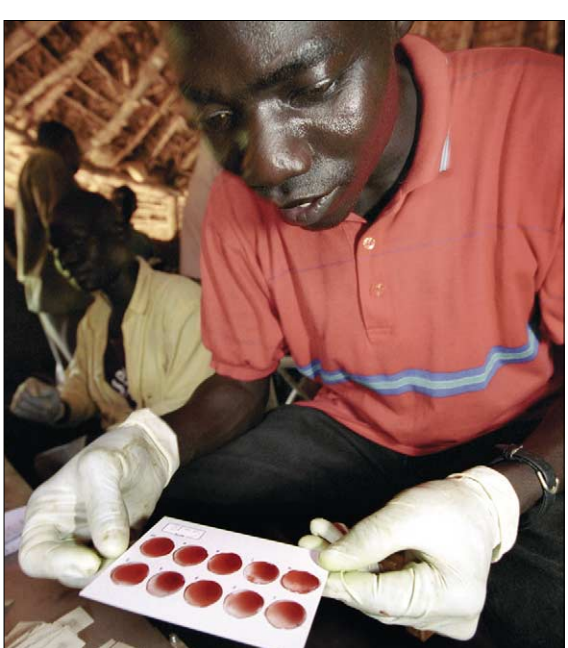
Fast, simple, and low-cost test for drug-resistant pathogens

Published online July 6, 2005
DOI:10.1016/S0140-6736(05)66833-X

See Research Letters page 486

More and more pathogens, including protozoa and bacteria, show resistance against a growing number of drugs.[1] Apart from the inconvenience for patients and the costs of unsuccessful use of medication, this trend increases the risk of spreading resistant pathogens through the population, leading to more patients and the risk of outbreaks of epidemics. If the resistance profile of the pathogen was known before medication is started, a correct choice for different medication could often be made. Thus only fast, cheap, and specific tests enabling the characterisation of the resistance profile of pathogens can revert these adverse effects. The contribution of Mhairi Stewart and colleagues[2] in today's *Lancet* is a fine example of such a test to detect arsenical drug resistance in *Trypanosoma brucei*. *T brucei* is a major parasite causing sleeping disease in sub-Saharan Africa, the second most common epidemic-causing pathogen in this area after malaria, with an estimated 300 000 infected patients. The test can be done on thin blood-smears incubated for only 1 min with a fluorescent dye and examination with a microscope.

Although the test looks elegant, its applicability in the field is not straightforward. The time-dependence of the assay might easily result in erroneous interpretation in the absence of precise timers. Furthermore, in chronic *T brucei gambiense* sleeping sickness, the parasite load is often too low to detect a single trypanosome within a minute. One solution might be the prior concentration of parasites or their propagation in laboratory animals. Propagation in laboratory animals will also ensure that *T brucei gambiense* parasites will appear in the long slender form, which is imperative for the correct working of the test. The assay, albeit in a modified format, might prove efficient in a laboratory setting.



Panos Pictures

Screening for sleeping sickness

Whilst Stewart and colleagues' test is very useful, it remains limited to *Trypanosoma* parasites that have become resistant against arsenical drugs through a defective plasma-membrane P2 adenosine-transporter encoded by the *TbAT1* gene.[3] This transporter molecule is vital for the uptake of the drug into the parasite, yet other transporters may also play a role.[4] The fluorescent dye in the test uses the same transporter-molecule pathway, thus directly indicating the presence or absence of the transporter molecule.

Another example of a known pathway occurs in multidrug-resistant cancer cells. In these pathways, membrane-bound transporter molecules play a pivotal role. The transporters might be channels that allow drugs to enter the cell or pumps that export the drugs from the cytoplasm to the extracellular space.[5]

In general, knowledge about the mechanism of resistance is often lacking because drug pathways are not known in enough detail.[6] Sensitive predictive tests to detect the resistance state of parasites can only be developed if the pathway of the pharmacology of the drug and the mechanism underlying the resistance trait is known. Therefore, to prevent future spreading of resistance in parasites, research must focus on elucidation of the pharmacology of drugs and related resistance mechanisms. For the P2 transporter, the underlying mechanism of resistance might often be found in the genome. Sequencing of the genomes of many parasites (for the *Trypanosoma* genome, see reference 7), and research to find genomic differences between different strains of trypanosomes by fine-scale genomic fingerprinting[8–11] related to pathogen-specific traits, will further add to our knowledge of the biology of the parasite and drug resistance. Furthermore the functional genomic (transcriptomic) and proteomic expression of the genome might also elucidate many such pathways. How-

ever, there is still much research to be done to maximise the effective drug treatment of parasites.

Marinus F W te Pas, Filip Claes, Philippe Büscher
Wageningen University and Research Centre, Animal Sciences Group, ID-Lelystad, Division of Animal Resource Development, Animal Genomics Group, 8200 AB Lelystad, Netherlands (MFWteP); and Prince Leopold Institute of Tropical Medicine, Department of Parasitology, Antwerp, Belgium (FC, PB)
Marinus.tepas@wur.nl

We declare that we have no conflict of interest.

1 Anene BM, Onah DN, Nawa Y. Drug resistance in pathogenic African trypanosomes: what hope for the future? *Vet Parasitol* 2001; **96:** 83–100.
2 Stewart ML, Krishna S, Burchmore RJS, et al. Detection of arsenical drug resistance in *Trypanosoma brucei* with a simple fluorescence test. *Lancet* 2005; **366:** DOI:10.1016/S0140-6736(05) 66793-1.
3 Matovu E, Geiser F, Schneider V, et al. Genetic variants of the *TbAT1* adenosine transporter from African trypanosomes in relapse infections following melarsoprol therapy. *Mol Biochem Parasitol* 2001; **117:** 73–81.
4 Matovu E, Stewart ML, Geiser F, et al. Mechanisms of arsenical and diamine uptake and resistance in *Trypanosoma brucei*. *Eukaryot Cell* 2003; **2:** 1003–08.
5 Shabbits JA, Krishna R, Mayer LD. Molecular and pharmacological strategies to overcome multidrug resistance. *Expert Rev Anticancer Ther* 2001; **1:** 585–94.
6 Majiwa P, Konde V, de Souza M, Peregrine A, Maina M. Molecular approaches to the identification of DNA markers for drug resistance in *Trypanosoma congolense*. *ICPTV Newsletters* 2000; no 2: http://www.icptv.org/newsletters/newsletter2/molecular.html (accessed May 5, 2005).
7 Wellcome Trust Sanger Institute. The *Trypanosoma brucei* genome project. Jan 16, 2004: http://www.sanger.ac.uk/Projects/T_brucei (accessed April 27, 2005).
8 Agbo EC, Majwa PAO, Büscher P, Claassen E, te Pas MFW. *Trypanosoma brucei* genomics and the challenge of identifying drug and vaccine targets. *Trends Microbiol* 2003; **11:** 322–29.
9 Agbo EC, Clausen PH, Büscher P, MajiwaPA, Claassen E, te Pas MFW. Population genetic structure and cladistic analysis of *Trypanosoma brucei* isolates. *Infect Genet Evol* 2003; **3:** 165–74.
10 Agbo EC, Duim B, Majiwa PAO, Büscher P, Claassen EJHM, te Pas MFW. Multiplex-endonuclease genotyping approach (MEGA): a tool for the fine-scale detection of unlinked polymorphic DNA markers. *Chromosoma* 2003; **111:** 518–24.
11 Agbo EC, Majiwa PAO, Claassen EJHM, te Pas MFW. Molecular variation of *Trypanosoma brucei* subspecies as revealed by AFLP fingerprinting. *Parasitology* 2002; **124:** 349–58.

Surveillance of HIV and tuberculosis drug resistance

See **Research Letters** page 488

Knowing the proportion of tuberculosis patients living with HIV is useful for health planning and for epidemiological tracking of HIV. Theoretically, this statistic can be obtained without much effort, because diagnostic HIV testing is part of the routine medical management of tuberculosis.[1] Tuberculosis clinics should therefore be able to provide information on HIV epidemiology at regular intervals from their service statistics.

In the reality of most health-care systems in the world, the situation is, however, more complex. Even if diagnostic HIV testing is offered to tuberculosis patients, testing and counselling of acceptable quality might not be available. Patients may have little motivation to attend these services, unless they have the prospect of accessing antiretroviral therapy if and when needed.

AP

In today's *Lancet*, Lisa Nelson and colleagues present the results of a survey of HIV prevalence in tuberculosis patients in Botswana.[2] The investigators used unlinked anonymous HIV testing. The ethics of using this method for HIV prevalence surveys have been discussed extensively. Most of what we know about the epidemiology of HIV originates in a process of removing some blood from a sample taken for other purposes, stripping it of all identifying markers, and testing it for HIV without the consent of the individual who provided the sample.[3] The approach has been generally accepted as ethical, although there have been dissenting voices.[4]

Blood testing is not a routine requirement for the diagnosis and management of tuberculosis in low-income countries. This fact has constrained the possibility of using unlinked anonymous testing for HIV surveillance in tuberculosis patients, although it can be done.[5] Nelson and colleagues overcame this constraint by testing sputum submitted for tuberculosis drug-resistance surveillance. Available sputum tests lack sufficient sensitivity for diagnostic HIV testing, but they are adequate for epidemiological surveillance in settings where HIV prevalence is above 10%.[1]