

# Performance Evaluation of Particle Enhanced Immunoturbidometric Method For Ferritin Reagent

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# Clinical Application of Ferritin Measurement

**MEASUREMENT OF SERUM FERRITIN CONCENTRATION GIVES A QUANTITATIVE DETERMINATION OF THE MOBILIZABLE STORAGE IRON.**

**FERRITIN MEASUREMENT IS INDICATION OF IRON, DEFICIENCY, REPLETION AND OVERLOAD**

**A DECREASED FERRITIN LEVEL INDICATES TISSUE IRON DEPLETION AND IS PARTICULARLY USEFUL IN THE EARLY DETECTION OF IRON DEFICIENCY ANEMIA WHICH IS THE MOST COMMON DEFICIENCY DISORDER IN THE INDUSTRIALIZED WORLD.**

**INCREASED SERUM FERRITIN CONCENTRATIONS CAN BE SUGGESTIVE OF IRON OVERLOAD IN CONJUNCTION WITH IRON STORAGE DISORDERS LIKE HEREDITARY OR ACQUIRED HEMOCHROMATOSIS.**

**THEY CAN ALSO BE USED TO EVALUATE CLINICAL CONDITIONS NOT RELATED TO IRON STORAGE INCLUDING CHRONIC LIVER DISEASE, INFECTIONS, INFLAMMATION AND MALIGNANCY**

# Ferritin

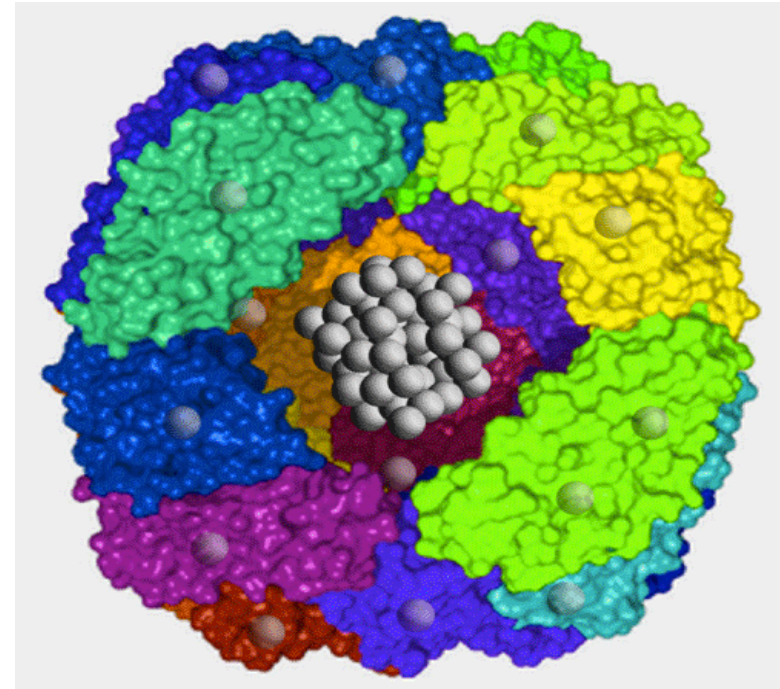
**It Is Major Storage Protein Reserve Of Iron In The Body.**

**Ferritin Is Spherical In Shape.**

**Free Iron Ferritin Is Synthesized As Apoferritin. Chemically Iron Is Incorporated As Mineral Ferrihydrite Which Is Attached In The Inner Wall Of The Sphere.**

**Each Ferritin Molecule Has A Potential Capacity To Incorporate As Many As 4500 Iron Atoms.**

**The Iron Storage Is Initiated With  $\text{Fe}^{2+}$  Ions Being Oxidized By Ferritin Itself.**

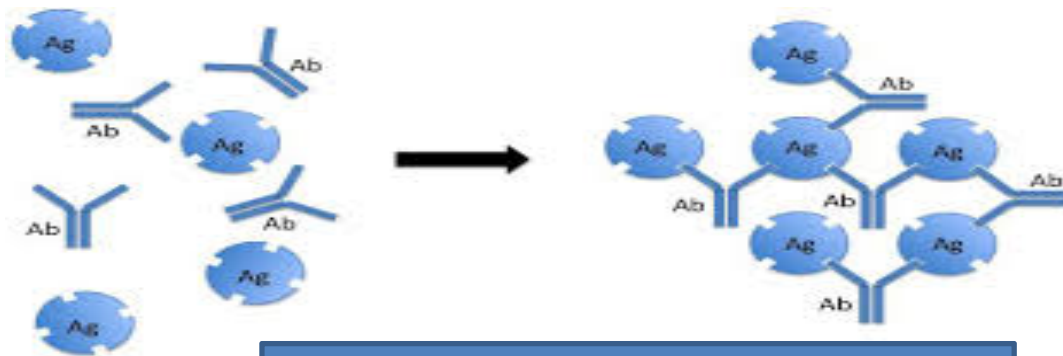


Within Run Imprecision of Commonly Used Method For Ferritin  
Measurement

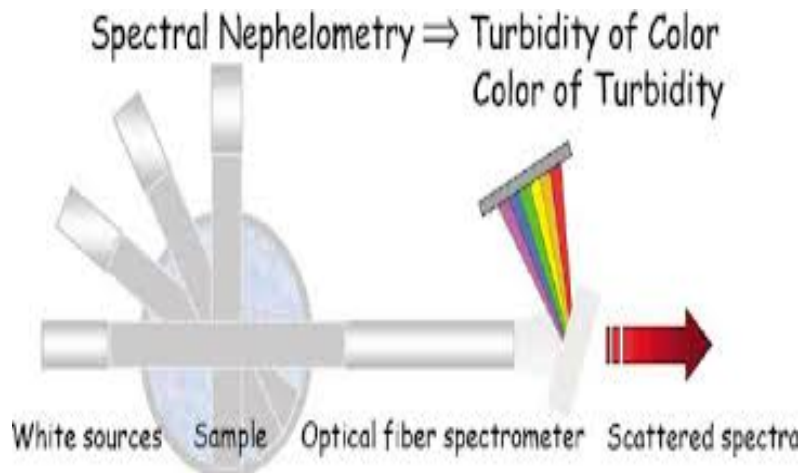
Method Type and Subtype	Number of Entries	Mean	STD	95% Confidence Interval	
<b>Labelled –Non Radiometric</b>					
EIA	31	5.8	2.5	4.43	6.98
Fluorimetric	7	8.3	6.3	3.66	12.95
ELISA	25	7.5	3.3	6.24	8.00
Chemiluminescent	25	4.8	2.3	3.88	5.72
MEIA ( Microarticle Enzyme Immunoassy)	5	4.3	1.8	2.64	5.87
RPIA Radial Partition Immunoassay	1	4.9			
<b>Labelled Radiometric</b>					
RIA	27	7.5	4.0	5.97	8.98
IRMA	32	6.7	3.0	5.65	7.74
<b>Agglutination</b>					
Turbidimetric	13	4.3	3.1	2.57	2.94
Nephelometric	3	2.3	0.4	1.84	2.70
LPIA	1	3.1			
<b>Others</b>	1	5.4			
<b>All</b>	171	6.2	3.4	5.67	6.70

## Between Run Imprecision of Commonly Used Method For Ferritin Measurement

Method Type And SubType	Number of Entries	Mean	STD		
				Lower Limit	Upper Limit
<b>Labelled Non Radiometric</b>					
EIA	24	8.1	3.7	7.21	10.06
Fluorimetric	5	24.8	41.5	0.00	61.21
ELISA	25	10.1	3.6	8.68	11.52
Chemiluminescent	17	6.9	2.6	5.70	8.14
MEIA	4	5.2	0.4	4.76	5.59
<b>Labelled Radiometric</b>					
RIA	22	10.2	4.3	8.15	11.76
IRMA	25	8.7	3.3	7.45	10.00
<b>Aggultination</b>					
Turbidometric	10	4.3	3.0	2.41	6.10
Nephelometric	3	4.1	0.1	3.99	4.21
LPIA	1	2.8			
All	136	8.9	8.7	7.44	10.35

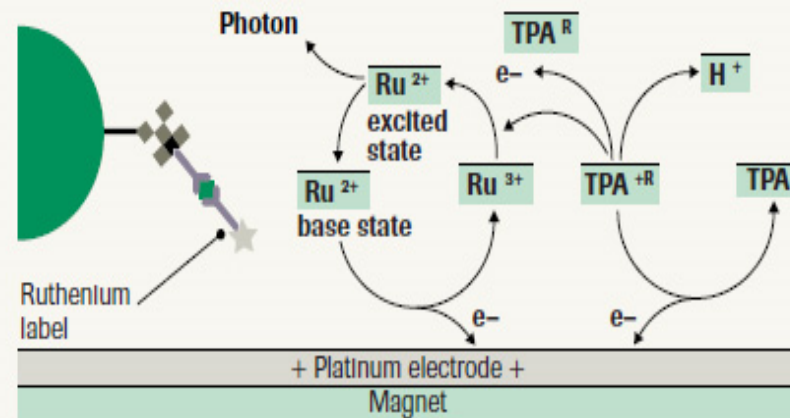


Immunoturbidometric



Nephelometry

Reaction phase-light generation

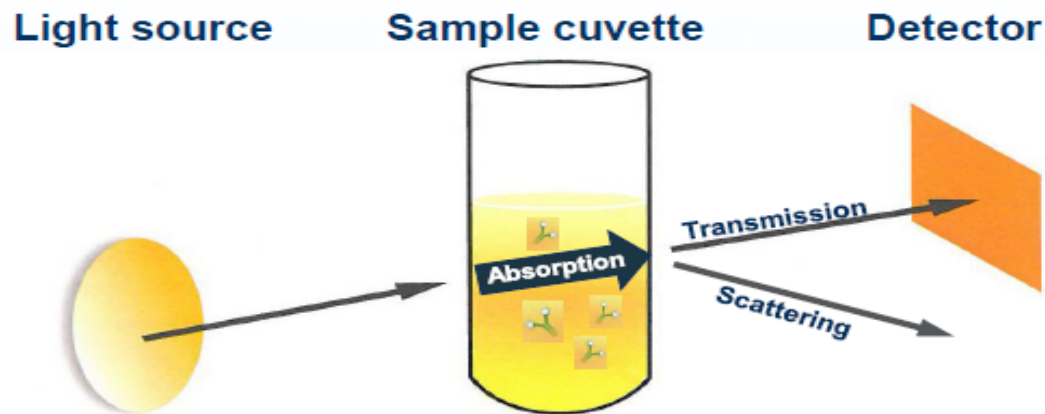


Chemiluminescence

Enlisting Advantage of Three Most Popular Methods for Ferritin Measurement

Nephelometry	Immunoturbidometry	Chemiluminescence
High Sensitivity	Comparatively Sensitive For Required Diagnostic Range	Extremely Sensitive Absence of Interfering Emission Wide Measuring Range
Longer Duration of Time for Getting Results	Fast And Rapid Performing Time	Slow Analysis Time
Not Cost Effective	Cost Effective	High Cost Not Cost Effective
Require Skilled Personal		
Dedicated Instrument And Reagent Is Required	It Could Be Performed On Routine Laboratory Instruments	Dedicated Instrument Is Required
	Longer Reagent Stability	
	Requires Approve Optic System	

## Turbidimetry uses the same measuring principle as photometry

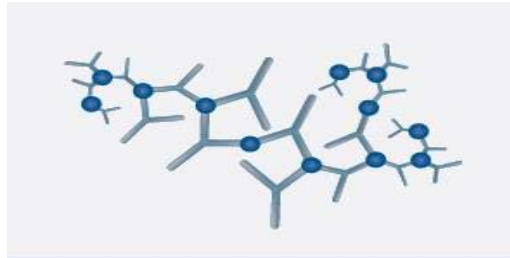


**Incident light entering the cuvette will be subjected to three reactions:**

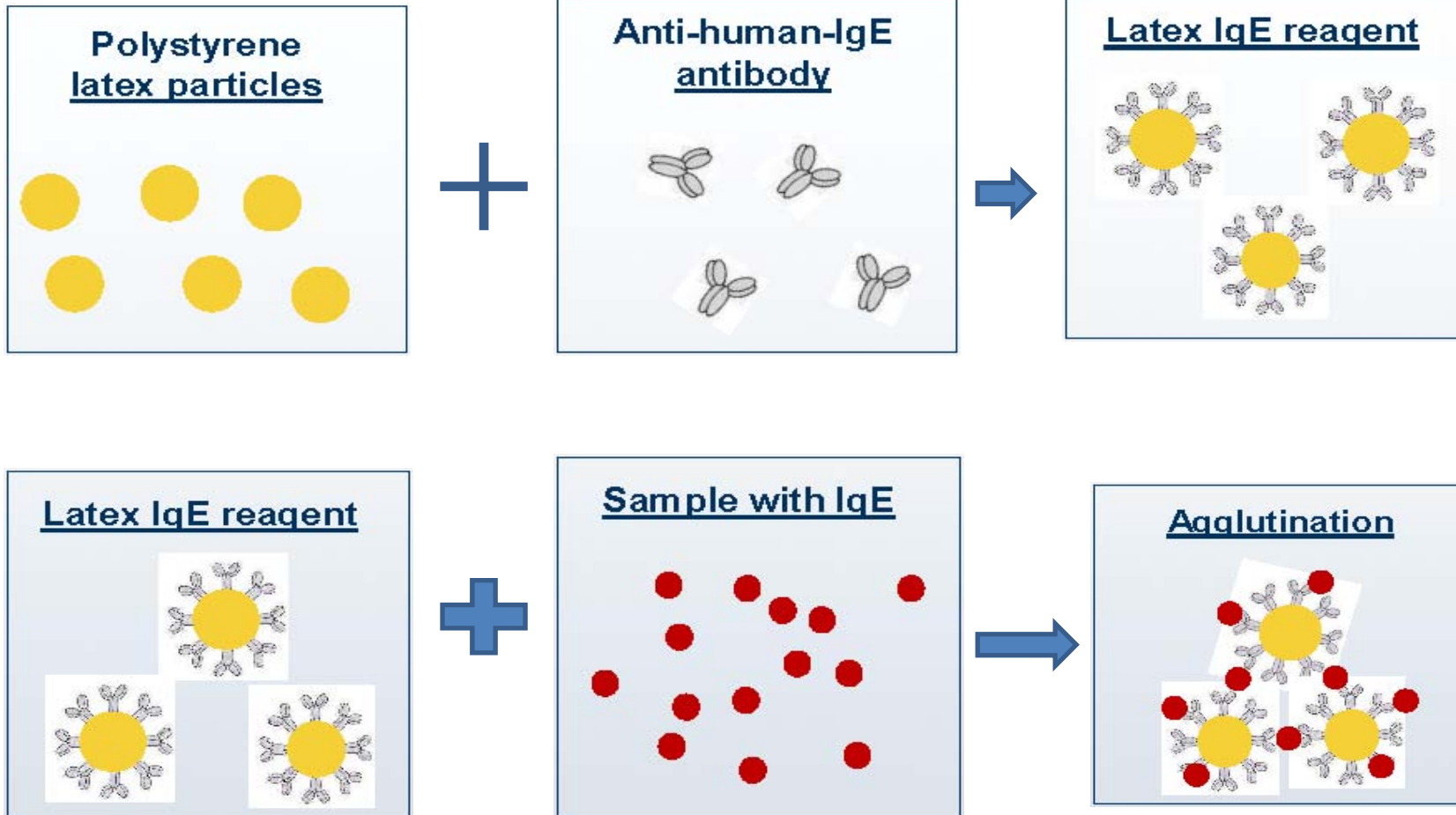
1. Some of the light will be absorbed (blocked) by the particles
2. Some will be transmitted through the cuvette
3. Some will be scattered in various directions



Direct Immunoturbidometric Reagent  
Antibodies Form Immunocomplexes By Direct Attachment To  
Corresponding Antigen Causing Turbidity



## Principle of Immunoturbidometri Reagent



## Factors Affecting Optical Properties of Suspension and Particle Size

عواملی که خواص نوری و اندازه ذرات را در یک محلول مایع که ذرات معلق دارند می تواند تغییر دهد

- **1. Ratio Of Concentration of Solution Mixed**
- 2. Order Of Mixing Solution**
- 3. Rate of Mixing**
- 4. Stability Of Colloidal Particle**
- 5. Time Required To attain Maximum Turbidity**

# Instrumental Factors In Optimizing Immunoturbidometric Reagents

**Latex Particle Optimization With Antibody Is Subject To An  
Extensive Regimen Including Following :**

**Forces Affecting Both Colloidal Stability and Antigen Antibody Interaction**

**Particle Size**

**Antibody Concentration**

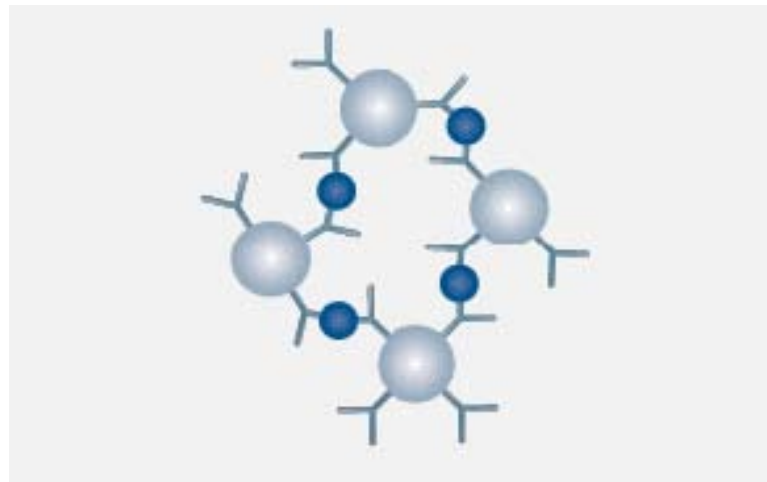
**PH and Ionic Strength**

**A.Acid Chemisty of Particle Surface**

**Careful Control Of These Parameters Was Found To Be Necessary To Achieve The Desired  
Effects Of Balancing High Colloidal Stability In The Absence Of Antigen But Promoting A  
Rapid, Sensitive, And Dose-Dependent Agglutination With Pathological Serum Samples.**

**Each antibody system is unique, a similar optimization should be performed in diagnostic  
immunoassays of this type to maximize their clinical utility**

Particle Enhanced Immunturbidometric  
Are Particles Coated With Antibodies from Complexes With Antigen In  
Sample Causing Turbidity



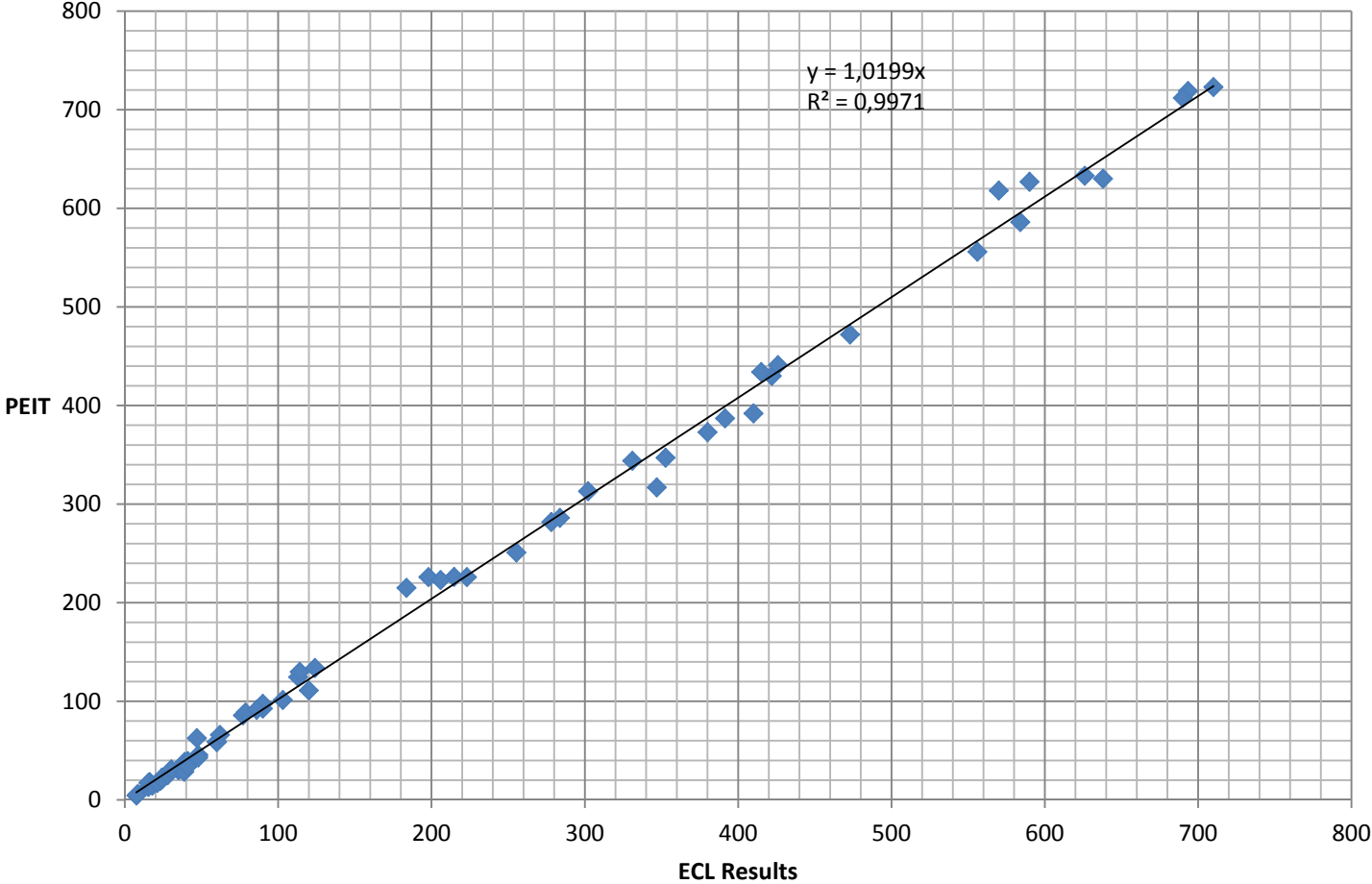
# Reference Interval Information

Variable Discussed	ECL	PEIT	LTT
Reference Interval			
4- months to 16 years	15-150 ug/L	15-150 ug/L	7-140
Adult Women <50 years	15-150 ug/L	15-150 ug/L	20-200
Adult Women > 50 yeas and Men	30- 400 ug/L	30-400 ug/L	20-250

# Performance Characteristic

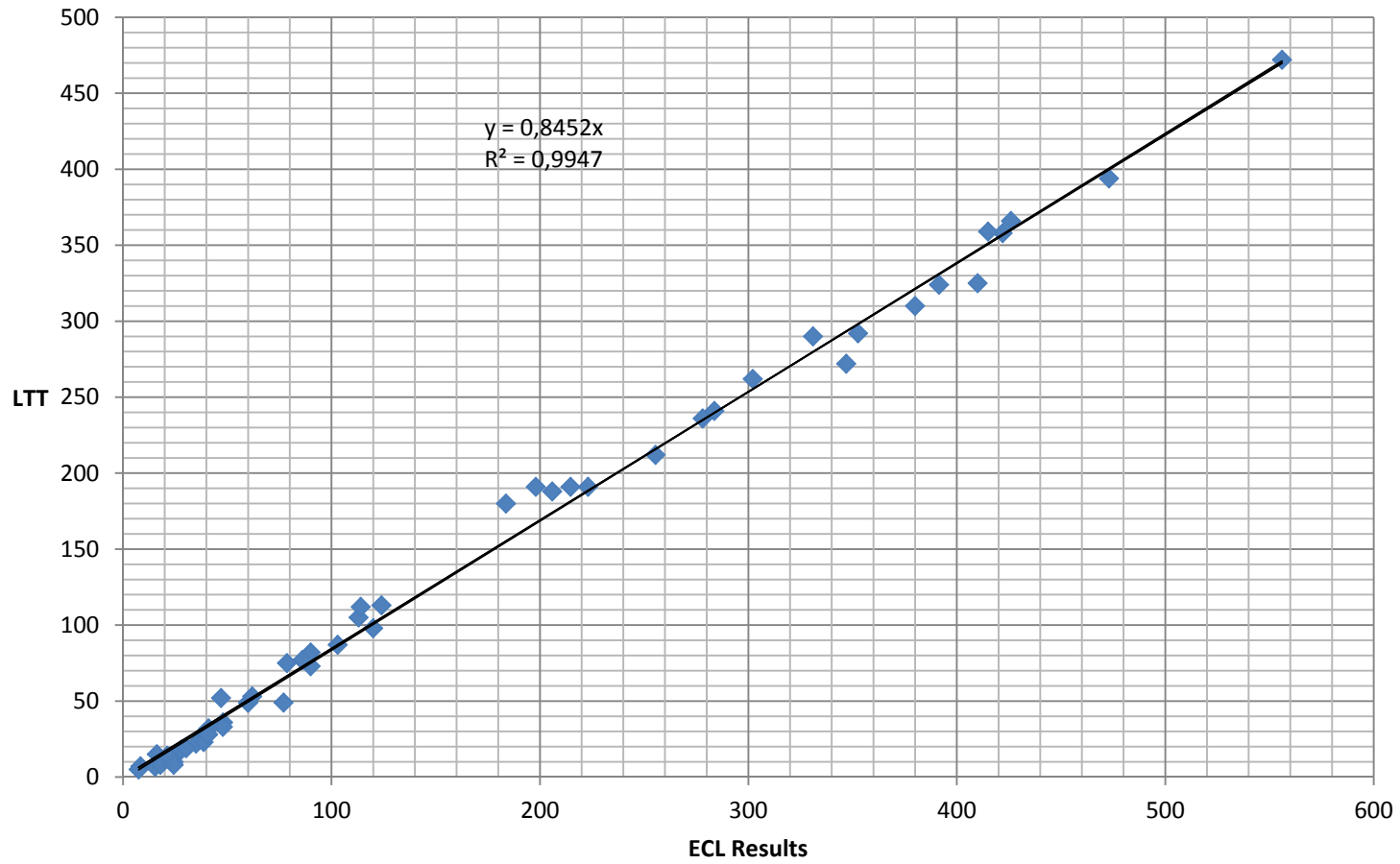
Variable	LTT	PEIT	ECL
Sensitivity	4ug/L	10ug/L	0.5ug/L
Measuring Range	4-500ug/L	10-700ug/L	005-2000 ug/L
Metrological Traceability	WHO94/572	WHO94/572	WHO94/572
Ascorbic Acid	-	30mg/dl	-
Bilirubin	62mg/dl	60 mg/dl	65mg/dl
Hb	10 g/L	1000 mg/dl	<0.5g/dl
Lipemic		10% up Triglyceride Level of 1400 mg/dl and Ferritin Concentration of 180 ug/dl	
Hook Effect	30000 ug/L	30000 ug/L	100000 ng/ml

# Regression Coefficient ECL vs PEIT

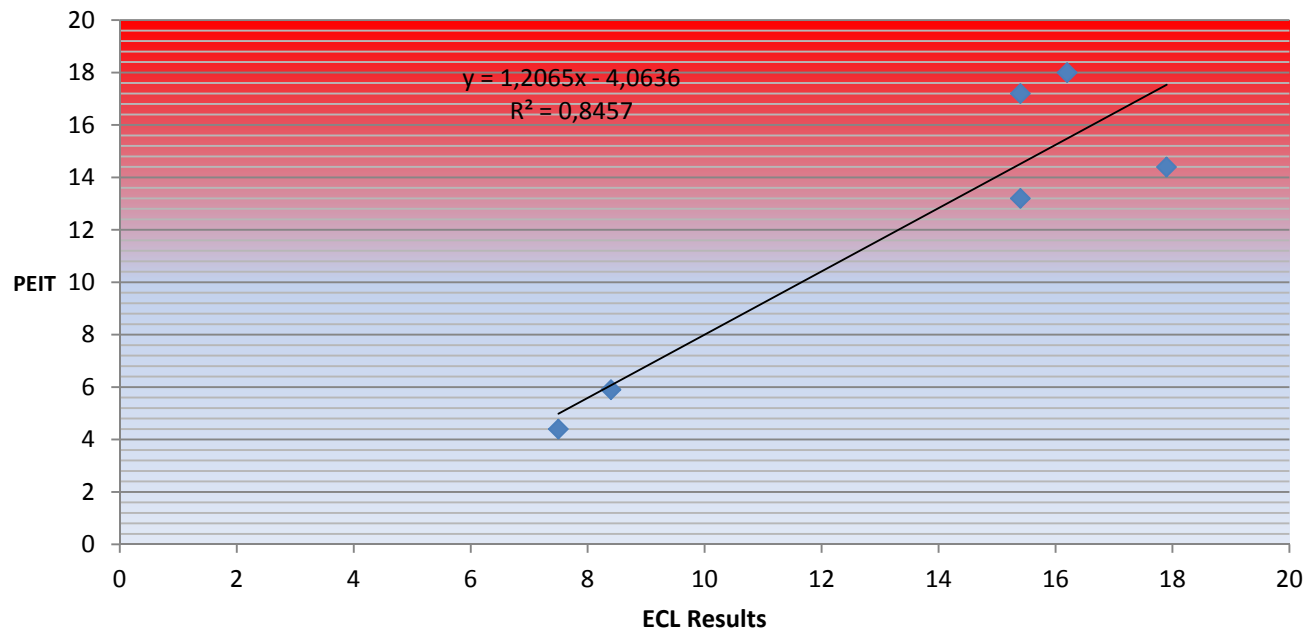




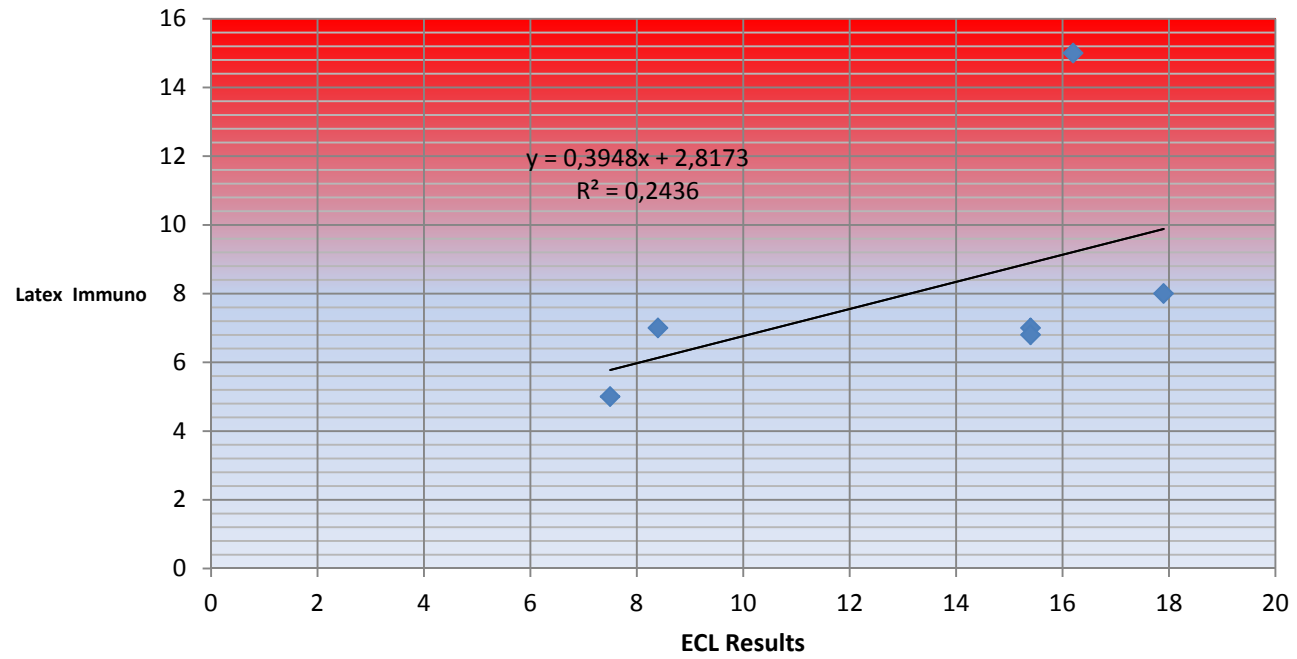
# Regression Coefficient of ECL vs LTT



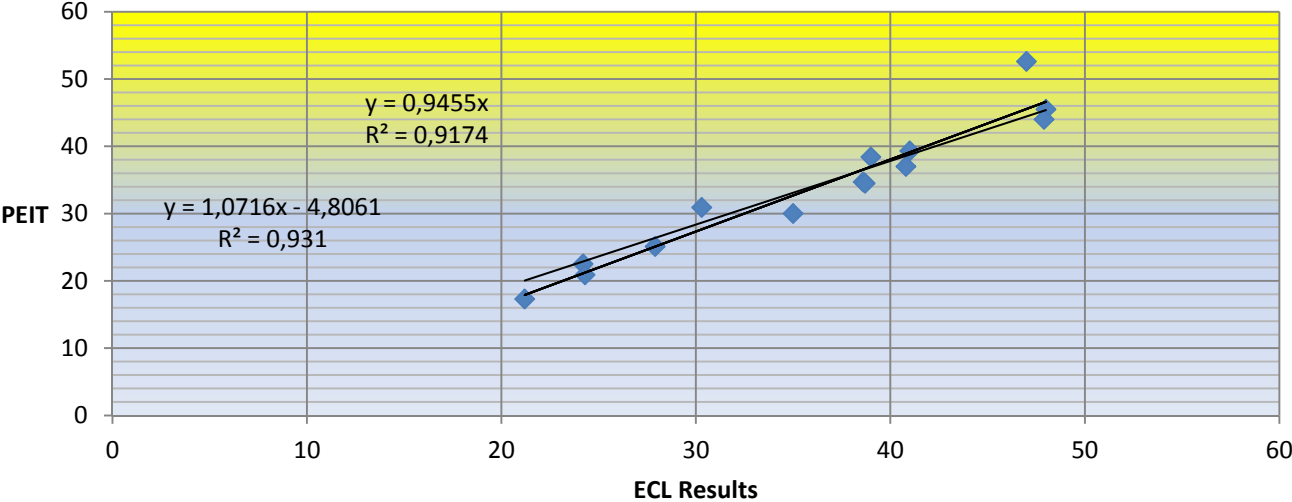
Regression Coefficient ECL vs PEIT In Critical Diagnostic Range



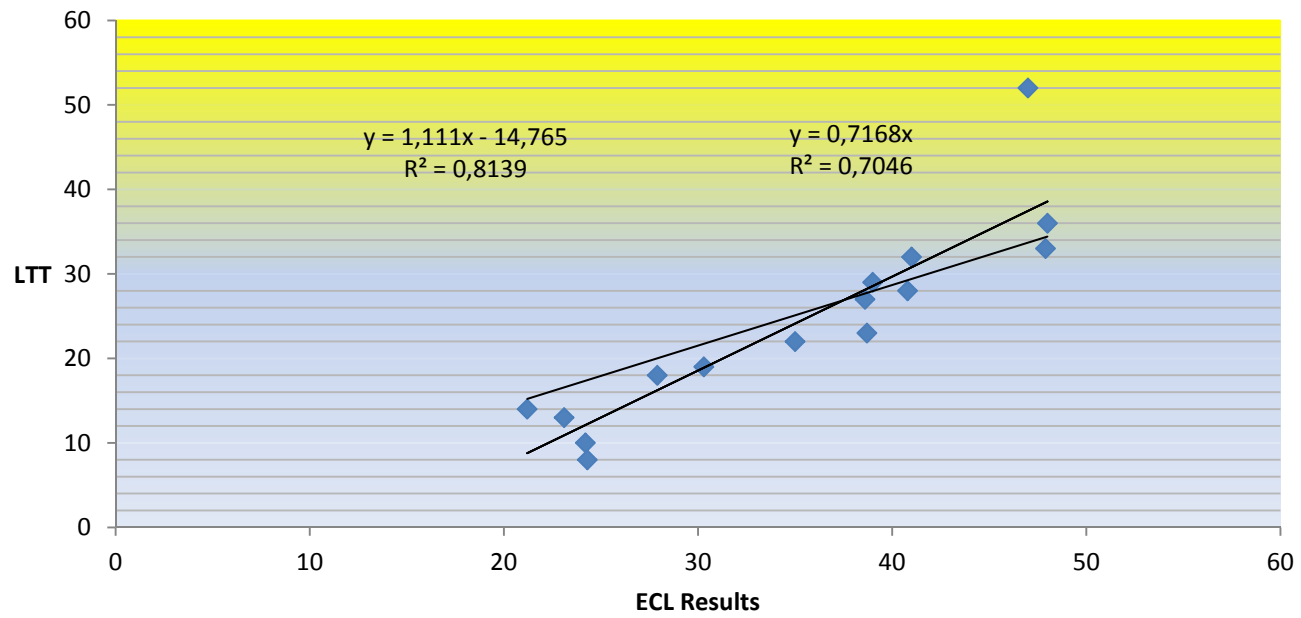
### Regression Coefficient ECL vs LTT In Critical Diagnostic Range



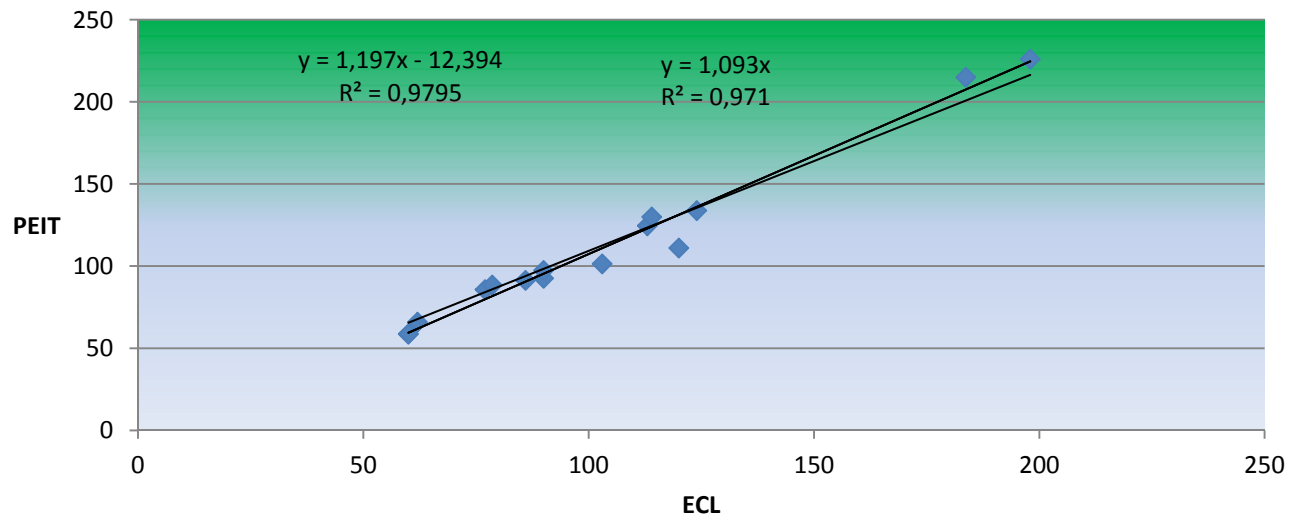
Regression Coefficient ECL vs PEIT In Normal Monitoring Range



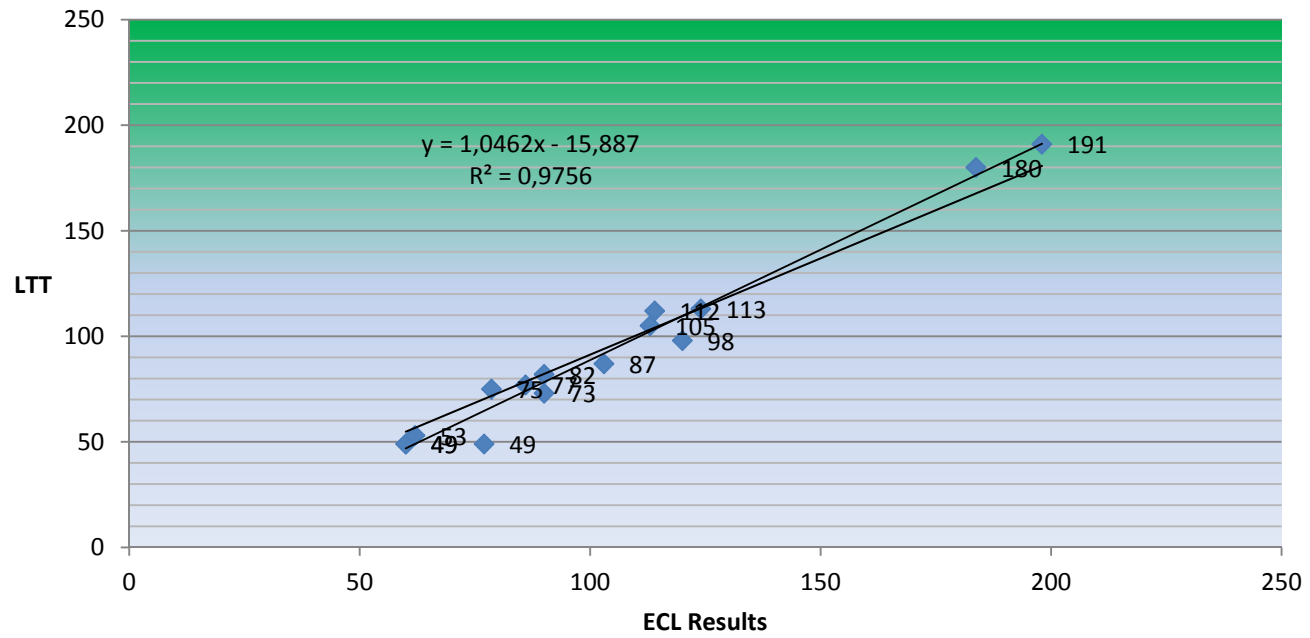
Regression Coefficient ECL vs LTT In Normal Monitoring Range



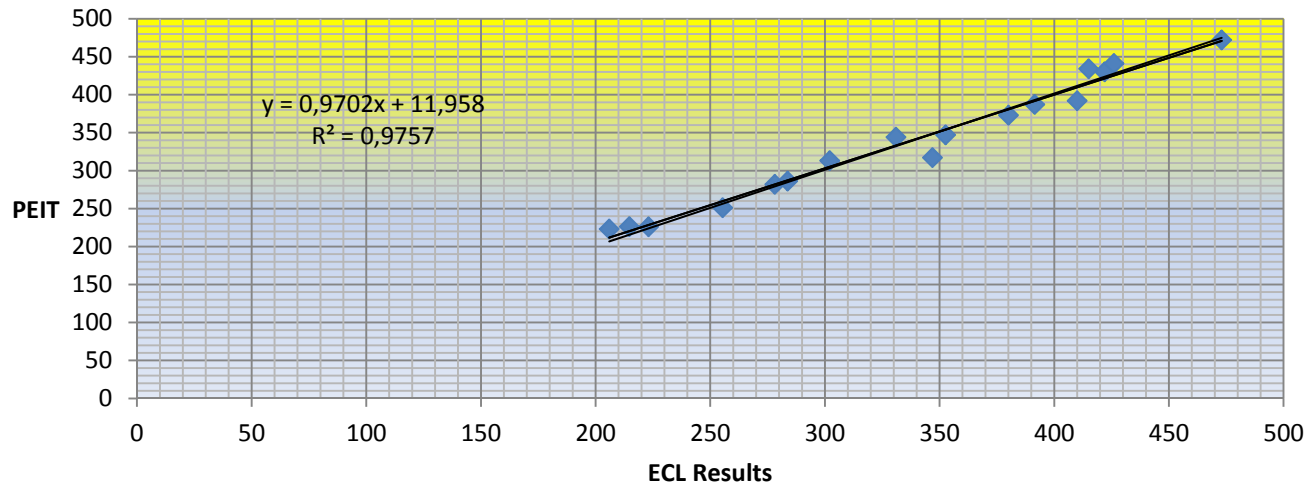
## Regression Coefficient ECL vs PEIT In Normal Range



Regression Coefficient ECL vs LTT In Normal Range

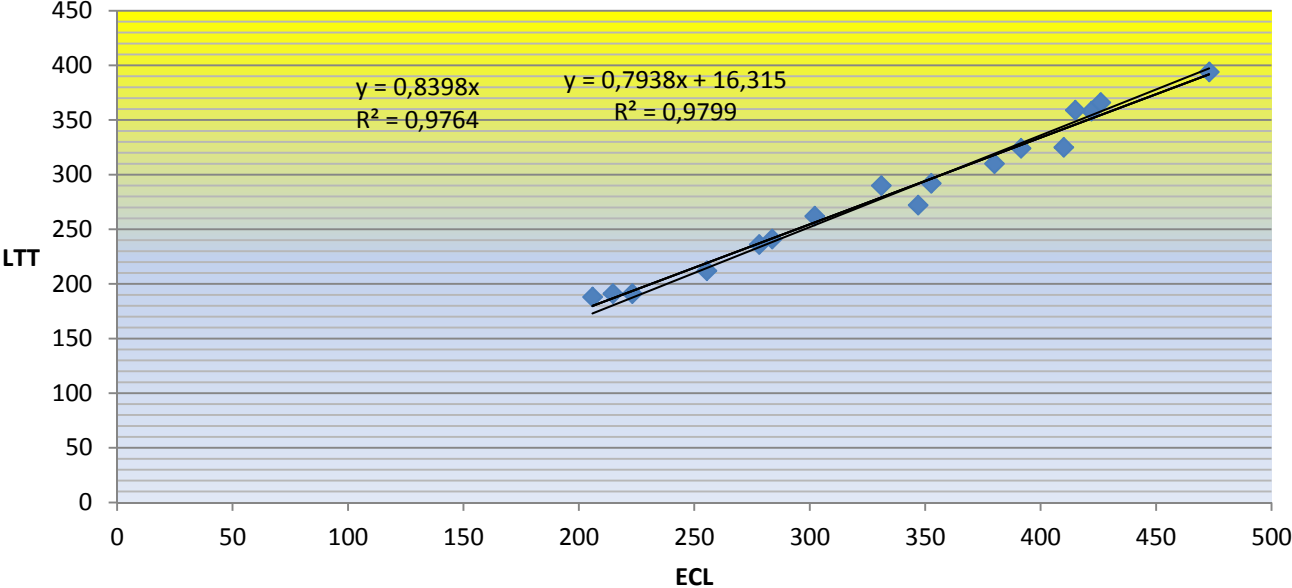


**Regression Coefficient ECL vs PEIT In Conflicting Range**

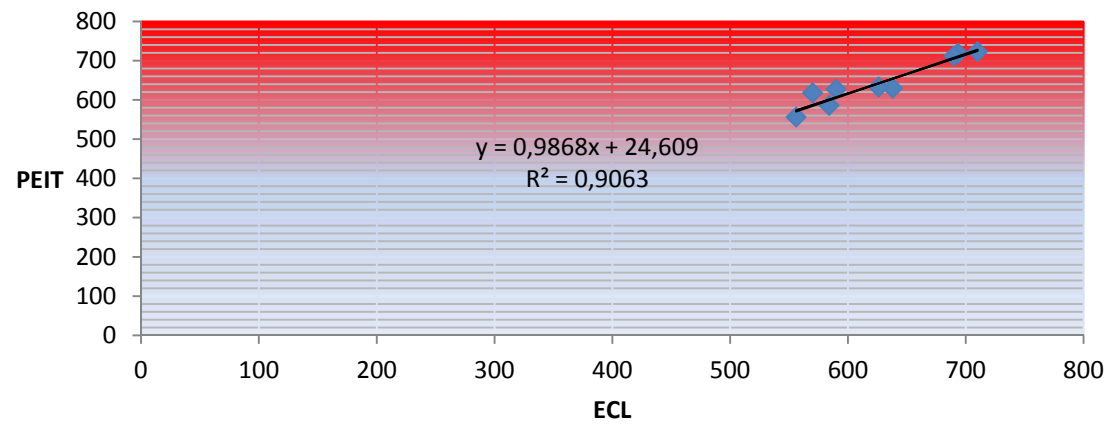




### Regression Coefficient ECL vs LTT In Conflicting Range



## Regression coefficient ECL vs PEIT In Higher Than Normal Range



## Comparing Reference Range Regression Coefficient At Different Level

Reference Range Interval Addressed	Regression Coefficient Value
Critical Range –ECL vs PEIT	0.845
Critical Range –ECL vs LTT	0.243
Critical Range –ECL vs PEIT Normal Monitoring Range	0.917
Critical Range –ECL vs LTT Normal Monitoring Range	0.813
Normal Range ECL vs PEIT	0.979
Normal Range ECL vs LTT	0.975
Conflicting Zone ECL vs PEIT	0.975
Conflicting Zone ECL vs LTT	0.979
Higher than Normal Range	0.906
Overall ECL vs PEIT	0.997
Overall ECL vs LTT	0.994

## Conclusion

Both the Version of Immunoturbidometric Analysis seem to be compatible to Each Other Other than Critical Range When Compared With ECL Results but there should be more Number Of Samples Analyzed For Such Conclusion In That Particular Range Of Reference Intervals

One Should Remember That Immunoturbidometric Reagents Results Needs Optimized Optical Systems And Proper Setting On Instruments For Compatible Results

Thank YOU