Alinity i Total β-hCG (β-hCG)-25

Prepared by: Yusra Othman /Director/Supervisor-Chem **Date:** May/26/2024
signature/title

Reviewed by: Jordan Dillard /Instructor **Date:** June 26 2024
signature/title

Approved by: Sanford N. Bailey, M.D /Chairman **Date:** June 28 2024
signature/title

BIENNIAL REVIEW:

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INTENDED USE

The Alinity i Total β-hCG assay is a chemiluminescent microparticle immunoassay (CMIA) used for the quantitative and qualitative determination of beta-human chorionic gonadotropin (β-hCG) in human serum and plasma for the early detection of pregnancy on the Alinity i analyzer.

SUMMARY AND EXPLANATION OF THE TEST

Human chorionic gonadotropin (hCG) is a sialoglycoprotein with a molecular weight of

approximately 46,000 daltons.[1] HCG is initially secreted by the trophoblastic cells of the placenta shortly after implantation of the fertilized ovum into the uterine wall.[2, 3] The rapid rise in hCG serum levels after conception makes it an excellent marker for early confirmation of pregnancy.

Physiologically, hCG appears to maintain the corpus luteum, thereby allowing synthesis of progesterone and estrogens that support the endometrium. As uncomplicated pregnancies progress, the placenta assumes the production of these hormones. The serum hCG levels increase to a peak concentration, then decrease and plateau. HCG circulates as the intact molecule in the serum of normal women who have an uncomplicated pregnancy. The subunits are cleaved rapidly and cleared by the kidney.[4]

The placental hormone, hCG, is similar to luteinizing hormone (LH), follicle stimulating hormone (FSH), and thyroid stimulating hormone (TSH). All are glycoproteins consisting of two noncovalently bound dissimilar subunits, designated alpha and beta, with attached carbohydrate sidechains. The alpha subunits of these glycoproteins are very similar. In contrast, the beta subunit portions determine the biological and immunochemical specificities.[5, 6] The beta subunits of hCG and LH exhibit considerable homology in amino acid content. Amino acid residues specific for the beta subunit of hCG confer the immunochemical specificity.[7]

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

This assay is a two-step immunoassay for the quantitative and qualitative determination of β-hCG in human serum and plasma using chemiluminescent microparticle immunoassay (CMIA) technology.

Sample and anti-β-hCG coated paramagnetic microparticles are combined and incubated. The β-hCG present in the sample binds to the anti-β-hCG coated microparticles. The mixture is washed. Anti-β-hCG acridinium-labeled conjugate is added to create a reaction mixture and incubated. Following a wash cycle, Pre-Trigger and Trigger Solutions are added.

The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is a direct relationship between the amount of β-hCG in the sample and the RLUs detected by the system optics.

For additional information on system and assay technology, refer to the Alinity ci-series Operations Manual, Section 3.

REAGENTS

Kit Contents

Alinity i Total β-hCG Reagent Kit 07P51

Volumes (mL) listed in the table below indicate the volume per cartridge.

REF	07P5121	07P5131
Tests per cartridge	100	600
Number of cartridges per kit	2	2
Tests per kit	200	1200
MICROPARTICLES	6.6 mL	32.1 mL
CONJUGATE	4.2 mL	9.0 mL
MICROPARTICLES Anti-β-hCG (mouse, monoclonal) coated microparticles in TRIS buffer with protein (bovine) stabilizers. Minimum concentration: 0.06% solids. Preservatives: antimicrobial agents.		
CONJUGATE Anti-β-hCG (mouse, monoclonal) acridinium-labeled conjugate in MES buffer with protein (bovine) stabilizers. Minimum concentration: 2.9 µg/mL. Preservative: antimicrobial agent.		

Warnings and Precautions

- IVD
- For *In Vitro* Diagnostic Use
- Rx ONLY

Safety Precautions

CAUTION: This product requires the handling of human specimens. It is recommended that all human-sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.[8], [9], [10], [11]

Safety Data Sheets are available at www.abbottdiagnostics.com or/and SDS folder.

For a detailed discussion of safety precautions during system operation, **refer to the Alinity ci-series Operations Manual, Section 8.**

Reagent Handling

Upon receipt, gently invert the unopened reagent kit by rotating it over and back for a full 180 degrees, 5 times with green label stripe facing up and then 5 times with green label stripe facing down. This ensures that liquid covers all sides of the bottles within the cartridges. During reagent shipment, microparticles can settle on the reagent septum.

- Place a check in the square on the reagent kit to indicate to others that the inversions have been completed.

- After mixing, place reagent cartridges in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate.
- If a reagent cartridge is dropped, place in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate.
- Reagents are susceptible to the formation of foam and bubbles. Bubbles may interfere with the detection of the reagent level in the cartridge and cause insufficient reagent aspiration that may adversely affect results.

For a detailed discussion of reagent handling precautions during system operation, refer to the Alinity ci-series Operations Manual, Section 7.

Reagent Storage

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Unopened	2 to 8°C	Until expiration date	Store in upright position. If cartridge does not remain upright, gently invert the cartridge 10 times and place in an upright position for 1 hour before use.
Onboard	System Temperature	30 days	
Opened	2 to 8°C	Until expiration date	Store in upright position. If cartridge does not remain upright during storage, discard the cartridge. Do not reuse original reagent caps or replacement caps due to the risk of contamination and the potential to compromise reagent performance.

Reagents may be stored on or off the system. If removed from the system, store reagents with new replacement caps in an upright position at 2 to 8°C. For reagents stored off the system, it is recommended that they be stored in their original trays or boxes to ensure they remain upright.

For information on unloading reagents, **refer to the Alinity ci-series Operations Manual, Section 5.**

Indications of Reagent Deterioration

Deterioration of the reagents may be indicated when:

- a calibration error occurs
- a control value is out of the specified range

Associated test results are invalid, and samples must be retested. Assay recalibration may be necessary.

For troubleshooting information, **refer to the Alinity ci-series Operations Manual, Section 10**.

INSTRUMENT PROCEDURE

The Alinity i Total β-hCG assay file must be installed on the Alinity i analyzer prior to performing the assay.

For detailed information on assay file installation and viewing and editing assay parameters, refer to **the Alinity ci-series Operations Manual, Section 2.**

For information on printing assay parameters, **refer to the Alinity ci-series Operations Manual, Section 5.**

For a detailed description of system procedures, **refer to the Alinity ci-series Operations Manual.**

Alternate Result Units

Edit assay parameter "Result Units" to select an alternate unit.

Conversion formula:

(Concentration in Default result unit) x (Conversion factor) = (Concentration in Alternate result unit)

Default Result Unit	Conversion Factor	Alternate Result Unit
mIU/mL	1	IU/L

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

The specimen types listed below were verified for use with this assay.

Specimen Types	Collection Tubes
Serum	Serum
	Serum separator
Plasma	Dipotassium EDTA
	Tripotassium EDTA
	Lithium heparin
	Lithium heparin plasma separator
	Sodium heparin

Specimen Conditions

- For accurate results, serum and plasma specimens should be free of fibrin, red blood cells, and other particulate matter. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.
- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

Preparation for Analysis

- Follow the tube manufacturer's processing instructions for collection tubes. Gravity separation is not sufficient for specimen preparation.
- Inadequate centrifugation or the presence of fibrin or particulate matter in the sample may cause an erroneous result.
- Specimens should be free of bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross-contamination.

To ensure consistency in results, recentrifuge specimens prior to testing if

- they contain fibrin, red blood cells, or other particulate matter.

NOTE: If fibrin, red blood cells, or other particulate matter are observed, mix by low speed vortex or by inverting 10 times prior to recentrifugation.

Prepare frozen specimens as follows:

- Frozen specimens must be completely thawed before mixing.
- Mix thawed specimens thoroughly by low speed vortex or by inverting 10 times.
- Visually inspect the specimens. If layering or stratification is observed, mix until specimens are visibly homogeneous.
- If specimens are not mixed thoroughly, inconsistent results may be obtained.
- Recentrifuge specimens.

Recentrifugation of Specimens

- Transfer mixed specimens to a centrifuge tube and centrifuge.
- Transfer clarified specimen to a sample cup or secondary tube for testing. For centrifuged specimens with a lipid layer, transfer only the clarified specimen and not the lipemic material.

Specimen Storage

Specimen Type	Temperature	Maximum Storage Time	Special Instructions
Serum/Plasma	2 to 8°C	7 days	If testing will be delayed more than 7 days, specimens should be frozen at -10°C or colder.
	-10°C or colder	12 months	Specimens stored frozen at -10°C or colder for 12 months showed no performance difference.

If testing will be delayed more than 24 hours, remove serum or plasma from the clot, serum separator or red blood cells.

Avoid multiple freeze/thaw cycles.

Specimen Shipping

Package and label specimens in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances.

PROCEDURE

Materials Provided

07P51 Alinity i Total β-hCG Reagent Kit

Materials Required but not Provided

- Alinity i Total β-hCG assay file
- 07P5101 Alinity i Total β-hCG Calibrators
- 07P5110 Alinity i Total β-hCG Controls or other control material
- 09P1540 Alinity i Multi-Assay Manual Diluent
- Alinity Trigger Solution
- Alinity Pre-Trigger Solution
- Alinity i-series Concentrated Wash Buffer

For information on materials required for operation of the instrument, **refer to the Alinity ci-series Operations Manual, Section 1.**

For information on materials required for maintenance procedures, **refer to the Alinity ci-series Operations Manual, Section 9.**

Assay Procedure

For a detailed description of how to run an assay, **refer to the Alinity ci-series Operations Manual, Section 5.**

- If using primary or aliquot tubes, refer to the Alinity ci-series Operations Manual, Section 4 to ensure sufficient specimen is present.
- To minimize the effects of evaporation, verify adequate sample cup volume is present prior to running the test.

Maximum number of replicates sampled from the same sample cup: 10

Priority:

- Sample volume for first test: 75 µL
- Sample volume for each additional test from same sample cup: 25 µL

≤ 3 hours on the reagent and sample manager:

- Sample volume for first test: 150 µL
- Sample volume for each additional test from same sample cup: 25 µL

> 3 hours on the reagent and sample manager:

- Replace with a fresh aliquot of sample.

- Refer to the Alinity i Total β-hCG calibrator package insert and Alinity i Total β-hCG control package insert for preparation and usage.
- For general operating procedures, refer to the Alinity ci-series Operations Manual, Section 5.
- For optimal performance, it is important to perform routine maintenance as described in the Alinity ci-series Operations Manual, Section 9. Perform maintenance more frequently when required by laboratory procedures.

Sample Dilution Procedures

Samples with a β-hCG value exceeding 15 000.00 mIU/mL (15 000.00 IU/L) are flagged with the code "> 15 000.00 mIU/mL" (> 15 000.00 IU/L) and may be diluted with either the Automated Dilution Protocol or the Manual Dilution Procedure.

Automated Dilution Protocol

The system performs a **1:15** dilution of the sample and automatically calculates the concentration by multiplying the result by the dilution factor.

Manual Dilution Procedure

Suggested dilution: 1:15

It is **recommended that dilutions not exceed 1:75.**

Add 20 µL of the sample to 280 µL of Alinity i Multi-Assay Manual Diluent.

The operator must enter the dilution factor in the Specimen or Control tab of the Create Order screen. The system will use this dilution factor to automatically calculate the concentration of the sample and report the result. The result should be > 467.00 mIU/mL (> 467.00 IU/L) before the dilution factor is applied.

If the operator does not enter the dilution factor, the result must be manually multiplied by the appropriate dilution factor before reporting the result. If a diluted sample result is < 467.00 mIU/mL (< 467.00 IU/L), do not report the result. Rerun using an appropriate dilution.

NOTE: A printed or displayed result of < 7000.00 mIU/mL (1:15 Automated Dilution Protocol) indicates the need to retest the sample at a lower dilution or undiluted. The result and interpretation should not be reported.

For detailed information on ordering dilutions, refer to the Alinity ci-series Operations Manual, Section 5.

Calibration

For instructions on performing a calibration, **refer to the Alinity ci-series Operations Manual, Section 5.**

Each assay control must be tested to evaluate the assay calibration.

Once a calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:

- A reagent kit with a new lot number is used.
- Daily quality control results are outside of statistically-based quality control limits used to monitor and control system performance, as described in the Quality Control Procedures section of this package insert.
    - **If statistically-based quality control limits are not available**, then the calibration should **not exceed a 30-day** limit for recalibration frequency.

This assay may require recalibration after maintenance to critical parts or subsystems or after service procedures have been performed.

Quality Control Procedures

The recommended control requirement for the Alinity i Total β-hCG assay is that a single sample of each control level be tested once every day testing performed.

To establish **statistically-based** control limits, each laboratory should establish its own concentration target and ranges for new control lots at each clinically relevant control level. This can be accomplished by assaying **a minimum of 20 replicates over several (3-5)** days

and using the reported results to establish the expected average (target) and variability about this average (range) for the laboratory. Sources of variation that should be included in this study in order to be representative of future system performance include:

- Multiple stored calibrations
- Multiple reagent lots
- Multiple calibrator lots
- Multiple processing modules (if applicable)
- Data points collected at different times of the day

Refer to published guidelines for information or general control recommendation, for example Clinical and Laboratory Standards Institute (CLSI) Document C24-A3 or other published guidelines, for general quality control recommendations.[12]

- If quality control results do not meet the acceptance criteria defined by laboratory QC procedure, sample results may be suspect. Follow the established quality control procedures to troubleshoot. Recalibration may be necessary. **For troubleshooting information, refer to the Alinity ci-series Operations Manual, Section 10.**
- Review quality control results and acceptance criteria following a change of reagent or calibrator lot.

Due to variation in analyte composition and/or matrices, external quality control materials and proficiency survey samples, may not elicit identical results across all hCG assays. Non-Abbott Quality Control and proficiency testing material may contain high levels of free beta-subunit hCG molecules. Non-Abbott Quality Control and proficiency testing material may generate different results when comparing a whole molecule hCG assay to a total β-hCG assay. Each laboratory needs to determine the suitability of each control material for specific immunoassays and validate the material prior to use.

Quality Control Guidance

Refer to “Basic QC Practices” by James O Westgard, Ph.D. for guidance on laboratory quality control practices.[13]

Verification of Assay Claims

For protocols to verify package insert claims, refer to Verification of Assay Claims in the Alinity ci-series Operations Manual.

RESULTS

Calculation

The Alinity i Total β-hCG assay utilizes a 4 Parameter Logistic Curve fit data reduction method (4PLC, Y-weighted) to generate a calibration curve.

For information on alternate result units, refer to the INSTRUMENT PROCEDURE, Alternate Result Units section of this package insert.

Interpretation of Results

For qualitative interpretation of the Alinity i Total β-hCG test results, specimens with β-hCG levels **less than or equal to 5.00 mIU/mL** (5.00 IU/L) will be reported in the INTERPRETATION field on the test results screen or printout as **"NEGATIVE**".

Specimens with β-hCG levels **greater than or equal to 25.00** mIU/mL (25.00 IU/L) will be reported as "**POSITIVE"**.

Specimens with β-hCG levels greater than 5.00 mIU/mL (5.00 IU/L) and less than 25.00 mIU/mL (25.00 IU/L) will be reported with concentrations only. No interpretation will be reported for these results.

≤ 5.00 Neg

5-25 Equivocal, indicative of early pregnancy

≥ 25 Positive

Flags

Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the Alinity ci-series Operations Manual, Section 5.

Measuring Interval

Measuring interval is defined as the range of values in mIU/mL (IU/L) which meets the limits of acceptable performance for linearity, imprecision, and bias.

The measuring interval of the Alinity i Total β-hCG assay is **2.42 to 15 000.00** mIU/mL (2.42 to 15 000.00 IU/L).

LIMITATIONS OF THE PROCEDURE

- This assay is capable of detecting whole molecule (intact) hCG as well as free β-hCG subunits.
- For diagnostic purposes, hCG results should always be used in conjunction with other data; e.g., patient's medical history, symptoms, results of other tests, clinical impressions, etc. Ectopic pregnancy cannot be distinguished from normal pregnancy by hCG measurements alone. The results from this or any other diagnostic kit should be used and interpreted only in the context of the overall clinical picture.*14*, *15*
- If the hCG level is inconsistent with, or unsupported by, clinical evidence, results should be confirmed by an alternate hCG method. This may include the qualitative testing of urine. The absence of urinary hCG may suggest a falsely elevated serum result. Results may also be confirmed by performing serial dilutions of the sample. Usually, but not always, samples that contain interfering substances exhibit nonlinear results when diluted.*16*
- The Alinity i Total β-hCG assay is cleared for use in the early detection of pregnancy **only**. It is not approved for any other uses such as tumor marker screening, tumor marker monitoring, etc. and it should not be performed for any other uses.

Infrequently, hCG levels may appear consistently elevated and could be due to, but not limited to, the presence of the following:[16], [17], [18], [19], [20]

- heterophilic antibodies
- nonspecific protein binding
- hCG-like substances
- trophoblastic or nontrophoblastic neoplasms

- As with any immunochemical reaction, unknown interferences from medications or endogenous substances may affect results.
- Elevated hCG levels have been associated with some pathological conditions (e.g., trophoblastic and nontrophoblastic neoplasms) and the results of this test should not be used in the diagnosis of these abnormal states. There have been reports of people receiving unnecessary medical treatment and surgery, including chemotherapy and hysterectomy, when hCG results were used in the diagnosis of abnormal conditions.[21], [22]
- Interfering substances (such as heterophilic antibodies, non-specific proteins, or hCG-like substances) may falsely depress or falsely elevate results. These interfering substances may cause false results over the entire range of the assay, not just at low levels, and may indicate the presence of hCG when there is none.
- Detection of very low levels of hCG does not rule out pregnancy. Low levels of hCG can occur in apparently healthy, nonpregnant subjects. Because hCG values double approximately every 48 hours in a normal pregnancy, patients with very low levels of hCG should be resampled and retested after 48 hours.[18], [23], [24]
- Post-menopausal specimens may have low levels of hCG levels unrelated to pregnancy. It is good clinical practice to resample and retest after 48 hours, or to test with an alternate hCG method.
- Because of the high degree of sensitivity of the assay, specimens tested as positive during initial days after conception may later be negative due to natural termination of the pregnancy. Natural termination occurs in 22% of clinically unrecognized pregnancies and 31% of pregnancies overall.[25]
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits such as Alinity i Total β-hCG that employ mouse monoclonal antibodies. Additional information may be required for diagnosis.[26], [27]
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference, and anomalous values may be observed. Additional information may be required for diagnosis.[17], [18]

EXPECTED VALUES

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

It is recommended that each laboratory determine its own reference range based upon its particular locale and population characteristics.

Because hCG is normally synthesized and secreted by cells of the placenta or its precursor, levels of the hormone in normal, non-pregnant individuals are low to undetectable. Concentrations of β-hCG measured in the sera of non-pregnant individuals, as reported in the literature, are **< 5 mIU/mL**. The concentration of β-hCG in maternal serum rises rapidly during early pregnancy. β-hCG levels between 5 mIU/mL and 25 mIU/mL may be indicative of early pregnancy. Values for β-hCG generally peak during the first trimester and decline slowly throughout the remainder of the pregnancy.[2, 21, 28, 29]

The study was conducted based on guidance from the Clinical and Laboratory Standards Institute (CLSI) document EP28-A3c.[30]

Human serum specimens were collected **from non-pregnant, pre-menopausal**, peri-menopausal, and post-menopausal females and were evaluated using the Alinity i Total β-hCG assay. The results are summarized in the following table.

Age (years)	n	Menopausal Status	Reference Interval mIU/mL (IU/L) (2.5 - 97.5 percentile)
18 - 41	128	pre-menopausal	< 2.42
42 - 55	140	peri-menopausal	< 2.42 - 4.87
> 55	137	post-menopausal*	< 2.42 - 7.60

* Post-menopausal is defined as female subjects who had not had a menstrual period for 12 months or more.

SPECIFIC PERFORMANCE CHARACTERISTICS

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

The Alinity i analyzer, and the ARCHITECT i System utilize the same reagents and sample/reagent ratios.

Unless otherwise specified, all studies were performed on the Alinity i analyzer.

Precision

Within-Laboratory Precision

A study was performed based on guidance from CLSI EP05-A2. Testing was conducted using 2 lots each of Alinity i Total β-hCG Reagent Kits, Alinity i Total β-hCG Calibrators, and Alinity i Total β-hCG Controls, and 2 instruments. Seven panels were assayed in a minimum of 2 replicates at 2 separate times per day on 20 different days.[31]

Panel Member	n	Mean mIU/mL (IU/L)	Within-Run (Repeatability) SD	Within-Run (Repeatability) %CV	Within-Laboratory (Total)[a] SD (Range[b])	Within-Laboratory (Total)[a] %CV (Range[b])
1	398	25.35	0.844	3.3	1.293 (1.154 - 1.416)	5.1 (4.6 - 5.5)
2	399	765.76	11.099	1.4	14.809 (11.542 - 17.734)	1.9 (1.5 - 2.3)
3	399	4971.95	73.079	1.5	110.173 (85.849 - 131.611)	2.2 (1.7 - 2.7)
B	400	5.33	0.269	5.0	0.408 (0.357 - 0.463)	7.6 (7.1 - 8.2)
C	400	165.16	3.680	2.2	4.853 (4.386 - 5.639)	2.9 (2.7 - 3.3)
D	399	9421.08	194.693	2.1	265.152 (214.698 - 339.368)	2.8 (2.2 - 3.5)
E	400	13069.37	314.717	2.4	412.842 (372.088 - 484.306)	3.2 (2.8 - 3.6)

[a] Includes within-run, between-run, and between-day variability.

[b] Minimum and maximum SD or %CV for each reagent lot and instrument combination.

In addition, 9 samples were assayed in replicates of at least 22 on 2 runs in a single day on 2 instruments and reagent lots. The number of replicates within negative and positive concentrations are shown in the table below.

Mean Concentration mIU/mL (IU/L)	n	Negative ≤ 5 mIU/mL (IU/L)	> 5 and < 25 mIU/mL (IU/L)[a]	Positive ≥ 25 mIU/mL (IU/L)
0.01	176	176	0	0
3.15	176	176	0	0
4.06	176	176	0	0
6.57	176	0	176	0
8.47	176	0	176	0
21.10	175	0	175	0
24.00	176	0	139	37

Mean Concentration mIU/mL (IU/L)	n	Negative ≤ 5 mIU/mL (IU/L)	> 5 and < 25 mIU/mL (IU/L)[a]	Positive ≥ 25 mIU/mL (IU/L)
28.41	176	0	0	176
29.94	176	0	0	176
2.32[b]	401	401	0	0
5.33[b]	400	106	294	0
25.35[b]	398	0	173	225

[a] Status not determined. Redraw is recommended after 48 hours to determine status.

[b] These samples are from the 20-day precision study.

Lower Limits of Measurement

A study was performed based on guidance from CLSI EP17-A2. Testing was conducted using 3 lots of Alinity i Total β-hCG Reagent Kits on each of 2 instruments over a minimum of 3 days. The maximum observed Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) values are summarized below.*32*

	mIU/mL (IU/L)
LoB[a]	0.20
LoD[b]	0.67
LoQ[c]	**2.42**

[a]The LoB represents the 95th percentile from n ≥ 60 replicates of zero-analyte samples.

[b]The LoD represents the lowest concentration at which the analyte can be detected with 95% probability based on n ≥ 60 replicates of low-analyte level samples.

[c] The LoQ was determined from n ≥ 60 replicates of low-analyte level samples and is defined as the lowest concentration at which a maximum TEa (Total Error allowable) of 25% was met.

Linearity

A study was performed based on guidance from CLSI EP06-A.

This assay is linear across the measuring interval of **2.42 to 15 000 mIU/mL** (2.42 to 15 000 IU/L).*33*

Analytical Specificity

A study was performed based on guidance from CLSI EP07-A2. Samples containing the cross-reactants listed below were tested with the Alinity i Total β-hCG assay on the Alinity i analyzer. The cross reactivity was calculated as a percent interference for samples with a β-hCG concentration > 25 mIU/mL (> 25 IU/L) and was shown to be less than 10% for each cross reactant. Samples with a β-hCG concentration ≤ 1.2 mIU/mL (≤ 1.2 IU/L) were

evaluated for concentration and the results were ≤ 1.2 mIU/mL (≤ 1.2 IU/L) for each cross reactant. See the table below.[34]

		β-hCG concentration	
		> 25 mIU/mL (> 25 IU/L)	**≤ 1.2 mIU/mL (≤ 1.2 IU/L)**
Cross-Reactant	**Cross-Reactant Concentration**	**% Interference**	**Mean Concentration (mIU/mL) (IU/L)**
TSH	100 µIU/mL	-0.6	Not detectable
LH	500 mIU/mL	3.0	Not detectable
FSH	500 mIU/mL	-1.0	Not detectable
hCG alpha subunit	500 mIU/mL	-1.2	Not detectable

Interference

Potentially Interfering Substances

A study was performed based on guidance from CLSI EP07-A2.[34]

An interference of greater than 10% is considered significant.

		% Interference	
Potentially Interfering Substance	**Interferent Concentration**	**7.00 mIU/mL (IU/L)**	**50.00 mIU/mL (IU/L)**
Bilirubin (Conjugated)	≤ 20 mg/dL	-3.6	-3.0
Bilirubin (Unconjugated)	≤ 20 mg/dL	-1.4	-0.5
Hemoglobin	≤ 500 mg/dL	-1.2	-1.4
Total Protein	≤ 12 g/dL	8.4	-10.3
Triglycerides	≤ 3000 mg/dL	-2.9	1.4
Rheumatoid Factor (RF)	≤ 194 IU/L	0.8	-0.4
Acetaminophen	≤ 20 mg/dL	-2.1	-0.7
Acetylcysteine	≤ 167 mg/dL	-0.1	-1.9
Acetylsalicylic Acid	≤ 66 mg/dL	0.6	-3.2
Ampicillin	≤ 53 mg/L	-0.3	-1.5
Ascorbic Acid	≤ 6 mg/dL	1.2	-0.1
Atropine	≤ 20 mg/dL	-2.2	-2.2
Ca-Dobesilate	≤ 200 mg/L	-2.1	-1.8
Caffeine	≤ 20 mg/dL	-2.1	-0.7
Cyclosporine	≤ 5 mg/L	-0.5	-0.5
Cefoxitin	≤ 660 mg/L	-3.2	-0.5
Doxycycline	≤ 30 mg/L	-0.6	-0.9

Potentially Interfering Substance	Interferent Concentration	% Interference 7.00 mIU/mL (IU/L)	% Interference 50.00 mIU/mL (IU/L)
Ethanol	≤ 1%	-0.4	0.3
EDTA	≤ 80 mg/dL	-1.6	-1.7
Ibuprofen	≤ 50 mg/dL	-0.8	0.2
Levodopa	≤ 20 mg/L	0.4	7.3
Methyldopa	≤ 15 mg/L	-1.0	0.0
Metronidazole	≤ 120 mg/L	1.2	0.4
Phenylbutazone	≤ 400 mg/L	5.6	-0.7
Rifampicin	≤ 64 mg/L	-4.4	-2.9
Sodium Heparin	≤ 3000 U/L	-1.9	0.2
Sodium Salicylic Acid	≤ 70 mg/dL	-1.3	-0.4
Theophylline	≤ 40 mg/L	-1.3	0.7
Gentisic Acid	≤ 20 mg/dL	-2.1	-2.1

Matrix Comparison

Matched sample sets spanning the measuring interval were tested. A Weighted Deming regression was performed comparing the first replicate of the collection tube to the mean of the serum tube concentration. See the table below.

Collection Tube	n	Correlation Coefficient	Intercept	Slope	Concentration Range mIU/mL (IU/L)
Dipotassium EDTA	45	1.00	0.62	0.98	5.09 - 11 194.83
Lithium heparin	45	1.00	0.84	1.01	5.08 - 11 731.58
Sodium heparin	45	1.00	0.77	1.01	5.06 - 11 163.25
Lithium heparin (separator tube)	45	1.00	0.76	1.01	5.00 - 11 245.48
Serum (separator tube)	45	1.00	0.18	1.02	4.80 - 12 049.91
Tripotassium EDTA	45	1.00	0.66	0.94	4.87 - 10 073.85

Method Comparison

Quantitative

A study was performed using serum specimens based on guidance from CLSI EP09-A3 using the Weighted Deming regression method.[35]

	n	Correlation Coefficient	Intercept	Slope	Concentration Range mIU/mL (IU/L)
Alinity i Total β-hCG vs ARCHITECT Total β-hCG	210	1.00	0.12	1.01	2.40 - 14 866.03

Qualitative

A total of 381 Alinity i Total β-hCG qualitative results were analyzed for concordance with the ARCHITECT Total β-hCG assay. Samples below the lower limit of the measuring interval were included in the analysis.

	ARCHITECT Total β-hCG mIU/mL (IU/L)		
Alinity i Total β-hCG mIU/mL (IU/L)	**Positive ≥ 25.00**	**> 5.00 - < 25.00[a]**	**Negative ≤ 5.00**
Positive ≥ 25.00	201	0	0
> 5.00 - < 25.00[a]	1	44	3
Negative ≤ 5.00	0	0	132

[a] Status not determined. Redraw is recommended after 48 hours to determine status.

Concentration Values of Discordant Specimens	
Alinity i Total β-hCG mIU/mL (IU/L)	**ARCHITECT Total β-hCG mIU/mL (IU/L)**
24.42	26.41
5.25	4.58
5.06	4.62
5.16	4.80

Carryover

Carryover from a sample containing 1 000 000 mIU/mL β-hCG to an adjacent 0 mIU/mL β-hCG sample was less than 7.5 mIU/mL β-hCG.

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