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 α_2-adrinergic 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 δ 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 µL of platelet-poor plasma (PPP) or platelet-rich plasma (PRP) sample 20 µL of R1 diluted in saline solution Measurement 600 sec at 37°C with mixture OD at 660 nm	**Weak/Normal**

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[1]	18-25°C [1]	-20°C [2]	On board			
Epinephrine 40 µmol/L	Reconstitute R1 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[3]	Epi Epi2[4] Epi3[4] Epi4[4] Epi5[4]	i8 EU EV EW EX
	Dilute to 1/20 R1 reconstituted in saline solution (1 vol. R1 , 19 vol. saline solution)	NA	NA	NA	10h			
Saline solution	Ready to use	NA	NA	NA	NA	Reagents Zone[3]	Sal.PPP	FG

[1]On a closed vial, free of any contamination or evaporation [2]Freeze rapidly after stabilization (see freezing/thawing recommendations for lyophilized HYPHEN BioMed reagents). [3] It is recommended to transfer to 4 mL cup. [4] 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 inter-change 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.

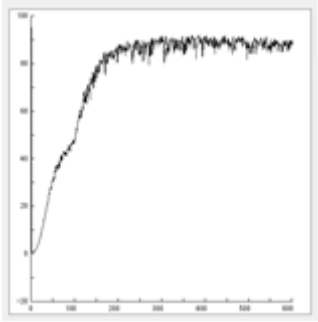
7. Performance and characteristics:

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%, 97.5th percentile).
The reference range measured is 68.8 – 99.8 % of maximum aggregation (median: 87.2% of maximum aggregation).

Specificity

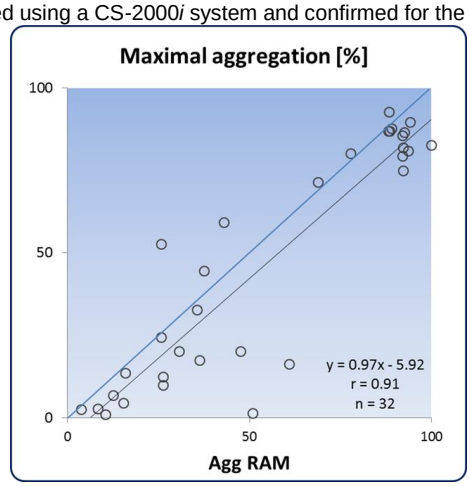
The mean value measured out of 150 normal platelet-rich plasmas is 87.0 % of the maximum aggregation.

Interferences

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

[1]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.
[2]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.
[3]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*
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

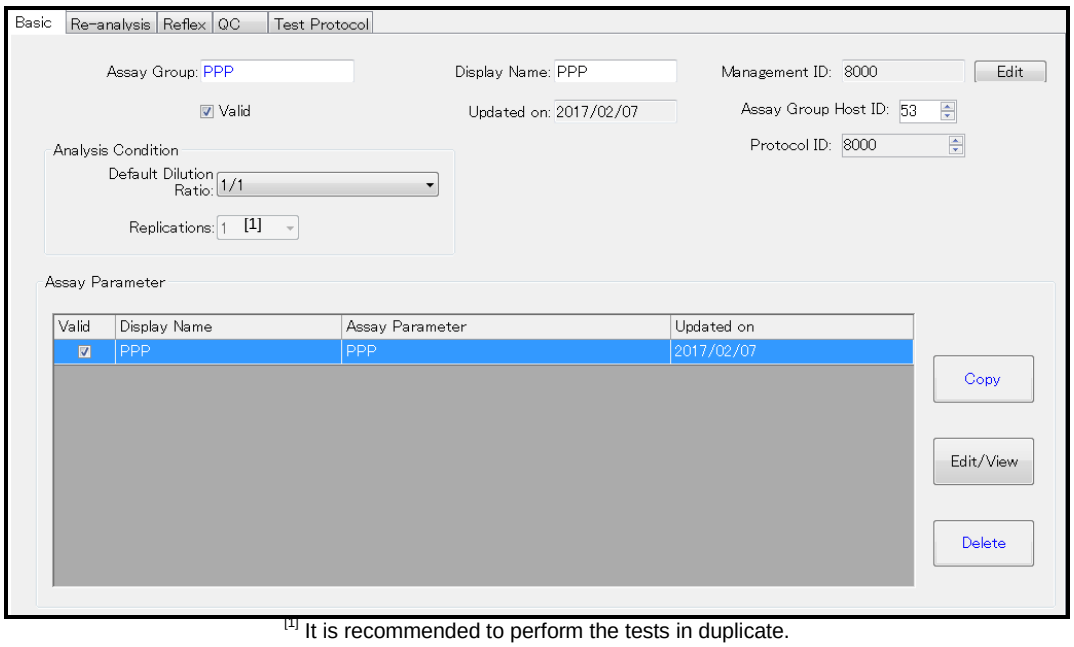
2- Program the specific configuration according to the settings described below, and check compliance by re-reading them.

3- Return to the usual laboratory language via:
Menu > Settings > System settings > Language

Assay Group Setup - [PPP]

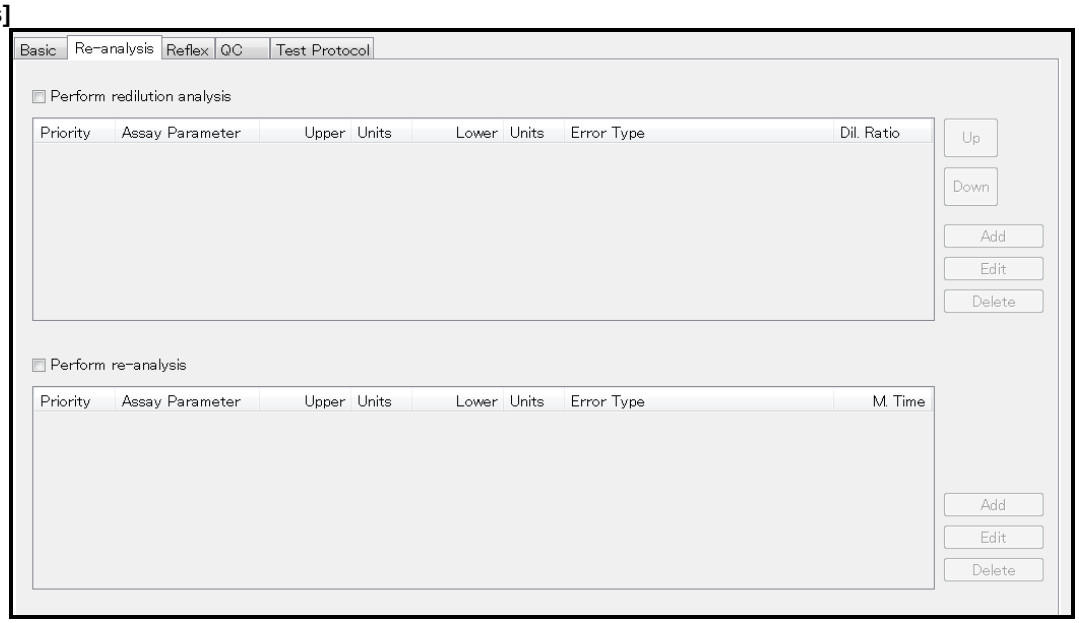
▲ [Main Menu] - [Settings] - [Assay Group Settings] - **[PPP]**

► **[Basic]**



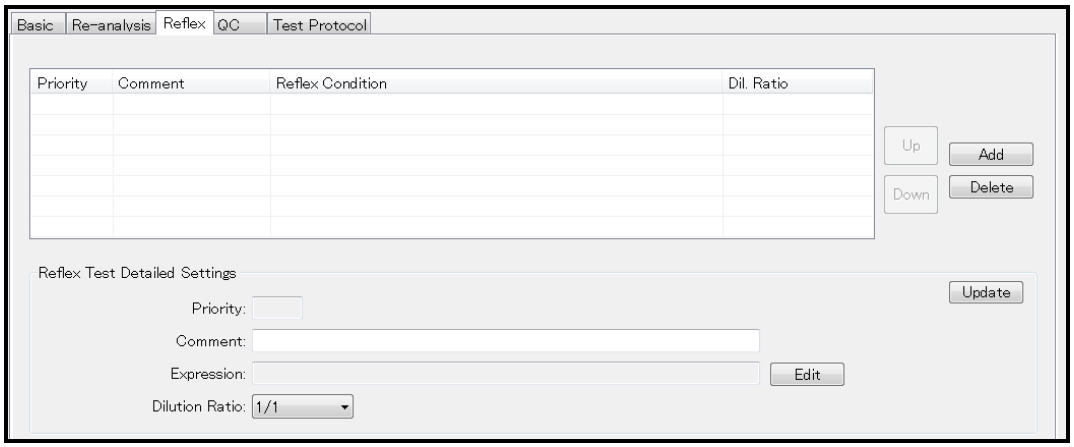
[1] It is recommended to perform the tests in duplicate.

► **[Re-analysis]**



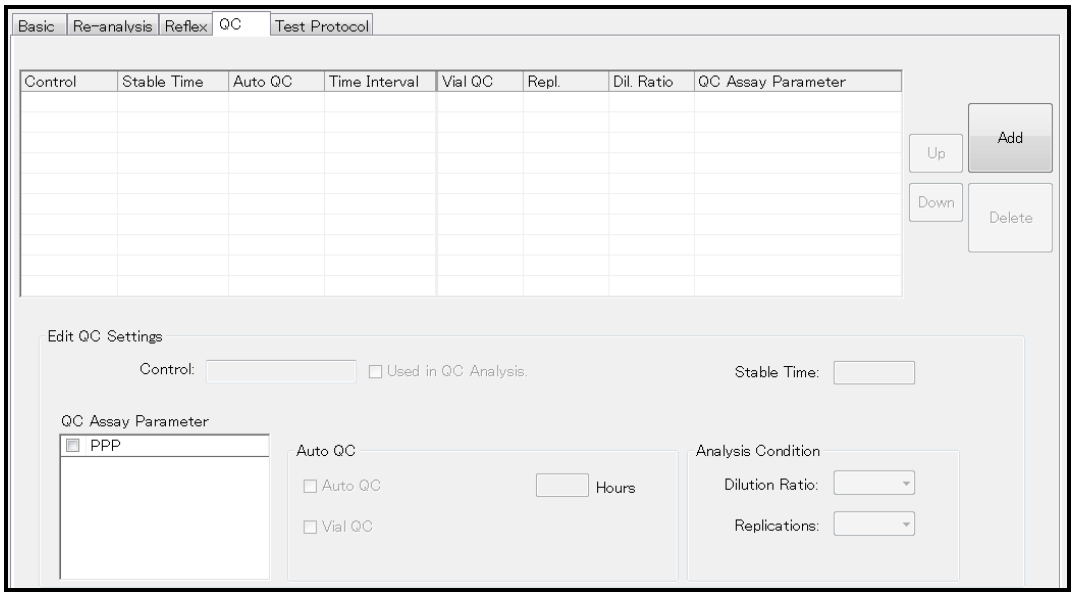
NOTE: Please adjust the parameters of the Re-analysis conditions, if necessary.

► **[Reflex]**



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

► **[QC]**



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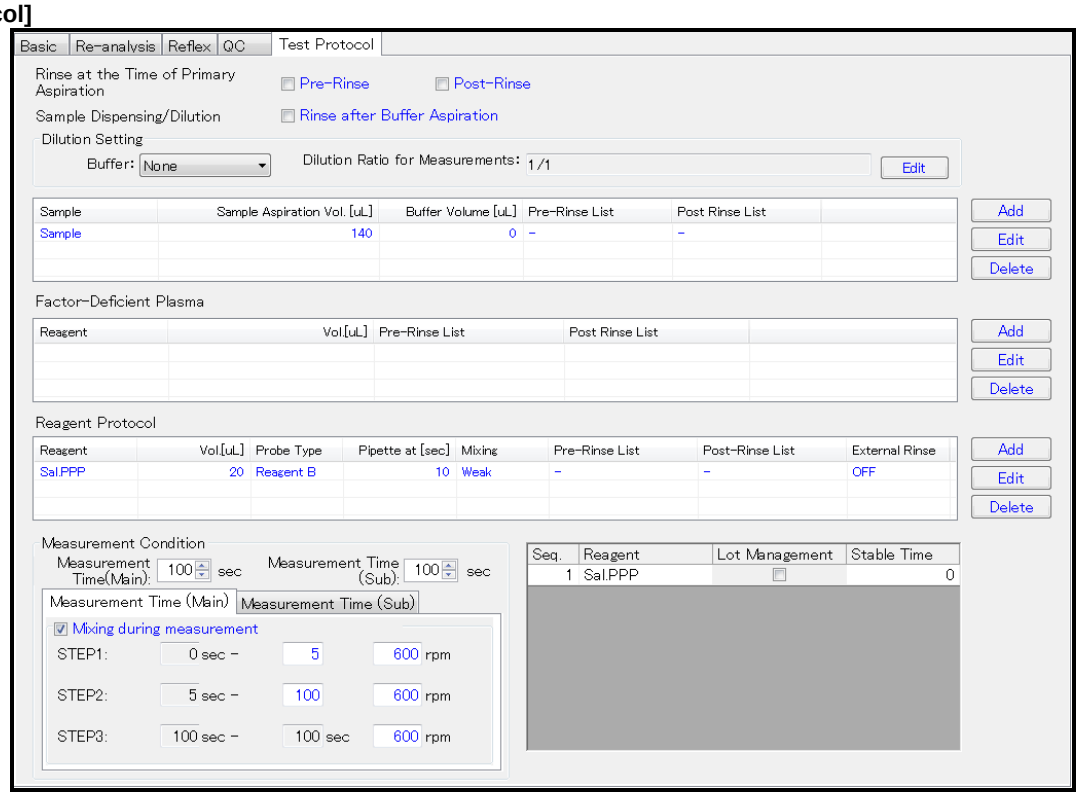

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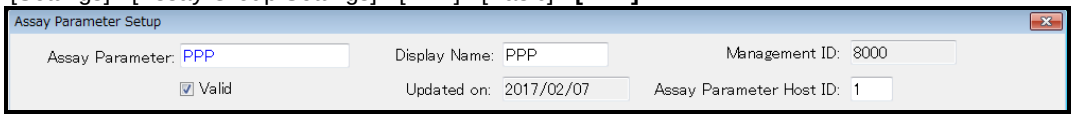

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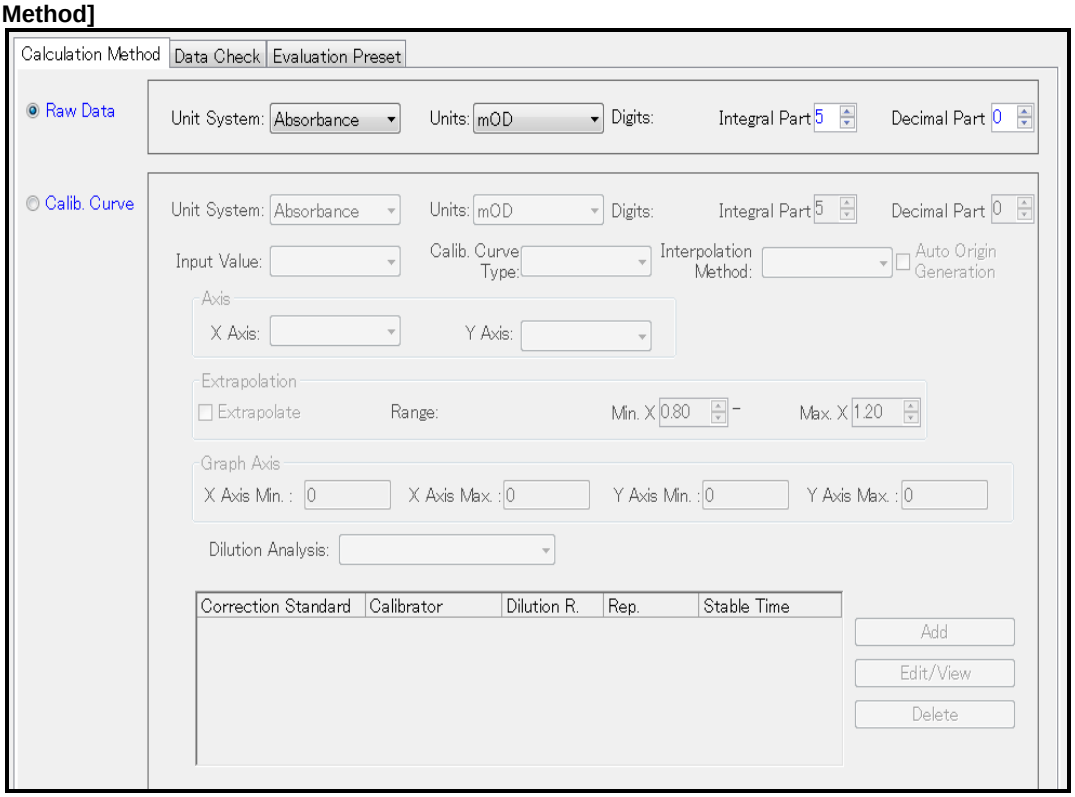

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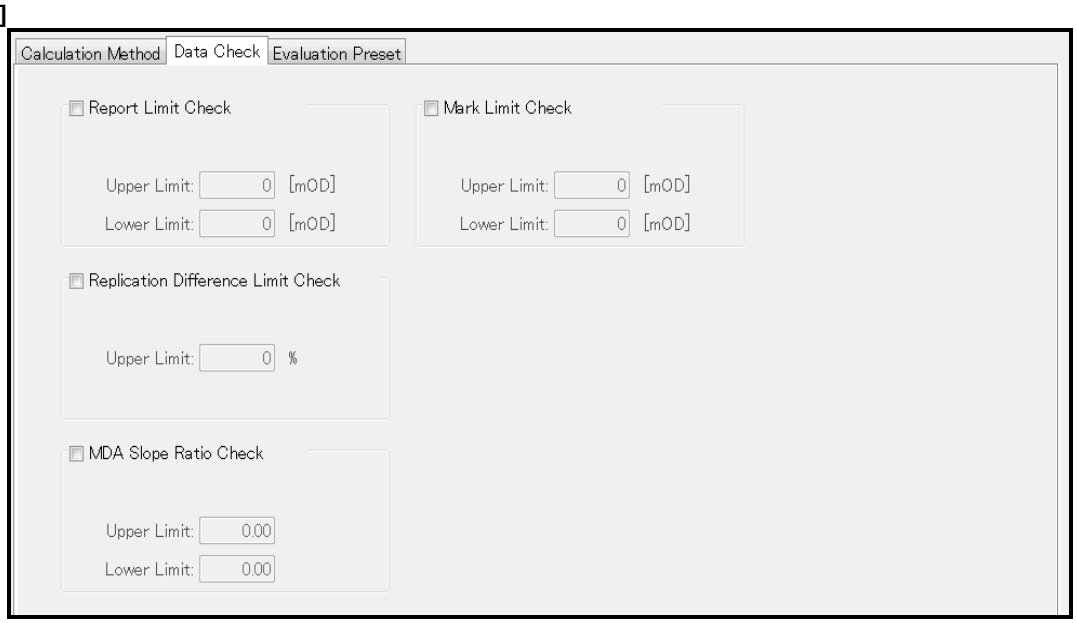

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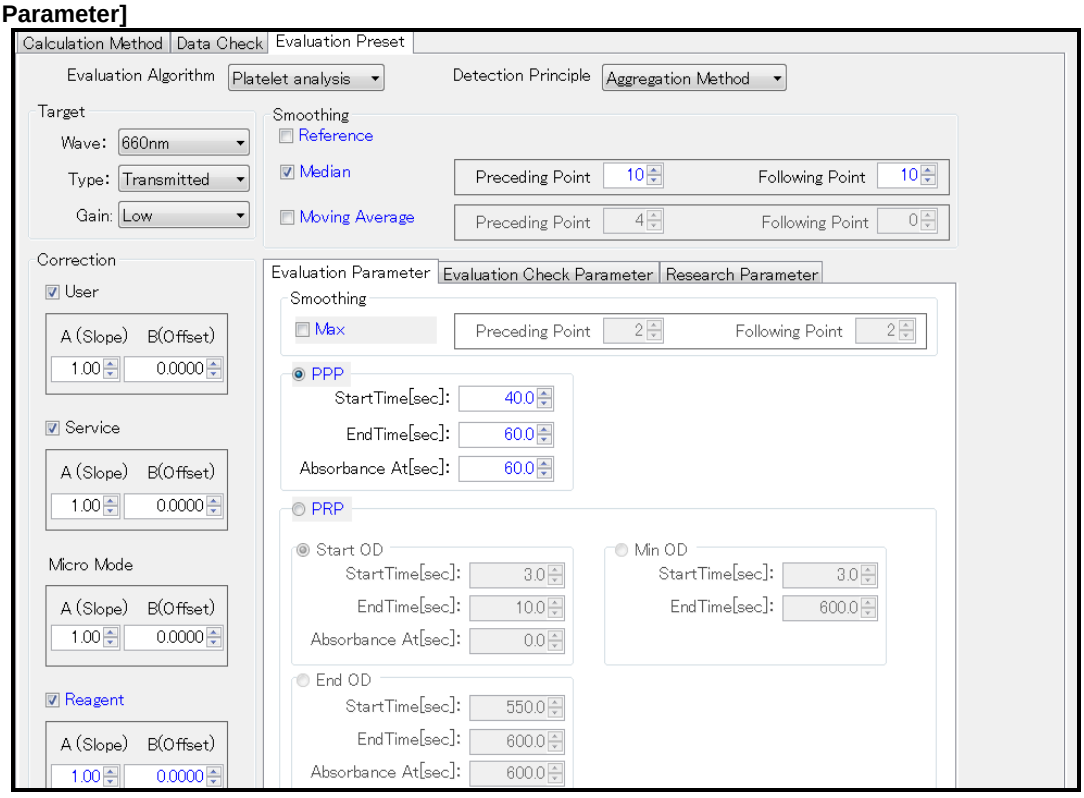
▼ **[Evaluation Parameter]**



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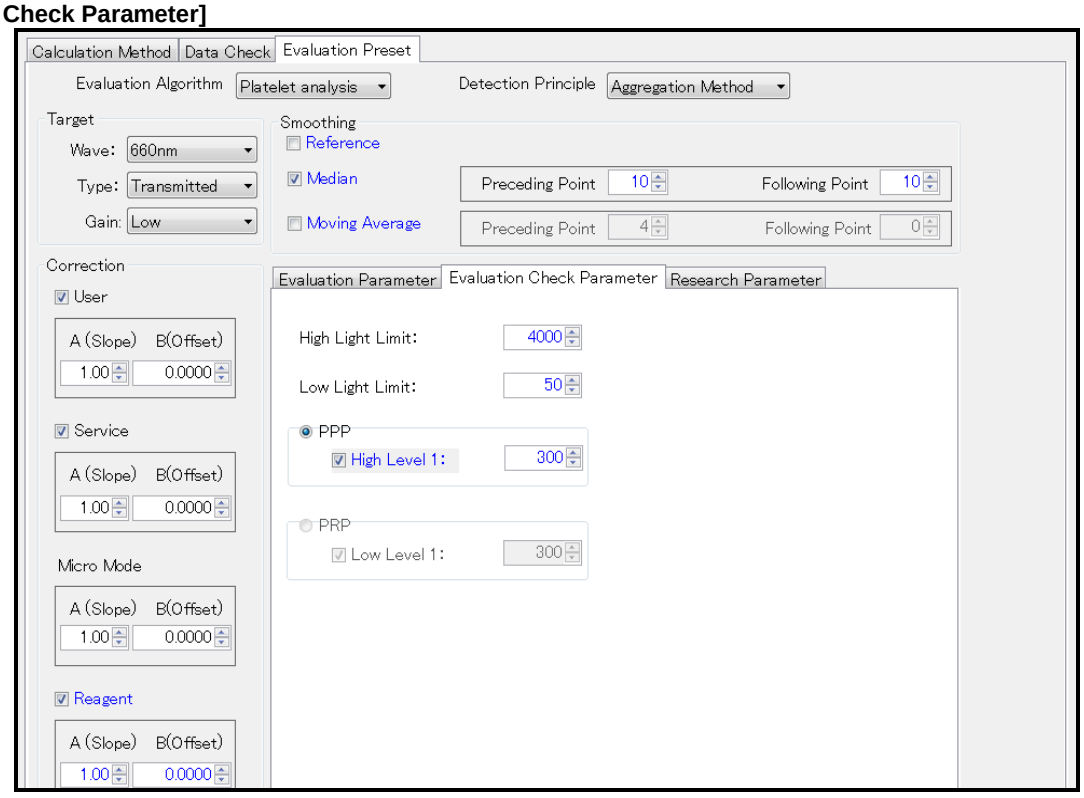

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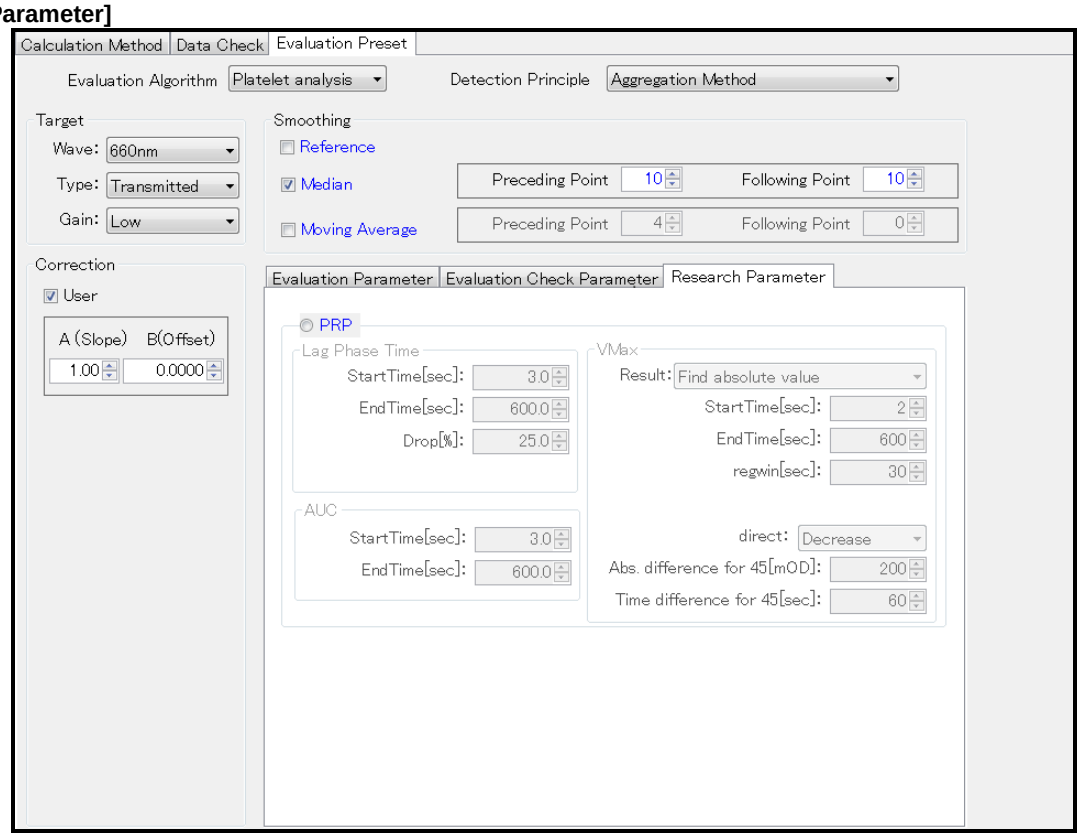

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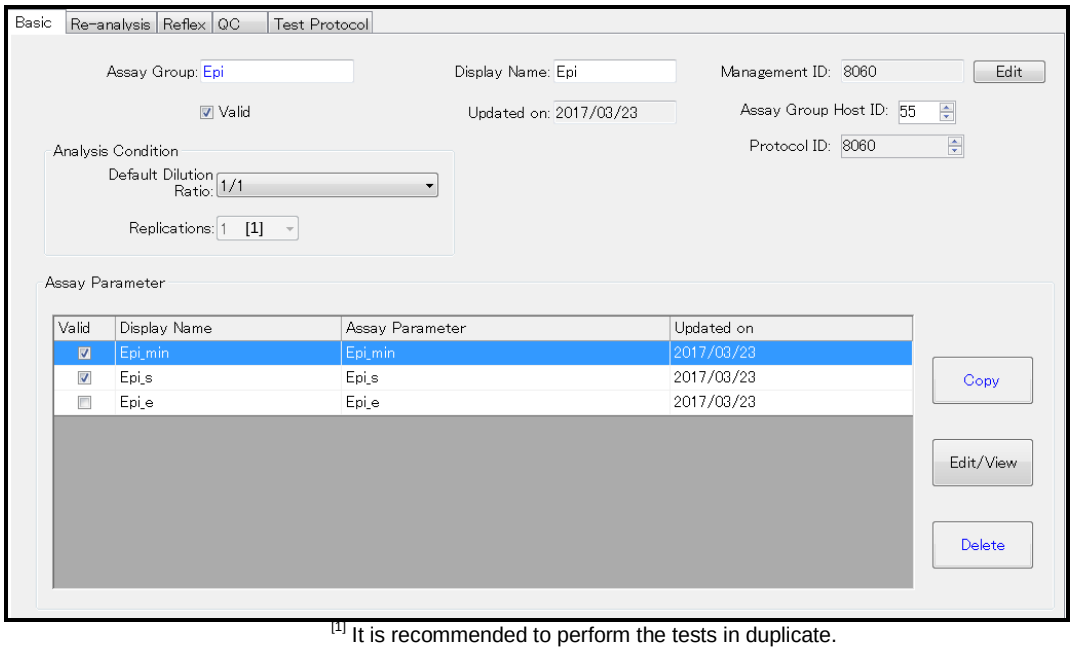
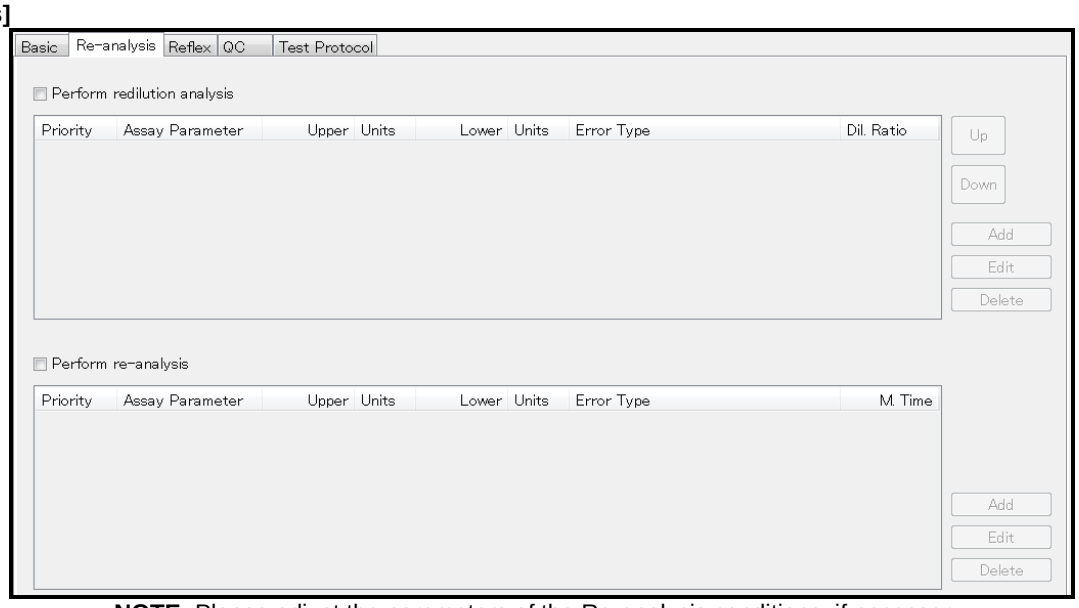


[1] It is recommended to perform the tests in duplicate.

► **[Re-analysis]**



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

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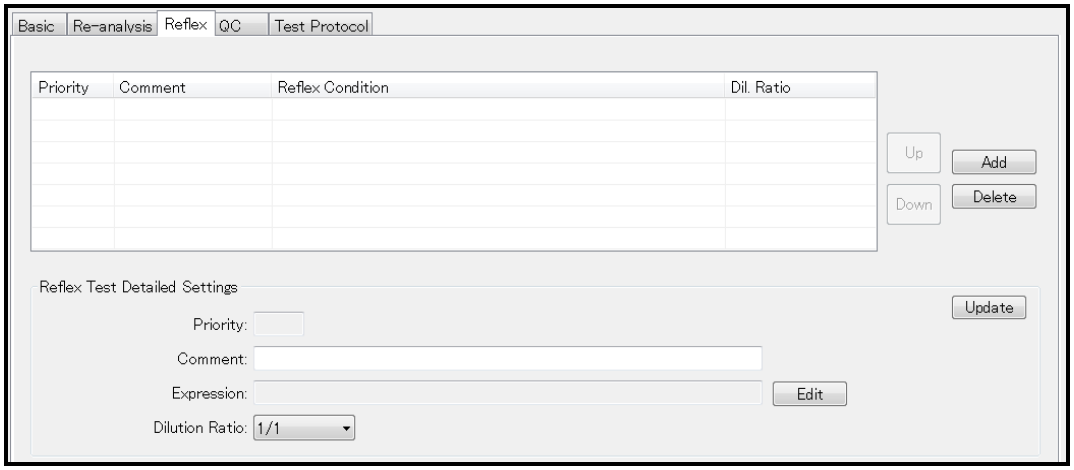

NOTE: This tab allows defining the Reflex options. Please adjust the parameters of the Reflex conditions, if necessary.

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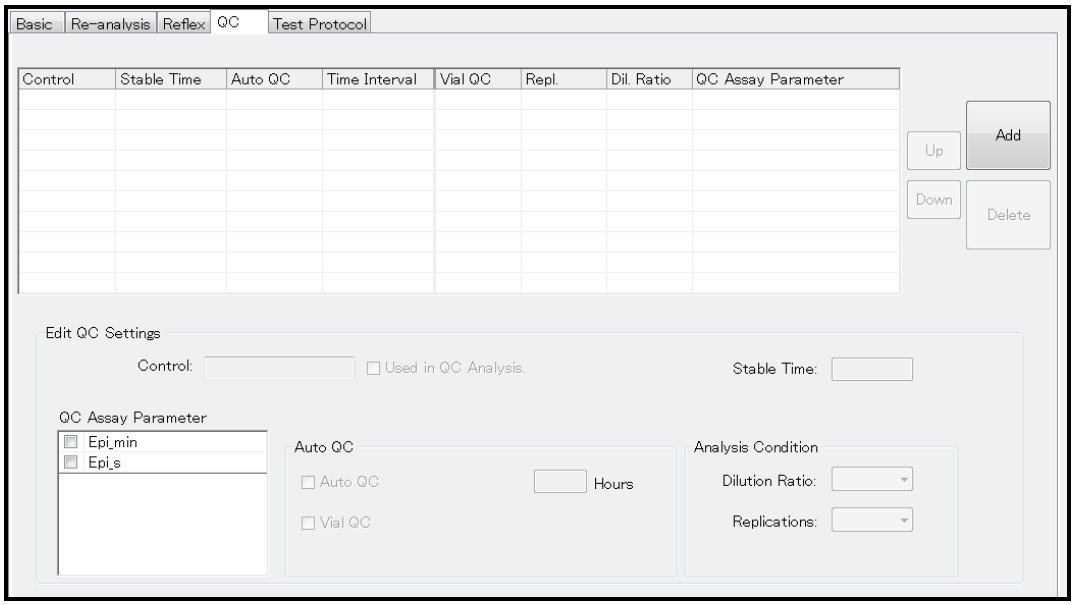

NOTE: This tab allows defining the QC options. Please adjust the parameters of the QC conditions, if necessary.

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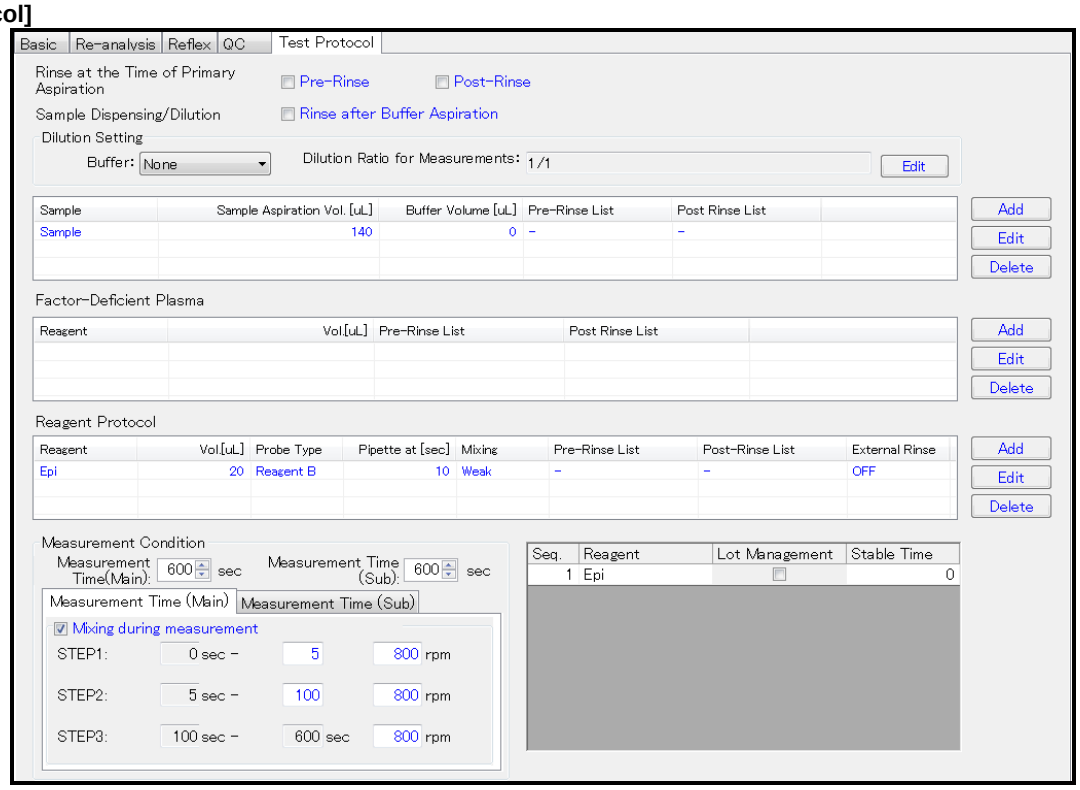

Assay Parameter Setup - [Epi_min]

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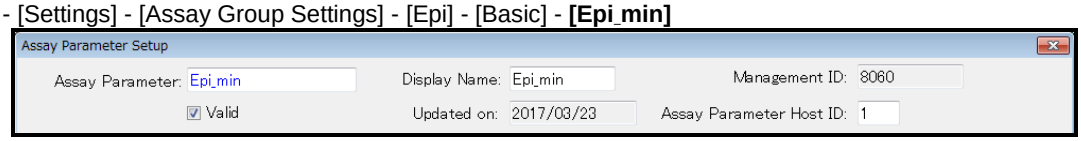

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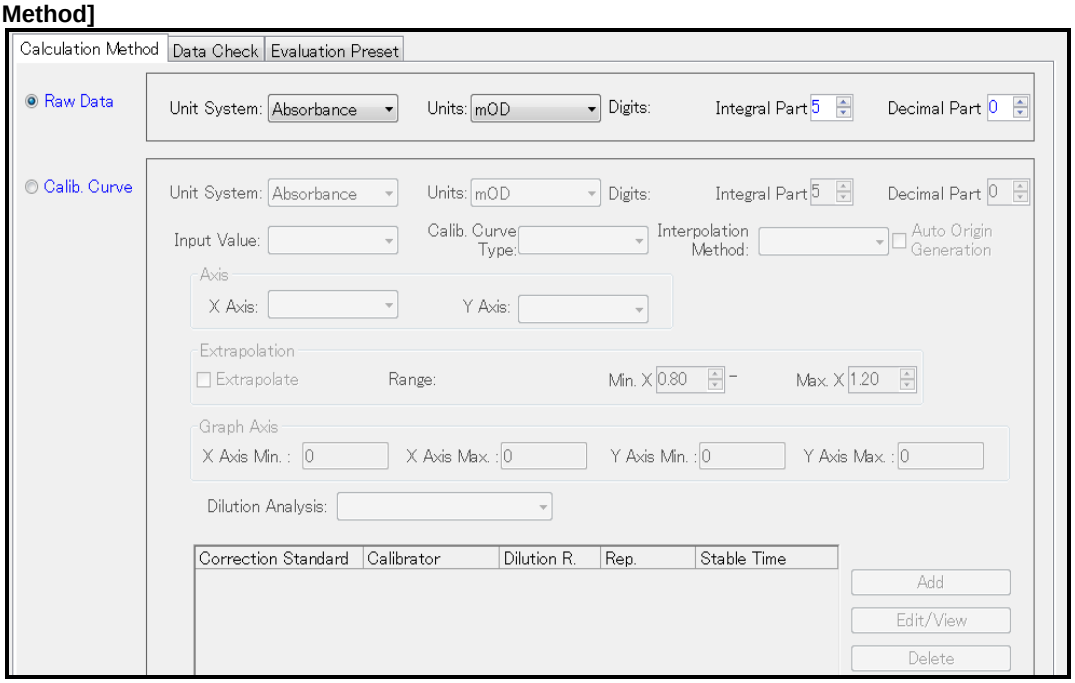

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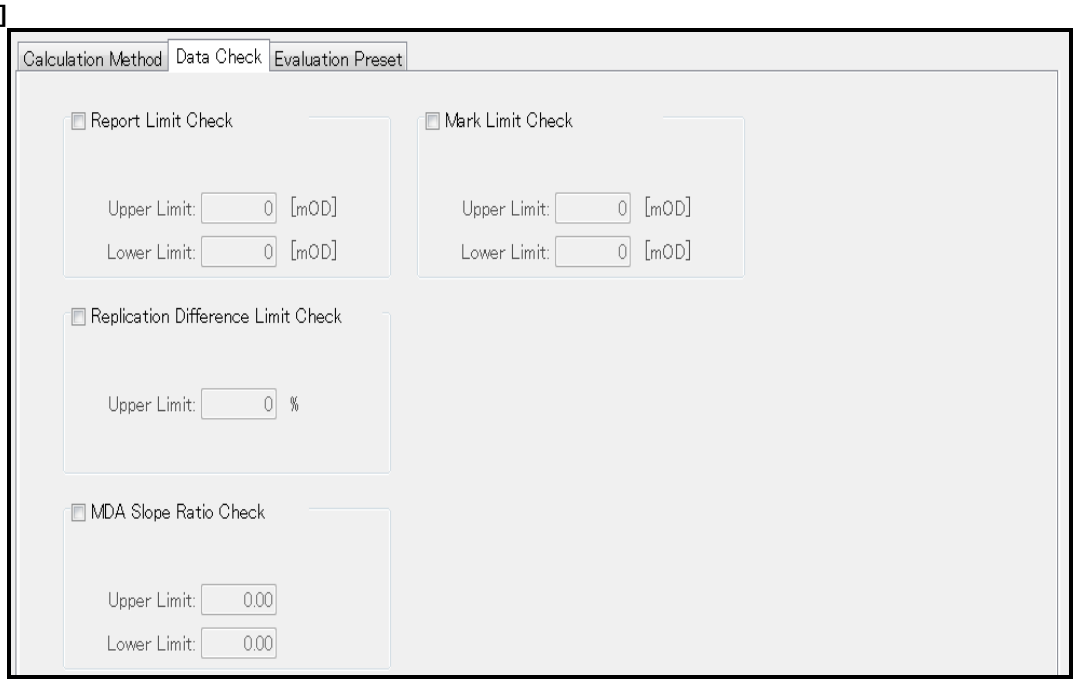

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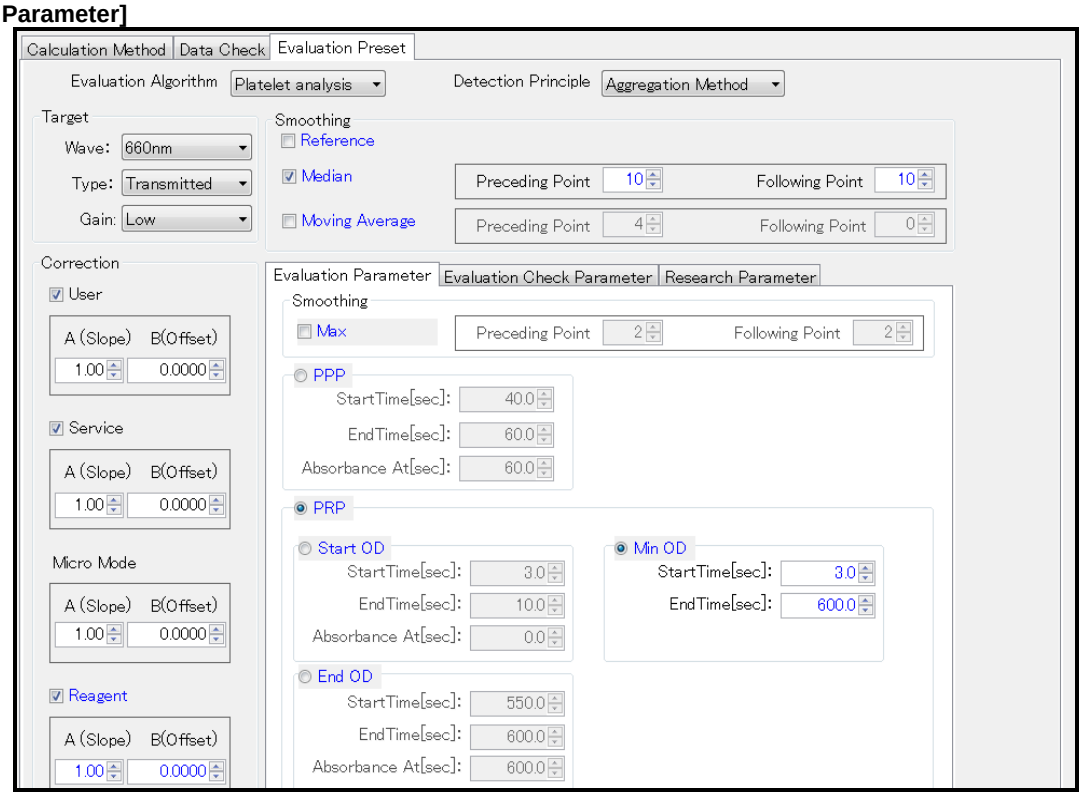

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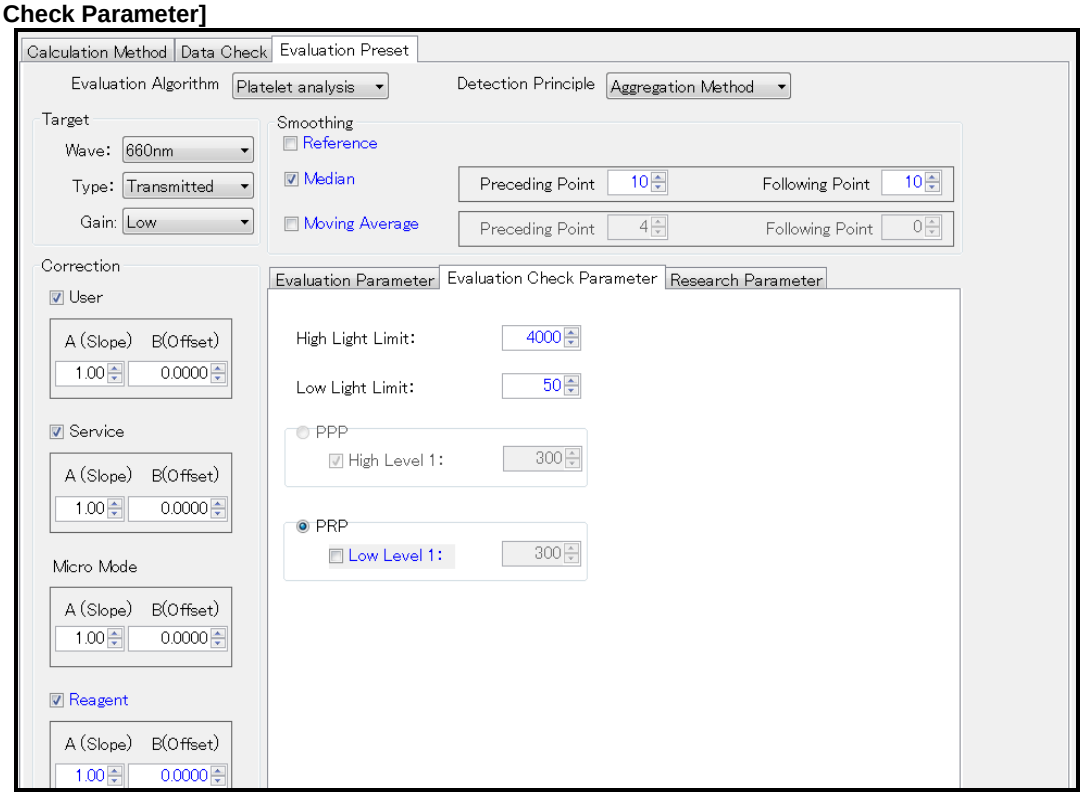

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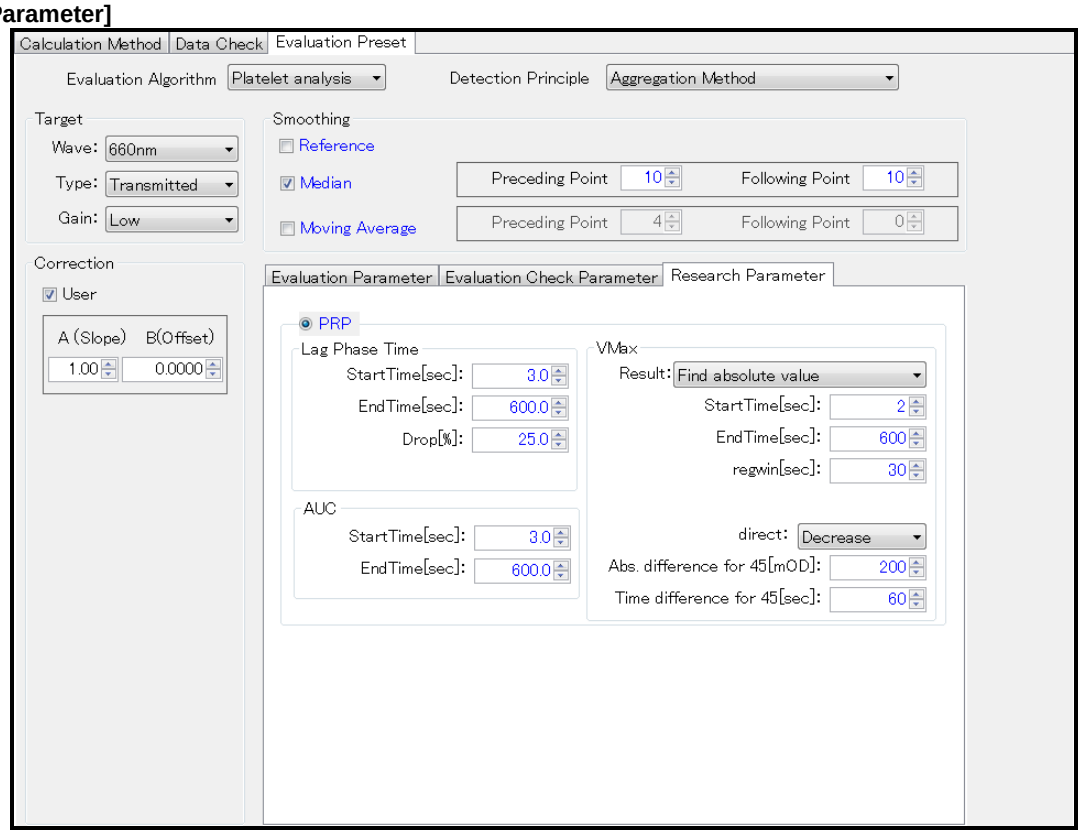

Assay Parameter Setup - [Epi_s]

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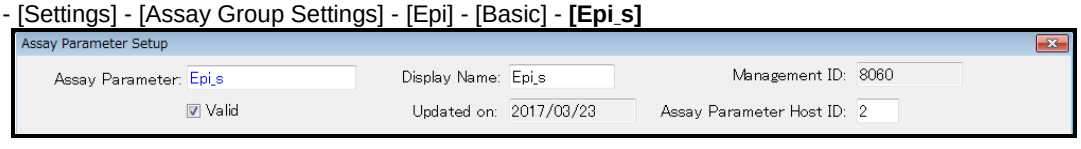

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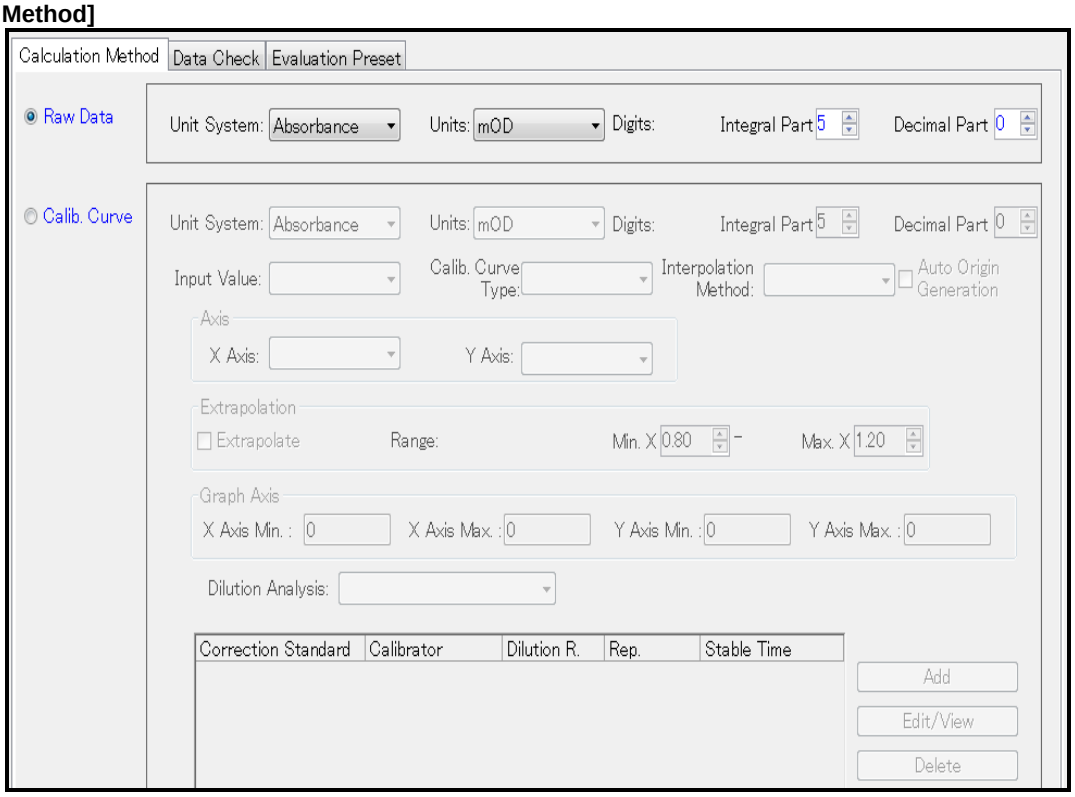

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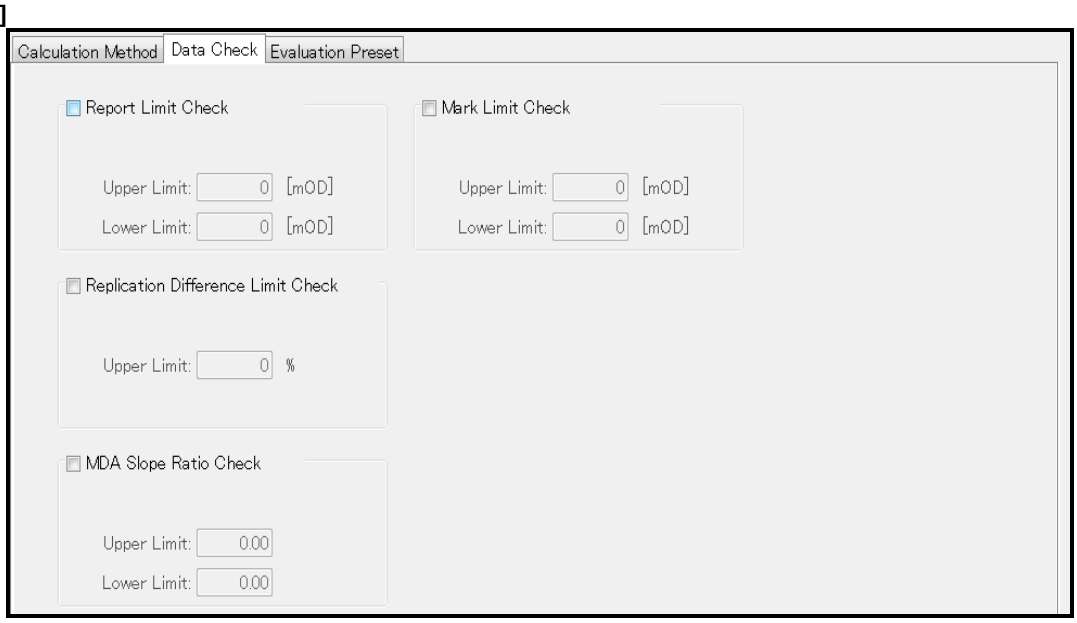

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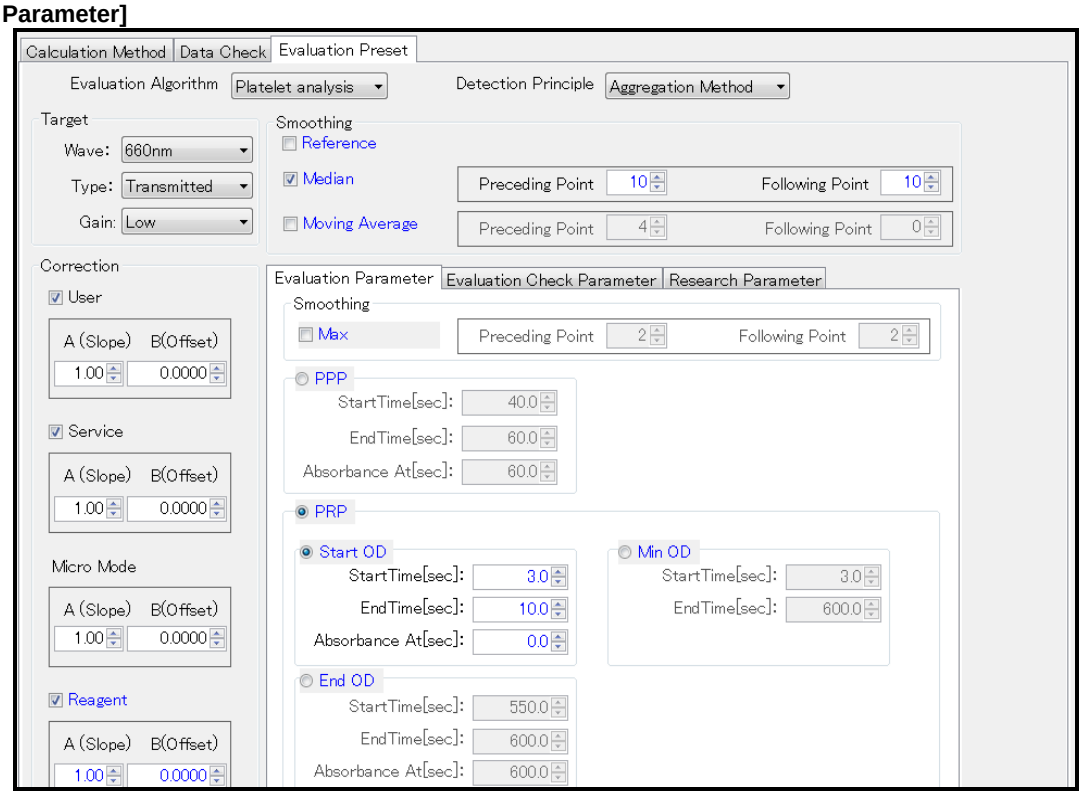

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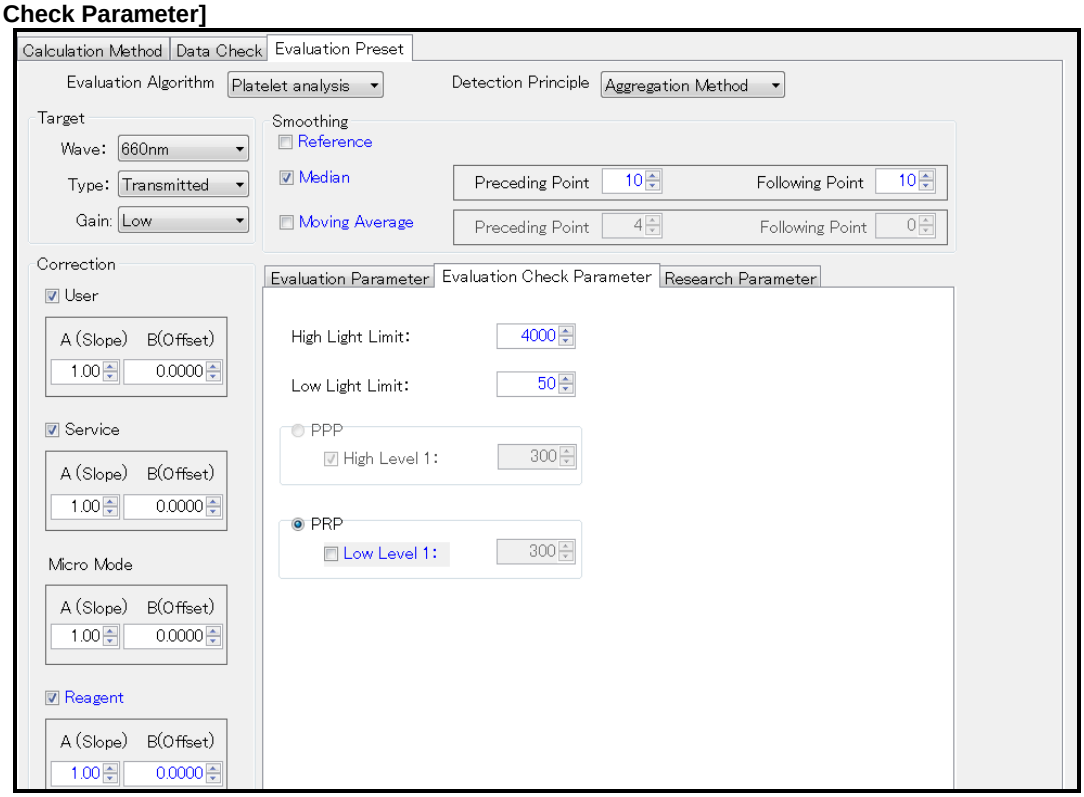

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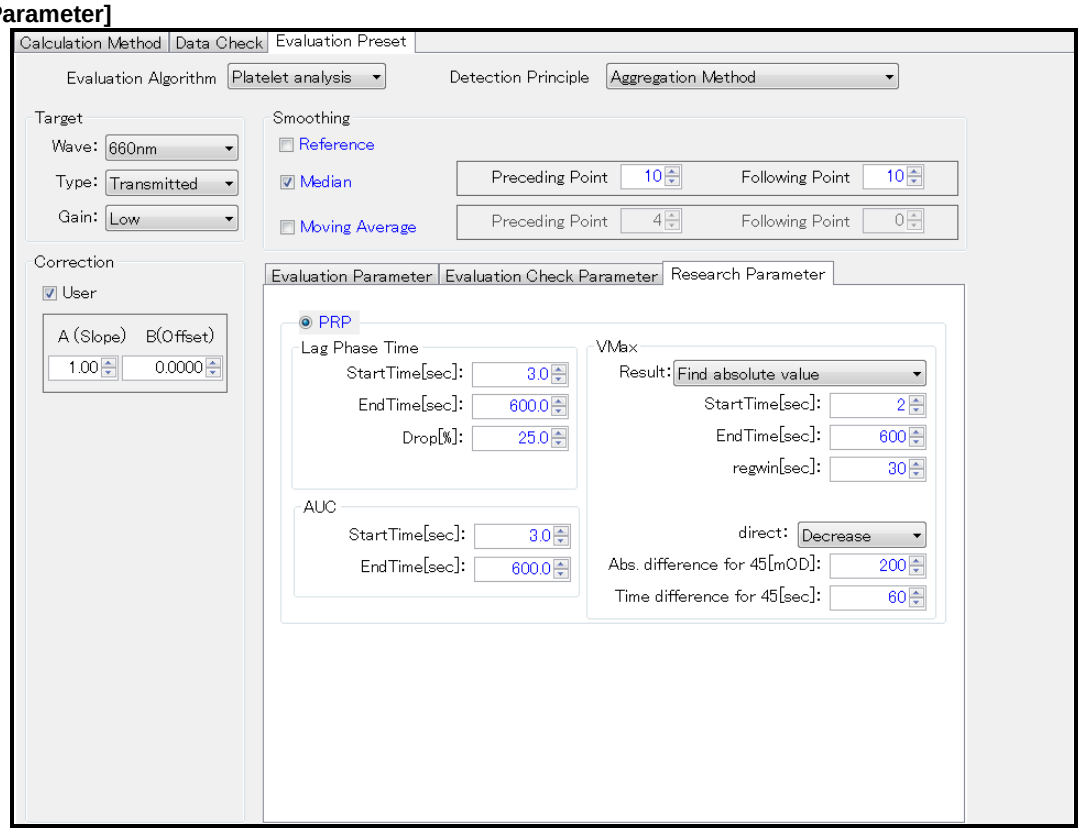

Assay Parameter Setup - [Epi_e]

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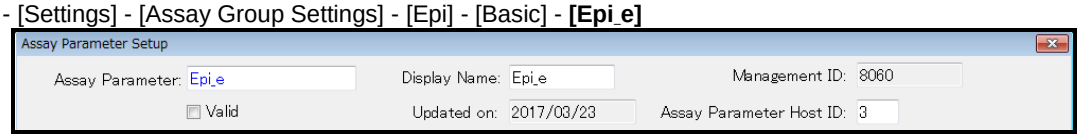

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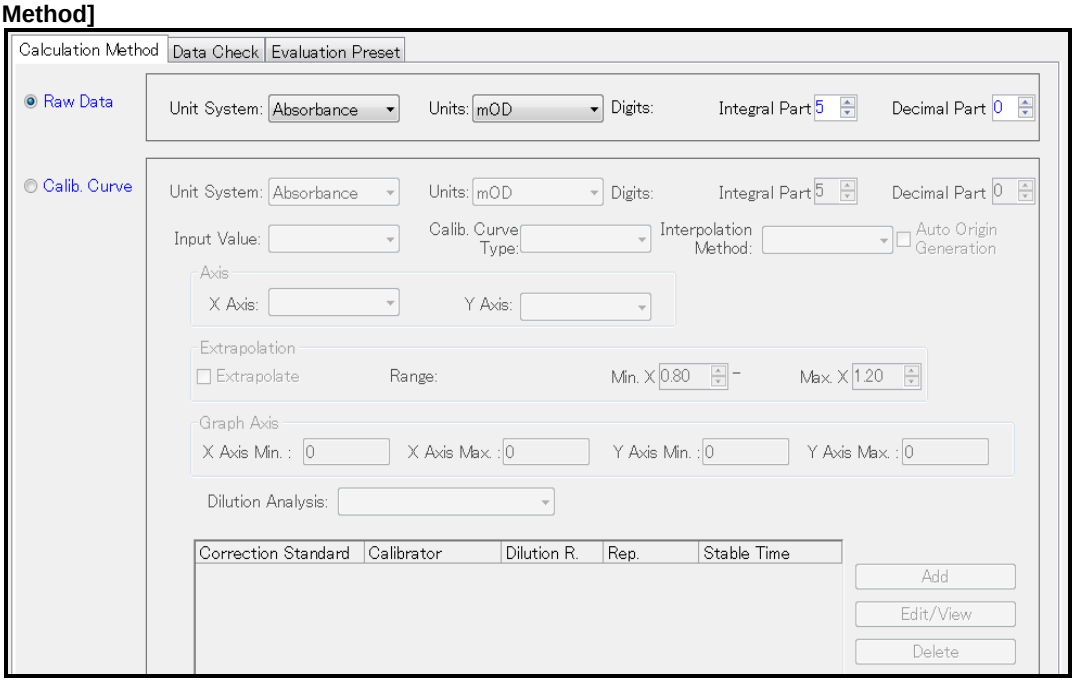

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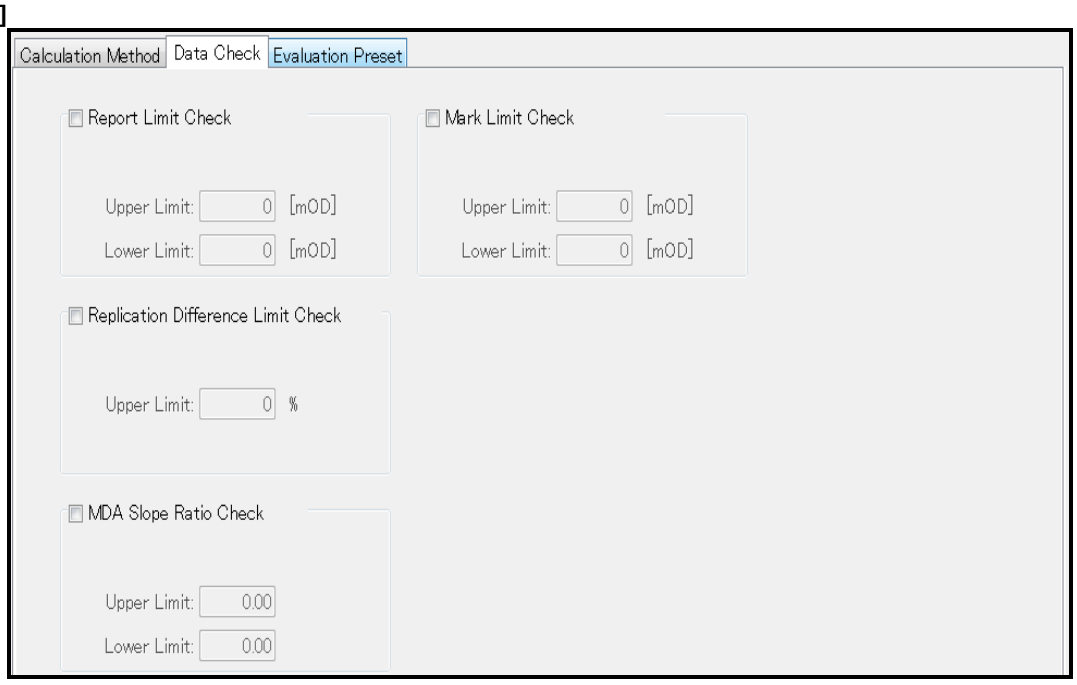

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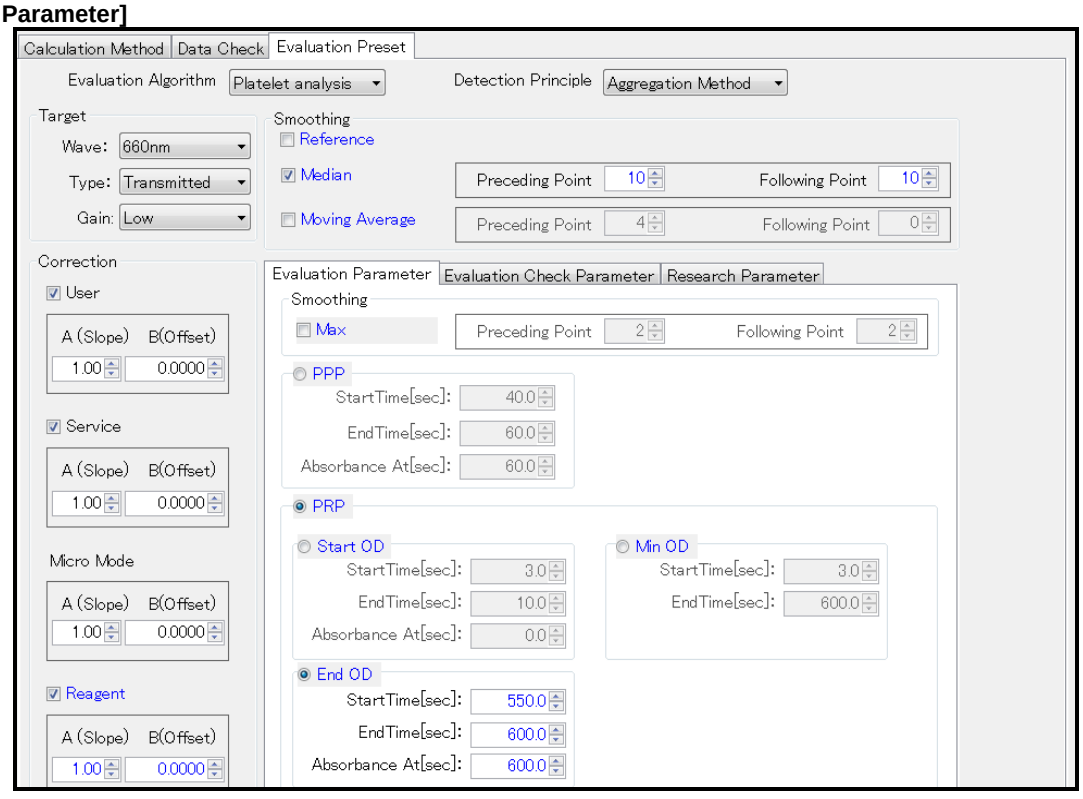

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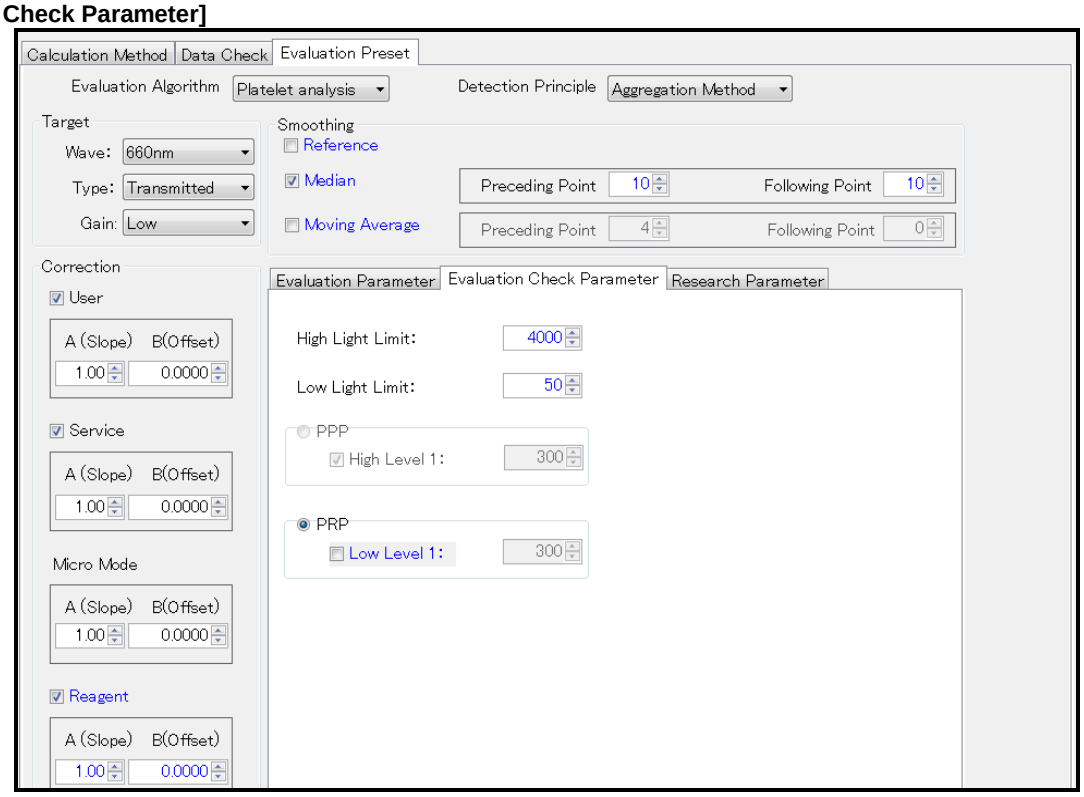

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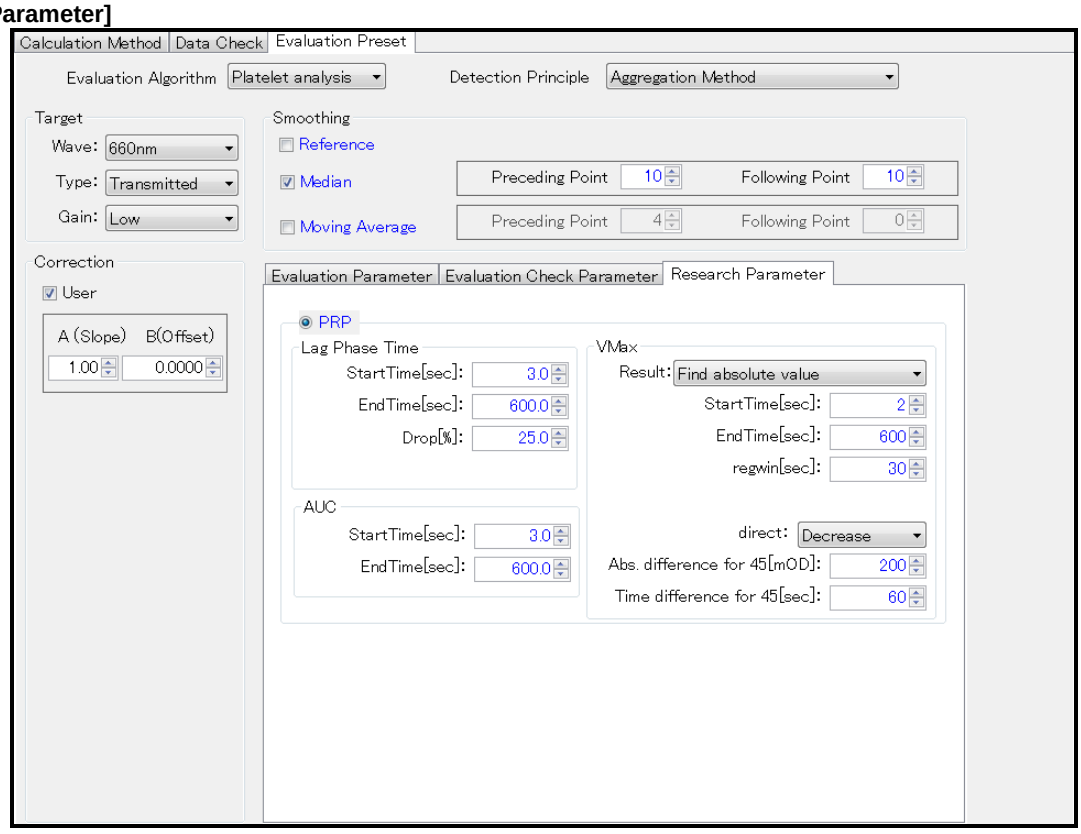
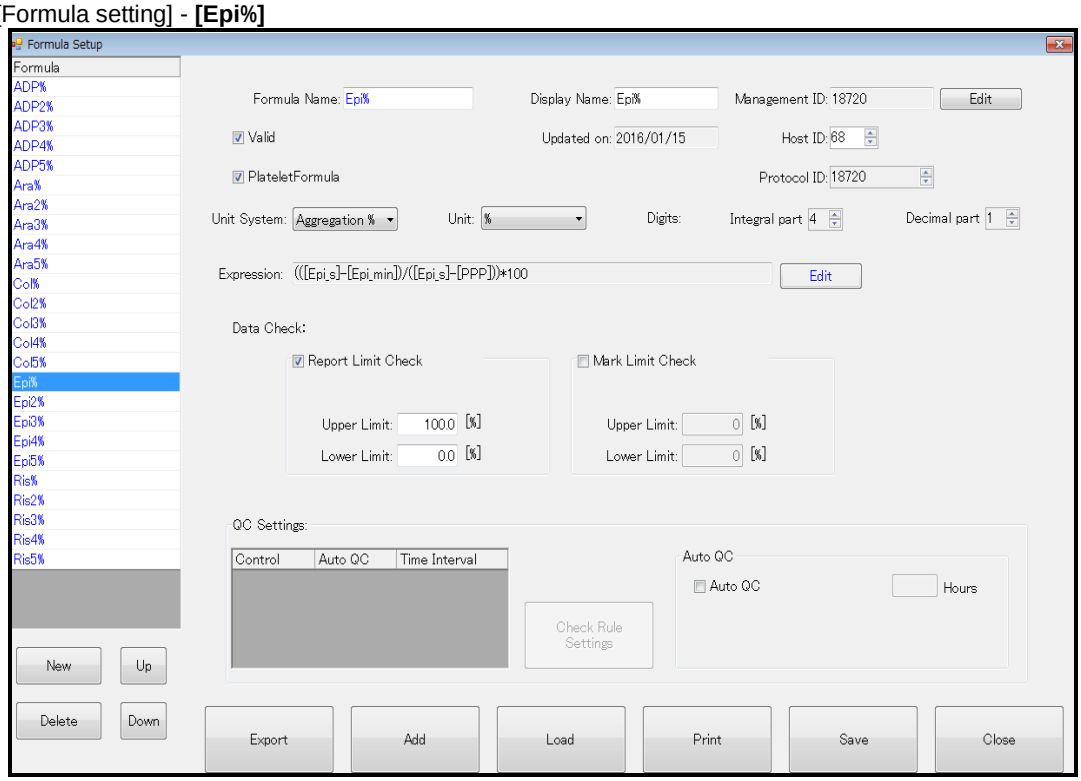


Formula setting - [Epi%]

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



Formula

Management ID	Assay Parameter	Formula
18720	Epi%	(([Epi_s]-[Epi_min])/([Epi_s]-[PPP]))*100
18721	Epi2%	(([Epi2_s]-[Epi2_min])/([Epi2_s]-[PPP]))*100
18722	Epi3%	(([Epi3_s]-[Epi3_min])/([Epi3_s]-[PPP]))*100
18723	Epi4%	(([Epi4_s]-[Epi4_min])/([Epi4_s]-[PPP]))*100
18724	Epi5%	(([Epi5_s]-[Epi5_min])/([Epi5_s]-[PPP]))*100