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Lyme disease and new molecular biological detection methods

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Abstract

Molecular biology is a crucial tool for understanding the structures, functions, and internal controls within cells. Its applications span from diagnosing diseases to developing new medicines and improving our understanding of cellular physiology. However, diagnosing Lyme disease presents unique challenges as the bacteria responsible for the disease are difficult to observe directly in body tissues and are slow-growing in laboratory environments. Lyme disease can affect multiple body systems and exhibit a range of non-specific symptoms, which can complicate diagnosis. Common laboratory diagnostics also have high rates of false positives in contaminated areas. To overcome these limitations, scientists have focused on developing fast and accurate diagnostic methods using molecular biology. Researchers have identified Borrelia burgdorferi, a type of gram-negative spirochete bacteria, as the primary cause of Lyme disease, but other species can also cause the disease. Accurate molecular tests have been designed to identify specific strains of Borrelia with precision. This study reviewed 131 related articles from Scopus, ISI, and PubMed databases and reported methods for creating accurate molecular tests to detect disease agents. These developments represent a significant step towards a more effective diagnosis and treatment of Lyme disease. The study analyzed the varied approaches and techniques outlined in the literature to create a cohesive understanding of the most effective methods for designing molecular tests. Ultimately, the study reported on the optimal methods for designing and implementing accurate molecular tests to diagnose and isolate disease agents.

Keywords: *Borrelia Burgdorferi,* Lyme Disease, Molecular Biology, OspA protein, OspB protein.

Introduction

Molecular biology is a crucial tool for understanding the structures, functions, and internal controls within cells. Its applications span from diagnosing diseases to developing new medicines and improving our understanding of cellular physiology. However, diagnosing Lyme disease presents unique challenges as the bacteria responsible for the disease are difficult to observe directly in body tissues and are slow-growing in laboratory environments. Lyme disease can affect multiple body systems and exhibit a range of non-specific symptoms, which can complicate diagnosis. Common laboratory diagnostics also have high rates of false positives in contaminated areas. To overcome these limitations, scientists have focused on developing fast and accurate diagnostic methods using molecular biology. Researchers have identified Borrelia burgdorferi, a type of gram-negative spirochete bacteria, as the primary cause of Lyme disease, but other species can also cause the disease. Accurate molecular tests have been designed to identify specific strains of Borrelia with precision. This study reviewed 131 related articles from Scopus, ISI, and PubMed databases and reported methods for creating accurate molecular tests to detect disease agents. These developments represent a significant step towards a more effective diagnosis and treatment of Lyme disease. The study analyzed the varied approaches and techniques

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outlined in the literature to create a cohesive understanding of the most effective methods for designing molecular tests. Ultimately, the study reported on the optimal methods for designing and implementing accurate molecular tests to diagnose and isolate disease agents.[1-3]

1- Borrelia Phylogenetic

Borrelia, like other spirochetes, is helical in shape and consists of a protoplasmic cylinder, a peptidoglycan-cytoplasmic membrane complex, a flagellum, and a periplasmic area surrounded by two layers of the outer membrane.[4] Flagellar filaments are absent from the outer membrane of most bacteria; but, in Borrelia, they are inserted at the end of the protoplasm and totally confined in the periplasmic space, where their rotational motion drives the group's peculiar left-handed corkscrew movement.[5,6] Borrelia's distinctive left-handed corkscrew motion is powered by the rotational action of its flagella.[7] Using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), more than 100 protein species have been identified in Borrelia burgdorferi.[8] Thirteen of these proteins were highlighted through labeling with 125I, or biotin, with apparent molecular weights of 22, 24, 29, 31, 34, 37, 39, 41, 52, 66, 70, 73, and 93 kDa. 28 The outer surface proteins (OSPs), OSP-A and OSP-B, also referred to as 31 kDa and 34 kDa, respectively, have been identified as crucial components of the spirochete responsible for Lyme disease.[9] Western blotting revealed that the OSP-A and OSP-B of US isolates were more homogenous than those of European strains.[10] It is important to note that the spirochete responsible for Lyme disease is made up of at least 20 genotypes within the Lyme disease complex.[11,12] Borrelia afzeli, Borrelia garinii, Borrelia bavariansis, Borrelia bergdorferi senses strict, and Borrelia spirmani are the five recognized species linked to Lyme disease in humans.[13] While other species such as Borrelia lusitaniae, Borrelia bissettii, and Borrelia valaisiana are hardly ever isolated from humans, their pathogenicity remains unclear. Borrelia bergdorferi, a typical spirochete, is motile, host-associated, and requires cultivation. The Borrelia genus is remarkable because of its highly unusual genome. It consists of a linear chromosome (approximately 910 kbp) and several linear and circular plasmids containing over 600 kbp of DNA.[7] The linear chromosomes in the Lyme disease spirochete are of similar size, approximately 910 kbp, as those found in the B31 strain.[14] Linear replicons have covalently closed telomeres.[11,15] Lipoprotein genes expressed in the outer membrane are mainly located on plasmids, while chromosomes contain most of the housekeeping genes in Borrelia. The plasmid diversity of Borrelia varies among strains and is a natural occurrence. Unique to Borrelia are several regions of its genome, such as a chromosome containing a single gene encoding 16S rRNA (rrs) separated from a pair of randomly repeated 23S (rrlA and rrlB) and 5S rRNA genes (rrfA and rrfB). This distinct rRNA gene organization is frequently used as a target for molecular analysis of Borrelia.[16] Ten different genomic groups related to the Borrelia burgdorferi sensu lato complex have been identified worldwide.[17-19] In Europe and Asia, various Borrelia groups have been identified, including Borrelia burgdorferi sensu stricto, Borrelia garinii, Borrelia garinii (type NT29), Borrelia afzelii, Borrelia valaisiana (group VS116), Borrelia lusitaniae (group PotiB2), Borrelia japonica, Borrelia tanukii, and Borrelia turdae. In the United States, Borrelia burgdorferi s.s., Borrelia andersonii (group DN127), 21038, CA55, and 25015 are also present. Borrelia japonica, discovered in Japan, is not known to be pathogenic to humans, while group VS116 (Borrelia valaisiana) has not been reported to have pathogenic potential.[20]

2- Pathogenesis

Borrelia burgdorferi causes Lyme disease, and failure of several systems leads in a wide range of clinical symptoms. Localized skin lesions are typically the first clinical signs of the disease, followed by Borrelia spread to other organs, early spreading erythema migrans (Lyme neuroborreliosis, Lyme carditis, erythema migrans multifocals, Borrellian lymphoma), or persistent sexually transmitted diseases (chronic Lyme arthritis, chronic acrodermatitis, late neurological symptoms).[13] Borrelia species induce a variety of clinical presentations, including Borrelia afzeli, which causes cutaneous symptoms, Borrelia garinii, which causes central nervous system diseases, and Borrelia bergdorferi sensu stricto, which causes Lyme arthritis.[13] Borrelia burgdorferi is primarily found in the midgut of

ticks. After being ingested by feeding ticks, the bacteria replicate and cause changes in gene expression, leading to changes in lipoprotein expression that increase colonization and chemotaxis. For instance, Borrelia burgdorferi expresses outer surface protein A (OspA) in the tick midgut. When ticks start feeding, OspA expression down-regulates and OspC expression up-regulates.[20] OspA enhances bacterial binding at the tick midgut OspA receptor, whereas OspC is a potential plasminogen receptor that plays a role in tissue colonization.[21] After approximately 36 hours, the bacteria migrate to the salivary glands and are transmitted to the host via saliva.[22] Host release and infection start in Borrelia burgdorferi are determined by migration and adhesion.[23,24] The bacteria can swim through the host matrix, penetrate between cells, and enter capillaries. Furthermore, Borrelia burgdorferi can colonize large joints, the heart, and other tissues of the host.[25-30]

3- Geographic distribution and gene bank

GenBank storage was used as a reference in Table 1 of the study.

4- Whole Genome-Based Genotyping

4-1- Whole Genome Based Restriction

To enable growth in Borrelia cultures, a large restriction fragment pattern (LRFP) is necessary. This involves whole-genome restriction analysis[31] using various restriction enzymes, such as MluI, ApaI, KspI, SmaI, and XhoI, to restrict genomic DNA. MluI-based constraints are typically used to determine the identification potential of Borrelia species.[32] In Lyme disease, periodic electrical changes are used to separate large DNA molecules, while pulsed-field gel electrophoresis separates restricted genomic DNA. MluI-based restriction enables the detection of Borrelia species and identifies subgroups within each species. The MluI-LRFP pattern observed in Borrelia afzelii isolates is fairly uniform, with the majority of isolates belonging to the Borrelia afzeli Mla1 subgroup (>99%), and the remaining isolates distributed among Borrelia afzeli Mla2, Mla3, and Mla4 subgroups.[31] Borrelia garinii and Borrelia burgdorferi sensu stricto isolates, on the other hand, have a diversified and limited pattern that is split into seven (Mlg1–7) and fifteen (Mlb1–15) subgroups, respectively.[31] These subgroups, which were linked to reservoir host, geographic location, and clinical presentation, showed differences in abundance. However, due to the approach required to study them, there are few available studies on this subject.[31] Borrelia valaisiana, Borrelia lusitaniae, and Borrelia spielmanii have two subgroups each and display a highly uniform restriction pattern. Although many strains were analyzed, there are still many strains that were not studied.[31]

Table 1. Geographic distribution and gene bank

Genospecies	Geographic origin	Accession No
B. burgdorferi sensu stricto	United States	*AY586362*
B. burgdorferi sensu stricto	United States	*AY586363*
B. burgdorferi sensu stricto	Holland	*AY586364*
B. burgdorferi sensu stricto	Switzerland	*AY586365*
B. burgdorferi sensu stricto	France	*AY586366*
B. burgdorferi sensu stricto	France	*AY586367*
B. burgdorferi sensu stricto	France	*AY586368*
B. burgdorferi sensu stricto	Switzerland	*AY586369*
B. garinii	Japan	*AY586370*
B. garinii	Japan	*AY586371*
B. garinii	Switzerland	*AY586372*
B. garinii	Germany	*AY586373*
B. garinii	Switzerland	*AY586374*
B. garinii	Japan	*AY586375*
B. garinii	Russia	*AY586376*
B. garinii	Holland	*AY586377*
B. afzelii	Switzerland	*AY586384*
B. afzelii	Denmark	*AY586383*
B. afzelii	Holland	*AY586384*
B. afzelii	Sweden	*AY586385*
B. afzelii	Germany	*AY586386*
B. lusitaniae	Portugal	*AY586378*
B. lusitaniae	Portugal	*AY586379*
B. lusitaniae	Portugal	*AY586380*
B. valaisiana	Switzerland	*AY586381*
B. valaisiana	England	*AY586382*
B. Burgdorferi sensu lato	United States	*AY586383*
B. japonica	Japan	*AY586387*
B. japonica	Japan	*AY586388*
B. bissettii	United States	*AY586389*
B. bissettii	United States	*AY586390*
B. bissettii	United States	*AY586391*
B. andersoni	United States	*AY586392*

4-2- Plasmid Analysis

To determine the specifications of plasmids in Borrelia culture, the gel insert method is utilized, which has been previously described. This involves extracting genomic borrelial DNA.[31-34] Typically, bacterial cells are embedded in agarose blocks at a density of 109 mL and then lysed using lysozyme and proteinase K.[31–34] Pulsed-field gel electrophoresis (PFGE) is used to separate chromosomal and plasmid DNA for 0.9–3 seconds with a run time of 37 hours.[35,36] To calculate the relative molecular size of a linearized plasmid, the appropriate pulse markers are employed. PFGE is only able to separate linear plasmids and may have difficulties with circular Borrelia plasmids. Based on the number and molecular weight of the plasmids in each cell, the linear plasmid profile of a specific strain can be determined.[37] The number and size of linearized plasmids vary among different Borrelia species.[38] The observation of several plasmids with identical molecular weights that only PCR could differentiate was observed. Furthermore, certain plasmids may be misplaced during extended periods of culture.[39] Plasmid characterization may become more difficult if the copy count of a particular plasmid is low and falls below PFGE sensitivity, rendering the plasmid difficult to detect and plasmid fingerprinting more challenging.[38] Several papers said that each strain harbored one large plasmid and numerous minor plasmids, whilst others stated that strains harbored multiple large plasmids or plasmid dimers.

5- Next Generation Sequencing

Next-generation sequencing allows for the sequencing of whole bacterial genomes from multiple isolates in a single day. However, one major limitation of this method, aside from the cost, is the lack of available data analysis and interpretation tools.[40–42] Several platforms, including 454 (FLX Titanium), Illumina (HiSeq, MiSeq, GA), SOLiD (4, 5500), Helicos, Ion Torrent, PacBio, and Starlight, are available for next-generation sequencing.

NCBI Genome4 currently has complete or substantially complete genome sequences for 42 Borrelia burgdorferi sensu stricto, 9 Borrelia afzelii, and 40 Borrelia garinii strains. In a study by Troy et al., next-generation sequencing was used to provide insight into the spread of Borrelia burgdorferi sensu stricto during infection.[43] The researchers used massively parallel sequences associated with transposon mutations to reveal the exact pathogenesis of Borrelia burgdorferi sensu stricto. The findings confirmed that Borrelia burgdorferi sensu stricto is a highly successful pathogen that can survive population bottlenecks at the inoculation site, causing widespread and long-term infection throughout the mammalian host.[43]

5-1- PCR-Based Typing

RFLP analysis by PCR permits amplification of either rrsrrlA (16S-23S) or rrfA-rrlB (5S-23S rRNA) spacers, followed by restriction endonuclease digestion and fragment analysis by dissecting gel electrophoresis.[44,45] This technique can be used to input cultured spirochetes (one-step PCR) or crude spirochetes from clinical or tick samples (nested PCR). Amplification of the rrfA-rrlB spacer results in an amplicon size of 225-266 bp. Two commonly used restriction enzymes in RFLP analysis are MseI and DraI. This method can be utilized to diagnose clinical specimens directly and to identify strains, reservoir hosts, or ticks.[45–51] Clarity of restriction digests can be visualized using gels containing 16% acrylamide - 0.8% bisacrylamide.[44] The rRNA spacer rrs-rrlA is amplified with a 941 bp amplicon size using nested PCR. Borrelia burgdorferi sensu stricto strains can be classified into three ribosomal spacer types using HinfI or Msel RFLP analysis: RST1, RST2, and RST3.[48] According to scientific literature, Borrelia burgdorferi sensu stricto RST type is correlated with virulence.[48-52] Patients infected with the RST1 strain showed a larger proportion of positive blood culture results, multiple erythematous migratory lesions, and more severe symptoms than those infected with Borrelia burgdorferi sensu stricto RST2 or RST3 isolates.[53]

5-2- Outer Surface Protein C (OspC) Analysis

The gene encoding OspC is essential for in vitro growth and is located in the single-copy circular plasmid, cp26.[53] OspC is a significant antigen in the humoral IgM immune response. It is responsible for causing infectious neck wounds in vertebrates and plays a crucial role in transmitting Borrelia from ticks to vertebrates.[54–57] Genotyping involves amplifying and sequencing a ~600 bp

region of the OspC gene.[58] This method groups Borrelia burgdorferi sensu lato strains into 21 different genetic variants. There is a correlation between different types of ribosomal spacers and OspC genotypes in Borrelia burgdorferi sensu stricto. Specifically, RST1 corresponds to OspC genotypes A and B, RST2 corresponds to OspC types F, H, K, and N, and RST3 corresponds to the remaining 10 OspC types including D, E, G, and I.[52,59-61] When RST and OspC genotypes of isolates are compared, we find that RST may miss changes within groups, but OspC genotypes may result in tiny groups. Both typing systems provide more information about clinical correlations. OspC and other outer surface proteins are variable and widely used in interspecies population studies.[62]

5-3-Real-time PCR and Melting Temperature Analysis

Real-time PCR, in conjunction with the determination of the melting temperature of target DNA amplification, is an effective method for identifying Borrelia. This is because a DNA fragment's melting temperature is determined by characteristics such as its nucleotide sequence, length, and GC concentration.[63,64] To detect bacteria on the surface of seeds, it is crucial to follow the correct protocol.

Various Borrelia genes, including hbb, p66, recA, and ospA, have been utilized to distinguish between different Borrelia species.[65-67] While differences in the hbb gene can distinguish most Borrelia species that cause Lyme disease, it is not possible to differentiate between Borrelia spirmani and Borrelia valaisiana using this method.[68]

5-4- Flagellin Based Typing

The flagellin gene, which is highly conserved and located on the chromosome, exhibits significant differences between various Borrelia bergdorferi sensurat species. Due to its easy accessibility, this gene is often employed for diagnostic purposes, with its diversity facilitating the identification of Borrelia species.[69,70]

Jaulhace et al. utilized an oligonucleotide typing method involving a PCR fragment of the flagellin gene to represent seven Borrelia species, including Borrelia garinii, Borrelia afzelii, Borrelia burgdorferi sensu stricto, Borrelia japonica, Borrelia andersoni, Borrelia valaisiana, and Borrelia bissettii.[71] Although this technique is essential for confirming the presence of Borrelia in clinical specimens, it is technically demanding.

6- Multilocus Sequence Typing

Urwin and Maiden first proposed multilocus sequence typing (MLST), which has since evolved to eliminate bias by amplifying, sequencing, and analyzing internal portions of housekeeping genes scattered throughout the genome.

MLST is a molecular typing tool utilized for population studies, epidemiological surveys, phylogenetic analysis, and evolutionary studies.[72,73] Within Borrelia, MLST schemes exist for spirochetes, including clpA (Clp protease subunit A), clpX (Clp protease subunit X), nifS (aminotransferase), pepX (dipeptidyl aminopeptidase), pyrG (CTP synthase), recG (DNA recombinase), rplB (50S ribosomal protein), and uvrA (exonuclease ABC).[74] The Borrelia MLST scheme is available on the MLST Network platform (http://www.mlst.net/). This method has proven useful in distinguishing between different strains of Borrelia garini and Borrelia bavariensis with regards to some epidemiological data, such as choice of host (birds and small mammals, respectively).[51] In summary, MLST represents an exciting alternative pathway for conducting Borrelia taxonomy as well as phylogenetic and ecological studies of spirochetes.

7- Diagnosis

The diagnosis of Lyme disease generally involves a combination of clinical evaluation, analysis for potential tick bites, and laboratory testing.[75]

7-1-Clinical Symptoms

The clinical manifestations of Lyme disease are diverse and may impact the skin, eyes, heart, musculoskeletal system, and nervous system [Table 2]. Due to these various symptoms, Lyme disease is frequently included in differential diagnoses.[76-78]

7-2- Lab Diagnosis

A microbiological diagnostic assay is typically necessary to confirm a Lyme disease diagnosis, except in cases where erythema migrans presents clinically.[79,80] For surveillance purposes, the CDC and the European Union Concerted Action on Lyme Borreliosis (EUCALB) have established a case definition of Lyme disease. This definition requires

either a physician-diagnosed erythema migrans with single lesions measuring at least 5 cm in diameter or at least one late joint, neurologic, or cardiac manifestation with laboratory confirmation (EUCALB, CDC).

Table 2. Main clinical manifestations of Lyme Boeerliosis[78]

Organ system	Clinical feature
Skin	Erythema Migrans Erythema Migrans Multiple Lesions Borrelial lymphocytoma Acrodermatitis Chronica Atrophicans
Nervous	System Meningitis Meningoencephalitis Meningo-Radiculoneuritis Encephalomyelitis Cerebral Vasculitis Pheripheral Neuropathy
Musculoskeletal	Arthritis Myositis
Heart	Carditis
Eye	Conjunctivitis, Endophtalmitis, Anophthalmitis

While not intended to be completely specific or sensitive for clinical diagnosis, this definition can serve as a basis for differential diagnosis and underscores the significance of laboratory testing, particularly in cases of extra cutaneous Lyme Borreliosis. Over the past several years, laboratory tests for the disease have seen significant advancements. Even so, clinical specimens, obtained through skin biopsy or cutaneous lavage specimens from Erythema migrans lesions and blood from patients with early-disseminated disease, remain the standard for diagnosis of Lyme disease.[76,77]

Secondary Erythema migrans lesions (nearly 90%), initial Erythema migrans lesions (50%), and substantial volumes of blood or plasma specimens (48%), have all been reported to be positive in patients with early Lyme Borreliosis.[78] Most detection assays for this disease amplify specific Borrelia burgdorferi using PCR nucleic acid sequences obtained from blood, CSF, joint fluid, or tissue biopsy samples. The advancement of conventional and nested PCR assays has aided in the improvement of detection rates, with detection techniques spanning from Southern hybridization and gel electrophoresis to real-time PCR. Both chromosomal targets and plasmid targets have been utilized, each with its own advantages. Plasmid targets, such as ospA, ospC, and vlsE, are present in multiple copies within each bacterium, making them more sensitive than single-copy chromosomal targets like fla, recA, rpoB, 16S and 23S ribosomal DNA, and rDNA intergenic spacers.

The availability of several specific sequences in databases makes it easy to select the best DNA sequences for amplification.[80] PCR can be used to confirm Erythema migrans lesions before serum antibodies appear and without the delay of culture isolation. Skin biopsy samples from patients with Erythema migrans have a high PCR detection rate of Borrelia, with a median sensitivity of around 70%, while joint samples from patients with Lyme arthritis (LA) have a median sensitivity of up to 80%.[80,81] However, a negative PCR test result cannot exclude Lyme disease. To obtain reliable and consistent PCR results, it is crucial to use the right methods to collect, transport, and prepare DNA from clinical samples, as the number of spirochetes in the infected tissues or bodily fluids of patients is typically very low.[80]

Contamination leading to false positive results is a limitation of methods for amplification of nucleic acids. In assays that require maximum sensitivity to diagnose infections caused by *Borrelia burgdorferi*, amplicon contamination is a significant issue.[82] Real-time PCR provides the advantage of monitoring the exponential phase of the reaction compared to conventional PCR, which can only identify the plateau phase. PCR provides quantitative information only during the few cycles when the amount of DNA climbs logarithmically from just above the background to the plateau; this typically corresponds to 4 to 5 cycles out of 40. However, identification of the log-linear region of PCR amplification is relatively easy since real-time PCR monitors the entire PCR reaction.[83]

The principle of real-time PCR has been utilized in various reports for qualitative diagnosis, but only one study has reported its application in the quantitative clinical diagnosis of a pathogen.[83] This study describes the development of a constant PCR measure for the precise quantitative recognition of Borrelia burgdorferi, which

provides an easy and reliable diagnostic tool for this bacterium. Furthermore, using the mouse Lyme disease model, it was shown that there are two different inbred strains of mice with different disease susceptibilities, i.e., antibiotic-treated mice and untreated mice.[84]

8- Compare of Clinical Diagnosis Tests Accuracy

8-1- Enzyme Immunoassays (EIA)

This study reviewed 23 separate studies that evaluated the effectiveness of first-tier serological tests, such as ELISA, for diagnosing Lyme disease with well-defined whole-cell targets. These tests included both FDA-authorized tests and in-house tests. The research found that the test performance was highly variable, with poor sensitivity for stage 1 Lyme disease, similar to the two-tiered tests. However, sensitivity improved as Lyme disease progressed. The overall specificity was also found to vary between studies and by test, more so than the two-tier tests.

To further analyze the variation between studies, the researchers evaluated 53 data lines specific to the ELISA function during the initial stages of Lyme disease. The Immunetics 1 C6 Borrelia burgdorferi ELISATM kit contained four data lines for three trials, while unlicensed C6 ELISAs contained seven data lines for four studies.

This study found that whether the C6 ELISA was authorized explained 27% of the heterogeneity among studies. Commercial ELISAs had slightly higher sensitivity (91% compared to 64%) and similar specificity (97% compared to 97%) over all stages of Lyme disease. One early Lyme disease whole-cell sonication (WCS) ELISA used ten strains from six investigations and three commercial test kits: the Lime Stat Test Kit, the VIDAS Lime Screen II, and the Wampole Bb ELISA test systems. These kits spanned six lines and three studies but performed differently than the four internal WCS ELISAs, and the authors did not provide an explanation for the divergent results.

Researchers conducted experiments on patients with early Lyme disease using recombinant and/or chimeric proteins from Osp A-F (primarily A and C) as targets. Sensitivities ranged from 0% to 86% and were based on in-house ELISAs with small sample sizes in each study.

In one study, PEG-peptide conjugates were successfully used in an ELISA assay, demonstrating 100% sensitivity and specificity in a small sample set.[85] Another test, the Borrelia burgdorferi strain B31 and B126 indirect hemagglutination antibody (IHA), has similar sensitivity (46%) but high specificity (98-99%) in detecting early Lyme illness.[86] However, the IHA test exhibited increased sensitivity and specificity for experiments used in later stages of the disease when compared to early Lyme disease results.

The Immunetics 1C6 Borrelia burgdorferi ELISATM outperformed various other ELISAs, including in-house C6 ELISAs, VIDAS Lyme Screen II, Wampole Bb (IgG/IgM) ELISA test system, and in-house ELISAs that used different recombinant and chimeric Osp targets, in a meta-regression controlled for the late stage of Lyme disease. However, the evaluation of reactivity in individuals previously vaccinated with the discontinued Osp A vaccine was not included in the meta-analyses. Furthermore, a WCS ELISA showed a false positive rate of 95%, while a recombinant Osp A ELISA showed a 5% false positive rate.[87]

8-2- Immunoblots Methods

Nine studies were conducted to compare clinical diagnoses of various Lyme diseases with several commercial Western blot tests. These tests included the Marblot test strip framework by MarDx, the B. burgdorferi western smear test kit by Boston Biomedica Inc., the Borrelia Spot Smudge Test by Immuno Speck, and the Borrelia burgdorferi B31 Virablot by Viramed Biotech. Additionally, the sensitivity of a few recombinant targets was examined using a single in-house immunoblot. In four further examinations, the effectiveness of the MarDx Lyme Sickness Marblot Strip Test Framework was evaluated among select lyme illness groups, including those with early or late-stage Lyme disease.[88-92] A meta-regression analysis revealed that in patients with Lyme disease, significantly better diagnostic tests were performed while controlling for the group. Interestingly, the evaluation of results for IgM, IgG, or both didn't significantly affect the sensitivity or specificity of the diagnostic tests. Two independent studies evaluated the BBI Western smear test

on a similar CDC test board. However, there was a slight difference in the classification criteria used. One study employed the BBI criteria, which required IgM to have 2+ bands of 23, 39, 41, and 83 kDa, while IgG needed 3+ bands of 20, 23, 31, 34, 35, 39, and 83 kDa. The formulation for positive samples was different when utilizing the CDC criteria (which need IgG with 5+ bands of 18, 23, 28, 30, 39, 41, 45, 58, 66, and 83-93 kDa, and IgM with 2+ bands of 23, 39, and 41 kDa). The CDC criteria had a sensitivity of 77% and 93% for IgM and IgG blots, respectively, while the BBI criteria had a specificity of 93% and 100% for IgM and IgG. Although the difference in specificity was not significant, there was a slight loss in specificity and a gain in sensitivity with the BBI criteria. In a study analyzing the General Biometric Inc. Borrelia Dot Blot IgG/IgM test, sensitivity increased by 50% (95% CI 19, 87) in stage 1, 70% in stage 2, and 100% in stage 3.[93] Another small study found that the Viramed Biotech Borrelia burgdorferi B31 IgG/IgM Virablot was as sensitive and specific as other immunoblots.[94] However, in a study evaluating a single in-house recombinant immunoblot, sensitivities ranged from 7% to 60% for various targets (data not shown), and the assay failed.

8-3. PCR Tests for Direct Detection

Six studies[95–100] investigated the isolation of Borrelia burgdorferi using culture and PCR techniques in various human samples from patients with early and disseminated Lyme disease. Unfortunately, no meta-analyses were available due to insufficient data for each location strategy. The Barbour-Stoner-Kelly (BSK) medium, which has been modified by some authors to improve its sensitivity, is the most commonly used medium.[101,102] In three studies, the sensitivity of this method was reported to be 27%, 71%, and 94% in isolating Borrelia burgdorferi from patients with early Lyme disease (stage 1).[30–32] However, the extremely high sensitivity reported in one of the studies suggests the possibility of laboratory contamination. Two investigations[97,98] found sensitivities ranging from 62% to 81% in biopsy samples from patients with early Lyme illness.

Phillips et al. evaluated an aMPMo medium for detecting Borrelia burgdorferi in the blood of Lyme disease patients who had previously been treated for the disease but had since relapsed, with both sample sizes being small.[103] In these patients, they reported a sensitivity of 91.5%; however, two studies failed to replicate these findings and both demonstrated that the BSK-H culture was superior.[102,104] Information on the use of PCR to identify Borrelia burgdorferi in early Lyme disease was gathered from three studies (eight lines of data), utilizing blood and tissue biopsies as samples, and various primers were the focus of each PCR.[99,100] Eshoo et al. established the Borrelia burgdorferi genotype using blood tests and multi-loci PCR with eight distinct loci, with a 62% awareness and a 100% specificity.[104] Liveris et al. used a nested PCR assay to detect Borrelia burgdorferi in serum and biopsy samples, reporting sensitivities of 40.6% and 42.6%, respectively.[95] They also demonstrated a qPCR with a sensitivity of 33.8% on plasma samples. Two nested PCR sets were explored in neurological lyme disease, focusing on the Osp A quality. Samples taken from the cerebral spinal fluid in both early and late cases had low sensitivity, even lower than the two-tier test regime, assays, or immunoblots reported for early lyme disease. Sensitivity was reported to be 37.5%–50% in acute cases and 12.5-25% in late cases.[100]

Conclusions

Molecular biology has emerged as a viable option for identifying and treating Lyme disease. With the abundance of scientific resources, modern technologies, accurate instructions, well-equipped laboratories, and skilled personnel in this field, molecular biology offers a viable diagnosis method for the disease. However, despite some promising results, there have been instances of false and weak outcomes with this method, indicating the need for further research and improvements to the process. Nevertheless, at present, molecular biology provides the most reliable and effective methods and tests for diagnosing Lyme disease.

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Competing interests

The authors declare that they have no competing interests.

Abbreviations

Centers for Disease Control and Prevention: CDC; Sodium dodecyl sulfate-polyacrylamide gel electrophoresis: SDS-PAGE; Outer surface proteins: OSPs; Restriction fragment length polymorphism: RFLP; Pulsed-field gel electrophoresis: PFGE; multilocus sequence typing: MLST; Lyme arthritis: LA; European Union Concerted Action on Lyme Borreliosis; EUCALB; Whole-cell sonication: WCS; Enzyme-linked immunosorbent assays: ELISA; Indirect hemagglutination antibody: IHA; Barbour-Stoner-Kelly: BSK.

Authors' contributions

All authors read and approved the final manuscript. All authors take responsibility for the integrity of the data and the accuracy of the data analysis.

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The study was conducted in accordance with the Declaration of Helsinki.

Consent for publication

By submitting this document, the authors declare their consent for the final accepted version of the manuscript to be considered for publication.

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