

SUMMARY OF SAFETY AND EFFECTIVENESS DATA

I. GENERAL INFORMATION

Device Generic Name:	Anti-c-KIT Rabbit Monoclonal Antibody for Immunocytochemical Staining
Device Trade Name:	Ventana® Medical Systems' PATHWAY Anti-c-KIT (9.7) Primary Antibody
Applicant Name and Address:	Ventana Medical Systems, Inc. 1910 Innovation Park Drive Tucson, AZ 85705
Premarket Approval Application Number:	P020055
Date of Panel Recommendation:	None
Date of Notice of Approval To the Applicant:	August 11, 2004

II. INDICATIONS FOR USE

This antibody is intended for in vitro diagnostic (IVD) use. Ventana® Medical Systems' PATHWAY Anti-c-KIT (9.7) Primary Antibody is intended for laboratory use, via light microscopy, for the qualitative detection of KIT protein in formalin-fixed, paraffin-embedded gastrointestinal stromal tumors (GISTs) using either an automated immunohistochemistry staining system or a manual assay. It is indicated as an aid in the diagnosis of GIST within the context of the patient's clinical history, tumor morphology, and other diagnostic tests evaluated by a qualified pathologist. It may be used after the diagnosis of GIST as an aid in the selection of GIST patients who may qualify for imatinib mesylate (Gleevec®/Glivec®) therapy.

III. CONTRAINDICATIONS None

IV. WARNINGS AND PRECAUTIONS

Warnings and Precautions for use of the device are stated in the product labeling.

V. DEVICE DESCRIPTION

Reagent Provided

PATHWAY Anti-c-KIT (9.7) Primary Antibody (clone 9.7) consists of one dispenser of c-KIT primary antibody (rabbit monoclonal) and contains approximately 5 ml (50

test) of prediluted reagent. The dispenser contains approximately 25 µg of antibody in Tris buffer, pH 7.5, with carrier protein, non-ionic detergent, and 0.09% sodium azide as a preservative.

In addition, the following materials and reagents are necessary to perform the assay:

- Negative tissue control slide (normal colon tissue)
- Positive tissue control slide (GIST tissue)
- Microtome
- Microscope slides, silanized or polylysine-coated
- Drying oven capable of maintaining a temperature of $70^{\circ}\text{C} \pm 5^{\circ}\text{C}$.
- Bar code labels for Automated Slide Stainer use (Ventana Medical Systems, Inc. Cat. No. 451-801 for negative control and any one of Cat. No. 451-001 through 451-451 for PATHWAY Anti-c-KIT (9.7) Primary Antibody)
- Xylene (histological grade)
- Ethanol or reagent alcohol (histological grade)
- Deionized/distilled water
- Ventana Automated Slide Stainer:
 - -Ventana NexES® Slide Staining System, or
 - -Ventana BenchMark™ Slide Staining System, or
 - -Ventana BenchMark™ XT Slide Staining System
- Ventana Medical Systems iVIEW DAB Detection Kit
- Ventana APK Wash Solution Concentrate (10X) (NexES IHC automated slide stainers)
- Ventana EZ Prep™ Solution Concentrate (10X)
- Ventana Cell Conditioning 1 (CC1) Solution Pre-dilute (BenchMark and BenchMark XT automated slide stainers)
- Ventana Low Temperature Liquid Coverslip™ Solution Pre-dilute (NexES IHC automated slide stainers, or Ventana High Temperature Liquid Coverslip Solution Pre-dilute (BenchMark and BenchMark XT automated slide stainers)
- CONFIRM™ Negative Control Rabbit Ig
- Mounting Medium: Permanent Mounting Medium for use with DAB
- Cover Glass
- Light microscope (20-80X)
- Staining jars or baths
- Timer (capable of 2-10 minute intervals)
- Wash bottles
- Absorbent wipes
- Ventana Hematoxylin or Nuclear Fast Red Counterstain
- Ventana Bluing Reagent
- Decloaking Chamber, Digital Pressure Cooker (Biocare Medical) (NexES IHC automated slide stainers)
- Tissue Tek™ slide rack (Biocare Medical)

Principle of Device Methodology

PATHWAY Anti-c-KIT (9.7) Primary Antibody is a rabbit monoclonal antibody intended to qualitatively detect the expression of KIT protein in formalin-fixed, paraffin-embedded normal and neoplastic tissue, using either a Ventana automated immunohistochemistry staining system or a manual assay. PATHWAY Anti-c-KIT (9.7) Primary Antibody is a rabbit monoclonal antibody which binds a site on the internal domain of the KIT oncoprotein in paraffin-embedded tissue sections. This reagent is supplied as an IgG antibody from cell culture supernatant diluted in a tris buffer solution to a concentration of approximately 3 µg/mL. Sodium azide is added at 0.09% as a preservative.

The antibody may be used on an automated immunohistochemistry system or manually. The presence of bound antibody is detected by DAB precipitation from the avidin-biotin based *i*View binding reagent (secondary antibody conjugated to biotin) to the monoclonal antibody. The presence of staining with a score of 1+ or greater is considered to be positive, while no or faint staining below 1+ is considered negative. A positive score is used in conjunction with other clinical and histopathological findings to make a diagnosis of GIST.

The remainder of the reagents necessary to obtain results is recommended in the “Materials and Reagents Needed But Not Provided” section of the product labeling. The system is used with antigen enhancement reagent, endogenous peroxidase blocker, biotinylated goat anti-rabbit IgG, streptavidin-horseradish peroxidase conjugate, DAB chromogenic substrate, copper enhancing reagent, and hematoxylin counterstain. The additional reagents are not part of the PATHWAY Anti-c-KIT Primary Antibody device, but are purchased separately.

The specific antibody is localized by a biotin-conjugated secondary antibody formulation that recognizes rabbit and mouse immunoglobulins. This step is followed by the addition of a streptavidin-enzyme conjugate that binds to the biotin present on the secondary antibody. The specific antibody-secondary antibody-streptavidin-enzyme complex is then visualized with a precipitating enzyme reaction product. The visualization of the bound PATHWAY Anti-c-KIT (9.7) Primary Antibody in tissue specimens is facilitated using Ventana’s *i*VIEW DAB Detection, an indirect avidin-biotin system coupled to an enzyme. Visualization occurs through the localization of a DAB precipitate. Each step is incubated for a precise time and temperature. At the end of each incubation step, sections are washed to stop the reaction and remove unbound material that would hinder the desired reaction in subsequent steps. Results are interpreted using a light microscope and aid in the differential diagnosis of pathophysiological processes, which may or may not be associated with a particular antigen. Detailed instructions in the package insert for use of control materials, reagents and how to interpret their results are provided to insure adequate assay control.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are no FDA approved or cleared in vitro diagnostic immunohistochemical assays for detection of KIT protein in normal and neoplastic tissue.

Analysis of c-KIT status could be performed via KIT gene mutation analysis of genomic tumor DNA, as was done for 121 subjects enrolled in the CSTIB2222 clinical trial.¹⁰ KIT gene mutational analysis is most commonly performed using polymerase chain reaction (PCR), for which there are no commercially available assays.

Analyte Specific Reagents for c-KIT immunohistochemistry and flow cytometry and c-KIT assays for research are currently marketed by several companies, some of which are listed in Table 1.

Table 1. Commercially Available Assays for c-KIT

Company	Application	Intended Use
BioGenex	IHC	Analyte Specific Reagent
Cell Marque	IHC	Analyte Specific Reagent
DakoCytomation	IHC	Analyte Specific Reagent
Biocare Medical	IHC	Research Use Only
BioGenex	IHC	Research Use Only
Chemicon	IHC	Research Use Only
Lab Vision/NeoMarkers	IHC	Research Use Only
Zymed Laboratories	IHC	Research Use Only
Beckman Coulter	Flow Cytometry	Analyte Specific Reagent
Becton, Dickinson and Company	Flow Cytometry	Analyte Specific Reagent
Caltag Laboratories	Flow Cytometry	Analyte Specific Reagent

VII. MARKETING HISTORY

PATHWAY[®] Anti-c-KIT (9.7) Primary Antibody has not been marketed. Marketing of a Class I Exempt version of the device under the CONFIRM[™] brand name began in June 2004 in the United States, Europe, and Japan. The device has not been withdrawn from any market for any reason of safety and effectiveness.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Ventana[®] Medical Systems' PATHWAY Anti-c-KIT (9.7) Primary Antibody is indicated as an aid in the selection of GIST patients who may qualify for imatinib mesylate (Gleevec[®]) therapy. Patients falsely assigned as positive following assessment would be considered eligible for treatment. Because the design of the clinical studies did not include treatment of patients with negative assay results, the risks or benefits of treatment in this patient population are unknown. The risks of Gleevec treatment to c-KIT-positive patients included dermatologic reactions, fluid retention and edema, gastrointestinal irritation and bleeding, anemia, neutropenia, and

thrombocytopenia. Liver and kidney toxicity and immunosuppression may result from long-term use. Women of childbearing age should be advised to avoid becoming pregnant because of the potential for teratogenic effects.

Patients falsely assigned as negative might be less likely to receive the benefits of therapy with Gleevec.

The labeling recommends that the testing laboratory employ positive and negative tissue controls to reduce the potential for an erroneous test result. The strong and weakly positive internal controls of mast cells and interstitial cells of Cajal (ICC), present in the patient sample also aid in the control of the immunohistochemistry assay and reduce the potential for erroneous results.

IX. SUMMARY OF PRECLINICAL STUDIES

Preclinical testing of the Ventana® PATHWAY Anti-c-KIT (9.7) Primary Antibody included clinical and analytical specificity, purity of reactivity, reproducibility, and stability/stress studies.

1) Antibody Specificity

a) Clinical Specificity/Tour of Normal Tissues Throughout the Body

To describe the specificity of positive staining in normal tissues, twenty-eight (28) normal tissue types (of 30 recommended in FDA's special control for immunocytochemistry devices' Guidance for Submission of Immunohistochemistry Applications to the FDA) in tissue arrays containing 81 specimens were stained using the Ventana BenchMark automated staining instrument using Ventana PATHWAY (9.7) Primary Antibody and the polyclonal c-KIT antibody submitted in the original submission to examine c-KIT expression in these tissues. Parathyroid, pituitary, and mesothelial tissues were not included since there is no published evidence that c-KIT would be detected there. Bone marrow was also not included for unspecified reasons. Slides were blinded to observers and read by three independent qualified readers.

Acceptance criteria were that specimens stained with PATHWAY 9.7 monoclonal antibody must demonstrate $\geq 90\%$ agreement with those stained by the original polyclonal antibody.

Results: Mast cell staining was identified in colon, esophagus, kidney, lung, prostate, skin, small intestine, stomach, tonsil and uterus. Membrane staining of ductal cells was observed (unexpectedly) in breast.

Agreement (specimens positive by both antibodies plus specimens negative by both antibodies divided by total specimens) was 100%, 95% CI = 96.5-100%. Seventy-eight specimens were not stained by either antibody, and three specimens (breast) were stained by both antibodies.

Conclusions: Ventana PATHWAY (9.7) Primary Antibody performed acceptably on normal tissue samples and the acceptance criteria were met. The results for the Ventana PATHWAY (9.7) Primary Antibody are published in its package insert so that users will know what to expect with regard to c-KIT staining in normal tissues in the background of tumor specimens.

b) Clinical Specificity/Tour of Tumors

To describe the specificity of positive staining in cancer tissues, a tissue array containing 49 tumor specimens including 18 different neoplastic tumor types (at least two specimens of each tumor type and including tumors relevant to the differential diagnosis of GIST, leiomyoma and sarcoma) were stained with PATHWAY 9.7 monoclonal and the original polyclonal antibodies using the Ventana BenchMark automated staining instrument. Slides were blind-labeled and read by three independent qualified readers.

Acceptance criteria were that PATHWAY 9.7 monoclonal must show $\geq 90\%$ agreement with the original polyclonal antibody.

Results: Agreement (specimens positive by both antibodies plus specimens negative by both antibodies divided by total specimens) was 97.9%. The single discrepancy was a breast cancer with weak membrane staining with the original polyclonal.

FDA originally requested 60 different tumor tissues representing 20 tumor types to be tested. For the original submission, 50 tissues were tested. Ventana believes that the use of 49 specimens representing 18 tumor types is adequate and not significantly different from what was previously tested.

Conclusions: Acceptance criteria were met, and the Ventana PATHWAY (9.7) Primary Antibody performed equivalently to the original polyclonal antibody. Scientific Reviewer, Dr. Elizabeth Mansfield concurred that the smaller number of tissues tested was acceptable because this was a comparison study to demonstrate equivalence of antibodies raised against the same antigen. The staining of these neoplasms was stated in the Specificity section of the Ventana PATHWAY Anti-c-KIT (9.7) Primary Antibody package insert, so that users will know what staining to expect from this product.

c) Purity of Staining of the Ventana PATHWAY (9.7) Primary Antibody

To examine the purity of staining of the Ventana PATHWAY (9.7) Primary Antibody Sodium Dodecyl Sulfate (SDS)-Polyacrylamide Gel Electrophoresis (PAGE) and Western Blotting were employed. GIST 822 cell line lysate was blotted and probed with the Ventana PATHWAY (9.7) Primary Antibody, and with antibody plus immunogenic peptide, and examined with chemiluminescent detection. The antibody was expected to detect a doublet at 140-145 kDa, and the antibody plus peptide was expected to show no or faint

bands due to competition for binding by the peptide.

Two bands (doublet) at 140-145 kDa were observed and no other bands were detected when the monoclonal antibody was used. When the monoclonal plus peptide were used no staining was detected, indicating complete competition for binding.

Conclusion: Dr. Mansfield concluded this was not a strict measure of purity but of specificity (purity of staining). It was her judgement that the antibody is sufficiently pure, given that it is a monoclonal antibody, that no other demonstration of purity should be requested.

d) Proof of c-KIT specificity of PATHWAY 9.7 Monoclonal Antibody – Lack of Cross-Reactivity with Closely Related Types of Tyrosine Kinase Receptors

To demonstrate antibody specificity and lack of cross-reactivity with related tyrosine kinase receptors, cell line lysates containing Platelet Derived Growth Factor-Alpha (PDGFRa), Macrophage Colony Stimulating Factor Receptor (c-FMS), and FMS-like Tyrosine Kinase (Flt-3) in addition to a lysate containing c-KIT were run on SDS-PAGE and Western Blotted electrophoretically. One of the blots was probed with Ventana PATHWAY (9.7) Primary Antibody, and demonstrated the expected doublet at 140-145 kilo Daltons in c-Kit-expressing cell line lysate, and no bands in lysates with other related receptors. Other blots were probed with antibodies specific for PDGFRa, Flt-3, and c-FMS. For each related receptor type, the staining demonstrated the presence of the related receptor type in the appropriate lysate, and the absence of staining with Ventana PATHWAY (9.7) Primary Antibody in the same lysates. The same test was also performed in the presence of c-KIT immunizing peptide.

Results: No reaction of the Ventana PATHWAY (9.7) Primary Antibody with any of the related tyrosine kinase receptors was noted, while the reactivity of the Ventana c-KIT (9.7) Monoclonal Antibody was completely blocked by c-KIT immunizing peptide.

Conclusion: This is a convincing demonstration of the specificity and lack of cross-reactivity of the Ventana PATHWAY (9.7) Primary Antibody to other related receptor types. The blocking of the reactivity by the immunizing peptide demonstrated that the antibody is specific for its immunogen, and thus detects c-KIT. No additional data were recommended to be requested by Dr. Mansfield.

e) Specificity of PATHWAY (9.7) Monoclonal Antibody on cells

Three lots of Ventana PATHWAY (9.7) Primary Antibody were tested for specific staining on neoplastic (GIST) and normal (colon) tissue sections. Ability of the immunizing peptide to block antibody binding on tissue sections was also examined. Slides were stained on the Ventana BenchMark

automated staining instrument, blind-labeled and read by three independent qualified readers.

Acceptance criteria were that staining must be $\leq 1+$ in the presence of blocking peptide, and $\geq 3+$ in the absence of blocking peptide.

The antibody lots performed identically. Staining of GIST and normal colon sections was 4+ intensity. Mast cells and Interstitial Cells of Cajal (ICC) were detected in normal colon. Staining of GIST and colon sections was intensity = 0 with the addition of the immunizing peptide.

Results: Acceptance criteria were met, and the Ventana PATHWAY (9.7) Primary Antibody stained GIST and colon cells as expected.

Conclusion: The Ventana PATHWAY (9.7) Primary Antibody reactivity is completely blocked by the peptide used to immunize the rabbit to create it. It, therefore, gives expected reactivity and specificity.

2) Reproducibility

To determine the performance characteristic of reproducibility for declaration in the product labeling, FDA requested that the following reproducibility studies be repeated with the new PATHWAY (9.7) Monoclonal Antibody on the Ventana BenchMark automated staining instrument, and by manual staining.

a) Inter- and intra-run reproducibility on automated platform

For the determination of inter-run reproducibility on the automated platforms, 5 GIST specimens and one leiomyosarcoma specimen were assessed on Ventana automated staining instruments on 3 days, using 3 different instruments (NexES, BenchMark, and BenchMark XT), and the manual version of the assay. The same specimens were tested in nine replicates using the same three different instruments to determine intra-run reproducibility. All instruments used the same protocol (NexES 9.1 paraffin recipe *iView* DAB detection protocol). Deparaffinization and antigen enhancement were performed manually for the NexES instruments, and the manual protocol was performed according to the Ventana PATHWAY (9.7) Monoclonal Antibody package insert. Slides were blind-labeled and scored by three independent qualified readers.

Acceptance criteria were that 90% of samples demonstrate specific staining intensity scores within 0.5 scoring grade across automated and manual staining platforms. Non-specific staining must be ≤ 0.5 scoring grade or \leq staining intensity of negative control for all samples.

Results: Acceptance criteria were met for all platforms for inter-run and intra-run reproducibility.

Conclusion: This was not an extensive reproducibility study. However, because no samples deviated from expectations except for one falsely positive

leiomyosarcoma sample that was found to be sectioned too thick (had high non-specific staining), it is reasonable to expect that the assay is reproducible. No further studies were requested. The results were summarized under the Performance characteristics section of the package insert, so that users will know what to expect with regard to reproducibility performance.

b. Lot-to-lot reproducibility

To determine lot-to-lot reproducibility, 4 lots of Ventana PATHWAY (9.7) Primary Antibody were used to stain a tissue array containing 5 GIST specimens and an array containing 60 normal and neoplastic tissues. The study director confirmed the diagnoses and expected staining of the 60 tissues. The GIST specimens were stained in duplicate with the four lots of Ventana PATHWAY (9.7) Monoclonal Antibody, with the original polyclonal antibody, and in singlicate with negative control antibody. The other tissue specimens were stained in singlicate with Ventana PATHWAY (9.7) Primary Antibody, original polyclonal antibody, and negative control antibody. The staining took place on the Ventana BenchMark automated staining instrument using the PATHWAY protocol. The method used for the negative control was not specified. Slides were stained and blind labeled, then scored by three independent qualified readers.

Acceptance criteria were that $\geq 90\%$ of specimens demonstrate specific staining intensity scores within 0.5 stain intensity across all lots of Ventana PATHWAY (9.7) Primary Antibody, and within 1.0 stain intensity of the original polyclonal antibody. Non-specific staining must be ≤ 0.5 scoring grade or \leq intensity of the negative control.

Results: A single reader on a single GIST specimen did not meet acceptance criteria, with a variation between lots of 1.0. Thus ~58/60 (96.7%) of cases conformed to acceptance criteria for GIST. For other normal and neoplastic tissues, all tissues were negative except normal breast (stained 1.75-2.0 with all lots), GIST, and seminoma, which are known to express c-KIT. Overall 98% of cases met acceptance criteria.

Conclusion: This study demonstrates adequate lot-to-lot reproducibility for the Ventana PATHWAY (9.7) Primary Antibody.

c. Manual vs. automated reproducibility

To determine manual vs. automated reproducibility, a multi tissue block (MTB) containing 5 c-KIT positive GIST specimens, and a separate leiomyosarcoma specimen were used to compare staining reproducibility of three automated platforms (NexES, BenchMark, BenchMark XT) against manual staining. Single GIST MTB and leiomyosarcoma slides were stained with negative control reagent. The NexES protocol was used for both the Ventana PATHWAY (9.7) Primary Antibody and the original polyclonal c-KIT antibodies. Deparaffinization and antigen enhancement were performed manually for NexES and manual assays. The manual assay was as described

in the package insert. Slides were blind-labeled and read by 3 independent qualified readers.

Acceptance criteria were that $\geq 90\%$ of specimens have staining scores within 0.5 scoring grade across automated platforms and the manual assay. Non-specific staining must be ≤ 0.5 scoring grade or \leq staining intensity of negative control.

Results: The acceptance criteria were met for all platforms and manual staining with both antibodies.

Conclusion: Platform-to-platform performance reproducibility is equivalent between the 3 different platforms and manual staining with Ventana PATHWAY (9.7) Primary Antibody.

3) Antibody Stability and Stress testing

To determine the stability of the Ventana PATHWAY Anti-c-KIT (9.7) Primary Antibody, 3 lots of product were stored at 2-8° C for 18 months. Testing intervals were day 0, week 1, and months 1, 2, 3, 5 and at appropriate intervals through month 18. In addition, to stress the product stability was analyzed after a mock shipping stress (2 days at 45° C followed by continuous storage at 2-8° C for 18 months) tested at day 3, week 1, and months 1, 2, 3, 6 and at appropriate intervals through month 18. Additional stress testing included tests performed at 37° C and 45° C continuous testing sampled at week 1, and months 1, 2, and 3, or until failure. Two freeze/thaw cycles were also tested from -20° C to room temperature at month 2. On-board open-bottles were tested stored at room temperature for one week after opening. Intended storage is at 2-8° C.

The tissue used for stability studies was paraffin-embedded GIST and normal colon tissue. Normal stomach tissue will be incorporated into the study in the future to increase the probability that the 2+ interstitial cells of Cajal (ICC) will be present. Testing of Ventana® Medical Systems' PATHWAY Anti-c-KIT (9.7) Primary Antibody included antigen enhancement, stained on the Ventana BenchMark® automated staining instrument and was visualized using Ventana's iView DAB detection kit. The slides were stained in triplicate for evaluation. Cells expected to be negative in the GIST and normal tissue slides are used as a negative tissue control to look for unintended cross-reactivity.

The test parameters measured were staining intensity of GIST tumors, mast cells and ICC at the time points indicated. Slides were masked and scored in comparison with rated reference slides of intensity levels of 0, 1, 2, 3, and 4+. Passing criteria were set as maintaining intensity when compared to day 0 slides for each lot. Should the sample time point fail, the assay shall be repeated once. A second failure constitutes a product failure.

Results and Conclusions: Dating for this product has been set at 5 months from manufacture when stored at 2-8° C. Real-time stability and stress testing are on-going.

4) Overall Conclusions for Preclinical Studies: The results of the preclinical testing performed on Ventana PATHWAY Anti-c-KIT (9.7) Primary Antibody demonstrate that this product is reproducible and is specific to c-KIT expression with preclinical performance characteristics appropriate to aid in the assessment of patients considered for Gleevec® (imatinib mesylate) therapy.

X. SUMMARY OF CLINICAL STUDIES

Two studies were conducted with clinical samples to determine the agreement between PATHWAY Anti-c-KIT (9.7) Primary Antibody vs. the investigational immunocytochemical antibody (ICA) used in the Gleevec clinical trial using clinical cases involved in the differential diagnosis of GIST with known diagnoses.

THE OBJECTIVE OF THE CLINICAL STUDIES

The objective of the clinical studies was to demonstrate acceptable agreement between the PATHWAY Anti-c-KIT (9.7) Primary Antibody and the investigational immunocytochemical antibody (ICA) used in the Gleevec clinical trial using clinical cases with known diagnoses that would be appropriate for the differential diagnosis of GIST. The acceptance criterion was 75% overall agreement defined as the total number of specimens that agreed with the ICA results divided by the total number of samples tested.

All clinical testing was done in-house at Ventana.

STUDY POPULATIONS

Study #1. A multi-tissue array of 129 United States GIST cases obtained from Diomedea in Maryland. Thirty-five of the specimens were collected during the Gleevec clinical trial.

Study #2. Two micro arrays comprised of 63 non-GIST sarcoma cases and 13 GIST cases present in duplicate or triplicate obtained from Dr. Ray Tubbs at the Cleveland Clinic Foundation of Cleveland, Ohio.

STUDY SUMMARIES

STUDY #1

A total of 129 GIST cases assembled into a tissue array were obtained from Diomedea (Maryland). Sections of each tissue array were stained with both of the antibodies (PATHWAY 9.7 and ICA Polyclonal), and with a negative control rabbit IgG antibody. The Ventana antibody and the control were used with the BenchMark instrument, and the ICA antibody and its control were run on the DAKO Autostainer. The PATHWAY and the original antibody were run using the same protocol on the

BenchMark instrument. The ICA antibody was run on the DAKO Autostainer with the protocol recommended for the DAKO (ICA) antibody. The slides were blinded and read by three independent qualified readers.

STUDY #2

A cohort of 63 non-GIST sarcoma cases and 13 GIST cases assembled into tissue arrays was obtained from Dr. Ray Tubbs of the Cleveland Clinic. The samples were obtained with IRB approval and the diagnostic information was provided by Dr. Tubbs. The data was reviewed by the study director to confirm the presence of cases representing non-GIST sarcomas and GIST. Sections of each tissue array were stained with both of the antibodies (PATHWAY 9.7 and ICA Polyclonal), and with a negative control rabbit IgG antibody. The Ventana antibody and the control were used with the BenchMark instrument, and the ICA antibody and the control were run on the DAKO Autostainer. The PATHWAY and the original antibody were run using the same protocol on the BenchMark instrument. The ICA antibody was run on the DAKO Autostainer with the protocol recommended for the DAKO (ICA) antibody. The slides were blinded and read by three independent qualified readers.

RESULTS OF THE CLINICAL STUDIES

STUDY #1. PATHWAY Anti-c-KIT (9.7) Primary Antibody vs. ICA Using a GIST Tissue Micro array with cases of known mutational status

A total of 129 cases was a part of the original GIST micro array. However, about one-fourth of the cores were variably lost to each of the three qualified readers. Since one of the reader's results was considerably different than the other two, the results are presented separately by qualified reader.

Qualified Reader #1

		ICA Polyclonal		
		Positive	Negative	Total
PATHWAY Anti-c-KIT (9.7)	Positive	86	0	86
	Negative	1	9	10
	Total	87	9	96

Positive Agreement = 99% (86/87) with 95% Clopper-Pearson Lower Confidence Bound (LCB) = 95%

Negative Agreement = 100% (9/9) with 95% LCB = 72%

Overall Agreement = 99% (95/96)

Qualified Reader #2

		ICA Polyclonal		
		Positive	Negative	Total
PATHWAY	Positive	67	0	67
Anti-c-KIT	Negative	15	9	24
(9.7)	Total	82	9	91

Positive Agreement = 82% (67/82) with 95% LCB = 73%

Negative Agreement = 100% (9/9) with 95% LCB = 72%

Overall Agreement = 83.5% (76/91)

Qualified Reader #3

		ICA Polyclonal		
		Positive	Negative	Total
PATHWAY	Positive	85	0	85
Anti-c-KIT	Negative	1	9	10
(9.7)	Total	86	9	95

Positive Agreement = 99% (85/86) with 95% LCB = 95%

Negative Agreement = 100% (9/9) with 95% LCB = 72%

Overall Agreement = 99% (94/95)

Results and Conclusions:

Ten (10) of the 95 cases with a core evaluated by at least 2 of the qualified readers, did not demonstrate over-expression of c-KIT protein. These results demonstrate that the absence of c-KIT over expression alone cannot be used to eliminate a diagnosis of GIST, and reinforce the importance of considering the patient's complete histopathologic profile and clinical history before making a diagnosis.

All of the qualified readers met study criterion of the $\geq 75\%$ overall agreement and thus demonstrated acceptable agreement with the ICA. This data demonstrates that the PATHWAY Anti-c-KIT (9.7) Primary Antibody may be used to select GIST patients for therapy with the drug, Gleevec.

STUDY 2. PATHWAY Anti-c-KIT (9.7) Primary Antibody vs. ICA Using GIST/Non-GIST Sarcoma Tissue Micro array

Micro arrays comprised of 63 non-GIST sarcoma cases and 13 GIST cases were also studied by 3 qualified readers. However, about one-fifth of cores was variably lost to the readers or contained no tumor. Two GIST and two non-GIST tumors had no evaluable cores. These results are presented separately by pathologist, also.

Qualified Reader #1

		ICA Polyclonal		
		Positive	Negative	Total
PATHWAY	Positive	11	0	11
Anti-c-KIT	Negative	0	61	61

(9.7)	Total	11	61	72
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Positive Agreement = 100% (11/11) with 95% LCB = 76%
 Negative Agreement = 100% (61/61) with 95% LCB = 95%
 Overall Agreement = 100% (72/72)

Qualified Reader #2

		ICA Polyclonal		
		Positive	Negative	Total
PATHWAY	Positive	10	0	10
Anti-c-KIT	Negative	4	54	58
(9.7)	Total	14	54	68

Positive Agreement = 71% (10/14) with LCB = 46%
 Negative Agreement = 100% (54/54) with LCB = 95%
 Overall Agreement = 94% (64/68)

Qualified Reader #3

		ICA Polyclonal		
		Positive	Negative	Total
PATHWAY	Positive	11	0	11
Anti-c-KIT	Negative	2	58	60
(9.7)	Total	13	58	71

Positive Agreement = 85% (11/13) with 95% LCB = 59%
 Negative agreement = 100% (58/58) with 95% LCB = 95%
 Overall Agreement = 97.2% (69/71)

Results and Conclusions:

Two of the concordant positive specimens consisted of one spindle cell melanoma specimen and one synovial sarcoma specimen. C-KIT expression has been reported in approximately 20% of malignant melanomas (18). There is controversy in the literature regarding c-KIT expression in synovial sarcoma (32).

Four cases, all GISTs, had discrepant results according to at least one reader. For two of these cases, the discrepancy was noted with two of the readers. One reader had no discrepant results. These results demonstrate comparable performance between the PATHWAY Anti-c-KIT (9.7) Primary Antibody and the ICA and between the three different qualified readers.

All of the qualified readers met study criterion of the $\geq 75\%$ overall agreement and thus demonstrated acceptable agreement with the ICA. This data demonstrates that the PATHWAY Anti-c-KIT (9.7) Primary Antibody may be used to select GIST patients for therapy with the drug, Gleevec.

Conclusion of the Clinical Studies

By identifying c-KIT positive GIST tissue, the Ventana PATHWAY Anti-c-KIT (9.7) Primary Antibody was demonstrated to be an effective aid in the assessment of patients being considered for therapy with Gleevec

XI. OVERALL CONCLUSIONS DRAWN FROM ALL OF THE STUDIES

The results of the preclinical and clinical testing performed on Ventana PATHWAY Anti-c-KIT (9.7) Primary Antibody demonstrate that this product is reproducible and is specific to c-KIT expression with analytical and clinical performance characteristics appropriate to aid in the assessment of patients considered for Gleevec® (imatinib mesylate) therapy.

Risk Benefit Analysis

The testing performed on Ventana PATHWAY Anti-c-KIT (9.7) Primary Antibody indicates that the assay performs consistently and that the assay results are clinically relevant in the assessment of patients considered for c-KIT targeted therapy.

Patients falsely assigned as c-KIT-positive following assessment would be considered eligible for treatment. Because the design of the clinical studies did not include treatment of patients with negative assay results, the risks or benefits of treatment in this patient population are unknown. The risks of Gleevec treatment to c-KIT-positive patients included dermatologic reactions, fluid retention and edema, gastrointestinal irritation and bleeding, anemia, neutropenia, and thrombocytopenia. Liver and kidney toxicity and immunosuppression may result from long-term use. Women of childbearing age should be advised to avoid becoming pregnant because of the potential for teratogenic effects.

False negative test results would potentially exclude the patient from treatment.

Based on the information in the studies provided, the FDA has concluded that the benefits of using the Ventana PATHWAY Anti-c-KIT (9.7) Primary Antibody for its intended use outweigh the risks associated with using it.

Safety

The Ventana PATHWAY Anti-c-KIT (9.7) Primary Antibody is an *in vitro* diagnostic test and does not contact the patient. Instructions for the safe use of the product are included in the package insert.

Effectiveness

The results of testing performed with the Ventana PATHWAY Anti-c-KIT (9.7) Primary Antibody indicated that the assay is effective in aiding in the assessment of patients considered for c-KIT targeted therapy.

CDRH has, therefore, concluded that the device is safe and effective for the stated indication.

X. PANEL RECOMMENDATIONS

In accordance with the provisions of section 515(c)(2) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Immunology Advisory Panel, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XI. CDRH DECISION

CDRH issued an approval order for the applicant's PATHWAY Anti-c-KIT (9.7) Primary Antibody on August 11, 2004.

The applicant's manufacturing facility was inspected in June 4, 2004 and found to be in compliance with the Quality System Regulation (21 CFR 820).

XII. APPROVAL SPECIFICATIONS

Directions for use: See labeling

Hazards to Health from Use of the Device: See Indications, Warnings and precautions in the labeling.

Postapproval Requirements and Restrictions: See approval order.