

Spectroquant[®] COD Cell Tests – Chemistry Presentation

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Merck KGaA

ISPRA Roma, Rete Referenti Area A, Riunione.

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Measurement of Oxygen Demand (COD)

Normative COD Methods Examples	Introduced last update	Measuring ranges	Chloride tolerance	Temp	Time
Open refluxion and titration					
- ISO 6060	1989-11	30 to 700 mg/l	1000 mg/l	148 ± 3°C	10 + 110 min
- APHA 5220 B	22 th ed	50 to 900 mg/l	no information	no information	2h
- EPA 410.2	1978	5 to 50 mg/l	no information	no information	2h
- EPA 410.1	1978	> 150 mg/l	2000 mg/l	no information	2h
- EPA 410.3	1978	250 to 800 mg/l	4000 to 20000 mg/l	no information	2h
- DIN 38409 H41-1 (+ -2)	1980-12	15 to 300 mg/l	< 1000 mg/l (>1000 mg/l)	148 ± 3°C	10 + 110 min
- DIN 38409 H44-1 (+ -2)	1992-05	5 to 50 mg/l	< 300 mg/l (> 300 mg/l)	152 ± 3°C	2h
Closed refluxion and titration					
- APHA 5220 C	22 th ed	40 to 400 mg/l	no information	150 ± 2°C	2h
- ISO 15705	2002-11	to 1000 mg/l	1000 mg/l	150 ± 5°C	2h ± 10 min
Photometric analysis					
- ISO 15705	2002-11	to 50 mg/l	1000 mg/l	150 ± 5°C	2h ± 10 min
- ISO 15705	2002-11	to 150 mg/l	1000 mg/l	150 ± 5°C	2h ± 10 min
- ISO 15705	2002-11	to 1000 mg/l (1500 mg/l)	1000 mg/l	150 ± 5°C	2h ± 10 min
- APHA 5220 D	22 th ed	to 90 mg/l	no information	150 ± 2°C	2h
- APHA 5220 D	22 th ed	100 to 900 mg/l	no information	150 ± 2°C	2h
- EPA 410.4 (manual)	1978	20 to 900 mg/l	no information	150°C	2h
- EPA 410.4 (automated)	1993-08	3 to 900 mg/l	no information	150°C	2h

Photometric determination of COD means two different methods (principles)

The concentration of the unconsumed **yellow $\text{Cr}_2\text{O}_7^{2-}$** ions is determined photometrically. This method gives the calibration curve a **negative** gradient. This is the case for the following five items:

- 1.14560.0001	4.0 – 40.0 mg/l	340 nm
- 1.01796.0001	5.0 – 80.0 mg/l	340 nm
- 1.14540.0001	10 – 150 mg/l	445 nm
- 1.09772.0001 (Hg free)	10 – 150 mg/l	445 nm
- 1.14895.0001	15 – 300 mg/l	445 nm
- 1.14690.0001	50 – 500 mg/l	445 nm

The concentration of the **green Cr^{3+}** ions is determined photometrically. This method gives the calibration curve a **positive** gradient. This is the case for the following four items:

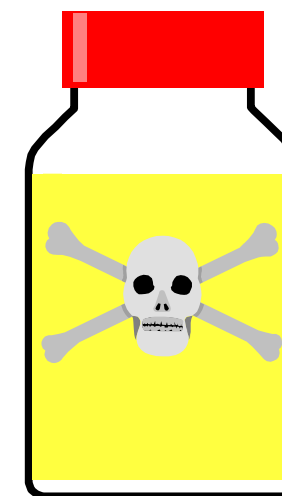
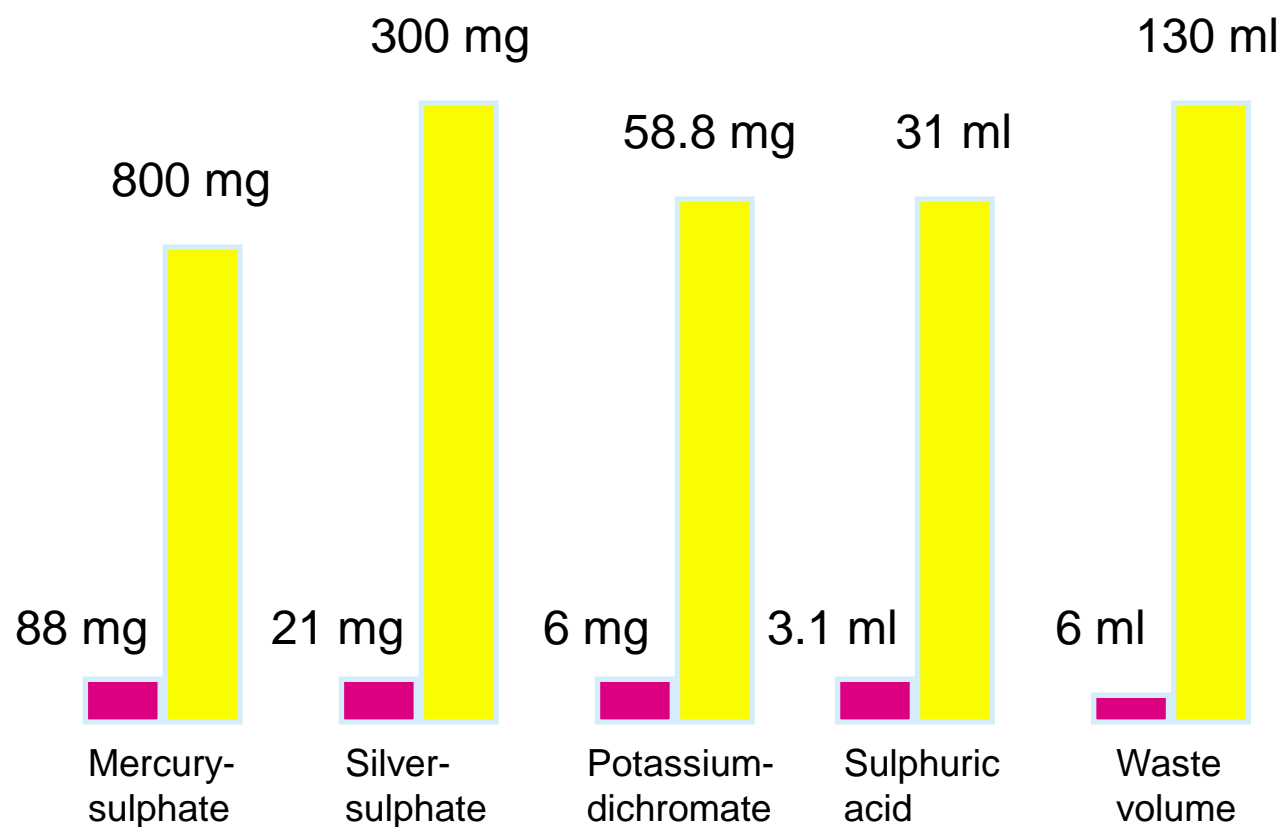
- 1.14541.0001	25 – 1500 mg/l	605 nm
- 1.09773.0001 (Hg free)	100 – 1500 mg/l	605 nm
- 1.14691.0001	300 – 3500 mg/l	605 nm
- 1.14555.0001	500 – 10000 mg/l	605 nm
- 1.01797.0001	5000 – 90000 mg/l	605 nm


Both methods corresponds to ISO 15705 and are analogous to EPA 410.4 +APHA 5220 D.

Reasons why the ISO 15705 was established

- Minimise the handling of toxic chemicals by laboratory staff:
 - The ISO 6060 (open refluxion and titration) is using toxic reagents like
 - Potassium dichromate
 - Mercury sulfate
 - Silver sulfate
 - Sulfuric acid
 - These reagents must be very carefully handled from the laboratory staff.
- The **same** above **reagent** are used in the **cell tests** but in a **closed tube**!
- **Minimise toxic waste**
 - The quantity of used reagents from COD Cell Tests according ISO 15705 in comparison with the ISO 6060 is about **10 times less**!

Spectroquant® - Less toxic waste!



 COD Standard method ISO 6060 (open reflux, titrimetric)

 COD Cell test Merck 1.14540

Reasons why the ISO 15705 focused on commercial available cell tests

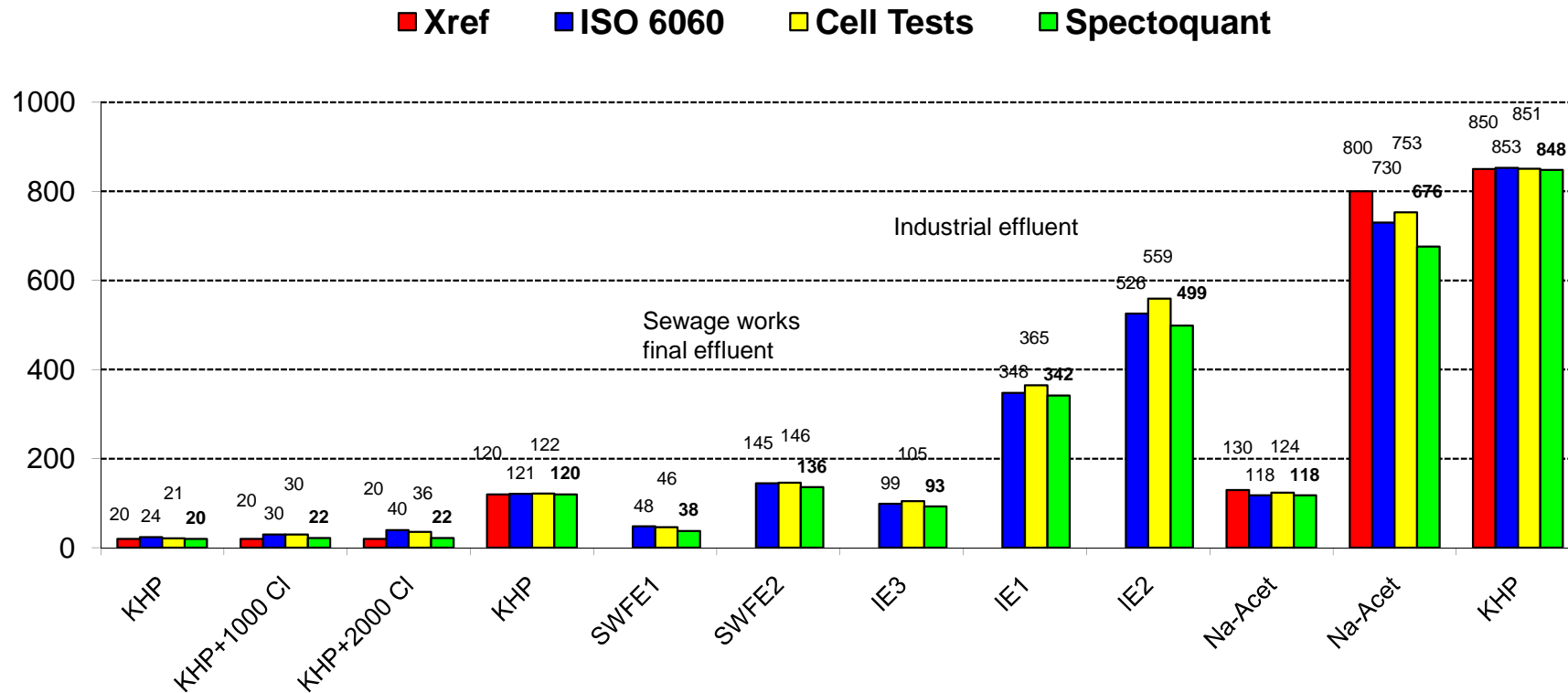
- Many users are already using the COD Cell Tests
 - COD Cell Test are worldwide used for the control of i.e. waste water.
 - The COD Cell Tests are giving good results and they are available in a high quality from different suppliers



The idea of the ISO 15705 was to use commercial available cell tests which have been on the market already !!!

Ring trial data for ISO 15705 titration method – Σ Cell Tests - Spectroquant®

Comparison: titration method (18 results) – Cell Tests (135 results)
two Merck laboratories (8 results) using Spectroquant®



Conclusion: the results from titration and COD cell tests are comparable

Statistical data from the ring trial used from the ISO 15705

Table G.1 — Summary of results of interlaboratory trial using the small-scale sealed-tube ST-COD method

Sample	<i>L</i>	<i>N</i>	NAP	X_{ref}	\bar{X}	Rec	SDr	CVr	SDR	CVR
Sewage Works Final Effluent 1	35	136	6,62		46,2	—	3,88	8,40	7,47	16,16
KHP 20	35	135	5,93	20	21,1	105,4	3,62	17,17	4,32	20,52
Industrial Effluent 1	32	124	4,03		365,2	—	9,09	2,49	16,59	4,54
KHP 850	32	123	12,20	850	851,1	100,1	10,27	1,21	14,85	1,75
KHP 20 + chloride 1 000	35	131	6,11	20	30,3	151,4	4,22	13,94	8,48	28,00
Industrial Effluent 2	32	124	7,26		558,8	—	10,11	1,81	39,01	6,98
KHP 120	35	136	4,41	120	122,1	101,7	3,54	2,90	5,03	4,12
Industrial Effluent 3	35	136	1,47		105,0	—	3,94	3,76	7,63	7,27
KHP 20 + chloride 2 000	33	127	20,47	20	36,3	181,7	5,68	15,63	10,52	28,96
Sodium acetate 130	35	136	5,15	130	124,0	95,4	4,08	3,29	6,40	5,17
Sewage Works Final Effluent 2	34	132	7,58		145,6	—	5,74	3,94	10,36	7,11
Sodium acetate 800	32	124	3,23	800	753,2	94,2	11,88	1,58	46,19	6,13
Key										
<i>L</i> is the number of laboratories (for this level);										
<i>N</i> is the number of values;										
NAP is the percentage of outliers;										
X_{ref} is the reference value, in milligrams per litre;										
\bar{X} is the total mean value, in milligrams per litre;										
Rec is the recovery, in percent;										
SDr is the repeatability standard deviation, in milligrams per litre;										
CVr is the repeatability coefficient of variation, in percent;										
SDR is the reproducibility standard deviation, in milligrams per litre;										
CVR is the reproducibility coefficient of variation, in percent;										
KHP is potassium hydrogen phthalate.										
NOTE All KHP and sodium acetate concentrations given in the sample column are in milligrams per litre ST-COD. All chloride concentrations given in the sample column are in milligrams per litre of chloride.										

Existing Spectroquant® COD Cell Tests for water and wastewater analysis from MM

What are the Chloride levels which doesn't interfere at the today's portfolio?

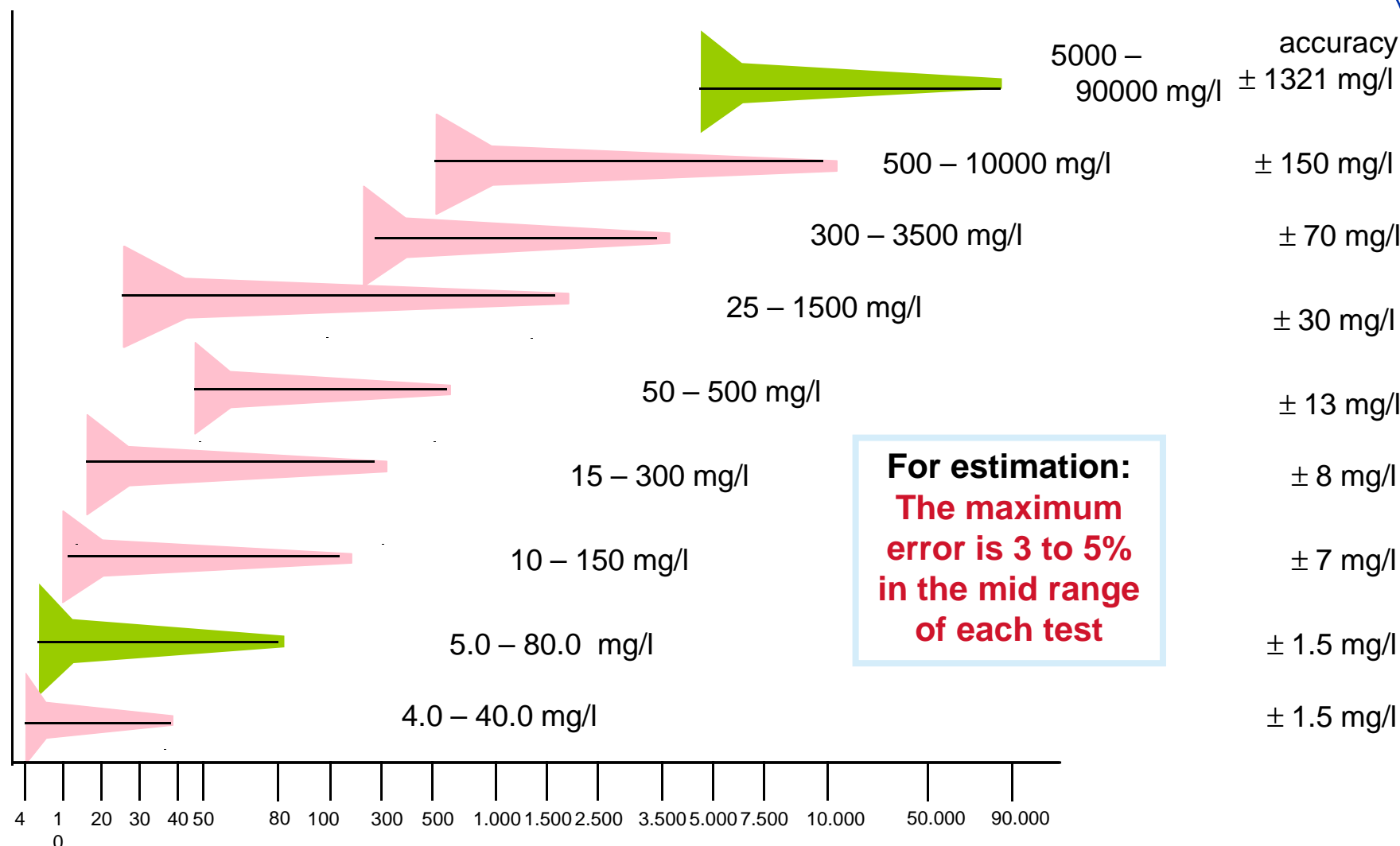
- The tolerated chloride levels depends on the measuring ranges:

COD Measuring ranges	Sample volume	Chloride tolerance which doesn't interfere	item#
4.0 – 40.0 mg/l	3.0 ml	2000 mg/l	1.14560.0001
5.0 – 80.0 mg/l	2.0 ml	2000 mg/l	1.01796.0001
10 – 150 mg/l	3.0 ml	2000 mg/l	1.14540.0001
15 – 300 mg/l	2.0 ml	2000 mg/l	1.14895.0001
50 – 500 mg/l	2.0 ml	2000 mg/l	1.14690.0001
25 – 1500 mg/l	3.0 ml	2000 mg/l	1.14541.0001
300 – 3500 mg/l	2.0 ml	2500 mg/l	1.14691.0001
500 – 10000 mg/l	1.0 ml	5000 mg/l	1.14555.0001
5000 – 90000 mg/l	0.10 ml	50000 mg/l	1.01797.0001

How to choose the correct measuring range for COD?

- We have nine different measuring ranges available, plus two mercury free COD Cell Tests
 - Select the range according to **the customers requirements from the samples**
 - **Avoid dilution** because this increases the error
- The absolute error depends on the measuring range, as shown on next page
- Just by choosing the correct range, the error can be minimized
- Be aware that
 - **Smaller quantities (e.g. 0.10 ml) require a homogenous sample.** A proper **sample preparation** therefore is essential.
 - For quantities below 1.0 ml a **proper micro pipette** is required too. A glass pipette with a size of 1.0 ml and a 0.1 ml graduation is not sufficient enough to add 0.10 ml of volume precisely.
 - A micro pipette is also required when adding reagent 2 from CombiCheck (also 0.10 ml volume).

Quality data for the different COD measuring ranges



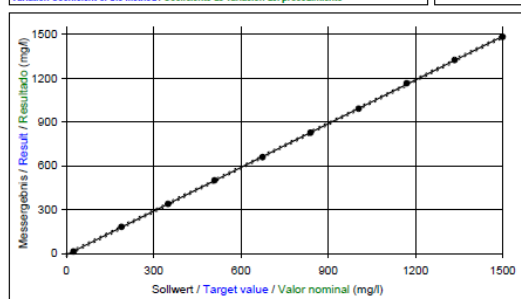
What kind of data can you find on the Spectroquant® lot certificate?

① M

Chargenzertifikat Lot Certificate / Certificado del lote

Spectroquant® CSB-Küvettestest
Spectroquant® COD Cell Test / Spectroquant® Test en cubetas DQO

Art.Nr. / Cat.No. / Art. Nro.		n = 10	
Messbereich Measuring Range / Intervalo de medida	25 - 1500 mg/l CSB / COD / DQO	Sollwert Target value Valor nominal (Standard / Patron) mg/l CSB/COD/DQO	Messergebnis Result / Resultado (Standard / Patron) mg/l CSB/COD/DQO
Charge-Nr. / Lot no. / Lote nro.	HC122887	25	14
Verwendbarkeit Expiry date / Fecha de caducidad	06/14	190	182
Standard / Standard / Patron	Potassium hydrogen phthalate 1.02400	350	340
Photometer / Photometer / Fotómetro	Referenz / Reference / Referencia	610	601
Wellenlänge / Wavelength / Longitud de onda	825 nm	675	669
Küvette / Cell / Cubeta	16 mm (round / round / redonda)	840	827
Prüfer / Tester / Verificador	Hr. May	1.005	992
Datum / Date / Fecha	27.06.2011	1.170	1.156
Datst / File / Fichero	1145410001_HC122887_EN	1.335	1.325
		1.500	1.483
Kalibrierfunktion / Calibration Function / Función de calibración DIN 38402 AS1 / ISO 8466-1		Sollwert Target value Valor nominal	Chargenwert Lot value Valor del lote
Steigung / Slope / Pendiente	± Toleranz / Tolerancia	1,00 ± 0,03	1,00
Ordinatenabschnitt / Ordinate segment / Intersección en ordenadas		0,010 ± 0,010 A	-10
Reag.blindwert / Reagent blank / Valor en blanco del react.	± Toleranz / Tolerancia	± 25 mg/l	± 10 mg/l
Vertrauensbereich (95% Wahrscheinlichkeit) Confidence Interval (p=95%) / Intervalo de confianza (95 % de probabilidad)			± 4,0 mg/l
Verfahrensstandardabweichung Standard Deviation of the Method / Desviación estándar del procedimiento			± 0,5%
Verfahrensvariationskoeffizient Variation Coefficient of the Method / Coeficiente de variación del procedimiento			± 0,5%



Merck KGaA
Dr. S. Frey

Qualitätskontrolle
Quality control / Control de calidad

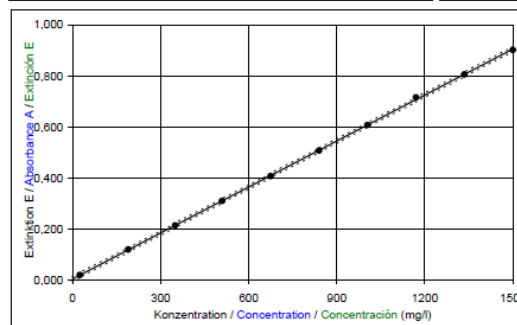
Laborleiter / Head of Lab.
Jefe de laboratorio

② M

Chargenzertifikat Lot Certificate / Certificado del lote

Spectroquant® CSB-Küvettestest
Spectroquant® COD Cell Test / Spectroquant® Test en cubetas DQO

Art.Nr. / Cat.No. / Art. Nro.		n = 10	
Messbereich Measuring Range / Intervalo de medida	25 - 1500 mg/l CSB / COD / DQO	Konz. / Conc. (Standard / Patron) mg/l CSB/COD/DQO	Extinktion Absorbance Extinción
Charge-Nr. / Lot no. / Lote nro.	HC122887	25	0,021
Verwendbarkeit Expiry date / Fecha de caducidad	06/14	190	0,121
Standard / Standard / Patron	Potassium hydrogen phthalate 1.02400	350	0,215
Photometer / Photometer / Fotómetro	Referenz / Reference / Referencia	510	0,311
Wellenlänge / Wavelength / Longitud de onda	825 nm	675	0,408
Küvette / Cell / Cubeta	16 mm (round / round / redonda)	840	0,508
Prüfer / Tester / Verificador	Hr. May	1.005	0,508
Datum / Date / Fecha	27.06.2011	1.170	0,716
Datst / File / Fichero	1145410001_HC122887_EN	1.335	0,906
		1.500	0,902
Kalibrierfunktion / Calibration Function / Función de calibración DIN 38402 AS1 / ISO 8466-1		Sollwert Target value Valor nominal	Chargenwert Lot value Valor del lote
Steigung / Slope / Pendiente	± Toleranz / Tolerancia	0,000500 ± 0,000018	0,000500
Ordinatenabschnitt / Ordinate segment / Intersección en ordenadas		0,010 ± 0,010 A	0,006
Reag.blindwert / Reagent blank / Valor en blanco del react.	± Toleranz / Tolerancia	± 25 mg/l	± 14 mg/l
Vertrauensbereich (95% Wahrscheinlichkeit) Confidence Interval (p=95%) / Intervalo de confianza (95 % de probabilidad)			± 5,8 mg/l
Verfahrensstandardabweichung Standard Deviation of the Method / Desviación estándar del procedimiento			± 0,8%
Verfahrensvariationskoeffizient Variation Coefficient of the Method / Coeficiente de variación del procedimiento			± 0,8%



Merck KGaA
Dr. S. Frey

Qualitätskontrolle
Quality control / Control de calidad

Laborleiter / Head of Lab.
Jefe de laboratorio

First page

- Contains the comparison of the target values and measure values

Second page

- Contains the comparison of the concentration and absorbance values

What kind of data can you find on the Spectroquant[®] lot certificate?

Spectroquant[®] CSB-Küvettestest

Spectroquant[®] COD Cell Test / Spectroquant[®] Test en cubetas I

Art.Nr. / Cat.No. / Art. Nro.	1.14541.0001
Messbereich Measuring Range / Intervalo de medida	25 - 1500 mg/l CSB / COD / DQO
Charge-Nr. / Lot no. / Lote nro.	HC122887
Verwendbarkeit Expiry date / Fecha de caducidad	06/14
Standard / Standard / Patrón	Potassium hydrogen phthalate 1.02400
Photometer / Photometer / Fotómetro	Referenz / Reference / Referencia
Wellenlänge / Wavelength / Longitud de onda	605 nm
Küvette / Cell / Cubeta	16 mm (rund / round / redonda)
Prüfer / Tester / Verificador	Hr. Maly
Datum / Date / Fecha	27.06.2011
Datei / File / Fichero	1145410001_HC122887_EN

The left picture shows all relevant lot specific data to identify the QC data belonging to the item#.

- Name of the test kit
- Item #
- Measuring range
- Lot #
- Expiry date
- The used standard
- The reference photometer
- The used wavelength
- The cell diameter
- The analyst
- The date of analysis
- The file name for download from Internet

What kind of data can you find on the Spectroquant® lot certificate?


page 1		page 2	
Target value Standard mg/l COD	Results Standard mg/l COD	Concentration Standard mg/l COD	Absorbance
25	14	25	0,021
190	182	190	0,121
350	340	350	0,215
510	501	510	0,311
675	659	675	0,408
840	827	840	0,508
1.005	992	1.005	0,608
1.170	1.166	1.170	0,716
1.335	1.325	1.335	0,806
1.500	1.483	1.500	0,902

The left picture shows the lot specific comparison

- Page 1
 - Contains the comparison of the target values and the measured values.
- Page 2
 - Contains the comparison of the concentration and the absorbance values
- Why is this information important for customers?

1145410001_HC122887_EN

What kind of data can you find on the certificates of quality from Spectroquant®?



Qualitätszertifikat
Certificate of quality · Certificado de calidad

Eignung der Spectroquant® Testsätze zur Selbstüberwachung
Applicability of Spectroquant® Test Kits for Self-Monitoring.
Aptitud de los equipos de ensayo Spectroquant® para autovigilancia

Die Verfahrenskennzahlen für den unten genannten Testsatz wurden gemäß ISO 8466-1 und DIN 38402 A51 "Kalibrierung von Analyseverfahren" bei der Produktionsendkontrolle ermittelt.
The characteristic data of the procedure of the following test kit were determined in accordance with ISO 8466-1 and DIN 38402 A51 "Calibration of analysis methods" during the production control process.
Los datos característicos del procedimiento para el equipo de ensayo abajo citado se determinaron según ISO 8466-1 y DIN 38402 A51 "Calibración de procedimientos analíticos" durante el control final de producción.

Spectroquant® CSB-Küvettest, Art.-Nr. 1.14541
Spectroquant® COD Cell Test, Cat. No. 1.14541
Spectroquant® Test en cubetas DQO, Art. Núm. 1.14541

Messbereich / Measuring Range / Intervalo de medida	25 – 1500 mg/l CSB / COD / DQO
Empfindlichkeit: 0.010 E (Extinktion) = Sensitivity: 0.010 A (absorbance) = Sensibilidad: 0.010 A (absorbancia) =	17 mg/l CSB / COD / DQO
Nachweisgrenze Lower Limit of Detection (LLD) Límite de detección	6.9 mg/l CSB / COD / DQO
Bestimmungsgrenze Method Detection Limit (MDL) Límite de determinación	18 mg/l CSB / COD / DQO
Vertrauensbereich (95 % Wahrscheinlichkeit) (Mittelwert aller Chargen) Confidence Interval (P = 95 %) (average value of lots) Intervalo de confianza (95 % de probabilidad) (valor medio de todos los lotes)	± 13 mg/l CSB / COD / DQO
Verfahrensstandardabweichung (Mittelwert aller Chargen) Standard Deviation of the Method (average value of lots) Desviación estándar del procedimiento (valor medio de todos los lotes)	± 5.2 mg/l CSB / COD / DQO
Verfahrensvariationskoeffizient (Mittelwert aller Chargen) Variation Coefficient of the Method (average value of lots) Coefficiente de variación del procedimiento (valor medio de todos los lotes)	± 0.68 %
Anzahl Produktionschargen zur Berechnung Number of Lots for calculation Número de lotes de producción para el cálculo	55
Genauigkeit / Accuracy / Exactitud	± 29 mg/l CSB / COD / DQO

Merck KGaA, Darmstadt, 20.09.2011

Ralf Ott

Ralf Ott

Merck KGaA, 64271 Darmstadt, Germany

The certificate of quality show the background of our norms we follow for the calibration.

It is describe as following

- The characteristic data of the procedure of the following test kit were determined in accordance with ISO 8466-1 and DIN 38402 A51 "Calibration of analysis methods" during the production control process.
- Additionally data like sensitivity, LLD, MDL and accuracy are supplied.
- The certificates of quality are authorized by the head of the R&D lab.

What kind of data can you find on the certificates of quality from Spectroquant®?

Measuring range	25 – 1500 mg/l COD
Sensitivity: 0.010 A (absorbance) =	17 mg/l COD
Lower Limit of Detection (LLD)	6.9 mg/l COD
Method Detection Limit (MDL)	18 mg/l COD
Confidence Interval (P = 95 %) (average value of lots)	± 13 mg/l COD
Standard Deviation of the Method (average value of lots)	± 5.2 mg/l COD
Variation Coefficient of the Method (average value of lots)	± 0.68 %
Number of Lots for calculation	56
Accuracy	± 29 mg/l COD

The certificate of quality summarize all important lot data.

- The CoQ shows the quality of our production and gives and information about how precise are the test kits on the long term and what is the typical deviation customers can expect.
- This example summarize the last 56 lots from ~ 10 years.

What norms are we following during the validation of a Spectroquant[®] method

During the development of a new test or method the following **characteristic statistics** are measured or determined:

- Lower Level of Detection (LLD) according to APHA 1030 C
- Method Detection Level (MDL) according to APHA 1030 C
- Sensitivity = slope (b) measure or calculate per definit. of ISO 8466-1
- Calculate blank a = reagent blank =
ordinate segment (E_0) according to ISO 8466-1
- Confidence Interval VB (P = 95 %) according to ISO 8466-1
- Standard deviation of the method (S_{x_0}) according to ISO 8466-1
- Coefficient of variation of the method (V_{x_0}) according to ISO 8466-1
- Accuracy (A) own calculation (confidence interval plus
the estimation of the blank value error)
- Precision of the results according the APHA 1020 B
- Recovery rate in % according the APHA 1020 B
- Measuring range (working range) according to the ISO 8466-1

ISO 15705: Nov. 2002 – the first ISO norm for Cell Tests - COD



- ISO 15705 was established in **Nov. 2002.**
- ISO 15705 is the first norm describing the use of **commercially available COD cell tests.**

ISO 15705: Nov. 2002 – the first ISO norm for Cell Tests - COD



Certificate

ISO conformity of Merck COD Cell Tests

Cat.-No.	Measuring range
1.14560.0001	4.0 – 40.0 mg/l COD
1.01796.0001	5.0 – 80.0 mg/l COD
1.14540.0001	10 – 150 mg/l COD
1.14895.0001	15 – 300 mg/l COD
1.14690.0001	50 – 500 mg/l COD
1.14541.0001	25 – 1500 mg/l COD
1.14691.0001	300 – 3500 mg/l COD
1.14555.0001	500 – 10000 mg/l COD
1.01797.0001	5000 – 90000 mg/l COD

Hereby we confirm, that the above mentioned Merck COD Cell Tests fully comply with

ISO 15705

Water quality — Determination of the chemical oxygen demand Index (ST-COD) — Small-scale sealed-tube method

	ISO 15705	Merck
Reagents	Potassium dichromate Mercury sulfate Silver sulfate Sulfuric acid	Potassium dichromate Mercury sulfate Silver sulfate Sulfuric acid
Digestion temperature	150 °C ± 5 °C	148 °C ± 3 °C
Digestion time	120 ± 10 min	120 min
Wavelength and measuring range for photometric determination	to 50 mg/l O ₂ 348 ± 20 nm to 150 mg/l O ₂ 440 ± 20 nm to 1500 mg/l O ₂ 600 ± 20 nm	4.0 – 40.0 mg/l O ₂ 340 nm 5.0 – 80.0 mg/l O ₂ 340 nm 10 – 150 mg/l O ₂ 445 nm 15 – 300 mg/l O ₂ 445 nm 50 – 500 mg/l O ₂ 445 nm 25 – 1500 mg/l O ₂ 605 nm 300 – 3500 mg/l O ₂ 605 nm 500 – 10000 mg/l O ₂ 605 nm 5000 – 90000 mg/l O ₂ 605 nm
Accepted chloride levels	1000 mg/l Cl ⁻	minimum 2000 mg/l Cl ⁻

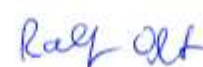
Merck KGaA, Darmstadt, Germany

Darmstadt, 30.04.2011

I.V.


Gunter Decker
Product management

I.A.


Ralf Ott
Head Photometry Lab

- Merck **certificate** of conformity **covers all our COD measuring ranges.**

Eugenio Sironi

EUGENIO.SIRONI@MERCKGROUP.COM

Market Manager Italia – Lab Essentials
Merck KGaA

Grazie