Close-up of HIV-2 qualitative RNA and viral load testing

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July 2019—Qualitative HIV-2 RNA testing to resolve discordant HIV-2 results may be warranted but seldom results in confirmation of HIV-2 infection, Linda M. Styer, PhD, illustrated with data at the 2019 HIV Diagnostics Conference in March. She also presented an overview of eight years of HIV-2 viral load testing at New York State's Wadsworth Center, where she is a research scientist in the Bloodborne Viruses Laboratory.

HIV-2 is rare and has its highest prevalence in West Africa. The most recent U.S. data (1987–2009) found that 66 percent of the 166 confirmed cases of HIV-2 in the United States were located in the Northeast, with 46 percent of those cases in New York City. "This is why in New York we are concerned about HIV-2," said Dr. Styer, who is also an assistant clinical professor, Biomedical Sciences Department, School of Public Health, University at Albany, State University of New York.

Dr. Styer's laboratory developed an HIV-2 real-time PCR assay to detect HIV-2 RNA and an internal control virus (mouse hepatitis virus) in serum or plasma. The assay starts with a lysis step and the addition of 0.1 to 0.9 mL of patient serum or plasma and 20 µL of the internal control virus. The total nucleic acid present in the samples is extracted using BioMérieux's Nuclisens EasyMag extraction system, followed by a random hexamer reverse transcription reaction to generate cDNA, which is then amplified in a real-time PCR reaction.

The assay has two primer-TaqMan probe sets, Dr. Styer said. One detects the HIV-2 long terminal repeat region, which is present at both ends of the HIV-2 genome. The second set detects the internal control.

"We use a high-positive control, low-positive control, and negative control, and we also look at our internal control," she said. The limit of detection for the highest volume of plasma used is seven international units (IU)/mL. A validation study was published in 2013 (Styer LM, et al. *J Clin Virol.* 2013;58[suppl 1]:e127–e133).

The HIV diagnostic testing algorithm recommended by the Centers for Disease Control and Prevention and the Association of Public Health Laboratories differentiates between HIV-1 and HIV-2 antibodies but indeterminate HIV-2 antibody results are possible. "This is where HIV-2 nucleic acid testing can be important," Dr. Styer said.

One instance in which HIV-2 NAT can be useful is when there is unconfirmed reactivity to HIV-2 antibodies, which can occur when the Bio-Rad Geenius HIV 1/2 Supplemental assay provides a result of HIV indeterminate or HIV-2 indeterminate because of reactivity to only one of the two HIV-2 antigen bands (gp36 and gp140).

"It can also occur if you are running the Bio-Rad BioPlex 2200 HIV Ag-Ab assay where you have HIV-2 antibody reactivity but the Geenius HIV 1/2 Supplemental assay does not confirm that reactivity," she said.

HIV-2 NAT is also useful in cases in which the result is HIV positive, untypable. "Generally, this result occurs because of cross-reactivity between HIV-1 and HIV-2 antibodies, which often resolves to reactivity to either HIV-1 or HIV-2 antibodies later on."

It is also possible for a patient to be dually infected with HIV-1 and HIV-2, though this is extremely rare in the U.S.

Many public health laboratories lack access to HIV NAT because of the expense of maintaining a high-complexity test for the few specimens that require NAT, which can delay diagnosis and treatment (Wesolowski LG, et al. *J Clin Virol.* 2015;65:6–10).

The APHL/CDC HIV NAT Referral Project addressed this need by providing a mechanism for U.S. public health laboratories to send their HIV specimens that require NAT to one of two laboratories, the Bloodborne Viruses Laboratory at the Wadsworth Center or the Florida Bureau of Public Health Laboratories. In June 2016, the Wadsworth Center began offering HIV-2 RNA testing for all participating U.S. public health laboratories based on specific results: HIV antigen/antibody reactive followed by Geenius HIV-2 or HIV indeterminate, or a result on the

BioPlex HIV Ag-Ab assay that was reactive for HIV-2 antibody but not confirmed by Geenius results (nonreactive or indeterminate).

The Wadsworth Center laboratory received 90 specimens for HIV-2 qualitative RNA testing between June 2016 and December 2018. Forty-eight specimens were submitted as part of the APHL/CDC Demonstration Project, and 42 specimens came from New York State clinicians, laboratories, and other contracted facilities.

Dr. Styer presented the test results in groups. Group one, the largest, contained 56 specimens with unconfirmed HIV-2 antibody reactivity. The results for 45 specimens in this group were HIV antigen/antibody reactive, Geenius HIV-2 indeterminate, and HIV-1 RNA not detected. "We did not detect HIV-2 RNA in any of those," she said. "Unfortunately, we didn't get the Geenius banding pattern on every one of those specimens so it's hard to know what band was reactive in that HIV-2 indeterminate result." For seven specimens they knew the banding pattern; all were gp140 (HIV-2 antigen) reactive.

Eight specimens were Geenius HIV indeterminate, and HIV-2 RNA was not detected in any of them. In two specimens the Geenius banding pattern was known: gp36 (HIV-2 antigen) and gp41 (HIV-1 antigen). Three specimens in group one had an undifferentiated result on the BioPlex HIV Ag-Ab assay and an HIV-2 antibody not confirmed result on the Geenius assay. No HIV-2 RNA was detected.

"There was one patient for whom we received five specimens that were all HIV-2 indeterminate on Geenius, and those specimens were collected over a 17-month period," Dr. Styer said. "So there are some patients who have very long-term HIV-2 indeterminate results."

Dr. Styer

Group two consisted of eight specimens on which HIV-2 nucleic acid testing was performed to rule out dual HIV-1, HIV-2 infections. All eight specimens were Geenius HIV-positive, untypable, and some had detectable HIV-1 RNA. "Once again we did not detect HIV-2 RNA in these specimens, suggesting that these are most likely cross-reactivity from people we are testing early in their infection process," Dr. Styer said.

In group three, in which NAT was performed to "resolve HIV status," there were 21 specimens in which the laboratory did not detect HIV-2 RNA. These specimens were submitted for HIV-2 RNA testing for multiple reasons: "Some were Geenius untypable in the initial test, but by the time we received the specimen for HIV-2 nucleic acid testing, it had resolved to HIV-1 positive," Dr. Styer said.

One specimen was from a patient from Africa who was diagnosed as HIV-1 positive by Geenius but for whom no HIV-1 nucleic acid was detected in the HIV-1 viral load assay, so the aim was to rule out HIV-2. "We also had several specimens submitted from physicians who were confused about the Geenius result, which is a theme," she said. "The individual analyte results were HIV-1 reactive, HIV-2 indeterminate, and they thought they needed to go on for HIV-2 nucleic acid testing when in fact they did not." Specimens with these results have a final assay interpretation of HIV-1 positive on Geenius and should be referred to care for a HIV-1 infection, Dr. Styer tells CAP TODAY. According to the Geenius package insert, the HIV-2 indeterminate result in these specimens is "likely due to cross-reactivity of HIV-1 antibodies on HIV-2 antigens and confirmation of HIV-2 is not required."

Other specimens were submitted to rule out HIV-2 infection in a pregnant patient from Africa and in a physician who exhibited symptoms similar to acute HIV infection after a needlestick injury received while treating a patient from Africa.

There's another source of confusion with this category, Dr. Styer said. "Clinicians are confused about the detection ability of the HIV antigen/antibody test. They think it detects both HIV-1 and HIV-2 antigens. It only detects HIV-1 antigen, and therefore there is no way the initial HIV screening test can detect an acute HIV-2 infection."

Group four consisted of five specimens submitted to confirm HIV-2 diagnosis. All were Geenius HIV-2 positive. "And aha! We did detect HIV-2 RNA, finally, in three out of the five specimens," Dr. Styer said.

"We found that the HIV-2 RNA test helped exclude infection in specimens with unresolved HIV-2 antibody reactivity," she said in summary. "The question is, are there too many unnecessary tests? Probably yes. Some could be cleared up if we got rid of the confusion about the detection ability of the antigen/antibody test." Also leading to unnecessary tests are the misunderstanding around the Geenius results versus the interpretation, and false reactivity of the Geenius gp140 band.

In her second session, Dr. Styer shared data to show that HIV-2 viral load testing is a valuable tool for monitoring treatment response and that while HIV-2 viral load values are generally thought to be low, values above 10,000 IU/mL are not uncommon.

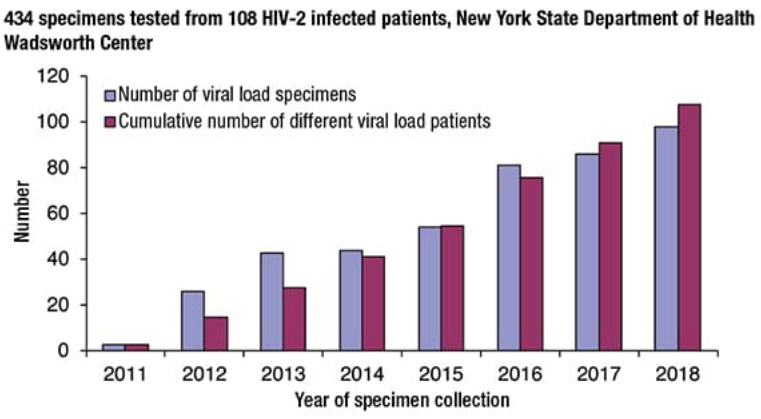
"Since 2011, we have tested 434 specimens from 108 HIV-2 infected patients," Dr. Styer said. The Bloodborne Viruses Laboratory uses the same HIV-2 RT-PCR assay but with a few differences, such as testing only freshly collected plasma, using two new defined volumes of 0.2 and 0.9 mL, and incorporating a set of five standards with values set by the HIV-2 RNA international standard. "There are criteria for the linearity of that standard curve and also the predicted viral load values of the high and low positive controls."

The assay also uses a no-reverse transcription control, which "allows us to detect HIV-2 DNA that may be contaminating our plasma samples," Dr. Styer said. "Initially, we didn't think we would continue using this control, but it has become useful."

The HIV-2 viral load assay has the same limit of detection as the qualitative assay—7 IU/mL—but with a lower limit of quantification (41 IU/mL [0.9 mL], 185 IU/mL [0.2 mL]).

The number of viral load specimens the laboratory tests each year has increased consistently since 2011, with almost 100 specimens tested in 2018. And every year new viral load patients are added to the lab's testing population. "It doesn't look like we're plateauing out yet. We anticipate that we will continue to add more patients as they are diagnosed," Dr. Styer said.

The laboratory confirms each patient's HIV-2 infection status with antibody testing, or with HIV-2 RNA testing for those for whom sufficient sample is not left over for antibody testing, which was the case for 23 of the 108 patients.



Samples from an additional 47 patients were tested with Bio-Rad's Multispot HIV-1/HIV-2 Rapid Test, which confirmed HIV-2 infection in 46 samples. "There was one HIV undifferentiated result, and it turned out to be a true dual infection," Dr. Styer said. "We detected both HIV-1 and HIV-2 RNA."

Specimens from the remaining 38 patients were tested with the Geenius assay, and 33 specimens "had the expected result: either HIV-2 positive with HIV-1 cross-reactivity or HIV-2 positive," Dr. Styer said.

The other five specimens yielded unusual test results. One specimen was HIV positive, untypable, with an additional result of an HIV-2 viral load of 3,000 IU/mL, which confirmed HIV-2 infection. A second specimen had a result of HIV-2 indeterminate with only the gp36 (HIV-2 antigen) band. “We did detect HIV-2 DNA in that specimen, so that’s another confirmed infection,” she said.

A third specimen had an initial result of HIV indeterminate with visible gp36 (HIV-2 antigen) and gp41 (HIV-1 antigen) bands. Ten months later, the laboratory received another sample from the patient; this time it was Geenius HIV-2 positive.

Dr. Styer elaborated on two cases with highly unusual results. The first case was a Geenius negative HIV-2 viral load specimen, received in March 2017 and in which no HIV-2 RNA was detected. “That can happen; it’s probably just someone who is not infected,” she said. But in August 2017, HIV-2 RNA was detected in a new specimen from the same patient, though it was still Geenius negative.

“That raised some alarm in the laboratory,” she said. “Did we mess something up? What’s going on?” The laboratory then tested both specimens using the antigen/antibody test, and both were reactive, with very low signal-to-cutoff of 13.3 and 18.3. “It was odd.”

Two more of the patient’s specimens arrived for viral load testing. In October 2017, the specimen was again Geenius negative with no HIV-2 RNA viral load detected, and a similar antigen/antibody test result. One year later, the laboratory detected a higher level of HIV-2 RNA of 200 IU/mL on a new specimen, but still with a Geenius negative result.

“We called a physician to find out what was going on with this patient,” Dr. Styer said. The staff learned that the female patient from Cape Verde, off the west coast of Africa, had been diagnosed with HIV-2 in 2011 by Western blot performed by the CDC. Five bands were positive on that Western blot, “so it was a nice, strong result.”

The patient was on antiretroviral treatment and had no immune system abnormalities. “There’s nothing to explain why she had not completely seroconverted on the Geenius assay,” Dr. Styer said. “This was our first indication that sometimes you don’t always get the result you expect.”

The second case presented in October 2018 when the laboratory detected a fairly strong HIV-2 viral load of 1,200 IU/mL in a sample, but the Geenius result was HIV-1 positive. “That was very odd,” she said.

The antigen/antibody test was reactive with a strong, positive signal-to-cutoff, and HIV-1 RNA was not detected. “What clued us in that something was weird about this was that the Geenius results were a little bit odd,” Dr. Styer said. “There was a really strong gp36 band, no gp140 band, and two very light HIV-1 bands.” With two HIV-1 bands and only one HIV-2 band, the Geenius reader interpreted the result as HIV-1 positive.

They repeated the test. This time, the gp36 and gp140 bands were present as were the two HIV-1 bands. “Now the interpretation was HIV-2 positive with HIV-1 cross-reactivity.”

The laboratory ran a third test on the same specimen “and it went back to HIV-1 positive because that gp140 band disappeared. This was very weird. It still boggles the mind.”

Dr. Styer said she called the physician again and found out that the male patient was from Ivory Coast and had been tested with HIV-1 viral load since 2004, indicating that he was misdiagnosed at that point as HIV-1 positive, “and of course the HIV-1 viral load had been undetectable ever since.”

The physician said that in March 2018, they repeated the diagnostic testing and this time got a Geenius HIV-2 positive result, which is why they sent a specimen for HIV-2 viral load testing. “The patient is currently on antiretroviral treatment, but the physician indicated he will probably switch it up,” since he now knows which type of HIV infection the patient has.

The two cases “were definite eye-openers and made us realize that you have to look at the full clinical picture of a

patient to know what the next test should be," Dr. Styer said.

She returned to talking about the full population of 108 HIV-2 viral load patients. Sixty-seven were residents of New York City, five were from elsewhere in the state, and 36 were from out of state, among them 26 from a contracted facility in Massachusetts. Mean age is 55, and the laboratory tested slightly more female than male patients. Sixty-seven percent immigrated from West Africa.

The laboratory was able to get a detectable viral load within the viral load reporting range for 42 percent of the 434 specimens tested. The average HIV-2 viral load was 2,526 IU/mL; the highest was 750,000 IU/mL.

"It's important to realize that while patients infected with HIV-2 generally have low viral loads, it is possible to get very high viral loads," Dr. Styer said. "We have had nine specimens with the viral load above 50,000 IU/mL from eight different patients."

The laboratory detected HIV-2 RNA in 22 percent of specimens but at levels less than the limit of quantification. HIV-2 RNA was undetected in 33 percent of specimens. Three percent were indeterminate because of DNA contamination or PCR inhibition.

Fifty-three of the 108 patients provided at least three specimens for HIV-2 viral load testing, and Dr. Styer grouped those patients into viral load patterns. The most frequent pattern, representing 15 patients (28 percent of population), was one in which HIV-2 RNA was detected in at least one specimen but the value was lower than the lower limit of quantification.

"We have also seen evidence of successful treatment," Dr. Styer said, pointing to 13 patients who saw a greater than 1.5 log drop in their HIV-2 viral load values. "We don't get a full clinical or treatment history on these patients, but luckily we have some information to know when treatment was started in certain patients. It correlates nicely with when their viral load drops"—a good thing to see when a viral load test is run, she noted, because it shows the right thing is being detected.

Ten patients had consistent HIV-2 viral load values. "In some of these cases, I know that patients are on treatment but the treatment just isn't working."

Seven patients had fluctuating HIV-2 viral load values. "Some of those patients are on treatment but are not very adherent," Dr. Styer said, adding that she spoke with the physicians. "It definitely shows up in their viral load results."

In six patients, no HIV-2 RNA was detected, and in two patients increasing viral load values were detected.

Dr. Styer highlighted three unusual HIV-2 viral load cases. The first was a patient in which HIV-2 DNA was detected repeatedly in his plasma samples. The black male patient, age 50 to 60, had been on hemodialysis for a long period and had a kidney transplant in 2015. "Possibly there was some cell lysis that was allowing DNA to get into the plasma. This made us realize that the no-reverse transcription control is important to make sure we don't report out overinflated viral loads for this patient."

In two cases there were extremely high HIV-2 viral loads. A black male, age 15 to 19, was put on antiretroviral treatment immediately after the lab tested two specimens with an HIV-2 viral load value of 250,000 IU/mL. The patient showed a fairly good response to treatment when his next specimen was tested, but has since shown poor response.

The second case involved a patient with an HIV-2 viral load value of 750,000 IU/mL. "When we received that result, I called the physician right away," Dr. Styer said. The patient was a black female, age 30 to 35, who was admitted to the hospital with neurological symptoms. She underwent a cerebrospinal fluid draw for encephalitis testing, and physicians noticed the patient had symptoms of cytomegalovirus retinitis, an opportunistic infection associated with AIDS or compromised immune function. The patient was tested for HIV and diagnosed with HIV-2 infection, immediately placed on treatment, and had an excellent response. "Her viral load is now undetectable," Dr. Styer

said.

The patient's CSF specimen was sent to Dr. Styer's laboratory for HIV-2 RNA testing. "We found quite a bit of it, 7,000 IU/mL, in her CSF specimen," suggesting that the HIV-2 infection may have contributed to her neurological symptoms.

"I think we've shown with our testing program that viral load testing is a vital part of the clinical care of these HIV-2 infected patients," Dr. Styer said. "Although we generally think of HIV-2 as having very low viral loads, it's important to remember that they occasionally can have very high viral loads."

Amy Carpenter Aquino is CAP TODAY senior editor.