

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE**

A. 510(k) Number:

k092399

B. Purpose for Submission:

New device

C. Measurand:

Anti-mutated Citrullinated Vimentin IgG

D. Type of Test:

Semi-quantitative and qualitative ELISA

E. Applicant:

ORGENTEC Diagnostika GmbH

F. Proprietary and Established Names:

Anti-mutated Citrullinated Vimentin IgG EIA

G. Regulatory Information:

1. Regulation section
21 CFR§ 866.5775 – Rheumatoid factor immunological test system
2. Classification
Class II
3. Product code
OQZ, Anti-mutated Citrullinated Vimentin IgG
4. Panel
Immunology (82)

H. Intended Use:

1. Intended use(s)
Anti-MCV[®] IgG EIA is an indirect solid phase enzyme immunoassay (ELISA) for the qualitative and semi-quantitative measurement of IgG class autoantibodies against mutated citrullinated vimentin (MCV) in human serum.
2. Indication(s) for use:
The assay is intended for in vitro diagnostic use only as an aid in the diagnosis of Rheumatoid Arthritis in conjunction with other laboratory and clinical findings.
3. Special conditions for use statement(s)
For prescription use only
4. Special instrument requirements
ELISA plate reader at 450 nm

I. Device Description:

Each Anti-MCV[®] EIA Kit contains the following components:

1. Divisible microplate consisting of 12 modules of 8 wells each, coated with mutated citrullinated Vimentin (MCV).
2. Calibrators with Anti-MCV[®] IgG antibodies (A-F) in a serum/buffer matrix (PBS, BSA, NaN₃ <0.1% (w/w)): 0; 20; 40; 100; 300; and 1000 U/mL.
3. Anti-MCV positive and negative controls in a serum/buffer matrix (PBS, BSA,

NaN₃ <0.1% (w/w)) one vial (1.5 mL). Ready to use.

4. Sample buffer (Tris,NaN₃ <0,1% (w/w)),yellow, concentrate (5x). 1 vial, 20 mL.
5. Enzyme conjugate solution (PBS, Proclin 300 <0.5% (v/v)), (light red) containing polyclonal rabbit anti-human IgG; labeled with horseradish peroxidase.
5. TMB substrate solution.
6. Stop solution (contains acid).
7. Wash solution (PBS, NaN₃ <0.1% (w/w)), concentrate (50x).

J. Substantial Equivalence Information:

1. Predicate device name(s):
Eurodiagnostica Immunoscan Anti-CCP (Cyclic Citrullinated Peptide) IgG Antibody Test
2. Predicate 510(k) number(s):
k052133
3. Comparison with predicate:

Similarities		
Item	Predicate	Device
	Immunoscan RA Anti-CCP	ORGENTEC Anti-MCV
Indication for use	Aid in the diagnosis of Rheumatoid Arthritis (RA) in conjunction with other laboratory and clinical findings.	Same
Method	ELISA	Same
Solid phase	Microwells	Same
Controls	Negative control Positive control	Same
Sample matrix	human serum or plasma	Same
Test results	Qualitative and Semi-Quantitative	Same
Storage	2-8°C (35 - 46°F)	Same
Conjugated antibody	Horse Radish Peroxidase (HRP) labeled polyclonal anti-human IgG	Same
Type of substrate	TMB (3,3',5,5'-Tetramethylbenzidine)	Same
Open Vial stability	30 days	Same

Differences		
Item	Predicate	Device
Analyte	Anti-CCP	Anti-MCV
Antigen	Cyclic citrullinated peptide	Mutated citrullinated vimentin
Sample dilution factor	1:100	1:50

Differences		
Item	Predicate	Device
Total incubation time	60 minutes at room temperature (18-28°C)	120 minutes at room temperature (18-25°C)
Cut-off	≥ 25 U/mL	≥ 20 U/mL
Calibrator	Anti-CCP IgG calibrators (25; 50; 200; 800; and 1600 U/mL)	Anti-MCV IgG calibrators (0; 20; 40; 100; 300; and 1000 U/mL)

K. Standard/Guidance Document Referenced (if applicable):

Not applicable

L. Test Principle:

Anti-MCV[®] IgG EIA is an indirect solid phase enzyme immunoassay. Mutated citrullinated vimentin (MCV) is bound to microwells. Antibodies against this antigen, if present in diluted serum or plasma, bind to the respective antigen. Washing of the micro-wells removes unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human IgG immunologically detects the bound patient antibodies forming a conjugate/antibody/antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow color is measured photometrically at 450 nm. The amount of color is directly proportional to the concentration of IgG antibodies present in the original sample.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Statistics for Coefficients of Variation (CV) were calculated for each of seven samples from the results of 16 determinations in a single run for Intra-Assay precision. The set of same 7 specimens were used for the intra- and inter-assay imprecision studies. Run-to-run precision was calculated from the results obtained by one operator for 5 different runs performed on 5 different days with single determinations of each sample. The acceptance criteria are the imprecision should be less than 15%.

Table 1. Intra-assay Precision

Intra-Assay Precision				
Sample	n	Mean [U/mL]	SD	CV [%]
1	20	3.2	0.32	10.2
2	20	21.8	1.47	6.7
3	20	17.3	1.1	6.3
4	16	20.2	1.1	5.3
5	16	111.0	10.2	9.2
6	16	451.6	34.8	7.7
7	16	806.2	73.1	9.1

Table 2. Inter-assay Precision

Inter-Assay Precision			
Sample	Mean [U/mL]	SD	CV [%]
1	5.0	0.76	15.3
2	20.1	2.9	14.2
3	22.7	1.4	6.2
4	27.0	3.7	13.6
5	118.8	7.6	6.4
6	548.1	25.1	4.6
7	981.5	68.7	7.0

Inter-Lot precision was calculated from the results of four different kit lots with single determinations using twelve samples.

Table 3. Inter-lot Precision

Inter-Lot			
Sample	Mean [U/mL]	SD	CV [%]
1	6.9	0.9	13.0
2	17.2	2.5	14.4
3	20.4	1.7	8.4
4	21.9	2.2	10.2
5	27.6	2.0	7.4
6	130.4	17.1	13.1
7	150.3	7.8	5.2
8	255.1	9.4	3.7
9	255.7	27.1	10.6
10	327.0	4.8	1.5
11	652.2	120.3	18.4
12	1043.2	17.3	1.7

Inter-Laboratory precision was calculated for each of twenty-two samples tested in single determination with one lot by three individuals in three different laboratories. Results are shown in the below tables.

Table 4. Inter-laboratory Precision

Inter-Laboratory			
Sample No.	Mean [U/mL]	SD	CV [%]
1	4.6	0.5	11.2
2	6.9	1.0	14.5
3	4.1	0.6	13.3
4	5.5	0.8	14.9
5	19.9	0.6	2.8
6	18.5	0.6	3.1
7	39.5	4.0	10.2
8	37.3	3.8	10.2
9	78.4	3.8	4.8
10	73.3	2.5	3.4
11	141.6	2.9	2.8

Inter-Laboratory			
Sample No.	Mean [U/mL]	SD	CV [%]
12	138.7	3.8	2.7
13	173.8	8.0	4.6
14	171.8	18.4	10.7
15	234.6	17.3	7.4
16	242.4	28.7	11.8
17	222.4	20.9	9.4
18	214.1	17.0	7.9
19	266.6	21.8	8.2
20	245.4	5.6	2.3
21	487.1	11.9	2.4
22	538.3	69.1	12.8

The specification for acceptance is CV = <15%. The specification was met.

b. Linearity/assay reportable range:

Four patient samples containing high levels of antibody were serially diluted 6-9 times in buffer throughout the dynamic range of the assay to demonstrate the upper end of linearity. Specifications are that linear regression coefficient (R²) should be ≥ 0.990 and recovery is 100%±20%. Results met the criteria and were considered linear. The calculated values together with the recovery and the linear regression coefficient (R²) are shown in the table below.

Table 5. Summary of Linearity

	Sample 1	Sample 2	Sample 3	Sample 4
Concentration U/mL	882.8	932.1	727.9	901.3
Regression R ²	0.9963	0.9993	0.9999	0.9971
Average % Recovery	90	102	97	106
Range of % Recovery	81-100	93-109	84-100	93-108

Reportable range

3.2-900 U/mL

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Traceability:

There is no reference standard for anti-MCV. The values for calibrators (A-F) and controls (C1 and C2) are arbitrary units.

Reagent stability

Each kit lot produced by ORGENTEC is tested throughout its complete shelf life. The data presented in the stability studies derives from the stability testing of three random kit lots. The assay kit was stable for 18 months under 2-8°C. The diluted sample buffer and wash buffer are stable for at least 30 days when stored at 2-8°C.

Sample stability

Twelve samples with anti-MCV concentrations ranging from 17.6 to 959.7 U/mL were tested in duplicate to determine stability of storage at 2-8°C for 5 days. Results met acceptance criteria with % recovery ranged from 94% to

100%.

d. *Detection limit:*

i. Limit of Blank (LoB)

Sample Buffer was diluted according to instructions for use and measured 32 times on one plate. Calibrators and Controls were analyzed in duplicate. The detection limit was calculated as the mean of the optical densities (OD) of the Sample Buffer plus 3 SD and expressed in U/mL. Acceptance Criteria: The analytical sensitivity has to be ≤ 1 U/mL. The (mean + 3 SD) was 0.044 OD for the Sample Buffer, which corresponded to an analytical sensitivity of 0.6 U/mL.

ii. Limit of Detection (LoD)

The limit of detection was calculated from LoB and LoQ using the following formula $LoD = \mu B + 1.645 \delta B + 1.645 \delta S$. (Note: B = Blank; S = Lowest Sample).

The $LoD = 0.032OD \text{ Mean of Blank} + 1.645 \times 0.007OD (0.012OD) = 0.044OD = 3.0 \text{ U/mL}$.

iii. Limit of Quantitation (LoQ)

Limit of quantitation was determined from the coefficient of variation of four very low serum samples run in replicates of 16 in three assay runs. The lowest concentration which could be measured with a coefficient of variation below 20% is 3.2 U/mL.

e. *Analytical specificity:*

i. Interference

Interference due to bilirubin, hemoglobin and lipemia was evaluated using a negative serum, a low positive serum and a high positive serum spiked with the respective interfering substance in increasing concentrations. Hemoglobin up to 1000 mg/dL, bilirubin up to 40 mg/dL, and lipemia (i.e. triglyceride concentration) up to 3000 mg/dL in human serum do not interfere with Anti-MCV[®] ELISA results.

ii. Cross reactivity

A series of patient samples from other potentially cross-reacting and similar symptoms to Rheumatoid Arthritis were evaluated for reactivity in the Anti-MCV[®] assay. No positive results were found in these samples. The results of the testing are summarized below.

Table 6. Cross-reactivity

Condition	Total No.	Positives No.	Positives %	Clinical Specificity %
Blood donors	443	4	0.9%	99.1%
Psoriasis Arthritis	10	0	0.0%	100.0%
Juvenile Arthritis	15	0	0.0%	100.0%
Scleroderma	8	0	0.0%	100.0%
Sicca Symptomatic	6	0	0.0%	100.0%
Sjögren Syndrome	50	2	4.0%	96.0%
UCTD	25	1	4.0%	96.0%
MCTD	6	0	0.0%	100.0%
SLE	97	3	3.1%	96.9%
APS	20	0	0.0%	100.0%

Condition	Total No.	Positives No.	Positives %	Clinical Specificity %
Celiac Disease	75	1	1.3%	98.7%
Morbus Crohn	1	0	0.0%	100.0%
Microscopic Polyangiitis	30	2	6.7%	93.3%
Morbus Wegener/Vasculitis	7	0	0.0%	100.0%
Polymyositis/Dermatomyositis	3	0	0.0%	100.0%
Thyroiditis	104	3	2.9%	97.1%
div. Infectious Diseases	25	0	0.0%	100.0%
Diabetes Mellitus	40	2	5.0%	95.0%
Non-RA:	965	18	1.9%	98.1%

f. Assay cut-off:

Values ≥ 20 U/mL are considered positive, values < 20 U/mL are considered negative

2. Comparison studies:

a. Method comparison with predicate device:

Five hundred and fifty five (555) sera with concentrations ranged from Anti MCV IgG 1.5 - 900 U/mL were tested by the ORGENTEC Anti-MCV[®] ELISA in single measurement and by the Immunoscan Anti-CCP IgG ELISA. The quantitative results were calculated from a calibration curve on the basis of tested calibrators. In the ORGENTEC Anti-MCV[®] assay quantitative values above or equal to 20 U/mL were considered positive, values below 20 U/mL were considered negative. In the Immunoscan Anti-CCP IgG assay quantitative values above or equal 25 U/mL were considered positive, values below 25 U/mL were considered negative.

A summary analysis of the results are shown in the following Tables:

Table 7. A Summary of Data Analysis of the Method Comparison

Comparative Method			
Anti-CCP			
ORGENTEC	Positive	Negative	Total
Positive	231	10	241
Negative	9	305	314
Total	240	315	555
Positive Percent Agreement:	96.3%	C.I. (95%) = 93.0 – 98.3%	
Negative Percent Agreement:	96.8%	C.I. (95%) = 94.2 – 98.5%	
Overall Agreement:	96.6%	C.I. (95%) = 94.7 – 97.9%	

b. Matrix comparison:

Use human serum only.

3. Clinical studies:

Patient samples were collected from various types of sites and commercial sources that provided known disease characteristics and sufficient number of samples to cover the range of rheumatological and non-rheumatological conditions to demonstrate the performance of this test. Following is a summary of the groups and sources identified for this study.

Inclusion criteria

- i. Clinically characterized Rheumatoid Arthritis (according to American College of Rheumatology (ACR) criteria) were defined clinically RA-positive
- ii. Disease Control characterized non-RA samples from diseases with clinical symptoms similar to RA, grouped as: rheumatological/SLE/other autoimmune disease/non-inflammatory disease samples. They were defined clinically RA-negative.
- iii. Negative control group
Healthy individuals (blood donors), grouped “normal”. They were defined RA-negative.

Exclusion criteria

- i. Patient with any condition that would prevent participation in the study and completion of the study procedures.
- ii. Patient is not willing to sign an informed consent.

Summary of the Anti-MCV IgG frequency distribution among the study subjects

Table 8. Observed Frequencies of Anti-MCV IgG for Disease Group (U/mL)

	<0.6	0.6 to 3.5	3.6 to 15	16 to 20	21 to 25	31 to 40	41 to 80	81 to 1000	>1000	Totals
Rheumatoid arthritis	1	10	75	9	24	27	66	201	77	490
Rheumatological SLE	1	16	93	7	2	0	1	0	0	120
Other autoimmune	1	4	85	4	3	0	0	0	0	97
Non-inflammatory	4	42	184	6	0	1	3	0	0	240
Normal	0	12	50	1	0	1	1	0	0	65
Totals	1	32	191	9	0	1	0	0	0	234
	8	116	678	36	29	30	71	201	77	1246

a. *Clinical Sensitivity and Clinical specificity*

Studies were performed to evaluate the sensitivity and specificity of the Anti-MCV[®] (ELISA) test when compared to the predicate assay Anti-CCP IgG assay (Immunoscan, Eurodiagnostica) using a mix of clinically diagnosed Rheumatoid Arthritis disease state samples and a presumed normal asymptomatic blood bank population and other arthritic and autoimmune patient samples obtained from hospital labs and autoimmune clinics.

The sensitivity performance of the ORGENTEC Anti-MCV[®] assay was established using four hundred and ninety (n=490) samples from clinically diagnosed patients as having Rheumatoid Arthritis disease that were obtained from a variety of clinical sources (hospitals and autoimmune clinics). The patients collectively included 124 males and 366 females. The age ranged from 19 to 92 years. The specificity performance of the ORGENTEC Anti-MCV[®] assay was established using 234 “presumed normal” sera obtained from blood donor centers age 24 to 82 years, 522 other samples from patients with arthritic and autoimmune disease conditions, obtained from a variety of clinical sources

(hospitals and autoimmune clinics) age 2 to 92 years. The samples collectively included 166 males and 590 females.

Based on clinical diagnosis, three hundred ninety-eight of the 490 Rheumatoid Arthritis disease diagnosed sera were positive in the ORGENTEC Anti-MCV[®] assay and ninety-two tested as negative, thus yielding a clinical diagnostic sensitivity of 81.2% (95% CI 77.8 – 84.7%). This data is consistent with reported data of sensitivities ranging from 69 % to 82 % for commercial Anti-MCV IgG assay for the diagnosis and follow up of RA Disease patients (Table in Egerer et al., Deutsches Ärzteblatt International Dtsch Arztebl Int 2009; 106 (10):159–63).

The specificity of the ORGENTEC Anti-MCV[®] assay was 98% (95% CI 96.7 – 98.9%)

A summary of analyses of the results is shown in the following Tables:

Table 9. Summary of Data Analysis of Clinical Studies

ORGENTEC	Clinical Diagnosis		Total
	Disease	Non-Disease	
Positive	398	15	413
Negative	92	741	833
Total	490	756	1246
Clinical Sensitivity:	81.2%	C.I. (95%) = 77.8 – 84.7%	
Clinical Specificity:	98.0%	C.I. (95%) = 96.7 – 98.9%	
Clinical Agreement:	91.5%	C.I. (95%) = 89.7 – 92.9%	

On a subset of n=399 specimens for which measurement data of the predicate were available, the assay performance data compared to the clinical diagnosis are summarized in the following Table 10:

Table 10. Assay Performance of the Predicate Compared to Clinical Diagnosis

Predicate Device	Clinical Diagnosis		Total
	Disease	Non-Disease	
Positive	239	2	241
Negative	82	76	158
Total	321	78	399
Clinical Sensitivity:	74.5%	C.I. (95%) = 69.7 – 79.2%	
Clinical Specificity:	97.4%	C.I. (95%) = 91.0 – 99.7%	
Clinical Agreement:	78.9%	C.I. (95%) = 74.9 – 82.0%	

b. Other clinical supportive data

None

4. Clinical cut-off:

Same as assay cut-off.

5. Expected values/Reference range:

A series of 209 assumed normal blood donor samples ages 18 to 69 years were collected from various blood banks. These 209 samples were tested in the ORGENTEC Anti-MCV[®] assay to determine a normal range and cut-off for the assay.

The combined mean concentration of Anti-MCV[®] antibodies was 5.6 U/ml. The mean+2SD was 12.8 U/mL, and mean+3SD = 16.5 U/mL.

Based on these results the cut-off was determined to be ≥ 20 U/ml.

At a cut-off of ≥ 20 U/ml, 1 sample was positive for a specificity of 99.5%.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.