3) Allison KH, Hammond EH, Dowsett M, et al. Estrogen and Progesterone Receptor Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Guideline Update. *Arch Pathol Lab Med.* 2020-144(5):545-63.

CYG.48950 Predictive Marker Testing - Decalcified Specimens **Phase I**

If the laboratory performs in situ hybridization (ISH) for predictive markers on decalcified specimens, the assay was validated for decalcified specimens or the results include a disclaimer noting that these assays have not been validated on decalcified specimens.

NOTE: Decalcification may adversely affect patient results. If the assay has not been validated for decalcified specimens, a disclaimer must be included in the patient report, such as, "This assay has not been validated on decalcified tissues. Results should be interpreted with caution given the possibility of false negative results on decalcified specimens."

Use of decalcification solutions with strong acids is not recommended.

REFERENCES
1) Darvishian F et al. Impact of decalcification on receptor status in breast cancer. *The Breast Journal* 2011. 17:689-91.
2) Hanna W et al. Testing for HER2 in breast cancer: current pathology challenges faced in Canada. *Curr Oncol* 2012. 19:315-323.
3) Gertych A et al. Effects of tissue decalcification on the quantification of breast cancer biomarkers by digital image analysis. *Diag Pathol* 2014. 9:213.

DIGITAL IMAGE ANALYSIS

This section applies to laboratories using digital image analysis to evaluate specific features in a specimen or tissue section image following enhancement and processing of that image, including but not limited to morphometric analysis, ISH and cytogenetics (evaluation of metaphase chromosomes).

If predictive marker testing is performed, additional requirements in the Predictive Markers section also apply.

Inspector Instructions:

READ	• Sampling of validation and calibration policies and procedures • Sampling of validation/calibration records • Sampling of specimen analysis policies and procedures • Sampling of patient digital image analysis reports for completeness
ASK	• What is your course of action if calibration is unacceptable?
DISCOVER	• Select a representative case and follow the entire process from receipt to final reporting

CYG.49470 Preanalytic Testing Phase Validation **Phase II**

There are records showing that the preanalytic phase of the test system has been validated for each assay, including fixation and processing.

NOTE: Applicable requirements under the "Test Method Validation and Verification-Nonwaived Tests" section of the All Common Checklist must be followed.

REFERENCES
1) Hipp J, Bauer TW, Bui MM, et al. *CAP Pathology Resource Guide: Digital Pathology.* Version 7.0(2). Northfield, IL: College of American Pathologists; 2017.

CYG.49475 Calibration **Phase II**

Each instrument is calibrated in accordance with the specifications of the instrument.

REFERENCES
1) Hipp J, Bauer TW, Bui MM, et al. *CAP Pathology Resource Guide: Digital Pathology.* Version 7.0(2). Northfield, IL: College of American Pathologists; 2017.

CYG.49480 Quality Control - Digital Image Analysis **Phase II**

Control materials are run concurrently with patient specimens to ensure appropriate functionality of the digital image system.

NOTE: Controls are samples that act as surrogates for patient/client specimens. They are periodically processed like a patient/client sample to monitor the ongoing performance of the analytic process. Controls should check test performance at relevant decision points for the digital image analysis system.

For qualitative tests, a positive and a negative control may be sufficient. For quantitative or semiquantitative tests, controls at more than one level should be used.

Evidence of Compliance:
- ✓ Records of QC results

REFERENCES
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Medicare, Medicaid and CLIA programs; CLIA fee collection; correction and final rule. *Fed Register*. 2003(Jan 24): [42CFR493.1256(d)(3)(i)].
2) Clinical and Laboratory Standards Institute. *Statistical Quality Control for Quantitative Measurement Procedures, Principles and Definitions.* 4th ed. CLSI guideline C24. Clinical and Laboratory Standards Institute, Wayne, PA, 2016.

CYG.49485 Area of Analysis **Phase II**

A qualified pathologist selects or confirms the appropriate areas for analysis prior to reporting results, as applicable.

NOTE: Specimens that do not represent "in situ" samples embedded in paraffin may not require pathologist review. Examples include cultured preparations and direct preparations of liquid specimens including blood, urine, pleural fluid, etc.

CYG.49490 Analysis Guidelines **Phase II**

There are written guidelines for identification of appropriate areas and cells for analysis.

NOTE: Evaluation of heterogeneous cell populations requires use of specific guidelines and procedures to ensure analysis of the appropriate areas and/or cells, particularly if there is background or nonspecific staining, or if there is cell debris, endogenous pigment, and/or artifacts of aging, sectioning or preparation.

Test results may be affected by fixation parameters, including time of fixation, type of fixative used, hemorrhage, necrosis, and autolysis of tissue.

CYG.49495 Final Report Elements - Digital Image Analysis **Phase II**

The final report includes the specimen source, name of the vendor and imaging system used, probe, and the detection method, as well as any limitations of the test result, if applicable.