



EL-ANCA™

**An enzyme immunoassay for the detection and measurement
of anti-neutrophil cytoplasmic autoantibodies**

**Anti-MPO (Myeloperoxidase)
and
Anti-PR3 (Proteinase 3)**

Instruction Manual

Catalog Nos:

104-114 (EL-ANCA™: anti-MPO; 1-plate kit)

104-115 (EL-ANCA™: anti-PR3; 1-plate kit)

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INTRODUCTION

Names: TheraTest EL-ANCA™: anti-MPO and TheraTest EL-ANCA™: anti-PR3

Intended Use

FOR IN VITRO DIAGNOSTIC USE ONLY

“The EL-ANCA™: anti-MPO and EL-ANCA™: anti-PR3 are intended for use in clinical laboratories as *in vitro* diagnostic tests for the detection and measurement of autoantibodies in human serum directed against human neutrophil myeloperoxidase and proteinase 3, respectively. Measurement of anti-MPO aids in the diagnosis of microscopic polyangiitis. Measurement of anti-PR3 aids in the diagnosis of Wegener’s granulomatosis.

Summary and Explanation of the Tests

On indirect immunofluorescence (IIF) two main patterns generated by anti-neutrophil cytoplasmic antibodies (ANCA) were identified in the serum of patients with various vasculitic syndromes. One pattern was perinuclear (P-ANCA), which was subsequently shown in ELISA tests to be represented mainly by antibodies against myeloperoxidase (MPO-ANCA) and one was cytoplasmic (C-ANCA), mainly represented in ELISA tests by anti-proteinase 3 antibodies (PR3-ANCA).¹ ANCA was first described by IIF in Wegener’s granulomatosis² and subsequently in microscopic polyangiitis, Churg-Strauss syndrome and idiopathic rapidly progressing glomerulonephritis (iRPGN).

Vasculitides are syndromes associated with blood vessel inflammation. The most common is **Wegener’s Granulomatosis** in which characteristic pathologic vascular inflammatory lesions develop at multiple sites: skin, kidneys, lungs, ear, nose, throat, eyes, muscles, joints, central nervous system and gut. The disease may be lethal if not treated and effective treatment consists of cytotoxic drugs and corticosteroids. Another condition is **Microscopic Polyangiitis** characterized most often only by kidney inflammation. Some patients may also develop inflammation in the lungs as well as fever and other general symptoms. Treatment with cytotoxic drugs is required. Overall about 90% of the patients with these two conditions have one of the two ANCA specificities, MPO or PR3.⁴⁻⁶

Churg-Strauss Syndrome is a related but relatively benign condition manifested as asthma, eosinophilia (increased number of blood eosinophilic leucocytes) and vasculitic inflammation in two or more non-pulmonary organs. The idiopathic **Rapidly Progressing Glomerulonephritis** is characterized by vascular inflammation in the kidney.

EL-ANCA™:anti-MPO and EL-ANCA™:anti-PR3 kits are solid phase enzyme immunoassays. Antigen-precoated microplate wells are incubated with Calibrator, Controls, and serum Specimens. The antigens are purified from human neutrophils. During the incubation, relevant antibody present in the test sample binds to the antigen-coated wells. The wells are then washed to remove unbound (irrelevant) antibodies after which the Enzyme Conjugate (horseradish peroxidase labeled goat anti-human IgG) is incubated in the wells. After another wash step of the wells to remove unbound Enzyme Conjugate, Chromogen (substrate) is added and autoantibodies bound to the solid phase of each well are measured using a spectrophotometric plate reader.

REAGENTS SUPPLIED IN THE KIT*

- 1. MPO Specimen Diluent:** 100 mL of Buffer containing stabilizer, preservative, and yellow dye. Ready for use.
- 2. PR3 Specimen Diluent:** 100 mL of Buffer containing stabilizer, preservative, and yellow dye. Ready for use.
- 3. Wash Buffer (10X):** 100 mL of 10X concentrated buffer.
- 4. Chromogen:** 27 mL of Buffered Substrate and Chromogen (3,3',5,5'-tetramethylbenzidine or TMB). Protect from light. Ready for use.
- 5. Stop Reagent:** 27 mL of 2M Phosphoric Acid. Ready for use.

6. **ANCA anti-IgG Conjugate:** 27 mL of Anti-human IgG (Fc γ -specific) conjugated with horseradish peroxidase, preservative, and green dye. Ready for use.
7. **MPO Calibrator**:** 1.8 mL of Human serum with anti-MPO autoantibodies in buffer. Ready for use.
8. **PR3 Calibrator**:** 1.8 mL of Human serum with anti-PR3 autoantibodies in buffer. Ready for use.
9. **Positive Control**:** 0.35 mL of Human serum with anti-MPO and/or anti-PR3 autoantibodies in stabilizing buffer.
10. **Negative Control**:** 0.35 mL of Human serum without anti-MPO or anti-PR3 autoantibodies.
11. **Coated Wells:** 96 Plate wells coated with MPO and/or plate wells coated with PR3. Wells are printed with the name of the antigen to indicate the coated antigen present: MPO or PR3. Ready for use.

*Positive Control(s), Calibrator(s), Specimen Diluent(s), and Coated Wells will differ among the 3 different kits.

**Reagents containing sodium azide

WARNINGS AND PRECAUTIONS

FOR IN VITRO DIAGNOSTIC USE

Reagents Containing Human Source Material. Treat as potentially infectious.

Each serum/plasma donor unit used in the preparation of this product has been tested by an FDA approved method and found non-reactive for the presence of HBsAg, antibody to HCV and antibody to HIV 1/2. While these methods are highly accurate, they do not guarantee that all infected units will be detected. This product may also contain other human source material for which there is no approved test. Because no known test method can offer complete assurance that hepatitis B virus, hepatitis C virus (HCV), Human Immunodeficiency Virus (HIV) or other infectious agents are absent, all products containing human source material should be handled in accordance with good laboratory practices using appropriate precautions as described in the Centers for Disease Control and Prevention/National Institutes of Health "BMBL" publication, "Biosafety in Microbiological and Biomedical Laboratories," 4th ed., 1999, available at the web sites: <http://bmb1.od.nih.gov> and www.cdc.gov/OD/ohs/biosfty/bmb14/bmb1toc.htm.

Stop Reagent (2M Phosphoric Acid)

May cause severe burns on contact with skin. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes.

European Community Hazardous Substance Risk Phrases (Council Directive 88/379/EEC).

R34 – Causes burns. **S26** – In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. **S36/37/39** – Wear suitable protective clothing, gloves and eye/face protection. **S45** – In case of accident or if you feel unwell, seek medical advice immediately (show label where possible).

Chromogen

Irritant! This product contains 3,3',5,5'-tetramethylbenzidine (TMB) ($\leq 0.05\%$), a chromogenic indicator of horseradish peroxidase activity. It has shown neither mutagenic nor carcinogenic effects in laboratory experiments (9).

Hazardous Substance Risk & Safety Phrases:

R36/37/38 – Irritating to eyes, respiratory system, and skin. Avoid inhalation and direct contact.

S24/25 – Avoid contact with skin or eyes.

S26 – In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

S36 – Wear suitable protective clothing.

S51 – Use only in well-ventilated areas.

Reagents Containing Sodium Azide

Calibrators and Controls contain sodium azide which can react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush drain with large quantities of water to prevent azide build-up.

Hazardous Substance Risk & Safety Phrases:

R22 - Harmful if swallowed.

R36/37/38 - Irritating to eyes, respiratory system, and skin. Avoid inhalation and direct contact.

S26 - In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

S28 - After contact with skin, wash immediately with plenty of water.

S36/37/39 - Wear suitable protective clothing, gloves and eye/face protection

S46 - If swallowed, seek medical advice immediately and show this container label.

General Precautions and Information

1. Specimen Diluent*, Wash Buffer, Chromogen, and Stop Reagent from different lots can be interchangeably. All other reagents are test and lot specific and therefore not interchangeable.

*NOTE: MPO Specimen Diluent (Cat. No. 5146) can only be used with MPO materials

AND

PR3 Specimen Diluent (Cat. No. 5147) can only be used with PR3 materials.

2. The Stop Reagent can irritate eyes and mucous membranes.
3. Do not allow Chromogen to come in contact with metal or oxidizing agents.
4. Handle patient sera and kit reagents with appropriate precautions. Do not pipette by mouth.
5. Do not use test components beyond expiration date.
6. Avoid microbial contamination of the reagents. If solutions become turbid, they should not be used.
7. Avoid exposure of reagents to excessive heat or light during storage.
8. Use disposable labware or wash all glass and plastic thoroughly according to standard laboratory practice.

SPECIMEN REQUIREMENTS

Collection and Storage of Serum

A whole blood specimen should be obtained using acceptable medical techniques to avoid hemolysis. Avoid using sera that are lipemic, hemolyzed, and/or icteric. The presence of rheumatoid factor-IgM, -IgG, or -IgA does not affect the results. The blood should be allowed to clot and the serum separated by centrifugation. Whole blood can be stored at 18-25°C for 24 hours before the separation of serum. The test serum should be clear, i.e., without cells. Serum samples may be stored at 2-8°C for up to 28 days prior to testing. Freeze-thawing of sera may result in variable loss of autoantibody activity. Testing of heat-inactivated serum is not recommended.

PROCEDURE

Materials Required but not Provided

In addition to the reagents supplied, the following materials are required:

1. Calibrated micropipettors with disposable plastic tips, which deliver 10 µL and 100 µL.

2. Calibrated adjustable multichannel pipettors.
3. Pipette tips.
4. Deionized or distilled water.
5. Test tubes (12 x 75 mm) or 1.2 mL minitubes.
6. Timer.
7. Pipettes (1-mL, 5-mL, and 10-mL).
8. Pipette reagent reservoirs.
9. Single (450 nm) or dual (450 nm test, 620-690 nm reference) wavelength spectrophotometer (ELISA reader) for 96-well microtiter plates.
10. Clean wash bottle, vacuum aspiration system or automated plate washer.

Reagent Preparation

1. **Coated Wells:** Testing could be done for one or both antigens, MPO and PR3; suggested plate arrangements of wells in the plate are shown on the Data Sheet accompanying the kit. The entire strip of wells may be employed or individual wells may be used as desired.
2. **1X Wash Buffer:** Wash Buffer (10X) must be diluted 1:10 with deionized or distilled water prior to use. Prepare 1X Wash Buffer by pouring the contents of the bottle labeled 10X Wash Buffer into a clean one-liter volumetric container. Rinse the bottle with deionized or distilled water to remove residual buffer and redissolve any existing crystals (VERY IMPORTANT). Add the rinse to the one-liter container. Add deionized or distilled water until a total volume of 1.0 liter is reached; mix thoroughly. Diluted Wash Buffer is stable for 8 weeks at 2-8°C.
3. **Specimens, Positive Control and Negative Control:** Specimens and Controls must be diluted 1:101 prior to use. Pipette 10 µL of the appropriate sample into 1 mL of Specimen Diluent. Unused diluted sample is discarded when the assay is completed. The range of acceptable values for each Control is reported on the Lot Number-specific Data Sheet accompanying each kit.

Assay Procedure

1. Allow all reagents and patient sera to equilibrate to room temperature (18-25°C). Upon removal from 2-8°C storage, the plate should equilibrate to room temperature in its sealed foil pouch to protect wells from condensation.
2. Dispense 1 mL of Specimen Diluent in each dilution tube.
3. Dilute Controls and Patient Specimens 1:101 in the Specimen Diluent and mix until homogeneous without foaming (e.g., add 10 µL of appropriate specimen to 1 mL of diluent).
4. Select the appropriate plate format and determine the correct number of strips (see layout on the Data Sheet accompanying the kit). Remove the appropriate number of strips to be used and label strip tabs to avoid confusion. Place the remaining strips in the foil pouch with the desiccant, reseal pouch and return it to refrigerator for future use.
5. Load wells: Add Specimen Diluent (Blank), Calibrator(s), and diluted Controls and Patient Specimens to appropriate wells; use a multichannel pipettor to facilitate transfer of diluted samples.
6. Incubate the wells for 30-35 minutes at room temperature (18-25°C).
7. Discard well contents by decanting or aspirating with a vacuum device.
8. Wash plate wells 3X as follows. Fill all wells with 1X Wash Buffer (300 µL per well). Empty wells by shaking plate over a disposal container or aspirate well contents. Repeat two more times for a total of three washes. Remove all residual liquid from the wells by inverting and blotting the plate on absorbent paper.

9. Immediately pipette 100 μ L of IgG Enzyme Conjugate into each well. Use a multichannel pipettor for this step.
10. Incubate plate for 30-35 minutes at room temperature (18-25°C).
11. Discard Tracer (Enzyme Conjugate) from wells and wash plate 3X as described in Step 8.
12. Immediately pipette 100 μ L of Chromogen into each of the wells (use a multichannel pipettor). Incubate plate at room temperature (18-25°C) for 15 \pm 1 minutes.
13. Pipette 100 μ L of Stop Reagent into each of the wells (multichannel pipettor) and mix by gently tapping the side of the plate. The color will change from blue to yellow.
14. Measure the absorbance of each well at 450 nm. Absorbance values should be measured within 30 minutes of completing the assay. If a dual wavelength ELISA reader is used, set the test wavelength at 450 nm with the reference between 620 and 690 nm.

Procedural Comments

1. Storage of wells

The foil pouch must be cut at one end so that it may be resealed. Wells that are not used during the assay should be promptly resealed in the foil pouch with desiccant and stored at 2-8°C.

2. Washing

Each column of wells may be washed using a multichannel pipettor or a repetitive pipettor. The well contents may be aspirated or shaken into a disposal container. Alternatively, commercial semi-automated washing systems may be used. When using either washing technique, the wells should be blotted thoroughly on absorbent paper after the last wash.

Attention: *Incomplete washing of wells may lead to decreased precision.*

3. Pipetting

To avoid cross-contamination and sample carryover, the Positive Control, Negative Control, Calibrators and Test Specimens MUST be pipetted using separate pipette tips. When testing multiple specimens, a calibrated multichannel pipettor should be used to pipette the IgG Enzyme Conjugate, Wash Buffer, Chromogen and Stop Reagent. To avoid false positive results, it is strongly recommended to use aerosol-barrier tips for dispensing Chromogen.

4. Measurement of Absorbance Values

Absorbance values should be measured within 30 minutes after completion of assay. If the absorbance value exceeds the limit of detection for the instrument, an approximate value may be obtained by one of two methods:

a. Dilute the end product (developed well) with deionized or distilled water to bring the absorbance value within the capacity of the reader. Multiply the measured value by the dilution factor. LIMITATION: Patient antibody levels may exceed the available antigenic sites of the well.

b. Repeat the assay testing the Specimen at a 1:1010 dilution, or greater, in Specimen Diluent. Multiply the measured value by the additional dilution factor beyond 1:101 (i.e., value \times 10 for 1:1010 dilution). LIMITATION: Approximation of measurement is subject to the intrinsic nonlinearity of (diluted) serum antibodies.

QUALITY CONTROL

1. Analysis of Diluent Blank

If the OD₅₄₀ value of the Diluent Blank exceeds 0.2, the assay should be repeated. If a more definitive explanation is needed, please contact the manufacturer.

2. Analysis of Positive and Negative Controls

A Positive and Negative Control should be run for each analyte being tested in every assay. The Positive and Negative Control values should fall within the ranges provided on the lot-specific Data Sheet accompanying the kit. If the values are not in agreement with those on the Data Sheet, the assay is not valid and the results should not be reported.

RESULTS

Calculation of Results

1. Determination of net absorbance values

The net absorbance value for each sample well is calculated by subtracting the absorbance value of the Diluent Blank well from the absorbance value of each of the sample wells, i.e., Calibrator wells, Control wells and Specimen wells.

EXAMPLE:

Absorbance for Diluent Blank well = 0.050

Absorbance for specimen (Specimen #1) = 1.100

Net absorbance for Specimen #1 = $1.100 - 0.050 = 1.050$

2. Calculation of autoantibody Unit activity (Units / mL)

Autoantibody activity is calculated as follows:

$$\text{Conversion Factor (CF)} = \frac{\text{Autoantibody Units of Calibrator}}{\text{Absorbance value of Calibrator}}$$

$$\text{Autoantibody Units of Specimen (U/mL)} = \text{CF} \times \text{Net Abs value of Specimen}$$

3. The autoantibody Unit value is unique for each Calibrator; the autoantibody Units for the Calibrator are reported on the lot specific Data Sheet accompanying the kit. The Conversion Factor for the Calibrator must be calculated each time the Calibrator is used for testing. Calculation of data can be facilitated by the use of computer software. However, when using software, check first that manual and computer calculations yield the same results.

Limitations of the Procedure

1. The Positive Control and the Calibrator for a specific autoantibody may contain other autoantibodies, i.e., these reagents may not be monospecific.
2. The EL-ANCA™ should not be performed on microbially contaminated samples. This method has been tested using serum samples only. The performance using other types of specimens has not been determined.
3. Diagnosis should not be made solely on the basis of a positive test result. The data provided by the patient history and other clinical information available to the physician is essential in the diagnosis of autoimmune disease.
4. Disease activity cannot be established based on the level of autoantibody activity obtained; the levels vary widely between patients.

EXPECTED VALUES

The results should be considered normal and abnormal (positive) as follows

Test	Percentile	Normal	Equivocal	Abnormal
Anti-MPO	99-100	≤ 20 U/mL	21-25 U/mL	> 25 U/mL
Anti-PR3	99-100	≤ 10 U/mL	11-20 U/mL	> 20 U/mL

Note: Normal values for special populations should be validated by the individual laboratory.

From data on 37 specimens expected to have anti-MPO, the relative sensitivity for EL-ANCA™:anti-MPO was 100% ([91,100], for 95% C.I.) and the range of positive values was between 30 U/mL and 386 U/mL. Anti-MPO and anti-PR3 autoantibodies are rarely found in healthy individuals (125 blood bank donors tested), specificity of 100% ([97,100] for 95% C.I.), and in patients with other autoimmune diseases (75 tested), specificity 93% ([85,98] for 95% C.I.) (Table 1).

Out of 43 specimens expected to have anti-PR3, the relative sensitivity of EL-ANCA™: PR3 was 100% ([92,100] for 95 % C.I.) and the range of positive values was from 23 U/mL to 608 U/mL. Based on 125 blood bank donors the specificity was 100% ([91,100] for 95% C.I.); based on 75 patients with other collagen vascular diseases the specificity was 99% ([93,100] for 95 % C.I.) (Table 1).

The anti-MPO and anti-PR3 antibodies are rarely found together in the same patient. Indeed, out of 37 specimens positive by EL-ANCA™:anti-MPO, 4 were equivocal by EL-ANCA™:anti-PR3, but none fell into the abnormal range. Also out of 43 specimens positive by EL-ANCA™:anti-PR3, only 2 were positive by EL-ANCA™:anti-MPO (Table 1).

Table 1. Relative sensitivity and specificity for EL-MPO-ANCA and EL-PR3-ANCA

Group	No.	MPO Pos. [†]	MPO Equiv. [†]	PR3 Pos.	PR3 Equiv.
Expected* MPO positive	37	37	0	0	4
Expected* PR3 positive	43	2**	0	43	0
Blood Bank Donors	125	0	0	0	0
Seropositive RA	25	0	0	0	0
SLE	25	4	1	1	2
Scleroderma	25	1	0	0	0

[†]Pos. = Positive; Equiv. = Equivocal

* Expected based on a test already marketed for *in vitro* diagnostic use

** Also expected to be positive

When the results are equivocal, it is recommended that they be reported as equivocal and that the test be repeated at a later date on a different bleeding.

GUIDE TO INTERPRETATION

A published multicenter study on the basis of a standardized ELISA for detection of anti-MPO and anti-PR3 antibodies on 169 newly diagnosed and 189 historical patients with idiopathic

systemic vasculitis or iRPGN showed the following sensitivity and specificity³ for MPO-ANCA and PR3-ANCA. Results were compared with those of 184 disease and 740 healthy controls. The sensitivity in WG was: anti-PR3 66%, anti-MPO 24% (total about 90%). In microscopic polyangiitis (MPA) the sensitivity was anti-PR3 26%, anti-MPO 58% (84% total). Sensitivity in iRPGN was: anti-PR3 50%, anti-MPO 64% (total about 84%). The specificity of assays (related to disease controls) was: anti-PR3, 87%; anti-MPO, 91%. Therefore it is expected that about 10-15% of patients with vasculitides may not have ANCA by ELISA. This is also true for IIF.^{3,6} Thus, the tests are helpful in diagnosis when they are positive, but absence of ANCA does not exclude the presence of a vasculitic syndrome.

MPO-ANCA and PR3-ANCA have also been described in a variety of other inflammatory conditions, but at frequencies much less than that seen in Wegener's or MPA. These other inflammatory conditions include inflammatory bowel disease, post-streptococcal and other forms of glomerulonephritis or they may be drug induced by hydralazine, procainamide, penicillamine, or phenytoin.^{4,7,8} Other inflammatory conditions have to be considered in the differential diagnosis.

PERFORMANCE DATA

Antigen Specificity. The antigen specificity was determined by antigenic competition with MPO and PR3 on MPO- and on PR3-coated wells. On qualified specimens there was 95% inhibition of anti-MPO binding to solid-phase MPO by free MPO and 3% inhibition by free PR3; there was 55% inhibition of anti-PR3 binding to solid-phase PR3 by free PR3 and 1% inhibition by free MPO.

Precision. Intra-assay precision for EL-ANCATM:anti-MPO and EL-ANCATM:anti-PR3 was determined on 20 replicates of 3 specimens. The inter-assay precision was determined by testing 3 specimens in 20 different runs (**Table 2**).

Table 2. Precision studies.

Intra-assay variation	Inter-assay variation

Test	Level	Mean U/mL	CV	Level	Mean U/mL	CV
Anti-MPO	Low	54	8.5%	Low	50	11.1%
Anti-MPO	Moderate	101	4.4%	Moderate	107	9.7%
Anti-MPO	High	201	2.9%	High	218	11.2%
Anti-PR3	Low	25	8.3%	Low	31	6.8%
Anti-PR3	Moderate	52	4.9%	Moderate	59	6.0%
Anti-PR3	High	75	3.5%	High	78	6.0%

Agreement. The TheraTest EL-ANCA™: anti-MPO and anti-PR3 tests were compared to other EIA tests for MPO-ANCA and PR3-ANCA on a total of 280 specimens: 125 blood bank donors, 37 expected positive for anti-MPO antibodies, 43 expected positive for anti-PR3 antibodies, 25 with SLE, 25 with scleroderma, and 25 with rheumatoid arthritis (RA). There were four discrepant samples that were discrepant for anti-MPO in the SLE group (see **Table 1**). No discrepancy was observed for anti-PR3. Results are summarized in the following two tables (**Tables 3 and 4**). The calculated agreement with 95% C.I. is presented in **Table 5**.

Table 3. Agreement between EL-ANCA™:anti-MPO and another EIA test

		Another EIA anti-MPO test for Clinical Laboratory Use		
		Positives	Negatives*	Total
EL-ANCA™:	Positives	40	4	44
	Negatives*	0	236	236
Anti-MPO		Total	40	240
			240	280

Included 1 equivocal by both tests

Table 4. Agreement between EL-ANCA™:anti-PR3 and another EIA test

		Another EIA anti-PR3 test for Clinical Laboratory Use		
		Positives	Negatives*	Total
EL-ANCA™:	Positives	46	0	46
	Negatives	0	234	234
Anti-PR3		Total	46	280
			234	280

*Included 1 equivocal by both tests and 5 equivocal by EL-ANCA™ among specimens from patients.

Table 5. Agreement between EL-ANCA and another EIA ANCA test kit

Test	Number	Agreement	Range, 95% C.I.
Anti-MPO	280 (all specimens)	99%	[96 – 100]
Anti-MPO	44 (positives)	91%	[78 – 97]*
Anti-MPO	236 (negatives)	98%	[96 – 100]
Anti-PR3	280 (all specimens)	100%	[99 – 100]
Anti-PR3	46 (positives)	100%	[92 – 100]
Anti=PR3	234 (negatives)	100%	[98 – 100]

*The small disagreement is due to the fact that the TTL test is slightly more sensitive, although it has the same specificity as evidenced by the results obtained with blood bank donors (**Table 1**).

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TROUBLESHOOTING

Problem	Possible Causes	Solution
Control values out of range.	<ol style="list-style-type: none"> 1. Incorrect temperature, timing or pipetting; reagents not mixed. 2. Cross-contamination of Controls. 3. Improper dilution. 4. Optical pathway not clean. 5. Wavelength of filter incorrect. 6. Diluent Blank >0.2 	<ol style="list-style-type: none"> 1. Check that temperature was correct. Check that time was correct. See "Poor Precision" (below) No. 2-4. Repeat test. 2. Pipette carefully. 3. Repeat test. 4. Check for moisture or dirt. Wipe bottom and reread. 5. Change filter to 450 ± 5 nm. 6. Check for contamination of Chromogen or Conjugate solution.
All test results negative.	<ol style="list-style-type: none"> 1. One or more reagents not added, or added in wrong sequence. 2. Improper dilution of Wash Buffer. 3. Antigen-coated plate inactive. 	<ol style="list-style-type: none"> 1. Recheck procedure. Check for unused solutions. Repeat test. 2. Repeat test. 3. Check for obvious moisture in unused wells. Rerun test with Controls only for checking activity of coated wells..
All test results yellow. Scattered false positives	<ol style="list-style-type: none"> 1. Contaminated Chromogen. 2. Contaminated buffers/reagents. 3. Wash Buffer (1X) contaminated. 4. Improper dilution of serum. 5. Contaminated pipette 	<ol style="list-style-type: none"> 1. Check absorbance of unused Chromogen. 2. Check all solution for turbidity. 3. Use clean container. Check quality of water used to prepare buffer. 4. Repeat test. 5. Use aerosol-barrier tips for Chromogen
Poor precision.	<ol style="list-style-type: none"> 1. Pipettor delivery CV greater than 5%. 2. Serum or reagents not mixed sufficiently; reagents not at room temperature prior to addition. 3. Reagent addition taking too long; inconsistency in timing intervals, air bubbles. 4. Air currents blowing over plate during incubations. 5. Optical pathway not clean. 6. Instrument not equilibrated before readings were taken. 7. Washing not consistent; trapped bubbles; liquid left in wells at end of wash cycle. 8. Improper pipetting. 	<ol style="list-style-type: none"> 1. Check calibration of pipettor. Use reproducible technique. 2. Mix all reagents gently but thoroughly and equilibrate to room temperature. 3. Develop consistent uniform technique and avoid splashing, or use multi-channel device or autodispenser to decrease addition time. 4. Cover plate or place in chamber. 5. Wipe bottom of plate with soft tissue. Check instrument light source and detector for dirt. 6. Check instrument manual for warm up procedure. 7. Use only acceptable washing devices. Lengthen timing delay on washing devices. Check that all wells are filled. 8. Avoid air bubbles in pipette tips.



(GB)(USA)(CDN) Expiry date (D)(A)(B)(CH) Verfallsdatum (F)(B)(CH)(CDN) Date de péremption (I)(CH) Data di scadenza (E) Fecha de caducidad (P) Data de validade (NL) Uiterste gebruiksdatum (DK) Udløbsdato (S) Utgångsdatum



(GB)(USA)(CDN) Consult instructions for use (D)(A)(B)(CH) Bitte Gebrauchsanweisung einsehen (F)(B)(CH)(CDN) Consultez la notice d'utilisation (I)(CH) Consultare le istruzioni per l'uso (E) Consulte las instrucciones de utilización (P) Consulte as instruções de utilização (NL) Raadpleeg de gebruiksaanwijzing (DK) Se brugsanvisningen (S) Läs anvisningarna före användning



(GB)(USA)(CDN) In Vitro Diagnostic Medical Device (For In Vitro Diagnostic Use) (D)(A)(B)(CH) Medizinisches In-vitro-Diagnostikum (zur In-vitro-Diagnostik) (F)(B)(CH)(CDN) Dispositif médical de diagnostic in vitro (Pour usage diagnostique in vitro) (I)(CH) Dispositivo medico per diagnostica in vitro (per uso diagnostico in vitro) (E) Dispositivo médico de diagnóstico in vitro (para uso diagnóstico in vitro) (P) Dispositivo médico para diagnóstico in vitro (Para utilização de diagnóstico "in vitro") (NL) Medisch hulpmiddel voor diagnostiek in vitro (Voor diagnostisch gebruik in vitro) (DK) Medicinsk udstyr til in vitro-diagnostik (Udelukkende til in vitro diagnostisk anvendelse) (S) Medicinteknisk produkt avsedd för in vitro-diagnostik (För in vitro-diagnostiskt bruk)



(GB)(USA)(CDN) Lot / Batch Number (D)(A)(B)(CH) Charge / Chargennummer (F)(B)(CH)(CDN) Lot / Code du lot (I)(CH) Lotto / Numero lotto (E) Lote / Código de lote (P) Lote / Código do lote (NL) Lot-/Partijnummer (DK) Lot / Batchkode (S) lot / Satskod



(GB)(USA)(CDN) Manufactured by (D)(A)(B)(CH) Hergestellt von (F)(B)(CH)(CDN) Fabriqué par (I)(CH) Prodotto da (E) Fabricado por (P) Fabricado por (NL) Vervaardigd door (DK) Fabrikation af (S) Tillverkad av



(GB)(USA)(CDN) Catalogue Number (D)(A)(B)(CH) Bestell-Nummer (F)(B)(CH)(CDN) Numéro de référence (I)(CH) Numero di riferimento (E) Número de referencia (P) Número de referência (NL) Referentienummer (DK) Referencenummer (S) Katalognummer



(GB)(USA)(CDN) Store at between (D)(A)(B)(CH) Lagerung bei zwischen (F)(B)(CH)(CDN) Conserver à entre (I)(CH) Conservare a tra (E) Conservar a temp. entre (P) Armazene a entre (NL) Bewaar bij tussen (DK) Opbevares mellem (S) Förvaras vid



(GB)(USA)(CDN) Contains sufficient for x tests (D)(A)(B)(CH) Inhalt ausreichend für x Tests (F)(B)(CH)(CDN) Contient suffisant pour x tests (I)(CH) Contenuto sufficiente per x test (E) Contiene suficiente para x pruebas (P) Contém suficiente para x testes (NL) Bevat voldoende voor x bepalingen (DK) Indeholder tilstrækkeligt til x prøver (S) Innehållet räcker till x analyser

Abbreviated Test Procedure

- 1. Dilute Controls and Specimens 1:101 with Specimen Diluent.**
- 2. Pipette 100 μ L of Calibrator(s) and diluted Controls and Specimens into appropriate wells (see Data Sheet for configuration); add Specimen Diluent only to one well (Blank).**
- 3. Incubate for 30-35 minutes at room temperature (18-25°C).**
- 4. Wash the wells three times with 1X Wash Buffer.**
- 5. Add 100 μ L IgG Enzyme Conjugate to each well.**
- 6. Incubate for 30-35 minutes at room temperature (18-25°C).**
- 7. Wash the wells three times with 1X Wash Buffer.**
- 8. Add 100 μ L Chromogen to each well.**
- 9. Incubate for 15 \pm 1 minutes at room temperature (18-25°C).**
- 10. Add 100 μ L Stop Reagent to each well.**
- 11. Read the absorbance at 450 nm, reference 620-690 nm within 30 minutes.**

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