

## **Application guide for the Platelet function test by automated aggregation with Epinephrine (AG002K)**

This application guide describes the parameters and the procedure for measuring epinephrine-induced platelet aggregation, using the Epinephrine kit (AG002K) for optimal performance on Sysmex CS-5100. This application guide is based on the Sysmex CS-5100 system with the software version 00-13. This document is the only valid source of information for the assay application in the Sysmex CS-5100 system. HYPHEN BioMed has validated the instructions provided for the combination of reagents, instrument, software and customizable features of this system in order to optimize the performance of the product and comply with specifications. **The modification of these parameters may affect performance and results. If the application is modified, the user shall be responsible for the validation of the modifications and their impact on all the results.** For further information, please refer to the analyzer and reagents instruction manual. A specific configuration is required to perform the test.

### **1. Test Principle:**

When added to platelet-rich plasma (PRP), epinephrine binds to the  $\alpha_2$ -adrennergic receptors on the platelet surface, inducing an initial wave of reversible aggregation. This primary aggregation leads to exposure of fibrinogen receptors and inhibition of adenylate cyclase activity. A second wave of aggregation, clearly distinct from the first, is triggered by ADP release from  $\delta$  granules and by thromboxane  $A_2$  synthesis. Epinephrine is considered to be a weak agonist as the effects it produces are partial and reversible.

### **2. Test synopsis:**

140 $\mu$ L of platelet-poor plasma (PPP) or platelet-rich plasma (PRP) sample 20 $\mu$ L of <span style="border: 1px solid black; padding: 0 2px;">R1</span> diluted in saline solution Measurement 600 sec at 37°C with mixture OD at 660 nm	<b>Weak/Normal</b>
---	--------------------

### **3. Reagents and material required but not provided:**

- Distilled water
- Saline solution (0.9% NaCl)
- 4mL Cup (424-1160-8)
- SB Cuvette (064-1041-9) and SB Kit tool (063-4151-5)
- Laboratory materials and equipment

#### 4. Preparation and stability of reagents:

The stability must be verified and adjusted according to the exact laboratory working conditions.

Reagents	Preparation	Stability				Position on board	Name of reagent	Reagent ID
		2-8°C <sup>[1]</sup>	18-25°C <sup>[1]</sup>	-20°C <sup>[2]</sup>	On board			
Epinephrine 40 µmol/L	Reconstitute <b>R1</b> with exactly 0.625 mL of distilled water. Shake vigorously until complete dissolution. Allow to stabilize 30 min at 18-25°C shaking the vial occasionally.	7 days	24h	2 months	NA	Reagents Zone <sup>[3]</sup>	Epi Epi2 <sup>[4]</sup> Epi3 <sup>[4]</sup> Epi4 <sup>[4]</sup> Epi5 <sup>[4]</sup>	i8 EU EV EW EX
	Dilute to 1/20 <b>R1</b> reconstituted in saline solution (1 vol. <b>R1</b> , 19 vol. saline solution)	NA	NA	NA	10h			
Saline solution	Ready to use	NA	NA	NA	NA	Reagents Zone <sup>[3]</sup>	Sal.PPP	FG

<sup>[1]</sup> On a closed vial, free of any contamination or evaporation <sup>[2]</sup> Freeze rapidly after stabilization (see freezing/thawing recommendations for lyophilized HYPHEN BioMed reagents). <sup>[3]</sup> It is recommended to transfer to 4 mL cup. <sup>[4]</sup> For multiple concentrations. Do not use a non-validated concentration.

#### 5. Recommendations:

**Homogenize well** the reagents **prior to each use**. To obtain a homogeneous reactivity, it is essential to allow the temperature of the reagents **stabilize for at least 30 minutes on board the analyzer** prior to any use. **Do not interchange reagent vials from different batches.**

Quality control tests must be performed regularly, and with each change of reagent batch, after any major maintenance work on the analyzer, and when the results do not comply with the expected values for the method.

The performance may vary slightly depending on the analyzer used. Each laboratory may establish its own acceptance range.

#### 6. Results:

- The results are expressed in % of maximum aggregation.
- It is recommended to make duplicate measurements.

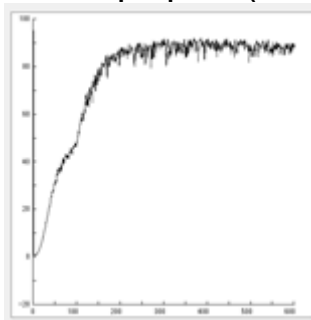
#### 7. Performance and characteristics:

##### Precision

Samples	Intra-assay		
	n	Mean (% maximum aggregation)	CV (%)
Normal	30	86.0	3.0
Abnormal	30	35.7	7.6

The acceptable variability (imprecision) must be such that the coefficient of variation (CV) of the analytical system on the same normal sample is less than 20% and the abnormal samples less than 30% for the maximum aggregation measurement (%).

**Aggregation curve with Epinephrine (For information only)**



**Range of measurement**

Measurement Principle	Analytical measurement range
Aggregation test (optical transmission aggregometry)	0-100% of maximum aggregation

**Expected values**

The values obtained on healthy subjects vary from one laboratory to another, each laboratory must determine its own reference ranges.

The reference range was established on health adult subjects (n=150) using Sysmex CS-5100, 2000*i*, 2100*i*, 2400, 2500 (Central 90%, 97.5<sup>th</sup> percentile).

The reference range measured is 68.8 – 99.8 % of maximum aggregation (median: 87.2% of maximum aggregation).

**Specificity**

The mean value measured out of 150 normal platelet-rich plasmas is 87.0 % of the maximum aggregation.

**Interferences**

No interference up to <sup>[1]</sup>		
Intralipids <sup>[2]</sup>	193 mg/dL	
Hemoglobin <sup>[3]</sup>	100 mg/dL	1 g/L
Bilirubin (Free)	28 mg/dL	479 µmol/L
Bilirubin (Conjugated)	28 mg/dL	336 µmol/L

<sup>[1]</sup>Intralipids and Interference Check.A Plus were used in Interference Studies. Clinical samples may exhibit different behavior because Intralipids and Interference Check.A Plus are artificial materials.

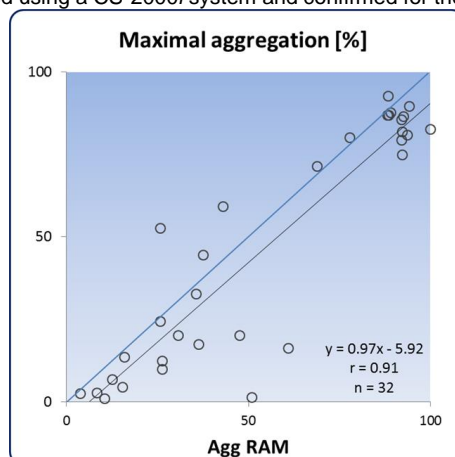
Interference Studies have been established using the CS-2000*i* and 5100 System.

<sup>[2]</sup>Platelet Poor Plasma (PPP) samples with high intralipids levels may give an error code [7030.0000.0000 / Ana\_PPP\_High1]. Accordingly, the associated result may give an error code [7030.0000.0000 / Ana\_PPP\_High1] and [7050.0000.0000 / Ana\_Tube\_Position]. The cause of the error code is excess turbidity.

<sup>[3]</sup>Refer to ISTH recommendation (Cattaneo, et al, 2013), "hemolyzed samples may affect platelet aggregation and should be discarded."

### Comparison of Methods:

The comparison of the methods was established using a CS-2000*i* system and confirmed for the CS-5100 system.



Epinephrine (Helena) on Helena AggRAM		vs	Epinephrine (HYPHEN BioMed) on Sysmex CS-2000 <i>i</i>	
Number of samples		n	32	
Linear regression			$y = 0.97x - 5.92$	
Coefficient of correlation		r	0.91	

## 8. Setup of test:

The special position may contain a maximum of 48 (24x2) cuvettes with magnetic stir bar (SBC) to be inserted with the SBC tool.

Place the PPP and PRP on the sample rack side by side in the order PPP and then PRP. Thus, the PPPs will be in odd numbered positions while the PRP will be in the even numbered positions. Touch the agonist button to start the measurement on the PRP- Once the PPP and PRP analysis has ended, the instruments calculates the percentage of maximum aggregation (% Max aggregation) automatically.

ID Management	Tests	Comments
8000	PPP	Setup of test for PPP
8060	Epi	Setup of test for Epinephrine
8070	Epi2	Setup of test for multiple concentrations
8080	Epi3	
8090	Epi4	
8100	Epi5	

### To program the specific instrument configuration, you should:

**1- Select the instrument's English user language via:**

**Menu > Settings > System settings > Language**

**2- Program the specific configuration according to the settings described below, and check compliance by re-reading them.**

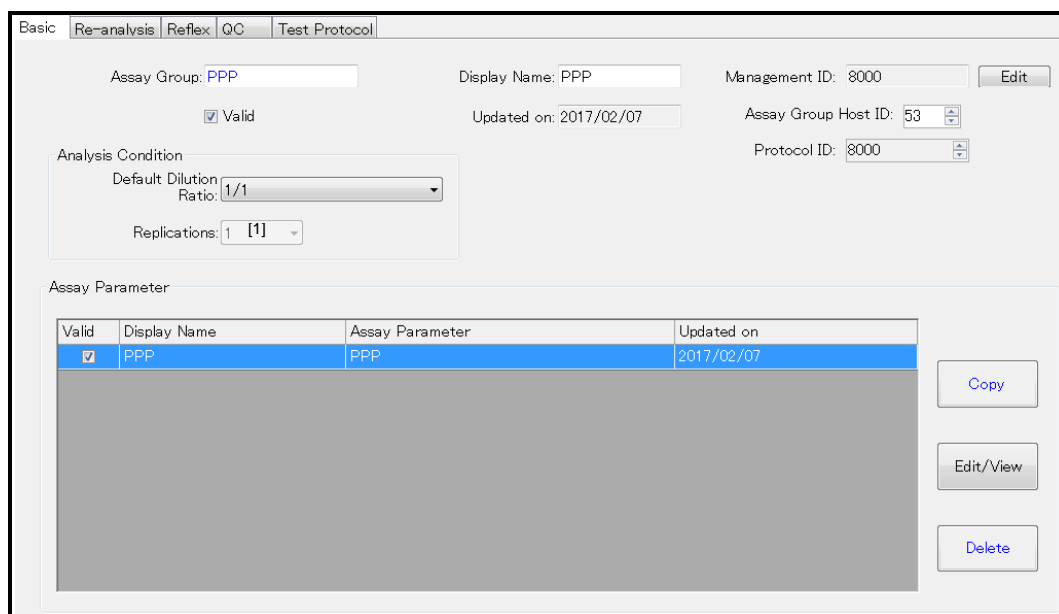
**3- Return to the usual laboratory language via:**

**Menu > Settings > System settings > Language**

## Assay Group Setup - [PPP]

▲ [Main Menu] - [Settings] - [Assay Group Settings] - [PPP]

► [Basic]



Valid	Display Name	Assay Parameter	Updated on
<input checked="" type="checkbox"/>	PPP	PPP	2017/02/07

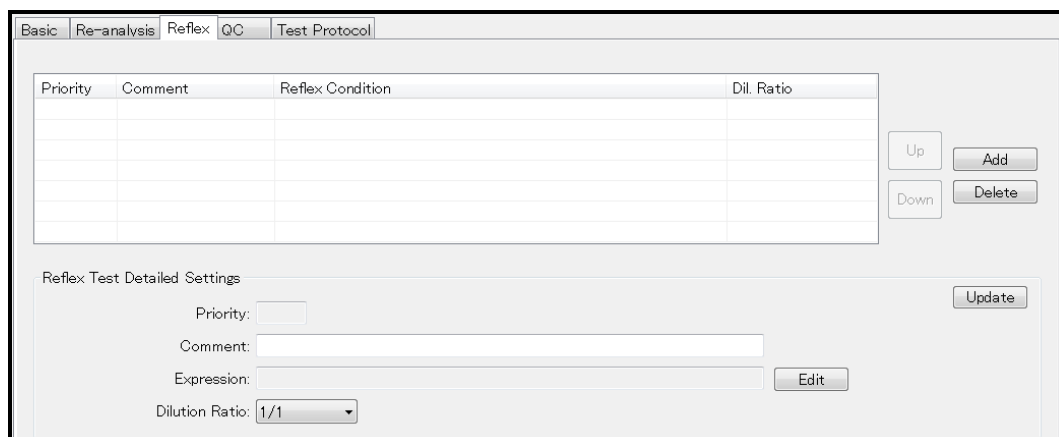
<sup>[1]</sup> It is recommended to perform the tests in duplicate.

► [Re-analysis]



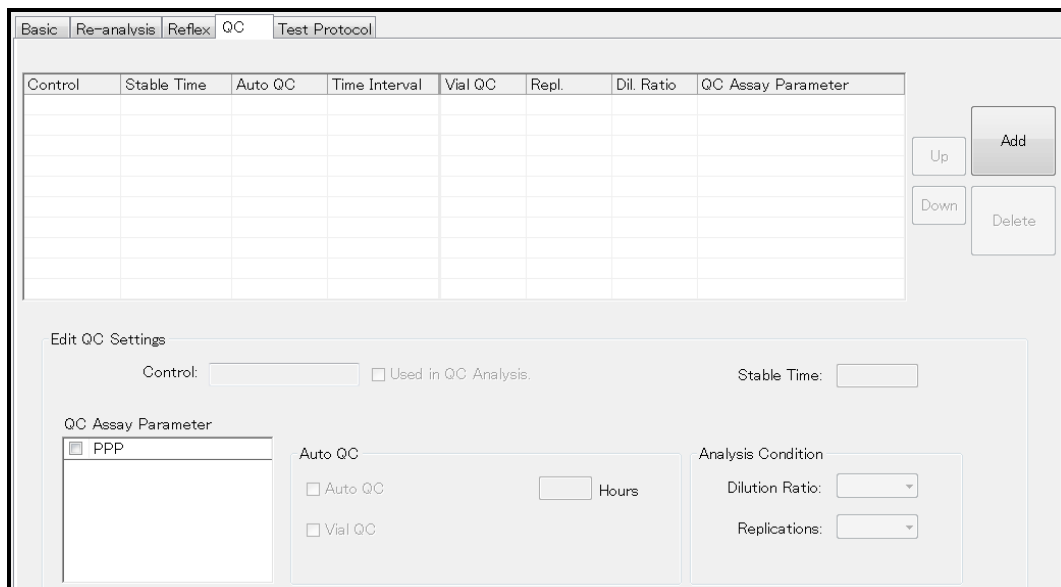
**NOTE:** Please adjust the parameters of the Re-analysis conditions, if necessary.

► [Reflex]



**NOTE:** This tab allows defining the Reflex options. Please adjust the parameters of the Reflex conditions, if necessary.

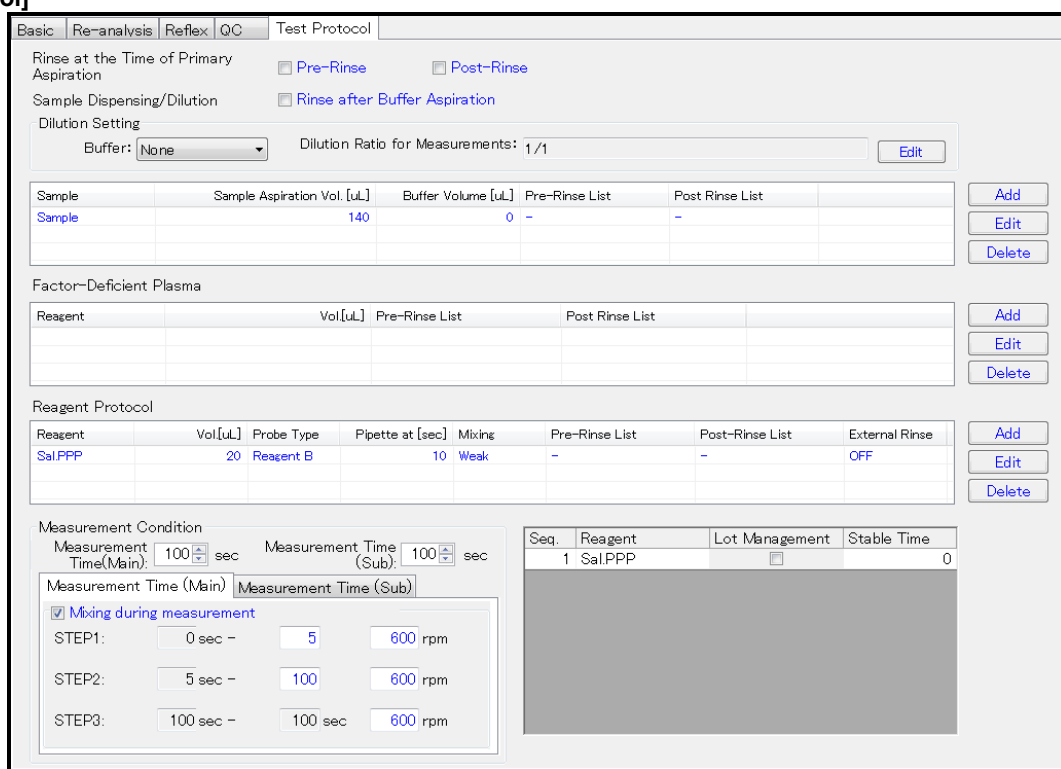
► [QC]



The QC tab interface includes a table with columns: Control, Stable Time, Auto QC, Time Interval, Vial QC, Repl., Dil. Ratio, and QC Assay Parameter. To the right of the table are buttons for 'Up', 'Down', 'Add', and 'Delete'. Below the table is the 'Edit QC Settings' section, which contains fields for 'Control', 'Used in QC Analysis', 'Stable Time', 'QC Assay Parameter' (with a dropdown for 'PPP'), 'Auto QC' (with checkboxes for 'Auto QC' and 'Vial QC'), 'Analysis Condition' (with dropdowns for 'Dilution Ratio' and 'Replications'), and a 'Hours' field.

**NOTE:** This tab allows defining the QC options. Please adjust the parameters of the QC conditions, if necessary.

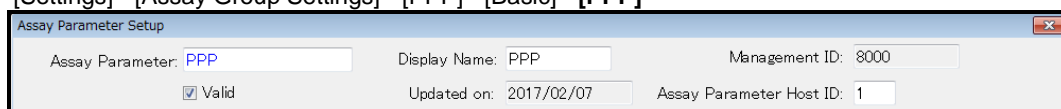
► [Test Protocol]



The Test Protocol tab interface includes several sections: 'Rinse at the Time of Primary Aspiration' with checkboxes for 'Pre-Rinse' and 'Post-Rinse'; 'Sample Dispensing/Dilution' with a checkbox for 'Rinse after Buffer Aspiration'; 'Dilution Setting' with a 'Buffer' dropdown and 'Dilution Ratio for Measurements' field; a table for 'Sample' with columns for 'Sample', 'Sample Aspiration Vol. [uL]', 'Buffer Volume [uL]', 'Pre-Rinse List', and 'Post Rinse List'; a 'Factor-Deficient Plasma' section with a table for 'Reagent', 'Vol [uL]', 'Pre-Rinse List', and 'Post Rinse List'; a 'Reagent Protocol' section with a table for 'Reagent', 'Vol [uL]', 'Probe Type', 'Pipette at [sec]', 'Mixing', 'Pre-Rinse List', 'Post-Rinse List', and 'External Rinse'; and a 'Measurement Condition' section with fields for 'Measurement Time (Main)' and 'Measurement Time (Sub)', a 'Mixing during measurement' checkbox, and a table for 'STEP' settings (STEP1, STEP2, STEP3) with columns for 'Time', 'Speed', and 'RPM'. A 'Seq.' table is also present with columns for 'Seq.', 'Reagent', 'Lot Management', and 'Stable Time'.

**Assay Parameter Setup - [PPP]**

▲ [Main Menu] - [Settings] - [Assay Group Settings] - [PPP] - [Basic] - [PPP]



The Assay Parameter Setup window displays the following information: 'Assay Parameter: PPP', 'Display Name: PPP', 'Management ID: 8000', 'Valid' checkbox, 'Updated on: 2017/02/07', and 'Assay Parameter Host ID: 1'.

▶ **[Calculation Method]**

Calculation Method

Data Check

Evaluation Preset

Raw Data

Unit System: Absorbance

Units: mOD

Digits:

Integral Part 5

Decimal Part 0

Calib. Curve

Unit System: Absorbance

Units: mOD

Digits:

Integral Part 5

Decimal Part 0

Input Value:

Calib. Curve Type:

Interpolation Method:

☐ Auto Origin Generation

Axis

X Axis:

Y Axis:

Extrapolation

☐ Extrapolate
 Range:
 Min. X 0.80 - Max. X 1.20

Graph Axis

X Axis Min. : 0

X Axis Max. : 0

Y Axis Min. : 0

Y Axis Max. : 0

Dilution Analysis:

Correction Standard	Calibrator	Dilution R.	Rep.	Stable Time
<div> <div>Add</div> <div>Edit/View</div> <div>Delete</div> </div>				

► [Data Check]

Calculation Method	Data Check	Evaluation Preset
<div><input type="checkbox"/> Report Limit Check</div> <div>Upper Limit: <input type="text" value="0"/> [mOD]</div> <div>Lower Limit: <input type="text" value="0"/> [mOD]</div> <div><input type="checkbox"/> Replication Difference Limit Check</div> <div>Upper Limit: <input type="text" value="0"/> %</div> <div><input type="checkbox"/> MDA Slope Ratio Check</div> <div>Upper Limit: <input type="text" value="0.00"/></div> <div>Lower Limit: <input type="text" value="0.00"/></div> <div><input type="checkbox"/> Mark Limit Check</div> <div>Upper Limit: <input type="text" value="0"/> [mOD]</div> <div>Lower Limit: <input type="text" value="0"/> [mOD]</div>		

► [Evaluation Preset]  
▼ [Evaluation Parameter]

Calculation Method | Data Check | Evaluation Preset

Evaluation Algorithm: Platelet analysis | Detection Principle: Aggregation Method

Target  
Wave: 660nm | Type: Transmitted | Gain: Low

Smoothing  
☐ Reference  
☒ Median  
☐ Moving Average

Preceding Point: 10 | Following Point: 10  
Preceding Point: 4 | Following Point: 0

Correction  
☒ User  
A (Slope) B(Offset)  
1.00 0.0000

☒ Service  
A (Slope) B(Offset)  
1.00 0.0000

Micro Mode  
A (Slope) B(Offset)  
1.00 0.0000

☒ Reagent  
A (Slope) B(Offset)  
1.00 0.0000

Evaluation Parameter | Evaluation Check Parameter | Research Parameter

Smoothing  
☐ Max  
Preceding Point: 2 | Following Point: 2

☒ PPP  
StartTime[sec]: 40.0  
EndTime[sec]: 60.0  
Absorbance At[sec]: 60.0

☐ PRP  
☒ Start OD  
StartTime[sec]: 3.0  
EndTime[sec]: 10.0  
Absorbance At[sec]: 0.0

☐ Min OD  
StartTime[sec]: 3.0  
EndTime[sec]: 600.0

☐ End OD  
StartTime[sec]: 550.0  
EndTime[sec]: 600.0  
Absorbance At[sec]: 600.0

▼ [Evaluation Check Parameter]

Calculation Method | Data Check | Evaluation Preset

Evaluation Algorithm: Platelet analysis | Detection Principle: Aggregation Method

Target  
Wave: 660nm | Type: Transmitted | Gain: Low

Smoothing  
☐ Reference  
☒ Median  
☐ Moving Average

Preceding Point: 10 | Following Point: 10  
Preceding Point: 4 | Following Point: 0

Correction  
☒ User  
A (Slope) B(Offset)  
1.00 0.0000

☒ Service  
A (Slope) B(Offset)  
1.00 0.0000

Micro Mode  
A (Slope) B(Offset)  
1.00 0.0000

☒ Reagent  
A (Slope) B(Offset)  
1.00 0.0000

Evaluation Parameter | Evaluation Check Parameter | Research Parameter

High Light Limit: 4000  
Low Light Limit: 50

☒ PPP  
☒ High Level 1: 300

☐ PRP  
☒ Low Level 1: 300



▼ [Research Parameter]

Calculation Method | Data Check | Evaluation Preset

Evaluation Algorithm: Platelet analysis | Detection Principle: Aggregation Method

Target  
Wave: 660nm  
Type: Transmitted  
Gain: Low

Smoothing  
☐ Reference  
☒ Median  
☐ Moving Average

Preceding Point: 10 | Following Point: 10  
Preceding Point: 4 | Following Point: 0

Correction  
☒ User  
A (Slope): 1.00 | B (Offset): 0.0000

Evaluation Parameter | Evaluation Check Parameter | Research Parameter

PRP  
Lag Phase Time  
StartTime[sec]: 3.0 | EndTime[sec]: 600.0 | Drop[%]: 25.0  
VMax  
Result: Find absolute value  
StartTime[sec]: 2 | EndTime[sec]: 600 | regwin[sec]: 30  
direct: Decrease  
Abs. difference for 45[mOD]: 200  
Time difference for 45[sec]: 60

## Assay Group Setup - [Epi]

▲ [Main Menu] - [Settings] - [Assay Group Settings] - [Epi]

► [Basic]

Basic | Re-analysis | Reflex | QC | Test Protocol

Assay Group: Epi | Display Name: Epi | Management ID: 8060 | Edit

☒ Valid | Updated on: 2017/03/23 | Assay Group Host ID: 55 | Protocol ID: 8060

Analysis Condition  
Default Dilution Ratio: 1/1  
Replications: 1 [1]

Assay Parameter

Valid	Display Name	Assay Parameter	Updated on
<input checked="" type="checkbox"/>	Epi_min	Epi_min	2017/03/23
<input checked="" type="checkbox"/>	Epi_s	Epi_s	2017/03/23
<input type="checkbox"/>	Epi_e	Epi_e	2017/03/23

Copy  
Edit/View  
Delete

<sup>(1)</sup> It is recommended to perform the tests in duplicate.

► [Re-analysis]

[illegible]

**NOTE:** Please adjust the parameters of the Re-analysis conditions, if necessary.

► [Reflex]

Basic

Re-analysis

Reflex

QC

Test Protocol

Priority	Comment	Reflex Condition	Dil. Ratio

Up

Add

Down

Delete

Reflex Test Detailed Settings

Priority:

Comment:

Expression:

Dilution Ratio:

1/1

Update

Edit

**NOTE:** This tab allows defining the Reflex options. Please adjust the parameters of the Reflex conditions, if necessary.

► [QC]

Basic

Re-analysis

Reflex

QC

Test Protocol

Control	Stable Time	Auto QC	Time Interval	Vial QC	Repl.	Dil. Ratio	QC Assay Parameter

Up

Add

Down

Delete

Edit QC Settings

Control:

☐ Used in QC Analysis.

Stable Time:

QC Assay Parameter

☒ Epi\_min

☒ Epi\_s

Auto QC

☐ Auto QC

☐ Vial QC

Hours

Analysis Condition

Dilution Ratio:

Replications:

**NOTE:** This tab allows defining the QC options. Please adjust the parameters of the QC conditions, if necessary.

► [Test Protocol]

Basic | Re-analysis | Reflex | QC | Test Protocol

Rinse at the Time of Primary Aspiration ☐ Pre-Rinse ☐ Post-Rinse

Sample Dispensing/Dilution ☐ Rinse after Buffer Aspiration

Dilution Setting  
Buffer: None Dilution Ratio for Measurements: 1/1 Edit

Sample	Sample Aspiration Vol. [uL]	Buffer Volume [uL]	Pre-Rinse List	Post Rinse List
Sample	140	0	-	-

Add Edit Delete

Factor-Deficient Plasma

Reagent	Vol[uL]	Pre-Rinse List	Post Rinse List

Add Edit Delete

Reagent Protocol

Reagent	Vol[uL]	Probe Type	Pipette at [sec]	Mixing	Pre-Rinse List	Post-Rinse List	External Rinse
Epi	20	Reagent B	10	Weak	-	-	OFF

Add Edit Delete

Measurement Condition

Measurement Time(Main): 600 sec Measurement Time (Sub): 600 sec

Measurement Time (Main) Measurement Time (Sub)

☒ Mixing during measurement

STEP1: 0 sec - 5 800 rpm

STEP2: 5 sec - 100 800 rpm

STEP3: 100 sec - 600 sec 800 rpm

Seq.	Reagent	Lot Management	Stable Time
1	Epi	<input type="checkbox"/>	0

## Assay Parameter Setup - [Epi\_min]

▲ [Main Menu] - [Settings] - [Assay Group Settings] - [Epi] - [Basic] - [Epi\_min]

Assay Parameter Setup

Assay Parameter: Epi\_min Display Name: Epi\_min Management ID: 8060

☒ Valid Updated on: 2017/03/23 Assay Parameter Host ID: 1

► [Calculation Method]

Calculation Method | Data Check | Evaluation Preset

☒ Raw Data Unit System: Absorbance Units: mOD Digits: Integral Part 5 Decimal Part 0

☐ Calib. Curve Unit System: Absorbance Units: mOD Digits: Integral Part 5 Decimal Part 0

Input Value:  Calib. Curve Type:  Interpolation Method:  ☐ Auto Origin Generation

Axis  
X Axis:  Y Axis:

Extrapolation  
☐ Extrapolate Range: Min. X 0.80 Max. X 1.20

Graph Axis  
X Axis Min.: 0 X Axis Max.: 0 Y Axis Min.: 0 Y Axis Max.: 0

Dilution Analysis:

Correction Standard	Calibrator	Dilution R.	Rep.	Stable Time

Add Edit/View Delete

► [Data Check]

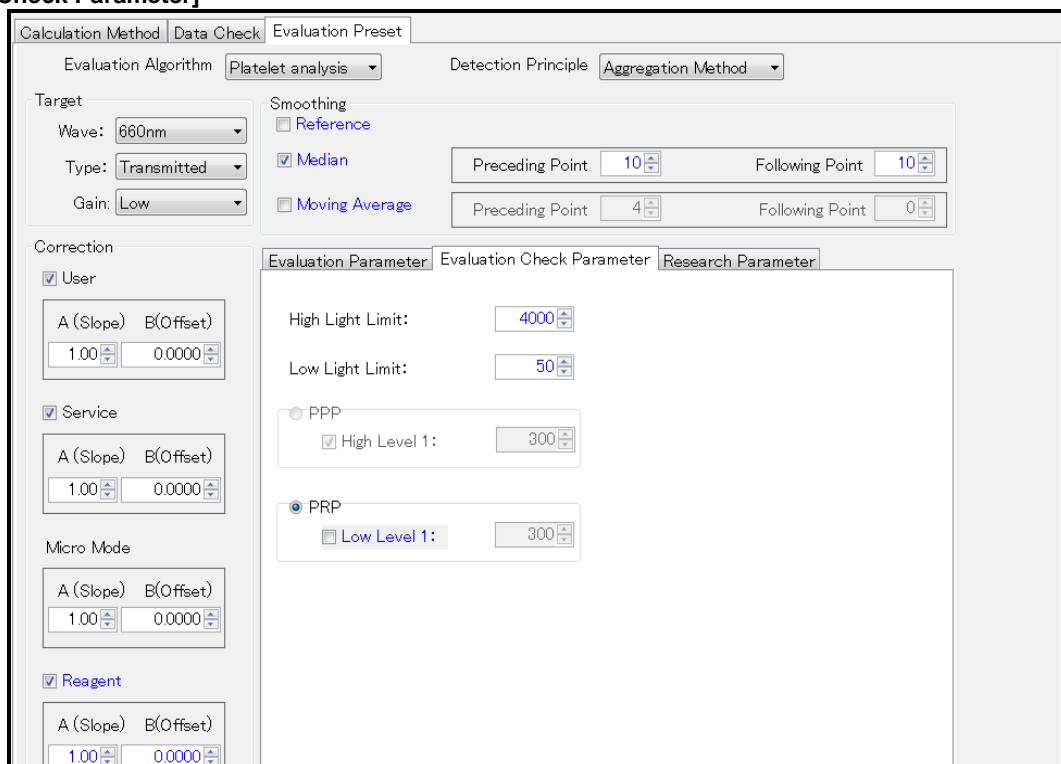
Calculation Method	Data Check	Evaluation Preset
<div> <input type="checkbox"/> Report Limit Check           <div>             Upper Limit: <input type="text" value="0"/> [mOD]              Lower Limit: <input type="text" value="0"/> [mOD]           </div> </div> <div> <input type="checkbox"/> Mark Limit Check           <div>             Upper Limit: <input type="text" value="0"/> [mOD]              Lower Limit: <input type="text" value="0"/> [mOD]           </div> </div>		
<div> <input type="checkbox"/> Replication Difference Limit Check           <div>             Upper Limit: <input type="text" value="0"/> %           </div> </div>		
<div> <input type="checkbox"/> MDA Slope Ratio Check           <div>             Upper Limit: <input type="text" value="0.00"/>              Lower Limit: <input type="text" value="0.00"/> </div> </div>		

► [Evaluation Preset]

▼ [Evaluation Parameter]

Calculation Method	Data Check	Evaluation Preset
Evaluation Algorithm: <b>Platelet analysis</b> Detection Principle: <b>Aggregation Method</b>		
<div> <div> <b>Target</b>            Wave: <b>660nm</b>            Type: <b>Transmitted</b>            Gain: <b>Low</b> </div> <div> <b>Smoothing</b>  <input type="checkbox"/> Reference  <input checked="" type="checkbox"/> Median  <input type="checkbox"/> Moving Average           </div> <div>             Preceding Point: <input type="text" value="10"/>      Following Point: <input type="text" value="10"/>              Preceding Point: <input type="text" value="4"/>      Following Point: <input type="text" value="0"/> </div> </div>		
<div> <div> <b>Correction</b>  <input checked="" type="checkbox"/> User  <div>           A (Slope) B(Offset)  <input type="text" value="1.00"/> <input type="text" value="0.0000"/> </div> </div> <div> <input checked="" type="checkbox"/> Service  <div>           A (Slope) B(Offset)  <input type="text" value="1.00"/> <input type="text" value="0.0000"/> </div> </div> <div> <input checked="" type="checkbox"/> Reagent  <div>           A (Slope) B(Offset)  <input type="text" value="1.00"/> <input type="text" value="0.0000"/> </div> </div> </div>		
<div> <b>Evaluation Parameter</b>    <b>Evaluation Check Parameter</b>    <b>Research Parameter</b> </div> <div> <b>Smoothing</b>  <input type="checkbox"/> Max      Preceding Point: <input type="text" value="2"/>      Following Point: <input type="text" value="2"/>  <input checked="" type="radio"/> PPP            StartTime[sec]: <input type="text" value="40.0"/>            EndTime[sec]: <input type="text" value="60.0"/>            Absorbance At[sec]: <input type="text" value="60.0"/>  <input checked="" type="radio"/> PRP  <div> <input checked="" type="radio"/> Start OD      <input checked="" type="radio"/> Min OD  <div>           StartTime[sec]: <input type="text" value="3.0"/>      StartTime[sec]: <input type="text" value="3.0"/>            EndTime[sec]: <input type="text" value="10.0"/>      EndTime[sec]: <input type="text" value="600.0"/>            Absorbance At[sec]: <input type="text" value="0.0"/>      EndTime[sec]: <input type="text" value="600.0"/> </div> </div> <div> <input type="radio"/> End OD            StartTime[sec]: <input type="text" value="550.0"/>            EndTime[sec]: <input type="text" value="600.0"/>            Absorbance At[sec]: <input type="text" value="600.0"/> </div> </div>		

### ▼ [Evaluation Check Parameter]



Calculation Method | Data Check | Evaluation Preset

Evaluation Algorithm: Platelet analysis | Detection Principle: Aggregation Method

Target: Wave: 660nm | Type: Transmitted | Gain: Low

Smoothing: ☐ Reference | ☒ Median | Preceding Point: 10 | Following Point: 10  
☐ Moving Average | Preceding Point: 4 | Following Point: 0

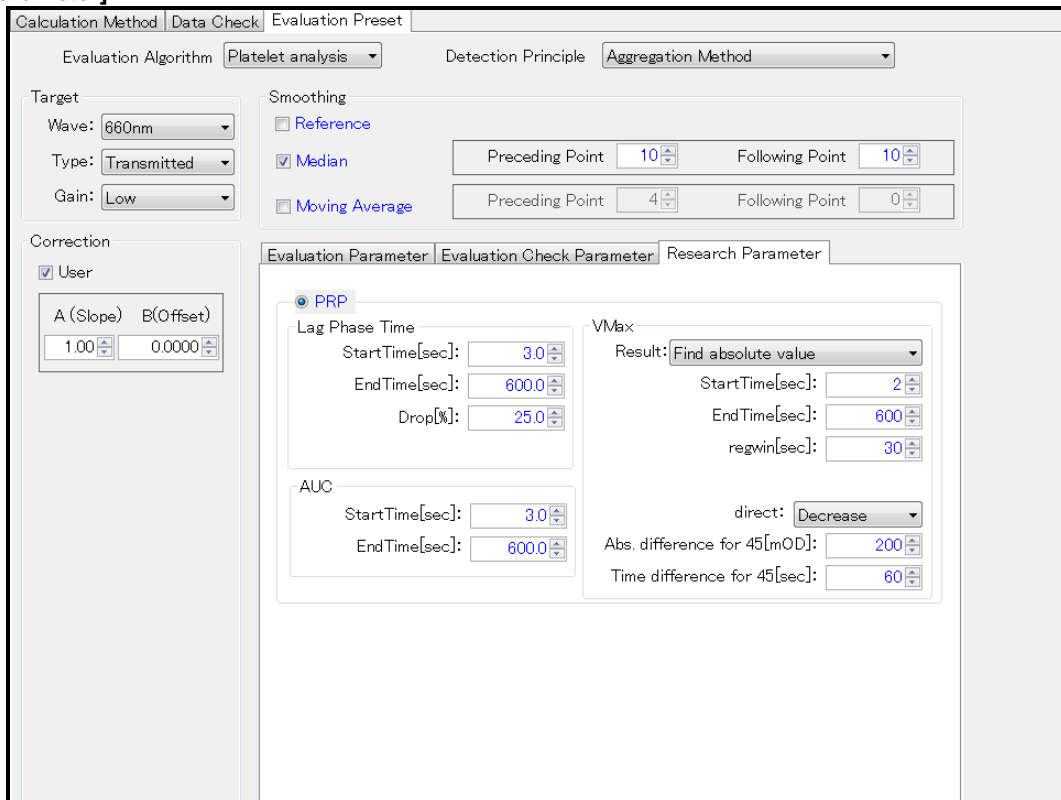
Correction: ☒ User  
 A (Slope) B(Offset): 1.00 | 0.0000  
☒ Service  
 A (Slope) B(Offset): 1.00 | 0.0000  
 Micro Mode  
 A (Slope) B(Offset): 1.00 | 0.0000  
☒ Reagent  
 A (Slope) B(Offset): 1.00 | 0.0000

Evaluation Parameter | Evaluation Check Parameter | Research Parameter

High Light Limit: 4000  
 Low Light Limit: 50

PPP: ☒ High Level 1: 300  
 PRP: ☒ Low Level 1: 300

### ▼ [Research Parameter]



Calculation Method | Data Check | Evaluation Preset

Evaluation Algorithm: Platelet analysis | Detection Principle: Aggregation Method

Target: Wave: 660nm | Type: Transmitted | Gain: Low

Smoothing: ☐ Reference | ☒ Median | Preceding Point: 10 | Following Point: 10  
☐ Moving Average | Preceding Point: 4 | Following Point: 0

Correction: ☒ User  
 A (Slope) B(Offset): 1.00 | 0.0000

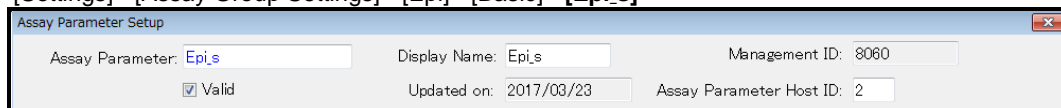
Evaluation Parameter | Evaluation Check Parameter | Research Parameter

PRP  
 Lag Phase Time  
 StartTime[sec]: 3.0 | EndTime[sec]: 600.0 | Drop[%]: 25.0  
 AUC  
 StartTime[sec]: 3.0 | EndTime[sec]: 600.0

VMax  
 Result: Find absolute value  
 StartTime[sec]: 2 | EndTime[sec]: 600 | regwin[sec]: 30  
 direct: Decrease  
 Abs. difference for 45[mOD]: 200  
 Time difference for 45[sec]: 60

## Assay Parameter Setup - [Epi.s]

▲ [Main Menu] - [Settings] - [Assay Group Settings] - [Epi] - [Basic] - [Epi.s]



Assay Parameter Setup

Assay Parameter: Epi.s | Display Name: Epi.s | Management ID: 8060  
☒ Valid | Updated on: 2017/03/23 | Assay Parameter Host ID: 2

► [Calculation Method]

Calculation Method | Data Check | Evaluation Preset

☒ Raw Data

Unit System: Absorbance Units: mOD Digits: Integral Part 5 Decimal Part 0

☐ Calib. Curve

Unit System: Absorbance Units: mOD Digits: Integral Part 5 Decimal Part 0

Input Value: Calib. Curve Type: Interpolation Method: ☐ Auto Origin Generation

Axis

X Axis: Y Axis:

Extrapolation

☐ Extrapolate Range: Min. X 0.80 - Max. X 1.20

Graph Axis

X Axis Min.: 0 X Axis Max.: 0 Y Axis Min.: 0 Y Axis Max.: 0

Dilution Analysis:

Correction Standard	Calibrator	Dilution R.	Rep.	Stable Time

Add  
Edit/View  
Delete

► [Data Check]

Calculation Method | Data Check | Evaluation Preset

☒ Report Limit Check

Upper Limit: 0 [mOD]  
Lower Limit: 0 [mOD]

☐ Mark Limit Check

Upper Limit: 0 [mOD]  
Lower Limit: 0 [mOD]

☐ Replication Difference Limit Check

Upper Limit: 0 %

☐ MDA Slope Ratio Check

Upper Limit: 0.00  
Lower Limit: 0.00

► [Evaluation Preset]  
▼ [Evaluation Parameter]

Calculation Method | Data Check | Evaluation Preset

Evaluation Algorithm: Platelet analysis | Detection Principle: Aggregation Method

Target  
Wave: 660nm | Type: Transmitted | Gain: Low

Smoothing  
☐ Reference  
☒ Median | Preceding Point: 10 | Following Point: 10  
☐ Moving Average | Preceding Point: 4 | Following Point: 0

Correction  
☒ User  
A (Slope) B(Offset)  
1.00 0.0000  
☒ Service  
A (Slope) B(Offset)  
1.00 0.0000  
Micro Mode  
A (Slope) B(Offset)  
1.00 0.0000  
☒ Reagent  
A (Slope) B(Offset)  
1.00 0.0000

Evaluation Parameter | Evaluation Check Parameter | Research Parameter

Smoothing  
☐ Max | Preceding Point: 2 | Following Point: 2  
☐ PPP  
StartTime[sec]: 40.0  
EndTime[sec]: 60.0  
Absorbance At[sec]: 60.0  
☒ PRP  
☒ Start OD  
StartTime[sec]: 3.0  
EndTime[sec]: 10.0  
Absorbance At[sec]: 0.0  
☐ Min OD  
StartTime[sec]: 3.0  
EndTime[sec]: 600.0  
☐ End OD  
StartTime[sec]: 550.0  
EndTime[sec]: 600.0  
Absorbance At[sec]: 600.0

▼ [Evaluation Check Parameter]

Calculation Method | Data Check | Evaluation Preset

Evaluation Algorithm: Platelet analysis | Detection Principle: Aggregation Method

Target  
Wave: 660nm | Type: Transmitted | Gain: Low

Smoothing  
☐ Reference  
☒ Median | Preceding Point: 10 | Following Point: 10  
☐ Moving Average | Preceding Point: 4 | Following Point: 0

Correction  
☒ User  
A (Slope) B(Offset)  
1.00 0.0000  
☒ Service  
A (Slope) B(Offset)  
1.00 0.0000  
Micro Mode  
A (Slope) B(Offset)  
1.00 0.0000  
☒ Reagent  
A (Slope) B(Offset)  
1.00 0.0000

Evaluation Parameter | Evaluation Check Parameter | Research Parameter

High Light Limit: 4000  
Low Light Limit: 50  
☐ PPP  
☒ High Level 1: 300  
☒ PRP  
☐ Low Level 1: 300

▼ [Research Parameter]

Calculation Method | Data Check | Evaluation Preset

Evaluation Algorithm: Platelet analysis | Detection Principle: Aggregation Method

Target  
Wave: 660nm | Type: Transmitted | Gain: Low

Smoothing  
☐ Reference  
☒ Median  
☐ Moving Average

Preceding Point: 10 | Following Point: 10  
Preceding Point: 4 | Following Point: 0

Correction  
☒ User  
A (Slope) B (Offset)  
1.00 | 0.0000

Evaluation Parameter | Evaluation Check Parameter | Research Parameter

PRP  
Lag Phase Time  
StartTime[sec]: 3.0 | EndTime[sec]: 600.0 | Drop[%]: 25.0  
AUC  
StartTime[sec]: 3.0 | EndTime[sec]: 600.0

VMax  
Result: Find absolute value  
StartTime[sec]: 2 | EndTime[sec]: 600 | regwin[sec]: 30  
direct: Decrease  
Abs. difference for 45[mOD]: 200  
Time difference for 45[sec]: 60

Assay Parameter Setup - [Epi.e]

▲ [Main Menu] - [Settings] - [Assay Group Settings] - [Epi] - [Basic] - [Epi.e]

Assay Parameter Setup

Assay Parameter: Epi.e | Display Name: Epi.e | Management ID: 8060  
☐ Valid | Updated on: 2017/03/23 | Assay Parameter Host ID: 3

► [Calculation Method]

Calculation Method | Data Check | Evaluation Preset

Raw Data  
Unit System: Absorbance | Units: mOD | Digits: Integral Part 5 | Decimal Part 0

Calib. Curve  
Unit System: Absorbance | Units: mOD | Digits: Integral Part 5 | Decimal Part 0  
Input Value: | Calib. Curve Type: | Interpolation Method: | Auto Origin Generation  
X Axis: | Y Axis: |  
Extrapolation  
☐ Extrapolate | Range: Min. X 0.80 | Max. X 1.20  
Graph Axis  
X Axis Min.: 0 | X Axis Max.: 0 | Y Axis Min.: 0 | Y Axis Max.: 0  
Dilution Analysis: |  
Correction Standard | Calibrator | Dilution R. | Rep. | Stable Time  
Add  
Edit/View  
Delete



► [Data Check]

Calculation Method | Data Check | **Evaluation Preset**

☐ Report Limit Check

Upper Limit:  [mOD]  
Lower Limit:  [mOD]

☐ Mark Limit Check

Upper Limit:  [mOD]  
Lower Limit:  [mOD]

☐ Replication Difference Limit Check

Upper Limit:  %

☐ MDA Slope Ratio Check

Upper Limit:   
Lower Limit:

► [Evaluation Preset]

▼ [Evaluation Parameter]

Calculation Method | Data Check | Evaluation Preset

Evaluation Algorithm: **Platelet analysis** | Detection Principle: **Aggregation Method**

Target  
Wave: **660nm**  
Type: **Transmitted**  
Gain: **Low**

Smoothing  
☐ Reference  
☒ Median  
☐ Moving Average  
Preceding Point:  | Following Point:   
Preceding Point:  | Following Point:

Correction  
☒ User  
A (Slope) B (Offset)  
   
☒ Service  
A (Slope) B (Offset)  
   
Micro Mode  
A (Slope) B (Offset)  
   
☒ Reagent  
A (Slope) B (Offset)

Evaluation Parameter | **Evaluation Check Parameter** | Research Parameter

Smoothing  
☐ Max  
Preceding Point:  | Following Point:

☒ PPP  
StartTime[sec]:   
EndTime[sec]:   
Absorbance At[sec]:

☒ PRP  
☒ Start OD  
StartTime[sec]:   
EndTime[sec]:   
Absorbance At[sec]:   
☒ End OD  
StartTime[sec]:   
EndTime[sec]:   
Absorbance At[sec]:   
☒ Min OD  
StartTime[sec]:   
EndTime[sec]:

### ▼ [Evaluation Check Parameter]

Calculation Method | Data Check | Evaluation Preset

Evaluation Algorithm: Platelet analysis | Detection Principle: Aggregation Method

Target  
Wave: 660nm | Type: Transmitted | Gain: Low

Smoothing  
☐ Reference  
☒ Median  
☐ Moving Average

Preceding Point: 10 | Following Point: 10  
Preceding Point: 4 | Following Point: 0

Correction  
☒ User  
A (Slope) B(Offset)  
1.00 0.0000

☒ Service  
A (Slope) B(Offset)  
1.00 0.0000

Micro Mode  
A (Slope) B(Offset)  
1.00 0.0000

☒ Reagent  
A (Slope) B(Offset)  
1.00 0.0000

Evaluation Parameter | Evaluation Check Parameter | Research Parameter

High Light Limit: 4000  
Low Light Limit: 50

PPP  
☒ High Level 1: 300

PRP  
☒ Low Level 1: 300

### ▼ [Research Parameter]

Calculation Method | Data Check | Evaluation Preset

Evaluation Algorithm: Platelet analysis | Detection Principle: Aggregation Method

Target  
Wave: 660nm | Type: Transmitted | Gain: Low

Smoothing  
☐ Reference  
☒ Median  
☐ Moving Average

Preceding Point: 10 | Following Point: 10  
Preceding Point: 4 | Following Point: 0

Correction  
☒ User  
A (Slope) B(Offset)  
1.00 0.0000

Evaluation Parameter | Evaluation Check Parameter | Research Parameter

PRP

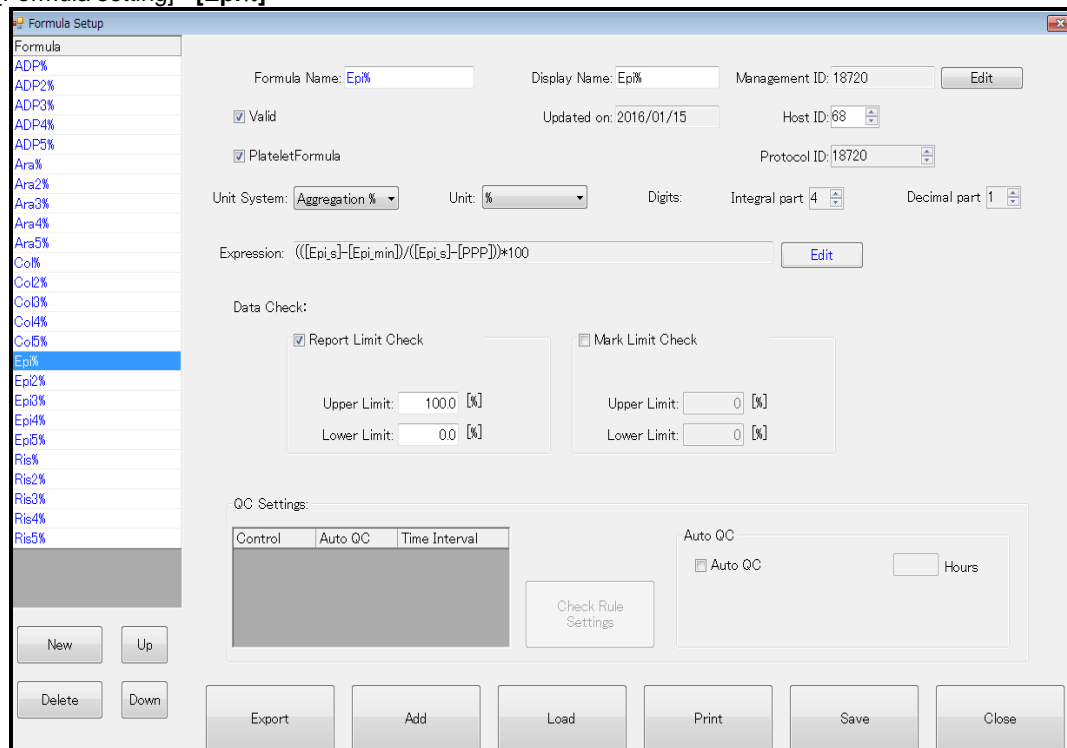
Lag Phase Time  
StartTime[sec]: 3.0  
EndTime[sec]: 600.0  
Drop[%]: 25.0

AUC  
StartTime[sec]: 3.0  
EndTime[sec]: 600.0

VMax  
Result: Find absolute value  
StartTime[sec]: 2  
EndTime[sec]: 600  
regwin[sec]: 30  
direct: Decrease  
Abs. difference for 45[mOD]: 200  
Time difference for 45[sec]: 60

## Formula setting - [Epi%]

[Main Menu] - [Formula setting] - [Epi%]



The screenshot shows the 'Formula Setup' window for the 'Epi%' formula. The left pane lists various formulas, with 'Epi%' selected. The main area contains the following settings:

- Formula Name:** Epi%
- Display Name:** Epi%
- Management ID:** 18720
- ☒ **Valid**
- Updated on:** 2016/01/15
- Host ID:** 68
- ☒ **PlateletFormula**
- Protocol ID:** 18720
- Unit System:** Aggregation %
- Unit:** %
- Digits:** Integral part 4, Decimal part 1
- Expression:**  $(([\text{Epi}_s] - [\text{Epi}_{\text{min}}]) / ([\text{Epi}_s] - [\text{PPP}])) * 100$
- Data Check:**
  - ☒ **Report Limit Check**
    - Upper Limit: 100.0 [%]
    - Lower Limit: 0.0 [%]
  - ☐ **Mark Limit Check**
    - Upper Limit: 0 [%]
    - Lower Limit: 0 [%]
- QC Settings:**
  - Control, Auto QC, Time Interval
  - Auto QC: ☐ Auto QC, [ ] Hours
  - Check Rule Settings

Buttons at the bottom include: New, Up, Delete, Down, Export, Add, Load, Print, Save, Close.

## Formula

Management ID	Assay Parameter	Formula
18720	Epi%	$(([\text{Epi}_s] - [\text{Epi}_{\text{min}}]) / ([\text{Epi}_s] - [\text{PPP}])) * 100$
18721	Epi2%	$(([\text{Epi2}_s] - [\text{Epi2}_{\text{min}}]) / ([\text{Epi2}_s] - [\text{PPP}])) * 100$
18722	Epi3%	$(([\text{Epi3}_s] - [\text{Epi3}_{\text{min}}]) / ([\text{Epi3}_s] - [\text{PPP}])) * 100$
18723	Epi4%	$(([\text{Epi4}_s] - [\text{Epi4}_{\text{min}}]) / ([\text{Epi4}_s] - [\text{PPP}])) * 100$
18724	Epi5%	$(([\text{Epi5}_s] - [\text{Epi5}_{\text{min}}]) / ([\text{Epi5}_s] - [\text{PPP}])) * 100$