Update of the Belgian guidelines for HER2 testing in breast cancer

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This update of the Belgian guidelines for HER2 testing is based on the updated recommendations recently published by the American Society of Clinical Oncology and the College of American Pathologists.[1-3]
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Introduction

In 2007, a joint Expert Panel convened by the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) developed guidelines for HER2 testing in breast cancer.[4] HER2 is amplified and/or overexpressed in 15-20% of primary breast cancers, a well established prognostic and predictive marker, and the most efficient therapeutic pathway until now. Since the 2007 ASCO/CAP guidelines, a vast amount of literature has been published on new diagnostic strategies, unusual HER2 genotypic abnormalities and new HER2-targeted therapeutic options. Therefore, an update of the guidelines to ensure accurate and standardised HER2 testing was needed.

Only the major changes and updates compared to the 2007 guidelines will be discussed.

Specimens to be tested

All newly diagnosed breast cancer patients must have a HER2 test performed. Patients who develop metastatic disease must have a HER2 test performed on a metastatic sample if a tissue sample is available. Both core biopsy and excision specimen of the primary tumour can be tested. Whereas the core biopsy may be a better specimen for analysis because of better tissue fixation, the excision specimen is a more representative sampling of the tumour. However, the most important issue remains adherence to pre-analytical requirements in all specimen types.

Testing of core biopsy is cautiously recommended, with caveats as limited tissue, equivocal result or histopathological discordance (*Table 1* lists the histopathological discordances and retesting requirements). In these situations, repeat testing on the excision specimen is recommended. This may also require reviewing the core biopsy, which is not infrequently performed in a different centre. Retesting may thus have significant financial and other implications.[5]

Overall, primary testing on the excision specimen, provided correct adherence to pre-analytical requirements, could be a more pragmatic approach.

Cytology specimens can be tested as well, if fixed in formalin.

Immunohistochemical (IHC) algorithm

New criteria for the immunohistochemical scores have been defined, with changes in both the staining intensity and in the percentage of positive cells.

For a positive 3+ score, the cut-off of 10% positive cells is reintroduced, referring to the Food and Drug Administration (FDA) criteria and inclusion criteria of the initial clinical trials.

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Table 1. Histopathologic features suggestive of possible HER2 test discordance.

A new HER2 test should be ordered if the following histopathologic findings occur and the initial HER2 test was positive: Histologic grade 1 carcinoma or the following types: • Infiltrating ductal or lobular carcinoma, ER and PgR positive • Tubular (at least 90% pure) • Mucinous (at least 90% pure) • Cribriform (at least 90% pure) • Adenoid cystic carcinoma (at least 90% pure) and often triple negative
If the initial HER2 test result in a core needle biopsy specimen of a primary breast cancer is negative, a new HER2 test must be ordered on the excision specimen if one of the following is observed: • Tumour is grade 3 • Amount of invasive tumour in the core biopsy is small • Resection specimen contains high-grade carcinoma that is morphologically distinct from that in the core • Core biopsy result is equivocal for HER2 after testing by both ISH and IHC • There is doubt about the specimen handling of the core biopsy (long ischemic time, short fixation time, different fixative) or the test is suspected by the pathologist to be negative on the basis of testing error

The equivocal category of 2+ score is broadened at the lower end, by including incomplete membrane staining. It is to be expected that this will outnumber the 0.15% of cases with positive cells between 10% and 30% that migrate from the 2+ to the 3+ category.[6]

The negative categories of score 1+ and 0 have been redefined as well, with less stringent criteria for score 0. This implies a narrowing of the negative 1+ category.

The new guidelines have introduced new categories of intensity of staining that are not well defined and more prone to observer variability. Moreover, not all staining patterns are included in the IHC algorithm, e.g. weak/moderate staining in less than 10% of tumour cells. Some rare HER2-amplified breast cancers show intense but incomplete staining. The authors suggest considering these cancers as equivocal and requesting reflex testing. To overcome these possible areas of confusion, we propose the IHC algorithm in *Table 2*.

In Situ Hybridisation (ISH) algorithms

The new guidelines have included bright-field ISH and separate algorithms for single and dual probe ISH assay (*Figures 1* and *2*). The cut-off for a positive ISH test based on HER2/CEP17 ratio has been changed to 2.0, referring to the FDA criteria and inclusion criteria of the initial clinical trials.

ISH testing interpretation is described more elaborately. The pathologist should scan the entire ISH slide, or alternatively use the IHC slide to identify areas of potential HER2 amplification, prior to counting at least 20 non-overlapping cells in two separate areas of invasive cancer.

In case a second population of aggregated tumour cells with increased HER2 signals/cells is found, a separate counting of at least 20 cells within this population should be reported. The former definition of heterogeneity is revoked: any discrete contiguous amplified population should be reported if it represents more than 10% of the total tumour population on the ISH slide. Since this 10% rule is dependent on the size of the tested sample, we think it is clinically sounder to report the ISH result in every discrete aggregated amplified

Table 2. IHC algorithm.

IHC 3+ = circumferential membrane staining that is complete and intense, within >10% of tumour cells
IHC 1+ = incomplete membrane staining that is faint/barely perceptible, within >10% of tumour cells
IHC 0 = no staining observed or incomplete membrane staining that is faint/barely perceptible, within ≤10% of tumour cells
IHC 2+ = all other categories
Note: *intense staining = unequivocal membrane staining at low magnification (x2.5/x5)* *weak/moderate staining = unequivocal membrane staining at medium magnification (x10/x20)* *faint/barely perceptible staining = unequivocal membrane staining at high magnification (x40)*

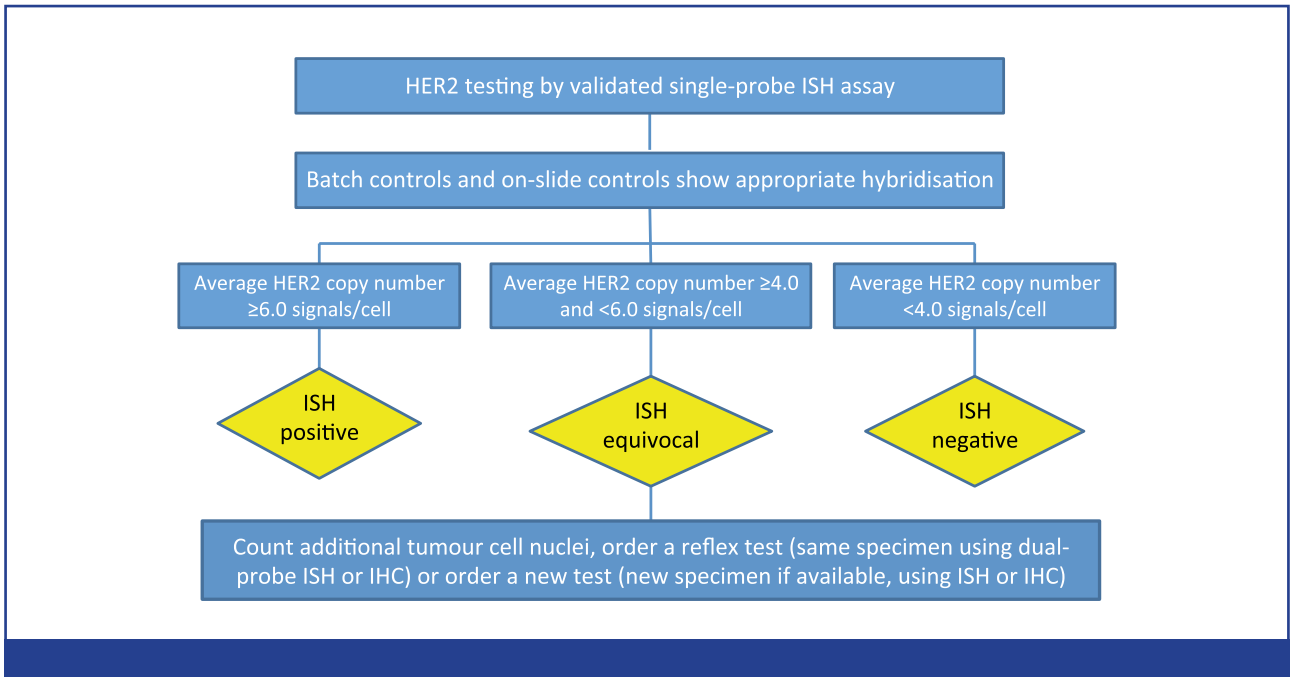

Figure 1. Single-probe ISH assay algorithm.

tumour cell population with at least 20 countable cells, while also reporting the relative proportion of this amplified population in the total tumour population. Cases containing amplified and non-amplified areas should be considered HER2 positive.

The dual ISH assay methodology discriminates cases first according to HER2/CEP17 ratio, and secondly according to HER2 copy number. The updated ISH algorithm allows clarification of polysomy and monosomy cases.

In case of a HER2/CEP17 ratio between 1.8 and 2.2, it is recommended to count 20 extra nuclei. It is unclear whether the mean value of 40 cells or the highest count of 20 cells should be withheld, although it seems logical to choose an unequivocal option if possible. An important new category of indeterminate HER2 result is defined if technical issues prevent one or both tests from being reported as positive, negative or equivocal.

IHC negative cases and double equivocal cases

Whereas the change in cut-off for the positive 3+ category is evidence based, the rationale behind the modification of the other categories is not very clear. Broadening of the equivocal category will lead to more reflex testing and an increase in double equivocal cases. Redefining the negative categories blurs the biologically and clinically relevant distinction between IHC 0 and IHC 1+ categories.[7,11]

The 2013 update does not recommend testing of all IHC negative (score 0-1+) cases, stating that the true frequency of false negatives may be well below the intrinsic analytical variability of existing HER2 assays. The authors refer to data showing that the risk of false positive testing declines considerably with proficiency testing and accreditation systems.

In case of double (IHC and ISH) equivocal cases, the 2013 ASCO/CAP update states that the oncologist may consider HER2-targeted therapy. In Belgium however, this therapy is only reimbursed by health insurance for ISH positive cases and for ISH equivocal cases with a HER2 IHC score 3+ using a validated IHC test. Before defining a HER2-ISH status as equivocal, 20 additional cells should be counted by the first observer. If still equivocal, 20 additional cells should be counted by a second observer for a maximum of 60 cells, to confirm a true equivocal test result. Then, several options of additional testing are proposed. The most pragmatic approach is to perform additional testing on the excision specimen in case of primary testing of the core biopsy, or testing another tissue block in case of primary testing of the excision specimen, or other available specimens in case of a small (single block) tumour (e.g. lymph node or visceral metastasis).

It should be emphasised however that repeat testing should be used sparingly, since reimbursement criteria allow only one test per patient per year.

Quality assurance

In the 2013 guideline update on HER2 testing, recom-

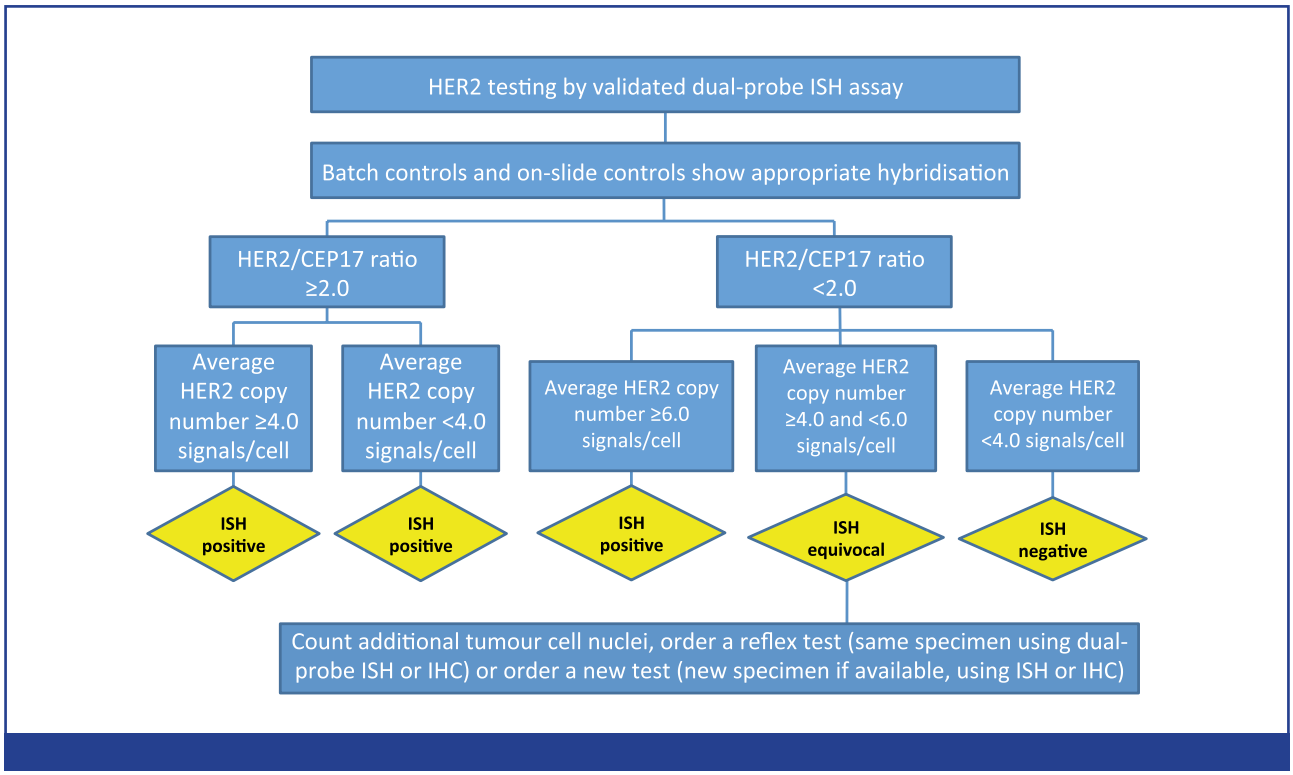

Figure 2. Dual-probe ISH assay algorithm.

mendations for quality assurance are very similar to those described in 2007.[2,3] A survey performed by the Belgian Working Group for Breast Pathology in 2012 showed that the recommended quality measures had not been implemented in all laboratories performing HER2 testing in Belgium at that time. Laboratories accredited according to the ISO15189 norm obtain significantly better results at external quality assessment rounds for HER2 testing (cfr infra). This should encourage all labo-ratories to implement the quality assurance measures described in the guidelines.

Optimal tissue handling requirements

The time from removal of the tumour to incision of the specimen and fixation in formalin should be as short as possible, preferably less than one hour. HER2 fluorescent in situ hybridisation interpretation starts to be compromised with delays to formalin fixation of more than one hour. The expression of oestrogen and progesterone receptors begins to diminish when the delay is two hours and one hour, respectively.[8] Since formalin penetrates slowly into tissue (1 mm/hour), the pathologist should slice the tissue (at five to ten mm intervals) to allow optimal fixation of the tumour.

Most laboratories are well aware of this requirement, but will have to enhance communication with surgeons and radiologists, in order for them to understand the major implications on ultimate treatment decisions. The pathology request form should provide a space to indicate date and time of resection/biopsy and the time when formalin fixation of the tissue started. The information provided allows determining the fixation delay ('cold ischaemic time') and the fixation duration, which should be at least six to eight hours. Shorter fixation times will lead to decreased oestrogen receptor detection.[9] The recommended duration of fixation has been extended from maximum 48 to 72 hours, in concordance with the recommendations for ER and PgR testing.[10] If accreditation has been obtained according to fixation time of 6-48 hours, this extended fixation time will have to be revalidated.

Initial and continued test validation

Validation steps must be performed before the test is routinely used and revalidated if the procedure is changed, the extent of revalidation depends on the extent of the changes. Validation requires parallel testing of samples with an alternative validated method in the same laboratory or with a validated method in another laboratory. This information is available in most laboratories, since IHC and ISH have been performed in parallel for many years in Belgium.

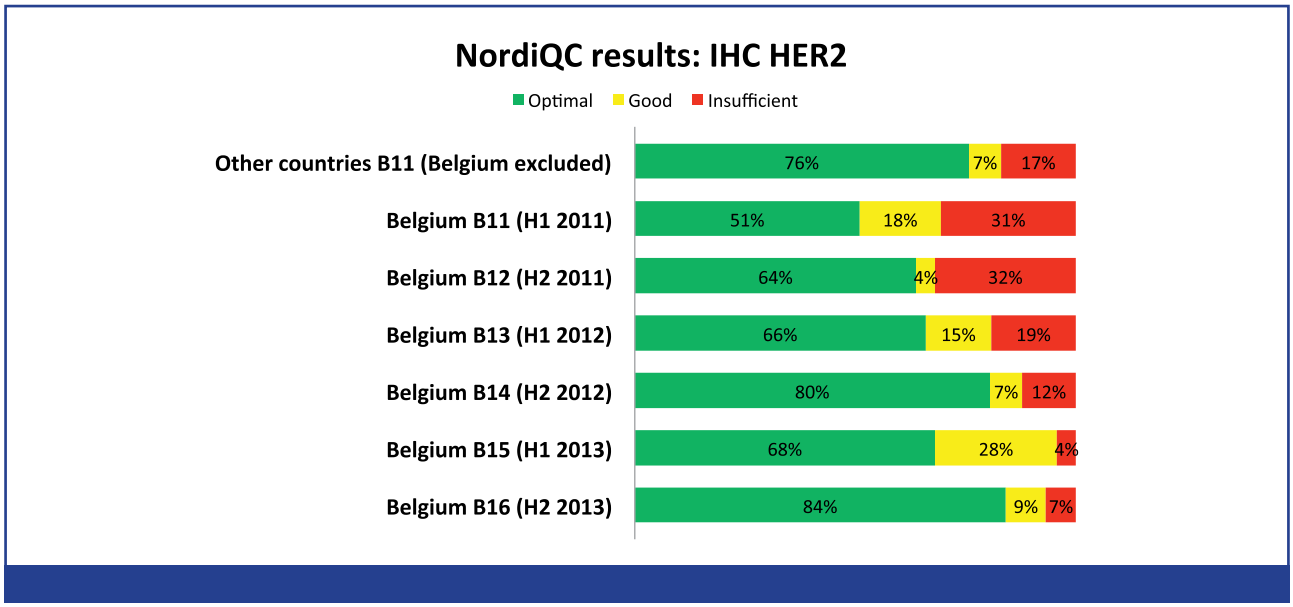

Figure 3. NordiQC results 2011-2013: Graph showing anonymised results of subsequential NordiQC runs. Top bar: test results of run B11 of all participating labs (excl. Belgium); the subsequential graphs represent Belgian results only. Green: optimal results; Yellow: good results; Red: insufficient results (i.e. 'Borderline staining' and 'Poor staining' according to the NordiQC assessment methods).

The number of tests required for an initial validation is 20 negative and 20 positive samples for FDA approved methods and 40 negative and 40 positive samples for laboratory developed tests. The best practice is to report validation results for positive and negative cases separately and preferably for each IHC scoring category. The observed concordance may drop below 95% when only a finite number of cases are evaluated. Routine periodic performance needs to be monitored and concordance should be >95% in both positive and negative categories.

Optimal internal quality assurance procedures

Laboratories should perform ongoing quality control and equipment maintenance, and provide initial and ongoing laboratory personnel training and competency assessment.

Standardised operating procedures should be used, including routine use of control materials.

External and internal controls should be reviewed and documented with each test.

The use of external controls with known HER2 levels alongside the assay procedure is mandatory. Positive (IHC score 3+, high level HER2 gene amplification) and negative (IHC score 0-1+, no HER2 gene amplification) controls are a minimal requirement. An additional control close to cut-off values is strongly recommended, e.g. a tumour with IHC score 2+ and HER2/CEP17 between 2.0 and 2.5; this control will be more informative about the sensitivity of the technique than the IHC 3+ block. A composite control tumour block can be made by every laboratory. Laboratories not wanting to waste tumour tissue in the control block can use normal breast tissue as a negative control and high grade HER2 positive ductal carcinoma in situ as a positive control. Ideally, control tumour blocks are placed on the same slide as the patient's tumour to detect, for example, failure to apply reagents.

Normal breast tissue can be used as internal control: normal ducts should show no more than incomplete weak membrane IHC staining and normal disomic ISH result.

Ongoing competency assessment of pathologists reporting the test results is necessary.

Optimal external proficiency assessment

All laboratories should successfully complete external proficiency testing programs with at least two testing events a year as offered by CAP, NordiQC and others. Continued participation has shown to increase the accuracy of the assays in the laboratories.

In Belgium, the number of participants in the NordiQC breast cancer runs was consistent over the last three years, varying from 41 to 49 labs. In run B11, the quality of IHC HER2 testing in Belgian labs was clearly inferior to that of labs in other countries (206 participants) (*Figure 3*). Almost one third of Belgian participating labs achieved an insufficient assessment, indicating an

Table 3. HER2 test reporting.

Must report **HER2 test result as positive** for HER2 if: *ISH positive based on:* Single probe average HER2 copy number ≥6.0 signals/cell Dual probe HER2/CEP17 ratio ≥2.0 Dual probe HER2/CEP17 ratio <2.0 with an average HER2 copy number ≥6.0 signals/cell
Must report **HER2 test result as equivocal** and order reflex test if: IHC 2+ *ISH equivocal based on:* Single probe ISH average HER2 copy number ≥4.0 and <6.0 signals/cell Dual probe HER2/CEP17 ratio <2.0 with an average HER2 copy number ≥4.0 and <6.0 signals/cell
Must report **HER2 test result as negative** if a single (or both) test(s) show: IHC 1+ or IHC 0 *ISH negative based on:* Single probe average HER2 copy number <4.0 signals/cell Dual probe HER2/CEP17 ratio <2.0 with an average HER2 copy number <4.0 signals/cell
Must report **HER2 test result as indeterminate** if technical issues prevent one or both tests from being reported as positive, negative or equivocal. Conditions may include: inadequate specimen handling, artifacts, analytic testing failure. Another specimen should be requested for testing to determine HER2 status. Reason for indeterminate testing should be noted in a comment in the report.

elevated risk of false positive or false negative scoring. Over the last two years, an overall increase in the IHC HER2 testing quality has been observed with 84% of the participating labs achieving an optimal score in the second half of 2013. Data from run B12 and B14 show better results by BELAC (ISO15189) accredited labs compared to non accredited labs (data not shown).

Optimal laboratory licensing and accreditation

Since March 1st 2013, the Scientific Institute of Public Health (ISP/WIV) is responsible for the licensing of pathology laboratories, on behalf of the Minister of Public Health. The 2013 CAP/ASCO HER2 guideline update emphasises the need for testing laboratories to follow all accreditation requirements set by CAP or equivalent accreditation authorities in order to ensure reliability of the tests. CAP-accreditation includes onsite inspection (external audit) every other year and annual self inspection (internal audit), reviewing, among others, the laboratories' external proficiency results. All Belgian pathology laboratories have to implement a quality management system in order to be licensed. Performing regular internal audits is part of any quality management system. The Praktijkrichtlijn/Directive Pratique, developed by the Commission of Anatomic Pathology details the procedure of performing internal audits. A file for prospective recording of IHC and ISH results is extremely useful to yearly audit the HER2-positive results in an unselected breast cancer population. This should show that HER2 gene amplification occurs in 15-20% of patients with primary breast cancer.[2,3]

Belgian reimbursement criteria and testing algorithm

Most Belgian laboratories still perform HER2 IHC as a screening tool for ISH testing. Unlike other countries, an IHC 3+ score is insufficient for reimbursement of HER2-targeted therapy. As quality assurance measures still lack wide implementation, the risk of false positives was considered too high to abolish this criterion.

The above mentioned IHC algorithm is recommended. ISH testing of all equivocal and positive cases is still recommended as well. ISH testing of all IHC negative cases is not mandatory. However, a proportion of the IHC negative cases should be tested to document concordance rates. Belgian health insurance reimburses HER2 targeted therapy for ISH positive cases; for ISH equivocal cases, a validated HER2 IHC score 3+ is needed for reimbursement.

Reporting elements for both IHC and ISH assays are summarised in *Table 3*.

Conclusion

The update of the Belgian guidelines for HER2 testing in breast cancer is largely based on the update of the ASCO/CAP recommendations. Where necessary, clarifi-

Key messages for clinical practice

1. The update of the Belgian guidelines on HER2 testing in breast cancer is based on the ASCO/CAP guideline update.
2. New algorithms for IHC and ISH analysis are proposed.
3. Testing of all metastatic lesions is emphasised.
4. Quality assurance remains crucial for accurate HER2 testing.

cations and adjustments to Belgian regulations are made. Although the quality of HER2 testing has improved since the publication of the previous guidelines, there is still ample room for continuing efforts in order to ensure quality assured HER2 testing in breast cancer.

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