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Update on immunological tests for lymphatic filariasis

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Until recently, the TropBio antigen enzyme-linked immunosorbent assay and the immunochromatographic test rapid-card test were the only commercially available diagnostic tests for lymphatic filariasis. The new Brugia Rapid antibody-detection dipstick is a welcome addition, but there is an urgent need to develop more cost-effective, accurate and standardized immunological tests for use in the global filariasis elimination program.

The mosquito-borne parasitic disease lymphatic filariasis currently infects around 120 million people and is targeted for elimination as a public health problem by the year 2020 [1,2]. Three filaroid species cause lymphatic filariasis. Bancroftian filariasis is caused by *Wuchereria bancrofti*, which occurs in many tropical and subtropical areas and accounts for 90% of cases. Brugian filariasis is caused by *Brugia malayi*, which occurs in southern and eastern Asia, and *Brugia timori*, which occurs in Timor and nearby islands, and accounts for the remaining 10% of cases [3].

The evolution of filariasis testing

Traditionally, diagnosis of lymphatic filariasis depended on the detection of microfilariae (Mf) in blood samples. Mf detection is insensitive if the Mf density is low and, although this can be improved by using concentration methods [4], the nocturnal periodicity of Mf in many endemic areas demands blood sample collection late at night – a situation not well tolerated by community members or health workers. A third of active cases are

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amicrofilaraemic; therefore, basing prevalence estimates on Mf detection alone underestimates the burden of infection [4].

Bancroftian filariasis diagnosis was revolutionized by the introduction of circulating filarial antigen (CFA) enzyme-linked immunosorbent assays (ELISA) in the early 1990s [5] and the immunochromatographic test (ICT) rapid card test for CFA in the mid-1990s (now marketed as Binax Filariasis NOW®) [6]. CFA tests have excellent sensitivity and specificity, are not affected by periodicity, and detect both microfilaraemic and amicrofilaraemic cases [4,7]. The ICT test can be performed in the field despite limited formal training, with results available almost immediately. It has become the preferred method for mapping endemicity by the Global Program to Eliminate Lymphatic Filariasis, which was launched in 2000 [1,2].

Brugian filariasis diagnostic challenges

Until recently, there was no comparable rapid test for Brugian filariasis and diagnosis relied upon the traditional method of nocturnal Mf detection with its poor sensitivity. A recently released dipstick test called 'Brugia Rapid', which was developed in Malaysia, shows 97% sensitivity, 99% specificity, 97% positive predictive value and 99% negative predictive value. It detects IgG4 antibody, not filarial antigen, and is not specific to *Brugia* species, reacting with most cases (but interestingly not all) of *Wuchereria bancrofti* but not with other common helminth and protozoal parasites [8]. Performance of the test requires several steps that make it not as convenient as the ICT test and a single-step cassette modification is under development. Although not a substitute for a CFA test, it is a useful addition to the very limited array of available filariasis diagnostic tools. An ELISA version of the test, used for batch testing in a laboratory environment, is also available [9,10].

The need for improved diagnostic modalities

An ideal diagnostic test for filariasis should have the following characteristics: high sensitivity and specificity across a wide range of parasite prevalence levels and parasite loads; availability both in a field-user-friendly format and for use in laboratory-based batch testing; availability as a standardized quality assured kit; and cost effective and affordable to those countries where it will be used.

There is a need for further research and development as all three parasites are targeted for global elimination. There is concern that the currently available tools might have severe limitations for certifying cessation of transmission when parasite prevalence and antigen levels are extremely low. Despite the widely held belief that transmission will cease once the Mf prevalence is reduced to 1%, there is very little scientific justification for this. *Aedes polynesiensis*, a major vector in the Pacific, can transmit parasites even when Mf prevalence and density are extremely low. This has been demonstrated by the studies on Maupiti Island in French Polynesia, where Mf levels were reduced to well below 1% after 34 years of mass community drug therapy, yet transmission continues [11]. In such situations, the only way to stop resurgence might be to eradicate the parasite completely.

The TropBio ELISA, which was developed 13 years ago using a monoclonal antibody against the Og4C3 antigen produced using the cattle parasite *Onchocerca gibsoni*, is regarded as the 'gold standard' for filariasis diagnosis but its effectiveness at very low Mf densities has been questioned [12]. The need for pre-treatment of serum by boiling, the long incubation times required for the slow-binding IgM, and a three-step detection and indicator system means that the assay is cumbersome by standards of today. Using *Wuchereria bancrofti* antigens to raise an IgG monoclonal capture antibody, together with a more direct detection system, would lead to shorter incubation times and a more convenient, and hopefully more sensitive, assay. As yet, there is no *Brugia* antigen test on the horizon, and if the mapping of *Brugian* endemicity can be successfully accomplished with the Brugia Rapid antibody test, such a test might not be required.

There is currently no alternative to the Binax Filariasis NOW® rapid test available and this leaves the elimination program in a vulnerable situation should supply difficulties arise. This was graphically illustrated recently, when a relatively minor production change in the layout of the card introduced a potential error into interpreting results. Although it only took a few months to overcome, it did cause confusion, and the coordinators of the elimination program recommended that the use of the test be suspended until the situation was resolved [13,14].

Studies have shown that filarial antigen tests can remain positive for at least three years after mass drug administration, making them unsuitable for monitoring the effectiveness of control measures [15]. Alternatives to antigen-based tests include the possibility of using antibody tests as an index of on-going transmission. Methods for filarial antibody detection have been available since the 1960s, mostly based upon crude filarial antigens prepared from a wide variety of filaroid species [3,16]. The use of the IgG4 isotype reduces crossreactions with non-filaroid helminths, but a recent study has shown that there is still concern, especially with *Strongyloides* [17]. Several recombinant antigens are available [9,16–20]. One of these, Bm14, does not crossreact with *Strongyloides* and might form the basis for a standardized ELISA that will detect antibody to all three species [17]. A method has also been devised for the detection of IgG4 antibody in urine [21] and this approach compared favourably with the ICT test during a filariasis survey in Sri Lanka [22]. Clearly, this approach, which is less invasive than blood sampling, is more desirable in certain situations, for example in young children or where the risk of blood-borne pathogens is high. The problem with urine testing is that one can never be quite sure about the origin of the sample. A much better alternative would be the use of an oral fluid dipstick, if such a test could be devised.

Conclusion

An important factor in the long-term success of the global program to eliminate lymphatic filariasis is the availability of effective tools for diagnosis and monitoring. At present, only three tests are commercially available, which

have placed the program in a vulnerable position should they prove ineffective or the supply is interrupted. There are three elements to successful research and development: expertise, commitment and funding. There is no doubt that the filariasis research community has expertise and commitment, and several groups around the world are committed to developing newer and better filariasis diagnostic tests. It is hoped that funding bodies will understand the benefit of investing in that expertise and commitment to accelerate the day when humankind is free from the ravages of this dreadful disease.

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Letter

Analyzing the structure of biodiversity

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Thierry de Meeûs *et al*. [1] recently discussed some of the limitations of the biological species concept, and they argue that the nonexistence of a single-species concept that satisfactorily reflects the complexity of natural diversity illustrates the futility of the search for such a concept. de Meeûs *et al*. consider the species as a taxonomic category (which was also stated by Kunz [2] from a parasitological context). We believe that this consideration could mean: (i) identification of the concept with the object to which it refers (i.e. the species entity); (ii) negation of the said object; or (iii) recognition of an object, although it cannot be conceptualized because of a lack of well-defined limits. The first is the product of confusion, the second of radicalization because the definition involves adoption of a purely nominalist principle, and the third is a hypothesis that requires confirmation. We think it is premature to invalidate any of the species concepts, or to suggest that classic concepts such as the biological species concept are not useful at a time when we are just beginning to understand the genetics of the speciation process.

The term 'species' can have three different definitions: (i) a species is a taxonomic rank in Linnean hierarchy; (ii) a species is a taxon (i.e. a concept that refers to a natural group); and (iii) a species is the natural group itself [3]. Taxonomic categories and taxa are abstract and arbitrary

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