Abbott RealTi*me* IDH1

en

REF 08N90-090

51-608283/R4

Rx ONLY

REF 08N90-090

51-608283/R4

NOTE: Changes Highlighted

Key to Symbols Used

REF	Reference Number
IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
In Vitro Test	In Vitro Test
For In Vitro Diagnostic Use	For In Vitro Diagnostic Use
Rx ONLY	For Prescription Use Only
	Use by
CONTROL –	Negative Control
CONTROL +	Positive Control
Σ	Contains sufficient for <n> tests
	Temperature Limit
	Consult instructions for use
	Warning
	Manufacturer
OLIGONUCLEOTIDE REAGENT 1	Oligonucleotide Reagent 1
OLIGONUCLEOTIDE REAGENT 2	Oligonucleotide Reagent 2
DNA POLYMERASE	DNA Polymerase
ACTIVATION REAGENT	Activation Reagent

CUSTOMER SERVICE: 1-800-553-7042

This package insert must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

NAME

Abbott RealTi*me* IDH1

INTENDED USE

Abbott RealTi*me* IDH1 is an *in vitro* polymerase chain reaction (PCR) assay for the qualitative detection of single nucleotide variants (SNVs) coding five IDH1 R132 mutations (R132C, R132H, R132G, R132S, and R132L) in DNA extracted from human blood (EDTA) or bone marrow (EDTA). Abbott RealTi*me* IDH1 is for use with the Abbott *m*2000*rt* System.

INDICATION FOR USE

Abbott RealTi*me* IDH1 is indicated as an aid in identifying acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) patients with an isocitrate dehydrogenase-1 (IDH1) mutation for treatment with TIBSOVO® (ivosidenib) or in identifying AML patients for treatment with REZLIDHIA™ (olutasidenib).

This test is for prescription use only.

SUMMARY AND EXPLANATION OF THE TEST

Abbott RealTi*me* IDH1 detects single nucleotide variants (SNVs) coding five IDH1 mutations (R132C, R132H, R132G, R132S, and R132L) 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 IDH1 mutations detected by the Abbott RealTi*me* IDH1 assay.

Table 1. Mutations Detected by the Abbott RealTi*me* IDH1 Assay

Codon	IDH1 Mutation	SNV
R132	R132C	TGT
	R132H	CAT
	R132G	GGT
	R132S	AGT
	R132L	CTT

IDH1 (isocitrate dehydrogenase-1) is a cytoplasmic enzyme involved in regulation of cellular metabolism. IDH1 catalyzes the oxidative decarboxylation of isocitrate to produce α-ketoglutarate (α-KG). Mutations in codon R132 of IDH1 causes the enzyme to acquire a neomorphic activity that converts α-KG to D-2-hydroxyglutarate (2-HG) resulting in highly elevated levels of 2-HG, a rare metabolite normally present at very low levels in healthy cells.[1,2] 2-HG acts as an oncometabolite that is associated with altered gene expression, DNA and histone hypermethylation, and blocked differentiation of hematopoietic progenitor cells.[3–7]

Mutations in codon R132 of IDH1 can be found in several cancer types, including acute myeloid leukemia (AML), in which 6% to 10% of patients possess an IDH1 mutation.[6,8,9] Multiple IDH1 amino acid changes have been identified at the arginine residue of codon R132 and include: R132H, R132C, R132L, R132G, and R132S. R132H and R132C are the most prevalent, occurring in over 50% of AML patients with IDH1 mutations.[10–12]

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

Abbott RealTi*me* IDH1 consists of two kits:

- Abbott RealTi*me* IDH1 Amplification Reagent Kit (List No. 08N90-090)
- Abbott RealTi*me* IDH1 Control Kit (List No. 08N90-080)

Specimens for Abbott RealTi*me* IDH1 are processed manually using Abbott *m*Sample Preparation System$_{DNA}$ (List No. 06K12-24) reagents to isolate and purify sample DNA. The Abbott RealTi*me* IDH1 amplification reagents are combined into two 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 *m*2000*rt* instrument for amplification and detection of IDH1 mutations. The specimen result is automatically reported on the Abbott *m*2000*rt* 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 RealTi*me* IDH1 are contained in an assay application specification file, which is loaded onto the Abbott *m*2000*rt* 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 *m*Sample Preparation System$_{DNA,}$ 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.

Abbott

REAGENT PREPARATION AND REACTION PLATE ASSEMBLY

The Abbott RealTi*m*e IDH1 Oligonucleotide Reagents (Oligonucleotide Reagent 1 and Oligonucleotide Reagent 2) are each manually combined with DNA Polymerase and Activation Reagent to create 2 unique master mixes. These master mixes are added to 2 separate wells of the Abbott 96-Well Optical Reaction Plate along with aliquots of the extracted DNA sample. After manual application of the Abbott Optical Adhesive Cover, the plate is transferred to the Abbott *m*2000*rt* instrument.

AMPLIFICATION/DETECTION

Each Abbott RealTi*m*e IDH1 master mix is designed to amplify and detect 2 or 3 IDH1 amino acid mutations (codon with mutant nucleotide underlined). Oligonucleotide 1 master mix amplifies and detects R132C (TGT) and R132H (CAT). Oligonucleotide 2 master mix amplifies and detects R132G (GGT), R132S (AGT), and R132L (CTT). Refer to **Table 2**. In addition, both master mixes are designed to amplify and detect a region of the IDH1 gene outside of codon 132, which serves as an endogenous internal control (IC).

Table 2. IDH1 Mutations Detected by each Master Mix

Master Mix	IDH1 Mutation	SNV
Oligonucleotide 1	R132C	TGT
	R132H	CAT
Oligonucleotide 2	R132G	GGT
	R132S	AGT
	R132L	CTT

During the amplification reaction on the Abbott *m*2000*rt* instrument, the target DNA is amplified by DNA Polymerase in the presence of primers, deoxyribonucleoside triphosphates (dNTPs), and magnesium chloride ($MgCl_2$). 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 RealTi*m*e IDH1, 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 IDH1 mutation and IC targets takes place simultaneously in the same PCR well.

IDH1 products are detected during the annealing/extension step by measuring the real-time fluorescence signals of the IDH1 mutation and IC-specific probes, respectively. The IDH1 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, 2 PCR reactions are evaluated. Abbott RealTi*m*e IDH1 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 IDH1 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 RealTi*m*e IDH1 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 RealTi*m*e IDH1. Refer to the **SPECIAL PRECAUTIONS** section of this package insert.

REAGENTS

Abbott RealTi*m*e IDH1 Amplification Reagent Kit (List No. 08N90-090)

1. **OLIGONUCLEOTIDE REAGENT 1** **Abbott RealTi*m*e IDH1 Oligonucleotide Reagent 1**
(List No. 8N90A) (1 vial, 0.905 mL)
<0.1% synthetic oligonucleotides and <1% dNTP, in a buffered solution with a reference dye. Preservatives: sodium azide and 0.15% ProClin® 950.

2. **OLIGONUCLEOTIDE REAGENT 2** **Abbott RealTi*m*e IDH1 Oligonucleotide Reagent 2**
(List No. 8N90B) (1 vial, 0.905 mL)
<0.1% synthetic oligonucleotides and <1% dNTP, in a buffered solution with a reference dye. Preservatives: sodium azide and 0.15% ProClin 950.

3. **DNA POLYMERASE** **Abbott RealTi*m*e IDH1 DNA Polymerase**
(List No. 8N90E) (2 vials, 0.051 mL per vial)
DNA Polymerase (5.4 to 5.9 Units/µL) in a buffered solution with stabilizers.

4. **ACTIVATION REAGENT** **Abbott RealTi*m*e IDH1 Activation Reagent**
(List No. 8N90M) (1 vial, 0.930 mL)
50 mM magnesium chloride in a buffered solution. Preservatives: sodium azide and 0.15% ProClin 950.

Abbott RealTi*m*e IDH1 Control Kit (List No. 08N90-080)

1. **CONTROL** **–** **Abbott RealTi*m*e IDH1 Negative Control**
(List No. 8N90Z) (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.

2. **CONTROL** **+** **Abbott RealTi*m*e IDH1 Positive Control**
(List No. 8N90W) (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

IVD **In Vitro Diagnostic Medical Device**
For In Vitro Diagnostic Use

Abbott RealTi*m*e IDH1 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 *m*Wash 2_{DNA} sample preparation reagent. **Do not use ethanol that contains denaturants.**

Safety Precautions

Refer to the Abbott *m*2000*rt* Operations Manual, **Hazards** section, for instructions on safety precautions.

The Abbott RealTi*m*e IDH1 Oligonucleotide Reagent 1, Oligonucleotide Reagent 2, Activation Reagent, Positive Control, and Negative Control contain the following components:

- 2-methyl-2H-isothiazol-3-one
- Sodium azide

The following warnings apply:

Warning

H317	May cause an allergic skin reaction.
EUH032	Contact with acids liberates very toxic gas.
P261	Avoid breathing mist/vapours/spray.
P280	Wear protective gloves/protective clothing/eye protection.
P272	Contaminated work clothing should not be allowed out of the workplace.
P302+P352	IF ON SKIN: Wash with plenty of water.
P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
P362+P364	Take off contaminated clothing and wash it before reuse.
P501	Dispose of contents / container in accordance with local regulations.

Important information regarding the safe handling, transport, and disposal of this product and the Abbott *m*Sample Preparation System$_{DNA}$ is contained in the Safety Data Sheets.

SPECIAL PRECAUTIONS

Abbott RealTi*me* IDH1 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 RealTi*me* IDH1 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 specimens. Refer to the Abbott *m*2000*rt* 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 all completed, interrupted, or aborted Abbott *m*2000*rt* instrument runs, dispose of the Abbott 96-Well Optical Reaction Plate in a sealed plastic bag according to the Abbott *m*2000*rt* Operations Manual. The gloves used to handle the plate should be disposed of in a waste container.
- Decontaminate and dispose of all specimens, reagents, and other potentially biohazardous materials in accordance with local, state, and federal regulations.[13,14]
- 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.

Aerosol Containment

To reduce the risk of nucleic acid contamination due to aerosols formed during manual pipetting, aerosol barrier pipette tips must be used. The pipette tips must be used only one time. Clean and disinfect spills of specimens and reagents as stated in the Abbott *m*2000*rt* Operations Manual and the instructions in the **POST PROCESSING PROCEDURES** section.

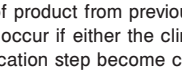
Contamination and Inhibition

The following precautions should be observed to minimize the risks of DNase 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 product).
- 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.

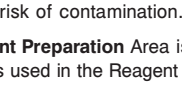
STORAGE INSTRUCTIONS

Abbott RealTi*me* IDH1 Amplification Reagent Kit (List No. 08N90-090)



The Abbott RealTi*me* IDH1 Amplification Reagent Kit (List No. 08N90-090) must be stored at −25°C to −15°C when not in use. Care must be taken to separate the Abbott RealTi*me* IDH1 Amplification Reagent Kit that is in use from direct contact with samples and controls.

Abbott RealTi*me* IDH1 Control Kit (List No. 08N90-080)



The Abbott RealTi*me* IDH1 Control Kit (List No. 08N90-080) must be stored at −25°C to −15°C.

SHIPPING CONDITIONS

Component	Shipping Condition
Abbott RealTi*me* IDH1 Amplification Reagent Kit	Dry Ice
Abbott RealTi*me* IDH1 Control Kit	Dry Ice

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 Customer Service.

SPECIMEN COLLECTION, STORAGE, AND TRANSPORT TO THE TEST SITE

Blood and Bone Marrow Aspirate Specimen Collection and Storage

Human blood (EDTA) and bone marrow aspirate (EDTA) specimens may be used with the Abbott RealTi*me* IDH1 assay. Follow the manufacturer's instructions for processing collection tubes. After collection, specimens may be stored:

- At 15°C to 30°C for up to 48 hours
- At 2°C 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°C to 30°C or at 2°C to 8°C. Once thawed, if specimens are not being processed immediately, they can be stored at 2°C 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 RealTi*m*e IDH1 application specification file must be installed on the Abbott *m*2000*rt* instrument from the Abbott RealTi*m*e IDH1 *m*2000*rt* Application CD-ROM prior to performing the assay. For detailed information on application specification file installation, refer to the Abbott *m*2000*rt* Operations Manual, **Operating Instructions** section.

ABBOTT REALTI*M*E IDH1 ASSAY PROCEDURE

Materials Provided

- Abbott RealTi*m*e IDH1 Amplification Reagent Kit (List No. 08N90-090)

Materials Required But Not Provided

- Abbott RealTi*m*e IDH1 Control Kit (List No. 08N90-080)

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 *m*Sample Preparation System$_{DNA}$ (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 (sterile, polypropylene, 10.8 mm diameter, 44 mm length)
- 2.0 mL screw caps (sterile, polypropylene)
- 50 mL polypropylene 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
- 1.5 mL PCR-grade microtubes
- Microcentrifuge racks
- Vortex mixer
- Centrifuge capable of 1200 to 1500 g with rotor to accommodate Abbott 96-Well Optical Reaction Plate
- Heat blocks, 56°C ± 3°C and 75°C ± 5°C, suitable for 2.0 mL microtubes
- Molecular Biology Grade Water

Amplification Area

- Abbott *m*2000*rt* Instrument (List No. 09K15-01 (with System Software Version 8.0 or higher)
- Abbott RealTi*m*e IDH1 *m*2000*rt* Application CD-ROM (List No. 08N90-000)
- Abbott *m*2000*rt* 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 this package insert carefully before processing samples. The Abbott RealT*m*e IDH1 Amplification Reagent Kit is intended for use with the Abbott RealTi*m*e IDH1 Control Kit and the Abbott *m*Sample Preparation System$_{DNA}$ for sample processing, and the Abbott *m*2000*rt* instrument for amplification and detection.
- For a detailed description of how to operate the Abbott *m*2000*rt* instrument, refer to the Abbott *m*2000*rt* Operations Manual, **Operating Instructions** section.
- Laboratory personnel must be trained to operate the Abbott *m*2000*rt* 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 from kit lot Y.
- In the Abbott RealTi*m*e IDH1 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 RealTi*m*e IDH1 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 RealTi*m*e IDH1 Positive Control and Negative Control are integral to the performance of the Abbott RealTi*m*e IDH1 assay. Abbott RealTi*m*e IDH1 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 *m*Wash 2 Buffer$_{DNA}$. **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 *m*2000*rt* 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 RealTi*m*e IDH1 assay protocol includes the following steps:

A. Manual preparation (ie, DNA extraction) of samples (specimens and controls) using the Abbott *m*Sample Preparation System$_{DNA}$.

B. PCR assay setup using the sample eluates and the Abbott RealTi*m*e IDH1 Amplification Reagent Kit.

C Amplification/detection on the Abbott *m*2000*rt* 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 System$_{DNA}$ contains 4 sets of reagents. Each set of reagents can support preparation (ie, 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 IDH1 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°C to 30°C or at 2°C to 8°C.
   - Once thawed, if the amplification reagents are not being used immediately, they can be stored at 2°C 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°C 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°C to 30°C or at 2°C 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°C 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°C to 30°C or at 2°C to 8°C.
   - Once thawed, if blood or bone marrow aspirate specimens are not being processed immediately, they can be stored at 2°C 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 56°C ± 3°C
   b. Set one heat block at 75°C ± 5°C

   If only one heat block is available, then set to 56°C ± 3°C and equilibrate to 75°C ± 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 *m*Sample Preparation System$_{DNA}$ reagent bottles from the kit, and gently invert each bottle except *m*Microparticles$_{DNA}$ 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 *m*Sample Preparation System$_{DNA}$ 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 *m*Wash 2$_{DNA}$ working solution by adding 70 mL of 190 to 200-proof (95% to 100%) ethanol to the *m*Wash 2$_{DNA}$ 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°C to 30°C.

DNA Extraction

Lysis

7. Label a 2.0 mL tube for each patient specimen and assay control.
   - One IDH1 Negative Control and one IDH1 Positive Control must be processed alongside the patient specimens to be tested.
8. Resuspend *m*Microparticles$_{DNA}$ 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 *m*Microparticles$_{DNA}$ to each tube.
9. Add 1.3 mL of *m*Lysis$_{DNA}$ to each 2 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 3 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 200 µL.
    - For bone marrow aspirate specimens add 100 µL.
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 3 ± 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 *m*Lysis$_{DNA}$.
    - 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 *m*Wash 1$_{DNA.}$
    - 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 and for each tube:
    - Add 900 µL of *m*Wash 1$_{DNA.}$
    - 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 and for each tube:
    - Add 900 µL of *m*Wash 2_{DNA}.
    - 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 and for each tube:
    - Add 900 µL of *m*Wash 2_{DNA}.
    - 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 and for each tube:
    - Add 500 µL of *m*Elution Buffer$_{DNA}$.
    - 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°C to -15°C or colder for up to 2 months, and should not undergo more than 5 freeze/thaw cycles.
    - 15°C to 30°C for 24 hours;
    - 2°C to 8°C for 14 days;

    Frozen DNA eluates can be thawed at 15°C to 30°C or at 2°C to 8°C prior to PCR setup. Storage of DNA eluates must not exceed a cumulative total of 24 hours at 15°C to 30°C or 14 days at 2°C to 8°C prior to PCR setup.

Amplification Area

Abbott *m*2000*rt* Initiation and Test Order Creation

48. Switch on and initialize the Abbott *m*2000*rt* instrument.

49. Create an Abbott *m*2000*rt* test order. Refer to the **Operating Instructions** section of the Abbott *m*2000*rt* Operations Manual. From the Protocol screen, select the appropriate application specification file corresponding to the Abbott RealTi*m*e IDH1 assay.

50. A 12-sample setup (2 controls and 10 specimens) 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.

Example Plate Setup

	1	2	3	4	5	6	7	8
A	NEG	NEG	#7	#7				
B	POS	POS	#8	#8				
C	#1	#1	#9	#9				
D	#2	#2	#10	#10				
E	#3	#3						
F	#4	#4						
G	#5	#5						
H	#6	#6						

Oligo 1 master mix (columns 1 and 3)

Oligo 2 master mix (columns 2 and 4)

Oligo 1 master mix (columns 3 and 4)

Oligo 2 master mix (columns 3 and 4)

Figure 1 represents a partial image of the Abbott 96-Well Optical Reaction Plate.

Each control or sample must be placed in 2 adjacent wells, as indicated in Figure 1 and as described below:
- Oligo 1 master mix may only be added to columns 1, 3, 5, 7, 9 and 11.
- Oligo 2 master mix may only be added to columns 2, 4, 6, 8, 10 and 12.

Negative Control (NEG), Positive Control (POS), and specimens may be placed as indicated or in place of any of the specimen positions, following the 2 adjacent well requirement.

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

Sample Preparation Area

Preparation of the sample

51. Label a 1.5 mL PCR grade microtube for each specimen and control.

52. Vortex each specimen and control eluate tube for 10 seconds. After vortexing, tap each tube on the bench to bring the liquid to the bottom of the tube.

53. Dilute each specimen and control eluate by combining 40 µL of molecular biology grade water with 20 µL of sample eluate in a new labeled 1.5 mL PCR grade microtube. Mix by vortexing for 3 to 5 seconds, and tap each tube on the bench to bring the liquid to the bottom of the tube.

    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 (*e.g.*, 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 Reagent, 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 pipettor designated for master mix reagent use only.

- Prepare Oligo 1 master mix by adding the following to a new DNA Polymerase vial:

  a. 895 µL of Oligonucleotide Reagent 1.

  b. 250 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."

- Prepare Oligo 2 master mix by adding the following to a new DNA Polymerase vial:

  a. 895 µL of Oligonucleotide Reagent 2.

  b. 250 µL of Activation Reagent.

  Vortex vial 3 times for 2 to 3 seconds. Minimize the creation of foam or bubbles. Label as "Oligo 2 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 *m*2000*rt* protocol (PCR Run Initiation, step 65) 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

54. 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.
55. Prior to use, mix the amplification master mix by gently pipetting up and down 5 times. Ensure that no foam or bubbles are created.
56. Pipette 45 µL of the master mix into the required number wells of the Abbott 96-Well Optical Reaction Plate according to the test order.
57. 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°C to 8°C or 60 days at -25°C to -15°C.
    - Frozen master mixes cannot undergo more than 5 freeze/thaws
58. Visually verify that 45 µL of master mix have been dispensed into each designated well.
59. Prior to use, vortex each diluted DNA eluate tube for 10 seconds. After vortexing, tap each tube on the bench to bring the liquid to the bottom of the tube.
60. Pipette 15 µL of diluted DNA eluate into each well designated for a sample according to the test order. Change pipette tips between wells.
61. Visually verify that 60 µL total have been dispensed into each designated well.
62. Seal the Abbott 96-Well Optical Reaction Plate with the Abbott Optical Adhesive Cover according to the instructions in the Abbott *m*2000*rt* Operations Manual.
63. Ensure that all material is at the bottom of each well. If needed, centrifuge 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

64. Transfer the Abbott 96-Well Optical Reaction Plate from the Abbott Splash-Free Support Base to the Abbott *m*2000*rt* instrument.
65. Select the Abbott *m*2000*rt* test order created in **Abbott *m*2000*rt* Initiation and Test Order Creation**, Step 49. Initiate the run, as described in the Abbott *m*2000*rt* Operations Manual, **Operating Instructions** section.
66. At the completion of the run, assay results are reported on the Abbott *m*2000*rt* 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 *m*2000*rt* Operations Manual, along with the gloves used to handle the plate.
2. Clean the Abbott Splash-Free Support Base before next use, according to Abbott *m*2000*rt* Operations Manual.
3. Decontaminate and dispose of all specimens, controls, 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, clear and clean all work areas. Decontaminate work area according to laboratory guidelines.

QUALITY CONTROL PROCEDURES

Abbott *m*2000*rt* Optical Calibration

Refer to the **Calibration Procedures** section in the Abbott *m*2000*rt* Operations Manual for a detailed description of how to perform an Abbott *m*2000*rt* Optical Calibration. Optical calibration of the Abbott *m*2000*rt* instrument is required for the accurate measurement and discrimination of dye fluorescence during the Abbott RealTi*m*e IDH1 assay.

The following Abbott *m*2000*rt* Optical Calibration Plates are used to calibrate the Abbott *m*2000*rt* instrument for the Abbott RealTi*m*e IDH1 assay:

- FAM™ Plate (Carboxyfluorescein)
- VIC™ Plate (Proprietary dye)
- NED™ Plate (Proprietary dye)
- 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 RealTi*m*e IDH1 controls need to be processed alongside the specimens prior to running the amplification portion of the assay.

The Positive Control is formulated with DNA containing IDH1 mutation and IC sequences. R132H, R132L, and R132G signals should be detected for the Positive Control. In addition, the IC signal should be detected in both reactions for the Positive Control.

The Negative Control is formulated with DNA containing the IC sequence. Only the IC signal should be detected for the Negative Control in both reactions.

IDH1 mutations (R132C, R132H, R132G, R132S, and R132L) should not be detected in the Negative Control. IDH1 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 *m*2000*rt* instrument according to the Abbott *m*2000*rt* 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 of the Negative Control or 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 (e.g. 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 *m*2000*rt*, 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 R132H, R132C, R132G, R132S, or R132L in the swab sample(s). If contamination is not present, CN values for R132H, R132C, R132G, R132S, or R132L 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 IDH1 mutation is detected on equipment, follow the cleaning and decontaminating guidelines given in the equipment operations manual. If IDH1 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 IDH1 mutation(s) is no longer detected.

INTERPRETATION OF RESULTS

The Abbott *m*2000*rt* instrument automatically reports the results on the *m*2000*rt* workstation. Examples of assay results and interpretations are provided in **Table 3**.

The Abbott RealTi*m*e IDH1 assay controls are used to establish run validity for the Abbott RealTi*m*e IDH1 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 3. Examples of Assay Results

Sample ID	Result	Interpretation
Sample 1	R132H[a]	Mutation Detected
Sample 2	Not Detected	Not Detected

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

Results from both 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 RealTi*m*e IDH1 assay are listed in **Appendix 1** and **Appendix 2**. For each error code, probable causes and corrective actions specific to the Abbott RealTi*m*e IDH1 are also listed. Information for additional error codes is presented in the Abbott *m*2000*rt* 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 *m*2000*rt* 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 *m*2000*rt* 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 RealTi*m*e IDH1 is for use with human blood (EDTA) and bone marrow aspirate (EDTA) specimens only.
- Optimal 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 RealTi*m*e IDH1 assay is limited to personnel who have been trained in the procedures of molecular diagnostic assays, the Abbott *m*2000*rt* instrument, and the manual sample preparation method for Abbott RealTi*m*e IDH1.
- A "Not Detected" result does not preclude the presence of IDH1 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 RealTi*m*e IDH1 is designed to detect IDH1 R132C, R132H, R132G, R132S, and R132L mutations. Specimens with results reported as "Not Detected" may contain mutations that are not targeted by the assay.
- 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 IDH1 Oligonucleotide Reagents from Amplification kit lot X with the DNA Polymerase from Amplification kit lot Y.

SPECIFIC PERFORMANCE CHARACTERISTICS

Bone marrow aspirate and blood were tested in all key analytical studies. For IDH1 mutations, AML clinical specimens were used, when possible; otherwise cell lines with IDH1 mutations were used. Five separate cell lines were used in the analytical studies, each cell line contained a single IDH1 mutation and represented all 5 IDH1 mutations that can be detected by Abbott RealTi*m*e IDH1 including R132C, R132H, R132L, R132G, and R132S. When contrived specimens were used for the Within-Laboratory Repeatability, Within-Laboratory Lot-to-Lot Reproducibility, Within-Laboratory Specimen Handling Reproducibility, Extracted Clinical Specimen DNA Stability, Potentially Interfering Substances, Potentially Interfering Drugs, and Potentially Interfering Microorganisms studies, cell lines were spiked into blood or bone marrow to target specific mutation percentages. When contrived specimens were used for the Analytical Sensitivity – Limit of Detection, Characterization of IDH1 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 RealTi*m*e IDH1 is designed such that quantitation of DNA prior to PCR amplification is not required. A fixed amount of diluted eluate (15 µL) from sample extraction is used in each PCR reaction. In order to evaluate the typical DNA yield from AML specimens, an analysis was performed on DNA extracted from 277 AML clinical specimens (198 bone marrow aspirate specimens and 79 blood specimens) using the Abbott *m*Sample Preparation System$_{DNA}$. 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 10 ng to 839 ng per PCR reaction, which represents an IC CN range of 14.64 CN to 21.01 CN. The gDNA input range observed for whole blood was 11 ng to 492 ng per PCR reaction, which represents an IC CN range of 15.41 CN to 20.94 CN. Abbott RealTi*m*e IDH1 assay performance was verified across a range of 10 ng to 850 ng gDNA input per PCR reaction.

ANALYTICAL SENSITIVITY

Limit of Detection

Analytical sensitivity was assessed using eluates generated from IDH1 mutation positive AML clinical specimens and contrived specimens. The IDH1 percent mutation ranged from 0.25% to 10% for 10 ng gDNA input and from 0.13% to 10% mutation for 85 ng gDNA input. A 28-member panel was generated for each of the 5 SNVs targeted by the assay. IDH1 R132C mutation positive panel members were prepared from AML clinical specimen eluates, both blood and bone marrow aspirate. IDH1 R132H, R132L, R132G, and R132S mutation positive panel members were prepared from eluates of IDH1 mutation positive cell lines spiked into blood or bone marrow. Each panel member was tested with 2 Abbott RealTi*m*e IDH1 Amplification Reagent Kit lots with 4 runs per lot over a minimum of 4 days and 5 replicates per run for a total of 40 replicates per panel member per mutation and 200 replicates for the 5 mutations combined.

The analysis demonstrated a detection rate of 100% (200/200) at mutation levels of 2% and higher for all IDH1 mutations combined. The analysis demonstrated a detection rate of 98% (196/200) or greater at mutation levels of 1% and higher for all IDH1 mutations combined. Refer to **Table 4** for results.

Table 4: Limit of Detection Study Results for all IDH1 Mutations* Combined

gDNA Input per Reaction	Sample Type	Mutation %	Detection Ratio	Detection Rate
85 ng	Blood	10%	200/200	100%
85 ng	Blood	5%	200/200	100%
85 ng	Blood	2%	200/200	100%
85 ng	Blood	1%	196/200	98%
85 ng	Blood	0.5%	180/200	90%
85 ng	Blood	0.25%	156/200	78%
85 ng	Blood	0.13%	129/200	64.5%
85 ng	Bone Marrow	10%	200/200	100%
85 ng	Bone Marrow	5%	200/200	100%
85 ng	Bone Marrow	2%	200/200	100%
85 ng	Bone Marrow	1%	197/197	100%
85 ng	Bone Marrow	0.5%	191/200	95.5%
85 ng	Bone Marrow	0.25%	165/200	82.5%
85 ng	Bone Marrow	0.13%	129/200	64.5%
10 ng	Blood	10%	200/200	100%
10 ng	Blood	5%	200/200	100%
10 ng	Blood	4%	200/200	100%
10 ng	Blood	2%	200/200	100%
10 ng	Blood	1%	199/200	99.5%
10 ng	Blood	0.5%	166/200	83%
10 ng	Blood	0.25%	72/200	36%
10 ng	Bone Marrow	10%	200/200	100%
10 ng	Bone Marrow	5%	200/200	100%
10 ng	Bone Marrow	4%	200/200	100%
10 ng	Bone Marrow	2%	200/200	100%
10 ng	Bone Marrow	1%	200/200	100%
10 ng	Bone Marrow	0.5%	156/200	78%
10 ng	Bone Marrow	0.25%	61/200	30.5%

* Includes R132C, R132H, R132L, R132G, and R132S, 40 replicates per mutation.

In a second study, analytical sensitivity was assessed using eluates generated from IDH1 mutation positive AML clinical specimens. The IDH1 percent mutation ranged from 0.25% to 5% for 10 ng gDNA input. A 10-member panel was generated for each of 4 SNVs (R132H, R132L, R132G, and R132S) evaluated in this study and was prepared from AML clinical specimen eluates, both blood and bone marrow aspirate. Each panel member was tested with 2 Abbott RealTi*m*e IDH1 Amplification Reagent Kit lots with 4 runs per lot over 4 days and 5 replicates per run for a total of 40 replicates per panel member and 160 replicates for the 4 mutations combined.

The analysis demonstrated a detection rate of 100% (160/160) at mutation levels of 1% and higher for the 4 IDH1 mutations combined. Refer to **Table 5** for results.

Table 5. Limit of Detection Study Results for 4 IDH1 Mutations* Combined

gDNA Input per Reaction	Sample Type	Mutation %	Detection Ratio	Detection Rate
10 ng	Blood	5%	160/160	100%
10 ng	Blood	2%	160/160	100%
10 ng	Blood	1%	160/160	100%
10 ng	Blood	0.5%	131/160	81.9%
10 ng	Blood	0.25%	46/160	28.8%
10 ng	Bone Marrow	5%	160/160	100%
10 ng	Bone Marrow	2%	160/160	100%
10 ng	Bone Marrow	1%	160/160	100%
10 ng	Bone Marrow	0.5%	123/160	76.9%
10 ng	Bone Marrow	0.25%	51/160	31.9%

* Includes R132H, R132L, R132G, and R132S, 40 replicates per mutation.

Characterization of IDH1 Mutation Detection Across a Low to High Range of DNA Input Levels

This study evaluated the detection of IDH1 mutations targeted by the assay at several gDNA inputs across a range of 1 ng to 850 ng. For each IDH1 SNV, the following gDNA inputs were tested at 2% mutation level in both blood and bone marrow aspirate: approximately 1 ng, 25 ng, 200 ng, 400 ng, and 850 ng. A total of 50 panel members (5 panel members x 5 mutations x 2 sample types [blood and bone marrow]) were tested. Three replicates per panel member per mutation were tested for a total of 15 replicates for the 5 mutations combined.

All panel members reported “Mutation Detected” at a 100% (15/15) detection rate when total gDNA input levels were 25 ng, 200 ng, 400 ng and 850 ng per reaction. When the gDNA input level was 1 ng per reaction, an overall detection rate of 0% (0/15) 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. Refer to **Table 6** for results.

The analysis demonstrated a detection rate of 100% at mutation levels of approximately 2% when the total gDNA input was approximately 25 ng, 200 ng, 400 ng and 850 ng per reaction for all mutations tested.

Table 6. Detection of 2% Mutation Level for a Range of DNA Inputs for all IDH1 Mutations* Combined

Sample Type	gDNA Input per Reaction (ng)	Number Valid Replicates	Number of Replicates Mutation Detected	Valid Replicate Detection Rate (%)	Total Number of Replicates	Overall Detection Rate (%)
Blood	1	0	0	N/A	15	0
Blood	25	15	15	100	15	100
Blood	200	15	15	100	15	100
Blood	400	15	15	100	15	100
Blood	850	15	15	100	15	100
Bone Marrow	1	0	0	N/A	15	0
Bone Marrow	25	15	15	100	15	100
Bone Marrow	200	15	15	100	15	100
Bone Marrow	400	15	15	100	15	100
Bone Marrow	850	15	15	100	15	100

* Includes R132C, R132H, R132L, R132G, and R132S, 3 replicates per mutation

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 RealTi*m*e IDH1 target using a panel representing IDH1 SNVs targeted by the assay, IDH1 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 85 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 85 ng of total DNA. Panel Members included wild type HP DNA (R132) and wild type HP DNA plus one of 7 DNA plasmids coding for each of the 5 IDH1 SNVs targeted by the assay (R132C, R132H, R132L, R132G, and R132S) or 2 IDH1 SNVs not targeted by the assay (R132P, R132V). The prepared panel members were directly tested using the Abbott RealTi*m*e IDH1 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. Five 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 IDH1 mutation. Two panel members not targeted by the assay (R132P mutation, and R132V mutation) reported “Not Detected” with a 100% (40/40) rate of correct sample interpretation. R132H CN values were observed for the wild type HP DNA panel member with a correct final interpretation of “Not Detected” in all replicates. The assay data reduction algorithm employs multiple criteria to determine the final mutation status.

The Abbott RealTi*m*e IDH1 assay demonstrated a 100% (40/40) correct sample interpretation rate using a panel representing IDH1 SNVs targeted by the assay, IDH1 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 IDH1 mutation negative and IDH1 R132H mutation positive blood and bone marrow aspirate specimens. For the IDH1 mutation positive panel members, a R132H positive cell line was spiked in blood and bone marrow targeting a 6% 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 RealTi*m*e IDH1 assay was observed in the presence of the potentially interfering substances for all IDH1 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 IDH1 mutation negative and IDH1 R132C mutation positive blood and bone marrow aspirate specimens. For the IDH1 mutation positive panel members, a R132C positive cell line was 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:

Potentially Interfering Drug Pools			
Pool 1	**Pool 2**	**Pool 3**	**Pool 4**
Vancomycin Hydrochloride	Levofloxacin	Linezolid	Meropenem
Busulfan	Acyclovir	Arsenic Trioxide	Azacytidine
Decitabine	Cyclophosphamide	Cytarabine	Daunorubicin Hydrochloride
Hydroxyurea	Doxorubicin Hydrochloride	Etoposide	Fludara
Mitoxantrone Hydrochloride	Idarubicin Hydrochloride	Lomustine	Melphalan
Everolimus	Vincristine Sulfate	Erythropoietin	Filgrastim
Dexamethasone	Mercaptopurine	Mycophenolate Mofetil	Tacrolimus
Imipenem	Prednisone	AG-221	AG-120

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 EP07-A2.[15] The analysis demonstrated the percent agreement was 100% (3/3) for each sample and test condition.

No interference of the Abbott RealTi*m*e IDH1 assay was observed in the presence of the potential interfering drugs for all IDH1 mutation positive and negative samples for either blood or bone marrow aspirate specimens.

Potentially Interfering Microorganisms

Potentially interfering microorganisms were added to IDH1 mutation negative and IDH1 R132C mutation positive blood and bone marrow aspirate specimens. For the IDH1 mutation positive panel members, a R132C positive cell line was 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:

Potentially Interfering Microbe Pools			
Pool 1	**Pool 2**	**Pool 3**	**Pool 4**
Staphylococcus aureus (MRSA; COL)	*Streptococcus agalactiae*	*Enterococcus faecalis*	*Pseudomonas aeruginosa*
Staphylococcus aureus (CoNS)	*Streptococcus pneumoniae*	*Enterobacter cloacae*	*Acinetobacter baumannii*
Staphylococcus epidermidis	*Klebsiella pneumoniae*	*Aspergillus terreus*	varicella zoster virus
Escherichia coli	*Candida albicans*	influenza A H3	respiratory syncytial virus type A
Serratia marcescens	herpes simplex virus type 1 MacIntyre		respiratory syncytial virus type B
cytomegalovirus	herpes simplex virus type 2 MS		

Potentially interfering microorganisms were chosen based on clinical presentation in AML patients.[16] The analysis demonstrated the percent agreement was 100% (3/3) for each sample and test condition.

No interference or cross reactivity of the Abbott RealTi*m*e IDH1 assay was observed in the presence of the potentially interfering microorganisms for all IDH1 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 an 8-member panel. The panel contained IDH1 mutation negative blood and bone marrow aspirate specimens as well as blood and bone marrow aspirate specimens spiked with R132C or R132G 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 two master mixes. Each operator tested the panel using 2 lots of grouped reagents–2 lots of Abbott *m*Sample Preparation System$_{DNA}$, Abbott RealTi*m*e IDH1 Amplification Reagent Kits, and Abbott RealTi*m*e IDH1 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 *m*2000*rt* instruments. Each reagent lot was assigned to its own instrument.

All IDH1 mutation positive panel members reported "Mutation Detected" with a 100% percent agreement across all 32 replicates, and all IDH1 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 RealTi*m*e IDH1 assay demonstrated a percent agreement of 100% with expected results for each panel member by operator, by 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 for mutation positive panel members are shown in **Table 7**. 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 as well as 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 7. Analysis of dCN Values for Mutation Positive Panel Members

					Within-Run Variability		Between-Run Variability		Between-Lot Variability		Between-Operator Variability		Total	
Panel	**Mutation**	**Sample Type**	**N**	**Mean**	**SD**	**% CV**	**SD**	**% CV**	**SD**	**% CV**	**SD**	**% CV**	**SD**	**% CV**
3	R132C	Blood	32	9.32	0.310	3.3	0.248	2.7	0.111	1.2	0.080	0.9	0.420	4.5
4	R132G	Blood	32	6.00	0.163	2.7	0.000	0.0	0.097	1.6	0.058	1.0	0.190	3.2
7	R132C	Bone Marrow	32	7.83	0.154	2.0	0.174	2.2	0.283	3.6	0.283	3.6	0.366	4.7
8	R132G	Bone Marrow	32	5.60	0.170	3.0	0.000	0.0	0.000	0.0	0.008	0.1	0.170	3.0

Table 8. Analysis of Oligo IC CN for Positive and Negative Controls and Mutant CN values for Positive Controls

				Within-Run Variability	Between-Run Variability	Between-Lot Variability	Between-Operator Variability	Total
Control	**Value**	**N**	**Mean**	**SD**	**SD**	**SD**	**SD**	**SD**
IDH1 Negative Control	OLIGO1 IC CN	16	23.32	0.631	0.291	0.242	0.343	0.961
	OLIGO2 IC CN	16	22.01	0.224	0.000	0.077	0.114	0.262
IDH1 Positive Control	OLIGO1 IC CN	16	23.23	0.746	0.268	0.245	0.185	0.830
	OLIGO2 IC CN	16	22.02	0.173	0.000	0.068	0.000	0.186
	R132H CN	16	26.08	0.683	0.370	0.000	0.205	0.808
	R132L CN	16	24.04	0.305	0.000	0.212	0.040	0.371
	R132G CN	16	24.13	0.195	0.116	0.035	0.079	0.243

Within-Laboratory Lot-to-Lot Reproducibility

The within-laboratory lot-to-lot reproducibility was evaluated using 3 unique lots of Abbott *m*Sample Preparation System$_{DNA}$ kits and 3 unique lots of Abbott RealTi*m*e IDH1 Amplification Reagent Kits. An 8-member panel contained IDH1 mutation negative blood and bone marrow specimens as well as blood and bone marrow specimens spiked with R132C or R132G 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 two master mixes. For each panel member, 10 replicates were prepared using 3 unique lots of Abbott *m*Sample Preparation System$_{DNA}$ for a total of 30 extractions per panel member. Each extraction was tested using 3 unique lots of Abbott RealTi*m*e IDH1 Amplification Reagent Kit for a total of 9 unique sample preparation/amplification kit combinations. Three *m*2000*rt* instruments were used, one for each lot of Abbott RealTi*m*e IDH1 Amplification Reagent Kit. Thus, for each panel member, a total of 90 replicates were tested.

Mutation positive panel members demonstrated ≥ 99% agreement overall with expected results. All mutation negative panel members demonstrated 100% agreement overall with expected results. The percent agreement by Abbott *m*Sample Preparation System$_{DNA}$ lot was 97% or greater for each panel member. The percent agreement by Abbott RealTi*m*e IDH1 Amplification Reagent Kit lot was 97% or greater for each panel member.

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 for mutation positive panel members are shown in **Table 9**. "Not Detected" replicates were excluded since dCN value was not generated. 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 as well as the mean and SD of mutant CN values and the between-replicate, between-amplification lot and between-sample prep lot variability for Positive Controls is shown in **Table 10.**

Table 9. Analysis of dCN Values for Mutation Positive Panel Members

Panel	Mutation	Sample Type	N	Mean	Between-Replicate Variability SD	Between-Amplification Lot Variability SD	Between-Sample Prep Lot Variability SD	Total SD
3	R132C	Blood	89*	8.39	0.309	0.363	0.981	1.051
4	R132G	Blood	89*	6.16	0.160	0.198	0.096	0.272
7	R132C	Bone Marrow	90	6.92	0.301	0.270	0.291	0.441
8	R132G	Bone Marrow	90	5.61	0.157	0.149	0.320	0.376

*One replicate was Not Detected and excluded from the analysis since dCN value was not generated.

Table 10. Analysis of Oligo IC CN for Positive and Negative Controls and Mutant CN values for Positive Controls

Control	Value	N	Mean	Between-Replicate Variability SD	Between-Amplification Lot Variability SD	Between-Sample Prep Lot Variability SD	Total SD
IDH1 Negative Control	OLIGO1 IC CN	36	22.71	0.466	0.234	0.176	0.532
	OLIGO2 IC CN	36	21.78	0.306	0.281	0.124	0.434
IDH1 Positive Control	OLIGO1 IC CN	36	22.61	0.555	0.210	0.155	0.613
	OLIGO2 IC CN	36	21.82	0.176	0.243	0.077	0.310
	R132H CN	36	25.20	0.513	0.179	0.105	0.553
	R132L CN	36	24.04	0.214	0.221	0.050	0.311
	R132G CN	36	24.18	0.205	0.154	0.049	0.261

Within-Laboratory Specimen Handling Reproducibility

The within-laboratory specimen handling reproducibility of Abbott RealTi*m*e IDH1 was evaluated using two operators. Each operator tested a 10-member panel containing three mutation positive (R132C, R132H and R132S) AML blood specimens and AML bone marrow aspirate specimens, in addition to two mutation positive (R132L and R132G) contrived blood specimens and contrived bone marrow aspirate specimens. The contrived specimens were prepared from normal blood or bone marrow spiked with IDH1 mutation positive cell line targeting 6% mutation level. Each operator tested the panel using the same lots of Abbott RealTi*m*e IDH1 Amplification Reagent Kit and Abbott RealTi*m*e IDH1 Control Kit and unique lots of Abbott mSample Preparation System$_{DNA}$. Each operator performed 1 run per day over a total of 5 days. A run consisted of sample preparation and PCR. Two replicates of each panel member were tested in each run, for a total of 20 replicates per panel member (10 replicates per operator). One 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 two *m*2000*rt* instruments. Each Abbott *m*Sample Preparation System$_{DNA}$ lot was assigned to its own instrument.

In the within-laboratory specimen handling reproducibility, all IDH1 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 11**. The mean and SD of IC CN values and the between-run and between-operator variability for Positive and Negative Controls as well as the mean and SD of mutant CN values and the between-run and between-operator variability for Positive Controls are shown in **Table 12**.

Table 11. Analysis of dCN values for Panel Members

						Within-Run Variability	Between-Run Variability	Between-Operator Variability	Total
Panel	Value	Mutation	Sample Type	N	Mean (dCN)	SD	SD	SD	SD
1	dCN	R132C	Blood	20	3.75	0.130	0.188	0.237	0.329
2	dCN	R132H	Blood	20	2.86	0.074	0.039	0.000	0.084
3	dCN	R132S	Blood	20	3.33	0.108	0.045	0.000	0.117
4	dCN	R132L	Blood	20	4.07	0.173	0.110	0.347	0.403
5	dCN	R132G	Blood	20	4.91	0.156	0.083	0.239	0.292
6	dCN	R132C	Bone Marrow	20	3.97	0.068	0.189	0.441	0.485
7	dCN	R132H	Bone Marrow	20	3.23	0.051	0.083	0.000	0.098
8	dCN	R132S	Bone Marrow	20	2.53	0.088	0.128	0.090	0.159
9	dCN	R132L	Bone Marrow	20	4.17	0.110	0.172	0.240	0.315
10	dCN	R132G	Bone Marrow	20	4.47	0.112	0.091	0.193	0.241

Table 12. Analysis of Oligo IC CN for Positive and Negative Controls and Mutant CN values for Positive Controls

				Between-Run Variability	Between-Operator Variability	Total
Control	Value	N	Mean	SD	SD	SD
IDH1 Negative Control	OLIGO1 IC CN	10	23.28	0.443	0.530	0.691
	OLIGO2 IC CN	10	22.00	0.162	0.000	0.162
IDH1 Positive Control	OLIGO1 IC CN	10	23.27	0.556	0.366	0.666
	OLIGO2 IC CN	10	22.05	0.230	0.000	0.230
	R132C CN	10	24.60	0.229	0.000	0.229
	R132H CN	10	26.24	0.411	0.466	0.621
	R132L CN	10	24.40	0.225	0.000	0.225

Reproducibility I

Reproducibility of the Abbott RealTi*m*e IDH1 assay was evaluated at 3 external sites by testing IDH1 wild type (WT) mutation negative and IDH1 mutation positive blood and bone marrow specimens. The 4 member panel contained IDH1 wild type (WT) mutation negative and IDH1 R132C mutation positive blood and bone marrow specimens. For the IDH1 mutation positive panel members, a R132C positive cell line was spiked in blood and bone marrow targeting a 6% mutation level. At each site, a technologist 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 *m*Sample Preparation System$_{DNA}$ for a total of 3 unique lots. The same lot of Abbott RealTi*m*e IDH1 Amplification Reagent Kit and Abbott RealTi*m*e IDH1 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 13**. 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 14**.

Table 13. Reproducibility I Overall Precision

						Within-Run Variability	Between-Run Variability	Between-Site Variability	Total
Panel	**Mutation**	**Specimen Type**	**Value**	**N**	**Mean**	**SD**	**SD**	**SD**	**SD**
1	WT	Bone Marrow	OLIGO1 IC CN	36	15.89	0.400	0.324	1.272	1.372
	WT	Bone Marrow	OLIGO2 IC CN	36	15.89	0.402	0.288	1.218	1.315
2	R132C	Bone Marrow	dCN	36	7.29	0.346	0.232	0.741	0.851
3	WT	Blood	OLIGO1 IC CN	36	16.56	0.382	0.077	0.887	0.969
	WT	Blood	OLIGO2 IC CN	36	16.53	0.357	0.045	0.810	0.887
4	R132C	Blood	dCN	36	7.99	0.293	0.218	0.542	0.654

Table 14. Reproducibility I Analysis of Oligo IC CN for Positive and Negative Controls and Mutant CN values for Positive Controls

				Between-Run Variability	Between-Site Variability	Total
Control	**Value**	**N**	**Mean**	**SD**	**SD**	**SD**
IDH1 Negative Control	OLIGO1 IC CN	18	22.25	0.424	0.494	0.651
	OLIGO2 IC CN	18	21.44	0.166	0.255	0.305
IDH1 Positive Control	R132H CN	18	25.46	0.335	0.324	0.466
	R132L CN	18	24.05	0.169	0.133	0.215
	R132G CN	18	24.33	0.147	0.276	0.313
	OLIGO1 IC CN	18	21.29	0.899	0.226	0.927
	OLIGO2 IC CN	18	21.15	0.584	0.221	0.625

Reproducibility II

Reproducibility of the Abbott RealTi*m*e IDH1 assay was evaluated at 3 external sites by testing DNA eluate extracted from IDH1 wild type (WT) mutation negative and IDH1 mutation positive blood and bone marrow specimens. The panel members included 5 IDH1 SNVs and wild type specimens targeting 25 ng and 85 ng gDNA input levels. IDH1 mutation positive panel members were prepared to target a mutation percentage of approximately 2% or 15%. The mutation positive panel members were prepared from eluates of IDH1 mutation positive cell lines spiked into blood or bone marrow. Panel members were tested using 3 unique lots of Abbott RealTi*m*e IDH1 Amplification Reagent Kit. At each site, 2 technologists 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.

The eluate reproducibility analysis demonstrated 100% agreement for each panel member at Site 1 and Site 3, and a range of 76% to 100% agreement for each panel member at Site 2. For Site 2, agreement rates for panel members 3, 4, 5, 8, 9, 10, 12 were 100%, for panel member 1 was 86%, for panel member 2 was 91%, for panel member 6 was 98%, for panel member 7 was 76%, and for panel member 11 was 98%.

The eluate reproducibility analysis demonstrated 100% (60/60) agreement for each panel member tested with Abbott RealTi*m*e Amplification Reagent Kit lot 2, a range of 90% to 100% agreement for Abbott RealTi*m*e Amplification Reagent Kit lot 1, and a range of 86% to 100% agreement for Abbott RealTi*m*e Amplification Reagent Kit lot 3.

The eluate reproducibility analysis demonstrated showed a range of 92% to 100% overall agreement 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 15**. "Not Detected" replicates were excluded since dCN values were not generated. 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 16**.

Table 15. Reproducibility II Overall Precision

						Within-Run Variability	Between-Run Variability	Between-Lot Variability	Between-Operator Variability	Between-Site Variability	Total
Panel	**Mutation**	**Specimen Type**	**Value**	**N**	**Mean**	**SD**	**SD**	**SD**	**SD**	**SD**	**SD**
1	R132H	Bone Marrow	dCN	169[a]	6.05	0.351	0.243	0.061	0.218	0.298	0.567
2	R132C	Bone Marrow	dCN	173[b]	6.43	0.693	0.547	0.120	0.498	0.628	1.213
3	R132L	Bone Marrow	dCN	178[c]	5.65	0.307	0.132	0.000	0.076	0.418	0.541
4	R132S	Bone Marrow	dCN	177[d]	3.41	0.116	0.046	0.140	0.057	0.304	0.361
5	R132G	Bone Marrow	dCN	178[c]	6.48	0.224	0.155	0.056	0.150	0.130	0.342
6	R132H	Blood	dCN	179[e]	3.54	0.307	0.549	0.000	0.550	0.000	0.835
7	R132C	Blood	dCN	164[f]	8.33	0.527	0.212	0.000	0.000	0.738	0.931
8	R132L	Blood	dCN	180	2.71	0.207	0.240	0.000	0.159	0.710	0.811
9	R132S	Blood	dCN	180	5.04	0.295	0.152	0.043	0.071	0.382	0.513
10	R132G	Blood	dCN	179[g]	5.62	0.296	0.112	0.121	0.086	0.179	0.392
11	WT	Bone Marrow	OLIGO1 IC CN	179[h]	19.00	0.761	0.294	0.289	0.216	0.434	0.992
	WT	Bone Marrow	OLIGO2 IC CN	179[h]	19.47	0.515	0.413	0.000	0.000	0.662	0.935
12	WT	Blood	OLIGO1 IC CN	179[g]	19.23	1.496	0.000	0.548	0.499	0.345	1.704
	WT	Blood	OLIGO2 IC CN	179[g]	19.57	0.873	0.706	0.000	0.000	1.357	1.761

Note: "Not Detected" replicates were excluded since dCN values were not generated.

[a] 8 replicates were Not Detected, 3 replicates were invalid.

[b] 5 replicates were Not Detected, 7 replicates were invalid.

[c] 2 replicates were invalid.

[d] 3 replicates were invalid.

[e] 1 replicate was Not Detected.

[f] 14 replicates were Not Detected, 2 replicates were invalid.

[g] 1 replicate was invalid.

[h] 1 replicate was Mutation Detected

Table 16. Reproducibility II Analysis of Oligo IC CN for Positive and Negative Controls and Mutant CN values for Positive Controls

				Between-Run Variability	Between-Lot Variability	Between-Operator Variability	Between-Site Variability	Total
Control	**Value**	**N**	**Mean**	**SD**	**SD**	**SD**	**SD**	**SD**
IDH1 Negative Control	OLIGO1 IC CN	60	23.13	0.642	0.000	0.000	0.658	0.919
	OLIGO2 IC CN	60	22.02	0.134	0.039	0.156	0.328	0.389
IDH1 Positive Control	R132H CN	60	25.75	0.612	0.000	0.478	0.629	0.999
	R132L CN	60	23.81	0.158	0.066	0.085	0.409	0.451
	R132G CN	60	24.57	0.159	0.092	0.060	0.398	0.443
	OLIGO1 IC CN	60	22.78	0.927	0.000	0.206	0.355	1.014
	OLIGO2 IC CN	60	21.82	0.568	0.000	0.167	0.294	0.661

Reproducibility III

Reproducibility of the Abbott RealTi*m*e IDH1 assay was evaluated at 3 external sites by testing DNA eluate extracted from IDH1 mutation positive blood and bone marrow AML clinical specimens. The 4-member panel contained the R132C IDH1 SNV mutation at different targeted mutation percentage levels while maintaining a targeted gDNA input level of 25ng; the targeted mutation percentages were either approximately 2% or 6%, with the low mutation percentage representing samples near the assay limit of detection. Panel members were tested using 3 unique lots of Abbott RealTi*m*e IDH1 Amplification Reagent Kit. At each site, 2 technologists performed 2 amplification/detection runs over 5 non-consecutive 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.

The AML clinical specimen eluate reproducibility analysis demonstrated 100% (60/60) agreement for each panel member at Site 1 and Site 3, and a range of 98% to 100% agreement for each panel member at Site 2. For Site 2, agreement rates for panel members 1, 2, 3 were 100%, for panel member 4 was 98%.

The AML clinical specimen eluate reproducibility analysis demonstrated 100% (60/60) agreement for each panel member tested with Lot 2 and Lot 3 of the Abbott RealTi*m*e Amplification Reagent Kit, and a range of 98% to 100% agreement for Lot 1 of the Abbott RealTi*m*e Amplification Reagent Kit.

The AML clinical specimen eluate reproducibility analysis demonstrated a range of 99% to 100% overall agreement for each panel member for all sites and lots combined.

In Reproducibility III, 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.**

Table 17. Reproducibility III Overall Precision

						Within-Run Variability	Between-Run Variability	Between-Lot Variability	Between-Operator Variability	Between-Site Variability	Total
Panel	**Mutation**	**Specimen Type**	**Value**	**N**	**Mean**	**SD**	**SD**	**SD**	**SD**	**SD**	**SD**
1	R132C	Bone Marrow	dCN	180	7.80	0.215	0.354	0.770	0.043	0.250	0.910
2	R132C	Bone Marrow	dCN	180	6.92	0.213	0.368	0.725	0.189	0.066	0.864
3	R132C	Blood	dCN	180	8.09	0.225	0.331	0.687	0.177	0.071	0.815
4	R132C	Blood	dCN	179[a]	7.27	0.196	0.373	0.705	0.274	0.000	0.866

[a] 1 replicate was Not Detected and was excluded since a dCN value was not generated.

The mean and SD of IC CN values and between-run, within-run, between-lot, between-operator and between-site variability for the Positive and Negative Controls are shown in **Table 18**.

Table 18. Reproducibility III Analysis of Oligo IC CN for Positive and Negative Controls

				Within-Run Component	Between-Run Component	Between-Lot Component	Between-Tech Component	Between-Site Component	Total
Control	**Value**	**N**	**Mean**	**SD**	**SD**	**SD**	**SD**	**SD**	**SD**
IDH1 Negative Control	IDH1 OLIGO1 IC CN	60	23.00	0.000	0.262	0.225	0.188	0.234	0.399
	IDH1 OLIGO2 IC CN	60	22.10	0.000	0.142	0.106	0.110	0.086	0.226
IDH1 Positive Control	IDH1 OLIGO1 IC CN	60	22.99	0.000	0.297	0.326	0.175	0.387	0.518
	IDH1 OLIGO2 IC CN	60	22.11	0.000	0.163	0.193	0.108	0.139	0.308

COMPARISON TO NGS

A retrospective correlation analysis was performed using a data set of 305 clinical patients with valid IDH1 mutation testing results from both the Abbott RealTi*m*e IDH1 assay and one of two laboratory validated NGS (Next Generation Sequencing) methods. The data set included IDH1 mutation positive patients and IDH1 mutation negative patients, with similar numbers of blood and bone marrow samples from the 305 patients (285 blood samples and 271 bone marrow samples). The analysis evaluated agreement for the 5 IDH1 mutations that the Abbott RealTi*m*e IDH1 assay is designed to detect.

The correlation analysis of the 305 patient results, using the Abbott RealTi*m*e IDH1 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% (201/201) Positive Percent Agreement (PPA) with a 95% exact CI of (98.18%, 100.00%). The analysis demonstrated a 96.15% (100/104) Negative Percent Agreement (NPA) with a 95% exact CI of (90.44%, 98.94%).

The correlation of interpretation analysis was performed by sample type. For the blood sample type, the analysis demonstrated a 100.00% (194/194) Positive Percent Agreement (PPA) with a 95% exact CI of (98.12%, 100.00%). The analysis demonstrated a 98.90% (90/91) NPA with a 95% exact CI of (94.03%, 99.97%). For the bone marrow sample type, the analysis demonstrated a 100.00% (193/193) PPA with a 95% exact CI of (98.11%, 100.00%). The analysis demonstrated a 94.87% (74/78) NPA with a 95% exact CI of (87.39%, 98.59%).

An analysis demonstrating mutation identity agreement for samples identified as IDH1 "Mutation Detected" by the Abbott RealTi*m*e IDH1 assay is shown in **Table 20.**

Table 19. Correlation of Interpretation of Abbott RealTi*m*e IDH1 Results with NGS Methods Results

	NGS Method		
Abbott RealTi*m*e IDH1	**Mutation Detected**	**Mutation Not Detected**	**Total**
Mutation Detected	201	4	205
Mutation Not Detected	0	100	100
Total	201	104	305

Table 20. Correlation of Mutation Identity of Abbott RealTi*m*e IDH1 Results with NGS Method Results

	NGS Method						
Abbott RealTi*m*e IDH1	**Not Detected**	**R132C**	**R132H**	**R132G**	**R132S**	**R132L**	**Total**
R132C	0	122	0	0	1	0	**123**
R132H	2	0	50	0	0	0	**52**
R132G	2	1	0	11	0	0	**14**
R132S	0	0	0	0	10	0	**10**
R132L	0	0	0	0	0	6	**6**
Total	**4**	**123**	**50**	**11**	**11**	**6**	**205**

CONCORDANCE BETWEEN BLOOD AND BONE MARROW SPECIMEN TYPES

The Abbott RealTi*m*e IDH1 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 RealTi*m*e IDH1. 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 were seven subjects who had Abbott RealTi*m*e IDH1 mutation detected results in bone marrow and the mutation was not detected in blood. There was one subject who had Abbott RealTi*m*e IDH1 mutation detected result in blood and the mutation was not detected in bone marrow. The analysis demonstrates concordance between specimen types, with a slightly higher IDH1 mutation positive detection rate observed in bone marrow.

Table 21. Abbott RealTi*m*e IDH1 Concordance of Results from Blood versus Bone Marrow

	Blood		
Bone Marrow	**Mutation Detected**	**Not Detected**	**Total**
Mutation Detected	203	7	210
Not Detected	1	130	131
Total	204	137	341

Overall Agreement (OA): 97.65% (333/341) 95% CI (95.89%, 99.12%)

Average Positive Agreement (APA): 98.07% (406/414) 95% CI (96.56%, 99.26%)

Average Negative Agreement (ANA): 97.01% (260/268) 95% CI (94.82%, 98.87%)

CLINICAL STUDIES

Clinical Studies for TIBSOVO

The safety and effectiveness of the Abbott RealTi*me* IDH1 assay were demonstrated through testing of specimens from patients enrolled in Study AG120-C-001 (ClinicalTrials.gov Identifier NCT02074839). Study AG120-C-001 was an open-label, single-arm, multicenter clinical trial of TIBSOVO (ivosidenib) of 174 adult patients with relapsed or refractory Acute Myeloid Leukemia (R/R AML) and one of 5 IDH1 mutations in codon R132, who were assigned to receive a 500 mg daily dose. IDH1 mutations were identified by a local or central diagnostic test and retrospectively confirmed using the Abbott RealTi*me* IDH1 assay. TIBSOVO was given orally at a starting dose of 500 mg daily until disease progression, development of unacceptable toxicity, or undergoing hematopoietic stem cell transplantation. Twenty-one of the 174 patients (12%) went on to stem cell transplant following TIBSOVO treatment.

The baseline demographic and disease characteristics are shown in **Table 22**. The study participants were predominantly white (62%) with a median age of 67 years. Enrollment according to gender was equivalent. All 5 IDH1 mutations in codon R132 were detected among patients enrolled into the trial. The predominant IDH1 positive mutation was R132C (59%) followed by R132H (25%).

Table 22. Baseline Demographic and Disease Characteristics in Patients with Relapsed or Refractory AML

Demographic and Disease Characteristics	**TIBSOVO (500 mg daily) N=174**
Demographics	
Age (Years) Median (Min, Max)	67 (18, 87)
Age Categories, n (%)	
<65 years	63 (36)
≥65 years to <75 years	71 (41)
≥75 years	40 (23)
Sex, n (%)	
Male	88 (51)
Female	86 (49)
Race, n (%)	
White	108 (62)
Black or African American	10 (6)
Asian	6 (3)
Native Hawaiian/Other Pacific Islander	1 (1)
Other/Not provided	49 (28)
Disease Characteristics	
ECOG PS, n (%)	
0	36 (21)
1	97 (56)
2	39 (22)
3	2 (1)
IDH1 Mutation Variant, n (%)[a]	
R132C	102 (59)
R132H	43 (25)
R132G	12 (7)
R132S	10 (6)
R132L	7 (4)
Cytogenetic Risk Status, n (%)	
Intermediate	104 (60)
Poor	47 (27)
Missing/Unknown	23 (13)
Relapse Type, n (%)	
Primary refractory	64 (37)
Refractory relapse	45 (26)
Untreated relapse	65 (37)

Table 22 (continued). Baseline Demographic and Disease Characteristics in Patients with Relapsed or Refractory AML

Demographic and Disease Characteristics	TIBSOVO (500 mg daily) N=174
Relapse Number, n (%)	
0	64 (37)
1	83 (48)
2	21 (12)
≥3	6 (3)
Prior Stem Cell Transplantation for AML, n (%)	40 (23)
Transfusion Dependent at Baseline,[b] n (%)	110 (63)
Median Number of Prior Therapies (Min, Max)	2 (1, 6)
Type of AML, n (%)	
De novo AML	116 (67)
Secondary AML	58 (33)

ECOG PS: *Eastern Cooperative Oncology Group Performance Status*.

[a] Using confirmatory Abbott RealTi*m*e IDH1 assay testing results.

[b] Patients were defined as transfusion dependent at baseline if they received any transfusion occurring within 56 days prior to the first dose of TIBSOVO.

Blood and bone marrow aspirate specimens from R/R AML patients being considered for treatment were tested. Patients with an IDH1 mutation positive result were eligible for enrollment in the drug trial if they met other eligibility criteria. Patients with an IDH1 mutation negative result at screening were ineligible for drug trial enrollment. Conclusions supporting TIBSOVO efficacy were based on patients with IDH1 mutation positive results as detected by the Abbott RealTi*m*e IDH1 assay.

Efficacy for TIBSOVO was established on the basis of the rate of complete remission (CR) plus complete remission 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 23.** The median follow-up was 8.3 months (range, 0.2 to 39.5 months) and median treatment duration was 4.1 months (range, 0.1 to 39.5 months).

Table 23: Efficacy Results in Patients with Relapsed or Refractory AML

Endpoint	TIBSOVO (500 mg daily) N=174
CR[a] n (%)	43 (24.7)
95% CI	(18.5, 31.8)
Median DOR[b] (months)	10.1
95% CI	(6.5, 22.2)
CRh[c] n (%)	14 (8.0)
95% CI	(4.5, 13.1)
Median DOR (months)	3.6
95% CI	(1.0, 5.5)
CR+CRh[d] n (%)	57 (32.8)
95% CI	(25.8, 40.3)
Median DOR (months)	8.2
95% CI	(5.6, 12.0)

CI: confidence interval.

[a] CR (complete remission) was defined as <5% blasts in the bone marrow, no evidence of disease, and full recovery of peripheral 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.

[c] CRh (complete remission with partial hematological recovery) was defined as <5% blasts in the bone marrow, no evidence of disease, and partial recovery of peripheral blood counts (platelets >50,000/microliter and ANC >500/microliter).

[d] CR+CRh rate appeared to be consistent across all baseline demographic and baseline disease characteristics with the exception of number of prior regimens.

For patients who achieved a CR or CRh, the median time to CR or CRh was 2 months (range, 0.9 to 5.6 months). Of the 57 patients who achieved a best response of CR or CRh, all achieved a first response of CR or CRh within 6 months of initiating TIBSOVO.

Among the 110 patients who were dependent on red blood cell (RBC) and/or platelet transfusions at baseline, 41 (37.3%) became independent of RBC and platelet transfusions during any 56-day post baseline period. Of the 64 patients who were independent of both RBC and platelet transfusions at baseline, 38 (59.4%) remained transfusion independent during any 56-day post baseline period.

MDS SubStudy

The safety and effectiveness of the Abbott RealTi*me* IDH1 assay were demonstrated through testing of specimens from patients with relapsed or refractory myelodysplastic syndrome (R/R MDS) enrolled in Study AG120-C-001. Study AG120-C-001 was a Phase 1, open-label, single-arm, multicenter clinical trial to assess the safety and clinical activity of TIBSOVO (AG-120) in which adult patients (age 18 and over) with R/R MDS with an isocitrate dehydrogenase-1 (IDH1) mutation were assigned to receive TIBSOVO 500 mg once daily (QD) dose orally. IDH1 mutations were detected in peripheral blood or bone marrow by a local or central diagnostic test and confirmed retrospectively using the Abbott RealTi*me*™ IDH1 assay. The baseline demographics and disease characteristics are shown in **Table 24**. The median age of the 18 eligible participants was 74 years with more males (78%) than females (22%). All 5 IDH1 mutations in codon R132 were detected among patients enrolled into the trial.

Table 24. Baseline Demographic and Disease Characteristics in Patients with Relapsed or Refractory MDS (Study AG120-C-001)

Demographic and Disease Characteristics	**TIBSOVO (500 mg daily) N=18**
Demographics	
Age (Years) Median (Min, Max)	74 (61, 82)
Age Categories, n (%)	
<65 years	3 (17)
≥65 years to <75 years	7 (39)
≥75 years	8 (44)
Sex, n (%)	
Male	14 (78)
Female	4 (22)
Race, n (%)	
White	14 (78)
Black or African American	1 (6)
Not Reported	3 (17)
Disease Characteristics	
ECOG PS, n (%)	
0	5 (28)
1	10 (56)
2	3 (17)
IDH1 Mutation, n (%)[1]	
R132C	9 (50)
R132H	5 (28)
R132G	2 (11)
R132L	1 (6)
R132S	1 (6)
Cytogenetic Risk Status, n (%)	
Good	4 (22)
Intermediate	8 (44)
Poor	5 (28)
Missing	1 (6)
Baseline Bone Marrow Blasts, n (%)	
< 5%	7 (39)
≥ 5%	11 (61)
Prior Therapies	
Intensive chemotherapy	3 (17)
Non-intensive chemotherapy	15 (83)
1 line of HMA-based therapy	14 (78)
2 lines of HMA-based therapy	1 (6)

ECOG PS: *Eastern Cooperative Oncology Group Performance Status.*

[1] Using confirmatory Abbott RealTi*me* IDH1 assay testing results.

Blood and bone marrow aspirate specimens from R/R MDS patients being considered for treatment were tested. Patients with an IDH1 R132 mutation positive result were eligible for enrollment in the AG120-C-001 trial if they met other eligibility criteria. Patients with an IDH1 mutation negative result at screening were ineligible for enrollment.

Efficacy was established on the basis of the rate of complete remission (CR) or partial remission (PR) as per the 2006 International Working Group response criteria for MDS, the duration of CR+PR, and the rate of conversion from transfusion dependence to transfusion independence. All observed responses were CRs. The efficacy results are shown in **Table 25**. The median follow-up was 27.1 months (range 3.7 to 88.7 months) and median duration of exposure to TIBSOVO was 8.3 months (range 3.3 to 78.8 months).

Table 25. Efficacy Results in Patients with Relapsed or Refractory MDS (Study AG120-C-001)

	TIBSOVO (500 mg daily)
Endpoint	**N=18**
CR[1] n (%)	7 (38.9)
95% CI	(17.3, 64.3)
DOCR[2] (months) median (range)	NE (1.9, 80.8+[3])

CI: confidence interval, CR: complete remission, NE: not estimable, derived based on Kaplan-Meier method.

[1] CR responders with baseline bone marrow blast <5% was 43% (3/7).

[2] Duration of CR (DOCR) = date of first documented CR (lasted at least 4 weeks) to date of first documented confirmed relapse or death, whichever is earlier.

[3] + indicates censored observation.

For patients who achieved a CR, the median time to CR was 1.9 months (range, 1.0 to 5.6 months).

Among the 9 patients who were dependent on red blood cell (RBC) and/or platelet transfusions at baseline, 6 (67%) became independent of RBC and platelet transfusions during any 56-day post-baseline period. Of the 9 patients who were independent of both RBC and platelet transfusions at baseline, 7 (78%) remained transfusion independent during any 56-day post-baseline period.

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

CLINICAL STUDIES FOR REZLIDHIA

The safety and effectiveness of the Abbott RealTi*me*™ IDH1 assay were demonstrated through testing of specimens from patients enrolled in Study 2102-HEM-101 (NCT02719574). Study 2102-HEM-101 was an open-label, single-arm, multicenter clinical trial in 147 adult patients with relapsed or refractory AML with one of 5 IDH1 mutations in codon R132. IDH1 mutations were identified by a local diagnostic test and subsequently centrally confirmed using the Abbott RealTi*me* IDH1 assay. REZLIDHIA™ was given orally at a dose of 150 mg twice daily until disease progression, unacceptable toxicity, or hematopoietic stem cell transplantation. Sixteen of the 147 patients (11%) underwent stem cell transplantation following REZLIDHIA treatment.

The baseline demographic and disease characteristics are shown in **Table 26.** The median age of study participants was 71 years with an approximately equal number of males and females. All 5 IDH1 mutations in codon R132 were detected among patients enrolled into the trial.

Table 26. Baseline Demographics and Disease Characteristics in Patients with Relapsed or Refractory AML (Study 2102-HEM-101)

Demographic and Disease Characteristics	REZLIDHIA (150 mg twice daily) N=147
Demographics	
Age (Years) Median (Min, Max)	71 (32, 87)
Age Categories, n (%)	
<65 years	37 (25)
≥65 years to <75 years	65 (44)
≥75 years	45 (31)
Sex, n (%)	
Male	74 (50)
Female	73 (50)
Race, n (%)	
White	67 (46)
Black or African American	5 (3)
Asian	5 (3)
Native Hawaiian/Other Pacific Islander	0 (0)
Other/Not provided	70 (48)
Disease Characteristics	
ECOG PS, n (%)	
0	45 (31)
1	76 (52)
2	23 (16)
IDH1 Mutation[1], n (%)	
R132C	85 (58)
R132H	35 (24)
R132G	12 (8)
R132S	11 (7)
R132L	4 (3)
Type of AML, n (%)	
De novo AML	97 (66)
Secondary AML	50 (34)
Cytogenic Risk Status[2], n (%)	
Favorable	6 (4)
Intermediate	107 (73)
Poor	25 (17)
Unknown	9 (6)
Relapsed/Refractory Patient Category, n (%)	
Primary Refractory	46 (31)
Untreated relapse[3]	81 (55)
Refractory relapse[3]	20 (14)
Relapse Number, n (%)	
0	46 (31)
1	87 (59)
2	11 (8)
≥3	3 (2)

Table 26 (continued). Baseline Demographics and Disease Characteristics in Patients with Relapsed or Refractory AML (Study 2102-HEM-101)	
Prior Stem Cell Transplantation for AML, n (%)	17 (12)
Transfusion Dependent at Baseline[4] , n (%)	86 (59)
Median Number of Prior Therapies (Min, Max)	2 (1,7)

[1] Using centrally confirmed IDH1 assay testing results.

[2] Cytogenetic risk categorization was investigator reported by NCCN or ELN guideline

[3] May be first or subsequent relapse

[4] Transfusion-Dependent at Baseline is defined as receiving a transfusion within 8 weeks prior to first dose of olutasidenib or noting transfusion dependence prior to coming on study.

Blood and bone marrow aspirate specimens from R/R AML patients being considered for treatment were tested. Patients with an IDH1 mutation positive result were eligible for enrollment in the drug trial if they met other eligibility criteria. Patients with an IDH1 mutation negative result at screening were ineligible for drug trial enrollment. Patients used to support conclusions regarding REZLIDHIA efficacy were based on the IDH1 mutation positive specimen results as detected by the Abbott RealTi*me* IDH1 assay.

Efficacy for REZLIDHIA was established on the basis of the rate of complete remission (CR) plus complete remission 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 27**.

Table 27. Efficacy Results in Patients with Relapsed or Refractory AML (Study 2102-HEM-101)	
Endpoint	**REZLIDHIA (150 mg twice daily) N=147**
CR+CRh [1, 2] n (%)	51 (35)
95% CI	(27, 43)
Median DOCR+CRh[3](months)	25.9
95% CI	(13.5, NR)
CR[1] n (%)	47 (32)
95% CI	(25, 40)
Median DOCR[3] (months)	28.1
95% CI	(13.8, NR)
CRh[1] n (%)	4 (2.7)
95% CI	(0.7, 6.8)
Observed DOCRh[3] (months)	1.8, 5.6, 13.5, 28.5+

CI: confidence interval; NR = not reached

[1] CR (complete remission) was defined as <5% blasts in the bone marrow, no blasts with Auer rods, no extramedullary disease, and full recovery of peripheral blood counts (platelets >100,000/microliter and absolute neutrophil counts [ANC] >1,000/microliter); CRh (complete remission with partial hematologic recovery) was defined as < 5% blasts in the bone marrow, no evidence of disease, and partial recovery of peripheral blood counts (platelets > 50,000/microliter and ANC > 500/microliter).

[2] CR+CRh rate was consistent across all baseline demographic and baseline disease characteristic subgroups with the exception of IDH1 R132H mutation (CR+CRh 17%).

[3] Duration of response is defined as the time from the date of the first response to the date of the relapse or death. Patients who did not relapse were censored at the date of last response assessment. + indicates censored observation.

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

BIBLIOGRAPHY

1. Gross S, Cairns RA, Minden MD, et al. Cancer-associated metabolite 2-hydroxyglutarate accumulates in acute myelogenous leukemia with isocitrate dehydrogenase 1 and 2 mutations. *J Exp Med*. 2010;207:339–44.
2. Cairns RA, Mak TW. Oncogenic isocitrate dehydrogenase mutations: mechanisms, models, and clinical opportunities. *Cancer Discov.* 2013;3:730-41.
3. Dang L, White DW, Gross S, et al. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature.* 2010;465(7300):966.
4. Clark O, Yen K, Mellinghoff IK, et al. Molecular Pathways: Isocitrate Dehydrogenase Mutations in Cancer. *Clin Cancer Res* 2016;22(8):1837-42.
5. Lu C, Ward PS, Kapoor GS. et al. IDH mutation impairs histone demethylation and results in a block to cell differentiation. *Nature* 2012; 483(7390):474-8.
6. Medeiros BC, Fathi AT, DiNardo CD, et al. Isocitrate dehydrogenase mutations in myeloid malignancies. *Leukemia* 2016; doi: 10.1038/leu.2016.275.
7. Dang, L, Yen K, Attar EC. IDH mutations in cancer and progress toward development of targeted therapeutics. *Annals of Oncology* 2016;27:599-608.
8. Bullinger L, Döhner K, Döhner H. Genomics of Acute Myeloid Leukemia Diagnosis and Pathways. *J Clin Oncol*. 2017; doi: 10.1200/JCO.2016.71.2208
9. Abbas S, Lugthart S, Kavelaars FG, et al. Acquired mutations in the genes encoding IDH1 and IDH2 both are recurrent aberrations in acute myeloid leukemia: prevalence and prognostic value. *Blood* 2010;116(12):2122-6.
10. Mardis ER, Ding L, Dooling DJ, et al. Recurring mutations found by sequencing an acute myeloid leukemia genome. *N Engl J Med*. 2009;361(11):1058–66.
11. Paschka P, Schlenk RF, Gaidzik VI, et al. IDH1 and IDH2 mutations are frequent genetic alterations in acute myeloid leukemia and confer adverse prognosis in cytogenetically normal acute myeloid leukemia with NPM1 mutation without FLT3internal tandem duplication. *J Clin Oncol*. 2010;28;(22):3636-43.
12. Chotirat S, Thongnoppakhun W, Promsuwicha O, et al. Molecular alterations of isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2), metabolic genes and additional genetic mutations in newly diagnosed acute myeloid leukemia patients. *J Hematol Oncol*. 2012;5:5.
13. Clinical and Laboratory Standards Institute. *Clinical Laboratory Waste Management: Approved Guideline –Third Edition*. CLSI Document GP5-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2011;22(3):1-23,32-44.
14. US Environmental Protection Agency. *EPA Guide for Infectious Waste Management* Publication No. EPA/530-SW-86-014; Washington, DC: US Environmental Protection Agency; 1986:1-1–5-5,R1-R3,A1-A24.
15. Clinical and Laboratory Standards Institute. Interference Testing in Clinical Chemistry; *Approved Guideline-Second Edition*. CLSI document EP07-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2005;25(27).
16. Sung L, Langer BJ, Gerbing RB, Alonzo TA, Feusner J. Microbiologically documented infections and infection-related mortality in children with acute myeloid leukemia. *Blood* 2007;110(10):3532-9.

TECHNICAL ASSISTANCE

For technical assistance, call Abbott Technical Services at 1-800-553-7042 or visit the Abbott Website at www.molecular.abbott.

Abbott Molecular Inc. is the legal manufacturer of the:

Abbott RealTi*m*e IDH1 Amplification Reagent Kit (List No. 08N90-090)

Abbott RealTi*m*e IDH1 Control Kit (List No. 08N90-080)

Abbott Molecular Inc.
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Appendix 1. List of Abbott RealTi*me* IDH1 Specific Error Codes for Positive Control and Negative Control

Code	Message Text	Probable Causes	Corrective Actions
4926	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 to avoid contamination. 3. Ensure that the correct sample preparation procedure is followed. 4. Ensure that the sample is correctly labeled and tested.
4927	IC cycle number is greater than the maximum	• Insufficient DNA extracted • Insufficient reagent(s) pipetted • Sample preparation procedure errors • Wrong sample loaded onto reaction plate • No or insufficient sample loaded • Sample preparation reagent, master mix, or sample contaminated with DNase	1. Ensure that the correct amounts of Positive Control and Negative Control are used. 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 sample is correctly labeled and tested. 5. Ensure that the correct amount of eluate is used. 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 • Positive Control was extracted • 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 a new vial of Negative Control is used. 4. Ensure that the correct sample preparation procedure is followed. 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 • Sample preparation procedure errors • Wrong sample loaded	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.
4930	Mutation cycle number is greater than the maximum	• Insufficient DNA extracted • Negative Control was extracted • Insufficient reagent(s) pipetted • Sample preparation procedure errors • Wrong sample loaded onto reaction plate • No or insufficient sample loaded • Sample preparation reagent, master mix, or sample contaminated with DNase	1. Ensure that the correct amount of Positive Control is used. 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 sample is correctly labeled and tested. 5. Ensure that the correct amount of eluate is used. 6. Follow package insert for good laboratory practices to avoid DNase contamination.

Appendix 2. List of Abbott RealTi*me* IDH1 Specific Error Codes for Samples

Code	Message Text	Probable Causes	Corrective Actions
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[a]	Refer to Constituent Result error code[a]
4935	See Constituent Result for Associated Error	• Refer to Constituent Result error code[a]	Refer to Constituent Result error code[a]

[a] Constituent Result error codes are found associated with individual IC (OLIGO1 or OLIGO2) or mutation (R132C, R132H, R132G, R132S, and R132L) results.