

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

A. 510(k) Number:

k031363

B. Analyte:

Estrogen Receptor protein on formalin-fixed paraffin-embedded breast cancer specimens

C. Type of Test:

Computer-assisted image analyzer for immunohistochemistry (immunocytochemistry)

D. Applicant:

Cell Analysis, Inc.

E. Proprietary and Established Names:

QCA (Version 3.1) a video microscopy software system for quantitative estrogen receptor immunohistochemistry

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:

The QCA device is intended to detect and classify cells of clinical interest based on recognition of cellular areas of particular color and chromatic intensity. In this software application, the QCA device is intended to measure and quantitate the percentage and intensity of positively stained nuclei in formalin-fixed, paraffin-embedded tissue specimens immunohistochemically stained for estrogen receptors.

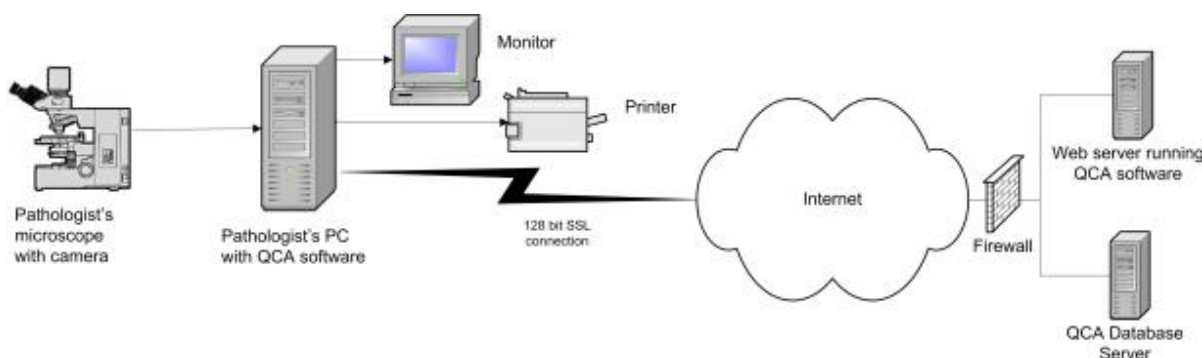
1. Indication(s) for use:
It is indicated for use as an aid in the management, prognosis and prediction of therapy outcomes of breast cancer when used with reagents validated for those indications.
2. Special condition for use statement(s):
The QCA system is an adjunctive computer-assisted methodology to assist the reproducibility of a qualified pathologist in the acquisition and measurement of images from microscopic slides of breast cancer specimens stained for the presence of estrogen (ER) nuclear receptor protein. The accuracy of the test result depends upon the quality of immunohistochemical staining. It is the

responsibility of a qualified pathologist to employ appropriate morphological studies and controls to assure the validity of the QCA ER scores.

3. Special instrument Requirements:
QCA (Quantitative Cellular Assessment)

H. Device Description:

QCA is a standalone, automated intelligent cell assessment software device that analyzes digital images of cells of interest by pixel color attributes and pixel area detection algorithms. The software system utilizes a pathologist's own personal computer, light microscope, digital camera, printer, and Internet connection.



It is specifically designed to help pathologists make objective measurements of the estrogen receptor nuclear antigens visualized by immunohistochemistry (IHC). The system is essentially software that analyzes images captured by a pathologist through a video camera using the pathologist's own microscope and desktop computer. The system requires competent human intervention at all steps in the analysis process. After the pathologist chooses appropriate fields for analysis, enters necessary settings, and masks areas of non-tumor if desired, the system will automatically derive an overall score of the field of interest. Should the pathologist disagree with the score, s/he can adjust QCA settings so that the system derives a score that matches their professional assessment. In this software application, the QCA device is intended to measure and quantitate the percentage and intensity of positively stained nuclei in formalin-fixed, paraffin-embedded tissue specimens immunohistochemically stained for estrogen receptors.

The QCA system is an adjunctive computer-assisted methodology to assist the reproducibility of a qualified pathologist in the acquisition and measurement of images from microscopic slides of breast cancer specimens stained for the presence of estrogen (ER) nuclear receptor protein. The accuracy of the test result depends upon the quality of immunohistochemical staining. It is the responsibility of a qualified pathologist to employ appropriate morphological studies and controls to assure the validity of the QCA ER scores.

I. Substantial Equivalence Information

1. Predicate device name(s)
ChromaVision Medical Systems, Inc. ACIS (Automated Cellular imaging System) ER software application
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 for estrogen receptor nuclear protein. | Examines formalin-fixed paraffin-embedded breast cancer specimens stained for estrogen receptor nuclear protein. |
| The method of assessment/analysis by the software: colorimetric pattern recognition by microscopic examination of digital images by hue and intensity. | The method of assessment/analysis by the software: colorimetric pattern recognition by microscopic examination of digital images by hue and intensity. |
| B. Differences | |
| User-purchased instrumentation and QCA software obtained via the internet | ACIS instrument hardware and software |

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 QCA ER application was evaluated for precision in simulated clinical settings. Precision was assessed via two precision studies, each study with an increasing level of variation in study design.

Precision Study #1- Within-Image reproducibility Study

To document the within-image reproducibility of the QCA system, ten different breast cancer cases/slides that had been subjected to the same ER antibody staining protocol (as mentioned below in the Appendix) were chosen to perform the following reproducibility study.

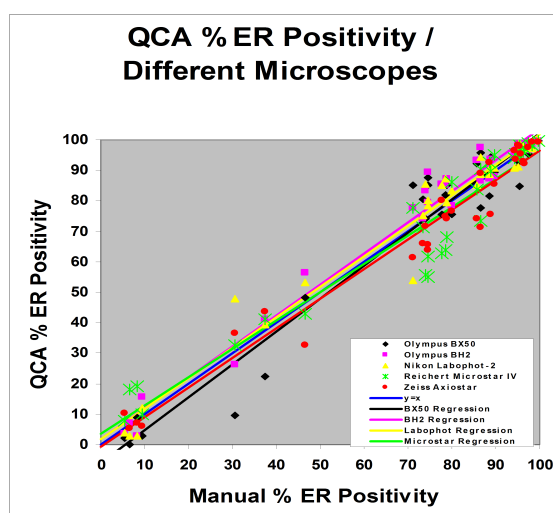
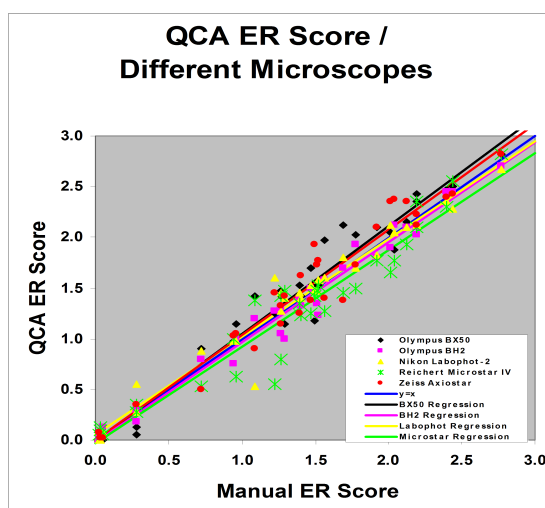
An individual microscopic field from each of ten different slides was repeatedly captured 10 times using the QCA system. Every result

within each set of 10 images was absolutely identical with respect to QCA ER score and percentage positivity (data not shown). This experimental design tested the reproducibility of the microscope/camera systems as well as that of the software itself.

Precision Study #2- Between Hardware Reproducibility Study

Inter-microscope Variability Study

To study the inter-microscope variability, one pathologist conducted the following experiment. First, the QCA system was installed in 5 different pathologists' offices, one with an Olympus BX50 microscope, another with an Olympus BH-2 microscope, another with a Nikon Labophot-2 microscope, another with a Reichert Micro Star IV microscope, and the last with a Zeiss Axiostar Plus microscope. Having previously shown that four images per case yield results within one standard deviation of the true mean for QCA score and percent positivity, four areas on each of the 32 breast cancer cases/slides were first chosen and marked by the pathologist. Then the same pathologist manually assessed the 4 areas to derive the manual percent positivity and score (using the formula described on page 4, Manual evaluation). This pathologist then captured and analyzed the same 4 images of each of the 32 cases/slides on each of the five systems. For each case, appropriate negative and positive controls were also captured. As described on page 4 (QCA evaluation), the final QCA score and percent positivity for each case/slide was the cumulative assessment of 4 images taken for each case. The next two figures show the regression results of QCA ER score and QCA percent positivity against those of the manual results.



Inter-microscope Variability Study Data

| | BX50 | | BH2 | | Labophot | | Microstar | | Axiostar | |
|---------------|---------|--------|--------|--------|----------|--------|-----------|--------|----------|--------|
| | %Pos | Score | %Pos | Score | %Pos | Score | %Pos | Score | %Pos | Score |
| n | 32 | 32 | 32 | 32 | 32 | 32 | 32 | 32 | 32 | 32 |
| Corr Coef | 0.976 | 0.980 | 0.988 | 0.989 | 0.982 | 0.980 | 0.964 | 0.962 | 0.980 | 0.977 |
| Slope (m) | 1.081 | 1.065 | 1.018 | 0.984 | 0.999 | 0.963 | 0.930 | 0.952 | 0.972 | 1.037 |
| m low 95% | 0.991 | 0.983 | 0.959 | 0.929 | 0.927 | 0.891 | 0.834 | 0.852 | 0.899 | 0.953 |
| m high 95% | 1.171 | 1.146 | 1.077 | 1.038 | 1.071 | 1.035 | 1.026 | 1.053 | 1.046 | 1.121 |
| Intercept (b) | -6.125 | -0.015 | 1.634 | -0.016 | 1.831 | 0.058 | 3.481 | -0.027 | -0.822 | 0.001 |
| b low 95% | -13.134 | -0.142 | -2.969 | -0.101 | -3.830 | -0.054 | -4.042 | -0.183 | -6.553 | -0.129 |
| b high 95% | 0.884 | 0.111 | 6.237 | 0.068 | 7.492 | 0.170 | 11.005 | 0.128 | 4.910 | 0.131 |
| SE | 7.331 | 0.169 | 4.814 | 0.113 | 5.920 | 0.150 | 7.868 | 0.209 | 5.994 | 0.174 |

b. *Linearity/assay reportable range:*

Not applicable.

c. *Traceability (controls, calibrators, or method):*

The analytical traceability of the system depends on the ER IHC assay used. The pathologist is responsible for running appropriate controls and assuring that the QCA 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 IHC ER assay run. The pathologist is responsible for running appropriate controls and assuring that the QCA 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 receptor IHC assay.

2. Comparison studies:

a. *Method comparison with predicate device:*

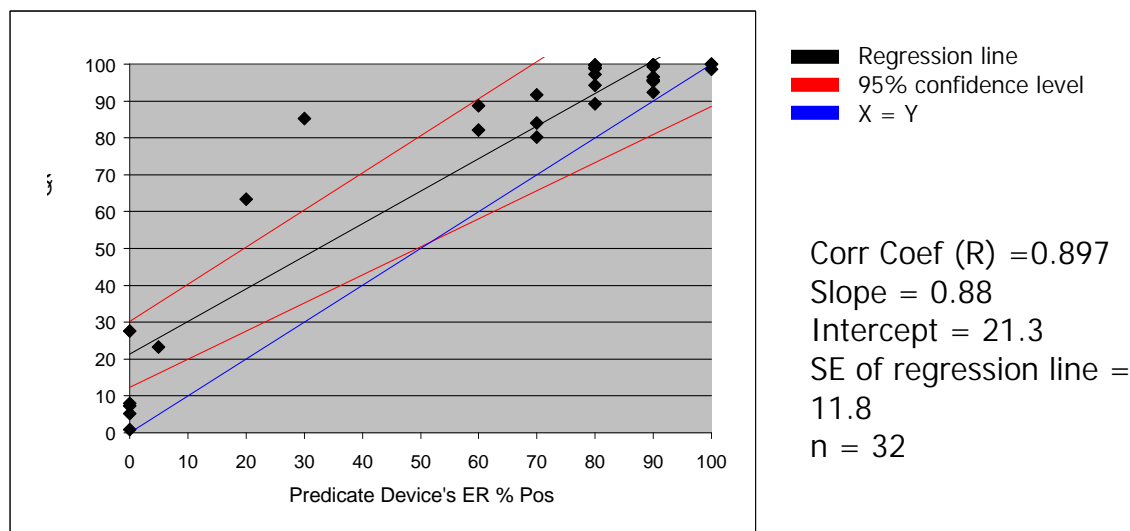
The substantial equivalence studies were based on comparison to conventional manual microscopy for the two test results: percent positivity and QCA score for intensity of nuclear staining. For all comparison studies the primary estrogen receptor antibody used was the DakoCytomation 1D5 clone (FDA 510(K) cleared). The detection system was the labeled Streptavidin-Biotin peroxidase system (LSAB2), also purchased from DakoCytomation Corporation.

The immunohistochemistry staining procedure used in all studies for this submission.

1. Formalin-fixed, paraffin-embedded breast tumor tissue blocks were sectioned at 5 microns in thickness.

2. These tissue sections were affixed onto glass slides by baking in a dry oven at 60°C for 30 minutes.
 3. The slides were de-paraffinized through xylene and hydrated through 100%, 95% and 70% ethyl alcohol then finally in distilled water.
 4. Antigen retrieval was achieved by immersing slides in a jar with 1mM EDTA pH7.5 solution. This jar was placed in a steamer and steamed for 30 minutes. It was then allowed to cool for 20 minutes.
 5. Slides were immersed in 3% hydrogen peroxide and protein blocking solution (DakoCytomation) for 10 minutes each to block the non-specific antigen binding sites and to neutralize the endogenous peroxidase activity.
 6. The slides were incubated with DakoCytomation 1D5 ER monoclonal antibody (1/25 dilution) at room temperature for 30 minutes.
 7. These slides were then incubated with biotinylated secondary antibody for 30 minutes
 8. The slides were then incubated with streptavidin-horseradish peroxidase conjugate for 30 minutes
 9. 3.3' diaminobenzidine (DAB) Chromogen was added and allowed to develop color for 5 minutes.
 10. The slides were counter-stained with Gill 3 hematoxylin for 5 minutes.
 11. Slides were dehydrated and cover slipped.
- QCA ER Percent Positivity vs. Predicate Device ER Percent Positivity Evaluation

We performed regression analysis comparing the predicate device results to those of QCA for percent positivity. The predicate device's percent positivity values on 32 cases were provided by a CLIA-approved laboratory on a case-by-case (slide-by-slide) basis only. The values were provided in 10% increments on 31 cases. One case was reported at 5%. The QCA percent positivity was the cumulative assessment by QCA of all 6 images taken of each of the same 32 cases. The following figure shows the regression results of the predicate device's ER percent positivity against QCA's ER percent positivity. The regression statistics are shown in the legend.



Comparison to manual method.

- QCA ER Percent Positivity vs. Manual Percent Positivity Evaluation

Manual evaluation: As manual inspection of IHC slides remains the most widely utilized method by a wide margin*, therefore this must be considered the standard of current pathology practice. However, it is also recognized that manual inspection suffers from considerable inter-observer variability.

QCA chose tissue specimens from 32 consecutive invasive breast cancers received in a pathology practice over a three and a half month period. Based on professional judgment, three representative images of each ER slide were digitally captured by each of two pathologists. These 192 different images (3 images x 2 pathologists x 32 cases = 192 images) were then first randomly mixed and then screened by each of three different pathologists to ensure blinding of results. The three then manually assessed the percentage of tumor cell nuclei with weak positive ER IHC staining (% weak positivity), the percentage of tumor cell nuclei with moderate positive staining (% moderate positivity), and the percentage of tumor cell nuclei with strong staining (% strong positivity) for each slide. From these determinations, a total percentage of tumor cell nuclei with any degree of positivity (% total positivity) was calculated for each slide.

$(\% \text{ total positivity}) = (\% \text{ weak positivity}) + (\% \text{ moderate positivity}) + (\% \text{ strong positivity})$

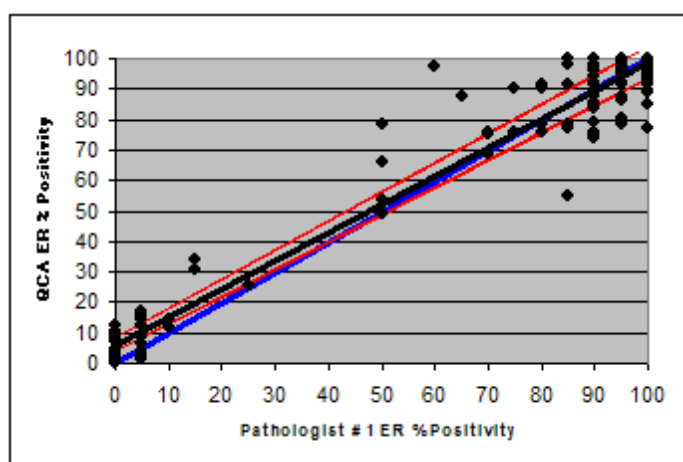
A completely ER-negative tumor was scored as 0%, and a tumor showing any degree of positive ER staining of all tumor cells (regardless whether the staining is weak, moderate, or strong) was scored as 100%.

QCA evaluation: The same 192 images were then assessed with the QCA software by one pathologist without any manual adjustments of the nuclear thresholds. The pathologist did mask nine of the images to exclude areas of non-tumorous cells.

Different from the manual study, instead of counting the percent of positively stained nuclei, QCA software evaluates individual “nuclear pixels” and automatically assigns a staining intensity score 0, 1, 2, or 3 to each pixel. Each pixel’s individual score is automatically determined based on the negative control and positive control provided by the pathologist at the beginning of the testing. Any degree of staining above the negative control will be assigned by QCA as a “positive” pixel. QCA will calculate the “% weak positivity” (as the number of weakly stained pixels against the total number of nuclear pixels), % moderate positivity, and % strong positivity. Using the same formula, the total percent of positively stained pixels (% total positivity) can be calculated as follows: (% total positivity) = (% weak positivity) + (% moderate positivity) + (% strong positivity).

Regression analysis was performed by using individual pathologist’s (manually assessed positively stained nuclei) manual ER percent positivity against QCA’s (nuclear pixel) ER percent positivity. The results of three pathologists are shown on the next page.

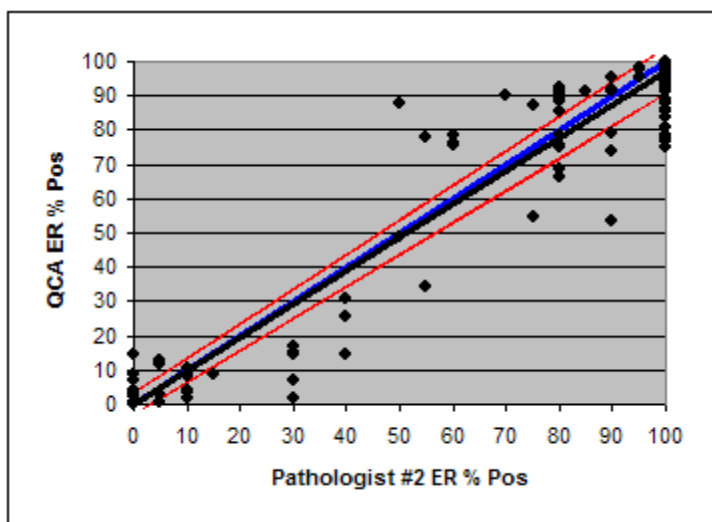
* Layfield LJ, Gupta D, Mooney EE. _ Assessment of Tissue Estrogen and Progesterone Receptor Levels: A Survey of Current Practice, Techniques, and Quantitation Methods. *Breast J.* 2000; 6:189-196.



■ Regression line
 ■ 95% confidence level
 ■ X = Y

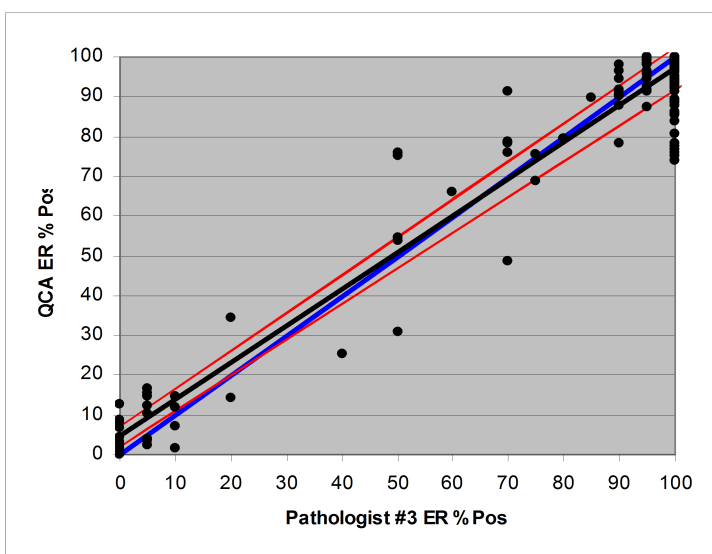
Pathologist #1

Corr Coef (R) = 0.957
 Slope = 0.92
 Intercept = 6.18
 SE of regression line = 7.09
 N=192



Pathologist #2

Corr Coef (R) = 0.934
 Slope = 0.97
 Intercept = 0.15
 SE of regression line = 8.81
 N = 192



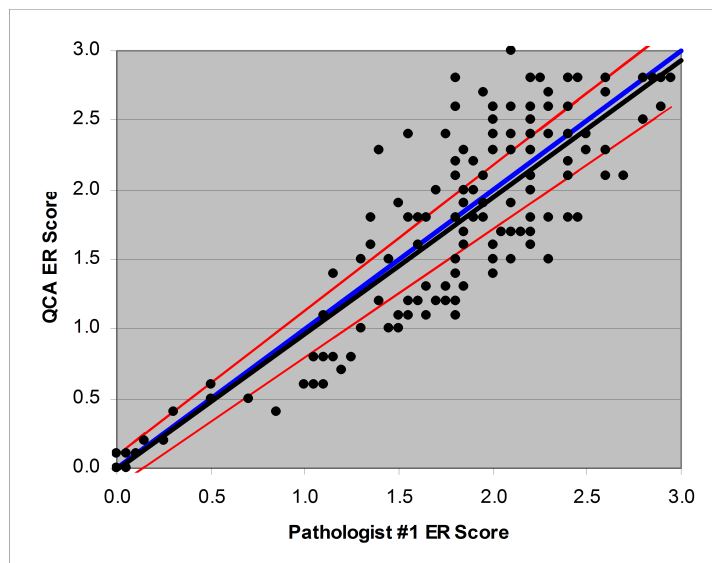
Pathologist #3

Corr Coef (R) = 0.925
 Slope = 0.92
 Intercept = 4.62
 SE of regression line = 7.36
 N = 107

- QCA ER Score vs. Manual Score Evaluation

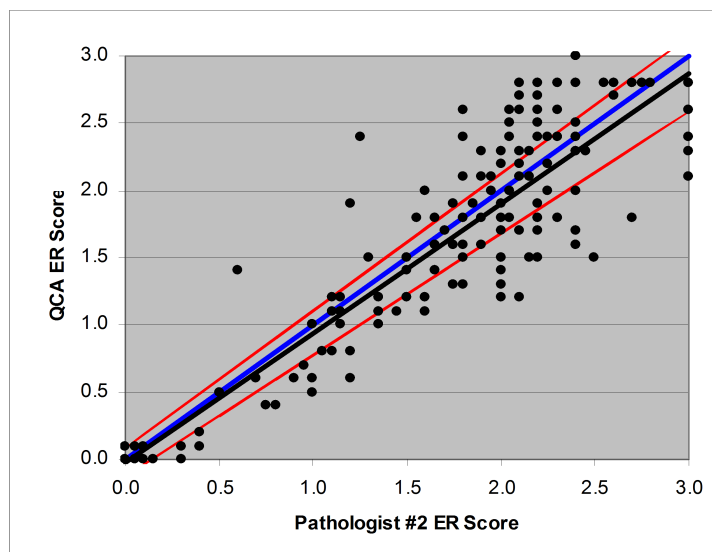
Using the same 32 consecutive invasive breast cancer slides mentioned in the outline, a different scoring system that additionally incorporates the intensity of the ER staining, the intensity score, was also generated by the same manual inspection method (Manual ER Score) and by QCA software (QCA ER Score). Both manual and QCA scores were calculated using the same formula: Intensity Score = {(% weak positivity x 1) + (% moderate positivity x 2) + (% strong positivity x 3)} / 100%. As mentioned above, the manual score is nuclei based and QCA is pixel based. This Intensity Score is adopted and modified from the concept of HSCORE*, which is currently used in many pathology laboratories for ER scoring.

* HSCORE = $\sum (I + 1) \times PC$, where I and PC represent the intensity and the percentage of cells that stained at each positive intensity category, respectively. (McCarty KS Jr., et al. Cancer Res. 1986;46(8 Suppl):4244s-4248s.)



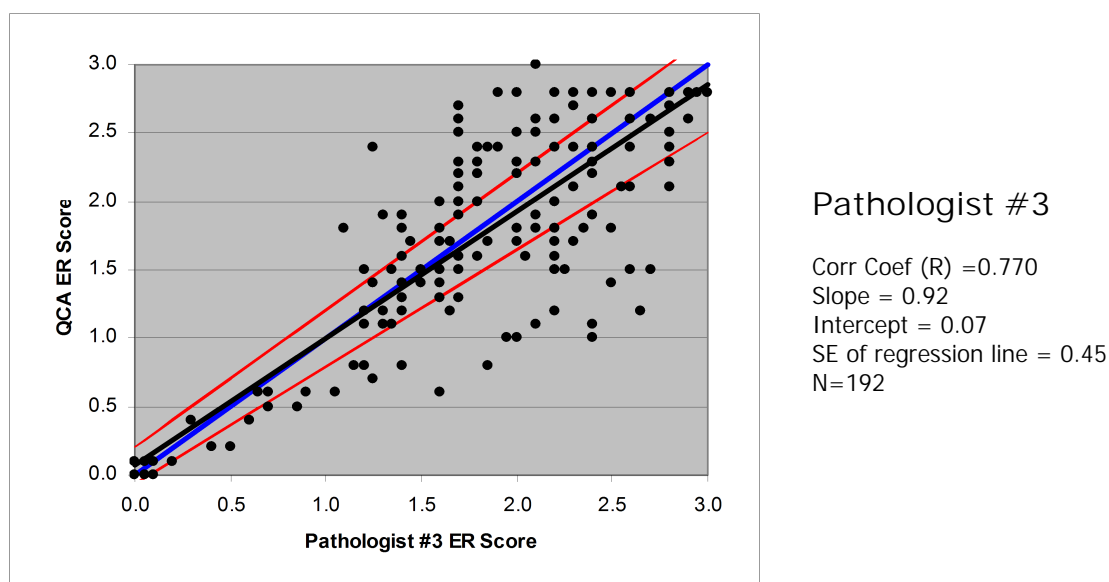
Pathologist #1

Corr Coef (R) = 0.849
 Slope = 0.98
 Intercept = -0.01
 SE of regression line = 0.37
 N=192



Pathologist #2

Corr Coef (R) = 0.854
 Slope = 0.92
 Intercept = -0.02
 SE of regression line = 0.36
 N=192



- Qualitative Percent Positivity Comparison Study

The following agreement tables include the data from the original 32 invasive breast carcinomas and an additional 120 cases (4 images per case/slide according to our determination of the optimum number of images to consider per case study). These cases include both invasive and in-situ breast cancers that were processed and stained according to the protocol listed in the Appendix. The QCA final percent positivity for each of the 152 (32 + 120) cases/slides was calculated based on the cumulative assessment of all tumor cell nuclear pixels taken from each case (see QCA Evaluation, page 4 above). The manual percent positivity for each of the 152 total cases was calculated based on the same formula shown on page 4 (manual evaluation). Interpretations of **positive** breast tumor **ER status** vary from 1% to 10% of percent positivity in different pathology laboratories. We used $\geq 5.0\%$ and $\geq 1.0\%$ positivity as example cut-off values, and derived the following qualitative agreement tables to compare the manual against the QCA methods.

| 1.0% \geq Positive | | Manual | |
|----------------------------------------|----------|---------------|----------|
| | | Positive | Negative |
| QCA | Positive | 149 | 0 |
| | Negative | 0 | 3 |

| 5.0% \geq Positive | | Manual | |
|----------------------------------------|----------|---------------|-----------|
| | | Positive | Negative |
| QCA | Positive | 136 | 2 |
| | Negative | 2 | 12 |

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 IHC test used. 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 IHC test used. 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 the estrogen receptor IHC assay.

5. Expected values/Reference range:

Not Applicable.

M. Instrument Name:

QCA (Quantitative Cellular Assessment)

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: Yes

3. Sample Identification

Not Applicable. It is laboratory-dependent. The user provides their own hardware. The QCA is accessed via the internet.

4. Specimen Sampling and Handling

Not Applicable. It is laboratory-dependent. The user provides their own hardware. The QCA is accessed via the internet.

5. Assay Types

Computer-assisted digital image analysis of formalin-fixed paraffin-embedded breast tissue stained by immunohistochemistry reaction for estrogen receptor nuclear protein.

6. Reaction Types:

Light microscopy

7. Calibration:

The QCA software employs laboratory-stained negative and 3+ ER stained control slides for every different staining run to calibrate the computer-assisted detection system.

8. Quality Control:

The accuracy of the system depends on the pathologist's following usual immunohistochemistry (IHC) staining quality control procedures to assure the validity of the staining.

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 the results of the comparison and reproducibility studies described in this 510(k) submission, it is concluded that the QCA system is as safe and effective (therefore substantially equivalent) as the predicate device as an aid in the assessment of specimens from breast cancer patients stained for the nuclear estrogen receptor protein.