FOR INFORMATION ONLY. WHEN PERFORMING THE ASSAY ALWAYS REFER TO PACKAGE INSERT SUPPLIED WITH THE KIT

ROMA™

(HE4 EIA + ARCHITECT CA125 II™)

Prod. No. 404-10US

Instructions for use. 2011-09

PRECAUTION: ROMA (HE4 EIA + ARCHITECT CA 125 II) should not be used without an independent clinical /radiological evaluation and is **not** intended to be a screening test or to determine whether a patient should proceed to surgery. Incorrect use of ROMA (HE4 EIA + ARCHITECT CA 125 II) carries the risk of unnecessary testing, surgery, and/or delayed diagnosis.

INTENDED USE

For In Vitro Diagnostic Use Only.
The Risk of Ovarian Malignancy Algorithm (ROMA™) is a qualitative serum test that combines the results of HE4 EIA, ARCHITECT CA 125 II™ and menopausal status into a numerical score.

ROMA is intended to aid in assessing whether a premenopausal or postmenopausal woman who presents with an ovarian adnexal mass is at high or low likelihood of finding malignancy on surgery. ROMA is indicated for women who meet the following criteria: over age 18; ovarian adnexal mass present for which surgery is planned, and not yet referred to an oncologist. ROMA must be interpreted in conjunction with an independent clinical and radiological assessment. The test is not intended as a screening or stand-alone diagnostic assay.

SUMMARY AND EXPLANATION OF THE ASSAY

The Risk of Ovarian Malignancy Algorithm (ROMA™) is a qualitative serum test combining the results of HE4 EIA, ARCHITECT CA 125 II™ and menopausal status into a numerical score. ROMA was developed in a training set using separate logistic regression equations for premenopausal and postmenopausal women:

Premenopausal woman:

Predictive Index (PI) = -12.0 + 2.38*LN[HE4] + 0.0626*LN[CA 125]

Postmenopausal woman:

Predictive Index (PI) = -8.09 + 1.04*LN[HE4] + 0.732*LN[CA 125]

ROMA = exp(PI) / [1 + exp(PI)] *10

ROMA is used to stratify women into likelihood groups for finding cancer on surgery. In order to provide a specificity level of 75%, a cut point of ≥ 1.31 was used for premenopausal women and ≥ 2.77 was used for postmenopausal women who present with an ovarian adnexal mass. Women with ROMA results above these cut points is at high likelihood of finding malignancy on surgery.

The HE4 EIA is an enzyme immunometric assay for the quantitative determination of human epididymis protein 4 (HE4) in human serum. ROMA can only be used with the HE4 EIA assay value obtained from the manual HE4 EIA method from Fujirebio Diagnostics.

The ARCHITECT CA 125 II assay is a chemiluminescent Microparticle immunoassay (CMIA) for the quantitative determination of OC 125 defined antigen in human serum and plasma on the ARCHITECT i System. ROMA can only be used with the ARCHITECT CA125 II assay value obtained from ARCHITECT *i*2000SR.

HE4 and CA125 are detected in elevated concentrations in serum from women with ovarian cancer. 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% (1). The authors concluded that the sensitivity of the HE4 is comparable to that of CA125, but that HE4 is less frequently elevated in women with nonmalignant disease. In a study by Moore et al., evaluating nine known biomarkers for ovarian cancer, HE4 showed the highest sensitivity for the detection of ovarian cancer, particularly in early stage disease. 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% (2). A study by Montagnana et al. assessed serum levels of both HE4 and CA125 in healthy controls and in patients diagnosed with a malignant pelvic mass and revealed that HE4 had a significantly higher area under the curve than CA125 (0.99 vs. 0.91) with a sensitivity and specificity of 98 and 100%, respectively (3). Huhtinen et al. reported that serum concentration of HE4 was significantly higher in patients with endometrial and ovarian cancer than in patients with ovarian endometriomas or other types of endometriosis (4). A study by Montagnana et al. confirmed that HE4 has a significantly higher area under the curve compared to

CA125 for differentiating ovarian cancer from ovarian endometriomas (5). These studies suggest that HE4 is valuable for discriminating ovarian cancers from benign ovarian masses. Several studies have indicated that using HE4 alone or including HE4 in multivariate analysis of ovarian cancer likelihood may improve the accuracy for detection of ovarian cancer at an earlier stage (3, 6-12).

Ovarian cancer is the fourth most common cause of cancer-related death in women worldwide. In the United States, annual incidence is about 25,000 with an annual mortality of 14,000 (13). The symptoms of ovarian cancer are related to the presence of adenxal 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 likelihood for ovarian malignancy, in order to ensure proper management (14). Since the initial report in 1988, clinical impression, serum CA125 and ultrasound along with CT scan, MRI and CT/PET have been the standards in the determination of whether an adnexal mass is suspicious for malignancy (15). Although the literature is replete with papers describing which modality is the more accurate, the combination of physical examination, CA125 and imaging affords the highest positive predictive value (16-18).

To improve the management of patients presenting with adnexal mass, the results of HE4 EIA may be used in conjunction with the results of ARCHITECT CA 125 II as an aid in assessing the likelihood of finding malignancy on surgery in premenopausal and postmenopausal women presenting with an adnexal mass. An additional use of the HE4 EIA is as an aid in monitoring recurrence or progressive disease in patients with epithelial ovarian cancer (8, 19). The results should be used in conjunction with other clinical methods used for monitoring ovarian cancer.

PRINCIPLE OF THE TEST

The HE4 EIA is an enzyme immunometric assay for the quantitative determination of HE4 in human serum. The HE4 EIA is a solid-phase, non-competitive immunoassay based upon the direct sandwich technique using two mouse monoclonal antibodies, 2H5 and 3D8, directed against two epitopes in the C-WFDC domain of HE4. Calibrators, controls and patient samples are incubated together with biotinylated Anti-HE4 monoclonal antibody (MAb) 2H5 in streptavidin coated microstrips. HE4 present in calibrators or samples is adsorbed to the streptavidin coated microstrips by the biotinylated Anti-HE4 MAb during the incubation. The strips are then washed and incubated with HRP labeled Anti-HE4 MAb 3D8. After washing, buffered Substrate/Chromogen reagent (hydrogen peroxide and 3, 3', 5, 5' tetra-methyl-benzidine) is added to each well and the enzyme reaction is allowed to proceed. During the enzyme reaction a blue color will develop if antigen is present. The intensity of the color is proportionate to the amount of HE4 present in the samples. The color intensity is determined in a microplate spectrophotometer at 620 nm (or optionally at 405 nm after addition of Stop Solution).

The ARCHITECT CA 125 II assay is a chemiluminescent Microparticle immunoassay (CMIA) for the quantitative determination of OC 125 defined antigen in human serum and plasma on the ARCHITECT *i*2000SR System[1]. The ARCHITECT CA 125 II assay is a two-step immunoassay to determine the presence of OC 125 defined antigen in human serum and plasma, using CMIA technology with flexible assay protocols, referred to as Chemiflex. In the first step, sample and OC 125 coated paramagnetic microparticles are combined. OC 125 defined antigen present in the sample binds to the OC 125 coated microparticles. After washing, M11 acridinium-labeled conjugate is added in the second step. Pre-Trigger and Trigger Solutions are then added to the reaction mixture; the resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of OC 125 defined antigen in the sample and the RLUs detected by the ARCHITECT *i*2000SR optical system[1].

[1]ROMA (HE4 EIA +ARCHITECT CA 125 II) has been validated for use on the ARCHITECT i2000SR system for the CA 125 testing used in the ROMA equation. Other ARCHITECT i System platforms have not been validated for ROMA testing.

The Risk of Ovarian Malignancy Algorithm (ROMA™) is a qualitative serum test that combines the results of HE4 EIA, ARCHITECT CA125 II™ and menopausal status into a numerical score. Refer to the *Calculation of Results* section of this package insert.

WARNINGS AND PRECAUTIONS

For In Vitro Diagnostic Use:

- For professional use only
- Follow the instructions in the package inserts for HE4 EIA and ARCHITECT CA 125 II, respectively. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in the package inserts.

HE4 EIA KIT STORAGE AND HANDLING

For detailed instructions on HE4 EIA Kit storage and handling including specific instructions for all kit components, refer to the package insert.

- When stored and handled as directed, the HE4 EIA Kit is stable until the expiration date stated on the label outside of the kit box.
- Do not use the HE4 EIA Kit beyond the expiration date.
- The HE4 EIA Kit must be stored at 2-8°C. Do not freeze. Return to 2-8°C immediately after use.
- The HE4 EIA Kit reagents should be allowed to reach room temperature (20–25°C) prior to use.
- Do not mix identical reagents from kits having different lot numbers.

ARCHITECT CA 125 II KIT STORAGE AND HANDLING

For detailed instructions on ARCHITECT CA 125 II Kit storage and handling including specific instructions for all kit components, refer to the package inserts.

- When stored and handled as directed, ARCHITECT CA 125 II Kits are stable until the expiration date stated on the label outside of the kit box.
- Do not use ARCHITECT CA 125 II Kits beyond the expiration date.
- The ARCHITECT CA 125 II Kits must be stored at 2-8°C and may be used immediately after removal from 2-8°C storage.
- The ARCHITECT CA 125 II Reagent Kit must be stored at 2-8°C in an upright position.
- Do not mix identical reagents from kits having different lot numbers.

SPECIMEN COLLECTION AND HANDLING

ROMA is intended for use with serum (including serum collected in separator tubes (SST)). Plasma and other body fluids have not been validated for use with ROMA. Collect blood by venipuncture and follow the tube manufacturer's processing instructions for collection tubes.

Serum can be stored at 2-8°C for 3 days before being tested. For longer periods store samples at -40°C or colder.

Multiple freeze/thaw cycles of specimens should be avoided. Bring frozen samples to room temperature and mix *thoroughly* by gently inverting multiple times before

analysis. Samples that contain gross particulates should be centrifuged at 10.000 x g for 10 minutes prior to use to eliminate any particulate matter that may have developed from the thawing process.

For further instructions on specimen collection and handling, refer to the current individual package inserts for HE4 EIA and ARCHITECT CA 125 II.

MATERIALS REQUIRED

- 2K45 ARCHITECT CA 125 II Instructions For Use
- 2K45 ARCHITECT CA 125 II Reagent Kit
- 2K45-01 ARCHITECT CA 125 II Calibrators
- 2K45-10 ARCHITECT CA 125 II Controls
- ARCHITECT *i*2000SR System
- 404-10US HE4 EIA Instructions For Use
- 404-10US HE4 EIA Kit
- 404-10US ROMA (HE4 EIA + ARCHITECT CA 125 II) Instructions For Use
- C900-395 ROMA (HE4 EIA + ARCHITECT CA 125 II) Calculator Tool (CD-ROM)
  - Microsoft® Windows 7, Vista® or XP (Service Pack 2)

CALCULATION OF RESULTS

Calculation using the ROMA™ (HE4 EIA + ARCHITECT CA 125 II™) Calculator tool

1. Install the ROMA (HE4 EIA + ARCHITECT CA 125 II) Calculator Tool following "Installation and Troubleshooting" instructions. See "Readme" document on CD.
2. Launch the FDI ROMA Calculator Tool using the desktop icon or through the "Programs" menu.
3. A pop-up window containing "Warnings and Precautions" and "Warranty" information will appear. Read all statements. Once statements are understood, either click on "Do Not Accept" to terminate the Calculator Tool application or "Accept" to continue using the application.
4. If "Accept" is chosen, a second pop-up window appears with a "Warning" for use. Again, read the statement. Once the statement is understood, either click on "Do Not Accept" to terminate the Calculator Tool application or "Accept" to continue using the application.
5. If "Accept" is chosen, the Calculator Tool opens for use.
6. Enter the correct assay data into appropriate data fields. Enter only numerical data. For data entry HELP, hold cursor over the center of the entry field in question. Instructions will appear to the right of the cursor.

a. ARCHITECT CA125 II Value obtained from the ARCHITECT *i*2000SR
NOTE: single assay measurements are used for ARCHITECT CA 125 II Value entry.
b. HE4 EIA Value obtained from the manual HE4 EIA method from Fujirebio Diagnostics.
NOTE: it is recommended that the assay is performed in duplicate. Use the mean of the sample replicates for HE4 EIA Value entry.

If non-numerical data or data out of range is entered, pop-up windows will appear as an alert to correct the associated data entry error.

7. Once data points are correctly entered, click on the "Calculate Likelihood" button in the lower left corner of the Calculator Tool faceplate.
8. Results from calculations will be displayed in the right side of the Calculator Tool faceplate:
   a. Inputs for ARCHITECT CA125 II Value and HE4 EIA Value will be verified and repeated
   b. Premenopausal LIKELIHOOD
   c. Premenopausal ROMA Score
   d. Postmenopausal LIKELIHOOD
   e. Postmenopausal ROMA Score
9. Record the ROMA (HE4 EIA + ARCHITECT CA 125 II) calculated results as needed.
   Results are not saved by the Calculator Tool.
10. To continue using the Calculator Tool, over-write the input values with new data values as instructed in step 6 and continue through steps 7, 8, and 9.
11. To close the ROMA (HE4 EIA + ARCHITECT CA 125 II) Calculator Tool application, click on the white X in the red box in the upper right corner of the faceplate.
12. To re-open and use the ROMA (HE4 EIA + ARCHITECT CA 125 II) Calculator Tool, go to Step 2.

LIMITATIONS OF THE PROCEDURE

The Risk of Ovarian Malignancy Algorithm (ROMA) uses the combination of HE4 EIA and ARCHITECT CA 125 II assay values that depend on the premenopausal or postmenopausal status of a woman. The premenopausal or postmenopausal status must be based on ovarian function determined with information available from clinical evaluation and medical history.

- ROMA cannot be used as absolute evidence for the presence or absence of malignant disease.
- ROMA should not be used as a cancer screening test.
- ROMA has only been evaluated in women who will undergo a surgical intervention and is only intended for use in this population.

- ROMA should not be used without an independent clinical evaluation and is not intended to determine whether a patient should proceed to surgery. A low likelihood ROMA result, in the setting of a positive initial cancer risk assessment, should not preclude oncology referral.
- ROMA has not been validated for the following groups: women previously treated for malignancy, women currently being treated with chemotherapy, pregnant women and women < 18 years of age.

HE4 EIA results should not be used interchangeably with other manufacturers' methods for HE4 determinations in the ROMA calculation. Use only with the HE4 EIA assay value obtained from the manual HE4 EIA method from Fujirebio Diagnostics. ARCHITECT CA 125 II results should not be used interchangeably with other manufacturers' methods for CA 125 determinations in the ROMA calculation. Use only with the ARCHITECT CA125 II assay value obtained from ARCHITECT i2000SR. Values obtained from non-designated methods or instrument platforms may produce incorrect ROMA results.

An error in the calculation of results could lead to inaccurate likelihood of malignancy assessment and improper management of the patient.

Anti-reagent antibodies (human anti-mouse antibody (HAMA) or heterophilic antibodies) in the patient sample may occasionally interfere with the assay, even though specific blocking agents are included in the buffers.

Specimens containing levels of Rheumatoid Factor (RF) above 250 IU/mL may interfere with the ROMA result.

Risk of ovarian malignancy algorithm (ROMA)

Refer to the *Calculation of Results* section of this package insert.

ROMA takes into account the results of HE4 EIA and the results of ARCHITECT CA 125 II as well as the menopausal status of the woman. The ROMA value is used to aid in assessing whether a woman is at high or low likelihood of finding malignancy on surgery.

The effectiveness of ROMA was determined in a prospective, multi-center, blinded clinical trial for premenopausal and postmenopausal women presenting with an adnexal mass requiring surgical intervention.

A total of 461 women were evaluable in the study. For each patient, an initial cancer risk assessment (ICRA) was completed by a non-gynecological oncologist, providing the assessment of the patient's mass as benign (negative) or malignant (positive) based upon the information available to the non-gynecological oncologist during their work-up of the patient. The corresponding histopathology reports were collected after surgery.

Using a preoperatively collected serum sample, ROMA was determined and the patient was stratified into a low or a high likelihood group for finding malignancy on surgery.

The histopathological classifications of the 461 evaluable patients are summarized in the table below:

Histopathological classification	All n=461		Premenopausal n=240		Premenopausal n=221	
	N	%	N	%	N	%
Benign Pathology	375	81.3	220	91.7	155	70.1
Low Malignant Potential (LMP)/ Borderline	18	3.9	7	2.9	11	5.0
Epithelial Ovarian Cancer	48	10.4	9	3.7	39	17.6
Non-Epithelial Ovarian Cancer	2	0.4	0	0.0	2	0.9
Other Gynecological Cancer	10	2.2	3	1.2	7	3.2
Other Cancer	7	1.5	1	0.4	6	2.7
Metastatic Cancer	1	0.2	0	0.0	1	0.5

Use of ROMA for stratification into low likelihood and high likelihood groups for finding malignancy on surgery

The following cut-points were used in order to provide a specificity level of 75%:

Premenopausal women:

ROMA score ≥ 1.31 = High likelihood of finding malignancy

ROMA score < 1.31 = Low likelihood of finding malignancy

Postmenopausal women:

ROMA score ≥ 2.77 = High likelihood of finding malignancy

ROMA score < 2.77 = Low likelihood of finding malignancy

The reported result should include both the premenopausal and postmenopausal likelihood result and associated ROMA score on a scale of 0-10.

The stratification of patients presenting with an adnexal mass into high likelihood of harboring malignant disease (epithelial ovarian cancer (EOC), borderline or low malignant potential (LMP) tumors and other gynecological or non-gynecological cancers) using ROMA results above the cut-point ≥ 1.31 for premenopausal and ≥ 2.77 for postmenopausal women by histopathology is shown in the table below:

	Premenopausal n = 240	**Postmenopausal n = 221**	**All n = 461**
All EOC[1]	9/9 (100%)	36/39 (92.3%)	45/48 (93.8%)
EOC Stage I+II	3/3 (100%)	6/9 (66.7%)	9/12 (75%)
EOC Stage III+IV	5/5 (100%)	29/29 (100%)	34/34 (100%)
LMP Tumors	4/7 (57.1%)	9/11 (81.8%)	13/18 (72.2%)
Other Cancer[2]	2/4 (50%)	11/16 (68.7%)	13/20 (65.0%)
All cancer and LMP Tumors	15/20 (75%)	56/66 (84.8%)	71/86 (82.6%)

[1] 2 EOC patients were unstaged, [2]non-epithelial ovarian cancer, other gynecologic, and non-gynecologic cancers.

The performance of ROMA for stratification into low likelihood and high likelihood groups for premenopausal and postmenopausal women with epithelial ovarian cancer (EOC) only is shown in the table below:

	Premenopausal n=229		Postmenopausal n=194	
	Estimate	**95 % CI**	**Estimate**	**95 % CI**
Sensitivity	100.0% (9/9)	70.1% – 99.2%	92.3% (36/39)	79.7% – 97.2%
Specificity	74.5% (164/220)	68.4% – 79.8%	76.8% (119/155)	69.5% – 82.7%
TP-FP [1]	74.5%	68.7% – 80.4%	69.1%	58.2% – 80.0%
PPV [2]	13.8% (9/65)	7.5% – 24.2%	50.0% (36/72)	38.7% – 61.2%
NPV [3]	100.0% (164/164)	97.7% – 99.9%	97.5% (119/122)	93.0% – 99.1%
Prevalence	3.9% (9/229)		20.1% (39/194)	

[1]TP-FP = True Positive rate – False Positive rate, [2]PPV = Positive Predictive Value, [3]NPV = Negative Predictive Value

Adjunctive use of ROMA with Initial Cancer Risk Assessment (ICRA) for stratification into low likelihood and high likelihood groups of harboring malignancy

The performance for the adjunctive use of ROMA with ICRA (ROMA and/or ICRA being positive for high likelihood of finding malignancy on surgery) was evaluated by calculating sensitivity, specificity, PPV (positive predictive value) and NPV (negative predictive value). Adding ROMA to ICRA produced a statistically significant improvement in the negative predictive value. The NPV for correctly classifying benign patients into the low likelihood group increased from 93.2 to 96.2%, making the adjunctive use of ROMA with ICRA effective in ruling out malignancy.

Total counts for premenopausal and postmenopausal women combined:

Malignancy by Pathology[1]					No Malignancy by Pathology[1]				
		ICRA					ICRA		
		Positive (High Likelihood)	Negative (Low Likelihood)	Total			Positive (High Likelihood)	Negative (Low Likelihood)	Total
ROMA	Positive (High Likelihood)	58	13	71	ROMA	Positive (High Likelihood)	28	64	92
	Negative (Low Likelihood)	5	10	15		Negative (Low Likelihood)	31	252	283
	TOTAL	63	23	86		TOTAL	59	316	375

[1]All malignancies found including EOC, LMP, non-epithelial ovarian cancer, other gynecologic, and non-gynecologic cancers.

Performance for premenopausal and postmenopausal women combined with 95% Confidence Intervals (CI):

	ICRA		ROMA		Adjunctive	
	Estimate	95 % CI	Estimate	95 % CI	Estimate	95 % CI
Sensitivity	73.3%	63.1% – 81.4%	82.6%	73.2% – 89.1%	88.4%	79.9% 93.5%
Specificity	84.3%	80.2% – 87.6%	75.5%	70.9 – 79.5%	67.2%	62.3% 71.8%
TP-FP [1]	57.5%	47.3% – 67.8%	58.0%	48.7% – 67.3%	55.6%	47.1% 64.0%
PPV [2]	51.6%	42.9% – 60.3%	43.6%	36.2% – 51.2%	38.2%	31.7% 45.1%
NPV [3]	93.2%	90.0% – 95.4%	95.0%	91.9% – 96.9%	96.2%	93.1% 97.9%
Prevalence	18.7%					

[1]TP-FP = True Positive rate – False Positive rate
[2]PPV = Positive Predictive Value
[3]NPV = Negative Predictive Value

EXPECTED VALUES

The distribution of ROMA determined in 120 healthy premenopausal women and 120 healthy postmenopausal women is shown in the table below:

	All Healthy Subjects	**Premenopausal Healthy Subjects**	**Postmenopausal Healthy Subjects**
N	240	120	120
ROMA Result			
Mean (SD)	1.19 (0.76)	0.94 (0.75)	1.44 (0.68)
Median	0.98	0.72	1.30
Range (min-max)	0.22-4.58	0.22-4.51	0.39-4.58
Reference Interval (5^{th}–95^{th} percentile)	0.39-2.75	0.33-2.36	0.61-2.75
ROMA Likelihood (n, %)			
High Likelihood	25 (10.4%)	19 (15.8%)	6 (5.0%)
Low Likelihood	215 (89.6%)	101 (84.2%)	114 (95.0%)

In this study, 95% of the premenopausal healthy female subjects had ROMA results equal to or below 2.36 and 95% of the postmenopausal healthy female subjects had ROMA results equal to or below 2.75.

The distribution of ROMA determined in non-ovarian malignancy conditions is shown in the table below:

	Bladder Cancer	**Breast Cancer**	**Endometrial Cancer**	**GI Cancer**	**Lung Cancer**
N	40	40	40	39	40
ROMA					
Mean (SD)	5.45 (3.09)	4.52 (3.07)	5.44 (2.99)	3.56 (2.81)	4.70 (2.45)
Median	5.36	2.88	5.26	2.32	4.60
Range (min-max)	0.38-10.0	0.60-9.93	0.67-9.99	0.55-9.24	0.74-9.63
Reference Interval (5^{th}–95^{th} percentile)	0.77-9.85	1.32-9.88	1.29-9.90	0.97-9.02	0.98-9.14
ROMA Likelihood (n, %)					
High Likelihood	31 (77.5%)	26 (65.0%)	33 (82.5%)	21 (53.8%)	31 (77.5%)
Low Likelihood	9 (22.5%)	14 (35.0%)	7 (17.5%)	18 (46.2%)	9 (22.5%)

The distribution of ROMA determined in benign conditions is shown in the table:

	Benign Gyneco-logical Disease	Other Benign Disease	Conges-tive Heart Failure	Hyperten-sion	Pregnant
N	381	40	40	40	38
ROMA					
Mean (SD)	1.55 (1.20)	2.05 (1.47)	3.09 (1.82)	2.34 (1.72)	1.01 (0.59)
Median	1.16	1.72	2.53	1.84	0.88
Range (min-max)	0.19-8.56	0.15-6.97	0.83-7.93	0.33-8.38	0.28-3.47
Reference Interval (5^{th}–95^{th} percentile)	0.43-3.72	0.54-4.95	1.08-5.95	0.83-5.17	0.34-1.94
ROMA Likelihood (n, %)					
High Likelihood	94 (24.7%)	15 (37.5%)	17 (42.5%)	12 (30.0%)	7 (18.4%)
Low Likelihood	287 (75.3%)	25 (62.5%)	23 (57.5%)	28 (70.0%)	31 (81.6%)

It is recommended that each laboratory establish its own reference value for the population of interest.

PERFORMANCE CHARACTERISTICS

Lot-to-Lot Precision

A study was performed as described per the National Committee for Clinical Laboratory Standards NCCLS (CLSI) guideline EP5-A2 (20). A panel of five serum samples was tested and evaluated using both premenopausal and postmenopausal forms of the ROMA equation, using three lots of HE4 EIA Kits and three lots of ARCHITECT CA 125 II Reagent and Calibrator Kits, evaluating two measurements of each panel, at two separate times per day for 5 days. Data from this study is summarized in the table below.[1]

Sample	Menopausal State	n	Mean ROMA Result	Between Lots (SD)	Between Lots (CV %)	Total (SD)	Total (CV %)
1	Pre	60	0.66	0.023	3.5	0.051	7.7
	Post	60	1.05	0.014	1.3	0.043	4.1
2	Pre	60	1.32	0.023	1.8	0.060	4.5
	Post	60	2.55	0.012	0.5	0.043	1.7
3	Pre	60	2.81	0.000	0.0	0.150	5.2
	Post	60	4.83	0.007	0.1	0.085	1.8
4	Pre	60	1.28	0.025	1.9	0.051	4.0
	Post	60	2.39	0.022	0.9	0.046	1.9
5	Pre	60	8.66	0.000	0.0	0.071	0.8
	Post	60	8.73	0.018	0.2	0.043	0.5

[1]Representative data; results in individual laboratories may vary from these data.

Reproducibility

A study was performed as described per the National Committee for Clinical Laboratory Standards NCCLS (CLSI) guideline EP5-A2 (20). A panel of five serum samples was tested and evaluated using both premenopausal and postmenopausal forms of the ROMA equation, using one lot of HE4 EIA Kits and one lot of ARCHITECT CA 125 II Reagent and Calibrator Kits, at three sites, evaluating two measurements of each panel, at two separate times per day for 6 days. Data from this study is summarized in the table below.[1]

Sample	Menopausal State	n	Mean ROMA Result	Between Sites (SD)	Between Sites (CV %)	Total (SD)	Total (CV %)
1	Pre	72	0.56	0.107	19.0	0.143	25.9
	Post	72	0.96	0.083	8.6	0.107	11.2
2	Pre	72	1.16	0.168	14.6	0.195	16.9
	Post	72	2.39	0.124	5.2	0.153	6.4
3	Pre	72	2.66	0.143	5.4	0.297	11.2
	Post	72	4.75	0.116	2.5	0.186	3.9
4	Pre	72	1.13	0.180	16.0	0.232	20.7
	Post	72	2.25	0.149	6.6	0.183	8.1
5	Pre	72	8.59	0.046	0.5	0.178	2.1
	Post	72	8.72	0.048	0.6	0.086	1.0

[1]Representative data; results in individual laboratories may vary from these data.

Analytical specificity

Studies were performed to compare sera containing the listed substances at the indicated concentrations with control sera to determine if potential interference is observed impacting ROMA. Data from this study is summarized in the table below.[1]

Interferent	Concentration	Percent Difference From Control (%)					
		ROMA (Low)		ROMA (Med)		ROMA (High)	
		PRE[2]	POST[2]	PRE[2]	POST[2]	PRE[2]	POST[2]
Hemoglobin	5 mg/mL	-6.7	-3.4	0.5	0.6	4.0	1.0
Bilirubin (Conjugated)	20 mg/dL	2.3	1.2	-3.9	0.0	-5.5	-1.6
Bilirubin (Unconjugated)	20 mg/dL	2.8	2.3	3.9	1.9	-4.7	-1.2
Protein	12 g/dL	-3.7	-2.0	1.4	-0.8	-0.3	-1.1
Lipid	3 g/dL	8.8	3.2	-4.0	-4.3	-1.0	-1.5
Human Anti-Mouse Antibodies (HAMA)	1000 ng/mL	7.7	9.2	-3.2	-0.9	0.9	-0.3
Rheumatoid Factor (RF)	1000 IU/mL	1.9	28.2[3]	-1.0	6.0	2.7	-0.6
	500 IU/mL	-6.9	12.6[3]	2.6	3.6	-2.5	-1.5
	250 IU/mL	-0.6	-0.6	0.6	0.8	2.6	0.0

[1]Representative data; results in individual laboratories may vary from these data.
[2]Analyzed using both premenopausal and postmenopausal forms of the ROMA equation
[3]Specimens with Rheumatoid factor greater than 250 IU/mL interference with the test by more than 10%

WARRANTY

The performance data presented here were obtained using the assay procedure indicated. Any change or modification of the procedure not recommended by Fujirebio Diagnostics may affect the results, in which event Fujirebio Diagnostics disclaims all warranties expressed, implied or statutory including the implied warranty of merchantability and fitness for use.

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