- Stability testing with storage of the array and the liquid reagent for 180 days.
- Lowest concentration of DNA that can be detected per reaction mixture.
- Potential of compounds found in blood to interfere with the test. The compounds tested were bilirubin, cholesterol, and heparin.

To reiterate FDA's position on validation on biomarkers and other types of tests, FDA recommends that, "[f]or pivotal studies that require regulatory action for approval or labeling ... the bioanalytical methods should be fully validated" (224).

d. Validation PCR Reaction Diagnostic Test in a PMA Submission

The validation of a test for biomarkers taking the form of mutations in the *BRCA1* gene and *BRCA2* gene, are described. This example is from PMA no. P140020. The test is sensitive to mutations that are point mutations, deletions, and insertions. The goal of the diagnostic test is to aid in selecting ovarian cancer patients who are likely to be treatable with the drug, olaparib. The test includes a risk, in that a false positive will be treated with olaparib, and thus be susceptible to adverse drug reactions from olaparib. This risk compels the need to review this diagnostic test by way of a PMA submission.

According to the FDA reviewer "[a]ccuracy of the ... test was verified by comparison against a validated next generation sequencing ... assay ... [a]ll specimens were tested in a blinded manner. The specimens covered a range of variants, including single nucleotide variants, deletions ... and insertions." In this writing, the term variants is equivalent to the term, mutations. In granting approval to the application (225), the FDA reviewer concluded that the Sponsor's test was 100% accurate, writing that, "Percent Positive Agreement (PPA) = 100%."

The medical device under review took the form of a kit that included a blood collection tube and instructions, and information on using machines and reagents for analyzing variants in the genomic DNA of patients. The PMA submission required that the machines include a specific DNA-sequencing machine (Qiagen's QIAsymphony SP®) and a specific machine for imaging electrophoresis gels (Invitrogen's E-Gel Safe Imager®).

Validation of the blood samples, reagents, and machines, took these forms:

- Tests on metabolites found in blood (IgG, hemoglobin, albumin, bilirubin).
- Tests on additives used in blood processing (EDTA).
- Tests on chemicals used in handling the machines (ethanol, bleach). The goal was to determine how these added chemicals could interfere with the in vitro diagnostic test that was the subject of the PMA.

In performing the validation, IgG, hemoglobin, albumin, and bilirubin were intentionally added to (spiked) genomic DNA samples acquired from the blood cells of patients.

Regarding the results, the FDA reviewer wrote, "[t]reatment with each potentially interfering substance ... with the exception of IgG at 60 g/L, did not affect the performance of the test." Further commenting on the interfering properties of IgG, the reviewer wrote that, "[s]amples with IgG at 60 g/L yielded a no call rate of 33%, which failed to meet the

[224]U.S. Department of Health and Human Services. Food and Drug Administration. Guidance for industry. Bioanalytical method validation; 2013 (28 pp.).

[225]Premarket approval submission P140020 was approved on Dec. 19, 2014 by Alberto Gutierrez, Office of In Vitro Diagnostics and Radiological Health, FDA.