

SUMMARY OF SAFETY AND EFFECTIVENESS DATA

I. GENERAL INFORMATION

| | |
|---|---|
| Device Generic Name: | DakoCytomation Mouse Anti-Human c-Kit Immunohisto - Chemistry Kit |
| Device Trade Name: | DakoCytomation c-Kit pharmDx™ |
| Applicant Name and Address: | DakoCytomation California, Inc. 6392 Via Real Carpinteria, CA 93013 |
| Premarket Approval Application Number: | P040011 |
| Date of Panel Recommendation: | None |
| Date of Notice of Approval to the Applicant: | June 27, 2005 |

II. INDICATIONS FOR USE

The c-Kit pharmDx™ assay is a qualitative immunohistochemical (IHC) kit system used on the Dako Autostainer, for the identification of c-kit protein/CD117 antigen (c-kit protein) expression in normal and neoplastic formalin-fixed paraffin-embedded tissues for histological evaluation. The c-Kit pharmDx™ rabbit polyclonal antibodies specifically detect the c-kit protein in CD117 antigen-expressing cells.

The c-Kit pharmDx™ is indicated as an aid in the differential diagnosis of gastrointestinal stromal tumors (GIST). After diagnosis of GIST, results from c-Kit pharmDx may be used as an aid in identifying those patients eligible for treatment with Gleevec™/Glivec® (imatinib mesylate).

Results from hematoxylin and eosin (H&E) stains and a panel of antibodies can aid in the differential diagnosis of GIST. Interpretation must be made by a qualified pathologist, within the context of a patient's clinical history, proper controls, and other diagnostic tests

III. CONTRAINDICATIONS: None

IV. WARNINGS AND PRECAUTIONS

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

V. DEVICE DESCRIPTION

Reagents Provided.

The DakoCytomation c-Kit pharmDx™ assay kit contains 2 vials of immunohistochemical reagents and 10 control slides bearing two formalin-fixed, paraffin-embedded cell line preparations. The first vial of immunohistochemical reagent consists of 9 mL of polyclonal

rabbit anti-human c-kit IgG in a Tris-HCl buffer, containing stabilizing protein and 0.015 mol/L sodium azide. The second vial contains 9 mL of polyclonal rabbit IgG at a greater than or equal to the positive anti-c-Kit concentration in a Tris-HCl buffer, containing stabilizing protein and 0.015 mol/L sodium azide. Positive and negative c-kit cell lines are provided on each control slide to be used as performance controls for the kit reagents. Each slide contains sections of two pelleted, formalin-fixed paraffin-embedded cell lines, which represent a moderate level of c-kit/CD117 protein expression and no c-kit/CD117 expression. The IHC staining scores of the cell pellets are 2+ and 0.

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

- Wash Buffer (code S3006)
- Dual Endogenous Enzyme Block (code S2003)
- Target Retrieval Solution (code S1699 & S1700)
- EnVision+ Rabbit (code K4002 & K4003)
- DAB+ (code K3467 & K3468)

Note: To ensure proper staining results, only these staining reagents should be used with c-Kit pharmDx™. Deviations from this recommended protocol have not been validated.

- Ammonium hydroxide, 15 mol/L diluted to 0.037 mol/L
- Hematoxylin, alcohol or water-based such as DakoCytomation's Hematoxylin (code S3301 or S3302)
- Coverslips
- Water bath, capable of maintaining 95–99°C or pressure cooker, capable of heating up to 121°C
- Distilled or deionized water (reagent-quality water)
- Drying oven, capable of maintaining 56–60°C
- Ethanol, absolute and 95%
- Light microscope (4x–40x objective magnification)
- Mounting medium, such as DakoCytomation's Faramount (code S3025)
- or DakoCytomation's Glycergel (code C0563) or DakoCytomation's Ultramount (code S1964)
- Positive and negative tissues to use as process controls (see Quality control section)
- Slides, Fisher's SuperFrost Plus, poly-L-lysine-coated slides, charged slides,
- or DakoCytomation's Silanized Slides (code S3003) (see Specimen preparation section)
- Staining jars or baths
- Timer (capable of 2–30 minute intervals)
- Wash bottle
- Xylene, toluene, or xylene substitutes
- 1 mL pipette
- Humid Chamber

Principle of Device Methodology

The DakoCytomation c-Kit pharmDx™ assay is a standard immunohistochemical (IHC) assay to identify the c-kit/CD117 receptor expression in normal and neoplastic tissues. The c-Kit pharmDx™ IHC kit contains antibodies and control slides to complete an IHC staining procedure for formalin-fixed and paraffin-embedded gastrointestinal stromal tumor (GIST) specimens. c-Kit pharmDx™ assay contains rabbit polyclonal antibodies which binds a site on the internal domain of the c-kit oncoprotein in paraffin-embedded tissue sections and specifically detects the c-kit/CD117 protein in c-kit-expressing cells. The kit also contains a vial of matched negative control reagent and control slides. The remainder of the reagents necessary to obtain results is recommended in the “Materials required, but not supplied” section of the product labeling and must be purchased separately. The system is used with antigen enhancement reagent, endogenous peroxidase blocker, ready-to-use visualization reagent based on dextran technology, diaminobenzidine (DAB) chromogen substrate, and hematoxylin counterstain.

Following incubation with the primary polyclonal antibodies to human c-kit/CD117 protein, which binds to the antigen in or on the cells, this kit recommends using a ready-to-use visualization reagent based on dextran technology. This reagent consists of both secondary goat anti-rabbit antibody molecules and horseradish peroxidase molecules linked to a common dextran polymer backbone, thus eliminating the need for sequential application of link antibody and peroxidase conjugate. This reagent binds to the c-Kit antibody that bound to the c-kit protein in or on the cell. The enzymatic conversion of the subsequently added DAB chromogen results in formation of a visible reaction product at the antigen site. 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. The specimen is then counterstained and coverslipped. Results are interpreted by a pathologist using a light microscope. 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. Control slides, containing two formalin-fixed paraffin-embedded human cell lines with staining intensity scores of 2+ and 0, are provided for quality control of the kit reagent performance. Detailed instructions in the package insert for use of reagents, control materials and how to interpret their results are provided also to insure adequate assay control. The kit is available in manual and automated configurations.

VI ALTERNATIVE PRACTICES AND PROCEDURES

Multiple methods exist for the identification of c-Kit protein GIST tissues. Analysis of c-KIT status could be performed via KIT gene mutation analysis of genomic tumor DNA, as was done for the 129 subjects in the US Diomedea multi-tissue array. C-Kit gene mutational analysis is most commonly performed using polymerase chain reaction (PCR), for which there are no commercially available assays.

VII. MARKETING HISTORY

The DakoCytomation c-Kit pharmDx™ assay is currently marketed as a standard, immunohistochemical assay intended to identify c-kit protein/CD117 antigen expression in formalin-fixed paraffin-embedded normal and neoplastic tissue specimens in Europe and South America. Less than 200 kits have been distributed world wide since August of 2004. The device has not been withdrawn from any market for any reason of safety and effectiveness.

VII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

The DakoCytomation c-Kit pharmDx™ assay 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 DakoCytomation c-Kit pharmDx™ assay to verify and validate the assay performance included assay sensitivity, specificity, precision/reproducibility, robustness, assay time and temperature, assay working stability, reagent chemistries, and shelf-life and stability.

1) Assay Positive Agreement

To describe the agreement of positive staining in tissues taken from GIST tumors used in a clinical study protocol, 36 GIST tumor specimens of various positive staining intensity including 2 that were negative were stained with DakoCytomation c-Kit pharmDx™ assay using the DAKO Autostainer as well as the manual methodology. Slides were blind-labeled and read by one independent qualified reader.

Acceptance criteria were that DakoCytomation c-Kit pharmDx™ assay must produce immunostaining scores ≥ 0.5 intensity in $\geq 50\%$ of the tumor cells in GIST tissue sections of formalin-fixed paraffin-embedded tissues. In negative control tissues, no specific staining of

targeted negative control cells should be observed..

Results: There was 100% agreement of the DakoCytomation c-Kit pharmDx™ assay performed manually and with the DAKO Autostainer as compared to the original c-Kit result.

Conclusion: The acceptance criteria were met, and the DakoCytomation c-Kit pharmDx™ assay performed in 100% agreement to the original clinical testing result. The assay positive agreement was thus 100%.

2) Assay Specificity

a) Clinical Specificity/Tour of 30 Normal Tissues Throughout the Body

To describe the specificity of positive staining in normal tissues, three each of thirty (30) normal tissue types (the 30 recommended in FDA's special control for immunocytochemistry devices' Guidance for Submission of Immunohistochemistry Applications to the FDA, June 3, 1998) from 3 independent sources embedded into 15 multi-tissue blocks were stained using the DakoCytomation c-Kit pharmDx™ assay on the DAKO Autostainer. Slides were blinded to observers and read by one certified pathologist.

Results: Mast cells stained where present in the tissues. Positive anti-c-kit staining was identified in breast, brain/cerebellum, colon, kidney, pancreas, skin, and small intestine.

Conclusion: DakoCytomation c-Kit pharmDx™ assay performed acceptably on normal tissue samples. They have characterized the performance of this product with thirty normal tissues from three independent sources. The results for the DakoCytomation c-Kit pharmDx™ assay 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) Antibody Specificity

To demonstrate the specificity of the DakoCytomation c-Kit pharmDx™ assay, the following different approaches were taken, several at the request of the FDA during the review.

The c-kit antibody was tested for reactivity against cell lines expressing c-kit protein.

In additional studies, the c-kit antibody was tested using Western Blotting to look for cross-reactivity to proteins that share structural homology, such as; Platelet derived Growth Factor Receptor (PDGFRa), macrophage colony stimulating factor receptor (c-FMS); and FMS-like tyrosine kinase 3 (FLT-3).

Furthermore, using Western blotting the DakoCytomation pharmDx antibody was tested with a lysate of the adenocarcinoma cell line HS, which does not express the c-kit gene.

Results: In Western blotting of a lysate of the small cell lung carcinoma cell line SY that over-expresses c-kit mRNA, the DakoCytomation c-Kit pharmDx™ primary antibody labeled a band of 145 kD corresponding to the c-kit protein. The labelled band was rather broad, from 120 to 155 kD. An additional band of 100 kD was also labelled. A second anti-c-kit antibody, likewise, labelled a 100 kD protein. This labelling was abolished when the antibody was incubated with the synthetic c-kit peptide antigen used for immunization.

No cross-reactivity was observed with the homologous proteins

Conclusion: The DakoCytomation c-Kit pharmDx™ primary antibody reacted with the c-Kit antigen. It did not cross-react with closely related proteins or with any other proteins in a transformed cell line.

3) Assay Precision/Reproducibility.

a) Within and Between Day and Between Technician and Interpreter Reproducibility

To demonstrate that the DakoCytomation c-Kit pharmDx™ assay gave consistent manual staining results within and between technician and interpreter and between day, three different technicians stained the same formalin-fixed paraffin-embedded tissue slides over a period of three days, excluding weekends. The slides were repeated in triplicate on the first day and in singlicate over the next two days. Tested specimens included one low and one medium-expressing GIST tissue and DakoCytomation c-Kit pharmDx™ prototype control slides consisting of a section of a formalin-fixed paraffin-embedded block made from one GIST positive and one GIST negative cell line.

The acceptance criteria were that the staining intensity for a specimen shall vary by no more than ± 0.5 intensity grade within a run, between runs and between technicians specific staining intensity. All grading was done by two reviewers.

Results: The acceptance criteria were met in all cases.

Conclusion: Even though few specimens were tested in an unmasked protocol, a sufficient variety of tissues tested demonstrated acceptable manual staining reproducibility within and between day and between three technicians and two interpreters.

b) Between-Day and Between Technician Reproducibility Study

To further demonstrate DakoCytomation c-Kit pharmDx™ assay between-day and between technician reproducibility, reproducibility was tested by manual methodology at three laboratories by two technicians in each laboratory over 3 days with 5 different specimens (4 positive and 1 negative in each laboratory) of various staining intensity scores randomized and masked.

Results: Excellent reproducibility (100%) was seen for positive versus negative results (0 vs. 1+, 2+ and 3+). Staining intensity varied by no more than ± 1 staining intensity grade with the following exceptions. At sites 1 and 3, one slide (each) varied by two intensity grades. This was a result of tissue disruption at site 1. Overall, excellent reproducibility (100%) was seen for positive versus negative results.

Conclusion: DakoCytomation c-Kit pharmDx™ assay provided reproducible results between days and between technicians within each of three laboratories.

c) Inter-laboratory and manual vs. automated reproducibility of staining

To demonstrate inter-laboratory reproducibility, immunohistochemical staining of 30 randomized and masked specimens of various expression levels of c-kit (10 negative and 20 positive) was performed at three geographically separated laboratories. Cut slides were forwarded to each testing laboratory for manual and automated staining and evaluation by a pathologist.

Results: At two sites inter-laboratory percent agreement was 100% for a dichotomous positive/negative determination where 0 was negative and 1+, 2+ and 3+ were positive for c-Kit protein expression for both manual and automated testing procedures. At site 2, duplicate slides of two tissues, designated before the study to have been negative, were found to be, in actuality, positive. Follow-up analysis revealed that a small area (~20% of the tumor cells) was actually staining positively. This section of tumor was not seen in the specimens tested at the other two laboratories.

Conclusion: Overall, inter-laboratory percent agreement was substantial to almost perfect for positive/negative determination of c-kit/CD117 protein expression in both manual and automated testing procedures. This study, thus, demonstrated agreement in reproducibility of results between three laboratories and concordance between manual and automated assay procedures.

e) Intra-run Reproducibility (Manual Staining)

To demonstrate intra-run reproducibility at each of the study sites, 5 slides of a same positive specimen was included in the set of 30 specimens stained manually. Staining intensity and percent of tumor staining were assessed.

Results: The tissue result remained positive at each study site, and the staining intensity remained within +/- 1 intensity grade variation.

Conclusion: DakoCytomation c-Kit pharmDx™ assay provided reproducible positive intra-run results at three different laboratories

f) Lot-to-Lot Reproducibility

To demonstrate lot-to-lot reproducibility, three kit lots were made from one lot of primary antibody raw material according to manufacturing procedures in three separate manufacturing runs. The three lots were tested on 20 formalin-fixed paraffin-embedded tissues: 10 non-GIST sarcomas and 10 GIST specimens as well as a control slide consisting of a positive (2+) and negative slice of formalin-fixed paraffin-embedded cell culture blocks. Paired slides were stained using the negative control reagent as negative control. Staining was performed on the DAKO Autostainer.

Required acceptance criteria were within ± 1.0 intensity grade on all similar specimens between lots. Desired acceptance criteria were within + 0.5 intensity grade on all similar specimens between lots.

Results: The acceptance criteria were met for all three lots gave comparable scoring. All tumor cells in the sarcoma specimens gave negative results, while infiltrating mast cells stained positively

and acted as an internal control. All paired negative control reagent stained negatively.

Conclusion: The protocol and acceptance criteria were appropriate to the lot-to-lot study. The required and desired acceptance criteria were met. This study demonstrated acceptable lot-to-lot reproducibility for the DakoCytomation c-Kit pharmDx™ assay.

4) Assay Robustness

To determine the incubation tolerances (desired and required incubation limitations) for every step of the DakoCytomation c-Kit pharmDx™ assay, a prototype tissue culture positive (2+) and negative control slide (formalin-fixed and paraffin-embedded) and one low and one medium expressing formalin-fixed and paraffin-embedded GIST tissue were tested at varying specified incubation times throughout the manual assay procedure. Two reviewers graded the specimens.

The acceptance criteria were that the assay shall be sufficiently robust such that the immunostaining of the specimens shall not vary by more than ± 0.5 specific staining intensity between the control assay run on that specimen and an alternate assay run on the same specimen.

Results: These studies determined the incubation times finally settled upon in the step-by-step product instructions: **Dual Endogenous Enzyme Block** incubate for 5 (± 1) minutes; **Target Retrieval Solution heated in the Water Bath at 95–99°C** incubate for 20(± 1) minutes; **Target Retrieval Solution heated in the Pressure Cooker (121°C)** for 5 minutes; **Primary Antibody or Negative Control Reagent incubated at 30(± 1) minutes**; **Labelled Polymer - Horseradish peroxidase detection system incubate at 30(± 1) minutes**; **DAB+ Substrate-Chromogen Solution incubate for 10(± 1) minutes**; **Counterstain incubate for 2-5 minutes** depending on the counterstain used, **Buffer Incubation = 5 (± 1) minutes**.

Conclusion: Assay robustness tolerances were determined by extensive testing.

5) Verification of assay time and temperature

To verify that the total assay time and temperature tolerances for the manual and automated DakoCytomation c-Kit pharmDx™ assay met specified acceptance criteria, a prototype tissue culture positive (2+) , negative control slide (formalin-fixed and paraffin-embedded) , one low and one medium expressing (formalin-fixed and paraffin-embedded GIST) tissue were tested at varying specified incubation times throughout the manual assay procedure. Two reviewers graded the specimens.

Acceptance Criteria:

Required acceptance criteria were that the staining intensity of the specimens tested shall vary by no more than ± 0.5 specific staining intensity between the control assay run on the specimen and the test assay run on the same specimen.

When processing < 25 slides according to the defined assay protocol, it is desired that the immunohistochemical staining procedure should take ≤ 2.5 hours.

When processing < 25 slides according to the defined assay protocol, it is required that the

immunohistochemical staining procedure should take ≤ 3.5 hours.

(Assay protocol = Target Retrieval through counterstain.)

When processing ≤ 25 slides according to defined assay protocol, it is required that all processing and staining procedures should take no more than one day. The detection system is desired to work at the temperature range of 18-27°C. The detection system must work at room temperature (20-25°C) without a humidity chamber. The detection system must work at room temperature (20-25°C) in a humidity chamber.

Results: All design specification requirements met the acceptance criteria. It was observed, however, that although testing the assay manually at room temperature without the use of a humid chamber passed the acceptance criteria, there was some unacceptable edge effect staining seen on the specimens in comparison with the use of a humid chamber. It was thus decided that the manual assay product labeling will recommend using a humid chamber.

Conclusion: The required and desired acceptance criteria were met. All design specification requirements for the DakoCytomation c-Kit pharmDx™ assay met the acceptance criteria.

6) Assay working stability

To determine the working stability of the DakoCytomation c-Kit pharmDx™ assay as well as those of prepared recommended ancillary reagents when stored for extended periods at room temperature, the unopened kit and prepared ancillary reagents (chromogen and wash buffer) were left at room temperature (RT) for extended periods of time and tested for acceptable performance. The purpose of one half of this protocol is to leave the unopened kit at room temperature for 48 and 96 hours to simulate what may happen if the kit were received by an end user on Friday and not stored as directed in the refrigerator until Saturday or Monday. A prototype tissue culture positive (2+) and negative control slide (formalin-fixed and paraffin-embedded) and four formalin-fixed and paraffin-embedded GIST tissues of varying staining intensity were tested at varying specified incubation times at RT. All grading of specimens was completed by two reviewers.

Required acceptance criteria were that the kit should perform with the same immunostaining intensity as a reference kit not stored for extended periods at room temperature used on a serial section of the same tissue sample.

Results: The unopened DakoCytomation c-Kit pharmDx™ assay met acceptance criteria for up to 5 days at RT. The prepared chromogen could be left at RT for 7 days, and the prepared wash buffer was stable for 5 days at RT.

Conclusion: The required working stability acceptance criteria were met by the products tested.

7) Lot-to-Lot Interchangeability of Ancillary Reagents.

To demonstrate lot-to-lot interchangeability, three lots of each of these ancillary reagents were tested manually on a prototype tissue culture positive (2+) and negative control slide (formalin-fixed and paraffin-embedded) and one low and one medium expressing formalin-fixed and paraffin-embedded GIST tissue. All grading of specimens was completed by two reviewers.

The acceptance criteria were that the assay shall be sufficiently robust so that the immunostaining intensity of a specimen shall not vary by more than 0.5 intensity grade on all similar specimens within the same run for multiple lots of ancillary reagents not included in this test kit.

Results: The acceptance criteria were met for all lots tested. They all gave comparable scoring.

Conclusion: The use of multiple lots of ancillary reagents was verified for use with the DakoCytomation c-Kit pharmDx™ assay.

8) Device Stability/Shelf Life.

To determine the expiration date of the DakoCytomation c-Kit pharmDx™ assay, one lot only of the pre-diluted positive primary and negative control reagent antibodies were tested because the antibody dilutions are the same as two previously approved protocols which already have extensive stability information. These individual lots plus control slides sectioned in 4 and 5 micrometer sections from three different freshly manufactured control tissue culture cell line blocks were stored at 2 – 8°C and tested at day 0, 17, 33, 50, 80, 160 and 12 and 18 months. The positive primary and negative control reagent antibodies were also tested on one positive GIST tissue. Paired sections were stained using the negative control reagent as negative control. Staining was performed using the manual product and the formulation for the DAKO Autostainer.

Required acceptance criteria were:

For the positive prediluted antibody: Specific staining of target cells at an IHC intensity grade of 2.0 – 4.0. Results should be within +0.5 grade of the reference control assay. Rejection criteria for the primary antibody are an IHC intensity less than 2.0 or greater than 4.0.

For the Negative control reagent: No specific staining of GIST tissues, background staining ≤ 1.0 . Results should be within +0.5 grade of the reference control assay. Rejection criteria for the negative control reagent antibody are non-specific background staining > 1.0 .

For the Control Slides: Specific staining of the positive cell line pellet should be 2.5 ± 0.5 . No specific staining of the negative cell line pellet. Non specific background staining of ≥ 1.0 . Results should be within + 0.5 intensity grade of the reference control product.

Results: The prediluted antibodies in the DakoCytomation c-Kit pharmDx™ assay demonstrated real-time stability when stored at 2 – 8°C for 18 months. The stability of the control slides sectioned at 4 micrometers was less robust than control slides sectioned at 5 micrometers. Thus, it was decided to manufacture all control slides sliced at 5 micrometers. The five micrometer slides demonstrated real-time 13 month stability when sectioned from a 6 month old control block and stored at 2 – 8°C. When the sections were sliced from 9 month-old control cell blocks, the real-time stability was only 7 months. Thus, it was decided that no greater than 6 month-old cell blocks should be used for manufacture. Since the results of no specific negative staining were reported in the stability study, it was requested that notations for such reporting be added to the stability protocol. The sponsor

attested to the fact that negative tissues had been present in the stability study and had performed acceptably. They agreed to provide space for its reporting in future studies.

Conclusion: The DakoCytomation c-Kit pharmDx™ assay Kits K1906 for manual use and K1907 for Autostainer use were demonstrated to be stable for 13 months when control slides are sectioned at a thickness of 5 micrometers from a 6 month-old control cell block and all components are stored at 2 – 8°C. Based on the data presented in the report, the sponsor is requesting a 12 month expiration date for the DakoCytomation c-Kit pharmDx™ assay. The FDA approves the protocol for stability testing with the addition of reporting of the results of specific negative background staining.

X. SUMMARY OF CLINICAL STUDIES

Two studies were conducted using clinical cases with known diagnoses involved in the differential diagnosis of GIST to determine the agreement between DakoCytomation c-Kit pharmDx™ assay and the investigational immunocytochemical protocol used in the Novartis Gleevec® clinical trial (NCTP).

The first comparison study was conducted with multiple-tissue arrays (MTAs), which included a large sample of GIST cases as well as a collection of various types of sarcomas, to demonstrate the agreement between the two heat-induced epitope retrieval (HIER) protocols of the DakoCytomation c-Kit pharmDx™ assay plus recommended ancillary reagents vs. the reference assay: a simulation of the Novartis clinical trial protocol (NCTP). The NCTP employed the identical DakoCytomation primary antibody at a dilution of 1:250 without HIER. The DakoCytomation c-Kit pharmDx assay specifies the use of two optional HIER protocols: 1) heating in a waterbath at 95-99°C for 20 (\pm 1) minutes and 2) heating in a pressure cooker (121°C) for 5 minutes. The use of HIER enables the pharmDx primary antibody to be used at a dilution of 1:500. This clinical study demonstrated that the performance of the DakoCytomation c-Kit pharmDx™ assay was comparable to that of the NCTP with mostly positive specimens.

Since there were few negative clinical samples in the first study, a second study of DakoCytomation c-Kit pharmDx assay negative clinical specimens was requested. The second study followed the identical protocol of the first study. The testing was performed on 80 additional c-kit negative specimens, including leiomyomas, leiomyosarcomas, and other sarcomas. The acceptance criteria for study #2 were that it was required that the DakoCytomation c-Kit pharmDx assay demonstrate $\geq 90\%$ concordance with the NCTP. It was desired that the DakoCytomation c-Kit pharmDx assay demonstrate $\geq 95\%$ concordance with the NCTP. This study demonstrated that the performance of the DakoCytomation c-Kit pharmDx™ assay was comparable to that of the NCTP with all negative specimens.

THE OBJECTIVE OF THE CLINICAL STUDIES

The main objective of these studies using clinical samples with known diagnoses appropriate for the differential diagnosis of GIST was to determine the acceptability of DakoCytomation c-Kit pharmDx assay as an alternative to the NCTP used in the Gleevec clinical trial as an aid in the selection of patients for treatment with Gleevec™/Glivec (imatinib mesylate). All testing for the clinical study was done in-house at DakoCytomation, Inc., California.

A secondary objective was to demonstrate the equivalence of staining when the two recommended protocols for heat-induced epitope retrieval (HEIR) were compared on the same formalin-fixed paraffin-embedded tissues. Briefly, water bath (95–99°C) for 20 minutes and pressure cooker (121°C) for 5 minutes of heating, both followed by 20 minutes of cool down, were used as heat sources. The remaining steps of the staining procedure were identical.

STUDY POPULATIONS

Study #1

A total of 276 paraffin-embedded specimens were collected and prepared into 2 sets of tissue arrays by Diomedea (Maryland). One multi-tissue array contained 35 specimens collected during the Novartis Gleevec clinical trial (US array, containing 129 cases). The second array contained specimens that were previously tested for CD117 expression using the established study procedure (Basel array, containing 147 cases). Comparisons are missing for approximately 50 samples in each pairing due to no evidence of tumor, no sample, or insufficient sample for evaluation. Tumors tested came from the primary lesions found in stomach, esophagus, small intestine (small bowel) colon, duodenum, mesenterium, mesocolon, and rectum. Additional specimens came from lymph node, or other metastasis, primarily from lung, or liver. Tumors not designated as GIST included leiomyomas, leiomyosarcomas, sarcomas with low malignant potential (LMP) or sarcomas with high malignant potential (HMP).

Study #2

At the request of the FDA in an e-mail sent to the sponsor on May 6, 2004, the sponsor tested an additional 80 formalin-fixed paraffin embedded c-kit negative specimens, including leiomyomas, leiomyosarcomas, and other sarcomas that were difficult to differentiate from GIST tumors and thus involved in the differential diagnosis of GIST were tested.

STUDY SUMMARIES

All of the tissues were stained using the c-Kit pharmDx™ assay after the use of the two methods of heat induced epitope retrieval (HIER) recommended in the product labeling. These assays were compared to a concurrently run simulation of the Novartis Clinical Trial Protocol (NCTP) that had been used previously to select patients for the clinical trial that obtained FDA approval for the use of Gleevec/Glivec with diagnosed GIST patients. The NCTP used a 2X higher concentration of DakoCytomation primary polyclonal antibody against c-kit (A4502) (the same reagent used in c-Kit pharmDx) and no HIER. The results of the specimens from Study #1 were also compared to the original CD117 assay results performed at the time the tissues were made into the multi-tissue array.

RESULTS OF THE CLINICAL STUDIES

Since all of the tissues in both studies were treated according to the same protocols, the results from all of the tissues in studies 1 and 2 were combined for the concurrent comparison between the NCTP and the c-Kit pharmDx™ assay. The results using pressure cooker as the heat source are listed in Table 2 and similar results (overall agreement =98.7%), were seen when a

water bath was used as a heat source. Percent positive and negative agreements were similar for the two comparisons.

Table 2. 2x2 table of c-Kit pharmDx™ protocol with pressure cooker test results compared to the concurrent NCTP test result

| c-Kit pharmDx™ Pressure Cooker Test Result | Concurrent NCTP Assay Test Results | | |
|---|---|---------------------------|--------------|
| | Positive n (%) | Negative n (%) | Total |
| Positive | 188 (60.8) | 3 (0.9) | 191 |
| Negative | 1 (0.3) | 117 (37.9) | 118 |
| Total | 189 | 120 | 309 |

Percent Positive Agreement = 99.5% (95% exact CI: 97.1% - 100%)

Percent Negative Agreement = 97.5% (95% exact CI: 92.9% - 99.5%)

Overall Agreement = 98.7% (95% exact CI: 96.7% - 99.7%)

A subset of these specimens (35 cases) represented tissues from patients participating in the Novartis Gleevec clinical trial. Of these patients 21 were treated with Gleevec™ (58.3%). When the same specimens were retested with the simulated NCTP and c-Kit pharmDx™ 20 were found to be positive with all three procedures, whereas 1 specimen was found to be negative. Among the 147 patients treated with Gleevec™ in the Novartis Gleevec Clinical Trial, it was reported that 54% had a partial response, and 28% had stable disease.⁷

For a subset of 259 specimens that were presented in Table 4, the CD117 test result was determined at the time the specimens were put into the MTAs. Thirty-five of the cases were enrolled in the Gleevec clinical trials (n=35), and the remainder were tested in the same laboratories that had participated in the Gleevec trials. Results from 179 specimens (the total was less than 259 due to specimen loss or lack of tumor in the MTAs) are presented in Table 3 below. These specimens demonstrated an overall agreement to the original CD117 status of 94.9% (95% exact CI: 90.7% - 97.7%).

Table 3: 2x2 table of pressure cooker test results compared to Original test result

| Pressure cooker test result | Original tissue CD117 status | | |
|--|-------------------------------------|-----------------------|------------------------|
| | Positive n | Negative n | Total n (%) |
| Positive | 136 | 3 | 139 (78%) |
| Negative | 6 | 34 | 40 (22%) |
| Total | 142 | 37 | 179 |

Percent positive agreement= 95.7% (95% exact CI: 91.0% - 98.4%)
Percent negative agreement= 91.9% (95% exact CI: 78.1% - 98.3%)
Overall agreement= 94.9% (95% exact CI: 90.7% - 97.7%)

Conclusions of the Clinical Studies

These studies demonstrated that on real GIST clinical specimens the DakoCytomation c-Kit pharmDx™ assay gave acceptable comparisons between the two recommended HIER methods, to the concurrent simulated NCTP, and to the original CD117 assay result of several years ago. It was thus demonstrated to be an effective and reproducible aid in the assessment of patients being considered for therapy with Gleevec®/Glivec™ (imatinib mesylate).

IX. OVERALL CONCLUSIONS DRAWN FROM ALL OF THE STUDIES

The results of the pre-clinical and clinical testing performed on DakoCytomation c-Kit pharmDx™ assay demonstrate that the assay is reproducible and is specific to c-Kit expression with performance characteristics appropriate to aid in the assessment of patients considered for Gleevec® (imatinib mesylate) therapy.

Risk Benefit Analysis

The testing performed on the DakoCytomation c-Kit pharmDx™ assay 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 DakoCytomation c-Kit pharmDx™ assay for its intended use outweigh the risks associated with using it.

Safety

The DakoCytomation c-Kit pharmDx™ assay 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 on the DakoCytomation c-Kit pharmDx™ assay indicate 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 Devices 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

FDA issued an approval order on June 27, 2005.

The applicant's manufacturing and control facilities were inspected on June 4, 2004 and the facilities were 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, Contraindications, Warnings, Precautions and Adverse Events in the labeling.

Postapproval Requirements and Restrictions: See approval order.