510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
DEVICE AND INSTRUMENT TEMPLATE

A. 510(k) Number:
K033200

B. Analyte:
Estrogen and Progesterone Receptors on formalin-fixed paraffin-embedded breast tissue specimens of the primary tumor from breast cancer patients

C. Type of Test:
Computer-assisted image analyzer for estrogen/progesterone receptor immunohistochemistry (immunocytochemistry)

D. Applicant:
Applied Imaging Corporation

E. Proprietary and Established Names:
Applied Imaging Ariol™ with ER/PR Application

F. Regulatory Information:
1. Regulation section:
   21 CFR §864.1860 Immunohistochemistry reagents and kits
2. Classification:
   Class II
3. Product Code:
   NQN (Microscope, Automated, Image Analysis, Immunohistochemistry, Operator Intervention, Nuclear Intensity and Percent Positivity)
4. Panel:
   Pathology 88

G. Intended Use:

Ariol™ is an automated scanning microscope and image analysis system. It is intended for *in vitro* diagnostic use as an aid to the pathologist in the detection, classification, and counting of cells of interest based on particular color, intensity, size, pattern, and shape.

This particular Ariol software application is intended to measure, count, and quantitate the percentage and intensity of positively stained nuclei in formalin-fixed paraffin-embedded tissue specimens immunohistochemically stained for Estrogens Receptors or Progesterone Receptors (ER/PR).

1. Indication(s) for use:
   ER/PR results are indicated for use as and aid in the management, prognosis, and prediction of therapy outcomes of breast cancer.
2. Special instrument Requirements:

Applied Imaging Ariol™ automated scanning microscope and image analysis system.

H. Device Description:

The Ariol is comprised of a computer, monitor, keyboard, mouse, printer, installed software, and a microscope with motorized stage, focus and filterwheels. The Ariol displays images of tissue areas on the monitor. It is the user's responsibility to review the Ariol-generated results and designate the final result on the report form. Automatic relocation, capture and archiving of the cell images are performed by the instrument based upon operator selection.

The Ariol™ system uses the Kisight (nuclear IHC) assay to analyze slides from tissue sections immunohistochemically stained for the presence of the Estrogen or Progesterone receptors (ER or PR). The workflow for the Kisight assay is as follows. First, the user trains the Kisight classifiers. There are classifiers for both 5x and 20x magnifications. The classifiers must be trained on both immunopositive and immunonegative cells as determined by the user. The 5x Kisight classifiers consist of color classifiers that measure the intensity of the brown color. The 20x Kisight classifiers consist of color and shape classifiers for the nuclei. Training provides sufficient information to allow the system to score all other cell nuclei. The classification procedure for Kisight is detailed as follows.

Once the classifiers have been determined, the system is ready to begin automatic scoring of test slides. The default assay for Kisight consists of three scan passes for each slide. The first scan pass, performed at a 1.25x magnification, allows the system to locate the tissue on the slide, and create scan regions over the tissue for the next pass.

The second pass is performed at a 5x magnification over the tissue region. During this pass, the system automatically selects suggested regions for the next scan pass. The suggested regions are chosen based on the areas with the most intense brown staining as determined by the 5x classifier.

After the second pass, the system pauses, allowing the user to define the invasive tumor regions for the final pass at a 20x magnification. The user may choose the suggested regions selected automatically by the system, or define his/her own regions. It is up to the user to ensure that the regions selected are in areas of invasive tumor and not other tissue types; the system is not capable of making this distinction automatically.

Both the first and second scan passes can be configured to limit the number and size of regions found for the next pass. The default settings are for 5 regions that are limited to 12 frames in size. The regions are selected by intensity of staining so the brownest regions are selected first. The user may change the default number and sizes using the Configure dialog in the Assay panel of the Scan application.

In the final pass, the system acquires 20x images of the specified regions, and performs the automatic scoring. Based on the 20x shape and color classifiers trained by the user, the system is able to identify and count immunopositive and immunonegative nuclei. The system then reports a percent positivity for each region to aid the pathologist in the diagnosis. It is the user's responsibility to review the Ariol-generated result and designate the final result on the report form.

I. Substantial Equivalence Information:

1. Predicate device name(s)
   ChromaVision Medical Systems, Inc. ACIS (Automated Cellular Imaging System) software application for Estrogen and Progesterone Receptors
2. Predicate K number(s):
   k012138
3. Comparison with predicate:

DEVICE	PREDICATE
A. Similarities	
Histologic observation by a pathologist through a controlled microscope/digital camera combination	Histologic observation by a pathologist through a controlled microscope/digital camera combination
Examines formalin-fixed paraffin-embedded breast cancer specimens stained by immunohistochemistry for Estrogen and Progesterone Receptor proteins.	Examines formalin-fixed paraffin-embedded breast cancer specimens stained by immunohistochemistry for Estrogen and Progesterone Receptor proteins.
B. Differences	
Ariol™ instrument and software version v1.1	ACIS instrument hardware and software
The method of assessment/analysis by the software is colorimetric and morphometric pattern recognition by microscopic examination of prepared cells by size, shape, hue, and intensity.	The method of assessment/analysis by the software is colorimetric pattern recognition by microscopic examination of prepared cells by hue and intensity.

J. Standard/Guidance Document Referenced (if applicable):
None

K. Test Principle:
Method of cell detection is by colorimetric pattern recognition by microscopic examination of prepared cells by size, shape, hue, and intensity as observed by an automated computer controlled microscope and/or by visual observation by a health care professional.

L. Performance Characteristics (if/when applicable):

1. Analytical performance:
   a. *Precision/Reproducibility:*
      The Ariol™ ER and PR applications were evaluated for precision in simulated clinical settings. Precision was assessed via three precision studies, with each study exhibiting an increasing level of variation in study design. The precision studies included five slides (5) for ER assessment and five (5) slides for PR assessment. The ten slides were prepared from formalin-fixed, paraffin-embedded sections, and all tissue samples were derived from patients with infiltrating breast cancer. The slides were stained with DakoCytomation ER stain (product code N1575) and DakoCytomation PR stain (product code M3569). Following incubation with the primary mouse antibodies to human ER and PR receptor proteins, the test employs a

ready-to-use visualization reagent based on dextran technology. The Dako Envision + System Peroxidase (DAB) kit consists of both secondary goat anti-mouse antibody molecules and horseradish peroxidase molecules linked to a common dextran polymer backbone. The slides were stained by following the directions in the DakoCytomation package inserts.

Precision Study #1- Within-run, within-instrument

The 10 slides (5 ER and 5 PR) were representative of the following five levels of positive staining cells (one each): negative, very low positive (0.5% to 5%), low positive (6% to 10%), positive (11% to 20%), and strongly positive (greater than 20%). The 10 slides were rerandomized for the purpose of masking each time before they were run, and were scanned and quantitated with one Ariol system three times in one day. A pathologist also manually estimated the percent of positive staining on each of the 10 slides. This was done only once, as it would not be expected that a manual result would   change over multiple runs performed on the same day.

Precision Study #2- Between-run, within-instrument

The same 10 slides described in Precision Study #1 were used for Precision Study #2. In this study, the same Ariol instrument was used to scan and quantitate the slides over three different days, with at least two days between each run and with re randomization of the slides each time. The same pathologist (as in Precision Study #1) also manually estimated the percent of positive staining on each of the 10 slides on three different days, with slide rerandomization between each day.

Precision Study #3- Between-instrument

The same 10 slides were evaluated for Precision Study #3. In this study, the 10 slides were scanned and quantitated for the percentage of positive staining cells by three different Ariol instruments. The same pathologist (as in Precision Studies 1 and 2) manually estimated the percent of positively staining slides re randomized on each of three different days for comparison with Ariol results performed on three instruments.

PRECISION STUDY RESULTS
The following data demonstrated results that were substantially equivalent to the manual readings. Further, the Ariol exhibited very low standard deviations (SDs) across the various runs, days, and instruments.

TABLE 1
ER PRECISION
Ariol and Manual Method

Precision Study #1: Within Day, Between Run, Within Instrument

Slide #	Slide ID	Pathologist Score* Ψ	Overall Ariol Score #1	Overall Ariol Score #2	Overall Ariol Score #3	Ariol Mean	Path Mean	Ariol SD	Ariol %CV
1	327	0	0	0	0	0.00	0	0.00	n/a
2	323	1	0.96	0.81	1.46	1.08	1	0.34	31.5%
3	407	5	5.08	6.22	5.80	5.70	5	0.58	10.2%
4	415	14	13.68	11.77	13.19	12.88	14	0.99	7.7%
5	337	95	95.77	95.81	95.82	95.80	95	0.03	0.3%

Score = % cells positive Ψ = Pathologist performed manual reading only once in this study. Path = pathologist

Precision Study #2: Between Day, Within Instrument

Slide #	Slide ID	Pathologist Mean	Pathologist Minimum	Pathologist Maximum	Overall Ariol Mean	Overall Ariol Minimum	Overall Ariol Maximum	Ariol SD	Ariol %CV
1	327	0	0	0	0.00	0	0	0.00	n/a
2	323	0.5	0	1	1.31	0.78	1.81	0.52	39.7%
3	407	5.67	5	7	5.70	5.15	6.13	0.50	8.8%
4	415	12.67	12	14	12.78	11.00	14.82	1.92	15.0%
5	337	95	95	95	94.77	92.07	96.71	2.41	2.5%

Precision Study #3: Between Instrument

Slide #	Slide ID	Pathologist Mean	Pathologist Minimum	Pathologist Maximum	Overall Ariol Mean	Overall Ariol Minimum	Overall Ariol Maximum	Ariol SD	Ariol %CV
1	327	0	0	0	0	0	0	0.00	n/a
2	323	0.67	0	1	1.25	0.96	1.45	0.26	20.8%
3	407	5	5	5	5.18	5.08	5.30	0.11	2.1%
4	415	13.33	12	14	13.43	11.80	14.82	1.53	11.4%
5	337	95	95	95	94.98	92.46	96.71	2.23	2.3%

TABLE 2
PR PRECISION
Ariol and Manual Method

Precision Study #1: Within Day, Between Run, Within Instrument

Slide #	Slide ID	Pathologist Score* Ψ	Overall Ariol Score #1	Overall Ariol Score #2	Overall Ariol Score #3	Ariol Mean	Path Mean	Ariol SD	Ariol %CV
1	328	0	0	0	0	0.00	0	0.00	n/a
2	430	1	1.14	1.42	1.14	1.23	1	0.16	13.0%
3	402	5	5.40	6.33	5.50	5.74	5	0.51	8.9%
4	434	15	11.43	13.72	14.04	13.06	15	1.42	10.9%
5	338	95	94.18	94.81	94.97	94.65	95	0.42	0.4%

Precision Study #2: Between Day, Within Instrument

Slide #	Slide ID	Pathologist Mean	Pathologist Minimum	Pathologist Maximum	Overall Ariol Mean	Overall Ariol Minimum	Overall Ariol Maximum	Ariol SD	Ariol %CV
1	328	0	0	0	0.00	0	0	0.00	n/a
2	430	1.67	1	3	1.36	1.00	1.60	0.32	23.5%
3	402	5	5	5	5.27	5.00	5.43	0.24	4.6%
4	434	15	15	15	14.76	12.97	16.87	1.97	13.3%
5	338	95	95	95	93.37	92.44	95.19	1.58	1.7%

Precision Study #3: Between Instrument

Slide #	Slide ID	Pathologist Mean	Pathologist Minimum	Pathologist Maximum	Overall Ariol Mean	Overall Ariol Minimum	Overall Ariol Maximum	Ariol SD	Ariol %CV
1	328	0.	0	0	0.00	0	0	0.00	n/a
2	430	1.	1	1	1.05	1.00	1.14	0.08	7.6%
3	402	5	5	5	5.73	5.39	6.41	0.59	10.3%
4	434	14	12	15	12.52	11.43	14.44	1.67	13.3%
5	338	95	95	95	95.09	94.18	95.89	0.86	0.9%

   b. *Linearity/assay reportable range:*
      Not applicable.
   c. *Traceability (controls, calibrators, or method):*
      The analytical traceability of the system depends on the ER or PR IHC assay
      used. The pathologist is responsible for running appropriate controls and
      assuring that the Ariol device is within control in its analysis.
   d. *Detection limit (functional sensitivity:*
      Not applicable
   e. *Analytical specificity*
      The specificity of the test result is dependent on the analytical performance
      of the ER or PR IHC assay run. The pathologist is responsible for running
      appropriate controls and assuring that the Ariol device is within control in
      its analysis.
   f. *Assay cut-off:*
      It has been customary for the medical doctor to choose the cutoff to be used
      with the Estrogen or Progesterone Receptor IHC assay.
2. Comparison studies:
   a. *Method comparison with predicate device:*
      The substantial equivalence studies were based on comparison to
      conventional manual microscopy performed using the reagents from and in
      accordance with the instructions given with the FDA-cleared
      DakoCytomation Estrogen Receptor (K993957) and Progesterone Receptor
      (K020023) IHC tests.

      Concordance between Ariol and manual methods was calculated at
      thresholds of 1%, 5%, and 10% positive tumor cells for ER or PR positive
      status. Approximately 75 clinical slides of known scoring intensity for ER
      and PR (total 150 slides) were obtained from a commercial vendor. All

slides were randomized, blinded, and read manually by each of three pathologists.

All slides were bar-coded, re-randomized to ensure blinding, and scanned by an Ariol system. The Ariol then presented scan modes and interpretations three times, once each to the same three pathologists who performed the manual readings.

The percent concordance between Ariol and the manual method is presented in the following table.

CONCORDANCE BETWEEN ARIOL AND MANUAL METHOD

% Nuclei stained	Range of concordance
ER staining	
1%	93.2 - 95.9%
5%	95.9 – 97.3%
10%	94.5 – 98.6%
PR staining	
1%	94.8 – 96.1%
5%	84.4 – 88.3%
10%	88.3 – 94.8%

Further, the data were evaluated in such as way that the three pathologists could be compared with one another. The data are presented in the following table.. These indicate that the concordances between Ariol and manual methods using three pathologists, and manual method against itself using three pathologists, were comparable.

CONCORDANCE BETWEEN 3 PATHOLOGISTS MANUAL RESULTS

% Nuclei stained	Range of concordance
ER staining	
1%	94.5 - 95.9%
5%	94.5% - 97.3%
10%	93.2 - 97.3%
PR staining	
1%	95.9% - 98.7%
5%	88.3% - 90.9%
10%	83.1% - 89.6%

b. *Matrix comparison:*
   Not applicable. Only one specimen type used.

3. Clinical studies:
   a. *Clinical sensitivity:*
      The clinical sensitivity of the test system is dependent on the analytical performance of the ER or PR IHC assay run. The pathologist is responsible for performing appropriate controls to assure the performance of the assay and test system.
   b. *Clinical specificity:*
      The clinical specificity of the test system is dependent on the analytical performance of the ER or PR IHC assay run. The pathologist is responsible for performing appropriate controls to assure the performance of the assay and test system.
4. Clinical cut-off:
   It is customary for the medical doctor to choose the clinical cutoff to be used with Estrogen and Progesterone Receptor IHC assays.
5. Expected values/Reference range:
   Not Applicable.

M. Instrument Name:
Applied Imaging Ariol™

N. System Descriptions:
See (H) Device Description.
1. Modes of Operation:
   Semi-automated computer-assisted interpretation.
2. Software:
   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types
3. Sample Identification:
   Bar-coding of the microscope slides is done before the slides are loaded into the instrument.
4. Specimen Sampling and Handling:
   The microscope slides to be examined are loaded into the Ariol™ and are scanned automatically. The Ariol™ constructs video images of the scanned data for the pathologist to examine and interpret.
5. Assay Types:
   Computer-assisted image analysis of formalin-fixed paraffin-embedded breast tissue stained by immunohistochemistry reaction for Estrogen or Progesterone receptor nuclear proteins.
6. Reaction Types:
   Light microscopy
7. Calibration:
   The Ariol™ instrument employs laboratory-stained positive and negative training slides for every different staining run to calibrate the computer-assisted detection system.

8. Quality Control:
   The accuracy of the system depends on the laboratory following the quality control instructions recommended in the labeling of the accessory immunohistochemistry (immunocytochemistry) kit used with the Ariol™.

O. Other Supportive Instrument Performance Characteristics Data Not Covered In The "L. Performance Characteristics" Section Of The SE Determination Decision Summary.

P. Conclusion:
   Based on information in the submission, the Ariol image analyzer with ER/PR Application can be recommended as substantially equivalent to the predicate device regulated under 21 CFR §864.1860 Immunohistochemistry reagents and kits (class II; product code – NQN; product name - microscope, automated, image analysis, immunohistochemistry, operator intervention, nuclear intensity and percent positivity).