K990618

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510(k) SUMMARY
Ventana PGR Primary Antibody (Clone 1A6)

Submitted By:
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Date: 21 July 1999

This summary of 510(k) safety and effectiveness information is being submitted in accordance with the requirements of SMDA 1990 and 21 CFR 807.92. The assigned 510(k) number is K990618.

Name of the Device:

Trade Name:	Ventana PGR Primary Antibody (Clone 1A6)
Common Name:	Antibody for aid in the detection of progesterone receptor (PGR) antigen in histological tissue sections
Classification:	To the best of our knowledge PGR antibody for aid in the detection of PGR antigen in histological tissue sections is classified as class II and codified at 21 CFR 864.1860; Medical Devices; Classification/Reclassification of Immunohistochemistry Reagents and Kits, June 3, 1998.

Predicate Device: Ventana PGR primary antibody (clone 1A6) is substantially equivalent to commercially available Abbot Laboratories' PgR-ICA Monoclonal, Immunocytochemical Assay for the Detection of Human Progesterone Receptor in Breast Tumor Tissue, approved by the FDA as PMA # 920006 and downclassified to class II by 21 CFR 864.1860 Immunohistochemistry reagents and kits on June 3, 1998.

Description: Ventana's PGR Primary Antibody (clone 1A6) is a monoclonal antibody which specifically binds to progesterone receptor antigen located in the nuclear region of a variety of normal and neoplastic tissues. The dispenser contains approximately 5 µg (50 test) or 25 µg (250 test) of mouse anti-human PGR monoclonal antibody. The total protein concentration of the reagent is approximately 10 mg/ml. Specific antibody concentration is approximately 1 µg/ml. Clone 1A6 is immunoglobulin class IgG_1, light chain kappa. There is no known irrelevant antibody in the preparation.

Intended Use: Ventana PGR primary antibody (clone 1A6) is intended for laboratory use for the qualitative detection of progesterone receptor (PGR) antigen in sections of formalin fixed, paraffin embedded normal and neoplastic tissue on a Ventana automated immunohistochemistry slide staining device. It is indicated as an aid in the management, prognosis and prediction of

therapy outcome of breast cancer within the context of the patient's clinical history and other diagnostic tests evaluated by a qualified pathologist.

Technology of the device and predicate device: The demonstration of antigens in tissue and cells by immunostaining is a process involving first the binding of an antibody to an antigen of interest and second, visualization of the bound primary antibody by an indirect biotin-avidin system coupled to an enzyme. Ventana PGR primary antibody (clone 1A6) is located by a biotin conjugated secondary antibody formulation which recognizes rabbit or mouse immunoglobulins. This step is followed by the addition of a streptavidin-enzyme conjugate which binds to the biotin on the secondary antibody. The primary antibody-secondary antibody-avidin enzyme complex is visualized by using a precipitating enzyme generated product. Ventana PGR Primary Antibody (clone 1A6) was developed for use on Ventana's automated slide staining devices. Briefly, the procedure is as follows:

1. Pretreatment of tissues or cells on a microscope slide with an antigen-enhancement procedure is required.
2. An inhibitor solution is added to the slide on which antigen-enhanced tissues or cells are affixed. The inhibitor reduces the endogenous peroxidase activity in the tissue. This step takes 4 minutes at 37° C. The slide is washed with buffer and covered with liquid coverslip.
3. Optimized PGR Primary Antibody (clone 1A6) is applied, mixed and incubated for 4 to 32 minutes at 37° C. The slide is washed with buffer and covered with liquid coverslip.
4. Streptavidin-enzyme reagent is added to the slide, mixed and incubated at 37° C. (The time of incubation is indicated in each detection kit insert.) The slide is washed with buffer and covered with liquid coverslip.
5. The chromogenic enzyme substrate is applied to the slide and mixed. The solutions are incubated with mixing at 37° C. (The time of incubation is indicated in each detection kit insert.) The slide is washed and ready for counterstaining, copper enhancement, or can be prepared for microscopic reading immediately.

Abbot PgR-ICA Monoclonal detects progesterone receptor in frozen tissue sections affixed to glass slides in a technically similar fashion to the immunostaining process used for staining paraffin embedded tissue. The frozen sections are fixed in formaldehyde, methanol and acetone, then treated with Blocking Reagent to prevent nonspecific binding. The primary antibody (the Ig fraction of a rat monoclonal antibody specific for PgR receptor) is then added to the tissue. Visualization of the bound primary antibody is via an indirect peroxidase anti-peroxidase (PAP) system. PgR-ICA Monoclonal is located by a goat anti-rat bridging antibody. Anti-Rat PAP complex which recognizes the bridging antibody is then added. This step is followed by the addition of a precipitating enzyme generated product (hydrogen peroxidase) and a chromogen substrate solution, containing DAB. The resulting insoluble reddish brown precipitation allows visualization of the bound primary antibody[2]. The two devices are similar in that both systems specifically bind to PGR proteins in breast carcinoma tissues, both employ similar detection chemistry principles for visualization of the product, and both aid in the diagnosis and prognosis of breast carcinoma.

Performance Data:

1. Tissue Specificity: The staining patterns of 78 normal tissues were examined. These included adrenal, bone marrow, breast, cerebrum, cervix, colon, endometrium, esophagus, heart, kidney, liver, lung, mesothelium, ovary, pancreas, peripheral nerve, pituitary, prostate, salivary gland, skeletal muscle, skin, small intestine, spleen, stomach, testis, thyroid, tonsil, and thymus. Tissues not tested as recommended in "FDA Guidance for Submission of Immunohistochemistry Applications to the FDA", were cerebellum and parathyroid. Staining was considered to be appropriate, with the exception of the unexpected negative staining of one case of breast tissue, one case of endometrium, and all three cases of ovary. The pathologist noted however, that in the case of the breast, the tissue was poorly preserved, so the negative staining may have been due to improper fixation. Positive nuclear staining was found in the lobular and ductal cells of the breast (2 of 3 cases), glandular epithelia and fibromuscular cells of the cervix (3 of 3 cases), and glandular epithelia, stromal and smooth muscle cells of the endometrium (2 of 3 cases) and secretory cells of the adenohypophysis of one of the two cases of the pituitary tissues. All other tissues were negative.

The staining patterns of 19 neoplastic tissues were also evaluated by a qualified pathologist. Neoplastic tissues examined were lung, prostate, colon, lymphoma, bladder, stomach, cervix, and ovary. Positive nuclear staining occurred in the surrounding normal tissue of two cases of cervical carcinoma. All other tissues were negative.

Prior to staining with Ventana PGR primary antibody (Clone 1A6), the dried, deparaffinized sections used for the specificity studies were treated for antigen enhancement. Slides containing the tissue sections were placed in citrate buffer (pH 5.75) and heated under pressure in a microwave for approximately 30 minutes. The slides were allowed to stand for an additional 30 minutes (approximate), then transferred to fresh citrate buffer until ready to run on the Ventana automated slide stainer. Optimized PGR Primary Antibody (clone 1A6) was applied, mixed and incubated 32 minutes at 37° C. Endogenous biotin was blocked using Ventana Medical Systems' Endogenous Biotin Blocking Kit (catalogue number 250-050). Detection used was Ventana's DAB Kit (catalogue number 250-001). DAB Detection includes: 1) An inhibitor solution, added to reduce the endogenous peroxidase activity in the tissue. 2) Universal biotinylated secondary antibody. 3) Avidin-HRPO. 4 and 5) Basic DAB solution and hydrogen peroxide. The two reagents are applied together and mixed on the specimen slide. And 6) Copper enhancement. The incubation time for each reagent was pre-programmed at Ventana.

2. Validation of Assay Performance by Comparison with an FDA approved chemical cytosol receptor assay for PGR, a Serum Binding Assay/ Dextran Coated Charcoal (SBA/DCC). The safety and effectiveness of Ventana's PGR Primary Antibody have been examined through clinical testing by Ventana Medical Systems, Inc. Ventana verified that PGR Primary Antibody detects PGR in breast carcinoma tissue in an equivalent manner to SBA/DCC by examining 100 cases of breast cancer for expression of PGR. The cases were randomly chosen from an archived collection of breast carcinoma biopsies (300 cases total) at one investigative site. The study was retrospective in design, using archived tissue samples embedded in paraffin blocks that had

concurrent PGR SBA/DCC analyses in the patient file. For antigen enhancement, the investigator used a 10 minute autoclave steam heating procedure and the Biogenex Citra antigen retrieval solution. The staining protocol was identical to that used in the specificity studies.

Quality control procedures included a positive tissue control (uterus) placed on every slide run in addition to the case tissue. Each case had two slides stained with Ventana PGR Primary Antibody (Clone 1A6) and one slide with Ventana Negative Control Reagent Ig (catalogue # 250-2014). For the test to be considered valid, the positive control tissue was expected to exhibit nuclear staining of the endometrial glands and stroma. These components were to be negative when stained with Negative Control Reagent Ig. In addition, a negative tissue control slide (PGR-negative breast carcinoma) was included for every batch of samples processed and run on the Ventana automated slide staining device. This negative tissue control was stained with PGR Primary Antibody (Clone 1A6) to ensure that the antigen enhancement and other treatment procedures did not create false positive staining. The study cases were evaluated within the context of the performance of the controls.

Staining of PGR primary antibody (clone 1A6) was assessed both as a percentage of cells stained and by staining intensity. Scoring of tumor cell percentages was estimated and categorized into the following cell count ranges: 0, 1-10, 11-25, 26-75, and over 75. If the scorer was uncertain of the category, 100 tumor cells in each of three fields at 40X magnification were counted and the values averaged for a true count percentage. Two cases were excluded from analysis. One was due to inappropriate staining of internal negative control tissue. The second was excluded because of inappropriate staining of the negative reagent control. The emphasis in data analysis was on percent positive cells rather than stain intensity. Scoring of the benign elements was also performed to aid in discrepancy resolution. The pathologist scoring the IHC was masked to the results of the PGR SBA/DCC. Results were as follows:

Table 1: PGR IHC vs. PGR SBA/DCC

% Positive Cells	TP	TN	FP	FN	TOTAL
Raw PGR $\geq$ 1%	49	20	20	9	98
Raw PGR $\geq$ 11%	36	35	5	22	98
Resolved PGR $\geq$ 1%	49	25	20	4	98
Resolved PGR $\geq$ 11%	36	40	5	17	98

Scoring Criteria: PGR IHC positivity was compared to PGR SBA/DCC at different cut-off values to determine the cut-off value for PGR Primary Antibody (Clone 1A6) that best agreed with the SBA/DCC results. Sensitivity, specificity, and concordance of the PGR test, relative to that of the PGR SBA/DCC test, was in best agreement with SBA/DCC using a cut-off value of $\geq$ 11% compared to $\geq$ 1%. Cell intensity within a patient tissue was variable and was not used as a criteria for positivity. PGR SBA/DCC was scored as positive at greater than or equal to 10 femtomoles (fm).

Discrepancy Resolution: Because SBA/DCC cannot distinguish between positivity of pathological and benign elements, the protocol allowed for the resolution of false negative PGR IHC to true negative if benign elements were positive and pathological elements did not stain. The results of these analyses are noted as "Resolved".

Agreement: PGR IHC with Ventana's PGR Primary Antibody showed the best agreement with PGR SBA/DCC ($\chi^2 = 35.91$, $P < 0.0001$) when $\geq 11\%$ of tumor cell nuclei were scored as positive. 78% (76/98) of the cases were concordant, with a 95% confidence interval of 68 – 85%.

A possible explanation for the negative SBA/DCC results of the 5 false positive cases is progesterone receptor degradation due to poor tissue handling during sample collection[2]. The majority of the false negative cases were due to the cut-off value chosen. 13 of the 17 false negatives (76%) did stain positively for PGR, but at a level below the cut-off (from $\geq 11\%$ positive staining of tumor cell nuclei): However the cost of using the lower cut-off ($\geq 1\%$) was 15 more false positive results. The 4 false negative cases that exhibited no staining for PGR using IHC are most likely due to pretreatment procedures such as sample handling or fixation[2].

Sensitivity: Resolved sensitivity relative to PGR SBA/DCC at a cut off of $\geq 11\%$ was 67.9% (36/53), with a 95% confidence interval of 54 – 80%.

Specificity: Resolved specificity relative to PGR SBA/DCC at a cut off of $\geq 11\%$ was 88.9% (40/45), with a 95% confidence interval of 76 – 96%.

Conclusions: Ventana claims that PGR Primary Antibody (Clone 1A6) is substantially equivalent to Abbott PgR-ICA Monoclonal, subject of P920006, which was downclassified from class III to Class II as a result of 21 CFR 864.1860, Immunohistochemistry Reagents and Kits. Performance characteristics of the Ventana PGR Primary Antibody were validated in a comparison with an FDA approved chemical cytosol receptor assay for PGR, serum binding assay/dextran coated charcoal (SBA/DCC). Demonstrated performance characteristics were: 67.9% relative sensitivity, 88.9% relative specificity, and 78% concordance.

Bibliography:

1. Bevitt, D. J. et. al. New monoclonal antibodies to oestrogen and progesterone receptors effective for paraffin section immunohistochemistry. J. Pathol. 183: 228-232, 1997.

2. Abbott PgR-ICA Monoclonal Package Insert. Abbott Laboratories Diagnostics Division. Customer Support Center (USA) 1-800-323-9100.

DEPARTMENT OF HEALTH & HUMAN SERVICES

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JUL 23 1999

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Re: K990618
Trade Name: Ventana PGR Primary Antibody (Clone 1A6)
Regulatory Class: II
Product Code: MXZ
Dated: May 4, 1999
Received: May 5, 1999

Dear Ms. Frederick:

We have reviewed your Section 510(k) notification of intent to market the device referenced above and we have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into either class II (Special Controls) or class III (Premarket Approval), it may be subject to such additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 895. A substantially equivalent determination assumes compliance with the Current Good Manufacturing Practice requirements, as set forth in the Quality System Regulation (QS) for Medical Devices: General regulation (21 CFR Part 820) and that, through periodic QS inspections, the Food and Drug Administration (FDA) will verify such assumptions. Failure to comply with the GMP regulation may result in regulatory action. In addition, FDA may publish further announcements concerning your device in the Federal Register. Please note: this response to your premarket notification submission does not affect any obligation you might have under sections 531 through 542 of the Act for devices under the Electronic Product Radiation Control provisions, or other Federal laws or regulations.

Under the Clinical Laboratory Improvement Amendments of 1988 (CLIA-88), this device may require a CLIA complexity categorization. To determine if it does, you should contact the Centers for Disease Control and Prevention (CDC) at (770) 488-7655.

This letter will allow you to begin marketing your device as described in your 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific advice for your device on our labeling regulation (21 CFR Part 801 and additionally 809.10 for in vitro diagnostic devices), please contact the Office of Compliance at (301) 594-4588. Additionally, for questions on the promotion and advertising of your device, please contact the Office of Compliance at (301) 594-4639. Also, please note the regulation entitled, "Misbranding by reference to premarket notification"(21 CFR 807.97). Other general information on your responsibilities under the Act may be obtained from the Division of Small Manufacturers Assistance at its toll-free number (800) 638-2041 or (301) 443-6597, or at its internet address "http://www.fda.gov/cdrh/dsma/dsmamain.html".

Sincerely yours,

Steven Gutman

Steven I. Gutman, M.D, M.B.A.
Director
Division of Clinical
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Enclosure

510(k) Number (if known): K990618

Device Name: Ventana PGR Primary Antibody (Clone 1A6)

Indications For Use:

Ventana Medical Systems' PGR Primary Antibody (Clone 1A6) may be used in immunohistochemical methods to aid in the identification of progesterone receptor (PGR) antigen in normal and neoplastic cells as an aid in the diagnosis of PGR positive tumors. The anti-PGR is intended for laboratory use to qualitatively stain PGR antigen in sections of formalin fixed, paraffin embedded tissue on a Ventana automated slide staining device. Detection chemistries used by Ventana to detect PGR are DAB, AEC, Alkaline phosphatase red and blue. Light microscopy is used to detect the staining of cell components. It is indicated for use as an aid in the management, prognosis and prediction of therapy outcome of breast cancer.

(PLEASE DO NOT WRITE BELOW THIS LINE-CONTINUE ON ANOTHER PAGE IF NEEDED)

Concurrence of CDRH, Office of Device Evaluation (ODE)

Peter E. Maldm

(Division Sign-Off)
Division of Clinical Laboratory Devices
510(k) Number K990618

Prescription Use ✓ OR **Over-The-Counter Use** ___
(Per 21 CFR 801.109)

(Optional Format 1-2-96)