

The main activities of the MolDia unit are developing new diagnostic tests and strategies for individual as well as population-wide diagnosis of a broad spectrum of infectious diseases, based on the latest knowledge and advanced genomics and proteomics techniques. We provide genotyping services both within the STI and externally.

Diagnostic services

The unit provides molecular diagnostic services for the STI-MEDDIA Department, handling patient samples that come from all over Europe. Accredited tests are offered for differentiating species using polymerase chain reaction (PCR), namely, *Entamoeba*, *Plasmodium* and *Leishmania*. The performance of our *P. falciparum* PCR assays was compared with results from other diagnostic laboratories in a World Health Organization (WHO) collaborative study to harmonise results for detection and quantification of *P. falciparum* DNA by nucleic acid amplification technique (NAT)-based assays. Currently we are working on introducing quantitative PCR (qPCR) assays. New tests are continually being devised, such as PCR-based detection of *Trypanosoma cruzi* and food-borne trematodes.

The unit also provides genotyping services commissioned from external partners. The task most frequently requested in the last biennium was differentiating *P. falciparum* and *P. vivax* clones in samples from regulatory in vivo drug efficacy trials of antimalarials. PCR adjustment of cure rates based initially on blood slide microscopy and clinical assessment is now considered necessary because, particularly in areas of high malaria transmission, superinfection with additional parasites frequently occurs during trial follow-up lasting 28 days or longer. Towards the end of the treatment period, antimalarial drug levels can fall below curative levels, allowing any new infections emerging from the liver to establish themselves. Genotyping is carried out to distinguish recrudescence from new infection, and PCR-corrected cure rates have become accepted as the endpoints for regulatory clinical trials and for antimalarial drug monitoring.

In an effort to harmonise genotyping procedures worldwide, STI scientists have been developing in collaboration with other specialised genotyping laboratories a number of recommendations and standard protocols published at the WHO, Medicines for Malaria Venture (MMV) and STI websites.

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Technicians: S. Steiger, D. Müller
Funding: MMV

Developing new genotyping tools

PCR-based detection of food-borne trematodes

We have developed sensitive and specific diagnostic PCR tests for detecting and differentiating *Opisthorchis viverrini* and *Haplorchis taichui*. These tests were vali-

dated against purge results and applied in a molecular epidemiological field study conducted in Laos (see section 9).

Real-time PCR for highly sensitive detection of *P. falciparum* in blood donations

As a potential screening test for *Plasmodium* parasites in blood donors, we have developed a highly sensitive and specific quantitative PCR assay (qPCR) that is fast and robust. It detects all four *Plasmodium* species found in humans in addition to *P. knowlesi*, a parasite of primates also found occasionally in travellers from South-East Asia. Advantages of qPCR over standard nested PCR lies in the fully standardised protocol, safe handling with minimal risk of contamination and increased sensitivity and specificity by using a fluorescent dye-labelled specific probe as well as specific PCR primers. This generic *Plasmodium* qPCR assay targets the 18S ribosomal RNA (rRNA) gene, which is present in the genome of Plasmodia in several copies. The primers and probe were designed from regions of the 18S rRNA gene that are conserved among the four human *Plasmodium* species but differ substantially from homologue sequences in humans and other protozoan parasites detectable in blood. The detection limit of this qPCR assay was 50 parasites per millilitre of whole blood. This sensitivity is 100-fold superior to thick blood film.

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Collaboration: Helsinki University Central Hospital, Finland

Developing diagnostic peptides for serological detection of helminthic infections

After an assembly of the *Schistosoma mansoni* genome was released in July 2006, and with the release of the annotated genome expected for 2008, a wealth of information is now becoming available to researchers. Bioinformatic screening of publicly available sequence databases followed by peptide synthesis constitutes a new approach to identifying *in silico* a broad variety of potential diagnostic targets. Using structure and antigenicity predicting bioinformatic programmes we have selected 288 peptides for chemical synthesis and determination of their immunological reactivity. Screening of these synthetic peptides is currently performed using a high-throughput platform technology. Custom-made peptide microarrays are produced commercially. Thirty-amino acid long peptides are synthesised and spotted in duplicate arrays of 96 peptides. Arrays are probed with immune sera and Cy-5-labelled secondary antibodies and scanned in an Axon microarray scanner. GenePix Pro software is used to analyse and record the intensity of signals. Immunological screening with individual patient sera is carried out to prioritise and select the best candidates for diagnostic peptides showing the highest sensitivity and specificity. This pilot project aims at establishing and validating the microarray platform for screening diagnostic targets. It will pave the way for further devel-

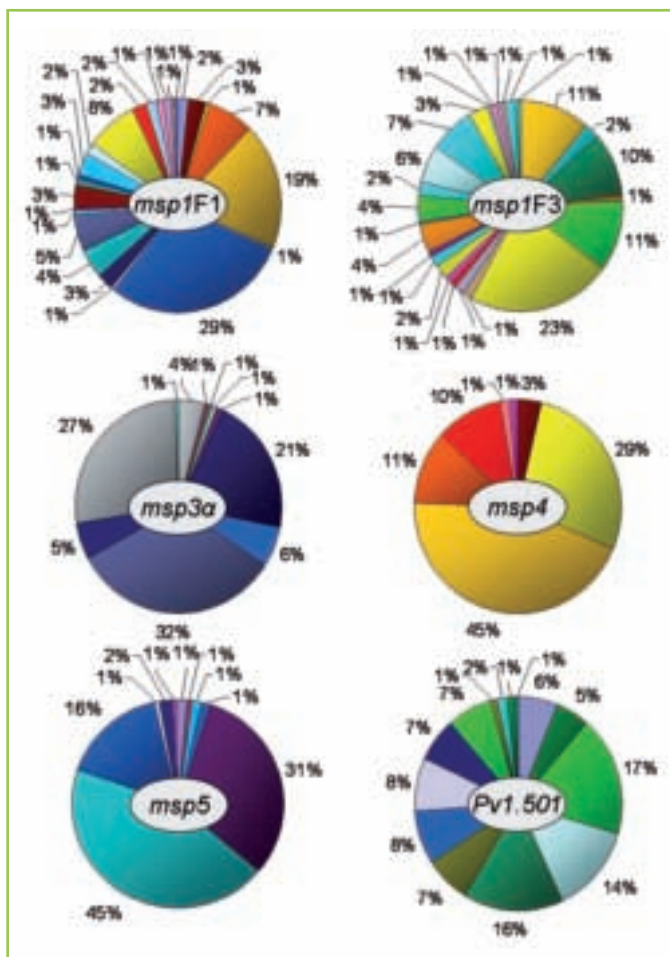


Figure 1: Allelic frequencies of six different molecular markers for genotyping *P. vivax*. Blood samples derive from cross-sectional surveys in three villages in Papua New Guinea.

opments in identifying highly specific diagnostic antigens also for other major human helminths.

In collaboration with N. Müller, Institute of Parasitology, University of Bern, we carried out a selection of peptides for *Echinococcus* serological diagnosis. Peptide microarray screening revealed a number of promising candidates whose diagnostic potential is currently under evaluation.

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 Collaboration: Institute of Parasitology, University of Bern (N. Müller)
 Funding: Fonds zur Förderung von Lehre und Forschung, Basel; Velux Foundation, Zurich; Rudolf Geigy Foundation, Basel

Molecular monitoring tools for *P. vivax*

P. vivax is the second most frequent cause of human malaria. While *P. falciparum* is dominant in large parts of Africa, *P. vivax* causes over 50% of all malaria cases outside Africa. In total, over 2.5 billion inhabitants of the Middle East, Asia, Eastern Africa, Central and South

America, and Oceania are exposed to *P. vivax*, suffering an estimated 71–390 million cases per year. In these areas *P. vivax* causes significant economic and societal damage. Moreover, there is increasing evidence that severe illness and death due to *P. vivax* may be more common than previously appreciated. Nevertheless, while considerable effort has been made in recent decades to understand and control *P. falciparum*, few resources have been channelled into *P. vivax* vaccine and drug research and development. *P. vivax* may thus be the most common “neglected disease”.

Many antimalarial interventions are accompanied by molecular monitoring of parasite infections, and a number of molecular typing techniques based on different polymorphic marker genes are currently being applied. We have developed a genotyping technique that provides a fast and precise means of studying *P. vivax* infection dynamics that involves following individual clones over time. The method was tested with samples from an *in vivo* drug efficacy study. PCR fragments were sized using capillary electrophoresis to determine the extent of fragment length polymorphism of seven potential genetic markers (five genes of merozoite surface proteins – *msp* – and two microsatellites) in 108 *P. vivax*-positive blood samples from three villages in Papua New Guinea.

Two fragments of *msp1* showed the greatest diversity in the study area with 28 and 25 different alleles found, followed by two microsatellites. *msp3*, *msp4* and *msp5* revealed limited polymorphism (see Figure 1). Even the most diverse markers showed allele frequencies of up to 16 or 24%. To reduce the theoretical probability of superinfection with parasites having the same genetic characteristics as parasites present at baseline, we propose to combine at least two markers for genotyping individual *P. vivax* infections.

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 Collaboration: Papua New Guinea Institute of Medical Research, Goroka (I. Müller)
 Funding: Swiss National Science Foundation

A new tool for molecular monitoring of HIV drug resistance

Genetic mutations in the target genes of anti-HIV drugs associated with viral drug resistance threaten the integrity of any combination treatment, which is the standard of care for HIV patients. Key mutations thus need to be identified to enable adequate therapeutic decisions for individuals as well as populations. Genotypic analysis of the respective HIV genes provides information about the presence or absence of patterns of mutations that are known to be responsible for drug failure. These patterns can be shaped by patients’ previous antiretroviral therapy or by selection pressure on the viral population as well as by transmission of already drug-resistant HIV variants.

In 2006, as a feasibility study, we began to develop a prototype version of a microarray for typing point muta-

tions (single-nucleotide polymorphisms, or SNPs) in a gene called HIV reverse transcriptase (RT). The principle of the genotyping-on-chip concept was established and validated previously by researchers at the STI who developed a malaria chip to detect therapy resistance for current antimalarials. By applying the same technology to genotype HIV, we aim to devise a low-cost genotyping method tailored for HIV mutations associated with antiretroviral failure, particularly for drugs available in resource-limited settings. Transfer of such a tool would make it possible to identify at point of care the major molecular markers for resistance to the locally applied drugs. An affordable, reliable and sustainable technique specifically addresses needs of countries where costs of currently established laboratory techniques are beyond the scope of local health facilities. Our final goal is to transfer the technology from Basel to the Ifakara Health Research and Development Center (now Ifakara Health Institute), Tanzania, and later to other interested parties.

Validation of genotyping on microarray was done in collaboration with T. Klimkait, Institute of Medical Microbiology, University of Basel. Our HIV chip currently provides data on 20 SNPs that are associated with drug resistance. We plan to upgrade the existing prototype chip to cover more mutations in the RT gene by specifically targeting sequences of the A, C and D subtypes of HIV-1, the predominant subtypes in Tanzania. These SNPs will cover resistance mutations to all five first-line drugs used within the Tanzanian National AIDS Control Programme. We plan to further extend the HIV microarray to genotyping SNPs in the protease gene.

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 Students: J. Gare, S. Zürcher
 Collaboration: Institute of Medical Microbiology, University of Basel (T. Klimkait); Ifakara Health Institute (E. Mossdorf)
 Funding: STI Seed Money, Stipendienkommission Basel-Stadt

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