Journal of Mass Spectrometry and Advances in the Clinical Lab 28 (2023) 27–29

Contents lists available at ScienceDirect

Journal of Mass Spectrometry and Advances in the Clinical Lab

journal homepage: www.sciencedirect.com/journal/journal-of-mass-spectrometry-and-advances-in-the-clinical-lab

Opinion

Molecular diagnostics and the laboratory developed test: A tale of success and the potential impacts of increased regulation

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In the earliest days of molecular diagnostics, before significant regulatory oversight of molecular testing by the U.S. Food and Drug Administration (FDA), laboratories were faced with acquiring the expertise to develop Southern blot transfer assays and eventually polymerase chain reaction (PCR) based assays for the detection of target nucleic acids, DNA and RNA, in patient samples. These technologies are now considered some of the most rudimentary techniques used in initial molecular diagnostics. The universal nature of these technologies led to a rapid penetration of this testing in the fields of genetic diseases, hematology, infectious disease, and oncology. Once hailed as the "homebrew" test, the laboratory developed test (LDT) became the backbone of the molecular diagnostics industry as vendors were too slow to manufacture kits for financially unbeneficial tests and even slower to obtain IVD clearance for other tests. Southern blot transfer analysis was routinely used to test for fragment size changes (e.g., *FMR1* gene for Fragile X Syndrome) or structural gene rearrangements (e.g., *T*-cell receptor or immunoglobulin heavy chain genes for the diagnosis of B- or *T*-cell lymphoma), and those tests became a standard of practice. As the field matured and clinical applications became more a part of the standard-of-care, the technologies rapidly evolved to higher complexity multiplex assays, microarrays, and massively parallel sequencing, which provide much more information in a single test. Reagent kits and instrumentation that automated many of these processes became commercially available; however, few, to this day, have obtained FDA approval.

Under the Clinical Laboratory Improvement Amendments (CLIA-88), laboratories were allowed to use LDTs as long as their analytical performance was validated, including the assessment of sensitivity, specificity, accuracy, and precision. However, few guidelines existed to address what needed to be done as part of this performance characterization. In response, the College of American Pathologists developed checklists to guide laboratories in best practices and quality assurance. Vendors, recognizing that test volumes at the time were low, realized that development and submission of test kits for FDA approval was not realistic. In 1994, the Association for Molecular Pathology (AMP) was founded, providing guidance for clinical practice and education in this new field of laboratory medicine. AMP became the go-to organization for establishing best testing practices, coordinating specimen exchanges, and providing expertise to regulatory agencies and other societies regarding this new modality of diagnostic testing. Other professional societies then became involved in the development of practice guidelines, QC materials, and training/educational materials. These and other technological developments enabled the democratization of molecular testing, such that PCR became a household conversation piece during the recent COVID-19 pandemic.

FDA oversight of this testing was limited, as CLIA regulations ensured best practices were in place. The FDA, however, maintained a policy of enforcement discretion of LDTs when laboratory tests were not meeting industry standards or federal regulations. A widely cited example by the FDA of "lab tests gone wrong" was reported in the August 2007 issue of MMWR, a publication from the Centers for Disease Control and Prevention. It reported on three pertussis outbreaks in the states of Massachusetts, New Hampshire, and Tennessee. In each outbreak, the CDC laboratory was unable to confirm the presence of pertussis in patient samples using culture, serology, and PCR testing; this indicated that false positive results had led to a false claim of an outbreak occurring. In any type of outbreak scenario, the consequences of under- or over-reporting positive results for the pathogen in question are significant. Over-reporting could lead to the implementation of strict mitigation strategies, including isolation of positive individuals, limiting visitors into the healthcare facility, unnecessary treatment or vaccination strategies, etc. On the other hand, under-reporting would lead to continued spread of the pathogen, increased morbidity and mortality, and the potential for biological variation to occur in the pathogen of interest.

At the time, the CDC laboratory was developing a new PCR test for B. pertussis and had not completed validations for the limit of detection or sensitivity of their test comparable to the other laboratories. The CDC assay was much less sensitive than the test being performed by at least one laboratory, and evidence of adequate validation by the CDC,

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https://doi.org/10.1016/j.jmsacl.2023.01.007
Received 20 October 2022; Received in revised form 23 January 2023; Accepted 23 January 2023
Available online 1 February 2023

including sensitivity, specificity, precision, and accuracy, was not made available for review. When the comparison test data was presented, the CDC stated that they were not considered a clinical laboratory and, as such, did not have to go through the rigorous validations that a clinical laboratory was required to. This lack of capability was further highlighted by the recent COVID-19 pandemic, when the SARS-CoV-2 test developed by the CDC was approved by the FDA and, after distribution to public health laboratories around the country, had to be withdrawn because it did not work as designed.

At the time, it was not appreciated that asymptomatic individuals could be "carriers" of low levels of pathogen and have the ability to infect others, despite ours and others' efforts to make this known. We only recently came to fully appreciate this respiratory viral phenomenon during the COVID-19 pandemic.

Subsequent to the MMWR article, there was a misleading article in the New York Times, and two rebuttals in the Dark Report addressing the nuances of PCR testing. Comparative studies at the time, with blinded samples tested between one of the laboratories mentioned and the CDC laboratory, showed a failure of the CDC test to have similar performance characteristics as the assay that many labs around the country were using, including lack of sensitivity of the CDC test. This was most likely due to inadequate validation performed by the CDC laboratory at the time, and was made known to the FDA numerous times after their citation of the report. Nonetheless, rigorous validation of the performance characteristics of molecular tests of any type must be conducted and documented regardless of the laboratory, costs, and effort required. This example, which continues to be cited incorrectly by the FDA, highlights the importance for all laboratories performing testing on patient samples to invest the proper resources in the validation of new tests and technologies, especially when the laboratory performing them does not have the adequate expertise to do so. Over the next two decades, the FDA attempted to increase oversight of LDTs in order to gain regulatory control of a rapidly expanding test market that was minimally impacted by the IVD process. Congress, however, never gave the FDA authority to regulate these laboratory-developed tests, and the Secretary of Health and Human Services issued a regulation in 1992 clearly stating that these tests fell under the jurisdiction of the Centers for Medicare and Medicaid Services, not the FDA. High-volume infectious disease testing for Chlamydia trachomatis and Neisseria gonorrhoeae became one of the first FDA-approved molecular tests, followed by viral load testing for HIV-1 and HCV. Various proposed changes to legislation included the registration of all LDTs by performing laboratories with the FDA and the submission of validation data by laboratories to the FDA for review and approval. At the time of the writing of this manuscript, the proposed Verifying Accurate Leading-edge IVCT Development Act of 2022 ("VALID Act") once again includes increased regulation of LDTs by the FDA, without consideration for the impact such legislation would have on clinical laboratories performing these tests and the ever-growing patient population dependent on them. The current proposed VALID Act, like past proposals to increase oversight of LDTs, would have tremendous impact on the laboratory community and the services provided. The LDT is not solely a molecular diagnostic phenomenon, as many sections of the clinical laboratory, including chemistry, hematology, flow cytometry, microbiology, histology, and cytology, all use the equivalent of some type of LDT in the test menus they offer. This implies that any additional oversight of LDTs would impact the entire diagnostic testing industry rather than just one sector, and would increase overall costs of testing.

With regards to molecular diagnostic testing, the field of genomic medicine continues to evolve at an unprecedented rate. Not only are new tests constantly being developed, but new and more complex technologies are also being developed to deliver their results. For example, it took over ten years (1990–2003) to complete the first draft of the human genome, which was approximately 90 % complete. Today, with the development of next generation or massively parallel sequencing capabilities, laboratories are able to produce a whole genome sequencing result in just a few days. The rapid evolution of polymerase chain reaction (PCR) technology from end-point PCR through real-time PCR and now digital PCR has resulted in faster, cheaper, and more precise detection and quantification of targets of interest. As we have seen during the pandemic, point-of-care or at-home PCR testing devices are also now a reality. The ability to produce rapid test results at home will have a significant impact on our healthcare system, as was witnessed during the COVID-19 pandemic. These tests would most likely not be LDTs, but instead manufactured by a vendor who would seek approvals or waivers. All of these tests and technologies must be vetted and validated for clinical utility and analytical performance before being used for clinical testing. Under the current Clinical Laboratory Improvement Amendments (CLIA) oversight, the LDT becomes invaluable to the healthcare system, and changes to this oversight could have detrimental impacts on patient care.

The LDT has allowed laboratories to perform testing that is considered to be of clinical necessity in a timely fashion, as new discoveries are made that significantly impact patient care. These LDTs often outperform vendor-developed tests that later receive FDA approval. In our own molecular diagnostics laboratory, approximately 25 % of clinical tests offered are FDA approved, while the other 75 % of the test menu is an LDT that undergoes stringent validation, typically beyond the requirements of CLIA and CAP, before being used to produce results on a patient sample. Although there are many guidelines for the validation of LDTs, few give specific details with regards to acceptable specimen types and numbers that should be tested before using the assay for patient testing. Our laboratory usually conducts validation studies of a test's performance using an increased number of samples (depending on the complexity of the test) and increased number of sample types to determine how well the test works in different situations. The ability of clinical laboratories to rapidly develop, validate, and implement LDTs was evident during the early days of the COVID-19 pandemic. Many laboratories were able to establish testing for the SARS-CoV-2 virus using tests that were granted FDA Emergency Use Authorization (EUA) status. While being granted EUA, this status is not a stamp of approval from the FDA, but more of a registration mark of the test. To gain EUA status, vendors only needed to show minimal performance data for their test in order to gain approval for use. EUA status was important when testing was needed quickly, as in the early days of the pandemic. However, the EUA-labeled test may not be as good as an LDT developed by a laboratory with more rigorous validation. This became apparent during the pandemic when so many tests were receiving EUA status based on minimal validation data. If our clinical testing laboratories were not familiar with the process of validating LDTs, the pandemic would have resulted in much higher numbers of deaths and prolonged mitigation strategies. The delays due to inadequate testing capacity and improperly designed CDC tests undoubtedly had a negative impact on the national response to this pandemic.

The range of complexity of the technology used in molecular diagnostics spans from relatively simple PCR-based tests to more highly complex next-generation sequencing assays for whole exome sequencing. The number of different instruments and technologies used by laboratories adds to the oversight burden, as there are few FDA-approved instruments for routine molecular diagnostic testing, in part because of the rapid development and evolution of these new technologies. Approving instruments for single tests also puts an unnecessary burden on the FDA and laboratories that may have equivalent instruments capable of performing the same tests. In doing so, the FDA creates excessive work for itself and a monopoly that laboratories must deal with. No laboratory test is perfect, and even the best tests may be prone to pre-analytical, analytical, and post-analytical variables. Nevertheless, properly validated LDTs have been a major success for our healthcare system. Updating current CLIA regulations could enhance oversight to ensure that laboratories are performing high-quality testing; however, more regulations from additional agencies will impede innovation, limit patient access to testing, and increase costs. Increased

regulatory oversight of LDTs would slow the development and validation of clinical testing in laboratories. Moreover, the associated costs for more laboratory or clerical work would result in fewer test offerings and, thus, limited access to state-of-the-art testing for patients. The LDT has become a political issue due to recent negative attention from the media, such as the MedPage article titled:"FDA is Letting Harmful Lab-Developed Tests Fall Through the Cracks". This piece was written by two non-clinical and non-laboratory experienced individuals who are once again citing a single failure as a reason to increase regulatory burdens on our healthcare system, despite the fact that LDTs positively impact millions of patients on a daily basis. In contrast, the Wall Street Journal recently published a Commentary piece titled: "The FDA's Lab-Test Power Grab" written by Brian Harrison and Bob Charrow, former chief of staff at HHS and HHS general counsel, respectively, arguing against further FDA oversight of the LDT.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Further readings

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