The true Incidence of Lyme Disease in the UK

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Lyme borreliosis is an emerging zoonotic disease in the UK and of increasing concern to health authorities and to the public alike. Nationally reported Lyme borreliosis cases are significantly understated, partly because serology testing has notoriously low rates of sensitivity, and partly because reporting by diagnosing physicians in cases of *erythema migrans* (bull's-eye rash) is optional in the UK and, therefore, a negligible number of cases is actually reported. This report outlines an analysis of available data, and extrapolates an estimation of the likely true annual incidence of the disease.

The two national reference laboratories in the UK which test for Lyme borreliosis reported, in 2014, a total of 954 newly diagnosed cases. By extrapolating the percentage of false negatives resulting from the low sensitivity of the diagnostic tests, we have estimated that over 6,000 blood tests each year are reported, falsely, as having a negative result after serology testing by the NHS. By further extrapolating the percentage of cases diagnosed by *erythema migrans* which go unreported, we believe there are around 45,000 new cases of Lyme disease each year in the UK, which equates to an incidence rate of 69 per 100,000 of the population. This implies that one person in every 20 can expect to catch Lyme disease at one point in their lives. Up to a third of these may develop lifelong chronic illness.

If we believe the current 26% annual growth rate implicit in the Public Health England data, there will be over a million new cases of Lyme borreliosis per year by 2030. With the World Health Organisation and the European Centre for Disease Control, however, reporting an annual average growth rate in new cases of Lyme borreliosis Europe-wide as high as 65% for the last 20 years [15], we ask what are the social and economic implications of leaving such a large number of patients undiagnosed and untreated?

Introduction

Lyme borreliosis definition

Lyme borreliosis is the commonest vector-borne disease in the UK and considered an emerging zoonotic disease. It is the most common tick-borne infectious disease in the most regions worldwide. The causative agent, the spirochaete Borrelia burgdorferi, is transmitted to the host by infected ticks of the Ixodes genus. Lyme borreliosis is a multi-system disorder that is treatable with antibiotics, but may lead to a chronic inflammatory disease affecting multiple organ systems, including the neurological system, the heart, and the joints.

Lyme disease diagnosis

In the United Kingdom, a Lyme disease diagnosis is normally made on the basis either of the distinctive *erythema migrans* (the "bull's-eye rash") or of a positive result in both stages of two-tier blood testing (ELISA followed by western blot). Rarely, it can also be diagnosed by analysis of cerebrospinal fluid. When two-tier testing is carried out, the first step (ELISA testing) is performed by the nearest microbiology laboratory to the patient's place of residence. If positive, blood samples from patients in England, Wales and Northern Ireland are forwarded to the Rare and Imported Pathogens Laboratory (RIPL) in Porton Down, Salisbury, part of Public Health England (PHE), whilst samples from Scotland are forwarded to the National Lyme Borreliosis Testing laboratory (NLBTL), Raigmore Hospital, Inverness.

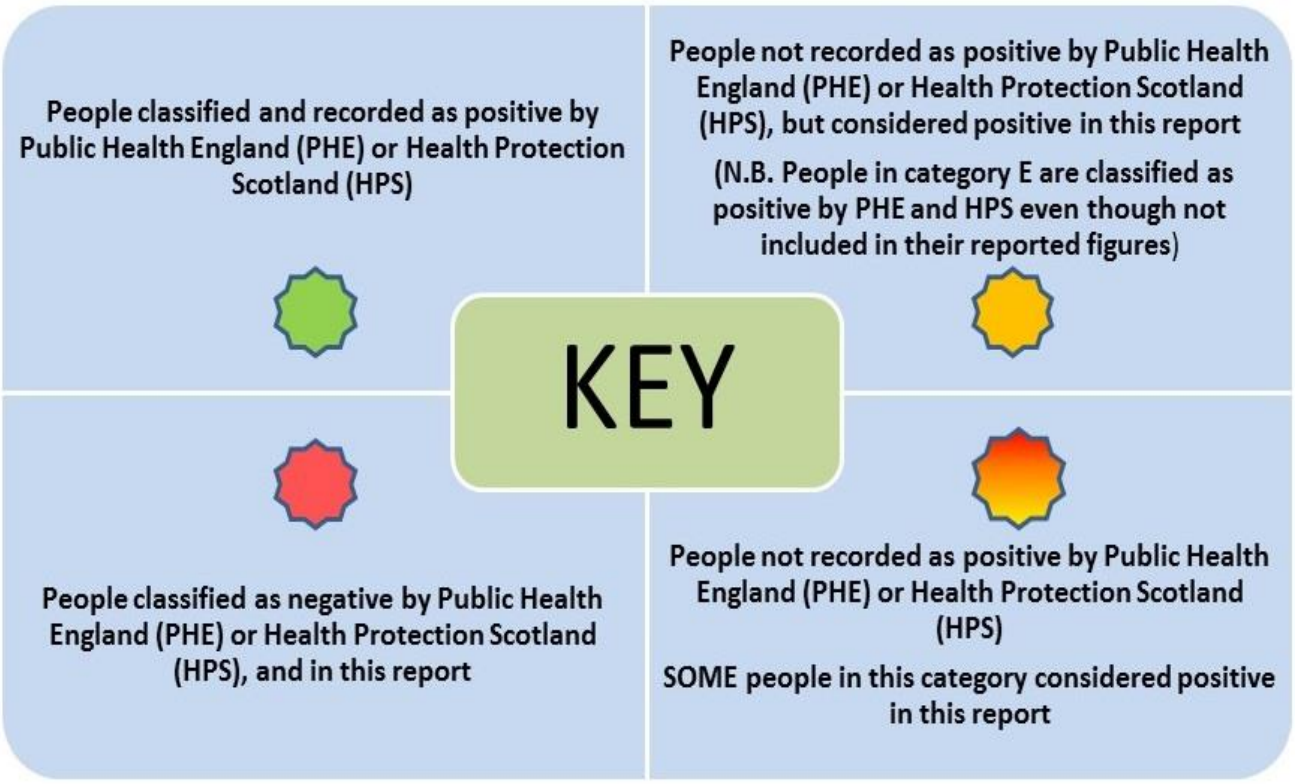
Lyme disease data capture

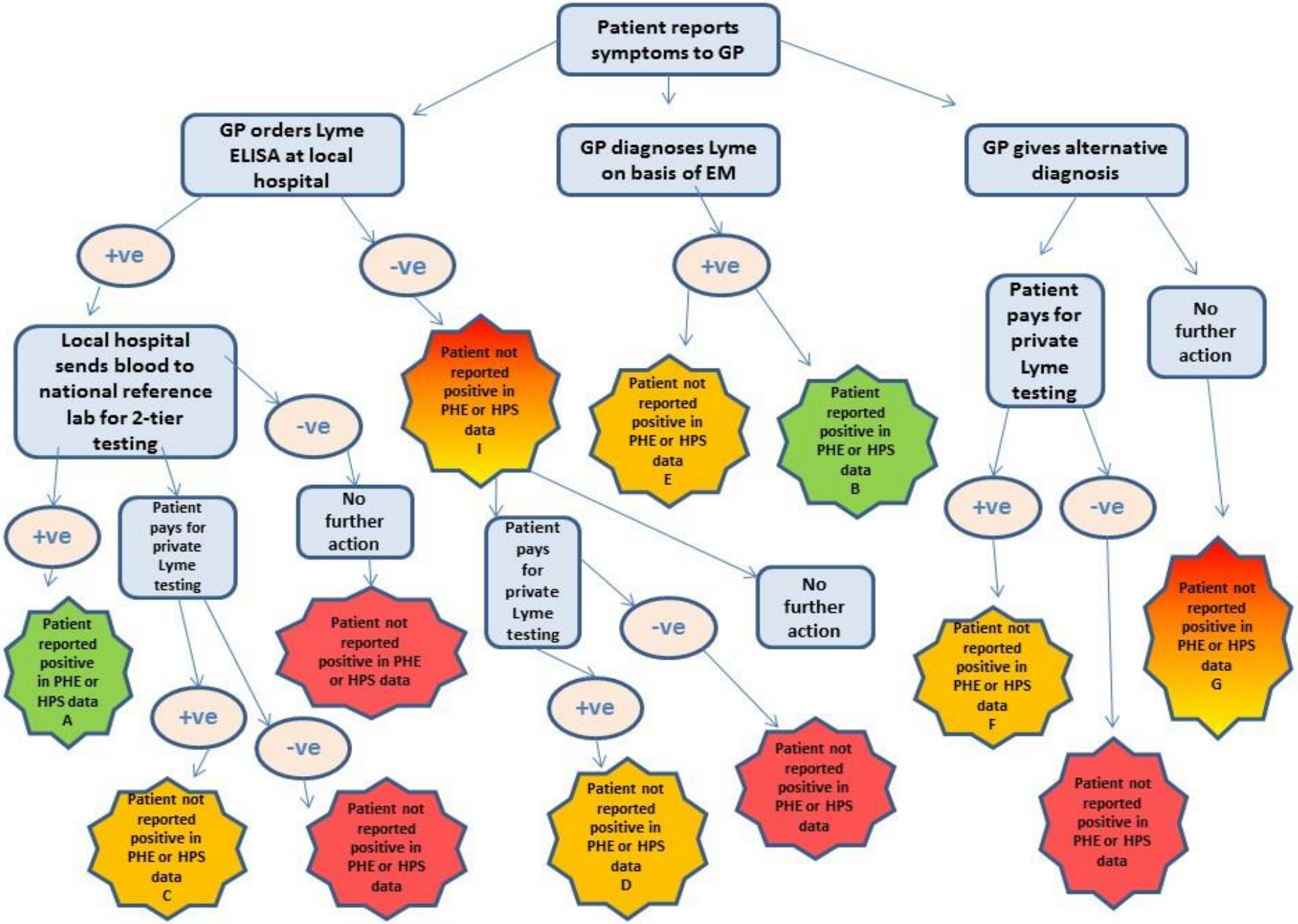
Cases of confirmed Lyme borreliosis in Wales, England and Northern Ireland are recorded by Public Health England (PHE), and confirmed cases in Scotland are recorded by Health Protection Scotland (HPS).

The most recent epidemiology report on Lyme disease from Public Health England was published in 2013. It clarifies the latest position regarding reporting, which has changed several times in recent years and thus makes analysis of data trends fraught with difficulty: 'Cases of Lyme borreliosis are not statutorily notifiable by medical practitioners in England, Wales and Northern Ireland.' In other words, reporting of diagnoses made on the basis of *erythema migrans* (data categories E and B in figure 1) is optional.

Reporting of ELISA blood test results to PHE is now obligatory: 'However, since October 2010 under the Health Protection (Notification) Regulations 2010, every microbiology laboratory (including those in the private sector) in England is required to notify all laboratory diagnoses of borreliosis to the Health Protection Agency (now Public Health England). Previously, reporting by laboratories was on a voluntary basis.' [Source: Lyme borreliosis epidemiology and surveillance, Published 1 May 2013, Public Health England: https://www.gov.uk/government/publications/lyme-borreliosis-epidemiology/lyme-borreliosis-epidemiology-and-surveillance]

Figure 1: Diagnostic pathways for UK Lyme borreliosis patients (with key)




Patient reports symptoms to GP
GP orders Lyme ELISA at local hospital
GP diagnoses Lyme on basis of EM
GP gives alternative diagnosis
+ve
-ve
+ve
Patient pays for private Lyme testing
No further action
Local hospital sends blood to national reference lab for 2-tier testing
-ve
Patient not reported positive in PHE or HPS data I
Patient not reported positive in PHE or HPS data E
Patient reported positive in PHE or HPS data B
+ve
-ve
No further action
+ve
Patient pays for private Lyme testing
Patient reported positive in PHE or HPS data A
No further action
Patient not reported positive in PHE or HPS data
Patient pays for private Lyme testing
-ve
No further action
+ve
-ve
Patient not reported positive in PHE or HPS data F
Patient not reported positive in PHE or HPS data G
+ve
-ve
Patient not reported positive in PHE or HPS data C
Patient not reported positive in PHE or HPS data
+ve
Patient not reported positive in PHE or HPS data D
Patient not reported positive in PHE or HPS data
Patient not reported positive in PHE or HPS data

Scotland is even more restrictive, not only in data gathering but even in samples testing. As Mavin, Watson and Evans state, 'There was a significant change in testing protocols in July 2012. ....in accordance with the British Infection Association position statement published in 2011, samples from patients with: a clear recent history of a tick bite with EM; tick bite only; or no clinical details, were no longer routinely tested.' This means that data from patients whose doctors do not provide case histories are excluded from Lyme disease records even if they manifest symptoms.

Analysis of diagnostic and reporting pathways

The steps in the UK patient's diagnostic pathway are shown in Figure 1.

Public Health England AND Health Protection Scotland capture two categories of patients with Lyme disease; those who test positive to two-tier testing, and those whose doctors optionally report their diagnosis on the basis of *erythema migrans*.

To estimate the true incidence of Lyme disease in the UK, we first need to assess the pathway to diagnosis (or non-diagnosis or mis-diagnosis) followed by UK patients. Figure 1 highlights in amber four data categories (denoted B, C, D and E) leading to diagnosis with Lyme disease whose patient numbers are not captured in PHE or HPS Lyme disease epidemiology data for UK patients. These patients are diagnosed by private laboratories (categories C, D and E), or by *erythema migrans* which is not reported by their doctor to PHE (category B), and this report aims to estimate as accurately as possible the total number of patients in these categories.

In addition to these patients, there are others who are not diagnosed at all and who are given an alternate diagnosis by their doctors. In the third part of this report, we discuss these numbers.

Part 1: National reference laboratories

Published figures based on positive two-tier serology testing

Public Health England has published complete annual figures on Lyme disease samples tested, and found positive, as in Figure 2.

Figure 2: Lyme borreliosis test results from RIPL

	Samples tested by RIPL for Lyme disease	Samples positive for Lyme disease
2013	12,299	878
2014	13,069	730

Thus, in the most recent published figures (2014), 730 western blot samples were found positive for Lyme disease, constituting 5.6% of the total of samples tested.

In Scotland, numbers sent to Health Protection Scotland (HPS) from the NLBTL have been reported and analysed more recently in a 2015 report by Mavin, Watson and Evans, *Distribution and presentation of Lyme borreliosis in Scotland – analysis of data from a national testing laboratory*. Additional information is buried in a report on the NLBTL website [http://www.hps.scot.nhs.uk/ewr/article.aspx#images] .

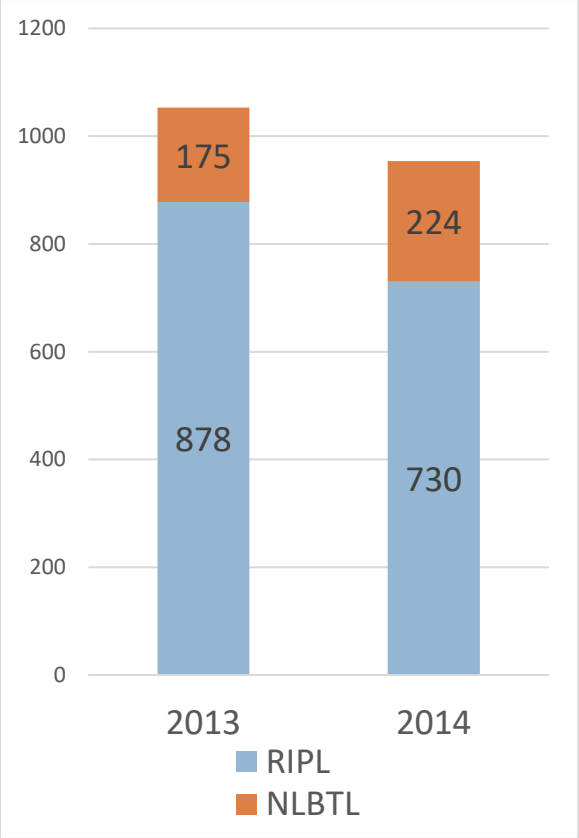
Figure 3: Lyme borreliosis testing results from NLBTL

	Samples tested by NLBTL for Lyme disease	Samples positive for Lyme disease
2013	4630	175
2014	Not published	224

These figures reveal that the 175 samples found positive for Lyme disease in 2013 were just 3.8% of the total tested, whilst total samples tested in 2014 have not been published.

On the basis of these figures, aggregated totals for nationally reported Lyme disease diagnoses based on positive blood tests in the United Kingdom were 1,053 in 2013 and 954 in 2014 (figure 4).

Figure 4: Samples found positive for Lyme borreliosis, UK, 2013 and 2014



A discussion and refinement of estimates of patients with false negative test results

The sensitivity and specificity of a diagnostic kit depends not only on the inherent qualities of the test kit, but the way the results are interpreted. Samples are considered positive if the result is positive in both the ELISA test and the immunoblot test. Even if automated and read consistently by a machine, the sensitivity of the readings can be calibrated differently from one laboratory to another. The interpretation of positive bands is a topic of debate and laboratories vary in their interpretation of which IgG and IgM bands they consider to indicate a positive result.

For these reasons, independent verifications of the sensitivity of the test kits used by RIPL and NLBTL can only be a rough approximation of the true performance of these tests. We have, however, used available research to make the most accurate assessment currently possible.

False negatives from RIPL

The Rare and Imported Pathogens Laboratory (RIPL) Elisa test for Borrelia burgdorferi, used as step one of two-tier testing, is the C6 ELISA made by Immunetics. In its official documentation (http://www.invitech.co.uk/downloads/instructions/CF-E601-801_C6_for_Europe_PI.pdf), Immunetics reports an assessment of its sensitivity as follows: 'The C6 Lyme ELISA™ Kit was evaluated in comparison with the Two-Tier protocol on the Lyme patient group. Overall, the C6 Lyme ELISA™ detected 74.9% of the Lyme patients, compared with 55.3% found positive by the Two-Tier protocol (Tables 2 and 3).' The sensitivity of the C6 Lyme ELISA was found to be 74.9%.

From this, one concludes that 25% of patients tested by the ELISA test at RIPL will be given a false negative result in the ELISA test.

The sensitivity and specificity of the ViraMed immunoblot test, which is used by RIPL as step two of the two-tier testing, has been evaluated and described in the research paper '2-tiered antibody testing for early and late Lyme disease using only an immunoglobulin G blot with the addition of a VlsE band as the second-tier test', by Branda JA, Aguero-Rosenfeld ME, Ferraro MJ, Johnson BJ, Wormser GP, Steere AC [17]. This research is posted on the ViraMed website as an independent assessment of their test kit.

The two-tier testing system (which is used at RIPL) was replicated using the ViraMed immunoblot by teams at Massachusetts General Hospital and Westchester Medical Center in the USA. The criteria for a positive serology result were the same as those required by the CDC: 'Although Western blots were performed on all serum samples, a positive result by 2-tiered testing required both a positive or equivocal EIA result and a positive immunoblot. Furthermore, patient samples that met IgM criteria, but not IgG criteria, were only considered positive if the duration of illness was < 1 month.'

The researchers concluded that 'With standard 2-tiered IgM and IgG testing, 31% of patients with active *erythema migrans* (stage 1), 63% of those with acute neuroborreliosis or carditis (stage 2), and 100% of those with arthritis or late neurologic involvement (stage 3) had positive results.'

In the UK, Lyme borreliosis diagnosis and treatment guidelines issued by Public Health England advise that samples should only be sent for serology testing from patients who can give a credible account of risk of a recent tick bite and present with symptoms of Lyme borreliosis: 'Before diagnostic tests are requested, a patient's risk of exposure to ticks should be properly assessed and the clinical history evaluated for features compatible with Lyme borreliosis (LB). Tests should not be requested if there is no significant risk of a patient having LB.' Patient support groups confirm anecdotally that this aspect of the guideline is followed by GPs and, on this basis, we shall assume that most patients whose blood is sent to RIPL for diagnostic testing is from patients in the acute phase of the illness.

Since the blood samples tested for Lyme disease each year will be from people who recall or are at risk of having a tick bite and are showing symptoms of Lyme borreliosis, we can assume they will be in the acute phase of the illness. The research referenced above [17] has found that the rate of positive results obtained when testing in this phase is 31%. On this basis, we conclude that 69% of new Lyme borreliosis patients whose blood is tested by this method yield a false negative result, when tested by this method at RIPL.

If we assume that the 730 samples which RIPL found positive for Lyme disease in two-tier testing in 2014 represent only (75% of 31% =23%) of the real total, this equates to 3,144 actual positive samples.

False negatives from NLBTL

The National Lyme Borreliosis Testing Laboratory (NLBTL) uses a screening enzyme assay Enzygnost Lyme link made by Siemens as step one of its two-tier testing. In an independent assessment of this test and a similar one by DADE Behring [11,] 56.1% of patients with culture confirmed *erythema migrans* were found to be positive by this test. This implies that 46% of patients with Lyme borreliosis will obtain a false negative result.

Step two of the two-tier testing at NLBTL is performed using the Borrelia RecomLine IgG test by Mikrogen. The company's own literature on sensitivity and specificity of this test reports a 53% sensitivity rate for its IgG immunoblot assay in patients with Lyme borreliosis demonstrated by *erythema migrans* (http://www.biochemmack.ru/upload/iblock/490/490b280b1ff75c2760d439c794835268.pdf). Whilst this sensitivity increases to 86% if IgM and IgG markers are read in combination, NLBTL only uses the IgG markers. This implies that, of all genuinely positive samples tested, 47% of them will achieve a false negative result.

If we assume that the 224 samples which NLBTL found positive for Lyme disease in two-tier testing in 2014 represent (56% of 53% =30%) of the true total, then this equates to a total of 755 true positive samples tested.

Aggregated totals at national level

The total false negatives for Lyme borreliosis which we have estimated so far, from the two UK national reference laboratories (RIPL and NLBTL), are 3,899 for 2014. We have not yet considered the false negative tests obtained in standard NHS laboratories around the country which, since they are negative, are of course excluded from forwarding to the national reference laboratories for confirmatory testing. This group of false negative samples corresponds to the false negative (not real negative) patients in data category H in figure 1.

Part 2: Regional NHS testing centres

False negatives at standard NHS testing laboratories

These category H samples, tested at NHS hospitals around the country, are analysed using either the LIAISON Borrelia IgM Quant made by Diasorin, or the VIDAS Lyme IgM and IgM Biomerieux, both of which are ELISA tests. The Diasorin Lyme ELISA has been evaluated independently by Marangoni *et al* [11] and found to have a sensitivity rate, when the IgM line was used alone (as is used in practise in standard NHS laboratories), of 24.2%. Thus the rate of false negatives found when using this test in this way is 75.8%. The Biomerieux VIDAS Lyme IgM and IgG ELISA test has been evaluated independently by Henningsson *et al* [12] and they found it to have a sensitivity of 86%. This implies a false negative rate of 14%.

Since we lack data on how many samples per year are tested using either kit, we cannot calculate a weighted

average and will have to approximate with a simple average. This implies an average sensitivity rate of 55% and a false negative rate of 44%.

On this basis, we can extrapolate from the 3,899 assumed real positives among samples sent to the national testing laboratories in 2014 that, if all the true samples from standard NHS testing laboratories were forwarded, the complete number would have been be 7,076 for 2014. This corresponds to the false negative (but not the real negative) patients in data category I in figure 1.

Part 3: diagnosis by EM rash

An analysis of figures based on *erythema migrans*

National reporting of patients diagnosed with Lyme disease on the basis of *erythema migrans* is optional throughout the UK. Actual reported numbers are entirely made up of patients whose doctors have not followed the guidelines (which state that *erythema migrans* is diagnostic of Lyme borreliosis), and have additionally ordered western blot testing; they are included in the total positive patient numbers reported by PHE or HPS, but only if they *also* test positive by two-tier serology testing at one of the two national reference laboratories.

Published data on the number of patients with *erythema migrans,* who are additionally tested at national reporting laboratories with positive results, is also patchy. The most recent annual report, from 2014, simply provides a link to a separate report referring to the third quarter of 2015. This, the most recent published source available, is the Public Health England quarterly zoonoses report published on 20th November 2015 [19] which reports that RIPL confirmed 421 cases of Lyme borreliosis in the third quarter of 2015 and that 65 of them had *erythema migrans*: in this instance, this correlates to 16% of the patients included in reported figures having *erythema migrans*. This data refers to England and Wales only, to samples sent to RIPL but not to NLBTL, and to just a three month period of one year. It is therefore risky to extrapolate this percentage as a typical figure. The comparable zoonoses report from 2013 and previous years makes no reference to the number of patients tested by serology who also had *erythema migrans*.

The free-text comments section of our online survey of 500 patients contained a number of claims by respondents that, despite having an *erythema migrans* recognised by their doctor, they then received a negative blood test from RIPL or NLBTL and were told by their doctor that they did not have Lyme borreliosis.

This lack of adequate data means that any attempt to calculate the number of *erythema migrans* cases in the UK, and thus to pinpoint the complete number of Lyme borreliosis cases which occur annually in the UK, must rely heavily on estimations. Given that fact that patients with *erythema migrans* should not, according to current national guidelines, be tested by the national reference laboratories, and given the dearth of information on the number of patients who are tested under such circumstances, we have worked on the assumption that the patients diagnosed by serology are a distinct group from the patients diagnosed by *erythema migrans*. The margin of error created by the small percentage of patients who may be double counted, as a result, will be more than offset in our calculations by the considerably larger range in percentages cited as the overall proportion of Lyme borreliosis patients who do in fact have *erythema migrans* as a presenting symptom.

The British Infection Association issued a paper in 2011 titled 'The epidemiology, prevention, investigation and treatment of Lyme borreliosis in United Kingdom patients: a position statement' [13] which cites 90% as the number of patients who present with *erythema migrans*. The paper states: 'In European prospective studies EM was shown to occur as the presenting sign in about 90% of patients with Lyme borreliosis and can be accompanied by "viral-like" ("flu-like") symptoms…'

Public Health England in its 'Suggested referral pathway for patients with symptoms related to Lyme disease' estimates a lower rate of *erythema migrans*, stating 'Note that up to a third of cases of Lyme disease do NOT have a classical rash, if any at all, and absence of rash or any recollection of a tick bite does not exclude the diagnosis.' This rate of around 66% of patients presenting with EM appears to underlie the statement made by PHE, on the NHS Choices website, that it believes there are between 2,000 and 3,000 cases of Lyme disease in the UK each year. This is explained in the report *Lyme borreliosis epidemiology*

and surveillance, Published 1 May 2013 by PHE: 'Reporting levels have improved, but the data remain incomplete because they do not include cases diagnosed and treated on the basis of clinical features such as *erythema migrans* (the early rash of Lyme borreliosis), without laboratory tests. It is estimated that between 1,000 and 2,000 additional cases of LB occur each year in England and Wales.'

In Scotland, as Mavin, Watson and Evans report, a questionnaire was set to patients and 594 replies were received, reporting that 48% had an *erythema migrans*. As the authors commented, 'The low number of patients with EM (48%) was surprising and is much lower than that documented in other studies (69.1 to 89.3%).' (Christova I, Komitova R. Clinical and epidemiological features of Lyme borreliosis in Bulgaria. Wien Klin Wochenschr 2004; Mehnert WH, Krause G. Surveillance of Lyme borreliosis in Germany, 2002 and 2003; Bacon RM, Kugeler KJ, Griffith KS et al. Lyme disease – United States, 2003-2005).

A refinement of estimates of *erythema migrans* diagnoses (category E)

It is interesting that patients diagnosed by *erythema migrans* formed only 15% of the total in our survey. The absolute number of survey respondents in this category was 101. There are various potential explanations for this. It may be that the true incidence of *erythema migrans* among Lyme borreliosis patients in the UK genuinely is even lower than the 48%found by the Mavin, Watson and Evans study [1], or far lower than the 66% inferred from the PHE estimates of 2,000 to 3,000 unreported Lyme disease cases each year, or indeed drastically lower than the 89.3% found in other studies conducted in Germany, Bulgaria and the United States by Mehnert and Krause, by Christova and Komitova, and by Bacon, Kugeler and Griffith *et al* respectively[3-5] or by the British Infection Association position statement [13]. It may instead be that some NHS doctors are reluctant to diagnose Lyme borreliosis by *erythema migrans* alone, in which case, cases may go undiagnosed and patients themselves may be unaware that they have Lyme borreliosis.

Let us assume that doctors follow PHE guidelines correctly, and do not send blood samples from patients with EM for testing, but instead diagnose and treat Lyme borreliosis on the basis of the rash. Using as our base figure firstly the 954 cases of Lyme borreliosis diagnosed by the two UK national reference laboratories in 2014, and secondly the total estimated number of positive patients with false negative results, we can make two sets of extrapolations of the unreported numbers of patients diagnosed by EM. Figures 7 and 8 show a low, a middle and two high end percentages based solely on the number of cases diagnosed by blood test at RIPL and NLBTL in 2014. The median estimate of 2,806 lies at the middle of the range of total UK cases of Lyme disease estimated by PHE and published on the NHS Choices website.

Extrapolations based on the higher figures we estimated, which include cases of Lyme borreliosis with false negative serology results, are shown in figures 9 and 10. Using this input data, the low end estimate is 13,634, the median is 20,812 the high end estimates are 66,131 to 70,760.

Figure 7: Extrapolations of patients diagnosed by EM and total patient numbers, using PHE and NLBTL published data for 2014

	Mavin ,Watson & Evans [2]	PHE assumed figure	German, Bulgarian and US studies 3-5	BIA position statement [13]
% of patients with EM	48.1%	66.0%	89.3%	90%
Implied % of total patients diagnosed by national reference laboratories' serology NOT EM	51.9%	34.0%	10.7%	10.0%
Published no. of UK patients diagnosed by national reference laboratories' serology, 2014	954	954	954	954
UK patients diagnosed by EM, extrapolated from national reference labs' data	884	1,852	7,962	8,586
ESTIMATED TOTAL PATIENTS DIAGNOSED BY POSITIVE SEROLOGY OR EM	**1,838**	**2,806**	**8,916**	**9,540**

Figure 8: Graphic representation of estimate ranges using PHE and NLBTL published data for 2014

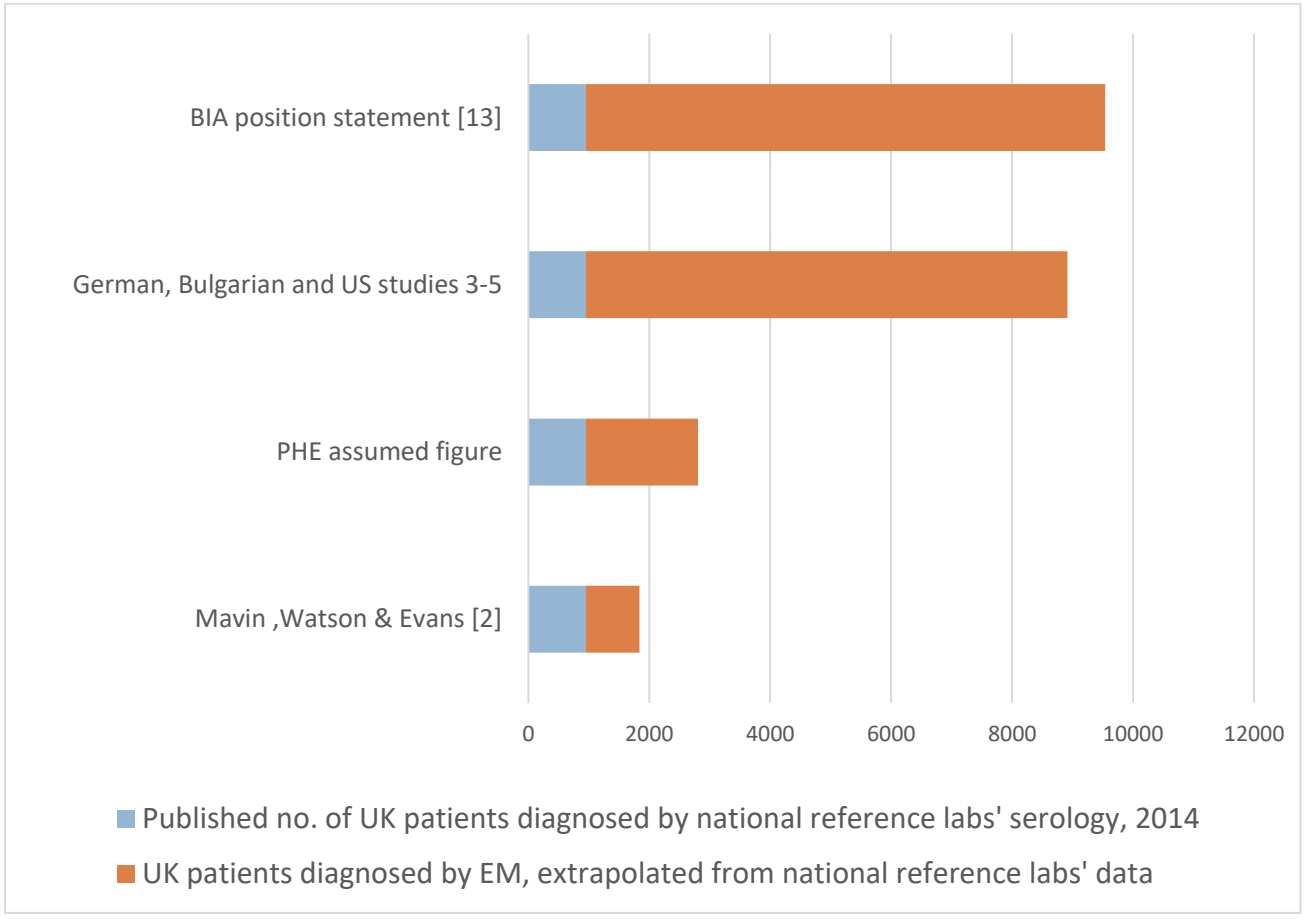
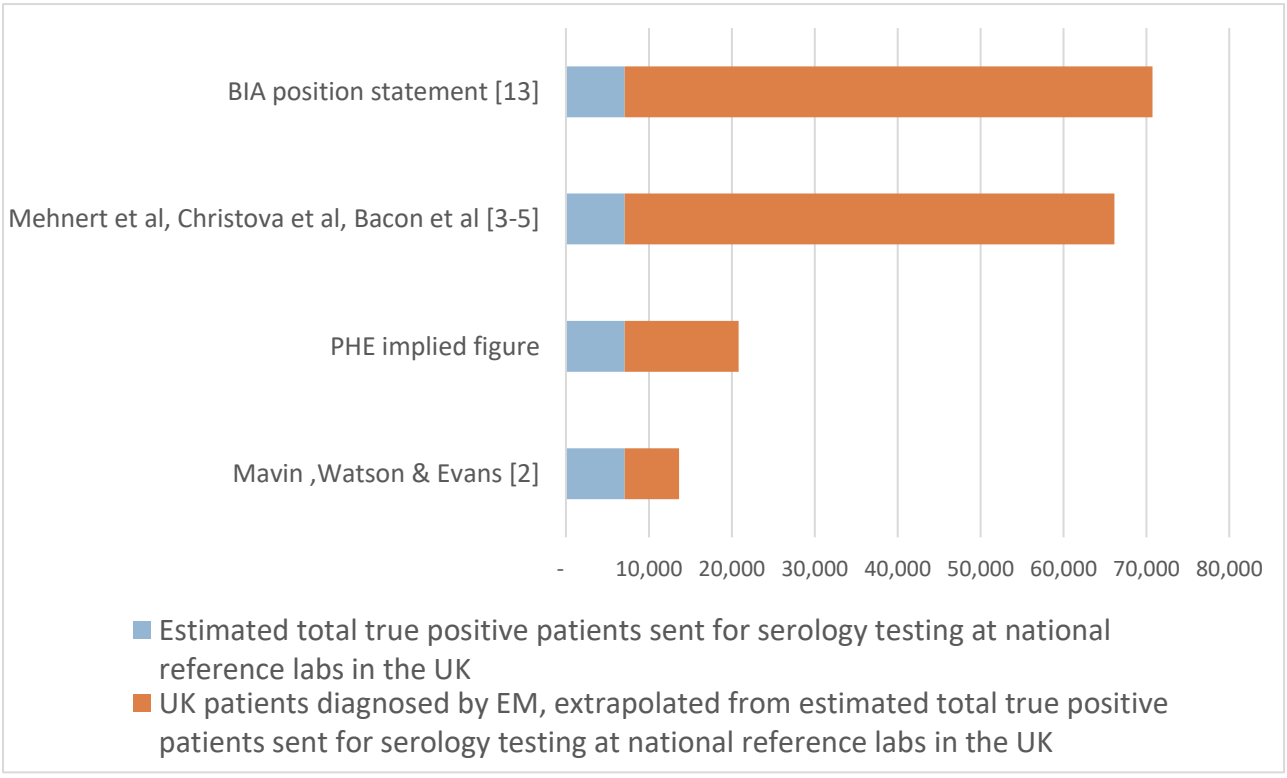


Figure 9: Extrapolations of patients diagnosed by EM and total patient numbers, using survey data of patients diagnosed by private blood test

	Mavin ,Watson & Evans [2]	PHE assumed figure	Mehnert et al, Christova et al, Bacon et al [3-5]	BIA position statement [13]
% of patients with EM	48.1%	66.0%	89.3%	90%
Implied % of total patients diagnosed by national reference lab serology NOT EM	51.9%	34.0%	10.7%	10.0%
Estimated total true positive patients sent for serology testing at national reference laboratories in the UK	7,076	7,076	7,076	7,076
UK patients diagnosed by EM, extrapolated from estimated total true positive patients sent for serology testing at national reference laboratories in the UK	6,558	13,736	59,055	63,684
ESTIMATED TOTAL PATIENTS DIAGNOSED BY POSITIVE SEROLOGY OR EM	**13,634**	**20,812**	**66,131**	**70,760**

Figure 10: Graphic representation of estimate ranges using survey data of patients diagnosed by private blood test



Conclusions

The total number of positive cases of Lyme borreliosis, diagnosed by the two UK national reference laboratory serology tests in 2014 (the most recent year for which we have published data) is 945. Based upon independent assays of the sensitivity of all the tests used at each step of Lyme borreliosis diagnostic testing in standard NHS laboratory tests and national reference laboratory tests, we have calculated that the total number of false negative results is likely to be 6,346, bringing the true total of positive samples sent for serology testing to 7,076.

In accordance with PHE guidelines, patients diagnosed by EM are not tested by serology and their numbers are not recorded by any national body. Based on the PHE statement that *erythema migrans* incidence in Lyme borreliosis patients is around 66%, we can extrapolate this number to a total of 20,812 of Lyme borreliosis in the UK in 2014. If, on the other hand, we use the British Infection Association position statement of 2011, which reports incidence of *erythema migrans* in Lyme borreliosis patients at 90%, then our extrapolated total number of Lyme borreliosis patients in the UK in 2014, and thus a good proxy for the annual number of new cases of Lyme borreliosis in the UK each year, is 70,760.

Estimates of *erythema migrans* incidence are based on research papers from different geographic regions. Reasons for the significant variations might be explained by variations in the prevalence of different strains of Borrelia burgdorferi, or variations in the appearance and recognition rate of *erythema migrans*. Since detailed data has not been gathered throughout the United Kingdom, we feel it would be conservative to consider a median rate based on the existing evidence; on this basis, we conclude that approximately 45,000 is the likely number of new cases of Lyme borreliosis diagnosed each year in the United Kingdom. This equates to a rate of 69 cases per 100,000 of the population.

Discussion

The range in the estimates produced by this research is large, and principally derives from a considerable lack of consensus in the scientific community regarding the true incidence of *erythema migrans* as a symptom of Lyme borreliosis. One fact is clear: there is no doubt that a great many Lyme disease patients in the UK go unreported, and many more go undiagnosed.

Considerable numbers of false negative results are reported, based on independent assays of the various diagnostic tests used in standard NHS testing laboratories and the two national references laboratories. The low sensitivities of some of the tests used are of particular concern.

The lack of data regarding the true number of patients alternatively diagnosed with conditions which mimic Lyme borreliosis, such as chronic fatigue syndrome or fibromyalgia, is of interest. Reliable data in this arena could potentially have a significant upward impact upon the estimated total number of Lyme borreliosis patients.

Testing samples for Lyme borreliosis alone misses many people who are bitten by a tick and may have other tick-borne diseases but not Lyme borreliosis: there is substantial overlap in symptoms between several tick-borne diseases, and GPs in the UK are not fully trained to recognise them or to distinguish between them. PHE issues recommendations that patients with a suspected history of tick bites are tested for some other tick-borne infections but, according to anecdotal evidence from patients, this rarely happens, in many cases even when patients explicitly request such additional testing.

With an incidence rate of 69 per 100,000 of the population and a UK average life expectancy of 81.5 years (according to the Office for National Statistics), 1 person in 20 can be expected to catch Lyme borreliosis at some point in their lives [20]. As the NHS choices website states: 'A few people with Lyme disease go on to develop long-term symptoms similar to those of fibromyalgia or chronic fatigue syndrome.' The International Lyme and Associated Diseases Society (ILADS) most recent treatment guidelines contain an assessment of the evidence using GRADE methodology and found treatment failure rates ranging from 16% to 39% for early treatment [16]. Aucott *et al* [18] report 36% of 63 patients with *erythema migrans,* recruited for a prospective cohort study, developed chronic symptoms leading to "significantly lower life functioning", which they call

Post Treatment Lyme Disease Syndrome. The reasons for the development of chronic symptoms are poorly understood, but are thought by some professional associations to be dependent on failure to diagnose and treat the infection early on.

For this reason, one of the major questions to consider with regard to this research is the effect of such a large proportion of Lyme borreliosis patients remaining undiagnosed. In how many patients will their condition self-resolve, and how many will go on to become chronically ill? What is the annual cost to the NHS of managing symptoms and complications in undiagnosed or misdiagnosed patients? And what should national healthcare authorities do to prevent and reduce the growth in chronically ill patients?

Appendix 1: An analysis of the Lyme borreliosis growth rate

Public Health England lacks consistent year-on-year data regarding incidence of Lyme borreliosis. The published data [14] stop in 2011, and reporting requirements underwent changes during the period of data captured. For this reason the data are not consistent year-on-year. The average annual growth rate in incidence of Lyme borreliosis implied by this set of data is 26%; this could, however, be as much a function of improving and varying data reporting, and of changes in testing policy, as of genuine spread of the disease.

Figure 14: Growth in Lyme borreliosis as reported to Public Health England to 2011

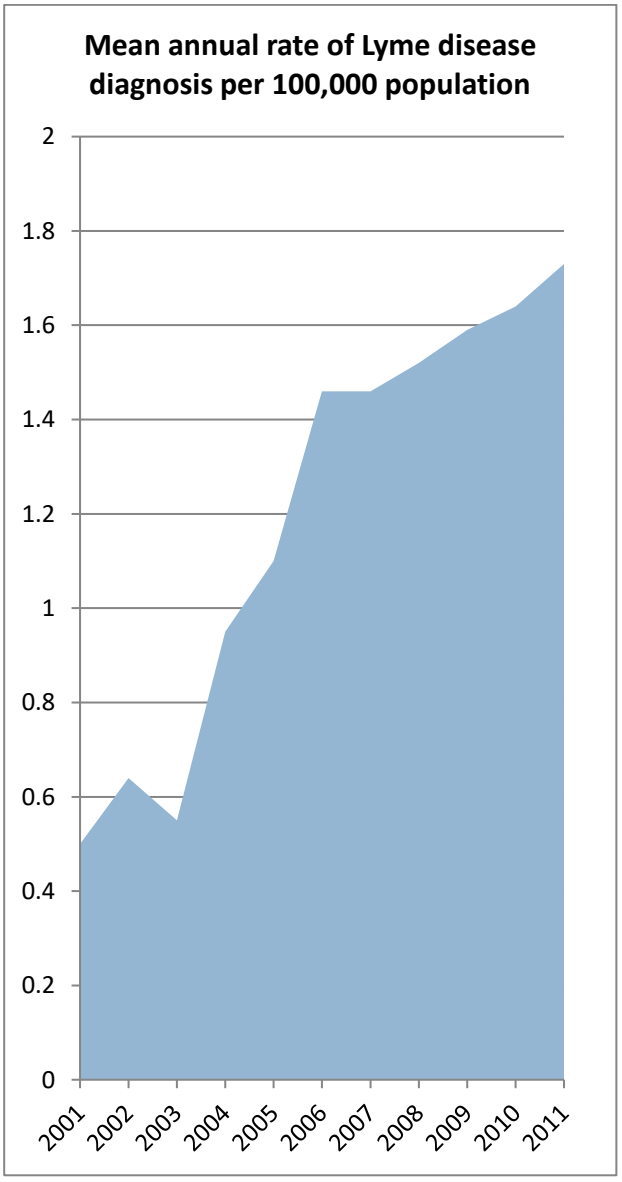


Figure 15: Source data for figure 14

Years	Total reports received	Mean annual range total	Mean annual rate per 100,000 popn	YOY growth
1997 to 2000	803	201 (148 to 322)	0.38	
2001	268	268	0.5	31.6 %
2002	340	340	0.64	28.0 %
2003	292	292	0.55	-14.1 %
2004	500	500	0.95	72.7%
2005	595	595	1.1	15.8 %
2006	768	768	1.46	32.7 %
2007	797	797	1.46	0.0%
2008	813	813	1.52	4.1%
2009	863	863	1.59	4.6%
2010	905	905	1.64	3.1%
2011	959	959	1.73	5.5%

The aggregated data gathered by the World Health Organisation from national reporting agencies Europe-wide is similarly plagued with misleading inadequacies in data capture. Whilst actual national numbers and incidence rates appear to be drastically understated in most European countries, improvements in data capture in some countries, over some years, give the impression that the growth rate in Lyme borreliosis incidence has been as high as 65% annually for the last 20 years. [15]

If we extrapolate numbers of new Lyme borreliosis cases in accordance with the 26% growth rate implied by PHE statistics, we predict that there will be over a million new cases of Lyme borreliosis per year by 2030 (figure 16).

This is clearly an area in which improved reporting requirements, both nationally and Europe-wide, could yield beneficial information for public health services.

Figure 16: Extrapolation of Lyme borreliosis incidence in the UK using the growth rate implied by PHE data

	Lyme borreliosis new cases
2016	45,000
2017	56,700
2018	71,442
2019	90,017
2020	113,421
2021	142,911
2022	180,068
2023	226,885
2024	285,875
2025	360,203
2026	453,856
2027	571,858
2028	720,542
2029	907,882
2030	1,143,932

Appendix 2: The diagnostic laboratories included in this survey

The patient survey reviewed in this paper considers patients tested for Lyme borreliosis specifically by the following testing laboratories: the Rare and Imported Pathogens Laboratory (RIPL), part of Public Health England (PHE); the National Lyme Borreliosis Testing laboratory (NLBTL), part of NHS Highlands; IGeneX, a private diagnostic laboratory in the USA; ArminLabs, a private diagnostic laboratory in Germany; and BCA Lab (formerly known as Infectolab), a private diagnostic and research laboratory in Germany.

Rare and Imported Pathogens Laboratory (RIPL)

Two-tier testing is used. This laboratory tests samples in the first stage using the ELISA immunoassay made by Immunetics, which tests for the C6 protein. The confirmatory immunoblot assay is the Borrelia ViraStripe IgM/IgG test manufactured by ViraMed. Both tests are CE marked and testing is automated.

National Lyme Borreliosis Testing laboratory (NLBTL)

Two-tier testing is used. Samples are first screened using the enzyme immunoassay Enzygnost Lyme link IgG/VlsE made by Siemens. If positive, the second stage confirmatory test is the Borrelia RecomLine IgG test made by Mikrogen. Both these tests are CE marked and testing is automated.

ArminLabs

This lab uses several tests. The Borrelia EliSpot (T-Cell-Spot / IGRA: Interferon-Gamma-Release Assay) is more sensitive than a conventional ELISA, and detects the secreted protein instead of the mRNA. Antigens used are the Borrelia burgdorferi B31-reference strain (Borrelia burgdorferi sensu stricto), the Borrelia burgorferi Peptide-Mix (OspA from Borrelia b. sensu stricto, Borrelia afzelii, Borrelia garinii + OspC native + DbpA recombinant), and Borrelia burgdorferi LFA-1 (Lymphocyte Function Antigen 1): Own body protein + Borrelia burgdorferi sensu stricto (shared epitope). [6] This test is CE marked but testing is not automated. The SeraSpot anti-borrelia IgG/IgM test, made by Seramun Diagnostica GmbH is also used as a confirmatory test in place of the western blot. [7] The SeraSpot Micro Array analyzes the following Borrelia Burgdorferi IgG- and IgM-antibodies and Borrelia Burgdorferi subspecies: VlsE (B.b. afzelii), p39 (B.b. afzelii), p58 (B.b. garinii), p100 (B.b. afzelii), OspC (B.b. afzelii + B.b. garinii + B.b. sensu stricto), DbpA (B.b. afzelii + B.b. garinii + B.b. sensu stricto). This test is CE marked and testing is automated.

ArminLabs also offers Polymerase Chain Reaction (PCR) testing of samples for Borrelia Burgdorferi is a molecular biology technique which amplifies a few DNA molecules into thousands to millions of copies of a special DNA sequence. This test is not CE marked.

BCA Lab

The tests offered by BCA Lab are the following: LymeSpot Revised; Borrelia EliSpot; Borrelia IgG and IgM-EIA; Borrelia IgG and IgM-Blot; and DNA-PCR for Borrelia. BCA Lab uses CE marked EliSpot tests and also a version of the test developed in-house, called LymeSpot revised, which provides more detailed information on the activity of the infection. This test can help to identify whether an active (specific effector cells) or a latent (specific memory cells) phase of the infection is present. This is used to plan treatment, not used alone for diagnosis.

While the existing EliSpot test is exclusively based on the production of γ-Interferon, the new LymeSpot also determines Cytokine IL-2 production. In accordance with the traffic light principle, an active infection (predominantly effector cells) shows green in the test result, meaning that treatment of the infection is necessary. If the ratio of γ-Interferon and Interleukin-2 is reversed, a latent disease can be assumed, manifesting itself as the colour red in the laboratory test (predominantly memory cells). In this case, a mandatory anti-infective treatment would no longer be needed. [7]

IGeneX

IGeneX uses ELISA testing and western blot testing. The IgG Immunoblot (western blot) is a test developed in-house by IGeneX and used exclusively by that laboratory. It has FDA approval for in-house use for diagnostic purposes but not the more rigorous approval for sale to other laboratories. It is a qualitative test and is generally more sensitive and specific than the ELISA. This test is used if the Lyme IgG/IgM antibody serology or Lyme IgG/IgA/IgM IFA is

equivocal or positive. The somewhat specific Lyme antibodies of importance are against the following molecular weights of the B. burgdorferi antigens: 23-25 kDa (Osp C); 31 kDa (Osp A); 34 kDa (Osp B); 39 kDa; 41 kDa (common of flagella-bearing organisms); and 83-93 kDa. The term "kDa" refers to kilodalton for molecular weight designations. The term "Osp" refers to Outer Surface Protein of the bacteria.

From the IgeneX website:

'There are currently multiple criteria that support a positive blot. "Positive" means consistent with the presence of antibody against B. burgdorferi. The CDC/ASTPHLD criteria are very conservative and require 5 of 10 bands for a positive result; equivocal or borderline results are not recognized. Unfortunately, not all Lyme patients have similar immune systems: only approximately 70% of those with Lyme disease generate a strong enough antibody response to appear on a western blot. IGeneX criteria of 2 starred bands is >96% specific for exposure to B. burgdorferi.

IGeneX has several years of clinical data that support more liberal reporting criteria.10 In addition, current studies show that the CDC/ASTPHLD criteria miss some patients with culture-proven *erythema migrans* (EM).5,11 Both the IGeneX and the CDC/ASTPHLD criteria are included on the IGeneX report form sent to the physician. 3,5,8,9 The CDC/ASTPHLD criteria for positive results are 5 of the following 10 antigenic bands: 18 kDa, 23-25 kDa (Osp C); 28 kDa, 30 kDa, 39 kDa; 41 kDa, 45 kDa, 58 Kda, 66 KDa, and/or 83-93 kDa. IGeneX criteria for positive result is 2 of the following 6 bands: 23-25kDa, 31 kDa (Osp A), the 34 kDa (Osp B), 39 kDa, 41kDa and/or the 83-93 kDa. 31kDa and 34kDa antigens are included to the criteria due to their importance in the recurrent and/or persistent disease period. IGeneX criteria of is 96% specific for exposure to B. burgdorferi.

A positive IgG result with clinical history may be indicative of Lyme disease. Patients with other spirochaetal disease and/or who test positive for rheumatoid factor or Epstein Barr virus may have cross-reacting antibodies. A positive response in this, as in any antibody assay, indicates sensitization, not necessarily active disease.'

Lyme Dot-blot Assay (LDA) is a qualitative immunoassay also offered by IGeneX for the direct detection of Borrelia burgdorferi specific antigens in urine using anti-Borrelia burgdorferi antibodies. The antigens present in urine are immobilized onto a membrane in a Dot-blot format and incubated with anti-Borrelia burgdorferi specific antibodies. After washing, the bound anti-Borrelia burgdorferi specific antibodies are reacted with anti-rabbit IgG, which is visualized by an enzyme/substrate reaction.

If the initial Lyme panel tests on blood samples are negative, including PCR, but symptoms for Lyme disease are present, the Lyme Dot-blot assay on urine, especially test panel #875 consisting of Lyme Dot-blot assay and PCR, can be helpful in making the diagnosis.

IGeneX also offers PCR testing. The website states:

'The standard PCR test is not always sensitive enough because of the very low numbers of organisms present. In addition, it is well known that the PCR sensitivity is reduced in the presence of inhibitors. Therefore, IGeneX has developed a nucleic acid-based Multiplex PCR diagnostic assay that simultaneously detects genomic and plasmid Borrelia burgdorferi DNA in clinical samples. The multiplex PCR offers enhanced performance, when compared to currently available microbiological, immunological, and amplified tests for the detection of microorganisms in test samples. While the genomic PCR gives excellent sensitivity and specificity if one recoverable bacterium or the DNA from one is obtained, the plasmid PCR does not require a whole organism but recognizes pieces or blebs of B. burgdorferi antigen. Dorward *et al* reported the presence of pieces or blebs of B. burgdorferi antigen in urine and other tissues. In addition, Nocton *et al* detected (by PCR) B. burgdorferi plasmid DNA in 96% of the patients with persistent Lyme arthritis. Based on this information, the plasmid PCR may have greater potential for sensitivity than the genomic PCR.'

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