



**Human Insulin Specific**

**250 Tubes**

**Cat. # HI-14K**

**HUMAN INSULIN SPECIFIC RIA KIT  
250 TUBES (Cat. # HI-14K)**

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**HUMAN INSULIN SPECIFIC RIA KIT  
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**I. INTENDED USE**

Insulin is a polypeptide hormone secreted from beta cells of the pancreas. The primary function of Insulin is to control blood glucose levels through its biochemical actions on cellular glucose uptake, glycogenesis, lipogenesis, and glucose oxidation. Insulin secretion into the bloodstream is predominantly controlled by the level of glucose in plasma but is also influenced by other factors, such as neural influences, intestinal hormones, and other beta cell secretory hormones. The measurement of in-vivo Insulin concentrations may aid in the diagnosis of conditions, such as nesidioblastosis, islet-cell tumors, and various insulin resistant conditions, such as diabetes mellitus. This Human Insulin Specific Kit is for the quantitative determination of Insulin in serum, plasma, and tissue culture media. This assay does not cross-react with Human Proinsulin (<0.2%) and therefore measures "true" Insulin levels. It is a completely homologous assay since the antibody was raised against purified Human Insulin and both the standard and the tracer are prepared with Human Insulin.

***This kit is for Research Use Only. Not for Use in Diagnostic Procedures.***

**II. PRINCIPLES OF PROCEDURE**

In radioimmunoassay, a fixed concentration of labeled tracer antigen is incubated with a constant dilution of antiserum such that the concentration of antigen binding sites on the antibody is limited, for example, only 50% of the total tracer concentration may be bound by antibody. If unlabeled antigen is added to this system, there is competition between labeled tracer and unlabeled antigen for the limited and constant number of binding sites on the antibody. Thus, the amount of tracer bound to antibody will decrease as the concentration of unlabeled antigen increases. This can be measured after separating antibody-bound from free tracer and counting one or the other, or both fractions. A standard curve is set up with increasing concentrations of standard unlabeled antigen and from this curve the amount of antigen in unknown samples can be calculated. Thus, the four basic necessities for a radioimmunoassay system are: a specific antiserum to the antigen to be measured, the availability of a radioactive labeled form of the antigen, a method whereby antibody-bound tracer can be separated from the unbound tracer, and finally, an instrument to count radioactivity.

The EMD Millipore Human Insulin assay utilizes <sup>125</sup>I-labeled Human Insulin and a Human Insulin antiserum to determine the level of Insulin in serum, plasma or tissue culture media by the double antibody/PEG technique<sup>1</sup>.

**III. REAGENTS SUPPLIED**

Each kit is sufficient to run 250 tubes and contains the following reagents.

**A. Assay Buffer**

0.05M Phosphosaline pH 7.4 containing 0.025M EDTA, 0.08% Sodium Azide, and 1% RIA Grade BSA  
Quantity: 40 mL/vial  
Preparation: Ready to use

**B. Human Insulin Antibody**

Guinea Pig anti-Human Insulin Specific antibody in Assay Buffer  
Quantity: 26 mL/vial  
Preparation: Ready to use

### III. REAGENTS SUPPLIED (continued)

#### C. <sup>125</sup>I-Insulin

<sup>125</sup>I-Insulin Label, HPLC purified (specific activity 367  $\mu\text{Ci}/\mu\text{g}$ )

Lyophilized for stability. Freshly iodinated label contains <5  $\mu\text{Ci}$  (185 kBq), calibrated to the 1st Monday of each month.

Quantity: 27 mL/vial upon hydration

Preparation: Contents Lyophilized. Hydrate with entire contents of Label Hydrating Buffer. Allow to set at room temperature for 30 minutes, with occasional gentle mixing.

#### D. Label Hydrating Buffer

Assay Buffer containing Normal Guinea Pig Serum as a carrier. Used to hydrate <sup>125</sup>I-Insulin.

Quantity: 27 mL/vial

Preparation: Ready to use

#### E. Human Insulin Standards

Purified Recombinant Human Insulin in Assay Buffer at the following concentration:

200  $\mu\text{U}/\text{mL}$

Quantity: 2 mL/vial

Preparation: Ready to use

#### F. Quality Controls 1 & 2

Purified Recombinant Human Insulin in Assay Buffer

Quantity: 1 mL/vial

Preparation: Ready to use

#### G. Precipitating Reagent

Goat anti-Guinea Pig IgG serum, 3% PEG and 0.05% Triton X-100 in 0.05M Phosphosaline, 0.025M EDTA,

0.08% Sodium Azide

Quantity: 260 mL/vial

Preparation: Ready to use; chill to 4°C.

### IV. STORAGE AND STABILITY

Refrigerate all reagents between 2 and 8°C for short-term storage. For prolonged storage (>2 weeks), freeze at  $\leq -20^\circ\text{C}$ . Avoid multiple (>5) freeze/thaw cycles. Refer to date on bottle for expiration when stored at  $\leq -20^\circ\text{C}$ . Do not mix reagents from different kits unless they have the same lot number.

### V. REAGENT PRECAUTIONS

#### A. Radioactive Materials

This radioactive material may be received, acquired, possessed and used only by research personnel or clinical laboratories for in-vitro research tests not involving internal or external administration of the material, or the radiation there from, to human beings or animals. Its receipt, acquisition, possession, use and transfer are subject to the regulations of the U. S. Nuclear Regulatory Commission (NRC) or of a State with which the Commission has entered into an agreement for the exercise of regulatory authority.

The following are suggested general rules for the safe use of radioactive material. The customer's Radiation Safety Officer is ultimately responsible for the safe handling and use of radioactive material.




## V. REAGENT PRECAUTIONS (continued)

1. Wear appropriate personal devices at all times while in areas where radioactive materials are used or stored.
2. Wear laboratory coats, disposable gloves, and other protective clothing at all times.
3. Monitor hands, shoes, and clothing and immediate area surrounding the workstation for contamination after each procedure and before leaving the area.
4. Do not eat, drink, or smoke in any area where radioactive materials are stored or used.
5. Never pipette radioactive material by mouth.
6. Dispose of radioactive waste in accordance with NRC rules and regulations.
7. Avoid contaminating objects such as telephones, light switches, doorknobs, etc.
8. Use absorbent pads for containing and easily disposing of small amounts of contamination.
9. Wipe up all spills immediately and thoroughly and dispose of the contaminated materials as radioactive waste. Inform Radiation Safety Officer.

## B. Sodium Azide

Sodium Azide has been added to all reagents as a preservative at a concentration of 0.08%. Although it is at a minimum concentration, Sodium Azide may react with lead and copper plumbing to form highly explosive metal azides. Dispose of unused contents and waste in accordance with international, federal, state, and local regulations.

### Full labels of hazardous components in this kit:

Ingredient, Cat #		Full Label	
<sup>125</sup> I-Insulin Tracer	9011		<b>Danger.</b> Harmful if swallowed. Causes serious eye damage. Harmful to aquatic life with long lasting effects. Avoid release to the environment. Wear eye protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Get medical advice/ attention.
Human Insulin Antibody	1014-K		<b>Warning.</b> Causes serious eye irritation. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
Precipitating Reagent	PR-UV		<b>Warning.</b> Causes serious eye irritation. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

## **VI. MATERIALS REQUIRED BUT NOT PROVIDED**

1. Borosilicate glass tubes, 12 x 75 mm. (NOTE: Polypropylene or polystyrene tubes may be used if the investigator finds that the pellet formation is acceptably stable in their system.)
2. 100  $\mu$ L pipet with disposable tips
3. 100  $\mu$ L & 1.0 mL repeating dispenser
4. Refrigerated swing bucket centrifuge capable of developing 2,000 - 3,000 xg. (Use of fixed-angle buckets is not recommended.)
5. Absorbent paper
6. Vortex mixer
7. Refrigerator
8. Gamma Counter

## **VII. SPECIMEN COLLECTION AND STORAGE**

1. A maximum of 100  $\mu$ L per assay tube of serum or plasma can be used, although, 50  $\mu$ L per assay tube is adequate for most applications. Tissue culture and other media may also be used.
2. Care must be taken when using heparin as an anticoagulant, since an excess will provide falsely high values<sup>2</sup>. Use no more than 10 IU heparin per mL of blood collected.
3. Specimens can be stored at 4°C if they will be tested within 24 hours of collection. For longer storage, specimens should be stored at  $\leq$  -20°C. Avoid multiple (>5) freeze/thaw cycles.
4. Avoid using samples with gross hemolysis or lipemia.

## VIII. ASSAY PROCEDURE

### Standard Preparation

Use care in opening the Standard vial.

Label six glass tubes 1, 2, 3, 4, 5, and 6. Add 1.0 mL Assay Buffer to each of the six tubes. Prepare serial dilutions by adding 1.0 mL of the 200uU/mL standard to tube 1, mix well and transfer 1.0 mL of tube 1 to tube 2, mix well and transfer 1.0 mL of tube 2 to tube 3, mix well and transfer 1.0 mL of tube 3 to tube 4, mix well and transfer 1.0 mL of tube 4 to tube 5, mix well and transfer 1.0 mL of tube 5 to tube 6, mix well.

Note: Do not use a Repeater pipette. Change tip for every dilution. Wet tip with Standard before dispensing. Unused portions of standard should be stored at  $\leq -20^{\circ}\text{C}$ . Avoid multiple freeze/thaw cycles.

Tube #	Standard Concentration	Volume of Assay Buffer to Add	Volume of Standard to Add
1	100 uU/mL	1.0 mL	1.0 mL of 200 uU/mL
2	50 uU/mL	1.0 mL	1.0 mL of 100 uU/mL
3	25 uU/mL	1.0 mL	1.0 mL of 50 uU/mL
4	12.5 uU/mL	1.0 mL	1.0 mL of 25 uU/mL
5	6.25 uU/mL	1.0 mL	1.0 mL of 12.5 uU/mL
6	3.125 uU/mL	1.0 mL	1.0 mL of 6.25 uU/mL

For optimal results, accurate pipetting and adherence to the protocol are recommended.

#### Assay Set-Up, Day One

1. Pipet 300  $\mu\text{L}$  of Assay Buffer to the Non-Specific Binding (NSB) tubes (3-4), 200  $\mu\text{L}$  to Reference (Bo) tubes (5-6), and 100  $\mu\text{L}$  to tubes 7 through the end of the assay.
2. Pipet 100  $\mu\text{L}$  of Standards and Quality Controls in duplicate (see flow chart).
3. Pipet 100  $\mu\text{L}$  of each Sample in duplicate. (NOTE: Smaller volumes of sample may be used when Insulin concentrations are anticipated to be elevated or when sample size is limited. Additional Assay Buffer should be added to compensate for the difference so that the volume is equivalent to 100  $\mu\text{L}$ , e.g., when using 50  $\mu\text{L}$  of sample, add 50  $\mu\text{L}$  of Assay Buffer). Refer to Section IX for calculation modification.
4. Pipet 100  $\mu\text{L}$  of hydrated  $^{125}\text{I}$ -Insulin to all tubes. Important: For preparation, see Section III, Part C.
5. Pipet 100  $\mu\text{L}$  of Human Insulin antibody to all tubes except Total Count tubes (1-2) and NSB tubes (3-4).
6. Vortex, cover, and incubate overnight (20-24 hours) at room temperature (22-25 $^{\circ}\text{C}$ ).

#### Day Two

7. Add 1.0 mL of cold (4 $^{\circ}\text{C}$ ) Precipitating Reagent to all tubes (except Total Count tubes).
8. Vortex and incubate 20 minutes at 4 $^{\circ}\text{C}$ .
9. Centrifuge, 4 $^{\circ}\text{C}$ , all tubes [except Total Count tubes (1-2)] for 20 minutes at 2,000-3,000 xg.

NOTE: If less than 2,000 xg is used or if slipped pellets have been observed in previous runs, the time of centrifugation must be increased to obtain a firm pellet (e.g., 40 minutes). Multiple centrifuge runs within an assay must be consistent.

Conversion of rpm to xg:

$$\text{xg} = (1.12 \times 10^{-5}) (r) (\text{rpm})^2$$

r = radial distance in cm (from axis of rotation to the bottom of the tube)

rpm = revolutions per minute

10. Immediately decant the supernate of all tubes except Total Count tubes (1-2), drain tubes for at least 15-60 seconds (be consistent between racks) and blot excess liquid from lip of tubes. NOTE: Invert tubes only one time. Pellets are fragile and slipping may occur.
11. Count all tubes in a gamma counter for 1 minute. Calculate the  $\mu\text{U}/\text{mL}$  of Human Insulin in unknown samples using automated data reduction procedures.

### VIII. ASSAY PROCEDURE (continued)

#### Assay Procedure Flow Chart

Day One						Day Two		
Set-up	Step 1	Steps 2 & 3	Step 4	Step 5	Step 6	Step 7	Step 8	Steps 9-11
Tube Number	Add Assay Buffer	Add Standard/ QC Sample	Add I-125 Human Insulin Tracer	Add Human Insulin Antibody	<b>Vortex, Cover, and Incubate 20-24 hrs at RT</b>	Add Precipitating Reagent	<b>Vortex, and Incubate 20 min. at 4°C</b>	<b>Centrifuge at 4°C for 20 min., Decant, and Count</b>
1,2	-	-	100 µL	-		-		
3,4	300 µL	-	100 µL	-		1.0 mL		
5,6	200 µL	-	100 µL	100 µL		1.0 mL		
7,8	100 µL	100 µL of 3.125 µU/mL	100 µL	100 µL		1.0 mL		
9,10	100 µL	100 µL of 6.25 µU/mL	100 µL	100 µL		1.0 mL		
11,12	100 µL	100 µL of 12.5 µU/mL	100 µL	100 µL		1.0 mL		
13,14	100 µL	100 µL of 25 µU/mL	100 µL	100 µL		1.0 mL		
15,16	100 µL	100 µL of 50 µU/mL	100 µL	100 µL		1.0 mL		
17,18	100 µL	100 µL of 100 µU/mL	100 µL	100 µL		1.0 mL		
19,20	100 µL	100 µL of 200 µU/mL	100 µL	100 µL		1.0 mL		
21,22	100 µL	100 µL of QC 1	100 µL	100 µL		1.0 mL		
23-24	100 µL	100 µL of QC 2	100 µL	100 µL		1.0 mL		
25,26	100 µL	100 µL of unknown	100 µL	100 µL		1.0 mL		
27-n	100 µL	100 µL of unknown	100 µL	100 µL	1.0 mL			



## IX. CALCULATIONS

### A. Explanation

The calculations for Human Insulin can be automatically performed by most gamma counters possessing data reduction capabilities or by independent treatment of the raw data using a commercially available software package<sup>3</sup>. Choose weighted 4-parameter or weighted log/logit for the mathematical treatment of the data.

NOTE: Be certain the procedure used subtracts the NSB counts from each average count, except Total Counts, prior to final data reduction.

### B. Manual Calculation

1. Average duplicate counts for Total Count tubes (1-2), NSB tubes (3-4), Total Binding tubes (reference, Bo) (5-6), and all duplicate tubes for standards and samples to the end of the assay.
2. Subtract the average NSB counts from each average count (except for Total Counts). These counts are used in the following calculations.
3. Calculate the percentage of tracer bound:  
 $(\text{Total Binding Counts} / \text{Total Counts}) \times 100$   
  
This should be 35-50%.
4. Calculate the percentage of total binding (%B/Bo) for each standard and sample:  
 $\%B/Bo = (\text{Sample or Standard} / \text{Total Binding}) \times 100$
5. Plot the % B/Bo for each standard on the y-axis and the known concentration of the standard on the x-axis using log-log graph paper.
6. Construct the reference curve by joining the points with a smooth curve.
7. Determine the  $\mu\text{U/mL}$  of Human Insulin in the unknown samples and controls by interpolation of the reference curve.

NOTE: When sample volumes assayed differ from 100  $\mu\text{L}$ , an appropriate mathematical adjustment must be made to accommodate for the dilution factor (e.g., if 50  $\mu\text{L}$  of sample is used, then calculated data must be multiplied by 2).

Conversion to SI units  
 $1 \mu\text{U Insulin} / \text{mL} = 6 \text{ pM}$

## X. INTERPRETATION

### Acceptance Criteria

1. The run will be considered accepted when all Quality Control values fall within the calculated Quality Control Range; if any QC's fall outside the control range, review results with supervisor.
2. If the difference between duplicate results of a sample is >10% CV, repeat the sample.
3. The limit of sensitivity for the Human Insulin assay is 2.715  $\mu\text{U}/\text{mL}$  (100  $\mu\text{L}$  sample size).
4. The limit of linearity for the Human Insulin assay is 200  $\mu\text{U}/\text{mL}$  (100  $\mu\text{L}$  sample size). Any result greater than 200  $\mu\text{U}/\text{mL}$  should be repeated on dilution using Assay Buffer as a diluent.

## XI. NORMAL FASTING RANGE

5-15  $\mu\text{U}/\text{mL}$

This range was determined from the analysis of blood drawn from 25 people after an 18 hour fast.

## XII. ASSAY CHARACTERISTICS

### A. Sensitivity

The lowest level of Insulin that can be detected by this assay is 2.715  $\mu\text{U}/\text{mL}$  when using a 100  $\mu\text{l}$  sample size.

### B. Performance

The following parameters of assay performance are expressed as Mean  $\pm$  Standard Deviation.

ED<sub>80</sub> = 6  $\pm$  1  $\mu\text{U}/\text{mL}$   
ED<sub>50</sub> = 22  $\pm$  3  $\mu\text{U}/\text{mL}$   
ED<sub>20</sub> = 84  $\pm$  15  $\mu\text{U}/\text{mL}$

### C. Specificity

The specificity (also known as selectivity) of an analytical test is its ability to selectively measure the analyte in the presence of other like components in the sample matrix.

Human Insulin	100%
Human Proinsulin (HPI)	<0.2%
Des 31,32 HPI	<0.2%
Des 64,65 HPI	76%
Canine Insulin	100%
Porcine Insulin	100%
Bovine Insulin	62%
Rat Insulin	0.1%
IGF	ND
Glucagon	ND
Somatostatin	ND
Pancreatic Polypeptide	ND

ND-not detectable

## XII. ASSAY CHARACTERISTICS (continued)

### D. Precision

Within and Between Assay Variation

Sample No.	Mean $\mu\text{U/mL}$	Within % CV	Between % CV
1	8	3.1	6.0
2	12	2.5	3.3
3	16	2.2	3.8
4	25	3.8	2.9
5	54	4.4	3.4

Within and between assays variations were performed on five human serum samples containing varying concentrations of Human Insulin. Data (mean and % CV) shown are from five duplicate determinations of each serum sample in five separate assays.

### E. Recovery

Spike & Recovery of Insulin in Human Serum

Sample No.	Insulin Added $\mu\text{U/mL}$	Observed $\mu\text{U/mL}$	Expected $\mu\text{U/mL}$	% Recovery
1	0	8	-	-
2	5	13	13	100
3	10	17	18	94
4	20	26	28	93
5	50	56	58	97

Varying concentrations of Human Insulin were added to five human serum samples and RIA determined the Insulin content. Mean of the observed levels from five duplicate determinations in five separate assays are shown. Percent recovery was calculated on the observed vs. expected.

## XII. ASSAY CHARACTERISTICS (continued)

### F. Linearity

Effect of Serum Dilution

Sample #	Volume Sampled	Observed $\mu\text{U}/\text{mL}$	Expected $\mu\text{U}/\text{mL}$	% Of Expected
1	100 $\mu\text{l}$	17	17	100
	75 $\mu\text{l}$	15		88
	50 $\mu\text{l}$	15		88
	25 $\mu\text{l}$	15		88
2	100 $\mu\text{l}$	42	42	100
	75 $\mu\text{l}$	40		95
	50 $\mu\text{l}$	39		93
	25 $\mu\text{l}$	36		86
3	100 $\mu\text{l}$	62	62	100
	75 $\mu\text{l}$	59		95
	50 $\mu\text{l}$	57		92
	25 $\mu\text{l}$	55		89
4	100 $\mu\text{l}$	83	83	100
	75 $\mu\text{l}$	86		104
	50 $\mu\text{l}$	87		105
	25 $\mu\text{l}$	90		108

Aliquots of pooled Human Serum containing varying concentrations of Insulin were analyzed in the volumes indicated. Dilution factors of 1, 1.33, 2 and 4 representing 100  $\mu\text{L}$ , 75  $\mu\text{L}$ , 50  $\mu\text{L}$  and 25  $\mu\text{L}$ , respectively, were applied in calculating observed concentrations. Mean Insulin levels and percent of expected for five separate assays are shown.

## XII. ASSAY CHARACTERISTICS (continued)

### G. Example of Assay Results

This data is presented as an example only and should not be used in lieu of a standard curve prepared with each assay.

Tube #	ID	CPM	Ave CPM	Ave Net	%	μU/mL
				CPM	B/Bo	
1	Totals	14879				
2	"	15738	15309			
3	NSB	683				
4	"	604	644			
5	Bo	5836				
6	"	5808	5822	5179		
<u>Standards</u>						
7	3.125 μU/mL	5262				
8		5039	5151	4507	87.0	
9	6.25 μU/mL	4530				
10		4666	4598	3955	76.4	
11	12.5 μU/mL	3949				
12		3775	3862	3219	62.2	
13	25 μU/mL	3100				
14		3087	3094	2450	47.3	
15	50 μU/mL	2006				
16		2100	2053	1410	27.2	
17	100 μU/mL	1426				
18		1477	1452	808	15.6	
19	200 μU/mL	1053				
20		997	1025	382	7.4	
<u>Controls/Unknown</u>						
21	QC 1	4033				
22		3999	4016	3373	65.1	11.4
23	QC 2	2075				
24		2157	2116	1473	28.4	48.86
25-n	Unknown					

### XIII. QUALITY CONTROLS

Good Laboratory Practice (GLP) requires that Quality Control (QC) specimens be run with each standard curve to check the assay performance. Two levels of controls are provided for this purpose. Quality control data is provided on an insert sheet within the protocol booklet. These quality controls and any other control materials should be assayed repeatedly to establish mean values and acceptable ranges. Each individual laboratory is responsible for defining their system for quality control decisions and is also responsible for making this system a written part of their laboratory manual. The ranges for Quality Control 1 and 2 are provided on the card insert or can be located at the EMD Millipore website [emdmillipore.com](http://emdmillipore.com) using the catalog number as the keyword.

Recommended batch analysis decision using two controls (Westgard Rules):<sup>4</sup>

1. When both controls are within  $\pm 2$  SD.  
Decision: Approve batch and release analyte results.
2. When one control is outside  $\pm 2$  SD and the second control is within  $\pm 2$  SD.  
Decision: Hold results, check with supervisor. If no obvious source of error is identified by the below mentioned check of systems, the supervisor may decide to release the results.

Technician check of systems:

1. Check for calculation errors
2. Repeat standards and controls
3. Check reagent solutions
4. Check instrument

### XIV. REPLACEMENT REAGENTS

Reagent	Cat#
<sup>125</sup> I-Insulin (<5 $\mu$ Ci, 185 kBq)	9011
Label Hydrating Buffer (27 mL)	LHB-P
Human Insulin Standards (2 mL each)	8014-K
Human Insulin Antibody (26 mL)	1014-K
Precipitating Reagent (260 mL)	PR-UV
QC 1&2 (1 mL each)	6000-K
Assay Buffer (40 mL)	AB-P

### XV. ORDERING INFORMATION

To place an order or to obtain additional information about our immunoassay products, please contact your Customer Service or Technical Support Specialist.

Contact information for each region can be found on our website:

[emdmillipore.com/contact](http://emdmillipore.com/contact)

#### Conditions of Sale

For Research Use Only. Not for Use in Diagnostic Procedures.

#### Safety Data Sheets (SDS)

Safety Data Sheets for EMD Millipore products may be ordered by fax or phone or through our website at [emdmillipore.com/msds](http://emdmillipore.com/msds).

### XVI. REFERENCES

1. Morgan, C.R. and Lazarow, A. Immunoassay of Insulin: Two antibody system. Plasma insulin levels in normal, Subdiabetic, and diabetic rats. *Diabetes* 12:115-126, 1963.
2. Thorell, J.I. *Scand. J. Clin. Lab. Invest.* 31:187, 1973.
3. Feldman, H. and Rodbard, D. "Mathematical Theory of Radioimmunoassay," in: W.D. Odell and Doughaday, W.H. (Ed.), Principles of Competitive Protein-Binding Assays. Philadelphia: J.B. Leppincott Company; pp 158-203, 1971.
4. Westgard, J.O., et. al. A multi-rule Shewhart chart for quality control in clinical chemistry. *Clin. Chem.* 27:493-501, 1981.