Monobind Inc.®
Lake Forest, CA 92630, USA

AccuBind®
ELISA Microwells

# Aldosterone
# AccuBind® ELISA Test System
## *Product Code: 10125-300*

## 1.0 INTRODUCTION

**Intended Use: The Quantitative Determination of Aldosterone Concentration in Human Serum or Plasma by a Microplate Enzyme Immunoassay, Colorimetric**

## 2.0 SUMMARY AND EXPLANATION OF THE TEST

Aldosterone is a steroid that is synthesized in the zona glomerulosa of the adrenal cortex. Like all steroids, aldosterone is derived from cholesterol through a series of enzymatic reactions.[1] It is considered the main mineralocorticoid hormone and acts in response to elevated potassium levels or lowered sodium levels in the blood. Aldosterone is the final product of the renin-angiotensin-aldosterone system (RAAS) and is essential in mediating blood-pressure and extracellular volume homeostasis. Increased blood aldosterone levels with normal to reduced blood renin abundance are increasingly associated with many cases of hypertension and congestive heart failure.[1,2] Additionally, aldosterone has been recognized to have adverse effects on endothelial, renal, and central nervous system tissues.[3]

Aldosterone is a key hormone involved in sodium conservation throughout the body. When aldosterone is released, it acts on the mineralocorticoid receptor (MR) which in turn activates specific amiloride-sensitive sodum channels (ENaC) to increase potassium excretion by the kidneys while sodium excretion is decreased. This results in a decrease of blood potassium while increasing sodium levels.[1]

Patients who produce high levels of aldosterone with low to normal renin levels are said to have primary hyperaldosteronism, or Conn's syndrome. Primary hyperaldosteronism is caused by benign adrenal tumors or adrenal gland hyperplasia in 99% of cases with <1% resulting from cancer or familial disorders.[4] Secondary hyperaldosteronism, however, is caused by an overactive RAAS and is far more common.[4] Primary hyperaldosteronism accounts for the cause of about 10-15% of hypertension patients while secondary hyperaldosteronism is commonly associated with cardiovascular injury and congestive heart failure.[3-5] Otherwise, many cases of hypertension can be treated by mediating the effect of aldosterone on sodium levels.[5,6]

Some pharmaceuticals are administered to hypertensive patients that reduce the effect of aldosterone. One such class is angiotensin-converting enzyme (ACE) inhibitors that reduce the production of aldosterone while another, MR antagonists, decrease its effectiveness.[1,2] Monitoring blood aldosterone levels is a key aspect of clinical mediation of high blood-pressure in many cardiovascular patients.

The Monobind Aldosterone Kit uses a specific monoclonal anti-aldosterone antibody, and does not require prior sample extraction of serum or plasma. Cross-reactivity to other naturally-occurring steroids is low. The employment of several serum references of known aldosterone concentration permits construction of a graph of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with aldosterone concentration.

## 3.0 PRINCIPLE

**Delayed Competitive Enzyme Immunoassay (TYPE 9):**
The essential reagents required for am enzyme immunoassay include antibody, enzyme-antigen conjugate and native antigen. Upon mixing the biotinylated antibody with a serum containing the antigen, a reaction results between the antigen and the antibody. The interaction is illustrated by the following equation:

$$Ag + Ab_{Btn} \rightleftharpoons AgAb_{Btn}$$

$Ab_{Btn}$ = Biotinylated antibody
Ag = Antigen (Variable Quantity) $AgAb_{Btn}$ = Immune Complex

After a short incubation, the enzyme conjugate is added. (This delayed addition permits an increase in sensitivity for low concentration samples). Upon the addition of the enzyme conjugate, competition reaction results between the enzyme analog and the antigen in the sample for a limited number of antibody binging sites (not consumed in the first incubation).

$$^{Enz}Ag + Ag + rAb_{Btn} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} AgAb_{Btn} + {}^{Enz}AgAb_{Btn}$$

$^{Enz}Ag$ = Enzyme-antigen Conjugate (Constant Quantity)
$^{Enz}AgAb_{Btn}$ = Enzyme-antigen Conjugate -Antibody Complex
$rAb_{Btn}$ = Biotinylated antibody not reacted in first incubation
$k_a$ = Rate Constant of Association
$k_{-a}$ = Rate Constant of Disassociation
$K = k_a / k_{-a}$ = Equilibrium Constant

A simultaneous reaction between the biotin attached to the antibody and the streptavidin immobilized on the microwell occurs. This effects the separation of the antibody bound fraction after decantation or aspiration.
$AgAb_{Btn} + {}^{Enz}AgAb_{Btn} + \underline{Streptavidin}_{CW} \Rightarrow$ immobilized complex
$\underline{Streptavidin}_{CW}$ = Streptavidin immobilized on well
Immobilized complex = sandwich complex bound to the solid surface

The enzyme activity in the antibody bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen con-centration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

## 4.0 REAGENTS

**Materials Provided**

**A. Aldosterone Calibrators - 1ml/vial (Lyophilized) - Icons A-F**
Six (6) vials of serum reference for aldosterone at concentrations of 0 (**A**), 25 (**B**), 125 (**C**), 250 (**D**), 500 (**E**), 1500 (**F**) in pg/ml. Store at 2-8°C. *Reconstitute each vial with 1.0ml of distilled or deionized water.* The reconstituted calibrators are stable for 30 days at 2-8°C. A preservative has been added. Concentrations can be expressed in ng/dl by dividing by 10.

**B. Aldosterone Control – 1ml/vial (Lyophilized) – Icon M**
One (1) vial of human serum based matrix containing Aldosterone at an established range. Store at 2-8°C. *Reconstitute each vial with 1.0ml of distilled or deionized water.* The reconstituted controls are stable for 30 days at 2-8°C. A preservative has been added.

**C. Aldosterone Enzyme Reagent – 7.0 ml/vial – Icon E**
One (1) vial containing Aldosterone (Analog)-horseradish peroxides (HRP) conjugate in a protein-stabilizing matrix with dye. Store at 2-8°C.

**D. Aldosterone Biotin Reagent – 7.0 ml/vial – Icon ∇**
One (1) vial containing biotinylated anti-aldosterone IgG conjugate in buffer, dye and preservative. Store at 2-8°C.

**E. Streptavidin Coated Plate – 96 wells – Icon ⇓**
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

**F. Wash Solution Concentrate – 20ml/vial – Icon**
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

**G. Substrate Reagent – 12 ml/vial – Icon $S^N$**
One (1) vial containing tetramethylbenzidine (TMB) and hydrogen peroxide ($H_2O_2$) in buffer. Store at 2-8°C.

**H. Stop Solution – 8ml/vial – Icon**
One (1) vial containing a strong acid (0.5M $H_2SO_4$). Store at 2-8°C.

**I. Product Instructions.**

**Note 1:** Do not use reagents beyond the kit expiration date.
**Note 2:** Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on label.**
**Note 3:** Above reagents are for a single 96-well microplate. For other kit configurations, refer to the chart at the end of the instructions.

### 4.1 Required But Not Provided:

1. Pipette capable of delivering 0.025ml (25µl) and 0.050ml (50µl) with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.050ml (50µl), 0.100ml (100µl) and 0.350ml (350µl) volumes with a precision of better than 1.5%.
3. Adjustable volume 0.050ml-1.0ml (50µl-1000µl) dispenser(s) for conjugate.
4. Microplate washer or a squeeze bottle (optional).
5. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
6. Absorbent Paper for blotting the microplate wells.
7. Plastic wrap or microplate cover for incubation steps.
8. Vacuum aspirator (optional) for wash steps.
9. Timer.
10. Quality control materials.

## 5.0 PRECAUTIONS

***For In Vitro Diagnostic Use***
***Not for Internal or External Use in Humans or Animals***

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

**Safe Disposal of kit components must be according to local regulatory and statutory requirement.**

## 6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum or heparinized plasma in type, and taken with the usual precautions in the collection of venipuncture samples. For accurate comparison to establish normal values, a fasting morning serum sample should be obtained. The blood should be collected in a redtop (with or without gel additives) venipuncture tube(s) or for plasma use evacuated tube(s) containing heparin. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

**In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.**

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml (50µl) of the specimen is required.

## 7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and high range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

## 8.0 REAGENT PREPARATION

1. **Wash Buffer**
   Dilute contents of wash solution concentrate to 1000ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at 2-30°C for up to 60 days.

***Note: Do not use reagents that are contaminated or have bacteria growth.***

## 9.0 TEST PROCEDURE

*Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).*
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.025 ml (25 µl) of the appropriate serum reference, control or specimen into the assigned well.
3. Add 0.050 ml (50µl) of the Aldosterone Biotin Reagent to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix.
5. Cover and incubate for 15 minutes at room temperature.
6. Add 0.050 ml (50µl) of Aldosterone Enzyme Reagent to all wells.
   **Add directly on top of the reagents dispensed in the wells**.
7. Swirl the microplate gently for 20-30 seconds to mix.
8. Cover and incubate for 45 minutes at room temperature.
9. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
10. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
11. Add 0.100 ml (100µl) of substrate solution to all wells. **Always add reagents in the same order to minimize reaction time differences between wells.**
    **DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION**
12. Incubate at room temperature for twenty (20) minutes.
13. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
14. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm. **The results should be read within fifteen (15) minutes of adding the stop solution.**

**Note**: Dilute the samples suspected of concentrations higher than 1500pg/ml 1:5 and 1:10 with Aldosterone '0' pg/ml calibrator.

## 10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of aldosterone in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding aldosterone concentration in pg/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Connect the points with a best-fit curve.
4. To determine the concentration of aldosterone for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in pg/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.215) intersects the dose response curve at (195pg/ml) aldosterone concentration (See Figure 1).

**Note:** Computer data reduction software designed for ELISA assay may also be used for the data reduction. If such software is utilized, the validation of the software should be

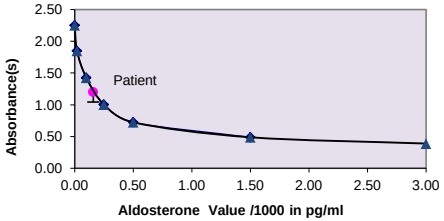
ascertained.

**EXAMPLE 1**

| Sample I.D. | Well Number | Abs (A) | Mean Abs (B) | Value (pg/ml) |
|---|---|---|---|---|
| Cal A | A1 | 2.611 | 2.611 | 0 |
| | B1 | 2.599 | | |
| Cal B | C1 | 2.314 | 2.326 | 25 |
| | D1 | 2.338 | | |
| Cal C | E1 | 1.529 | 1.533 | 125 |
| | F1 | 1.536 | | |
| Cal D | G1 | 1.008 | 1.040 | 250 |
| | H1 | 1.072 | | |
| Cal E | A2 | 0.583 | 0.584 | 500 |
| | B2 | 0.585 | | |
| Cal F | C2 | 0.220 | 0.211 | 1500 |
| | D2 | 0.203 | | |
| Pat# 1 | G2 | 1.222 | 1.215 | 195 |
| | H2 | 1.209 | | |

*The above table and data below is for example only. Do not use it for calculating your results.

**Figure 1**




Note: Multiply the horizontal values by 1000 to convert into pg/ml.

## 11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator 0 pg/ml should be ≥ 1.3.
2. All provided kit controls should produce results within the established ranges.
3. (Optional) 2 of 3 commercial quality control pools should be within the established ranges.

## 12.0 RISK ANALYSIS

*The SDS is available at https://www.monobind.com/safety-data-sheets and the Risk Analysis Form may be requested.*

### 12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch bottom of wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
11. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
12. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

### 12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional***.
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for AccuBind® ELISA procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays. *(Boscato LM Stuart MC.'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem 1988:3427-33).* For diagnostic purposes the results from this assay should be used in combination with clinical examination, patient's history, and, all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

## 13.0 EXPECTED RANGES OF VALUES

In agreement with established reference intervals for a "normal" adult population the expected ranges for the Aldosterone AccuBind® ELISA Test System are detailed in Table 1.

**TABLE 1**
**Expected Values for the Aldosterone Test System**

| Age | Posture Unspecified | Supine | Upright |
|---|---|---|---|
| 0-6 days | 50-1020 pg/ml | | |
| 1-3 weeks | 60-1790 pg/ml | | |
| 1-11 months | 70-990 pg/ml | | |
| 1-2 years | 70-930 pg/ml | | |
| 3-10 years | 40-440 pg/ml | | |
| 11-14 years | 40-310 pg/ml | | |
| 15 years and older | Less than or equal to 310 pg/ml | Less than or equal to 160 pg/ml | 40-310 pg/ml |

It is important to keep in mind that establishment of a range of values, which can be expected to be found by a given method for a population of "normal" persons, is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons, each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

## 14.0 PERFORMANCE CHARACTERISTICS

### 14.1 Precision

The within and between assay precision of the Aldosterone AccuBind® ELISA Test System were determined by analyses on six different levels of pool control sera. The number, mean values, standard deviation and coefficient of variation for each of these control sera are presented in Table 2.

**TABLE 2**
**Precision data for the Aldosterone Test System**

| | Mean Value (pg/ml) | Within-Run Precision | | Total Precision (n=80)* | |
|---|---|---|---|---|---|
| | | SD | CV% | SD | CV% |
| Sample 1 | 42.54 | 3.07 | 7.22 | 4.52 | 10.62 |
| Sample 2 | 125.47 | 4.74 | 3.78 | 13.44 | 10.71 |
| Sample 3 | 246.77 | 15.34 | 6.21 | 26.01 | 10.54 |
| Sample 4 | 501.77 | 14.28 | 2.85 | 42.58 | 8.49 |
| Sample 5 | 734.90 | 16.19 | 2.20 | 45.76 | 6.23 |
| Sample 6 | 1162.50 | 28.12 | 2.42 | 71.63 | 6.16 |

*As measured in forty experiments in duplicate over a twenty day period.

### 14.2 Sensitivity

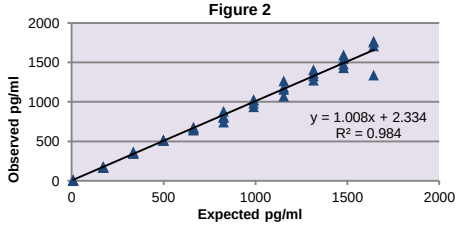
The Aldosterone AccuBind® ELISA Test System has a LoB of 14.39 pg/ml and a LoD of 22.18 pg/ml.

### 14.3 Accuracy

#### 14.3.1 Linearity

The linearity of the Aldosterone AccuBind® ELISA Test System was tested by diluting a human serum samples containing a high level of aldosterone (~1700 pg/ml) with the "0 pg/ml" serum reference. The system was determined to have excellent linearity up to 1700pg/ml with a slope of 1.008 and a correlation factor of 0.984. The expected values were compared to the observed concentrations of the samples and graphed in Figure 2.

**Figure 2**




#### 14.3.2 Recovery

The recovery of the Aldosterone AccuBind® ELISA Test System was calculated for five patient samples spiked with 100, 250, 550, 850, and 1250 pg/ml aldosterone. Recoveries were determined to be within 15% of the expected values for all samples.

#### 14.3.3 Method Comparison

The Aldosterone AccuBind® ELISA Test System was compared with another ELISA assay from a different manufacturer. Biological specimens from low, normal and relatively high aldosterone level populations were used. (The values ranged from 5 pg/ml - 850 pg/ml). The total number of such specimens was 63. The least square regression equation and the correlation coefficient were computed for this aldosterone EIA in comparison with the reference method.

**TABLE 3**

| Method | Mean (x) | Least Square Regression Analysis | Correlation Coefficient |
|---|---|---|---|
| This Method (y) | 207.6 | y= 0.703x+6.354 | 0.959 |
| Reference (x) | 286.4 | | |

Only slight amounts of bias between this method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

### 14.4 Specificity

The % cross reactivity of the aldosterone antibody to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of aldosterone needed to displace the same amount of labeled analog.

| Substance | %Cross Reactivity |
|---|---|
| Cortisol | <0.001 |
| Cortisone | 0.012 |
| Corticosterone | 0.010 |
| Progesterone | <0.001 |
| DHEA sulfate | 0.016 |
| Estradiol-17β | 0.008 |
| Estriol | 0.008 |

## 15.0 REFERENCES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## 16.0 AVAILABLE CONFIGURATIONS

Available test-system sizes and included materials are shown below. Each "pack size" has a unique item/SKU number, which includes the product code. For the standard pack size of 96-microwells, the product code will have the letter "A" added (e.g., 10125-300A) to make a SKU for sale. This test kit is currently available as per below (with contents shown in table):

| ITEM # | DESCRIPTION |
|---|---|
| 10125-300A | Aldosterone AccuBind ELISA Kit - 96 wells |
| 10125-300B | Aldosterone AccuBind ELISA Kit - 192 wells |

| Size | | 96(A) | 192(B) |
|---|---|---|---|
| Reagent (fill) | A) | 1ml set | 1ml set |
| | B) | 1 (1ml) | 2 (1ml) |
| | C) | 1 (7ml) | 2 (7ml) |
| | D) | 1 (7ml) | 2 (7ml) |
| | E) | 1 plate | 2 plates |
| | F) | 1 (20ml) | 1 (20ml) |
| | G) | 1 (12ml) | 2 (12ml) |
| | H) | 1 (8ml) | 2 (8ml) |

***Also Available:*** **QSure® Multi-Ligand Control**

**Revision: 3** **Date: 2025-NOV-14** **DCO: 1747**
**MP10125.3** **Product Code: 10125-300**


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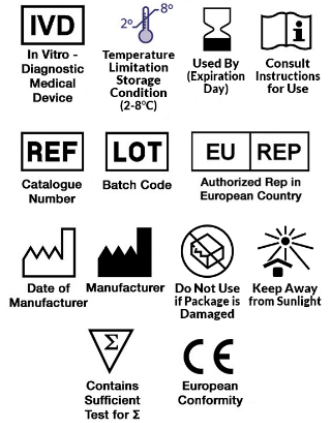
**Glossary of Symbols**
(ISO 15223)