

# QC challenges for molecular testing

By Clark A. Rundell, PhD, DABCC, FACB

Consistent and accurate results are necessary for the fulfillment of molecular testing's promise for improved disease detection and monitoring. Issues affecting quality in molecular testing include variable extraction efficiencies, false-positives due to contamination, PCR inhibition, wide variation of assay sensitivities, and lack of standardization.

The Clinical Laboratory Improvement Amendments (CLIA) mandate quality-assurance (QA) programs intended to monitor and control potential problems in these areas. The College of American Pathologists (CAP) checklist ([www.cap.org](http://www.cap.org)) is, in part, a listing of activities required for a laboratories compliance with the CLIA regulations.

## CAP checklist items [paraphrased] relating to molecular testing

### Validation:

- 1) The program must ensure quality throughout all phases of testing, including ... specimen processing ...;
- 2) Validation should include each of the possible reportable results (genotypes); and
- 3) New reagent lots and/or shipments should be checked against old reagent lots or with suitable reference material before use.

### Quality Control (QC):

"Controls are samples that act as surrogates for patient/client specimens. They are processed like a patient/client sample to monitor the ongoing performance of the entire analytic process in every run."

- 1) For qualitative tests, positive, negative, and sensitivity controls should be run for each assay;
- 2) Corrective action is necessary (i.e., if control is out, consider re-test of all samples since last acceptable control result);
- 3) Quality-control specimens should be tested in the same manner as patient samples;
- 4) Cut-off levels that distinguish wild type, heterozygote, and mutant should be verified;
- 5) The accuracy of endonuclease digests should be verified;
- 6) The fluorescence ratios for mutation identification should be validated; and
- 7) An internal standard or amplification control as well as a sensitivity control should be run in infectious-disease tests.

Unavailability of suitable reference materials has been a problem in satisfying these requirements and not unlike other testing technologies, molecular assay development has outpaced that of controls. The laboratory scientist's response has been to make the best practical use of the resources available.

## The need for controls

Previously tested patient samples were used for controls in the first immunoassay tests, and this is often the case in current molecular testing. Typically, molecular labs use a panel of patient samples from a colleague or cell lines to validate their new test and then, for daily controls, accumulate a bank of tested samples. Problems associated with this approach include failure to monitor the whole assay (i.e., the extraction), lack of rare mutations, inconsistent signal,

*The same CLIA regulations and need for good laboratory practice apply equally for molecular testing.*

potential infectivity, time needed to handle cell lines, and patient confidentiality and privacy. Patient materials cannot be used for quantitative PCR standard curves, nor do these permit monitoring of all analytes (mutations) in each run in multiplex assays. As stopgap measures, many molecular-testing labs make their own plasmid standard curves and for multiplex tests — such as cystic fibrosis — rotate one or two mutant samples per run.

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## Why take the risk?



Patient sample used as control **VS.**

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At left in the MMQCI research lab is Joan Gordon, president, with Steven Nesbitt, MS, scientist.

Testing of stable, comprehensive reference materials with those systems will generate the needed data-defining molecular-test characteristics even for qualitative tests. Most importantly, these data will lead to continued improvement in the robustness of test systems and improved patient care. □

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Improvement is indicated, however, since laboratories performing allergy testing or 30-analyte chemistry tests in compliance with CLIA regulations and good laboratory practice, validate each patient test run with reliable commercial controls that monitor the entire test and contain all reported analytes. The same CLIA regulations and need for good laboratory practice apply equally for molecular testing.

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Cost-effective materials for more comprehensive monitoring are increasingly available. A growing list of reference materials for genetic testing, including cell lines and synthetic multimutation commercial controls is available at [www.phppo.cdc.gov/dls/genetics/qcmaterials/materialsavailability.aspx](http://www.phppo.cdc.gov/dls/genetics/qcmaterials/materialsavailability.aspx), the Centers for Disease Control and Prevention (CDC) website.

**Data is needed**

The error rate of most molecular tests is not known. Effective and efficient quality schemes can and will be designed from definition of the medically allowable error and statistical knowledge of the characteristics of a test, including its rate of error (see [www.westgard.com](http://www.westgard.com)). Newer instrument systems provide fluorescent or densitometric signal intensities and signal ratios for genotyping.



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## Training for the laboratory worker

**W**hat do medical laboratorians have to do to prepare themselves for the molecular-testing world? What are the avenues down which they will need to travel in order to stay on top of the latest information that emanates from this "New World"? Here is some easily accessible assistance listed on the CDC's website to direct interested students of any age ([www.phppo.cdc.gov/dls/genetics/links.aspx](http://www.phppo.cdc.gov/dls/genetics/links.aspx)):

- The National Credentialing Agency for Laboratory Personnel conducts certification of medical laboratory personnel and other credential-related activities.
- The World of Genetic Societies' includes major organizations involved in graduate training in human genetics and provides career information, a guide to graduate and post-graduate training, and medical school curricula.
- The American Board of Pathology/American Board of Medical Genetics has a sub-specialty certification in molecular genetic pathology. The ABP offers primary certification through three routes: combined anatomic pathology and clinical pathology, anatomic pathology only, and clinical pathology only.
- American Association of Clinical Chemistry Certification in Molecular Diagnostics consists of an examination in molecular diagnostics designed to test knowledge of principles, concepts, methodologies, and usage of molecular biology techniques as applied to the clinical laboratory.
- The Association of Genetic Technologists dispenses information about education programs, certification programs and requirements, continuing education opportunities, protocol manuals, training guides, certification study guides, and professional publications.

There are many other resources available for those laboratory personnel who have the desire to become more involved in this exciting field of testing. □

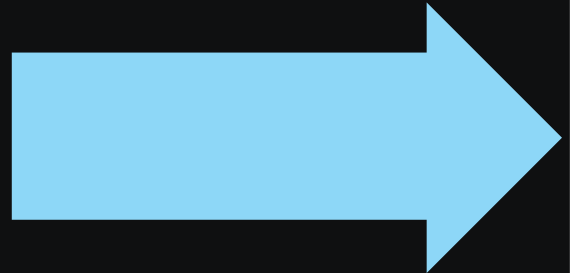
- Top 10 Reasons to Become an MLT/MT**
10. Each day in the laboratory is a day of discovery.
  9. You get to meet your patients, one cell at a time.
  8. Your education never ends.
  7. You get to be the cornerstone of all diagnoses.
  6. If you are prone to roam, you have a portable career.
  5. Great satisfaction comes from contributing to positive patient outcomes.
  4. You can meld your love of science and of patient care.
  3. You wear cool lab coats and look like a "Jedi Knight of Pathology."
  2. You are a detective who gets to grow germs for a living.
  1. You get to play with things your mother told you never to touch.

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