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Methods to Improve Confidence in the Accuracy of Molecular Testing for Multidrug-Resistant Tuberculosis.

Clin Chem 2026; 72(3): 390–7. <https://doi.org/10.1093/clinchem/hvaf178>

Guest: Dr. Denise O'Sullivan is a Principal Scientist in Analytical Microbiology at the National Measurement Laboratory in Guildford, United Kingdom.

Bob Barrett:

This is a podcast from *Clinical Chemistry*, a production of the Association for Diagnostics & Laboratory Medicine. I'm Bob Barrett.

In 2023, tuberculosis surpassed COVID-19 and reclaimed its historical place as the most common cause of infectious disease mortality worldwide, with an approximate 1.25 million deaths attributed to TB. Accurate diagnosis and appropriate treatment are essential to reduce this number and current guidelines recommend the use of rapid molecular tests that detect bacterial DNA. An important quality of these tests is the ability to identify drug-resistant strains allowing care providers to quickly discontinue ineffective treatment. This sounds simple in theory, but becomes challenging in practice, particularly in patients with mixed infections where both susceptible and resistant strains are present.

How do currently available molecular test methods perform in patients with mixed infections? The answer is that we don't really know, largely because we don't have reference standards that mimic co-infection with susceptible and resistant strains.

That, however, is changing. In a research article in the March 2026 issue of *Clinical Chemistry*, the authors described their creation of new multidrug-resistant TB reference materials and summarize the overall performance of molecular test methods used to test it.

Today we are joined by the article's lead author. Dr. Denise O'Sullivan is a Principal Scientist in Analytical Microbiology at the National Measurement Laboratory in Guildford, United Kingdom. She currently leads research focused on improving the accuracy of molecular methods ranging from applied diagnostics to metagenomic methods.

Dr. O'Sullivan, let's get basic first, why is accurate testing important for molecular diagnostics?

Denise O'Sullivan: So molecular diagnostic tests, they analyze the molecules that could be DNA, RNA, or proteins, which are present in a specimen, typically to identify a disease or the risk of developing a disease, and by studying these molecular mechanisms of disease, the tests can be used then to detect, diagnose, and monitor infectious diseases, but also things like oncology and genetic disorders.

So accuracy, and if we think of it like 'how close is the result of the true value?' is actually critical in molecular diagnostics as it enables early disease detection, effective monitoring, and better disease management.

So in infectious diseases, this can mean that pathogens, so those nasty things that are causing the disease, are identified at early stages and this could be even before physical symptoms appear.

High accuracy testing for detection of drug resistance, as is looked at in this study, reduces the likelihood of treatment failure, and this is actually critical for diseases like tuberculosis where treatment is lengthy, very complicated, because it requires multiple drugs. Improper therapy can lead to increased risk of developing further resistance and mortality.

And so molecular tests can actually offer a quicker method by which to detect the resistance in the case of *Mycobacterium tuberculosis*, which causes tuberculosis. This resistance is typically genotypic, which means it can be detected by molecular tests. So therefore, accurate testing improves the outcome for people with tuberculosis and quality control processes ensure that the tests are reliable, and this actually improves confidence in the result for the doctors, but also for the patients, and accurate molecular diagnostic testing requires appropriate quality assurance.

Bob Barrett: And how did this approach support quality assurance for multidrug-resistant tuberculosis testing?

Denise O'Sullivan: So this particular piece of work set about preparing material standards, which were made up of drug-resistant *Mycobacterium tuberculosis* and also a material standard that contained mixtures of a drug-resistant and a drug-sensitive *Mycobacterium tuberculosis*. We developed a reference measurement procedure to characterize these material standards so we knew what we had mixed together. We could understand how much TB was actually present in the material and what part of that contained the resistance, so we had really well-characterized material standards.

The reference measurement procedure that I mentioned, this is particularly a route which can be developed to support the standardization of molecular diagnostics. And so what you can do is apply this method to material standards. There does not need to be any available international reference standards. And this enables the assessment of the quality of the actual molecular diagnostic test. So in this case the detection of the multidrug-resistant TB, thereby improving accuracy and precision of the molecular diagnostic methods.

Bob Barrett: Doctor, why would one use digital PCR for this approach?

Denise O'Sullivan: So digital PCR is a method by which you can perform absolute counting of a target molecule. In this example that is counting of the *Mycobacterium tuberculosis*, but also counting of the drug-resistant *Mycobacterium tuberculosis*.

So what you do is it typically starts by adding your sample, which could be something like DNA or RNA, into reaction. And then in this reaction there is subdividing into tens of thousands of nanoliter reactions. This is really what separates digital PCR from many of the other PCR-based methods. We call this particular process 'partitioning.'

Now, in each of these partitions if there is your target molecule present, there would be a fluorescence and that can be measured. And then when there is no molecule present, there is no fluorescence.

So what we do is we actually count up the number of partitions that contain molecules and also that do not contain molecules. We apply then some statistics to use this to determine the concentration in our sample.

Digital PCR, the actual method itself, has the potential to be SI-traceable, and by this I mean it could be traceable to the SI unit, so the *Système international d'unités*. Our group and others in our community of national metrology institutes have shown that with careful measurement this method can be SI-traceable, and we've shown this when detecting things such as KRAS, which is a single nucleotide variant in colorectal cancer. These high accuracy measurements support the standardization of molecular diagnostics and enable them to be traceable to the SI.

Bob Barrett: Let's get to some of your findings, what were the key findings of this research?

Denise O'Sullivan: So in this research we prepared three drug-resistant standards and one material standard which contained a mixture of drug resistance and drug-sensitive *Mycobacterium tuberculosis*. All of the concentrations were known in the samples as we had applied our reference measurement

procedure to the materials. And we sent these materials to nine laboratories around Africa and Europe, and what we did was we asked them to perform their molecular diagnostic methods for TB, and if they had the methods available also to see if they could detect drug-resistant TB.

What we found was that all tests correctly identified that there was TB present; however, two out of the three tests identified that there was drug resistance, so that means one test missed the presence of drug resistance.

In this particular sample this contained lower concentrations of *Mycobacterium tuberculosis* and also was more complex because it was actually a mixture of drug-resistant and drug-sensitive TB. The approach that we used in this study allowed the assessment of the quality of these multiple drug-resistant TB molecular diagnostic tests and ultimately these approaches will improve the accuracy of molecular diagnostics as we've demonstrated. This will improve confidence in the results of the tests and lead to better test selection and improve treatment for patients.

Bob Barrett: Well, finally, Dr. O'Sullivan, let's widen the lens a little bit. Could this approach be applied to other scenarios or disease targets?

Denise O'Sullivan: Yes, absolutely, as long as we know what's actually causing the particular disease. So if we have knowledge as to the target, we can actually build and develop reference measurement procedures to detect that target. We and other colleagues have done this already for detection of SARS-CoV-2, human cytomegalovirus, HIV, as well as genetically modified organisms, so GMOs in foods.

Bob Barrett: That was Dr. Denise O'Sullivan from the National Measurement Laboratory in Guildford, United Kingdom. She wrote a research article in the March 2026 issue of *Clinical Chemistry* describing a new approach to ensure high quality testing for multidrug-resistant tuberculosis and she has been our guest in this podcast on that topic. I'm Bob Barrett. Thanks for listening.