The pathologist should scan the entire ISH slide prior to counting at least 20 cells or use IHC to define the areas of potential HER2 amplification. If there is a second population of cells with increased HER2 signals/cell and this cell population consists of more than 10% of tumor cells on the slide (defined by image analysis or visual estimation of the ISH or IHC slide), a separate counting of at least 20 non-overlapping cells must also be performed within this cell population and reported. For brightfield ISH, counting requires comparison between patterns in normal breast and tumor cells because artifactual patterns may be seen that are difficult to interpret. If tumor cell pattern is neither normal nor clearly amplified, test should be submitted for expert opinion.[10] The threshold for both HER2 IHC and HER2 FISH assays and the window for HER2 equivocal cases has been altered according to 2013 ASCO/CAP guidelines. The number of HER2 FISH equivocal and positive cases increased significantly after changes in the ISH scoring criteria because HER2 gene copy numbers became an important decision making factor regardless of HER2/CEN17 ratio of breast cancer patients.[11-13]

CEP17 signals may lead to discordant interpretations between the HER2/CEP17 ratio and absolute HER2 gene copy number in a significant proportion of cases.[14] Polysomy CEP17 can be observed either on its own, or in combination with HER2 gene equivocal status, or in combination with HER2 gene amplification. The index case belongs to the last category. A relatively high increase in HER2 gene copy number can be obtained as a result of polysomy 17 (6-7 copies) and are interpreted as positive based on absolute HER2 gene copy number. Polysomy 17 is relatively common in breast carcinomas. In a recently published series by Vanden et al, > 40% of breast carcinomas were found to harbor increased CEP17 copy number.[15] However, recent validation studies performed via comparative genomic hybridization have shown that "true" polysomy 17 is a rare event and that CEP17 amplification can mimic the presence of multiple copies of chromosome 17, leading to an overestimation of the incidence of polysomy. Recent studies show that the clinicopathologic impact of CEP17 alteration is not as strong as that of HER2 gene amplification and polysomic tumors possess pathologic features more similar to HER2 negative than to HER2 positive tumors. Polysomy 17 is a crucial cause of equivocal HER2 testing results by FISH, depend¬ing on which criterion (ratio vs. absolute number) is used for interpretation. Interestingly, CEP17 copy number may have a predictive therapeutic value; increased CEP17 copy number appears to be a predictive marker for anthracycline-based chemotherapy in breast cancer.[14] However a larger cohort study would be required to confirm this aspect.