Quantitative determination of the efficacy of drinking water disinfectants

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Foreword

The test procedure described in this paper was developed in the Drinking Water Resources and Water Treatment Department (FG II 3.3) of the German Federal Environment Agency with the collaboration of the Microbiological Risks Department (FG II 1.4). The test procedure has been presented to the Drinking Water Commission of the Federal Ministry of Health at the Federal Environment Agency and the appropriate working group of the German Technical and Scientific Association for Gas and Water (DVGW). Both bodies have approved the test procedure in principle.

Introduction

This test guideline describes a simulation process to determine whether a disinfectant that has been suggested for the purposes described in Section 1 is of sufficient bactericidal and virucidal effect for safe application in drinking-water supplies.

The procedure is conducted as laboratory test in a downgraded semi-industrial facility; it simulates application conditions in actual practice. If results show sufficient efficacy, the concentration of the tested disinfectant can be described as concentration necessary to achieve this sufficient effect. While this concentration applies, only to the specified experimental conditions, the test as such nonetheless can determine whether or not a disinfectant is generally suitable for use in drinking water disinfection. In practical applications, deviation from the sufficiently effective concentration may occur, depending on indication, application conditions and legal regulations. Determining the adequate doses for different water matices and application scenarios are a separate (next) step not addressed here.

1 Area of application

This test procedure defines minimum standards for sufficient bactericidal and virucidal effect of disinfectants that are introduced into water intended for drinking water production, or into drinking water in the distribution network.

A prerequisite for the determination of bactericidal or virucidal effect is that the substance is quantitatively measurable at reasonable cost, and that its effect can be neutralized (inhibited) within a short period of time (Annex C).

Under specific conditions (see Section 5.7) additional test conditions may be required for a final assessment.

2 Normative references

This test guideline contains specifications from standards that are identified by means of dated and undated references. These normative references are quoted at the respective points in the text, and the respective standards are also detailed below. In the case of dated references, alterations in or revisions of these standards only form part of this test guideline if they have been included in the guideline by means of alterations or revisions. With undated references, the latest edition of the respective standard applies.

DIN EN ISO 7899-1. Water quality - Detection and enumeration of intestinal enterococci in surface and waste water - Part 1: Miniaturized method (Most Probable Number) by inoculation in liquid medium (ISO 7899-1:1998)

DIN EN ISO 7899-2. Water quality - Detection and enumeration of intestinal enterococci - Part 2: Membrane filtration method (ISO 7899-2:2000)

DIN EN ISO 9308-1. Water quality - Detection and enumeration of Escherichia coli and coliform bacteria - Part 1: Membrane filtration method (ISO 9308-1:2000)

DIN EN ISO 9308-3. Water quality - Detection and enumeration of Escherichia coli and coliform bacteria in surface and waste water - Part 3: Miniaturized method (most probable number) by inoculation in liquid medium (ISO 9308-3:1998)

DIN EN ISO 8199. Water quality - General guidance on the enumeration of micro-organisms by culture

DIN EN 12671. Products for the treatment of water for human consumption − chlorine dioxide.

DIN EN ISO 7393-2. Water quality - Determination of free chlorine and total chlorine - Part 2: Colorimetric method using N,N-diethyl-1,4-phenylenediamine, for routine control purposes (ISO 7393-2:1985)

DIN EN ISO 10705-1. Water quality - Detection and enumeration of bacteriophages - Part 1: Enumeration of F-specific RNA bacteriophages (ISO 10705-1:1995)

DIN EN ISO 10705-2. Water quality - Detection and enumeration of bacteriophages. Enumeration of somatic coliphages

Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998 concerning the placing of biocidal products on the market (Biocidal Products Directive)

3 Definitions

The following definitions govern application in this test guideline:

3.1 Product

The definition of biocidal products pursuant to the Biocidal Products Directive applies:
"Active substances and preparations containing one or more active substances, put up in the form in which they are supplied to the user, intended to destroy, deter, render harmless, prevent the action of, or otherwise exert a controlling effect on any harmful organism by chemical or biological means."

3.2 Active Substance

The following definition of active biocidal substances pursuant to the Biocidal Products Directive applies:
"A substance or micro-organism, including a virus or a fungus, having general or specific action on or against harmful organisms."

3.3 Disinfectant

Product or active substance (3.1 and 3.2).

3.4 Test organism

Bacteria and bacteriophages (viruses) are used as test organisms in accordance with this guideline

3.5 Contact time

The period of contact between disinfectant and test organism.

4 Requirements

The laid down requirements apply only in connection with the underlying test procedure detailed in Section 5.

4.1 Bactericidal requirements

An active substance or product can only be described as having sufficient bactericidal effect for application in drinking water if the following criterion is met:
Ability of a product or active substance to achieve a reduction in the concentration of living, vegetative bacterial cells of *Eschericha coli and Enterococcus faecium* reference strains (see section 5.2.1), under the conditions specified in this guideline, of at least 2 log_{10} steps after a contact time of 10 minutes and 4 Log_{10} steps after 25 minutes.

Reference strains	Reduction | Contact time	Reduction | Contact time
Eschericha coli	2 Log_{10} steps | 10 minutes	4 Log_{10} steps | 25 minutes
Enterococcus faecium	2 Log_{10} steps | 10 minutes	4 Log_{10} steps | 25 minutes

4.2 Virucidal requirements

An active substance or product can only be described as having sufficient virucidal effect for application in drinking water if the following criterion is met:
Ability of a product or active substance to achieve a reduction in the concentration of bacteriophages (viruses) of MS2 *and* PRD1 reference strains (see section 5.2.1), under the conditions specified in this guideline, of at least 2 log_{10} steps after a contact time of 10 minutes and 4 Log_{10} steps after 25 minutes.

Reference strains	Reduction | Contact time	Reduction | Contact time
Bacteriophage MS2	2 log_{10} steps | 10 minutes	4 log_{10} steps | 25 minutes
Bacteriophage PRD1	2 log_{10} steps | 10 minutes	4 log_{10} steps | 25 minutes

5 Tests

5.1 Principle

Test organisms (bacteria and viruses) are introduced with continuous flow at the transfer point into a pipe through which a defined water sample (see Section 5.2.3.2) flows, and their concentration in the water sample is determined. The disinfectant to be tested is then injected into the volume flow. This marks the starting point of disinfection. Following three defined periods of contact between the test organism and the disinfectant, samples are taken at the sampling taps provided for this purpose. Immediately at sampling, the disinfection process in the sample is inhibited (neutralized) and the concentrations of test organisms then determined. The disinfectant concentration on all taps are recorded in parallel. In order to satisfy the efficacy requirements for a disinfectant prescribed in this test procedure, the above specified reduction of test organisms has to be achieved after 10 and 25 minutes.

5.2 Materials and reagents

5.2.1 test-Bacteria and test-viruses

The bactericidal effect is assessed using the following bacteria and viruses:

a) *Escherichia coli* A3, obtainable at the Federal Environment Agency[1].
b) *Enterococcus faecium,* obtainable at the Federal Environment Agency [1].
c) Bacteriophage MS2 (DSM[2] 13767)
d) Bacteriophage PRD1 (DSM[2] 19107)

Should other strains be used they must be cultivate under optimal growth conditions (temperature and atmosphere), similar to the test-bacteria an test-viruses. The precise process has to be documented in the test report (see Section 5.8).

5.2.2 Host bacteria for bacteriophages

Salmonella Typhimurium WG49 (NCTC[3] 12484) is used as host strain for the bacteriophages MS2 und PRD1.

5.2.3 Culture media and reagents

5.2.3.1 General

Reference is made in the test procedure to other standards. Equipment for the conduct of tests in accordance with these other standards is not listed.
The reagents must be of "analytically pure" grade quality and be appropriate for microbiological purposes. Pre-prepared media of the same composition, which are produced in accordance with the instructions of the manufacturer, can be used for production of culture media.

5.2.3.2 Defined water sample

The following basic conditions must be met by the defined water sample:

- temperature: 15 °C ± 2 °C

[1] Federal Environment Agency, FG II 1.4 Microbiological Risks, Corrensplatz 1, 14195 Berlin, Germany
[2] DSMZ - Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Inhoffenstraße 7 B, 38124 Braunschweig, Germany
[3] Health Protection Agency Culture Collections, Health Protection Agency, Centre for Emergency Preparedness and Response, Porton Down, Salisbury, SP4 0JG, United Kingdom

- pH value: 7.5 ± 0.2
- dissolved organic carbon (DOC): 2.0 mg/l ± 0.3 mg/l

The pH value is regulated through the addition of hydrochloric acid HCl or sodium hydroxide (NaOH). Only DOC that occurs naturally in water should be used. Production of the defined water sample takes place in a storage tank. Thorough mixing has to be ensured.

5.2.3.3 Lactose peptone bouillon

Lactose peptone bouillon, a liquid culture medium, is used for the propagation of *E. coli*.

Peptone obtained from casein (Sifin TN 1403)	17.0 g/l
Peptone obtained from soy flour (Sifin TN 1419)	3.0 g/l
Lactose (Merck 1.07657)	10.0 g/l
Sodium chloride (Merck 1.06404)	5.0 g/l
Demineralized water	1000 ml

Dissolve the substances in demineralized water in a flask (with a magnetic stir bar on a magnetic stirrer), adjust pH value to 7.2 ± 0.2 at 25°C, portion in 100 ml flasks, seal with a cellulose stopper and aluminium foil, and autoclave for 15 minutes at 121°C.

5.2.3.4 Glucose bouillon

Glucose bouillon, a liquid culture medium, is used for the propagation of *E. faecium.*

Peptone obtained from casein (Sifin TN 1403)	15,0 g/l
Meat extract (Sifin TN 1410)	4,8 g/l
D-glucose (Merck 1.08342)	7,5 g/l
Sodium chloride (Merck 1.06404)	7,5 g/l
Demineralized water	1000 ml

Dissolve the substances in demineralized water in a flask (with a magnetic stir bar on a magnetic stirrer), adjust pH value to 7.2 ± 0.2 at 25°C, portion in 100 ml flasks, seal with a cellulose stopper and aluminium foil and autoclave for 15 minutes at 121°C.

5.2.3.5 CASO-Agar (casein soy peptone agar)

Detection and enumeration of Escherichia coli and coliform bacteria (tryptone soy agar, *TSA*):

Casein (tryptically digested)	15g
Soy peptone	5g, NaCL 5g
Agar (in powder or flake form)	15g-25g depending on gel strength
Distilled water	1000ml

5.2.3.6 Neutralization medium

A substance that halts the effect of a disinfectant, including by-products, is called a neutralization medium or inhibitor. In order not to falsify measurements this reaction has to be concluded within a few seconds.
The inhibitability of a disinfectant is one of the acceptance criteria for active substances and products (Annex C).

Sodium thiosulphate can be used to neutralize (Annex D) oxidative chlorine compounds.

5.2.3.7 Chloroform

Chloroform ($CHCl_3$), 99 %, M = 119.38 g/mol is used for the production of phage suspension (see section 5.4.3).

5.3 Equipment

5.3.1 General

Reference is made in this test procedure to other standards. Here we list only equipment that is not within the scope of these other standards. The standard equipment of a microbiological laboratory is required. Special equipment and materials that are required to test the efficacy of disinfectants are listed in Annex A.

5.3.2 Centrifuge

Centrifuge with centrifuge tube that has a volume of 200 ml and a minimum rotational speed of 6000 rpm.

Incubator thermostatizable at (44 ± 0.5) °C
Incubator thermostatizable at (37 ± 2) °C

Autoclave
Operated at (121 + 3) °C and (115 + 3) °C
Photometer

5.4 Production of viral and bacterial suspension

5.4.1 General

Microbial strains have to be preserved in accordance with the requirements of EN 12353.

5.4.2 Bacterial suspension

5.4.2.1 *E. coli* A3

One strain *of E. coli* A3 from a working culture that has been frozen at -80° C (± 2 °C) is smeared on CASO-Agar (*casein soy peptone agar*) (5.2.3.5) and incubated for 8 h ± 1 hour at 36°C ± 2°C. The culture grown on the agar is retrieved with an inoculation loop and used for inoculation of 100 ml lactose peptone bouillon (5.2.3.3). The inoculated culture is incubated for 20 hours ± 4 hours at 36 °C ± 2 °C and retrieved through centrifugation (6000 rpm, 15 minutes). The supernatant is discarded after centrifugation and rinsed with a defined sterile water sample (6000 rpm, 15 minutes). The rinsed pellet is placed into a 100 ml sterile defined water sample. The bacterial suspension has to be preserved at 4 °C ± 2 °C and inoculated after two hours at the latest into the prepared storage vessel of the test facility (5.5.1).

5.4.2.2 *E. faecium* Teltow 11

One strain of *E. faecium* Teltow 11 from a frozen working culture is smeared on CASO-Agar and incubated for 7 ± 2 hours at 36°C ± 2°C. The culture grown on the agar is retrieved with an inoculation loop and used for inoculation of 100 ml glucose bouillon

(5.2.3.4). The inoculated culture is incubated for 20 hours ± 4 hours at 36°C ± 2°C and retrieved through centrifugation (6000 rpm, 15 minutes). The supernatant is discarded after centrifugation and rinsed with a defined sterile water sample (6000 rpm, 15 minutes). The rinsed pellet is placed into a 100 ml sterile defined water sample. The bacterial suspension has to be preserved at 4°C ± 2°C and inoculated after two hours at the latest into the prepared storage vessel of the test facility (5.5.1).

5.4.2.3 Salmonella thyphimurium strain WG49

The microbial and working cultures are produced in accordance with DIN EN ISO 10705-1.

5.4.3 Bacteriophage suspension

For production of the bacteriophage suspension for testing, the host-strain *Salmonella Typhimurium WG49* is cultivated (15 ± 2) hours in a thermomixer (80 rpm; (20 ± 4) hours; (36 ± 2) °C). TYGB is used as liquid medium (5.4.2.3).
25 ml TYGB are preheated to ambient temperature in a 300 ml Erlenmeyer flask, inoculated with 0.25 ml for (15 ± 2) h and incubated in the thermomixer for 90 minutes at (36 ± 2) °C.
Phages from a bacteriophage suspension are added from reference stock, so that a final concentration of plaque-forming units (pfu) of about 10^6 - 10^8/ ml is achieved. The suspension is then incubated for 4 to 5 hours (see above). 2.5 ml of chloroform (5.2.3.7) are then added under an extractor fan and thoroughly mixed. After sealing, the flask is stored overnight or at least for 4 hours at (5 ± 3) °C. The aqueous phase is transferred into a small pipe and centrifuged with 6000 rpm for 20 minutes. The supernatant is carefully decanted or pipeted off.
A decimal series of dilutions is produced and investigated in a plaque test (5.5.2.2) to determine the titre. The produced bacteriophage sylate is preserved until the test at (5 ± 3) °C, or frozen in 5 ml portions in cryotubes at (80 ±10) °C. In order to minimize the input of dissolve organic carbon (DOC) into the storage container, not more than 10 ml of phage suspension should be added (5.4.3). DOC can reduce the disinfectant by attritation.

5.5 Procedure

5.5.1 Preparation of the test facility

a) The storage container is filled with a defined water sample (5.2.3.2).
b) A volume flow of 400 litres per hour (± 20 l/h) is set.
c) The retention time of the defined water sample has to be constantly adjusted as follows:
    i. Sampling tap 1: 25 seconds ± 10 seconds (validating measurement 5.7.1)
    ii. Sampling tap 2: 10 minutes ± 30 seconds (test value 1)
    iii. Sampling tap 3: 25 minutes ± 60 seconds (test value 2)

    Determination of the retention time of the defined water sample in the disinfectant test rig is described in Annex B.
d) Test organisms are produced as described in Section 5.4 and placed into the storage container of the test rig. The bacterial suspensions (*E. coli, Enterococcus faecium*) are first added together with the bacteriophage MS2. Analysis of bacteriophage PRD1 for testing is conducted in a separate test preparation. In the bacterial-suspension storage container the concentration of bacteriophages must lie between (1×10^9 - 1×10^{11}) pfu /100 ml, and that of

bacteria between (1 x 10^8 - 5 x 10^9) CFU/ 100 ml. The test organisms are added to the defined water sample in a ratio of 1:1000, so that the concentration in the test facility (Tap 0) is between (1 x 10^5 - 5 x 10^6) CFU/ 100 ml or (1 x 10^6 - 1 x 10^8) pfu /100 ml.

e) The selected disinfectant concentration is added. The required physico-chemical parameters in the test rig have to be satisfied for at least 60 minutes before sampling can take place.
f) The disinfectant inhibitor is initially put into the sterile sampling vessel (5.2.3.6).

5.5.2 Procedure for determination of the bactericidal and virucidal effect of an active substance or product

Beginning with sampling taps 3 to 0 (Annex A), a 50 ml sampling vessel is held under the tap and filled under light shaking up to the 50 ml mark. Light shaking is intended to ensure good distribution of the inhibitor in the sample.
After filling, the vessel is quickly sealed, vigorously shaken two or three times and immediately placed onto a rapid mixer (fast 'vortex'). Thorough mixing of the sample with the inhibitor should be ensured, which is evidenced by deep vortex formation. The sample is then once more vigorously shaken.

Should larger sample volumes be required, a 1 litre glass bottle is held under the respective tap and filled under light shaking, leaving free a largish volume of air. After sampling the bottle is immediately sealed and vigorously shaken. The samples are stored in the refrigerator at (5 ± 3) °C until analysis.

The disinfectant concentration is measured after every microbiological sampling of taps 1 to 3, so that the attrition of Disinfectant can be measured.
The disinfectant concentration at time zero is calculated on the basis of the concentration of the storage vessel and the dilution factor arising in the volume flow of the defined water sample.

Before sampling, the rate of flow from every sampling tap has to be measured. Based on flow measurement, the precise contact time between test organism and disinfectant has to be calculated, taking account of the correction factor (see Annex B). Should contact times not comply with specifications (5.5.1c) the rate of flow has to be correspondingly adjusted.

Sampling is repeated three times at intervals of 30 to 60 minutes.

5.5.2.1 Test of complete neutralization of the disinfectant by neutralization medium

An additional sampling vessel with neutralization medium is prepared for each test and a sample taken at Tap 1. The sample is checked for reactive disinfectant residues. No disinfectant may be detected.

5.5.2.2 Analysis of microbiological samples

The bacteriological parameters of the samples (5.5.2) are checked as soon as possible, at least after 4 hours, the virological parameters at the latest after 36 hours. The samples must be stored in a cool place at (5 ± 3) °C.

The following detection methods have to be applied for test organisms:

a) *E. coli* A3 (DIN EN ISO 9308-1 and -3)
b) *E. faecium* Teltow 11 (DIN EN ISO 7899-1 and -2)

c) MS2 (DIN EN ISO 10705-1:2001)
d) PRD1 (DIN EN ISO 10705–2:2001)

For the detection of test bacteria, and depending on the expected concentration, the membrane filtration method (DIN EN ISO 9308-1 or DIN EN ISO 7899-2) or the MPN method (DIN EN ISO 9308-3 or DIN EN ISO 7899-1) is employed with corresponding dilution levels. With membrane filtration at least two discs per sample are prepared.

5.6 Calculation and presentation of results

5.6.1 Calculation of bacterial concentration

With the MPN method the result is directly obtained as MPN/ml or MPN/100 ml.
The calculation of results on the basis of the membrane filtration method takes place in accordance with DIN EN ISO 8199 in CFU/ 100 ml.

5.6.2 Calculation of bacteriophage concentration

If available, select discs with more than 30 well-separated plaques. Where the number of plaques is invariably less than 30 per disk, those disks with the largest sample volume should be selected. Disks with fewer than 10 plaques cannot be used for quantitative determination. On the basis of the number of enumerated plaques, calculate the number X of plaque-forming units of somatic coliphages in 1 ml of the sample as follows:

$$X = \frac{N}{(n_1 V_1 F_1) + (n_2 V_2 F_2)}$$

X number of plaque-forming units of somatic coliphages per millilitre (pfu/ml)
N total number of enumerated plaques of all disks
n_1, n_2 number of parallel determinations, related to each dilution F_1, F_2;
V_1, V_2 applied sample volumes in millilitres, related to F_1, F_2;
F_1, F_2 dilution or concentration factor, related to V_1, V_2
(F = 1 for an undiluted sample, F = 0.1 for tenfold dilution, F = 10 for tenfold concentration etc.).

When merely one dilution or concentration is enumerated, the formula is simplified to:

$$X = \frac{N}{nVF}$$

Example:
A sample was undiluted and diluted 1:10, and in each case two parallels investigated. Enumeration had the following result:

Dilution	Result pfu
undiluted	98; 91
-1	10; 5

Calculation of the result:
N = 98 + 91 + 10 + 5 = 204
Sample quantity V_1 = 1 ml, number of parallel determinations n_1 = 2, factor F_1 = 1, since undiluted,
Sample quantity V_2 = 1 ml, number of parallel determinations n_2 = 2, factor F_2 = 0.1, since diluted 1:10,

$$X = \frac{204}{(2 \cdot 1 \cdot 1) + (2 \cdot 1 \cdot 0.1)}$$

Result: 93 pfu/ml or $9.3 \cdot 10^2$ / 100 ml

5.6.3 Statement of results

The results of microbiological measurements are presented in tables and graphics. The graphical presentation encompasses the results of individual measurement series and the average value depending on contact time (Annex E). The detection limit has to be stated, and data recorded in the unit pfu per 100 ml or CFU per 100 ml.

Disinfection concentrations at the sampling taps 1 to 3 have to be presented in tabular form in mg/litre. In addition, disinfectant concentration in the storage container and computed concentration in volume flow have to be stated in mg/litre. Flow-rate measurement data and precise contact times (after correction) have also to be stated (Annex B).

5.7 Evaluation

If in application of this test procedure the requirements defined in Chapter 4 are met, the disinfectant in the applied concentration can be assumed to be of sufficient effect.
If in application of this test procedure the requirements defined in Chapter 4 are not met, the disinfectant is not suitable for use in the applied concentration in drinking water disinfection.

Since the reference concentration[4] that results from this test procedure corresponds to prescribed experimental conditions, the concentration of the disinfectant in waters with other physico-chemical properties (pH value, DOC, temperature) might have to be adjusted.
If the disinfectant is to be applied under conditions that deviate considerably (chemical composition of the defined water sample, temperature etc.) from test conditions, additional test conditions may be required in order to enable a conclusive comprehensive assessment of concerning sufficient efficacy.

5.7.1 Validating measurement

The validating measurement (5.5.1c) provides additional information on the kinetic with which an active substance or product achieves its effect. It is measured by sampling after 25 seconds ± 10 seconds on tap 1.

[4] If results show sufficient efficacy (see section 4), the concentration of the tested disinfectant can be described as concentration necessary to achieve this sufficient effect or reference concentration.

Very high efficacy in the test procedure at this point indicates that an excessively high concentration might have been applied. Measuring errors and uncertainties can also be more closely examined.

5.8 Test report

The test report must refer to this guideline.
The test report must at least include the following data:

a) Identification of the testing laboratory and the client.
b) Identification of the test conducted:
    1. Identification of product and active substance
    2. Batch number and use-by date (if available)
    3. Manufacturer, supplier, date of delivery (if available)
    4. Prescribed storage conditions
    5. Diluent recommend by the manufacturer for application of the product
    6. Active substance and its concentration
c) Test conditions:
    1. Date/dates of test (period of analysis)
    2. Physico-chemical data on the defined water sample (5.2.3.2)
    3. Concentration of active substance or product
    4. Formation of precipitates or flocculation is documented
    5. Incubation temperature
    6. Neutralization medium or inhibitor applied (5.2.3.6)
    7. Identity of additionally used bacterial and viral strains, together with the applied propagation process
d) Test results:
    1. All results listed in Section 5.6.3 have to be stated
    2. Reference concentration[5]
    3. Assessment of bactericidal and virucidal effect
    4. Number of reruns per test organism
    5. Specific comments
    6. Conclusion
    7. Place, date, signature

[5] If results show sufficient efficacy (see section 4), the concentration of the tested disinfectant can be described as concentration necessary to achieve this sufficient effect or reference concentration.

Annex A: Facility for testing the efficacy of drinking water disinfectants

1 Introduction and facility setup

The disinfectant test facility required under the terms of this test instruction has to be operated in continuous flow mode.
The test facility enables the taking of water samples at four sampling taps (Tap 0 to Tap 3 (see Figure 1). These taps are designed in such a way that retention and contact times can be realized:

Tap 0 = test organisms without disinfectant (start of disinfection)
Tap 1 = 25 seconds ± 10 seconds (validating measurement, 5.7.1)
Tap 2 = 10 minutes ± 30 seconds (test value 1)
Tap 3 = 25 minutes ± 60 seconds (test value 2)

The precise calculation and experimental determination of contact times is described in Annex B.
In phases in which the facility is not used, and at least three weeks before a disinfectant test, a low volume flow of drinking water must be passed through the facility.

The dosing of a defined water sample takes place unpressurized at a transfer point, from which the water is pumped into the facility. Measurement of the physico-chemical parameters pH value, conductivity, redox potential and temperature is carried out at the transfer point, so that characterization of the defined water sample is possible on inflow. Measurements of pressure and total flow have also to be conducted in the test facility. Pressure in the facility should be 3 bar, and may not fall below 2 bar.
Dosing of microbiological test organisms takes place directly at the base of the transfer-point pipe section by means of a peristaltic pump. The disinfectant itself is injected directly into the pipe system. Rapid and thorough blending is required. Before dosing, disinfectant-free concentration of the test organism is determined at Tap 0. A test organism concentration of 10^4-10^6 CFU or pfu per 100 ml has to be effected at Tap 0

The physico-chemical parameters pH value, conductivity, redox potential and temperature of the defined water sample in the outflow of the measuring section have also to be recorded.
The physico-chemical parameters determined at inflow and outflow are continuously recorded (for example, by way of a memograph).

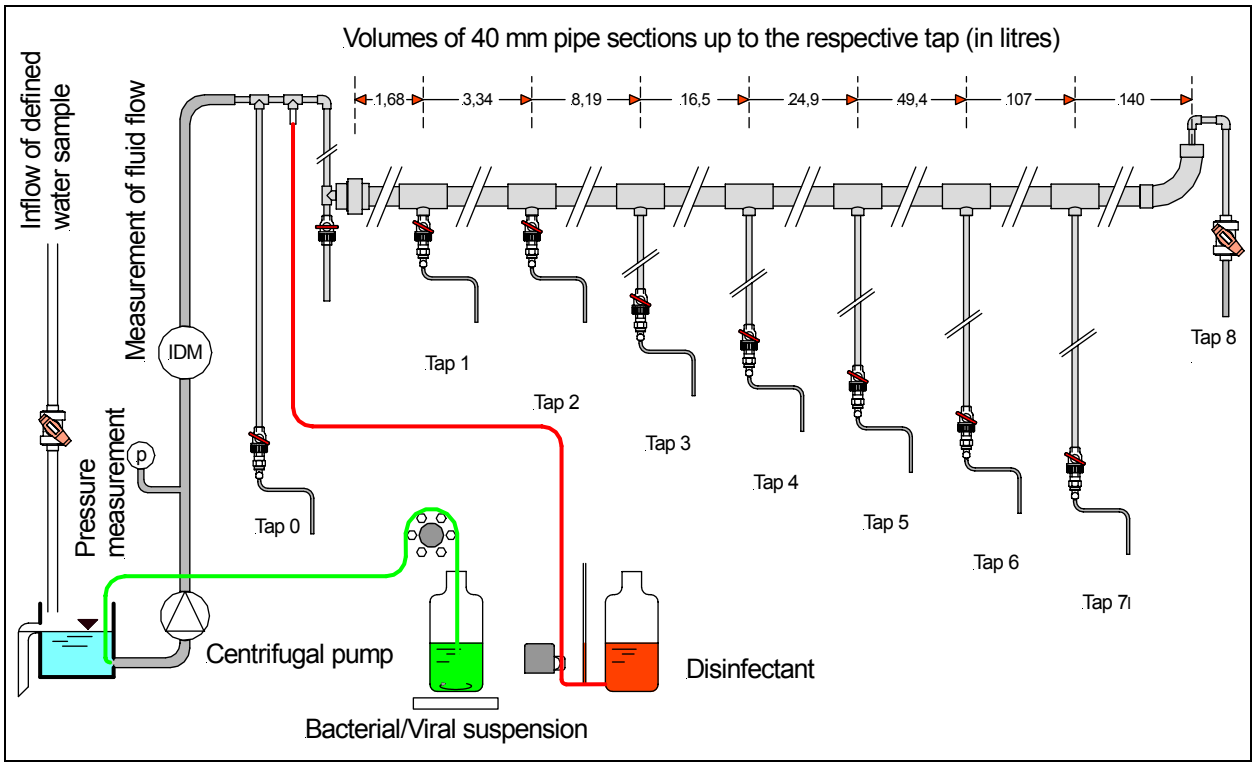


Figure 1) Schematic example of a disinfectant test facility. For the efficacy test stipulated in this guideline only Tap 0, Tap 1 and two further taps are needed that satisfy the prescribed contact times (5.5.1 c).

1.1 Further technical requirements

- The ratio between pipe inner surface and pipe volume must lie between 0.55 and 0.65.
- A flow rate of 0.016 m/s and 0.097 m/s must be achieved.
- In the storage vessel for the bacterial or viral suspension, the concentration of bacteriophages must be between1 x 10^9 - 1 x 10^{11}) pfu /100 ml, and that of bacteria between (1 x 10^8 – 5 x 10^9) CFU/ 100 ml. Test organisms are added to the defined water sample in a ratio of 1:1000, so that in the facility (Tap 0) the concentration amounts to between (1 x 10^5 - 5 x 10^6) CFU/ 100 ml or (1 x 10^6 - 1 x 10^8) pfu /100 ml.
- Test organisms are added to the defined water sample in a ratio of 1:1000, so that in the facility (Tap 0) the concentration lies between 5 x 10^4 and 1 x 10^7 or pfu /100 ml.
- Before the initial disinfectant test is carried out, it has to be confirmed that test organisms within the facility are not reduced. For this purpose, samples are taken from all taps and checked for test organisms.
- The sample volume must be at 50 ml, and be able to be taken within 20 to 40 seconds.
- The applied water pressure within the facility should be between 2 and 5 bar.

Annex B: Determination of contact time between organisms / viruses and disinfectant

1 Determination of retention time

Retention time / contact time is a decisive parameter for the assessment of the efficacy of a disinfectant.
Prior to microbiological tests, a method must therefore be developed with which determination of the retention time at the respective sampling tap is possible.
Determination of retention time takes place in two unrelated procedures that supplement each other. On the one hand it is calculated on the basis of flow rates and pipe diameters, while on the other it is determined experimentally by means of tracer tests.
Examination of the consistency between both methods on the basis of an appropriate comparison criterion, as well as the testing of the possibility for quick and as simple as possible determination of retention time at each sampling tap are necessary for the experimental procedure.

2 Notional calculation of contact time

For notional calculation of contact time in the test facility, the individual pipe sections are measured. On the basis of a given flow (in line with requirements in the guideline), contact time $t_{c,cal}$ is calculated in accordance with Equation (1). This equation is based on indirect proportionality between contact time and flow rate. A reduction in flow rate accordingly leads to longer contact time and *vice versa*. It is therefore possible to regulate contact time through a change in flow rate.

$$t_{C;calc} = \sum t_x + t_y + t_z = \sum \left(\frac{\left(r_x^{\,2} \cdot \pi\right) \cdot l_x}{Q_x} \right) + \frac{\left(r_y^{\,2} \cdot \pi\right) \cdot l_y}{Q_y} + \frac{\left(r_z^{\,2} \cdot \pi\right) \cdot l_z}{Q_z} \qquad (1)$$

$t_{C,calc}$: Contact time calculated [min]

t_x: Contact time in test section [min]

t_y: Contact time in subsection between test section and sampling tap [min]

t_z: Contact time in sampling tap [min]

r: Inner diameter of pipes [m]

l: Length of test section [m]

Q: Volume flow [l/h]; with $Q_y = Q_z$

3 Experimental determination of retention time (tracer tests)

For determination of retention time by means of tracer tests pulses of a saline solution NaCl are dosed into the test facility and conductivity changes at the sampling taps measured in the flow. For statistical evaluation of the results at least 10 saline dosages are required for each sampling point (Tap 1 to Tap 3) in each case. Total flow prior to input into the test section has to be adapted to requirements in the test procedure. Individual flow rates of all taps should be set at between 5 l/h and 15 l/h.

A comparison between notional retention time and measured retention time (tracer tests) enables sound calculation of actual contact times.

In order to obtain reliable comparison criteria, the plotted tracer curves are defined as the times of maximum conductivity as well as of 50% of throughput (50th percentile).

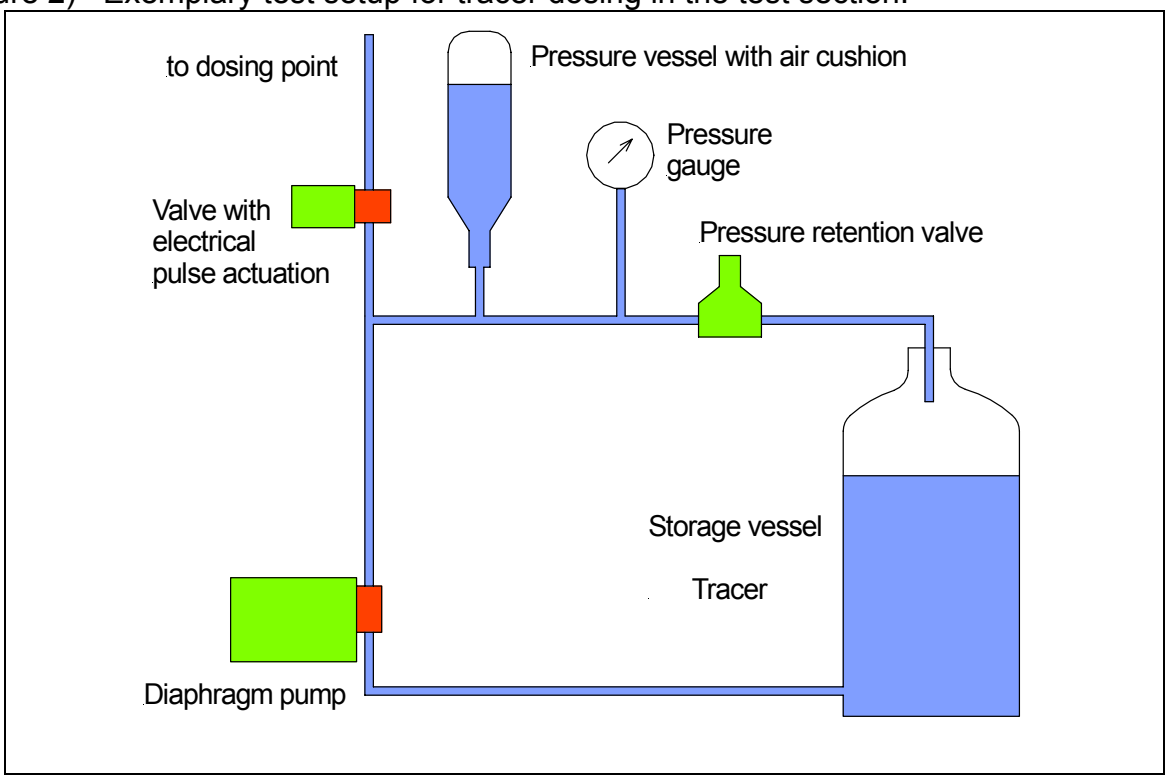
3.1 Test setup

Dosing of the saline solution is carried out with the aid of a diaphragm pump from a storage vessel. A solenoid valve with electrical pulse actuation enables pulsative dosing. Pulse intervals and the time when the solenoid valve is open can be infinitely varied by a control system (Figure 2).
In order that the complete conductivity curve can be recorded, with an increase in retention time from tap to tap pulse intervals must also be increased up to the next dosing.
In order to obtain evaluable measuring signals also in the case of taps with longer retention times, the times are increased during which the solenoid valve is open.

Table 1) Duration and intervals of electrical pulse actuation of a solenoid valve

Meauring point	Duration of pulse signal [s]	Pulse interval [min]
Tap 1	0.02	0.5
Tap 2	0.2	10
Tap 3	1.0	25

Figure 2) Exemplary test setup for tracer dosing in the test section:



3.1.1 Measurement and data recording

Once the saline solution has been introduced into the test facility (see Figure 2) conductivity at selected sampling taps can be measured.

A change in conductivity is measured with two electrodes. Recording of the measuring signal takes place with the aid of a measuring transducer. A conductivity value has to be recorded for each second (
Figure 3).
The recorded measuring signals are not calibrated, since for determination of the time values of respective comparison parameters (maximum, 50th percentile) merely the respective conductivity changes are evaluated related to a base signal, and as a result only the respective time values are required.

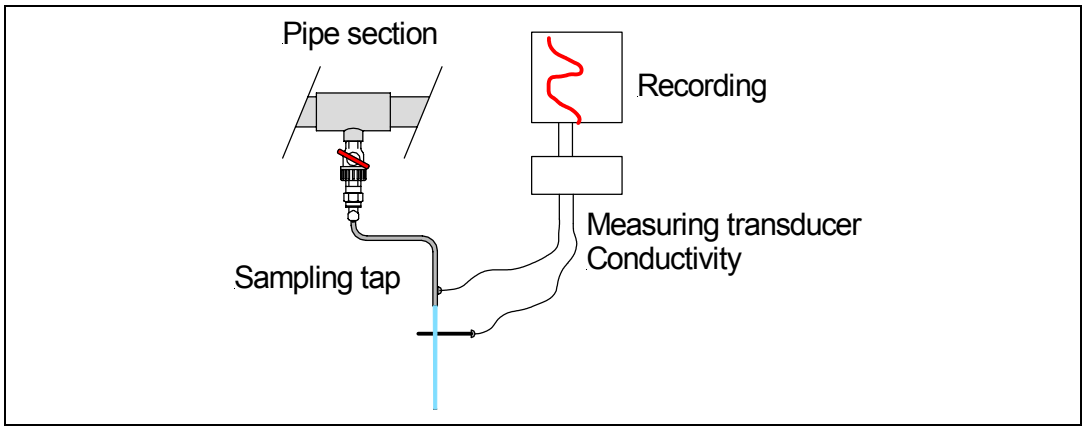


Figure 3) Measurement and data recording configuration

3.1.2 Data evaluation and visualization

For the purpose of graphical illustration recorded conductivity values are plotted over a period of time. Start time is the moment of dosing the tracer into the test section. Figure 4 displays a conductivity distribution curve.

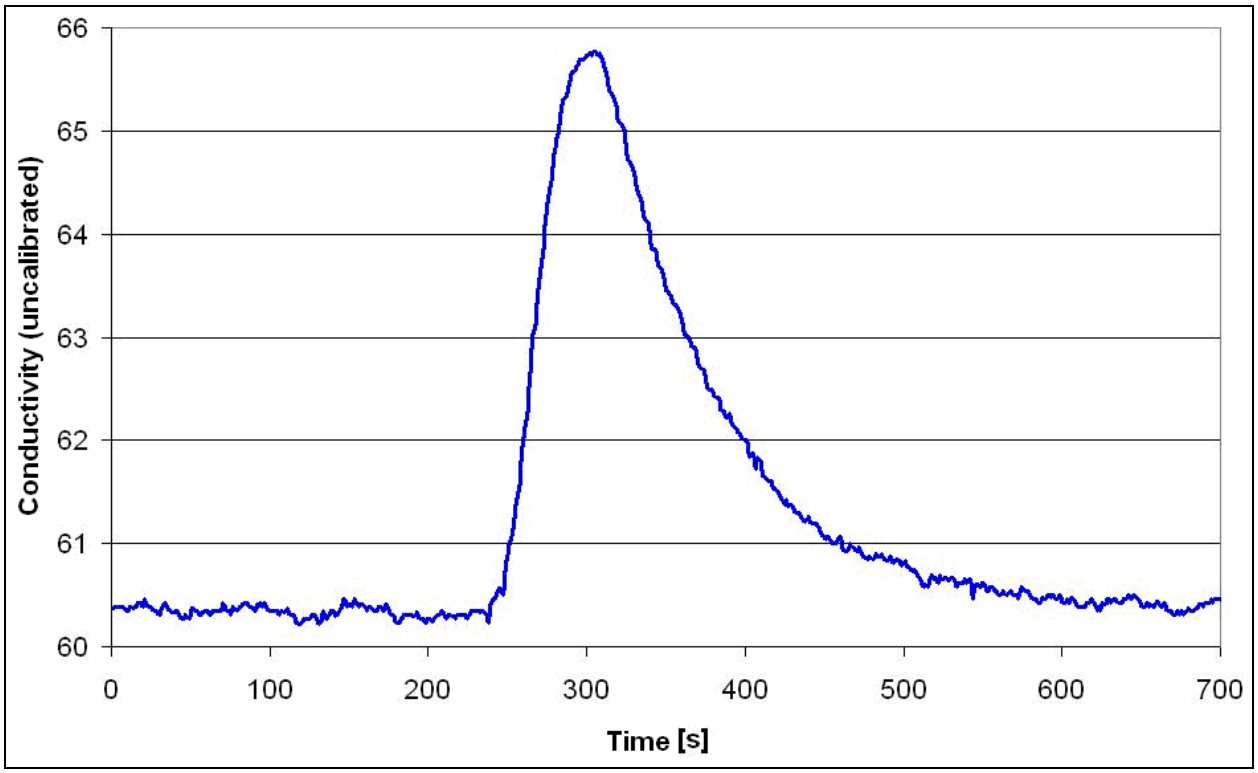


Figure 4) Exemplary graphical evaluation of tracer tests

This curve shows characteristic maximum conductivity, depending on the respective sampling taps, to which a precise time value can be allocated from raw data.
For further evaluation of this conductivity curve the zero baseline – that is background noise – must first be recorded. For this purpose, an average conductivity value is defined before the beginning of the peak.
With the aid of background noise, deviations of measured conductivity from this base signal are now determined.
The sum of all deviations gives the total peak area.
Dividing this value by two gives the value at which 50% of the saline solution has flowed through. A time value can also be allocated to this value from raw data.
The following figure illustrates the two parameters used for comparison.

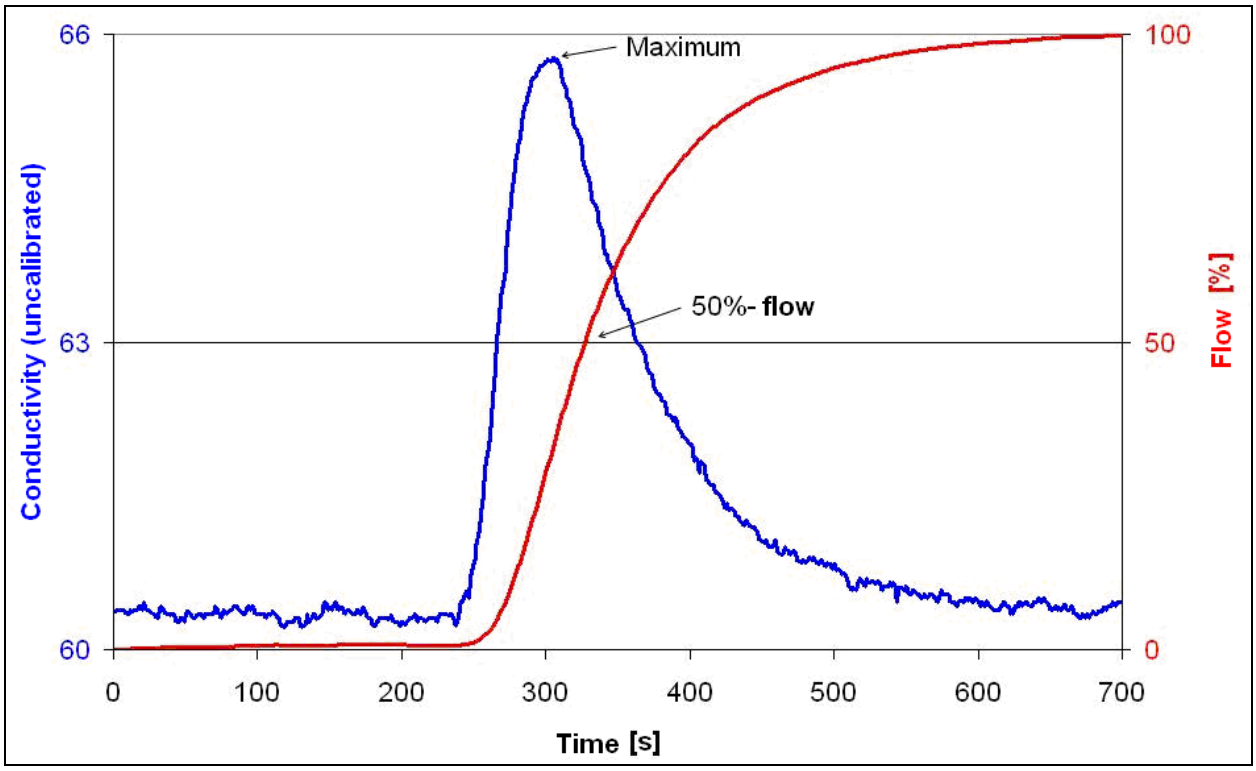


Figure 5) Exemplary graphical evaluation of tracer tests. Blue curve: Conductivity changes with maximum (left axis); red curve: cumulative curve with 50th percentile (right axis)

In order to be able to reliably evaluate respective conductivity changes, especially for the value of the 50th percentile and also in the case of long retention times, a trend line is added to the diagram (Figure 6, red line). This way, an individual conductivity value can be established for every point in time by means of a linear equation with a negative slope. At this Step a trend of the back round noise can be normalized.
On the basis of the method described above, the time value of the 50th percentile can then be calculated.

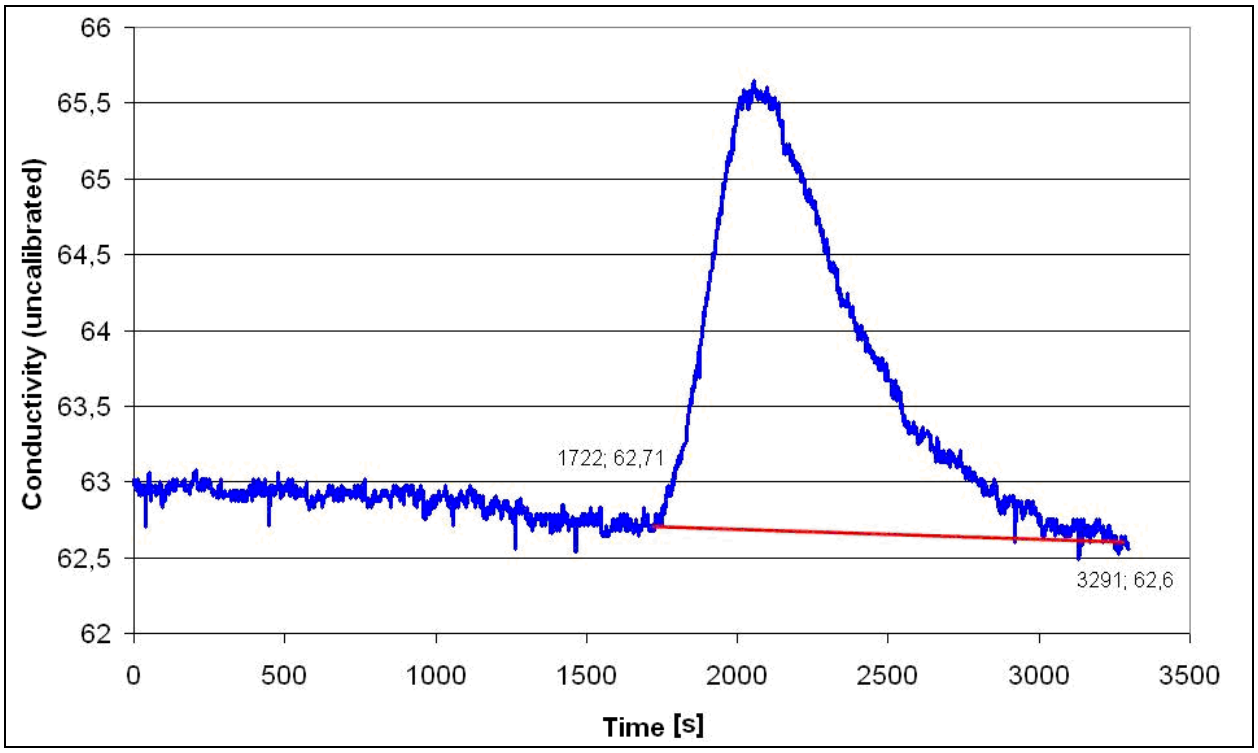


Figure 6) Exemplary graphical evaluation of tracer tests

Since every peak has to be individually evaluated, and at least 10 dosages of saline solution carried out at every tap, the average of all time values calculated at a tap and for a parameter is established. In order to be able to draw sound comparisons between the parameters, respective standard deviations must always be stated.

2.3 Comparison of methods for determination of retention time

3.1.3 Measurements

Varied results for calculated ($t_{calculated}$) retention times and measured ($t_{measured}$) retention times by means of trace tests are shown for the comparison parameters of maximum and 50th percentile of the tracer in Table 2. Standard deviations (s) arise for measured time values from the 10 to 15 dosages of saline solution at each tap.

Table 2) Exemplary results of tracer test for both comparison parameters

			Maximum		50th percentile	
Measuring point	**Flow rate [l/h]**	**$T_{calculated}$ [min]**	**$T_{measured}$ [min]**	**s [s]**	**$T_{measured}$ [min]**	**s [s]**
Tap 1	400	0.5	0.488	29.3	0.62	37.5
Tap 2	400	10.0	10.00	600.5	10.58	634.9
Tap 3	400	25.0	24.97	1498.3	26.17	1570.7

3.1.4 Interpretation and correction factors

In order to be able to make reliable statements on actual retention times for the test facility, without having to repeat tracer tests before each experiment, a correction factor c is applied (Equation 2) for every sampling point, which, when multiplied with retention time calculated on the basis of volume flow, gives actual retention time (t_{actual}) (Table 3).

$$c = \frac{t_{measured}}{t_{calculated}} \qquad (2)$$

Correction factors for the 50th percentile are deemed to be those factors that, when multiplied with notional retention time, best reflect actual retention time for each sampling tap.

Table 3) Exemplary calculation of actual retention time for each sampling tap

Measuring point	Determination of actual retention time
Tap 1	$T_{actual,\ Tap\ 1} = 1.118 \cdot t_{calculated,\ Tap\ 1}$
Tap 2	$T_{actual,\ Tap\ 2} = 1.176 \cdot t_{calculated,\ Tap\ 2}$
Tap 3	$T_{actual,\ Tap\ 3} = 0.852 \cdot t_{calculated,\ Tap\ 3}$

Contact time is required as a parameter for assessment of efficacy in the test facility. It therefore has to be determined prior to each testing for each tap. Since direct determination of contact time is not possible with an internal tracer during the efficacy test, it has to be computed on the basis of flow rates and pipe diameters. As this may contain errors, contact time is determined experimentally before or after the actual efficacy test by tracer tests in order to derive a correction factor for the result calculated from flow rates and pipe diameters. These are then used for quick and simple determination of retention times in the actual efficacy experiment.

Annex C: Acceptance criteria for drinking water disinfectants

Besides efficacy, drinking water disinfectants need to have further properties in order to be suitable for use in drinking-water supplies. These acceptance criteria need to be checked supplementary to efficacy testing.

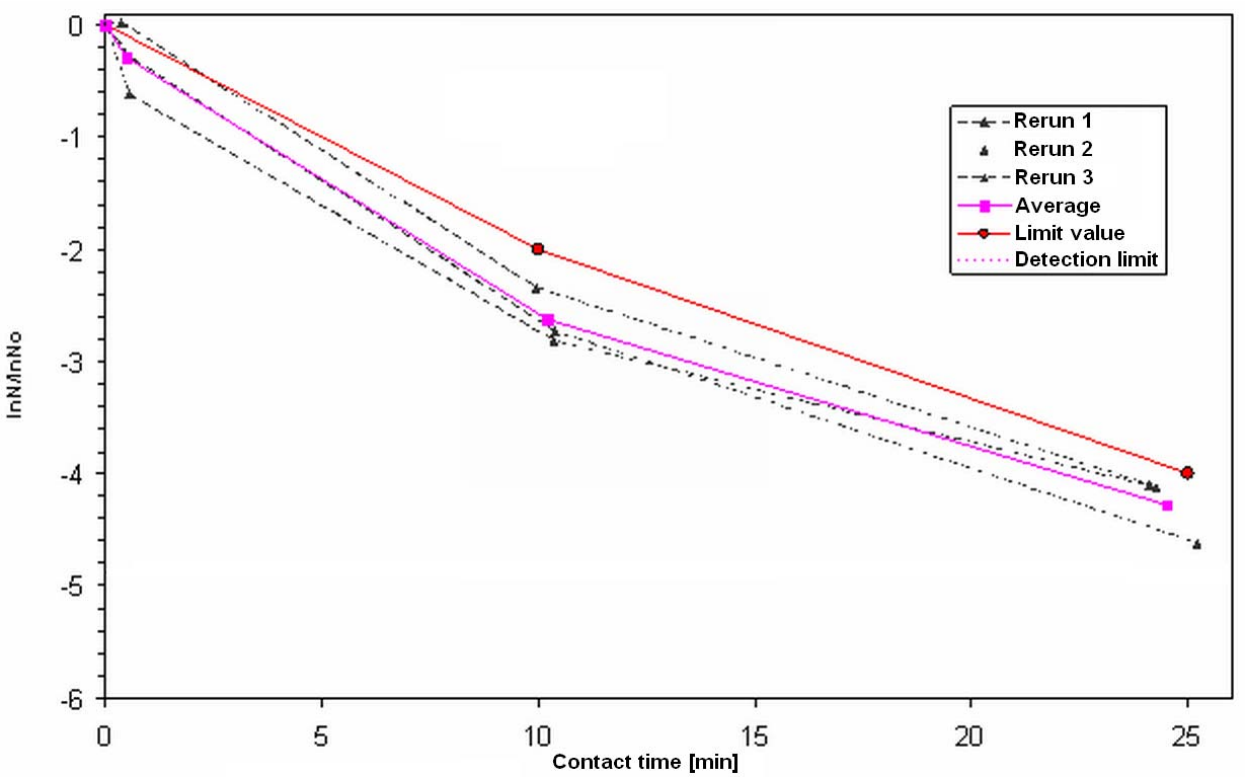
a) Inhibitability
b) On-site measurability
c) Carbon-free source
d) Disinfectant by-products of toxic relevance must be known
e) Simple dosing
f) Stable dosing solution

Annex D: Application of sodium thiosulphate for inhibition of oxidative chlorine compounds

With a sample volume of 50 ml, 40 µl of a 10% sodium thiosulphate solution is initially transferred into a small tube. The result is a final concentration of approximately 0.08 or 0.15 mg/l sodium thiosulphate.

With a sample volume of 1 litre, 300 µl of a 50% sodium thiosulphate solution is initially transferred into the sampling vessel.

Annex E: Exemplary presentation of results



The figure shows the typical course of an active substance or product that achieves the minimum efficacy for one of the four reference organisms in a test (Kapitel 4). The prescribed contact times are attained. The reduction attained is below the prescribed 2- and 4-log steps. The initial measurements after about 30 seconds are validating measurements (5.7.1).