acceptance criteria ... and demonstrated that IgG at ... 60 g/L interferes with the assay." The term "no call" has been defined as, "inconclusive results" (226), "indeterminable" (227), and as "clear results cannot be obtained" (228).

Validation also included tests on *run-to-run* variation, involving three independent runs on the same set of 20 samples of genomic DNA, runs using three lots of reagent, and runs using three different operators. Other validation tests involved an intentional deviation from the instructions. Specifically, alternative temperatures for conducting the PCR reaction were tested, where the annealing temperatures were altered by 1°C, 2°C, and 3°C. This type of validation is sometimes called *guardbanding* (229,230). Moreover, validation also involved tests for *cross-contamination* between runs, where two sequential samples were evaluated for inter-run contamination. The FDA reviewer commented that, "carryover events leading to miscall results were not observed." Furthermore, *stability* tests were also conducted on whole blood specimens and on reagents. For example, stability of genomic DNA was tested by storing patients' blood at 4°C for 14, 30, and 37 days.

To summarize, validation in the PMA submission for the BRCA1 and BRCA2 tests involved:

- spiking of patient samples with contaminants;
- an account of conditions that result in "no call" results;
- run-to-run variation, lot-to-lot variation, operator-to-operator variation;
- guardbanding;
- cross-contamination;
- stability tests.

e. Summary

One of the main themes in this textbook is the use of biomarkers as endpoints in FDA-regulated clinical trials for drugs. Because FDA recommends that biomarkers be validated when used in these clinical trials, the present material provides concrete guidance on procedures for validating in vitro diagnostic tests, including reagents and machines that are used to detect various biomarkers. The most useful source of guidance on validating biomarkers comes from FDA's approval package of medical devices taking the form of an in vitro diagnostic test, in response to 510(k) submissions and PMA submissions.

f. Distinctions Between 510(k) Submission and PMA Submission, for Medical Devices

FDA's regulation of drugs and medical devices find an intersection where a clinical trial in support of drug approval makes use of in vitro biomarker tests. Depending on the

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