Konelab/Gallery plus Method for the Determination of Cellulase Activity in Detergent Wash Liquid and Filter Dust

Zhou Yang
Unilever (Sichuan) Co. LTD, Meishan 620866, Sichuan, China
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Abstract: This method can use the automatic dilution method of equipment to formulate the standard curve, which is more convenient and accurate than the manual dilution calibration curve method. Manual dilution requires at least 7 gradient concentrations of solution, and the automatic dilution method only needs to configure two gradient concentrations of solution. This method is innovative in the following aspects: using the method of automatic dilution of equipment to make standard curves, more convenient and accurate than manual dilution method.
***Keywords*:** enzyme analytical, cellulase activity

1. Warning and safety precautions

The main hazards of the materials involved are indicated below. Users should refer to supplier

Safety information for further details. An assessment of the risk to employees, and safety precautions required should be carried out locally before commencement of work. In some countries there is a requirement to document this assessment.

Enzymes are irritating to eyes, respiratory system and skin. Moreover, they are respiratory sensitizers and may cause allergies with symptoms like hay fever and asthma. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. In case of inhalation, leave the workplace and warn colleagues. For more detailed information: see the document 'Instructions for the Safe Handling of Detergent Enzymes on Laboratory Scale'. This document can be obtained via EAU, UR&D Mumbai or the Enzyme Database.

2. Scope

This method is used for the determination of the cellulolytic activity in enzyme in Detergent Liquid and in filter dust.

3. Normative references

NA

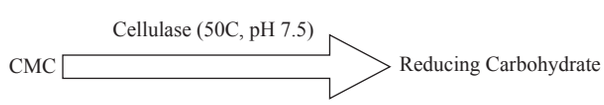
4. Definitions

The Cellulase activity is expressed in CCU and/ or RCU & CPCU. One Cellulase unit is defined as the amount of enzyme that produces, under the standard conditions (CMC, pH 7.5 and 50°C), an amount of reducing sugars per minute corresponding with 1μmol of Cellobiose

5. Principles

Cellulase (e.g.1, 4-endoglucanase, EC.3.2.1.4) hydrolyses cellulose by forming reducing carbohydrate. The substrate carboxymethyl cellulose (CMC) is a substituted form of cellulose. The reaction is stopped by an alkaline reagent containing PAHBAH and bismuth that forms complexes with reducing sugar. The complex formation results in colour production which can be read at 405 nm. The produced colour is proportional to the cellulose activity. Enzymatic reaction and measurement of absorbance proceeds automatically in the (Konelab/Gallery Plus) analyser.

6. Reactions



Detection reaction:

7. Reagents and materials

Sodium sulphite anhydrous AR: Merck Cat No. 61752290511730 CAS No:-7757-83-7 (CAUTION: Irritant; Avoid inhaling)

Para-hydroxybenzoic acid hydrazide (PAHBAH): Sigma Cat No. H9882, CAS No: -5351-23-5

Alternate Supplier: TCI H1275 Lot No.7BCHA-FA

Sodium Carboxymethylcellulose (CMC): Low viscosity Sigma Cat No. C5678, CAS No: -9004-32-4

Sodium Hydroxide pellet: Analytical grade or equivalent, Merck, CAS No. 1310-73-2 (CAUTION: Irritant; Corrosive; Avoid contact with skin and eyes)

Sodium Di-hydrogen phosphate, monohydrate: CAS No: -10049-21-5, Merck 61787405001730 GR or equivalent

Di-sodium hydrogen phosphate, di-hydrate: Merck Art NO. 1.93622.0521 LR or equivalent

Aces: Sigma A9758, CAS No.7365-82-4 AR

Potassium sodium tartrate, tetra hydrate: Merck Art. NO. 1.93626.0521 AR or equivalent

Bismuth (III) acetate: Sigma 401587, CAS NO. 22306-37-2

PEG 6000: Merck, Catalogue number: 807491 or equivalent

Tween 20: CAS No:- 9005-64-5 Merck 61773505001730 (AR)

HCL: 35% Merck CAS NO. 7647-01-0, Art. No 1.93401.0521 LR or equivalent

Enzyme reference standard and control samples Supplied by EAU, Mumbai on a yearly basis. A table with the actual activity values and units of the reference standard and control samples is enclosed with each shipment. The reference standard and control samples should be stored in desiccators at 2-8°C (refrigerator). Before opening, the bottles should be kept at room temperature for 15 minutes to avoid moisture uptake. (CAUTION: Harmful and irritant; Avoid dust formation)

Distilled water conductivity (0.1 – 0.5 μS/cm)

8. Apparatus and equipment

Konelab Arena 20: Thermo Fisher Scientific Suppliers

Gallery Plus: Thermo Fisher Scientific Suppliers

Analytical Balance: Balances with a least count 0.01mg

Top loading Balance: Balances with a least count 0.01mg

Micropipettes: 20 – 200ul, 100 – 1000ul, 1-5ml

Roller Mixer: 9 rollers (Stuart scientific manufacturer)

Heater Block: Stuart Scientific

Magnetic stirrer: Remi Magnetic Stirrers-MS 500 or equivalent

Filter paper: Whatman Filter Paper 41- ALDRICH catalogue number GF/C1822-150

Glass wares: Standard glass wares

Hot Plate: Standard hot plate

Disposable gloves: filter handling and reagent handling and Preparation.

9. Reagent solutions

9.1 CMC substrate (CMC-SUB)

Take 150 ml beaker and add 75 ml phosphate buffer 0.1M pH7.5

Heat the above buffer to a temperature up to 80°C

Weigh CMC…….1.0+/- 0.1 g.

Add the CMC slowly to the heated buffer during vigorous stirring for 10 min, turn off the heater and stir it for another 20mins.

Cool to room temperature and adjust pH to 7.5+/- 0.05 with 2M NaOH or 2M HCL.

Make up the volume to 100ml in a volumetric flask with phosphate buffer and store.

Stability: 4 days in refrigerated conditions

9.2 Aces Buffer 10 mM

Add 900ml demineralised water to 2 liter beaker.

Weigh out 1.822g +/- 0.005g Aces and transfer to above beaker, stir until the buffer is dissolved.

Adjust the pH to 7.5+/- 0.05 with 2M NaOH.

Make up the volume with demi-water in 1liter volumetric flask and stir.

Prepare the buffer quantity as required. Stability: Prepare fresh solution daily

9.3 Phosphate Buffer 0.1M pH 7.5 (PHOS-BUF)

Add 450mL demi-water to 500ml beaker.
Weigh out 1.125g sodium Di-hydrogen phosphate, monohydrate.
Weigh out 7.45 g Di-sodium hydrogen phosphate, di-hydrate.
Stir until the buffer dissolves and adjust the pH to 7.5+/- 0.05 with 2M NaOH or 2M HCL as appropriate.
Make up the volume with demi-water to the 500ml volumetric flask and mix.
Stability: 4 days in refrigerated conditions

9.4 Pahbah/Bismuth/Tartrate solution (PAHBAH/S)

Add 25.0 ml of 0.5M Sodium hydroxide solution to a 50 ml beaker.
Add potassium sodium tartrate, tetrahydrate……1.2500+/- g, stir till fully dissolved using a magnetic stirrer.
Add Bismuth acetate…….0.1380+/-0.001g,stir to dissolve completely.
Wrap immediately in aluminium foil to protect from light
Add PAHBAH……..0.5000+/-0.001g,stir for 15min to dissolve completely
Check if the powder is dissolved completely and solution should be colourless or very faint yellow
Reagent can be stored on board up to 8 hrs. Stability: Prepare fresh solution daily

9.5 NaOH 0.5M

Dissolve 2g NaOH in 75ml demi-water.
Cool down and make up to 100ml with demi-water. (Stability two months)

9.6 NaOH 2M

Dissolve 8g NaOH in 75ml demi-water.
Cool down and make up to 100ml with demi-water.

9.7 Celluclean Sample diluent (Only for Celluclean Filter Dust Analysis)

Add 800 mL demineralised water to a 1 L volumetric flask.
Weigh out 2.25 g Sodium di-hydrogen phosphate, monohydrate.
Weigh out 14.9 g Di-sodium hydrogen phosphate, di-hydrate.
Transfer the chemicals quantitatively to the volumetric flask.
Stir until everything is dissolved
Transfer 50 mL Tween 20 to the volumetric flask.
Transfer 45 g PEG 6000 to the volumetric flask.
Stir until everything is dissolved
Adjust the pH to 7.50 ± 0.05 with 2M NaOH
Fill the volumetric flask with demineralised water to the mark and mix by stirring.

9.8 Tween 20 (10% W/V) (Only for Carezyme 1000L Filter Dust Analysis)

Weigh 10g of Tween 20 in a 100 ml volumetric flask.
Dilute up to the mark with demineralised water and mix well.

9.9 Carezyme Sample diluent (Only for Carezyme1000L/Carezyme Premium4500L Filter Dust Analysis) Transfer 5 ml tween 20 (9.8) to a 1000ml volumetric flask

Fill the volumetric flask with Aces buffer 10mM, pH 7.5 to the 1000ml (Prepare the diluent quantity as required.)
Stability: Prepare fresh solution daily

10. Sampling technique

10.1 Sampling of detergent powder

See UMA-5861 which recommends a sample size for enzyme analysis of 50g to be dissolved in 5 liters.

10.2 Dust sampling (galley sampler)

Airborne dust or aerosol samples are collected according to the procedures given in SHE 29.

11. Procedures

11.1 Procedure Konelab/ Gallery Plus

Analytical protocols for different classes of Cellulases have been set up for the KONELAB / GALLERY PLUS and are as follows.

Enzyme	Formulation	Test definition	Calibration Range	QC Range
CAREZYME 1000L (RCU/l)	Product	CAREPR L	0-10	7.2-8.8
	Dust	CAREDU L	0-2	0.425-0.575

11.2 Preparation standard solutions for "CAREPR L" for Konelab/ Gallery Plus

Weigh to ± 0.0001g the amount of the reference standard appropriate for the enzyme to obtain the following Concentration of enzyme Solution. The correct weighment is given in the table enclosed with the latest shipment.

Name of enzyme	Solution	Concentration	Total Dissolution volume	Diluent
CAREZYME 1000L	Solution B	400 RCU/l	250ml	ACES

Note: The correct amount is given in the table enclosed with the latest shipment.

Pipette 50 ml of Solution B transfer to beaker containing 250ml of Aces buffer and stir for 10-15 minutes using a magnetic stirrer ("Solution C" is 80 RCU/l). (Calibration should be done as soon as possible after dilutions)

The above stock solution can be stored in refrigerator (Up to 8hrs)

11.3 Control solution (QC) for "CAREPR L" for Konelab/Gallery plus

Weigh to ± 0.0001g the amount of the reference standard appropriate for the enzyme under investigation to obtain a solution of exactly 160 RCU/l and transfer to beaker containing 250ml of Aces Buffer and stir for 10 - 15 minutes using a magnetic stirrer. (Note: The correct amount is given in the table enclosed with the latest shipment.)

Pipette out 5 ml of this solution to a to beaker containing 100ml of Aces buffer solution and mix.

The QC solution is now 8 RCU/l.

Insert a sample cup with the QC solution when requesting for calibration.

Store this solution in refrigerator (Up to 8 hrs).

11.4 Preparation standard solutions for "CAREDU L" for Konelab/ Gallery Plus

Weigh to ± 0.0001g the amount of the reference standard appropriate for the enzyme under investigation to obtain a solution of exactly 400 RCU/l and transfer to beaker containing 250ml of Aces buffer and stir for 10-15 minutes using a magnetic stirrer ("Solution B" is 400 RCU/l). (Note: The correct amount is given in the table enclosed with the latest shipment.)

Pipette 6.25 ml of Solution B transfer to beaker containing 500ml of Aces buffer and stir for 10-15 minutes using a magnetic stirrer ("Solution C" is 5 RCU/l).

The above solution can be stored in refrigerator for 8 hours.

11.5 Control solution (QC) for "CAREDU" for Konelab/ Gallery Plus

Weigh to ± 0.0001g the amount of the reference standard appropriate for the enzyme under investigation to obtain a solution of exactly 200 RCU/l and transfer to beaker containing 250ml of Aces buffer and stir for 10-15 minutes using a magnetic stirrer. (Solution G)

Note: The correct amount is given in the table enclosed with the latest shipment.

Name of enzyme	Dust QC	Concentration of control solution	Total Dissolution volume	Primary dilution (Solution H)	Diluent
Carezyme 1000 Liquid	0.5 RCU/l	200RCU/l (Solution G)	250ml	100X (1.25ml of Sol G to 500ml graduated flask) Total Dissolution Volume is 500ml) 0.5 RCU/l	Aces

11.6 Extraction procedure via Roller Mixer for CAREDU 1000 L /CPDU L(Carezyme Premium)

Place the whole filter (folded two times) with the dust side inwards into a 100ml jar.

Add 50ml of the Carezyme sample Diluent (9.9) to a whole filter. Use 25 ml for a half filter and 20 ml for filters that

are cut in 3 pieces.

Close the jar with a screw cap and rotate the jar on the roller mixer for 15 mins to dissolve the enzyme dust. Check if filter is placed correctly and is submerged into the extraction liquid with every rotation.

Take the filter out of the solution.

Fill 2 micro tubes with 1 ml of extract. (one for "test" and one for "blank" sample)

Centrifuge the both the micro tubes with the sample solutions for 3 mins at 14000 rpm.

Heat the solution from the "blank" micro tube at 100oC for 10 mins, cool the solution

Fill a sample cup and blank cup and place in a sample rack of the analyzer.

Dilute a sample solution that falls outside the range of the calibration curve with the Carezyme sample diluent (see 9.9).

Repeat point 11.16.5 to 11.16.8 for the diluted samples.

12. Calculation & expression of results

12.1 Calculations for product analysis

Calculate the Cellulase activity in CLCU/g or RCU/g or CPCU/g of a sample by dividing the printed value in CCU/L or RCU/l with the sample weight in g/l.

$$a = (T–B)/c$$

Where:

a = activity (CLCU/g or RCU/g or CPCU/g)

T = Test value from Konelab/Gallery Plus print out (CCU/L or RCU/l or CPCU/l)

B = Blank value from Konelab/Gallery Plus print out (CCU/L or RCU/l or CPCU/l)

c = concentration (g/l)

Note: For Carezyme Premium 4500L please analyse the Base product without enzyme(X) and Subtract the value from Test to get the final Value.

$$a = (T–X–B)/c$$

12.2 Calculations for filter dust analysis

12.2.1 Calculation of sampled air volume

$$va = (M_2 – M_1) \times K$$

$$K = \{760 – (P_1 + P_2)/2\}/760$$

Where:

K = factor only for Newton type samplers which corrects for the totaliser operating below ambient pressure.

va = "corrected" air volume through galley sampler (m^3)

M_1 = initial reading on totaliser (m^3)

M_2 = final reading on totaliser (m^3)

P_1 = initial vacuum guage reading (Newton samplers) (mm Hg)

P_2 = final vacuum guage reading (Newton samplers) (mm Hg)

Note: Apply the K factor only Newton or similar (local produced) type of Galley samplers and not for Barwit, F& J or other types.

12.2.2 Calculation weight of dust on Galley filters

$$W = (W_2 – W_1) \times 10^6$$

Where:

W = total weight on filter (µg)

W_1 =initial weight (g)

W_2 = final weight (g)

12.2.3 Calculation Cellulase activity on filter

$$q = (pA\text{-}pB) \times v_e \times Fx\ 10^{-3}$$

Where:

q = total cellulase activity on filter (CLCU or RCU or CPCU) pA = value of the active sample from Konelab/ Gallery Plus print out (CCU/L or RCU/l or CPCU/l) pB = value of the deactived sample from Konelab/ Gallery Plus print out (CCU/L or RCU/l or CPCU/l)

v_e = volume extraction solution (ml)

F = factor of 1, 2 or 3 to correct for 1, ½ or 1/3 filter respectively

Note: Apply a dilution factor when filter solutions (active) have been diluted.

12.2.4 Calculation airborne dust in the monitor air

$$\mu g/m3 = W/va$$

Where:

W= total weight on filter (µg) va = air volume through Galley sampler (m^3)

12.2.5 Calculation Cellulase activity in the monitored air

$$3 = q/va\ U/m$$

Where:

q = total cellulase activity on filter (CCU or RCU)

va = "corrected" air volume through Galley sampler (m^3)

Report the results in 10^{-3} CCU/m^3 rounded to two decimals (e.g. 0.212 × 10^{-3} CCU/ m^3)

12.2.6 Conversion of activity in the monitored air from U/m^3 to ng/m^3

$$\text{Enzyme level (ng/m}^3) = \frac{\text{Total Activity (U/m}^3) \times 4}{A}$$

Where A is Lower Action level in terms of U/m^3 equivalent to 4ng/m3 of total protein.

Carezyme 1000L A= 1.31 × 10^{-4} RCU/m^3

Celluclean 5000L A= 1.84 × 10^{-4} CLCU/m^3

Carezyme Premium 5000T A= 0.99 × 10^{-4} CPCU/m^3

Total Activity (U/m3) from 12.2.5

13. Raw and processed data

NA

14. Quality assurance and control

NA

15. Special cases and notes

15.1 Enzyme safety

Handling of enzymes and enzyme containing products should be done in a Powder Handling Booth with a suitable air extraction system with appropriate control measures installed.

Use a vacuum cleaner with HEPA filter for cleaning of powder spillages.

Under no circumstances grind enzymes or enzyme containing products into a fine powder!

More info available in the booklet "Instructions for safe handling of detergent enzymes on laboratory scale" ask your safety officer or contact your local ECC or EAU, Mumbai.

15.2 Instrument book for Konelab/ Gallery Plus

All Konelab/ Gallery Plus operating procedures for daily routine as well as instructions for more advanced users like database management and method programming are listed in the Konelab/ Gallery Plus Instrument Book. It is advised to

use this I-book as a guide when starting to use the Konelab/ Gallery Plus. For extra training contact the ECC in your region or EAU, Mumbai.

16. APPENDIX I: Set up

Programme characteristics for "CAREPR L" (Product mode)

Calibration range	0- 10 RCU/l
Reaction temperature	50°C
Reaction pH	7.5
Colorimetric detection	405nm

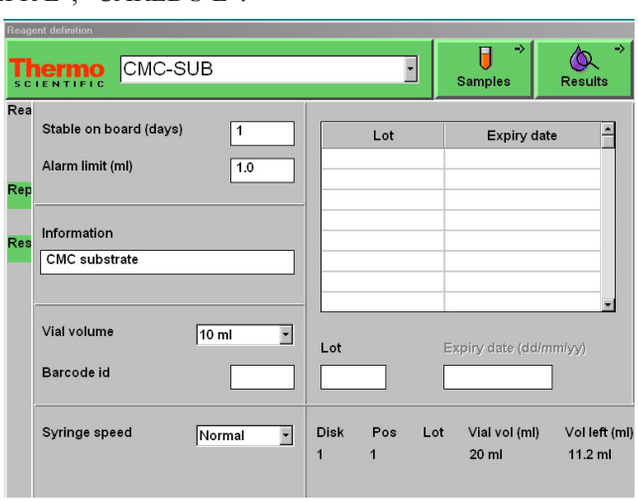
Programme characteristics for "CAREDU L" (Dust mode):

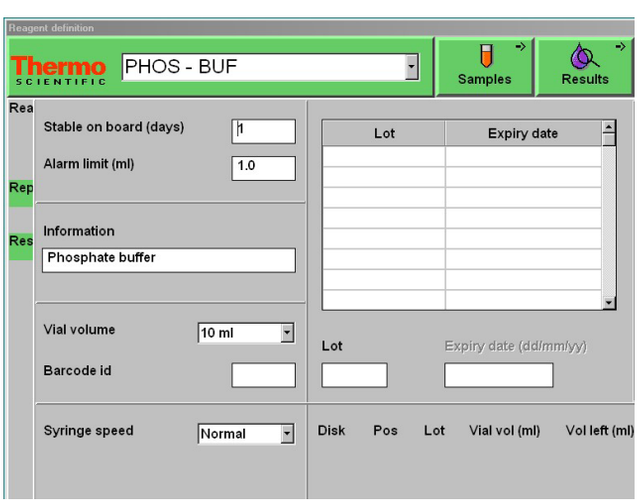
Calibration range	0 – 2 RCU/l
Reaction temperature	50°C
Reaction pH	7.5
Colorimetric detection	405nm

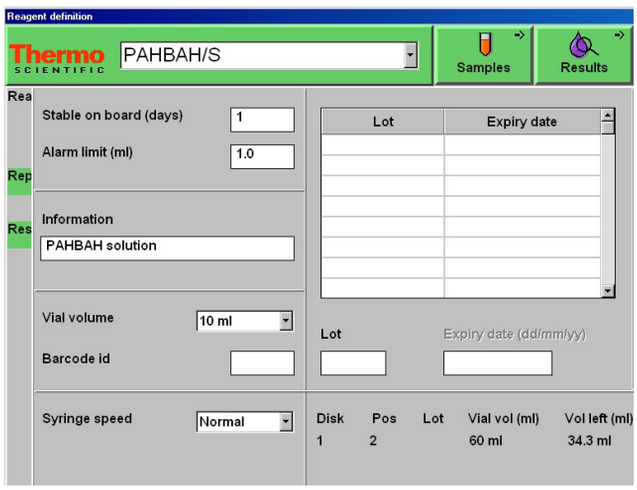
16.1 APPENDIX II: Konelab set up

To programme the Konelab properly, follow the step by step procedure as described in the Instrument Book (IB).

Konelab set up for "CAREPR L", "CAREDU L":

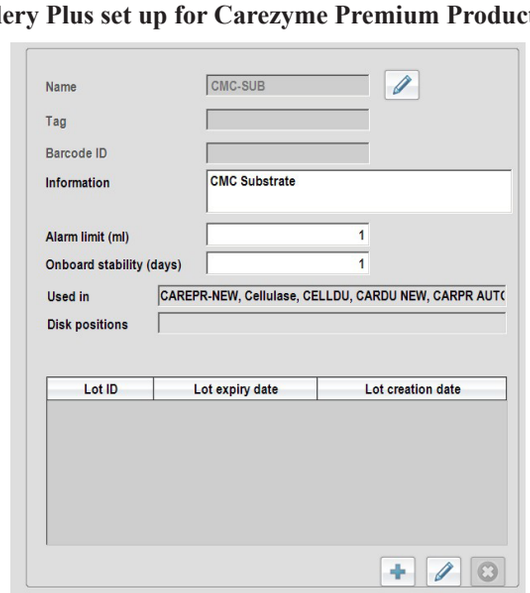


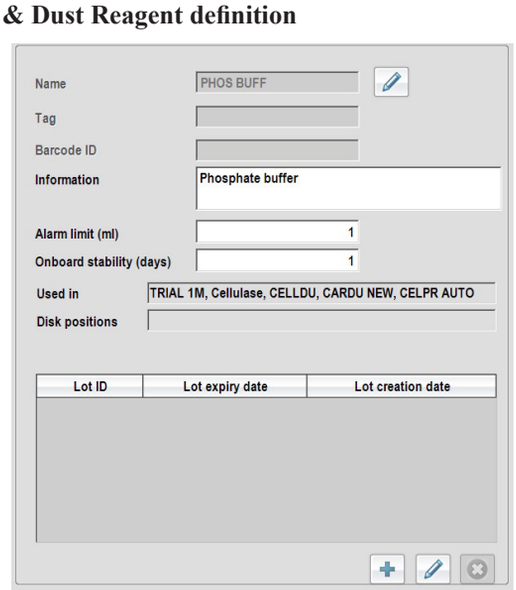


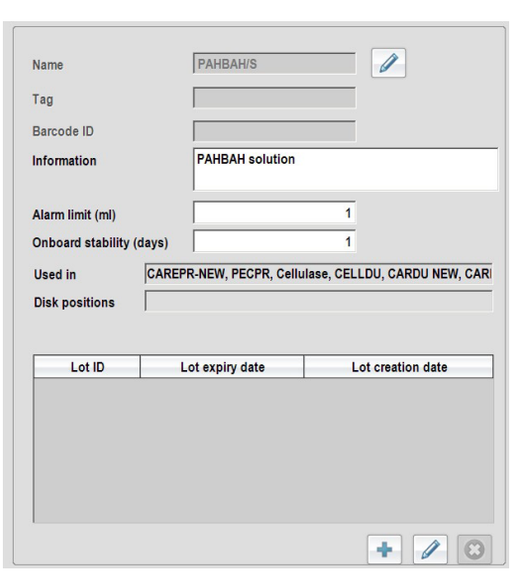


16.2 Gallery plus set up

16.2.1 Gallery Plus set up for Carezyme Premium Product & Dust Reagent definition





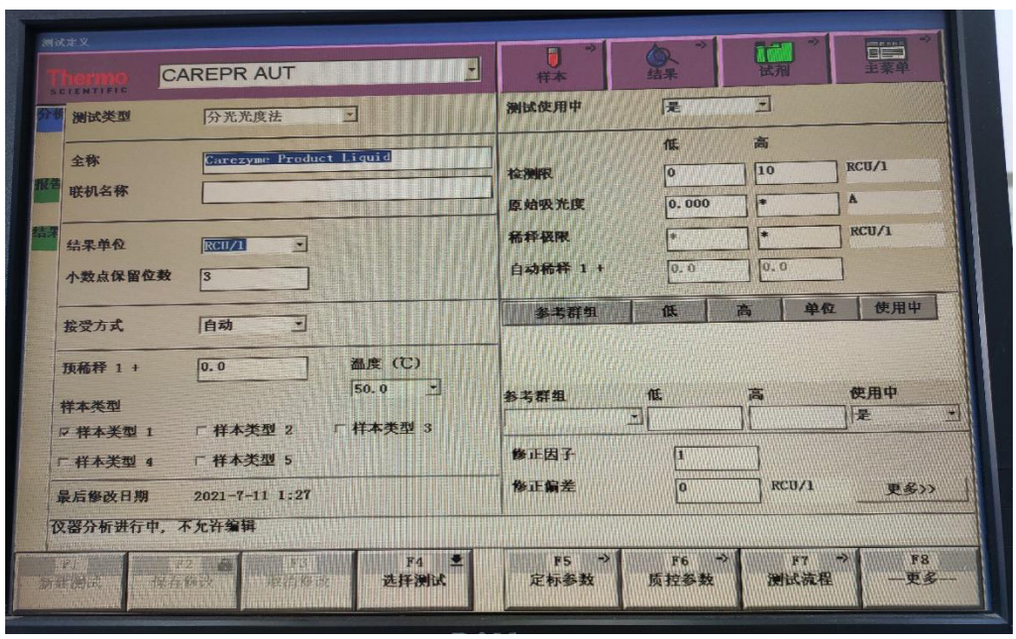


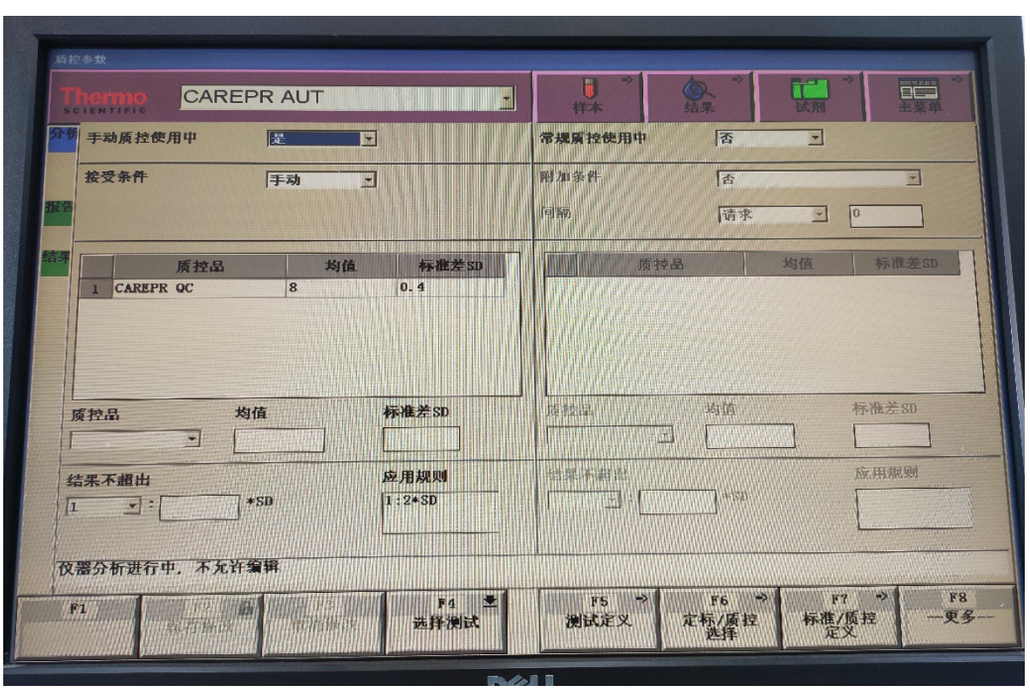
16.2.2 Gallery Plus set up for Carezyme Premium Product & Dust Wash

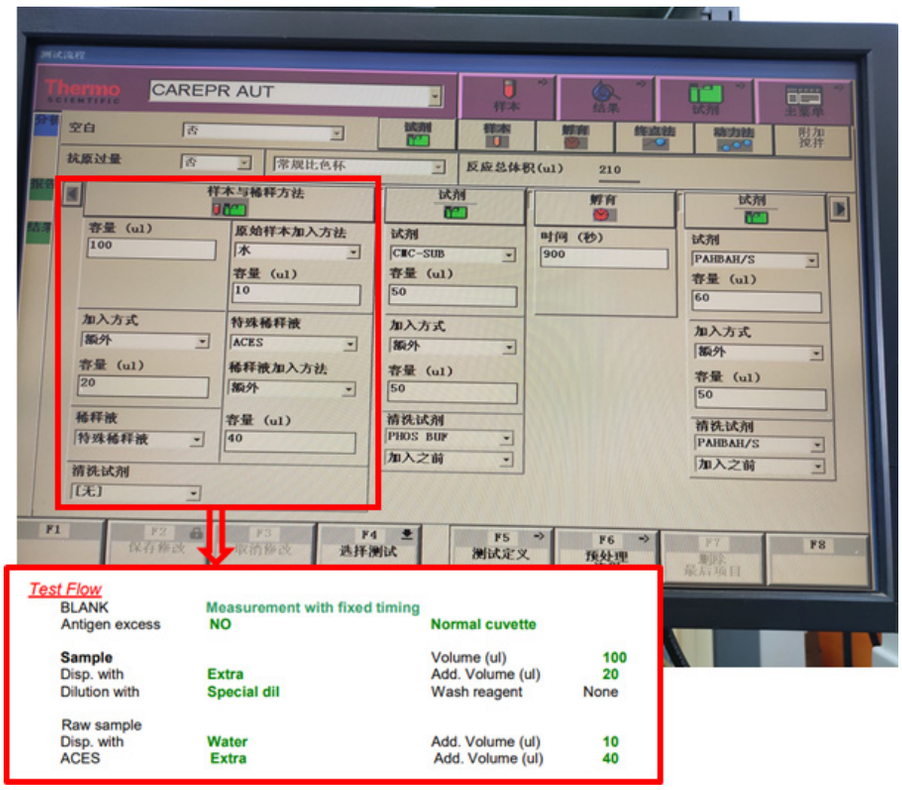
1 Test status | 2 Reagents | 4 Test definition | 5 Reagent definition | 6 Wash definition | 7 Profile definition

Preceding reagent	Following reagent	Wash type	Wash reagent	Tag
All	CMC-SUB	Reagent (small)	PHOS BUFF	☐
All	PAHBAH/S	Reagent (small)	PAHBAH/S	☐

16.2.3 Konelab set up for Carezyme product (CAREPR L)

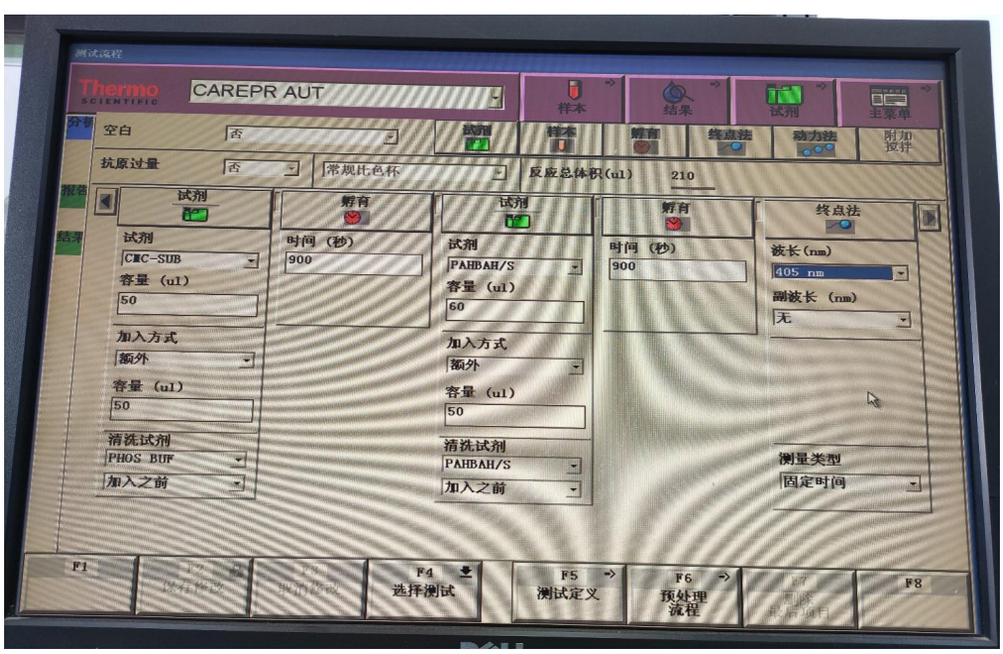


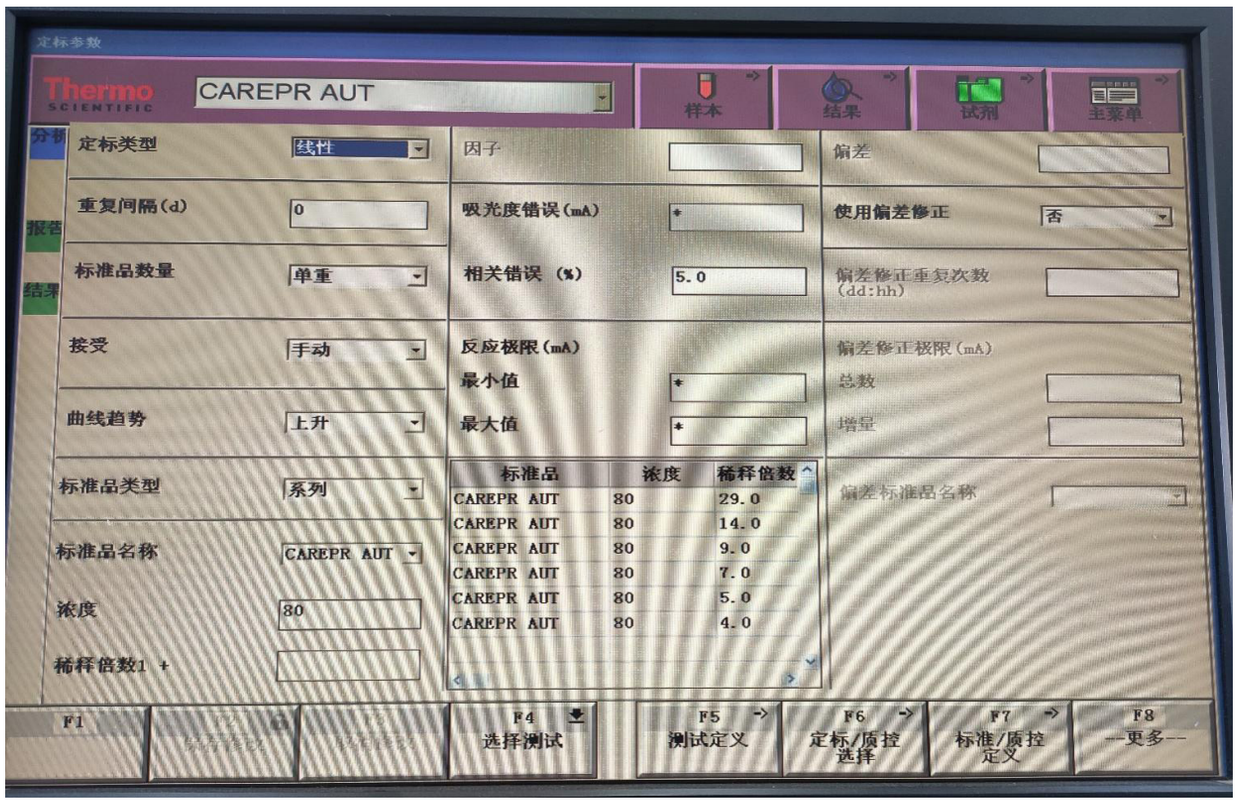


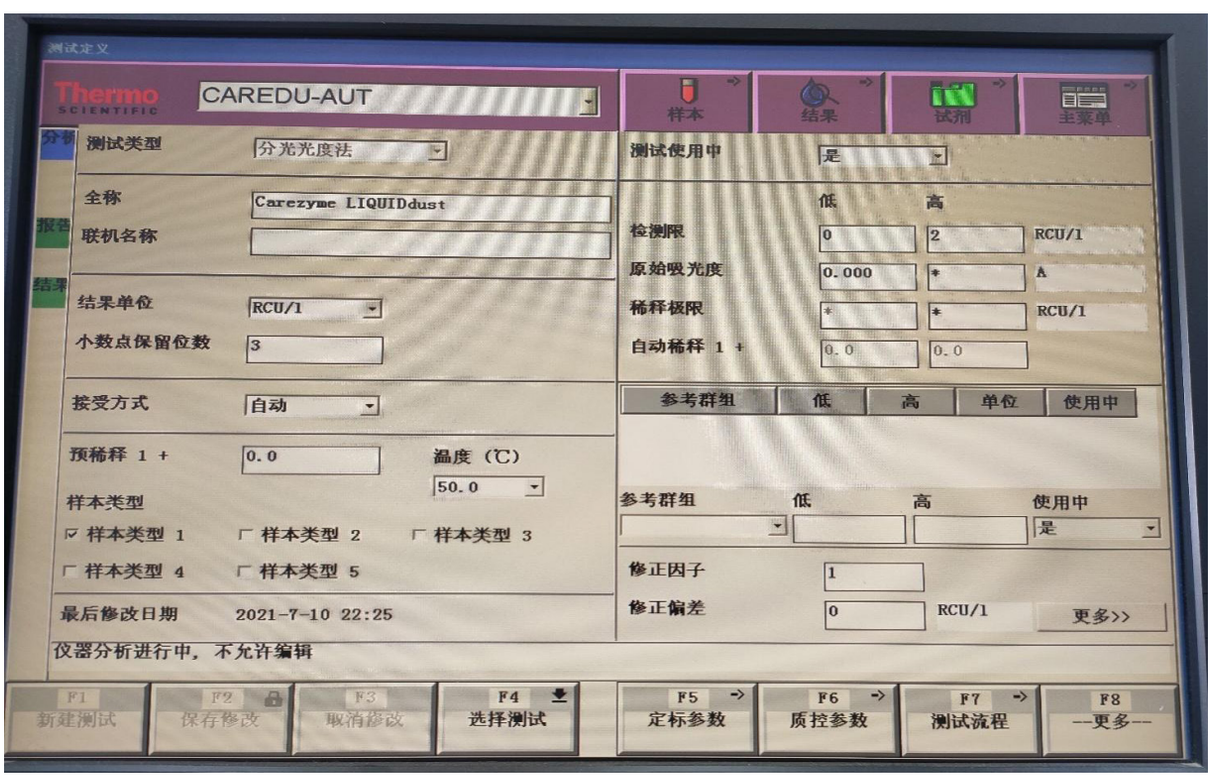


Test Flow

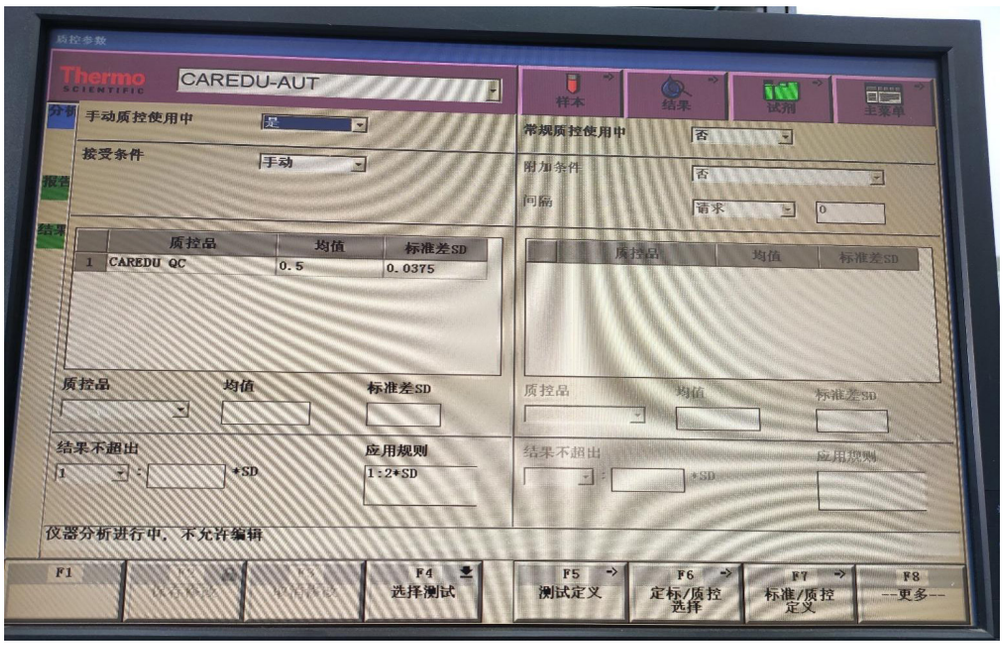
BLANK	Measurement with fixed timing		
Antigen excess	NO	Normal cuvette	
Sample		Volume (ul)	100
Disp. with	Extra	Add. Volume (ul)	20
Dilution with	Special dil	Wash reagent	None
Raw sample			
Disp. with	Water	Add. Volume (ul)	10
ACES	Extra	Add. Volume (ul)	40

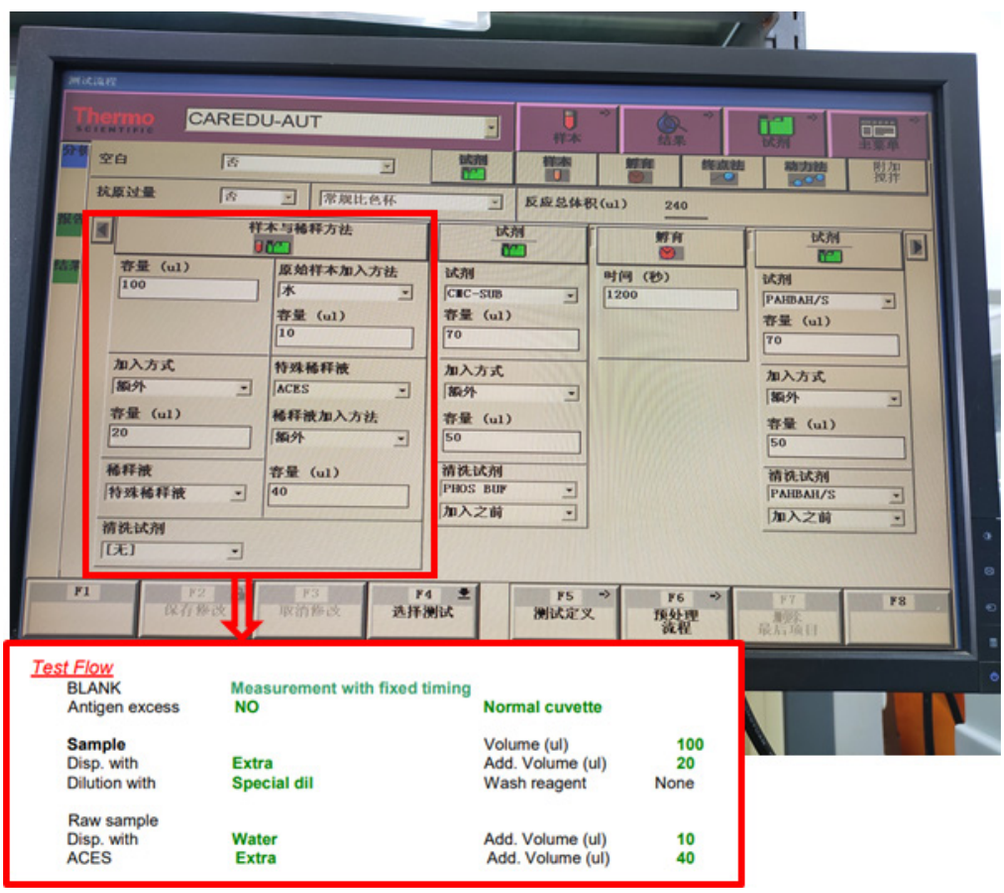



定标参数
Thermo SCIENTIFIC
CAREPR AUT
样本
结果
试剂
主菜单
定标类型
线性
因子
偏差
重复间隔(d)
0
吸光度错误(mA)
*
使用偏差修正
否
标准品数量
单重
相关错误 (%)
5.0
偏差修正重复次数 (dd:hh)
接受
手动
反应极限(mA)
最小值
*
偏差修正极限(mA)
总数
曲线趋势
上升
最大值
*
增量
标准品类型
系列
标准品名称
CAREPR AUT
浓度
80
稀释倍数1 +
标准品 浓度 稀释倍数
CAREPR AUT 80 29.0
CAREPR AUT 80 14.0
CAREPR AUT 80 9.0
CAREPR AUT 80 7.0
CAREPR AUT 80 5.0
CAREPR AUT 80 4.0
偏差标准品名称
F1
F4 选择测试
F5 测试定义
F6 定标/质控选择
F7 标准/质控定义
F8 --更多--


测试定义
Thermo SCIENTIFIC
CAREDU-AUT
样本
结果
试剂
主菜单
测试类型
分光光度法
测试使用中
是
全称
Carezyme LIQUIDdust
联机名称
低 高
检测限 0 2 RCU/1
原始吸光度 0.000 * A
稀释极限 * * RCU/1
自动稀释 1 + 0.0 0.0
结果单位
RCU/1
小数点保留位数
3
参考群组 低 高 单位 使用中
接受方式
自动
预稀释 1 +
0.0
温度 (℃)
50.0
样本类型
样本类型 1
样本类型 2
样本类型 3
样本类型 4
样本类型 5
参考群组 低 高 使用中
是
修正因子
1
修正偏差
0
RCU/1
更多>>
最后修改日期
2021-7-10 22:25
仪器分析进行中，不允许编辑
F1 新建测试
F2 保存修改
F3 取消修改
F4 选择测试
F5 定标参数
F6 质控参数
F7 测试流程
F8 --更多--

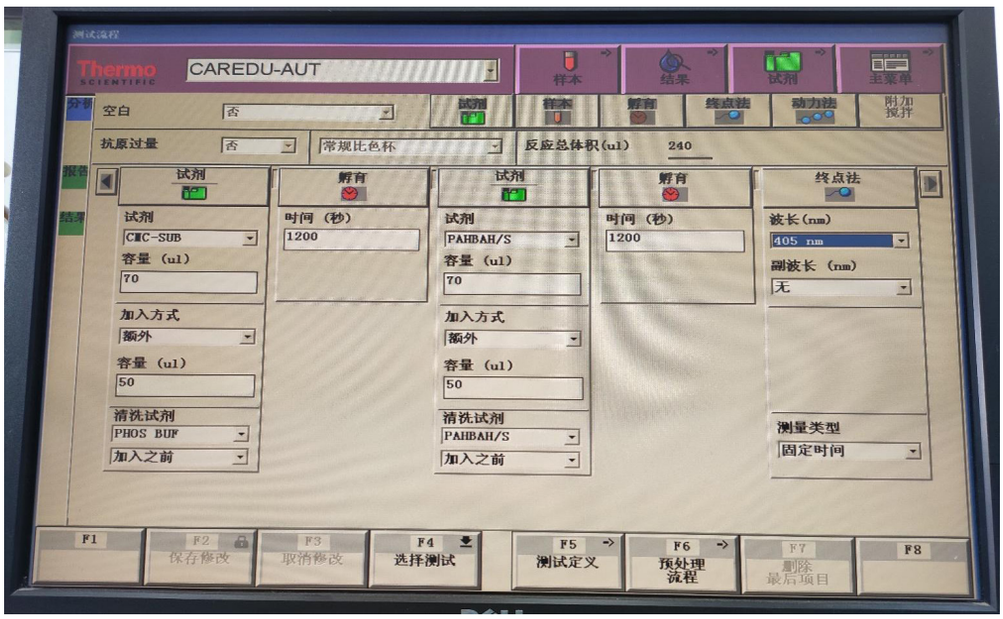
16.2.4 Konelab set up for Carezyme liquid Dust (CAREDU L)

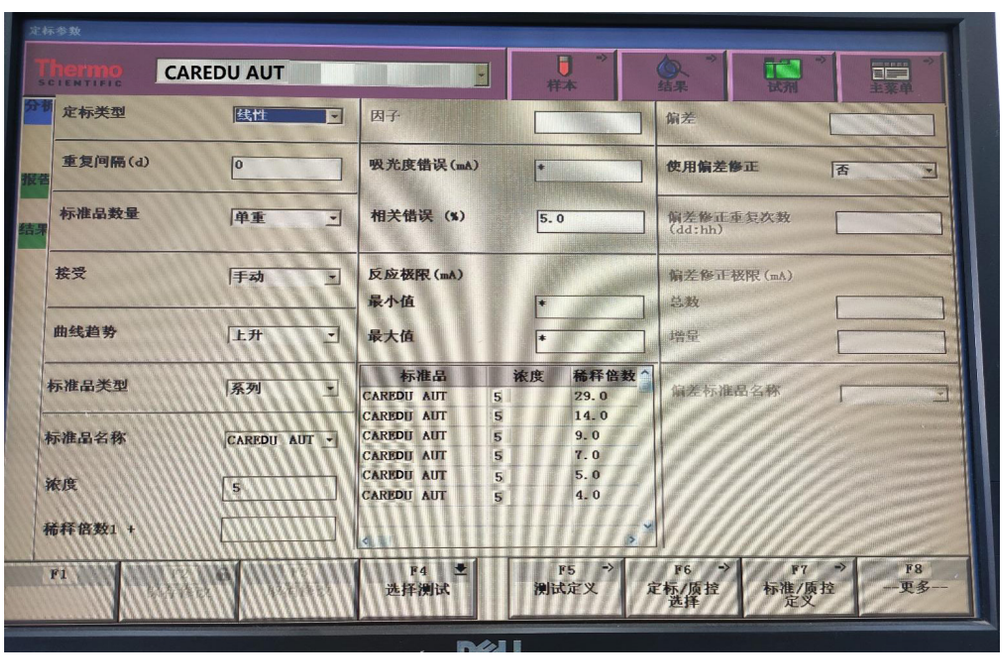




Test Flow

BLANK	Measurement with fixed timing		
Antigen excess	NO	Normal cuvette	
Sample		Volume (ul)	100
Disp. with	Extra	Add. Volume (ul)	20
Dilution with	Special dil	Wash reagent	None
Raw sample			
Disp. with	Water	Add. Volume (ul)	10
ACES	Extra	Add. Volume (ul)	40





References

[1] UMA 6368, April 2020, Author (year): Sheetal Hada (January 2012), page1-6.