IWW Rheinisch-Westfälisches Institut für Wasser
Beratungs- und Entwicklungsgesellschaft mbH

Final report of a comparison study for the evaluation of the equivalence of XEBIOS DIAGNOSTICS COLIKAT RAPID® and IDEXX LABORATORIES COLILERT-18®

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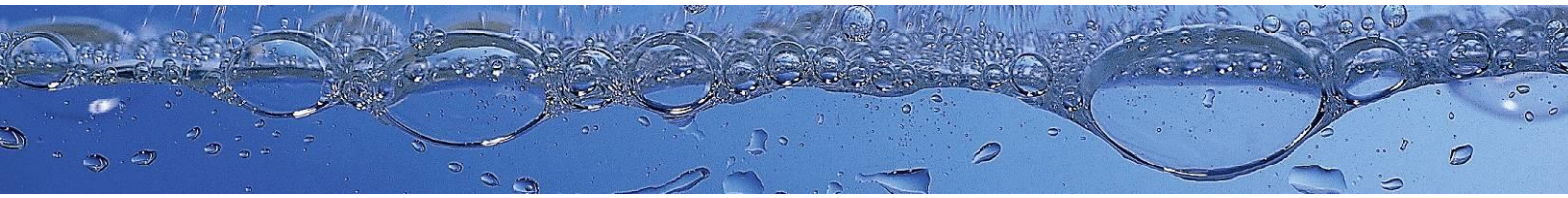

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Executive Summary

An independent validation study for the evaluation of the equivalence of two analytical testing systems for the enumeration of coliform bacteria and *E. coli* according to EN ISO 9308-2 has been carried out on behalf of XEBIOS DIAGNOSTICS GMBH. The intention of this study was the evaluation of the equivalence of the newly launched COLIKAT RAPID® method of XEBIOS DIAGNOSTICS GMBH with the established COLILERT-18®/QUANTI-Tray® method of IDEXX LABORATORIES INC.

12 national and international laboratories participated in the study, which was carried out according to the requirements of the standard method EN ISO 17994. Mainly drinking water samples were inoculated (spiked) with effluents from sewage water installations or surface waters to obtain samples with a suitable concentration of the target organisms (*E. coli*, coliform bacteria). With the help of the spiking procedure the concentration of target organisms in the samples were adjusted to 10 to 200 MPN/100 ml prior to the analysis.

Each sample for analysis was split into two sub-samples. If the results of the sub-sample analysed with COLIKAT RAPID® was not within the 95%-confidence interval of the results of the sub-sample analyses with COLILERT-18®/QUANTI-Tray®, all positive cultures of the cavities of both enumeration trays used were tested for false-positive results, using the MALDI-TOF-MS method. To reduce the enormous effort for the confirmation, the decision criteria was changed from the 95%-confidence interval to a 99%-confidence interval after the relative differences between the two methods stabilized due to a sufficiently high number of analysed samples. In total, 470 valid samples for coliform bacteria and 533 valid samples for *E. coli* were analysed simultaneously with both methods.

Without additional verification of the confirmed counts with MALDI-TOF-MS, the results of all valid pairs of samples (MPN between 1 and 200,5 MPN/100 ml) show lower (LO) and higher (HI) limits of the confidence interval according to EN ISO 17994

for the **enumeration of coliform bacteria** of

$LO_{Coliforms}$ = -1,28 % (target range: > -10 % and < 0 %)

$HI_{Coliforms}$ = 4,13 % (target range : > 0 % and < 10 %)

and for the **enumeration of *E. coli*** of

$LO_{E.\ coli}$ = 1,41 % (target range: > -10 % and < 0 %)

$HI_{E.\ coli}$ = 9,59 % (target range: > 0 % and < 10 %)

According EN ISO 17994 [5], both methods are considered equal, if the lower limit of the confidence interval LO shows a value between -10 % and 0 % and the higher limit of the confidence interval shows a value between 0 % and 10 %. Therefore, the study proved that **both methods are equivalent regarding the enumeration of coliform bacteria**.

The lower limit of the confidence interval LO for the enumeration of *E. coli* is 1,41 %, which is higher than 0 %. According to EN ISO 17994 [5], Section 7.3.5 this can be interpreted in a way that **COLIKAT RAPID® has a significantly higher recovery for *E. coli* compared to COLILERT 18/QUANTI-TRAY®.**

If the corrections resulting from the approximately 4.500 verification tests with MALDI-TOF-MS are taken into consideration, the lower and higher limits of the confidence intervals only change slightly.

In this case the lower and higher limits of the confidence interval for the **enumeration of coliform bacteria** are

$LO_{Coliforms}$ = -1,11 % (target range: > -10 % and < 0 %)

$HI_{Coliforms}$ = 4,17 % (target range: > 0 % and < 10 %)

and for the **enumeration of *E. coli*** they turned out as

$LO_{E.\ coli}$ = 1,30 % (target range: > -10 % and < 0 %)

$HI_{E.\ coli}$ = 9,53 % (target range: > 0 % and < 10 %)

The conclusion that both methods equivalent as regards coliform bacteria and the higher recovery of COLIKAT RAPID® for *E. coli* are absolutely not affected by the verification of positive cultures in the cavities by MALDI-TOF-MS. These verifications were carried out in case of deviating MPN values of COLIKAT RAPID® in comparison to the reference method for the respective sample-pairs. This is mainly a consequence of the excellent specificity of both methods.

Even in the case that only those samples are considered for the evaluation according to EN ISO 17994 [5] in which the results of both methods were between 10 and 200 MPN/100 ml (that means: only results in the statistically sound range) and the verification of the positive wells by MALDI-TOF is taken into account, the conclusion regarding the equivalence of the two methods does not alter.

In this case the lower and the higher limits of the confidence interval for the **enumeration of coliform bacteria** are

$LO_{Coliforms}$= -0,72 % (target range: > -10 % and < 0 %)

$HI_{Coliforms}$ = 4,36 % (target range: > 0 % and < 10 %)

and for the **enumeration of *E. coli*** they turned out as

$LO_{E.\ coli}$ = 1,53 % (target range: > -10 % and < 0 %)

$HI_{E.\ coli}$ = 7,34 % (target range: > 0 % and < 10 %)

To summarise and to conclude the entire set of results and evaluations of the study: It was clearly proven that both methods are equivalent regarding the enumeration of coliform bacteria and that COLIKAT RAPID® shows a significantly higher recovery of *E. coli* compared to COLILERT-18®/QUANT-TRAY®. This applies independently of the criteria for the selection of sample pairs taken into consideration for the evaluation of the equivalence according to the statistical procedure prescribed by EN ISO 17994.

For formal purposes: Only the officially signed German original version applies in case of any formal doubt. All data used for the calculations and evaluations are available for inspection and verification by third parties at:

https://owncloud.iwwtech.de/index.php/s/ZYA6XGRWnGMqPay (access to file: Xebios2021#)

IWW Rheinisch-Westfälisches Institut für Wasser
Beratungs- und Entwicklungsgesellschaft mbH

Mülheim an der Ruhr, January 12th, 2021

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1 Introduction and scope of the study

EN ISO 9308-2:2012 [1] describes a method for the enumeration of *Escherichia coli* and coliform bacteria by determining the most probable number (MPN) of the target organisms.

Until recently, the culture medium described in this ISO method and the accompanying MPN single use trays were patented products and therefore have been commercially available exclusively from IDEXX LABORATORIES INC. Since the related patents expired, other manufacturers or laboratories may as well produce and distribute the test system according to EN ISO 9308-2 [1], provided that all conditions and the procedure are in accordance with the standard method.

The legal compliance of the application of commercially available test systems produced by other manufacturers or the application of the reagent that has been prepared by the applying laboratory itself results from the direct reference of national drinking water legislation to EN ISO 9308-2 [1]: In Europe the member states are obliged to adapt the direct reference to EN ISO 9308-2 [1] into national legislation due to Council Directive 98/83/EG [3] and Commission Directive 2015/1787 EC [4]. For Germany as member state of the European Union the applicability of the standard method results from §15 (1a) No. 1 TrinkwV [2], that directly refers to EN ISO 9308-2 [1] as legally binding method for the enumeration of coliform bacteria and *Escherichia coli*. Such a method therefore is suitable for the examination of potable water according to the drinking water ordinance (TrinkwV [2]) § 15 (1a).

Recently, XEBIOS DIAGNOSTICS GMBH (XEBIOS) has developed a culture medium under the registered trademark „COLIKAT RAPID®“ that complies with EN ISO 9308-2 [1] according to the manufacturer’s information. Therefore, this product may also be used for the examination of potable water under compliance testing conditions. The validation of the ISO-standardised method applying the corresponding MPN-Trays has been carried out in the course of the ISO-standardization process for ISO 9308-2 [1].

As both products and methods formally comply with the requirements of EN ISO 9308-2, an evaluation of the equivalence of XEBIOS’s COLIKAT RAPID® with IDEXX’s COLILERT-18®/QUANTI-TRAY® method is not required legally.

However, XEBIOS decided to launch an international equivalence study according to EN ISO 17994:2014 [5] to validate that COLIKAT RAPID® meets the quality requirements of EN ISO 9308-2 [1] and to dispel possible doubts regarding the suitability of the culture medium for the purpose of drinking water analysis. For this reason XEBIOS asked IWW RHEINISCH-WESTFÄLISCHES INSTITUT FÜR WASSER BERATUNGS- UND ENTWICKLUNGSGESELLSCHAFT MBH (IWW) as independent and recognised consultant to carry out a study for the evaluation of the equivalence

of the COLIKAT RAPID® method with the IDEXX's COLILERT-18®/QUANTI-TRAY® method. The equivalence study has been carried out in accordance with the requirements of EN ISO 17994 "Water Quality – Requirements for the comparison of the relative recovery of microorganisms by two quantitative methods" [5].

2 Participating laboratories

The following 12 laboratories took part in the study. All labs are competent and accredited for the enumeration of *E. coli* and coliform bacteria according to EN ISO 9308-2.

Table 1: Participating laboratories to the equivalence study

Participating laboratory	Country	Responsible
1) De Watergroep / Vlaamse Maatschappij voor Watervoorziening CV	Belgium	TOON SCHOEMAKER
2) DREWAG - Stadtwerke Dresden GmbH	Germany	RONNY MORGENSTERN
3) Eurofins Umwelt Ost GmbH Jena	Germany	ALEXANDRA KELLNER
4) Hamburger Wasserwerke GmbH Hamburg Wasser	Germany	THOMAS MEIER
5) Hessenwasser GmbH & Co. KG	Germany	STEFFEN SCHNEIDER
6) IWW Rheinisch-Westfälisches Institut für Wasser Beratungs- und Entwicklungsges. mbH	Germany	BERND LANGE, JANINE WAGNER
7) Lehr- und Versuchsgesellschaft für innovative Hygienetechnik mbH Institut für angewandte Bau- und Bäderhygiene (LVHT)	Germany	MARKUS FUNKE
8) Niedersächsisches Landesgesundheitsamt (NLGA)	Germany	KATHRIN LUDEN
9) The National Institute of Public Health Czech Republic	Czech Republic	DANA BAUDISOVA
10) RheinEnergie AG Köln	Germany	IRIS HÜBNER
11) Westfälische Wasser- und Umweltanalytik GmbH	Germany	KIM DIEKERMANN
12) Zweckverband Bodensee-Wasserversorgung	Germany	JÜRGEN MEYER

The study has been carried out under the supervision and coordination of IWW (Project Supervisor: DIPL.-BIOL. BERND LANGE, Assistant Project Supervisor JANINE WAGNER). All results submitted to IWW by the participants were analysed and evaluated by LANGE und WAGNER.

3 Project meetings

Two kick-off project meetings were organised before the practical work of the study started. All relevant questions related to the organization of the study and the study design were discussed in detail. The meetings were organized as web-conferences with representatives of all participating laboratories.

In addition to the kick-offs, the preparation of samples suitable for the study, the data capturing and documentation for reporting purposes, the delivery of data and sample trays to IWW for verification and finally the time schedule of the study have been discussed and decided between participants and the coordinator in detail.

4 Sample preparation

Although both test methods are mainly used in the drinking water sector, drinking water is generally not suitable for carrying out a study to compare detection methods for *E. coli* and coliform bacteria, since these target organisms are normally not detectable in drinking water. Therefore, 670 potable water samples, 12 surface water samples and 4 bathing water samples have been inoculated (spiked) with effluents from sewage water installations or with surface waters. Sufficient numbers of target organisms are regularly present in these water matrices. They usually live in a broad natural population together with other microorganisms. Thus, a potential problem caused by an inoculation with a single pure culture could be avoided. In some cases the original samples (surface water) could be analysed without prior spiking. Due to significant regional differences in the waters used by the participating laboratories, a number of different water qualities were evaluated in this study.

5 Study implementation

All samples have been analysed according to EN ISO 9308-2 [1] in all participating laboratories.

Drinking water samples were inoculated with the respective target organisms to achieve a concentration of *E. coli* and coliform bacteria in between 10 und 200 MPN/100 ml.

If necessary, waste water effluents were pre-filtered. After this initial step dilution series of the effluents and the surface waters were prepared and the concentration of *E. coli* and coliform bacteria was determined. As IDEXX's QUANTI-TRAY 2000® und COLILERT-18® are generally recognised methods, they have been applied to carry out these pre-tests.

Based on the results derived by the pre-tests, up to 8 potable water samples, or in rare cases bathing water samples (each 250 ml), were inoculated so that the expected concentration of the target organisms was between 10 und 200 MPN/100 ml.

Due to the fact that the concentration of *E. coli* is usually significantly lower than the concentration of coliform bacteria in natural water samples, it was often necessary to inoculate the water samples for the study with different volumes of the inoculum to achieve the desired range of the concentration for both target organisms.

The samples were shaken thoroughly and separated into two sub-samples of 100 ml each. One sub-sample was then tested with XEBIOS's COLIKAT RAPID® and the other sub-sample with IDEXX's COLILERT-18®. The sub-samples were poured into the corresponding MPN-trays of the two manufacturers, sealed and incubated for 20 hours at (36 ± 2) °C. In case that the results were not clear after 20 hours both trays of the specific sample were incubated for additional 2 hours.

The evaluation was carried out according to EN ISO 9308-2 [1].

In case the results for both sub-samples of one sample showed significant deviations, all positive cavities of both sub-samples were controlled for false-positive results, using MALDI-TOF-MS analysis. As decision criterion for which sample pairs this applies to the 95%-confidence interval of the COLILERT-18® approach was used. That means that all positive cavities of both sub-samples were controlled if the result for the sub-samples tested with COLIKAT RAPID® was outside of the 95%-confidence interval of the result of the sub-sample tested with COLILERT-18®/QUANTI-TRAY®. To reduce the enormous effort for the confirmation, the decision criterion was changed from the 95%-confidence interval to a 99%-confidence interval after the relative differences between the two methods stabilized due to a high number of analysed samples.

Uniform Excel sheets were made available for the participating laboratories. They were mandatory to use in order to collect the individual data. The Excel sheets were configured to only allow the laboratory to enter the number of positive cultures in the cavities into the right cells of the table. All other cells in the table were password secured and locked for entries or editing. The calculation of the MPN and the verification whether the results of the analysis with COLIKAT

RAPID® were in the 95%-confidence interval determined by the results of the analysis with COLILERT-18®/QUANTI-TRAY® were carried out automatically by the Excel sheet. The Excel sheet was independently validated with test data by IWW in the preparation phase of the study.

Both trays of sample-pairs with results that showed a deviation from the designated range of the confidence interval were shipped to IWW using cooled transportation services in order to verify all positive cavities of these sample-pairs with MALDI-TOF-MS. The cavities with positive cultures (yellow or yellow + fluorescent) were marked with a waterproof pen on the tray by the laboratory, to cover the risk that the result would possibly change during the course of the shipment.

At IWW single-colonies from all positive cavities of all trays were spread out on chromogenic coliform agar (according to EN ISO 9308-1 [6]) and TSA-agar. The isolates were then used for identification with MALDI-Biotyper® of BRUKER DALTONIK GMBH (BRUKER). In total approximately 4500 cavities were verified with MALDI-TOF-MS.

All XEBIOS-materials, the COLIKAT RAPID® reagent and the accompanying enumeration-trays-51 and antifoam agent were provided to the 12 laboratories directly by XEBIOS so that all participating laboratories used a culture medium from a single batch. All IDEXX-materials were sourced by the laboratories themselves.

6 Identification of bacteria species with MALDI-TOF-MS

The identification of the isolates from the positive cavities in trays that needed verification (as described above) was carried out with a MALDI-Biotyper® of BRUKER Daltonik GmbH. A small portion of a single colony was transferred to a spot on a stainless steel target (MSP 96 target polished steel BC) or to a single-use target (MBT Biotarget 96) and 1µl Matrix (HCCA, portioned (α-Cyano-4-hydroxycinnamic acid, # 8255344, BRUKER Daltonik GmbH)) was pipetted onto the spot.

After the samples dried the target was inserted into the MALDI-Biotyper®. For the identification the BDAL-database with more than 8000 entries was utilized.

The MALDI-Biotyper® was calibrated every day and before the first identification was carried out the BRUKER Test Standard (BRUKER Bacterial Test Standard (# 8255343, BRUKER Daltonik GmbH) was applied.

7 Study evaluation

7.1 Abbreviations

A	(symbol for idea of) trial method (COLIKAT RAPID®)
a_i	test result (confirmed count) of method A (COLIKAT RAPID®) in sample i
B	(symbol of idea of) reference method (COLILERT-18®)
b_i	test result (confirmed count) of method B (COLILERT-18®) in sample i
D	highest acceptable deviation (upper and lower limit of the confidence interval) in the case of „no difference“ between method A and B [1]
k	coverage factor, which is used as multiplier for the standard uncertainty for the calculation of the extended uncertainty. EN ISO 17994 defines k = 2 as coverage factor.
n	number of samples
W	half width of the confidence interval
x	relative difference
x_i	value of the relative difference between a_i and b_i in sample i

7.2 Specifications according to EN ISO 17994

According to EN ISO 17994 [5], the data base to compare two quantitative methods must consist of pairs of confirmed counts (a_i, b_i) that were obtained by the analysis of 2 equally-sized fractions of one specific well homogenized sample. In order to carry out a comparison study, large numbers of corresponding pairs of confirmed counts are required for the evaluation of the equivalence.

The relative difference in percent x_i) for specific pairs of measurements is calculated according to

$$X_i = 100\ \%^* [\ln(a_i)-\ln(b_i)]$$

.

[1] EN ISO 17994 determines the highest acceptable deviation for the confidence interval for the evaluation of the performance of methods used for potable water in international interlaboratory evaluations to be D = 10 %.

From these values the average relative performance is calculated according to

$$\bar{x} = \frac{\sum x_i}{n} \quad .$$

The standard deviation is calculated according to

$$s = \sqrt{\frac{\sum (x_i - \bar{x})^2}{n-1}} \quad .$$

The standard uncertainty of the mean is calculated according to

$$s_{\bar{x}} = \frac{s}{\sqrt{n}}$$

and half width of the confidence interval is obtained by multiplying the standard uncertainty of the mean with the coverage factor k = 2:

$$W = k * s_{\bar{x}}$$

For the evaluation of the comparability of the trial method and the reference method the confidence interval around the mean is calculated by computing the lower limit and the upper limit:

Lower limit: $LO = \bar{x} - W$

Upper limit: $HI = \bar{x} + W$

According to EN ISO 17994 [5], two methods are considered to be “not different”[2] if the lower limit of the confidence interval *LO* has a value in between –10 % and 0 % and the upper limit of the confidence interval *HI* has a value in between 0 % und 10 %. The trial method has a (significantly) higher recovery as compared to the reference method if the lower limit of the confidence interval has a value above 0 %.[3]

[2] EN ISO 17994 Section 7 determines the terminology “not different”, “inconclusive”, “different” and “indifferent” for the evaluation of the compatibility of the trial method with the reference method. Whether or not one method can be named reference, “not different” is normally understood to mean that neither method gives significantly higher or significantly lower results than the other. Within this study the term “equivalent” shall have the synonymous meaning of “not different” as defined by EN ISO 17994 Sec. 7.

[3] EN ISO 17994 Section 7.3.5.

7.3 Study results for all samples without MALDI-TOF-MS verification

470 samples were included in the study for the evaluation of the equivalence of XEBIOS's COLIKAT RAPID® method with IDEXX's COLILERT-18®/QUANTI-TRAY® method regarding the enumeration of coliform bacteria. For *E.coli* 522 samples were analysed. According to EN ISO 17994 [5], such numbers of samples are more than sufficient to derive statistically sound statements about the equivalence of both methods within the ISO standard's test design. Including all samples - without verification of the results by means of MALDI-TOF-MS - the following average relative performances were obtained:

$\bar{x}_{Coliforms} = 1{,}42$

$\bar{x}_{E.coli} = 5{,}50$

Based on these results, the standard deviation

$Sc_{oliforms} = 29{,}35$

$s_{E.coli} = 46{,}70$

and the standard uncertainty of the mean was calculated:

$sx_{Coliforms} = 1{,}35$

$sx_{E.coli} = 2{,}04$

The half width of the confidence interval was obtained by multiplying the standard uncertainty of the mean and the coverage factor k = 2:

$W_{Coliforms} = 2{,}71$

$W_{E.coli} = 4{,}08$

For the evaluation of the comparability of the trial method (COLIKAT RAPID®) and the reference method (COLILERT-18®/QUANTI-TRAY®) the confidence interval around the mean is calculated by computing the lower limit and the upper limit:

Lower limit: $LO = \bar{x} - W$

Upper limit: $HI = \bar{x} + W$

The following range of the confidence interval was calculated for the enumeration of coliform bacteria:

$LO_{Coliforms} = -1{,}28\ \%$

$HI_{Coliforms} = 4{,}13\ \%$

The following range of the confidence interval was calculated for the enumeration of *E. coli*:

$LO_{E.\ coli}$ = 1,41 %

$HI_{E.\ coli}$ = 9,59 %

According to EN ISO 17994 [5], two methods are considered to be equivalent if the lower limit of the confidence interval LO is located between -10 % and 0 % and the upper limit of the confidence interval HI is located between 0 % and 10 %. **That means this study proves that XEBIOS's COLIKAT RAPID® method is <u>equivalent</u> to IDEXX's COLILERT-18®/QUANTI-TRAY® method regarding the enumeration of coliform bacteria.**

As the lower limit for the confidence interval for the enumeration of *E. coli* was 1,41 %, thus: above 0 %, **the study proves that XEBIOS's COLIKAT RAPID® method has a <u>significantly higher recovery</u> for the target organism *E. coli* as compared to IDEXX's COLILERT-18®/QUANTI-TRAY® method (reference method).**

7.4 Study results for all samples including MALDI-TOF-MS verification

Additionally, the equivalence of the two compared methods was evaluated after correction of the confirmed values in the data base taking into consideration the MALDI-TOF-MS verification results. In this case the following results were obtained.

First the average relative performances were calculated:

$\bar{x}_{Coliforms}$ = 1,53

$\bar{x}_{E.coli}$ = 5,24

Based on these results, the standard deviation

$s_{Coliforms}$ = 28,62

$s_{E.coli}$ = 46,9

and the standard uncertainty of the mean was calculated:

$sx_{Coliforms}$ = 1,32

$sx_{E.coli}$ = 2,06

The half width of the confidence interval was obtained by multiplying the standard uncertainty of the mean and the coverage factor k = 2:

$W_{Coliforms}$ = 2,64

$W_{E.coli}$ = 4,12

For the evaluation of the comparability of the trial method (COLIKAT RAPID®) and the reference method (COLILERT-18®/QUANTI-TRAY®) the confidence interval around the mean is calculated by computing the lower limit and the upper limit:

Lower limit: $LO = \bar{x} - W$

Upper limit: $HI = \bar{x} + W$

The following range of the confidence interval was calculated for the enumeration of coliform bacteria:

$LO_{Coliforms}$ = -1,11 %

$HI_{Coliforms}$ = 4,17 %

The following range of the confidence interval was calculated for the enumeration of *E. coli*:

$LO_{E. coli}$ = 1,30 %

$HI_{E. coli}$ = 9,53 %

According to EN ISO 17994 [5], two methods are considered to be equivalent if the lower limit of the confidence interval LO is located between -10 % and 0 % and the upper limit of the confidence interval HI is located between 0 % and 10 %. **That means this study proves that XEBIOS's COLIKAT RAPID® method is <u>equivalent</u> to IDEXX's COLILERT-18®/QUANTI-TRAY® method regarding the enumeration of coliform bacteria.**

As the lower limit for the confidence interval for the enumeration of *E. coli* was 1,30 %, thus: above 0 %, **the study proves that XEBIOS's COLIKAT RAPID® method has a <u>significantly higher recovery</u> for the target organism *E. coli* as compared to IDEXX's COLILERT-18®/QUANTI-TRAY® method (reference method).**

7.5 Study results for samples including MALDI-TOF-MS verification with 10 to 200 MPN/100ml

Additionally to the correction of the MPN after verification by MALDI-TOF-MS, sample pairs with concentrations outside the target concentration of 10 to 200 MPN/100 were excluded from the evaluation of the equivalence. In this case 432 samples were included into the calculation, for *E. coli* 360 samples were analysed. The following values were obtained for the evaluation of the equivalence of the two methods compared:

$\bar{x}_{Coliforms}$ = 1,82

$\bar{x}_{E.coli}$ = 4,44

Based on these results, the standard deviation

$s_{Coliforms}$ = 26,40

$s_{E.coli}$ = 27,54

and the standard uncertainty of the mean was calculated:

$sx_{Coliforms}$ = 1,27

$sx_{E.coli}$ = 1,45

The half width of the confidence interval was obtained by multiplying the standard uncertainty of the mean and the coverage factor k = 2:

$W_{Coliforms}$ = 2,55

$W_{E.coli}$ = 2,90

For the evaluation of the comparability of the trial method (COLIKAT RAPID®) and the reference method (COLILERT-18®/QUANTI-TRAY®) the confidence interval around the mean is calculated by computing the lower limit and the upper limit:

Lower limit: $LO = \bar{x} - W$

Upper limit: $HI = \bar{x} + W$

The following range of the confidence interval was calculated for the enumeration of coliform bacteria:

$LO_{Coliforms}$ = - 0,72 %

$HI_{Coliforms}$ = 4,36 %

The following range of the confidence interval was calculated for the enumeration of *E. coli*:

$LO_{E. coli}$ = 1,53 %

$HI_{E. coli}$ = 7,34 %

According to EN ISO 17994 [5], two methods are considered to be equivalent if the lower limit of the confidence interval LO is located between -10 % and 0 % and the upper limit of the confidence interval HI is located between 0 % and 10 %. **That means this study proves that – also in case that samples outside the targeted range for the MPN value of 10 to 200**

MPN/100 ml were eliminated from the population – XEBIOS's COLIKAT RAPID® method is equivalent to IDEXX's COLILERT-18®/QUANTI-TRAY® method regarding the enumeration of coliform bacteria.

As the lower limit for the confidence interval for the enumeration of *E. coli* was 1,53 %, thus: above 0 %, **the study proves that – also in case that samples outside the targeted range for the MPN value of 10 to 200 MPN/100 ml were eliminated from the population – XEBIOS's COLIKAT RAPID® method has a significantly higher recovery for the target organism *E. coli* as compared to IDEXX's COLILERT-18®/QUANTI-TRAY® method (reference method).**

In case the samples with a MPN value of 200,5 MPN/100 ml – that means samples at the upper counting range of the methods – were included in the calculations for the evaluation of the equivalence **this study proves that XEBIOS's COLIKAT RAPID® method is equivalent to IDEXX's COLILERT-18®/QUANTI-TRAY® method regarding the enumeration of coliform bacteria.** The lower limit auf the confidence interval was located at -1,54 %, the upper limit at 3,47 %. In this case **the study proves that XEBIOS's COLIKAT RAPID® method has a significantly higher recovery for the target organism *E. coli* as compared to IDEXX's reference method.** The upper limit of the confidence interval was 1,40 % and therefore above 0 %. In total this delimitation of the population comprised 468 samples for the enumeration of coliform bacteria and 362 samples for the enumeration of *E. coli*.

7.6 Equivalence for isolated analysis of the specific laboratories

Tables 2 to 5 comprise the individual results for the evaluation of the equivalence for each of the 12 participating laboratories. **Tables 2 and 3** reflect the equivalence under consideration of all samples - including the samples with results outside the targeted range of MPN values - without verification of the confirmed counts by MALDI-TOF-MS. According to EN ISO 17994 [5], at least 6 laboratories have to participate on an equivalence study. In this study 12 laboratories participated. Therefore, considerably more sample-pairs were taken into the evaluation as required by EN ISO 17994 [5] and the statistical power and reliability of the calculations were increased significantly.

For many participants of the study the equivalence could not finally be assessed based on their individual result because the number of samples within the specific single laboratory was not sufficient for a well-founded conclusion on the equivalence of the two methods compared.

However, this was also not to be expected for an equivalence study according to EN ISO 17994 [5], because a founded statistical statement can generally only be taken in the case of the simultaneous analysis of a high number of water samples. Only the simultaneous evaluation of all samples of all participating laboratories is intended and expedient pursuant to EN ISO 17994 [5].

This applies for both, the enumeration of coliform bacteria and the enumeration of *E. coli*. For the enumeration of coliform bacteria the two methods were classified as equivalent based on the individual data of one laboratory, the data set of one laboratory proved a higher recovery for coliform bacteria for XEBIOS's COLIKAT RAPID® method. For the enumeration of *E. coli* the datasets showed equivalence for two individual laboratories. For ten laboratories the results were inconclusive as the individual number of samples were insufficient because of the isolated view of the specific laboratories.

The evaluation of the equivalence under consideration of the verification of the confirmed counts with MALDI-TOF-MS for the individual laboratories is shown in **tables 4 and 5**. Also in case of delimitating the population to the verified confirmed counts, the equivalence could in many cases not finally be assessed for the individual laboratory results due to the aforementioned statistical restrictions. In this case the equivalence of the two methods compared could be shown for the individual samples of three laboratories, the data set of one laboratory proved a higher recovery for coliform bacteria for XEBIOS's COLIKAT RAPID® method. For the enumeration of *E. coli* the individual results of two of twelve participating laboratories showed equivalence. The individual results of ten laboratories were inconclusive due to the insufficient number of samples.

Table 2: **Equivalence of the methods for the enumeration of coliform bacteria in case of the individual analysis of the laboratories without verification of the confirmed counts with MALDI-TOF-MS**
(The numbering of the laboratories is randomly and is not comparable with table 1)

Coliform bacteria	Number of samples n	Mean value x	Standard deviation s	Standard uncertainty Sx	Half width of the confidence interval W	Confidence interval LO	Confidence interval HI
Lab A	42	-6,24	26,82	5,59	11,19	**-17,42**	**4,95**
Lab B	40	4,46	27,35	4,32	8,65	**-4,19**	**13,11**
Lab C	42	-8,52	31,88	4,92	9,84	**-18,36**	**1,32**
Lab D	32	11,07	41,41	7,32	14,64	**-3,58**	**25,71**
Lab E	34	-1,98	27,60	4,73	9,47	**-11,45**	**7,48**
Lab F	34	9,28	26,39	5,63	11,25	**-1,97**	**20,54**
Lab G	37	-1,15	39,13	6,43	12,87	**-14,01**	**11,72**
Lab H	23	6,72	19,44	4,05	8,11	**-1,39**	**14,83**
Lab I	45	-2,96	22,62	3,37	6,74	**-9,71**	**3,78**
Lab J	37	11,75	30,72	5,05	10,10	**1,65**	**21,85**
Lab K	56	1,03	25,27	3,38	6,75	**-5,72**	**7,79**
Lab L	40	-3,34	24,53	3,88	7,76	**-11,10**	**4,42**

Table 3: **Equivalence of the methods for the enumeration of *E. coli* in case of the individual analysis of the laboratories without verification of the confirmed counts with MALDI-TOF-MS**
(The numbering of the laboratories is randomly and is not comparable with table 1)

E. coli	Number of samples n	Mean value x	Standard deviation s	Standard uncertainty Sx	Half width of the confidence interval W	Confidence interval LO	Confidence interval HI
Lab A	48	-4,88	42,61	8,88	17,77	**-22,65**	**12,89**
Lab B	40	13,24	50,57	8,00	15,99	**-2,75**	**29,23**
Lab C	64	13,70	65,22	8,15	16,30	**-2,60**	**30,00**
Lab D	35	9,42	71,36	12,06	24,12	**-14,70**	**33,54**
Lab E	48	11,76	49,15	7,09	14,19	**-2,43**	**25,95**
Lab F	35	13,28	36,84	7,85	15,71	**-2,43**	**28,99**
Lab G	35	9,42	53,45	9,04	18,07	**-8,65**	**27,49**
Lab H	24	-1,91	19,87	4,06	8,11	**-10,02**	**6,20**
Lab I	48	3,19	41,52	5,99	11,99	**-8,80**	**15,18**
Lab J	43	1,31	25,57	3,90	7,80	**-6,49**	**9,11**
Lab K	56	-3,09	40,84	5,46	10,91	**-14,00**	**7,83**
Lab L	58	1,11	30,81	4,04	8,09	**-6,98**	**9,20**

Table 4: **Equivalence of the methods for the enumeration of coliform bacteria in case of the individual analysis of the laboratories with verification of the confirmed counts with MALDI-TOF-MS**
(The numbering of the laboratories is randomly and is not comparable with table 1)

Coliform bacteria	Number of samples n	Mean value x	Standard deviation s	Standard uncertainty Sx	Half width of the confidence interval W	Confidence interval LO	Confidence interval HI
Lab A	42	-6,53	26,92	5,87	11,75	**-18,28**	**5,22**
Lab B	40	5,84	25,89	4,09	8,19	**-2,34**	**14,03**
Lab C	42	-8,59	32,57	5,03	10,05	**-18,65**	**1,46**
Lab D	32	11,07	41,41	7,32	14,64	**-3,58**	**25,71**
Lab E	35	-0,62	27,70	4,68	9,37	**-9,98**	**8,75**
Lab F	34	10,17	24,00	5,12	10,23	**-0,06**	**20,40**
Lab G	37	0,72	37,23	6,12	12,24	**-11,52**	**12,96**
Lab H	30	6,44	18,63	3,80	7,61	**-1,17**	**14,05**
Lab I	45	-2,96	22,62	3,37	6,74	**-9,71**	**3,78**
Lab J	37	10,15	28,33	4,66	9,31	**0,84**	**19,46**
Lab K	56	0,70	24,13	3,22	6,45	**-5,75**	**7,15**
Lab L	40	-3,34	24,53	3,88	7,76	**-11,10**	**4,42**

Table 5: **Equivalence of the methods for the enumeration of *E. coli* in case of the individual analysis of the laboratories with verification of the confirmed counts with MALDI-TOF-MS**
(The numbering of the laboratories is randomly and is not comparable with table 1)

E. coli	Number of sam-ples n	Mean value x	Standard deviation s	Standard uncer-tainty Sx	Half width of the confidence interval W	Confidence interval LO	Confidence interval HI
Lab A	48	-4,90	42,48	8,86	17,72	**-22,62**	**12,81**
Lab B	40	13,24	50,57	8,00	15,99	**-2,75**	**29,23**
Lab C	64	13,13	65,46	8,18	16,37	**-3,23**	**29,50**
Lab D	35	10,80	72,60	12,27	24,54	**-13,74**	**35,34**
Lab E	40	8,14	43,96	6,95	13,90	**-5,76**	**22,04**
Lab F	35	13,00	34,50	7,35	14,71	**-1,71**	**27,71**
Lab G	35	9,94	53,57	9,06	18,11	**-8,17**	**28,05**
Lab H	30	-1,91	21,31	4,35	8,70	**-10,61**	**6,79**
Lab I	48	3,47	41,91	11,20	22,40	**-18,93**	**25,87**
Lab J	43	1,18	25,31	3,86	7,72	**-6,54**	**8,90**
Lab K	56	-2,62	41,22	5,51	11,02	**-13,64**	**8,40**
Lab L	58	1,31	30,61	4,02	8,04	**-6,73**	**9,35**

8 Discussion

This study clearly shows the equivalence of two testing methods for the enumeration of coliform bacteria and *E. coli* according to the evaluation procedure described in EN ISO 17994 [5]: XEBIOS's COLIKAT RAPID® method and IDEXX's COLILERT-18/QUANTI-Tray® method. 470 samples were analysed for the target organism "coliform bacteria" and 522 samples were analysed for the target organism *E. coli* simultaneously with both methods. If the result for a sample (MPN) obtained with XEBIOS's COLIKAT RAPID® method was out of the 95%/99%-confidence interval of the result for a sample obtained with IDEXX's COLILERT-18®/QUANTI-Tray® method, all positive cultures from the cavities were verified with MALDI-TOF-MS in order to exclude false-positive results from the analysis. The MPN of samples containing false-positive results was adjusted in this case. The number of verified cavities was approximately 4500.

The study clearly proved that the XEBIOS's COLIKAT RAPID® method is equivalent to the IDEXX's COLILERT-18/QUANTI-Tray® method for the enumeration of coliform bacteria. For the target organism *E. coli* the study clearly proved that XEBIOS's COLIKAT RAPID® has a significantly higher recovery compared to IDEXX's reference method COLILERT-18®/QUANTI-Tray®.

The results are robust even when different criteria for the evaluation of the equivalence were taken into consideration. Results from where all samples were considered – that means also those outside the statistically sound range of 10 to 200 MPN/100 ml – were comparable to results considering only those samples for which the MPN was within the target concentration. Moreover, after verification of the positive cavities (and correction of the confirmed counts) for samples analysed with COLIKAT RAPID® with a MPN value outside the confidence interval of the reference method (COLILERT-18®/QUANTI-Tray®), values for the confidence intervals only changed marginally.

For many participants of the study the equivalence could not finally be assessed based on their individual results because the number of samples within the specific single laboratory was not sufficient for a sound statistical conclusion on the equivalence of the two methods compared. However, this could not be expected for an equivalence study according to EN ISO 17994 [5], that requires the simultaneous analysis of a high number of water samples in order to derive a sound statistical evaluation of the equivalence of two testing methods for water samples.

9 Literature

[1] EN ISO 9308-2:2012: Water Quality – Enumeration of Escherichia coli and coliform bacteria - Part 2: Most probable number method

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[3] COUNCIL DIRECTIVE 98/83/EG of 3 November 1998 on the quality of water intended for human consumption; Official Journal L 330, 05.12.1998 P. 0032-0054.

[4] COMMISSION DIRECTIVE (EU) 2015/1787 of 6 October 2015 amending Annexes II and III to COUNCIL DIRECTIVE 98/83/EG of 3 November 1998 on the quality of water intended for human consumption; Official Journal L 260, 07.10.2015 P. 0006-0017.

[5] EN ISO 17994:2014: Water quality - Requirements for the comparison of the relative recovery of microorganisms by two quantitative methods

[6] 9308-1:2014: Water quality – Enumeration of Escherichia coli bacteria – Part 1: Membrane filtration method for waters with low bacterial background flora