New diagnostic tests for sleeping sickness

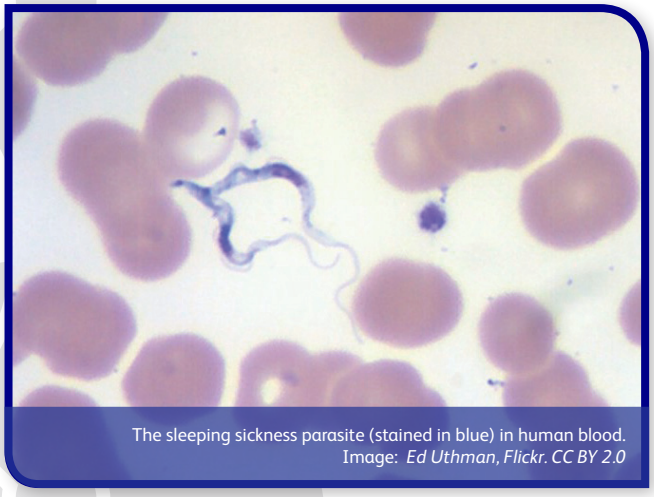
Researcher Dr Barrie Rooney at the University of Kent has developed a new diagnostic test for Human African Trypanosomiasis, or sleeping sickness. The prototype tests are currently undergoing clinical trials, and will contribute to World Health Organisation (WHO) efforts to control the disease and provide access to treatment.

Sleeping sickness is classified as a neglected tropical disease by the WHO, meaning that there is little investment in treatment from the pharmaceutical industry. There are between three and four thousand cases per year, concentrated in Central Africa. Diagnostic tests are needed to find people who carry the disease but show no symptoms, so they can be treated before the disease spreads. Existing tests require substantial infrastructure, including electricity, refrigeration and large teams of skilled people, which are difficult to access in the remote regions where the disease is found.

In contrast Rooney's new rapid diagnostic test is administered via a simple blood spot and does not require complex infrastructure.

Rooney used a BBSRC FLIP grant[1] to work in Professor Mark Smales' lab at the University of Kent to develop the technology underpinning the tests. A BBSRC follow-on fund pathfinder grant supported development of the prototype, which is undergoing testing at the Tropical Institute, Antwerp, followed by clinical trials with IRD in Marseilles. Further BBSRC follow-on funding is now being used to develop the test technology for related diseases, including those in animals. In 2016 Rooney won BBSRC's Social Innovator of the Year award for the research[2].

Rooney's research highlights the need for simple diagnostic tests to prevent the spread of these diseases and in healthcare more broadly. "Developing simple point-of-care tests is important these days for remote situations or even in the modern GP practice, so these are technologies that need to be developed to bring healthcare closer to the patient," says Rooney. "In developing countries, there is a need to address these diseases at sources, rather than letting them travel."



The sleeping sickness parasite (stained in blue) in human blood.
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A neglected tropical disease

Sleeping sickness is caused by *Trypanosoma* parasites. According to Rooney, "It's transmitted by the tsetse fly. In the past it was all across Central Africa – Democratic Republic of Congo (DRC), Uganda, South Sudan, Cameroon, Gabon, down to Angola as well. Now it tends to be populations of refugees, those affected by war. It's often the poorest people who are affected by it."

In 2014 there were 3,796 reported cases of sleeping sickness[3]. Overall, the number of cases reported each year is falling, but outbreaks do still occur. The sleeping sickness parasite can lay dormant in infected people for up to two years. During that time it can still be transmitted through the bite of the tsetse fly. As a result, sleeping sickness is often found in rural populations as these are most likely to be exposed to the tsetse fly.

Sleeping sickness can be treated. If it is identified early, treatment consists of a week of injections. By the time the sleeping symptoms occur the disease has reached the brain and patients require two weeks in hospital. By identifying and treating infected people as soon as possible, the WHO

IMPACT SUMMARY

Researchers at the University of Kent have developed an innovative new test for Human African Trypanosomiasis, or sleeping sickness.

The new diagnostic tests are much easier to use than traditional testing, as they do not require electricity, refrigeration or a large team of people.

The new tests are currently undergoing a clinical trial.

Sleeping sickness is classified as a neglected tropical disease by the World Health Organisation. It occurs in central African countries, usually amongst poor rural communities and those affected by war. There are around 3-4000 case reported per year.

Related diseases also affect animals, including livestock, and the researchers are aiming to develop tests for those forms of the disease.

The researchers have received a total of £319K from BBSRC to support the research

hopes to reduce the spread of the disease. For this reason, there is an urgent need for efficient new diagnostic tests.

Rapid diagnostic tests

Before starting this work, Rooney worked in industrial biotechnology and volunteered with MSF[4]. "I was familiar with the type of testing that was being done, and it didn't include new types of testing systems," says Rooney. "It was quite laborious. It required electricity, you had to bring generators up the Congo river, and you had to have a cold chain in extreme conditions. I thought it was time that new simpler tests were developed; rapid-type tests."

BBSRC FLIP funding enabled Rooney to work with Professor Mark Smales at Kent to address the need for a diagnostic test without the shortcomings of previous tests. The test developed by Rooney and colleagues at Kent relies on a system to produce 'recombinant antigens'; genetically engineered protein molecules. When added to a blood sample from someone carrying the sleeping sickness parasite these antigens will bind to specific molecules in the blood, signalling the presence of the disease. Uniquely, Rooney created a system to express the antigens using an organism closely related to the sleeping sickness parasite, rather than using the live, dangerous parasite itself, or one of the standard expression systems such as *E. coli* bacteria.

The tsetse fly is the insect that carries African trypanosomiasis, or sleeping sickness.
Image: Oregon State University. CC BY-SA 2.0

This is not new technology, but it is the first time it has been used for this application. "We chose a technology which had been looked at 15 years ago by German company Jena Biosciences[5]. They said they'd be very happy to support this work using their technology, and they would waive rights to their licence fee for this disease," says Rooney.

Through her work with MSF, Rooney was also able to gain access to samples held by the Institute of Tropical Medicine in Antwerp[6]; the World Reference Laboratory for sleeping sickness. Using these samples, Rooney could test the antigens she was producing from the expression system, and found that they worked as well as antigens taken from the disease-causing parasite[7].

The next step was to scale-up production of the diagnostic test and test it more widely. A BBSRC follow-on fund pathfinder grant[8] enabled Rooney to access the WHO sample bank for sleeping sickness, held at the Pasteur Institute in Paris[9], which contains 2000 samples from patients with the disease.

Rooney is also working with Belgian company Coris BioConcept[10] to scale-up production of the test for a clinical trial. Coris already produce a wide range of diagnostic tests, and have the expertise required to manufacture the sleeping sickness test. Collaborators at the IRD (Institut de recherche pour le développement) in Marseilles[11] will use the prototype tests from Coris in an EU-funded clinical trial to test their efficacy in patients.

Every living species

Closely-related disease-causing parasites are also found in animals, including livestock. "They've been around since before the dinosaurs and they've evolved to live in every living species you can think of," says Rooney.

The animal form of sleeping sickness is also transmitted by tsetse flies and can cause fever, weakness and weight loss. In some cases it can be fatal. Rooney has been awarded a BBSRC follow-on fund grant[12] to develop the diagnostic tests to help identify and treat sleeping sickness in livestock. "If you identify those animals and remove them, and ensure the stock is healthy it can improve the lives of people," she explains.

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