Weighing the risks in HIV, HCV algorithmic testing

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June 2023—For HIV and HCV algorithmic testing, the workflow options have risks to consider. Molecular testing performed as an automatic reflex on the same sample used for the serologic testing risks carryover contamination, and requiring a dedicated sample for the molecular assay risks incomplete testing.

Whatever approach the laboratory decides to take for HIV and HCV testing, don't assume full physician compliance with the complicated algorithms and don't "go it alone" when making lab-driven algorithms, said Neil W. Anderson, MD, D(ABMM), associate professor, Department of Pathology and Laboratory Medicine, Washington University School of Medicine in St. Louis, in an AACC session last year. He explained why and how the laboratory at Barnes-Jewish Hospital, where he is medical director of the molecular infectious disease laboratory and assistant medical director of the microbiology laboratory, made its own calls for both algorithms.

The testing algorithm for HIV begins with a fourth-generation HIV-1/2 antigen/antibody combination immunoassay to detect IgG and IgM to HIV-1 and HIV-2 and to detect the p24 antigen of HIV-1. "It's a specialized test, with both antibody and antigen detection," he noted.

If the fourth-generation Ag/Ab assay result is positive or reactive, "we perform the HIV-1/HIV-2 antibody differentiation immunoassay." If it's negative or indeterminate, "we need a third tiebreaker test, and that has to be an HIV-1 nucleic acid amplification test."

For HCV algorithmic testing, "there are additional nuances to keep in mind," Dr. Anderson said. It begins with HCV antibody testing. "All HCV serologic tests detect IgG. Some also detect IgM, but they don't differentiate between the two." The serologic assays are reported as reactive or nonreactive, though some manufacturers have indeterminate or equivocal callouts. In contrast to patients with HIV, HCV patients are serologically positive much later—typically around eight to 12 weeks post-infection. "And there's a lot more variability in time to positivity."

Dr. Anderson

With a nonreactive antibody test, the result is interpreted as likely negative for infection. "However, if there is a strong pretest probability of infection"—the patient is an IV drug user with reported recent use, for example—"we would recommend follow-up testing using a molecular approach, because there's a chance they may not have seroconverted yet."

A reactive antibody test could be a false-positive or the patient could have cleared the virus, which approximately 10 percent of HCV patients will do spontaneously. If the initial test is reactive, the next step is confirmatory HCV RNA-based testing. If that test is positive and a viral load is detected, "then that patient has HCV and testing is done." If no viral load is detected, they probably do not have HCV. "But there are some nuances there, and it helps to understand the viral load dynamics to understand why it's nuanced."

From the point at which the antibody is positive, HCV viral loads can fluctuate (within the first six months of infection), possibly falling to below detectable levels. A patient might test "not detected" despite having an active infection, he said. So if the patient has strong risk factors for HCV, "you're going to want to consider repeating that viral load, typically within six months."

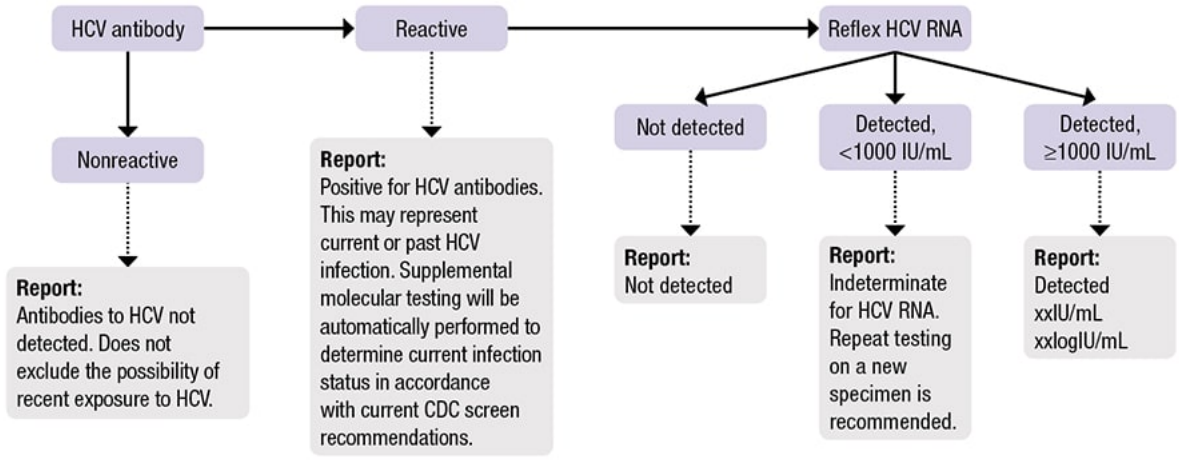
Algorithmic testing works best when performed automatically on a single specimen, but transferring specimens from the serologic to the molecular laboratory can be challenging, Dr. Anderson said, “even when all testing is available under the same roof.” Another challenge: the different specimen-handling practices of the two labs.

In one of the earlier studies of the potential for viral contamination of a total lab automation system, Bryan, et al., took environmental swabs followed by PCR for HBV and HCV from a chemistry TLA system during routine clinical use and after running a small number of high-titer HCV samples. Of 79 baseline swabs for nucleic acids performed on the TLA system, 10 were positive for HBV and eight for HCV, with tube decapping and tube manipulation the sites of greatest risk (Bryan A, et al. *Clin Chem.* 2016;62[7]:973–981).

“So are you going to require an additional dedicated sample for molecular testing, or are you going to perform all the testing on the same specimen?” Dr. Anderson asked, noting each lab will have its own “right answer.”

“If you’re going to do all the testing on one specimen, it’s easy to get all that testing done, but you do have that risk of contamination. If you require the dedicated specimen, you’re going to mitigate a lot of that contamination risk, but now you’re creating a different risk—you’re at risk for incomplete testing.” For laboratories that require additional specimens or orders, “I would recommend you have something in place to notify the provider, probably more actively than just reporting it in the electronic medical record. You might need a callback, so they know the testing on that patient is incomplete.”

Fig. 1. HCV reflex testing at Barnes-Jewish Hospital



“Reflex HCV RNA” order is only generated automatically following serology positivity (not orderable).

The provider orderable “HCV RNA” still requires a dedicated specimen though does NOT have an indeterminate category (all positive results reported).

Dr. Anderson explained how Barnes-Jewish Hospital, with its core lab on floor four and its molecular infectious disease lab on floor five, “did the math” this decision requires. HIV-1/2 differentiation assay testing is automatically performed for every reactive fourth-generation assay screen. “These instruments both live in our serology lab—they sit right next to each other. If we have a reactive fourth gen, our technologists know they need to do the differentiation assay.”

Making sure providers “stay on algorithm,” is important, he said. “We do not offer the HIV-1/HIV-2 differentiation assay [Geenius] as a standalone order because we don’t want people bypassing the route of testing they need to do. Because this is an automatic reflex, and it’s all done on the same specimen, our compliance here is virtually 100 percent.” Turnaround time for the differentiation assay, which is monitored, is one hour. “We had a lot of confusing instances where we would communicate the antigen/antibody result to our providers and then the differentiation assay required an additional specimen, or then the diagnosis was confirmed and we wanted to call that back too.” To prevent confusion and with the TAT now one hour, for most clinical locations the call is made only after the differentiation assay result is available. “And our providers are okay with that.” Obstetrics at their institution is one exception, he noted. “They want to know when the antigen/antibody assay results are available

because they have to make very quick decisions if labor is imminent."

The tiebreaker molecular testing isn't performed automatically. "It's orderable as a standalone test for our providers. Prior to 2016, this test could simply be added on to an in-lab specimen. A provider could see that it was needed, they would call the laboratory, we'd pull the specimen, and we would run it on the same specimen on which we ran the serologic testing." Physician compliance was 100 percent, based on a review of molecular testing from 2014 to 2016, with all molecular results reported within 10 days of the reactive Ag/Ab screen. "The vast majority of our patients were tested the same day. So overall this was reassuring," he said.

In 2016, considering evidence that specimen-to-specimen carryover may result in an erroneous diagnosis of HIV, a prevention policy was implemented. "The decision was made to cease doing any sort of molecular testing for HIV, HCV, or HBV on specimens other than dedicated specimens that were sent to the lab for that testing."

A one-year comparison of the data pre- and post-policy found that all patients who needed follow-up molecular testing received it. "The bad news was that we did have two patients with significant delays in follow-up testing—delays of almost half a year," and one of those patients had HIV. "So that's what we were balancing. In approximately one year's time we had a couple of patients who did not receive a timely diagnosis, versus every year or so—estimated based on the scale of our testing—we might have a false-positive that could lead to a misdiagnosed patient. Based on that analysis we decided we wanted to keep the practice we have, and we still require a dedicated tube for additional molecular testing for HIV."

Once the risk of delay became known, they implemented processes to mitigate the risk of loss to follow-up. Technologists now call the differentiation assay result to the physician who ordered the original test, following a script that emphasizes the need for an additional sample. The script is also added as a comment to the patient result. In addition, a daily report is compiled of all differentiation assay results and is made available to select HIV specialized providers. "There have been instances of patients they've tracked down after the fact because they realized they had a negative differentiation assay and needed that tiebreaker molecular testing."

"So we have some stopgaps."

HCV confirmatory molecular testing is also offered as a standalone test at Barnes-Jewish, rather than performed automatically. When Dr. Anderson and colleagues reviewed compliance with testing, they found that from 2010 to 2016, only 48 percent of HCV antibody-reactive specimens (1,329/2,792) received molecular follow-up within 30 days. "So less than half of patients were receiving testing to figure out whether they had active disease," he said. And of those who were serologically positive and did indeed receive molecular testing, only three quarters (2,715/3,607) had active infection, with the remainder false-positives or cases of cleared HCV. "If providers are actually using the serologic result alone to diagnose HCV, we're going to have a problem." Though similar to the challenge with HIV follow-up testing, given the comparative amount of patients who need HCV molecular testing, "the problem is far more significant," he said.

One option is to perform HCV molecular testing on a separately collected sample. This was their approach for a long period and is coupled with a comment that strongly emphasizes testing is incomplete and additional molecular testing is needed. "That's what we did for a long time—it's similar to what we do with our HIV testing." But by the time physicians saw the comment and would have ordered the molecular test, many of the patients seen in the ED were no longer available for the second draw.

A second option is to collect two specimens from each patient in case confirmatory testing is needed. The Barnes-Jewish laboratory performs about 200 HCV antibody tests a day, with 14 percent positivity. "So we'd be looking at about 180 tubes of plasma a day that we would be saving and eventually be discarding," Dr. Anderson said. "It would be a lot of wasted time and space." That leaves reflex testing, performed on the same tube. "And once again we're back to balancing that risk of incomplete testing versus contamination. But this is different from HIV—it's more dire. We know a lot of this reflexive testing isn't happening."

The literature on risk of HCV RNA contamination isn't fully consistent, he said. In one study, Rondahl, et al., tested

for contamination known HCV RNA-positive and RNA-negative samples (149 of each) in an alternating fashion by the Abbott anti-HCV assay in an Architect instrument. In subsequent retesting of the previously RNA-negative samples, six of the 149 were positive by the Roche Cobas TaqMan assay (Rondahl E, et al. *J Clin Virol.* 2014;60[2]:172–173). "What they found was a four percent carryover rate," Dr. Anderson said. "That's pretty astounding." The maximum viral load in the contaminated samples was 33 IU/mL. "So very low viral loads, which is important."

A larger study investigated the potential risk of HCV, HBV, and HIV nucleic acid cross-contamination on 480 negative specimens by a serology screening instrument that uses disposable tips for sample transfer before molecular testing. The negative plasma samples were subsequently tested with the Cobas HCV test, the Cobas HBV test, or the Cobas HIV test on the Cobas 6800. The authors found no evidence of cross-contamination in the 480 negative specimens on the serology module (Cobas e 602) after processing alongside of high-titer HCV, HBV, and HIV-spiked specimens (Rodriguez PL, et al. *Sex Transm Dis.* 2020;47[5S]:S32–S34).

"So what can we make of this?" Some instruments could have more risk than others, Dr. Anderson said. The 2014 study used serology screening instruments with a fixed needle for sample transfer; the 2020 study used the instrument with the disposable tip. "But one thing I would argue, as any of us in the clinical lab know, is it's more than just the instruments that could be contributing to this. How the tubes are handled, if there's any aliquotting steps, if they're going on an automated line where they're spun ahead of time—all of that could contribute." When it's multifactorial, Dr. Anderson added, "you need to do your own study."

That's what the Barnes-Jewish laboratory did. They prepared 10 negative contrived specimens from pooled serum from HCV-negative patients and 10 positive contrived specimens from negative pooled serum spiked with deactivated HCV control material to a concentration of 2.15×10^7 IU/mL. Aliquots from each pool were prepared into serum separator tubes, he said, "to mimic a real-world setting as much as possible," and numbered one to 20, alternating positive and negative. Sets of the same 20 alternating samples were distributed to the BJH core serology laboratory and the serology laboratories of two sister hospitals that send molecular testing to BJH. The labs were instructed to follow the normal process for serology testing, while maintaining the one to 20 order, after which the specimens were sent to the BJH molecular laboratory for testing. "We tried to blind them to the reason we were doing this."

Hospitals one and two performed serologic testing on the Abbott Architect, and hospital three used the Roche Cobas 8000. All molecular testing was done by the Cobas CAPTAQ HCV test on the TaqMan. Hospitals one and two had a zero percent contamination rate, with 10/10 samples negative by the CAPTAQ assay. The third hospital had a 10 percent contamination rate, with a single positive sample. "And it had a viral load of 21 IU/mL," Dr. Anderson said. "I interpreted this to mean that carryover contamination is indeed a risk in our hospital system. We can dissect this and try to figure out what we can do to mitigate the risk, but we need to accept that it can happen. However, there's something interesting": In the BJH study and in the others, the specimens with carryover contamination almost universally have very low viral loads. "And that's something we can work with, so we did implement a reflex algorithm" (**Fig. 1**).

In that algorithm, if serologic testing is reactive, it's reported as positive, "and we tell our providers we're going to perform reflexive molecular testing." A separate order, generated in Epic, that's unique to the reflex test is used. "We call it our reflex HCV RNA." If it's detected, with a viral load of more than 1,000 IU/mL, it's reported as detected, with the value. If it's detected and the value is less than 1,000 IU/mL, "there's a chance it could be contamination," he said, in which case it's called indeterminate, the value is not reported, and repeat testing on a new dedicated specimen is recommended. "We do see this happen occasionally with this reflex. I don't know if those patients are infected or not, but it's something we've used successfully. And it means the vast majority of our patients get the full testing."

HCV RNA testing not linked to the reflex algorithm can be ordered in Epic. "That does require a dedicated tube, and for that we would report values less than 1,000." This is important, he said, because in the setting of monitoring a known positive patient, values less than 1,000 regularly occur and are relevant.

A “major lesson” learned through this process, Dr. Anderson said, is that when designing algorithmic testing, collaborating with all stakeholders is a must, as is sizing up the full testing process. “You can validate each component of the assay, you can do all the carryover studies you want, but if you’re not taking into account the entire testing process, you’re not capturing the risk.”

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