Is the 99th Percentile Cutoff Still Relevant? A Single-Center Assessment of Different Thresholds for Diagnosing Antiphospholipid Syndrome

Article:
Alexis Dadelahi, Brandon S Walker, Dipanwita Banerjee, Michael Mahler, Abdulrahman Saadalla, Vijayalakshmi Nandakumar.
Is the 99th Percentile Cutoff Still Relevant? A Single-Center Assessment of Different Thresholds for Diagnosing Antiphospholipid Syndrome.
J Appl Lab Med 2025; 10(3): 605–18. https://doi.org/10.1093/jalm/jfae149

Guest: Dr. Vijayalakshmi Nandakumar from the University of Colorado School of Medicine and Exsera BioLabs.

Randye Kaye: Hello, and welcome to this edition of *JALM* Talk, from *The Journal of Applied Laboratory Medicine*, a publication of the Association for Diagnostics & Laboratory Medicine. I'm your host, Randye Kaye.

Antiphospholipid syndrome [APS] is a complex autoimmune disorder, characterized by laboratory evidence of antiphospholipid antibodies and complications such as thrombosis and adverse pregnancy outcomes.

Joint guidelines published in 2023 from the American College of Rheumatology and the European Alliance of Associations for Rheumatology defined classification criteria for antiphospholipid syndrome that involve a combination of clinical and laboratory criteria with a scoring system.

Key lab findings include the presence of lupus anticoagulant, anticardiolipin antibodies, or anti-beta-2 glycoprotein-1 antibodies. Traditionally detected using ELISA methods, these antibodies can now be identified with automated methods, such as chemiluminescent and fluorescence enzyme immunoassays.

To maximize specificity, the International Society on Thrombosis and Haemostasis recommends using the 99th percentile of a referenced population as the interpretive cutoff. However, comprehensive studies with enough healthy donors to accurately determine the 99th percentile and assess its applicability in real-world patient populations are still lacking.

The May 2025 issue of *JALM* features a study that defined the 99th percentile threshold for antiphospholipid antibodies and examined how varying cutoff values affected diagnostic performance using two different manufacturers' methods. The authors' findings highlight the assay-specific nature of these cutoffs and suggest that the 99th percentile may not always be appropriate.

The authors emphasize optimizing cutoffs for diagnostic performance and advocate using manufacturer-recommended cutoffs with rigorous validation, when clinical studies are impractical.

Today, we are joined by the article's corresponding author, Dr. Viji Nandakumar. Dr. Nandakumar is an Assistant Professor at the University of Colorado School of Medicine and the Medical Director of the Rheumatology subsection at Exsera BioLabs. Welcome, Dr. Nandakumar.

Viji Nandakumar: Hi. Thank you for having me.

Randye Kaye: To start, can you tell us why you conducted this study on antiphospholipid antibodies and what were your main goals?

Viji Nandakumar: Absolutely. So, as the Medical Director overseeing antiphospholipid antibody tests, I often get asked by clinician colleagues what exactly is the 99th percentile cutoff for your assays, and is my patient results above or below that line? So the 2023 ACR and the updated Sapporo criteria recommend using ELISA to test for these antibodies, especially at or above the 99th percentile.

So, our study are aimed to define this 99th percentile for anticardiolipin and anti-beta-2 glycoprotein-1 antibodies across two different platforms, and compare those results to the manufacturer-recommended cutoffs to assess their diagnostic performance for APS.

Randye Kaye: All right, thank you. So, what exactly is the 99th percentile? How is it determined and how is that different from the cutoffs that labs typically use for other tests?

Viji Nandakumar: That's a great question and one that comes up a lot. So now, labs typically set their test cutoffs in one of two ways. One is through what's called a reference range, where they look at a large group of healthy people and say, okay, here is what a normal looks like. That usually includes the middle 95% of values or the upper 97.5 percentile of a healthy population.

The other way is by using clinical decision limits, where they actually compare patient who have the disease with those who don't, and figure out the level that best separates the two groups. So, with antiphospholipid antibodies, it gets tricky. These antibodies can show up at low levels even in healthy people, and sometimes only transiently.

That's why guidelines recommend using the 99th percentile instead of the more typical 97.5th percentile. It's a way to improve specificity and avoid false positivity.

So, when we talk about 99th percentile in testing, we're basically saying that 99% of healthy people should fall below a certain value and only 1% would be above it. So, it's a way to flag results that are unusually high and possibly abnormal. That said, actually establishing that 99th percentile isn't so straightforward, as it may not be so feasible and resourceful.

You really need data from at least 300 healthy donors to attain adequate statistical power to establish such a cutoff. And even then, there is no universal agreement on a statistical approach, such as to remove outliers and other parameters to consider in order to establish the 99th percentile, and the practices may honestly differ between labs.

Randye Kaye: All right. Thank you. So you evaluated two different manufacturers' methods in your studies. So, how did they compare when it came to the 99th percentile cutoffs?

Viji Nandakumar: Yeah. So, this was actually one of the most interesting parts of the study. We found very significant differences between the two platforms. For the quantitative ELISA, the 99th percentile values derived based on healthy donors were actually so low that they fell at or even below the test's limit of quantification. Meaning, if we were to use that as our cutoff to identify APS patients, everyone would have looked positive, yielding zero specificity.

So, in that case, it was clear that the 99th percentile cutoff was inappropriate, and the cutoff actually needed to be much higher. Interestingly, the manufacturer's recommended cutoff for the test was already quite a bit higher than the 99th percentile, and that performed much better.

Now, for the Aptiva PMAT platform, which is a newer platform based on the particle-based multi-analyte technology, it was a different story. The 99th percentile cutoff were still on the lower side, but they worked pretty well. They gave us a diagnostic accuracy that was very close to what we saw with the manufacturer's recommended values. So, in that case, the 99th percentile was more aligned with the clinical expectations.

So, overall, it really shows how important it is to evaluate each test on its own, because the same threshold doesn't work equally well across platforms.

Randye Kaye: Okay. Thank you. So, the 99th percentile is intended to provide high specificity. So, why do you think it works better on one platform versus the other?

Viji Nandakumar: Great question. So here is the thing, not all tests are built the same, and there is considerable variability in the field.

Even if two tests use the same method, like ELISA, they might be designed to detect different parts of the antigenic sequence. So the 99th percentile might work well for one test that detects an antigenic sequence that is more prevalent in the healthy population, but not for another test that is only seen in a disease state.

So, some assays might even need a higher or a lower cutoff to strike the right balance between the sensitivity and specificity. So, in short, while the 99th percentile is a useful guideline, its effectiveness really depends on the specific test and how it is designed.

Randye Kaye: I see. So, can we talk a little bit more about the issues of sensitivity and specificity for these assays? What are the tradeoffs between these two concepts when you're working with 99th percentile cutoffs?

Viji Nandakumar: Definitely. It's a really important balance to understand any kind of diagnostic testing, and with APS, this gets even more complicated. That's because the antibodies we are testing for, what we call as aPL antibodies, can occasionally appear in healthy individuals, typically at low levels, and sometimes only transiently.

So, if your cutoff is too low, you might end up labeling a lot of healthy people as positive. So because of this, it may be beneficial to use the cutoff for aPL antibody testing that is optimized for higher specificity without significantly compromising sensitivity. So, this is where decision limits become important. In fact, most of the APS test kits on the market today, especially the FDA-cleared ones, use these kind of clinical decision-based cutoffs.

They've already gone through studies that evaluate how well they perform in actual patients. So, they are usually more fine-tuned for clinical use. And that's really one of the big takeaways from our study. The right cutoff isn't always the 99th percentile. It depends on the platform, the patient population, and what kind of diagnostic accuracy you are targeting.

Randye Kaye: One final question. Some labs may not have the resources like access to large antiphospholipid syndrome patient cohorts or the ability to run extensive reference interval studies. So, how would you recommend that they establish their own 99th percentile cutoffs?

Viji Nandakumar: That's a great question and a really common challenge for many labs. So, if a lab doesn't have the resources to run large-scale validation studies, I'd recommend sticking with the manufacturer-recommended cutoffs. Most of the aPL kits available today are FDA-cleared, and their cutoffs are

typically based on real clinical data, not just healthy individuals. These are what we call clinical decision limits, and they are designed to strike a better balance between sensitivity and specificity.

In fact, many manufacturers go beyond just the 99th percentile. They may set higher cutoffs based on what actually works best in diagnosing APS and if you're bringing in a new assay into your lab, I definitely suggest reaching out to the manufacturers. They can provide helpful background on how those thresholds are determined and why they are clinically relevant.

That said, if the lab does have small amount of internal data, maybe a few known APS or control cases, you can still do some internal validation to make sure the test performs well for your patient population.

So, at the end of the day, it's really about using the most evidence-based clinically-validated thresholds you have access to, not just sticking to a generic statistical number like the 99th percentile. Our study and others make it clear that cutoffs may need to be adjusted depending on the specific platform and context, and the 99th percentile guidelines may not always be applicable for all aPL testing platforms.

Randye Kaye: All right. Thank you so much for joining us today, doctor.

Viji Nandakumar: Thanks again for having me. Thank you.

Randye Kaye: That was Dr. Viji Nandakumar from the University of Colorado School of Medicine and Exsera BioLabs, describing the *JALM* article, "Is the 99th Percentile Cutoff Still Relevant? A Single-Center Assessment of Different Thresholds for Diagnosing Antiphospholipid Syndrome."

Thanks for tuning in to this episode of *JALM* Talk. See you next time. And don't forget to submit something for us to talk about.