INDICATION FOR USE
Abbott RealTime IDH2 is indicated as an aid in identifying acute myeloid leukemia (AML) patients with an isocitrate dehydrogenase - 2 (IDH2) mutation for treatment with IDHIFA® (enasidenib).

This test is for prescription use only.

SUMMARY AND EXPLANATION OF THE TEST
Abbott RealTime IDH2 detects single nucleotide variants (SNVs) coding nine IDH2 mutations (R140Q, R140L, R140G, R140W, R172K, R172M, R172G, R172S, and R172W) by using PCR technology with homogeneous real-time fluorescence detection. The assay uses human blood or bone marrow aspirate specimens and reports a qualitative result. Table 1 lists the IDH2 mutations detected by the Abbott RealTime IDH2 assay.

<table>
<thead>
<tr>
<th>Codon</th>
<th>IDH2 Mutation</th>
<th>SNV</th>
</tr>
</thead>
<tbody>
<tr>
<td>R140</td>
<td>R140Q</td>
<td>CAG</td>
</tr>
<tr>
<td></td>
<td>R140L</td>
<td>CTG</td>
</tr>
<tr>
<td>R140</td>
<td>R140G</td>
<td>GGG</td>
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<tr>
<td></td>
<td>R140W</td>
<td>TGG</td>
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<tr>
<td>R172</td>
<td>R172K</td>
<td>AAG</td>
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<tr>
<td></td>
<td>R172M</td>
<td>ATG</td>
</tr>
<tr>
<td>R172</td>
<td>R172G</td>
<td>GGG</td>
</tr>
<tr>
<td></td>
<td>R172S</td>
<td>AGT and ACG</td>
</tr>
<tr>
<td></td>
<td>R172W</td>
<td>TGG</td>
</tr>
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</table>

IDH2 (isocitrate dehydrogenase - 2) is a mitochondrial enzyme that helps break down nutrients and generate energy for the cell. The homodimeric enzyme catalyzes a reaction that converts isocitrate to α - ketoglutarate (α - KG) while reducing NADP to NADPH and liberating CO2. Because of its involvement in cellular energy production, IDH2 plays a role in the metabolism of glucose, fatty acids, and glutamine; and also contributes to the maintenance of normal cellular redox status.1

Mutations in the R140 and R172 codons of IDH2 are oncogenic and can be found in several cancer types, including acute myeloid leukemia (AML) in which 8% to 19% of patients possess an IDH2 mutation.1,2,3,4,15 Nine IDH2 amino acid changes have been identified in various cancer types: R140Q, R140L, R140G, R140W, R172K, R172M, R172G, R172S, and R172W.3,5,6,7 R140 mutations account for 80% of IDH2 mutations, with R140Q being the most prevalent, occurring in 30% to 50% of AML patients with IDH2 mutations.5,6,15 Mutations in R140 and R172 are associated with the ability of IDH2 to further process α - KG to generate 2-hydroxyglutarate (2-HG), an oncometabolite, which can result in concentrations that are orders of magnitude higher than normal.8, 9,10,11,12,13 Once produced, 2-HG alters the cells’ genetic programming, or epigenetics, resulting in increased numbers of quickly proliferating, early hematopoietic progenitor cells, and tumorigenesis.12,14

BIOLOGICAL PRINCIPLES OF THE PROCEDURE
Abbott RealTime IDH2 consists of two kits:
- Abbott RealTime IDH2 Amplification Reagent Kit (List No. 08N82 - 090)
- Abbott RealTime IDH2 Control Kit (List No. 08N82 - 080)

Specimens for Abbott RealTime IDH2 are processed manually using Abbott mSample Preparation System™ (List No. 06K12 - 24) reagents to isolate and purify sample DNA. The Abbott RealTime IDH2 amplification...
reagents are combined into four amplification master mixes. The purified DNA sample is combined with the master mixes in an Abbott 96-Well Optical Reaction Plate, and the plate is transferred to the Abbott m2000rt instrument for amplification and detection of IDH2 mutations. The specimen result is automatically reported on the Abbott m2000rt workstation at run completion. Assay controls are included within each run and are processed through the DNA extraction, amplification, and detection steps of the assay to assess run validity.

Software parameters specific to Abbott RealTime IDH2 are contained in an assay application specification file, which is loaded onto the Abbott m2000rt instrument by using a CD-ROM disk.

**DNA Extraction**

The purpose of DNA extraction is to isolate and purify genomic DNA from EDTA preserved blood or bone marrow aspirate specimens to make it accessible for amplification and to remove potential inhibitors of amplification. This process is accomplished by using the Abbott mSample Preparation System™ Kit, which uses magnetic particle technology to isolate and purify DNA. During the DNA extraction procedure, cells are lysed at an elevated temperature in a lysis buffer containing guanidine isothiocyanate. DNA is captured on magnetic microparticles, and inhibitors are removed by performing a series of washes with wash buffers. The bound DNA is eluted from the microparticles with elution buffer and is ready for PCR amplification.

**Reagent Preparation and Reaction Plate Assembly**

The Abbott RealTime IDH2 Oligonucleotide Reagents (Oligonucleotide Reagent 1, Oligonucleotide Reagent 2, Oligonucleotide Reagent 3, and Oligonucleotide Reagent 4) are each manually combined with DNA Polymerase, and Activation Reagent to create 4 unique master mixes. These master mixes are added to 4 separate wells of the Abbott 96-Well Optical Reaction Plate with aliquots of the extracted DNA sample. After manual application of the Abbott Optical Adhesive Cover, the plate is transferred to the Abbott m2000rt instrument.

**Amplification/Detection**

Each Abbott RealTime IDH2 master mix is designed to amplify and detect 2 or 3 IDH2 amino acid mutations (codon with mutant nucleotide underlined). Oligonucleotide 1 master mix amplifies and detects R140Q (CAG) and R140L (CTG). Oligonucleotide 2 master mix amplifies and detects R140G (GGG) and R140V (TGG). Oligonucleotide 3 master mix amplifies and detects R172K (AAQ) and R172M (ATG). Oligonucleotide 4 master mix amplifies and detects R172S (AGT and AGC), R172G (GGG), and R172W (TGG). Refer to Table 2. In addition, each master mix is designed to amplify and detect a region of the IDH2 gene outside of codon 140 and 172, which serves as an endogenous internal control (IC).

<table>
<thead>
<tr>
<th>Table 2. IDH2 Mutations Detected by each Master Mix</th>
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<tbody>
<tr>
<td><strong>Master Mix</strong></td>
</tr>
<tr>
<td>Oligonucleotide 1</td>
</tr>
<tr>
<td>Oligonucleotide 2</td>
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<tr>
<td>Oligonucleotide 3</td>
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<tr>
<td>Oligonucleotide 4</td>
</tr>
</tbody>
</table>

During the amplification reaction on the Abbott m2000rt instrument, target DNA is amplified by DNA Polymerase in the presence of primers, deoxyribonucleoside triphosphates (dNTPs), and magnesium chloride (MgCl2). The DNA Polymerase used in the assay is a thermophilic enzyme that has been chemically modified rendering it inactive.

During the amplification reaction of Abbott RealTime IDH2, DNA Polymerase is first activated at a high temperature. During each subsequent round of thermal cycling, a high temperature is used to melt double-stranded DNA strands, followed by a low temperature where primers anneal to their respective targets and are extended to generate double-stranded DNA products. Exponential amplification of the products is achieved through repeated cycling between high and low temperatures. Amplification of IDH2 mutation and IC targets takes place simultaneously in the same PCR well.

IDH2 products are detected during the annealing/extension step by measuring the real-time fluorescence signals of the IDH2 mutation and IC-specific probes, respectively. The IDH2 mutation and IC-specific probes are labeled with different fluorophores, allowing their signals to be distinguishable in a single PCR well.

**Assay Results**

For each patient sample, 4 PCR reactions are evaluated. Abbott RealTime IDH2 is a qualitative assay for which specimen interpretations are reported as “Mutation Detected” or “Not Detected.” For specimens with interpretations of “Mutation Detected”, the identity of the IDH2 mutation detected is reported. Refer to the **INTERPRETATION OF RESULTS** section for further details.

**PREVENTION OF NUCLEIC ACID CONTAMINATION**

The possibility of nucleic acid contamination is minimized because:

- Abbott RealTime IDH2 performs amplification and fluorescence detection in a sealed Abbott 96-Well Optical Reaction Plate.
- Detection is carried out automatically without the need to open the 96-Well Optical Reaction Plate.
- Aerosol barrier pipette tips are used for all pipetting. The pipette tips are discarded after use.
- Separate dedicated areas are used to perform Abbott RealTime IDH2. Refer to the **SPECIAL PRECAUTIONS** section of this package insert.

**REAGENTS**

Abbott RealTime IDH2 Amplification Reagent Kit (List No. 08N82-090)

1. **OLIGONUCLEOTIDE REAGENT 1** Abbott RealTime IDH2 Oligonucleotide Reagent 1 (List No. 8N82A) (1 vial, 0.880 mL)
   - 0.1% synthetic oligonucleotides and <1% dNTP, in a buffered solution with a reference dye. Preservatives: 0.084% sodium azide and 0.15% ProClin® 950.

2. **OLIGONUCLEOTIDE REAGENT 2** Abbott RealTime IDH2 Oligonucleotide Reagent 2 (List No. 8N82B) (1 vial, 0.880 mL)
   - 0.1% synthetic oligonucleotides and <1% dNTP, in a buffered solution with a reference dye. Preservatives: 0.084% sodium azide and 0.15% ProClin 950.

3. **OLIGONUCLEOTIDE REAGENT 3** Abbott RealTime IDH2 Oligonucleotide Reagent 3 (List No. 8N82C) (1 vial, 0.880 mL)
   - 0.1% synthetic oligonucleotides and <1% dNTP, in a buffered solution with a reference dye. Preservatives: 0.084% sodium azide and 0.15% ProClin 950.

4. **OLIGONUCLEOTIDE REAGENT 4** Abbott RealTime IDH2 Oligonucleotide Reagent 4 (List No. 8N82D) (1 vial, 0.880 mL)
   - 0.1% synthetic oligonucleotides and <1% dNTP, in a buffered solution with a reference dye. Preservatives: 0.084% sodium azide and 0.15% ProClin 950.

5. **DNA POLYMERASE** Abbott RealTime IDH2 DNA Polymerase (List No. 8N82E) (4 vials, 0.051 mL per vial)
   DNA Polymerase (5.4 to 5.9 Units/μL) in a buffered solution with stabilizers.

6. **ACTIVATION REAGENT** Abbott RealTime IDH2 Activation Reagent (List No. 8N82M) (1 vial, 1.200 mL)
   50 mM magnesium chloride in a buffered solution. Preservatives: 0.084% sodium azide and 0.15% ProClin 950.

Abbott RealTime IDH2 Control Kit (List No. 08N82-080)

- **CONTROL +** Abbott RealTime IDH2 Positive Control (List No. 8N82W) (5 vials, 0.210 mL per vial)
  <0.01% noninfectious plasmid DNA in a buffered solution with carrier DNA. Preservatives: sodium azide and 0.15% ProClin 950.

- **CONTROL -** Abbott RealTime IDH2 Negative Control (List No. 8N82Z) (5 vials, 0.210 mL per vial)
  <0.01% noninfectious plasmid DNA in a buffered solution with carrier DNA. Preservatives: sodium azide and 0.15% ProClin 950.
WARNINGS AND PRECAUTIONS

For In Vitro Diagnostic Use
Abbott RealTime IDH2 is for use with EDTA preserved human blood and EDTA preserved bone marrow aspirate.
Use only USP grade 190 to 200 proof ethanol (95 to 100% ethanol) to prepare the mLwash 300 sample preparation reagent. Do not use ethanol that contains denaturants.

Safety Precautions
Refer to the Abbott m2000rt Operations Manual, Hazards section, for instructions on safety precautions.
The Abbott RealTime IDH2 Oligonucleotide Reagent 1, Oligonucleotide Reagent 2, Oligonucleotide Reagent 3, Oligonucleotide Reagent 4, Activation Reagent, Positive Control, and Negative Control contain the following components:
- 2-Methyl-2H-isothiazol-3-one
- Sodium azide

The following warnings apply:
- H317 May cause an allergic skin reaction.
- EUH032 Contact with acids liberates very toxic gas.
- P261 Avoid breathing mist/vapours/spray.
- P272 Contaminated work clothing should not be allowed out of the workplace.
- P302+P352 IF ON SKIN: Wash with plenty of water.
- P338+P313 If skin irritation or rash occurs: Get medical advice/attention.
- P362+P334 Take off contaminated clothing and wash it before reuse.
- P501 Dispose of contents/container in accordance with local regulations.

Safety Data Sheet Statement: Important information regarding the safe handling, transport, and disposal of this product and the Abbott Sample Preparation System is dedicated to processing samples.

SPECIAL PRECAUTIONS

Abbott RealTime IDH2 is for use with EDTA preserved human blood and bone marrow aspirate specimens that have been collected and handled as described in the SPECIMEN COLLECTION, STORAGE, AND TRANSPORT TO THE TEST SITE section.
During preparation of samples, compliance with good laboratory practices is essential to minimize the risk of cross-contamination between samples, and the inadvertent introduction of nucleases into samples during and after the extraction procedure. Proper aseptic technique should always be used when working with nucleic acids. Amplification reactions such as PCR are sensitive to accidental introduction of product from previous amplification reactions. Incorrect results could occur if either the clinical specimen or the reagents used in the amplification step become contaminated.

Work Areas
It is recommended that 3 dedicated areas within the laboratory be used when performing the Abbott RealTime IDH2 assay. Physically separating the activities involved when performing PCR are measures taken to minimize the risk of contamination.
- The Reagent Preparation Area is dedicated to preparing reagents. All reagents used in the Reagent Preparation Area should remain in this dedicated area at all times. Pipettes, pipette tips, and vortex mixers used in the Reagent Preparation Area must remain in this area and not be moved to the other areas. Do not bring samples or amplification products into the Reagent Preparation Area.
- The Sample Preparation Area is dedicated to processing samples (specimens and controls) and to adding processed samples and controls to the Abbott 96-Well Optical Reaction Plate. All reagents used in the Sample Preparation Area should remain in this dedicated area at all times. Laboratory coats, pipettes, pipette tips, and vortex mixers used in the Sample Preparation Area must remain in this area and not be moved to the Reagent Preparation and Amplification Areas. Do not bring amplification product into the Sample Preparation Area.
- The Amplification Area is dedicated to the amplification and detection of amplified product. Laboratory coats and equipment used in the Amplification Area must remain in this area and not be moved to the Reagent Preparation and Sample Preparation Areas.
- Work areas and instruments must be considered potential sources of contamination. Change gloves after contact with potential contaminant (such as DNase, specimens, eluates, and/or amplified products) or before handling unopened reagents, controls, or specimen controls. Refer to the Abbott m2000rt Operations Manual and the instructions in the POST PROCESSING PROCEDURES section for cleaning procedures.
- If the assay procedure is incorrectly performed or is interrupted at any point so that the timing of the steps exceeds the required timing, dispose of all commodities and single-use reagents that have been used in the assay procedure according to the instructions in the POST PROCESSING PROCEDURES section. For an incomplete, interrupted, or aborted Abbott m2000rt Instrument, dispose of the Abbott 96-Well Optical Reaction Plate in a sealed plastic bag according to the Abbott m2000rt Operations Manual. The gloves used to handle the plate should be disposed of in a waste container.
- Decontaminate and dispose of all reagents, reagents, and other potentially hazardous materials in accordance with local, state, and federal regulations.
- All materials should be handled in a manner that minimizes the chance of potential contamination of the work area.

Note: Autoclaving the sealed reaction plate will not eliminate the amplified product and may contribute to the release of the amplified product by opening of the seal. The laboratory area can become contaminated with amplified product if the waste materials are not carefully handled and contained before and after processing.

Contamination and Inhibition
The following precautions should be observed to minimize the risks of contamination, cross-contamination between samples, and inhibition:
- Wear appropriate personal protective equipment at all times.
- Use powder-free gloves.
- Change gloves after contact with potential contaminants (specimens, eluates, and/or amplified products).
- Pipettes with aerosol barrier tips must be used for all pipetting. The length of the tip should be sufficient to prevent contamination of the pipette barrel. While pipetting, care should be taken to avoid touching the pipette barrel to the inside of the sample tube or container.

STORAGE INSTRUCTIONS
Abbott RealTime IDH2 Amplification Reagent Kit (List No. 08N82-090)
The Abbott RealTime IDH2 Amplification Reagent Kit (List No. 08N82-090) must be stored at –25 to –15°C when not in use. Care must be taken to separate the Abbott RealTime IDH2 Amplification Reagent Kit that is in use from direct contact with samples and controls.

Abbott RealTime IDH2 Control Kit (List No. 08N82-080)
-20°C to –15°C. The Abbott RealTime IDH2 Control Kit (List No. 08N82-080) must be stored at –25 to –15°C.

SHIPPING CONDITIONS

<table>
<thead>
<tr>
<th>Component</th>
<th>Shipping Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott RealTime IDH2 Amplification Reagent Kit</td>
<td>Dry Ice</td>
</tr>
<tr>
<td>Abbott RealTime IDH2 Control Kit</td>
<td>Dry Ice</td>
</tr>
</tbody>
</table>
INDICATION OF INSTABILITY OR DETERIORATION OF REAGENTS
When control values are out of the expected range, it may indicate deterioration of the reagents. Associated test results are invalid and samples must be retested. Refer to the QUALITY CONTROL PROCEDURES section of this package insert for details.

If you receive reagents or controls that are in a condition contrary to label recommendation, or that are damaged, contact Abbott Molecular Customer Service.

SPECIMEN COLLECTION, STORAGE, AND TRANSPORT TO THE TEST SITE

Blood and Bone Marrow Aspirate Sample Collection and Storage

Human blood (EDTA) and bone marrow aspirate (EDTA) specimens may be used with the Abbott RealTime IDH2 assay. Follow the manufacturer’s instructions for processing collection tubes. After collection, specimens may be stored:

- At 15 to 30°C for up to 48 hours
- At 2 to 8°C for up to 7 days
- At -20°C ± 5°C for longer term

Multiple freeze/thaw cycles should be minimized and should not exceed 3 freeze/thaw cycles. Document the number of freeze/thaws. Thaw specimens at 15 to 30°C or at 2 to 8°C. Once thawed, if specimens are not being processed immediately, they can be stored at 2 to 8°C for up to 24 hours.

Specimen Transport

Ship specimens with cold packs/boxes. The total time during transport should not exceed 48 hours. When shipment within 48 hours is not achievable, ship specimens on dry ice. Transport time on dry ice should not exceed 7 days provided that the dry ice is refilled during shipment and that the specimens remain frozen throughout transport. For domestic and international shipments, specimens should be packaged and labeled in compliance with applicable state, federal, and international regulations covering the transport of clinical, diagnostic, or biological specimens.

INSTRUMENT PROCEDURE

The Abbott RealTime IDH2 application specification file must be installed on the Abbott m2000rt instrument from the Abbott RealTime IDH2 m2000rt Application CD-ROM prior to performing the assay. For detailed information on application specification file installation, refer to the Abbott m2000rt Operations Manual, Operating Instructions section.

AABBOTT REALTIME IDH2 ASSAY PROCEDURE

Materials Provided

- Abbott RealTime IDH2 Amplification Reagent Kit (List No. 08N82-090)

Materials Required But Not Provided

- Abbott RealTime IDH2 Control Kit (List No. 08N82-080)

Reagent Preparation Area

- Calibrated precision pipettes capable of delivering 10 to 1000 µL
- 10 to 1000 µL PCR-grade aerosol barrier pipette tips for precision pipettes
- Vortex mixer

Sample Preparation Area

- Abbott mSample Preparation System (List No. 06K12-24)
- Magnetic rack (12-position) for 2.0 mL microtubes
- Abbott 96-Well Optical Reaction Plate (List No. 04J71-70)
- Abbott Splash-Free Support Base (List No. 9K31)
- Abbott Optical Adhesive Cover (List No. 04J71-75)
- Abbott Adhesive Cover Applicator (List No. 9K32)
- USP grade 190 to 200 proof (95 to 100%) ethanol
- Do not use ethanol that contains denaturants.
- 2.0 mL screw-cap microtubes (Sarstedt Cat. No. 72.693.005 or equivalent)
- 2.0 mL screw-caps (Sarstedt Cat. No. 65.716.725 or equivalent)
- 50 mL polypyrrole centrifuge tubes
- Graduated cylinder, 100 mL
- Serological pipettes
- Timer
- Calibrated precision pipettes capable of delivering 10 to 1000 µL
- 10 to 1000 µL PCR-grade aerosol barrier pipette tips for precision pipettes
- Microcentrifuge racks
- Vortex mixer
- Centrifuge capable of 1200 to 1500 g with rotor to accommodate Abbott 96-Well Optical Reaction Plate
- Heat blocks, 56 ± 3°C and 75 ± 5°C, suitable for 2.0 mL microtubes

Amplification Area

- Abbott m2000rt Instrument, List No. 09K15-01 (with System Software Version 8.0 or higher)
- Abbott RealTime IDH2 m2000rt Application CD-ROM (List No. 08N82-001)
- Abbott m2000rt Optical Calibration Kit (List No. 04J71-93)

Other Materials

- Lab coat
- Powder-free disposable gloves
- Protective eyewear
- Solid waste container
- Liquid waste container
- Sealable plastic bags

Procedural Precautions

- Read the instructions in the package insert carefully before processing samples. The Abbott RealTime IDH2 Amplification Reagent Kit is intended for use with the Abbott RealTime IDH2 Control Kit and the Abbott mSample Preparation System. For sample processing, and the Abbott m2000rt instrument for amplification and detection.
- For a detailed description of how to operate the Abbott m2000rt instrument, refer to the Abbott m2000rt Operations Manual, Operating Instructions section.
- Laboratory personnel must be trained to operate the Abbott m2000rt instrument. The operator must have thorough knowledge of the assay application run on the instrument and must follow good laboratory practices.
- Do not use kits or reagents beyond the expiration date.
- All kits should be stored at the proper temperature upon receipt. Refer to the STORAGE INSTRUCTIONS section for details. Do not use incorrectly stored reagents.
- Kit components from the same kit lot are intended to be used together. For example, do not use the Oligonucleotide Reagent from kit lot X with the DNA Polymerase Reagent from kit lot Y.
- In the Abbott RealTime IDH2 Amplification Reagent Kit, the Oligonucleotide Reagent and Activation Reagent vials are intended for single preparation only and should be discarded after use.
- In the Abbott RealTime IDH2 Control Kit, each Negative Control and Positive Control vial is intended for single use only and should be discarded after use.
- The Amplification Reagent Kit and Control Kit can be thawed and refrozen up to 3 times before use.
- The use of the Abbott RealTime IDH2 Positive Control and Negative Control are integral to the performance of the Abbott RealTime IDH2 assay. Abbott RealTime IDH2 controls must be processed alongside the specimens to be tested. Refer to the QUALITY CONTROL PROCEDURES section of this package insert for details.
- Use only USP Grade 190 to 200 proof (95 to 100%) ethanol to prepare the mWash 2DNA Buffer. Do not use ethanol that contains denaturants.
- Use aerosol-barrier pipette tips. The use of extended aerosol-barrier pipette tips is recommended to prevent contamination.
- Ensure that each sample eluate is transferred into the correct locations of the Abbott 96-Well Optical Reaction Plate designated by the test order. Refer to the Abbott m2000rt Initiation and Test Order Creation section of this package insert for details.
- To reduce the risk of nucleic acid contamination, clean and disinfect spills of specimens, reagents, and controls by using a detergent solution followed by a tuberculocidal disinfectant such as 1.0% (v/v) sodium hypochlorite or other suitable disinfectants. Refer to Safety Precautions for additional instructions.
ASSAY PROTOCOL
The Abbott RealTime IDH2 assay protocol includes the following steps:

A. Manual preparation (i.e., DNA extraction) of samples (specimens and controls) using the Abbott mSample Preparation SystemDNA Kit.

B. PCR assay setup using the sample eluates and the Abbott RealTime IDH2 Amplification Reagent Kit.

C. Amplification/detection on the Abbott m2000rt instrument.

Refer to the WARNINGS AND PRECAUTIONS section of this package insert for instructions before preparing samples. At least one Positive Control and one Negative Control must be included in each run.

Note: Each Abbott mSample Preparation SystemDNA Kit contains 4 sets of reagents. Each set of reagents can support preparation (i.e., DNA extraction) of up to 24 samples (patient specimens and/or assay controls). Discard any reagents remaining after 24 preparations.

Note: Per magnetic rack, a maximum of 12 samples (patient specimens and/or assay controls) can undergo DNA extraction. It is not recommended to perform DNA extraction in batch sizes that exceed 12 samples.

Note: Each Abbott RealTime IDH2 Amplification Reagent Kit supports testing of up to 24 samples (patient specimens and/or assay controls).

Reagent Preparation Area

Thawing of Amplification Reagents
New or previously prepared master mixes may be used (see Preparation of Amplification Master Mixes).

1. If a new master mix is needed, thaw the Oligonucleotide Reagents and Activation Reagent at 15 to 30°C or at 2 to 8°C.
   • Once thawed, if the amplification reagents are not being used immediately, they can be stored at 2 to 8°C for up to 24 hours until required for preparation of the amplification master mixes. OR
   • If previously prepared frozen master mixes are used, thaw the master mixes at 15 to 30°C for up to 30 minutes prior to PCR setup. Frozen master mixes should not undergo more than 5 freeze/thaw cycles.

Sample Preparation Area

Thawing of Assay Controls
2. Thaw assay controls at 15 to 30°C or at 2 to 8°C. One Positive Control and one Negative Control are required in each DNA extraction run; see the QUALITY CONTROL PROCEDURES section of the package insert.
   • Once thawed, if the assay controls are not being processed immediately, they can be stored at 2 to 8°C for up to 24 hours prior to DNA extraction.

Preparation of Archived Specimens
3. Thaw blood or bone marrow aspirate specimens at 15 to 30°C or at 2 to 8°C.
   • Once thawed, if blood or bone marrow aspirate specimens are not being processed immediately, they can be stored at 2 to 8°C for up to 24 hours.

Preparation of Heat Blocks
4. Turn on the temperature controlled dry heating blocks:
   a. Set one heat block at 50 ± 3°C
   b. Set another heat block at 75 ± 5°C
   If only one heat block is available then set to 56 ± 3°C and equilibrate to 75 ± 5°C after step 28.

Note: Check the temperature of the heating block(s). Do not proceed until the heating block(s) are at the correct temperature.

WARNING: To avoid personal injury, follow the manufacturer’s instructions for heat blocks. To avoid burns, turn off the power and allow the heat blocks to cool to 35°C or below before handling.

Preparation of DNA Extraction Reagents
5. Remove one set of Abbott mSample Preparation SystemDNA reagent bottles from the kit, and gently invert each bottle except mMicroparticlesDNA to ensure a homogeneous solution. If crystals are observed in any of the reagent bottles upon opening, allow the reagent to equilibrate at room temperature until the crystals disappear. Do not use the reagents without allowing the crystals to dissolve.

Note: Each set of Abbott mSample Preparation SystemDNA Reagents (labeled as “No Reuse”) may be used for multiple DNA extraction runs, depending on the number of samples processed per run.

6. Prepare the mWash 2DNA Buffer working solution by adding 70 mL of 190 to 200 proof (95 to 100%) ethanol to the mWash 2DNA bottle. Cap the bottle and gently invert 5 to 10 times to mix. Mark the bottle to indicate ethanol has been added, and assign an expiration date of 2 months from the date of preparation. Store at 15 to 30°C.

DNA Extraction

Lysis
7. Label a 2.0 mL Sarstedt tube or equivalent for each patient specimen and assay control.
   • One IDH2 Negative Control and one IDH2 Positive Control must be processed alongside the patient specimens to be tested.

8. Resuspend mMicroparticlesDNA by vortexing or vigorously shaking until particles are in suspension and settled particles are no longer seen on the bottom of the bottle. After the particles are resuspended, add 300 μL of mMicroparticlesDNA to each tube.

9. Add 1.3 mL of mLysisDNA to each 2.0 mL tube.

10. Prepare patient specimens and control.
    • Gently invert each patient specimen a minimum of 10 times to ensure homogeneity.
    • Vortex each assay control 3 times for 2 to 4 seconds before use. After vortexing, tap each vial on the bench to bring the liquid to the bottom of the vial.

11. Add patient specimen or controls to the appropriate tubes.
    • For controls add 200 μL.
    • For blood specimens add 100 μL.
    • For bone marrow aspirate specimens add 100 μL.
    The Positive Control and Negative Control vials are intended for single use only; remaining reagents after first use should be discarded.

12. Invert each tube three times and vortex for 30 seconds.

13. Place the tubes in the 56°C heating block for 7 ± 1 minutes.

14. Remove the tubes from the heating block and vortex for 5 seconds.

15. Place the tubes in the 56°C heating block for 8 ± 1 minutes.

Wash
16. Place the tubes in a magnetic rack for 1 minute to allow the particles to be captured on the sides of the tubes. With the tube in the magnetic rack, during the incubation, remove and discard the cap from each tube.

17. With the tube in the magnetic rack, use a fresh, sterile 1000 μL aerosol barrier pipette tip for each sample to carefully remove the liquid from each tube and discard the liquid into a designated guanidine waste container. Remove the liquid as completely as possible.

Do not disturb or aspirate the captured magnetic particles.
18. Place the tubes in a microcentrifuge rack and for each tube:
    • Add 900 μL of mLysisDNA.
    • Attach a new cap.
    • Vortex for 5 seconds.

19. Place the tubes in the 56°C heating block for 3 ± 1 minutes.

20. Place the tubes in a magnetic rack for 1 minute to allow the particles to be captured on the sides of the tubes. With the tube in the magnetic rack, during the incubation, remove and discard the cap from each tube.

21. With the tube in the magnetic rack, use a fresh, sterile 1000 μL aerosol barrier pipette tip for each sample to carefully remove the liquid from each tube and discard the liquid into a designated guanidine waste container. Remove the liquid as completely as possible.

Do not disturb or aspirate the captured magnetic particles.
22. Place the tubes in a microcentrifuge rack and for each tube:
    • Add 900 μL of mWash 1DNA.
    • Attach a new cap.
    • Vortex for 5 seconds.

23. Place the tubes in the 56°C heating block for 1 to 2 minutes.

24. Place the tubes in a magnetic rack for 1 minute to allow the particles to be captured on the sides of the tubes. With the tube in the magnetic rack, during the incubation, remove and discard the cap from each tube.
25. With the tube in the magnetic rack, use a fresh, sterile 1000 μL aerosol barrier pipette tip for each sample to carefully remove the liquid from each tube and discard the liquid into a designated guanidine waste container. Remove the liquid as completely as possible. **Do not disturb or aspirate the captured magnetic particles.**

26. Place the tubes in a microcentrifuge rack for each tube:
   - Add 900 μL of mWash 1<sub>DNA</sub>. 
   - Attach a new cap.
   - Vortex for 5 seconds.
27. Place the tubes in the 56 °C heating block for 1 to 2 minutes.
28. Place the tubes in a magnetic rack for 1 minute to allow the particles to be captured on the sides of the tubes. With the tube in the magnetic rack, during the incubation, remove and discard the cap from each tube.
29. With the tube in the magnetic rack, use a fresh, sterile 1000 μL aerosol barrier pipette tip for each sample to carefully remove the liquid from each tube and discard the liquid into a designated guanidine waste container. Remove the liquid as completely as possible. **Do not disturb or aspirate the captured magnetic particles.**

30. Place the tubes in a microcentrifuge rack for each tube:
   - Add 900 μL of mWash 2<sub>DNA</sub>. 
   - Attach a new cap.
   - Vortex for 5 seconds.
31. Place the tubes in a magnetic rack for 1 minute to allow the particles to be captured on the sides of the tubes. With the tube in the magnetic rack, during the incubation, remove and discard the cap from each tube.
32. With the tube in the magnetic rack, use a fresh, sterile 1000 μL aerosol barrier pipette tip for each sample to carefully remove the liquid from each tube and discard the liquid into a designated guanidine waste container. Remove the liquid as completely as possible. **Do not disturb or aspirate the captured magnetic particles.**

33. Place the tubes in a microcentrifuge rack for each tube:
   - Add 900 μL of mWash 2<sub>DNA</sub>. 
   - Attach a new cap.
   - Vortex for 5 seconds.
34. Place the tubes in a magnetic rack for 1 minute to allow the particles to be captured on the sides of the tubes. With the tube in the magnetic rack, during the incubation, remove and discard the cap from each tube.
35. With the tube in the magnetic rack, use a fresh, sterile 1000 μL aerosol barrier pipette tip for each sample to carefully remove the liquid from each tube and discard the liquid into a designated guanidine waste container. Remove the liquid as completely as possible. **Do not disturb or aspirate the captured magnetic particles.**

36. Place the tubes in the 75 °C heating block for 13 ± 1 minutes. 
**Note:** The tubes should not be capped.

**DNA Elution**

37. Place the tubes in a microcentrifuge rack for each tube:
   - Add 500 μL of mElution Buffer<sub>DNA</sub>. 
   - Attach a new cap.
   - Vortex for 5 seconds.
38. Place the tubes in the 75 °C heating block for 6 ± 1 minutes.
   - During the incubation, label new 2.0 mL tubes for the DNA eluates.
39. Vortex for 5 seconds.
40. Return the tubes in the 75 °C heating block for 6 ± 1 minutes.
41. Vortex for 5 seconds.
42. Return the tubes in the 75 °C heating block for 6 ± 1 minutes.
43. Vortex for 5 seconds.
44. Return the tubes in the 75 °C heating block for 6 ± 1 minutes.
45. Vortex for 5 seconds.

46. Place the tubes in a magnetic rack for 1 minute to allow the particles to be captured on the sides of the tubes. With the tube in the magnetic rack, during the incubation, remove and discard the cap from each tube.
47. With the tube in the magnetic rack, use a fresh, sterile 1000 μL aerosol barrier pipette tip for each sample to carefully transfer the eluted sample from each tube into a new, labeled 2.0 mL tube. **Do not disturb or aspirate the captured magnetic particles.**

DNA eluates for specimens or controls can be stored at:
- -25 to -15 °C or colder for up to 2 months, and should not undergo more than 5 freeze/thaw cycles;
- 15 to 30°C for 24 hours;
- 2 to 8°C for 14 days;
Frozen DNA eluates can be thawed at 15 to 30°C or at 2 to 8°C prior to PCR setup. Storage of DNA eluates must not exceed a cumulative total of 24 hours at 15 to 30°C or 14 days at 2 to 8°C prior to PCR setup.

**Amplification Area**

**Abbott m2000rt Initiation and Test Order Creation**

48. Switch on and initialize the Abbott m2000rt instrument.
49. Create an Abbott m2000rt test order. Refer to the Operating Instructions section of the Abbott m2000rt Operations Manual. From the Protocol screen, select the appropriate application specification file corresponding to the Abbott RealTime IDH2 assay.
50. A 12-sample (2 controls and 10 specimens) setup for the Abbott 96-well reaction plate is shown in Figure 1 below.

**Figure 1: Example of a 12 Sample Setup for Abbott 96-Well Optical Reaction Plate**

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<tbody>
<tr>
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<td>POS</td>
<td>NEG</td>
<td>POS</td>
<td>POS</td>
<td>POS</td>
<td>POS</td>
<td>POS</td>
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<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

Figure 1 represents a partial image of the Abbott 96-Well Optical Reaction Plate.
Each control or sample must be placed in 4 adjacent wells, as indicated in Figure 1 and as described below:
- Oligo 1 master mix may only be added to columns 1, 5, and 9.
- Oligo 2 master mix may only be added to columns 2, 6, and 10.
- Oligo 3 master mix may only be added to columns 3, 7, and 11.
- Oligo 4 master mix may only be added to columns 4, 8 and 12.

**Negative control (NEG), Positive Control (POS), and specimens may be placed anywhere on the plate, following the 4 adjacent well requirement.**

**Note:** Remove gloves before returning to the Reagent Preparation Area.

**Reagent Preparation Area**

**Preparation of the Amplification Master Mix**

A. If using previously prepared Oligonucleotide-specific master mixes, proceed to PCR Setup.
B. If combining multiple vials of Oligonucleotide-specific master mixes:
   - Only combine Oligonucleotide-specific master mixes from the same Amplification Reagent Kit lot.
   - Only combine Oligonucleotide-specific master mixes (eg, only combine Oligonucleotide 1 master mix with Oligonucleotide 1 master mix).
   - Only combine a volume of Oligonucleotide-specific master mix sufficient for current testing. Once combined, remaining volume of combined Oligonucleotide-specific master mixes should be discarded.
   - Combine Oligonucleotide-specific master mixes in a 2.0 mL microcentrifuge tube or 5 mL tube. The required volume of each Oligonucleotide-specific master mix is 45 μL × (total number of samples and controls + 1). Mix by gently pipetting up and down 5 times. Ensure that no foam or bubbles are created. Label the tube with the appropriate master mix designation (eg, Oligonucleotide 1 master mix).
C. If preparing new Oligonucleotide-specific master mixes:

1. Prior to opening the amplification reagents, tap each vial in an upright position on the bench to bring the liquid to the bottom.

2. Gently mix contents of Oligonucleotide Reagent and Activation Reagent vials by pipetting up and down 5 times prior to use.

   Note: To prepare master mixes, use a calibrated precision pipet designator for master mix reagent use only.

   • Prepare Oligo 1 master mix by adding the following to a new DNA Polymerase vial:
     a. 870 μL of Oligonucleotide Reagent 1.
     b. 275 μL of Activation Reagent.
   • Prepare Oligo 2 master mix by adding the following to a new DNA Polymerase vial:
     a. 870 μL of Oligonucleotide Reagent 2.
     b. 275 μL of Activation Reagent.
   • Prepare Oligo 3 master mix by adding the following to a new DNA Polymerase vial:
     a. 870 μL of Oligonucleotide Reagent 3.
     b. 275 μL of Activation Reagent.
   • Prepare Oligo 4 master mix by adding the following to a new DNA Polymerase vial:
     a. 870 μL of Oligonucleotide Reagent 4.
     b. 275 μL of Activation Reagent.

Vortex vial 3 times for 2 to 3 seconds. Minimize the creation of foam or bubbles. Label as “Oligo 1 master mix.”

Note: Oligonucleotide Reagent and Activation Reagent vials are intended for single preparation only. Remaining reagents after first preparation should be discarded.

Note: The Abbott m2000rt protocol (PCR Run Initiation, step 62) must be initiated within 60 minutes after preparation of the amplification master mixes.

Note: Remove gloves before returning to the Sample Preparation Area.

Sample Preparation Area

PCR Setup

51. Place an Abbott 96-Well Optical Reaction Plate onto an Abbott Splash-Free Support Base. DO NOT touch the surface or bottom of the plate.

52. Prior to use, mix the amplification master mix by gently pipetting up and down 5 times. Ensure that no foam or bubbles are created.

53. Pipette 45 μL of the master mix into the required number of wells of the Abbott 96-Well Optical Reaction Plate according to the test order.

54. Store the remainder of prepared master mixes (optional):

   • Store the remaining master mixes tightly capped and protected from light for up to 7 days at 2 to 8°C or 60 days at –25 to –15°C.
   • Frozen master mixes cannot undergo more than 5 freeze/thaws.

55. Visually verify that 45 μL of master mix have been dispensed into each designated well.

56. Prior to use, vortex each sample DNA elute tube for 10 seconds. After vortexing, tap each tube on the bench to bring the liquid to the bottom of the tube.

57. Pipette 15 μL of DNA elute into each well designated for a sample according to the test order. During the transfer of each elute, mix the reaction by pipetting up and down 3 to 5 times. Change pipette tips between wells.

58. Visually verify that 60 μL total have been dispensed into each designated well.

59. Seal the Abbott 96-Well Optical Reaction Plate with the Abbott Optical Adhesive Cover according to the instructions in the Abbott m2000rt Operations Manual.

60. Ensure that all material is at the bottom of each well. If needed, centrifugate the Abbott 96-Well Optical Reaction Plate in the Abbott Splash-Free Support Base at 1200 to 1500 g for 1 minute.

Amplification Area

PCR Run Initiation

61. Transfer the Abbott 96-Well Optical Reaction Plate from the Abbott Splash-Free Support Base to the Abbott m2000rt instrument.

62. Select the Abbott m2000rt test order created in Abbott m2000rt Initiation and Test Order Creation, Step 49. Initiate the run, as described in the Abbott m2000rt Operations Manual, Operating Instructions section.

63. At the completion of the run, assay results are reported on the Abbott m2000rt instrument. Refer to the INTERPRETATION OF RESULTS section of the package insert for further details.

POST PROCESSING PROCEDURES

1. Place the Abbott 96-Well Optical Reaction Plate in a sealable plastic bag and dispose according to the Abbott m2000rt Operations Manual, along with the gloves used to handle the plate.

2. Clean the Abbott Splash-Free Support Base before reuse, according to Abbott m2000rt Operations Manual.

3. Decontaminate and dispose of all specimens, chemical reagents, and other potentially contaminated materials in accordance with local, state, and federal regulations.

4. Remove and discard all disposables, as well as liquid and solid waste, in accordance with local, state, and federal regulations.

5. At the end of each run, clean and disinfect all work areas.

Decontaminate work area according to laboratory guidelines.

QUALITY CONTROL PROCEDURES

Abbott m2000rt Optical Calibration

Refer to the Calibration Procedures section in the Abbott m2000rt Operations Manual for a detailed description of how to perform an Abbott m2000rt Optical Calibration. Optical calibration of the Abbott m2000rt instrument is required for the accurate measurement and discrimination of dye fluorescence during the Abbott RealTime IDH2 assay. The following Abbott m2000rt Optical Calibration Plates are used to calibrate the Abbott m2000rt instrument for the Abbott RealTime IDH2 assay:

• FAM™ Plate (Carboxyfluorescein)
• VIC™ Plate (Carboxyfluorescein)
• NED™ Plate (Carboxyfluorescein)
• ROX™ Plate (Carboxy-X-rhodamine)
• Cy5 Plate (Cyanine)

Positive and Negative Controls

A Positive Control and a Negative Control are required in each run to verify that the sample processing, the amplification, and the detection steps are performed correctly. The Abbott RealTime IDH2 controls are processed alongside the specimens prior to running the amplification portion of the assay.

The Positive Control is formulated with DNA containing IDH2 mutation and IC sequences. R140Q, R140W, R172K, and R172W mutations should be detected for the Positive Control. In addition, the IC should be detected in all 4 reactions for the Positive Control. The Negative Control is formulated with DNA containing the IC sequence. Only the IC should be detected in all 4 reactions for the Negative Control.

IDH2 mutations (R140Q, R140L, R140G, R140W, R172K, R172M, R172G, R172S, and R172W) should not be detected in the Negative Control. IDH2 mutations detected in the Negative Control are indicative of contamination from other samples or amplified product introduced during sample processing or during preparation of the Abbott 96-Well Optical Reaction Plate. To remove contamination, clean the working area, the equipment, and the Abbott m2000rt instrument according to the Abbott m2000rt Operations Manual and the instructions in the POST PROCESSING PROCEDURES section. Following cleaning, repeat sample processing for specimens and controls.

IC results for the Negative Control and the Positive Control that are outside the validity limit may indicate the occurrence of inhibition during DNA extraction or during the amplification reaction steps of the assay. Repeat the processing for specimens (starting from specimen preparation) and controls (starting from DNA extraction).

If the result for the Negative Control or the Positive Control is out of range, the run is invalid and a flag is displayed for each specimen. Refer to “Assay Specific Error Codes” and “Repeating Invalid Runs or Invalid Samples.”
Monitoring the Laboratory for the Presence of Contamination

It is recommended that this be done when contamination is suspected (eg, by amplification product, patient specimen, or Positive Control). Contamination may be indicated by a Negative Control failure. It is very important to test all areas that may have been exposed to processed specimens and controls, and/or amplification product. This includes routinely handled objects such as pipettes, function keys for the Abbott m2000rt, magnetic racks, temperature blocks, laboratory bench surfaces, microcentrifuges, and centrifuge adaptors.

1. Add 0.6 mL molecular biology grade water to a 1.7 mL DNase-free microcentrifuge tube for each laboratory surface area to be monitored.

2. Saturate the cotton tip of an applicator (Puritan or equivalent) in the molecular biology grade water from the microcentrifuge tube.

3. Using the saturated cotton tip of the applicator, wipe the area to be monitored using a sweeping motion. Place the applicator into the microcentrifuge tube.

4. Swirl the cotton tip in molecular biology grade water 10 times, and then press the applicator along the inside of the tube so that the liquid drains back into the solution at the bottom of the microcentrifuge tube. Discard the applicator.

5. For each additional area to be monitored, repeat steps 2 through 4.

6. Test the samples according to the ASSAY PROTOCOL section of this package insert using a sample input volume of 200 μL for each swab sample.

7. Contamination is indicated by the presence of a positive CN value for R140Q, R140L, R140G, R140W, R172K, R172M, R172G, R172S, or R172W in the swab sample(s). If contamination is not present, CN values for R140Q, R140L, R140G, R140W, R172K, R172M, R172G, R172S or R172W will be -1 in the swab samples.

Note: The reported result and interpretation are not used to determine contamination because the IC signal may not be detected in the swab samples (ie, water).

8. If an IDH2 mutation is detected on equipment, follow the cleaning and decontaminating guidelines given in the equipment operations manual. If IDH2 is detected on surfaces, clean the contaminated areas with 1.0% (v/v) sodium hypochlorite solution, followed by 70% ethanol or water. Chlorine solutions may pit equipment and metal. Use sufficient amounts or repeated applications of 70% ethanol or water until chlorine residue is no longer visible.

9. Repeat testing of the contaminated area by following steps 1 through 6.

10. If the presence of contamination is detected again, repeat steps 8 and 9 until amplification of IDH2 mutation(s) is no longer detected.

INTERPRETATION OF RESULTS

The Abbott m2000rt instrument automatically reports the results on the m2000rt workstation. Examples of assay results and interpretations are provided in Table 3.

The Abbott RealTime IDH2 assay controls are used to establish run validity for the Abbott RealTime IDH2 assay. If an error code is generated for one or both assay controls, the run is invalid, and a "–QC" flag and/or "–QC" flag are displayed next to all specimen results.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Result</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>R140Q</td>
<td>Mutation Detected</td>
</tr>
<tr>
<td>Sample 2</td>
<td>Not Detected</td>
<td>Not Detected</td>
</tr>
</tbody>
</table>

a The displayed result will correspond to the mutation detected by the assay.

Results from all 4 PCR wells are evaluated for each specimen. If a mutation is detected, an interpretation of "Mutation Detected" will be displayed and the result field will display the identity of the mutation. If no mutations are detected, an interpretation of "Not Detected" will be displayed and the result field will display "Not Detected."

### Assay Specific Error Codes

Error codes specific to the Abbott RealTime IDH2 assay are listed in Appendix 1 and Appendix 2. For each error code, probable causes and corrective actions specific to the Abbott RealTime IDH2 are also listed. Information for additional error codes is presented in the Abbott m2000rt Operations Manual, Version 8.0 or higher, Troubleshooting and Diagnostics section.

### Repeating Invalid Runs or Invalid Samples

An invalid run in which an error code was generated for one or both assay controls should be repeated following the guidelines below.

- If the invalid run was due to an assignable error in plate setup or test order creation, repeat the run starting with Abbott m2000rt Initiation and Test Order Creation, by using the remaining eluates (controls and specimen(s)).
- If there was no assignable error, all of the controls and specimens must be reprocessed, beginning with sample preparation.

If an error code was generated for a patient specimen, the specimen should be retested following the guidelines below.

- If the error code was due to an assignable error in plate setup or test order creation, repeat the run starting with Abbott m2000rt Initiation and Test Order Creation, by using the remaining eluates (controls and specimen(s)).
- If there was no assignable error, the specimen that received the error code and controls must be reprocessed, beginning with sample preparation.

### LIMITATIONS OF THE ASSAY

- **FOR IN VITRO DIAGNOSTIC USE.**
- Abbott RealTime IDH2 is for use with human blood (EDTA) and bone marrow aspirate (EDTA) specimens only.
- Optional performance of this test requires appropriate specimen collection, handling, preparation, and storage (refer to the SPECIMEN COLLECTION, STORAGE, AND TRANSPORT TO THE TEST SITE section of this package insert).
- Use of the Abbott RealTime IDH2 assay is limited to personnel who have been trained in the procedures of molecular diagnostic assays, the Abbott m2000rt instrument, and the manual sample preparation method for Abbott RealTime IDH2.
- A "Not Detected" result does not preclude the presence of IDH2 mutations in the specimen. Assay results may be affected by inadequate specimen integrity, mutation content in the sample, and amount of amplifiable DNA.
- The instrument and assay procedures reduce the risk of contamination by amplification product. However, nucleic acid contamination from the positive control or specimens must be controlled by good laboratory practices and careful adherence to the procedures specified in this package insert.
- Abbott RealTime IDH2 is designed to detect IDH2 R140Q, R140L, R140G, R140W, R172K, R172M, R172G, R172S, and R172W mutations. Specimens with results reported as "Not Detected" may contain mutations that are not targeted by the assay.
- Analytical studies indicate that at high mutation percentage and high DNA input, the R140W probe can cross react with the R140Q mutation. In this case, the software reports the mutation which is present at the highest level. All IDH2 mutations were correctly called in the analytical studies.
- Components contained within a kit are intended to be used together. Do not mix components from different kit lots. For example, do not use the IDH2 Oligonucleotide Reagents from Amplification kit lot X with the DNA Polymerase Reagent from Amplification kit lot Y.
SPECIFIC PERFORMANCE CHARACTERISTICS

Bone marrow aspirate and blood were tested in all key analytical studies. For the most prevalent IDH2 mutations, AML clinical specimens were used, when possible; otherwise cell lines with IDH2 mutations were used. Ten separate cell lines were used in the analytical studies, each cell line contained a single IDH2 mutation and represented all 10 IDH2 mutations that can be detected by Abbott RealTime IDH2 including R140Q, R140L, R140G, R140W, R172K, R172M, R172G, R172S (cell lines for both R172S and R172Sc), and R172W. For the Repeatability, Lot-to-Lot Reproducibility, Extracted Clinical Specimen DNA Stability, Potentially Interfering Substances, Potentially Interfering Drugs, Potentially Interfering Microorganisms, and Analytical Carryover studies, cell lines were spiked into blood or bone marrow to target specific mutation percentages. For the Analytical Sensitivity–Limit of Detection, Characterization of IDH2 Mutation Detection at DNA Input Levels Across and Below Assay Range, and Activated Master Mix Use and Storage and Assembled Plate Stability studies, DNA eluates generated from blood or bone marrow spiked with cell lines were diluted to target specific mutation percentages and gDNA inputs. Functional equivalence studies were used to demonstrate that contrived specimens created using cell lines had comparable performance to clinical specimens in terms of limit of detection and precision.

Abbott RealTime IDH2 is designed such that quantitation of DNA prior to PCR amplification is not required. A fixed amount of eluate (15 μL) from sample extraction is used in each PCR reaction. In order to evaluate the typical DNA yield from AML specimens, DNA was extracted from 124 AML clinical specimens (74 bone marrow aspirate specimens and 50 blood specimens) with the Abbott mSample Preparation System DNA Kit. The DNA yield, as measured by the IC CN value, was similar for bone marrow and blood. The gDNA input range observed for bone marrow aspirate was 14 ng to 1,998 ng per 15 μL of eluate, which corresponds to an IC CN range of 15.01 CN to 22.00 CN. The gDNA input range observed for whole blood was 22 ng to 1,182 ng per 15 μL of eluate, which corresponds to an IC CN range of 15.77 CN to 21.50 CN. Abbott RealTime IDH2 assay performance was verified across a range of 10 ng to 2,000 ng gDNA input per PCR reaction.

ANALYTICAL SENSITIVITY

Limit of Detection

Analytical sensitivity was assessed using eluates generated from blood and bone marrow samples. The IDH2 percent mutation ranged from 0.25% to 10% for 10 ng gDNA input and from 0.13% to 10% mutation for 200 ng gDNA input. A 28 member panel was generated for each of the 10 SNVs targeted by the assay. IDH2 R140Q, R172K, and R140L mutation positive panel members were prepared from blood and bone marrow aspirate AML clinical specimen eluates. IDH2 R140G, R140W, R172M, R172S (AGT and AGC), R172W, and R172G mutation positive panel members were prepared from eluates of IDH2 mutation positive cell lines spiked into blood or bone marrow. Each panel member was tested with 2 Abbott RealTime IDH2 Amplification Reagent Kit lots with 5 runs per lot over a minimum of 5 days and 4 replicates per run for a total of 40 replicates per panel member per mutation and 400 replicates for the 10 mutations combined.

The analysis demonstrated a detection rate of 99.8% (399/400) or greater at mutation levels of 2% and higher for all IDH2 mutations combined. The analysis demonstrated a detection rate of 93.5% (374/400) or greater at mutation levels of 1% and higher for all IDH2 mutations combined. Refer to Table 4 for results.

<table>
<thead>
<tr>
<th>gDNA Input per Reaction</th>
<th>Sample Type</th>
<th>Mutation</th>
<th>Detection Ratio</th>
<th>Detection Rate</th>
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<tbody>
<tr>
<td>200 ng Blood</td>
<td>10%</td>
<td>400/400</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>200 ng Blood</td>
<td>5%</td>
<td>400/400</td>
<td>100</td>
<td></td>
</tr>
<tr>
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<td>1%</td>
<td>400/400</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>200 ng Blood</td>
<td>0.5%</td>
<td>400/400</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>200 ng Blood</td>
<td>0.25%</td>
<td>384/400</td>
<td>96</td>
<td></td>
</tr>
<tr>
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<td>283/400</td>
<td>70.8</td>
<td></td>
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<td>200 ng Bone Marrow</td>
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<td>301/400</td>
<td>75.3</td>
<td></td>
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<td>2%</td>
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<td>1%</td>
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<td>93.5</td>
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<td>67.8</td>
<td></td>
</tr>
<tr>
<td>10 ng Blood</td>
<td>0.25%</td>
<td>111/400</td>
<td>27.8</td>
<td></td>
</tr>
</tbody>
</table>

Table 4: Limit of Detection Study Results for all IDH2 Mutationsa Combined

Characterization of IDH2 Mutation Detection Across a Low to High Range of DNA Input Levels

This study evaluated the detection of IDH2 mutations targeted by the assay at several gDNA inputs across a range of 1 ng to 2,000 ng. For each IDH2 SNV, the following gDNA inputs were tested at 2% mutation level in both blood and bone marrow aspirate: approximately 1 ng, 50 ng, 600 ng, 1,000 ng, and 2,000 ng. A total of 100 panel members (10 per mutation) were each tested in triplicate. Three replicates per panel member per mutation were tested for a total of 30 replicates for the 10 mutations combined.

All panel members reported “Mutation Detected” at a 100% (30/30) detection rate when total gDNA input levels were 50 ng, 600 ng, 1,000 ng, and 2,000 ng per reaction. When the gDNA input level was 1 ng per reaction, an overall detection rate of 13% (4/30) was observed due to IC cycle number being nonreactive or greater than the maximum for non-detected replicates, as expected for the low input level. The valid replicates for the 1 ng per reaction input level reported “Mutation Detected” at a 100% detection rate. Refer to Table 5 for results.

The analysis demonstrated a detection rate of 100% at mutation levels of approximately 2% when the total gDNA was approximately 50 ng, 600 ng, 1,000 ng, and 2,000 ng per reaction for all mutations tested.

### Table 5. Detection of 2% Mutation Level for a Range of DNA Inputs for all IDH2 Mutations Combined

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>gDNA Input per Reaction (ng)</th>
<th>Number Valid Replicates</th>
<th>Number of Replicates Mutation Detected</th>
<th>Valid Replicate Detection Rate (%)</th>
<th>Total Number of Replicates</th>
<th>Overall Detection Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td>100</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>Blood</td>
<td>50</td>
<td>30</td>
<td>30</td>
<td>100</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>Blood</td>
<td>600</td>
<td>30</td>
<td>30</td>
<td>100</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>Blood</td>
<td>1,000</td>
<td>30</td>
<td>30</td>
<td>100</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>Blood</td>
<td>2,000</td>
<td>30</td>
<td>30</td>
<td>100</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>Bone Marrow</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td>100</td>
<td>30</td>
<td>13</td>
</tr>
<tr>
<td>Bone Marrow</td>
<td>50</td>
<td>30</td>
<td>30</td>
<td>100</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>Bone Marrow</td>
<td>600</td>
<td>30</td>
<td>30</td>
<td>100</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>Bone Marrow</td>
<td>1,000</td>
<td>30</td>
<td>30</td>
<td>100</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>Bone Marrow</td>
<td>2,000</td>
<td>30</td>
<td>30</td>
<td>100</td>
<td>30</td>
<td>100</td>
</tr>
</tbody>
</table>


**Limit of Blank**

The Limit of Blank (LoB) was verified by testing 10 normal blood specimens, 10 normal bone marrow aspirate specimens, and 2 blank samples (water). Each specimen or sample was tested in replicates of 4, yielding a total of 88 replicates (40 replicates for whole blood specimens, 40 replicates for bone marrow aspirate specimens, and 8 replicates for blank samples). The overall rate of correct sample interpretation was 100% (88/88) which demonstrated an acceptable rate of true negativity.

### ANALYTICAL SPECIFICITY

**Exclusivity**

This study evaluated the rate of correct sample interpretation for each Abbott RealTime IDH2 target using a panel representing IDH2 SNVs targeted by the assay, IDH2 SNVs not targeted by the assay, and wild type genomic DNA. Panel members were prepared such that each PCR reaction contained a total input of approximately 200 ng of wild type human placental (HP) DNA, or wild type HP DNA plus plasmid DNA resulting in a percent mutation of approximately 25% to 50% in 200 ng of total DNA. Panel Members included wild type HP DNA (R140, R172) and wild type HP DNA plus one of 12 DNA plasmids coding for each of the 10 IDH2 SNVs targeted by the assay (R140Q, R140L, R140G, R140W, R172K, R172M, R172G, R172S, R172Sc, R172St, R172W) or 2 IDH2 SNVs not targeted by the assay (R140P, R172T). The prepared panel members were directly tested using the Abbott RealTime IDH2 Amplification Reagent Kit. Forty replicates per panel member were tested.

The wild type HP DNA panel member reported “Not Detected” with a 100% (40/40) rate of correct sample interpretation. Ten panel members targeted by the assay each reported “Mutation Detected” with a 100% (40/40) rate of correct sample interpretation, which included the correct identity of the IDH2 mutation. Two panel members not targeted by the assay (R140P mutation, and R172T mutation) reported “Not Detected” with a 100% (40/40) rate of correct sample interpretation.

In this study, R140W probe cross reactivity was observed for the panel member containing the R140G mutation. When two mutations are detected, the software calls the mutation which is present at the highest level. As shown in this study the R140G mutation was called correctly for all 40 panel member replicates, thus a 100% rate of correct sample interpretation was demonstrated.

The Abbott RealTime IDH2 assay demonstrated a 100% (40/40) correct sample interpretation rate using a panel representing IDH2 SNVs targeted by the assay, IDH2 SNVs not targeted by the assay, and wild type genomic DNA.

**Potentially Interfering Substances**

Potentially interfering substances that may be found in clinical specimens were added to IDH2 mutation negative, IDH2 R140Q mutation positive, and IDH2 R172K mutation positive blood and bone marrow aspirate samples. For the IDH2 mutation positive panel members, R140Q or R172K positive cell lines were spiked in blood and bone marrow targeting a 5% mutation level. A total of 3 replicates of each test condition and each sample were evaluated for the presence of potential interference.

Test conditions included control diluents (one for bilirubin and one for all other substances) or 6 potentially interfering substances at study specified concentrations (hemoglobin, albumin, bilirubin, triglycerides, R(-)-2-hydroxyglutarate (2-HG), and EDTA). The analysis demonstrated the percent agreement was 100% (3/3) for each sample at each test condition.

No interference of the Abbott RealTime IDH2 assay was observed in the presence of the potentially interfering substances for all IDH2 mutation positive and negative samples for either blood or bone marrow aspirate specimens.
Potentially Interfering Drugs

Potentially interfering drugs that may be found in clinical specimens were added to IDH2 mutation negative, IDH2 R140Q mutation positive, and IDH2 R172K mutation positive blood and bone marrow aspirate specimens. For the IDH2 mutation positive panel members, R140Q or R172K positive cell lines were spiked in blood and bone marrow targeting a 6% mutation level. A total of 3 replicates of each test condition (control or Drug Pool 1 to 4) for each specimen was evaluated. The control condition tested specimens with no potentially interfering drugs added, whereas Drug Pool 1 to 4 tested specimens with added pooled drug combinations as described below.

### Abbott RealTime IDH2 Potentially Interfering Drug Pools

<table>
<thead>
<tr>
<th>Pool 1</th>
<th>Pool 2</th>
<th>Pool 3</th>
<th>Pool 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vancomycin Hydrochloride</td>
<td>Levofloxacin</td>
<td>Linezolid</td>
<td>Meropenem</td>
</tr>
<tr>
<td>Busulfan</td>
<td>Acyclovir</td>
<td>Arsenic Trioxide</td>
<td>Azacytidine</td>
</tr>
<tr>
<td>Decitabine</td>
<td>Cyclophosphamide</td>
<td>Cytarabine</td>
<td>Daunorubicin Hydrochloride</td>
</tr>
<tr>
<td>Hydroxyurea</td>
<td>Doxorubicin Hydrochloride</td>
<td>Etoposide</td>
<td>Fludara</td>
</tr>
<tr>
<td>Mitoxantrone Hydrochloride</td>
<td>Iadarubicin Hydrochloride</td>
<td>Lamustine</td>
<td>Melphalan</td>
</tr>
<tr>
<td>Everolimus</td>
<td>Vincristine Sulfate</td>
<td>Erythropeitin</td>
<td>Filgrastim</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>Mercaptopturate</td>
<td>Mycophenolate Mofetil</td>
<td>Tacrolimus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prednisone</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AG-221</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AG-12</td>
<td></td>
</tr>
</tbody>
</table>

Each drug was tested at three times the peak serum concentration (C.MAX), recommended dose as listed in the drug package inserts, or recommended concentration per Clinical and Laboratory Standards Institute EP7-A2.18 The analysis demonstrated the percent agreement was 100% (3/3) for each sample and test condition. No interference or cross reactivity of the Abbott RealTime IDH2 assay was observed in the presence of the potential interfering drugs for all IDH2 mutation positive and negative samples for either blood or bone marrow aspirate specimens.

### Potentially Interfering Microorganisms

Potentially interfering microorganisms were added to IDH2 mutation negative, IDH2 R140Q mutation positive, and IDH2 R172K mutation positive blood and bone marrow aspirate specimens. For the IDH2 mutation positive panel members, R140Q or R172K positive cell lines were spiked in blood and bone marrow targeting a 6% mutation level. A total of 3 replicates of each of 5 test conditions (control and Microbe Pools 1 to 4) were evaluated. The microbe pools were as follows:

### Abbott RealTime IDH2 Potentially Interfering Microbe Pools

<table>
<thead>
<tr>
<th>Pool 1</th>
<th>Pool 2</th>
<th>Pool 3</th>
<th>Pool 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus (MRSA; COL)</td>
<td>Streptococcus agalactiae</td>
<td>Enterococcus faecalis</td>
<td>Pseudomonas aeruginosa</td>
</tr>
<tr>
<td>Staphylococcus aureus (CoNS)</td>
<td>Streptococcus pneumoniae</td>
<td>Enterobacter cloacae</td>
<td>Acinetobacter baumannii</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>Klebsiella pneumoniae</td>
<td>Aspergillus terreus</td>
<td>varicella-zoster virus</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>Candida albicans</td>
<td>influenza A H3</td>
<td>respiratory syncytial virus type A</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>herpes simplex virus type 1 MucHyte</td>
<td>respiratory syncytial virus type B</td>
<td></td>
</tr>
<tr>
<td>cytomegalovirus</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Potentially interfering microorganisms were chosen based on clinical presentation in AML patients.19 The analysis demonstrated the percent agreement was 100% (3/3) for each sample and test condition. No interference or cross reactivity of the Abbott RealTime IDH2 assay was observed in the presence of the potentially interfering microorganisms for all IDH2 mutation positive and negative samples tested with either blood or bone marrow aspirate specimens.

### PRECISION

Within-Laboratory Repeatability

The within-laboratory repeatability was evaluated by two operators using a 12-member panel. The panel contained IDH2 mutation negative blood and bone marrow aspirate specimens as well as blood and bone marrow aspirate specimens spiked with R140Q, R140W, R172K, or R172W positive cell lines targeting a 6% mutation level. Positive mutations were chosen such that the panel contained a representative mutation amplified by each of the four master mixes. Each operator tested the panel using 2 lots of grouped reagents–2 lots of Abbott mSample Preparation System DNA kits, Abbott RealTime IDH2 Amplification Reagent Kits, and Abbott RealTime IDH2 Control Kits–for 4 runs per lot over 4 days. Each run consisted of 2 replicates for each panel member for a total of 32 replicates per panel member (16 replicates per lot). Testing was conducted using 2 m2000rt instruments. Each reagent lot was assigned to its own instrument.

All IDH2 mutation positive panel members reported “Mutation Detected” with a 100% percent agreement across all 32 replicates, and all IDH2 mutation negative panel members reported “Not Detected” across all 32 replicates. The percent agreement by operator was 100% (16/16) agreement for each panel member. The percent agreement by lot was 100% (16/16) agreement for each panel member. The Abbott RealTime IDH2 assay demonstrated a percent agreement of 100% with expected results for each panel member by operator, lot, and overall.

In within-laboratory repeatability, the mean and SD of dCN values and the within-run, between-run, between-lot and between-operator variability are shown in Table 6. The mean and SD of IC CN values and the within-run, between-run, between-lot and between-operator variability for Positive and Negative Controls are shown in Table 7. The mean and SD of mutant CN values and the within-run, between-run, between-lot and between-operator variability for Positive Controls are shown in Table 8.

### Table 6. Analysis of dCN Values for Mutation Positive Panel Members

<table>
<thead>
<tr>
<th>Panel</th>
<th>Mutation Status</th>
<th>Sample Type</th>
<th>N</th>
<th>Mean</th>
<th>Within-Run Variability</th>
<th>Between-Run Variability</th>
<th>Between-Lot Variability</th>
<th>Between-Operator Variability</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>03</td>
<td>R140Q</td>
<td>Blood</td>
<td>32</td>
<td>5.06</td>
<td>0.129</td>
<td>2.5</td>
<td>0.088</td>
<td>1.7</td>
<td>0.061</td>
</tr>
<tr>
<td>04</td>
<td>R140W</td>
<td>Blood</td>
<td>32</td>
<td>5.70</td>
<td>0.091</td>
<td>1.6</td>
<td>0.138</td>
<td>2.4</td>
<td>0.302</td>
</tr>
<tr>
<td>05</td>
<td>R172K</td>
<td>Blood</td>
<td>32</td>
<td>3.01</td>
<td>0.125</td>
<td>4.1</td>
<td>0.017</td>
<td>0.6</td>
<td>0.226</td>
</tr>
<tr>
<td>06</td>
<td>R172W</td>
<td>Blood</td>
<td>32</td>
<td>4.97</td>
<td>0.079</td>
<td>1.6</td>
<td>0.158</td>
<td>3.2</td>
<td>0.208</td>
</tr>
<tr>
<td>09</td>
<td>R140Q</td>
<td>Bone Marrow</td>
<td>32</td>
<td>5.49</td>
<td>0.126</td>
<td>2.3</td>
<td>0.153</td>
<td>2.8</td>
<td>0.185</td>
</tr>
<tr>
<td>10</td>
<td>R140W</td>
<td>Bone Marrow</td>
<td>32</td>
<td>5.67</td>
<td>0.328</td>
<td>5.8</td>
<td>0.117</td>
<td>2.1</td>
<td>0.269</td>
</tr>
<tr>
<td>11</td>
<td>R172K</td>
<td>Bone Marrow</td>
<td>32</td>
<td>3.75</td>
<td>0.172</td>
<td>4.6</td>
<td>0.207</td>
<td>5.5</td>
<td>0.113</td>
</tr>
<tr>
<td>12</td>
<td>R172W</td>
<td>Bone Marrow</td>
<td>32</td>
<td>4.33</td>
<td>0.064</td>
<td>1.5</td>
<td>0.318</td>
<td>7.3</td>
<td>0.302</td>
</tr>
</tbody>
</table>
Table 7. Analysis of Oligo IC CN Values for Positive and Negative Controls

<table>
<thead>
<tr>
<th>Panel</th>
<th>Mutation Status</th>
<th>Sample Type</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>SD</th>
<th>SD</th>
<th>SD</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDH2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Negative Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OLIGO1 IC CN</td>
<td></td>
<td>Blood</td>
<td>32</td>
<td>22.79</td>
<td>0.264</td>
<td>0.000</td>
<td>0.059</td>
<td>0.000</td>
<td>0.270</td>
</tr>
<tr>
<td>OLIGO2 IC CN</td>
<td></td>
<td>Blood</td>
<td>32</td>
<td>22.78</td>
<td>0.253</td>
<td>0.000</td>
<td>0.076</td>
<td>0.000</td>
<td>0.265</td>
</tr>
<tr>
<td>OLIGO3 IC CN</td>
<td></td>
<td>Blood</td>
<td>32</td>
<td>22.76</td>
<td>0.291</td>
<td>0.000</td>
<td>0.034</td>
<td>0.008</td>
<td>0.293</td>
</tr>
<tr>
<td>OLIGO4 IC CN</td>
<td></td>
<td>Blood</td>
<td>32</td>
<td>22.81</td>
<td>0.283</td>
<td>0.000</td>
<td>0.071</td>
<td>0.000</td>
<td>0.292</td>
</tr>
<tr>
<td>IDH2</td>
<td>Positive Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OLIGO1 IC CN</td>
<td></td>
<td>Bone Marrow</td>
<td>32</td>
<td>22.83</td>
<td>0.303</td>
<td>0.000</td>
<td>0.141</td>
<td>0.040</td>
<td>0.337</td>
</tr>
<tr>
<td>OLIGO2 IC CN</td>
<td></td>
<td>Bone Marrow</td>
<td>32</td>
<td>22.82</td>
<td>0.320</td>
<td>0.000</td>
<td>0.130</td>
<td>0.051</td>
<td>0.349</td>
</tr>
<tr>
<td>OLIGO3 IC CN</td>
<td></td>
<td>Bone Marrow</td>
<td>32</td>
<td>22.81</td>
<td>0.305</td>
<td>0.000</td>
<td>0.138</td>
<td>0.043</td>
<td>0.338</td>
</tr>
<tr>
<td>OLIGO4 IC CN</td>
<td></td>
<td>Bone Marrow</td>
<td>32</td>
<td>22.83</td>
<td>0.290</td>
<td>0.041</td>
<td>0.139</td>
<td>0.051</td>
<td>0.329</td>
</tr>
</tbody>
</table>

Table 8. Analysis of Mutant CN Values for Positive Control

<table>
<thead>
<tr>
<th>Panel</th>
<th>Mutation Status</th>
<th>Sample Type</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>SD</th>
<th>SD</th>
<th>SD</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDH2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R140Q CN</td>
<td></td>
<td>Blood</td>
<td>90</td>
<td>5.01</td>
<td>0.168</td>
<td>0.188</td>
<td>0.082</td>
<td>0.265</td>
<td></td>
</tr>
<tr>
<td>R140W CN</td>
<td></td>
<td>Blood</td>
<td>90</td>
<td>5.49</td>
<td>0.173</td>
<td>0.256</td>
<td>0.176</td>
<td>0.331</td>
<td></td>
</tr>
<tr>
<td>R172K CN</td>
<td></td>
<td>Blood</td>
<td>90</td>
<td>2.86</td>
<td>0.139</td>
<td>0.076</td>
<td>0.160</td>
<td>0.225</td>
<td></td>
</tr>
<tr>
<td>R172W CN</td>
<td></td>
<td>Blood</td>
<td>90</td>
<td>3.13</td>
<td>0.084</td>
<td>0.151</td>
<td>0.063</td>
<td>0.184</td>
<td></td>
</tr>
</tbody>
</table>

Within-Laboratory Lot-to-Lot Reproducibility

The within-laboratory lot-to-lot reproducibility was evaluated using 3 unique lots of Abbott mSample Preparation System DNA kits and 3 unique lots of Abbott RealTime IDH2 Amplification Reagent Kits. A 12-member panel contained IDH2 mutation negative blood and bone marrow specimens as well as blood and bone marrow specimens spiked with R140Q, R140W, R172K, or R172W positive cell lines targeting a 6% mutation level. Positive mutations were chosen such that the panel contained a representative mutation amplified by each of the four master mixes. For each panel member, 10 replicates were prepared using 3 unique lots of Abbott mSample Preparation System DNA kits for a total of 30 extractions per panel member. Each extraction was tested using 3 unique lots of Abbott RealTime IDH2 Amplification Reagent Kit for a total of 9 unique sample preparation/amplification kit combinations. Three m2000rt instruments were used, one for each lot of Abbott RealTime IDH2 Amplification Reagent Kit. Thus, for each panel member, a total of 90 replicates were tested.

All mutation positive panel members reported “Mutation Detected” with a 100% percent agreement across all 90 replicates, and all mutation negative panel members reported “Not Detected” with a 100% percent agreement across all 90 replicates. The percent agreement by Abbott mSample Preparation System lot was 100% (30/30) agreement for each panel member. The percent agreement by Abbott RealTime IDH2 Amplification Reagent Kit lot was 100% (30/30) agreement for each panel member. The Abbott RealTime IDH2 assay demonstrated a percent agreement of 100% with expected results for each panel member by sample preparation kit lot, amplification reagent kit lot, and overall.

In within-laboratory lot-to-lot reproducibility, the mean and SD of dCN values and the between-replicate, between-amplification lot and between-sample prep lot variability are shown in Table 9. The mean and SD of IC CN values and the between-replicate, between-amplification lot and between-sample prep lot variability for Positive and Negative Controls is shown in Table 10. The mean and SD of mutant CN values and the between-replicate, between-amplification lot and between-sample prep lot variability for Positive and Negative Controls is shown in Table 11.

Table 9. Analysis of dCN Values

<table>
<thead>
<tr>
<th>Panel</th>
<th>Mutation Status</th>
<th>Sample Type</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Between-Replicate Variability</th>
<th>Between-Amplification Lot Variability</th>
<th>Between-Sample Prep Lot Variability</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>03</td>
<td>R140</td>
<td>Blood</td>
<td>90</td>
<td>5.01</td>
<td>0.168</td>
<td>0.188</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>04</td>
<td>R140W</td>
<td>Blood</td>
<td>90</td>
<td>5.49</td>
<td>0.173</td>
<td>0.256</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>05</td>
<td>R172K</td>
<td>Blood</td>
<td>90</td>
<td>2.86</td>
<td>0.139</td>
<td>0.076</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>06</td>
<td>R172W</td>
<td>Blood</td>
<td>90</td>
<td>3.13</td>
<td>0.084</td>
<td>0.151</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bone Marrow</td>
<td>09</td>
<td>R140Q</td>
<td>3.48</td>
<td>0.212</td>
<td>0.200</td>
<td>0.209</td>
<td>0.358</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bone Marrow</td>
<td>10</td>
<td>R140W</td>
<td>5.90</td>
<td>0.134</td>
<td>0.201</td>
<td>0.029</td>
<td>0.242</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bone Marrow</td>
<td>11</td>
<td>R172K</td>
<td>3.81</td>
<td>0.195</td>
<td>0.068</td>
<td>0.091</td>
<td>0.226</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bone Marrow</td>
<td>12</td>
<td>R172W</td>
<td>4.38</td>
<td>0.205</td>
<td>0.162</td>
<td>0.064</td>
<td>0.269</td>
</tr>
</tbody>
</table>

Table 10. Analysis of IC CN Values for Positive and Negative Controls

<table>
<thead>
<tr>
<th>Panel</th>
<th>Mutation Status</th>
<th>Sample Type</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Between-Replicate Variability</th>
<th>Between-Amplification Lot Variability</th>
<th>Between-Sample Prep Lot Variability</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>03</td>
<td>OLIGO1</td>
<td>IC CN</td>
<td>54</td>
<td>23.13</td>
<td>0.387</td>
<td>0.460</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>04</td>
<td>OLIGO2 IC CN</td>
<td>54</td>
<td>23.19</td>
<td>0.567</td>
<td>0.548</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>05</td>
<td>OLIGO3 IC CN</td>
<td>54</td>
<td>23.16</td>
<td>0.569</td>
<td>0.544</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>06</td>
<td>OLIGO4 IC CN</td>
<td>54</td>
<td>23.25</td>
<td>0.617</td>
<td>0.616</td>
<td>0.041</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>07</td>
<td>OLIGO1</td>
<td>IC CN</td>
<td>54</td>
<td>23.14</td>
<td>0.394</td>
<td>0.485</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>08</td>
<td>OLIGO2 IC CN</td>
<td>54</td>
<td>23.16</td>
<td>0.381</td>
<td>0.510</td>
<td>0.086</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>09</td>
<td>OLIGO3 IC CN</td>
<td>54</td>
<td>23.14</td>
<td>0.426</td>
<td>0.556</td>
<td>0.121</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10</td>
<td>OLIGO4 IC CN</td>
<td>54</td>
<td>23.20</td>
<td>0.376</td>
<td>0.513</td>
<td>0.098</td>
</tr>
</tbody>
</table>
Within-Laboratory Specimen Handling Reproducibility

The within-laboratory specimen handling reproducibility of Abbott RealTime IDH2 was evaluated using two operators. Each operator tested a 4-member panel containing two mutation positive AML blood specimens and two mutation positive AML bone marrow aspirate specimens. Each operator tested the panel using the same lots of Abbott RealTime IDH2 Amplification Reagent Kit and Abbott RealTime IDH2 Control Kit and unique lots of Abbott mSample Preparation SystemDNA Kits. Each operator performed 1 run per day over a total of 5 days. A run consisted of sample preparation and PCR. Two (2) replicates of each panel member were tested in each run, for a total of 20 replicates per panel member (10 replicates per operator). One (1) replicate of each control was tested in each run, for a total of 10 replicates per control (5 replicates per operator). Testing was conducted using 2 m2000rt instruments. Each Abbott mSample Preparation SystemDNA Kit lot was assigned to its own instrument.

In the within-laboratory specimen handling reproducibility, all IDH2 mutation positive panel members were reported as “Mutation Detected” with 100% (10/10) agreement for each panel member by operator, and an overall agreement of 100% (20/20) for each panel member. The mean and SD of dCN values and the within-run, between-run and between-operator variability are shown in Table 12. The mean and SD of IC CN values and the between-run and between-operator variability for Positive and Negative Controls are shown in Table 13. The mean and SD of mutant CN values and the between-run and between-operator variability for Positive and Negative Controls are shown in Table 14.

Reproducibility I

Reproducibility of the Abbott RealTime IDH2 assay was evaluated at 3 external sites by testing IDH2 wild type (WT) mutation negative and IDH2 mutation positive blood and bone marrow specimens. The 6 member panel contained IDH2 wild type (WT) mutation negative and IDH2 R140Q and IDH2 R172K mutation positive blood and bone marrow specimens. For the IDH2 mutation positive panel members, R140Q or R172K positive cell lines were spiked in blood and bone marrow targeting a 6% mutation level. At each site, an operator performed 1 extraction run of the testing panel for 6 days. Each run contained 2 replicates of each panel member yielding a total of 12 replicates for each panel member or 36 replicates for each panel member for all 3 sites combined. Each run contained 1 replicate of each control yielding a total of 6 replicates for each control for 18 replicates for each control for all 3 sites combined. Each site used a unique lot of the Abbott mSample Preparation SystemDNA kit for a total of 3 unique lots. The same lot of Abbott RealTime IDH2 Amplification Reagent Kit and Abbott RealTime IDH2 Control Kit was used across all sites. The sample handling reproducibility analysis demonstrated 100% (12/12) agreement for each panel member by site, and 100% (36/36) overall agreement for each panel member.

In Reproducibility I, the mean and SD of dCN values and the within-run, between-run and between-site variability are shown in Table 15. The mean and SD of IC CN and mutant CN and the between-run and between-site variability for Positive and Negative Controls are shown in Table 16.

**Table 11. Analysis of Mutant CN Values for Positive Control**

<table>
<thead>
<tr>
<th>Panel</th>
<th>Value</th>
<th>N</th>
<th>Mean (dCN)</th>
<th>SD</th>
<th>SD</th>
<th>SD</th>
<th>SD</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDH2 Positive Control</td>
<td>R140Q CN</td>
<td>54</td>
<td>26.22</td>
<td>0.379</td>
<td>0.330</td>
<td>0.108</td>
<td>0.514</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R140W CN</td>
<td>54</td>
<td>26.03</td>
<td>0.402</td>
<td>0.484</td>
<td>0.149</td>
<td>0.646</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R172K CN</td>
<td>54</td>
<td>24.10</td>
<td>0.465</td>
<td>0.472</td>
<td>0.130</td>
<td>0.675</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R172W CN</td>
<td>54</td>
<td>25.31</td>
<td>0.371</td>
<td>0.438</td>
<td>0.101</td>
<td>0.583</td>
<td></td>
</tr>
</tbody>
</table>

**Table 12. Analysis of dCN Values for Panel Members**

<table>
<thead>
<tr>
<th>Panel</th>
<th>Value</th>
<th>Mutation Status</th>
<th>Sample Type</th>
<th>N</th>
<th>Mean (dCN)</th>
<th>Between-Replicate Variability</th>
<th>Between-Amplification Lot Variability</th>
<th>Between-Sample Prep Lot Variability</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>dCN</td>
<td>Positive</td>
<td>Blood</td>
<td>20</td>
<td>8.15</td>
<td>0.209</td>
<td>0.000</td>
<td>0.000</td>
<td>0.209</td>
</tr>
<tr>
<td></td>
<td>dCN</td>
<td>Positive</td>
<td>Bone Marrow</td>
<td>20</td>
<td>2.13</td>
<td>0.038</td>
<td>0.000</td>
<td>0.059</td>
<td>0.080</td>
</tr>
<tr>
<td></td>
<td>dCN</td>
<td>Negative</td>
<td>Blood</td>
<td>20</td>
<td>1.03</td>
<td>0.034</td>
<td>0.000</td>
<td>0.157</td>
<td>0.165</td>
</tr>
<tr>
<td></td>
<td>dCN</td>
<td>Negative</td>
<td>Bone Marrow</td>
<td>20</td>
<td>7.97</td>
<td>0.078</td>
<td>0.000</td>
<td>0.362</td>
<td>0.395</td>
</tr>
</tbody>
</table>

**Table 13. Analysis of Oligo IC CN Values for Positive and Negative Controls**

<table>
<thead>
<tr>
<th>Control</th>
<th>Value</th>
<th>N</th>
<th>Mean (CN)</th>
<th>Between-Run Variability</th>
<th>Between-Operator Variability</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDH2 Negative Control</td>
<td>OLIGO1 IC CN</td>
<td>10</td>
<td>22.49</td>
<td>0.167</td>
<td>0.325</td>
<td>0.375</td>
</tr>
<tr>
<td></td>
<td>OLIGO2 IC CN</td>
<td>10</td>
<td>22.51</td>
<td>0.185</td>
<td>0.306</td>
<td>0.357</td>
</tr>
<tr>
<td></td>
<td>OLIGO3 IC CN</td>
<td>10</td>
<td>22.47</td>
<td>0.223</td>
<td>0.344</td>
<td>0.409</td>
</tr>
<tr>
<td></td>
<td>OLIGO4 IC CN</td>
<td>10</td>
<td>22.58</td>
<td>0.220</td>
<td>0.359</td>
<td>0.421</td>
</tr>
<tr>
<td>IDH2 Positive Control</td>
<td>OLIGO1 IC CN</td>
<td>10</td>
<td>23.31</td>
<td>0.114</td>
<td>0.313</td>
<td>0.333</td>
</tr>
<tr>
<td></td>
<td>OLIGO2 IC CN</td>
<td>10</td>
<td>22.96</td>
<td>0.067</td>
<td>0.229</td>
<td>0.245</td>
</tr>
<tr>
<td></td>
<td>OLIGO3 IC CN</td>
<td>10</td>
<td>22.97</td>
<td>0.073</td>
<td>0.243</td>
<td>0.264</td>
</tr>
<tr>
<td></td>
<td>OLIGO4 IC CN</td>
<td>10</td>
<td>22.98</td>
<td>0.071</td>
<td>0.275</td>
<td>0.305</td>
</tr>
</tbody>
</table>

**Table 14. Analysis of Mutant CN Values for Positive Control**

<table>
<thead>
<tr>
<th>Control</th>
<th>Value</th>
<th>N</th>
<th>Mean (CN)</th>
<th>Between-Replicate Variability</th>
<th>Between-Operator Variability</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDH2 Positive Control</td>
<td>R140Q CN</td>
<td>10</td>
<td>26.03</td>
<td>0.114</td>
<td>0.363</td>
<td>0.380</td>
</tr>
<tr>
<td></td>
<td>R140W CN</td>
<td>10</td>
<td>26.45</td>
<td>0.187</td>
<td>0.387</td>
<td>0.430</td>
</tr>
<tr>
<td></td>
<td>R172K CN</td>
<td>10</td>
<td>25.45</td>
<td>0.112</td>
<td>0.400</td>
<td>0.415</td>
</tr>
<tr>
<td></td>
<td>R172W CN</td>
<td>10</td>
<td>25.31</td>
<td>0.135</td>
<td>0.304</td>
<td>0.332</td>
</tr>
</tbody>
</table>
Table 15. Reproducibility I Overall Precision

<table>
<thead>
<tr>
<th>Panel</th>
<th>Mutation</th>
<th>Specimen Type</th>
<th>Value</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Overall Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Within-Run Variability</td>
</tr>
<tr>
<td>1</td>
<td>WT</td>
<td>Blood</td>
<td>OLIGO1 IC CN 36 16.05 1.578 0.000 1.383 2.099</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>R140Q</td>
<td>Blood</td>
<td>dCN 36 4.13 0.148 0.106 0.187 0.261</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>R172K</td>
<td>Bone Marrow</td>
<td>dCN 36 2.17 0.111 0.084 0.219 0.263</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 16. Reproducibility I Analysis of Oligo IC CN and Mutant CN Values for Positive and Negative Controls

<table>
<thead>
<tr>
<th>Control</th>
<th>Value</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Between-Run Variability</th>
<th>Between-Site Variability</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDH2 Negative Control</td>
<td>OLIGO1 IC CN 18 22.99 0.177 0.087 0.264</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IDH2 Positive Control</td>
<td>R140Q CN 18 25.07 0.282 0.215 0.354</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Reproducibility II

Reproducibility of the Abbott RealTime IDH2 assay was evaluated at 3 external sites by testing DNA eluate extracted from IDH2 wild type (WT) mutation negative and IDH2 mutation positive blood and bone marrow specimens. The panel members included 10 IDH2 SNVs and wild type specimens targeting 50 ng and 200 ng gDNA input levels. IDH2 mutation positive panel members were prepared to target a mutation percentage of approximately 2% or 16%. The R140Q and R172K panel members for both blood and bone marrow were prepared using clinical specimens. The remaining mutation positive panel members were prepared from eluates of IDH2 mutation positive cell lines spiked into blood or bone marrow. Panel members were tested using 3 unique lots of Abbott RealTime IDH2 Amplification Reagent Kit. At each site, 2 operators performed 2 amplification/detection runs over a minimum of 5 days. Each run included 3 replicates of each panel member yielding a total of 180 replicates for each panel member for all 3 sites combined. There was a total of 241 replicates of each control for all 3 sites combined, which included 4 replicates of each control per run, plus 1 additional plate run by 1 site.

The eluate reproducibility analysis demonstrated 100% (60/60) agreement for each panel member at each site and 100% (60/60) agreement for each panel member tested with each Abbott RealTime Amplification Reagent Kit lot. The overall agreement was 100% (180/180) for each panel member for all sites and lots combined.

In Reproducibility II, the mean and SD of dCN values and the within-run, between-run, between-lot, between-operator and between-site variability are shown in Table 17. The mean and SD of IC CN and mutant CN values and the between-run, between-lot, between-operator and between-site variability for Positive and Negative Controls are shown in Table 18.
### Table 18. Reproducibility II Analysis of Oligo IC CN and Mutant CN Values for Positive and Negative Controls

<table>
<thead>
<tr>
<th>Control</th>
<th>Value</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>SD</th>
<th>SD</th>
<th>SD</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDH2 Negative</td>
<td>OLIGO1 IC CN</td>
<td>241</td>
<td>22.93</td>
<td>0.309</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.409</td>
</tr>
<tr>
<td></td>
<td>OLIGO2 IC CN</td>
<td>241</td>
<td>22.91</td>
<td>0.314</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.398</td>
</tr>
<tr>
<td></td>
<td>OLIGO3 IC CN</td>
<td>241</td>
<td>22.86</td>
<td>0.292</td>
<td>0.035</td>
<td>0.000</td>
<td>0.000</td>
<td>0.402</td>
</tr>
<tr>
<td></td>
<td>OLIGO4 IC CN</td>
<td>241</td>
<td>22.95</td>
<td>0.283</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.411</td>
</tr>
<tr>
<td>IDH2 Positive</td>
<td>R140Q CN</td>
<td>241</td>
<td>25.60</td>
<td>0.356</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.467</td>
</tr>
<tr>
<td></td>
<td>R140W CN</td>
<td>241</td>
<td>26.08</td>
<td>0.423</td>
<td>0.174</td>
<td>0.000</td>
<td>0.000</td>
<td>0.533</td>
</tr>
<tr>
<td></td>
<td>R172K CN</td>
<td>241</td>
<td>23.50</td>
<td>0.296</td>
<td>0.045</td>
<td>0.000</td>
<td>0.000</td>
<td>0.466</td>
</tr>
<tr>
<td></td>
<td>R172W CN</td>
<td>241</td>
<td>25.28</td>
<td>0.285</td>
<td>0.029</td>
<td>0.223</td>
<td>0.277</td>
<td>0.466</td>
</tr>
<tr>
<td></td>
<td>OLIGO1 IC CN</td>
<td>241</td>
<td>22.79</td>
<td>0.327</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.432</td>
</tr>
<tr>
<td></td>
<td>OLIGO2 IC CN</td>
<td>241</td>
<td>22.78</td>
<td>0.312</td>
<td>0.013</td>
<td>0.000</td>
<td>0.000</td>
<td>0.400</td>
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<tr>
<td></td>
<td>OLIGO3 IC CN</td>
<td>241</td>
<td>22.75</td>
<td>0.318</td>
<td>0.040</td>
<td>0.000</td>
<td>0.000</td>
<td>0.426</td>
</tr>
<tr>
<td></td>
<td>OLIGO4 IC CN</td>
<td>241</td>
<td>22.84</td>
<td>0.305</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.412</td>
</tr>
</tbody>
</table>

### COMPARISON TO NGS

A retrospective correlation analysis was performed using a data set of 173 clinical patients with valid IDH2 mutation testing results from both the Abbott RealTime IDH2 assay and one of two laboratory validated NGS (Next Generation Sequencing) methods. The data set included IDH2 mutation positive patients and IDH2 mutation negative patients, with similar numbers of blood and bone marrow samples from the 173 patients (167 blood samples and 150 bone marrow samples). The analysis evaluated agreement for the 9 IDH2 mutations that the Abbott RealTime IDH2 assay is designed to detect. The correlation analysis of the 173 patient results using the Abbott RealTime IDH2 assay and NGS assay qualitative interpretation ("Mutation Detected" or "Not Detected") as end points is shown in Table 19. The analysis demonstrated a 100.00% (93/93) Positive Percent Agreement (PPA) with a 95% exact CI of (96.11%, 100.00%). The analysis demonstrated a 98.75% (79/80) Negative Percent Agreement (NPA) with a 95% exact CI of (93.23%, 99.97%).

The correlation of interpretation analysis was performed by sample type. For the blood sample type, the analysis demonstrated a 100.00% (91/91) Positive Percent Agreement (PPA) with a 95% exact CI of (96.03%, 100.00%). The analysis demonstrated a 98.68% (75/76) Negative Percent Agreement (NPA) with a 95% exact CI of (92.89%, 99.97%). For the bone marrow sample type, the analysis demonstrated a 100.00% (78/78) Positive Percent Agreement (PPA) with a 95% exact CI of (95.01%, 100.00%).

An analysis demonstrating mutation identity agreement for samples identified as IDH2 "Mutation Detected" by the Abbott RealTime IDH2 assay is shown in Table 20. There were no samples with mutations R140G, R140L, R172G, R172M, R172S, or R172W identified in this data set.

### Table 19. Correlation of Interpretation of Abbott RealTime IDH2 Results with NGS Method Results

<table>
<thead>
<tr>
<th>NGS Method</th>
<th>Mutation Detected</th>
<th>Mutation Not Detected</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutation Detected</td>
<td>93</td>
<td>1</td>
<td>94</td>
</tr>
<tr>
<td>Mutation Not Detected</td>
<td>0</td>
<td>79</td>
<td>79</td>
</tr>
<tr>
<td>Total</td>
<td>93</td>
<td>80</td>
<td>173</td>
</tr>
</tbody>
</table>

### Table 20. Correlation of Mutation Identity of Abbott RealTime IDH2 Results with NGS Method Results

<table>
<thead>
<tr>
<th>NGS Method</th>
<th>R140Q</th>
<th>R140W</th>
<th>R172K</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not Detected</td>
<td>72</td>
<td>0</td>
<td>0</td>
<td>72</td>
</tr>
<tr>
<td>R140Q</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>R172K</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>1</td>
<td>73</td>
<td>1</td>
<td>94</td>
</tr>
</tbody>
</table>
CONCORDANCE BETWEEN BLOOD AND BONE MARROW SPECIMEN TYPES

The Abbott RealTime IDH2 assay is intended to be used on either blood or bone marrow aspirate. An analysis was performed to show concordance of results between matched blood and bone marrow specimen types tested by Abbott RealTime IDH2. Concordance between bone marrow specimens and blood specimens was determined at the subject level ("Mutation Detected" vs. "Not Detected"). Results are shown below in Table 21. There was one discordant call (R172K positive in blood was reported as mutation not detected in bone marrow). There was also one within-mutation detected discordant case (R140G positive result in blood was reported as R172K positive in bone marrow (data not shown)). These results demonstrate equivalency between specimen types.

<table>
<thead>
<tr>
<th>Bone Marrow</th>
<th>Mutation Detected</th>
<th>Not Detected</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>142</td>
<td>1</td>
<td>143</td>
</tr>
<tr>
<td>CRh</td>
<td>142</td>
<td>71</td>
<td>213</td>
</tr>
</tbody>
</table>

Average Positive Agreement = 99.65% (284/285); 95% CI = 98.87 – 100.00%
Average Negative Agreement = 99.29% (140/141); 95% CI = 97.58 – 100.00%
Overall Agreement = 99.53% (212/213); 95% CI = 97.41 – 99.99%

CLINICAL STUDIES

The safety and effectiveness of the Abbott RealTime IDH2 assay were demonstrated through testing of specimens from patients enrolled in Study AG221-C-001 (ClinicalTrials.gov Identifier: NCT01915498). Study AG221-C-001 was an open-label, single-arm, international, multicenter, two-cohort clinical trial of IDHIFA (enasidenib) on 199 adult patients with relapsed or refractory Acute Myeloid Leukemia (R/R AML) and one of 9 IDH2 mutations in codons R140 or R172, who were assigned to receive a 100 mg daily dose. Cohort 1 included 101 patients and Cohort 2 included 98 patients. IDH2 mutations were identified by a local diagnostic test and retrospectively confirmed by the Abbott RealTime IDH2 assay, or prospectively identified by the Abbott RealTime IDH2 assay. IDHIFA was given orally at starting dose of 100 mg daily until disease progression or unacceptable toxicity. Dose reductions were allowed to manage adverse events.

The baseline demographics and disease characteristics were similar in both study cohorts. The median age of study participants was 58 years and predominantly white (77%). Enrollment according to gender was equivalent. The predominant IDH2 positive mutation was R140Q (75%) followed by R172K (20%). Four (4) IDH2 mutations were not detected in patients enrolled into the trial (R140G, R172M, R172G, R172S).

Blood and bone marrow aspirate specimens from R/R AML patients being considered for treatment were tested. Patients with an IDH2 mutation positive result were eligible for enrollment in the drug trial if they met other eligibility criteria. Patients with an IDH2 mutation negative result were ineligible for drug trial enrollment. Patients in Cohort 2 were selected on the basis of the Abbott RealTime IDH2 assay. Patients in Cohort 1 used to support conclusions regarding IDHIFA efficacy were based on the IDH2 mutation positive specimen results as detected by the Abbott RealTime IDH2 assay.

Efficacy for IDHIFA was established on the basis of the rate of complete response (CR), complete response with partial hematologic recovery (CRh), the duration of CR/CRh, and the rate of conversion from transfusion dependence to transfusion independence. The efficacy results are shown in Table 22 and were similar in both cohorts. The median follow-up was 6.6 months (range, 0.4 to 27.7 months). Similar CR/CRh rates were observed in patients with either R140 or R172 mutation.

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>IDHIFA (100 mg daily)</th>
<th>N=199</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR (%)</td>
<td>37 (19)</td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>(13, 25)</td>
<td></td>
</tr>
<tr>
<td>Median DOR (months)</td>
<td>8.2</td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>(4.7, 19.4)</td>
<td></td>
</tr>
<tr>
<td>CRh (%)</td>
<td>9 (4)</td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>(2, 8)</td>
<td></td>
</tr>
<tr>
<td>Median DOR (months)</td>
<td>9.6</td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>(0.7, NA)</td>
<td></td>
</tr>
<tr>
<td>CR/CRh %</td>
<td>46 (23)</td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>(18, 30)</td>
<td></td>
</tr>
<tr>
<td>Median DOR (months)</td>
<td>8.2</td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>(4.3, 28)</td>
<td></td>
</tr>
</tbody>
</table>

CI: confidence interval; NA: not available
a CR (complete remission) was defined as <5% of blasts in the bone marrow, no evidence of disease, and full recovery of blood counts (platelets >100,000/microliter and absolute neutrophil counts [ANC] >1,000/microliter).
b DOR (duration of response) was defined as time since first response of CR or CRh to relapse or death, whichever is earlier.

For patients who achieved a CR/CRh, the median time to first response was 1.9 months (range, 0.5 to 7.5 months) and the median time to best response of CR/CRh was 3.7 months (range, 0.6 to 11.2 months). Of the 48 patients who achieved a best response of CR/CRh, 39 (85%) did so within 6 months of initiating IDHIFA.

Among the 47 patients who were dependent on red blood cell (RBC) and/or platelet transfusions at baseline, 53 (34%) became independent of RBC and platelet transfusions during any 56-day post baseline period. Of the 42 patients who were independent of both RBC and platelet transfusions at baseline, 32 (76%) remained transfusion independent during any 56-day post baseline period.

Refer to Drugs@FDA for the most recent IDHIFA product labeling.


### Appendix 1. List of Abbott RealTime IDH2 Specific Error Codes for Positive Control and Negative Control

<table>
<thead>
<tr>
<th>Code</th>
<th>Message Text</th>
<th>Probable Causes</th>
<th>Corrective Actions</th>
</tr>
</thead>
</table>
| 4926  | IC cycle number is less than the minimum         | • Sample preparation reagents, master mix, or sample contaminated with targets                                             | 1. Decontaminate work areas, pipettes, and equipment.  
      |                                                  | • Sample preparation procedure errors                                                                                | 2. Refer to this package insert for further instructions on precautions to avoid contamination.  
      |                                                  | • Wrong sample loaded onto reaction plate                                                                              | 3. Ensure that the correct sample preparation procedure is followed.  
      |                                                  | • Sample preparation procedure errors                                                                                | 4. Ensure that the sample is correctly labeled and tested. |
| 4927  | IC cycle number is greater than the maximum      | • Insufficient DNA extracted                                                                                               | 1. Ensure that the correct amounts of Positive Control and Negative Control are used.  
      |                                                  | • Insufficient reagent(s) pipetted                                                                                    | 2. Ensure that calibrated pipettes and the correct amount of assay reagents are used.  
      |                                                  | • Sample preparation procedure errors                                                                                | 3. Ensure that the correct sample preparation procedure is followed.  
      |                                                  | • Wrong sample loaded onto reaction plate                                                                              | 4. Ensure that the sample is correctly labeled and tested.  
      |                                                  | • No or insufficient sample loaded                                                                                    | 5. Ensure that the correct amount of eluate is used.  
      |                                                  | • Sample preparation reagent, master mix, or sample contaminated with DNase                                           | 6. Follow package insert for good laboratory practices to avoid DNase contamination.               |
| 4928  | Negative Control is reactive for a mutation      | • Sample preparation reagent, master mix, or sample contaminated with targets                                             | 1. Decontaminate work areas, pipettes, and equipment.  
      |                                                  | • Positive Control was extracted                                                                                       | 2. Refer to this package insert for further instructions on precautions for avoiding contamination.  
      |                                                  | • Sample preparation procedure errors                                                                                | 3. Ensure that a new vial of Negative Control is used.  
      |                                                  | • Wrong sample loaded onto reaction plate                                                                              | 4. Ensure that the correct sample preparation procedure is followed.  
      |                                                  | • Sample preparation procedure errors                                                                                | 5. Ensure that the sample is correctly labeled and tested.  
| 4929  | Mutation cycle number is less than the minimum   | • Sample preparation reagents, master mix, or sample contaminated with targets                                             | 1. Decontaminate work areas, pipettes, and equipment.  
      |                                                  | • Sample preparation procedure errors                                                                                | 2. Refer to this package insert for further instructions on precautions for avoiding contamination.  
      |                                                  | • Wrong sample loaded onto reaction plate                                                                              | 3. Ensure that the correct sample preparation procedure is followed.  
| 4930  | Mutation cycle number is greater than the maximum| • Insufficient DNA extracted                                                                                               | 1. Ensure that the correct amount of Positive Control is used.  
      |                                                  | • Negative Control was extracted                                                                                       | 2. Ensure that calibrated pipettes and the correct amount of assay reagents are used.  
      |                                                  | • Insufficient reagent(s) pipetted                                                                                    | 3. Ensure that the correct sample preparation procedure is followed.  
      |                                                  | • Sample preparation procedure errors                                                                                | 4. Ensure that the sample is correctly labeled and tested.  
      |                                                  | • Wrong sample loaded onto reaction plate                                                                              | 5. Ensure that the correct amount of eluate is used.  
      |                                                  | • No or insufficient sample loaded                                                                                    | 6. Follow package insert for good laboratory practices to avoid DNase contamination.               |
      |                                                  | • Sample preparation reagent, master mix, or sample contaminated with DNase                                           |  

<table>
<thead>
<tr>
<th>Code</th>
<th>Message Text</th>
<th>Probable Causes</th>
<th>Corrective Actions</th>
</tr>
</thead>
</table>
| 4931  | IC cycle number is non-reactive or greater than the maximum | • Poor sample quality  
• Insufficient DNA extracted  
• Insufficient reagent(s) pipetted  
• Sample preparation procedure errors  
• No or insufficient sample loaded  
• Sample preparation reagent, master mix, or sample contaminated with DNase | 1. Ensure that the specimen meets requirements.  
2. Ensure that calibrated pipettes and the correct amount of assay reagents are used.  
3. Ensure that the correct sample preparation procedure is followed.  
4. Ensure that the correct amount of eluate is used.  
5. Follow package insert for good laboratory practices to avoid DNase contamination. |
| 4932  | IC cycle number is less than the minimum          | • Sample preparation reagents, master mix, or sample contaminated with targets  
• Sample preparation procedure errors  
• Wrong sample loaded onto reaction plate | 1. Decontaminate work areas, pipettes, and equipment.  
2. Refer to this package insert for further instructions on precautions for avoiding contamination.  
3. Ensure that the correct sample preparation procedure is followed.  
4. Ensure that the sample is correctly labeled and tested. |
| 4933  | Cycle number difference is less than the minimum  | • Poor sample quality  
• Sample preparation reagents, master mix, or sample contaminated with targets  
• Sample preparation procedure errors  
• Wrong sample loaded onto reaction plate | 1. Decontaminate work areas, pipettes, and equipment.  
2. Refer to this package insert for further instructions on precautions for avoiding contamination.  
3. Ensure that the correct sample preparation procedure is followed.  
4. Ensure that the sample is correctly labeled and tested. |
| 4934  | See Constituent Result for Associated Error       | • Refer to Constituent Result error code<sup>a</sup> | Refer to Constituent Result error code<sup>a</sup> |
| 4935  | See Constituent Result for Associated Error       | • Refer to Constituent Result error code<sup>a</sup> | Refer to Constituent Result error code<sup>a</sup> |

<sup>a</sup> Constituent Result error codes are found associated with individual IC (OLIGO1, OLIGO2, OLIGO3, or OLIGO4) or mutation (R140Q, R140L, R140G, R140W, R172K, R172M, R172G, R172S, and R172W) results.