ARCHITECT

SYSTEM

en

HE4

REF 2P54

305-486 10/09

B2P540

HE4

Customer Service: Contact your local representative or find country specific contact information on www.abbottdiagnostics.com

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

Key to symbols used

REF	List Number	WARNING: SENSITIZER	Warning: Sensitizer
IVD	*In Vitro* Diagnostic Medical Device	REACTION VESSELS	Reaction Vessels
LOT	Lot Number	SAMPLE CUPS	Sample Cups
SN	Serial Number	REPLACEMENT CAPS	Replacement Caps
	Expiration Date	REAGENT LOT	Reagent Lot
	Consult instructions for use	CONTROL NO.	Control Number
	Manufacturer	SEPTUM	Septum
2°C 8°C	Store at 2-8°C	ASSAY CD-ROM	Assay CD-ROM

See **REAGENTS** section for a full explanation of symbols used in reagent component naming.

WARNING: HE4 assay values obtained with different assay methods cannot be used interchangeably due to differences in assay methods and reagent specificity. The results reported by the laboratory to the physician must include the identity of the HE4 assay used. If, in the course of monitoring a patient, the assay method used for determining serial HE4 levels is changed, additional sequential testing should be carried out. Prior to changing assays, the laboratory MUST confirm baseline values for patients being serially monitored. **The ARCHITECT HE4 assay should not be used as a cancer screening test.**

NAME

ARCHITECT HE4

INTENDED USE

The ARCHITECT HE4 assay is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of HE4 antigen in human serum.

The assay is to be used as an aid in monitoring recurrence or progressive disease in patients with epithelial ovarian cancer. Serial testing for patient HE4 assay values should be used in conjunction with other clinical methods used for monitoring ovarian cancer.

It is further intended to be used in conjunction with ARCHITECT CA 125 II as an aid in estimating the risk of epithelial ovarian cancer in premenopausal and postmenopausal women presenting with an adnexal mass who will undergo surgical intervention. The results must be interpreted in conjunction with other methods in accordance with standard clinical management guidelines.

SUMMARY AND EXPLANATION OF TEST

Human epididymis protein 4 (HE4) belongs to the family of whey acidic four-disulfide core (WFDC) proteins with suspected trypsin inhibitor properties. Other proteins in this family include SLPI, Elafin, and PS20 (WFDC1).[1] The HE4 gene codes for a 13 kD protein, although in its mature glycosylated form the protein is approximately 20-25 kD,[2] and consists of a single peptide containing 2 WFDC domains.[3] HE4 was first identified in the epithelium of the distal epididymis and originally predicted to be a protease inhibitor involved in sperm maturation.[4,5] HE4 has since been reported to be expressed in several normal tissues including epithelia of respiratory and reproductive tissues and also in ovarian cancer tissue.[2,3,6-9] In addition to expression on a cellular level, secreted HE4 has been detected in high levels in the serum of ovarian cancer patients. In a case-control study comparing patients with ovarian cancer to healthy and benign conditions, Hellström *et al.* found that HE4 detected ovarian cancer with 67% sensitivity at a specificity level of 96%.[10] In a subsequent study evaluating numerous known biomarkers for ovarian cancer, HE4 showed the highest sensitivity of any single marker. In this study, the combination of HE4 and CA 125 was a more accurate predictor of malignancy than either marker alone, with a sensitivity of 76% and a specificity of 95%.[11]

Ovarian cancer is the fourth most common cause of cancer-related death in women worldwide. In Europe, the mortality rate range is from 3.6 to 9.3 per 100.000 women.[12] The symptoms of ovarian cancer are related to the presence of adnexal masses and are often vague and unspecific. The primary goal of diagnostic evaluation of an adnexal mass is to determine whether it is benign or malignant. It is estimated that 5 to 10 percent of women in the United States will undergo a surgical procedure for a suspected ovarian neoplasm during their lifetime, and 13 to 21 percent of these women will be found to have an ovarian malignancy.[13] The American College of Obstetricians and Gynecologists Practice Bulletin published in 2007 states the following "Women with ovarian cancer whose care is managed by physicians who have advanced training and expertise in the treatment of women with ovarian cancer, such as gynecologic oncologists, have improved overall survival rates compared with those treated without such collaboration."[14] Since the majority of adnexal masses are benign, it is important to determine preoperatively whether a patient is at high risk for ovarian malignancy, in order to ensure proper management.[15] Since the initial report in 1988, clinical impression, serum CA 125 and ultrasound[15] along with other imaging modalities have been the standards in the determination of whether an adnexal mass is suspicious for malignancy. Studies have shown that the combination of physical examination, CA 125 and imaging affords the highest positive predictive value.[16-18]

To further improve the evaluation of patients presenting with an adnexal mass, a risk of ovarian malignancy algorithm (ROMA) has been developed using the ARCHITECT HE4 assay in conjunction with the ARCHITECT CA 125 II assay, as an aid in estimating the risk that the patient is harboring epithelial ovarian cancer.[19] The results must be interpreted in conjunction with other methods in accordance with standard clinical management guidelines.

The percentage increase in HE4 values has been used as an aid in monitoring recurrence or progressive disease in patients with invasive epithelial ovarian cancer. Currently there is no clinically accepted cut-off for use in monitoring cancer progression in epithelial ovarian cancer subjects with this assay.[20] Serial testing for patient HE4 assay values should be used in conjunction with other clinical methods used for monitoring ovarian cancer.[20-21]

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The ARCHITECT HE4 assay is a two-step immunoassay for the quantitative determination of HE4 antigen in human serum using CMIA technology with flexible assay protocols, referred to as Chemiflex.

In the first step, sample and 2H5 anti-HE4 coated paramagnetic microparticles are combined. HE4 antigen present in the sample binds to the anti-HE4 coated microparticles. After washing, 3D8 anti-HE4 acridinium-labeled conjugate is added to create a reaction mixture in the second step. Following another wash cycle, pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of HE4 antigen in the sample and the RLUs detected by the ARCHITECT *i** System optics.

For additional information on system and assay technology, refer to the ARCHITECT System Operations Manual, Section 3.

* *i* = immunoassay

REAGENTS

Reagent Kit, 100 Tests

ARCHITECT HE4 Reagent Kit (2P54)

- **MICROPARTICLES** 1 Bottle (6.6 mL) Anti-HE4 (mouse, monoclonal) coated microparticles in PBS buffer with protein (bovine) stabilizers and detergent. Minimum concentration: 0.1% solids. Preservative: ProClin 300.
- **CONJUGATE** 1 Bottle (5.9 mL) Anti-HE4 (mouse, monoclonal) acridinium-labeled conjugate in PBS buffer with protein (bovine) stabilizers and detergent. Minimum concentration: 50 ng/mL. Preservative: ProClin 300.

Assay Diluent

ARCHITECT *i* Multi-Assay Manual Diluent (No. 7D82-50)

- **MULTI-ASSAY MANUAL DILUENT** 1 Bottle (100 mL) ARCHITECT *i* Multi-Assay Manual Diluent containing phosphate buffered saline solution. Preservative: antimicrobial agent.

Other Reagents

ARCHITECT *i* Pre-Trigger Solution

- **PRE-TRIGGER SOLUTION** Pre-Trigger Solution containing 1.32% (w/v) hydrogen peroxide.

ARCHITECT *i* Trigger Solution

- **TRIGGER SOLUTION** Trigger Solution containing 0.35 N sodium hydroxide.

ARCHITECT *i* Wash Buffer

- **WASH BUFFER** Wash Buffer containing phosphate buffered saline solution. Preservatives: antimicrobial agents.

WARNINGS AND PRECAUTIONS

- **IVD**
- For *In Vitro* Diagnostic Use.
- Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

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[22]. Biosafety Level 2[23] or other appropriate biosafety practices[24,25] should be used for materials that contain or are suspected of containing infectious agents.
- The microparticles and conjugate contain methylisothiazolones, which are components of ProClin 300, and are classified per applicable European Community (EC) Directives as: Irritant (Xi). The following are the appropriate Risk (R) and Safety (S) phrases.

R43 May cause sensitization by skin contact.
S24 Avoid contact with skin.
S35 This material and its container must be disposed of in a safe way.
S37 Wear suitable gloves.
S46 If swallowed, seek medical advice immediately and show this container or label.

- For product not classified as dangerous per European Directive 1999/45/EC as amended - Safety data sheet available for professional user on request.
- For a detailed discussion of safety precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 8.

Handling Precautions

- Do not use reagent kits beyond the expiration date.
- **Do not pool reagents within a kit or between reagent kits.**
- Before loading the ARCHITECT HE4 Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment. For microparticle mixing instructions, refer to the **PROCEDURE, Assay Procedure** section of this package insert.
- **Septums MUST be used to prevent reagent evaporation and contamination and to ensure reagent integrity. Reliability of assay results cannot be guaranteed if septums are not used according to the instructions in this package insert.**
- To avoid contamination, wear clean gloves when placing a septum on an uncapped reagent bottle.
  - Once a septum has been placed on the reagent bottle, **do not invert the bottle** as this will result in reagent leakage and may compromise assay results.
  - Over time, residual liquids may dry on the septum surface. These are typically dried salts, and have no effect on assay efficacy.
- For a detailed discussion of handling precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 7.

Storage Instructions

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The ARCHITECT HE4 Reagent Kit must be stored at 2-8˚C in an upright position and may be used immediately after removal from 2-8˚C storage.
- When stored and handled as directed, the reagents are stable until the expiration date.
- The ARCHITECT HE4 Reagent Kit may be stored on board the ARCHITECT *i* System for a maximum of 30 days. After 30 days, the reagent kit must be discarded. For information on tracking onboard time, refer to the ARCHITECT System Operations Manual, Section 5.
- Reagents may be stored on or off the ARCHITECT *i* System. If reagents are removed from the system, store them at 2-8˚C (with septums and replacement caps) in an upright position. For reagents stored off the system, it is recommended that they be stored in their original trays and boxes to ensure they remain upright. **If the microparticle bottle does not remain upright (with a septum installed) while in refrigerated storage off the system, the reagent kit must be discarded.** For information on unloading reagents, refer to the ARCHITECT System Operations Manual, Section 5.

Indications of Reagent Deterioration

When a control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results are invalid and samples must be retested. Assay recalibration may be necessary. For troubleshooting information, refer to the ARCHITECT System Operations Manual, Section 10.

INSTRUMENT PROCEDURE

- The ARCHITECT HE4 assay file must be installed on the ARCHITECT *i* 2000 or *i* 2000_{SR} Systems from the ARCHITECT *i* System Assay CD-ROM - WW (excluding US)-Addition A, version 8.0 or higher, or on the ARCHITECT *i* 1000_{SR} System from the ARCHITECT *i* 1000_{SR} System Assay CD-ROM - WW (excluding US) Special Edition, version 7.0 or higher, before performing the assay. For detailed information on assay file installation and viewing and editing assay parameters, refer to the ARCHITECT System Operations Manual, Section 2.
- For information on printing assay parameters, refer to the ARCHITECT System Operations Manual, Section 5.
- For a detailed description of system procedures, refer to the ARCHITECT System Operations Manual.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

- Only human serum (including serum collected in serum separator tubes [SST]) may be used with the ARCHITECT HE4 assay. Other specimen collection tubes have not been tested with this assay.
- The ARCHITECT *i* System does not provide the capability to verify specimen type. It is the responsibility of the operator to verify that the correct specimen types are used in the ARCHITECT HE4 assay.

Specimen Conditions

- Do not use specimens with the following conditions:
  - heat-inactivated
  - pooled
  - grossly hemolyzed
  - obvious microbial contamination
  - cadaver specimens or body fluids other than human serum
- For accurate results, serum 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.
- Use caution when handling patient specimens to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
- For optimal results, inspect all specimens for bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.

Preparation for Analysis

- Follow the tube manufacturer's processing instructions for serum collection tubes. Gravity separation is not sufficient for specimen preparation.
- Mix thawed specimens thoroughly by low speed vortexing or by inverting 10 times. Visually inspect the specimens. If layering or stratification is observed, continue mixing until specimens are visibly homogeneous.
- To ensure consistency in results, it is recommended that specimens be transferred to a centrifuge tube and centrifuged at ≥ 10,000 RCF (Relative Centrifugal Force) for 10 minutes before testing if they contain fibrin, red blood cells, or other particulate matter. Transfer clarified specimen to a sample cup or secondary tube for testing.
- Centrifuged specimens with a lipid layer on the top must be transferred to a sample cup or secondary tube. Care must be taken to transfer only the clarified specimen without the lipemic material.

Storage

- Specimens may be stored on or off the clot or separator gel for
  - up to 24 hours at room temperature (study performed at 25˚C).
  - up to 4 days at 2-8˚C.
- If testing will be delayed more than 24 hours for specimens stored at room temperature, or more than 4 days for specimens stored at 2-8˚C, remove serum from the clot or separator gel and store at -10˚C or colder.
- Avoid more than three freeze/thaw cycles.

Shipping

- Before shipping specimens, it is recommended that specimens be removed from the clot or separator gel.
- When shipping, specimens must be packaged and labeled in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances.
- Specimens may be shipped on wet or dry ice. Do not exceed the storage time limitations listed above.

PROCEDURE

Materials Provided

- 2P54 ARCHITECT HE4 Reagent Kit

Materials Required but not Provided

- ARCHITECT *i* System
- 3K52 ARCHITECT *i* System **ASSAY CD-ROM** - WW (excluding US)-Addition A (for use with ARCHITECT *i* 2000 and *i* 2000_{SR} Systems)
- 1P61 ARCHITECT *i* 1000_{SR} System **ASSAY CD-ROM** - WW (excluding US) Special Edition
- 2P54-01 ARCHITECT HE4 Calibrators
- 2P54-10 ARCHITECT HE4 Controls
- 7D82-50 ARCHITECT *i* **MULTI-ASSAY MANUAL DILUENT**
- ARCHITECT *i* **PRE-TRIGGER SOLUTION**
- ARCHITECT *i* **TRIGGER SOLUTION**
- ARCHITECT *i* **WASH BUFFER**
- ARCHITECT *i* **REACTION VESSELS**
- ARCHITECT *i* **SAMPLE CUPS**
- ARCHITECT *i* **SEPTUM**
- ARCHITECT *i* **REPLACEMENT CAPS**
- Pipettes or pipette tips (optional) to deliver the volumes specified on the patient or control order screen.

For information on materials required for maintenance procedures, refer to the ARCHITECT System Operations Manual, Section 9.

Assay Procedure

- Before loading the ARCHITECT HE4 Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that have settled during shipment. After the first time the microparticles have been loaded, no further mixing is required.
  - **Invert the microparticle bottle 30 times.**
  - Visually inspect the bottle to ensure microparticles are resuspended. If microparticles remain adhered to the bottle, continue inverting the bottle until the microparticles have been completely resuspended.
  - **If the microparticles do not resuspend, DO NOT USE. Contact your local Abbott representative.**
  - Once the microparticles have been resuspended, place a septum on the bottle. For instructions on placing septums on bottles, refer to the **Handling Precautions** section of this package insert.
- Load the ARCHITECT HE4 Reagent Kit on the ARCHITECT *i* System.
  - Verify that all necessary assay reagents are present.
  - Ensure that septums are present on all reagent bottles.
- Order calibration, if necessary.
  - For information on ordering calibrations, refer to the ARCHITECT System Operations Manual, Section 6.
- Order tests.
  - For information on ordering patient specimens and controls and for general operating procedures, refer to the ARCHITECT System Operations Manual, Section 5.
- The minimum sample volume is calculated by the system and is printed on the Orderlist report. No more than 10 replicates may be sampled from the same sample cup. To minimize the effects of evaporation, verify adequate sample cup volume is present prior to running the test.
  - Priority: 75 µL for the first ARCHITECT HE4 test plus 25 µL for each additional ARCHITECT HE4 test from the same sample cup.
  - ≤ 3 hours on board: 150 µL for the first ARCHITECT HE4 test plus 25 µL for each additional ARCHITECT HE4 test from the same sample cup.
  - > 3 hours on board: Replace with a fresh sample (patient specimens, controls, and calibrators).
  - If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.
- Prepare calibrators and controls.
  - ARCHITECT HE4 Calibrators and Controls should be prepared according to their respective package inserts.
  - To obtain the recommended volume requirements for the ARCHITECT HE4 Calibrators and Controls, hold the bottles **vertically** and dispense a minimum of 6 drops of each calibrator or a minimum of 5 drops of each control into each respective sample cup.
- Load samples.
  - For information on loading samples, refer to the ARCHITECT System Operations Manual, Section 5.
- Press RUN.
- For additional information on principles of operation, refer to the ARCHITECT Operations Manual, Section 3.
- For optimal performance, it is important to perform routine maintenance as described in the ARCHITECT System Operations Manual, Section 9. When a laboratory requires more frequent maintenance, follow those procedures.

Specimen Dilution Procedures

Patient specimens with HE4 values exceeding 1500.0 pmol/L are flagged with the code "> 1500.0 pmol/L" and may be diluted with the Automated or Manual Dilution Protocol.

Automated Dilution Protocol

- If using the Automated Dilution Protocol, the system performs a 1:10 dilution of the specimen and automatically calculates the concentration of the specimen before dilution and reports the results.

Manual Dilution Protocol

- The suggested dilution for the ARCHITECT HE4 assay is 1:10.
- Add 50 µL of the patient specimen to 450 µL of the ARCHITECT *i* Multi-Assay Manual Diluent (7D82-50).
- The operator must enter the dilution factor in the Patient or Control order screen. The system will use this dilution factor to automatically calculate the concentration of the sample before dilution. This will be the reported result. The result should be within the measuring interval before the dilution factor is applied.
- For detailed information on ordering dilutions, refer to the ARCHITECT System Operations Manual, Section 5.

Calibration

- To perform an ARCHITECT HE4 calibration, test the calibrators A, B, C, D, E, and F in duplicate. The calibrators should be priority loaded.
- A single replicate of each ARCHITECT HE4 control level must be tested to evaluate the assay calibration.
  - Order controls as described in the **Assay Procedure** section.
  - Ensure that assay control values are within the concentration ranges specified in the control package insert.
- Calibration Range: 0.0 pmol/L - 1500.0 pmol/L.
- Once an ARCHITECT HE4 calibration is accepted and stored, all subsequent samples may be tested without further calibration unless one or more of the following occur:
  - A reagent kit with a new lot number is used.
  - Controls are out of range.
- For detailed information on how to perform an assay calibration, refer to the ARCHITECT System Operations Manual, Section 6.

QUALITY CONTROL PROCEDURES

The recommended control requirement for the ARCHITECT HE4 assay is that a single sample of each control be tested once every 24 hours each day of use. If your laboratory quality control procedures require more frequent use of controls to verify test results, follow those procedures. Additional controls may be tested in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's quality control policy.

The ARCHITECT HE4 control values must be within the acceptable ranges specified in the control package insert. If a control is out of its specified range, the associated test results are invalid and must be retested. Recalibration may be indicated.

Verification of Assay Claims

For protocols to verify package insert claims, refer to the ARCHITECT System Operations Manual, Appendix B. The ARCHITECT HE4 assay belongs to method group 1.

RESULTS

Calculation

The ARCHITECT HE4 assay uses a 4 Parameter Logistic Curve fit (4PLC, Y-weighted) data reduction method to generate a calibration curve.

Risk of Ovarian Malignancy Algorithm (ROMA)

ROMA is used to aid in the assessment of risk of epithelial ovarian cancer in patients presenting with an adnexal mass who will undergo surgical intervention.

Calculation of Predictive Index

A Predictive Index (PI) is calculated for premenopausal and postmenopausal patients separately using equations (1) and (2) below. To calculate the PI, obtain the menopausal status of the patient and the assay values from the ARCHITECT HE4 and ARCHITECT CA 125 II assays. Insert these values into the applicable equations below.

(1) Premenopausal

PI = -12.0 + 2.38*LN[HE4] + 0.0626*LN[CA125]

(2) Postmenopausal

PI = -8.09 + 1.04*LN[HE4] + 0.732*LN[CA125]

where, LN = Natural Logarithm. Do not use LOG = Log_{10}.

Calculation of ROMA Value

To calculate the ROMA value (*i.e.* Predictive Probability), insert the calculated value for PI into the equation below:

$$\text{ROMA value (\%)} = \frac{\text{Exp(PI)}}{1 + \text{Exp(PI)}} \times 100$$

where, $\text{Exp(PI)} = e^{PI}$

NOTE: These equations were used for the calculation of ROMA values with the ARCHITECT HE4 assay from 17.2 to 12,637.8 pmol/L and with the ARCHITECT CA 125 II assay from 3.9 to 14,163.0 U/mL.

The examples below should be used in order to validate calculations of PI and ROMA before reporting patient results:

Menopausal Status	ARCHITECT Values		PI Calculation	PI	ROMA (%)
	HE4 (pmol/L)	CA 125 II (U/mL)			
Pre-menopausal	37.5	74.9	-12.0+(2.38*3.624) +(0.0626*4.316)	-3.10388	4.3
	386.6	21.8	-12.0+(2.38*5.957) +(0.0626*3.082)	2.371517	91.5
Post-menopausal	66.7	11.3	-8.09+(1.04*4.200) +(0.732*2.425)	-1.94683	12.5
	383.1	22.7	-8.09+(1.04*5.948) +(0.732*3.122)	0.381799	59.4

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 ARCHITECT System Operations Manual, Section 5.

Measuring Interval

The measuring interval of the ARCHITECT HE4 assay is 20.0 pmol/L to 1500.0 pmol/L.

LIMITATIONS OF THE PROCEDURE

- The level of HE4 cannot be used as absolute evidence for the presence or absence of malignant disease and **the ARCHITECT HE4 assay should not be used as a cancer screening test.**
- ARCHITECT HE4 results should be used in conjunction with other clinical data; *e.g.*, symptoms, medical history, etc.
- If the ARCHITECT HE4 results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- ARCHITECT HE4 results should not be used interchangeably with other manufacturers' methods for HE4 determinations.
- ARCHITECT CA 125 II results should not be used interchangeably with other manufacturers' methods for CA 125 determinations in the ROMA calculation.
- Patients with confirmed ovarian cancer may have ARCHITECT HE4 assay values in the same range as healthy women. Certain histological types of ovarian cancer (*e.g.,* mucinous or germ cell tumors) rarely express HE4, therefore the use of the ARCHITECT HE4 assay is not recommended for monitoring of patients with known mucinous or germ cell ovarian cancer.[2] Conversely, elevated levels of HE4 antigen may be present in individuals with non-malignant disease.
- The ROMA has not been validated for the following patient groups: patients previously treated for malignancy, patients currently being treated with chemotherapy, and patients less than 18 years of age. Failure of the ARCHITECT HE4 and/or the ARCHITECT CA 125 II assays to perform as indicated, or error in the calculation of results, could lead to inaccurate risk assessment and improper management of the patient. Specifically, a falsely low result of the assay(s) could result in a determination that the patient is at lower risk of having epithelial ovarian cancer, which could triage the patient to a less specialized level of care.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA).[26,27] Specimens containing HAMA may produce anomalous values when tested with assay kits such as ARCHITECT HE4 that employ mouse monoclonal antibodies.[26]
- Heterophilic antibodies in human specimens can react with reagent immunoglobulins, interfering with *in vitro* immunoassays.[28] 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.
- Refer to the **SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS** section in this package insert for specimen limitations.

EXPECTED VALUES

It is recommended that each laboratory establish its own expected range which may be unique to the population it serves.

The distribution in percentage (%) of ARCHITECT HE4 assay values determined in 1626 female specimens is summarized in the table below.

		ARCHITECT HE4 Values (pmol/L)				
	n	0.0-70.0	70.1-140.0	140.1-500.0	500.1-1500.0	Greater than 1500.0
		Percentage Distribution (%)				
APPARENTLY HEALTHY						
Premenopausal	210	95.7	2.9	1.4	0.0	0.0
Postmenopausal	190	81.6	13.7	4.7	0.0	0.0
BENIGN CONDITIONS						
Gynecological Disease						
Premenopausal	306	91.2	6.9	1.3	0.3	0.3
Postmenopausal	213	73.2	19.3	6.6	0.9	0.0
Pregnancy	50	98.0	2.0	0.0	0.0	0.0
Non-Gynecological Disease	49	32.7	49.0	12.2	6.1	0.0
CHF [a]	44	34.1	36.4	27.3	2.3	0.0
CANCER						
Ovarian						
Premenopausal	67	40.3	25.4	19.4	13.4	1.5
Postmenopausal	247	24.3	17.0	30.0	17.8	10.9
Endometrial	50	52.0	26.0	18.0	2.0	2.0
Breast	50	38.0	38.0	12.0	6.0	6.0
Gastrointestinal	50	68.0	18.0	14.0	0.0	0.0
Lung	50	22.0	44.0	34.0	0.0	0.0
Bladder	50	24.0	28.0	34.0	10.0	4.0

[a] CHF = Congestive Heart Failure

Ninety-six percent of the apparently healthy premenopausal subjects had an ARCHITECT HE4 assay value at or below 70 pmol/L and 95% of the apparently healthy postmenopausal subjects had an ARCHITECT HE4 assay value at or below 140 pmol/L.

Risk Estimation in Patients Presenting with Adnexal Mass

The effectiveness of the ARCHITECT HE4 assay in combination with the ARCHITECT CA 125 II assay for risk estimation for epithelial ovarian cancer was determined in a retrospective, internal study for patients presenting with adnexal mass who underwent surgical intervention. An algorithm was developed for estimation of the risk of epithelial ovarian cancer (refer to the **RESULTS**, **Risk of Ovarian Malignancy Algorithm (ROMA)** section of this package insert). The algorithm takes into account the HE4 and CA 125 values as well as the menopausal status of the patient. The algorithm calculates a predictive probability of finding epithelial ovarian cancer on surgery. In the retrospective study a total of 494 patients were included and the predictive probability for ovarian cancer as well as the ability for separation into a low and a high risk group based on ROMA values was determined.

Stratification into Low Risk and High Risk Groups

The risk of ovarian malignancy algorithm was used to stratify patients into risk groups for finding epithelial ovarian cancer. The following cut-points were used in order to provide a specificity level of 75% for the ARCHITECT HE4 and ARCHITECT CA 125 II assay combination:

Premenopausal Patients

ROMA value ≥ 7.4% = High risk of finding epithelial ovarian cancer
ROMA value < 7.4% = Low risk of finding epithelial ovarian cancer

Postmenopausal Patients

ROMA value ≥ 25.3% = High risk of finding epithelial ovarian cancer
ROMA value < 25.3% = Low risk of finding epithelial ovarian cancer

The risk stratification into high risk of harboring epithelial ovarian cancer in all 494 patients (229 premenopausal and 265 postmenopausal) presenting with an adnexal mass using ROMA at a specificity level of 75% is summarized by menopausal status in the following table. The sensitivity for stratifying patients with stage I-IV epithelial ovarian cancer into the high risk group was 93% at a specificity of 75%, (*i.e.*, 75% of patients with benign adnexal mass were stratified into the low risk group). The positive and negative predictive values were 58% and 97%, respectively.

	Premenopausal Patients		**Postmenopausal Patients**	
	ROMA value < 7.4%	**ROMA value ≥ 7.4%**	**ROMA value < 25.3%**	**ROMA value ≥ 25.3%**
EOC [a] and LMP [b] Combined	7/34 (21%)	27/34 (79%)	12/116 (10%)	104/116 (90%)
LMP	5/16 (31%)	11/16 (69%)	3/6 (50%)	3/6 (50%)
Stage I-II EOC	1/7 (14%)	6/7 (86%)	7/28 (25%)	21/28 (75%)
Stage I-IIIC [c] EOC	1/8 (12%)	7/8 (88%)	7/39 (18%)	32/39 (82%)
Stage I-IV EOC	1/16 (6%)	15/16 (94%)	8/108 (7%)	100/108 (93%)
Stage III-IV EOC	0/9 (0%)	9/9 (100%)	1/80 (1%)	79/80 (99%)
Unstaged EOC	1/2 (50%)	1/2 (50%)	1/2 (50%)	1/2 (50%)
Benign	147/195 (75%)	48/195 (25%)	112/149 (75%)	37/149 (25%)

[a] EOC = Epithelial Ovarian Cancer
[b] LMP = Low Malignant Potential
[c] Stage I-IIIB and Stage IIIC (Omentum negative, lymph node positive) EOC.

SPECIFIC PERFORMANCE CHARACTERISTICS

Assay results obtained in individual laboratories may vary from data presented.

Precision

The ARCHITECT HE4 assay is designed to have an imprecision of ≤ 10% total CV.

A study was performed based on guidance from the National Committee for Clinical Laboratory Standards (NCCLS) document EP5-A2.[29] Testing was conducted using two lots of reagents, calibrators, and controls and three instruments. Three levels of controls and panels were assayed in replicates of two at two separate times per day for 20 different days. Each instrument used a single calibration curve throughout the study. The data are summarized in the following table.

Sample	Instrument	Reagent Lot	n	Mean (pmol/L)	Within-Run SD	Within-Run %CV	Total SD	Total %CV
Low Control	1	1	80	49.7	1.3	2.5	1.7	3.5
	2	2	80	49.4	1.5	3.0	1.6	3.3
	3	1	80	51.7	2.0	3.9	2.5	4.8
Medium Control	1	1	80	168.1	3.2	1.9	6.0	3.6
	2	2	80	174.4	3.9	2.2	5.4	3.1
	3	1	80	183.7	6.6	3.6	8.6	4.7
High Control	1	1	80	648.2	16.8	2.6	24.7	3.8
	2	2	80	687.3	16.3	2.4	22.5	3.3
	3	1	80	722.5	22.4	3.1	31.8	4.4
Panel 1	1	1	80	39.0	1.1	2.8	1.4	3.7
	2	2	80	38.4	1.2	3.1	1.5	3.8
	3	1	80	38.2	1.4	3.7	1.8	4.7
Panel 2	1	1	80	182.8	6.4	3.5	7.7	4.2
	2	2	80	189.7	5.5	2.9	5.9	3.1
	3	1	80	191.6	6.3	3.3	8.2	4.3
Panel 3	1	1	80	1080.3	30.2	2.8	42.7	4.0
	2	2	80	1114.7	32.7	2.9	37.0	3.3
	3	1	80	1121.8	55.0	4.9	55.0	4.9

Linearity

The ARCHITECT HE4 assay is designed to be linear across the measurement range of 20.0 to 1500.0 pmol/L.

Samples were prepared by mixing serum panels with diluted wash buffer and tested with the ARCHITECT HE4 assay. Based on a study performed by guidance from the NCCLS document EP6-A,[30] the ARCHITECT HE4 assay demonstrated linearity from 20.0 to 1500.0 pmol/L.

Sensitivity (LoB, LoD, LoQ)

The ARCHITECT HE4 assay is designed to have a Limit of Detection (LoD) of ≤ 15 pmol/L and a Limit of Quantitation (LoQ) of ≤ 20 pmol/L. The LoQ is defined as the lowest amount of analyte in a sample that can be accurately quantitated with a total allowable error of ≤ 30%.

A study was performed based on guidance from the NCCLS document EP17-A[31] with one zero-level HE4 sample and four low-level HE4 samples. The samples were tested in a minimum of five replicates once per day for three different days using three reagent lots and four instruments. The Limit of Blank (LoB) was 0.2 pmol/L, the LoD was 0.3 pmol/L, and the LoQ was 1.4 pmol/L.

Specificity

The tumor markers listed below were evaluated for cross-reactivity with the ARCHITECT HE4 assay. The tumor markers were prepared with ARCHITECT HE4 Calibrator A to achieve the concentrations indicated below. Reactivities greater than 15 pmol/L HE4 (LoD) were not observed.

Tumor Marker	Tumor Marker Concentration
CA 125, CA 15-3, and CA 19-9	up to 3500 U/mL
Carcinoembryonic Antigen (CEA)	up to 1000 µg/L
α-Fetoprotein (AFP)	up to 400 µg/L

Autodilution Verification

The ARCHITECT HE4 automated dilution method is designed to have an agreement of 100 ± 15% for each sample and a mean agreement of 100 ± 10% versus a manual dilution method when performed on samples with values > 1100 pmol/L.

The ARCHITECT HE4 assay was evaluated with the 1:10 autodilution method versus a 1:10 manual dilution method using human serum samples. Two replicates each of the autodiluted and manually diluted samples were assayed on three instruments using the ARCHITECT HE4 assay. The mean agreement by instrument was 101%, 99%, and 93% for instruments 1, 2, and 3, respectively. The data are summarized in the following table.

Sample	Instrument	Mean Manually Diluted Value (pmol/L)	Mean Automated Diluted Value (pmol/L)	% Agreement
1	1	1497	1521	102
	2	1421	1419	100
	3	1262	1225	97
2	1	1346	1408	105
	2	1305	1322	101
	3	1372	1202	88
3	1	1403	1469	105
	2	1382	1326	96
	3	1289	1173	91
4	1	1562	1499	96
	2	1485	1424	96
	3	1407	1277	91
5	1	1468	1516	103
	2	1460	1464	100
	3	1399	1331	95
6	1	1543	1491	97
	2	1488	1457	98
	3	1387	1314	95

$$\% \text{ Agreement} = \frac{\text{Mean Automated Diluted Value (pmol/L)}}{\text{Mean Manually Diluted Value (pmol/L)}} \times 100$$

Interference

The ARCHITECT HE4 assay is designed to have an individual recovery of 100 ± 15% and a mean recovery of 100 ± 10% when comparing potential interferents to the control results. The interference studies were performed based on guidance from the Clinical and Laboratory Standards Institute (CLSI) document EP7-A2.[32]

Potentially Interfering Therapeutic Agents and Endogenous Substances

Recovery studies were performed to compare sera containing the following therapeutic agents and endogenous substances at the indicated interferent concentrations with control sera. The data are summarized in the following tables.

Therapeutic Agent	Interferent Concentration	% Recovery Range	Mean % Recovery
Carboplatin	500 µg/mL	99 - 105	102
Cisplatin	165 µg/mL	97 - 98	98
Clotrimazole	0.3 µg/mL	99 - 103	101
Cyclophosphamide	500 µg/mL	101 - 106	104
Dexamethasone	10 µg/mL	97 - 105	101
Doxorubicin	1.16 µg/mL	100 - 105	103
Leucovorin	2.68 µg/mL	102 - 105	104
Melphalan	2.8 µg/mL	98 - 104	101
Methotrexate	45 µg/mL	96 - 100	98
Paclitaxel	3.5 ng/mL	101 - 102	102

Endogenous Substance	Interferent Concentration	% Recovery Range	Mean % Recovery
Bilirubin	20 mg/dL	91 - 102	98
Hemoglobin	500 mg/dL	104 - 113	108
Low Protein	3 g/dL	95 - 110	104
High Protein	12 g/dL	98 - 102	100
Triglycerides	3000 mg/dL	96 - 102	98

$$\% \text{ Recovery} = \frac{\text{HE4 Concentration (pmol/L) with Potential Interferent}}{\text{HE4 Concentration (pmol/L) in Control}} \times 100$$

Potentially Interfering Clinical Conditions

Six specimens positive for HAMA and six specimens positive for Rheumatoid Factor (RF) were evaluated at the indicated interferent concentration ranges. The data are summarized in the following table.

Clinical Condition	Interferent Concentration Range	% Recovery Range	Mean % Recovery
HAMA	54 - 327 ng/mL	93 - 114	102
RF	21 - 445 IU/mL	98 - 111	103

$$\% \text{ Recovery} = \frac{\text{Observed HE4 Concentration (pmol/L)}}{\text{Expected HE4 Concentration (pmol/L)}} \times 100$$

High Dose Hook

High dose hook is a phenomenon whereby very high level specimens may read within the dynamic range of the assay. For the ARCHITECT HE4 assay, no high dose hook effect was observed when samples up to approximately 94,000 pmol/L of HE4 antigen were assayed.

Method Comparison

The ARCHITECT HE4 assay is designed to have a slope of 1.0 ± 0.1 and a correlation coefficient (r) of ≥ 0.90 for serum specimens when compared to a commercially available EIA HE4. One hundred and ninety-three serum specimens were tested using the ARCHITECT HE4 assay and the EIA. The data are summarized in the following table.

ARCHITECT HE4 vs. EIA HE4

Regression Method	n	Slope (95% CI[a])	Intercept (95% CI[a])	Correlation Coefficient
Passing-Bablok[b]	193	0.96 (0.93 to 1.00)	-2.51 (-3.96 to -0.73)	0.97

ARCHITECT HE4 Specimen Range = 20.3 to 918.7 pmol/L
EIA HE4 Specimen Range = 21.9 to 762.3 pmol/L

[a] CI = Confidence Interval

[b] A linear regression method with no special assumptions regarding the distribution of the samples and measurement errors.[33]

BIBLIOGRAPHY

1. Bouchard D, Morisset D, Bourbonnais Y, *et al*. Proteins with whey-acidic-protein motifs and cancer. *Lancet Oncol* 2006;7:167-74.
2. Drapkin R, von Horsten HH, Lin Y, *et al*. Human epididymis protein 4 (HE4) is a secreted glycoprotein that is overexpressed by serous and endometrioid ovarian carcinomas. *Cancer Res* 2005;65(6):2162-9.
3. Bingle L, Singleton V, Bingle CD. The putative ovarian tumour marker gene HE4 (WFDC2), is expressed in normal tissues and undergoes complex alternative splicing to yield multiple protein isoforms. *Oncogene* 2002;21:2768-73.
4. Kirchhoff C, Habben I, Ivell R, *et al*. A major human epididymis-specific cDNA encodes a protein with sequence homology to extracellular proteinase inhibitors. *Biol Reprod* 1991;45:350-7.
5. Kirchhoff C. Molecular characterization of epididymal proteins. *Rev Reprod* 1998;3:86-95.
6. Galgano MT, Hampton GM, Frierson HF, Jr. Comprehensive analysis of HE4 expression in normal and malignant human tissues. *Mod Pathol* 2006;19:847-53.
7. Hough CD, Sherman-Baust CA, Pizer ES, *et al*. Large-scale serial analysis of gene expression reveals genes differentially expressed in ovarian cancer. *Cancer Res* 2000;60:6281-7.
8. Schummer M, Ng WV, Bumgarner RE, *et al*. Comparative hybridization of an array of 21 500 ovarian cDNAs for the discovery of genes overexpressed in ovarian carcinomas. *Gene* 1999;238:375-85.

9. Gilks CB, Vanderhyden BC, Zhu, S, *et al*. Distinction between serous tumors of low malignant potential and serous carcinomas based on global mRNA expression profiling. *Gynecol Oncol* 2005;96:684-94.
10. Hellström I, Raycraft J, Hayden-Ledbetter M, *et al*. The HE4 (WFDC2) protein is a biomarker for ovarian carcinoma. *Cancer Res* 2003;63: 3695-700.
11. Moore RG, Brown AK, Miller MC, *et al.* The use of multiple novel tumor biomarkers for the detection of ovarian carcinoma in patients with a pelvic mass. *Gynecol Oncol* 2008;108:402-8.
12. Bray F, Loos AH, Tognazzo S, *et al*. Ovarian cancer in Europe: Cross-sectional trends in incidence and mortality in 28 countries, 1953-2000. *Int J Cancer* 2005;113:977-90.
13. National Institutes of Health Consensus Development Conference Statement. Ovarian cancer: screening, treatment and follow-up. *Gynecol Oncol* 1994;55:S4-14.
14. ACOG Practice Bulletin No. 83. Clinical Management Guidelines for Obstetrician-Gynecologists. Management of adnexal masses. *Obstet Gynecol* 2007;110(1):201-14.
15. Finkler NJ, Benacerraf B, Lavin PT, *et al*. Comparison of serum CA 125, clinical impression, and ultrasound in the preoperative evaluation of ovarian masses. *Obstet Gynecol* 1988;72(4):659-64.
16. Maggino T, Gadducci A, D'Addario V, *et al*. Prospective multicenter study on CA 125 in postmenopausal pelvic masses. *Gynecol Oncol* 1994:54;117-23.
17. Roman LD, Muderspach LI, Stein SM, *et al.* Pelvic examination, tumor marker level, and gray-scale and doppler sonography in the prediction of pelvic cancer. *Obstet Gynecol* 1997:89(4);493-500.
18. DePriest PD, Shenson D, Fried A, *et al*. A morphology index based on sonographic findings in ovarian cancer. *Gynecol Oncol* 1993;51:7-11.
19. Moore RG, McMeekin DS, Brown AK, *et al.* A novel multiple marker bioassay utilizing HE4 and CA125 for the prediction of ovarian cancer in patients with a pelvic mass. *Gynecol Oncol* 2009;112:40-6.
20. Food and Drug Administration Website. *510(k) Substantial Equivalence Determination Decision Summary for K072939* (Fujirebio Diagnostics, Inc. HE4 EIA Kit). http://www.accessdata.fda.gov/cdrh_docs/reviews/K072939.pdf. Accessed October 06, 2009.
21. Allard J, Somers E, Theil R, *et al.* Use of a novel biomarker HE4 for monitoring of patients with epithelial ovarian cancer. *J Clin Oncol* 2008 ASCO Annual Meeting Proceedings 2008;26(15S May 20 Supplement):5535.
22. US Department of Labor, Occupational Safety and Health Administration, 29 CFR Part 1910.1030, Bloodborne pathogens.
23. US Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories*. 5th ed. Washington, DC: US Government Printing Office; January 2007.
24. World Health Organization. *Laboratory Biosafety Manual.* 3rd ed. Geneva: World Health Organization; 2004.
25. Clinical and Laboratory Standards Institute. *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline-Third Edition*. CLSI Document M29-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2005.
26. Primus FJ, Kelley EA, Hansen HJ, *et al*. "Sandwich" type immunoassay of carcinoembryonic antigen in patients receiving murine monoclonal antibodies for diagnosis and therapy. *Clin Chem* 1988;34(2):261-4.
27. Schroff RW, Foon KA, Beatty SM, *et al*. Human anti-murine immunoglobulin responses in patients receiving monoclonal antibody therapy. *Cancer Res* 1985;45:879-85.
28. Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. *Clin Chem* 1988;34(1):27-33.
29. National Committee for Clinical Laboratory Standards. *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition*. NCCLS Document EP5-A2. Wayne, PA: NCCLS, 2004.
30. National Committee for Clinical Laboratory Standards. *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline.* NCCLS document EP6-A. Wayne, PA: NCCLS, 2003.
31. National Committee for Clinical Laboratory Standards. *Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline*. NCCLS Document EP17-A. Wayne, PA: NCCLS, 2004.
32. Clinical and Laboratory Standards Institute. *Interference Testing in Clinical Chemistry; Approved Guideline-Second Edition*. CLSI Document EP7-A2. Wayne, PA: CLSI, 2005.
33. Passing H, Bablok W. A new biometrical procedure for testing the equality of measurements from two different analytical methods. *J Clin Chem Clin Biochem* 1983;21:709-720.

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