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**Diagnosing borreliosis**

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Abstract:	<p>Borrelia species fall into two groups, the <i>Borrelia burgdorferi</i> sensu lato (Bbsl) complex the cause of Lyme borreliosis (LB; also known as Lyme disease LD) and the relapsing fever group. Both groups exhibit inter- and intra-species diversity and thus, have variations in both clinical presentation and diagnostic approaches. A further layer of complexity is derived from the fact that ticks may carry multiple infectious agents and are able to transmit them to the host during blood feeding, with potential overlapping clinical manifestations. Besides this, pathogens like <i>Borrelia</i> have developed strategies to evade the host immune system, which allows them to persist within the host, including humans.</p> <p>Diagnostics can be applied at different times during the clinical course and utilise sample types, each with their own advantages and limitations. These differing methods should always be considered in conjunction with potential exposure and compatible clinical features. Throughout this review, we aim to explore different approaches providing the reader with an overview of methods appropriate for various situations. This review will cover human pathogenic members of Bbsl and relapsing fever borreliae, including newly recognised <i>B. miyamotoi</i> spirochetes.</p>

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4 **1 Diagnosing Borreliosis**

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5 22 **Introductory Remarks:**

6 23 *Borrelia* species fall into two groups, the *Borrelia burgdorferi* sensu lato (Bbsl)  
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8 24 complex the cause of Lyme borreliosis (LB; also known as Lyme disease LD) and  
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10 25 the relapsing fever group. Both groups exhibit inter- and intra-species diversity and  
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12 26 thus, have variations in both clinical presentation and diagnostic approaches. A  
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14 27 further layer of complexity is derived from the fact that ticks may carry multiple  
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16 28 infectious agents and are able to transmit them to the host during blood feeding, with  
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18 29 potential overlapping clinical manifestations. Besides this, pathogens like *Borrelia*  
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20 30 have developed strategies to evade the host immune system, which allows them to  
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22 31 persist within the host, including humans.  
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29 33 Diagnostics can be applied at different times during the clinical course and utilise  
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31 34 sample types, each with their own advantages and limitations. These differing  
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33 35 methods should always be considered in conjunction with potential exposure and  
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35 36 compatible clinical features. Throughout this review, we aim to explore different  
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37 37 approaches providing the reader with an overview of methods appropriate for various  
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39 38 situations. This review will cover human pathogenic members of Bbsl and relapsing  
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41 39 fever borreliae, including newly recognised *B. miyamotoi* spirochetes.  
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47 41 **Detection of *Borrelia* in the arthropod vector:**

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49 42 Various methods can be applied to detect the presence of *Borrelia* in vectors. Widely  
50  
51 43 used approaches that demonstrate significant sensitivity, specificity and reliability  
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53 44 include: multiple formats of PCRs, mostly nested PCR that target different genomic  
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55 45 loci, selection of which depends on the sample origin (template); reverse-line blotting  
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5 46 (RLB), based on hybridization of amplified selected *Borrelia* genes with spirochete-  
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7 47 specific probes; multilocus sequences analysis (MLSA) and multilocus sequence  
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9 48 typing (MLST), based on the sequence analysis of amplified fragments of spirochete  
10  
11 49 genome or microscopy with stained spirochetes in tick midgut or salivary glands  
12  
13 50 (Aguero-Rosenfeld, et al. 2005, Margos, et al. 2011). The most recently applied  
14  
15 51 techniques include next generation sequencing (NGS) and proteomic approaches.  
16  
17 52 Cultivation of *Borrelia* in commercial BSK (Barbour-Stoenner-Kelly) or home-made  
18  
19 53 MKP (modified Kelly- Pettenkoffer) media, that for a long time considered to be a  
20  
21 54 gold standard in LB diagnostics, is still widely used, but is rather time consuming and  
22  
23 55 challenging. The culture negative cases do not necessarily mean the absence of  
24  
25 56 spirochetes in a sample. The failure to culture the spirochetes might be caused by  
26  
27 57 multiple vector-, spirochete,- media- or cultivation conditions related factors (Cerar,  
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29 58 et al., 2008, Ružić-Sabljić, et al. 2014, Rudenko, et al., 2016). Nowadays, the priority  
30  
31 59 of all used techniques is re-directed form simple detection of pathogen in either  
32  
33 60 environmental sample or clinical sample, to simultaneous detection and identification  
34  
35 61 of spirochete species (or possible co-infection agents). Considering the high  
36  
37 62 possibility of the presence of multiple pathogens in tick vectors, the other question is  
38  
39 63 whether to use singleplex or multiplex formats for their detection/identification. Fluidic  
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41 64 microarrays allow the assessment of multiple tick-borne pathogens simultaneously  
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43 65 (Vayssier-Taussat, et al. 2013).  
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51 67 Use of proteomic methods to detect presence of some relapsing fever *Borrelia* in the  
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53 68 hemolymph of ticks provides additional options for borrelial detection in vectors  
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55 69 (Fotso Fotso, et al. 2014).  
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4 70 These methods provide invaluable research tools and facilitate epidemiological  
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6 71 studies, but their clinical relevance is debatable. Detection of a pathogen in the  
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8 72 vector does not imply that it has been successfully transmitted to the host upon  
9  
10 73 which the tick has fed. Transmission dynamics are complex and multi factorial and  
11  
12 74 beyond the scope of this review. Home use diagnostic kits are available and allow  
13  
14 75 individuals to test collected ticks for the presence of Lyme borreliae. The reliability of  
15  
16 76 these tests has been highly debated. Tick bites are frequently unnoticed and might  
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18 77 only demonstrate that you have been in a risk environment, but do not necessarily  
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20 78 correlate with any infectious consequences. That is why use of such tests is of  
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22 79 limited value for diagnosis, but can be useful for epidemiological studies.  
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29 81 Recommendation:

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31 82 Tick testing as supportive data for identification of LB endemic regions; correct  
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33 83 selection of PCR target based on the final goal of tests and sample nature; re-  
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35 84 analysis of tested sample targeting different genomic loci; consider the presence of  
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37 85 co-infection with multiple pathogens as highly possible.  
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### 42 87 **Clinical diagnosis of Lyme borreliosis and supportive diagnostic strategies**

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45 88 A reliable clinical diagnosis of LB is only evident to the non-expert physician when a  
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47 89 typical erythema migrans (EM) is present (Stanek and Strle 2003). Since the large  
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49 90 majority of LB symptoms have minimal diagnostic value because of their lack of  
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51 91 specificity, diagnosis of LB might be challenging for general practitioners in patients  
52  
53 92 without EM (Strle and Stanek 2009). Generally there exists a tendency towards  
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55 93 overdiagnosis of chronic Lyme disease (Czupryna, et al. 2016, Koedel, et al.  
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4 94 2015, Sigal 1996). Although different diagnostic approaches (mentioned later) have  
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6 95 been explored, to date the only recommended supportive tests used are serological  
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8 96 confirmation. Serological results alone are insufficient to distinguish whether the  
9  
10 97 patient suffers from an acute or re-infection that needs treatment, or is only  
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12 98 seropositive because of a past infection. This might be especially problematic for  
13  
14 99 individuals that are frequently exposed to ticks and therefore have at high risk of re-  
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16 100 infection. However even in low risk areas, the positive predictive value of serological  
17  
18 101 tests can be very low (Lantos, et al. 2015), meaning that clinical manifestations still  
19  
20 102 remain crucially important criteria for a reliable diagnosis of the disease. Factors that  
21  
22 103 need to be integrated for a reliable diagnosis are therefore the occurrence of  
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24 104 compatible symptoms, serological results and risk of tick exposure. Figure 1 provides  
25  
26 105 a diagnostic overview for LB.  
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33 107 **To date only serological tests are recommended to support the diagnosis of**  
34  
35 108 **Lyme borreliosis in the absence of EM**

36  
37 109 In cases where EM is clearly evident, serological tests are not needed and treatment  
38  
39 110 should start immediately (Stanek, et al. 2012). In patients who do not develop EM,  
40  
41 111 serological tests are recommended to support the diagnosis (Aguero-Rosenfeld, et  
42  
43 112 al. 2005). Initial problems with the specificity and sensitivity of serological tests have  
44  
45 113 resulted in controversial statements on their efficacy to support diagnosis of acute  
46  
47 114 LB. Recently, serological tests have been optimized switching from a single *Borrelia*  
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49 115 strain cell extract to a use of combination of more precisely chosen recombinant  
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51 116 antigens or synthetic peptides (Fang Ting, et al. 2000, Goettner, et al. 2005).  
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53 117 Previously a two-tier test approach, in which the presence of antibodies is first tested  
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4 118 by a highly sensitive ELISA and, in case of a positive result, further confirmed by a  
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6 119 highly specific immunoblot, was recommended (Branda, et al. 2010, Koedel, et al.  
7  
8 120 2015). Noteworthy, the reported accuracy of ELISAs and immunoblots varies  
9  
10 121 throughout Europe and a recent study revealed no overall benefit of two-tiered tests  
11  
12 122 over single tests (Leeflang, et al. 2016). Only early stage patients (symptoms <6  
13  
14 123 weeks) might still be seronegative, as they have not developed antibodies yet.  
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16 124 Therefore, diagnosis of LB should be re-evaluated in seronegative late stage  
17  
18 125 patients (Stanek, et al. 2012). Low antibody titers have been observed after antibiotic  
19  
20 126 treatment indicating that the induced B-cell immune response is probably not very  
21  
22 127 long-lived and robust. Especially patients where *Borrelia* took longer to disseminate  
23  
24 128 seem to develop long-lived antibody titers less efficiently (Aguero-Rosenfeld, et al.  
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26 129 1996, Elsner, et al. 2015, Hammers-Berggren, et al. 1994, Nowakowski, et al. 2003).  
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28 130 Recent mouse studies have shown that *Borrelia* have a direct effect on the mouse B-  
29  
30 131 cell response (Elsner, et al. 2015, Elsner, et al. 2015, Hastey, et al. 2012, Hastey, et  
31  
32 132 al. 2014). However, the underlying mechanism in humans requires further  
33  
34 133 investigation. Showing the induction of strain specific immunity (not non-  
35  
36 134 crossprotective), mouse and human studies together (Khatchikian, et al. 2014) may  
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38 135 explain reinfection of LB. Consequently, previous *Borrelia* infections must be taken  
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40 136 into account when considering serological testing (Nadelman and Wormser 2007).  
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138 Despite the described improvement of these tests, we still face the problem of non-  
139 standardization and inappropriate application of current serological tests (Ang, et al.  
140 2011, Leeflang, et al. 2016, Markowicz, et al. 2015, Muller, et al. 2012). Different (in-  
141 house) assays and result interpretation remain a major problem (Fallon, et al. 2014)



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4 142 that should be solved in the future by the implementation of a universal and  
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6 143 worldwide (or Europe/USA wide) diagnostic standard test, or as a minimum, use of  
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8 144 internationally agreed standards and participation in quality control schemes.  
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10 145 However, the problem remains (especially amongst high risk groups) to distinguish  
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12 146 between an acute and a resolved infection. Future studies should therefore focus on  
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14 147 the development of new strategies that would allow a yes or no result.  
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20 149 Noteworthy, serological tests should not be used as a proof of efficacy of the  
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22 150 antibiotic treatment, although antibody titers generally decrease after antibiotic  
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24 151 treatment, however patients may remain seropositive for years after the infection in  
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26 152 the absence of active disease (Aguero-Rosenfeld, et al. 1996,Glatz, et al.  
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28 153 2006,Hammers-Berggren, et al. 1994,Kalish, et al. 2001,Kowalski, et al.  
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30 154 2010,Lomholt, et al. 2000). Instead, the disappearance of symptoms is a more  
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32 155 reliable sign of cure.  
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38 157 When neuroborreliosis is suspected, detection of intrathecally produced anti-*Borrelia*  
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40 158 antibodies significantly supports the diagnosis. However, results might be negative at  
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42 159 early stages and more often in children (Christen, et al. 1993). Measurement of  
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44 160 *Borrelia*-specific antibodies in CSF cannot be used to assess the efficiency of  
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46 161 treatment (Koedel, et al. 2015).  
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51 163 Since antibiotic treatment is generally considered efficient, differential diagnosis is  
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53 164 crucial in case of a chronic course of the disease (Halperin 2015,Halperin  
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55 165 2016,Hjetland, et al. 2015,Markowicz, et al. 2015,Rebman, et al. 2015,Wills, et al.  
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4 166 2016). A chronic course has been observed in patients infected by *Borrelia*, viral and  
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6 167 non-viral pathogens, such as Epstein-Barr virus (glandular fever), *Coxiella burnetii*  
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8 168 (Q fever), or Ross River virus (epidemic polyarthritis) (Aucott, et al. 2013, Galbraith,  
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10 169 et al. 2011, Hickie, et al. 2006, Katz and Jason 2013) and the underlying causes are  
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12 170 not clear. In this context, also the general health status and/or the lifestyle of the  
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14 171 patient should be considered. In general, immunocompromised or otherwise not  
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16 172 completely healthy patients might be at higher risk to develop chronic symptoms  
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18 173 after treatment. Patients with hematological malignancies for example seem to suffer  
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20 174 more often from disseminated disease and more frequently require retreatment  
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22 175 (Maraspin, et al. 2015). In non-immunocompromised cases, where symptoms  
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24 176 continue to persist even after appropriate antibiotics treatment, it is currently not  
25  
26 177 recommended to prolong the treatment. Clinical studies have shown that the risk of  
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28 178 side effects outweighs any potential therapeutic benefits (Klempner, et al.  
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30 179 2001, Koedel, et al. 2015, Krupp, et al. 2003). In these cases, co-infections with other  
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32 180 tick borne diseases or other possible causes of the symptoms should be excluded  
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34 181 (Belongia 2002, Berghoff 2012, Godar, et al. 2015, Swanson, et al. 2006) and  
35  
36 182 symptomatic treatment considered (Koedel, et al. 2015). Only in late  
37  
38 183 neuroborreliosis, is prolongation of the antibiotic treatment justifiable in cases of  
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40 184 persistent cerebrospinal fluid (CSF) lymphocytic pleocytosis (Koedel, et al. 2015).  
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48 186 In rare cases, *Borrelia* can cause problems with the heart and vascular system and  
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50 187 might be considered as underlying cause of stroke-like symptoms in patients which  
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52 188 otherwise have no obvious risk for cardiovascular diseases (Allen and Jungbluth  
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54 189 2016, Zajkowska, et al. 2015). Full description of LB clinical manifestations and their  
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4 190 diagnosis have been recently reviewed by Stanek and co-workers (Stanek, et al.  
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6 191 2011).

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11 193 When encountering a tick bite, correct and early removal of the tick is a good way to  
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13 194 reduce probability of infection. In Europe, only about 2% (Wilhelmsson, et al. 2016)  
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15 195 and in USA, about 1% (Heymann and Ellis 2012) of patients bitten by a tick develop  
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17 196 LB. Detection of spirochete DNA in ticks alone does not necessarily means the  
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19 197 succesful pathogen transmission, which is why the value of this test has limited  
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21 198 diagnostic value for LB ((ESGBOR) 2013), but is useful for epidemiological studies  
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23 199 (Reye, et al. 2010) to define risk areas. In this context, next generation sequencing  
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25 200 is a new emerging technique that allows screening of the same tick in parallel for  
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27 201 various tick-borne pathogens, with the potential of getting more detailed information  
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29 202 about co-infections of ticks and identification of new yet unrecognised pathogens  
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31 203 (Michelet, et al. 2014,Vayssier-Taussat, et al. 2013). As transmission of *Borrelia* (and  
32  
33 204 indeed other pathogens) depends on the length of tick attachment, measurement of  
34  
35 205 scutal and coxial indexes can indicate duration of attachment (Crippa, et al.  
36  
37 206 2002,Gray, et al. 2005,Kahl, et al. 1998,Meiners, et al. 2006,Tijssse-Klasen, et al.  
38  
39 207 2011). In the absence of an EM and the presence of other LB related symptoms,  
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41 208 seroconversion can be used for supportive diagnosis. However, in the absence of  
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43 209 symptoms, seroconversion is no indication for antibiotic treatment as a study in a  
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45 210 Swiss risk group demonstrated that only 2% of patients who seroconverted  
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47 211 developed clinical LB (Fahrer, et al. 1991). Thus, as tick bite is a poor predictor of  
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49 212 disease, treatment is advisable only upon appearance of LB symptoms.  
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4 214 Recommendation:

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6 215 Clinical diagnosis alone, given a history of potential exposure and presence of EM,  
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8 216 can be sufficient, however clinical interpretation should generally be made in  
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10 217 conjunction with supporting laboratory findings to reach a reliable diagnosis.  
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16 219 **Alternative strategies explored for the diagnosis of Lyme borreliosis but not**  
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18 220 **on the list of recommended tests (ECDC 2016)**  
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21 221 Direct detection of *Borrelia* in the peripheral blood, other body fluids or tissues by  
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23 222 microscopy or molecular methods can be used as strong additional evidence in the  
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25 223 diagnosis of LB, but might have limited significance when used alone (Aguero-  
26  
27 224 Rosenfeld, et al. 2005). The sensitivity of PCR on skin biopsies is significantly higher  
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29 225 than some other molecular tools, however, recognition of the EM itself is the best  
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31 226 diagnosis for LB (Aguero-Rosenfeld, et al. 2005), nevertheless this provides useful  
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33 227 research data regarding strain prevalence, virulence and provides insights into  
34  
35 228 deciphering pathogenesis of LB (Strle, et al. 2013). Cultivation of *Borrelia* from  
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37 229 patient samples might be an alternative method to detect viable *Borrelia*, but is both  
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39 230 time consuming and challenging (Rudenko, et al. 2016). As such, cultivation is best  
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41 231 reserved as a research tool.  
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47 233 Lymphocyte transformation tests (LTT) have been explored for their potential to  
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49 234 overcome the diagnostic gap in LB patients without EM but before seroconversion  
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51 235 and in re-infected seropositive patients. This assay measures lymphocyte  
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53 236 proliferation *in vitro* after stimulation with *B. burgdorferi* specific antigens. Currently,  
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4 237 results are contradictory and consequently LTT is not recommended as a routine  
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6 238 diagnostic tool (Dessau, et al. 2014, Mygland, et al. 2010). T-cell ELISPOT is another  
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8 239 *in vitro* stimulation assay currently explored and improved (Jin, et al. 2013). More  
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10 240 direct methods measuring peripheral blood levels of specific cell subpopulations  
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12 241 (CD57+ cells (Marques, et al. 2009) or antigen-reactive cells (Tario, et al. 2015) by  
13  
14 242 flow cytometry, direct measure of CXCL13 levels in the CSF or metabolites within  
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16 243 serum (Molins, et al. 2015) are also not at a point yet to be used reliably for clinical  
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18 244 diagnosis. CD57 cell counts seem not to be reliable as a validation study found no  
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20 245 difference between patients and healthy controls (Marques, et al. 2009).  
21  
22 246 Demonstration of CSF CXCL13 as an activation marker is not specific for LB, its  
23  
24 247 absence is believed to have some value in excluding neuroborreliosis (Rupprecht, et  
25  
26 248 al. 2014) and it might become a valuable supportive tool to estimate treatment  
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28 249 efficiency in case of neuroborreliosis (Koedel, et al. 2015, Schmidt, et al. 2011, Senel,  
29  
30 250 et al. 2010). Problems with HLA types and identification of epitopes for antigen-  
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32 251 specific T-cell staining are challenges that need to be addressed to validate the  
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34 252 potential of *Borrelia* specific T-cell counts in peripheral blood to support diagnosis of  
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36 253 LB. Metabolite measurement is a future strategy under investigation but needs  
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38 254 further validation.  
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46 256 Generally, the detection of *Borrelia* DNA within ticks as well as other methods  
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48 257 discussed above should be considered as valuable research tools providing useful  
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50 258 information about the epidemiology of tick-borne diseases in general and LB  
51  
52 259 particularly. As with serological methods, their value is lower when used alone.  
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55 260 Combination of diagnostics, clinical and molecular tests provides a more robust and  
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4 261 timely diagnosis of disease. In any case, interpretation of tests results and clinical  
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6 262 diagnosis of LB remains controversial and should currently be restricted to experts.  
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10 264 Development and application of new molecular tools allow the detection and  
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12 265 differentiation among Lyme borreliosis or relapsing fever spirochetes, clearly  
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14 266 separating *B. burgdorferi* sensu lato spirochetes from recently described *B.*  
15  
16 267 *miyamotoi* (Margos, et al. 2008, Rudenko, et al. 2009, Venczel, et al. 2015).  
17  
18 268 Combination of multilocus PCR with electro spray ionisation and mass spectrometry  
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20 269 has recently been investigated for the detection and genotyping of *Borrelia* species  
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22 270 in whole blood (Eshoo, et al. 2012).  
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29 272 Recommendation:

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31 273 These tests are valuable research tools providing useful information about the  
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33 274 patient's immune response, but interpretation for clinical diagnosis has not been  
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35 275 clearly shown and should currently be restricted to specialised laboratories.  
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40 277 ***Diagnostics within symptomatic animals:***

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42 278 Veterinary infections are less well documented and benefit from laboratory  
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44 279 confirmation to ensure correct diagnosis. This is particularly important as EM lesions  
45  
46 280 have not been reported in animals and clinical signs are often common to several  
47  
48 281 pathologies. As for human cases, serology is the primary diagnostic approach used,  
49  
50 282 sometimes supported by use of PCR. Despite the absence of EM, cardiac,  
51  
52 283 neurological signs and lameness have been reported amongst companion animals  
53  
54 284 (Agudelo, et al. 2011, Hovius, et al. 1999, Krupka and Straubinger 2010). Most  
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4 285 veterinary cases have focussed upon lameness in dogs with positive serology,  
5  
6 286 though this does not necessarily establish borrelial causality for this condition. Rapid  
7  
8  
9 287 immunochematographic tests are often used in veterinary private practice to aid  
10  
11 288 diagnosis, however these assays have not necessarily undergone the rigorous  
12  
13 289 quality control applied to human serodiagnostic tests (Savić, et al. 2010).  
14  
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16 290

17  
18 291 **Relapsing fever diagnostics:**

19  
20 292 *Clinical diagnosis of relapsing fever infections:*

21  
22 293 In general, the clinical presentation of relapsing fever borreliosis is significantly  
23  
24 294 distinct from that of LB. The possible exception to this being the appearance of a  
25  
26 295 skin rash that challenges the previously believed “pathognomonic” EM, caused by  
27  
28 296 the borrelial agent carried by *Amblyomma americanum* ticks in the United States,  
29  
30 297 known as STARI (Borchers, et al. 2015, Masters, et al. 2008).  
31  
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33 298

34  
35 299 Human infection by recently described *B. miyamotoi* usually results in fever and  
36  
37 300 associated flu-like signs (headache, chills, fatigue, myalgia), occasionally with  
38  
39 301 neurological complications such as meningoencephalitis (Fonville, et al.  
40  
41 302 2014, Krause, et al. 2015).  
42  
43

44 303

45  
46 304 Relapsing fever, as its name suggests, results in relapsing febrile episodes  
47  
48 305 interspersed by afebrile periods. This is often accompanied by jaundice, muscle  
49  
50 306 pain, headaches and sometimes involvement of major organs (Borgnolo, et al.  
51  
52 307 1993). This clinical picture can often be mistaken for other infections such as malaria  
53  
54 308 that tend to overlap geographically in many endemic regions (Lundqvist, et al. 2010).  
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309

310 *Laboratory diagnostics for relapsing fever:*311 **Microscopy**

312 Though for LB, microscopy is not suitably sensitive for detection, this has been the  
313 diagnostic gold standard for detection of many relapsing fever spirochetes. Darkfield  
314 examination of unstained wet-preparations, Giemsa or silver-stained blood or tissue  
315 sections, or immunofluorescence methods have been successfully used. Despite its  
316 frequent use, even relapsing fever can be difficult to detect using microscopy with  
317 some species such as *B. crocidurae* typically producing lower blood burdens than  
318 others, like *B. duttonii*. For such cases, a centrifugation step to concentrate the  
319 sample can be beneficial (Larsson and Bergström 2008). Furthermore, detection is  
320 restricted to times of febrile episodes when spirochetes are present at detectable  
321 levels. On a cautionary note, various artefacts can share the size and helical shape  
322 of spirochetes when viewed by darkfield microscopy, but tend not to show the typical  
323 gyrating spirochete-characteristic movement. Microscopy will not provide information  
324 regarding the infecting species.

325

326 Recommendation:

327 Microscopic methods lack both sensitivity and specificity, but can add value when  
328 used in conjunction with other methods. Sample concentration can offer distinct  
329 benefits.

330

331 **Cultivation**



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3  
4 332 Cultivation methods for detection of *Borrelia* have been particularly challenging and  
5  
6 333 some members of the genus being particularly refractory to cultivation (Cutler, et al.  
7  
8 334 1994) whilst others are cultivable, but only in complex medium. Huge advances were  
9  
10 335 made with the formulation of BSK medium with a commercial variant BSK-H  
11  
12 336 supporting the growth of LB strains (Barbour 1984). Relapsing fever strains appear  
13  
14 337 more diverse in their requirements. *Borrelia miyamotoi* for instance appears to prefer  
15  
16 338 MKP medium (Wagemakers, et al. 2014) or high serum concentrations (Margos, et  
17  
18 339 al. 2015). On a cautionary note, these preferences might reflect batch variations of  
19  
20 340 composite ingredients that can vastly influence performance of these “home-made”  
21  
22 341 media (Cutler personal observation). Collectively, cultivation should be considered a  
23  
24 342 low yield procedure, but vital for recovery of much-needed strains for research  
25  
26 343 purposes (Ružić-Sabljić, et al. 2014).

30  
31 344

32  
33 345 Animal inoculation or xenodiagnosis (allowing infected ticks to feed upon a test  
34  
35 346 animal) has been used for primary recovery of isolates prior to cultivation in axenic  
36  
37 347 medium (Naddaf, et al. 2015, Schwan, et al. 2012). It must be remembered that some  
38  
39 348 species are refractory to growth in most animal models, such as *B. recurrentis*.

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42  
43 350 Recommendation:

44  
45 351 Cultivation is low yield, time consuming and expensive and thus poorly suited to  
46  
47 352 support diagnosis. Nevertheless, it still has a vital role for recovery of isolates for  
48  
49 353 research purposes.

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53 355 *Serological diagnosis:*

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4 356 For the relapsing fever group, specific serology can be undertaken using GlpQ  
5  
6 357 protein as antigen. GlpQ is absent from LB species, thus facilitating it's specificity for  
7  
8 358 diagnostic purposes (Fritz, et al. 2013). Alternatively, BipA can also serve as a  
9  
10 359 differential antigen present in relapsing fever spirochetes, but absent from the LB  
11  
12 360 group (Lopez, et al. 2010). As acutely presenting patients may not yet have had  
13  
14 361 sufficient time for seroconversion, serology is best reserved for retrospective  
15  
16 362 diagnosis.  
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20 363

#### 21 22 364 **PCR**

23  
24 365 PCR provides a valuable diagnostic approach in acutely ill patients (Mediannikov, et  
25  
26 366 al. 2014). This overcomes the poor sensitivity of microscopy and can either be used  
27  
28 367 to diagnose relapsing fever borreliosis, or to further characterise the infecting  
29  
30 368 spirochete. The absence of *GlpQ* in LB species makes it a specific target for  
31  
32 369 detection of relapsing fever spirochetes (Takano, et al. 2014). Other assays can  
33  
34 370 either speciate specific relapsing fever borreliae or be designed to detect a single  
35  
36 371 member of the relapsing fever clade such as *B. miyamotoi* (Elbir, et al. 2013, Reiter,  
37  
38 372 et al. 2015). The limitation of this approach is having an appropriate sample that is  
39  
40 373 likely to contain spirochetal DNA. Blood collected during febrile episodes and CSF  
41  
42 374 samples have given good results (Gugliotta, et al. 2013). Furthermore, in highly  
43  
44 375 relapsing fever endemic areas, it is possible to have positive PCR results unrelated  
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46 376 to current clinical pathology (Cutler, et al. 2010).  
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53 378 Recommendation:  
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4 379 PCR can provide useful supporting information, but multiple available assays must  
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6 380 be properly standardised, and are hampered by sample timing, type and quality.  
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### 10 382 **Next Generation Sequencing**

11  
12 383 NGS offers huge potential and data has only recently been forthcoming limiting  
13  
14 384 comprehensive appraisal at this stage. With the exception of dermato-borreliosis,  
15  
16 385 here the challenge is which diagnostic sample type to investigate for LB in the  
17  
18 386 absence of focal lesions. Sensitivity can be further improved, especially amongst  
19  
20 387 high levels of host DNA. Care should be taken to avoid bias when using target  
21  
22 388 enhancement strategies to amplify low copy number targets. Data analysis  
23  
24 389 represents an additional computational challenge. NGS methods combined with  
25  
26 390 bioinformatics tools might overcome the limitations of culture-connected techniques  
27  
28 391 or of some molecular protocols. However, the extreme diversity of spirochetes from  
29  
30 392 *B. burgdorferi* sensu lato complex reduce the usefulness of NGS as it doesn't  
31  
32 393 differentiate between the pathogenic to human spirochete strains from those that  
33  
34 394 were never connected with human LB. Additionally this offers a means of assessing  
35  
36 395 rank abundance, evolving genomic profiles such as those corresponding to vector  
37  
38 396 adaptations (Gatzmann, et al. 2015) and fluctuations over time providing valuable  
39  
40 397 insights into host-microbial interactions (Strandh and Råberg 2015).  
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49 399 To date enrichment techniques can only partially overcome sensitivity problems  
50  
51 400 caused by the giant excess of host DNA (vector, endosymbiont and other microbial  
52  
53 401 DNA) compared to the low proportion of target DNA (borreliac DNA in ticks is <0.01%  
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55 402 of total DNA within field-collected nymphal ticks) (Carpi, et al. 2015). This can impact  
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4 403 upon successful detection with only about a third of infected ticks revealing positive  
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6 404 *Borrelia* NGS data (Carpi, et al. 2015).  
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10  
11 406 Recommendations:

12  
13 407 NGS offers huge potential and data has only recently been forthcoming limiting  
14  
15 408 comprehensive appraisal at this stage. Sensitivity can be further improved,  
16  
17 409 especially amongst high levels of host DNA. Care should be taken to avoid bias  
18  
19 410 when using target enhancement strategies to amplify low copy number targets. Data  
20  
21 411 analysis represents an additional computational challenge.  
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#### 25 26 413 **Fact sheets and resources**

27  
28 414 Several excellent fact sheets have been produced by ECDC to provide information  
29  
30 415 on LB and tick-borne relapsing fever. Furthermore, more specific resources can be  
31  
32 416 obtained from European study group for Lyme borreliosis (ESGBOR;  
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34 417 [www.escmid.org/research\\_projects/study\\_groups/esgbor/](http://www.escmid.org/research_projects/study_groups/esgbor/)).  
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#### 38 39 419 **Knowledge gaps and future perspectives**

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41 420 The poor sensitivity of direct detection methods coupled with the poor predictive  
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43 421 value of indirect serological methods, particularly in less typical clinical  
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45 422 presentations, presents a significant diagnostic challenge. Serology is further  
46  
47 423 challenged by the requirement for sufficient time in order for the host to produce  
48  
49 424 antibody responses to enable detection. Detection of the host response to infections  
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51 425 provides a particularly attractive prospect for LB where organism loads are typically  
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53 426 low. Indeed, levels of CXCL13 have shown promise for neuroborreliosis, but require  
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4 427 further validation (Schmidt, et al. 2011, Senel, et al. 2010). It is possible that  
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6 428 signature biomarker profiles might have value, but whether this would vary too much  
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8 429 between individuals or indeed with differing genetic variants of borreliae awaits  
9  
10 430 investigation. Another diagnostic approach under exploration is based on targeted  
11  
12 431 proteomics. By selected reaction monitoring mass spectrometry, specific *Borrelia*  
13  
14 432 proteins can be detected and quantified in skin biopsies (Schnell, et al. 2015). The  
15  
16 433 powerful new emerging technologies provide insights into our understanding of the  
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18 434 dynamic interactions of borreliae with their vector, host and other organisms, with the  
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20 435 possibility of disclosing opportunities for future intervention.  
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#### 27 437 **Concluding remarks**

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29 438 During these brief guidelines, we have attempted to highlight the strengths and  
30  
31 439 limitations of various diagnostic methods used to diagnose borrelial infection. No  
32  
33 440 single approach is suitably robust for this purpose, thus making interpretation  
34  
35 441 challenging. Furthermore, laboratory diagnostics need to be viewed in conjunction  
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37 442 with potential exposure and compatible clinical features.  
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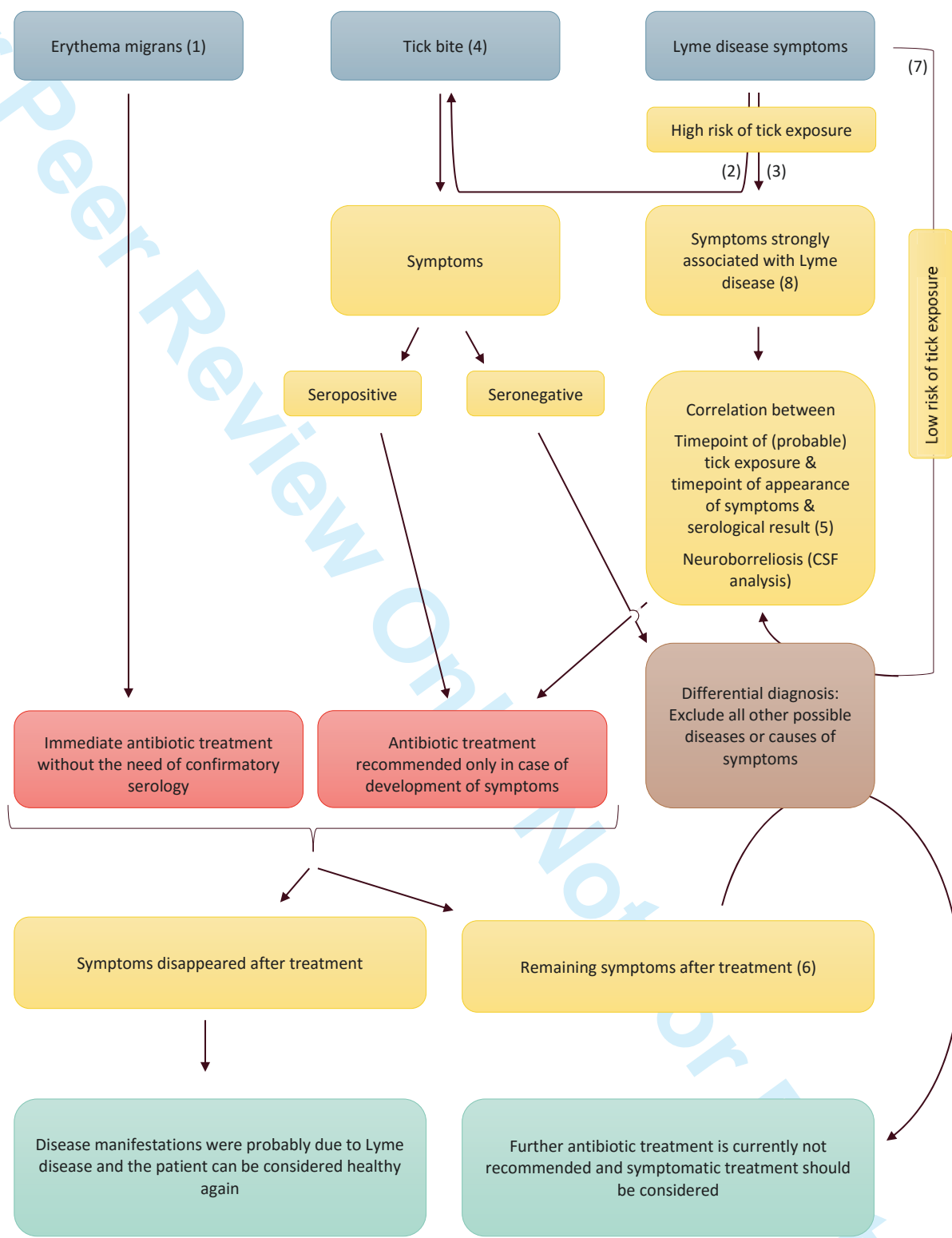
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3 **Figure 1. No diagnostic tests currently provide a yes or no result for acute**  
4 **Lyme borreliosis (LB) exist, thus clinical signs still remain the major factor for**  
5 **deciding whether antibiotic treatment is necessary.** In case of unclear  
6 symptoms, the risk of tick exposure and serological tests should be considered to  
7 support the diagnosis. Represented in blue are three possible scenarios for which LB  
8 should be considered: the patient presents with the characteristic skin manifestation  
9 erythema migrans (EM) or a recent tick bite. A third possibility is that the patient's  
10 symptoms might be compatible with LB. As can be readily deduced from this  
11 schematic representation (yellow: clinical decision), erythema migrans is the least  
12 complicated case and should be treated (red) immediately without need for further  
13 testing. The situation gets more complicated if the patient cannot remember a tick  
14 bite (which can occur in up to 2/3 of cases (Hofhuis, et al. 2013)) and/or has  
15 nonspecific symptoms. Green: Final outcome. **(1)** EM: Incubation time between 3  
16 days and 1 month. Red skin lesion that might in some cases be associated with  
17 slight itching or burning and that expands around the site of the tick bite. EM can be  
18 distinguished from a simple tick bite induced irritation of the skin by the fact that it  
19 has a minimum diameter of 5cm. Erythema migrans is often associated with non-  
20 specific symptoms like fatigue, headache, fever or malaise and can occur at different  
21 locations on the same patient (multiple erythema migrans) (Godar , et al. 2015) **(2)** In  
22 case a patient presents with symptoms that have been associated with, but are not  
23 clearly specific for Lyme disease, an assessment of the risk of prior tick exposure  
24 should be done. For this purpose the following questions might be considered: Does  
25 the patient pay attention to ticks? Did the patient maybe notice in the recent past an  
26 itching and scratched something small off from his body? Does the patient have pets  
27 which often have ticks? How much time does the patient spend outdoors in the  
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3 green? Has the patient recently been on holidays in a risk area? Season or weather  
4 conditions supporting high activity of ticks (might also be interesting to exclude other  
5 possible infections)? **(3)** Try to estimate based on the symptoms (early or late stage)  
6 the timepoint of infection and check if the season and/or weather conditions have  
7 been such that at the possible timepoint of infection ticks might have been active.  
8 Ticks are active during wet not too hot seasons of the year. For more information on  
9 factors affecting tick activity please refer to reference: (Medlock, et al. 2013). **(4)** If a  
10 patient shows up with a tick bite, appropriate and early removal of the tick can  
11 prevent transmission of Lyme disease, however since the transmission efficiency  
12 and kinetics depends on the *Borrelia* strain (Crippa , et al. 2002), an early  
13 transmission cannot reliably be excluded (Kahl , et al. 1998) and the patient should  
14 be monitored for the development of symptoms and treatment considered only if  
15 such appear. In case the tick has been damaged or removed late, a short-term  
16 prophylactic antibiotics (oral or cutaneous) treatment might be considered  
17 (Warshafsky, et al. 2010) (Piesman and Hojgaard 2012) (Piesman, et al. 2014).  
18 However due to the small time period during which this method is efficacious and  
19 due to the high number of patients that need to be treated for a successful outcome  
20 (Hofhuis , et al. 2013) controversial opinions exist on this procedure. **(5)** Please  
21 consider here the fact that patients are not necessarily protected after a first course  
22 of Lyme disease and re-infection can occur (Shapiro 2015) (Nadelman and Wormser  
23 2007) (Khatchikian , et al. 2014). In this case the interpretation of serological results  
24 might be complicated. **(6)** In case of persistent fluelike symptoms after appropriate  
25 treatment of erythema migrans, consider coinfections with other tick borne  
26 pathogens (Godar , et al. 2015). Make sure that treatment has been done in the  
27 correct way otherwise consider retreatment with appropriate method. In case of a  
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3 post treatment chronic course of Lyme disease other possible reasons for the  
4 symptoms should be excluded. **(7)** Make sure that the symptoms have only occurred  
5 after potential exposure to a tick bite and that they did not already exist before the  
6 tick exposure. In case of nonspecific disease manifestations, ask the patient if he  
7 might recall symptoms similar to erythema migrans in the past. **(8)** To have a better  
8 overview of the symptoms that are frequently associated with Lyme disease consult  
9 for example (Stanek , et al. 2012, Koedel , et al. 2015).  
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