

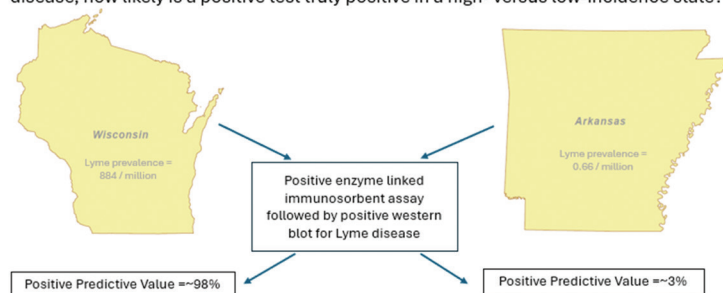


ORIGINAL ARTICLE

# How Prevalence Influences the Interpretation of Lyme Disease Test Results in a High-Incidence State (Wisconsin) Versus a Low-Incidence State (Arkansas)

## Graphical Abstract

Using the U.S. Centers for Disease Control's recommended testing algorithm for early Lyme disease, how likely is a positive test truly positive in a high- versus low-incidence state?



## Highlights

- This study uses publicly available data to conduct a thought experiment demonstrating the strong influence of prevalence on the positive predictive value (PPV) of Lyme disease serologic test results.
- A state with high Lyme disease incidence (Wisconsin) is compared with a state with low Lyme disease incidence (Arkansas).
- The PPV in Wisconsin is approximately 30 times higher than that in Arkansas.

## Authors

Dirk Haselow and Aaron Wendelboe

## Correspondence author

haselowdirk@uams.edu (DH)

## Brief Statement

This study demonstrates that in high-incidence states, such as Wisconsin, the PPV of serologic testing for early Lyme disease is high (98.1%). However, in low-incidence states, such as Arkansas, the PPV is so low in the context of early Lyme disease (3%) that we recommend that clinicians consider and work up other potential diagnoses first.

# How Prevalence Influences the Interpretation of Lyme Disease Test Results in a High-Incidence State (Wisconsin) Versus a Low-Incidence State (Arkansas)

Dirk Haselow<sup>1,\*</sup> and Aaron Wendelboe<sup>1</sup>

## Abstract

**Objective:** Clinicians' understanding of prevalence is key in diagnostic test interpretation. In high-prevalence scenarios, the post-test probability of a positive test result can approach 100%, whereas in low-prevalence situations, the post-test probability might only slightly exceed the initial low pre-test probability. Herein, we present an example of this issue in the context of interpreting Lyme disease test results in high- versus low-incidence states.

**Methods:** We present a thought experiment demonstrating the effects of prevalence on the interpretation of early Lyme disease serologic testing.

**Results:** A positive test result was found to be more than 30 times more likely to be a false positive than a true positive in Arkansas.

**Conclusions:** In locations with low Lyme disease incidence, efforts might be better spent on thoroughly working up alternative diagnoses before considering testing for Lyme disease. Furthermore, we suggest that clinicians in low-incidence states in the southern U.S. consider waiting before treating patients empirically with antibiotics for clinical scenarios consistent with early Lyme disease, because southern tick-associated rash illness in the southern U.S. commonly mimics the classic erythema migrans rash associated with Lyme disease, but is mild and self-limiting.

**Key words:** Lyme disease, prevalence, positive predictive value

\*Corresponding author:

E-mail: haselowdirk@uams.edu (DH)

<sup>1</sup>Department of Epidemiology, Fay Boozman College of Public Health, University of Arkansas for Medical Sciences, Little Rock, AR, USA

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## INTRODUCTION

Lyme disease is the most common tick-borne disease in the United States; whereas 62,551 cases were reported in 2022 [1], as many as 476,000 estimated cases occur each year [2]. This disease is caused by the parasite *Borrelia burgdorferi* and is transmitted by two hard ticks, most frequently the black-legged tick or deer tick (*Ixodes scapularis*) and the Western black-legged tick (*I. pacificus*). Infected ticks are most commonly found in forested areas in the

northeastern, north-central, and mid-Atlantic states, as well as in smaller areas within Pacific Coast states [3].

The United States' Centers for Disease Control and Prevention (CDC) has classified states and jurisdictions into high Lyme disease incidence ( $n = 15$ ) and low Lyme disease incidence ( $n = 36$ ) jurisdictions, as summarized in Table 1 [1]. In agreement with the distribution of the tick vectors, the high-incidence jurisdictions are in the Northeast, mid-Atlantic, and upper-Midwest portions of the U.S.

**TABLE 1** | States and jurisdictions classified as having high or low incidence of Lyme disease.

| High incidence       | Low incidence | Low incidence  |
|----------------------|---------------|----------------|
| Connecticut          | Alabama       | Missouri       |
| Delaware             | Alaska        | Montana        |
| District of Columbia | Arizona       | Nebraska       |
| Maine                | Arkansas      | Nevada         |
| Maryland             | California    | New Mexico     |
| Massachusetts        | Colorado      | North Carolina |
| Minnesota            | Florida       | North Dakota   |
| New Hampshire        | Georgia       | Ohio           |
| New Jersey           | Hawaii        | Oklahoma       |
| New York             | Idaho         | Oregon         |
| Pennsylvania         | Illinois      | South Carolina |
| Rhode Island         | Indiana       | South Dakota   |
| Virginia             | Iowa          | Tennessee      |
| West Virginia        | Kansas        | Texas          |
| Wisconsin            | Kentucky      | Utah           |
|                      | Louisiana     | Washington     |
|                      | Michigan      | Wyoming        |
|                      | Mississippi   |                |

The recommended method for Lyme disease diagnosis is assessment of antibody presence in serum through a two-tier test protocol [4]. Diagnostic tests using PCR methods, pathogen culture, or other methods of direct pathogen assessment exist but are fraught with interpretive challenges; consequently, these tests are recommended by the Infectious Disease Society of America (IDSA) only as adjunctive options for patients who have already received a positive serologic test result [5]. Although the updated surveillance case definition accounts for high-incidence and low-incidence jurisdictions [4], the accuracy of this two-tier serological testing protocol remains poor in low-incidence jurisdictions [6]. According to CDC's testing guidance, a patient should meet three pre-test criteria: (1) the patient has been in an area where Lyme disease is common, (2) the patient is likely to have been exposed to ticks, and (3) the patient has symptoms characteristic of Lyme disease [7].

In regions where Lyme disease is common, this disease is a major cause of acute and chronic morbidity. However, in regions where this disease is uncommon, it often poses a major cause of confusion and frustration for both patients and clinicians.

This confusion is driven by a well-known epidemiological principle in which the prevalence of a disease affects the positive predictive value (PPV) of a diagnostic test. Herein, we aim to illustrate how the low prevalence of Lyme disease is contributing to challenges

among both clinicians and patients living in low-incidence jurisdictions regarding the interpretation of positive test results according to the current two-tier test algorithm. We compare PPVs using the same test protocol for a hypothetical patient in a low-incidence state (Arkansas, ranked 49th in incidence in 2022) versus a high-incidence state (Wisconsin, ranked 5th in incidence in 2022).

## METHODS

Let us propose a thought experiment in which physician A sees patient A in Wisconsin, and physician B sees patient B in Arkansas. Both patients are 45-year-old men who report experiencing 2 days of 101.5° F fever and a bullseye rash (erythema migrans [EM]) after having camped in the woods the previous month, in August. Neither patient reports traveling out of their respective states for more than a year. Both patients recall removing embedded ticks. Both physicians determine that these presentations are consistent with classic Lyme disease and therefore order the two-stage serologic testing for Lyme disease. Both patients are empirically prescribed doxycycline, the drug of choice for Lyme disease, before the test results are available. Is this the proper course of action?

Within 48 hours, both patients' tests are determined to be positive according to the CDC's recommended testing algorithm. Before we critique either physician's management, we will calculate the expected findings of tier 1 and 2 testing.

## Sensitivity analysis

In an effort to acknowledge the reality that not all skin lesions/rashes resulting in care seeking during the tick season resemble EM, we conducted sensitivity analyses as follows, assuming that: (i) 40% of all rashes resemble EM in both states, (ii) 20% of all rashes resemble EM in both states, or (iii) 20% of all rashes resemble EM in Arkansas, and 40% of all rashes resemble EM in Wisconsin. We selected 20% as the lowest percentage of rashes resembling EM during the tick season, so that the initial group of patients in this sensitivity analysis entering tier 1 testing would at least exceed the current yearly Lyme disease case count in Wisconsin.

## RESULTS

According to the CDC Wide-ranging Online Data for Epidemiologic Research (Wonder) year-end reported cases of Lyme disease by state, Arkansas had 2 cases, whereas Wisconsin had 5,208 cases of Lyme disease reported in 2022 [8]. According to the USAFacts website, the populations of these states in 2022 were estimated to be 3,045,637 and 5,892,531, respectively, and the U.S. population was estimated to be 333,287,557 [9]. These values translate into case rates of 0.657 cases per million people in Arkansas and of 884 cases per million people in Wisconsin, a number approximately 1,345 times higher.

To estimate how many people might seek care for a rash in each state, we made several assumptions based on data from the National Center for Health Statistics. According to the 2019 National Ambulatory Medical Care Survey, among the 1,036,484,000 ambulatory care visits in the U.S., 10,295,000 were for skin lesions [10]. Among the latter visits, 50.3% of patients sought care from primary care providers (PCPs), and we estimate that 41.6% (5/12) of those visits occurred in the five high-incidence months for tick-borne diseases (May through September) [10]. By multiplying these numbers together, we estimate that 2,154,204 visits to PCPs for skin lesions occurred during May to September in the U.S. in 2022. Assuming that the same rate per capita in the U.S. can be applied to individual states, we estimate that 19,717 and 38,147 visits occurred in Arkansas and Wisconsin, respectively.

The complete two-tier testing algorithm is described on the CDC website [7]. In summary, the first test should comprise either an enzyme immunoassay or an immunofluorescence assay. The second test should be a western immunoblot assay to confirm first-tier tests yielding either positive or equivocal results. In early Lyme disease, as in this scenario, the sensitivity and specificity of the first-tier test have been reported to be 54.0% and 96.8%, respectively [11]. Similarly, the sensitivity and specificity of the second-tier test are 60.6% and 96.8%, respectively [11].

With these data inputs, we can proceed with the exercise and complete Table 2. For simplicity, we describe only the values for Arkansas here, but the corresponding values for Wisconsin can be calculated similarly. The population of patients who presented to a PCP with a rash illness ( $n = 19,717$ ) goes in cell i. The number of reported cases ( $n = 2$ ), which we will assume is the true number of cases, goes in cell g. The true number of patients without Lyme disease ( $n = 19,715$ ) corresponds to cell h and is calculated by subtracting cell g from cell i ( $19,717 - 2 = 19,715$ ). Notably, according to this information, the prevalence of Lyme disease among patients presenting with a rash illness is  $2/19,717 = 0.0001$ . To estimate the number of patients who have Lyme disease and also test positive (cell a, the true positives), we use the sensitivity of the test,  $0.54 = \text{cell a} / \text{total cases (cell g)}$ . Solving for cell a, we

obtain  $a = 0.54 \times 2 = 1.08$ . The number of patients who have Lyme disease but test negative, i.e., the false negatives, is found in cell d and is calculated by subtracting the number of patients who test positive (cell a) from the total cases (cell g). Solving for cell d, we obtain  $2 - 1.08 = 0.92$ . The specificity of the test is used to calculate the number of patients who do not have Lyme disease and also test negative, i.e., cell e divided by cell h. Solving for e, we obtain  $0.968 \times 19,715 = 19,184$ . The patients who do not have Lyme disease but test positive, i.e., the false positives, are found in cell b and are calculated by subtracting the total non-cases (i.e., those with negative test findings; cell e) from the total cases (cell h). Solving for b, we obtain  $19,175 - 19,084 = 631$ .

The following formulas reference cell locations in the table. Cells g and h were taken directly from the aforementioned references. Cell a = cell g \* sensitivity. Cell d = cell g - cell a. Cell e = cell h \* specificity. Cell b = cell h - cell e. Cell c = cell a + cell b. Cell f = cell d + cell e. The right half of Table 2 is completed similarly, with data specific to Wisconsin.

The false positive rate is defined as the number of patients who do not have the disease but test positive, divided by the number of all patients who test positive (cell b/cell c) =  $(631/632) = 99.8\%$  in Arkansas and  $27.2\%$  in Wisconsin. The false negative rate is the number of patients who have the disease but test negative, divided by all patients who test negative (cell d/cell f) =  $(0.92/19,085) = 0.005\%$  in Arkansas and  $6.9\%$  in Wisconsin.

The next step in the thought experiment is performing second-tier tests on the patients who tested positive in the first-tier tests. For Arkansas, we would take all patients who tested positive and place that information in Table 3. The number of true positives in cell a of Table 2 ( $n = 1.08$ ) is inserted into cell g of Table 3, and the number of false positives in cell b of Table 2 ( $n = 631$ ) is inserted into cell h of Table 3. The same process is used to complete the remainder of tier 2 testing in Table 3, except that 60.6% is used as the corresponding sensitivity (whereas the specificity remains unchanged at 96.8%). Likewise, the calculations for Wisconsin would use the true positives in cell a' of Table 2 ( $n = 2,813$ ) and insert them into cell g' of Table 3, the number of false positives in cell b'

**TABLE 2** | Theoretical results of first-tier Lyme disease testing in Arkansas and Wisconsin.

|                      | Arkansas (population 3.046 million, true incidence 0.657/million) |   |                            |   |        |   | Wisconsin (population 5.892 million, true incidence 884/million) |    |                            |    |        |    |
|----------------------|-------------------------------------------------------------------|---|----------------------------|---|--------|---|------------------------------------------------------------------|----|----------------------------|----|--------|----|
|                      | Has Lyme disease                                                  |   | Does not have Lyme disease |   | Total  |   | Has Lyme disease                                                 |    | Does not have Lyme disease |    | Total  |    |
| Tier 1 Test Positive | 1.08                                                              | a | 631                        | b | 632    | c | 2,812                                                            | a' | 1,054                      | b' | 3,866  | c' |
| Tier 1 Test Negative | 0.92                                                              | d | 19,084                     | e | 19,085 | f | 2,396                                                            | d' | 31,885                     | e' | 34,281 | f' |
| Total                | 2.00                                                              | g | 19,175                     | h | 19,717 | i | 5,208                                                            | g' | 32,939                     | h' | 38,147 | i' |

The letters that occur immediately after the numbers in the body of the table refer to specific cell locations and are included for clarity to facilitate reference back to calculations in the text.

of Table 2 ( $n = 1,054$ ) is inserted into cell h' of Table 3, and the total number of patients who tested positive in Wisconsin in cell c' of Table 2 ( $n = 3,866$ ) is inserted into cell i' of Table 3.

The performance values for second-tier testing for Arkansas are as follows:  $PPV = 0.65/21 = 3.1\%$ , negative predictive value (NPV)  $= 611/611 = 100\%$ . For Wisconsin, the tier 2  $PPV = 1,704/1,738 = 98.0\%$  and  $NPV = 1,020/2,128 = 47.9\%$ .

### Net Lyme disease testing algorithm characteristics in Arkansas

Whereas the performance values of the respective first- and second-tier testing aid in understanding the clinical value (or lack thereof) of the test results in low-incidence jurisdictions, for communication purposes, providing performance values for the testing algorithm as a whole can be helpful. The following formulas are used to calculate the net performance values when the tests are conducted in a series:

The net sensitivity after the two tiers of testing for Arkansas would be Table 3, cell a, divided by Table 2, cell g, or  $0.65/2 = 32.7\%$ . The net specificity in Arkansas would be (Table 2, cell e + Table 3, cell e) divided by Table 2, cell I  $= (19,084 + 611)/19,717 = 0.999$ . The net  $PPV =$  Table 3, cell a, divided by Table 3, cell c  $= 0.65/21 = 0.0314$ . The net  $NPV =$  (Table 2, cell e + Table 3, cell e)/(Table 2 cell f + Table 3, cell f)  $= (19,084 + 611)/(19,085 + 611) = 0.999$ . The net false positive rate is (Table 3, cell b/Table 3 cell c)  $= (20/21) = 95.2\%$ . The net false negative rate  $= ([\text{Table 2 cell d} + \text{Table 3 cell d}]/[\text{Table 2, cell f} + \text{Table 3, cell f}]) = (0.92+0.43)/(19,085+611) = 0.0007\%$ . The number of patients needed to benefit from Lyme testing in Arkansas  $= 1/(\text{net } PPV) = 1/0.0314 = 31.8$ . The ratio of false positives to true positives  $= 20/0.65 = 30.8$ .

### Net Lyme disease testing algorithm characteristics in Wisconsin

These net testing characteristics for Wisconsin are performed with the same methods as those used for Arkansas. The net sensitivity after the two tiers of testing for Wisconsin equals 32.7%. The net specificity in

Wisconsin would be 99.9%. The net  $PPV$  would be 0.981. The net  $NPV$  would be 0.904. The net false positive rate in Wisconsin is 1.96%. The net false negative rate in Wisconsin is 40.5%. The number of patients needed to benefit from Lyme testing in Wisconsin is 1.02. The ratio of false positives to true positives is  $34/1,704 = 0.02$ .

### Interpretation of the thought experiment

In Wisconsin, using the CDC's Lyme disease testing algorithm seems reasonable, because the net  $PPV$  reached 98.1%, and a relatively high  $NPV$  of 90.4% was maintained. Given the example scenario of early care seeking by patients with an EM rash, a true positive would be likely to be found nearly every time a positive test is encountered. However, Arkansas had 30 times more false positives than true positives. These findings prompt questions regarding whether the diagnostic workup should initially consider Lyme disease testing at all in this scenario.

### Sensitivity analysis

To acknowledge the reality that a minority of skin lesions/rashes during the tick season resemble EM, we ran the following sensitivity analysis. When we assumed that 40% of rashes resemble EM, the net  $PPVs$  increased from 3.1% to 7.5% and from 98.1% to 99.4% in Arkansas and Wisconsin, respectively. The net  $PPVs$  further increased to 13.9% for Arkansas and 99.9% for Wisconsin when we assumed that only 20% of all rashes during the tick season resemble EM.

## DISCUSSION

This thought experiment has several limitations. It attempted to account for the finding that clinically consistent symptomatology increases a patient's pre-test probability of having Lyme disease in either high- or low-incidence settings, by starting with patients seeking care in an ambulatory setting for a skin lesion or rash. However, a lower proportion of patients seeking care for skin lesions or rashes is likely to have EM-like rashes consistent with Lyme disease; moreover, that proportion might differ between states with high versus low Lyme disease incidence. In the absence of prevalence

**TABLE 3** | Theoretical results of second-tier Lyme disease testing in Arkansas and Wisconsin.

|                      | Arkansas (population 3.046 million, true incidence 0.657/million) |   |                            |   |       |   | Wisconsin (population 5.892 million, true incidence 884/million) |    |                            |    |       |    |
|----------------------|-------------------------------------------------------------------|---|----------------------------|---|-------|---|------------------------------------------------------------------|----|----------------------------|----|-------|----|
|                      | Has Lyme disease                                                  |   | Does not have Lyme disease |   | Total |   | Has Lyme disease                                                 |    | Does not have Lyme disease |    | Total |    |
| Tier 1 Test Positive | 0.65                                                              | a | 20                         | b | 21    | c | 1,704                                                            | a' | 34                         | b' | 1,738 | c' |
| Tier 1 Test Negative | 0.43                                                              | d | 611                        | e | 611   | f | 1,108                                                            | d' | 1,020                      | e' | 2,128 | f' |
| Total                | 1.08                                                              | g | 631                        | h | 632   | i | 2,813                                                            | g' | 1,054                      | h' | 3,866 | i' |

The letters that occur immediately after the numbers in the body of the table refer to specific cell locations and are included for clarity to facilitate reference back to calculations in the text.

estimates of EM rashes, either by state or as a proportion of all skin lesions, we conducted sensitivity analyses in which we assumed that 20%–40% of skin lesions during the tick season resembled EM and would reasonably prompt Lyme disease testing. Although the results demonstrated an increase in net PPV in both states, the largest magnitude was the increase in PPV from 3.1% to 13.9% in Arkansas. We believe that, even in this case, the overall PPV remains insufficiently high to have substantial clinical value.

This analysis did not attempt to address the limitations of currently recommended and/or available Lyme disease testing. Of note, neither CDC nor IDSA currently recommends direct pathogen testing strategies, because PCR testing, direct culturing of the parasite, direct visualization, and direct antigen testing are fraught with a variety of limitations and are generally not recommended [5]. Instead, they propose the aforementioned CDC-recommended two-tiered serologic testing approach, which is most useful in high-incidence settings. Furthermore, this serologic testing strategy has been well recognized to have poor sensitivity in the first 4 weeks after exposure and therefore might undercount cases that are tested quickly [5], because many patients require approximately 1 month to develop antibody levels that can be measured by the testing protocol. This physiological delay might provide additional rationale to wait several weeks before considering Lyme disease testing in low-incidence settings in the southern U.S. for patients presenting shortly after the development of the rash and within 1 month of exposure. For example, during the additional time physicians and patients spend watching and waiting, a southern tick-associated rash illness (STARI) diagnosis might potentially become apparent, if the fullest extent of the rash shows the smaller size and more rapid resolution characteristics consistent with STARI rather than EM [3].

Several contextual factors should be considered in interpreting diagnostic tests for Lyme disease, such as travel history, symptom duration, the presence of additional symptoms, and test timing. Although numerically estimating how each of these factors might change the PPV of the two-tier approach is beyond the scope of this article, these baseline estimates should aid healthcare providers in ranking the potential etiologies within the differential diagnosis.

The clinical management of rashes that resemble EM, of both Lyme disease and non-Lyme disease origin, is complex and has been addressed elsewhere [12–15]. Although consulting resources such as the CDC's map of Lyme disease incidence is helpful, we also recommend contacting local/state health departments to obtain current epidemiological information that might be more specific to each patient's situation. When the presentation is consistent and the disease is prevalent, empiric treatment of tick-borne illnesses such as Lyme disease or RMSF with doxycycline should be considered before Lyme disease serologic test results become available. However, empiric treatment of EM in areas where Lyme disease is uncommon might not be worthwhile, given the potential common adverse effects

of doxycycline treatment, such as gastrointestinal symptoms (e.g., nausea, vomiting abdominal pain, and diarrhea), sun sensitivity, heightened rates of *Clostridioides difficile* infection, or diminished effectiveness of oral birth control, as well as less common but severe adverse events, including psychiatric disorders, tooth staining in children, serious skin reactions, intracranial hypertension, hyperthyroidism, bone marrow suppression, or kidney damage [16].

In conclusion, Lyme disease, despite being the most common tick-borne disease in the U.S., is not equally common across states. Whereas this disease is highly endemic in some states in the upper Midwest and the Northeast, other states, such as those in the South, have low rates.

The main message of this exercise is to illustrate why the CDC/CSTE case definition suggests pursuing alternative diagnoses before conducting Lyme testing in low-incidence states, by reviewing the fundamental mathematical framework of incorporating disease prevalence in calculating the PPV of the recommended diagnostic process. The pre-test probability that a patient has Lyme disease is exceedingly low, and testing positive in a low-incidence setting/scenario minimally increases the likelihood of a true positive. Even when a patient's clinical picture is consistent with early Lyme disease, in the absence of travel to an endemic area—because, as in this thought experiment, false positive results might potentially outnumber true positive results by 30-fold—diagnostic efforts might be better spent on working up alternative diagnoses first.

For example, multiple tick- and non-tick-associated conditions mimic the rash associated with early Lyme disease. Such conditions not associated with tick-borne infection include insect bite hypersensitivity, cellulitis, drug allergy, ringworm, pityriasis rosea, granuloma annulare, and urticaria multiforme [15]. A major tick-associated illness that mimics Lyme disease in the southern U.S. is STARI [16]. In low-incidence areas, physicians might consider Lyme disease testing only after these conditions that mimic Lyme disease have been thoroughly considered and/or addressed, given the potential interpretive pitfalls in Lyme disease testing.

The poor prognostic value of testing for early Lyme disease in low-incidence jurisdictions can lead to the misdiagnosis and mismanagement of patients who do not have Lyme disease. Furthermore, even when other causes have been ruled out, and the physician decides to test for Lyme disease, in scenarios of potential early disease, particularly in the South, waiting for test results—rather than treating the patient empirically, as recommended in high-incidence states—might seem reasonable, because STARI, for which no diagnostic test is available, is the most likely Lyme disease mimic in the southern U.S. and is a mild, self-limiting illness [16].

#### DATA AVAILABILITY STATEMENT

This study is a thought experiment and as such utilizes no data not described in the references.

## ETHICS STATEMENT

No direct interactions with human or animal subjects were involved. Therefore, ethical approval and informed consent were not required.

## AUTHOR CONTRIBUTIONS

DH conceived of the manuscript. Both DH and AW were involved in design, analysis, interpretation, and writing.

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## CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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