pathogens

MDPI

Review

Beyond Tradition: Exploring Cutting-Edge Approaches for Accurate Diagnosis of Human Filariasis

Damian Pietrzak [1], Julia Weronika Łuczak [2] and Marcin Wiśniewski [1,*]

[1] Division of Parasitology and Parasitic Diseases, Department of Preclinical Sciences, Institute of Veterinary Medicine, Warsaw University of Life Sciences—SGGW, 02-786 Warsaw, Poland; damianpietrzak23@gmail.com
[2] Faculty of Animal Breeding, Bioengineering and Conservation, Warsaw University of Life Sciences—SGGW, 02-786 Warsaw, Poland; luczak.ju@icloud.com
* Correspondence: marcin_wisniewski@sggw.edu.pl

Abstract: Filariasis is recognised as a global public health threat, particularly in tropical and subtropical regions. It is caused by infection with a nematode parasite of the superfamily Filarioidea, including *Wuchereria bancrofti*, *Brugia malayi*, *Onchocerca volvulus*, and *Onchocerca lupi*. Three main types of filariasis have been classified: lymphatic filariasis, subcutaneous filariasis, and serous cavity filariasis. The symptoms exhibited by individuals afflicted with filariasis are diverse and contingent upon several variables, including the species of parasite, the host's health and immune response, and the stage of infection. While many classical parasitological techniques are considered indispensable tools for the diagnosis of parasitic infections in humans, alternative methods are being sought due to their limitations. Novel tests based on host–parasite interactions offer a rapid, simple, sensitive, and specific diagnostic tool in comparison to traditional parasitological methods. This article presents methods developed in the 21st century for the diagnosis of filariasis caused by invasion from *W. bancrofti*, *B. malayi*, *O. volvulus*, and *O. lupi*, as well as techniques that are currently in use. The development of modern diagnostic methods based on molecular biology constitutes a significant advancement in the fight against filariasis.

Keywords: filariasis; parasitology; Nematoda; molecular biology techniques; immunoenzymatic tests; diagnostics

check for updates

Citation: Pietrzak, D.; Łuczak, J.W.; Wiśniewski, M. Beyond Tradition: Exploring Cutting-Edge Approaches for Accurate Diagnosis of Human Filariasis. *Pathogens* **2024**, *13*, 447. https://doi.org/10.3390/pathogens13060447

Academic Editor: Alban Ramette

Received: 29 April 2024
Revised: 16 May 2024
Accepted: 23 May 2024
Published: 24 May 2024

Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Filariasis is a disease caused by parasites belonging to the phylum Nematoda, which is the third most species-rich in the animal kingdom [1,2]. It is estimated that there may be as many as 24,000 species of nematodes parasitizing vertebrates, the majority of which remain undescribed [3–5]. Filariasis of animals, especially mammals, can be transmitted to humans and is often characterised by the absence of specific symptoms [6,7]. Human infections with zoonotic filariasis are reported worldwide [8] and were first described in modern research over a century ago [9]. A number of different genera of filaria have been isolated from humans, including *Dirofilaria* [10,11], *Brugia* [12,13], *Onchocerca* [14,15], *Dipetalonema* [16,17], *Loa* [18,19], and *Mansonella* [20,21]. The location of these parasites within the human body varies from species to species. Filariasis affects a range of human tissues and often evinces little or no observable host response throughout their development [22,23]. An exception occurs when the parasite enters extremely sensitive tissues, such as the conjunctiva [24].

Blood-sucking insects, such as mosquitoes, blackflies, or midges, act as biological vectors [25]. Infection is transmitted through the bite of an infected insect as it feeds upon blood [26]. Thereafter, parasite larvae mature into adult forms within the host. These adult parasites then multiply, producing microfilariae, which are subsequently ingested by blood-sucking insects along with human blood. These microfilariae undergo subsequent stages of development within the insect's bodies [27–29].

Filariasis can be classified based on the location of the parasite within the host's body: lymphatic filariasis—*Wuchereria bancrofti*, *Brugia malayi*, *Brugia timori*; subcutaneous filariasis—*Loa loa*, *Onchocerca volvulus*; and serous cavity filariasis—*Mansonella* spp. [30,31].

2. Lymphatic Filariasis

The term lymphatic filariasis refers to infection with one of three nematode species: *W. bancrofti*, *B. malayi*, and *B. timori*. The majority of *W. bancrofti* infections occur in South and Southeast Asia, as well as sub-Saharan Africa. Central and South America and the Pacific islands are endemic regions. *B. malayi* has been reported in South and Southeast Asia, India, Indonesia, Thailand, Vietnam, Malaysia, and the Philippines. In contrast, *B. timori* is restricted to eastern Indonesia and East Timor (Figure 1) [32].

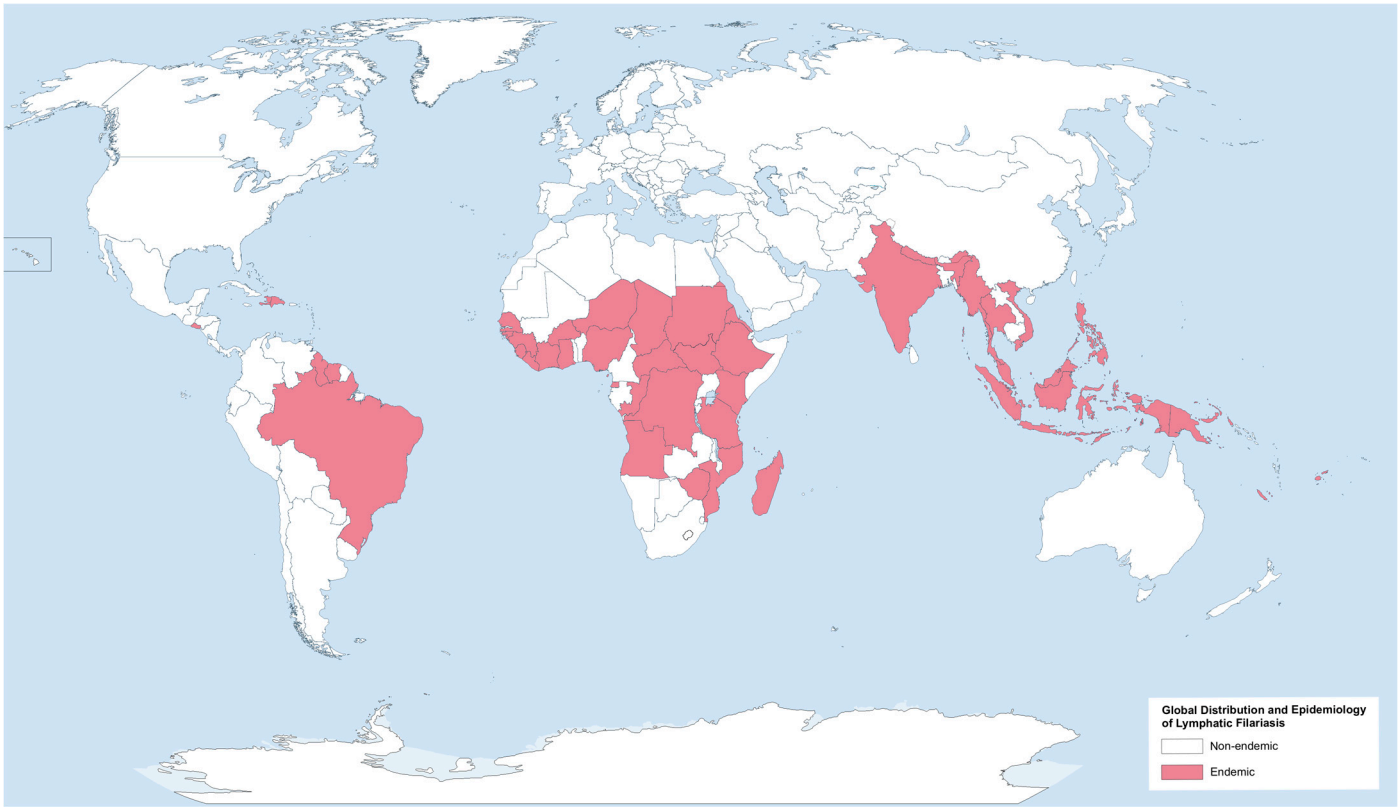


Figure 1. Global distribution and epidemiology of lymphatic filariasis. Created with GIMP 2.10.

Lymphatic filariasis is a long-lasting, chronic, and persistent disease, with the majority of infected individuals remaining asymptomatic. However, the disease can manifest in a wide range of clinical signs, including lymphedema of the legs, lymphangitis, elephantiasis, and, uniquely in *W. bancrofti*-infected individuals, hydrocele [33]. Asymptomatic infections are characterised by the presence of several regulatory immune processes driven by living parasites that promote their long-term survival [34–37]. The precise mechanisms by which clinical disease manifests remain uncertain, although it is likely to be associated with the host's inflammatory response to dead or dying adult parasites. The parasites' death results in inflammation of lymphatic vessels and lymph nodes, which may cause pain and swelling in the affected area [33,38]. The susceptibility to infection, host response, and the risk of developing pathological features are familial and probably related to host genetic predisposition [39–41].

Lymphatic filariasis has been recognised by the World Health Organization (WHO) as a neglected tropical disease. In 2000, the Global Programme to Eliminate Lymphatic Filariasis (GPELF) was established with the objective of halting transmission through mass

drug administration (MDA) of anthelmintics and alleviating the suffering of people affected by the disease through morbidity management and disability prevention (MMDP). Since the start of the GPELF, the number of infections has been reduced globally by 74% [42]. Despite the success of eradication programmes, by 2018, it was estimated that 51 million people continued to suffer from chronic symptoms of the disease, including lymphoedema, elephantiasis, and hydrocele [43]. This indicates that there is still a need for reliable and sensitive diagnostic methods to remain a priority [44].

3. Subcutaneous Filariasis

In the context of filariasis research, it is essential not to overlook the subcutaneous form of the disease, which presents distinct symptoms. This form is primarily characterised by cutaneous and subcutaneous symptoms resulting from nematode infections, including those caused by *O. volvulus*, *L. loa*, and *M. ozzardi* [45].

O. volvulus is predominantly impacting sub-Saharan Africa, Yemen, and regions of Central and South America [46,47]. *L. loa* primarily affects rural populations in Central and West Africa, with over 20 million patients chronically infected in regions with medium to high transmission rates [48], while *M. ozzardi*, one of three species in the genus *Mansonella* (the others being *M. perstans* and *M. streptocerca*) causing mansonellosis [49], is common across the neotropical region from southern Mexico to northwestern Argentina (Figure 2) [21].

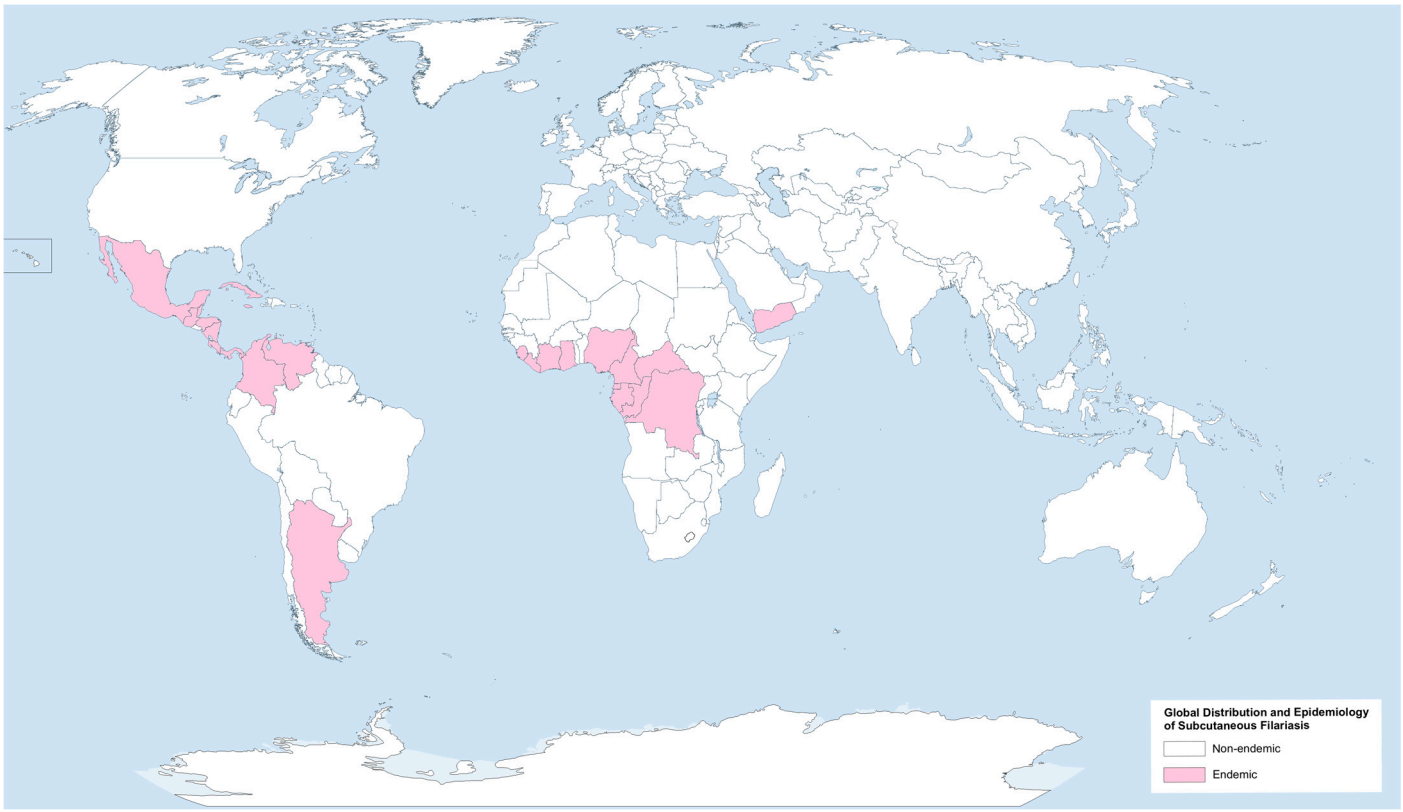


Figure 2. Global distribution and epidemiology of subcutaneous filariasis. Created with GIMP 2.10.

The symptoms include the appearance of papules, nodules, and scratches on the skin surface, which becomes dry and thickened, often accompanied by changes in skin pigmentation and intense pruritus [50]. *L. loa* infection, also known as African eye worm or loiasis, is currently not recognised by the World Health Organisation (WHO) as one of the key neglected tropical diseases. The typical clinical manifestation of loiasis, occurring in approximately 80% of individuals, is the migration of the adult parasite under the sclera or

subconjunctival eyelid tissue [51]. *M. ozzardi* infection can manifest as a skin rash, headache, fever, itching, lymphoedema (e.g., swelling of the arms or legs), and joint pain [52].

Both lymphatic filariasis and subcutaneous filariasis pose significant public health threats in endemic areas around the world. They have a crucial impact on the physical and mental health of patients and pose a challenge to health systems. To prevent further spread of the disease and to minimise the impact on affected communities, it is essential to implement comprehensive intervention in both diagnosis and treatment.

4. Parasitological Methods for Filariasis Diagnostics

Parasitological diagnosis is a process that involves laboratory procedures with the aim of detecting organisms in clinical specimens using morphological features and visual identification. The final identification is typically based on microscopic examination of stained slides, often utilising high-magnification techniques such as oil immersion [53]. Only a few procedures can be automated, and differences in morphological characteristics between species can be very difficult to distinguish. To become proficient in the morphological analysis of parasites using a microscope requires a considerable amount of training. Additionally, the identification of the organism is highly dependent on the skill of the person performing the examination. In order to provide accurate and effective diagnosis services in the area of medical parasitology, it is essential for laboratory professionals to possess a thorough understanding of parasite life cycles, epidemiology, invasiveness, geographic range, clinical symptoms, and the recommended therapies [54].

One of the traditional diagnostic methods for nematode infections involves the visual detection of microfilariae using stained thin and thick blood smear techniques. Morphological identification of microfilariae in blood or skin snips remains the gold standard for routine clinical diagnosis of most human filariasis. Important criteria for morphological analysis include the length and width of microfilariae and the presence or absence of a sheath [55]. The recommended staining methods for the detection of microfilariae in blood are as follows: Giemsa, Wright–Giemsa, and Delafield's haematoxylin [54,56]. Certain parasitic nematode species exhibit periodicity, whereby microfilariae are present within the blood at specific times of day, adapted to the feeding behaviour of the vectors [57]. One disadvantage of these tests is their sensitivity, as blood sampling must be conducted at specific time intervals that vary according to the parasite species. These species include *W. bancrofti*, *Brugia* spp., and *L. loa*. *W. bancrofti* and *Brugia* species exhibit nocturnal periodicity, with the optimal time for blood sampling occurring between 10 p.m. and 2 a.m. *L. loa* exhibits diurnal periodicity, with peak microfilariae detection periods occurring between 10 a.m. and 2 p.m. [58,59].

Other parasitological methods include Knott's technique and the membrane filtration technique, which facilitate the concentration of microfilariae present in blood samples taken from patients, enabling morphometric identification of circulating parasites. These procedures may enhance the detection of microfilariae in blood and in other bodily fluids. Knott's technique is an easy and inexpensive technique that is more commonly used. The test procedure involves drawing approximately 1 mL of venous blood into a tube containing either EDTA or citrate. The volume of 10 mL of 10% formalin is then added to the blood. The sample is centrifuged at 300× *g* for 2 min. The supernatant is removed and the resulting precipitate is analysed under the microscope. Furthermore, the precipitate can be spread on a slide, allowed to dry, and then stained with Giemsa or haematoxylin [31,55]. The modified Knott's test represents the most common approach. In this method, a 2% formalin is used instead of the conventional 10%. This concentration was determined to be optimal, as weaker solutions fail to form a satisfactory amount of precipitate after centrifugation, while stronger solutions precipitate haemoglobin [60]. The disadvantage of the method is the use of formaldehyde in the test, a substance contained in formalin that is mutagenic and genotoxic [61,62]. In 2012, the International Agency for Research on Cancer (IARC) classified formaldehyde as a human carcinogen [63]. Moreover, the European Chemicals Agency (ECHA) defines the substance as lethal if inhaled, toxic if swallowed and in contact

with the skin, and capable of causing severe skin burns and eye damage. Formalin also requires special precautions for handling, storage, and disposal. To enhance the utilisation of the Knott test, particularly among veterinarians, research is being conducted to identify a substitute for formalin [64,65]. The membrane filtration technique involves the collection of blood in a manner comparable to the Knott test; a volume equivalent to 10 mL of 10% Teepol saline solution is added to the drawn blood. The blood–Teepol mixture is passed through a suitable filter paper (Nuclepore® membrane) placed in a holder, using a syringe. Subsequently, 10 mL of water is passed through the filter, followed by 3 mL of methanol to fix the microfilariae. The filter is then placed on a slide and stained with Giemsa. The dried slide is analysed microscopically [31,55,66,67].

Skin snips, obtained by biopsy, are used to diagnose the presence of microfilariae *Onchocerca* spp. and *M. streptocerca*. For onchocerciasis, this method is considered the gold standard [68]. The sections should be thin, involving only the epidermis and superficial dermis. It is important to avoid heavy bleeding during skin biopsy to prevent contamination of the sample with peripherally circulating microfilariae. Usually, sections are taken from several sites on the body to increase the sensitivity of parasite detection. The periodicity of microfilariae in skin sections is not observed; therefore, they can be detected at any time [31,55]. This method is highly specific for active infection, whereas it is not sensitive in detecting early, light, and pre-symptomatic invasions. Furthermore, this test is not optimal for assessing the efficacy of macrofilaricidal therapies for onchocerciasis. A further limitation is the biopsy procedure, which is invasive, painful, and impractical for quick and easy diagnosis. The biopsy instruments used must be carefully disinfected between examinations to prevent the transmission of pathogens through the blood, such as HIV. The possibility of infecting subjects during biopsy collection has reduced the acceptance of skin testing among populations in endemic areas, especially in the context of countries with limited sanitation standards [69–71].

Although traditional microscopic examination has enabled significant developments in the field of parasitology, it poses a number of problems in the diagnosis of nematode infections. Performing blood sampling at unconventional times, requiring significant parasitological expertise from microscope operators, involving complex sample preparation steps, needing specialised equipment and reagents, and facing limitations in achieving easy and swift diagnosis all contribute to the challenges in this process. Furthermore, classical parasitological methods for diagnosing the presence of microfilariae are time-consuming and subjective, as their interpretation requires the experience of laboratory staff, and the often complicated handling of samples can lead to potential errors or loss of test material. Consequently, there is a continued demand for affordable diagnostic tests that allow rapid, easy, and sensitive diagnosis of the course of nematode infections. Molecular and immunoenzymatic methods have a significant impact on the development of diagnostic techniques. Figure 3 provides a summary of the parasitological methods for filariasis diagnostics.

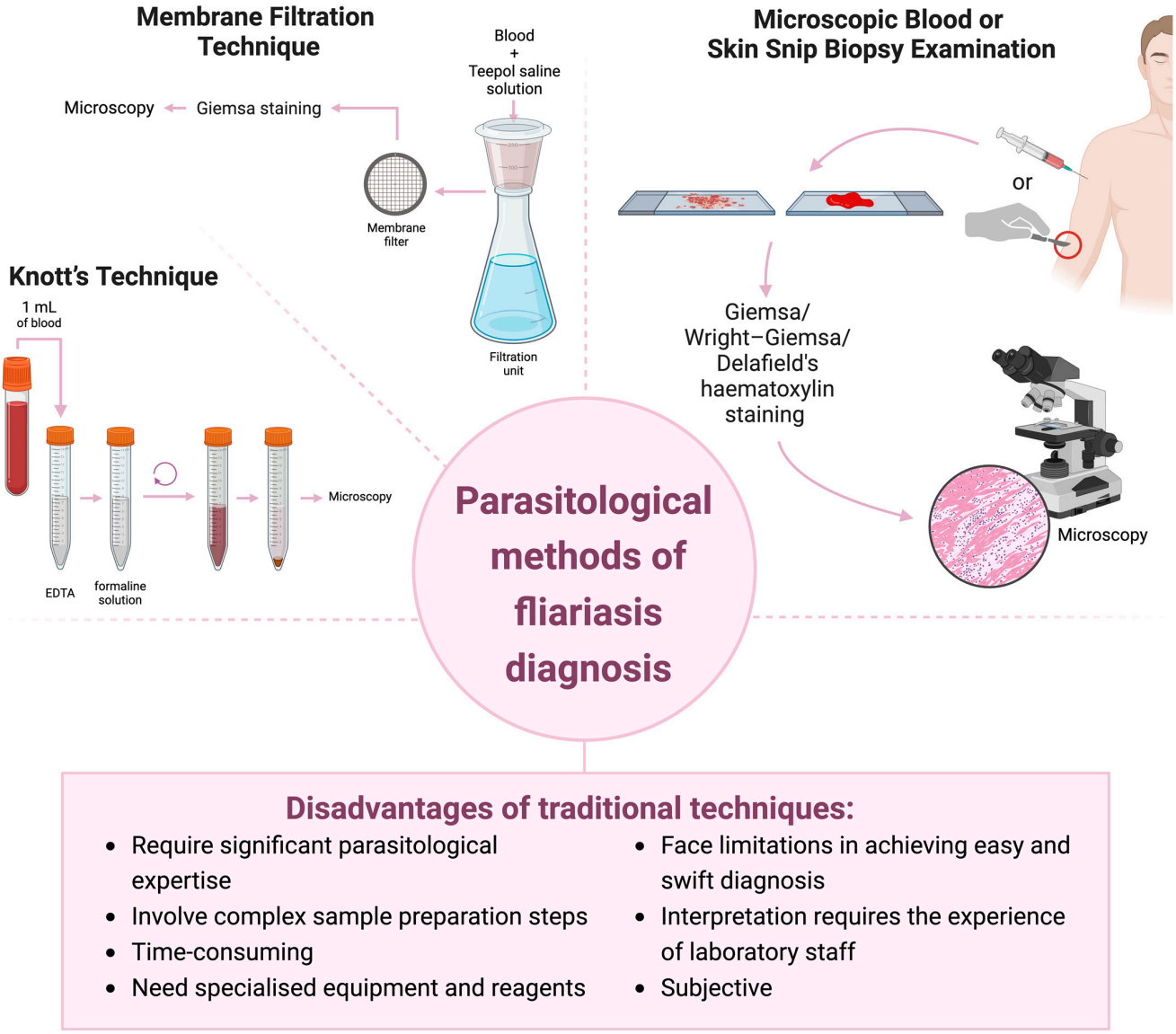


Figure 3. Parasitological methods for filariasis diagnostics. Created with BioRender.com.

5. Next-Generation Methods for Filariasis Diagnostics

5.1. Brugia malayi

Although *B. malayi* accounts for only 10% of lymphatic filariasis cases, it remains a significant threat to human health [72]. Its prevalence is predominantly recorded in Southeast Asia, encompassing countries such as the Philippines, Malaysia, Indonesia, South Korea, Vietnam, and India [55]. It is estimated that more than 70% of filariasis cases in Indonesia are caused by *B. malayi* [73]. The first published case involving human infection by *Brugia* spp. was identified in 1962 in a New York City resident who presented with painless swelling of the inguinal lymph nodes [74].

There are several vectors of *B. malayi*, including mosquito species of the genus *Aedes*, *Anopheles*, and *Mansonia*, among others, depending on the geographical area [75]. The length of *B. malayi* females ranges from 43 to 55 mm, while males are shorter, reaching up to 23 mm [76]. The microfilariae of *B. malayi* are up to 230 µm long, and on Giemsa-stained preparations, their sheath is stained pink to distinguish them from the colourless sheath of *W. bancrofti* [58].

As previously stated, the most prominent clinical feature of lymphatic filariasis is the development of severe lymphoedema of the extremities (elephantiasis) and, on rare occa-

sions, the genitalia (hydrocele), which is a consequence of vascular dysfunction [77]. Furthermore, tropical pulmonary eosinophilia, which is characterised by an eosinophilic pulmonary infiltrate, peripheral hypereosinophilia, wheezing, chest pain, splenic enlargement, and bloody sputum, is also associated with *W. bancrofti* and *B. malayi* infections [78–80].

5.1.1. Immunoenzymatic Tests

The Brugia Rapid™ (Reszon Diagnostics International Sdn. Bhd., Selangor, Malaysia) is a rapid strip test operating on the principle of immunochromatographic technology [81]. The test is based on the specific reaction of a recombinant BmR1 protein produced in *Escherichia coli* with antiparasitic antibodies found in the patient's serum. The antibodies react with the BmR1 antigen, which then binds to monoclonal anti-human IgG4 antibodies linked to colloidal gold [82]. The IgG4 enzyme-linked immunosorbent assay (ELISA), utilising the BmR1 antigen, demonstrated a specificity rate of 95.6% and a sensitivity rate of 96% [83]. For the Brugia Rapid™ test, the results indicated 97% sensitivity and 99% specificity [81]. Furthermore, a second validation study was conducted. The sensitivity of the strip test was found to be 93%. In the field study, compared to microscopic examination of thick blood smears, the sensitivity of the test was 87% and the specificity was 100%. The rapid strip test was therefore a useful diagnostic tool for filariasis in *B. malayi* endemic areas [84]. Other studies have also demonstrated the high sensitivity and specificity of the test in detecting infection with the parasite [85–88]. Based on the detection of specific IgG4 antibodies against recombinant BmR1 antigens, a Brugia Rapid™ test is commercially available from Reszon Diagnostics International Sdn. Bhd., Selangor, Malaysia. The WHO, in its Global Programme to Eliminate Lymphatic Filariasis, recommends the Brugia Rapid™ test for the detection of *B. malayi* invasion [89]. However, it should be noted that Brugia Rapid™ tests, which detect IgG4 antibodies specific to filaria, may generate false-positive results in patients who have been previously infected and have undergone treatment. This is because individuals affected by lymphatic filariasis may retain B-cell memory, leading to the detection of antibodies for some time after the elimination of the parasite. Currently, there is a lack of a commercial test for the detection of *B. malayi* antigens, which highlights the need for further research to improve the diagnosis of lymphatic filariasis [90].

Two-dimensional gel electrophoresis (2DE), 2DE-immunoblotting analysis of adult parasite extract, SDS-PAGE, liquid chromatography with tandem mass spectrometry (LC-MS/MS), and bioinformatics analysis enabled the identification of *B. malayi* antigens that induce the production of specific antibodies, as well as those involved in the formation of immune complexes (ICs) circulating in the blood of people with microfilaremia. This enabled the selection of 34 antibody-recognised proteins in the sera of individuals with microfilaremia who had no tissue pathology associated with parasite invasion. In addition, antigens specific to *B. malayi* microfilariae were detected, which were involved in the formation of ICs. The detection of the ICs in question may be a useful indicator for assessing the efficacy of anti-microfilariae drugs and may replace the inconvenient overnight blood sampling. These proteins have significant potential as diagnostic markers and vaccine markers. Therefore, it is crucial to continue research to gain a deeper understanding of their role in the immunopathology of filariasis and their potential therapeutic applications. Further research on these proteins may lead to the development of new diagnostic tests that can be used for the early detection of *B. malayi* and the identification of new therapeutic targets for antiparasitic drugs and potential vaccines. Furthermore, continued research on these antigens may lead to the development of new strategies to control and eradicate filariasis, which is crucial for public health, especially in endemic areas [91].

The recombinant protein BmALT-2, produced in *E. coli* strain BL21(DE3), was analysed for immunoreactivity by ELISA with human sera. This study aimed to assess the feasibility of developing a circulating filarial antigen (CFA) ALT-2 assay to detect early stages of *B. malayi* infection. The BmALT-2 protein plays an important role in filaria invasiveness and is highly expressed at the L3 stage of the parasite. The test has demonstrated high specificity, allowing further research to be conducted with the objective of developing an

effective, rapid, and specific strip test to detect *B. malayi* infection [92]. In 2017, a method for the production of BmALT-2 protein in a yeast expression system was developed. The glycosylated ALT-2 protein was demonstrated to exhibit greater immunoreactivity than its non-glycosylated form, and the eukaryotic expression system based on *Pichia pastoris* yeast was identified as the optimal platform for the large-scale production of the protein [93].

Another approach to the diagnosis of *B. malayi* infection is the production of human scFv-IgG1Fc antibodies to detect recombinant BmR1 and BmSXP antigens. The recombinant BmR1 and BmSXP proteins are transcribed from the *Bm17DIII* and *BmSXP-1* genes, respectively, and are recognised by antibodies in the serum of infected patients. Both proteins are secretory products of the parasite, but their functions have not been precisely defined. In this study, three different recombinant scFv-based immunoglobulin gamma IgG1 antibodies were produced (anti-BmR1 clone 4, anti-BmXSP clone 5B, and anti-BmXSP clone 2H2). The antibodies produced were found to have high specificity and were able to bind to the corresponding proteins even at relatively low concentrations. The conjugation of Fc to scFv ensures the binding stability and solubility of scFv, and the Fc part enables the attachment of signalling molecules (e.g., probes, gold, fluorescent dyes), opening up the possibility of their use in rapid lateral flow assays. These antibodies may be employed in the development of assays for the detection of *B. malayi* antigens, particularly rapid diagnostic tests with high specificity and sensitivity. Furthermore, they can be used to avoid the detection of previous past infection with the parasite [90,94].

5.1.2. Molecular Methods

A number of promising molecular techniques, including conventional polymerase chain reaction (cPCR) and real-time PCR, have been developed for the detection and differentiation of filarial nematodes, including *B. malayi* [95–97], loop-mediated isothermal amplification (LAMP) for the detection of *B. malayi* and *B. timori* [98], or pyrosequencing utilising SL and 5S rRNA as specific molecular markers [99]. However, highly specific and sensitive molecular tests are still required for the monitoring and detection of human infections.

In 2011, Hindson et al. developed the high-throughput droplet digital PCR (ddPCR) method. In the reaction, the mixture is split into tens of thousands of water-in-oil nanodroplets, and a single sample of genomic DNA and a subsequent primer–probe pair are subjected to simultaneous PCR amplification and quantitative evaluation of short regions of the amplicon [100]. ddPCR is a third-generation method and is more sensitive and capable of direct absolute quantitative detection than routine PCR and quantitative PCR (qPCR) [101]. Technologies have been employed to develop a duplex ddPCR assay with fluorescently labelled probes, which is capable of distinguishing and quantifying *W. bancrofti* and *B. malayi* in mosquito and mammalian blood samples and also detecting co-invasion [102]. The authors observed that the method is straightforward to perform and interpret, relatively inexpensive, and provides high specificity and sensitivity in detecting *W. bancrofti* (100% and 100%) and *B. malayi* DNA (98.3% and 100%).

In recent years, high-resolution melting (HRM)-PCR has become a widely used method in parasitological diagnostics [103–105]. In this method, products undergo thermal denaturation after the real-time PCR. Fluorescence changes caused by the release of intercalating dye from the DNA strand are monitored in real time. By comparing the resulting melting curves of unknown samples with the melting curve profiles of known isolates, it is possible to assign them to specific strains or species [106]. A real-time HRM PCR assay was developed for the detection and differentiation of *W. bancrofti*, *B. malayi*, *D. immitis*, and *B. pahangi* in vectors and blood samples. A single primer pair that is employed to amplify the arrays of all four species enables the detection of the 5S rRNA gene and spliced leader sequences [103]. Identification of each species is possible based on different melting temperatures [107]. In terms of cost per sample, the method is less expensive than multiplex real-time PCR methods. Furthermore, assays can be performed on a large number of samples simultaneously, with only very small sample volumes needed for a single

analysis [108]. Additionally, reactions can be performed on paraffin-embedded samples, thus providing an effective alternative diagnostic procedure [109].

5.1.3. Other Tests

Since the 1990s, a number of diagnostic techniques, such as the identification of specific antibodies and circulating antigens, and PCR protocols have been introduced to improve the detection of filariasis and increase the reliability of the results [110,111]. However, none of these methods is sensitive and precise enough to simultaneously identify all three aetiological factors of lymphatic filariasis. Consequently, it is relatively simple for the clinician to make an erroneous diagnosis, particularly when the patient's symptoms are atypical, especially in non-endemic areas. Although shotgun metagenomics has been extensively utilised for virus identification [112,113] and the detection of bacterial pathogens [114], its application in parasitic infections remains limited [115]. In the case of *B. malayi*, a case was described of a 34-year-old Chinese student who, despite repeated visits to hospitals in both the US and China, could not obtain a definitive diagnosis [116]. The patient exhibited no overt symptoms of lymphatic filariasis [117]. However, testing for filariasis was conducted via PCR of the conserved genes *B. malayi*, *B. timori*, and *W. bancrofti*. The results of the analysis demonstrated the absence of parasites in the patient's blood. Given the absence of a diagnosis and the patient's increased itching and swelling of the body, a metagenomic approach was finally applied in order to identify the pathogen. The results of this analysis indicated that the patient was infected with *B. malayi*. This provides hope for the diagnostic identification of not only typical lymphatic filariasis infections but also those that are cryptic [116].

Table 1 presents a comparative analysis of diagnostic methods for *B. malayi* infection, as previously outlined in the preceding sections.

Table 1. Immunoenzymatic, molecular, and other tests for the detection of *B. malayi* infection.

Type of Method	Method	Description	Article
Immunoenzymatic	Brugia Rapid™	Rapid diagnostic test utilising a recombinant BmR1 protein	[81,82]
	Immunoproteomic	Identification of antigens with potential diagnostic applications	[91]
	ELISA	Utilises recombinant BmR1 antigen	[83]
		Utilises recombinant BmALT-2 antigen	[92,93]
		Utilises human scFv-IgG1Fc antibodies to detect recombinant BmR1 and BmSXP antigens	[90,94]
Molecular	Duplex ddPCR	Amplification of HhaI repeat element	[102]
	Real-Time HRM-PCR	Amplification of 5S rRNA gene and spliced leader sequence SL1	[103]
Other	Metagenomic	Massively parallel sequencing	[116]

5.2. Onchocerca volvulus

Onchocerciasis in humans, primarily caused by *O. volvulus*, remains a significant neglected tropical disease, continuing to exhibit a high incidence and contributing to poverty in endemic areas [118]. As indicated by estimates from the Global Burden of Disease Study 2015, approximately 15.5 million people are currently living with onchocerciasis, including 12.2 million people with onchocerca skin disease (OSD) and 1.025 million people with vision loss (known as river blindness) [119].

This nematode is transmitted by the female whiteflies of the genus *Simulium* [120]. In the human host, the worms reach maturity after 1–3 years, and the adult worms (macrofilariae) reside in nodules under the skin, where females can live for up to 15 years. During this period, they are capable of producing millions of microfilariae that migrate under the skin of infected individuals. The males of *O. volvulus* have a length of 23 mm and a posterior end which curves ventrally. In contrast, females have a length ranging from 230 to 700 mm [121].

The most prominent clinical signs in individuals with onchocerciasis are skin lesions, including pruritic dermatitis and nodules [122]. Many patients suffer from visual disturbances commonly referred to as river blindness [123,124]. Additionally, disease has been linked to epilepsy, specifically onchocerciasis-associated epilepsy (OAE) [125,126] and nodding syndrome [127].

5.2.1. Immunoenzymatic Tests

The immunoenzymatic techniques used in the detection of *O. volvulus* have been extensively researched, which clearly demonstrates their significance. Despite the existing knowledge, there are ongoing endeavours to refine the existing tests and identify new diagnostic procedures. This continuous effort is understandable, as improvements in diagnostic efficiency and accuracy can be crucial for the treatment and control of onchocerciasis.

The OV-16 antibody test is the only WHO-approved method for the diagnosis of *O. volvulus* infection in humans [128]. The OV-16 ELISA test is based on the detection of antibodies that are specific to the recombinant OV-16 *O. volvulus* antigen. The assay was developed in the 1990s [129]. It has been observed that approximately 15–25% of individuals have genetic alterations that prevent the induction of an immune response to the OV-16 antigen [130]. Furthermore, this test is unable to distinguish between past and active infections, as parasitic antibody levels remain high even after the disease has ended [70]. Although the detection of anti-Ov16 IgG4 immunoglobulins is more sensitive and less invasive than microscopic analysis of skin biopsies [131], frequent false-negative results give a false sense of security and make it difficult for clinicians to make a diagnosis.

The OV-16 ELISA, developed by Cama et al. (2018), represents a modification of a protocol utilised by multiple laboratories engaged in evaluating the effectiveness of an onchocerciasis elimination programme in Guatemala [132,133]. The modifications involved a reduction in antigen concentration from 1.0 to 0.5 µg/well, an increase in incubation temperature from room temperature to 37 °C, and an optimisation of the reaction time of chromogenic substrate at four different times (15, 30, 45, and 60 min). The utilisation of specific incubation temperatures and a fixed time of 60 min for the reactivity of the chromogenic substrate proved to be significant factors in enhancing the accuracy of this assay. The specificity and sensitivity of the modified Ov16 IgG4 ELISA were reported to be 99.7% and 88.23%, respectively [134].

Additionally, there is a growing body of research aimed at the identification of new antigens that may enable more specific and sensitive diagnoses of *O. volvulus* infection. In 2020, Shintouo et al. designed and validated the recombinant chimeric antigen Ov-MANE1. An indirect ELISA was performed to quantify specific IgG antibodies present in the sera of infected and uninfected individuals. Furthermore, the OvMANE1 antigen did not cross-react with antibodies in sera collected from patients infected with related nematodes. Studies have demonstrated the considerable potential of OvMANE1 for use in the development of antigenic diagnostic tests and antibody capture reactions, which are essential for monitoring the progress of onchocerciasis eradication programmes [135]. Additionally, novel potential diagnostic peptides were produced and characterised, including OvNMP-48 [136]; OvMP-1, OvMP-2, OvMP-3, OvMP-23 [137]; OvHSP70 [138]; Ov58GPCR-ECD [139]; OVOC10469, OVOC3261 [140]; OV-17 and OV-33 [141]. These studies provide an important foundation for further attempts to use the identified proteins as specific biomarkers in the diagnosis of human onchocerciasis.

Another diagnostic approach is the use of recombinant antibodies as controls in serological tests. Among others, a recombinant human anti-Ov16 IgG4 clone has been identified and used to produce standards for the serological OV-16 ELISA. This provides a consistent and standardised control between tests. The anti-Ov16 IgG4 control can facilitate ELISA plate validation and normalisation of data for analysis and comparison. Furthermore, the utilisation of a singular positive control may facilitate comparisons of assay performance with novel rapid diagnostic tools, such as the Bioline® ONCHOCERCIASIS IgG4 rapid diagnostic assay, which allows for the significant characterisation of the limits of detection of specific antibodies [142].

The Bioline® ONCHOCERCIASIS IgG4 rapid diagnostic test (OV-16 RDT), manufactured by Abbot (Lake Forest, IL, USA), is a commercial, qualitative, rapid immunochromatographic test that detects IgG4 antibodies against the *O. volvulus* specific OV-16 antigen from patient blood [143]. The test was compared with the OV-16 ELISA and microscopic detection of microfilariae in skin sections. The OV-16 ELISA demonstrated a higher sensitivity of 83%, while the OV-16 RDT achieved a value of 74.8% and the examination of skin sections showed the lowest sensitivity of 71.4%. Moreover, the OV-16 RDT exhibited a greater degree of specificity (98.6%) compared to the OV-16 ELISA (84.8%) [144]. In other studies, the OV-16 RDT and OV-16 ELISA were observed to have a satisfactory percentage agreement of 99.2% in positive samples, identifying parasitic invasion. Furthermore, it was demonstrated that the OV-16 RDT could detect fewer individuals with *O. volvulus* infection than the OV-16 ELISA [145].

5.2.2. Molecular Methods

As with the detection of other nematodes causing filariasis, PCR can also be used to identify *O. volvulus*. These methods rely on the amplification of a repetitive 150 nucleotide sequence (designated O-150) and its subsequent detection with, for instance, a specific probe utilising the Southern blot technique [146–148]. In 2011, Fink et al. described a melting curve-based qPCR using degenerate primers to detect a 154 bp long O-150 repeat amplicon (O-150 qPCR) [149]. However, it has been observed that the long amplicon results in limited sensitivity, and the absence of a hybridisation probe may reduce the specificity of the assays [150]. Accordingly, Mekonnen et al. introduced a methodology utilizing a hydrolysis probe-based qPCR technique targeting the 5S rRNA gene of *O. volvulus* (O-5S qPCR), with the aim of refinement and optimization [151]. The new method was found to increase the detection limit by half compared to O-150 qPCR. The sensitivity of the assay was enhanced by the use of a highly stable hydrolysis probe that is 100% specific for *O. volvulus* [150].

A one-step method for detecting *O. volvulus* infection in skin scrapings based on the length polymorphism of the first internal transcribed spacer (ITS1) repeat of ribosomal DNA has also been described [152]. These regions are used to detect and differentiate filarial parasite species [153,154]. This approach allows the development of a single set of 'universal' primers that can amplify the DNA of various filarial species, and then differentiate them based on amplicon length or sequence polymorphism. The authors adapted this method to real-time polymerase chain reaction with melting curve analysis (qPCR-MCA), which allows the detection and differentiation of PCR products of different sizes in a single tube and during a single PCR. Limiting the assay to a single tube reduces the risk of error due to carryover or cross-contamination [152]. The qPCR-MCA assay was compared with the qPCR-O-150 and the O-150-PCR ELISA [155]. A total of 471 skin biopsies were analysed, with 47.5%, 43.5%, and 27.0% of them found to be positive for *O. volvulus* in the qPCR-O-150, qPCR-MCA, and O-150-PCR ELISA tests, respectively. A comparison of the sensitivity and specificity of the qPCR-MCA test with the qPCR-O-150 test as a comparator demonstrated values of 89.3% and 98.0%, respectively, while for the O-150-PCR ELISA, the values were 56.7% and 100%. The majority of qPCR-MCA misclassifications occurred in mixed infections, which highlights the need for specific qPCR tests.

LAMP was first described in 2000 [156]. LAMP is an alternative to cPCR methods that is characterised by ease of execution and the possibility of colorimetric readout [157]. This technique is particularly useful in resource-limited settings, where it does not require expensive equipment [158,159], which is usually not available in endemic locations, making diagnosis difficult. Additionally, LAMP is less sensitive to the inhibitors present in clinical samples and insects, which can inhibit Taq polymerases used in PCR. The technique relies on DNA synthesis, with automatic cyclic strand displacement guided by *Bst* DNA polymerase. The reaction proceeds without denaturation of the DNA template [160], thus allowing it to be carried out at isothermal temperatures. Using the reaction carried out, the matrix can be amplified up to 10^9–10^{10} times in 15 to 60 min. The amplified products consist of a series of parent DNA loops of different lengths [161]. The results are read by visual inspection of the turbidity due to precipitation of white magnesium pyrophosphate, which is a by-product of DNA synthesis [162], or by visual and UV inspection of DNA amplification using a fluorescent dye [163]. This method has also been applied in the diagnosis of *O. volvulus* infection. *COX1* sequences are amplified, utilizing DNA extracted from skin biopsies as the template, a material concurrently employed in the PCR O-150 procedure [164–166]. The LAMP test shows high specificity for the genus *Onchocerca*. However, it has been observed that the test can also detect other species, including *Onchocerca ochengi* [167]. Nevertheless, this is considered a significant problem because the *O. ochengi* is typically found in cattle, not in humans, and therefore, its presence in the biopsy is negligible [168]. The molecular testing still depends on the detection of *O. volvulus* DNA in the skin and therefore may not be reliable after treatment with antiparasitic drugs. Skin biopsies are also invasive and are becoming less common, with endemic communities often refusing the procedure [169].

An alternative approach is the detection of circulating DNA and microRNA (miRNA) of *O. volvulus*, where sampling is less invasive and diagnosis is not based on the temporal presence of microfilaremia [170]. miRNAs are short (18–22 nucleotides), single-stranded, non-coding RNAs responsible for regulating mRNA expression in both animal and plant cells [171]. The literature indicates that miRNAs may play a significant role in the parasite's adaptation to the host [172] as well as in modulating its innate immunity [173]. A number of attempts have been made to utilise circulating, body fluid-derived miRNAs and DNA as a diagnostic tool. miRNAs belonging to *O. volvulus* have been identified in the serum of infected individuals [174,175]. Unfortunately, the majority of studies indicate that they are not a sufficient diagnostic tool or biomarkers of infection [170,176,177].

5.2.3. Other Tests

With advances in technology and science, novel diagnostic methods for *O. volvulus* are emerging that transcend traditional approaches based on immunoenzymatic and PCR techniques. One such approach is the detection of N-acetyltyramine-O,β-glucuronide (NATOG) in urine, which has emerged as a highly promising biomarker specific for *O. volvulus* [178]. NATOG is a neurotransmitter-derived secretory molecule from *O. volvulus* [179]. The potential of NATOG as a diagnostic marker was investigated by assessing its stability under conditions resembling those in tropical environments and its adsorption characteristics. A further study was conducted to evaluate the use of lateral flow circulation (LC) with fluorescence to detect NATOG in urine and to analyse its levels in samples from individuals with nodules present but with no or very low microfilariae. The results were then compared with control groups to assess the diagnostic performance of this biomarker. The evaluation of the use of NATOG in rural areas in sub-Saharan Africa demonstrated that it retained its stability even after 24 h of incubation at 50 °C and exposure to sunlight for 36 h. Despite maintaining relatively high stability, the sensitivity of NATOG was found to be relatively low in patients with very low microfilaria levels in the skin. These findings indicate that NATOG is most likely to be most effective as a biomarker in cases of very advanced *O. volvulus* infections, where the parasite titre in the body is high [178].

Table 2 presents a comparative analysis of diagnostic methods for *O. volvulus* infection, as previously outlined in the preceding sections.

Table 2. Immunoenzymatic, molecular, and other tests for the detection of *O. volvulus* infection.

Type of Method	Method	Description	Article
Immunoenzymatic	OV-16 ELISA	Detection of antibodies specific to the recombinant OV-16 *O. volvulus*	[129]
	Modified OV-16 ELISA	Enhanced OV-16 ELISA with improved accuracy	[134]
	ELISA	Utilises recombinant chimeric antigen OvMANE1	[135]
		Utilises recombinant human anti-O16 IgG4 as a control	[142]
	Bioline® ONCHOCERCIASIS IgG4 (OV-16 RDT)	Rapid diagnostic test for the detection of anti-O16 IgG4 *O. volvulus*	[144,145]
Molecular	PCR	Amplification of a repetitive sequence (O-150)	[146–148]
	O-150 qPCR		[149]
	O-5S qPCR	Amplification of intergenic spacer region of the 5S rRNA	[151]
	qPCR-MCA	Amplification of ITS1 region	[152]
	LAMP	Amplification of *COX1* gene fragment	[167]
	Circulating DNA, miRNA profile analysis	Insufficient diagnostic tool	[170,176,177]
Other	LC-MS/MS	Detection of N-acetyltyramine-O, β-glucuronide in urine	[178]

5.3. *Onchocerca lupi*

In 1967, a previously unidentified *Onchocerca* spp. was discovered in wolves (*Canis lupus*) as *O. lupi* [180]. Studies have demonstrated the distinctive morphology of this species, as well as the fact that its 5Sr DNA spacer sequences do not resemble those of other nematodes of the genus *Onchocerca* [181]. *O. lupi* is typically associated with the disease onchocerciasis, which primarily affects dogs [182,183] and cats [184]. However, there have been an increasing number of reports of human infection with this parasite in various geographical locations around the world [185–187]. Adult male *O. lupi* is white in colour and measures between 43 and 50 mm in length, with a diameter of approximately 0.15 mm [182]. In contrast, it is challenging to ascertain the length of the female, as it is difficult to remove it entirely from the nodules. However, the longest fragments were reported to be between 100 and 165 mm [180,188].

The clinical signs observed in humans are highly variable and may include the formation of nodules on the conjunctiva occurring together with inflammation of varying degrees in the ocular region [186,187]. Additionally, the development of soft masses within the mid-ciliary canal, is observed in this species, which is a unique characteristic [189,190].

5.3.1. Immunoenzymatic Tests

The diagnosis of *O. lupi* infection is based on the detection of microfilariae within skin biopsies and the identification of ocular nodules located on the eyelids, conjunctiva, or sclera.

In particular, skin biopsy is an invasive and time-intensive procedure, thus necessitating the exploration of novel diagnostic modalities. Latrofa et al. (2021) employed

an immunoproteomic approach, integrating immunoblotting and mass spectrometry, to identify novel antigens that could be utilised in diagnostic tests. This study indicates that Ol-MJA and Ol-PARA, two linear protein peptides, may be suitable as promising candidates as biomarkers for diagnostic test development. Furthermore, Ol-MJA and Ol-PARA have been recognised by IgG antibodies in the sera of dogs infected with *O. lupi* [191]. In 2024, an indirect ELISA was conducted to evaluate the specificity and sensitivity of parasite detection using the previously characterised linear peptides of the Ol-MJA and Ol-PAR proteins, in addition to three newly identified peptides from Ol-MJA. The proposed assay based on the linear peptides of the Ol-MJA and Ol-PAR proteins demonstrated high immunoreactivity in all assays, including sera from dogs with low levels of *O. lupi* microfilaremia. Furthermore, the indirect ELISA exhibited high accuracy, with 100% specificity and sensitivity, ranging from 85.45% to 94.55%, depending on the peptide tested. Although *O. lupi* predominantly causes onchocerciasis in dogs and cats, the number of human infections is increasing. Therefore, the development of a non-invasive, rapid, specific, and sensitive tool for serodiagnosis of *O. lupi* infection is greatly needed, both for monitoring epidemiology and helping sick people and animals. Further research should concentrate on assessing the efficacy of the test in detecting *O. lupi* infection in cats and humans and improving this diagnostic method [192].

5.3.2. Molecular Methods

Mitochondrial markers have gained significant recognition in phylogenetic and epidemiological studies due to their clonality, the rare phenomenon of recombination, and their faster evolution than nuclear genes. This makes their analysis possible, enabling the distinction of closely related individuals from different geographical locations and the reconstruction of their phylogeny [193,194]. In individuals of the genus *Onchocerca*, the mitochondrial genes for NADH dehydrogenase subunit 5 (*ND5*) and cytochrome c oxidase subunit 1 (*COX1*) are amplified and sequenced [186,195–197]. Nevertheless, this method requires a considerable investment of labour and may be less sensitive than desired, particularly in the identification of microfilariae. This limits the scope of extensive epidemiological investigations which involve both vertebrates and potential vectors.

In 2018, a paper by Latrofa et al. was published in which a quantitative hybridisation probe-based qPCR assay was developed to detect *O. lupi* DNA in canine and feline samples and putative vectors. The selected target gene was *COX1*, which is considered to be a specific 'barcode' of filarial nematodes [198,199]. This gene has been demonstrated to have a high amplification efficiency and a high copy number, allowing the detection of minimal amounts of DNA [200]. These facts, combined with a skilfully designed probe that was 100% specific for *O. lupi* DNA, allowed the development of a reliable method. The assay is characterised by high sensitivity, enabling the detection of small quantities of DNA, coupled with high efficacy. The analytical specificity and sensitivity of the developed method were evaluated by examining genomic DNA from skin samples with different parasite numbers, through serial dilutions of microfilariae DNA and adult *O. lupi* specimens. The qPCR test was also highly specific in detecting *O. lupi* DNA in both co-infected samples from dogs and potential vector species. This obviates the need to verify the test result with the sequencing required by cPCR methods [201].

Despite obtaining rather impressive results, the above method was further modified, this time using human samples. The assay was modified to include primers designed for the genus *Onchocerca*, as well as two species-specific (*O. lupi* and *O. volvulus*) TaqMan probes targeting the *COX1* locus. This allowed for simultaneous detection and differentiation of *O. lupi* and *O. volvulus*. The newly developed qPCR method demonstrated high efficiency, with satisfactory limit of detection and no cross-reactivity with human DNA or other parasites tested. The results were consistent with those obtained using 12S and 16S PCR, confirming a 95% (20/21) agreement with microscopic analysis. It is noteworthy that the novel qPCR method was unable to detect a single case of *O. lupi* that was positive under the microscope. However, the authors hypothesise that the problem may not have originated in the method

itself but rather in a too low concentration of total DNA, resulting from an initially very small clinical sample. The analytical sensitivity of this reaction was comparable to that reported by Latrofa et al. (2018) [202].

The qPCR method allowed for the specific identification of *O. lupi* and *O. volvulus* parasites, even in cases where sequencing data were incomplete or absent, or where identification by microscopy was limited to genus determination only. Nevertheless, it is important to acknowledge that the efficacy of PCR assays could be influenced by a multitude of variables, including suboptimal sample storage and fixation, which can result in inaccurate DNA amplification [203].

Table 3 presents a comparative analysis of diagnostic methods for *O. lupi* infection, as previously outlined in the preceding sections.

Table 3. Immunoenzymatic, molecular, and other tests for the detection of *O. lupi* infection.

Type of Method	Method	Description	Article
Immunoenzymatic	Immunoproteomic	Integrates immunoblotting and mass spectrometry to identify novel peptides for diagnostic tests	[191]
	ELISA	Utilises linear peptides of the Ol-MJA and Ol-PAR proteins	[192]
Molecular	qPCR	Amplification of *COX1* gene fragment	[199,202]

5.4. Wuchereria bancrofti

Among the three human parasites responsible for lymphatic filariasis, *W. bancrofti* is the most prevalent. This cosmopolitan parasite is found in tropical, subtropical, and temperate regions, including South Asia, East Asia, Africa, the Western Pacific, and, to a lesser extent, certain locations in the Americas [204].

The life cycle of *W. bancrofti* is similar to other lymphatic filariasis-causing parasites. During a blood meal, an infected female mosquito introduces third-stage larvae of filariasis onto the skin of a human host, where they penetrate the bite wound. These larvae mature into adults, primarily inhabiting the lymphatic system. Females measure 80 to 100 mm in length, while males are smaller, measuring approximately 40 mm. Adult parasites produce microfilariae that are sheltered and exhibit higher nocturnal activity. These microfilariae migrate through the lymphatic and blood channels, actively traversing through these bodily fluids. The cycle then closes when the mosquito takes up microfilariae from the blood during a meal [77].

The invasion caused by *W. bancrofti*, although rarely leading to death, is considered a major cause of disability, permanent disability, and chronic morbidity [205].

5.4.1. Immunoenzymatic Tests

BinaxNOW® (Alere, Scarborough, ME, USA) is a commercial lateral flow assay (LFA) that was developed in the early 2000s. However, it has been observed that the test has low sensitivity and stability, a short shelf life (3 months), variable performance which significantly affects the results obtained, requires cold storage until use, and has a relatively high cost. Another issue with the test is the narrow time window for reading the test results. The manufacturer's instructions recommend reading the test after 10 min, and delaying this reading (e.g., after 20 min) often leads to false-positive results. Furthermore, cross-reactivity of the test with phyla other than *W. bancrofti* has been reported [206–211].

Another commercially available test that has replaced BinaxNOW® is the Alere Filariasis Test Strip (Alere, Scarborough, ME, USA). It was launched in 2013 and is recommended by the WHO for the diagnosis of filariasis caused by *W. bancrofti* in endemic areas [212]. Additionally, the STANDARD Q Filariasis Antigen test (SD Biosensor, Suwon, South Korea) is also commercially available [213]. All point-of-care (POC) tests discussed are based on

the technology of detecting circulating antigens (CFA) of *W. bancrofti* in the patient's blood. Antigen tests are more sensitive for filariasis infection than tests detecting microfilariae in blood collected at night (nocturnal periodicity of microfilariae in blood) [214]. The current antigen tests are less expensive, have a longer shelf life and higher analytical sensitivity compared to the previously used BinaxNOW test® [215,216].

BLF Rapid™ (Universiti Sains Malaysia, Gelugor, Malaysia) is a prototype immunochromatographic assay based on the detection of antiparasitic IgG4 antibodies against the recombinant BmSXP protein in sera from patients with filariasis caused by *W. bancrofti*. Independent evaluations have demonstrated a diagnostic sensitivity of 84% and specificity of 100%, indicating the test's potential as a diagnostic tool [217].

A recombinant monoclonal antibody (5B) specific to the BmSXP [218] antigen was utilised in the development of an ELISA. This enabled the detection of circulating parasite antigens in the serum of patients infected with *W. bancrofti*. The diagnostic specificity and specificity of the test were demonstrated to be 100% when tested with sera from healthy individuals and patients with other parasitic diseases. Consequently, the test has the potential to be a promising alternative test for the detection of the *W. bancrofti* antigen [219].

The recombinant protein Wb-Bhp-1, which is a homologue of the BmR1 protein used in tests to detect *B. malayi*, was evaluated for the diagnosis of *W. bancrofti* infection. The ELISA demonstrated variable sensitivity for the detection of microfilariae, which differed between sera from patients from different countries. Furthermore, low cross-reactivity was observed with samples from individuals with onchocerciasis or loiasis, which is necessary for the test to be useful for lymphatic filariasis serology in many areas of sub-Saharan Africa [220].

The recombinant protein HSP70 (BmHSP70) of *B. malayi* was characterised for its ability to diagnose the asymptomatic stage of *W. bancrofti* microfilariae infection. Based on ELISA, it was concluded that the antigen tested, in terms of sensitivity and specificity, could be used to diagnose the early stage of microfilariae infection [221].

The immunogenicity of recombinant *B. malayi* (Bm-TPP and BmAF-Myo) and *Wolbachia* (Wol Tl IF-1, wBm-LigA) proteins were analysed by immunoblotting techniques and the measurement of specific IgG antibodies by ELISA. The study included patient serum samples from the three main endemic zones of filariasis caused by *W. bancrofti*: endemic normal (EN), microfilariae carriers (MF), and chronic filarial patients (CP). The results of the analysis demonstrated that individuals from the endemic area (EN) of *W. bancrofti* had specific IgG antibodies directed against all four *B. malayi* and *Wolbachia* proteins tested. The strong reactivity of EN sera to all tested proteins suggests their potential immunoprotective nature, thereby indicating the potential use of recombinant *B. malayi* proteins as diagnostic markers or vaccine candidates against *W. bancrofti* [222].

5.4.2. Molecular Methods

Amplification-based molecular methods, such as PCR, have been demonstrated to be highly sensitive and specific for the detection of *W. bancrofti* microfilariae [223–225]. However, they are not routinely used due to the high cost and complexity of the procedures, which require trained personnel and sophisticated equipment that is difficult to access in endemic areas [226].

A potential solution is the utilisation of compact PCR-based methodologies and devices that are capable of detecting parasites at the site of patient sampling [227,228]. The method utilises a miniPCR® thermocycler (DBA miniPCR bio; Amplyus LLC., Cambridge, MA, USA), which is an inexpensive and portable device. However, this method requires electrophoretic analysis, which is not possible to perform under field conditions. In order to circumvent these steps, a combination of miniPCR and duplex lateral flow dipstick (DLFD) was selected for the rapid detection of *W. bancrofti* (and *B. malayi*) in human blood. In the laboratory evaluation, the miniPCR-DLFD and the stained blood smear, considered the gold standard for detecting MF, showed concordant results, confirming the efficacy of the miniPCR-DLFD test in the diagnosis of lymphatic filariasis. The detection limit of the

miniPCR-DLFD was 4 mg of *W. bancrofti* per ml of blood. Furthermore, no cross-reactivity was observed when tested with the other 11 parasites. The only identified limitation observed was the *HhaI* amplicons of *B. timori*, which demonstrated 96.77% identity with *B. malayi*. However, this does not impede the identification of *W. bancrofti*, and furthermore, the endemic region of *B. timori* is confined to East Timor and a few islands in eastern Indonesia only [229].

As previously stated in the context of *O. volvulus* detection, the LAMP assay also demonstrates significant advantages over cPCR. The reactions can be carried out using a straightforward apparatus, and the visualisation of amplicons is achieved through the use of pH-sensitive dyes or by observing the turbidity of the reaction mixture [162,230]. An additional advantage is that the lyophilised reagents do not require refrigeration, which ensures that they remain stable even at high temperatures. The LAMP technique is also used to identify *W. bancrofti* infestations. The LAMP assay, developed by Kinyatta (2021), demonstrates that this method can be both highly specific (97.3%) and sensitive (the lowest concentration detected was 10^{-6}, corresponding to 1 microfilariae per 200 µL). Moreover, the LAMP reaction has the advantage of greater specificity compared to cPCR methods due to the use of four to six primers (FIP, BIP, F3, B3, and/or 2 loop primers) to recognise six to eight different regions of interest in a sequence [231].

Table 4 presents a comparative analysis of diagnostic methods for *W. bancrofti* infection, as previously outlined in the preceding sections.

Table 4. Immunoenzymatic, molecular, and other tests for the detection of *W. bancrofti* infection.

Type of Method	Method	Description	Article
Immunoenzymatic	BinaxNOW®	Rapid diagnostic tests	[207]
	Alere Filariasis Test Strip		[212]
	STANDARD Q Filariasis Antigen Test		[213]
	BLF Rapid™		[217]
	ELISA	Utilises recombinant monoclonal antibody (5B) specific to the BmSXP	[218,219]
		Utilises recombinant Wb-Bhp-1 antigen	[220]
		Utilises recombinant BmHSP70 antigen	[221]
		Utilises recombinant Bm-TPPBmAF-Myo, Wol Tl IF-1, wBm-LigA antigens	[222]
Molecular	miniPCR-DLFD	Amplification of 181 bp region of *Ssp*I repetitive noncoding DNA sequence	[229]
	LAMP	Amplification of 18S rRNA fragment	[231]

6. Conclusions

Filariasis, caused by nematodes, remains a formidable challenge to global public health. Despite concerted efforts and international initiatives aimed at eradicating the disease, definitively halting its proliferation has remained elusive. A significant factor contributing to this persistence is the limited understanding of the intricate mechanisms by which parasites evade detection by the host immune system. Over the last several decades, molecular biology methods have developed rapidly, opening up new possibilities in the study of filariasis and other parasitic diseases. The introduction of modern DNA sequencing

techniques, such as next-generation sequencing (NGS), has enabled in-depth analyses of parasite genomes and their interactions with the host. Furthermore, PCR techniques allow rapid and sensitive detection of parasite genetic material even in small biological samples, which contributes to rapid disease diagnosis and surveillance. Molecular diagnostics plays a pivotal role in comprehending the intricate adaptive mechanisms of parasites and in developing more efficient methods for detecting and treating filariasis. Application of molecular techniques enables the identification of genetic markers of parasites and the analysis of their interactions with the host immune system. Moreover, molecular biology techniques not only facilitate the delineation of parasite genes but also enable the production of recombinant proteins with potential utility as diagnostic biomarkers or therapeutic targets. ELISA tests based on recombinant proteins enable more accurate and specific detection of antibodies against parasites, which can be crucial for rapid diagnosis and disease monitoring. By combining these advanced technologies, researchers aspire to devise comprehensive strategies for combating filariasis that are both efficacious and precise. Ongoing research endeavours aim to refine diagnostic tools and pioneer innovative therapies, thereby fortifying global efforts to safeguard the health of populations worldwide against this formidable parasitic threat.

Author Contributions: Conceptualization, D.P. and M.W.; writing—original draft preparation, D.P. and J.W.Ł.; writing—review and editing, M.W.; visualization, J.W.Ł.; supervision, M.W. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Andrássy, I. *Klasse Nematoda: (Ordnungen Monhysterida, Desmoscolecida, Araeolaimida, Chromadorida, Rhabditida)*; De Gruyter: Berlin, Germany, 1984; ISBN 3112484584.
2. Sommer, R.J. *Nematoda. Evolutionary Developmental Biology of Invertebrates 3*; Springer: Vienna, Austria, 2015; pp. 15–33.
3. Dobson, A.; Lafferty, K.D.; Kuris, A.M.; Hechinger, R.F.; Jetz, W. Homage to Linnaeus: How Many Parasites? How Many Hosts? *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 11482–11489. [CrossRef]
4. Blaxter, M.; Koutsovoulos, G. The Evolution of Parasitism in Nematoda. *Parasitology* **2015**, *142*, S26–S39. [CrossRef]
5. Schafer, W. Nematode Nervous Systems. *Curr. Biol.* **2016**, *26*, R955–R959. [CrossRef]
6. Khare, P. Incidental Diagnosis of Filariasis in Superficial Location by FNAC: A Retrospective Study of 10 Years. *J. Clin. Diagn. Res.* **2014**, *8*, FC05. [CrossRef]
7. Kaminsky, R.; Geary, T.G. Breaking the Silos—Obstacles and Opportunities for One Health in Filariases. In *Human and Animal Filariases*; Wiley: Hoboken, NY, USA, 2022; pp. 1–15.
8. Hanchinalmath, J.V.; D'mello, D.; Shivasharanappa, K.; Pramod, T.; Patil, S.J. Filaricidal Activity of Phytochemicals Against Lymphatic Filariasis. In *Neglected Tropical Diseases and Phytochemicals in Drug Discovery*; Wiley: Hoboken, NY, USA, 2021; pp. 245–269.
9. Addario, C. Su Di Un Nematode Dell'occhio Umano. *Ann. Ottalmol.* **1885**, *13*, 135–147.
10. Perles, L.; Dantas-Torres, F.; Krücken, J.; Morchón, R.; Walochnik, J.; Otranto, D. Zoonotic Dirofilariases: One, No One, or More than One Parasite. *Trends Parasitol.* **2024**, *40*, 257–270. [CrossRef]
11. Simón, F.; Siles-Lucas, M.; Morchón, R.; González-Miguel, J.; Mellado, I.; Carretón, E.; Montoya-Alonso, J.A. Human and Animal Dirofilariasis: The Emergence of a Zoonotic Mosaic. *Clin. Microbiol. Rev.* **2012**, *25*, 507–544. [CrossRef]
12. Orihel, T.C.; Beaver, P.C. Zoonotic Brugia Infections in North and South America. *Am. J. Trop. Med. Hyg.* **1989**, *40*, 638–647. [CrossRef]
13. Eberhard, M.L.; DeMeester, L.J.; Martin, B.W.; Lammie, P.J. Zoonotic Brugia Infection in Western Michigan. *Am. J. Surg. Pathol.* **1993**, *17*, 1058–1061. [CrossRef]
14. Lefoulon, E.; Giannelli, A.; Makepeace, B.L.; Mutafchiev, Y.; Townson, S.; Uni, S.; Verocai, G.G.; Otranto, D.; Martin, C. Whence River Blindness? The Domestication of Mammals and Host-Parasite Co-Evolution in the Nematode Genus Onchocerca. *Int. J. Parasitol.* **2017**, *47*, 457–470. [CrossRef]

15. Hotterbeekx, A.; Namale Ssonko, V.; Oyet, W.; Lakwo, T.; Idro, R. Neurological Manifestations in Onchocerca Volvulus Infection: A Review. *Brain Res. Bull* **2019**, *145*, 39–44. [CrossRef]
16. Huynh, T. Dipetalonema Reconditum in the Human Eye. *Br. J. Ophthalmol.* **2001**, *85*, 1384. [CrossRef]
17. John, M.; Mathew, S.; Sebastian, V.; Biswas, J.; Raman, M. Multiple Live Subconjunctival Dipetalonema: Report of a Case. *Indian J. Ophthalmol.* **2012**, *60*, 228. [CrossRef]
18. Antinori, S.; Schifanella, L.; Million, M.; Galimberti, L.; Ferraris, L.; Mandia, L.; Trabucchi, G.; Cacioppo, V.; Monaco, G.; Tosoni, A.; et al. Imported Loa Loa Filariasis: Three Cases and a Review of Cases Reported in Non-Endemic Countries in the Past 25 Years. *Int. J. Infect. Dis.* **2012**, *16*, e649–e662. [CrossRef]
19. Dieki, R.; Nsi-Emvo, E.; Akue, J.P. The Human Filaria Loa Loa: Update on Diagnostics and Immune Response. *Res. Rep. Trop. Med.* **2022**, *13*, 41–54. [CrossRef]
20. Kabego, L.; Kasengi, J.B.; Mirindi, P.; Ruhanya, V.; Lupande, D.; Bulabula, A.; Ngoma, P. Pulmonary Localization of Mansonella Perstans in a 16 Months-Old Male Patient in a Tertiary Care Hospital in Bukavu, Democratic Republic of Congo. *Germs* **2016**, *6*, 151–154. [CrossRef]
21. Ferreira, M.U.; Crainey, J.L.; Luz, S.L.B. Mansonella Ozzardi. *Trends Parasitol.* **2021**, *37*, 90–91. [CrossRef]
22. Hübner, M.P.; Layland, L.E.; Hoerauf, A. Lymphatic and Tissue Filariasis. In *Helminth Infections and Their Impact on Global Public Health*; Springer: Vienna, Austria, 2014; pp. 367–409.
23. Mackenzie, C.D. Human Filarial Infections: Reflections on the Current Understanding of Their Importance, Pathobiology, and Management. In *Human and Animal Filariases*; Wiley: Hoboken, NY, USA, 2022; pp. 33–73.
24. Orihel, T.C.; Eberhard, M.L. Zoonotic Filariasis. *Clin. Microbiol. Rev.* **1998**, *11*, 366–381. [CrossRef]
25. Anderson, R.C. *Nematode Parasites of Vertebrates: Their Development and Transmission*; Cabi: Wallingford, UK, 2000; ISBN 0851997864.
26. Paily, K.P.; Hoti, S.L.; Das, P.K. A Review of the Complexity of Biology of Lymphatic Filarial Parasites. *J. Parasites Dis.* **2009**, *33*, 3–12. [CrossRef]
27. Maizels, R.M.; Kurniawan, A.; Selkirk, M.E.; Yazdanbakhsh, M. Immune Responses to Filarial Parasites. *Immunol. Lett.* **1991**, *30*, 249–254. [CrossRef]
28. Korzeniewski, K. Choroby Skóry w Gorącej Strefie Klimatycznej. *Forum Med. Rodz.* **2013**, *7*, 185–197.
29. Simón, F.; González-Miguel, J.; Diosdado, A.; Gómez, P.J.; Morchón, R.; Kartashev, V. The Complexity of Zoonotic Filariasis Episystem and Its Consequences: A Multidisciplinary View. *BioMed Res. Int.* **2017**, *2017*, 6436130. [CrossRef]
30. Nanduri, J.; Kazura, J.W. Clinical and Laboratory Aspects of Filariasis. *Clin. Microbiol. Rev.* **1989**, *2*, 39–50. [CrossRef]
31. Shaukat, A.; Aleem, M.T.; Kanwal, A.; Kalim, A.; Kalim, F.; Memoon, A.; Siddique, M.H.; Samad, M.A.; Shaukat, R.; Shaukat, I. Recent Advances in Diagnosis of Filariasis. *Int. J. Agric. Biosci.* **2023**, *2*, 45–57. [CrossRef]
32. Ajendra, J.; Hoerauf, A.P.; Hübner, M. Biology of the Human Filariases. In *Parasitic Helminths and Zoonoses—From Basic to Applied Research*; IntechOpen: London, UK, 2022.
33. Dreyer, G.; Norões, J.; Figueredo-Silva, J.; Piessens, W.F. Pathogenesis of Lymphatic Disease in Bancroftian Filariasis: A Clinical Perspective. *Parasitol. Today* **2000**, *16*, 544–548. [CrossRef]
34. Hoerauf, A.; Satoguina, J.; Saeftel, M.; Specht, S. Immunomodulation by Filarial Nematodes. *Parasite Immunol.* **2005**, *27*, 417–429. [CrossRef]
35. Babu, S.; Blauvelt, C.P.; Kumaraswami, V.; Nutman, T.B. Cutting Edge: Diminished T Cell TLR Expression and Function Modulates the Immune Response in Human Filarial Infection. *J. Immunol.* **2006**, *176*, 3885–3889. [CrossRef]
36. Babu, S.; Blauvelt, C.P.; Kumaraswami, V.; Nutman, T.B. Regulatory Networks Induced by Live Parasites Impair Both Th1 and Th2 Pathways in Patent Lymphatic Filariasis: Implications for Parasite Persistence. *J. Immunol.* **2006**, *176*, 3248–3256. [CrossRef]
37. Babu, S.; Kumaraswami, V.; Nutman, T.B. Alternatively Activated and Immunoregulatory Monocytes in Human Filarial Infections. *J. Infect. Dis.* **2009**, *199*, 1827–1837. [CrossRef]
38. Pfarr, K.M.; Debrah, A.Y.; Specht, S.; Hoerauf, A. Filariasis and Lymphoedema. *Parasite Immunol.* **2009**, *31*, 664–672. [CrossRef]
39. Cuenco, K.T.; Halloran, M.E.; Lammie, P.J. Assessment of Families for Excess Risk of Lymphedema of the Leg in a Lymphatic Filariasis-Endemic Area. *Am. J. Trop. Med. Hyg.* **2004**, *70*, 185–190. [CrossRef]
40. Cuenco, K.T.; Halloran, M.E.; Louis-Charles, J.; Lammie, P.J. A Family Study of Lymphedema of the Leg in a Lymphatic Filariasis-Endemic Area. *Am. J. Trop. Med. Hyg.* **2004**, *70*, 180–184. [CrossRef]
41. Cuenco, K.T.; Ottesen, E.A.; Williams, S.A.; Nutman, T.B.; Steel, C. Heritable Factors Play a Major Role in Determining Host Responses to Wuchereria Bancrofti Infection in an Isolated South Pacific Island Population. *J. Infect. Dis.* **2009**, *200*, 1271–1278. [CrossRef]
42. World Health Organization. Global Programme to Eliminate Lymphatic Filariasis: Progress Report, 2021. *Wkly. Epidemiol. Rec.* **2022**, *97*, 513–524.
43. World Health Organization. Meeting of the International Task Force for Disease Eradication—29 October 2008. *Wkly. Epidemiol. Rec.* **2009**, *84*, 89–94.
44. Cromwell, E.A.; Schmidt, C.A.; Kwong, K.T.; Pigott, D.M.; Mupfasoni, D.; Biswas, G.; Shirude, S.; Hill, E.; Donkers, K.M.; Abdoli, A.; et al. The Global Distribution of Lymphatic Filariasis, 2000–2018: A Geospatial Analysis. *Lancet Glob. Health* **2020**, *8*, e1186–e1194. [CrossRef]
45. Mendoza, N.; Li, A.; Gill, A.; Tyring, S. Filariasis: Diagnosis and Treatment. *Dermatol. Ther.* **2009**, *22*, 475–490. [CrossRef]

46. Kapoor, U.; Sharma, V.; Chittoria, R.S. Onchocercoma in a United Nations Peacekeeper. *Med. J. Armed Forces India* **2015**, *71*, S104–S106. [CrossRef]
47. Nicholls, R.S.; Duque, S.; Olaya, L.A.; López, M.C.; Sánchez, S.B.; Morales, A.L.; Palma, G.I. Elimination of Onchocerciasis from Colombia: First Proof of Concept of River Blindness Elimination in the World. *Parasites Vectors* **2018**, *11*, 237. [CrossRef]
48. Ramharter, M.; Butler, J.; Mombo-Ngoma, G.; Nordmann, T.; Davi, S.D.; Zoleko Manego, R. The African Eye Worm: Current Understanding of the Epidemiology, Clinical Disease, and Treatment of Loiasis. *Lancet Infect. Dis.* **2024**, *24*, e165–e178. [CrossRef]
49. Ta-Tang, T.-H.; Crainey, J.; Post, R.J.; Luz, S.L.B.; Rubio, J. Mansonellosis: Current Perspectives. *Res. Rep. Trop. Med.* **2018**, *9*, 9–24. [CrossRef] [PubMed]
50. Tyring, S.; Lupi, O.; Hengge, U. (Eds.) *Tropical Dermatology*; Elsevier: Amsterdam, The Netherlands, 2006; ISBN 9780443067907.
51. Veletzky, L.; Hergeth, J.; Stelzl, D.R.; Mischlinger, J.; Manego, R.Z.; Mombo-Ngoma, G.; McCall, M.B.B.; Adegnika, A.A.; Agnandji, S.T.; Metzger, W.G.; et al. Burden of Disease in Gabon Caused by Loiasis: A Cross-Sectional Survey. *Lancet Infect. Dis.* **2020**, *20*, 1339–1346. [CrossRef] [PubMed]
52. Fischer, P.; Bamuhiiga, J.; Büttner, D.W. Occurrence and Diagnosis of Mansonella Streptocerca in Uganda. *Acta Trop.* **1997**, *63*, 43–55. [CrossRef] [PubMed]
53. Garcia, L.S. Diagnostic Medical Parasitology. In *Manual of Commercial Methods in Clinical Microbiology*; Wiley: Hoboken, NY, USA, 2001; pp. 274–305.
54. Garcia, L.S.; Procop, G.W. Diagnostic Medical Parasitology. In *Manual of Commercial Methods in Clinical Microbiology*; Wiley: Hoboken, NY, USA, 2016; pp. 284–308.
55. Mathison, B.A.; Couturier, M.R.; Pritt, B.S. Diagnostic Identification and Differentiation of Microfilariae. *J. Clin. Microbiol.* **2019**, *57*, 1–13. [CrossRef] [PubMed]
56. Orihel, T.C.; Ash, L.R.; Ramachandran, C.P.; Ottesen, E.A. *Bench Aids for the Diagnosis of Filarial Infections*; World Health Organization: Geneva, Switzerland, 1997; ISBN 9241544899.
57. Hawking, F. The 24-Hour Periodicity of Microfilariae: Biological Mechanisms Responsible for Its Production and Control. *Proc. R Soc. Lond. B Biol. Sci.* **1967**, *169*, 59–76. [CrossRef]
58. Ash, L.R.; Orihel, T.C. *Atlas of Human Parasitology*; American Society of Clinical Pathologists: Chicago, IL, USA, 1980; ISBN 0891890815.
59. Korzeniewski, K.; Pokorna-Kałwak, D. Diagnostyka Wybranych Chorób Pasożytniczych u Bezobjawowych Pacjentów Powracających z Tropiku. *Lekarz POZ* **2020**, *6*, 226–231.
60. Knott, J. A Method for Making Microfilarial Surveys on Day Blood. *Trans. R Soc. Trop. Med. Hyg.* **1939**, *33*, 191–196. [CrossRef]
61. National Toxicology Program. Final Report on Carcinogens Background Document for Formaldehyde. *Rep. Carcinog. Backgr. Doc.* **2010**, *10*, i-512.
62. Bernardini, L.; Barbosa, E.; Charão, M.F.; Brucker, N. Formaldehyde Toxicity Reports from in Vitro and in Vivo Studies: A Review and Updated Data. *Drug. Chem. Toxicol.* **2022**, *45*, 972–984. [CrossRef]
63. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Chemical Agents and Related Occupations. *IARC Monogr. Eval. Carcinog. Risks Hum.* **2012**, *100*, 9.
64. Genchi, M.; Ciuca, L.; Vismarra, A.; Ciccone, E.; Cringoli, G.; Kramer, L.; Rinaldi, L. Evaluation of Alternative Reagents on the Performance of the Modified Knott's Test. *Vet. Parasitol.* **2021**, *298*, 109555. [CrossRef] [PubMed]
65. Evans, C.C.; Bradner, J.L.; Savadelis, M.D.; Nelson, C.T.; Moorhead, A.R. Acetic Acid as an Alternative Reagent in the Modified Knott Test. *Vet. Parasitol.* **2019**, *276*, 108975. [CrossRef] [PubMed]
66. McMahon, J.E.; Marshall, T.D.C.; Vaughan, J.P.; Abaru, D.E. Bancroftian Filariasis: A Comparison of Microfilariae Counting Techniques Using Counting Chamber, Standard Slide and Membrane (Nuclepore) Filtration. *Ann. Trop. Med. Parasitol.* **1979**, *73*, 457–464. [CrossRef] [PubMed]
67. Chularerk, P.; Desowitz, R.S. A Simplified Membrane Filtration Technique for the Diagnosis of Microfilaremia. *J. Parasitol.* **1970**, *56*, 623. [CrossRef] [PubMed]
68. Centers for Disease Control and Prevention—Global Health, D. of P.D. and M. Parasites—Onchocerciasis (Also Known as River Blindness)-Resources for Health Professionals. Available online: https://www.cdc.gov/parasites/onchocerciasis/health_professionals/index.html (accessed on 25 March 2024).
69. Newell, E.D. Comparison of the Use of Skin Scarification and Skin Biopsies to Determine the Prevalence and Intensity of Onchocerca Volvulus Infection. *Ann. Trop. Med. Parasitol.* **1997**, *91*, 633–642. [CrossRef] [PubMed]
70. Vincent, J.A.; Lustigman, S.; Zhang, S.; Weil, G.J. A Comparison of Newer Tests for the Diagnosis of Onchocerciasis. *Ann. Trop. Med. Parasitol.* **2000**, *94*, 253–258. [CrossRef] [PubMed]
71. Boatin, B.A.; Toe, L.; Alley, E.S.; Nagelkerke, N.J.D.; Borsboom, G.; Habbema, J.D.F. Detection of Onchocerca Volvulus Infection in Low Prevalence Areas: A Comparison of Three Diagnostic Methods. *Parasitology* **2002**, *125*, 545–552. [CrossRef] [PubMed]
72. World Health Organization. Lymphatic Filariasis. Available online: https://www.who.int/news-room/fact-sheets/detail/lymphatic-filariasis (accessed on 7 April 2024).
73. Mulyaningsih, B.; Umniyati, S.R.; Hadisusanto, S.; Edyansyah, E. Study on Vector Mosquito of Zoonotic Brugia Malayi in Musi Rawas, South Sumatera, Indonesia. *Vet. World* **2019**, *12*, 1729–1734. [CrossRef] [PubMed]
74. Rosenblatt, P.; Beaver, P.C.; Orihel, T.C. A Filarial Infection Apparently Acquired in New York City. *Am. J. Trop. Med. Hyg.* **1962**, *11*, 641–645. [CrossRef] [PubMed]

75. Guptavanij, P.; Harinasuta, C.; Vutikes, S.; Deesin, T.; Surathin, K. The Vectors of Brugia Malayi in Southern Thailand. *Southeast Asian J. Trop. Med. Public Health* **1978**, *9*, 543–548.
76. Klion, A. Lymphatic Filariasis: Epidemiology, Clinical Manifestations, and Diagnosis. Available online: https://medilib.ir/uptodate/show/5675 (accessed on 7 April 2024).
77. Centers for Disease Control and Prevention-Global Health, D. of P.D. and M. DPDx-Laboratory Identification of Parasites of Public Health Concern-Lymphatic Filariasis. Available online: https://www.cdc.gov/dpdx/lymphaticfilariasis/ (accessed on 3 April 2024).
78. Webb, J.K.G.; Job, C.K.; Gault, E.W. Tropical Eosinophilia Demonstration of Microfilarae in Lung, Liver, and Lymph-Node. *Lancet* **1960**, *275*, 835–842. [CrossRef]
79. Neva, F.A.; Ottesen, E.A. Tropical (Filarial) Eosinophilia. *N. Engl. J. Med.* **1978**, *298*, 1129–1131. [CrossRef]
80. Ong, R.K.C.; Doyle, R.L. Tropical Pulmonary Eosinophilia. *Chest* **1998**, *113*, 1673–1679. [CrossRef]
81. Rahmah, N.; Taniawati, S.; Shenoy, R.K.; Lim, B.H.; Kumaraswami, V.; Anuar, A.K.; Hakim, S.L.; Hayati, M.I.N.; Chan, B.T.; Suharni, M.; et al. Specificity and Sensitivity of a Rapid Dipstick Test (Brugia Rapid) in the Detection of Brugia Malayi Infection. *Trans. R Soc. Trop. Med. Hyg.* **2001**, *95*, 601–604. [CrossRef]
82. Rahmah, N.; Shenoy, R.K.; Nutman, T.B.; Weiss, N.; Gilmour, K.; Maizels, R.M.; Yazdanbakhsh, M.; Sartono, E. Multicentre Laboratory Evaluation of Brugia Rapid Dipstick Test for Detection of Brugian Filariasis. *Trop. Med. Int. Health* **2003**, *8*, 895–900. [CrossRef]
83. Rahmah, N.; Lim, B.H.; Anuar, A.K.; Shenoy, R.K.; Kumaraswami, V.; Hakim, S.L.; Chotechuang, P.; Kanjanopas, K.; Ramachandran, C.P. A Recombinant Antigen-Based IgG4 ELISA for the Specific and Sensitive Detection of Brugia Malayi Infection. *Trans. R Soc. Trop. Med. Hyg.* **2001**, *95*, 280–284. [CrossRef]
84. Jamail, M.; Andrew, K.; Junaidi, D.; Krishnan, A.K.; Faizal, M.; Rahmah, N. Field Validation of Sensitivity and Specificity of Rapid Test for Detection of Brugia Malayi Infection. *Trop. Med. Int. Health* **2005**, *10*, 99–104. [CrossRef]
85. Lammie, P.J.; Weil, G.; Noordin, R.; Kaliraj, P.; Steel, C.; Goodman, D.; Lakshmikanthan, V.B.; Ottesen, E. Recombinant Antigen-Based Antibody Assays for the Diagnosis and Surveillance of Lymphatic Filariasis—A Multicenter Trial. *Filaria J.* **2004**, *3*, 9. [CrossRef]
86. Fischer, P.; Bonow, I.; Supali, T.; Rückert, P.; Rahmah, N. Detection of Filaria-Specific IgG4 Antibodies and Filarial DNA, for the Screening of Blood Spots for Brugia Timori. *Ann. Trop. Med. Parasitol.* **2005**, *99*, 53–60. [CrossRef]
87. Rahmah, N.; Lim, B.H.; Mehdi, R.; Nazmi, M.N. Treatment Follow-up of Brugia Malayi Microfilaraemic and Amicrofilaraemic Individ-Uals with Serological Evidence of Active Infection. *Malays. J. Microbiol.* **2005**, *1*, 42–47.
88. Melrose, W.; Rahmah, N. Use of Brugia Rapid Dipstick and ICT Test to Map Distribution of Lymphatic Filariasis in the Democratic Republic of Timor-Leste. *Southeast Asian J. Trop. Med. Public Health* **2006**, *37*, 22.
89. World Health Organization. *Monitoring and Epidemiological Assessment of Mass Drug Administration in the Global Programme to Eliminate Lymphatic Filariasis: A Manual for National Elimination Programmes*; World Health Organization: Geneva, Switzerland, 2011; ISBN 9789241501484.
90. Abdo, A.I.K.; Ngoh, Y.Y.; Lew, M.H.; Dass, S.A.; Rahumatullah, A.; Noordin, R.; Tye, G.J. Generation of Human ScFv–IgG1Fc Antibodies for Detection of Lymphatic Filarial Recombinant Antigens, BmR1 and BmSXP. *Biotechnol. Appl. Biochem.* **2022**, *69*, 70–76. [CrossRef]
91. Reamtong, O.; Rujimongkon, K.; Sookrung, N.; Saeung, A.; Thiangtrongjit, T.; Sakolvaree, Y.; Thammapalo, S.; Loymek, S.; Chaicumpa, W. Immunome and Immune Complex-Forming Components of Brugia Malayi Identified by Microfilaremic Human Sera. *Exp. Parasitol.* **2019**, *200*, 92–98. [CrossRef]
92. Ravishankaran, R.; Shridharan, R.N.; Vishal, L.A.; Meenakshisundaram, S.; Karande, A.A.; Kaliraj, P. Evaluation of Immuno Diagnostic Assay for the Exposure of Stage Specific Filarial Infection. *Acta Parasitol.* **2016**, *61*. [CrossRef]
93. Paul, R.; Saha, G.; Selvaraja, V.; Kaliraj, P. Cloning, Expression and Characterization of Brugia Malayi Abundant Larval Protein Transcript-2 (BmALT-2) Expressed in Pichia Pastoris. *Biotechnol. Biotechnol. Equip.* **2017**, *31*, 403–410. [CrossRef]
94. Khor, B.Y.; Lim, T.S.; Noordin, R.; Choong, Y.S. The Design of Target Specific Antibodies (ScFv) by Applying de Novo Workflow: Case Study on BmR1 Antigen from Brugia Malayi. *J. Mol. Graph. Model.* **2017**, *76*, 543–550. [CrossRef]
95. Thanchomnang, T.; Intapan, P.M.; Lulitanond, V.; Choochote, W.; Manjai, A.; Prasongdee, T.K.; Maleewong, W. Rapid Detection of Brugia Malayi in Mosquito Vectors Using a Real-Time Fluorescence Resonance Energy Transfer PCR and Melting Curve Analysis. *Am. J. Trop. Med. Hyg.* **2008**, *78*, 509–513. [CrossRef]
96. Intapan, P.M.; Thanchomnang, T.; Lulitanond, V.; Maleewong, W. Rapid Detection of Wuchereria Bancrofti and Brugia Malayi in Mosquito Vectors (Diptera: Culicidae) Using a Real-Time Fluorescence Resonance Energy Transfer Multiplex PCR and Melting Curve Analysis. *J. Med. Entomol.* **2009**, *46*, 158–164. [CrossRef]
97. Pilotte, N.; Torres, M.; Tomaino, F.R.; Laney, S.J.; Williams, S.A. A TaqMan-Based Multiplex Real-Time PCR Assay for the Simultaneous Detection of Wuchereria Bancrofti and Brugia Malayi. *Mol. Biochem. Parasitol.* **2013**, *189*, 33–37. [CrossRef] [PubMed]
98. Poole, C.B.; Tanner, N.A.; Zhang, Y.; Evans, T.C.; Carlow, C.K.S. Diagnosis of Brugian Filariasis by Loop-Mediated Isothermal Amplification. *PLoS Negl. Trop. Dis.* **2012**, *6*, e1948. [CrossRef] [PubMed]

99. Sanpool, O.; Tantrawatpan, C.; Thanchomnang, T.; Janwan, P.; Intapan, P.M.; Rodpai, R.; Lulitanond, V.; Taweethavonsawat, P.; Maleewong, W. Pyrosequencing Using SL and 5S RRNA as Molecular Markers for Identifying Zoonotic Filarial Nematodes in Blood Samples and Mosquitoes. *Vector-Borne Zoonotic Dis.* **2016**, *16*, 326–333. [CrossRef] [PubMed]
100. Hindson, B.J.; Ness, K.D.; Masquelier, D.A.; Belgrader, P.; Heredia, N.J.; Makarewicz, A.J.; Bright, I.J.; Lucero, M.Y.; Hiddessen, A.L.; Legler, T.C.; et al. High-Throughput Droplet Digital PCR System for Absolute Quantitation of DNA Copy Number. *Anal. Chem.* **2011**, *83*, 8604–8610. [CrossRef] [PubMed]
101. Day, E.; Dear, P.H.; McCaughan, F. Digital PCR Strategies in the Development and Analysis of Molecular Biomarkers for Personalized Medicine. *Methods* **2013**, *59*, 101–107. [CrossRef] [PubMed]
102. Jongthawin, J.; Intapan, P.M.; Lulitanond, V.; Sanpool, O.; Thanchomnang, T.; Sadaow, L.; Maleewong, W. Detection and Quantification of Wuchereria Bancrofti and Brugia Malayi DNA in Blood Samples and Mosquitoes Using Duplex Droplet Digital Polymerase Chain Reaction. *Parasitol. Res.* **2016**, *115*, 2967–2972. [CrossRef] [PubMed]
103. Thanchomnang, T.; Intapan, P.M.; Tantrawatpan, C.; Lulitanond, V.; Chungpivat, S.; Taweethavonsawat, P.; Kaewkong, W.; Sanpool, O.; Janwan, P.; Choochote, W.; et al. Rapid Detection and Identification of Wuchereria Bancrofti, Brugia Malayi, B. Pahangi, and Dirofilaria Immitis in Mosquito Vectors and Blood Samples by High Resolution Melting Real-Time P. *Korean J. Parasitol.* **2013**, *51*, 645–650. [CrossRef] [PubMed]
104. Pazoki-ghohe, H.; Haghparast-kenari, B.; Fakhar, M. Current and Novel Laboratory Diagnostic Methods and Identification of Causative Agents for Cutaneous Leishmaniasis. *J. Maz. Univ. Med. Sci.* **2016**, *25*, 350–366.
105. Ihsan, B.M.; Berbudi, A.; Agoes, R.; Hanif, M.I. Microfilaria Positification Test Using Real-Time PCR Technique with HRM (High-Resolution Melting). *Acta Parasitol.* **2022**, *67*, 496–503. [CrossRef]
106. Wittwer, C.T. High-Resolution DNA Melting Analysis: Advancements and Limitations. *Hum. Mutat.* **2009**, *30*, 857–859. [CrossRef] [PubMed]
107. Ririe, K.M.; Rasmussen, R.P.; Wittwer, C.T. Product Differentiation by Analysis of DNA Melting Curves during the Polymerase Chain Reaction. *Anal Biochem.* **1997**, *245*, 154–160. [CrossRef] [PubMed]
108. Nettuwakul, C.; Sawasdee, N.; Yenchitsomanus, P. Rapid Detection of Solute Carrier Family 4, Member 1 (SLC4A1) Mutations and Polymorphisms by High-Resolution Melting Analysis. *Clin. Biochem.* **2010**, *43*, 497–504. [CrossRef]
109. Wongkamchai, S.; Mayoon, B.; Kanakul, N.; Foongladda, S.; Wanachiwanawin, D.; Nochote, H.; Loymek, S. Rapid Differentiation of Filariae in Unstained and Stained Paraffin-Embedded Sections by a High-Resolution Melting Analysis PCR Assay. *Vector-Borne Zoonotic Dis.* **2015**, *15*, 473–480. [CrossRef] [PubMed]
110. Weil, G.J.; Lammie, P.J.; Weiss, N. The ICT Filariasis Test: A Rapid-Format Antigen Test for Diagnosis of Bancroftian Filariasis. *Parasitol. Today* **1997**, *13*, 401–404. [CrossRef] [PubMed]
111. Plichart, C.; Lemoine, A. Monitoring and Evaluation of Lymphatic Filariasis Interventions: An Improved PCR-Based Pool Screening Method for High Throughput Wuchereria Bancrofti Detection Using Dried Blood Spots. *Parasites Vectors* **2013**, *6*, 110. [CrossRef] [PubMed]
112. Naccache, S.N.; Peggs, K.S.; Mattes, F.M.; Phadke, R.; Garson, J.A.; Grant, P.; Samayoa, E.; Federman, S.; Miller, S.; Lunn, M.P.; et al. Diagnosis of Neuroinvasive Astrovirus Infection in an Immunocompromised Adult with Encephalitis by Unbiased Next-Generation Sequencing. *Clin. Infect. Dis.* **2015**, *60*, 919–923. [CrossRef] [PubMed]
113. Castellot, A.; Camacho, J.; Fernández-García, M.D.; Tarragó, D. Shotgun Metagenomics to Investigate Unknown Viral Etiologies of Pediatric Meningoencephalitis. *PLoS ONE* **2023**, *18*, e0296036. [CrossRef] [PubMed]
114. Gand, M.; Bloemen, B.; Vanneste, K.; Roosens, N.H.C.; De Keersmaecker, S.C.J. Comparison of 6 DNA Extraction Methods for Isolation of High Yield of High Molecular Weight DNA Suitable for Shotgun Metagenomics Nanopore Sequencing to Detect Bacteria. *BMC Genom.* **2023**, *24*, 438. [CrossRef] [PubMed]
115. McCarthy, C.B.; Diambra, L.A.; Rivera Pomar, R.V. Metagenomic Analysis of Taxa Associated with Lutzomyia Longipalpis, Vector of Visceral Leishmaniasis, Using an Unbiased High-Throughput Approach. *PLoS Negl. Trop. Dis.* **2011**, *5*, e1304. [CrossRef] [PubMed]
116. Gao, D.; Yu, Q.; Wang, G.; Wang, G.; Xiong, F. Diagnosis of a Malayan Filariasis Case Using a Shotgun Diagnostic Metagenomics Assay. *Parasites Vectors* **2016**, *9*, 86. [CrossRef] [PubMed]
117. Nutman, T.B. Insights into the Pathogenesis of Disease in Human Lymphatic Filariasis. *Lymphat. Res. Biol.* **2013**, *11*, 144–148. [CrossRef]
118. Boatin, B. The Onchocerciasis Control Programme in West Africa (OCP). *Ann. Trop. Med. Parasitol.* **2008**, *102*, 13–17. [CrossRef]
119. Vos, T.; Barber, R.M.; Bell, B.; Bertozzi-Villa, A.; Biryukov, S.; Bolliger, I.; Charlson, F.; Davis, A.; Degenhardt, L.; Dicker, D.; et al. Global, Regional, and National Incidence, Prevalence, and Years Lived with Disability for 301 Acute and Chronic Diseases and Injuries in 188 Countries, 1990–2013: A Systematic Analysis for the Global Burden of Disease Study 2013. *Lancet* **2015**, *386*, 743–800. [CrossRef] [PubMed]
120. Boussinesq, M.; Prod'hon, J.; Chippaux, J.P. Onchocerca Volvulus: Striking Decrease in Transmission in the Vina Valley (Cameroon) after Eight Annual Large Scale Ivermectin Treatments. *Trans. R Soc. Trop. Med. Hyg.* **1997**, *91*, 82–86. [CrossRef] [PubMed]
121. Neafie, R.C. Morphology of Onchocerca Volvulus. *Am. J. Clin. Pathol.* **1972**, *57*, 574–586. [CrossRef] [PubMed]
122. Sejvar, J.J.; Kakooza, A.M.; Foltz, J.L.; Makumbi, I.; Atai-Omoruto, A.D.; Malimbo, M.; Ndyomugyenyi, R.; Alexander, L.N.; Abang, B.; Downing, R.G.; et al. Clinical, Neurological, and Electrophysiological Features of Nodding Syndrome in Kitgum, Uganda: An Observational Case Series. *Lancet Neurol.* **2013**, *12*, 166–174. [CrossRef]

123. Newland, H.S.; White, A.T.; Greene, B.M.; Murphy, R.P.; Taylor, H.R. Ocular Manifestations of Onchocerciasis in a Rain Forest Area of West Africa. *Br. J. Ophthalmol.* **1991**, *75*, 163–169. [CrossRef] [PubMed]
124. Hoerauf, A. Science, Medicine, and the Future: Onchocerciasis. *BMJ* **2003**, *326*, 207–210. [CrossRef] [PubMed]
125. Boussinesq, M.; Pion, S.D.S.; Demanga-Ngangue; Kamgno, J. Relationship between Onchocerciasis and Epilepsy: A Matched Case-Control Study in the Mbam Valley, Republic of Cameroon. *Trans. R Soc. Trop. Med. Hyg.* **2002**, *96*, 537–541. [CrossRef]
126. Levick, B.; Laudisoit, A.; Tepage, F.; Ensoy-Musoro, C.; Mandro, M.; Bonareri Osoro, C.; Suykerbuyk, P.; Kashama, J.M.; Komba, M.; Tagoto, A.; et al. High Prevalence of Epilepsy in Onchocerciasis Endemic Regions in the Democratic Republic of the Congo. *PLoS Negl. Trop. Dis.* **2017**, *11*, e0005732. [CrossRef]
127. Colebunders, R.; Hendy, A.; van Oijen, M. Nodding Syndrome in Onchocerciasis Endemic Areas. *Trends Parasitol.* **2016**, *32*, 581–583. [CrossRef]
128. World Health Organization. *Guidelines for Stopping Mass Drug Administration and Verifying Elimination of Human Onchocerciasis: Criteria and Procedures*; World Health Organization: Geneva, Switzerland, 2016; ISBN 9789241510011.
129. Lobos, E.; Weiss, N.; Karam, M.; Taylor, H.R.; Ottesen, E.A.; Nutman, T.B. An Immunogenic Onchocerca Volvulus Antigen: A Specific and Early Marker of Infection. *Science* **1991**, *251*, 1603–1605. [CrossRef]
130. Gbakima, A.A.; Nutman, T.B.; Bradley, J.E.; McReynolds, L.A.; Winget, M.D.; Hong, Y.; Scott, A.L. Immunoglobulin G Subclass Responses of Children during Infection with Onchocerca Volvulus. *Clin. Diagn. Lab. Immunol.* **1996**, *3*, 98–104. [CrossRef]
131. Ogunrinade, A. Longitudinal Studies of Skin Microfilaria and Antibody Conversion Rates in Children Living in an Endemic Focus of Onchocerciasis in Nigeria. *J. Trop. Pediatr.* **2000**, *46*, 348–351. [CrossRef]
132. Lindblade, K.A.; Arana, B.; Zea-Flores, G.; Rizzo, N.; Porter, C.H.; Dominguez, A.; Cruz-Ortiz, N.; Unnasch, T.R.; Punkosdy, G.A.; Richards, J. Elimination of Onchocercia Volvulus Transmission in the Santa Rosa Focus of Guatemala. *Am. J. Trop. Med. Hyg.* **2007**, *77*, 334–341. [CrossRef]
133. Cruz-Ortiz, N.; Gonzalez, R.J.; Lindblade, K.A.; Richards, F.O.; Sauerbrey, M.; Zea-Flores, G.; Dominguez, A.; Oliva, O.; Catú, E.; Rizzo, N. Elimination of Onchocerca Volvulus Transmission in the Huehuetenango Focus of Guatemala. *J. Parasitol. Res.* **2012**, *2012*, 638429. [CrossRef] [PubMed]
134. Cama, V.A.; McDonald, C.; Arcury-Quandt, A.; Eberhard, M.; Jenks, M.H.; Smith, J.; Feleke, S.M.; Abanyie, F.; Thomson, L.; Wiegand, R.E.; et al. Evaluation of an OV-16 IgG4 Enzyme-Linked Immunosorbent Assay in Humans and Its Application to Determine the Dynamics of Antibody Responses in a Non-Human Primate Model of Onchocerca Volvulus Infection. *Am. J. Trop. Med. Hyg.* **2018**, *99*, 1041–1048. [CrossRef]
135. Shintouo, C.M.; Shey, R.A.; Nebangwa, D.N.; KEsoh, K.; Nongley, N.F.; Nguve, J.E.; Giron, P.; Mutesa, L.; Vanhamme, L.; Souopgui, J.; et al. In Silico Design and Validation of OvMANE1, a Chimeric Antigen for Human Onchocerciasis Diagnosis. *Pathogens* **2020**, *9*, 495. [CrossRef]
136. Lagatie, O.; Verheyen, A.; Nijs, E.; Batsa Debrah, L.; Debrah, Y.A.; Stuyver, L.J. Performance Evaluation of 3 Serodiagnostic Peptide Epitopes and the Derived Multi-Epitope Peptide OvNMP-48 for Detection of Onchocerca Volvulus Infection. *Parasitol. Res.* **2019**, *118*, 2263–2270. [CrossRef]
137. Lagatie, O.; Verheyen, A.; Nijs, E.; Van Dorst, B.; Batsa Debrah, L.; Debrah, A.; Supali, T.; Sartono, E.; Stuyver, L.J. Evaluation of the Diagnostic Performance of Onchocerca Volvulus Linear Epitopes in a Peptide Enzyme-Linked Immunosorbent Assay. *Am. J. Trop. Med. Hyg.* **2018**, *98*, 779–785. [CrossRef] [PubMed]
138. Ambe, L.A.; Limunga, E.; Mbah, C.E.; Adela, N.; Eric, N.; Ngoe, M.; Sone, B.; Lochnit, G.; Tachu, J.B.; Wanji, S.; et al. Identification and Characterization of Onchocerca Volvulus Heat Shock Protein 70 (OvHSP70) as Novel Diagnostic Marker of Onchocerciasis in Human Urine. *Pathogens* **2024**, *13*, 293. [CrossRef]
139. Shey, R.A.; Ghogomu, S.M.; Njume, F.N.; Gainkam, L.O.T.; Poelvoorde, P.; Mutesa, L.; Robert, A.; Humblet, P.; Munyampundu, J.-P.; Kamgno, J.; et al. Prediction and Validation of the Structural Features of Ov58GPCR, an Immunogenic Determinant of Onchocerca Volvulus. *PLoS ONE* **2018**, *13*, e0202915. [CrossRef]
140. Bennuru, S.; Oduro-Boateng, G.; Osigwe, C.; Del Valle, P.; Golden, A.; Ogawa, G.M.; Cama, V.; Lustigman, S.; Nutman, T.B. Integrating Multiple Biomarkers to Increase Sensitivity for the Detection of Onchocerca Volvulus Infection. *J. Infect. Dis.* **2020**, *221*, 1805–1815. [CrossRef] [PubMed]
141. Feeser, K.R.; Cama, V.; Priest, J.W.; Thiele, E.A.; Wiegand, R.E.; Lakwo, T.; Feleke, S.M.; Cantey, P.T. Characterizing Reactivity to Onchocerca Volvulus Antigens in Multiplex Bead Assays. *Am. J. Trop. Med. Hyg.* **2017**, *97*, 666–672. [CrossRef] [PubMed]
142. Golden, A.; Stevens, E.J.; Yokobe, L.; Faulx, D.; Kalnoky, M.; Peck, R.; Valdez, M.; Steel, C.; Karabou, P.; Banla, M.; et al. A Recombinant Positive Control for Serology Diagnostic Tests Supporting Elimination of Onchocerca Volvulus. *PLoS Negl. Trop. Dis.* **2016**, *10*, e0004292. [CrossRef]
143. Nana-Djeunga, H.C.; Sicard, C.M.; Mogoung-Wafo, A.E.; Chesnais, C.B.; Deléglise, H.; Touka-Nounkeu, R.; Domche, A.; Golden, A.; Klion, A.D.; Nutman, T.B.; et al. Changes in Onchocerciasis Ov16 IgG4 Rapid Diagnostic Test Results Over One-Month Follow-up: Lessons for Reading Timeframe and Decision-Making. *Am. J. Trop. Med. Hyg.* **2022**, *107*, 658–661. [CrossRef] [PubMed]
144. Hotterbeekx, A.; Perneel, J.; Mandro, M.; Abhafule, G.; Siewe Fodjo, J.N.; Dusabimana, A.; Abrams, S.; Kumar-Singh, S.; Colebunders, R. Comparison of Diagnostic Tests for Onchocerca Volvulus in the Democratic Republic of Congo. *Pathogens* **2020**, *9*, 435. [CrossRef] [PubMed]

145. Ekanya, R.; Beng, A.A.; Anim, M.A.; Pangwoh, Y.Z.; Dibando, O.E.; Gandjui, N.V.T.; Awah, A.R.; Amambo, G.N.; Nchanji, G.T.; Ndzeshang, B.L.; et al. Concordance between Ov16 Rapid Diagnostic Test (RDT) and Ov16 Enzyme-Linked Immunosorbent Assay (ELISA) for the Diagnosis of Onchocerciasis in Areas of Contrasting Endemicity in Cameroon. *Parasite Epidemiol. Control.* **2023**, *21*, e00290. [CrossRef] [PubMed]
146. Meredith, S.E.O.; Lando, G.; Gbakima, A.A.; Zimmerman, P.A.; Unnasch, T.R. Onchocerca Volvulus: Application of the Polymerase Chain Reaction to Identification and Strain Differentiation of the Parasite. *Exp. Parasitol.* **1991**, *73*, 335–344. [CrossRef] [PubMed]
147. Zimmerman, P.A.; Toe, L.; Unnasch, T.R. Design of Onchocerca DNA Probes Based upon Analysis of a Repeated Sequence Family. *Mol. Biochem. Parasitol.* **1993**, *58*, 259–267. [CrossRef] [PubMed]
148. Zimmerman, P.A.; Guderian, R.H.; Aruajo, E.; Elson, L.; Phadke, P.; Kubofcik, J.; Nutman, T.B. Polymerase Chain Reaction-Based Diagnosis of Onchocerca Volvulus Infection: Improved Detection of Patients with Onchocerciasis. *J. Infect. Dis.* **1994**, *169*, 686–689. [CrossRef]
149. Fink, D.L.; Fahle, G.A.; Fischer, S.; Fedorko, D.F.; Nutman, T.B. Toward Molecular Parasitologic Diagnosis: Enhanced Diagnostic Sensitivity for Filarial Infections in Mobile Populations. *J. Clin. Microbiol.* **2011**, *49*, 42–47. [CrossRef]
150. Taylor, S.; Wakem, M.; Dijkman, G.; Alsarraj, M.; Nguyen, M. A Practical Approach to RT-QPCR—Publishing Data That Conform to the MIQE Guidelines. *Methods* **2010**, *50*, S1–S5. [CrossRef]
151. Mekonnen, S.A.; Beissner, M.; Saar, M.; Ali, S.; Zeynudin, A.; Tesfaye, K.; Adbaru, M.G.; Battke, F.; Poppert, S.; Hoelscher, M.; et al. O-5S Quantitative Real-Time PCR: A New Diagnostic Tool for Laboratory Confirmation of Human Onchocerciasis. *Parasites Vectors* **2017**, *10*, 451. [CrossRef]
152. Cama, V.A.; Sleshi, M.; Thiele, E.A.; Abanyie, F.; Lakwo, T.; Mekasha, S.; Cantey, P.T.; Kebede, A. Detection of Onchocerca Volvulus in Skin Snips by Microscopy and Real-Time Polymerase Chain Reaction: Implications for Monitoring and Evaluation Activities. *Am. J. Trop. Med. Hyg.* **2016**, *94*, 906–911. [CrossRef]
153. Morales-Hojas, R.; Post, R.J.; Shelley, A.J.; Maia-Herzog, M.; Coscarón, S.; Cheke, R.A. Characterisation of Nuclear Ribosomal DNA Sequences from Onchocerca Volvulus and Mansonella Ozzardi (Nematoda: Filarioidea) and Development of a PCR-Based Method for Their Detection in Skin Biopsies. *Int. J. Parasitol.* **2001**, *31*, 169–177. [CrossRef]
154. Tang, T.-H.T.; López-Vélez, R.; Lanza, M.; Shelley, A.J.; Rubio, J.M.; Luz, S.L.B. Nested PCR to Detect and Distinguish the Sympatric Filarial Species Onchocerca Volvulus, Mansonella Ozzardi and Mansonella Perstans in the Amazon Region. *Mem. Inst. Oswaldo Cruz.* **2010**, *105*, 823–828. [CrossRef]
155. Prince-Guerra, J.L.; Cama, V.A.; Wilson, N.; Thiele, E.A.; Likwela, J.; Ndakala, N.; Muzinga wa Muzinga, J.; Ayebazibwe, N.; Ndjakani, Y.D.; Pitchouna, N.A.; et al. Comparison of PCR Methods for Onchocerca Volvulus Detection in Skin Snip Biopsies from the Tshopo Province, Democratic Republic of the Congo. *Am. J. Trop. Med. Hyg.* **2018**, *98*, 1427–1434. [CrossRef]
156. Notomi, T. Loop-Mediated Isothermal Amplification of DNA. *Nucleic Acids Res.* **2000**, *28*, 63e–663. [CrossRef]
157. Kaneko, H.; Kawana, T.; Fukushima, E.; Suzutani, T. Tolerance of Loop-Mediated Isothermal Amplification to a Culture Medium and Biological Substances. *J. Biochem. Biophys. Methods* **2007**, *70*, 499–501. [CrossRef]
158. Poole, C.B.; Li, Z.; Alhassan, A.; Guelig, D.; Diesburg, S.; Tanner, N.A.; Zhang, Y.; Evans, T.C.; LaBarre, P.; Wanji, S.; et al. Colorimetric Tests for Diagnosis of Filarial Infection and Vector Surveillance Using Non-Instrumented Nucleic Acid Loop-Mediated Isothermal Amplification (NINA-LAMP). *PLoS ONE* **2017**, *12*, e0169011. [CrossRef] [PubMed]
159. Avendaño, C.; Patarroyo, M.A. Loop-Mediated Isothermal Amplification as Point-of-Care Diagnosis for Neglected Parasitic Infections. *Int. J. Mol. Sci.* **2020**, *21*, 7981. [CrossRef] [PubMed]
160. Nagamine, K.; Watanabe, K.; Ohtsuka, K.; Hase, T.; Notomi, T. Loop-Mediated Isothermal Amplification Reaction Using a Nondenatured Template. *Clin. Chem.* **2001**, *47*, 1742–1743. [CrossRef]
161. Itou, T.; Markotter, W.; Nel, L.H. Reverse Transcription-Loop-Mediated Isothermal Amplification System for the Detection of Rabies Virus. In *Current Laboratory Techniques in Rabies Diagnosis, Research and Prevention*; Elsevier: Amsterdam, The Netherlands, 2014; pp. 85–95.
162. Mori, Y.; Nagamine, K.; Tomita, N.; Notomi, T. Detection of Loop-Mediated Isothermal Amplification Reaction by Turbidity Derived from Magnesium Pyrophosphate Formation. *Biochem. Biophys. Res. Commun.* **2001**, *289*, 150–154. [CrossRef]
163. Tomita, N.; Mori, Y.; Kanda, H.; Notomi, T. Loop-Mediated Isothermal Amplification (LAMP) of Gene Sequences and Simple Visual Detection of Products. *Nat. Protoc.* **2008**, *3*, 877–882. [CrossRef]
164. Lloyd, M.M.; Gilbert, R.; Taha, N.T.; Weil, G.J.; Meite, A.; Kouakou, I.M.M.; Fischer, P.U. Conventional Parasitology and DNA-Based Diagnostic Methods for Onchocerciasis Elimination Programmes. *Acta Trop.* **2015**, *146*, 114–118. [CrossRef]
165. Alhassan, A.; Osei-Atweneboana, M.Y.; Kyeremeh, K.F.; Poole, C.B.; Li, Z.; Tettevi, E.; Tanner, N.A.; Carlow, C.K.S. Comparison of a New Visual Isothermal Nucleic Acid Amplification Test with PCR and Skin Snip Analysis for Diagnosis of Onchocerciasis in Humans. *Mol. Biochem. Parasitol.* **2016**, *210*, 10–12. [CrossRef]
166. Amambo, G.N.; Innocentia, N.; Abong, R.A.; Fombad, F.F.; Njouendou, A.J.; Nietcho, F.; Ekanya, R.; Kien, C.A.; Ebai, R.; Lenz, B.; et al. Application of Loop Mediated Isothermal Amplification (LAMP) Assays for the Detection of Onchocerca Volvulus, Loa Loa and Mansonella Perstans in Humans and Vectors. *Front. Trop. Dis.* **2023**, *3*, 1016176. [CrossRef]
167. Lagatie, O.; Merino, M.; Batsa Debrah, L.; Debrah, A.Y.; Stuyver, L.J. An Isothermal DNA Amplification Method for Detection of Onchocerca Volvulus Infection in Skin Biopsies. *Parasites Vectors* **2016**, *9*, 624. [CrossRef]
168. Bwangamoi, O. Onchocerca Ochengi New Species, an Intradermal Parasite of Cattle in East Africa. *Bull. Epizoot. Dis. Afr.* **1969**, *17*, 321–335.

169. Wilson, N.O.; Badara Ly, A.; Cama, V.A.; Cantey, P.T.; Cohn, D.; Diawara, L.; Direny, A.; Fall, M.; Feeser, K.R.; Fox, L.M.; et al. Evaluation of Lymphatic Filariasis and Onchocerciasis in Three Senegalese Districts Treated for Onchocerciasis with Ivermectin. *PLoS Negl. Trop. Dis.* **2016**, *10*, e0005198. [CrossRef]
170. Macfarlane, C.L.; Quek, S.; Pionnier, N.; Turner, J.D.; Wanji, S.; Wagstaff, S.C.; Taylor, M.J. The Insufficiency of Circulating MiRNA and DNA as Diagnostic Tools or as Biomarkers of Treatment Efficacy for Onchocerca Volvulus. *Sci. Rep.* **2020**, *10*, 6672. [CrossRef]
171. Bartel, D.P. MicroRNAs. *Cell* **2004**, *116*, 281–297. [CrossRef]
172. Britton, C.; Winter, A.D.; Gillan, V.; Devaney, E. MicroRNAs of Parasitic Helminths—Identification, Characterization and Potential as Drug Targets. *Int. J. Parasitol. Drugs Drug. Resist.* **2014**, *4*, 85–94. [CrossRef]
173. Buck, A.H.; Coakley, G.; Simbari, F.; McSorley, H.J.; Quintana, J.F.; Le Bihan, T.; Kumar, S.; Abreu-Goodger, C.; Lear, M.; Harcus, Y.; et al. Exosomes Secreted by Nematode Parasites Transfer Small RNAs to Mammalian Cells and Modulate Innate Immunity. *Nat. Commun.* **2014**, *5*, 5488. [CrossRef]
174. Tritten, L.; Burkman, E.; Moorhead, A.; Satti, M.; Geary, J.; Mackenzie, C.; Geary, T. Detection of Circulating Parasite-Derived MicroRNAs in Filarial Infections. *PLoS Negl. Trop. Dis.* **2014**, *8*, e2971. [CrossRef]
175. Quintana, J.F.; Makepeace, B.L.; Babayan, S.A.; Ivens, A.; Pfarr, K.M.; Blaxter, M.; Debrah, A.; Wanji, S.; Ngangyung, H.F.; Bah, G.S.; et al. Extracellular Onchocerca-Derived Small RNAs in Host Nodules and Blood. *Parasites Vectors* **2015**, *8*, 58. [CrossRef]
176. Tritten, L.; O'Neill, M.; Nutting, C.; Wanji, S.; Njouendoui, A.; Fombad, F.; Kengne-Ouaffo, J.; Mackenzie, C.; Geary, T. Loa Loa and Onchocerca Ochengi MiRNAs Detected in Host Circulation. *Mol. Biochem. Parasitol.* **2014**, *198*, 14–17. [CrossRef]
177. Lagatie, O.; Batsa Debrah, L.; Debrah, A.; Stuyver, L.J. Plasma-Derived Parasitic MicroRNAs Have Insufficient Concentrations to Be Used as Diagnostic Biomarker for Detection of Onchocerca Volvulus Infection or Treatment Monitoring Using LNA-Based RT-QPCR. *Parasitol. Res.* **2017**, *116*, 1013–1022. [CrossRef]
178. Lagatie, O.; Njumbe Ediage, E.; Batsa Debrah, L.; Diels, L.; Nolten, C.; Vinken, P.; Debrah, A.; Dillen, L.; Silber, S.; Stuyver, L.J. Evaluation of the Diagnostic Potential of Urinary N-Acetyltyramine-O,β-Glucuronide (NATOG) as Diagnostic Biomarker for Onchocerca Volvulus Infection. *Parasites Vectors* **2016**, *9*, 302. [CrossRef]
179. Globisch, D.; Moreno, A.Y.; Hixon, M.S.; Nunes, A.A.K.; Denery, J.R.; Specht, S.; Hoerauf, A.; Janda, K.D. Onchocerca Volvulus—Neurotransmitter Tyramine Is a Biomarker for River Blindness. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 4218–4223. [CrossRef]
180. Rodonaja, T.E. A New Species of Nematode, Onchocerca Lupi n. Sp., from Canis Lupus Cubanensis. *Soobshchenyia Akad. Nauk. Gruz. SSR* **1967**, *45*, 715–719.
181. Egyed, Z.; Sréter, T.; Széll, Z.; Beszteri, B.; Oravecz, O.; Márialigeti, K.; Varga, I. Morphologic and Genetic Characterization of Onchocerca Lupi Infecting Dogs. *Vet. Parasitol.* **2001**, *102*, 309–319. [CrossRef]
182. Sréter, T.; Széll, Z. Onchocercosis: A Newly Recognized Disease in Dogs. *Vet. Parasitol.* **2008**, *151*, 1–13. [CrossRef]
183. Franchini, D.; Giannelli, A.; Di Paola, G.; Cortes, H.; Cardoso, L.; Lia, R.P.; Campbell, B.E.; Dantas-Torres, F.; Lenoci, D.; Assad, E.A.; et al. Image Diagnosis of Zoonotic Onchocercosis by Onchocerca Lupi. *Vet. Parasitol.* **2014**, *203*, 91–95. [CrossRef]
184. Labelle, A.L.; Daniels, J.B.; Dix, M.; Labelle, P. Onchocerca Lupi Causing Ocular Disease in Two Cats. *Vet. Ophthalmol.* **2011**, *14*, 105–110. [CrossRef]
185. Sréter, T.; Széll, Z.; Egyed, Z.; Varga, I. Subconjunctival Zoonotic Onchocerciasis in Man: Aberrant Infection with Onchocerca Lupi? *Ann. Trop. Med. Parasitol.* **2002**, *96*, 497–502. [CrossRef]
186. Otranto, D.; Dantas-Torres, F.; Gürlü, V.P.; Sakru, N.; Yakar, K.; Bain, O.; Lia, R.P.; Testini, G. First Evidence of Human Zoonotic Infection by Onchocerca Lupi (Spirurida, Onchocercidae). *Am. J. Trop. Med. Hyg.* **2011**, *84*, 55–58. [CrossRef]
187. Mowlavi, G.; Farzbod, F.; Kheirkhah, A.; Mobedi, I.; Bowman, D.D.; Naddaf, S.R. Human Ocular Onchocerciasis Caused by Onchocerca Lupi (Spirurida, Onchocercidae) in Iran. *J. Helminthol.* **2014**, *88*, 250–255. [CrossRef]
188. Komnenou, A.; Eberhard, M.L.; Kaldrymidou, E.; Tsalie, E.; Dessiris, A. Subconjunctival Filariasis Due to Onchocerca Sp. in Dogs: Report of 23 Cases in Greece. *Vet. Ophthalmol.* **2002**, *5*, 119–126. [CrossRef]
189. Hobohm, D.; Eberhard, M.L.; Chundu, K.; Mathison, B.A.; Cantey, P.T.; Feiz-Erfan, I.; Ostovar, G.A.; Bishop, H.S. Zoonotic Onchocerca Lupi Infection in a 22-Month-Old Child in Arizona: First Report in the United States and a Review of the Literature. *Am. J. Trop. Med. Hyg.* **2013**, *88*, 601–605. [CrossRef]
190. Dudley, R.W.R.; Smith, C.; Dishop, M.; Mirsky, D.; Handler, M.H.; Rao, S. A Cervical Spine Mass Caused by Onchocerca Lupi. *Lancet* **2015**, *386*, 1372. [CrossRef]
191. Latrofa, M.S.; Palmisano, G.; Annoscia, G.; Pierri, C.L.; Chandrashekar, R.; Otranto, D. Major Antigen and Paramyosin Proteins as Candidate Biomarkers for Serodiagnosis of Canine Infection by Zoonotic Onchocerca Lupi. *PLoS Negl. Trop. Dis.* **2021**, *15*, e0009027. [CrossRef]
192. Latrofa, M.S.; Louzada-Flores, V.N.; Maia, C.; Kelly, M.A.; Verocai, G.G.; Cantacessi, C.; Otranto, D. Development of an Indirect ELISA for the Serodiagnosis of Canine Infection by Onchocerca Lupi. *Sci. Rep.* **2024**, *14*, 3348. [CrossRef]
193. Le, T.H.; Blair, D.; McManus, D.P. Mitochondrial Genomes of Human Helminths and Their Use as Markers in Population Genetics and Phylogeny. *Acta Trop.* **2000**, *77*, 243–256. [CrossRef]
194. Rojas, A.; Dvir, E.; Farkas, R.; Sarma, K.; Borthakur, S.; Jabbar, A.; Markovics, A.; Otranto, D.; Baneth, G. Phylogenetic Analysis of Spirocerca Lupi and Spirocerca Vulpis Reveal High Genetic Diversity and Intra-Individual Variation. *Parasites Vectors* **2018**, *11*, 639. [CrossRef]
195. Labelle, A.L.; Maddox, C.W.; Daniels, J.B.; Lanka, S.; Eggett, T.E.; Dubielzig, R.R.; Labelle, P. Canine Ocular Onchocercosis in the United States Is Associated with Onchocerca Lupi. *Vet. Parasitol.* **2013**, *193*, 297–301. [CrossRef]

196. McLean, N.J.; Newkirk, K.; Adema, C.M. Canine Ocular Onchocerciasis: A Retrospective Review of the Diagnosis, Treatment, and Outcome of 16 Cases in New Mexico (2011–2015). *Vet. Ophthalmol.* **2017**, *20*, 349–356. [CrossRef]
197. Verocai, G.G.; Hassan, H.K.; Lakwo, T.; Habomugisha, P.; Katabarwa, M.N.; Begumisa, S.; Clouds, P.; Katamanywa, J.; Nahabwe, C.; Unnasch, T.R. Molecular Identification of Onchocerca Spp. Larvae in Simulium Damnosum Sensu Lato Collected in Northern Uganda. *Am. J. Trop. Med. Hyg.* **2017**, *97*, 1843–1845. [CrossRef]
198. Ferri, E.; Barbuto, M.; Bain, O.; Galimberti, A.; Uni, S.; Guerrero, R.; Ferté, H.; Bandi, C.; Martin, C.; Casiraghi, M. Integrated Taxonomy: Traditional Approach and DNA Barcoding for the Identification of Filarioid Worms and Related Parasites (Nematoda). *Front. Zool.* **2009**, *6*, 1. [CrossRef]
199. Latrofa, M.S.; Annoscia, G.; Colella, V.; Cavalera, M.A.; Maia, C.; Martin, C.; Šlapeta, J.; Otranto, D. A Real-Time PCR Tool for the Surveillance of Zoonotic Onchocerca Lupi in Dogs, Cats and Potential Vectors. *PLoS Negl. Trop. Dis.* **2018**, *12*, e0006402. [CrossRef]
200. Hillis, D.M.; Dixon, M.T. Ribosomal DNA: Molecular Evolution and Phylogenetic Inference. *Q. Rev. Biol.* **1991**, *66*, 411–453. [CrossRef]
201. Casiraghi, M.; Bazzocchi, C.; Mortarino, M.; Ottina, E.; Genchi, C. A Simple Molecular Method for Discriminating Common Filarial Nematodes of Dogs (Canis Familiaris). *Vet. Parasitol.* **2006**, *141*, 368–372. [CrossRef]
202. de Almeida, M.; Nascimento, F.S.; Mathison, B.A.; Bishop, H.; Bradbury, R.S.; Cama, V.A.; da Silva, A.J. Duplex Real-Time PCR Assay for Clinical Differentiation of Onchocerca Lupi and Onchocerca Volvulus. *Am. J. Trop. Med. Hyg.* **2020**, *103*, 1556–1562. [CrossRef]
203. Dietrich, D.; Uhl, B.; Sailer, V.; Holmes, E.E.; Jung, M.; Meller, S.; Kristiansen, G. Improved PCR Performance Using Template DNA from Formalin-Fixed and Paraffin-Embedded Tissues by Overcoming PCR Inhibition. *PLoS ONE* **2013**, *8*, e77771. [CrossRef]
204. Michael, E.; Bundy, D.A.P. Global Mapping of Lymphatic Filariasis. *Parasitol. Today* **1997**, *13*, 472–476. [CrossRef]
205. Dreyer, G.; Addiss, D.; Bettinger, J.; Dreyer, P.; Norões, J.; Rio, F. Treatment and Prevention of Problems Associated with Lymphatic Filariasis. In *Lymphoedema Staff Manual*; WHO: Geneva, Switzerland, 2000; pp. 1–60.
206. Simonsen, P.E.; Magesa, S.M. Short Communication: Observations on False Positive Reactions in the Rapid NOW® Filariasis Card Test. *Trop. Med. Int. Health* **2004**, *9*, 1200–1202. [CrossRef]
207. Rebollo, M.P.; Bockarie, M.J. Rapid Diagnostics for the Endgame in Lymphatic Filariasis Elimination. *Am. J. Trop. Med. Hyg.* **2013**, *89*, 3–4. [CrossRef]
208. World Health Organization. *Strengthening the Assessment of Lymphatic Filariasis Transmission and Documenting the Achievement of Elimination*; King, J., Ed.; WHO: Geneva, Switzerland, 2014; ISBN 9789241508797.
209. Gounoue-Kamkumo, R.; Nana-Djeunga, H.C.; Bopda, J.; Akame, J.; Tarini, A.; Kamgno, J. Loss of Sensitivity of Immunochromatographic Test (ICT) for Lymphatic Filariasis Diagnosis in Low Prevalence Settings: Consequence in the Monitoring and Evaluation Procedures. *BMC Infect. Dis.* **2015**, *15*, 579. [CrossRef]
210. Wanji, S.; Amvongo-Adjia, N.; Njouendou, A.J.; Kengne-Ouafo, J.A.; Ndongmo, W.P.C.; Fombad, F.F.; Koudou, B.; Enyong, P.A.; Bockarie, M. Further Evidence of the Cross-Reactivity of the Binax NOW® Filariasis ICT Cards to Non-Wuchereria Bancrofti Filariae: Experimental Studies with Loa Loa and Onchocerca Ochengi. *Parasites Vectors* **2016**, *9*, 267. [CrossRef]
211. Pantelias, A.; King, J.D.; Lammie, P.; Weil, G.J. Development and Introduction of the Filariasis Test Strip: A New Diagnostic Test for the Global Program to Eliminate Lymphatic Filariasis. *Am. J. Trop. Med. Hyg.* **2022**. [CrossRef]
212. World Health Organization. *Diagnostic Test for Surveillance of Lymphatic Filariasis: Target Product Profile*; World Health Organization: Geneva, Switzerland, 2021; ISBN 9789240018648.
213. Scott, J.L.; Mayfield, H.J.; Sinclair, J.; Martin, B.M.; Howlett, M.; Muttucumaru, R.; Won, K.Y.; Thomsen, R.; Viali, S.; Tofaeono-Pifeleti, R.; et al. Field Comparison of STANDARD™ Q Filariasis Antigen Test (QFAT) with Bioline Filariasis Test Strip (FTS) for the Detection of Lymphatic Filariasis in Samoa, 2023. *medRxiv* **2024**. [CrossRef]
214. Chesnais, C.B.; Awaca-Uvon, N.-P.; Bolay, F.K.; Boussinesq, M.; Fischer, P.U.; Gankpala, L.; Meite, A.; Missamou, F.; Pion, S.D.; Weil, G.J. A Multi-Center Field Study of Two Point-of-Care Tests for Circulating Wuchereria Bancrofti Antigenemia in Africa. *PLoS Negl. Trop. Dis.* **2017**, *11*, e0005703. [CrossRef]
215. Weil, G.J.; Ramzy, R.M.R. Diagnostic Tools for Filariasis Elimination Programs. *Trends Parasitol.* **2007**, *23*, 78–82. [CrossRef]
216. Chesnais, C.B.; Vlaminck, J.; Kunyu-Shako, B.; Pion, S.D.; Awaca-Uvon, N.-P.; Weil, G.J.; Mumba, D.; Boussinesq, M. Measurement of Circulating Filarial Antigen Levels in Human Blood with a Point-of-Care Test Strip and a Portable Spectrodensitometer. *Am. J. Trop. Med. Hyg.* **2016**, *94*, 1324–1329. [CrossRef]
217. Noordin, R.; Yunus, M.H.; Robinson, K.; Won, K.Y.; Babu, S.; Fischer, P.U.; Hisam, S.; Mahmud, R. Laboratory Evaluation of a Rapid IgG4 Antibody Test (BLF Rapid™) for Bancroftian Filariasis. *Am. J. Trop. Med. Hyg.* **2018**, *99*, 1587–1590. [CrossRef]
218. Rahumatullah, A.; Ahmad, A.; Noordin, R.; Lim, T.S. Delineation of BmSXP Antibody V-Gene Usage from a Lymphatic Filariasis Based Immune ScFv Antibody Library. *Mol. Immunol.* **2015**, *67*, 512–523. [CrossRef]
219. Rahumatullah, A.; Lim, T.S.; Yunus, M.H.; Noordin, R. Development of an Antigen Detection ELISA for Bancroftian Filariasis Using BmSXP-Specific Recombinant Monoclonal Antibody. *Am. J. Trop. Med. Hyg.* **2019**, *101*, 436–440. [CrossRef]
220. Greene, S.E.; Fischer, K.; Choi, Y.-J.; Curtis, K.C.; Budge, P.J.; Mitreva, M.; King, C.L.; Fischer, P.U.; Weil, G.J. Characterization of a Novel Microfilarial Antigen for Diagnosis of Wuchereria Bancrofti Infections. *PLoS Negl. Trop. Dis.* **2022**, *16*, e0010407. [CrossRef]
221. Ahmad, F.; Liebau, E.; Rathaur, S. Human Immune Response against Filarial HSP70 and Its Role in the Diagnosis of Lymphatic Filariasis. *Parasite Immunol.* **2023**, *45*, e12978. [CrossRef]

222. Jha, R.; Gangwar, M.; Chahar, D.; Setty Balakrishnan, A.; Negi, M.P.S.; Misra-Bhattacharya, S. Humans from Wuchereria Bancrofti Endemic Area Elicit Substantial Immune Response to Proteins of the Filarial Parasite Brugia Malayi and Its Endosymbiont Wolbachia. *Parasites Vectors* **2017**, *10*, 40. [CrossRef]
223. Nutman, T.B.; Ottesen, E.A.; Williams, S.A.; Zhong, M.; McCarthy, J.; Bierwert, L.; Lizotte-Waniewski, M.; Chanteau, S. A Polymerase Chain Reaction Assay for Detection of the Parasite Wuchereria Bancrofti in Human Blood Samples. *Am. J. Trop. Med. Hyg.* **1996**, *54*, 357–363. [CrossRef]
224. Zimmerman, P.A.; Kazura, J.W.; Alpers, M.P.; Bockarie, M.J.; Williams, S.A.; Fischer, P.; Griffin, L. Application of a Polymerase Chain Reaction-ELISA to Detect Wuchereria Bancrofti in Pools of Wild-Caught Anopheles Punctulatus in a Filariasis Control Area in Papua New Guinea. *Am. J. Trop. Med. Hyg.* **2000**, *62*, 363–367. [CrossRef]
225. Ramzy, R.M.R. Field Application of PCR-Based Assays for Monitoring *Wuchereria bancrofti* Infection in Africa. *Ann. Trop. Med. Parasitol.* **2002**, *96*, 55–59. [CrossRef]
226. Nuchprayoon, S. DNA-Based Diagnosis of Lymphatic Filariasis. *Southeast Asian J. Trop. Med. Public Health* **2009**, *40*, 904.
227. Zaky, W.I.; Tomaino, F.R.; Pilotte, N.; Laney, S.J.; Williams, S.A. Backpack PCR: A Point-of-Collection Diagnostic Platform for the Rapid Detection of Brugia Parasites in Mosquitoes. *PLoS Negl. Trop. Dis.* **2018**, *12*, e0006962. [CrossRef]
228. González-González, E.; Mendoza-Ramos, J.L.; Pedroza, S.C.; Cuellar-Monterrubio, A.A.; Márquez-Ipiña, A.R.; Lira-Serhan, D.; Trujillo-de Santiago, G.; Alvarez, M.M. Validation of Use of the MiniPCR Thermocycler for Ebola and Zika Virus Detection. *PLoS ONE* **2019**, *14*, e0215642. [CrossRef]
229. Phuakrod, A.; Sripumkhai, W.; Jeamsaksiri, W.; Pattamang, P.; Loymek, S.; Brindley, P.J.; Sarasombath, P.T.; Wongkamchai, S. A MiniPCR-Duplex Lateral Flow Dipstick Platform for Rapid and Visual Diagnosis of Lymphatic Filariae Infection. *Diagnostics* **2021**, *11*, 1855. [CrossRef]
230. Njiru, Z.K.; Mikosza, A.S.J.; Matovu, E.; Enyaru, J.C.K.; Ouma, J.O.; Kibona, S.N.; Thompson, R.C.A.; Ndung'u, J.M. African Trypanosomiasis: Sensitive and Rapid Detection of the Sub-Genus Trypanozoon by Loop-Mediated Isothermal Amplification (LAMP) of Parasite DNA. *Int. J. Parasitol.* **2008**, *38*, 589–599. [CrossRef]
231. Kinyatta, N. Development and Validation of Rapid Molecular Assays for Diagnosis and Surveillance of Wuchereria Bancrofti in Human Blood and Mosquito Vectors in Kenya. Ph.D. Thesis, University of Nairobi, Nairobi, Kenya, 2022.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.