Onchocerciasis and Lymphatic Filariasis
Background Information, and PATH Diagnostics

Written by Shira Gabry, 2015

PATH is the leader in global health innovation. An international nonprofit organization, we save lives and improve health, especially among women and children. We accelerate innovation across five platforms—vaccines, drugs, diagnostics, devices, and system and service innovations—that harness our entrepreneurial insight, scientific and public health expertise, and passion for health equity. By mobilizing partners around the world, we take innovation to scale, working alongside countries primarily in Africa and Asia to tackle their greatest health needs. Together, we deliver measurable results that disrupt the cycle of poor health.

Acronyms:

APOC: African Programme for Onchocerciasis Control
CDC: Center for Disease Control and Prevention
CDTI: community-directed treatment with ivermectin
CSA: Committee of Sponsoring Agencies
DEC: diethylcarbamazine
ELISA: enzyme linked immuno assay
FAO: Food and Agricultural Organization
GAELF: The Global Alliance to Eliminate Lymphatic Filariasis
GPELF: The Global Program to Eliminate Lymphatic Filariasis
GSK: GlaxoSmithKlein
JAF: Joint Action Forum
L3: third-stage larvae
LF: lymphatic filariasis
MDA: Mass drug administration
MDP: Mectizan Donation Program
NTD: Neglected Tropical Disease
NGDO: Non-Governmental Development Organization
NIH: National Institutes of Health
OCP: Onchocerciasis Control Program
OEPA: Onchocerciasis Elimination Program for the Americas
PENDA: Programme for the Elimination of Neglected Diseases in Africa
PCR: polymerase chain reaction
PTS: post-treatment surveillance
SAE: severe adverse effects
SD: Standard Diagnostics, Inc.
TCC: Technical Consulting Committee
UNDP: United Nations Development Program
WHO: World Health Organization

PATH Diagnostics Program

PATH develops innovative diagnostic tools for the most pressing global health issues by utilizing its expertise in technology and product development, and by executing field evaluations and business collaborations with leading diagnostics companies. The diagnostics program's portfolio comprises HIV/STIs, non-communicable diseases, neglected tropical diseases (NTDs), malaria, and tuberculosis. Because many developing countries face serious challenges in accurately identifying and diagnosing diseases due to their remote, rural settings, PATH focuses on creating rapid diagnostics tests that are low-cost, simple to use, and can diagnose disease at point-of-care (30).

Onchocerciasis biology and control efforts

Onchocerciasis is transmitted by female blackflies that have ingested microfilariae from an already infected person. Six to twelve days after the microfilariae are ingested, they develop into third-stage larvae (L3) and become infective. Onchocerciasis is spread to human hosts when the infective female blackfly takes a blood meal and introduces L3 into the skin (1). The L3 differentiate into adult worms within the body in 1-3 months. Groups of 2-3 adult female worms settle into nodules throughout the body and become inseminated by male worms that travel to the nodules. After insemination, female adult worms may release 1300-1900 microfilaria per day for 11-13 years. Without treatment, the microfilaria travel into the lymphatic system and into the sub-epidermis of the body, eventually making their way into the eye.

Picture 1: The life cycle of Onchocerca (CDC)

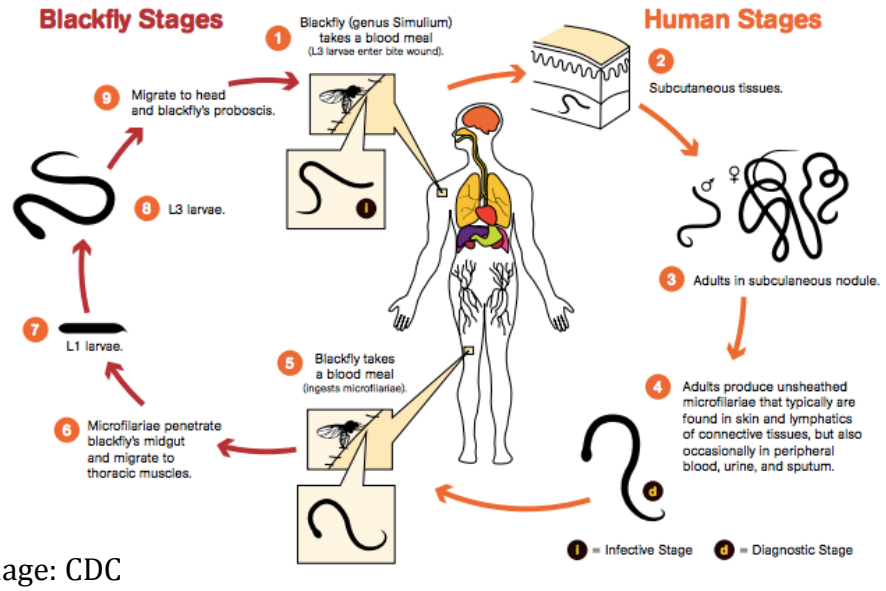


Image: CDC

When the microfilariae perish, the immune system reacts with an inflammatory response. The inflammatory response leads to symptoms that include severe itching, skins lesions and if repeated exposure without treatment occurs, eventual blindness (1). Onchocerciasis is treated with annual or twice-annual mass drug administration (MDA) of ivermectin. Depending on pre-treatment prevalence of infection and the severity of the infection, it is estimated that it will take approximately 15-20 years to achieve elimination after the start of MDA. Ivermectin acts by killing the microfilariae in the body, but the adult worms are not affected. As a result, treatment must continue until the adult worms perish (15-20 years), or the microfilariae levels are low enough that transmission is unlikely to occur frequently enough to initiate recrudescence or reemergence of the disease in the area (19).

Regions co-endemic with eye worm, a filarial infection caused by the *Loa loa* parasite, face the risk of severe adverse effects (SAE), including encephalitis and death, occurring if a person positive with *Loa loa* is treated with ivermectin. This presents a challenge to areas co-endemic with *Loa loa* in treating lymphatic filariasis (LF) and onchocerciasis (25).

Organized control efforts for onchocerciasis began in 1974 with the establishment of the Onchocerciasis Control Program (OCP). OCP was launched in a collaborative effort of four U.N. agencies: the World Health Organization (WHO), the UN Development Program (UNDP), the World Bank, and the Food and Agricultural Organization (FAO). OCP targeted 11 countries in Africa, and until MDP began, focused exclusively on vector control and insecticide spraying as the method of control. OCP was incredibly successful and ceased all programs in December of 2002 after achieving elimination in all target countries except for Sierra Leone, who was undergoing civil unrest (1,3,38).

In 1987, Merck, the pharmaceutical company that developed Mectizan, the non-generic version of ivermectin, decided to donate the medication to all who could benefit until onchocerciasis was eliminated. In order to coordinate the donations, they established the Mectizan Donation Program (MDP) (31). In the past 25 years since MDP's inception, more than 1.5 billion treatments have been released for onchocerciasis MDA (31).

In 1992, the Onchocerciasis Elimination Programme for the Americas (OEPA) was launched by the Carter Center to target endemic areas in Latin America for elimination with twice-annual MDA. OEPA has been incredibly effective with 11 out of 13 endemic areas having interrupted transmission by June of 2013, and with elimination having occurred in many of the 13 endemic areas since (37). After seeing OEPA and OCP's successes in treating onchocerciasis with ivermectin and the impact that treatment had on transmission in OCP and OEPA countries, the African Program for Onchocerciasis Control (APOC) was launched in 1995 by the WHO to continue this work in other endemic countries (4).

In working to determine the best way to distribute ivermectin in endemic communities, a team of African scientists developed community directed treatment with ivermectin (CDTI) as a low cost, feasible solution. CDTI relies on community participation and engagement to organize, develop and implement an ivermectin distribution system best fit for that particular community. APOC formally adopted CDTI in 1997, and has continued its practice ever since (44).

APOC is comprised of non-governmental development organizations (NGDO), ministries of health, affected communities and governing bodies (39). The Joint Action Forum (JAF) is the top governing body of APOC and determines APOC's general strategy. Under JAF is the Committee of Sponsoring Agencies (CSA), which is comprised of UNDP, the World Bank and the WHO. They assist JAF in major program and policy decisions and oversee the Technical Consulting Committee (TCC), The NGDO Coordination Group and general APOC management (39). APOC as it is currently structured will be shutting down in December of 2015, and conversations have been started to determine the next phase for onchocerciasis and NTD control and elimination. One possible solution is to develop a new entity known as the Program for the Elimination of Neglected Diseases in Africa (PENDA). PENDA would focus on coordinating the elimination of onchocerciasis and LF, among other NTDs in Africa (4). There is still a lot of uncertainty surrounding this transformation and what impact it will have on endemic countries who currently benefit from partnerships and support from APOC (10,18).

Current onchocerciasis diagnostic tools

Currently, there are a variety of diagnostic tests for onchocerciasis available on the market but there is no gold standard.

- **Skin-snip test:** The test most commonly used to detect onchocerciasis is skin-snip microscopy. Also called a sclerocorneal punch, the test requires cutting a small piece of skin

tissue from both sides of the pelvis and soaking the skin in saline solution until microfilariae emerge. The skin punch and the saline are then read under a microscope to identify microfilaria. An individual must have been infected for at least 18 months in order for an adequate number of microfilariae to be released to obtain a positive test result. Also, ivermectin treatment results in a lowering of microfilariae count, particularly in the months directly following treatment, resulting in low sensitivity of the test. This makes it inadequate for areas that have reached low prevalence. The skin snip is an unpopular test because it can be very painful (14).

    - **PCR test:** The polymerase chain reaction (PCR) test is a highly sensitive test that searches for parasitic DNA in residual skin shavings from the skin-snip microscopy, making it more sensitive than the skin-snip test, but still requires the presence of microfilariae in the skin. These tests can only be completed in areas with access to a laboratory with high infrastructure (24).

- **DEC patch test:** The DEC patch test requires that a patch soaked in a low level of diethylcarbamazine (DEC) be placed on the skin and observed after 24 hours to see if a skin reaction occurred. A skin reaction means a positive test result. DEC may also cause a reaction if the individual is positive for other parasites, increasing the risk of false positives (24). The DEC patch is only available for use from the WHO.
- **Nodule palpation:** Once the L3 larvae differentiate into adult worms and the female adult worms form nodules in the body, it is often possible to feel the nodules on the head and around the iliac crests. This method of diagnosis is inaccurate as it is subject to variation, nodules can easily be missed, and onchocerciasis infection without nodules does occur (11).
- **Ov16 ELISA:** The enzyme linked immuno assay (ELISA) is often used to confirm the results of other diagnostic tests. There are many different versions of ELISA tests for onchocerciasis, but all share the major component of testing for the Ov16 antibodies, antibodies that are produced during an onchocerciasis infection, using antigens in a laboratory setting. Different ELISAs prioritize high sensitivity whereas other ELISAs have a high level of specificity but lower sensitivity.

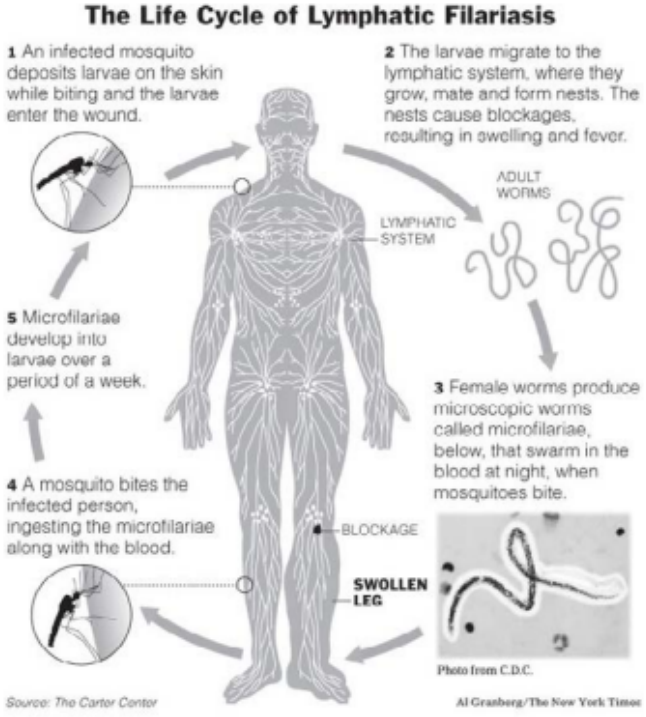


Image: Al Granberg, The New York Times.

Lymphatic filariasis biology and control efforts

Lymphatic filariasis (LF) is transmitted by three types of parasitic worms. *Wuchereria bancrofti* is the most common, and accounts for 90% of LF cases worldwide. *Brugia malayi* and *Brugia timori* are found in Southeast Asia, and account for the remainder of cases (40). LF is transmitted to human hosts through mosquitos. An infected mosquito introduces larvae into a person's skin through its bite and the larvae travel to the lymphatic system, differentiate into adult worms and mate. Similar to onchocerciasis, the female worms produce microfilariae and release them into the blood stream. Adult female worms produce microfilariae for 5-8 years. Once

microfilariae are present in the blood stream, mosquitos become infected by ingesting microfilariae from an infected person (26).

LF is more commonly known as elephantiasis due to the swelling of limbs that often occur in cases of long-term infection. Swelling and pain results from the settling of the nests of the adult worms in the lymphatic vessels as well as from the inflammatory immune response to the death of the adult worms. After frequent and long-term infection, the lymphatic vessels become permanently damaged, causing the retention of fluid in the limbs in the form of lymphedema (40). Males may experience hydrocele, the accumulation of fluid around the testicles, leading to sexual dysfunction and extreme discomfort. High rates of LF in a community can have a severe economic impact as it prevents those who experience symptoms from working. LF is the second-most common cause of long-term and permanent disability in the world (40). The WHO recommends a combined treatment of albendazole and ivermectin for LF. Since 1998, GlaxoSmithKline () has been donating albendazole for LF treatment with a commitment to provide treatment for Yemen and for African countries that are co-endemic for LF and onchocerciasis (9). MDP coordinates the co-administration of albendazole and Mectizan where LF and onchocerciasis are co-endemic. MDP has coordinated the provision of more than 665 albendazole million treatments for LF since 2000 (31).

In 2000, the WHO founded the Global Programme to Eliminate Lymphatic Filariasis (GPELF). GPELF was established with the goal of eliminating LF worldwide by the year 2020. Because the side effects and symptoms of LF cause severe disability and loss of productivity, part of the GPELF strategy is to address and treat those suffering from LF, while also working towards elimination. GPELF is a public-private partnership that focuses on mobilizing partners and coordinating MDA and treatment efforts (35). Also founded in 2000 was the Global Alliance to Eliminate Lymphatic Filariasis (GAELF). GAELF is a public-private partnership based at the Liverpool School of Tropical Medicine comprised of NGDOs, country government officials and other organizations. They provide support to GPELF through communications, technical and fundraising assistance (41). While GPELF and GAELF has achieved numerous successes in mapping, MDA, and treatment efforts since 2000, LF transmission is still occurring and it is unlikely that LF will be eliminated worldwide by 2020 (34).

Current LF diagnostic tools

Currently, there are a variety of diagnostic tests for LF available on the market but there is no gold standard.

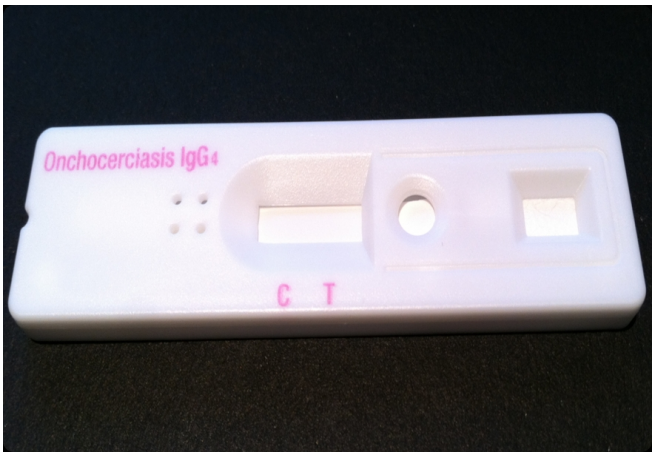
- **Filaria Detect™ IgG4 ELISA System:** The ELISA test for LF is currently being developed by InBios, a medical diagnostics company that specializes in infectious disease. The test detects the LF antibody by exposing a blood sample to the LF antigen and observing to see if binding occurs (36).
- **Night blood:** Before more technologically advanced point-of-care rapid tests were developed, LF could only be diagnosed by collecting the blood of an infected person at night and searching for microfilariae in the blood smear under a microscope. While night blood smears are no longer the first diagnosis tool used, they are sometimes used to confirm positive rapid test results (29). Collecting the blood at night is critical due to the periodicity of the LF microfilariae in the bloodstream.
- **BinaxNOW Filariasis:** BinaxNOW Filariasis ICT card test is a rapid diagnostic test that detects LF using blood, serum, or plasma. It tests for circulating filarial antigens and can provide results in 10 minutes (28). This test is the official test used by GPELF for mapping, to determine when to stop MDA and for post treatment surveillance (PTS). While the test is

effective, if not ready within the time frame, false positives are common (27). Additionally, in areas co-endemic with *Loa loa*, the BinaxNOW Filariasis test has been found to provide unreliable results (42).

- **Alere™ Filariasis Test Strip:** The Alere™ Filariasis Test Strip is similar to the BinaxNOW Filariasis in that it is a rapid diagnostic that diagnoses LF by detecting the circulating filarial antigens, but the test is a strip as opposed to a card. In a study comparing the BinaxNOW card test to the test strip in the lab and in the field, it was found that the test strip was a more sensitive test, particularly in field studies. The test strip also uses less blood than the card test, and has a longer shelf life at ambient temperatures (27). Like the BinaxNOW Filariasis test, the Alere™ Filariasis test strip has been found to provide unreliable results in areas co-endemic with *Loa loa*.
  (42)

PATH's work in onchocerciasis and LF

In 2010, PATH was awarded a grant from the Bill & Melinda Gates Foundation to develop an affordable rapid test to diagnose onchocerciasis. The Ov16 rapid test that PATH developed is a serology-based rapid test that detects exposure to *Onchocerca volvulus,* the parasite that causes onchocerciasis, by checking for Ov16 antibodies in a single drop of blood from a finger prick (43). The test result can be determined just 20 minutes after the test is taken, and the result remains valid for up to 24 hours. Unopened test kits can be kept in storage for up to two years. After developing the Ov16 rapid test and conducting field evaluations in Togo in early 2013, PATH refined the test utilizing data from the study and transferred the technology to Standard Diagnostics, Inc. (SD), a manufacturing company based in South Korea. After a second field study in 2014, again conducted in Togo, the SD BIOLINE Onchocerciasis IgG4 point-of-care rapid test launched in November 2014 (32).



PATH/A. Golden

The original grant has since been expanded to develop a dual-detection, or "biplex", point-of-care test for LF and onchocerciasis in collaboration with the National Institute of Allergy and Infectious diseases, part of the National Institutes of Health (NIH), the Centers for Disease Control and Prevention (CDC), and the Task Force for Global Health. This biplex test simultaneously detects antibodies generated in response to both the Wb123 antigen from *W. bancrofti* and the Ov16 antigen from *O. volvulus.* PATH will be starting field evaluations of the Ov16 Wb123 rapid test in the fall of 2015 and will continue their partnership with SD to manufacture and distribute the product (33).

Advantages of new diagnostics for onchocerciasis and LF

The WHO states the importance of diagnostic tools for serologic evaluation and PTS in their guidelines for the three phases of an onchocerciasis elimination program (19). Their recommendations specify that while their original criteria approved the use of the DEC patch test, skin snip or nodule palpation to determine absence of infection in children under the age of 5, an Ov16 antibody detection test is a more suitable approach for later phases and as elimination is reached. Also discussed in these guidelines is the requirement to continue MDA in places co-endemic with LF, if LF transmission is ongoing. As a result, interruption of transmission for onchocerciasis cannot be measured until after the cessation of MDA (19). This highlights the importance of the Ov16 Wb123 rapid test in its ability to integrate surveillance activities for both onchocerciasis and LF. Because the SD BIOLINE Onchocerciasis IgG4 rapid diagnostic test and the Ov16 Wb123 rapid test detect antibodies, they are useful in low-prevalence areas where other diagnostic tests are no longer able to accurately detect prevalence (33). Data to date indicate that the Ov16 Wb123 rapid test does not result in a significant number of false positives in individuals infected with *Loa loa* as do some other diagnostics. Additionally, both tests only require a minimally invasive finger prick to retrieve the small amount of blood required to successfully run the tests and no laboratory is necessary. They can easily be integrated into other NTD surveillance efforts and do not need to be timed with treatment (43).

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