new virus or one that resembled human T lymphocyte virus type (HTLV-1) as was recently discovered by Bob Gallo, in the United States.

Soon the lymphocytes started to die. This was not expected if the virus was a HTLV-11 type of virus. The latter immortalized the T lymphocytes. So were they dealing with a new virus which killed human T lymphocytes?

Luc Montagnier set up a new experiment whereby he set up new cultures of uninfected lymphocytes from a healthy blood donor and verified if he could infect them with Bru's virus. It was working. A new retrovirus was growing in these cultures.

Montagnier contacted Gallo. The latter promptly supplied the French team with sera containing antibodies against HTLV-1 as well as cells infected with the HTLV-1 virus. Jean-Claude Chermann checked if the lymphocytes infected with Bru's virus reacted with HTLV-1 antibodies. Bru's serum appeared to react against the cells containing HTLV-1. But Sophie Chamaret's findings suggested that the Bru's serum did not contain antibodies to the internal protein of HTLV-1. This observation was confirmed by Jean-Claude Chermann and his collaborator, Marie-Thérèse Nugeyze. Yet, the internal protein, called protein 24, was one of the most conserved throughout the retrovirus family. It seemed possible that the "Bru" virus was a new retrovirus which differed from the HTLV-1 virus.

On February 4, 1983, Charles Daguet, observed under the electron microscope retrovirus like particles. All experiments were repeated and the initial results confirmed.

The three scientists, Jean-Claude Chermann, Françoise Barré-Sinoussi and Luc Montagnier, named their virus LAV, for "lymphadenopathy-associated virus".

They began to isolate the virus from other AIDS patients. Soon the virus would also be isolated though under a different name, HTLV-3, by the world famous Robert Gallo and his team at the National Cancer Institute in the United States.

Today, the above mentioned viruses are known as the human immunodeficiency virus type-1 or HIV-1.

They had found the virus that caused AIDS.

Ryan FP. Virus X. Tracking the new killer plagues. Out of the present and into the future. Boston, 1997:247-267.

Now we had to prove that HIV-1 was also present in Africa and could cause AIDS over there. P. Piot was eager to go to Kinshasa, and see what was ongoing, but he didn't have the money. The director of ITM Prof. L. Eyckmans and members of the board at that time were initially not eager to finance a trip to Zaire for the study of HIV/AIDS. P. Piot had to find the money elsewhere.

1983 Start of a Zairian-American-Belgian collaborative project "Projet Sida" one of the most successful in elucidating the most fundamental of epidemiological questions of HIV/AIDS in Africa.

In the summer 1983, Thomas Quinn of the US National Institute of Allergy and Infectious Disease (NIAID) and Richard Krause, then director of NIAID, met at a café in Vienna with P. Piot. The topic of discussion: hints from a variety of sources that AIDS had struck in Zaire. T. Quinn and Krause had recently returned from Haiti, where they learned that many Haitians had worked in Zaire after it became independent from Belgium in 1960, and then were forced in the '70s to leave. This unified those three: we've got to get into Zaire. The trio met again that fall 1983 in Belgium to discuss the collaboration. They were joined by the CDC's Joseph McCormick. On 18 October 1983 P. Piot, J. McCormick and T. Quinn set out for Kinshasa. They obtained green light from the Minister of Health and met Dr. Kapita, who then was head of internal-medicine at Mama Yemo. P. Piot still confirms today, that thanks to Dr. Kapita, the whole project could start. B. Kapita was one of the first medical doctors in Zaire (now DRC) to recognize correctly AIDS cases. His observations combined with those of the "white doctors" was published in the Lancet⁵ in 1984. It took many years before the message of this article was fully understood. The message was two-fold: AIDS is not just a disease which occurred in promiscuous gay people at the West and East-coast of the United States, but is present in Africa and is mainly heterosexually transmitted!

P. Piot finally found his sponsors. CDC became the projects major funder: of its final US 4 million dollar budget, roughly 2.5 million US dollar came from CDC, 1 million US dollar from NIAID, and 0.5 million US dollar from ITM. "Projet SIDA" (Project AIDS) officially started in June 1984⁶.

This was the start of a Zairian-American-Belgian research program, one of the most successful in elucidating the most fundamental of epidemiological questions: how many people got infected? Who got the disease? What were the risk factors favoured of infection? What were the transmission modes? Was AIDS the same in Zaire as seen elsewhere? Could mosquitoes transmit HIV? How can we prevent disease? How can we slow down the disease?

Can mosquitoes transmit the HIV virus?

At the period 1981-1985 not so much information was available for the press and the public, since P. Piot, B. Colebunders (physician active in the Projet SIDA), Dirk Avonts (young physician) and Guido van der Groen were heavily involved in the HIV/AIDS fight, they all travelled many kilometres after their working hours to give talks, interviews to radio, TV, newspapers, in order to help inform the general public. One of the most frequent questions asked was: can mosquitoes transmit the HIV virus? Do we need to travel to Africa with a mosquito net to protect us against AIDS? The answer was "no", since we had no epidemiological evidence for mosquitoes transmitting HIV.

We answered many times: "feel free to use your mosquito net, but be careful underneath the net for mosquitoes with the size of 1m50 up to 2m! One sting can be enough to infect you." This to highlight that AIDS predominantly was sexually transmitted.

The amazing achievement of this project was, that nearly 300 Zairian scientists, doctors, technicians, logistic personnel were involved of which only seven were expatriates. One of them was the clinician Dr. Bob Colebunders from ITM and a new-comer to the Department of Microbiology Marie Laga.

Intermezzo of Hantavirus hunting

In 1983 G. van der Groen became head of the virology section in the Department of Microbiology at ITM. He became extremely interested in the hantaviruses, the etiologic agent of a disease in man called Haemorrhagic Fever with Renal Syndrome (HFRS), when he was working at the United States Army Medical Research Laboratories, Fort Detrick, Maryland, US, to get acquainted with the work in a high security laboratory to manipulate class four pathogens (the most dangerous and deadly viruses to work with). A parcel with serum samples arrived from Belgium with the request of Prof. Jan Desmyter (University Leuven, Belgium), to check the sera for antibodies against hantaviruses. After reading a recent article in the Lancet on a laboratory infection with hantaviruses in Japan, Prof. Desmyter suspected a laboratory infection with this virus in technicians who have manipulated infected laboratory rats in Belgium.

G. van der Groen's colleagues at Fort Detrick were looking at him: *"We have a Belgian here with us! So he is the right man to analyse these sera. Here is the protocol how to determine the antibodies against the Hantaan virus. Success!"*

The results were positive. As such we identified the first hantavirus laboratory infection in Belgium. He returned home with hantaviruses and reference sera and established a service for serologic and virologic characterisation of Hantaviruses in Belgium.

In close collaboration with J. Desmyter (Leuven), C. Van Ypersele,-de Strihou (Brussels), R. Verhaegen (Antwerp) and J. Clement (Belgian Army), they started a sero-epidemiologic study on the occurrence of hantaviruses in Belgium. We helped to initiate Hantaviruses research in collaboration with laboratories in Holland, France, Germany, Portugal, Switzerland, Austria, Sweden, USSR, Great Britain, Yugoslavia, Albania and Poland. In addition, a close collaboration was established with laboratories in the USA, Canada, Japan, Korea and China, Senegal and Tanzania. Many of our studies were focusing on: establishment of laboratory diagnostic methods, prevalence studies of hantavirus antibodies in man and rodents; virus isolations; virus characterisations by epitope mapping using monoclonal antibodies; correlation of the clinical course of HFRS and the serotype of hantaviruses.

Hantavirus hunting

During the summer of 1976 (same year ITM virus fighters discovered the Ebola virus), the Hantaan virus was discovered by H.W. Lee, South Korea. The Hantaan virus became known as the prototype virus of what later was called "the hantaviruses". The name of the virus was correlated with the Hantaan river on the 38th parallel in Korea. Why? First in a town, Songnaeri, the first Hantaan virus infected field mice was captured. Second the place where patients infected with Hantaan virus and developing the disease "Korean haemorrhagic fever (later renamed Haemorrhagic fever with renal syndrome (HFRS)) were found, was located upstream to the Hantaan river. Third, the name of the Hantaan river sounded more pleasant and was easier to pronounce than that of the local Korean city.

"It looks if though name of rivers were preferred to baptize new discovered viruses"

The official rule was to give the virus the name of the locality in which it was discovered for the first time. According this rule the virus discovered by H.W. Lee and his colleagues had to be named "Songnaeri virus". Impossible to pronounce.



Foto 8

Dr. Karl Johnson from the Centers for Disease Control, USA, leader of the International Committee studying the Ebola Haemorrhagic (EH) fever outbreak in 1976, in front of the map of DRC. The new virus was named Ebola according to the name of a small river in the endemic area. It was a nice name and could be pronounced easily. It was less stigmatizing for the DRC inhabitants, to use the name of a small river, than that of a village. We thought that on the map, the Ebola river was very close to the Yambuku village, in which the Ebola EH fever outbreak started. But we found out that the river was OUTSIDE the endemic area. The correct name of new virus had to be "Yambuku".

The Hantaan virus caused Haemorrhagic Fever with Renal Syndrome (HFRS) among soldiers in the Korean war. The South Korean scientist Ho-Wang Lee (H.W. Lee) was immediately accused by the North Koreans, that he was the inventor of a new biological weapon, used by the American Army against North Korea.

During the second World War, thousands of soldiers were dying from HFRS. The Japanese Germ Warfare Unit 731 and Soviet scientists carried out experiments on human subjects to study HFRS. The latter had been of interest to the military as a potential biological weapon, long before the etiologic agent, Hantaan virus was discovered.

HFRS has always been linked to war. The natural reservoir that carries the hantavirus is the field mouse. In wartime soldiers camp outdoors in large groups and may stay for a long time in one area. The field mice are chronically infected and the virus is continuously secreted in urine, saliva and faeces. The virus left in dirt and grass sticks to dust particles which are inhaled by the soldiers when they move through the field or are digging trenches.

H.W. Lee deserves a lot of credit for his discovery of the Hantaan virus. He succeeded where the Americans failed. Americans, armed with the most advanced research techniques tried very hard to characterize the etiologic agent of HFRS. They spent about US \$ 40 million over 20 years in their futile search for the elusive virus.

In 1961, cases of nephropathia epidemics, a milder form of HFRS, were described in Finland, Sweden and Norway. Our investigations in the 80's revealed also a mild form of HFRS in Belgium as well as in many neighbouring European countries. We have isolated new type of hantaviruses. Each rodent species carries a different hantavirus type.

In the spring of 1993, a strange disease broke out in a Navajo reservation at the "Four corners" (area in the US where four states New Mexico, Arizona, Colorado and Utah, meet). In three weeks time 21 people were hospitalized of whom 11 died. The patients first complained of fever, muscle pain, conjunctivitis, headaches and their lungs were filled with water. They died within a week from severe coughing and difficulty breathing. The pathogen causing the disease was a new member of the hantavirus family: "sin nombre virus", isolated from deer mouse.

For sure, new hantaviruses will continue to be discovered in different rodent species in different parts of the world. New diseases will occur with clinical signs, symptoms and case fatality rates different from HFRS and HPS. Virus fighters should remain vigilant.

Lee HW. Hantavirus hunting. Forty years of battling hantaviruses around the world. Seoul, 2004.

The coming of new staff members

Since 2000, Luc Kestens, Professor and Dr., is chairman of the Department of Microbiology. He started his career at PLITM in 1978 studying the immunopathology of schistosomiasis in mice. Since the early days of the HIV pandemic in 1983, he was intrigued by this immune deficiency disease and became progressively involved in many HIV research projects in ITM and in the field, predominantly Africa. Following the discovery of HIV in 1983, he led an international mission to Kivu in

1984 to study whether African Kaposi sarcoma (KS), an opportunistic malignancy seen in the first AIDS patients in the United States was also associated with HIV. African KS had been endemic in Kivu since many decades at the time of the study and if KS was really associated with HIV, than that was the place to investigate this. Fortunately this mission demonstrated for the first time that the endemic form of African KS was not associated with immune deficiency, in contrast to the epidemic form which started to appear in many other places in Africa. The mission lasted 3 months and was confronted with a high HIV seroprevalence (12.5%) in this area in 1984. Fortunately, it was shown later that all of these were false positives due to lack of specificity of the experimental first generation HIV tests. Apparently polyclonal B cell stimulation in malaria patients had induced cross-reactive antibodies. Since that expedition Luc Kestens' interest in HIV/AIDS never waned and since 1995 he heads the Immunology Unit of ITM whose main focus is the cellular immunology of HIV/AIDS.

Prof. Marie Laga joined the Division of Microbiology at ITM as a scientific collaborator in April 1984. In the period April 1984-September 1986, she was a research fellow at Kenya Medical Research Institute and WHO Collaborating Center for STD Research and Training at the University of Nairobi, Kenya. As a young ambitious physician with an extraordinary field experience in India, Zaire and Burundi she joined the Institute. Due to her extensive field experience, she was very efficient in her work in the Projet Sida. M. Laga together with the Zairian colleagues, proved that preventing and treating sexually transmitted diseases (STDs) decreases the incidence of new HIV infections. This was of crucial importance for the future worldwide intervention-prevention activities against HIV/AIDS. Projet Sida also rigorously measured both the prevalence and incidence rate of HIV infections in Kinshasa. In contrast with the USA and Europe, the Projet Sida study highlighted that HIV in Central Africa was spread predominantly by heterosexual transmission. It was clear from comparative studies that AIDS patients had had significant more ex partners as well as more contacts with prostitutes compared to the control group. Prospective studies in Kinshasa (Projet Sida) and Nairobi, Kenya by M. Laga and P. Piot, have shown that genital ulcers caused by *H. ducreyi* as well as *Chlamydia trachomatis* infections, can favour the transmission of HIV. The high prevalence of STI's in many urban populations of central Africa has increased the heterosexual transmission of HIV considerably. AIDS patients transmitted HIV more effectively than seropositive healthy HIV infected individuals. HIV infected pregnant women transmitted HIV to their baby's with a frequency of 40 up to 65% compared to 5-10% in Europe. The risk for HIV infection after blood transfusion was much higher in Africa compared to Europe. Up to 30% of AIDS patients in Africa have been infected through blood transfusion. Many of these observations of crucial importance for the future fight against HIV/AIDS worldwide have been generated during the Projet Sida. The results of this project formed the basis for the future prevention-intervention actions against HIV/AIDS which are still ongoing now.

Due to the rapidly expanding HIV/AIDS research activities we needed to reshuffle the virologic priorities. P. Piot and G. van der Groen decided to close down the high security laboratory in which we have manipulated successfully the most dangerous haemorrhagic fever viruses in the period 1978 up to 1985. The laboratory was re-equipped in order to grow and characterize HIV-1 viruses isolated from HIV-1 infected patients, as well as to optimize new serologic as well as molecular biological

techniques to diagnose and characterize retroviruses, of which HIV-1 was one of them. In 1984, G. van der Groen went to the laboratory of L. Montagnier to be acquainted with the necessary techniques and protocols to isolate and characterize HIV-1 strains.

Back at the Microbiology Division, we started to install the HIV-1 virus isolation protocols, as well as started the use of the first commercially available enzyme linked immunosorbent assays (ELISA) for the detection of HIV-1 antibodies in HIV infected patients. This was an enormous progress, since HIV infected patients at average remain healthy without any specific clinical sign or symptom the first 10 years to 12 years post infection. The test allowed detecting the infection much earlier.

In 1985 P. Piot became director of a new erected AIDS Reference Laboratory (ARL) at ITM, one of the seven ARL's which have been erected in Belgium. The aim of these laboratories was to collect epidemiological data on the HIV/AIDS epidemic in Belgium, as well as to optimize and improve the diagnosis and to develop new methods to improve the treatment and follow up of HIV infected and AIDS patients in Belgium.

In 1987 the clinic Leopold II at ITM was transferred to the Academic Hospital Antwerp.

A new unit to treat and care AIDS patients was erected under the leadership of Dr. Bob Colebunders. To promote the spread of information concerning HIV/AIDS prevention to the general public and to risk groups in Belgium, P. Piot erected different organisations such as Stag/AIDS telephone (1985), Inter Provincial AIDS Coordinate (IPAC) (1987), "De Witte Raven" (1990) (self help group of HIV infected patients). He also collaborated with a private initiative which focussed on Belgian homosexuals (AIDS team 1987). In 1980 P. Piot erected a special clinic for STD patients. Diagnosis, treatment of STD was considered a win in the fight against HIV. P. Piot deserved a lot of credit, because the STD clinic activities were initially paid with money they received for the AIDS Reference Laboratories (ARL) activities.

Jan Vielfont started to work at ITM in 1975, and was in the 100 year history of ITM, the first administrator-logistic officer appointed at departmental level of ITM. We thought this was essential for a good management for a very rapid expanding department, with so many multidisciplinary projects overseas. J. Vielfont was also the driving force behind a long series of international AIDS conferences organized in Africa.

1986 - 1992 Exponential growth of the multidisciplinary fight against HIV/AIDS tuberculosis and STD in the Division of Microbiology - under the leadership of P. Piot.

In 1986, P. Piot intensified his contacts with the University of Nairobi, Kenya where he was appointed as an associate professor at the Division of Microbiology. In 1987, he became professor of microbiology and head, Department of Microbiology at ITM as well as the director of the World Health Organisation Collaborating Centre on AIDS at ITM. In the meantime, Projet Sida continued and needed his and our input. Unfortunately, this project ended in 1991. P. Piot was co-investigator in a large project "Projet Retro-Ci" Abidjan, Ivory Coast in 1992. On top of that, P. Piot was involved in numerous consultancies involving clinical, epidemiological and laboratory

work on AIDS, tuberculosis and leprosy, neonatal conjunctivitis, reproductive tract infections in women, and low birth weight. Needless to say that the international networking of the Microbiology Division was expanding fast. In this time period, molecular techniques were introduced in the laboratories and initiated a tremendous progress in the knowledge of mycobacteria which infected HIV and AIDS patients. So far, the study of atypical mycobacteria was hampered by the slow growth of the mycobacteria in culture. Moreover, AIDS patients were sometimes infected with mycobacteria which did not grow at all in culture. The polymerase chain reaction (PCR) made the impossible possible. Instead of spending months to grow a large enough quantity of mycobacteria to extract a sufficient amount of DNA, the PCR allowed to amplify the DNA to millions of copies in 24 hrs, allowing much faster characterization.

F. Portaels and her co-workers designed already in 1989 the use of DNA probes to examine mycobacteria isolated from AIDS patients. With their skills in molecular biology, they made a fast progress in genetic characterization, resistance monitoring and identification of multidrug resistant tuberculosis, as well as seroepidemiology of *M. ulcerans* (Ulcus Buruli) and lepra work.

In collaboration with Innogenetics NV, a Belgian biotech company, we developed a new assay (INNOLIA) to confirm initial antibody positive samples in the ELISA screening assays. We have isolated SIV_{cpz} from wild life chimpanzees and in collaboration with Innogenetics NV, this virus has been characterized as new retrovirus, genetically close to the HIV-1 found in humans. This was the first evidence that HIV-1 was transmitted to humans through a zoonose: the first HIV-1 strains of African origin were isolated; reanalysis of human sera collected in 1976 in Zaire during the Ebola outbreak, were HIV antibody positive. This proved that HIV was already circulating in 1976 in Zaire, before it appeared in 1981 for the first time at the West Coast of USA; it took five years after discovery of AIDS in the USA, to convince Lancet to accept a paper describing the natural history of HIV infection in Zaire, highlighting heterosexual transmission was the main route; evidence for transmission by blood transfusion; association tuberculosis and AIDS; risk factors female to male transmission; in collaboration with Innogenetics NV HIV-1_{ANT70} was isolated and characterized. The antibodies induced by this virus were not picked up by existing commercially available HIV antibody tests. New tests were produced which picked them up.

Belgian virologist Martine Peeters played a key role in the elucidation of the ancestry of HIV-1

In 1989 Martine Peeters, a dynamic Belgian virologist and colleagues described for the first time ever the isolation and partial characterization of an HIV-1 related virus occurring naturally in chimpanzees in Gabon¹, West equatorial Africa.

Genetic analysis of this chimpanzee virus has shown that the chimpanzee lentivirus was closely related to the HIV-1 found in humans. The virus was called SIV_{cpz-gab}, with SIV standing for simian immunodeficiency virus, cpz (chimpanzee), gab (Gabon). This was at the time news! Was the chimpanzee carrying the ancestor of HIV-1? Intrigued by this question, Martine Peeters as a young PhD student, joined our team at PLITM, and continued her search for other HIV-1 like chimpanzee viruses. Forty-four wild captured chimpanzees were tested for HIV and SIV antibodies, 34 of which lived in captivity in Belgium, and 10 in captivity in Côte d'Ivoire. One chimpanzee was confirmed HIV-1 antibody positive. Out of this chimpanzee a second HIV-1 related virus was isolated called SIV_{cpz-ant} (ant = Antwerpen²). The positive chimpanzee was a young male estimated to be 5 years old. It was impounded by customs officers in Brussels upon illegal arrival in Belgium from Zaire, where it had been captured for sale as a pet animal. On arrival, the chimpanzee was estimated to be between 24 and 30 months old. It was not used subsequently in medical experiments and did not receive

injections with infected material.

Soon after the SIV_{cpz-ant} virus isolation, the chimpanzee was transported to a special facility in the Netherlands where, in close collaboration with ITM, it was still studied for many years. It allowed Dr. Pascale Onda, at that time PhD student in Immunology Unit, Department Microbiology, to study in great detail the mechanisms by which SIVcpz in chimpanzees continue to multiply, without causing the disease AIDS. The animal is still alive today and shows no sign of AIDS-like disease. The follow-up of the animal stopped and is enjoying his retirement after the many contribution he has given to science.

Soon after the discovery of SIVcpz-ant, Martine Peeters discovered a third SIVcpz representation: SIV_{cpz-qab2}³. The SIVcpz isolates differed from each other as well as from the reference HIV-1 strain⁴.

As such, the ancestry of HIV-1 (human immunodeficiency virus-1) has been traced to SIVcpz (simian immunodeficiency virus) infecting chimpanzees (pan troglodytes) in west central Africa., but the origin of SIVcpz itself remains unknown. Martine Peeters in close collaboration with American researchers continued the study of SIVs in different wild captured non human primates. In phylogenetic analyses (analysis of how different parts of the genome (genetic information) of different viruses, differ or resemble each other), SIVcpz clustered in the *pol* (polymerase) region closely with SIVrcm from red-capped mangabeys (*Cercocetus torquatus*) and in the *env* (envelope) region closely with SIVgsm from greater spot-nosed monkeys (*Cercopithecus nictitans*).

Other features of the genomic structure of SIVcpz, SIVrcm and SIVgsm did not differ significantly. This is consistent with a more recent origin of SIVcpz by recombination between ancestors of SIVs infecting red-capped mangabeys and greater spot-nosed monkeys, the ranges of which overlap with chimpanzees (*P.t. troglodytes*) in west central Africa.

Because chimpanzees are known to hunt smaller monkey species, the simplest explanation appears to be that both SIVrcm and SIVgsm have been acquired by chimpanzees and recombined in the same host. Recombination means that chimpanzees have hunted, killed and consumed simultaneously two different monkey species of which one was SIVrcm, the other SIVgsm infected. The two viruses entered simultaneously the same host cell in the chimpanzee. Inside this host cell part of the SIVrcm genome was recombined with a part of the SIVgsm, resulting in a new SIVcpz virus. If you compare SIVrcm with a woman and SIVgsm with a man, due to recombination a new virus will be formed which above the belt looks like a woman, and under the belt like a man... This new virus is SIVcpz with biological properties which differ from SIVrcm and SIVgsm. The latter was capable of spreading to humans. Consequently, HIV-1 disease in humans is a zoonose (transfer of a virus from an animal host to humans).

It will be important to examine whether chimpanzees' predatory behaviour has led to other SIV infections of chimpanzees and whether the resulting chimpanzee-adapted SIVs are more likely to infect humans⁵.

Cross-species transfer of SIVs will continue to occur between monkeys as well as between monkeys and chimpanzees, and last but not least, between chimpanzees and man. It means we should be prepared to discover new SIVcpz strains as well as new SIVcpz introduction into humans. Consequently, it is not excluded to discover new HIV-1 strains which represent new members in the subgroups (M, N, O, N) of HIV-1 or even representatives of a new group of HIV-1 type.

1. Peeters M et al. Isolation and partial characterization of an HIV-related virus occurring naturally in chimpanzees in Gabon. AIDS 1989;3:625-630. 2. Peeters M et al. Isolation and characterization of a new chimpanzee lentivirus (simian immunodeficiency virus isolate cpz-ant) from a wild-captured chimpanzee. AIDS 1992;6:447-451. 3. Peeters M et al. Phylogenetic analysis of a new chimpanzee lentivirus SIVcpz-gab2. AIDS Res Human Retrov 1994;10:1371-1373. 4. Vanden Haesevelde MM et al. Sequence analysis of a highly divergent HIV-1-related lentivirus isolated from a wild captured chimpanzee. Virology 196;221:346-350. 5. Bailes E et al. Hybrid origin of SIV in chimpanzees. Science 2003;300:1713.

S.R. Pattyn, pioneer of bacteria and virus fighting retired in 1992. P. Piot left ITM to become associate director of Global Programme on AIDS and later on, in 1995, executive director of the Joint United Nations Programme on HIV/AIDS. Dr. Wouter Janssens and Ing. Leo Heyndrickx joined the Division of Microbiology. They were recruited to strengthen the molecular biology of retroviruses in the Division of Microbiology.

1993 – 2000 Impact of the first-ever external evaluation (1995) on the research efficiency of the new reorganized Department of Microbiology

The internal structure of PLITM, with fifteen small and relatively autonomous units, was no longer adapted to goal-oriented scientific policies, multidisciplinary synergy and cost effect management. Therefore, the units were effectively integrated in five new departments. Each department was requested to conduct an internal evaluation of past achievements and to establish its own policy plan for the period 1996 – 2001, in order to prepare an in-depth departmental audit by the SAB late 1996. G. van der Groen was elected as chairman of the restructured Department of Microbiology. Based on the recommendation of the SAB, the department was restructured, weak departmental research activities were ended (mycology transferred to Institute of Public Health (IPH). See frame article: "The Vanbreuseghem Mycotheque") and the available institutional means (personnel and lab space) were reallocated. The Department was renamed in Department of Microbiology. The new Department of Microbiology consisted of a Sexual Transmitted Disease (STD)/HIV Research and Intervention, Mycobacteriology, Immunology and Virology Unit under the leadership of respectively, M. Laga, F. Portaels, L. Kestens and G. van der Groen. The Virology Unit consisted of three subunits: virology, molecular biology and the AIDS Reference Laboratory. All units were supported by an administrative and logistic team of the department under the leadership of J. Vielfont.

The "Vanbreuseghem Mycotheque"¹

Raymond Vanbreuseghem (RV) (°31.12.1909 Monceau sur Sambre - † 27.11.1993 Brussels) graduated as doctor of medicine at the State University of Liège in 1934. After receiving his diploma from the Institute of Tropical Medicine in Antwerp, he worked from 1935 to 1946 in the former Belgian Congo. Returning to Europe, he went to Paris when he had the opportunity of working with famous mycologists such as Maurice Langeron and his friend Emile Rivalier. It was during his stay in Paris, that in 1947, he isolated and collected the first fungi, which at the beginning were mainly dermatophytes. He developed a new technique for the isolation of fungi from soil². This technique was used by mycologists worldwide and expanded enormously the identification of new fungi species. Over a period of fifty years, RV collected and characterized with the help of his collaborators Prof. Dr. Charles DeVroey, Prof. Dr. Mitsuo Takashio and Prof. Dr. D. Swinne, 12.145 fungi isolates.

This unique "Vanbreuseghem Mycotheque" or "RV collection" from the Institute of Tropical Medicine in Antwerp was transferred to the Laboratory of Mycology (IPH, Brussels. Head laboratory: Dr. Nicole Nollard) and integrated into the Belgian co-ordinated collections of micro-organisms and - in effect - represents RV's legacy.

The RV collection of *Cryptococcus neoformans* is the largest, with 2.549 isolates from all over the world and both varieties, *neoformans* and *gattii*, are well represented. Although *Cryptococcus* is a worldwide mycotic disease, most cases nowadays are associated with AIDS. Prof. Dr. D. Swinne is actually the curator of the collection.

In conclusion, and I quote Prof. Dr. D. Swinne: "this RV collection is not only a witness of the past, but is biological material for the present and the future. It's part of world heritage" end quote.

¹. <http://bccm.belspo.be/newsletter/7-00/bccm02.htm>

². L. Eyckmans. Bull Seanc. Acad. r. Sci. Outre-Mer 1994;40:86-91.

Policy plans of the Department of Microbiology for the period 1996-2000

The mission statement of the department was: "to contribute to the control of HIV/AIDS, sexually transmitted infections and tuberculosis worldwide and in particular in developing countries".

Each department had essential three tasks: research, education and service. For the period 1996-2000, the main research objectives were: to identify and develop strategies for the prevention and control of sexual transmitted infections and HIV/AIDS in developing countries (*STD/HIV Research and Intervention Unit*); to study the phenotypic and genotypic diversity of HIV and its impact on vaccine development, antiviral therapy and diagnosis (*Virology Unit*); to study the immunologic defence mechanisms which correlate with protection against HIV/AIDS and its implications on prevention and treatment (*Immunology Unit*); to optimize diagnosis and control of tuberculosis and non-tuberculosis mycobacteria (NTM); to develop methods for rapid detection and worldwide surveillance of multiresistant tuberculosis; to develop new methods for the identification of the reservoir of *M. ulcerans* (etiologic agent of Ulcus Buruli) and to better understand the transmission mechanisms of *M. ulcerans* (*Mycobacteriology Unit*); Multiple national as well as international collaborations were erected in each of the units with research centres as well as with industry (Innogenetics NV, VIRCO-TIBOTEC, Janssen Pharmaceutica, Beckton-Dickinson, Chiron).

Research, education and service results obtained in the period 1996-2000

The results obtained in the period 1996-2000 have been published in 222 articles published in international peer reviewed journals with an average impact factor of 3.8. In addition, 103 scientific articles have been published in international and national journals. During this period, eight young scientists have defended their PhD thesis successfully.

The Department of Microbiology had an important input in the modification and upgrading of an existing Master course into a Master's Course in Disease Control (MDC). M. Laga, head of the STD/HIV Research and Intervention Unit was co-ordinator of MDC, its erection in October 1998. Some of the academicians and scientists were nominated since as guest professors and/or lecturers at the Vrije Universiteit Brussel (VUB) and at the Universiteit Antwerpen (UA). We also supervised the thesis work of many students in the "Master" course of ITM, as well as from VUB or UA, as well as PhD thesis students.

The structures in Africa are not always there to receive the brains that are trained

The Department of Microbiology at ITM has trained and is still training a lot of very talented young scientists from Africa. The most talented were able to perform a PhD thesis, obtained a Doctors title and became internationally highly respected scientists. One of them was Dr. John Nkengasong from Cameroon, described by John Cohen in the highly respected journal *Science*¹, as an example of an outstanding young well trained scientist, contributing to the development of Africa.

John Nkengasong, son of a Cameroonian subsistence farmer, graduated from the University of Yaounde in 1987. He won a scholarship to study in Belgium at the ITM where he, in, 1992, obtained a Master in Tropical Biomedical Sciences (MScBT), as well as a Masters in Medical and Pharmaceutical Research at the Free University of Brussels (Vrije Universiteit Brussel – VUB). Under the supervision of Peter Piot and myself at PLITM, John Nkengasong obtained his PhD in virology at the VUB in 1995.

John Nkengasong was the first African who was put in charge of the direct supervision of the virologic laboratory activities at PLITM. We really had a lot of confidence in him and we were proud of his accomplishments there. John badly wanted to return to Africa, but he could not find an attractive job in Cameroon, his homeland. "The structures in Africa are not always there to receive the brains that are trained" he said. When the chance to head the virology lab at the Projet Retro-Ci, Abidjan, Côte d'Ivoire (Ivory Coast) came up, John jumped on it.

There, John as a well trained virologist with an extensive experience in the study of the genetic,

antigenic and biological diversity of HIV strains of African origin, was hired in 1996 to build a virology lab from scratch. As such, he became in charge of one of the best equipped HIV labs in sub-Saharan Africa. A key part of John's work at that time was measuring quantitatively the amount of HIV virus circulating in the patient (viral load), which was an essential parameter for treatment of patients with antiretroviral drugs. John and his co-workers also have analysed the genetic diversity (subtypes) of HIV-1 and HIV-2 circulating in vaccine developers. Unfortunately, due to political instability, the Projet Retro-Ci in Abidjan was stopped and John Nkengasong and his colleagues had to move to the Centers of Disease Control (CDC) in Atlanta, Georgia, United States. Currently John Nkengasong is the team leader of the Global AIDS Program Laboratory, Global AIDS Program (GAP), CDC. He oversees the laboratory activities in all 25 GAP countries. These activities include lab monitoring for anti retrovirals (ARVs), early diagnostics in programs or mother-to-child transmission, surveillance of ARV drug resistance, measuring HIV incidence using novel approaches... etc.

John Nkengasong is a nice example of an excellent well-trained person who has given a lot back to the continent where he got his basic training. In future we need to assure better the continuity of the career of young African scientists who come to Belgium for study and training. This can be done by a better integration of his or her PhD thesis work into the research priorities not only of the host laboratory in Belgium, but also of the center in Africa where the candidate studied or worked before his or her departure to Belgium. "Sandwich PhD's" should be promoted, whereby a part of the thesis is effectuated in Belgium as well as in the homeland of the candidate: long-term planning is needed as well as a budget to assure the young Doctor in Science, can return to his country and has a budget to continue his/her career in his/her homeland for at least five years, during which he or she will be able to find the necessary additional funding to proceed further .

1. Cohen JC. Building a World-class lab from scratch. Science 2000;288:2157

At 04.09.2000, the AIDS Reference Laboratory (ARL) obtained an official accreditation certificate (NBN-EN-45001) N° 194-T. The external committee for quality control (Beltest) expressed their appreciation and were, quote: *"impressed by the practical skills, knowledge and commitment of each of the ARL personnel members. It was clearly shown that they managed well and performed accurately what had been written up in the standard operation procedures"*.

The Virology Unit was a "World Health Organization Centre for Transfusion Transmissible Infections" (1985-2002) as well as a member of the UNAIDS HIV variability network.

In 1999, the Mycobacteriology Unit was acknowledged as an International Reference Centre for Tuberculosis. They were also nominated in 1999 as the worldwide coordinator of the WHO/International Union Against Tuberculosis and Lung Disease (IUATLD); Supranational Reference Laboratory for Buruli Ulcer; EC Reference Laboratory for Zoonoses (TB); European Reference Laboratory for bovine tuberculosis.

At the national level, the mycobacteria unit was acknowledged as:

Flemish Reference Laboratory for Tuberculosis and Mycobacteria: National Reference laboratory for Tuberculosis and Mycobacteria.

The Immunology Unit started already in 1985, routine determination of CD4 T-cell subset in HIV infected patients, as a service to the outpatient clinic of ITM. An increase of 140% was observed in number of samples analyzed between January 1996 and December 2000.

The STD/HIV Research and Intervention Unit provided technical assistance to the HIV/AIDS intervention projects of Family Health International (US); to STI/HIV prevention and control in Cambodia. T. Delvaux served as advisor to Directorate-

General for International Cooperation (DGIC) on matters of reproductive health and rights and its integration with HIV/AIDS prevention and control and STI care. M. Laga served as an advisor to the DGIC and cabinet of the state secretary on matters of HIV/AIDS policies within the Belgian development co-operation. Since 1998, the unit assumed the co-ordination and supervision of an HIV prevention project among migrants, predominantly from sub-Saharan Africa in the Flanders region.

Foundation of the “first” departmental logistic and administrative unit

The logistic/administrative team co-ordinated by J. Vielfont was the backbone of the Department of Microbiology, co-ordinating and carrying out all logistics, financial and administrative aspects of all research and intervention projects of the Virology and STD/HIV Research and Intervention Unit. Later on this service was extended to the other two units. This included logistic and financial coordination and follow up of a large number of projects with international partners, preparation of supplies and shipment overseas, reception of collaborators and trainees visiting ITM, organisation of meetings, workshops and training sessions related to the projects, but also all secretarial support, data entry and quality control and production of audio-visual aids when needed. The team was also very instrumental in the organisation of international conferences on AIDS in Africa, on request of UNAIDS: Aids in Africa, Kampala-Uganda (1995), Abidjan – Ivory Coast (1997), Lusaka-Zambia (1999), Ouagadougou-Burkina Faso (2001). Jan Vielfont was also the interlocutor of the Department of Microbiology to communicate with the central administration. Because of the efficiency of J. Vielfont's logistic and administrative team, academic and scientific staff of the department were left with more time for research, education and service. In the hundred year long history of ITM, the erection of a departmental logistic and administrative unit was considered as an innovation. Later on, it has inspired other departments to follow this initiative, resulting in an improved managerial interaction with the central administration of the Institute. For further reading on the research, education and service activities in the period the author refers to ⁷.

2001 – 2005 New policy plans of the Department of Microbiology

2001 Audit report by the Scientific Advisory Board

After the fundamental reorganisation of the PLITM in 1996, the research, education and service achievements of the Department of Microbiology for the period 1996 – 2000 were critically evaluated by the external Scientific Advisory Board (SAB).

The Audit report of 16-17 July 2001 for the period 1996-2000 on the research, services and education of the Department of Microbiology, can be summarized as follows:

Among the educational activities the course “Masters in Disease Control” was evaluated as excellent.

For the research and service activities the SAB has given the following appreciation: Quote *“The Department of Microbiology as a whole, through its laboratory and fieldwork, is of true international level and definitely belongs to the top 20% worldwide. PLITM should maximally support the Department of Microbiology with its*

own funds and do whatever is possible to attract external funds from Belgian as well as international sources". End quote. Encouraged by the positive evaluation of the SAB, the Department of Microbiology was able to employ a senior epidemiologist, Anne Buvé.

Anne Buvé, epidemiologist joined the STD/HIV Research and Intervention Unit. She was acting head of the STD/HIV Research and Intervention Unit during the sabbatical leave of M. Laga, who spent two years (2001-2003) in Abidjan, Ivory Coast, as director of Projet Retro-Ci, a large center for Disease control (CDC) funded HIV/AIDS intervention program (prevention, care and capacity building).

New policy plans for the period 2001-2005

The Scientific Advisory Board has also given green light to proceed with the new policy plans of the Department of Microbiology. The Department had to describe the future objectives as well as to describe the studies and activities which were planned in order to fulfil each of the objectives. In the following paragraphs the policy plans, as agreed on by the SAB, are briefly described.

The mission statement of the Department of Microbiology which was: "to contribute to the control of HIV/AIDS, sexual transmitted infections and tuberculosis worldwide and in particular in developing countries", remained the same for the period 2001-2005.

The internal collaboration between the different units was strengthened considerably. The research policy plans were focused on five objectives.

Objective 1: Development and evaluation of strategies to prevent and treat STD and HIV/AIDS in developing countries

The Department of Microbiology continued the studies to better understand the different dynamics of HIV/STI epidemics in Africa. The different speed of HIV/AIDS spread in Africa is related to a variety of factors (virologic, host dependent, cultural, social, economic, genetic ...). The better we start to know these factors, the more efficacious will become our prevention-intervention strategies to slow down or stop the spread of HIV/AIDS.

The evaluation and implementation of interventions among sex workers, youth, mother-to-child transmission and care and support for people living with HIV/AIDS will continue and strengthened⁸.

For the period 2001-2005, the Department, in close collaboration with the Centers for Disease Control, Atlanta, US, intended to set up interventions in Kisumu, Kenya, targeting youth. It was decided to set up cohort studies on the interaction between Herpes Simplex Virus 2 (HSV-2) and HIV (Rationale: it was hypothesized that HSV-2 was a strong biological co-factor for the sexual transmission of HIV, and that HIV influences the susceptibility to HSV-2, resulting in a mutual reinforcement of the two epidemics, and one of the possible driving forces of those explosive epidemics in Southern Africa).

While in the previous period 1996-2000, the Department obtained disappointing results of phase III trial with N-9, the unit remained fully committed to continue to contribute to the development and evaluation of new vaginal microbicides. Further identification and preparation of phase III trial sites was set up in close collaboration with the Contraceptive Research and Development Program (CONRAD). The phase III results of a new promising microbicide was expected to be known in 2010. We aim to improve the diagnosis of *T. vaginalis* in order to better understand the

epidemiology/transmission of this sexual transmitted infection. A new simple and cheap test for the diagnosis of *C. trachomatis* and *N. gonorrhoeae* will be evaluated, as well as the introduction of this simplified technology within health programmes in developing countries in Africa. Mother-to-child transmission of HIV can be reduced with 50% with the short course of anti-retroviral therapy of mother and child around time of delivery. It was decided to study how to integrate such a mother-to-child HIV prevention programme within the existing mother-to-child health services in Africa. Due to the high adequate antiretroviral treatment (HAART) becoming available for HIV+ patients in developing countries, the demand for capacity building was overwhelming. The key issue was how to deliver HAART in a safe and responsible way within the context of existing health services in Africa. Therefore it was decided to study models for care and support for people living with HIV/AIDS in developing countries.

Objective 2: To study cellular and humoral immunity against HIV. Implications for vaccine development, antiviral therapy and diagnosis of HIV.

To fulfil this objective, the following studies were planned for the period 2001-2005: continuation of the study of the genetic variability of HIV-1; development of a well-standardized high-throughput system to quantify antibodies in sera of HIV infected or HIV vaccinated individuals, which neutralize in vitro primary HIV-1 virus isolates, as well as the development of a more sensitive method, the enzyme-linked immunospot (ELISPOT) to measure cellular anti-HIV responses quantitatively (Rationale: Since they don't know yet the correlates of protective immunity against HIV-1 in vaccinated individuals, they need to develop standardized protocols and assays to measure neutralising antibody responses as well as CTL and T-helper responses in sera of anti-HIV vaccinated individuals, in order to compare the strength of the induced immunity and to correlate it with the efficacy of a vaccine); identification and isolation of cross-neutralizing human monoclonal antibodies (able to neutralize a wide variety of genetic different HIV-1 virus), which could be used as therapeutic agents in combination or instead of the known existing anti-retroviral drugs; identification of epitopes of HIV-1 which were able to induce cross neutralising antibodies, to be included into experimental vaccines; validation of the viral fitness of and how it correlates with disease progression and transmission; development of an in vitro model for immunotherapy using dendritic cells; evaluation of new "prime boost" vaccination strategies using immunostimulating complexes (ICSOMs) to protect monkeys challenged with SHIV viruses; generation of pseudoviruses and validation of the use of them in measuring protective humoral and cellular immune responses. (Rationale: Pseudoviruses are not infectious for the laboratory worker, and can be used in bio-safety level two type of laboratory, which is less demanding in safety precautions and consequently can be used in developing country settings.); consolidation of the successful method previously developed in the department for the preclinical evaluation of anti-retroviral products intended to be active very early in the infection (morning after pill; microbicides); expanding and upgrading the biosafety level 3 laboratoria to work with HIV/AIDS and tuberculosis of ITM.

Objective 3: To increase the availability and follow up on highly active anti-retroviral therapy (HAART) in developing countries

(Rationale: only in 1996 HAART became available and was mainly used in the developed part of the world. Expertise was lacking how to install, implement and follow up HAART, inside the existing or not existing health services in developing countries). Therefore, the following studies-actions

were planned for the period 2001-2005: giving input in the Directorate-General for Development Cooperation (DIGIS) integral AIDS policy; in collaboration with the Departments of Public Health and Clinical Sciences: Beleidsvoorbereidend Onderzoek (BVO) to advise on the organisation and financing of HAART in developing countries; improving laboratory skills and methods to better follow the efficacy of HAART therapy (CD4+ cell counting: resistance monitoring: viral load monitoring)

Objective 4: To contribute to the better control of tuberculosis and other mycobacterial diseases in humans and animals worldwide and in particular in developing countries using new as well as conventional tools and strategies.

The following studies-actions were planned for the period 2001-2005 to fight tuberculosis: to optimize the techniques for direct smear sputum examination and interpretation of the results; to develop and evaluate methods for rapid diagnosis of multi-drug resistant TB; to develop new genetic markers and techniques for molecular-epidemiology studies of tuberculosis (Rationale: which strains circulate where? How they correlate with drug resistance?); to consolidate the surveys and surveillance of *M. tuberculosis* drug-resistance and to improve the role of the supranational Reference Laboratory Network; to evaluate standard regimens in areas with high and low prevalence of MDR-TB, using drug susceptibility testing; to continue and consolidate MDR-TB treatment trials; to facilitate the discovery of new antimycobacterial drug candidates, in collaboration with the biotech companies TIBOTEC-VIRCO (Mechelen – Belgium). (Rationale: Given the HIV-pandemic, coinciding with the increasing incidence of MDR-TB, makes the discovery of new anti-TB drugs a top priority. Having access to a large bank of MDR-TB strains will help the evaluation of the new drugs. Rapid MDR-TB will allow also to select better treatment regimens. The Mycobacteriology Unit has an enormous collection which is still expanding.); to set up of well-characterized strain collection of *M. tuberculosis* complex strain; and to develop and use better transport media for clinical specimens and better conservation techniques for isolated strains.

The following studies-actions were planned for the period 2001-2005 to fight Non tuberculosis mycobacterial diseases (NMD): to develop new techniques for the identification of the natural reservoir of *M. ulcerans*, and to understand better the transmission of *M. ulcerans*. (Rationale: Buruli Ulcer caused by *M. ulcerans*, is the most frequently observed mycobacterial disease in humans after tuberculosis and leprosy; to improve the diagnosis of bovine paratuberculosis. (Rationale: Bovine tuberculosis as well as NMD are frequent in animals, and diseases in animals may have an impact on human health (zoonoses); to optimize molecular techniques to detect *M. leprae* DNA in the environment. (Rationale: the prevalence of clinical leprosy has decreased very considerably worldwide since the introduction of the WHO multidrug therapy regimens. That was the reason the unit stopped their research activities on leprosy during the period 1996-2000. However, the detection rate on an annual basis has remained astonishingly stable at about 500 000 to 700 000 new cases per year during the last 15 years. The possibility of existence of an extra human reservoir of *M. leprae* in animals or in the environment remains a possibility and should be explored)⁹.

Objective 5: Study of the immunologic interactives between HIV, HTLV-1 and parasite infections

To fulfil this objective, the following studies-actions were planned: to start and consolidate ongoing collaboration with centres in Senegal, Peru and Zambia.

Education policy plans 2001-2005

Since PhD students are the “oxygen” for a dynamic and fruitful research in the Department of Microbiology, a lot of effort will be spent to recruit good and highly motivated PhD students as well as to spend a lot of time and energy in coaching these students in performing their research activities, as well as in writing and defending their PhD theses. For each PhD student, it is expected that they have published at least two articles as leading author in international highly respected peer reviewed scientific journals, before they defend their thesis in public.

In order to attract and sensibilize young students to come and perform a thesis at the Department of Microbiology, some members of the academic staff of the Department will continue and consolidate their guest professorships at Flemish universities. This implies teaching 60 hours a year of immunology, infectious diseases and molecular biology of HIV in the Masters of Biomedical Sciences at the University of Antwerp. In the same context, the guest professorship at the Free University of Brussels (VUB) of two members of the academic staff of the Department of Microbiology will be extended. Each of them will continue to spend 20hrs on HIV/AIDS and tuberculosis. The aim of the courses is to help the student to understand “why it is so difficult to fight HIV/AIDS and tuberculosis”. These courses fit into a Master & PhD Program in Medical and Pharmaceutical Research at the Free University of Brussels. The expected outcome is to obtain a positive evaluation by the students as well as by the educational board of the respective universities and to recruit a number of talented youngsters to start successfully a PhD thesis at the Department of Microbiology, ITM. Last but not least, some of the academic staff members of the Department of Microbiology will be involved in the creation of a new Master in Tropical Biomedical Sciences, in close collaboration with the University of Antwerp and the Free University of Brussels.

Service policy plans 2001-2005

Activities which includes the following national reference tasks: confirmation or exclusion of HIV infection; development or optimization of new techniques for the diagnosis and follow-up of an HIV infection in patients; evaluation and quality controls of HIV diagnostic assays; making available new epidemiological data on the HIV/AIDS epidemic in Belgium. During the period 2001-2005, it was expected to establish a central operational electronic data handling system for the seven AIDS Reference Laboratories in Belgium; to extend the quality accreditation status of Beltest n° 194; to extend the annual updates on the HIV epidemiology in Belgium.

Consolidation of the immunologic diagnostics service for the polyclinic of ITM which implies quantitation of the number of CD4⁺ lymphocytes of HIV infected patients under anti-retroviral treatment. It is expected to obtain the Beltest quality control accreditation for the quantitative CD4⁺ lymphocyte assay. Continuation of making recommendations to the National Program for Lymphocyte Characterization in Belgium.

Consolidation of the international reference tasks on HIV diagnostics on behalf of the World Health Organization, which implies the critical evaluation of the operational characteristics of commercially available diagnostic assays for monitoring HIV-1 &

HIV-2 antibodies and/or antigens in sera of HIV infected patients. Expected outcome to have the evaluation results published in World Health Organization publications being distributed worldwide; to obtain the prolongation of the contract as World Health Organization collaborating center for Transfusion Transmitted Infections.

Consolidation of the international reference tasks related to tuberculosis and the Buruli Ulcer disease. This implies the continuation and consolidation of the following reference tasks: supranational reference laboratory for tuberculosis and multiple drug resistant tuberculosis; national reference centre for tuberculose and mycobacteria; Flemish reference centre for tuberculosis and mycobacteria; international World Health Organization reference laboratory for Buruli ulcer; worldwide coordinator of the WHO/IUATLD supranational reference laboratory network for tuberculosis drug resistance surveillance. It was expected to obtain quality control accreditation for the supranational reference laboratory of tuberculosis and multi-drug resistant tuberculosis, and to have the status of the other reference centres confirmed and prolonged.

Consolidation of the reference laboratory of sexual transmitted infections in support of the extensive field studies organized by the STD/HIV Research and Intervention Unit¹⁰.

2004 Reorganization of the Virology Unit in the Department of Microbiology

The Virology Unit was restructured in 2004 following the retirement of Prof. Dr. G. van der Groen.

Katrien Fransen joined the Department of Microbiology in 1989 as a scientific assistant. She was the driving force behind the successful accreditation of the AIDS Reference Laboratory. She was appointed as Director of the ARL and Dr. G. Vanham as Professor Head of the Virology Unit. Guido Vanham joined the Department of Microbiology in 1989 as Qualified Specialist in Internal Medicine.

This restructuring resulted in a significant extension of the research topics and of the scientific and technical staff.

Summary of research, service, education activities in the Department of Microbiology

Virology Unit

Research with regard to HIV-vaccine development continued in the Virology Unit. Dr. Janssens and collaborators use phage display technology to characterize epitopes of broad cross-neutralizing antibodies. Dr. Helen Donners undertook a training course at Scripps (La Jolla) to identify and purify the broad-cross neutralizing antibody fragments themselves, using a modification of phage display. Ing. Leo Heyndrickx further developed a high-throughput screening assay, based on single-round infection with pseudoviral particles in collaboration with TIBOTEC. Moreover, our joint project with Prof Leroux from Ghent University on *in vivo* activity of neutralizing antibodies in humanized SCID mice was successfully continued.

The search for microbicides, women-controlled preventive HIV medicines, has gained a lot of interest. Condom use is male controlled and often not negotiable for women

who are economically, socially or religiously dependent. For the most vulnerable population groups, including adolescent girls, sex workers and displaced people, there is an urgent need for the development of an HIV microbicide. The Virology Unit therefore developed an *in vitro* co-culture model of CD4+ T cells and monocyte-derived dendritic cells (MO)-DCI), the latter representing mucosal interstitial dendritic cells. The Unit further extended their model with an endocervical epithelial layer, thus closely mimicking the cells that play a crucial role during sexual transmission *in vivo*. Several new non-nucleoside reverse transcriptase inhibitors (NN-RTI), developed by the Center for Molecular Design of Janssen Pharmaceutica, were found to be extremely potent in our model. Moreover, HIV infection of the target cells could be fully prevented at compound concentrations for below immune suppressive concentrations. The Unit already tested *in vitro* a gel formulation of the NN-RTI TMC120, a product that is currently under evaluation for use as a microbicide in humans. Full prevention of HIV infection was obtained *in vitro*, offering hope for the potency of this compound *in vivo*. A first PhD thesis on the role of non-nucleoside reverse transcriptase inhibitors as potential microbicides was delivered by Yven Van Herreweghe and part of Harr Njai's thesis will also relate to this topic.

Immunotherapy offers a fascinating opportunity to complement or replace drug therapy. Our approach is based on electroporation of non-infectious but fully antigenic HIV m-RNA in either dendritic cells or B cells. Two PhD students, Ellen Van Gulck and Glenn Van Den Bossche, are working in this field.

The Unit is continuing research on the role of HIV fitness in disease progression and the shape of the epidemic. Kevin Ariën finished one study on the differential fitness of HIV subtypes and another on viral attenuation in the Antwerp epidemic over time. Relative HIV viral fitness showed that the "old viruses" (1986-1989) were significantly more fit than the recent ones (2002-2003), both in activated T cells (model for "pathogenic fitness") and in co-cultures of monocyte-derived dendritic cells and T cells ("transmission model"). Moreover, the less fit viruses were more susceptible to suppression by reverse transcriptase inhibitors and co-receptor blockers. These data are in favour of "viral attenuation" occurring over time, probably as a consequence of multiple transmissions. Harr Njai concentrated on the importance of viral recombination between subtype A and G in fitness.

A new fundamental research topic is on interactions between viral and interferon-induced proteins by functional proteomics, coordinated by Dr Janssens. Dr Janssens also started a new collaboration with the Gambia to characterize drug-resistant mutations in HIV-2 and to develop new tests. Ms. Sabelle Jallow received an ITM scholarship to perform her PhD on this topic. Based on the experience of our AIDS Reference Laboratory (ARL), Dr. Fransen continued the 2nd year of the capacity strengthening project at the National Reference Laboratory for HIV/STD, D.R. Congo. Under her guidance, Amber Litzroth developed and validated a new test for cost-effective diagnosis of HIV infections in newborns and monitoring viral load under therapy. Obviously, the ARL also continued its national and international reference tasks.

In addition to the HIV-related work, we have an ongoing project on HTLV-1 (Human T Lymphotropic Virus) in Lima, Peru. Tine Verdonck investigates the clinical and epidemiological aspects, whereas Ivan Best focuses on immunological and

virological aspects. A major achievement in 2004 was our successful application for a grant from the Flemish Interuniversity Council (VLIR) to reinforce the project with molecular virology and human genetics in collaboration with the University of Antwerp and the Universidad Peruana Cayetano Heredia in Peru.

Immunology Unit

The restructuring of the Virology Unit had important implications for the Unit of Immunology. Dr. Guido Vanham succeeded Prof. van der Groen as head of Department of Microbiology in January 2004. As a consequence he left the Unit of Immunology. Several research topics formerly associated with the Immunology Unit were transferred to the Virology Unit.

The Unit of Immunology, in its new form, continued to focus its research on the cellular immunology of HIV. The study of resistance to HIV in persistently HIV-exposed seronegative (ESN) subjects in Abidjan Ivory Coast continues, focusing on the identification and characterisation of HIV-specific T cell responses in ESN subjects.

Ms. Dominique Beels performed research in 2004 for her Master thesis on constructing new HIV-1 pseudovirus particles for the evaluation of humeral responses in HIV-1 infected subjects, in collaboration with Ing. Leo Heyndrickx (see Virology Unit). In December 2004, she successfully applied for an IWT PhD scholarship to extend her study on pseudovirus particles for the evaluation of cellular responses to HIV-1.

Antiretroviral (ARV) treatment for HIV is now more frequently used in developing countries. There is a great need for alternative and affordable tests for viral load and CD4, essential laboratory tools to monitor efficacy of ARV. The Immunology Unit received a prestigious grant from The American Dorus Duke Charity Foundation to develop alternatives for viral load & CD4 counting. Dr. Pascale Ondo and Chris Vereecken invested a lot of research energy to develop an alternative flow-based test to measure CD4 cells and HIV-p24 (alternative test for HIV viral load on the same instrument).

The Immunology Unit considerably reinforced the capacity of the Immunology laboratory of the virology and bacteriology Unit at the Centre Hospitalier Universitaire (Prof. S. Mboup) in Dakar, Senegal in the context of the ITM/DGDC programme. Several missions to Dakar were undertaken by Prof. Luc Kestens, Dr Pascale Ondo and Dr Wim Jennes to this end. Camara Makhtar, a sandwich PhD student, started his training in Antwerp in July 2003 and is supervised by Wim Jennes. Since the establishment of a CD4 training platform in the ITM laboratory, we provided training to scientists and technicians from the Tropical Disease Research Centre in Ndola (Zambia), from Guinée-Conakry and from NGO's such as MSF. Luc Boel visited the National Reference Laboratory for HIV/STD, D.R. Congo in December to evaluate and improve the quality of CD4 counting in the reference laboratory.

In 2004, the Immunology Unit renewed its agreement with the World Health Organization (WHO) as a WHO collaborating Centre for support to laboratory

diagnostics in HIV/AIDS (see further). Evaluations of new alternative techniques for CD4 counting were performed to this end.

The routine CD4 counting activities, which take place in the Immunology Unit since 1985 as a service to the out-patient clinic of ITM received the national BELTEST accreditation in 2004.

STD/HIV Research and Intervention Unit

Since its creation the STD/HIV Research and Intervention Unit focuses its research and service delivery activities on strategies for the prevention and control of HIV infection and other sexually transmitted infections (STIs), in particular in low resource settings. The unit continues to pursue these objectives but since 2002 is also involved in issues related to antiretroviral treatment in low resource countries. In collaboration with the Departments of Public Health and Clinical Sciences, the unit carries out policy research on obstacles and bottlenecks for scaling up access to antiretrovirals. This ITM/DGDC policy research programme also includes exploration of the effects of programmes for the prevention of mother-to-child transmission on quality of obstetric care. In addition one staff member of the unit is developing research on the management of TB-HIV co-infected patients.

The unit also studies the epidemiology and impact of other “neglected” STIs in developing countries. Research on the epidemiology of different trichomonads in women in Zambia, Tanzania and The Gambia is progressing. A population based study including assessment of the HSV-2 infection has been conducted in Western Kenya and a study is planned on the prevalence and risk factors for HSV-2 infection in female commercial sex workers in Cambodia.

In 2004 a phase 1 trial was completed with a vaginal gel containing TMC-120, a NNRTI developed by TIBOTEC (Johnson & Johnson). In addition, the STD laboratory has been contracted to provide technical assistance and assure the external quality control on the HIV and STI diagnosis in phase III clinical trials of vaginal microbicides, initiated by CONRAD (USA) and Family Health International (USA) in several sub-Saharan Africa Countries.

The unit maintains its commitment to the prevention of HIV infection and other reproductive health problems among vulnerable groups in the population. The target population includes sex workers in Abidjan (Ivory Coast) and Cambodia, young people in a rural area in Western Kenya and migrants from sub-Saharan Africa in Flanders. Interventions among sex workers consist of services for the treatment of STIs and other health problems and condom promotion. In Cambodia where abortion is legal, the unit has supported the set up of safe abortion services and is evaluating the effects of these on the use of contraceptives. The youth intervention programme in Western Kenya is a multicomponent programme that seeks to bring about behavioural change at the individual, family and community level. The programme will be evaluated by a pre- and post-intervention population based survey on sexual behaviour, HIV infection and other STI's. An anthropologist has joined the team in

Antwerp to support the youth interventions through research, more especially on the relation between sexual behaviour and means of support.

The collaboration between the unit and the National Centre for HIV, AIDS and Dermatology in Cambodia has been strengthened through a joint EuropeAid project that has been approved for funding by the European Commission. The contract between ITM and the European Commission came into effect on 1st February 2004 and the aim is to strengthen HIV prevention among young people and care of HIV-infected people in three operational districts in Cambodia.

In 2004 we held a two day retreat to assess the activities of the unit and discuss future directions. The unit's activities are very varied but at the retreat it became apparent that the potential for cross fertilisation between different projects was underutilised. Several projects have an important component of innovative interventions and are grappling with the issue of evaluation of the effects of the interventions.

The STI laboratory of the unit obtained accreditation by BELTEST for the testing of *Chlamydia trachomatis* and *Herpes Simplex virus 1* and *2* by molecular techniques. In December the laboratory was audited in Good Clinical Laboratory Practice by the Director of Regulatory Affairs and Quality Assurance of Family Health International.

M. Laga of the Unit, was asked to provide technical assistance to ACHAP, a Gates funded HIV/AIDS program in Botswana. The aim was to review their current activities and provide guidance for the future, mainly in terms of strengthening the prevention component. With the support of ACHAP, the government of Botswana has been very successful in rolling out ARV throughout the country, with a current estimated coverage of around 50% (the highest on the African continent). The main challenges are to keep the number of new HIV infections low by strengthening the primary prevention activities, as well as by using the opportunities in expanded care to maximise the "Prevention for Positives" strategies, including stronger involvement of people living with HIV.

M. Laga was also invited to participate in the mid-term review of TASO, a large Ugandan NGO, providing Care and Support services for PLWA. A team of consultants headed by JSI, was asked to review progress according to the targets set in the strategic plan (2002-2006), as well assess the justification, relevance and impact of the new activities such as ARV roll out and child and orphan care.

The Unit also provided technical expertise for UNAIDS led series of workshops on "AIDS in Africa: Scenarios for the future" as well as "Strategic and Technical Advisory Committee for HIV/AIDS" to evaluate HIV/AIDS work at WHO.

Mycobacteriology Unit

As the reference centre of an international network, the Mycobacteriology Unit continued its contribution to the world-wide monitoring of resistant tuberculosis, against first- as well as second-line drugs. The results vary strongly according to the region. Apart from economic reasons, efficiency of TB control programmes and the (sometimes chaotic) drug prescription policies appear to be major factors in the spread of drug-resistant TB. The unit also continued its work on the simplification,

standardisation and field-evaluation of techniques for the determination of drug sensitivity, with emphasis on cheap methods. In the field of TB treatment our unit contributed to the evaluation of standardised (re)treatment schedules for Multi Drug Resistant TB that gave very promising results so far. We participated in a multi-centre study on a gatifloxacin-containing drug regimen for shortening the duration of treatment of pulmonary TB. Furthermore, capacity strengthening of local laboratories through training and quality controls remained one of the goals in 2004.

Buruli ulcer (BU) remained the second most important research subject in 2004. The intense cooperation with the National Reference Laboratory of Benin has continued, and an external quality control of this lab proved excellent. In the search for the reservoir of *Mycobacterium ulcerans*, the role of various animal species was further investigated (fish, amphibians and molluscs)¹¹.

2. Actual situation in the world of the dangerous liaison HIV/AIDS & tuberculosis

2005 marks a pivotal moment in international efforts to fight extreme poverty. I quote: *“During the United Nations (UN) Millennium Summit in 2000, 147 heads of state gathered and adopted the Millennium Development Goals (MDG) to address extreme poverty in its many dimensions – income, poverty, hunger, disease, lack of adequate shelter and exclusion while promoting education, gender equality and environmental sustainability, with quantitative targets set for the year 2015”*. This highlights that the HIV/AIDS & tuberculosis fighters of the Department of Microbiology represents only a very small link in the long multidisciplinary chain of activities required to reach the MDG's. If the MDG's are to be met, 2005 must be a major increase in effort. Therefore it is considered a pivotal year¹².

HIV and *M. tuberculosis* annually cause 3.1 million and two million deaths respectively. In 2004, 600 000 individuals, doubly infected with HIV and *M. tuberculosis* died. Since World War I, approximately 150 million people have succumbed to these two infections, more total deaths than in all wars in the last 2000 years.

In 2004, 38.4 million people were infected with HIV, 2 billion with *M. tuberculosis*, and 15 million with both. In 2004, 5 million and 50 million were newly infected with HIV and *M. tuberculosis* respectively, with 2 million new double infections¹³. Compared to previous years, there were more new HIV infections 4.9 million and 3.1 million deaths in 2004. At December 2004, 39.4 million people were living with HIV, of which just under one half are women. Feminization of the epidemic¹⁴.

Where are we now in our fight against HIV/AIDS?

Good news

Worldwide, the number of people receiving counseling and testing services has doubled since 2001; the number of women offered services to prevent mother-to-child transmission has increased with 70% since 2001; the number of young people who have received AIDS education has doubled; the proportion of commercial companies in high-prevalence countries that have adopted HIV/AIDS policies in the

work environment has increased by 75% in the last year; the number of individuals receiving anti-retroviral therapy increased from an estimated 400 000 in June 2004 to 700 000 by December 2004; the survival rates of patients on antiretroviral therapy in low and middle income countries are 90% one year and 80% two years after initiating treatment; the availability of AIDS treatment has increased AIDS awareness in the community stimulated demand for HIV testing, and helped to ease the stigmatization of people living with HIV; in Sub-Saharan Africa, utilization of antiviral therapy more than doubled in the second half of 2004 and the number of service delivery points greatly expanded. For the first time in the 100-year history of ITM, two brand new fully equipped biosafety laboratories type three were inaugurated to cope with the enormous workload of the HIV/AIDS & tuberculosis research activities.

Bad news

AIDS undermines the hope of achieving the MDG's; only 12% of those who need antiretroviral therapy were receiving it as at December 2004; as many as 100 000 health and community workers must be trained to expand access to antiretroviral therapy; the average annual US\$ 300 per patient per year still inhibits broader access; of the total HIV infected 15% of those are children; currently not sufficiently help to address the growing crisis of orphaned children; acute shortage of trained personnel who possess requisite skills and expertise; lack of efficient microbicides to protect women; the first two HIV vaccines tested in phase three trials in human volunteers failed; increasing but still inadequate financial resources.

Millennium Development Goals 2015

- 1 Reduce extreme poverty and hunger by half relative to 1990.
- 2 Achieve universal primary education.
- 3 Promote gender equality and improvement of women.
- 4 Reduce child mortality by two thirds relative to 1990.
- 5 Improve maternal health, including reducing maternal mortality by three-quarters relative to 1990.
- 6 Prevent the spread of HIV/AIDS, malaria, tuberculosis and other diseases. To have by 2015, halted and begun to reverse the incidence rate of HV/AIDS. Between 1990 and 2015, to halve the TB prevalence and death rates. By 2005, to detect 70% of new smear-positive cases and successfully treat 85% of these cases.
- 7 Ensure environmental sustainability.
- 8 Develop a global partnership for development.

J.D. Sacks, JW McArthur. The Millennium Project: a plan for meeting the Millennium Development Goals. Lancet 2005;365:347-353.

Conclusion: AIDS epidemic still outpacing response.

Where are we now in our fight against tuberculosis?

Good news

The global tuberculosis prevalence has declined by more than 20% since 1990 and the incidence rates are now falling or stable in five of the six regions of the world. Worldwide, the incidence rate was growing at a maximum around 1.5% per year in 1995, but less than 1% per year by 2003, assuming strong commitment and resources are sustained; four regions, the America's, Eastern Mediterranean, South East Asia and Western Pacific are on track to reach the United Nations MDG's goal

of reducing TB incidence; direct observed treatment schedules (DOTS), the internationally approved treatment strategy for TB, has been found to achieve a 95% cure rate, even in the poorest countries; the cost of a six to eight month course of treatment has been brought down to US\$ 10 per patient¹⁵.

Bad news

The glaring exception is Africa, where TB incidence rates have tripled since 1990 in countries with high HIV prevalence and are still rising across the continent at a rate of 3-4 % annually; the second exception is Europe, where there are high levels of multidrug resistant TB and slow advances in DOTS in countries of the former Soviet-Union (*drug resistance occurs when patients do not take their medicines regularly or do not complete their course of treatment*); drug resistant TB requires extensive chemotherapy (up to two years of treatment), which is \pm 100 times the cost of the normal treatment, and is also more toxic to patients.

Conclusion: The fight against tuberculosis remains a paradox. Each year, near 2 million people die of TB, despite the availability of inexpensive treatments that are effective in up to 95% of cases.

Urgent action is immediately required

The fight against HIV/AIDS & tuberculose is not just in the hands of “bacteria and virus fighters”, but needs also a sustained political commitment for the coming decennia. So far, political commitment to the AIDS response remains inadequate in many countries.

“It is time for Governments to translate commitment into concrete action” said UN secretary-general Koffi Anan in his report of 2nd June 2005 entitled: “AIDS epidemic still outpacing response”.

“The AIDS epidemic has entered a new and critical phase, and so must be the response. The only way we will get ahead of the epidemic is, if there is a universal access to HIV prevention and treatment. This needs to be the world’s immediate goal” said Dr. Peter Piot UNAIDS Executive Director.

Is the Department of Microbiology on the right track?

It is encouraging to observe that the main research, education and service policies of the Department of Microbiology fit perfectly in the field of prevention and treatment as was mentioned by P. Piot. The Department of Microbiology in close collaboration with CONRAD, a United States based non-governmental organization (NGO) and the European Microbicides Programme (EMPRO) are for the moment world leaders in the organization of microbicide phase three trials. They plan the start of the trials with two or three very promising microbicides in 2007. The outcome is expected in 2010. In addition, the Department of Microbiology in collaboration with industry (TIBOTEC NV (Mechelen, Belgium); Janssen Pharmaceutica (Beerse, Belgium), developed a new generation of powerful-non-nucleoside reverse transcriptase inhibitors (NNRTI) as powerful microbicides. The Department performed the first phase I study in women sponsored by TIBOTEC NV (Johnson-Johnson) with their product TMC120. Very few side effects were observed and almost all women

finished their one week vaginal gel use. Further testing is ongoing. In addition, the Department developed a new innovative method to test *in vitro* antiviral activity of microbicides as well as of microbicides in a gel formulation. The gel formulation of the TMC120 induced free full prevention of HIV infection *in vitro*, offering hope for the potency of this compound *in vivo*. Several new non-nucleoside reverse transcriptase inhibitors, developed by the Center for Molecular Design of Janssen Pharmaceutica, were found to be potent in our model. This opens real perspectives for the development of potent microbicides in the near future.

Also the policy plans of the Mycobacteriology Unit in the Department of Microbiology fit within the worldwide priorities of the “fight against tuberculosis”.

Actually, worldwide 38 different types of experimental vaccines are produced and in phase I clinical trials in humans. It is expected that in the period 2015-2020, clinical phase III results of 4 to 5 experimental vaccines will be known. From these results, the best will be selected and larger group of volunteers vaccinated, and the vaccine will be optimized.

One of the problems in vaccine developments the lack of immunological correlates of protection (ie markers that unequivocally predict protective efficacy of a vaccine candidate). It is still an open question if neutralizing antibodies or cellular immune responses or both will correlate with protection. Therefore, the Department of Microbiology is involved in the design of two different vaccine concepts, one inducing neutralizing antibodies, the other inducing a cellular immune response. In addition, standardized and highly reproducible methods to quantify neutralizing antibody and cellular immune response are also needed. Such methods will allow to compare the strength of the immune responses induced by different types of vaccines. This is essential for vaccine development. Such methods are under development in the Department of Microbiology.

The Department of Microbiology has strengthened its commitment to the prevention of HIV infection and other reproductive health problems among vulnerable groups in the population, such as sex workers, young people and pregnant women in developing countries. Simultaneously, the Department of Microbiology is heavily involved in capacity building in order to implement and follow-up highly active anti-retroviral therapy, inside the existing or poor functioning health services in developing countries. Last but not least, despite the Department of Microbiology lacks sufficient institutional funding and regularly nice jobs and salaries in foreign countries are offered, majority of the personnel at the Department enjoy their job here in Antwerp, and remain highly motivated. A strong social-political engagement unifies them and makes them not to forget the people in the developing.

This altogether, makes that the Department of Microbiology is on the right track.

Is the financing of the Millennium Development Goals feasible?

The core challenge of the MDG's is in the financing and implementation of the interventions with the combined donor-country Gross National Product (GNP) at roughly US\$ 30 trillion ($30 \cdot 10^{12}$ or 30 000 billion (1 billion = 10^9)). 0.7% GNP would be about 200 billion per year, compared with present aid flow at about US\$ 70 billion per year. The UN Millennium projects findings show that the additional US\$ 130 billion per year would be more than enough to scale up the critical interventions needed to achieve the MDGs in well governed developing countries. To implement the MDGs in all low income and middle income countries will cost in 2006 = US\$ 135 (0.45% of GNP) billion and in 2015 = US\$ 195 (0.65% of GNP) billion.

As part of the MDGS, the package HIV & tuberculosis & malaria treatment, prevention, health mothers, child vaccinations, childhood disease treatment in 83 low income countries in 2015 will cost US\$ 40 (0.13%) billion (low estimate) up to US\$ 50 (0.16%) billion a year.

Conclusion: the financing of the MDGs is perfectly feasible. There is enough money available, but it is not allocated. It seems not yet to be considered as a priority¹⁶.

What will we win if we manage to reach the MDGs?

It will bring tremendous benefits worldwide: 500 million people will be lifted out of poverty in 2015 and tens of million lives will be saved, with a great proportion of the improvements taking place in Africa; 300 million will no longer suffer from hunger. From now up to 2015 roughly 30 million fewer children will die before their fifth birthday. More than 2 million mothers will be saved. Achieving the goals will also mean safe drinking water for another 250 million people and the benefits of basic sanitation to 650 million, allowing them to lead healthier and more dignified lives. The achievement of MDGs could put the world a path to ending absolute poverty. This accomplishment will be central to global security¹⁷.

Key question

How to convince political leaders and decision makers to allocate a sustained sufficient annual budget for at least one decennium to implement the programmes necessary to reach the eight millennium goals.

3. The future 2006 - 2015

What will be the long-term agenda we need to start tackling now, to fight HIV/AIDS successfully?

For the long-term agenda to fight HIV/AIDS I refer to an excellent special lecture entitled "The status of the response: what will it take to turn the epidemic around"?, given recently by Peter Piot, UNAIDS, Executive Director, at the 32rd International AIDS Society Conference¹⁸ on HIV Pathogenesis and Treatment in Rio de Janeiro, July 27, 2005.

According to Peter Piot it is clear that we have entered a new era in the response to AIDS – the era of implementation on a large scale. We are in the middle of that and so our short-term agenda is crystal clear.

Our long-term journey is less clear. According to Peter Piot, five areas are of crucial importance for the future agenda: 1. Universal access to HIV prevention and treatment; 2. Science and technology innovation in the AIDS field; 3. How do we organize ourselves for the long run?; 4. Leadership and political commitment; 5. Money.

1. Universal access to HIV prevention and treatment

Universal access to HIV prevention and treatment is crucial since we aim to keep the current and future generations HIV free, to ensure a discrimination-free life for all those living with HIV and last but not least to care for the orphans left behind.

The '3 by 5' Initiative (to treat 3 million HIV⁺ with antiretroviral therapy in 2005) was a bold first step towards universal access to HIV treatment. WHO and UNAIDS reported recently, 51 countries have now doubled the number of patients benefiting from antiretroviral therapy since December 2003, largely because of the funding provided by US PEPFAR and the Global Fund and in some cases by the governments of these countries. So let's build on '3 by 5' and joining forces for universal treatment access, which is now endorsed by the richest nations of the world, at the G8 Summit in Gleneagles.

The long-term complexities of antiretroviral therapy on a large scale are enormous and hardly addressed and sometimes Peter Piot wonders whether planners have internalized that the purpose of large-scale antiretroviral therapy is for people to stay alive and well for 30, 40, 50 years, just as long as those who are not HIV positive. Planning for this goal belongs, in a real sense, far more in the ministries of finance than in the public health community because we are talking really about very, very significant amounts of money. Some key issues are of importance in the universal access to HIV treatment. The first is pricing. Multiple sources of production and generic competition clearly have driven some prices down. But at the same time, development of new more potent antiretroviral drugs will continue to be crucial if we are to avoid a massive failure of therapy 10 to 20 years down the road. We know that historically patent protection has been the most effective incentive for industrial innovation. So how do we solve this dilemma between the need for universal access to the products of innovation, and, on the other hand, the need for continuing innovation, which is only possible with the kind of incentives offered by patent protection. Access for all can only work if governments and consumers in rich countries are willing to subsidize poorer countries directly through paying higher prices, or indirectly through subsidizing the R & D for products needed by poorer countries.

Second, with millions soon on antiretroviral therapy in developing countries, a sustainable supply of drugs may become a problem. We're increasingly getting reports of stock-outs of even first-line regimen molecules – even in countries like India that have a major generic production capacity. Stock-outs are of great concern not only because of the impact on the health of individuals, but also because of the worsening impact on viral resistance. SO that's why the production capacity of both the research-based and generic sectors of the pharmaceutical industry is of crucial importance. This is also a good reason for encouraging local production in the bigger markets for AIDS drugs.

Third, how will we guarantee long-term funding for treatment in poor countries? Millions of people's lives will soon directly depend on this. There's no precedent in international development, in international cooperation, for such long-term predictable funding. To meet these likely challenges, we need bolder international agreements than we have today. But at the same time, domestic funding of antiretroviral therapy should grow over time even in low-income countries. They all should embark on schemes that offer universal medical coverage, even if this is not feasible from day one.

Let us now turn to universal access to HIV prevention. With 5 million new HIV infections per year, HIV prevention must also be made available universally. It is no less essential that HIV treatment, even reaching or sustaining universal HIV treatment will be impossible without effective HIV prevention. It is certainly not one against the other – both are needed. Last month, the UNAIDS Programme Coordinating Board, which is the body that sets AIDS policy in the UN system, for the first time agreed on a comprehensive package of proven and effective methods for HIV prevention based on evidence and human rights principles. We now need collectively to move this to the point where there is firm political and community support for universal access to HIV prevention just as there is now for HIV treatment. HIV prevention actually poses equally formidable challenges in the long-term as HIV treatment. HIV treatment is for life, but so is HIV prevention, a point we tend to forget. HIV prevention is for life – throughout the life of a person, the life of a generation, and the life of the next generations. This means that we must not only accelerate HIV prevention in the short term, but we must take far more seriously the matter of bringing about sustainable changes in societal norms and values, as well as in the structural forces that make people more vulnerable to HIV. Peter Piot mentioned a whole list of these societal issues, including such issues as decriminalization of homosexuality; accepting harm reduction as a paramount principle whether as it relates to injection drug users or to sex workers; promoting full rights for women; making violence against women and sexual minorities not just illegal but socially unacceptable; and empowering teenage girls to say no to unwanted sex. These things are usually relegated to the bottom of HIV prevention strategies, together with human rights, and with no funding attached to them. We in the AIDS movement must make strong strategic alliances with the movements to combat poverty and to promote education and gender equality.

In terms of HIV prevention, we must ensure that the supply of prevention commodities, such as male and female condoms, keep pace with expanding HIV programmes. The reality today is that there are true shortages of condoms in many countries! And that the supply is getting problematic.

And we should not assume that antiretroviral therapy will automatically strengthen HIV prevention. There's definitely no evidence for that. It will require specific efforts, as recognized by the UNAIDS working group for HIV prevention. Otherwise both prevention and treatment will suffer.

2. Science and technology

Scientific advances on AIDS have been remarkable but there's still a huge agenda ahead of us.

First on the non-biomedical side, a much better understanding of the long-term societal impact of the AIDS epidemic is urgently needed in every region, and even at the level of most countries. UNAIDS embarked on such long-term scenario building in Africa and will expand this to other regions. This is important because such scenarios guide us to be more strategic in our investments today, they illustrate for example the absolute need to join prevention and treatment, and they offer great opportunities for top-level advocacy in dealing with the economy and the stability of the state.

Second, science and technology innovations in the AIDS field are crucial, be it for the development of new antiretroviral drugs, a vaccine or microbicides. There's a clear need for much higher investments. But the key question is whether the incentives for such huge investments will continue. SO again, we must strike a good balance between the imperative of making available universally every advance in technologies and the need to protect intellectual property, so that there is ongoing robust R & D for AIDS.

It's frustrating that even microbicide development is still not adequately funded, when it's only a matter of \$280 million annually, just double the current investment. The Global HIV Vaccine Enterprise is a recently established organization which improves the international collaboration in the development of an HIV vaccine. This is the kind of consortia that we need more of.

3. How do we organize ourselves for the long run?

Three issues here are considered important by Peter Piot. One, the growing pressure in many countries and even among some funders to consider AIDS as just another infectious disease and to merge the programmes, the research and everything with those for other infectious diseases. This would be disastrous. It would set the clock back 20 years. The current momentum would be lost, specific funding would wane and the strong engagement of communities and those infected would vanish. For a strong response to AIDS to be sustainable, distinctive AIDS programmes are needed, backed by top political commitment. Two, at the international level, we're getting our act together, we are preparing for more effective long-term action through the Global Task Team on Improving AIDS Coordination among Multilateral Institutions and International Donors. Coordination of all AIDS actors is not just a daydream of UN bureaucrats, it will truly save lives. There is still too much wastage of resources because of institutional rivalry and isolated initiatives. It's time for the scientific and academic community to join such united efforts.

And the third issue in terms of organizational challenges is that there is a huge challenge of institutional and human capacity, particularly in the countries most affected by the AIDS epidemic, notably in Africa. So we must invest much, more in capacity building, which means that we cannot be satisfied by quarterly reports because the results of these investments will not be evident for years. We must find ways to reverse the aggressively protect capacity in countries where the epidemic has not advanced far. Preserving existing capacity means keeping people alive as one of the measures. In other words, we come back to universal access, including for professionals. But preserving capacity is just as much about HIV prevention. But above all, we need to think out of the box. This is not just about training doctors and nurses or building and equipping clinics, as much as that is necessary, but it's as much to do with strengthening community action, community groups, and people living with HIV. People living with HIV are a vastly under-utilized capacity. In so many countries, from Swaziland to Kenya to Uganda, groups of people living with HIV can be agents, not only for change, but agents for treatment adherence, for nutritional support and so on. And addressing capacity constraints is not something that can wait until we have finished our little projects here and there. Our ability to get ahead of the epidemic depends on overcoming these constraints, and they had to be tackled right now.

4. Leadership and political commitment

This is possibly the most critical challenge, because as we all know, the half life of political commitment can be extremely short. There are innumerable examples of major issues that were in the global political spotlight that have since diminished in political favor, from child survival to population to the overall environmental movement. And also there are significant, competing, and very important global and local issues from climate change to terrorism, from extreme poverty to regional conflicts. So how do we keep AIDS at the top of the political agenda? I believe we need to move the response to AIDS into another league, on a par with other critical global issues, such as climate change and extreme poverty, and not stay in our AIDS ghetto. Debating AIDS belongs as much in the UN Security Council as it belongs in scientific conferences.

Sustaining political commitment also means that there is a never-ending need for activism. And it means we must broaden our constituencies through new alliances. New alliances with people with whom we may not agree on 100% of things, but if we can move together and we agree on the basic goals, we can really make a big impact. But are we ready to do that and what price?

5. Money

Despite the greatly increased funding for the AIDS response, the financing gap is becoming wider and wider. And that's because the needs are growing, particularly in terms of treatment. We estimate that for 2008 alone, \$22 billion will be needed. Where will that money come from? How are we even going to sustain what's available today? The replenishment conference of the Global Fund will be held in September and is a major challenge. Political sustainability is the basis for financial sustainability, as we all know. But sustaining the billions need requires, in the first place, results. We need to be able to demonstrate that the money available now produces results in terms of saving lives, both in terms of preventing new infections and keeping people alive and well. And we need to do a better job in terms of maintaining support from mainstream public opinion. And we need to diversify sources of funding. And finally, we need to emphasize the need to maintain special funding for AIDS for many years.

In everything we do in tackling AIDS there are a few non-negotiables. One is the promotion and protection of human rights. Two is equality between men and women. Three, that science is the basis of our policies and work. And four, accountability, not only to the funders, those who give us money, but to the people for whom we work.

And finally, the world must accept the exceptionalism of AIDS. There is simply no precedent in the history for such a crisis. And please let's not have an illusion that in a few years, one fine day the world will return to what it was before AIDS. No, AIDS has simply rewritten the rules. And to prevail, we too, must rewrite these rules. An exceptional threat demands exceptional action, be it on financing, development, trade rules, activist strategies, public service delivery or fiscal ceilings. So let us now design these longer-term strategies as otherwise we risk discouragement and demobilization. And we will achieve at best short-term results. Addressing AIDS in the long-term will require even more of the best brains, of the most creative entrepreneurs, and of the most determined leaders.

I sincerely hope that these wise words of our highly appreciated ex-colleague Peter Piot, will stimulate the best brains, at the ITM to continue their efforts and commitment, in order to reach one of the very important millennium goals which is in 2015 to halt and begin to reverse the incidence rate of HIV/AIDS.

In this context it was very encouraging to read in the "Launch of the 2005 AIDS epidemic update"¹⁹, the very welcome news that in several countries HIV infection rates have fallen recently.

This news is especially encouraging because these countries are of the regions most severely affected by AIDS - sub-Saharan Africa and the Caribbean.

Adult HIV infection rates in Kenya have gone from a peak of 10% in the late 1990s to 7% in 2003.

In Zimbabwe, levels of infection among pregnant women fell from 26% in 2002 to 21% in 2004. It is the first country in Southern Africa where we have seen such a decline on a national scale.

In several Caribbean islands, such as Barbados, the Bahamas and Bermuda, there has been a significant decline in prevalence among adults.

In Haiti, the country worst affected by AIDS outside Africa, HIV prevalence among pregnant women in urban areas has declined from 9% in 1993 to 3.7% in 2004.

In the two African countries, the declines in HIV rates have been due to changes in behavior, including increased use of condoms, people delaying the first time they have sexual intercourse, and people having fewer sexual partners. HIV information campaigns and voluntary HIV testing and counseling have encouraged these changes. In other words, HIV prevention efforts are working.

For Africa this is only the second set of times that we have seen sustained declines in national HIV rates. The first and largest turnaround was in Uganda, beginning nearly a decade ago.

We have also seen remarkable turnarounds in Brazil and Thailand - and so the overall lesson is very clear: **AIDS is a problem with a solution. And we are finally starting to see the return on our investments in HIV prevention.**

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