Response to Reviews for EDL Submission

Immunohistochemical testing (IHC) for the detection of estrogen (ER) and progesterone (PgR) receptors

Pre-submission ID Number: 63
Full Submission ID Number: 33

Reviewer’s questions in bold font

#Reviewer 1

1. **Inadequate Quality Assurance/Quality Control data provided that is important to emphasize when recommending use of any IHC IVD.** Include that the documented recommended methods of quality assurance by ASCO/CAP are: to control pre-analytic, analytic and post-analytic variables including tissue cold ischemic time, fixation time; to use negative and positive controls with every test run and include positive and negative controls on the test slide, and report out positive internal control staining on test tissue as quality assurance; to use only an approved antibody clone (as of 2010 publication: the ER/PR PharmDX assay (ER-2.123 and 1D5, and PR-1294) is one of these, but also included are ER-6F11, ER-SP1, ER-1D5 and PR-1A6 and PR-1A2, per 2010 testing guidelines). Since 2010, another popular rabbit monoclonal antibody is in wide use (ER-EP1), and a ready-to-go PR antibody from Ventana (PR-1E2) is the most widely used.

2. **Inadequate Quality Assurance/Quality Control data provided that is important to emphasize specifically with this higher-priced IVD, compared with other ready-to-use antibody assays e.g.** The ER/PR PharmDX assay kit includes batch control slides to run with tests and antibodies are pre-dilute. What other advantages does this assay have over others, in particular ready-to-use assays?

3. **Include quality assurance data comparing the different ER and PR assays in real-time use across hundreds of different laboratories (the CAP proficiency testing data from 2015 of over 1000 labs evaluating ER and PR using the most widely used assays, and the NordiQC data from the last five years can be used obtaining testing results from >300 different labs – report the QA data for the ER/PR PharmDX assay, ER-1D5 (Biocare Medical and Dako), ER-SP1 (available from multiple different vendors), and ER-6F11 (Leica), ER-EP1 (a widely used rabbit monoclonal antibody that has become very popular since the ER testing guidelines were published; from Dako), and PR-16 (Leica), PR-636 (Dako), PR-1294 (Dako), PR-1E2 (Ventana), PR-1A6 (Leica). http://www.nordiqc.org/downloads/assessments CAP data: Leung W,**

The present submission to EDL represents a proposal to enlist ER an PgR IHC in the IVD essential list (WHO EDL) and not an exercise to suggest a specific or a selection of branded tests; accordingly, comparisons across the different diagnostics are not required unless a specific warning is to be addressed. The submission to the EDL is not intended to propend for one brand or support a single or few branded devices but to enlist the device as essential for cancer care; for the same reason, no comparison between ready-to-go and laboratory- built tests will be fully commented, as the submission is intended for a HER2 IHC respecting the performance requirements and QA parameters. WHO grants a prequalification process to acknowledge and characterize the single brands, to be reported as “prequalified” device for the UN- Organizations documents. Thus, the comparison across the different devices is out of the intention of the present submission. The predicate device submitted serves as an example and class- representor; however, the submission is not intended to be limited to the predicate device from Dako.

It is essential to comment more on the QA, as suggested by the reviewer. Guidelines recommendations are provided by ASCO/CAP for the IHC testing of ER and PgR in breast cancer (Hammond MEH, Arch Pathol Lab Med. 2010;134:907–922; Hammond MEH, JCO 2010). As part of the QC, it must be ensured that the control check is executed across all the value chain, as issues have been described from the pre- to the post- analytic phases. In particular, ASCO/ CAP recommends: to control pre-analytic, analytic and post-analytic variables including fixation type and time; to use negative and positive controls with every test run and include positive and negative controls on the test slide, and report out positive internal control staining on test tissue as quality assurance; to use only an approved antibody clone/assay and avoid lab-developed assays if robust validation is not available or not feasible in the specific setting. It is essential to endorse the use of approved and validated antibody clones such as ER/PR PharmDX assay (ER-2.123 and 1D5, and PR-1294), ER-6F11, ER-SP1, ER-1D5, PR-1A6, PR-1A2, ER-EP1 and a ready-to-go PR antibody from Ventana (PR-1E2), as the most currently used.

According to ASCO/CAP, positive for ER or PgR is stated if finding of major/equal to 1% of tumor cell nuclei are immunoreactive to the ER or PgR clone; negative for ER or PgR if finding of less than 1% of tumor cell nuclei are immunoreactive in the presence of evidence that the sample can express ER or PgR (positive intrinsic controls are seen). As pointed out by the guidelines, the internal control is an essential part of the procedure, as non- reactive clones or issues in the preparation of the sample impairing the IHC reaction must be considered. As a general rule of QA, if the rate of positive/ negative ER stains is outside 70/30%, a quality check of the laboratory must be audited. This is particularly critical for settings and countries in which population-based analyses of breast cancer phenotype are not largely available e.g. to confirm if higher rates of triple- negative and endocrine- refractory breast tumors is reliably higher than the average known rates or an artifact, related to quality issues and IHC operational inefficiencies.
For instance, evidence from clinical trials can be paradigmatic. Central assessment of ER and PgR status of tumors included in the Breast International Group (BIG) 1–98 trial showed that locally tested ER-negative tumors tend to show ER positivity in a relatively high number of cases (70%), more pronounced for PgR (Viale G, JCO 2007). The ECOG E2197 study reported a concordance of 90% (ER) and 84% (PgR) at re-test in a centralized laboratory (Badve SS; JCO 2008). Similar discordance rates were reported from the ALTTO trial (Gelbert RD, Breast 2009) and, more recently, from EORTC 10041/BIG 03-04 MINDACT trial (Viale G, Annals of Oncol 2014), with a concordance for ER and PgR IHC tests of 97.6 and 89.6 %, respectively, showing an ameliorated results across the participating centers, probably as a result of an automatization of some critical steps, like the routine use of autostainers among all participating laboratories (Dekker TJA, Breast Cancer Res Treat. 2015).

Positive controls generally suggested are samples from normal breast tissues or endometrium, expressing ER and PgR at 90-100%; breast cancer samples can be variably positive to hormone receptors and are not the recommended samples of control. Essential elements of quality assurance and proficiency are: confirmation of accuracy; demonstration of reproducibility; concordance of results (intra- and inter- pathologists); comprehensive training of pathologist and other laboratory personnel and designation of an in-house expert as reference pathologist, serving as tie- breaker. All these steps must be ensured and tracked by a quality standard operational procedure under an accreditation scheme and using validated and approved IVD, to avoid false negative results or an excess or positive result, both impairing on the optimal treatment of patients. For instance, the heterogeneity in the reported prevalence of triple-negative breast cancer and, in general, the prevalence of various hormone receptor positive tumor types are likely to be multifactorial and to be related to a pre-/in-/ post-analytical issues and reported to the population clinical and demographic data (e.g. a reference center for BRCA1-mutated patients with an excess of triple- negative results), before confirming a real population-based difference in breast cancer phenotype distribution (Chatterjee S, JGO 2017). Eventually, continued enrollment of laboratories into quality control schemes is essential for achieving and maintaining the quality standard of care for breast cancer ER/PR testing.

4. A generic $10 per assay and $2000 for the autostainer was listed as the assay costs. This is inadequate information and in my opinion, does not reflect the actual cost that a patient typically pays in low and middle-income countries (at least $30.00, and hundreds of dollars in Europe and the USA). I understood where this number came from as I read the application for a
panel of IHC to diagnose solid tumors, it is reported as the cheapest possible cost per slide of an antibody test, using a concentrated antibody and generic reagents for testing in a publication of how to set up a 10 antibody IHC panel in low resource settings in the most cost-efficient way. In general, the issues of quality control for ER, PR and HER2 testing are so challenging and so important that it is hard to endorse use of antibodies that must be diluted, and also to endorse manual staining for ER, PR and HER2. It may be a realistic true price for ER and PR testing using concentrated antibody purchases, but not with the assay being put forwards here, and it doesn’t take account of quality assurance testing and validation testing required with each batch of antibodies bought. (http://mjpath.org.my/2018/v40n2/immunohistochemistry-bench.pdf). A table comparing the typical reagent/kit costs in different regions of the world (Europe, USA, South America, Africa and South East Asia) would really more adequately demonstrate the cost of this assay. Dako should be able to provide this information. Kit cost does vary according to region sold and country sold in, and whether bought in bulk or not, but price for a 50 test kit would at least allow comparisons. The table should include the top assays sold by the most popular vendors (use the ready-to-go antibody assays, e.g. Dako’s EP1, Ventana’s SP1 assay, and the ready-to-go PR assays, eg. Ventana’s PR-1E2).

<table>
<thead>
<tr>
<th>Country</th>
<th>IVD</th>
<th>Cost</th>
<th>Source</th>
</tr>
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<tbody>
<tr>
<td>Italy</td>
<td>Monoclonal rabbit anti human Estrogen Receptor α, clone EP1 (n=60 tests)</td>
<td>480 Euro</td>
<td><a href="https://www.unibs.it/sites/default/files/ricerca/allegati/Listino-%20Dako.pdf">https://www.unibs.it/sites/default/files/ricerca/allegati/Listino-%20Dako.pdf</a> (price list for 2015)</td>
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<tr>
<td></td>
<td>Monoclonal Mouse Anti-Human Estrogen Receptor α, Clone 1D5 (n=60)</td>
<td>480 Euro</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Progesterone Receptor, polyclonal (n=250)</td>
<td>1360 Euro</td>
<td></td>
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<tr>
<td></td>
<td>Monoclonal Mouse Anti-Human Progesterone Receptor, Clone PgR 636 (n=60)</td>
<td>480 Euro</td>
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<tr>
<td></td>
<td>ER-PR pharmDx™ Kit, for Dako Autostainer (n=60) **the kit contains anti-ER and anti-PgR antibodies, negative control reagents, control slides, reagents and wash buffer</td>
<td>6000 Euro</td>
<td></td>
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<tr>
<td></td>
<td>Autostainer Link 48 Staining System</td>
<td>63492 Euro</td>
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5. My interpretation of the data is that it is very hard to justify recommending this particular assay from Dako, since costs are obviously higher for the FDA-approved assay, robustness is not as high as for other ready-to-go assays available from Dako and from Ventana. **However, there should be a general recommendation for this category of assay (ER/PR IHC assay with no specific vendor or assay favoured).**

The submission is intended for the “class” of IVD and not as a prequalification process for the selection of a specific device. The predicate device Dako was used as “an example” and no brand is supposed to appear in the EDL, that should look like “ER and PgR IHC for breast cancer”.

#Reviewer 2

1. **The minimum number of cases required per year to maintain competency of the technologists and the pathologist has not been indicated in the submission. The minimum requirement for test validation before introduction of test has not been indicated. Inclusion of subscription to an external Quality assurance program and proficiency testing has not been indicated. Level of Laboratory accreditation before introduction of test into practice has not been indicated.**

The number of cases to maintain competency and optimize performance is to be determined locally; for in-home built ER and PgR IHC test, a specific accreditation process exists and recommendations from ASCO/CAP. Training for IHC testing is part of the general pathology training, as included in the core
curriculum for pathologists; despite the kit being specific for ER and PgR, the technique of analysis is used for different indications and the principle is the same: to study, assess, detect and quantify cellular and tissue antigens. The requirements under a functional quality management system in the laboratory are determined per accreditation schemes and is discussed for the QC per ASCO/CAP.