SIEMENS Healthineers

Direct Bilirubin 2 (DBil_2)

Current Revision and Date[a]	Rev. 04, 2024-01	
Product Name	Atellica CH Direct Bilirubin 2 (DBil_2)	REF 11097532 (1792 tests)
Abbreviated Product Name	Atellica CH DBil_2	
Test Name/ID	DBil_2	
Systems	Atellica CH Analyzer	
Materials Required but Not Provided	Atellica CH CHEM CAL Atellica CH BILI CAL	REF 11099411 REF 11537243
Specimen Types	Serum and plasma (lithium heparin)	
Sample Volume	14.3 µL	
Measuring Interval	0.1–15.0 mg/dL (2–256 µmol/L)	

[a] A vertical bar in the page margin indicates technical content that differs from the previous version.

CE 0197

Intended Use

The Atellica® CH Direct Bilirubin 2 (DBil_2) assay is for *in vitro* diagnostic use in the quantitative determination of direct bilirubin in human serum and plasma (lithium heparin) using the Atellica® CH Analyzer. Such measurements are useful in the screening of liver function disorders or in the diagnosis of jaundice.

Summary and Explanation

The Atellica CH Direct Bilirubin_2 (DBil_2) assay is based on a chemical oxidation method using vanadate as an oxidizing agent.[1]

Principles of the Procedure

The bilirubin is oxidized by vanadate at about pH 3 to produce biliverdin. In the presence of detergent and vanadate, conjugated (direct) bilirubin is oxidized. This oxidation reaction causes a decrease in the optical density of the yellow color, which is specific to bilirubin. The decrease in optical density at 451/545 nm is proportional to the direct bilirubin concentration in the sample. The concentration is measured as an endpoint reaction.

Reaction Equation

Conjugated Bilirubin + VO^{3-} ⟶ Biliverdin

Reagents

Material Description	Storage	Stability[a]
Atellica CH DBil_2	Unopened at 2–8°C	Until expiration date on product
Pack 1 (P1)	Onboard per well	30 days
Well 1 (W1) Reagent 1 (R1) 23.5 mL Tartrate buffer (pH 2.9) (0.1 mol/L); detergent		
Well 2 (W2) Reagent 1 (R1) 23.5 mL Tartrate buffer (pH 2.9) (0.1 mol/L); detergent		
Pack 2 (P2)		
Well 1 (W1) Reagent 2 (R2) 8.8 mL Phosphate buffer (pH 7.0) (10 mmol/L); sodium metavanadate (4 mmol/L)		
Well 2 (W2) Reagent 2 (R2) 8.8 mL Phosphate buffer (pH 7.0) (10 mmol/L); sodium metavanadate (4 mmol/L)		

[a] Refer to *Storage and Stability*.

Warnings and Precautions

For *in vitro* diagnostic use.

For Professional Use.

CAUTION

Federal (USA) law restricts this device to sale by or on the order of a licensed healthcare professional.

Safety data sheets (SDS) available on siemens-healthineers.com.

Reagent 1 (R1) contains hydroxylammonium chloride. May produce an allergic reaction.

Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner and in compliance with prevailing regulatory requirements.

Note For information about reagent preparation, refer to *Preparing the Reagents* in the *Procedure* section.

Storage and Stability

Unopened reagents are stable until the expiration date on the product when stored at 2–8°C.

Do not use products beyond the expiration date printed on the product labeling.

Onboard Stability

Reagents are stable onboard the system for 30 days per well. Discard reagents at the end of the onboard stability interval. Do not use products beyond the expiration date printed on the product labeling.

Specimen Collection and Handling

Serum and plasma (lithium heparin) are the recommended sample types for this assay.

Collecting the Specimen

- Observe universal precautions when collecting specimens. Handle all specimens as if they are capable of transmitting disease.[2]
- Follow recommended procedures for collection of diagnostic blood specimens by venipuncture.[3]
- Follow the instructions provided with your specimen collection device for use and processing.[4]
- Allow blood specimens to clot completely before centrifugation.[5]
- Keep tubes capped at all times.[5]

Storing the Specimen

Samples should be stored at 4°C and analyzed within 5 days. Bilirubin is extremely photosensitive. Care should be taken to protect sample from both daylight and fluorescent light to avoid photodegradation. Specimens may be stored frozen for up to 3 months at -70°C with no light exposure.[6,7]

The handling and storage information provided here is based on data or references maintained by the manufacturer. It is the responsibility of the individual laboratory to use all available references and/or its own studies when establishing alternate stability criteria to meet specific needs.

Transporting the Specimen

Package and label specimens for shipment in compliance with applicable federal and international regulations covering the transport of clinical specimens and etiological agents.

Preparing the Samples

This assay requires 14.3 µL of sample for a single determination. This volume does not include the unusable volume in the sample container or the additional volume required when performing duplicates or other tests on the same sample. For information about determining the minimum required volume, refer to the online help.

Note Do not use specimens with apparent contamination.

Before placing samples on the system, ensure that samples are free of:

- Bubbles or foam.
- Fibrin or other particulate matter.

Note Remove particulates by centrifugation according to CLSI guidance and the collection device manufacturer's recommendations.[5]

Note For a complete list of appropriate sample containers, refer to the online help.

Procedure

Materials Provided

The following materials are provided:

REF	Contents	Number of Tests
11097532	**Pack 1 (P1)** Well 1 (W1) 23.5 mL of Atellica CH DBil_2 Reagent 1 Well 2 (W2) 23.5 mL of Atellica CH DBil_2 Reagent 1 **Pack 2 (P2)** Well 1 (W1) 8.8 mL of Atellica CH DBil_2 Reagent 2 Well 2 (W2) 8.8 mL of Atellica CH DBil_2 Reagent 2	4 x 448

Materials Required but Not Provided

The following materials are required to perform this assay, but are not provided:

REF	Description	
	Atellica CH Analyzer[a]	
11099411 11537243	Atellica CH CHEM CAL (calibrator) Atellica CH BILI CAL (calibrator)	12 x 3.0 mL calibrator CAL 6 x 3.0 mL calibrator CAL Calibrator lot-specific value sheet CAL LOT VAL
	Commercially available quality control materials	

[a] Additional system fluids are required to operate the system: Atellica CH Diluent, Atellica CH Wash, Atellica CH Conditioner, Atellica CH Cleaner, Atellica CH Reagent Probe Cleaner 1, Atellica CH Reagent Probe Cleaner 2, Atellica CH Reagent Probe Cleaner 4, Atellica CH Lamp Coolant, and Atellica CH Water Bath Additive. For system fluid instructions for use, refer to the Document Library.

Assay Procedure

The system automatically performs the following steps:

1. For serum/plasma, dispenses 50 µL of primary sample and 200 µL of Atellica CH Diluent into a dilution cuvette.
2. Dispenses 80 µL of Reagent 1 into a reaction cuvette.
3. Dispenses 14.3 µL of pre-diluted sample into a reaction cuvette.
4. Measures the absorbance after sample addition.
5. Dispenses 20 µL of Reagent 2 into a reaction cuvette.
6. Mixes and incubates the mixture at 37°C.
7. Measures the absorbance after Reagent 2 addition.
8. Reports results.

Test Duration: 10 minutes

Preparing the Reagents

All reagents are liquid and ready to use.

Preparing the System

Ensure that the system has sufficient reagent packs loaded in the reagent compartment. For information about loading reagent packs, refer to the online help.

Performing Calibration

For calibration of the Atellica CH DBil_2 assay, use Atellica CH CHEM CAL or Atellica CH BILI CAL. Use the calibrators in accordance with the calibrator instructions for use.

Calibration Frequency

Perform a calibration if one or more of the following conditions exist:

- When changing lot numbers of primary reagent packs.
- At the end of the lot calibration interval, for a specified lot of calibrated reagent on the system.
- At the end of the pack calibration interval, for calibrated reagent packs on the system.
- When indicated by quality control results.
- After major maintenance or service, if indicated by quality control results.

At the end of the onboard stability interval, replace the reagent pack on the system with a new reagent pack. Recalibration is not required, unless the lot calibration interval is exceeded.

Stability Interval	Days
Lot Calibration	60
Pack Calibration	30
Reagent Onboard Stability	30

For information about lot calibration and pack calibration intervals, refer to the online help.

Follow government regulations or accreditation requirements for calibration frequency. Individual laboratory quality control programs and procedures may require more frequent calibration.

Performing Quality Control

For quality control of the Atellica CH DBil_2 assay, use at least two levels (low and high) of the appropriate quality control material of known analyte concentration. For assistance in identifying a quality control material, refer to the *Atellica CH Quality Control Material Supplement* available on siemens-healthineers.com. Additional quality control material can be used at the discretion of the laboratory. Use the quality control material in accordance with the quality control instructions for use.

For the assigned values, refer to the lot-specific value sheet provided.

In addition, perform quality control:

- Following a valid calibration.
- With use of a new lot of reagent.
- When troubleshooting test results that do not match clinical conditions or symptoms.

Follow government regulations or accreditation requirements for quality control frequency. Individual laboratory quality control programs and procedures may require more frequent quality control testing.

Acceptable performance is achieved when the analyte values obtained are within the expected control interval for the system, as indicated by the manufacturer of the control material or within the interval determined by an internal laboratory quality control procedure.

Follow your laboratory's quality control procedures if the results obtained do not fall within the acceptable limits. For information about entering quality control definitions, refer to the system operating instructions.

Taking Corrective Action

If the quality control results do not fall within the assigned values, do not report results. Perform corrective actions in accordance with established laboratory protocol. For suggested protocol, refer to the online help.

Results

Calculation of Results

The system determines the result using the calculation scheme described in the online help. The system reports results in mg/dL (common units) or µmol/L (SI units), depending on the units defined when setting up the assay.

Conversion formula: mg/dL x 17.1 = µmol/L

For information about results outside the specified measuring interval, refer to *Measuring Interval*.

Interpretation of Results

Results of this assay should always be interpreted in conjunction with the patient's medical history, clinical presentation, and other findings.

Limitations

The Atellica CH DBil_2 assay is limited to the detection of direct bilirubin in human serum and plasma (lithium heparin).

A number of substances cause physiological changes in serum or plasma analyte concentrations. A comprehensive discussion of possible interfering substances, their serum or plasma concentrations, and their possible physiological involvements is beyond the scope of this document. Consult listed reference for specific details on known potential interfering substances.[8]

Assay performance characteristics have not been established in neonates and cord blood samples. Therefore, users are responsible for establishing their own performance characteristics in these populations.

When samples with a direct bilirubin concentration of 1.0 mg/dL (17.6 µmol/L) are spiked at a therapeutic eltrombopag concentration of 25 µg/mL (56.5 µmol/L) a positive bias of 3.9% is observed. No significant interference is observed when samples with a direct bilirubin concentration of 5.0 mg/dL (85.5 µmol/L) are spiked at a supraphysiological eltrombopag concentration of 75 µg/mL (170 µmol/L), therefore spiking studies at this level of direct bilirubin have not been tested at the lower therapeutic concentration of eltrombopag.[9]

As with any chemical reaction, you must be alert to the possible effect of unknown interferences from medications or endogenous substances. The laboratory and physician must evaluate all patient results in light of the total clinical status of the patient.

Expected Values

Reference Interval

A reference interval for healthy adults was established in accordance with CLSI Document EP28-A3c and verified on the Atellica CH Analyzer.[10]

The reference interval for direct bilirubin is ≤ 0.3 mg/dL (≤ 5 µmol/L) for adults.[11]

As with all *in vitro* diagnostic assays, each laboratory should determine its own reference interval for the diagnostic evaluation of patient results. Consider these values as guidance only.[10]

Performance Characteristics

Measuring Interval

The Atellica CH DBil_2 assay is linear from 0.1–15.0 mg/dL (2–256 µmol/L). The system flags all values that are outside the specified measuring interval.

Extended Measuring Interval

An automatic repeat condition for this assay extends the measuring interval to 22.5 mg/dL (385 µmol/L) for serum and plasma. You may configure the system to trigger an automatic repeat. Automatic repeat results will be flagged **Autorepeat**.

Detection Capability

Detection capability was determined in accordance with CLSI Document EP17-A2.[12] The assay is designed to have a limit of blank (LoB) ≤ limit of detection (LoD) and LoD ≤ 0.1 mg/dL (≤ 2 µmol/L).

The LoD corresponds to the lowest concentration of direct bilirubin that can be detected with a probability of 95%. The LoD for the Atellica CH DBil_2 assay is 0.0 mg/dL (0 µmol/L), and was determined using 120 determinations, with 60 blank and 60 low level replicates, and a LoB of 0.0 mg/dL (0 µmol/L).

Assay results obtained at individual laboratories may vary from the data presented.

Precision

Precision was determined in accordance with CLSI Document EP05-A3.[13] Samples were assayed on an Atellica CH Analyzer in duplicate in 2 runs per day for 20 days (N ≥ 80 for each sample). The following results were obtained:

			Repeatability		Designed to be ≤	Within-Lab Precision		Designed to be ≤
Sample Type	N	Mean mg/dL (µmol/L)	SD[a] mg/dL (µmol/L)	CV[b] (%)	CV (%)	SD mg/dL (µmol/L)	CV (%)	CV (%)
QC	80	0.4 (7)	0.00 (0.0)	0.0	5	0.00 (0.0)	0.0	6
Plasma	80	3.5 (60)	0.04 (0.7)	1.0	4	0.05 (0.9)	1.6	5
Serum	80	11.9 (203)	0.02 (0.3)	0.2	4	0.6 (1.0)	0.5	5

[a] Standard deviation.

[b] Coefficient of variation.

Assay results obtained at individual laboratories may vary from the data presented.

Based on internal testing, the overall reproducibility is estimated to be ≤ 9.0% CV for samples tested and includes multiple reagent lots, instruments, days, and replicates.[9] Performance of the assay at individual laboratories may vary.

Assay Comparison

The Atellica CH DBil_2 assay is designed to have a correlation coefficient of > 0.950 and a slope of 1.0 ± 0.10 compared to ADVIA® Chemistry 1800 DBIL_2. Assay comparison was determined using the Deming linear regression model in accordance with CLSI Document EP09-A3.[14] The following results were obtained:

Specimen	Comparative Assay (x)	Regression Equation	Sample Interval	N[a]	r[b]
Serum	ADVIA Chemistry 1800 DBIL_2	y = 1.03x + 0.0 mg/dL (y = 1.03x + 0 µmol/L)	0.1–14.8 mg/dL (2–253 µmol/L)	103	1.000

[a] Number of samples tested.
[b] Correlation coefficient.

The agreement of the assay may vary depending on the study design, comparative assay, and sample population. Assay results obtained at individual laboratories may vary from the data presented.

Specimen Equivalency

Specimen equivalency was determined using the Deming linear regression model in accordance with CLSI Document EP09-A3.[14] The following results were obtained:

Specimen (y)	Reference Specimen (x)	Regression Equation	Sample Interval	N[a]	r[b]
Lithium heparin plasma	Serum	y = 1.00x + 0.0 mg/dL (y = 1.00x + 0 µmol/L)	0.1–10.3 mg/dL (2–176 µmol/L)	50	0.999

[a] Number of samples tested.
[b] Correlation coefficient.

Agreement of the specimen types may vary depending on the study design and sample population used. Assay results obtained at individual laboratories may vary from the data presented.

Interferences

Hemolysis, Icterus, and Lipemia (HIL)

The Atellica CH DBil_2 assay is designed to have ≤ 10% interference from hemoglobin and lipemia. Interfering substances at the levels indicated in the table below were tested in accordance with CLSI Document EP07-A2 using the Atellica CH DBil_2 assay.[15]

Bias is the difference in the results between the control sample (does not contain the interferent) and the test sample (contains the interferent) expressed in percent. Bias > 10% is considered interference. Analyte results should not be corrected based on this bias.

Substance	Substance Test Concentration Common Units (SI Units)	Analyte Concentration	Percent Bias
Hemoglobin	1000 mg/dL (0.621 mmol/L) 1000 mg/dL (0.621 mmol/L)	1.0 mg/dL (17 µmol/L) 4.7 mg/dL (80 µmol/L)	-10 -10
Lipemia (Triglycerides Concentrate)	750 mg/dL (8.5 mmol/L) 750 mg/dL (8.5 mmol/L)	1.0 mg/dL (17 µmol/L) 4.7 mg/dL (80 µmol/L)	0 2

Assay results obtained at individual laboratories may vary from the data presented.

Non-Interfering Substances

The following substances do not interfere with the Atellica CH DBil_2 assay when present in human serum and plasma (lithium heparin) at the concentrations indicated in the table below. Bias due to these substances is ≤ 10% at an analyte concentration of 1.0 mg/dL (17 µmol/L). These data were generated on the ADVIA Chemistry system with assay reaction conditions that are equivalent to those on the Atellica CH Analyzer.[9]

Substance	Substance Test Concentration Common Units (SI Units)	Analyte Concentration	Percent Bias
Ascorbic Acid	50 mg/dL (2.8 mmol/L)	1.0 mg/dL (17 µmol/L)	0

Assay results obtained at individual laboratories may vary from the data presented.

Standardization

The assay is traceable to the AACC reference method, which uses reference materials from the National Institute of Standards and Technology (NIST SRM 916).

Assigned values for calibrators are traceable to this standardization.[9]

Technical Assistance

According to EU regulation 2017/746, any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the EU Member State in which the user and/or patient is established.

For customer support, contact your local technical support provider or distributor.

siemens-healthineers.com

References

1. Tokuda K, Tanimoto K. New method of measuring serum bilirubin using vanadic acid. *Jpn J Clin Chem*. 1993;22(2):116–122.
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3. Clinical and Laboratory Standards Institute. *Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard—Sixth Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2007. CLSI Document GP41-A6.
4. Clinical and Laboratory Standards Institute. *Tubes and Additives for Venous and Capillary Blood Specimen Collection; Approved Standard—Sixth Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2010. CLSI Document GP39-A6.
5. Clinical and Laboratory Standards Institute. *Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests; Approved Guideline—Fourth Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2010. CLSI Document GP44-A4.
6. Burtis CA, Ashwood ER, Bruns DE, Tietz NW. *Textbook of Clinical Chemistry and Molecular Diagnostics*. 4th ed. Elsevier Saunders; 2006:1165–1208.
7. Young DS, *Effects of Preanalytical Variables on Clinical Laboratory Tests*. 2nd ed. Washington, DC: AACC Press; 1997:3–88,4–492.
8. Young DS. *Effects of Drugs on Clinical Laboratory Tests*. 3rd ed. Washington, DC: AACC Press; 1990.
9. Data on file at Siemens Healthcare Diagnostics.

10. Clinical and Laboratory Standards Institute. *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline—Third Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2010. CLSI Document EP28-A3c.
11. Tietz NW. *Clinical Guide to Laboratory Tests.* 3rd ed. Philadelphia, PA: WB Saunders Company; 1995: 88–91.
12. Clinical and Laboratory Standards Institute. *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline—Second Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2012. CLSI Document EP17-A2.
13. Clinical and Laboratory Standards Institute. *Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline—Third Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2014. CLSI Document EP05-A3.
14. Clinical and Laboratory Standards Institute. *Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline—Third Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2013. CLSI Document EP09-A3.
15. Clinical and Laboratory Standards Institute. *Interference Testing in Clinical Chemistry; Approved Guideline—Second Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2005. CLSI Document EP07-A2.

Definition of Symbols

The following symbols may appear on the product labeling:

Symbol	Symbol Title	Source	Symbol	Symbol Title	Source
	Manufacturer	5.1.1[a]		Authorized representative in the European Community	5.1.2[a]
	Use-by date	5.1.4[a]		Authorized representative in Switzerland	Proprietary
	Catalog number	5.1.6[a]		Batch code	5.1.5[a]
	Consult Instructions for Use	5.4.3[a]		Contains sufficient for <n> tests	5.5.5[a]
 < .../*eifu* >	Internet URL address to access the electronic instructions for use	Proprietary		Version of Instructions for Use	Proprietary
	*In vitro* diagnostic medical device	5.5.1[a]		Revision	Proprietary
RxOnly	Prescription device (US only)	FDA[b]		Unique Device Identifier	5.7.10[c]
	CE Marking with Notified Body	EU IVDR[d]		CE Marking	EU IVDR[d]
	Temperature limit	5.3.7[a]		Keep away from sunlight	5.3.2[a]
	Upper limit of temperature	5.3.6[a]		Lower limit of temperature	5.3.5[a]

Symbol	Symbol Title	Source	Symbol	Symbol Title	Source
	Do not re-use	5.4.2[a]		Do not freeze	Proprietary
	Recycle	1135[e]		This way up	0623[e]
	Biological risks	5.4.1[a]		Caution	5.4.4[a]
UNITS C	Common Units	Proprietary	UNITS SI	International System of Units	Proprietary
YYYY-MM-DD	Date format (year-month-day)	N/A	YYYY-MM	Date format (year-month)	N/A
	Document face up[f]	1952[e]		Handheld barcode scanner	Proprietary
	Target	Proprietary		Mixing of substances	5657[g]
CHECKSUM	Variable hexadecimal number that ensures the Master Curve and Calibrator definition values entered are valid.	Proprietary		Interval	Proprietary
MATERIAL ID	Unique material identification number	Proprietary	MATERIAL	Material	Proprietary
CONTROL TYPE	Type of control	Proprietary	CONTROL NAME	Name of control	Proprietary
CONTROL LOT VAL	Quality control lot value	Proprietary	CAL LOT VAL	Calibrator lot value	Proprietary

[a] International Standard Organization (ISO). ISO 15223-1 Medical Devices- Symbols to be used with medical device labels, labelling and information to be supplied.
[b] Federal Register. Vol. 81, No 115. Wednesday, June 15, 2016. Rules and Regulations: 38911.
[c] ISO 15223-1:2020-04
[d] IVDR REGULATION (EU) 2017/746
[e] International Standard Organization (ISO). ISO 7000 Graphical symbols for use on equipment.
[f] Indicates Assay-eNote
[g] International Electrotechnical Commission (IEC). IEC 60417-1 Graphical symbols for use on equipment – Part 1: Overview and Application

Legal Information

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