

# Titration of whole blood and blood plasma for acid-base analysis according to Joergensen and Stirum

Of interest to:  
 Pharmaceutical industry, Biochemistry, biology  
 | 4, 8

## Summary

The presented application bulletin describes the apparatus and methods that are used for acid-base analysis of whole blood and blood plasma by Joergensen and Stirum. Evaluation of the measured data is performed with a software sold by the Komstar AG.

## Apparatus and accessories

- 2.808.0010 808 Titrande
- 2.801.0040 801 Magnetic Stirrer
- 2.840.0100 840 Touch Control
- 6.3026.150 Exchange Unit 5 mL
- 6.1418.110 Titration vessel lid
- 6.9914.153 Titration vessel w. thermostat jacket
- 6.1801.050 PVC tubing 2 m
- 6.1446.000 SGJ stopper (3 pieces)
- 6.0256.100 LL flat-membrane electrode
- 6.2104.020 Electrode cable 1 m, F
- 6.1543.040 Micro tip M6 thread
- 6.2308.020 Electrolyte 3 mol/L KCl
- 6.1903.000 Stirring bar / 8 mm (10 pieces)

A thermostated waterbath with a circulation pump is needed in order to adjust the temperature of the titration vessel to 37 °C.

## Reagents

- Hydrochloric acid c(HCl) = 0.1 mol/L

## Ready-to-use solutions:

Buffer solution	6.2307.100 Buffer solution pH 4 6.2307.110 Buffer solution pH 7
Cleaning solution for the electrode (Pepsin/HCl)	e.g. VWR International order number 83603.260

The electrode should be cleaned several hours in the cleaning solution to prevent fouling.

## Analysis

2.0 mL of whole blood or blood plasma are transferred to the titration vessel that is thermostated to 37 °C. While stirring with the magnetic stirrer the pH-value is determined with the flat-membrane electrode. After that hydrochloric acid (c(HCl) = 0.1 mol/L) is added in 0.200 mL portions while simultaneously recording the pH-value. In case of whole blood 1.200 mL hydrochloric acid is added, in case of blood plasma only 0.600 mL.

## Parameters method "Whole blood"

### Options

Statistics	.....off
Show 'Direct parameters'	.....off
Save automatically	
Save determination automatically	.....off
Send PC/LIMS report	.....off
Note	.....
Display options	
Automatically after loading the method	..... off
Start options	
Method check at start	..... on
Sample Processor	
Rack reset	.....off
Increase sample variable automatically	..... on
Request rack position at start	.....off
Stop options	
Pumps off	..... on
Stirrer off	.....on
Set remote lines	.....*****
Sample data	
Identification 1	
Display	..... on
Label	.....Patient
Identification 2	
Display	.....off
Label	.....Identifikation 2
Fixed sample size	.....on
Sample size	.....2
Sample size unit	..... ml
Limits sample size	
Monitoring sample size limits	..... off
<b>01 STIR</b>	
Stirrer on	
Control device	..... Titrande 1
Stirrer	.....1
Status/Duration	..... on
Stirring rate	..... 15
<b>02 WAIT</b>	
Wait	
Hold sequence	.....off
Waiting time	..... 60 s
Message	..... off

### 03 MET pH

#### Monotonic pH titration

##### Start conditions

Start volume . . . . . 0.00000 mL  
 Dosing rate . . . . . maximum  
 Pause . . . . . 0 s

##### More start conditions

Start meas. value . . . . . off  
 Start slope . . . . . off  
 Dosing rate . . . . . 5.00 mL/min

##### Initial measured value

Signal drift . . . . . off  
 Waiting time min. . . . . 0 s  
 Waiting time max. . . . . 1 s

##### Titration parameters

Titration rate . . . . . User  
 Temperature . . . . . 37°C

##### User-defined parameters

Volume increment . . . . . 0.2 mL  
 Dosing rate . . . . . 2 mL/min  
 Signal drift . . . . . 5 mV/min  
 Waiting time min . . . . . 10 s  
 Waiting time max. . . . . 60 s

##### Stop conditions

Stop volume . . . . . 1.2 mL  
 Stop meas. value . . . . . off  
 Stop EP . . . . . off  
 Volume after EP . . . . . off  
 Stop time . . . . . off  
 Filling rate . . . . . maximum

##### Pot. evaluation

EP criterion . . . . . 0.50  
 EP recognition . . . . . off  
 Set windows . . . . . off

##### Control device

Control device . . . . . Titrand 1

##### Sensor

Measuring input . . . . . 1  
 Sensor . . . . . pH electrode  
 Temp. meas. . . . . automatic

##### Dosing device

Dosing device . . . . . 1  
 Titrant . . . . . HCl

##### Stirrer

Stirrer . . . . . 1  
 Stirring rate . . . . . 15  
 Switch off auto. . . . . on

### 04 EVAL FIX-EP

#### Evaluation fixed endpoints

Fixed quantity . . . . . Volume  
 Fixed EP1 at . . . . . 0.2 mL  
 Fixed EP2 at . . . . . 0.4 mL  
 Fixed EP3 at . . . . . 0.6 mL  
 Fixed EP4 at . . . . . 0.8 mL  
 Fixed EP5 at . . . . . 1.0 mL  
 Fixed EP6 at . . . . . off  
 Fixed EP7 at . . . . . off  
 Fixed EP8 at . . . . . off  
 Fixed EP9 at . . . . . off

### 05 CALC

#### Calculation

##### R1

Result name . . . . . pH 0.0 ml  
 Calc. formula R1 . . . . . 1M.MSM  
 Decimal places . . . . . 2  
 Result unit . . . . .  
 Note . . . . .  
 Result limits  
     Monitoring result limits . . . . . off  
 Result options  
     Variable for mean . . . . . SMN1  
     Save result as titer . . . . . off  
     Save result as common variable . . . . . off  
     Display result . . . . . on

Save result in result silo . . . . . off  
 Precision . . . . . Round

##### R2

Result name . . . . . pH 0.2 ml  
 Calc. formula R2 . . . . . 1E.FM1  
 Decimal places . . . . . 2  
 Result unit . . . . .  
 Note . . . . .  
 Result limits  
     Monitoring result limits . . . . . off  
 Result options  
     Variable for mean . . . . . SMN2  
     Save result as titer . . . . . off  
     Save result as common variable . . . . . off  
     Display result . . . . . on  
     Save result in result silo . . . . . off  
     Precision . . . . . Round

##### R3

Result name . . . . . pH 0.4 ml  
 Calc. formula R3 . . . . . 1E.FM2  
 Decimal places . . . . . 2  
 Result unit . . . . .  
 Note . . . . .  
 Result limits  
     Monitoring result limits . . . . . off  
 Result options  
     Variable for mean . . . . . SMN3  
     Save result as titer . . . . . off  
     Save result as common variable . . . . . off  
     Display result . . . . . on  
     Save result in result silo . . . . . off  
     Precision . . . . . Round

##### R4

Result name . . . . . pH 0.6 ml  
 Calc. formula R4 . . . . . 1E.FM3  
 Decimal places . . . . . 2  
 Result unit . . . . .  
 Note . . . . .  
 Result limits  
     Monitoring result limits . . . . . off  
 Result options  
     Variable for mean . . . . . SMN4  
     Save result as titer . . . . . off  
     Save result as common variable . . . . . off  
     Display result . . . . . on  
     Save result in result silo . . . . . off  
     Precision . . . . . Round

##### R5

Result name . . . . . pH 0.8 ml  
 Calc. formula R5 . . . . . 1E.FM4  
 Decimal places . . . . . 2  
 Result unit . . . . .  
 Note . . . . .  
 Result limits  
     Monitoring result limits . . . . . off  
 Result options  
     Variable for mean . . . . . SMN5  
     Save result as titer . . . . . off  
     Save result as common variable . . . . . off  
     Display result . . . . . on  
     Save result in result silo . . . . . off  
     Precision . . . . . Round

##### R6

Result name . . . . . pH 1.0 ml  
 Calc. formula R6 . . . . . 1E.FM5  
 Decimal places . . . . . 2  
 Result unit . . . . .  
 Note . . . . .  
 Result limits  
     Monitoring result limits . . . . . off  
 Result options  
     Variable for mean . . . . . SMN6  
     Save result as titer . . . . . off  
     Save result as common variable . . . . . off

Display result ..... on  
 Save result in result silo ..... off  
 Precision ..... Round  
**R7**  
 Result name ..... pH 1.2 ml  
 Calc. formula R7..... 1M.MCM  
 Decimal places ..... 2  
 Result unit .....  
 Note .....  
 Result limits  
     Monitoring result limits ..... off  
 Result options  
     Variable for mean. .... SMN7  
     Save result as titer ..... off  
     Save result as common variable ..... off  
     Display result ..... on  
     Save result in result silo ..... off  
     Precision ..... Round

### Parameters method "Blood plasma"

The parameters of the method for blood plasma are almost identical to those used for the analysis of whole blood. Here only the differences are listed:

#### 04 EVAL FIX-EP

Evaluation fixed endpoints  
     Fixed quantity. .... Volume  
     Fixed EP1 at. .... 0.2 mL  
     Fixed EP2 at. .... 0.4 mL  
     Fixed EP3 at. .... off  
     Fixed EP4 at. .... off  
     Fixed EP5 at. .... off  
     Fixed EP6 at. .... off  
     Fixed EP7 at. .... off  
     Fixed EP8 at. .... off  
     Fixed EP9 at. .... off

#### 05 CALC

Calculation

**R4**  
 Result name ..... pH 0.6 ml  
 Calc. formula R4..... 1M.MCM  
 Decimal places ..... 2  
 Result unit .....  
 Note .....  
 Result limits  
     Monitoring result limits ..... off  
 Result options  
     Variable for mean. .... SMN4  
     Save result as titer ..... off  
     Save result as common variable ..... off  
     Display result ..... on  
     Save result in result silo ..... off  
     Precision ..... Round

no results R5-R7

### Parameters method "Rinsing"

#### Options

Statistics ..... off  
 Show 'Direct parameters' ..... off  
 Save automatically  
     Save determination automatically ..... off  
     Send PC/LIMS report ..... off  
 Note .....  
     Display options  
         Automatically after loading the method ... off  
 Start options  
     Method check at start. .... on  
     Sample Processor  
     Rack reset. .... off  
     Increase sample variable automatically ..... on  
     Request rack position at start. .... off  
 Stop options  
     Pumps off. .... on  
     Stirrer off. .... on  
     Set remote lines ..... \*\*\*\*\*  
 Sample data  
     Identification 1  
         Display ..... off  
         Label ..... Identifikation 1  
     Identification 2  
         Display ..... off  
         Label ..... Identifikation 2  
     Fixed sample size ..... off  
     Sample size unit ..... g  
     Limits sample size  
         Monitoring sample size limits ..... off

#### 01 ADD

Dosing  
     Dosing parameters  
         Volume ..... 3.5 mL  
         Dosing rate. .... 3 mL/min  
         Filling rate ..... maximum  
 Control device  
     Control device. .... Titrand 1  
 Dosing device  
     Dosing device. .... 1  
     Titrant. .... HCl  
     Tandem dosing device  
         Dosing device. .... off

#### Stirrer

Stirrer ..... off

#### 02 ADD

Dosing  
     Dosing parameters  
         Volume ..... 3 mL  
         Dosing rate. .... 3 mL/min  
         Filling rate ..... maximum  
 Control device  
     Control device. .... Titrand 1  
 Dosing device  
     Dosing device. .... 1  
     Titrant ..... HCl  
     Tandem dosing device  
         Dosing device. .... off

#### Stirrer

Stirrer ..... off

### Parameters method "Calibration"

#### Options

Statistics ..... off  
 Show 'Direct parameters' ..... off  
 Save automatically  
     Save determination automatically ..... off  
     Send PC/LIMS report ..... off

Note .....  
     Display options  
         Automatically after loading the method. . . . off

Start options  
     Method check at start ..... on  
     Sample Processor  
         Rack reset. .... off  
         Increase sample variable automatically . . . on  
         Request rack position at start. .... off

Stop options  
     Pumps off ..... on  
     Stirrer off ..... on  
     Set remote lines ..... \*\*\*\*\*

Sample data  
     Identification 1  
         Display ..... off  
         Label ..... Identifikation 1  
     Identification 2  
         Display ..... off  
         Label ..... Identifikation 2  
     Fixed sample size ..... off  
     Sample size unit ..... g  
     Limits sample size  
         Monitoring sample size limits ..... off

#### 01 CAL

pH pH calibration  
 Calibration parameters  
     Signal drift ..... 2.0 mV/min  
     Waiting time min ..... 10 s  
     Waiting time max. .... 110 s  
     Temperature. .... 37 °C  
     Sample Processor ..... None  
     Buffers  
         Buffer type ..... Metrohm  
         Number of buffers. .... 2  
     Stirrer control  
         Stir solution during measurement ..... on  
 Control device  
     Control device ..... Titrand 1  
 Sensor  
     Measuring input ..... 1  
     Sensor ..... pH electrode  
     Temp. meas. .... automatic  
 Stirrer  
     Stirrer ..... 1  
     Stirring rate ..... 8

### Examples:

Example results for a whole blood analysis:

V (HCl) [mL]	pH
0.0	7.39
0.2	7.06
0.4	6.74
0.6	6.46
0.8	6.18
1.0	5.89
1.2	5.59

Example results for a blood plasma analysis:

V (HCl) [mL]	pH
0.0	7.48
0.2	6.71
0.4	6.22
0.6	5.77

Evaluation of the measured data is performed with the software BUFFY™ sold by Komstar AG.:

#### KOMSTAR AG

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