OPEN

Immunohistochemical Performance of Estrogen and Progesterone Receptor Antibodies on the Dako Omnis Staining Platform: Evaluation in Multicenter Studies

David Hicks, MD,* Patrizia Dell'Orto, DSc,† Mary Falzon, MC,‡ Kirsten D. Hoff, PhD,§
Yaron Y. Levy, PhD,§ Loralee McMahon, MS,* Keith Miller, FIBMS,‡ Leila Russo, MD,†
and Giuseppe Viale, MD†

Abstract: The analysis of estrogen receptor (ER) and progesterone receptor (PR) expression levels by immunohistochemistry is an important part of the initial evaluation of breast cancer and critically important in treatment planning. Anti-ERα (clone EP1) and anti-PR (clone PgR 1294) antibodies are in development for the Dako Omnis automated staining platform. These antibodies are not yet commercially available and are in performance evaluation, including the 4 international, multicenter studies reported here. For each antibody, a reproducibility study and a method comparison study was done in a randomized manner in order to test the antibodies under conditions closest to real-world user conditions. The reproducibility studies included 5 staining runs on the Dako Omnis with 20 formalin-fixed and paraffin-embedded human breast carcinoma specimens in 3 independent laboratories, and the method comparison studies included several hundred specimens stained on the Dako Omnis and on the Autostainer Link 48 platforms. Stained slides were evaluated for nuclear ER or PR expression according to American Society of Clinical Oncology/College of American Pathologists guidelines ($\geq 1\%$ cut-off for positive) by pathologists who were blinded from the staining method and specimen ID. For both anti-ERα (clone EP1) and anti-PR (clone PgR 1294) on the Dako Omnis, high reproducibility agreement rates were obtained on the interrun, interlaboratory, and interobserver endpoints. High concordance rates were observed between the specimens stained on the Dako Omnis platform and the Autostainer Link 48 platform. Staining quality was excellent for both anti-ERα (clone EP1) and anti-PR (clone PgR 1294) on the Dako Omnis. These results suggest that these antibodies are reliable and reproducible tools for immunohistochemistry analysis of ER and PR expression levels in formalin-fixed and paraffin-embedded breast carcinoma tissues on the Dako Omnis platform.

Key Words: immunohistochemistry, estrogen receptor-α, progesterone receptor, breast cancer, in vitro diagnostic medical devices

(Appl Immunohistochem Mol Morphol 2017;25:313–319)

For the last 30 years, determination of estrogen receptor (ER) and progesterone receptor (PR) expression in breast carcinomas has played an important role in the management of breast cancer because these biomarkers are highly predictive for a clinical benefit from targeted endocrine therapy.[1–3] The clinical importance of ER, in particular, in breast cancer management has made its assessment mandatory for every newly diagnosed patient,[4] and thereby a successful example of the use of biomarkers in guiding targeted cancer therapy.

In current clinical practice, the analysis of ER and PR by immunohistochemistry (IHC) is an important part of the initial pathologic evaluation of breast cancer,[5,6] and the recent inclusion of these biomarkers in the clinicopathologic surrogate definition of intrinsic breast cancer subtypes by the St. Gallen expert consensus[7] emphasizes the importance of standardization of preanalytical, analytical, as well as postanalytical variables in order to help ensure the reliability, quality, and accuracy of the results.[8] Evidence-based consensus guidelines from the joint recommendations of the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) have attempted to address the important issue of assay standardization, and

have stressed the importance of quality control and quality assurance programs for laboratories performing ER and PR assessment.[6]

In recent years, improvements have been made in IHC methodology with the introduction of polymer-based detection,[9] the development of highly sensitive and specific monoclonal antibody reagents,[10,11] and the introduction of new automated test platforms that provide unparalleled accuracy, efficiency, and reproducibility.[12] With the introduction of new test platforms and new IHC regents for ER and PR analysis, there is a need for rigorous evaluation and technical validation of their performance before their clinical implementation.

Here, we describe a series of multicenter studies that examined the performance characteristics of rabbit monoclonal anti-ER (clone EP1) antibody and mouse monoclonal anti-PR (clone PgR 1294) antibody for a new automated staining platform (Dako Omnis). These antibodies are in development and are not currently commercially available.

MATERIALS AND METHODS

Tissues

The specimens were residual tissue specimens from individual breast cancer patients whose identities were not traceable. Tissue blocks were obtained from Dako (Carpinteria, CA and Glostrup, Denmark) and from a commercial source (Cureline, South San Francisco, CA) who in turn collected them from at least 15 different hospitals or clinical sites. All tissues were formalin-fixed and paraffin-embedded and the majority were fixed for 6 to 72 hours according to ASCO/CAP fixation guidelines. Study activities were performed in accordance with the ethical principles that have their origin in the Declaration of Helsinki and Good Clinical Practice. Approval of the study protocols by ethics committees was not required as no human subjects were directly involved. Quality control was performed on the specimens to confirm a diagnosis of invasive breast cancer, and the specimens were prescreened to determine the extent of nuclear ER or PR expression (see Table 1 for antibodies used for prescreening).

Antibodies and Reagents

The antibodies used for these studies are shown in Table 1. The antibodies were configured as FLEX Ready-to-Use and used with the EnVision FLEX, High pH visualization system according to the manufacturer's instructions for use. At each testing laboratory, reagents from 1 lot of reagents was used for staining on 1 Dako Omnis automated staining platform. All antibodies and associated reagents were obtained from Dako (Glostrup, Denmark).

Overall Study Design, Stratification of Specimens, and Observer Blinding

Slides with tissue sections (4 μm thick) were prepared from each specimen at Dako and labeled with the study number, and a unique randomization number that was not related to the specimen ID. The slides were distributed to each testing laboratory for staining in a prespecified randomized order according to the protocol for each study. Also provided were prestained hematoxylin and eosin sections from each specimen, and unstained tissue control slides with positive control (cervix) and negative control (colon epithelium) tissues. The tissue controls were fixed in the same manner as the test tissues.

The method comparison studies tested the concordance (agreement) between automated staining on the Dako Omnis platform with either the anti-ER clone EP1 or anti-PR clone PgR 1294 and staining on the Autostainer Link 48 instrument (clone EP1 for ER; clone PgR 636 for PR). Tissue specimens were stratified into 2 groups of 125 specimens for the ER study and 3 groups of 100 specimens for the PR study in order to ensure an even distribution of specimens to the various testing laboratories (UCL, IEO, URMC) with regards to ER or PR expression.

The reproducibility studies tested the interrun (day-to-day), interlaboratory, and interobserver reproducibility of automated staining on the Dako Omnis platform with either the anti-ER clone EP1 or anti-PR clone PgR 1294. The studies were conducted at 3 laboratories; each performed 5 staining runs on 20 specimens on 5 nonconsecutive days over a period of at least 20 days. At each laboratory, 1 trained pathologist evaluated the sections

TABLE 1. Antibodies Used for Specimen Prescreening and in the Studies

Antigens	Clones	Name	Platform	Use (Prescreening, Study)
ERα	EP1	Monoclonal rabbit anti-human	Dako Omnis	Repro, Comp
ERα	EP1	Monoclonal rabbit anti-human	Dako AS Link 48	Prescreening, Comp
PR	PgR 1294	Monoclonal mouse anti-human	Dako Omnis	Prescreening, Repro, Comp
PR	PgR 636	Monoclonal mouse anti-human	Dako AS Link 48	Prescreening, Comp
PR	PgR 1294	ER/PR pharmDx Kit	Dako AS Link 48	Prescreening
None	—	Universal Negative Control reagent—rabbit	Dako Omnis	Repro, Comp
None	—	Negative Control reagent—rabbit	Dako AS Link 48	Comp
None	—	Universal Negative Control reagent–mouse	Dako Omnis	Repro, Comp
None	—	Negative Control reagent—mouse	Dako AS Link 48	Comp

AS indicates Autostainer; Comp, method comparison study; ER, estrogen receptor; PR, progesterone receptor; Repro, reproducibility study.

stained at that laboratory. When the scoring was complete, the stained slides from laboratories 1 (UCL) and 2 (IEO) were forwarded to laboratory 3 (URMC) where they were evaluated by 3 independent pathologists.

IHC

Deparaffinization and antigen retrieval were done using EnVision FLEX High pH Target Retrieval Solution for Autostainer Link 48 or Dako Omnis. Control tissues and universal negative control antibody reagents (Table 1) were processed in parallel with tissues exposed to the primary antibodies. Stained tissue sections were dehydrated and coverslipped following routine procedures. The slides, which were blinded for specimen ID and staining method, were evaluated by a pathologist in the randomized order using a bright field microscope. Specimens were evaluated for several parameters related to staining quality, including artifacts and levels of background cytoplasmic staining in tumor and nontumor cells. Nuclear ER or PR staining was scored using the ASCO/CAP guideline,[6] which defines a positive result as nuclear staining of ER or PR in ≥1% of invasive breast carcinoma tumor cells. For the method comparison studies, nuclear ER or PR staining was also recorded for other cell types if present, including ductal carcinoma in situ (DCIS) and nontumor cells.

Data Collection and Statistical Analysis

Study data were entered directly into an online electronic data capture system (Viedoc; Pharma Consulting Group, Uppsala, Sweden). Once the last data were entered and the study databases were locked, the data were unblinded for specimen ID using the randomization key for each study. Two-sided Wilson Score 95% confidence intervals were calculated for the study endpoints using JMP software (SAS Institute, Cary, NC).

RESULTS

Histopathology of Breast Cancer Study Tissues

After staining but before assessment of ER or PR status, the tissue specimens were evaluated to ensure that they contained an adequate amount of invasive breast carcinoma tissue to obtain a reliable ER or PR status and acceptable morphology. Tissues not meeting these criteria were excluded from the study. For the ER and PR reproducibility studies, all 20 specimens in each study were acceptable and were included. Of the 250 specimens in the ER method comparison study, 11 were excluded due to inadequate invasive breast carcinoma tissue, and one due to retraction artifacts. Of the 300 specimens in the PR method comparison study, 8 were excluded due to inadequate invasive breast carcinoma tissue, and 3 due to unacceptable artifacts.

Overall Staining Quality

Invasive tumor cells across all tissues in these studies showed a spectrum of staining characteristics that ranged from an absence of ER/PR staining (IHC negative) to nuclear staining in a few cells (IHC positive if nuclear staining was present in >1% of invasive tumor cells) to diffuse strong nuclear reactivity across the tumor, which was comparable between the Dako Omnis and the Autostainer Link 48 staining platforms. Normal breast elements, when present in the section with invasive tumor, showed variable nuclear staining in virtually all cases regardless of the staining platform.

The specimens included in the studies were stratified to ensure that they represented the range of ER or PR expression encountered in clinical settings. The proportion of specimens in each study in various expression categories is shown in Table 2. The reproducibility studies included approximately 20% to 25% of specimens with invasive carcinoma around the 1% cutoff, and 11.8% (ER) and 14.2% (PR) for the method comparison studies.

Representative examples of the range of nuclear staining in invasive breast carcinoma tumor cells using the anti-ERα clone EP1 and anti-PR clone PgR 1294 antibodies on the Dako Omnis platform are shown in Figures 1 and 2. Occasional, scattered weak nuclear, and/or cytoplasmic staining of non-neoplastic cells including stromal cells, lymphocytes, and other cells was observed, and was similar between the 2 staining platforms (data not shown).

Across the 4 studies approximately 1% of the slides stained with the negative control reagents showed weak nuclear staining of either single or a very few invasive tumor cells but all were judged as acceptable. No slides stained with the negative control antibodies had any cytoplasmic staining of invasive tumor cells or cytoplasmic staining of nontumor cells (data not shown).

Results From the ER studies

In the method comparison study, sections from 250 tissue blocks were stained at 2 centers (IEO and URMC; 125 specimens each) with the anti-ERα clone EP1 antibody on the 2 staining platforms (Dako Omnis or Autostainer Link 48), and the percent agreement in ER status (positive or negative) was compared. The reproducibility study looked at interrun, interlaboratory, and interobserver reproducibility on sections from 20 tissue blocks stained with the anti-ERα clone EP1 antibody on the Dako Omnis platform in 5 runs at 3 centers (UCL, EIO, and URMC). The results from these studies are summarized in Table 3. High agreement rates were obtained for all study endpoints.

ER Staining Quality With Dako Omnis

The ER staining observed with the Dako Omnis platform showed crisp nuclear expression that varied in terms of the proportion of positive tumor cells and the intensity of signal across the different cases in this study (Fig. 1). Cytoplasmic staining in tumor cells was seen only in cases where there was diffuse strong nuclear staining present in the breast carcinoma.

For each ER-stained invasive carcinoma specimen included in the method comparison study, the pathologists recorded the percentage of positive cells and the staining intensity (Figure, Supplemental Digital Content 1, http://links.lww.com/AIMM/A101), as well as staining of DCIS

TABLE 2. Breast Cancer Specimens Used in the Studies by ER or PR Expression Level

Study	Specimen Category (Percent Cells With Nuclear ER or PR Staining) [N (%)]				Total [N (%)]
	0% (Negative)	> 0 < 1% (Negative)	≥ 1 ≤ 10% (Positive)	> 10% (Positive)	
ER reproducibility*	8 (40)	0 (0)	4 (20)	8 (40)	20 (100)
ER method comparison†	102 (42.8)	13 (5.5)	15 (6.3)	108 (45.4)	238 (100)
PR reproducibility*	8 (40)	1 (5)	4 (20)	7 (35)	20 (100)
PR method comparison†	70 (24.2)	17 (5.9)	24 (8.3)	178 (61.6)	289 (100)

*The median % positive cells for the 15 observations on the Dako Omnis platform (5 runs × 3 laboratories) was used to determine specimen category.
†Specimen category was determined by the 1 observation on the Dako Omnis platform.
ER indicates estrogen receptor; PR, progesterone receptor.

components, if present on the section (Figure, Supplemental Digital Content 2, http://links.lww.com/AIMM/A102). The results were highly comparable between the 2 staining platforms in terms of the proportion of positive tumor cells, with a slight tendency for a higher staining intensity for cases run on the Dako Omnis platform compared with the Autostainer Link 48 platform. The pattern of DCIS staining was similar between the 2 staining platforms.

Results From the PR Studies

In the method comparison study, sections from 300 tissue blocks were stained at 3 centers (IEO, UCL, and URMC; 100 specimens each) with anti-PR clone PgR 636 antibody on the Autostainer Link 48 platform and anti-PR clone PgR 1294 antibody on the Dako Omnis platform and

the percent agreement in PR status (positive or negative) was compared. The reproducibility study looked at inter-run, interlaboratory, and interobserver reproducibility on sections from 20 tissue blocks stained with the anti-PR clone PgR 1294 antibody on the Dako Omnis platform in 5 runs at 3 centers (UCL, EIO, and URMC). The results from these studies are summarized in Table 4. High agreement rates were obtained for all study endpoints.

PR Staining Quality With Dako Omnis

The PR staining observed with the Dako Omnis platform also showed crisp nuclear expression that varied in terms of the proportion of positive tumor cells and the intensity of signal across the different cases in this study (Fig. 2). Cytoplasmic staining in tumor cells tended to be
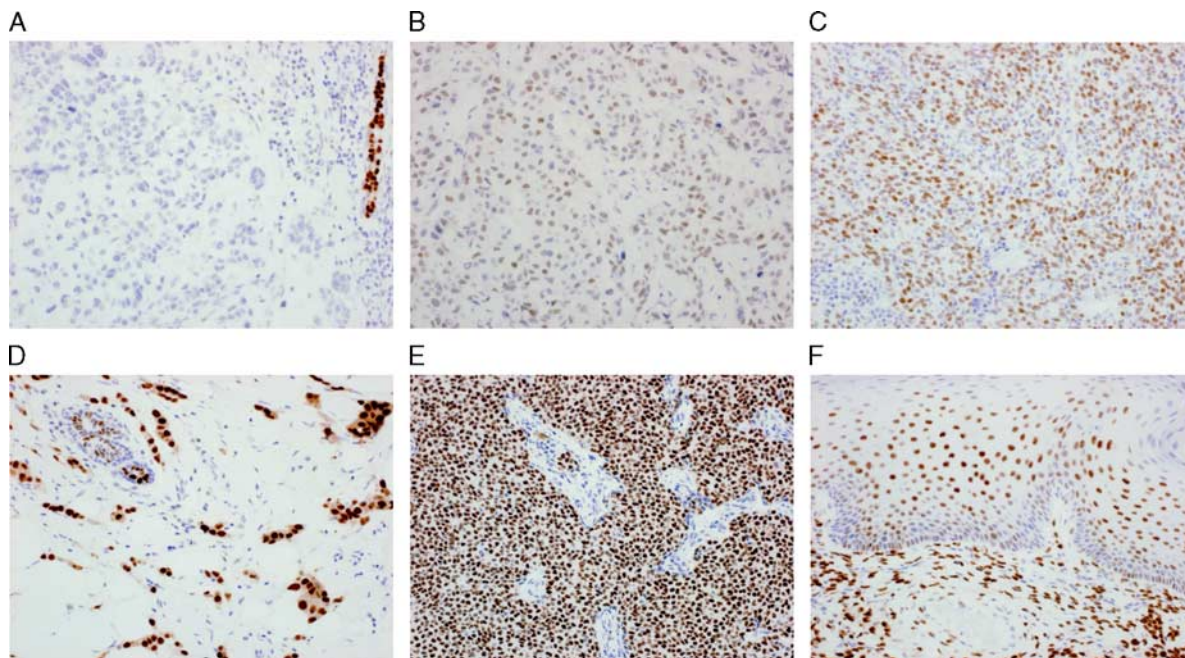
FIGURE 1. Staining of estrogen receptor using rabbit monoclonal antibody anti-ERα clone EP1 on the Dako Omnis platform. A, Negative staining of breast carcinoma; positive staining of normal glands; (B) positive staining of breast carcinoma (weak intensity); (C) positive staining of breast carcinoma (moderate intensity); (D) positive staining of breast carcinoma (strong intensity); positive staining of normal glands; (E) positive staining of breast carcinoma (strong intensity); (F) positive control tissue (cervix) with negative endothelial cells. ER indicates estrogen receptor.
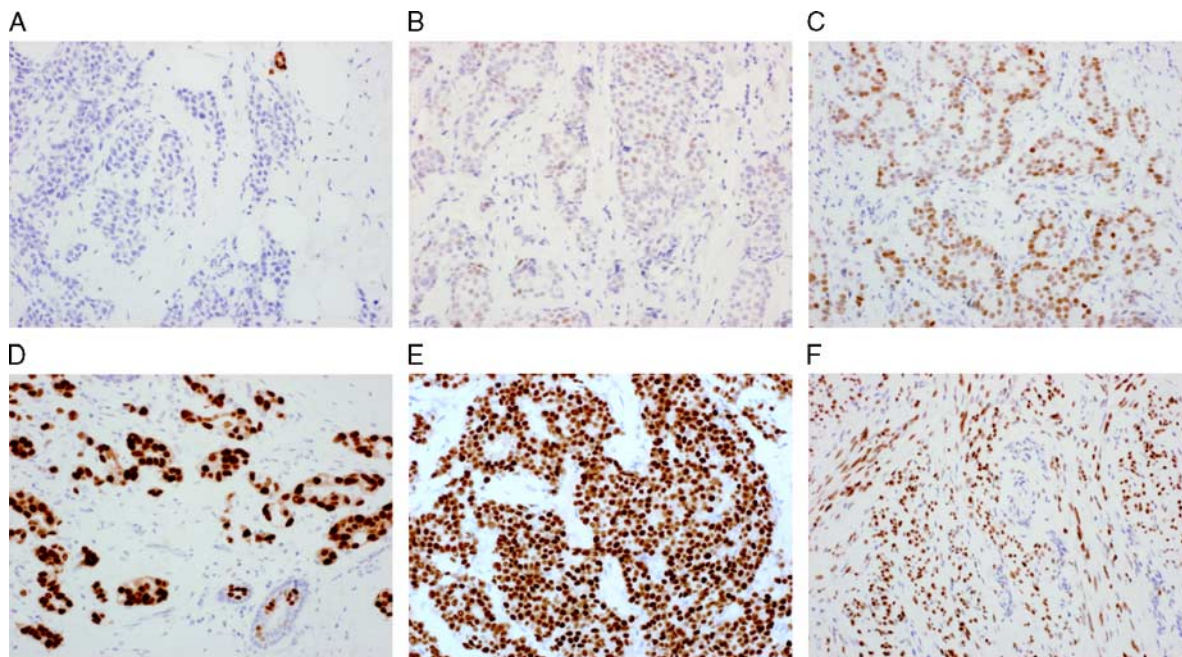
FIGURE 2. Staining of progesterone receptor using mouse monoclonal antibody anti-PR clone PgR 1294 on the Dako Omnis platform. A, Negative staining of breast carcinoma; positive staining of normal glands; (B) positive staining of breast carcinoma (weak intensity); (C) positive staining of breast carcinoma (moderate intensity); (D) positive staining of breast carcinoma (strong intensity); positive staining of normal glands; (E) positive staining of breast carcinoma (strong intensity); (F) positive control tissue (cervix) with negative endothelial cells. PR indicates progesterone receptor.

seen only in cases where there was diffuse strong nuclear staining present in the breast carcinoma.

For each PR-stained invasive carcinoma specimen included in the method comparison study, the pathologists recorded the percentage of positive cells and the staining intensity (Figure, Supplemental Digital Content 3, http://links.lww.com/AIMM/A103), as well as staining of DCIS components, if present on the section (Figure, Supplemental Digital Content 4, http://links.lww.com/AIMM/A104). The results were highly comparable between the 2 staining platforms in terms of the proportion of positive tumor cells, with a slight tendency for a higher staining intensity for cases run on the Autostainer Link 48 platform compared to the Dako Omnis platform. The pattern of DCIS staining was similar between the 2 staining platforms.

DISCUSSION

Accurate, reliable, and reproducible evaluation of the ER status in breast cancer is critically important to help ensure appropriate treatment planning.[13] IHC is the main method currently used for this evaluation, and all laboratories that perform IHC assays for ER and PR should closely follow quality control and assurance measures as outlined in published guidelines.[5,6,14] Breast cancer treatment is predicated on the ability of IHC to provide an accurate assessment of the expression of these biomarkers in formalin-fixed, paraffin-embedded breast cancer tumor tissue.[5] Ideally, the assessment and interpretation of ER/PR staining would be standardized, to help ensure that results are reproducible between different observers and different laboratories regardless of reagents

TABLE 3. Results of the Estrogen Receptor Studies

Study	Endpoints	Agreement (%)	95% Confidence Interval
Method comparison	Overall agreement	95.8	92.4-97.7
	Positive agreement	100	96.7-100
	Negative agreement	91.9	85.8-95.6
Reproducibility	Interrun: overall agreement	97.5	94.7-98.8
	Interlaboratory: overall agreement	94.3	90.9-96.5
	Interlaboratory: negative agreement	98.2	93.7-99.5
	Interlaboratory: positive agreement	91.7	86.5-95.0
	Interobserver: negative agreement	98.8	96.4-99.6
	Interobserver: positive agreement	98.1	96.0-99.1

TABLE 4. Results of the Progesterone ReceptorStudies

Study	Endpoint	Agreement (%)	95% Confidence Interval
Method comparison	Overall agreement	97.6	95.1-98.8
	Positive agreement	96.7	93.3-98.4
	Negative agreement	100	95.4-100
Reproducibility	Interrun: overall agreement	98.8	96.4-99.6
	Interlaboratory: overall agreement	93.2	89.7-95.6
	Interlaboratory: negative agreement	99.2	95.6-99.9
	Interlaboratory: positive agreement	88.3	82.3-92.5
	Interobserver: negative agreement	96.9	94.1-98.4
	Interobserver: positive agreement	98.2	96.2-99.2

or staining platform used. However, a number of studies have documented significant discordant results as well as interlaboratory variability in ER assay results that have been attributed to a variety of causes, including sensitivity and specificity of antibody reagents,[15–18] insufficient antigen retrieval,[19–22] and differing threshold and interpretation criterion.[20,23] These studies highlight the importance of rigorous and thorough evaluation of all aspects of testing, including technical validation of the performance of any new IHC staining platforms or new IHC reagents for ER and PR analysis before their clinical implementation.

In the present study we investigated new ER and PR assays and a novel automated staining platform in a large cohort of primary breast cancer cases obtained from at least 15 different hospitals or clinical sites, which would likely be representative of breast cancer cases seen in the general population across a number of different institutions and clinical sites. Our results show excellent concordance between the Dako Omnis staining platform compared with the existing marketed staining platform (Autostainer Link 48). There was excellent interrun, interobserver, and interlaboratory reproducibility for both ER (clone EP1) and PR (clone PgR 1294) antibodies employed on the Dako Omnis staining platform across the 3 different study laboratories. The staining results showed high specificity with crisp, distinct nuclear reactivity for both ER and PR antibodies both in tumor cells and normal luminal cells with very low background.

Although anti-ER clone EP1 and anti-PR clone PgR 1294 for Dako Omnis were not directly compared in the present studies to the Dako ER/PR pharmDx kit, indirect inference can be made based on the reported high degree of concordance (~95%) between clone EP1 and the ER component of the pharmDx kit (a mixture of 2 mouse monoclonal antibodies) using the Allred scoring system to determine the cutoff for positivity.[11] For detection of PR, the present study used clone PgR 1294 on the Dako Omnis, which is the same antibody used as the PR component of the pharmDx kit, but compared it with clone PgR 636 in order to consistently use the ASCO/CAP recommended cut-off of ≥ 1% positive tumor cells for positive assessment. Our results are consistent with those of Gill et al,[24] who showed 96% agreement between anti-PR clone PgR 1294 and clone PgR 636.

For this study cohort, the proportions of specimens with invasive carcinoma around the 1% cutoff (derived from the actual percent-positive-cells scoring from the study data) was around 20% to 25% for the reproducibility studies with a mean of 11.8% (ER) and 14.2% (PR) for the method comparison studies, suggesting that this is a challenging and robust data set with which to make comparison between different staining platforms and antibody reagents. As has been previously described, the overall concordance would be expected to be higher in unequivocally positive or negative cases,[25] whereas tumors in the low/borderline steroid hormone receptor group would be more challenging to reach a high degree of concordance.[21] In a recent report among 1700 consecutive cases of invasive ductal carcinoma from a single institution, only 32 (1.9%) of cases fell into the ER positive 1% to 10% category,[26] suggesting that the number of borderline cases in the current study is overrepresented compared with the general breast cancer population. In the cohort of breast cancer cases presented here, a high degree of concordance was demonstrated between anti-ER clone EP1 and anti-PR clone PgR 1294 used on Dako Omnis and the existing anti-ER and anti-PR Dako products for Autostainer Link 48. In addition, excellent reproducibility of the new anti-ER and anti-PR reagents was observed on the Dako Omnis across multiple runs, testing laboratories, and evaluating pathologists (observers).

The ability to detect a range of levels of expression of ER and PR, particular for cases with low levels of expression, is important and desirable for any biomarker assay for hormone receptors and may have prognostic significance. In a retrospective study of 1424 consecutive patients with HER2-negative breast cancer and low endocrine receptor expression, patients with ER/PR of 1% to 10% had a slight but not statistically significant, better prognosis than the ER/PR < 1% group.[27] These authors concluded that further studies are needed to identify the appropriate clinical approach in this subset of patients with low ER/PgR expression (ER/PgR 1% to 10%), HER2-negative early breast cancer. Thus, the accurate assessment of ER/PR, particularly for cases with low levels of expression, remains important for both treatment planning and furthering our understanding of the biology of breast tumors with different levels of ER/PR expression.[26]

The data presented here suggests that immunohistochemical analysis of ER and PR expression levels using monoclonal antibody clones EP1 and PgR 1294 on the Dako Omnis automated staining platform is reliable and reproducible across staining runs, testing laboratories, and evaluating pathologists.

ACKNOWLEDGMENTS

REFERENCES

1. Fisher B, Redmond C, Brown A, et al. Treatment of primary breast cancer with chemotherapy and tamoxifen. *N Engl J Med.* 1981;305: 1–6.
2. Fisher B, Redmond C, Brown A, et al. Influence of tumor estrogen and progesterone receptor levels on the response to tamoxifen and chemotherapy in primary breast cancer. *J Clin Oncol.* 1983;1: 227–241.
3. Mohsin SK, Weiss H, Havighurst T, et al. Progesterone receptor by immunohistochemistry and clinical outcome in breast cancer: a validation study. *Mod Pathol.* 2004;17:1545–1554. Erratum in: *Mod Pathol* 2005 Mar;18:461. Qian, Zho [corrected to Qian, Zhang].
4. Harris L, Fritsche H, Mennel R, et al. American Society of Clinical Oncology 2007 update of recommendations for the use of tumor markers in breast cancer. *J Clin Oncol.* 2007;25:5287–5312.
5. Allred DC, Carlson RW, Berry DA, et al. NCCN Task Force Report: estrogen receptor and progesterone receptor testing in breast cancer by immunohistochemistry. *J Natl Compr Canc Netw.* 2009;7(suppl 6):S1–S21. Quiz S22-S23.
6. Hammond ME, Hayes DF, Dowsett M, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. *J Clin Oncol.* 2010;28: 2784–2795.
7. Goldhirsch A, Winer EP, Coates AS, et al. Personalizing the treatment of women with early breast cancer: highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. *Ann Oncol.* 2013;24:2206–2223.
8. Lee M, Lee CS, Tan PH. Hormone receptor expression in breast cancer: postanalytical issues. *J Clin Pathol.* 2013;66:478–484.
9. Charbgoo F, Mirshahi M, Sarikhani S, et al. Synthesis of a unique high-performance poly-horseradish peroxidase complex to enhance sensitivity of immunodetection systems. *Biotechnol Appl Biochem.* 2012;59:45–49.
10. Rossi S, Laurino L, Furlanetto A, et al. Rabbit monoclonal antibodies: a comparative study between a novel category of immunoreagents and the corresponding mousemonoclonal antibodies. *Am J Clin Pathol.* 2005;124:295–302.
11. Badve S, Vladislav IT, Spaulding B, et al. EP1: a novel rabbit monoclonal antibody for detection of oestrogen receptor α. *J Clin Pathol.* 2013;66:1051–1057.
12. Bánkfalvi A, Boecker W, Reiner A. Comparison of automated and manual determination of HER2 status in breast cancer for diagnostic use: a comparative methodological study using the Ventana BenchMark automated staining system and manual tests. *Int J Oncol.* 2004;25:929–935.
13. Burstein HJ, Prestrud AA, Seidenfeld J, et al. American Society of Clinical Oncology clinical practice guideline: update on adjuvant endocrine therapy for women with hormone receptor-positive breast cancer. *J Clin Oncol.* 2010;28:3784–3796.
14. Yaziji H, Taylor CR, Goldstein NS, et al. Consensus recommendations on estrogen receptor testing in breast cancer by immunohistochemistry. *Appl Immunohistochem Mol Morphol.* 2008;16: 513–520.
15. Maderia KP, Daltoe RD, Sirtoli GM, et al. Comparison of immunohistochemical analysis with estrogen receptor SP1 and 1D5 monoclonal aitnbodies in breast cancer. *Pathol Res Pract.* 2012;208:657–661.
16. McCullough AE, Dell'orto P, Reinholz MM, et al. Central pathology laboratory review of HER2 and ER in early breast cancer: an ALTTO trial [BIG 2-06/NCCTG N063D (Alliance)] ring study. *Breast Cancer Res Treat.* 2014;143:485–492.
17. Welsh AW, Harigopal M, Wimberly H, et al. Quantitative analysis of estrogen receptor expression shows SP1 antibody is more sensitive than 1D5. *Appl Immunohistochem Mol Morphol.* 2013;21: 139–147.
18. Rocha R, Nunes C, Rocha G, et al. Rabbit monoclonal antibodies show higher sensitivity than mouse monoclonals for estrogen and progesterone receptor evaluation in breast cancer by immunohistochemistry. *Pathol Res Pract.* 2008;204:655–662.
19. Rhodes A, Jasani B, Balaton AJ, et al. Study of interlaboratory reliability and reproducibility of estrogen and progesterone receptor assays in Europe. Documentation of poor reliability and identification of insufficient microwave antigen retrieval time as a major contributory element of unreliable assays. *Am J Clin Pathol.* 2001;115:44–58.
20. Parker RL, Huntsman DG, Lesack DW, et al. Assessment of interlaboratory variation in the immunohistochemical determination of estrogen receptor status using a breast cancer tissue microarray. *Am J Clin Pathol.* 2002;117:723–728.
21. Wasielewski RV, Hasselmann S, Rüschoff J, et al. Proficiency testing of immunohistochemical biomarker assays in breast cancer. *Virchows Arch.* 2008;453:537–543.
22. Rhodes A, Sarson J, Assam EE, et al. The reliability of rabbit monoclonal antibodies in the immunohistochemical assessment of estrogen receptors, progesterone receptors, and HER2 in human breast carcinomas. *Am J Clin Pathol.* 2010;134:621–632.
23. Viale G, Slaets L, Bogaerts J, et al. High concordance of protein (by IHC), gene (by FISH; HER2 only), and microarray readout (by TargetPrint) of ER, PgR, and HER2: results from the EORTC 10041/BIG 03-04 MINDACT trial. *Ann Oncol.* 2014;25: 816–823.
24. Gill MS, Paish EC, Ronan J, et al. Comparison of the PharmDx immunohistochemical system with standard methods for assessing estrogen and progesterone receptors in invasive carcinoma of the breast. *Appl Immunohistochem Mol Morophol.* 2013;21:90–93.
25. Wells CA, Sloane JP, Coleman D, et al. Consistency of staining and reporting of oestrogen receptor immunocytochemistry within the European Union—an interlaboratory study. *Virchows Arch.* 2004; 445:119–128.
26. Zhang Z, Wang J, Skinner KA, et al. Pathological features and clinical outcomes of breast cancer according to levels of oestrogen receptor expression. *Histopathology.* 2014;65:508–516.
27. Balduzzi A, Bagnardi V, Rotmensz N, et al. Survival outcomes in breast cancer patients with low estrogen/progesterone receptor expression. *Clin Breast Cancer.* 2014;14:258–264.