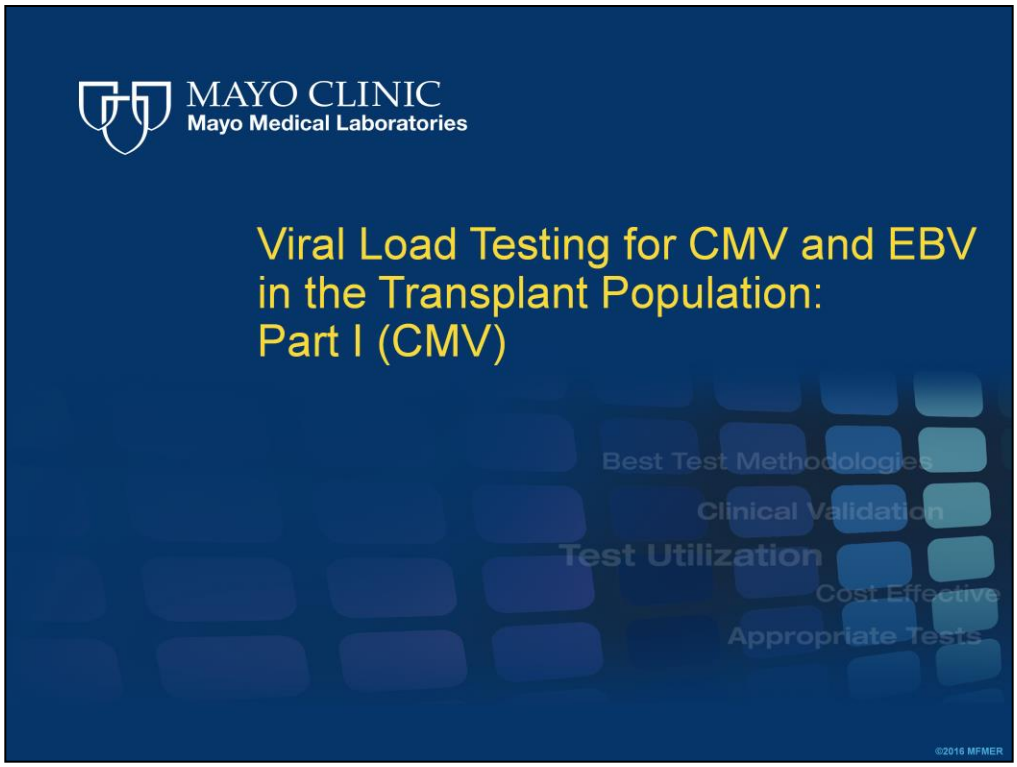

Welcome to Mayo Medical Laboratories Hot Topics. These presentations provide short discussions of current topics and may be helpful to you in your practice. Cytomegalovirus and Epstein-Barr virus are viral pathogens that may cause significant morbidity and mortality following transplantation. This 2-part presentation discusses the clinical presentations of infection, reviews the available testing for these viruses, and provides guidance on selecting the appropriate tests for your patients. Part 1 will focus on CMV in the transplant population and will include a case study that highlights the use and interpretation of lab tests in the diagnosis and monitoring of CMV disease.

Matt Binnicker, PhD
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Viral Load Testing: Part 1 CMV

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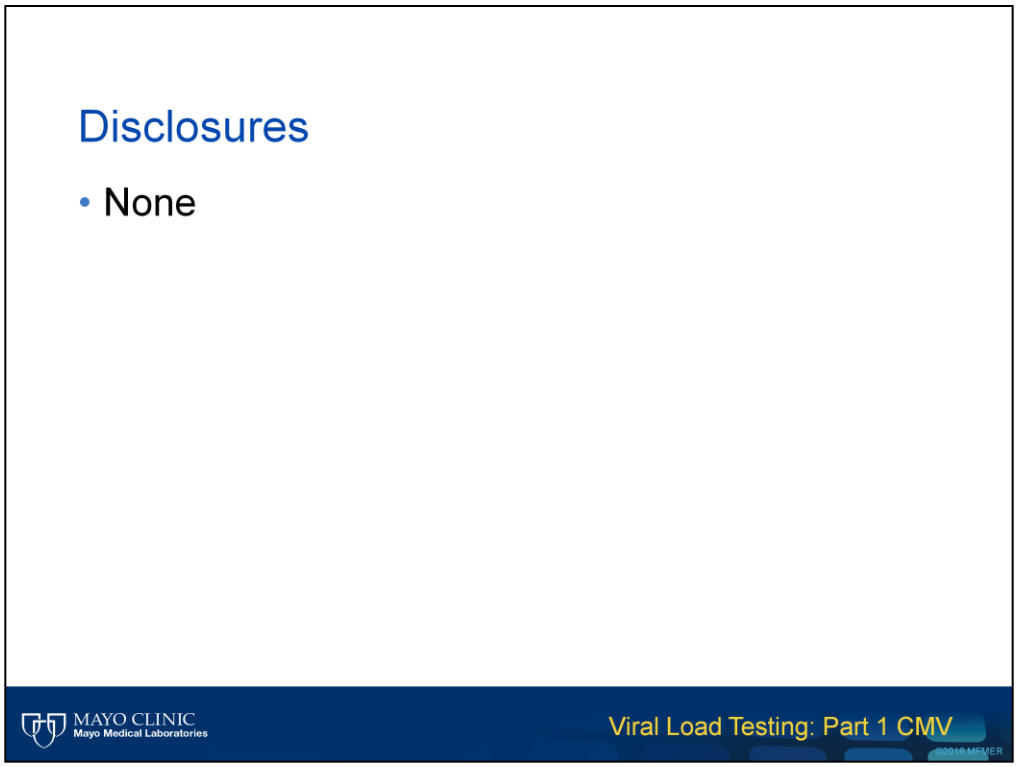
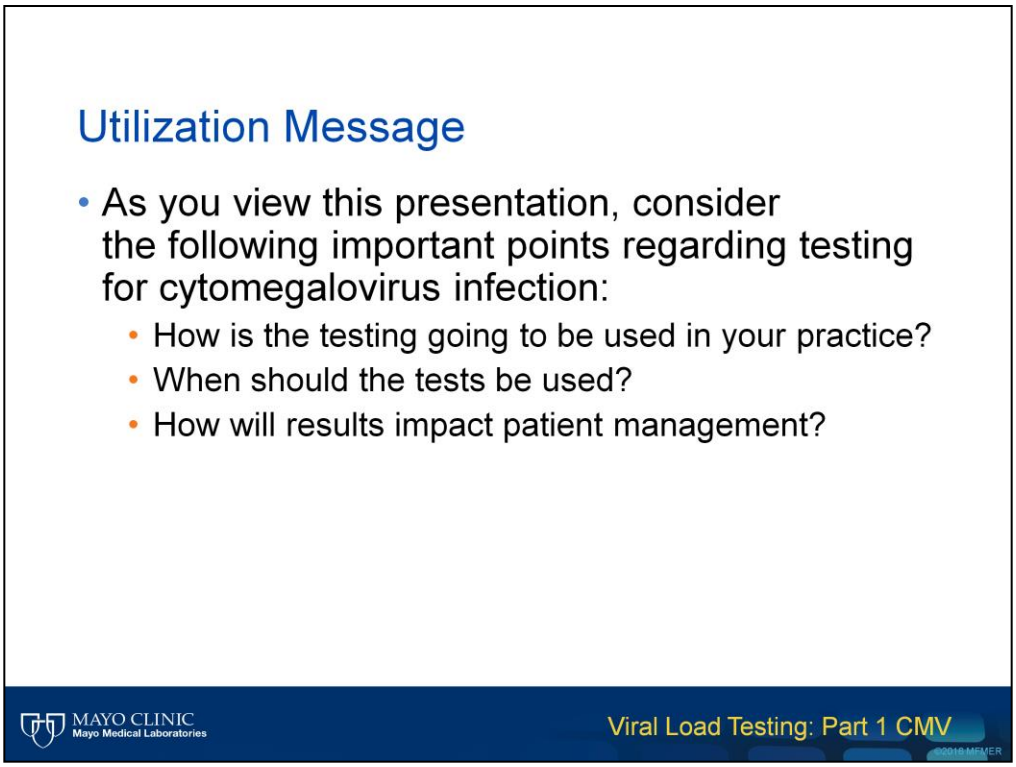
Our speaker for this program is Dr. Matt Binnicker, Associate Professor of Laboratory Medicine and Pathology and Director of the Clinical Virology Laboratory in the Division of Clinical Microbiology at Mayo Clinic in Rochester, Minnesota.

Dr. Binnicker, thank you for presenting today.

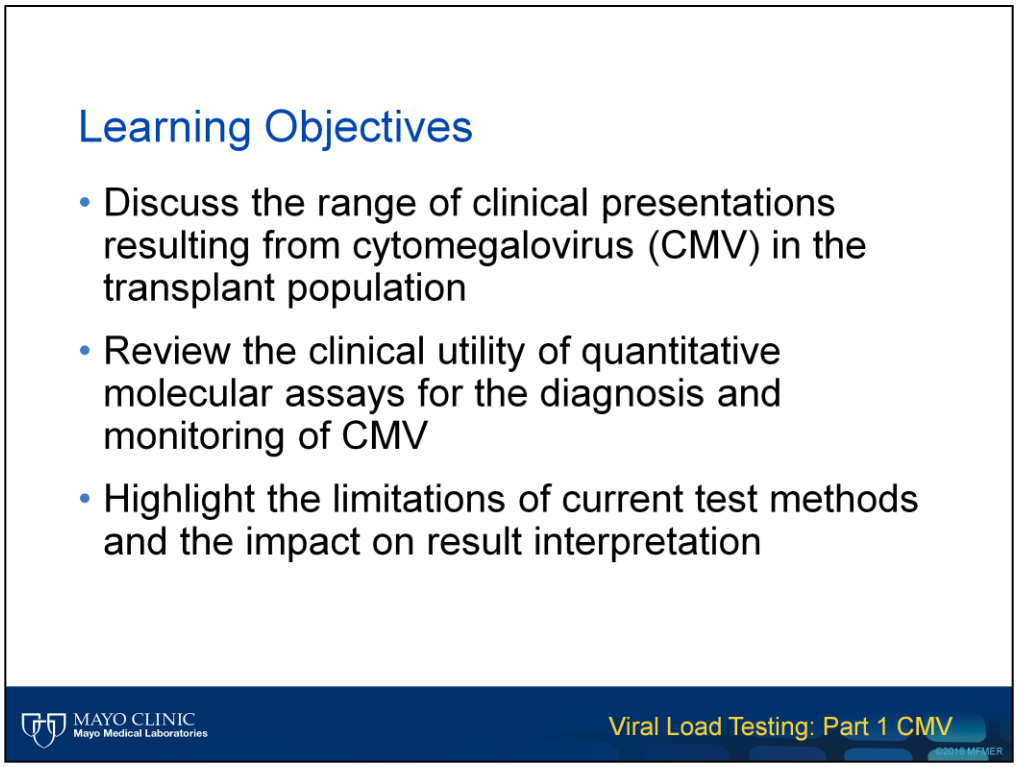
Thanks for the introduction, and thanks for joining for me for this update on viral load testing in the transplant population.



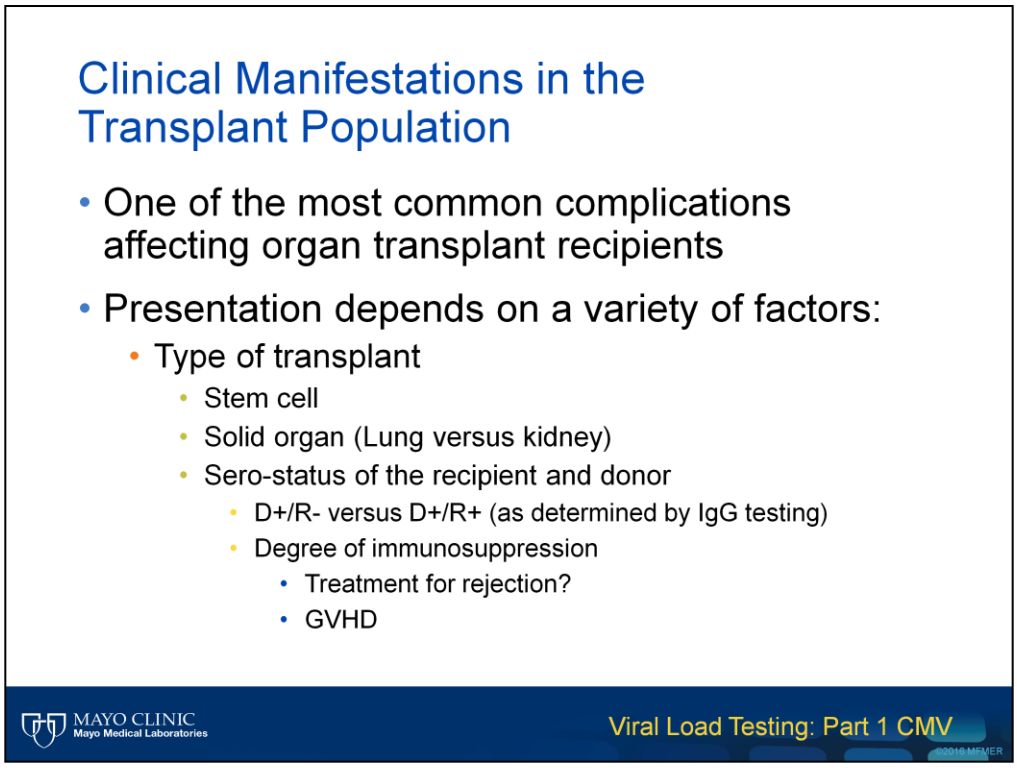
Before I begin, I should mention that I don't have any corporate or financial conflicts of interest to disclose.



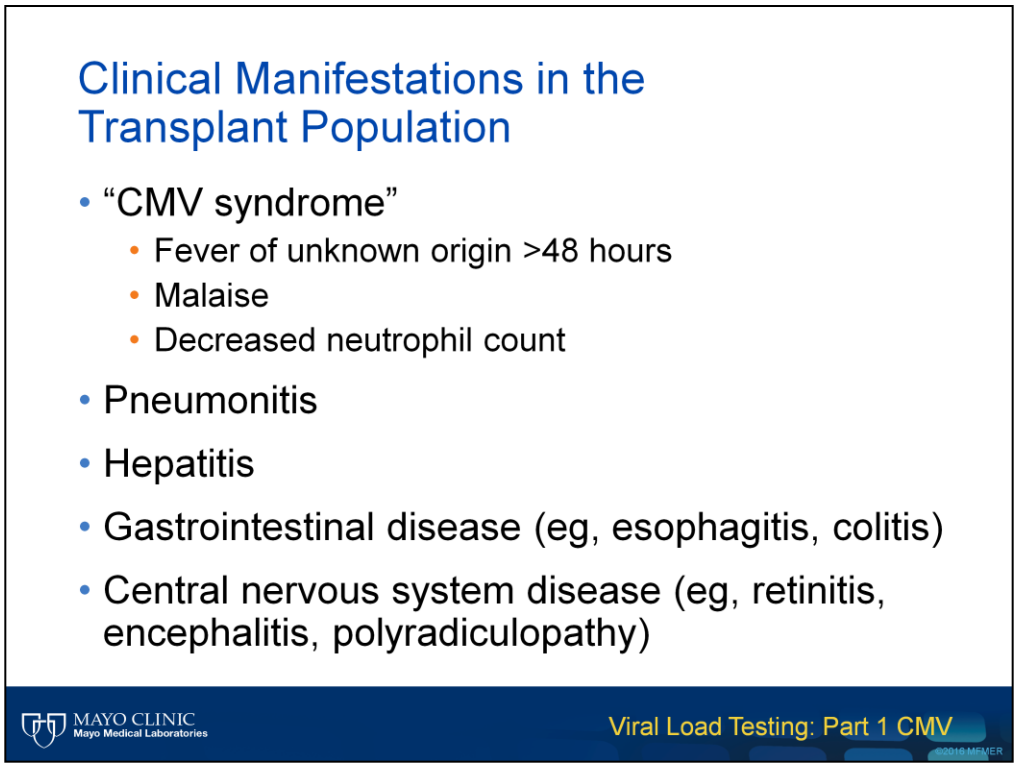
Also, I'd encourage you to consider several points in regards to test utilization as you view this presentation. First, how is the testing that we'll discuss going to be used in your practice? Second, when should the tests be ordered and, finally, how will the results impact patient management?



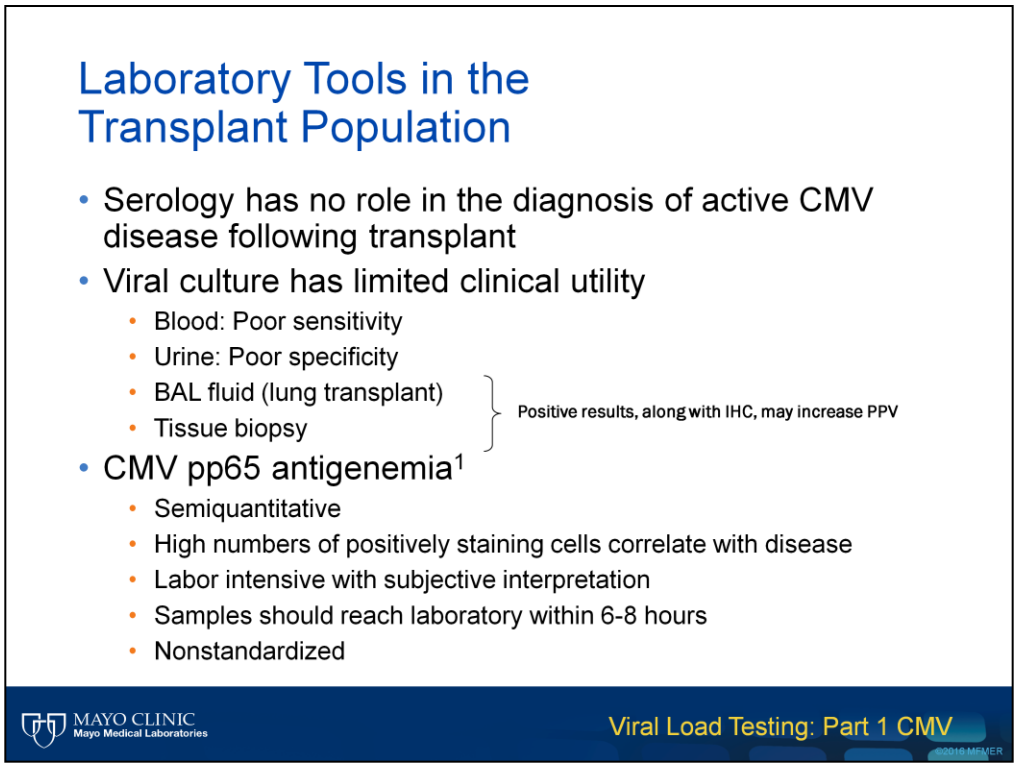
In part 1 of this series, we're going to focus on a significant cause of morbidity and mortality in the transplant population, cytomegalovirus. We're going to discuss the range of clinical presentations resulting from CMV in the transplant population, and review the clinical utility of quantitative molecular assays for the diagnosis and monitoring of this virus. And finally, I'll highlight the limitations of current test methods and the impact these limitations may have on result interpretation. So let's get started.



Cytomegalovirus or CMV is one of the most common viruses affecting transplant recipients. CMV is a member of the herpes virus family, and a high percentage of the population has been infected with CMV by adulthood, so it can cause either an acute or reactivated infection in transplant recipients. The clinical presentation following CMV infection ultimately depends on a variety of factors. First, it's important to consider the type of transplant – for example, did the patient receive a stem cell or solid organ transplant? Second, what was the serostatus of the recipient and donor prior to the transplant? This is determined by serologic testing for IgG-class antibodies to determine if the patient has been exposed to the virus. The highest risk for disease following transplant is when the donor is seropositive for CMV, while the recipient is seronegative, which is noted as a D+/R- classification. A third factor that may influence the clinical presentation is the degree of immunosuppression. For example, is the patient severely immunosuppressed and being treated for possible rejection, or is there concern for graft versus host disease? Remember, in transplant recipients, it's really a balancing act between maintaining enough immunosuppression to prevent rejection, but not too much immunosuppression so that the patient succumbs to opportunistic infections.



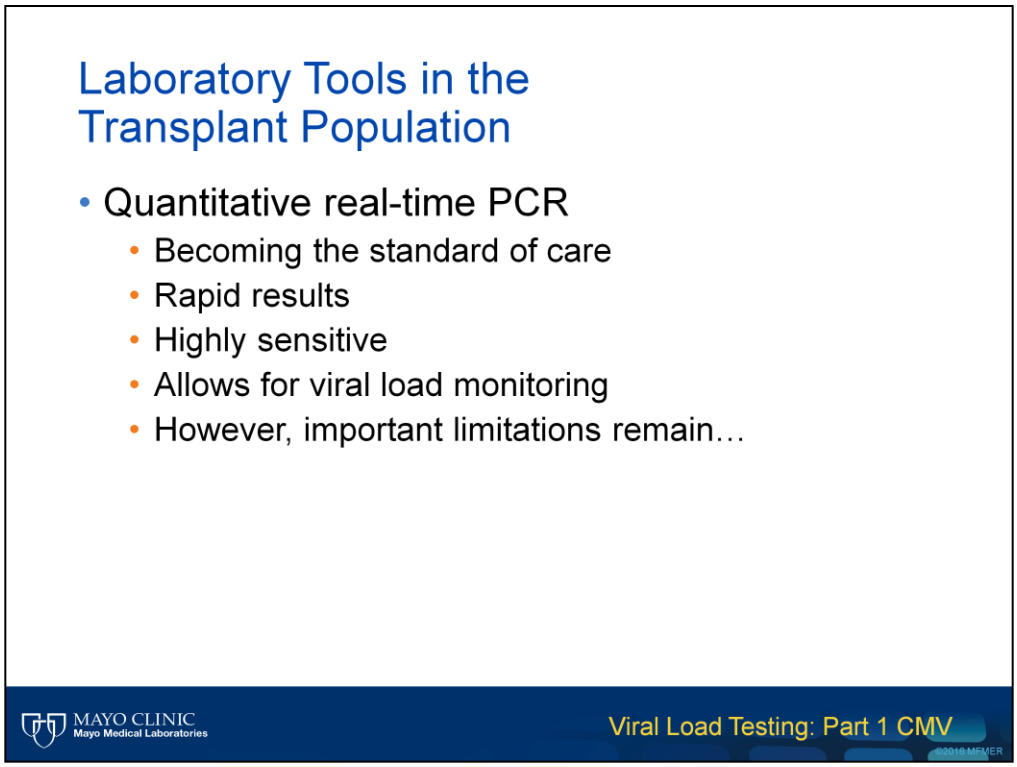
In patients that develop disease due to CMV, there are a number of clinical manifestations that may occur. A common presentation is known as CMV syndrome, in which patients may develop a fever, generalized malaise, and a decreased neutrophil count. Other manifestations include pneumonitis, hepatitis, gastrointestinal illness, and in some cases, central nervous system disease.



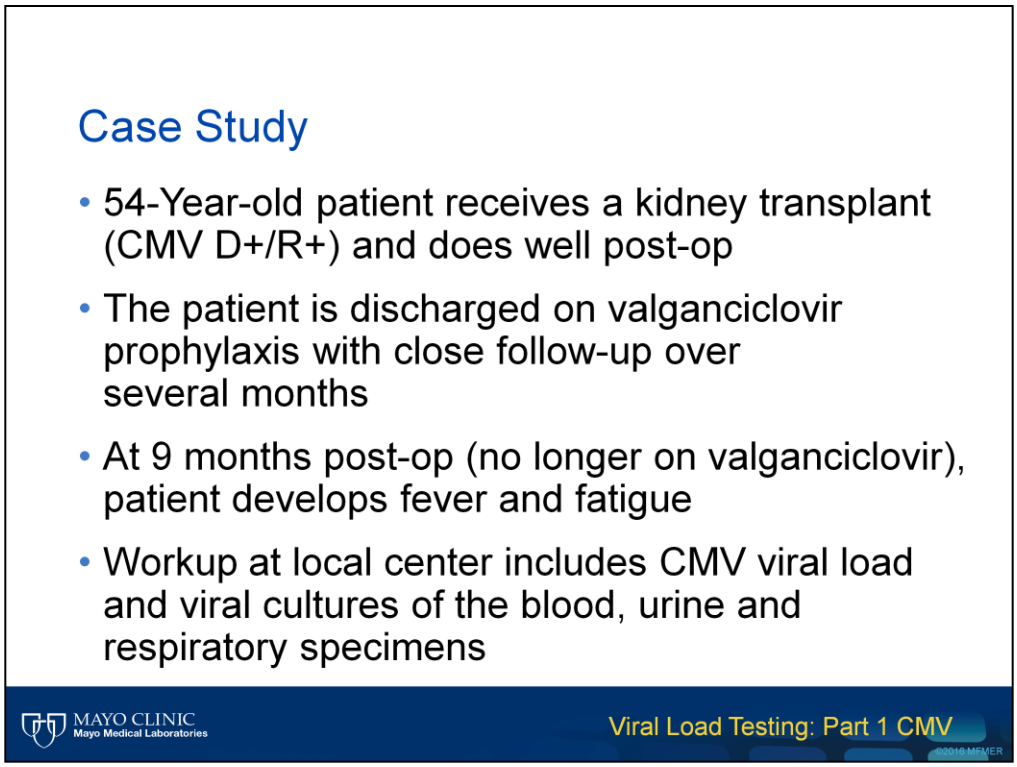
When CMV disease is suspected in a transplant patient, there are several laboratory tools that can be used to assist in establishing the diagnosis. Although serology is commonly used to determine the donor and recipient's serostatus prior to transplantation, serology generally has no role in the diagnosis of active CMV disease in the post-transplant setting.

Viral culture is a method that has been used for decades in the diagnosis of viral infections, but this approach has limited clinical utility for diagnosing CMV disease in transplant patients. This is due o several important limitations, including poor sensitivity in specimen types such as blood, and poor specificity for disease in samples like urine. Recovery of the virus in certain sources, such as BAL fluid in lung transplant recipients, or tissue from an affected organ, may have a higher positive predictive value, but often needs to be accompanied by immunohistochemistry to confirm tissue invasive disease.

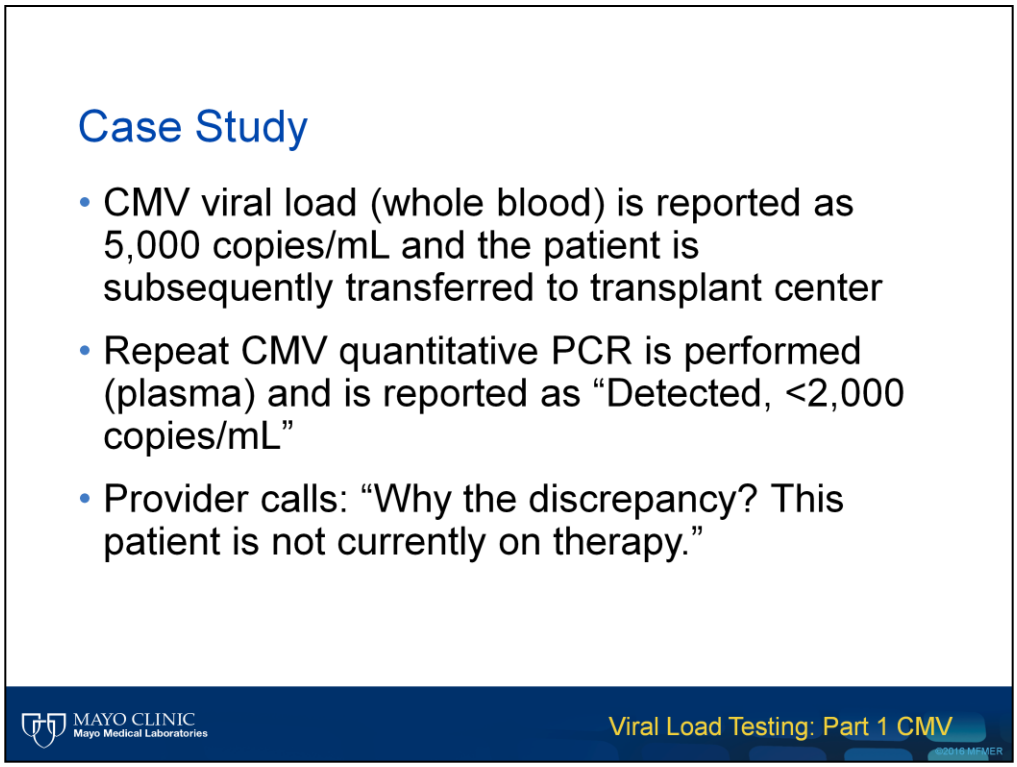
Pp65 antigenemia is another method for diagnosing CMV, but this approach has become uncommon in most testing laboratories. Although this test is semiquantitative and results correlate fairly well with disease, it is labor intensive, subjective, and isn't standardized among testing labs.



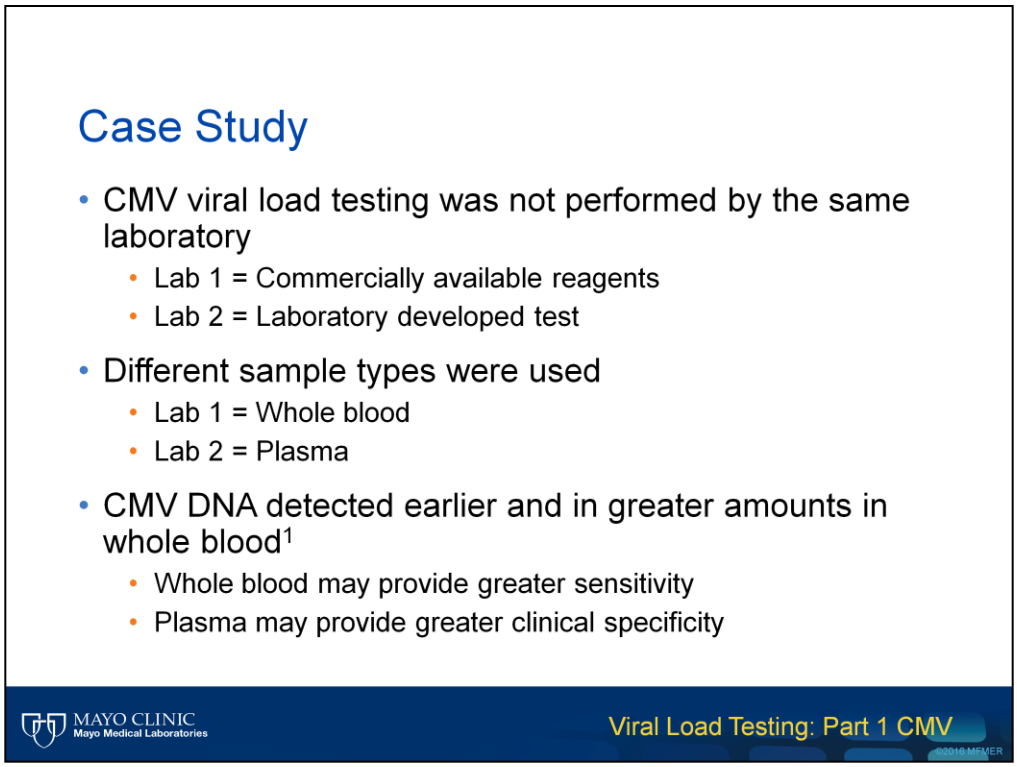
That brings us to quantitative real-time PCR, which has become the standard of care for diagnosing and monitoring transplant patients with CMV disease. This approach provides rapid results, is highly sensitive, and due to the potential to quantitate results, allows providers to monitor the patient's viral load after treatment has been initiated. However, I should emphasize that there are important limitations with quantitative real-time PCR, and to help illustrate some of these limitations, I'd like to review a brief patient case.



In this case, a 54-year-old patient undergoes a kidney transplant, for which the CMV serostatus pretransplant was donor positive, recipient positive. The patient did well postoperatively, and was ultimately discharged on valganciclovir prophylaxis with close follow-up over several months. At 9 months postop, the patient develops fever and fatigue, and has a number of lab tests performed at a local center, including CMV viral load testing, as well as viral cultures of the blood, urine, and respiratory specimens.

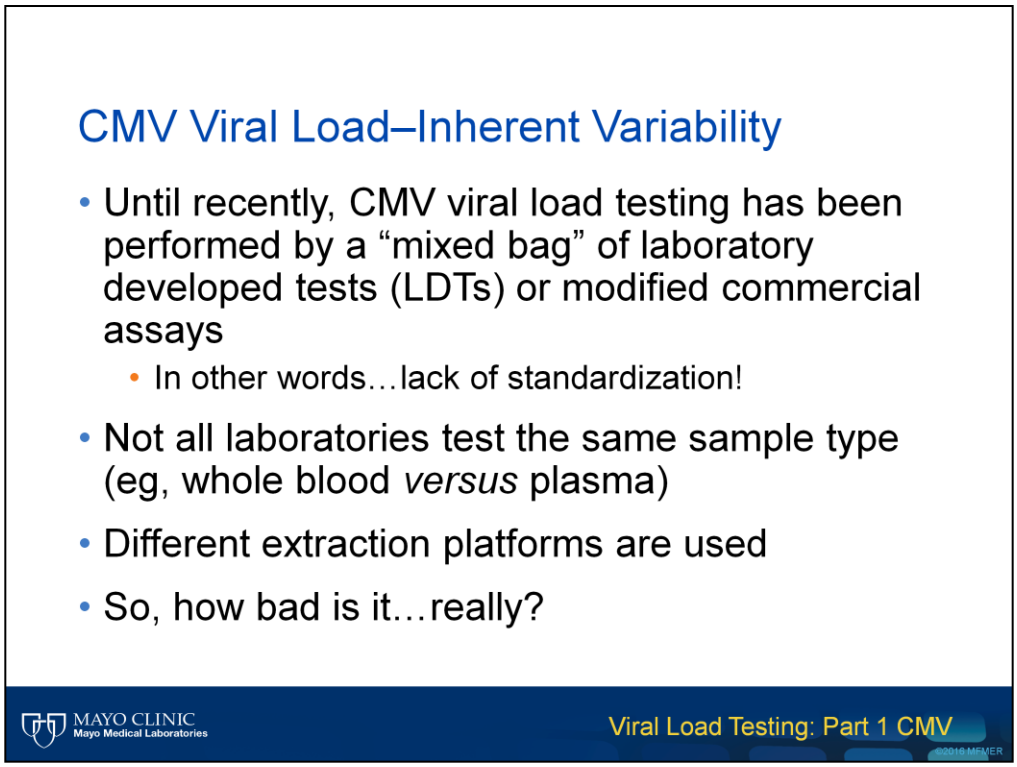


The results of the viral cultures came back negative, but the patient's CMV viral load on a whole blood sample was reported as positive at 5,000 copies/mL. Due to this, the patient was transferred to a transplant center for further evaluation, where the CMV quantitative PCR was repeated, but the repeat test was performed on plasma and reported as "Detected, but less than 2,000 copies/mL." Confused by the 2 viral load results, the provider calls the laboratory, questions the discrepancy, and wonders how the viral load has dropped by more than 2-fold when the patient is not currently on therapy?

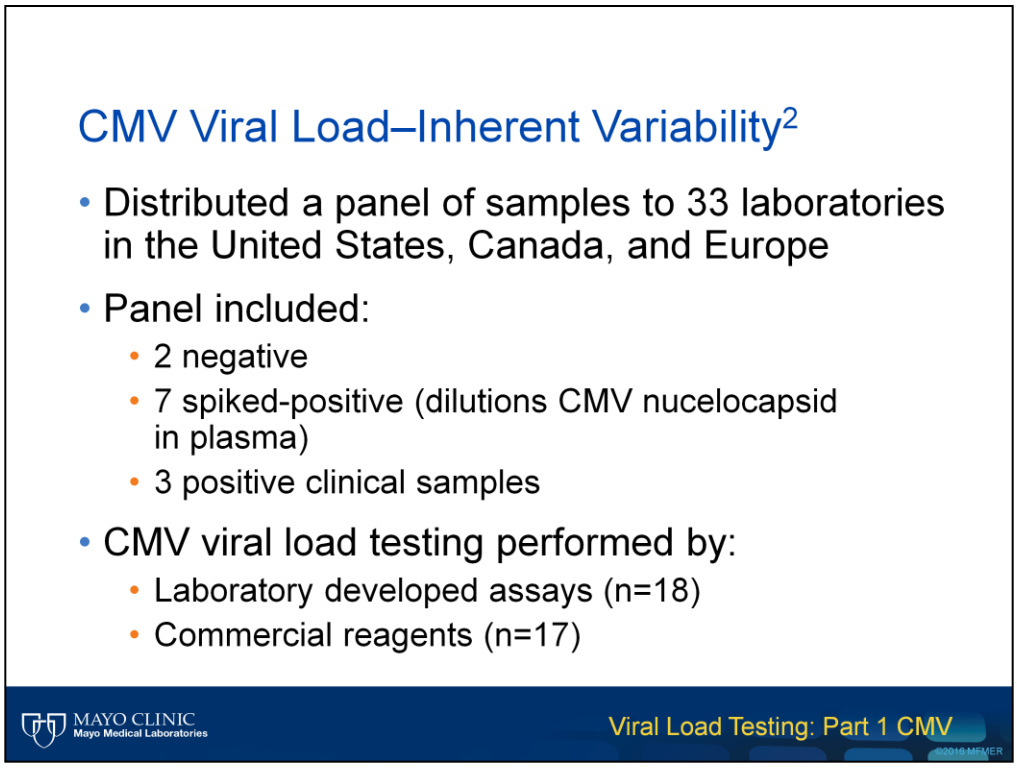


To help address these questions, we have to look at some of the details regarding the testing that was performed. First, it's important to highlight that the CMV viral load testing was not performed in the same laboratory; the first test was performed in a lab using commercially available reagents, while the second laboratory uses a laboratory developed test. Second, the 2 labs perform testing on different sample types, with lab 1 testing whole blood, and lab 2 using plasma. This is an important point, as previous studies have demonstrated that testing whole blood for CMV may provide greater sensitivity for detecting the virus, but testing plasma may yield greater clinical specificity; in other words, detecting the virus in plasma may correlate more closely with clinical disease compared to detecting CMV in whole blood.

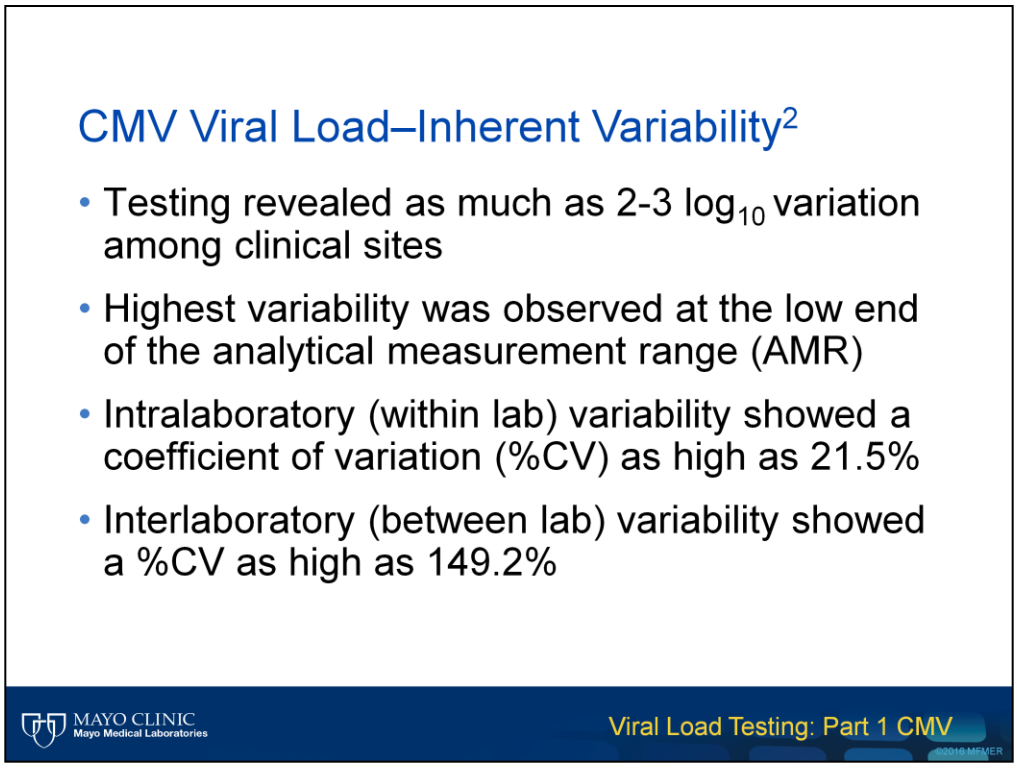
So although these points may begin to help us understand the challenges associated with monitoring CMV, you're probably still left asking the question, "Why are viral loads so different?"



Unfortunately, there isn't an easy answer to this question. It really boils down to there being a lot of inherent variability in the process, and the fact that until recently, CMV viral load testing has been performed using a "mixed bag" of laboratory developed tests or modified commercial assays. So, in other words, there has been a lack of standardization in how labs have been testing for CMV viral loads. In addition, not all labs test the same sample type (eg, whole blood versus plasma) and as we pointed out earlier in the presentation, this can introduce variability in the results that are generated. Even details down to the level of which platform or instrument a lab is using to extract viral nucleic acid from clinical samples can have a significant impact on the results that are generated. With all of these potential sources of variability, I think it's important for us to spend just a few minutes discussing how much variability in CMV viral load testing potentially exists.



In 2009, a study was published that assessed intra- and interlaboratory variability. This group distributed a panel of samples to 33 labs in the United States, Canada, and Europe. Among the participating laboratories, CMV viral load testing was performed by laboratory developed tests in 18 labs, and commercially available reagents in 17 labs. The results of this study showed that there was as much as 2 to 3 log variation in CMV viral loads among the clinical sites. The highest variability in results was observed with specimens containing CMV at the low end of the analytical measurement range. Surprisingly, the intralaboratory variability, as measured as the percent coefficient of variation, was as high as 21.5%. This means that when a sample was tested in replicates within the same lab, using the same instrumentation, there was a substantial amount of variability. And the interlaboratory variability, or the difference in results between one lab and another, showed a percent CV as high as 149.2%! This study, as well as others, have shown that the variability in CMV quantitative real-time PCR results is real, and in some situations, significant.



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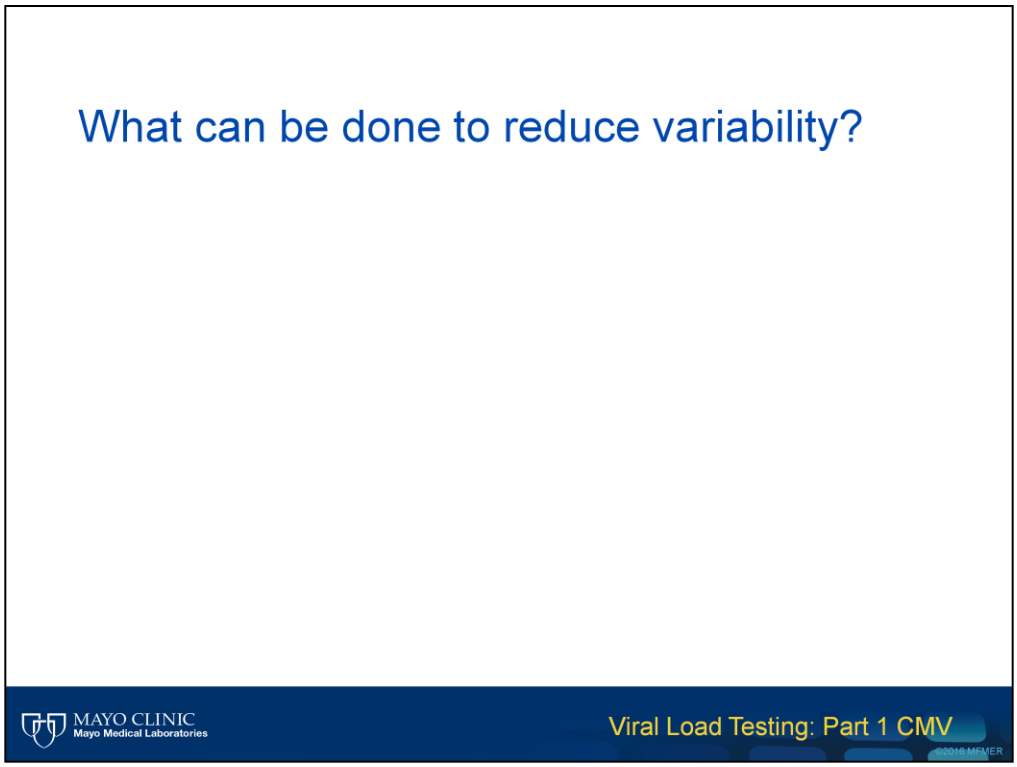
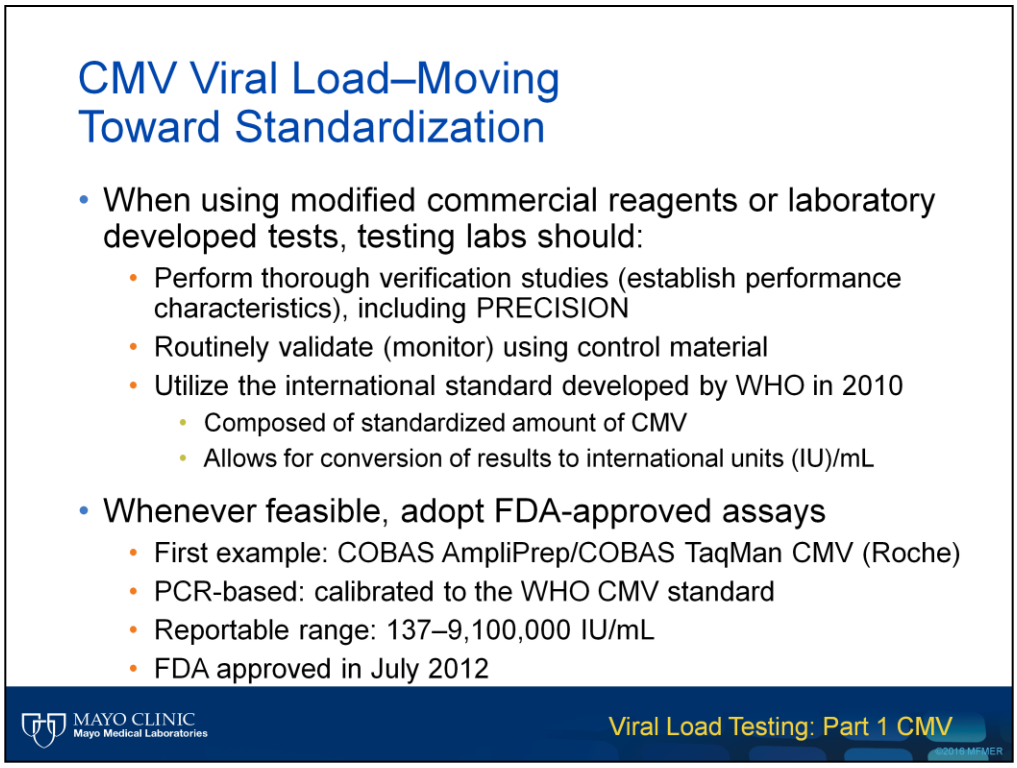
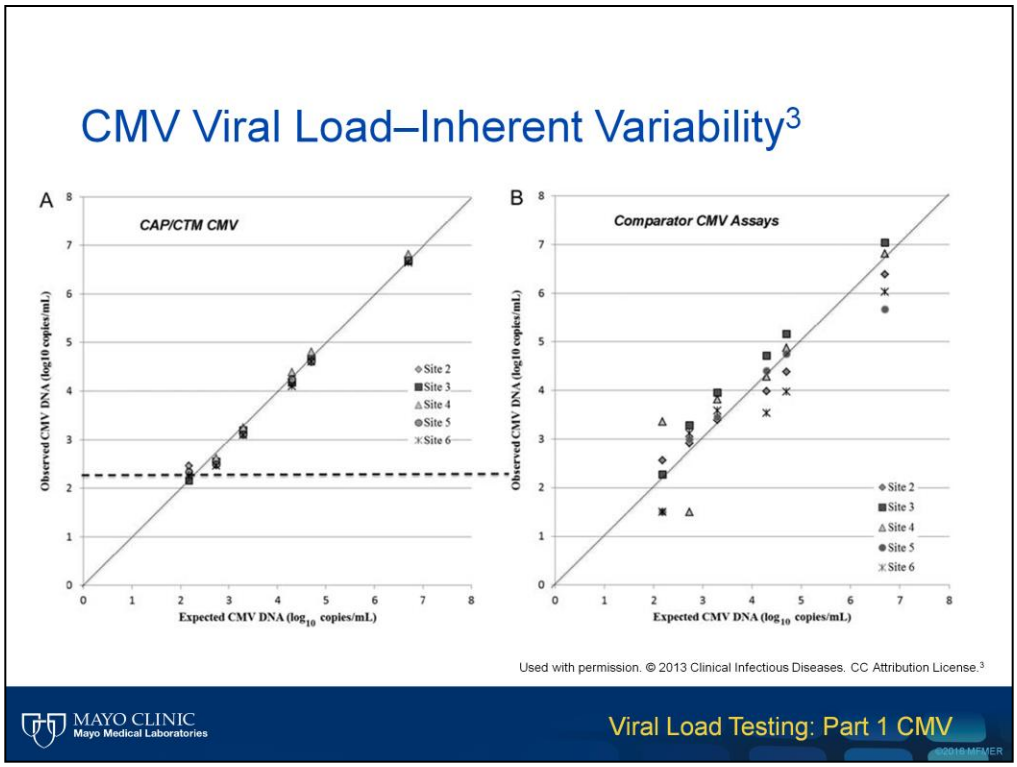


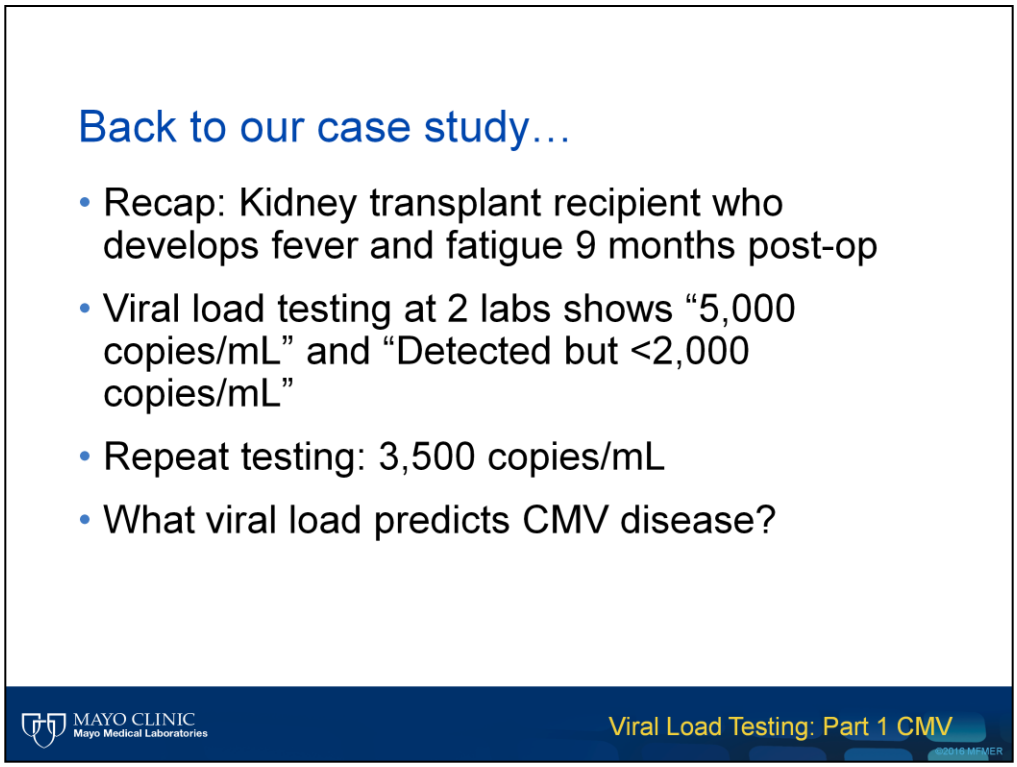
So what can be done to help reduce this variability?



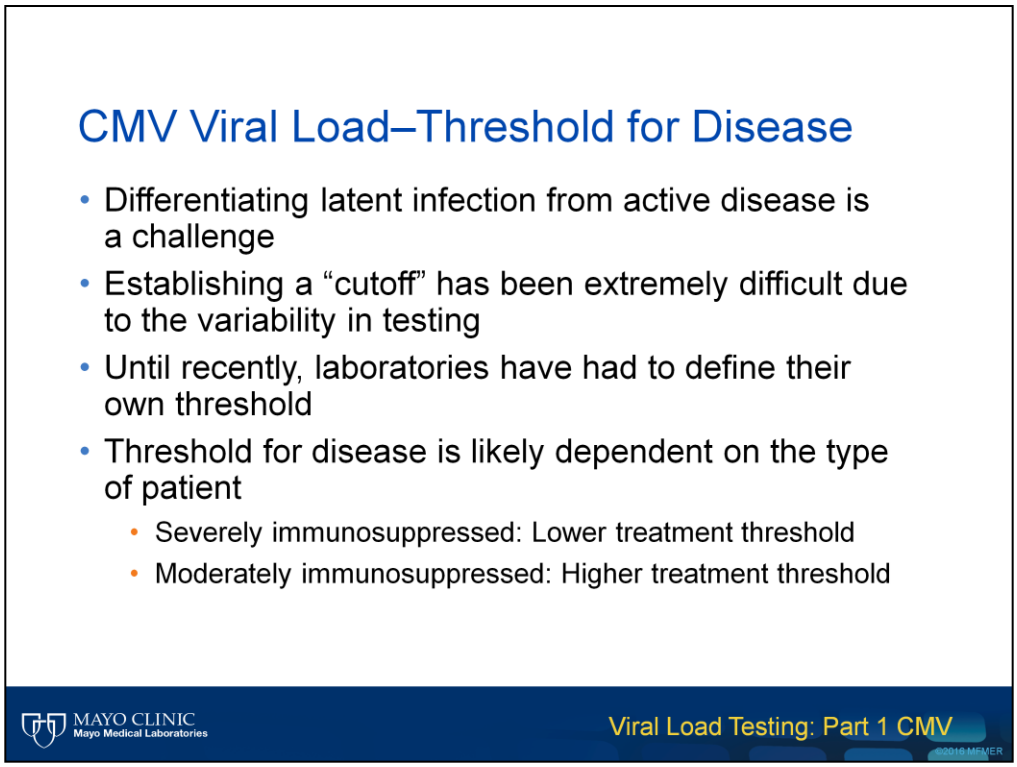
Well, when a laboratory is using modified commercial reagents or laboratory developed tests, they should perform thorough verification studies, including a robust assessment of the quantitative precision of the assay. Testing labs should also routinely monitor the performance of their test using control material and, whenever possible, utilize the international standard developed by the World Health Organization (WHO). This international standard is composed of known amounts of CMV, and allows for conversion of results to international units per milliliter. Importantly, there are now several commercially-available tests that are FDA-approved for CMV viral load testing in transplant patients and, whenever feasible, labs should consider adopting these tests. The first example was the Cobas AmpliPrep TaqMan assay, which was approved by the FDA in July of 2012. This is a PCR-based assay calibrated against the WHO CMV standard, and it has a reportable range of 137 to 9.1 million international units per mL. The Qiagen artus CMV assay was approved in 2014, and is another option for FDA-approved CMV quantitation. So does using a standardized, FDA-approved test make a difference?



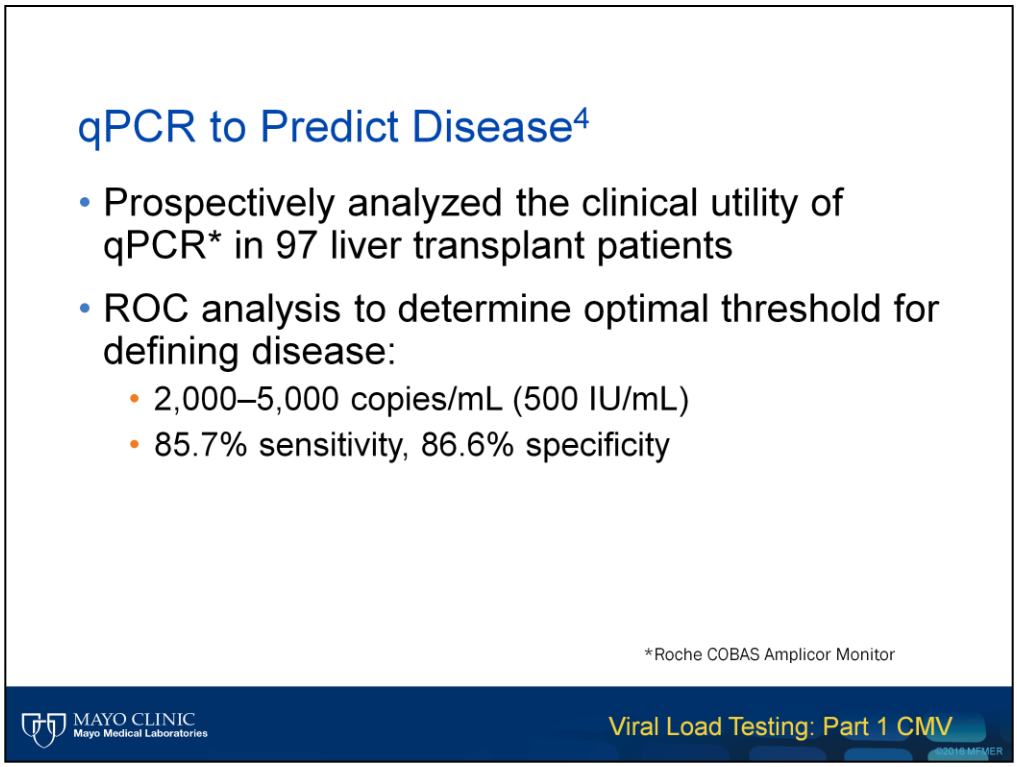
A study published in 2013 addressed this question by having a panel of samples tested at numerous sites by either the FDA-approved Cobas AmpliPrep TaqMan assay or other non-FDA-approved tests. As you can see on the left-hand side of this screen, samples tested by the Cobas AmpliPrep TaqMan assay showed a relatively low amount of variability across the measurement range. In contrast, testing by the non-FDA approved assays showed a higher degree of scatter, or more variability, as depicted on the right-hand side of the screen.



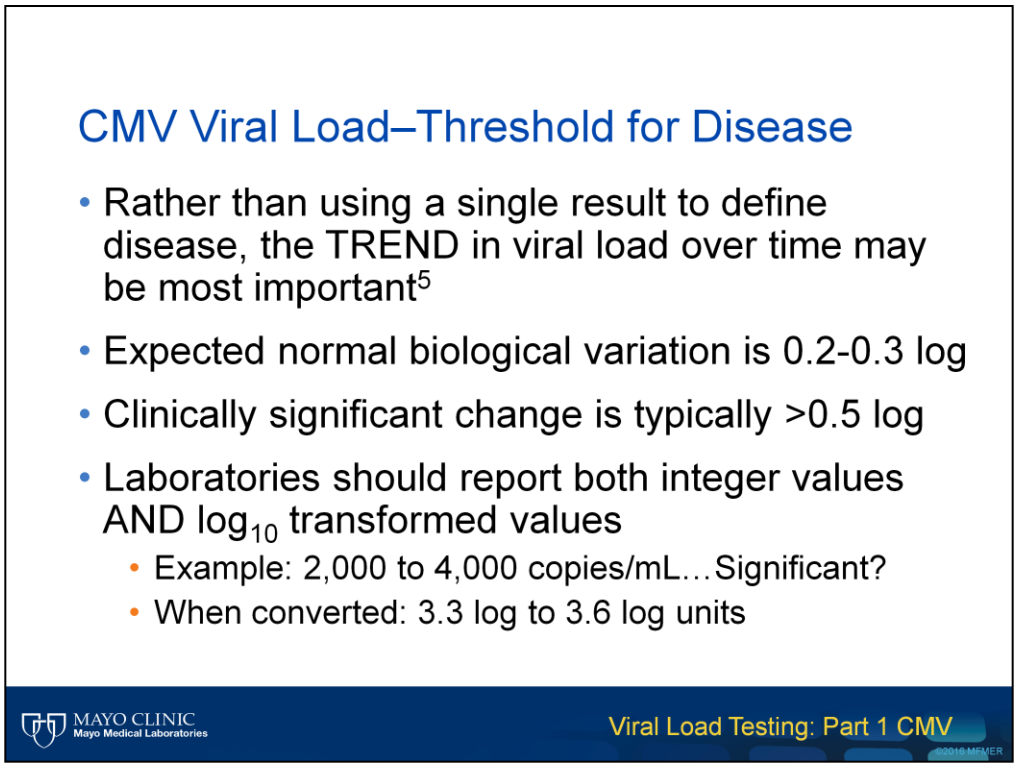
Now that we have a better appreciation for some of the limitations of viral load testing and how we can help reduce variability, let’s return to our patient case for an update. You’ll remember that our patient was a kidney transplant recipient who developed fever and fatigue 9 months postop, and viral load testing at 2 different labs showed results of 5,000 copies/mL and “detected, but less than 2,000 copies/mL." A third sample, this time a plasma specimen, was collected and tested with a result of 3,500 copies/mL. This raises a common question, which is “What viral load predicts CMV disease?” In other words, is there a level of CMV viremia that is associated with clinical disease rather than simply detecting transient viral nucleic acid or latent virus?



Unfortunately, differentiating between latent infection and active CMV disease is a challenge, and establishing a 'cutoff' to define disease has been extremely difficult due to the variability that we've discussed. In essence, because of the mixed bag of testing strategies, laboratories have had to define their own threshold, which is often dependent on a number of factors, including the type of patient population that is being tested. For example, a severely immunosuppressed patient will likely have a lower treatment threshold, while an individual that is only moderately immunosuppressed may require a much higher viral load before CMV disease is diagnosed and treated.



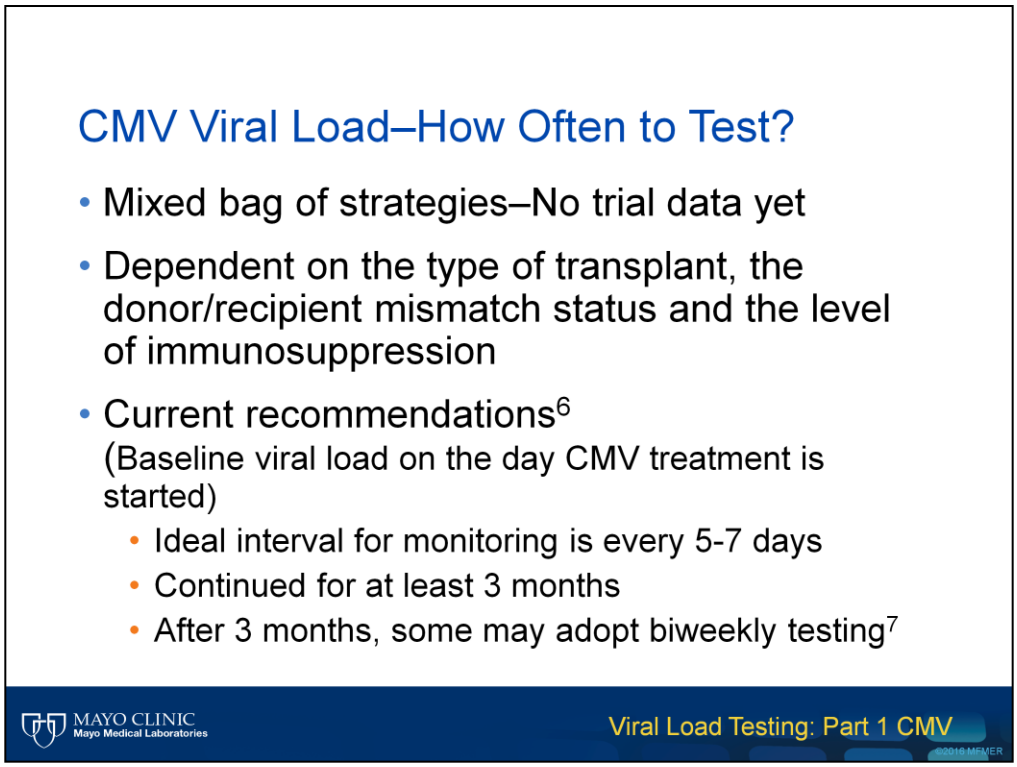
A few studies have attempted to define a threshold for CMV disease, but have yielded mixed results. One study published in 1999 prospectively analyzed the clinical utility of a commercially available quantitative PCR assay in a cohort of 97 liver transplant patients. This group performed a ROC analysis of the results, and their results suggested that a viral load between 2,000 and 5,000 copies/mL, which corresponds to approximately 500 IU/mL, showed a sensitivity of 85.7% and specificity of 86.6% for defining CMV disease. While these results are beneficial, they are not generalizable to other commercial tests or lab developed tests.



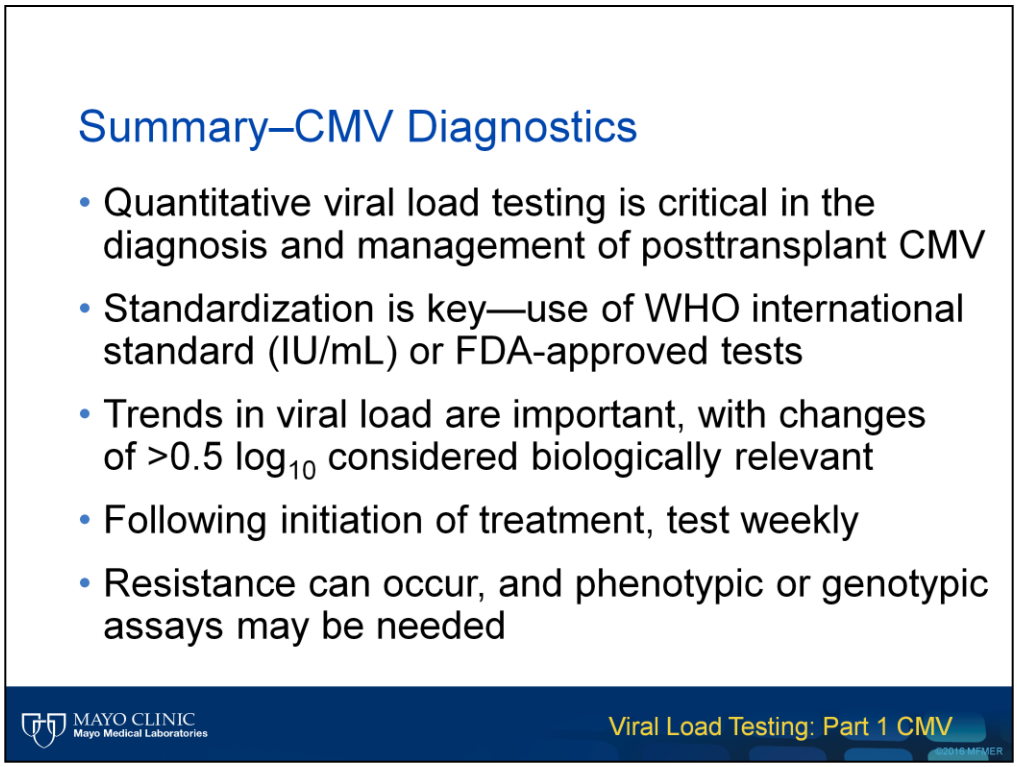
Rather than using a single result to define disease, it is currently suggested to monitor the ***trend*** in viral load ***over time***. In other words, how does a patient's viral load change over the course of weeks or months following an initial diagnosis or initiation of treatment?

It's also important to highlight that there is an expected biological variation of between 0.2 and 0.3 log; this means that the same sample tested by the same lab may show variation in results of up to 0.3 log. Alternatively, different samples collected from the same patient over a single day may show the same degree of variation. Because of this, it is generally thought that a difference of at least 0.5 log between samples is needed to be considered a clinically significant change.

I also want to emphasize that laboratories performing viral load testing for CMV should report results in both integer **AND** log transformed values. Why is this important? Well, let's take a look at an example, in which a patient is initially tested for CMV with a viral load of 2,000 copies, and then a week later is retested with a result of 4,000 copies/mL. Some might interpret this as a significant change due to the apparent doubling of the virus in just a week's time. But when we convert these values to log, we see that the difference is only 0.3 log, which as we learned earlier in the presentation, is within the range of normal biologic variability.



Finally, let's discuss how often patients should be tested by a CMV viral load assay. Unfortunately, there aren't a lot of data yet to guide us in this area, and the interval for testing may be dependent on the type of transplant, the donor/recipient serostatus, and the patient's level of immunosuppression. Generally, the recommendation is to perform a baseline viral load on the day CMV treatment is started and, subsequently, monitor results every 5 to 7 days over a period of at least 3 months. After 3 months, some may choose to adopt testing once every 2 weeks. This is certainly an area where more studies are needed to better define the testing interval and duration following a diagnosis of CMV in transplant patients.



In summary, quantitative viral load testing is critical in the diagnosis and management of CMV in the posttransplant setting. Due to the inherent variability associated with testing, standardization is key! Labs should attempt to adopt the WHO international standard, or implement FDA-approved tests, whenever possible. Monitoring trends in viral load is recommended over using a single result in time, and changes of at least 0.5 log between samples is usually needed to be considered clinically significant. Once a patient has initiated treatment, the current recommendation is to test weekly over a period of at least 3 months. And finally, although we didn't cover antiviral resistance in detail in this presentation, it is important to point out that this can occur. Resistance would typically be suspected if a patient is on treatment and shows an initial decline in viral load, and then demonstrates an increase in viral load while still on therapy. When this happens, phenotypic, or more commonly, genotypic assays may be needed to determine if the virus has acquired mutations associated with antiviral resistance.

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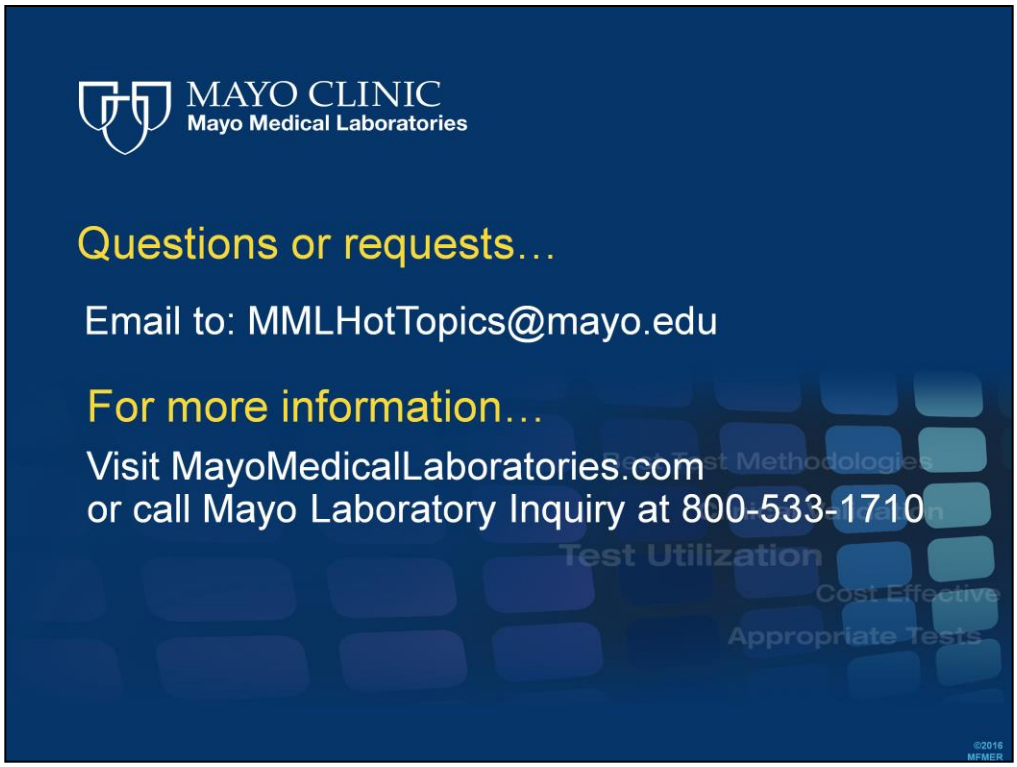

I'd like to thank you for taking the time to join me for this update on CMV viral load testing, and I'd encourage you to dial in for part 2 of this series, in which we'll cover how quantitative PCR can be used to diagnose and monitor Epstein Barr virus in transplant patients.