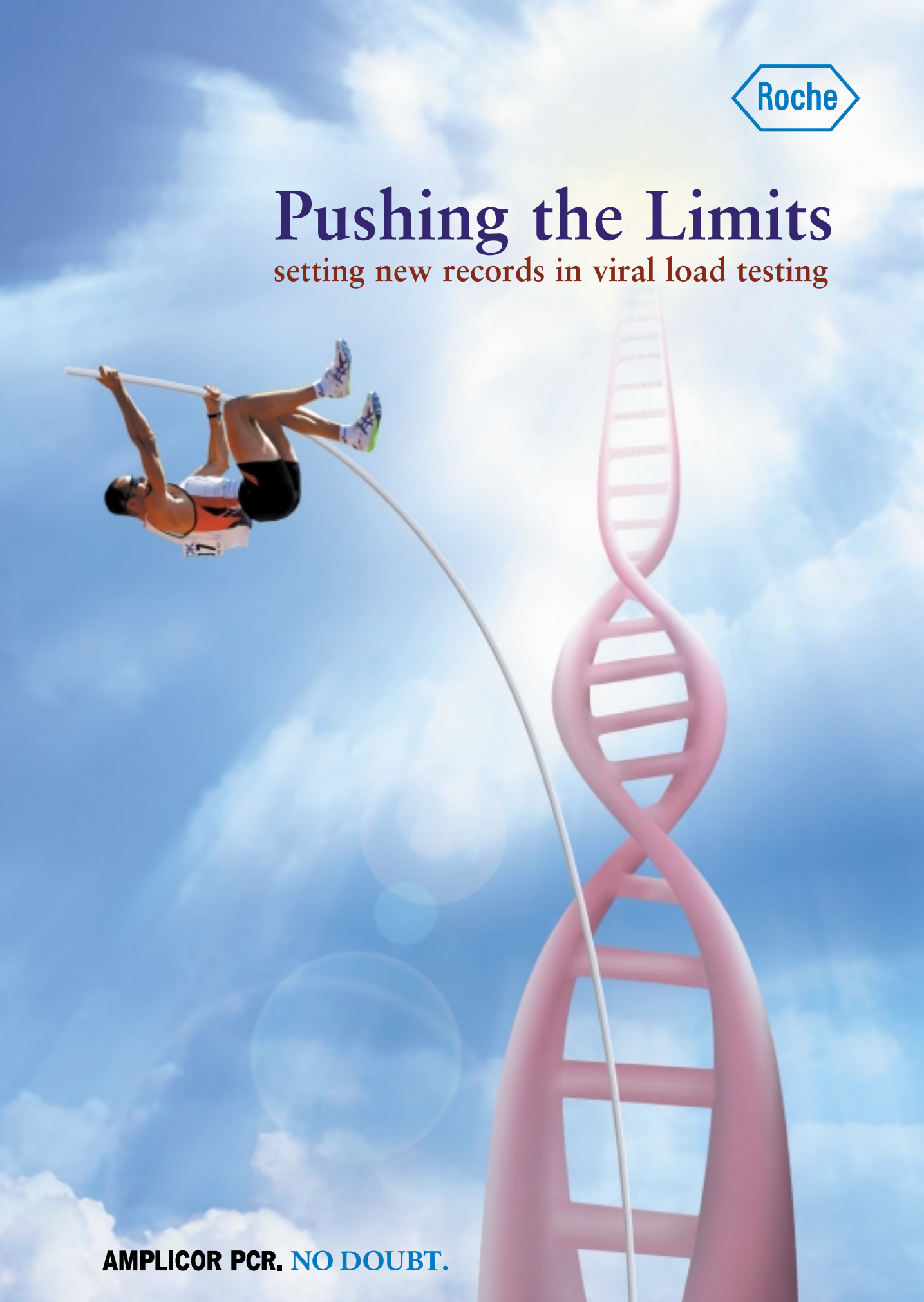

Roche
Pushing the Limits
setting new records in viral load testing
AMPLICOR PCR. NO DOUBT.

Contents

Chair – Professor Christine Rouzioux

CHU Necker-Enfants Malades, Paris, France

Message from the Chair

Since the discovery of HIV almost 20 years ago, scientific research has been crucial to improving our understanding of the virus. Unfortunately, as our knowledge has grown, so the HIV/AIDS epidemic has spread across the globe. While the gap between our knowledge and its impact on the epidemic is clearly of concern, the enthusiasm and mobilisation of researchers facing this complex situation is still very much alive.

This Barcelona meeting is an opportunity to build and reinforce international solidarity, which is still too disorganised. More than ever, symposia such as these are needed to bring together industry, clinicians and researchers, to encourage interactivity, and to allow all involved to tackle problems that are relevant to both industrialised and developing countries. Currently, the greatest concern is related to therapeutic options for patients in industrialised countries. Their situation stresses the absolute necessity to find alternative and innovative therapeutic approaches, since it is obvious that antiretroviral therapies cannot be tolerated by these patients in the long-term.

It is now clear that in order to push the boundaries of therapeutic approaches, we must expand the limits of knowledge on HIV. The challenge relating to HIV reservoirs is not easily overcome, nor is the challenge of genetic diversity of HIV-1 and HIV-2, which becomes more complex every day. Future objectives include the development of new markers, such as proviral DNA, expanding the value of existing markers, such as plasmatic HIV RNA, and extending the usefulness of RT/PCR. Achieving these goals is certain to improve the clinical management of people infected with HIV, including those in developing countries.

Professor Christine Rouzioux

Christine Rouzioux
Professor of Virology, CHU Necker-Enfants Malades, Paris, France

Performance indicators: the predictive and clinical value of HIV-1 quantitive proviral DNA assays

Christine Rouzioux was awarded her PhD in 1985 and became Assistant Professor of Virology in 1986 and then Professor of Virology in 1990 at the Necker University School of Medicine in Paris, France.

Her main areas of published research on HIV include:

- *the first isolation and characterisation of HIV-1, then HIV-2 (as part of Luc Montagnier's group from 1983 to 1988)*
- *development of the first HIV serological test*
- *first studies on HIV serology in African patients*
- *first epidemiological studies in patients with haemophilia*
- *studies on vertical transmission of HIV — estimation of the timing of mother-to-child transmission*
- *diagnosis of HIV in neonates and infants, studies conducted in France, Europe and Africa*

Currently, her research activities focus on HIV pathophysiology and therapeutics in adults and children, with an emphasis on cohort studies.

The contribution of highly active antiretroviral therapy (HAART) to the reduction in morbidity and mortality of HIV-infected patients constituted a major step in the management of HIV infection. Yet, despite the effectiveness of HAART in blocking HIV replication, it soon became apparent that the virus could not be eradicated due to a lack of direct impact of HAART on latently infected cells. The level of proviral DNA in peripheral blood mononuclear cells (PBMC) provides a measure of latent infection, which can vary considerably between patients. Whereas some patients exhibit less than 100 copies of proviral DNA per million PBMC, others have proviral DNA levels that are ten to 100 times higher. In order to assess the clinical value of proviral DNA, we and others have evaluated this marker in the context of the natural history of HIV infection, as well as in response to HAART.

In the SEROCO cohort study, investigators analysed the predictive value of proviral DNA. The results showed that the level of proviral DNA was predictive of the risk of disease progression to AIDS, independent of the number of CD4+ cells or the level of HIV RNA in plasma. Hence, proviral DNA levels bring different and complementary information to the study of the physiopathology of HIV infection. Whereas plasma HIV RNA levels are indicative of viral production, proviral DNA levels represent the capacity to produce virus. The French PRIMO cohort study evaluated subjects with primary HIV infection and found that latently infected cells are established at the very beginning of HIV infection. Long-term asymptomatic subjects were evaluated in the French ALT cohort study, which confirmed the predictive value of proviral DNA on the risk of progression to AIDS and showed a protective role of the specific humoral and cellular immune response.

Several teams have evaluated the impact of HAART on the level of proviral DNA in HIV-infected subjects. The PRIMOFERON study (peg-interferon and HAART) showed a significant impact of HAART on proviral DNA levels when treatment was initiated during primary infection. The ANRS 079 trial (IL-2 and HAART) demonstrated the absence of expansion of the stock of HIV-infected cells induced by IL-2. PRIMSTOP (HAART and structured treatment interruptions) found rebounds of proviral DNA parallel to those observed for HIV RNA in plasma. Finally, a study carried out at the Necker Hospital on the follow-up of patients treated for over 5 years, who presented continuous control of viral replication, showed that the effect of antiviral drugs on the stock of HIV-infected cells occurred mainly during the first 2 years of treatment, while 100% of the patients presented with lipodystrophy after 5 years of HAART.

Taken together, the results of these studies has led to the conclusion that latently infected cellular reservoirs, as measured by the level of proviral DNA in PBMCs, represents a major obstacle to developing a cure for HIV infection. Nonetheless, it is possible to live with the virus provided that expansion of latently infected cellular reservoirs is controlled. This undoubtedly constitutes a new objective for the management of HIV infection — associating antiretroviral treatments with immunotherapy or programmed interruptions, with the aim of extended long-term follow-up of infected patients. Currently, reducing the risk of treatment-related adverse effects by limiting extended exposure to HAART or by alternating 'on/off' periods of drug regimens is not realistic. However, it is reasonable to expect that studies in which proviral DNA is analysed will provide new insight into the best time to initiate, stop, or restart therapy.

Notes

Clive Loveday
International Clinical Virology Centre, Buckinghamshire, UK

Tracking global diversity: viral subtypes and their implications for HIV viral load and resistance

Clive Loveday joined the International Clinical Virology Centre at the beginning of this year. The organisation is a new charitable trust composed of administrative offices, a clinical area, a teaching centre and academic laboratories overseen by independent charity trustees. It has been founded with donations and academic and commercial research grants. The aims of the charity are to research real-time, quality-assured, molecular technologies to enhance patient care, the understanding of viral infections, and to educate all those involved with these technologies.

Previously he was the Foundation Professor of Retrovirology at the Royal Free Hospital Medical School in London, focusing on issues relating to viral load, resistance and subtype diversity in HIV-1 infection. He has worked in HIV/AIDS for 17 years, initially undertaking the clinical care of patients with HIV/AIDS in the early 1980s, and later as a clinical virologist establishing PCR technologies to diagnose and monitor patients. He carried out the first UK trial of AZT (1986), developed viral load and resistance assays to support patient care (1989), served as a clinical virologist on Concorde and Delta trials, and carried out early work defining the dynamics of HIV-1 turnover (1994). Currently he has academic awards from the MRC, EuroSIDA, SPREAD and INITIO, and serves on advisory boards for the MRC, UK Department of Health, British HIV Association resistance group, PENTA, EuroSIDA, European resistance group (EuroGuidelines), and IAS USA faculty and resistance group.

He has published over 100 papers in the field, is a Fellow of The Royal College of Pathologists, and holds membership of the British Pharmacological Society, British Society of General Microbiologists, International AIDS Society and Chaos Theory and Nonlinear Dynamics Society.

HIV-1 is characterised by error-prone replication during reverse transciption of HIV-1 RNA to cDNA, resulting in the generation of genetically diverse subpopulations (quasispecies), which gradually accumulate in the infected host over time. This serves as a mechanism for virus evolution, with selection of quasispecies in response to altered environmental pressures in the host. The complexity of this process should not be underestimated: in established infection, broad genetic heterogeneity is seen within a host (intra-host variability), between hosts (inter-host variability) and in different communities. HIV-1 genetic subtypes in different geographical regions represent extreme forms of this process where different subtypes have evolved in different and distinct genetic environments over time.

By 1993, subtypes of HIV-1 were classified by phylogenetic analysis of genetic sequences into at least ten different subtypes and could be placed in distinct geographical areas. Since then, it has become clear that changes in national barriers and worldwide movement of populations has resulted in the inexorable march of viruses from endemic to new geographical regions. The presence of multiple subtypes in any given community has revealed the ability of HIV-1 to genetically recombine in one host. The intrinsic evolution capacity of this species has resulted in the selection of recombinant forms that are biologically more successful than the existing viruses.

The implications for management of the epidemic and clinical care of infected patients are being considered in the light of these facts as part of the SENTRY project.

Molecular technologies for diagnosis, viral quantification and drug resistance have become the cornerstone of patient care. Most of these assays were developed in the late 1980s using laboratory strains of HIV-1 subtype B. Thus, clinical laboratories have been using assays fixed in time and space to monitor viruses that are genetically mobile in time and space. Our group has been contributing nationally and internationally to the surveillance of HIV-1 diversity with a view to characterising the distribution and ongoing spread of subtypes throughout the world. Further, the SENTRY project is revealing a significant prevalence of all non-B subtypes in the UK, and is exploring the possible implications for diagnosis, monitoring and correlation to treatment response within our patients. These studies have been developed to help the scientific community to understand the biology of these viruses, the clinicians to design better treatment regimens, and the technologists to develop the next generation of molecular tests to ensure optimal result integrity in spite of continued evolution of the virus in real-time.

Notes

Raising the standards: improving the sensitivity of viral load testing

Brooks Jackson is Professor and Chairman of Pathology at the Johns Hopkins Medical Institutions. Dr Jackson received his MD and MBA degrees from Dartmouth College and received his residency training in Clinical Pathology and fellowship training in Transfusion Medicine at the University of Minnesota. Dr Jackson is Director of the clinical HIV laboratory at Johns Hopkins Hospital and has been involved in numerous clinical HIV therapeutic and prevention trials in the Unites States, Uganda and China. He is a funded investigator in the NIAID-sponsored adult and paediatric AIDS Clinical Trials Groups and the HIVNET and HIV Prevention Trials Network. Dr Jackson is the Protocol Chair of several adult and perinatal HIV prevention trials in the United States and Uganda, including the HIVNET 012 perinatal nevirapine trial.

Brooks Jackson
Johns Hopkins Medical Institutions, Baltimore, USA

Background. The need to quantify very low levels of HIV-1 virus may prove useful in monitoring viral suppression and determining the relative efficacy of different drug regimens. The primary objective of our study was to determine whether the sensitivity of the Roche AMPLICOR™ HIV Monitor test could be enhanced by modifying the 1.0 or 1.5 version of the Ultra Sensitive assay.

Methods. The methodology for this testing format was largely adapted from S Yerly *et al.* (2000). The modified Ultra Sensitive assay was customised from the Roche AMPLICOR™ HIV-1 Monitor Ultra Sensitive 1.5 version of the assay. The modifications include a plasma centrifugation of larger plasma volume ('ultraspin': 1 ml plasma at 25,000 xg, 80 min, 4°C), resuspension in 55 µl of diluent, a reduction in quantitation standard to 6.6 µl, and an extension in substrate incubation time from 10 min to 15 min. The linearity of the modified assay was determined using serial dilutions of the Virology Quality Assurance (VQA) standards. We also tested 27 positive and 35 negative patient samples (as detected by the 1.0 or 1.5 version Ultra Sensitive assay) with the modified 1.5 version Ultra Sensitive assay. In addition, we tested 40 replicates each of a four and ten copy VQA standard by both assays to compare sensitivities. For specificity, 40 different plasma samples from HIV antibody negative individuals were tested.

Results. The modified Ultra Sensitive assay appears to be linear to approximately 12 copies/ml and detected 60.5% vs 24.4% of the four copy standard compared with the 1.5 Ultra Sensitive assay, and 86.7% vs 45.9% for the ten copy standard. In addition, 76–80% of non-detectable patient samples (using the Ultra Sensitive assay) were detectable on the modified Ultra Sensitive assay. All 27 patient samples detected by the Ultra Sensitive assay were detectable by the modified Ultra Sensitive assay. Forty HIV antibody-negative patient samples run on the Ultra Sensitive and modified Ultra Sensitive assay showed no detectable RNA.

Conclusions. This modified Ultra Sensitive assay appears to be at least twice as sensitive as the Ultra Sensitive assay, detecting HIV RNA in significantly more patients who were undetectable by the Ultra Sensitive assay.

Notes

Thomas Myers
Associate Director, Program in Core Research, Roche Molecular Systems, Inc., Alameda, USA

Going the distance: extending the usefulness of RT/PCR

Thomas Myers joined Roche Molecular System's Program in Core Research as a Research Investigator in 1991, going on to positions as Senior Research Investigator/Research Leader and his current position as Associate Director. He is a recognised expert in PCR technology, holding seven patents in the field, and has written more than forty-five articles, chapters and papers in his distinguished career. In October 2001, Dr Myers was the recipient of the first Roche Molecular Systems 'President's Award for Innovation' in recognition of his pioneering contributions to establishing Roche's RT/PCR technology.

Prior to joining Roche, Dr Myers was a postdoctoral fellow with Cetus Corporation, where he worked with David H Gelfand, in the Department of Core Technology within the PCR Division of the company developing PCR methodologies (1990–1991). Prior to joining Cetus Corporation, Dr Myers held a postdoctoral fellowship with Robert A Bambara, in the Biochemistry Department of the University of Rochester in New York studying the enzymology of mammalian DNA replication (1987–1990). Dr Myers received his PhD in Chemistry from Wayne State University in Michigan, investigating proteins involved with the replication of bacteriophage T7 (1981–1987).

Dr Myers holds memberships of the American Chemical Society, American Society for Biochemistry and Molecular Biology, and the American Society for Microbiology and is a frequent speaker at conferences around the world.

The process of RT/PCR has become increasingly important in nucleic acid-based diagnostics and molecular biology research in general. However, performing sensitive, long-range RT/PCR has remained a problematic and time-consuming procedure, especially in the clinical laboratory setting. We previously found that the implementation of an RT/PCR system using a mixture of thermostable DNA polymerases could alleviate many of the challenges of performing highly sensitive long RT/PCR. These mixtures typically contained a DNA polymerase devoid of proofreading ability blended with low levels of an archaeal DNA polymerase exhibiting 3'→5'-exonucleolytic or proofreading activity. In order to circumvent the need to blend enzymes, a family of mutant enzymes was constructed using structure-based design tools. Amino acid residues involved in substrate binding were identified and mutants were generated with a range of reduced proofreading activities. These designer DNA polymerases use a chimeric DNA polymerase, which contains the 5'→3'-nuclease domain from *Thermus sp.* ZO5, and the 3'→5'-exonuclease and DNA polymerase domains from *Thermotoga maritima*. Additionally, in contrast to the archaeal DNA polymerases, this family of enzymes retains the ability to incorporate dUTP, allowing for Amperase/UNG carryover contamination control and enhanced specificity due to dUTP/UNG-mediated hot start.

Using these chimeric DNA polymerases in high temperature long RT/PCR, we have designed a single buffer assay to sensitively and specifically amplify HIV-1 targets over 1.7 kb in length critical for HIV resistance genotyping. These amplicons may be used for sequencing by conventional methodology or for genotyping by hybridisation-based techniques. As a model system for high throughput diagnostic laboratories, we have extended our RT/PCR assay by adding a chemically and genetically-modified thermostable DNA polymerase to the RT/PCR that upon thermal 'reactivation' efficiently incorporates ribonucleotides into the amplicon during the later cycles of the PCR amplification. The labelled PCR product is simply fragmented by heating in alkali and hybridised to a microarray. The entire process allows for a rapid format that requires minimal 'hands-on-time' and is easily automated. The RT/PCR assay, coupled with the Affymetrix GeneChip® array technology, forms the basis of an HIV-1 genotyping assay for the detection of mutations associated with antiretroviral drug resistance.

Notes

Notes

Pushing the Limits
setting new records in viral load testing

Name:
Affiliation:
Question:

AMPLICOR PCR. NO DOUBT.

Pushing the Limits
setting new records in viral load testing

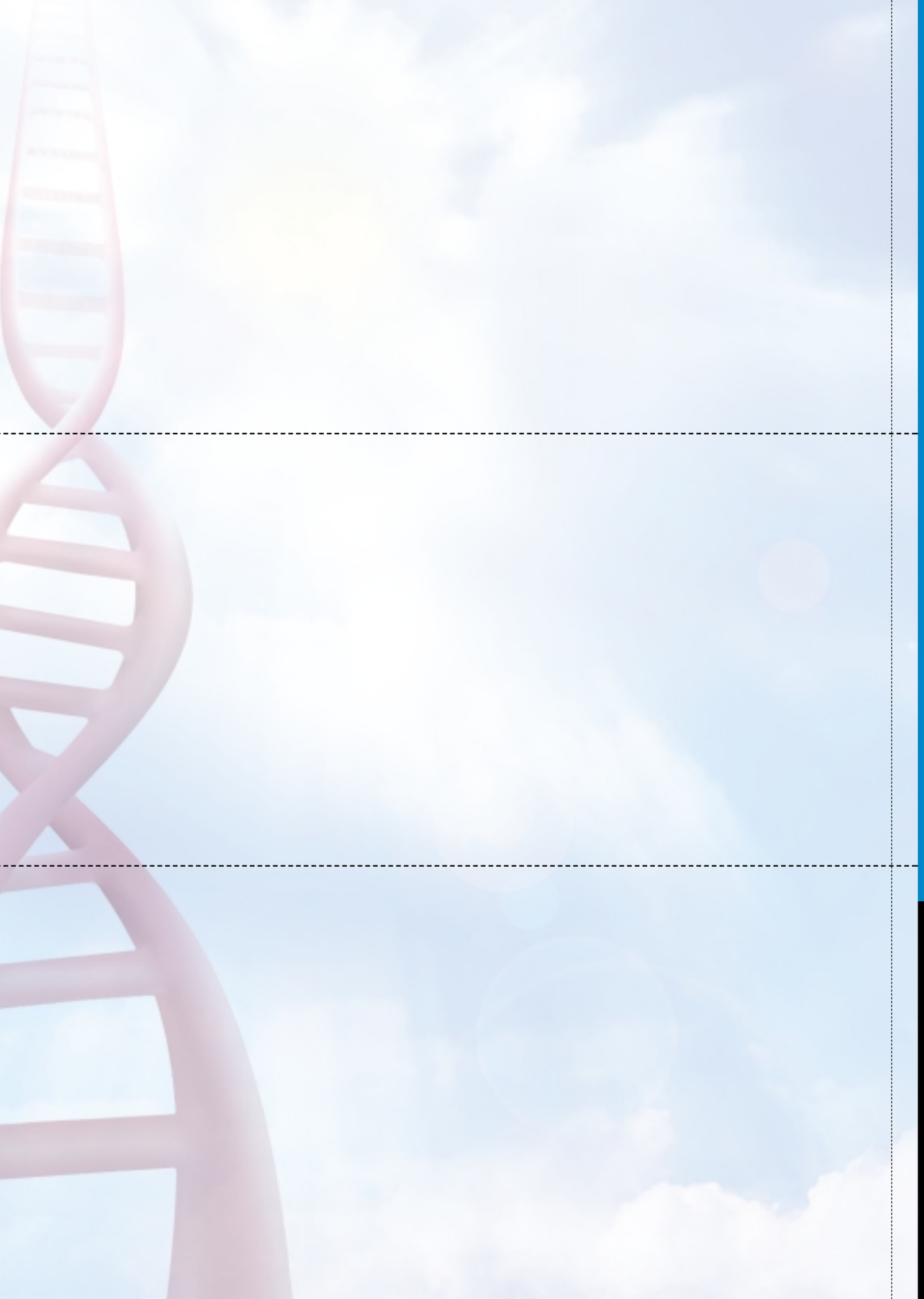
Name:
Affiliation:
Question:

AMPLICOR PCR. NO DOUBT.

Pushing the Limits
setting new records in viral load testing

Name:
Affiliation:
Question:

AMPLICOR PCR. NO DOUBT.



Roche

THE MOST POWERFUL WEAPON AGAINST HIV ISN'T A DRUG OR A LAW OR EVEN A LIFESTYLE.

IT'S THE TRUTH.

It's a fact: effective management of HIV-1 infected patients starts with accurate HIV-1 viral load management. And the Roche COBAS AMPLICOR HIV-1 MONITOR* Test, v1.5 provides this precise information, with reliable quantitation down to 50 copies/mL. For more information, contact your local Roche sales representative. Because while drugs, laws, and lifestyles may change, the power of the truth never will. **AMPLICOR PCR. NO DOUBT.**

*Pending FDA approval. © 2001 Roche Molecular Systems, Inc. COBAS AMPLICOR, AMPLICOR and NO DOUBT are trademarks of a member of the Roche Group.

Designed and produced by MediTech Media Ltd [UK] +44 (0)20 7398 0500

2048RO