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# NordVal International Certificate

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| Issued for: | **BRT Inhibitor Test** |
| NordVal No: | 051 |
| First approval date: | 01 March 2019 |
| Renewal date: | 01 March 2023 |
| Valid until: | 01 March 2025 |

## BRT Inhibitor Test

**Manufactured and supplied by:**

Analytik in Milch Produktions- und Vertriebs-GmbH
Kaiser-Ludwig-Platz 2
80336 München
Germany

fulfils the validation requirements of the NordVal Validation Protocol 2. The BRT Inhibitor Test is a brilliant black reduction test for detection of antibiotic residues.

The method is tested for penicillins, cephalosporins, macrolides, sulfonamides, tetracyclines, aminoglycosides and Chloramphenicol in raw bovine milk on microtiter plates. Detection capability for clear results and presumptive results of photometric and visual reading is stated. In order for the method to be applicable in the EU, the detection capabilities for the substances of interest must be below given EU Maximum Residue Limits (MRL). The detection capabilities and associated MRLs for the substances tested are given in Table 2. Examples of the probability of detection (POD) / dose-response curves are given in the certificate for illustration. POD curves for all substances are given in the validation report, which is enclosed as an annex.

The performance of the method was tested in a comprehensive validation study carried out at the laboratory of Milchprüfring Bayern e. V. and tested in a large proficiency test.

The production of BRT Inhibitor Test is ISO 9001:2015-accredited by LGA InterCert GmbH.

Date: 27 February 2023

Yours sincerely,

Hilde Skår Norli
Chair of NordVal International

Eystein Oveland
NMKL Executive Director

## PRINCIPLES OF THE METHOD

The BRT Inhibitor Test is a Brilliant Black Reduction test (BRT) containing the test bacteria G. *stearothermophilus* var. *calidolactis* C953, the redox indicator brilliant black, nutrients and other supplements. Antibiotic residues present in a sample can inhibit the growth of the test bacteria, thus preventing or decelerating the reduction of the colour indicator brilliant black and the consecutive colour change of the test medium from blue to yellow.

Milk samples (100 µl milk volume), positive and negative controls are added to the plates. Incubation is performed at 65 ℃ for 2 h 15 min ± 15 min until the complete discoloration of the negative control (colour change from blue to yellow) indicates the ideal reading time. Thereafter, milk is rinsed off the cavities for easier detection. The plates can be assessed with 2 different reading methods: visual examination or photometric evaluation using the measuring wavelength of 450 nm and the reference wavelength of 620 nm.

The recorded absorption values of the analysed samples are converted into relative percentage values by setting the average absorption level of the negative controls (yellow colour after incubation) as 0 % and that of the positive controls (blue colour after incubation) as 100 %, the absorption levels of the samples are set in relation to negative (0 %) and positive (100 %) controls.

The conversion formula is as follows: (S-NC)/(PC-NC) x 100 = X %
where

| | |
|---|---|
| S | is the analysed sample´s absorption level |
| NC | is the average of the four negative controls' absorption levels |
| PC | is the average of the four positive controls' absorption levels |
| X | is the relative percentage value of the analysed sample |

Results are referred to as either Class A or Class B as shown in **Table 1**.

**Table 1. Relation of reading systems and classes of results.**

| | Classes of results | | |
|---|---|---|---|
| Reading system | Positive Class A Clear | Positive Class B Presumptive | Negative |
| Visual | 1 | 2 | 0 |
| Photometric | ≥ 65 % | 40 % - < 65 % | < 40 % |

## SAMPLES USED IN THE STUDY

High-quality raw ex-farm bulk milk proven free of antibiotic residues was spiked with antibiotics. To verify the correct concentration of the stock solutions and the spiked raw milk samples, serial dilutions of the prepared positive samples were analysed with microbiological inhibitor and, when applicable, with receptor tests or analysed by LC-MS/MS.

### Batch-to-batch variation

Potential deviations in the detection capabilities of different plate batches were evaluated statistically, and it was shown that there were no statistically significant differences between the batches of plates.

## METHOD PERFORMANCE CHARACTERISTICS

### Selectivity

Marker substances of commonly used classes of veterinary drugs other than antibiotics were analysed with photometric reading in order to determine the selectivity of the BRT Inhibitor Test. The investigated compounds included the anti-inflammatories Flunixin, Metamizole (NSAIDs) and Prednisolone (glucocorticoid) as well as antiparasitic substances (Triclabendazole and Deltamethrin). Furthermore, the polyether-antibiotic Monensin, used for ketosis treatment in dairy cows, was tested. The substances were spiked at a concentration of 100 x EU MRL and inoculated with 6 replicates. None of the tested substances inhibited the growth of the test germs, leading to negative results (both Class A and Class B). Thus, no false positive results were observed.

### Detection capability (CCβ)

For the determination of the detection capability (CCβ), three different batches of plates were used at all times. The lowest concentration obtaining a minimum of 95 % positive results was considered as detection limit (CCß). Based on the different interpretation methods, CCß A and CCß B (for Class A results: clear; and Class B results: presumptive, respectively - Table 1) were established in parallel for each substance. Different substances were tested. Table 2 shows the results. The substances exceeding the EU MRLs or having high detection capability (Chloramphenicol) are marked in red and written in italic.

In the EU legislation, maximum residue limits (MRLs) are stated for milk, concentrations of veterinary drugs below these limits are accepted. According to this, the BRT Inhibitor Test plates are applicable for the detection of the penicillin compounds: Benzylpenicillin, Ampicillin, Amoxicillin, Cloxacillin, Dicloxacillin, Nafcillin and Oxacillin, for the cephalosporins: Cefapirin, Cefoperazone, Cefazolin and Cefalonium; and for Neomycin. The method was found not to be applicable for the detection of macrolides, sulfonamides, tetracyclines and aminoglycosides, other than Neomycin, at the maximum permitted EU residue level (MRL). Further, for chloram-phenicol, the EU minimum required performance limit (MPRL) is 0.3 µg/kg and hence the method is not applicable for this substance (Commission Decision of 13 March 2003 amending Decision 2002/657/EC).

**Table 2. Substances tested, EU Maximum Residue Limit (MRL) and detection limits by photometric and visual reading for CCβ A (>65 %) and CCβ B (40-65 %).**

| Group of antibiotics | Substance | MRL EU* [µg/kg] | CCβ A [µg/kg] | CCβ B [µg/kg] | CCβ A [µg/kg] | CCβ B [µg/kg] |
|---|---|---|---|---|---|---|
| | | | Photometric | | Visual | |
| | Benzylpenicillin | 4 | 2.5 | 2 | 2.5 | 2 |
| | Ampicillin | 4 | 3.5 | 3 | 3.5 | 3 |
| | Amoxicillin | 4 | 3 | 2.5 | 3.5 | 3 |
| Penicillins | Cloxacillin | 30 | 25 | 20 | 30 | 25 |
| | Dicloxacillin | 30 | 15 | 12.5 | 15 | 12.5 |
| | Nafcillin | 30 | 15 | 10 | 15 | 8 |
| | Oxacillin | 30 | 10 | 8 | 12 | 10 |
| | Cefalexin | 100 | 400 | 300 | 500 | 400 |
| | Cefapirin | 60 | 6 | 5 | 8 | 5 |
| | Cefoperazone | 50 | 35 | 25 | 35 | 25 |
| Cephalosporins | Cefazolin | 50 | 9 | 7 | 9 | 7 |
| | Cefquinome | 20 | 500 | 300 | 600 | 300 |
| | Ceftiofur | 100 | 200 | 150 | 250 | 200 |
| | Cefalonium | 20 | 14 | 12 | 16 | 12 |
| | Erythromycin | 40 | 100 | 50 | 100 | 60 |
| Macrolides | Tylosin | 50 | 75 | 40 | 100 | 30 |
| | Sulfadiazine | 100 | >800 | 100 | >800 | 100 |
| | Sulfadimethoxin | 100 | >800 | 200 | >800 | 200 |
| | Sulfamethazine | 100 | 1,000 | 300 | >1,000 | 200 |
| Sulfonamides | Sulfathiazol | 100 | 400 | 60 | 400 | 60 |
| | Sulfadoxin | 100 | >1,500 | 400 | >1,500 | 400 |
| | Sulfamethoxypyridazine | 100 | 500 | 100 | 500 | 100 |
| | Chlortetracycline | 100 | >1,000 | 800 | >1,000 | 800 |
| Tetracyclines | Oxytetracycline | 100 | 800 | 400 | 800 | 400 |
| | Tetracycline | 100 | 1,000 | 600 | 1,000 | 600 |
| | Dihydrostreptomycin | 200 | 600 | 400 | 500 | 400 |
| Aminoglycosides | Streptomycin | 200 | 1,500 | 600 | 1,500 | 600 |
| | Gentamicin | 100 | 200 | 100 | 150 | 80 |
| | Neomycin | 1,500 | 400 | 200 | 300 | 200 |
| Fenicol | Chloramphenicol | - | 7,000 | 4,000 | 5,000 | 3,500 |

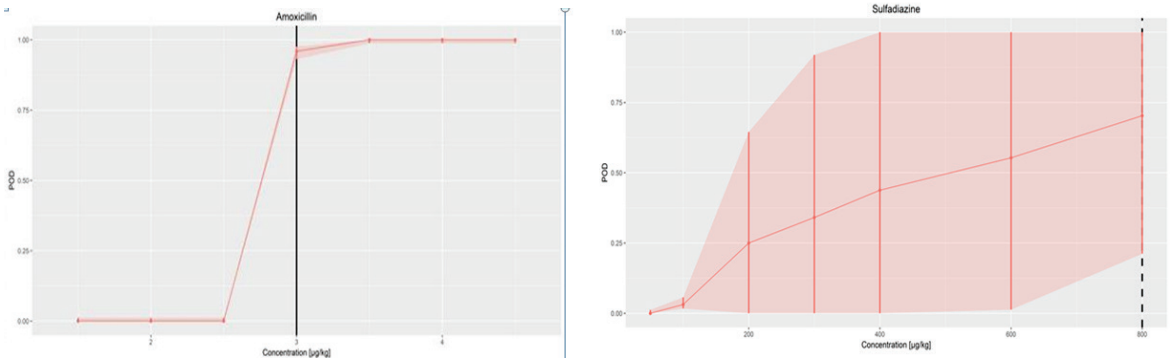
*Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin.

**Probability of detection (POD) / dose-response**

Probability of detection was established based on the Class A results with both photometric and visual evaluation (results are presented in the validation report, Annex 1, Table 2). For combined photometric and visual readings of the class A results, lower and upper CIs were calculated (presented in the validation report, Figure 3 and in its annex, Figure 1).

Bactericidal substances like beta-lactams mostly exhibited steeply increasing dose-response curves and narrow CIs (example **Figure 1a**, **Table 3**). In contrast, bacteriostatic substances displayed more consistent curve increments and often showed bigger variations between the two reading methods (example **Figure 1b**, **Table 4**). The width of the confidence interval provides information about the uncertainty of the measurement as the wider the confidence interval the greater the uncertainty. Compared to photometric reading, the visual interpretation leads to bigger variances in the results and thus to wider CIs.

**Figure 1. Dose-response curves for Amoxicillin (a) and Sulfadiazine (b), combined results for photometric and visual reading.**




In **Table 3**, showing results of the dose-response study for Amoxicillin, it can be seen that at 2.5 µg/kg for photometric reading none of the samples were clearly positive (absorbance level ≥ 65 %), but 98 % of the results were presumptive positive having absorbance levels of 40 % - < 65 %.

For visual reading at the same concentration, none of the samples were clearly positive (Class A), but 74 % of the samples were presumptive (Class B). At 3.0 µg/kg, photometric reading gave 100 % clear positive results (Class A) and thus, the same (100 %) when clear positive and presumptive results were combined (CCβ B). At the same concentration for visual reading, 13 results were presumptive positive and adding this to the clear positive results (179 samples; 0.93 %) a total of 100 % CCβ B was obtained. At 3.5 µg/kg all samples were detected clear positive with visual reading.

**Table 3. Dose-response for Amoxicillin**

| Read-ing system | Conc µg/kg | No. of total results | No. of results (pos. Class A) | Share of CCβ A | Lower 95%-CI (CCβ A) | Upper 95%-CI (CCβ A) | No. of results (pos. Class B) | Share of CCβ B | No. of results (nega-tive) |
|---|---|---|---|---|---|---|---|---|---|
| Photo | 1.5 | 128 | 0 | 0.00 | - | - | 0 | 0.00 | 128 |
| | 2 | 128 | 0 | 0.00 | - | - | 6 | 0.05 | 122 |
| | 2.5 | 128 | 0 | 0.00 | - | - | 126 | 0.98 | 2 |
| | 3 | 128 | 128 | 1.00 | - | - | 0 | 1.00 | 0 |
| | 3.5 | 128 | 128 | 1.00 | - | - | 0 | 1.00 | 0 |
| | 4 | 128 | 128 | 1.00 | - | - | 0 | 1.00 | 0 |
| | 4.5 | 128 | 128 | 1.00 | - | - | 0 | 1.00 | 0 |
| Visual | 1.5 | 192 | 0 | 0.00 | - | - | 0 | 0.00 | 192 |
| | 2 | 192 | 0 | 0.00 | - | - | 0 | 0.00 | 192 |
| | 2.5 | 192 | 0 | 0.00 | - | - | 142 | 0.74 | 50 |
| | 3 | 192 | 179 | 0.93 | - | - | 13 | 1.00 | 0 |
| | 3.5 | 192 | 192 | 1.00 | - | - | 0 | 1.00 | 0 |
| | 4 | 192 | 192 | 1.00 | - | - | 0 | 1.00 | 0 |
| | 4.5 | 192 | 192 | 1.00 | - | - | 0 | 1.00 | 0 |
| Photo + visual | 1.5 | 320 | 0 | 0.00 | 0.00 | 0.01 | 0 | - | 320 |
| | 2 | 320 | 0 | 0.00 | 0.00 | 0.01 | 6 | - | 314 |
| | 2.5 | 320 | 0 | 0.00 | 0.00 | 0.01 | 268 | - | 52 |
| | 3 | 320 | 307 | 0.96 | 0.93 | 0.98 | 13 | - | 0 |
| | 3.5 | 320 | 320 | 1.00 | 0.99 | 1.00 | 0 | - | 0 |
| | 4 | 320 | 320 | 1.00 | 0.99 | 1.00 | 0 | - | 0 |
| | 4.5 | 320 | 320 | 1.00 | 0.99 | 1.00 | 0 | - | 0 |

In **Table 4**, showing results of the dose-response study for Sulfadiazine, it can be seen that even at the highest concentration tested (800 µg/kg), the ratio of samples detected as clearly positive (absorbance level ≥ 65 %; Class A results) did not reach more than 74 % for photo-metric reading and 68 % for visual reading.

The sum of the presumptive and clear positive results (positive Class A and positive Class B) gives 100 % CCß B at 200 µg/kg (CCβ B) for both photometric and visual reading.

**Table 4. Dose-response for Sulfadiazine.**

| Reading system | Conc µg/kg | No. of total results | No. of results (pos. Class A) | Share of CCβ A | Lower 95%-CI (CCβ A) | Upper 95%-CI (CCβ A) | No. of results (pos. Class B) | Share of CCβ B | No. of results (negative) |
|---|---|---|---|---|---|---|---|---|---|
| Photo | 50 | 128 | 0 | 0.00 | - | - | 12 | 0.09 | 116 |
| | 100 | 128 | 0 | 0.00 | - | - | 123 | 0.96 | 5 |
| | 200 | 128 | 3 | 0.02 | - | - | 125 | 1.00 | 0 |
| | 300 | 128 | 0 | 0.00 | - | - | 128 | 1.00 | 0 |
| | 400 | 128 | 12 | 0.09 | - | - | 116 | 1.00 | 0 |
| | 600 | 128 | 49 | 0.38 | - | - | 79 | 1.00 | 0 |
| | 800 | 128 | 95 | 0.74 | - | - | 33 | 1.00 | 0 |
| Visual | 50 | 192 | 0 | 0.00 | - | - | 44 | 0.23 | 148 |
| | 100 | 192 | 10 | 0.05 | - | - | 176 | 0.97 | 6 |
| | 200 | 192 | 77 | 0.40 | - | - | 115 | 1.00 | 0 |
| | 300 | 192 | 109 | 0.57 | - | - | 83 | 1.00 | 0 |
| | 400 | 192 | 128 | 0.67 | - | - | 64 | 1.00 | 0 |
| | 600 | 192 | 128 | 0.67 | - | - | 64 | 1.00 | 0 |
| | 800 | 192 | 130 | 0.68 | - | - | 62 | 1.00 | 0 |
| Photo + visual | 50 | 320 | 0 | 0.00 | 0.00 | 0.01 | 56 | - | 264 |
| | 100 | 320 | 10 | 0.03 | 0.02 | 0.06 | 299 | - | 11 |
| | 200 | 320 | 80 | 0.25 | 0.00 | 0.65 | 240 | - | 0 |
| | 300 | 320 | 109 | 0.34 | 0.00 | 0.92 | 211 | - | 0 |
| | 400 | 320 | 140 | 0.44 | 0.00 | 1.00 | 180 | - | 0 |
| | 600 | 320 | 177 | 0.55 | 0.01 | 1.00 | 143 | - | 0 |
| | 800 | 320 | 225 | 0.70 | 0.21 | 1.00 | 95 | - | 0 |

**Specificity -** Rate of positive results not caused by residues of veterinary drugs

In order to demonstrate that the BRT Inhibitor Test performs properly with a broad range of samples, 704 ex-farm bulk milk samples were examined. Two out of 704 samples (0.28 %) were detected positive by the BRT Inhibitor Test. Both samples were confirmed to contain Cloxacillin. Thus, the rate of positive results not caused by residues of veterinary drugs was 0 %, as all positive samples detected were confirmed to contain antibiotic inhibitors (**Table 5**).

**Table 5. The specificity tested in 704 samples.**

| | Negative samples | | Positive samples | | | |
|---|---|---|---|---|---|---|
| Total no. samples | No. | Rate | No. | Rate | False positive | Con-firmed positive |
| 704 | 702 | 99.72 % | 2 | 0.28 % | 0 % | 100 % |

**False-negative rate (FN) / False-positive rate (FP)**

In this validation, 154 test plates were analysed, including 616 positive control samples and 3,080 samples of negative raw milk, in total giving 3,080 (positive control) and 15,400 (negative milk) readings, respectively, with combined photometric evaluation and visual reading.

No false-positive results were observed when analysing the results of the negative milk samples by photometric evaluation (**Table 6**). One sample was classified false-negative by 1 out of 3 persons performing visual reading, whereas none of the other readers (technicians or photometric) assessed this sample as positive, the negative result is considered as outlier and might have been caused by a typing error.

**Table 6. Rates of false-negative and false-positive results of the 3,080 positive controls and 15,400 negative milk samples.**

| | Rate of false results [%] | | | | |
|---|---|---|---|---|---|
| Type of milk sample | ELISA 1 | ELISA 2 | Visual 1 | Visual 2 | Visual 3 |
| Positive control | 0 | 0 | 0 | 0 | 0.2 |
| Negative control milk | 0 | 0 | 0 | 0 | 0 |

The maximum relative percentage value obtained with photometric reading for negative samples was 33 %, whereas the minimum relative percentage value for positive samples was 93 %.

These values demonstrate that with the chosen thresholds for photometric reading (65 % Class A; 40 % Class B, Table 1) the false interpretation of positive as well as negative samples can be avoided.

## INTERLABORATORY STUDY

The BRT Inhibitor Test was validated in an international proficiency test. 61 laboratories belonging to 55 companies originating from 10 countries participated in the examination of BRT Inhibitor Test plates provided for the interlaboratory study.

15 randomised and coded lyophilised UHT-milk samples were analysed - 8 samples contained antibiotics at MRL level, 7 samples consisted of inhibitor-free milk (**Table 7**), giving a total of 945 results. No false-positive or false-negative results were observed.

**Table 7. The antibiotics analysed by 61 laboratories and the percentage of correct and false results.**

| Antibiotic | MRL (µg/kg) | No. samples | BRT Inhibitor test | |
|---|---|---|---|---|
| | | | Correct result (%) | False result (%) |
| Benzylpenicillin | 4 | 2 | 100 | 0 |
| Ampicillin | 4 | 2 | 100 | 0 |
| Cefapirin | 60 | 2 | 100 | 0 |
| Cloxacillin | 30 | 2 | 100 | 0 |
| Inhibitor-free milk | - | 7 | 100 | 0 |

## CONCLUSION

The BRT Inhibitor Test (microtiter plate format) was tested for detection of antibiotic residues in milk. For use and interpretation of the results the MRLs of the respective countries must be considered. The method is only applicable for substances with detection capability below the MRL or MPRL (where no MRL exists).

## ANNEX 1

Validation Report BRT Inhibitor Test.

# Validation Report
# BRT Inhibitor Test

## 1) Introduction

The BRT Inhibitor Test (AiM – Analytik in Milch GmbH, www.aimbavaria.com) is a microbiological inhibitor test for the qualitative broad-spectrum detection of antibiotic residues in cow milk. The validation study was carried out at the laboratory of Milchprüfring Bayern e. V. (MPR Bayern, www.mpr-bayern.de), a large raw milk testing laboratory performing 1.8 million inhibitor tests per year, under the conduct of Silvia Orlandini (AEOS) and Christian Baumgartner (MPR Bayern), in accordance with the Commission Decision 2002/657/EC and the CRL Guidelines (Anonymous, 2010).

## 2) Test Principle, Test Procedure, Reading Methods and Plate Batches

The BRT Inhibitor Test (Figure 1) is a Brilliant Black Reduction Test (BRT) containing the test bacteria *G. stearothermophilus* var. *calidolactis* C953, the redox indicator brilliant black, nutrients and other supplements. Antibiotic residues present in a sample can inhibit the growth of the test bacteria, thus preventing or decelerating the reduction of the color indicator brilliant black and the consecutive color change of the test medium from blue to yellow. The BRT Inhibitor Test detects a broad spectrum of antibiotics, but is especially sensitive to beta-lactams.
The test is designed in particular to satisfy the demands of milk quality regulation in Germany and is used for quality evaluation and payment for tank milk. It is recognized as official inspection method for the detection of inhibitors in tank milk and is part of the official register of inspection procedures according to § 64 LFGB (German Food, Feed and Consumer Goods Code, L 01.01-5). Furthermore, it is produced according to Commission Decision 91/180/EEC.

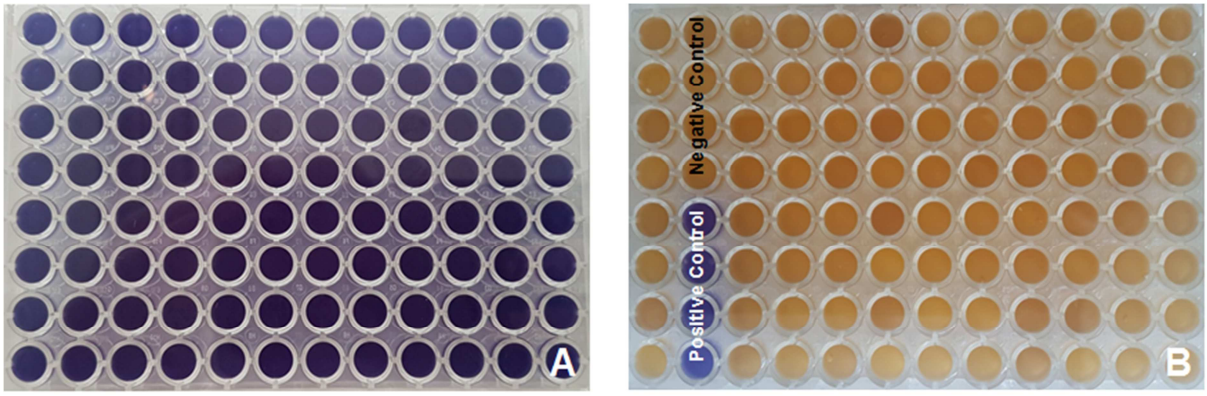



Figure 1. BRT Inhibitor Test plates before (A) and after (B) incubation

Within the framework of this validation, the BRT Inhibitor Test was evaluated in microtiter plate format. The plates were stored refrigerated (6 - 10 °C) until use. Additional to the samples (100 µl milk volume), each plate contained four positive (raw milk spiked with 4 µg/kg Penicillin G, remaining blue after incubation) and four negative controls (inhibitor-free raw milk, turning yellow after incubation) in order to enable a correct evaluation. According to the manufacturer´s instructions, the plates were incubated at 65 °C in a temperature-surveilled water bath until the complete

discoloration of the negative control (color change from blue to yellow, Figure 1) indicated the ideal reading time (2 hrs 15 ± 15 min). Thereafter, the milk was rinsed off the cavities and the plates were assessed with 2 different reading methods: Visual examination performed by 3 technical assistants trained particularly for this purpose and photometric evaluation, using 2 instruments (ELISA reader (Multiskan Ascent V1.24, Thermo Labsystems)).
The photometric measurements were evaluated conforming to the relativized absorption method described by Beer and Suhren (1993). Accordingly, the measuring wavelength of 450 nm and the reference wavelength of 620 nm were chosen for reading. The recorded absorption values of the analyzed samples were converted into relative percentage values by setting the average absorption level of the negative controls (yellow color after incubation) as 0% and that of the positive controls (blue color after incubation) as 100%, other absorption levels (samples) were set in relation to negative (0%) and positive (100%) control.
The conversion formula is as follows:

$$(S\text{-}NC)/(PC\text{-}NC) \times 100 = X\ \%$$

where
*S* is the analyzed sample´s absorption level
*NC* is the average of the four negative controls absorption levels
*PC* is the average of the four positive controls absorption levels
*X* relative percentage value of the analyzed sample

The photometric evaluation was regarded as reference method in this validation study as it provides objective, comparable and documented results and is commonly used by large laboratories.
The interpretation of the samples reading results was carried out in two different ways, in compliance with the method L 01.01-5 (§ 64 LFGB, inspection tests for milk quality payment) all samples exhibiting at least the color of the positive control respectively exceeding the threshold value of 65% (photometric evaluation) were interpreted as positive (indicated as class A) as well as all samples displaying a color which was clearly different from the negative control or exceeding the threshold value of 40% (indicated as class B), according to L 01.00-11 (§ 64 LFGB, German Food, Feed and Consumer Goods Code, MRL Screening test). All samples gaining <40% by photometric evaluation or appeared as yellow as the negative control were categorized as negative (Table 1).
For statistical purposes, the “quantitative” relative percentage photometric results were converted to the same format as the “qualitative” visual data. Thus, the number 1 was assigned to any photometric percentage value of ≥65%, whereas results with percentages in the range of 40% - <65% were referred to with 2 and negative results with 0, equaling <40% (Table 1).

Table 1. Relation of reading systems and classes of results

| | Classes of Results | | |
|---|---|---|---|
| Reading System | Positive Class A | Positive Class B | Negative |
| Visual (V) | 1 | 2 | 0 |
| ELISA (E) | ≥65% | 40% - <65% | <40% |

60,340 data results were obtained from the evaluation of 154 BRT Inhibitor Test plates and treated statistically using "R" software (Version 3.5.0 (2018-04-23)). The confidence interval (CI) was calculated according to the AOAC approach for qualitative data.

For the validation, 7 batches of plates (Table 2) were provided by the manufacturer.

Table 2. BRT Inhibitor Test plate batches provided for the validation

| Batch | Range of Batch Numbers |
|---|---|
| A | 20709235 - 20713911 |
| B | 20516031 - 20524980 |
| C | 20801052 - 20812126 |
| D | 21301001 - 21308901 |
| E | 21409615 - 21410635 |
| F | 21601002 - 21610752 |
| G | 22801005 - 22814753 |

### 3) Raw Milk Samples

A large quantity of high quality raw ex-farm bulk milk was collected, analyzed for milk quality and components (Table 3) and proven to be free of antibiotic residues by analysis with highly sensitive microbial inhibitor tests (BRT hi-sense and BRT ultra-sense, AiM GmbH, Munich, Germany) and receptor tests (BetaStar® 100, Neogen Corporation, Lansing, USA; Charm® MRL Beta-lactam Test, Charm Sciences Inc., Lawrence, USA; SNAP® Beta-Lactam ST-Test, IDEXX GmbH, Ludwigsburg, Germany). Additionally, the raw milk was tested with the AiM Penase Test (AiM GmbH, Munich, Germany) and proven to be free of penicillinase. Thereafter, the raw milk was aliquoted, frozen and stored until use. For the establishment of the rate of positive results not caused by residues of veterinary drugs, 704 ex-farm bulk milk samples, originating from routine milk quality payment testing, were analyzed with the BRT Inhibitor Test.

Table 3. Analysis results of the raw milk batch used for the validation samples

| Type of Milk | FC [g/100 ml][a] | PC [g/100 ml][b] | pH | SCC/ml[c] | CFU/ml[d] |
|---|---|---|---|---|---|
| Blank raw Milk | 4.17 | 3.51 | 6.65 | 77,000 | 5,000 |

[a] Fat content; [b] Protein content; [c] Somatic cell count; [d] Colony forming units

For the preparation of positive samples, blank raw cow milk was defrosted, spiked with a highly concentrated stock solution to obtain the desired level of antibiotic residue and frozen again. When required, the milk samples were defrosted overnight at 6 - 8 ℃ and used the next day. To verify the co rrect concentration of the stock solutions and the spiked raw milk samples, serial dilutions of the prepared positive samples were analyzed with microbiological inhibitor and - if available for a certain substance - receptor tests, then the obtained results were compared with the detection limits of the individual tests. No receptor tests were available for Erythromycin, Tylosin, Neomycin and Gentamicin, therefore the correct concentrations of the individual stock solutions were verified with LC - MS/MS analysis.
The approach of using a single batch of raw milk as base for the preparation of the spiked milk samples enhances the comparability of results obtained on different validation days and thus objectifies the assessment of the validation results, as irregularities are not attributable to deviating milk qualities. Ex-farm tank milk was chosen as basic matrix for the validation as this is the target product of the BRT Inhibitor Test.

## 4) Detection Capability

Materials and Methods

Involved in the validation study were 30 antibiotic compounds (Table 6), the concentrations of the samples to analyze were chosen according to the manufacturer´s specification. The choice of increment from concentration to concentration depended on the spiked standards´ concentrations (Table 4) as well as on practical aspects as two classes of results had to be considered, leading to two different detection limits (CCß A and CCß B).

Table 4. Correlation of concentration and increment of the spiked raw milk samples

| Concentration [µg/kg] | Increment [µg/kg] |
|---|---|
| 1-10 | 1 |
| 11-20 | 2 |
| 21-50 | 5 |
| 51-100 | 10 |
| 101-250 | 25 |
| 251-500 | 50 |
| 501-1,000 | 100 |
| 1,000-5,000 | 500 |

Correlated with the proximity to the respective EU Maximum Residue Limit (MRL) for antibiotic residues in milk, the standards were measured with 20, 40 or 60 replicates (Table 5).

Table 5. Number of replicates depending on the proximity to the respective MRL

| Closeness to MRL | No. of Replicates |
|---|---|
| ≤0.5 MRL | 20 |
| >0.5 MRL and <0.9 MRL | 40 |
| ≥0.9 MRL and ≤ MRL | 60 |
| > MRL | 20 |

For the determination of the detection capability, three different batches of plates were used at all times. The lowest concentration obtaining a minimum of 95% positive results was considered as detection limit (CCß). Based on the different interpretation methods, CCß A and CCß B (Section 2) were established in parallel for each substance. The detection limits determined with photometric evaluation were considered as reference values.

Results and Discussion

Table 6. Established detection limits (photometric reading) compared with the EU MRL levels. Marked in red are the substances exceeding the EU MRLs.

| Group of Antibiotics | Substance | MRL EU [µg/kg] | CCß A [µg/kg] | CCß B [µg/kg] |
|---|---|---|---|---|
| **Penicillins** | Benzylpenicillin | 4 | 2.5 | 2 |
| | Ampicillin | 4 | 3.5 | 3 |
| | Amoxicillin | 4 | 3 | 2.5 |
| | Cloxacillin | 30 | 25 | 20 |
| | Dicloxacillin | 30 | 15 | 12.5 |
| | Nafcillin | 30 | 15 | 10 |
| | Oxacillin | 30 | 10 | 8 |
| **Cephalosporins** | Cefalexin | 100 | 400 | 300 |
| | Cefapirin | 60 | 6 | 5 |
| | Cefoperazone | 50 | 35 | 25 |
| | Cefazolin | 50 | 9 | 7 |
| | Cefquinome | 20 | 500 | 300 |
| | Ceftiofur | 100 | 200 | 150 |
| | Cefalonium | 20 | 14 | 12 |
| **Macrolides** | Erythromycin | 40 | 100 | 50 |
| | Tylosin | 50 | 75 | 40 |
| **Sulfonamides** | Sulfadiazine | 100 | >800 | 100 |
| | Sulfadimethoxin | 100 | >800 | 200 |
| | Sulfamethazine | 100 | 1,000 | 300 |
| | Sulfathiazol | 100 | 400 | 60 |
| | Sulfadoxin | 100 | >1,500 | 400 |
| | Sulfamethoxypyridazine | 100 | 500 | 100 |
| **Tetracyclines** | Chlortetracycline | 100 | >1,000 | 800 |
| | Oxytetracycline | 100 | 800 | 400 |
| | Tetracycline | 100 | 1,000 | 600 |
| **Aminoglycosides** | Dihydrostreptomycin | 200 | 600 | 400 |
| | Streptomycin | 200 | 1,500 | 600 |
| | Gentamicin | 100 | 200 | 100 |
| | Neomycin | 1,500 | 400 | 200 |
| **Fenicol** | Chloramphenicol | - | 7,000 | 4,000 |

The particular sensitivity of *G. stearothermophilus* for beta-lactams and especially for penicillins is reflected in the detection limits of the different groups of antibiotics. All penicillins were detected below MRL as well as 4 out of 7 cephalosporins. Out of the groups of the macrolides, sulfonamides and aminoglycosides Tylosin, Sulfadiazine, Sulfathiazol, Sulfamethoxypyridazine and Gentamicin conformed with the regulatory limits with CCß B, but not with CCß A, whereas for Neomycin CCß A and CCß B were defined to be below MRL. The detection limits of other tested substances exceeded the respective MRLs. Additionally, for a low number of substances (Sulfadiazine, Sulfadimethoxin, Sulfadoxin and Chlortetracycline) CCß A could not be determined as the positive response was below 65% relative absorption at the highest concentrations tested.
Chloramphenicol, for which no MRL is established – it is prohibited for use in food producing animals (Commission Regulation (EU) No 37/2010) – can be tested positive at 4,000 µg/kg (CCß B) or 7,000 µg/kg (CCß A). The detection limits for the BRT Inhibitor Test established with the reference method (photometric evaluation) are reported in Table 6, detection limits established with visual reading are reported in Annex Table 1.
In conclusion, the most important antibiotics used in Germany for the treatment of dairy cows (penicillins and cephalosporins), are detected predominantly below EU MRL. A broad range of other inhibitors can be identified as well, however, mostly in concentrations exceeding the regulatory limit.

## 5) Dose-Response Curves

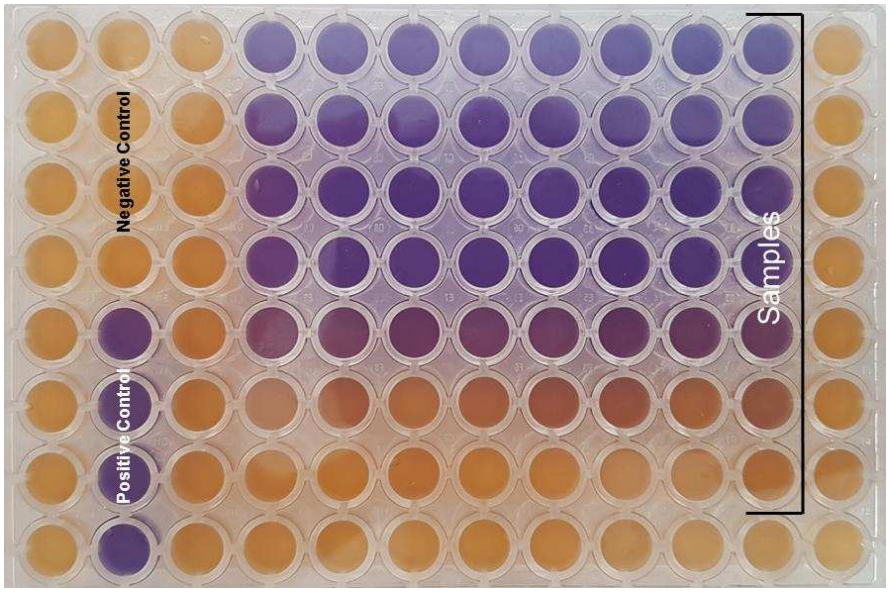



Figure 2. Incubated BRT Inhibitor Test plate inoculated with four positive and four negative controls as well as raw milk samples spiked with 7 different concentrations of an antibiotic substance

## Materials and Methods

Dose-response curves were established for all antibiotics analyzed in the validation based on the class A results (Section 2) obtained within the framework of the detection capability study with both photometric and visual evaluation. For this purpose, 7 samples containing increasing concentrations were examined for each substance, with the aim of identifying the concentrations resulting in approximately 25%, 50%, 75% and 100% positive rates and to determine the highest concentration with 0% positive results. Furthermore, lower and upper CIs were calculated for the class A results under consideration of both reading systems and included in the dose-response curves (Figure 2 and Annex Table 2).

## Results and Discussion

Figure 3 and Annex Figure 1 depict dose-response curves of all substances included in the validation of the BRT Inhibitor Test. The response rates generated with the respective concentrations of each substance are specified in Annex Table 2. It was not always possible to obtain dose-response curves entirely corresponding to the requirements of 0%, 25%, 50%, 75% and 100% positive results. Bactericidal substances like beta-lactams mostly exhibited steeply increasing dose-response curves. For Benzylpenicillin, e. g., the positive response rate was 0% at 1.5 µg/kg, 76% at 2 µg/kg already and 100% of samples were detected positive at 2.5 µg/kg. Substances like the sulfonamides and tetracyclines displayed more consistent curve increments, probably due to their bacteriostatic character. Sulfadoxin obtained 0% positive results at 100 µg/kg, 7% at 400 µg/kg, 31% respectively 39% at 600 µg/kg and 800 µg/kg. At 1,000 µg/kg, the positive response was 47% and with the highest concentration analyzed (1,500 µg/kg) 63% - no CCß A was established for this substance.

Principally, the confidence interval is narrow at the concentrations of the CCß and at concentrations close to 0% of positive results. Bactericidal substances tend to exhibit narrow CIs also at concentrations in between 0% and CCß, which indicates that most results of samples with different concentrations are interpreted in the same way with the different reading systems (photometric and visual) and individual readers.

In contrast, bacteriostatic substances often show bigger variations in the results at the concentrations below the CCß. The bacteriostatic activity causes different degrees of inhibition and consequently of color development, which can be more difficult to interpret by human eye. While the interpretation with photometric reading systems is well standardized, the visual interpretation leads to bigger variances in the results and thus to wider CIs.

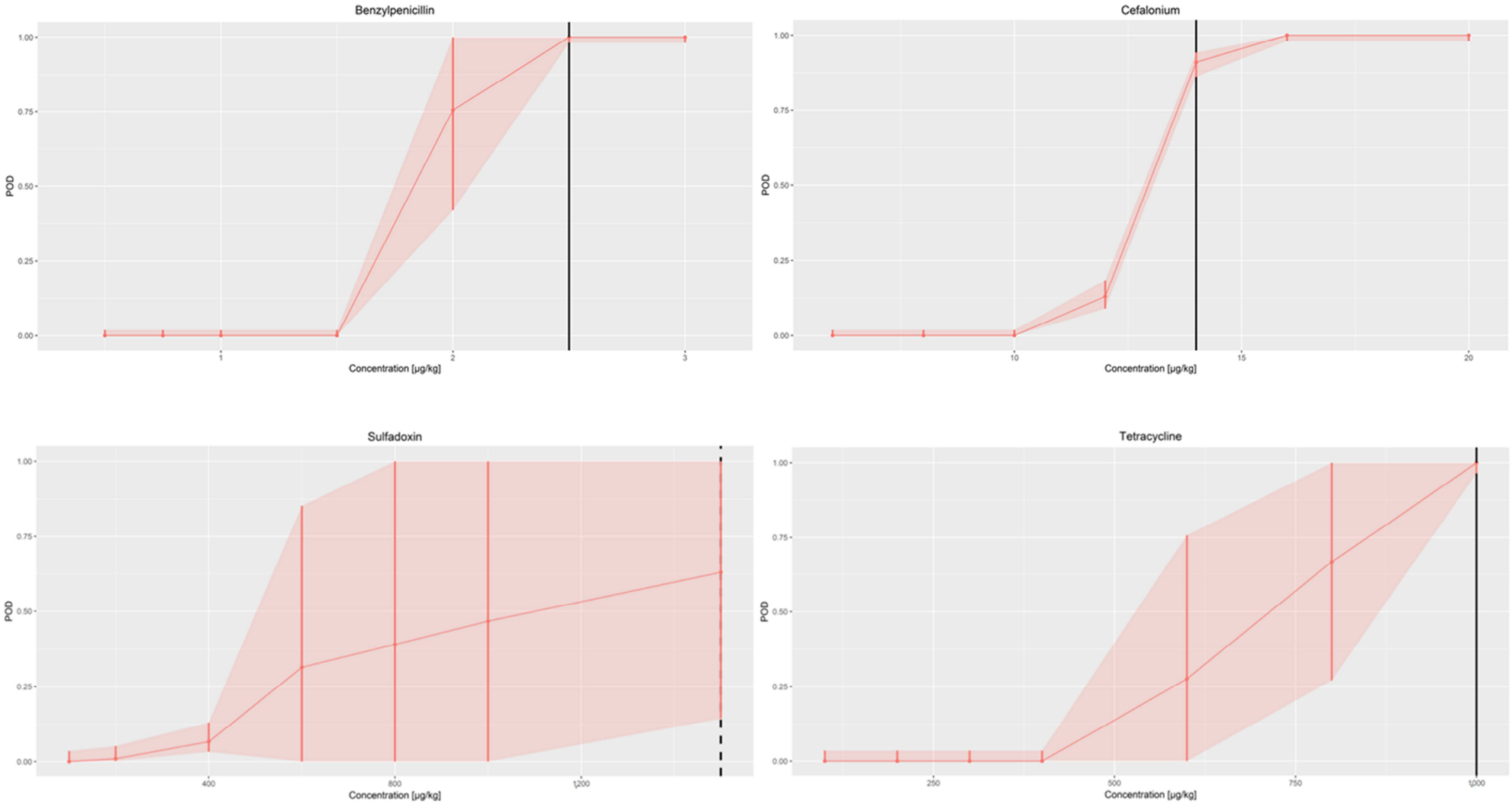



Figure 3. Dose-response curves of the bactericidal antibiotics Benzylpenicillin and Cefalonium and of the bacteriostatic substances Sulfadoxin and Tetracycline.
Red line = dose-response curve; red shade = CI; Black line = CCß A (photometric reading); Dotted line = highest concentration analyzed

## 6) Selectivity

Materials and Methods

Marker substances of commonly used classes of veterinary drugs other than antibiotics were analyzed with photometric reading in order to determine the selectivity of the BRT Inhibitor Test. The investigated compounds included the anti-inflammatories Flunixin, Metamizole (NSAIDs) and Prednisolone (glucocorticoid) as well as antiparasitic substances (Triclabendazole and Deltamethrin). Furthermore, the polyether-antibiotic Monensin, used for ketosis treatment in dairy cows, was tested. The substances were spiked at a concentration of 100 x EU MRL and inoculated with 6 replicates (Table 7).

Table 7. Selectivity: Concentrations of analyzed substances and test results

| Use | Drug Class | Substance | MRL [µg/kg] | Concentration [µg/kg] | False positive Results |
|---|---|---|---|---|---|
| Anti-inflammatory Substances | NSAID | Flunixin | 40 | 4,000 | 0/6 |
| | NSAID | Metamizole | 50 | 5,000 | 0/6 |
| | Glucocorticoid | Prednisolone | 6 | 600 | 0/6 |
| Antiparasitics | Antihelminthic | Triclabendazole | 10 | 1,000 | 0/6 |
| | Ectoparasite | Deltamethrin | 20 | 2,000 | 0/6 |
| Ketosis Treatment | Polyether-Antibiotic | Monensin | 2 | 200 | 0/6 |

Results and Discussion

Highly concentrated samples of Flunixin, Metamizole, Prednisolone, Triclabendazole, Deltamethrin and Monensin did not inhibit the growth of the test germs, leading to negative results (both class A and class B). Thus, no false positive results were observed, signifying a high specificity of the BRT Inhibitor Test for the detection of antibiotics opposed to other classes of veterinary drugs.

## 7) Batch-to-Batch Variability

Materials and Methods

In order to evaluate potential deviations in the detection capabilities of different plate batches statistically, Fisher's exact tests (method: two-sided) were applied at the concentration of the CCß A obtained with photometric reading. Contingency tables were created for the datasets of ELISA reader 1 and ELISA reader 2 to provide a basic picture of the interrelation between the two variables plate batches and number of results (class A) per batch. Due to the duration of the validation study and the limited shelf-life of the BRT Inhibitor Test plates, two sets of plate batches (A, B, C and D, E, F) had to be used.

The Fisher´s test was selected because the test is more precise than Chi square for this number of observations, the null hypothesis is based on the batches independence (the probability of the results is the same for the different batches). The Fisher's exact test was applied only to the analytes for which a CCß A could be

determined. If the significance level is α = 0.05 and the p-value <0.05, the null hypothesis is rejected, which would mean that there is a probability for batch-to-batch differences concerning the detection capability at the CCß A.

Results and Discussion

The Fisher´s test examinations for the concentrations at CCß A indicate that there are no significant differences in between the detection sensitivities of the different plate batches used in the validation. Most substances realized p-value = 1, for Cloxacillin, Oxacillin, Ceftiofur and Sulfathiazol the p values were 0.05 ≤ p <1 (Table 8). In addition to the p-values (CCß A), Annex Table 3 comprises the numbers of results per class (1-2-0), plate batch and individual ELISA reader at the concentration of the CCß A.

Table 8. Contingency table created with the Fisher Test for the concentration at CCß A obtained with photometric reading

| Group of Antibiotics | Substance | MRL | CCß A [µg/kg] | p value | | Group of Antibiotics | Substance | MRL | CCß A [µg/kg] | p value | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | ELISA 1 | ELISA 2 | | | | | ELISA 1 | ELISA 2 |
| Penicillins | Benzylpenicillin | 4 | 2.5 | 1 | 1 | Sulfonamides | Sulfadiazine | 100 | NA | - | - |
| | Ampicillin | 4 | 3.5 | 1 | 1 | | Sulfadimethoxin | 100 | NA | - | - |
| | Amoxicillin | 4 | 3 | 1 | 1 | | Sulphamethazine | 100 | 1,000 | 1 | 1 |
| | Cloxacillin | 30 | 25 | 0.33 | 1 | | Sulfathiazol | 100 | 400 | 1 | 0.06 |
| | Dicloxacillin | 30 | 15 | 1 | 1 | | Sulfadoxin | 100 | NA | - | - |
| | Nafcillin | 30 | 15 | 1 | 1 | | Sulfamethoxypyridazine | 100 | 500 | 1 | 1 |
| | Oxacillin | 30 | 10 | 0.20 | 1 | Tetracyclines | Chlortetracycline | 100 | NA | - | - |
| Cephalosporines | Cefalexin | 100 | 400 | 1 | 1 | | Oxytetracycline | 100 | 800 | 1 | 1 |
| | Cefapirin | 60 | 6 | 1 | 1 | | Tetracycline | 100 | 1,000 | 1 | 1 |
| | Cefoperazone | 50 | 35 | 1 | 1 | Aminoglycosides | Dihydrostreptomycin | 200 | 600 | 1 | 1 |
| | Cefazolin | 50 | 9 | 1 | 1 | | Streptomycin | 200 | 1,500 | 1 | 1 |
| | Cefquinome | 20 | 500 | 1 | 1 | | Gentamicin | 100 | 200 | 1 | 1 |
| | Ceftiofur | 100 | 200 | 0.11 | 0.11 | | Neomycin | 1,500 | 400 | 1 | 1 |
| | Cefalonium | 20 | 14 | 1 | 1 | Fenicols | Chloramphenicol | - | 7,000 | 1 | 1 |
| Macrolides | Erythromycin | 40 | 100 | 1 | 1 | | | | | | |
| | Tylosin | 50 | 75 | 1 | 1 | | | | | | |

Significance levels: Low: p <0.05; Medium: p <0.01; High: p <0.001

## 8) False-Positive and False-Negative Rate

Materials and Methods

With each BRT Inhibitor Test plate used during the validation study, 4 positive and negative control samples (Section 2) as well as additional 16 negative raw milk samples, adding up to 20 negative milk samples, were inoculated. By means of these samples, the rates of false-positive and false-negative results were established. Within the framework of this validation, 154 test plates were analyzed, including 616 positive control samples and 3,080 samples of negative raw milk in total. Thus, 3,080 (positive control) respectively 15,400 (negative milk) results were obtained with photometric evaluation (2 readers) and visual reading (3 technicians, Table 9).

Table 9. Numbers of positive and negative samples and obtained results used for the establishment of the false-negative and false-positive rates

| Type of Milk Sample | No. Plates | No. Samples | No. Results ELISA Readers | No. Results Visual | Total No. Results |
|---|---|---|---|---|---|
| Positive Control | 154 | 616 | 1,232 | 1,848 | 3,080 |
| Negative Milk | | 3,080 | 6,160 | 9,240 | 15,400 |

Results and Discussion

No false-positive results were observed when analyzing the results of the negative milk samples by photometric evaluation or visual reading, indicating a false-positive rate of 0%.

With photometric evaluation no false-negative result was obtained out of 3,080 results for the positive control samples. Only 1 sample was classified false-negative by 1 out of 3 examining technicians, leading to a false-negative rate of 0.2% for this person (Table 10). As none of the other readers (technicians and ELISA readers) assessed this sample as positive, the negative result is considered as outlier and might have been caused by a typing error.

Table 10. Rates of false-negative and false-positive results of all applied reading methods and readers

| | Rate of false Results [%] | | | | |
|---|---|---|---|---|---|
| Type of Milk Sample | ELISA 1 | ELISA 2 | Visual 1 | Visual 2 | Visual 3 |
| Positive Control | 0 | 0 | 0 | 0 | 0.2 |
| Negative Milk | 0 | 0 | 0 | 0 | 0 |

The maximum relative percentage value (Section 2) obtained with photometric reading for negative samples was 33%, whereas the minimum relative percentage value for positive samples was 93% (Table 11). These values demonstrate that with the chosen thresholds for photometric reading (65% class A; 40% class B; Table 1) the false interpretation of positive as well as negative samples can be avoided.

Table 11. Minimum and maximum photometric percentage results obtained with two photometric instruments

| Type of Milk Sample | No. Results ELISA Readers | Photometric Values Min/Max [%] |
|---|---|---|
| Positive Control | 1,232 | 93 |
| Negative Milk | 6,160 | 33 |

In conclusion, the absence of false-negative and false-positive results indicates that the validity of positive as well as negative results obtained for raw milk samples by analysis with the BRT Inhibitor Test is very high.

## 9) Rate of Positive Results not caused by Residues of Veterinary Drugs

Materials and Methods

In order to demonstrate that the BRT Inhibitor Test performs properly with a broad range of samples, the rate of positive results not caused by residues of veterinary drugs was established by analyzing 704 ex-farm bulk milk samples, originating from routine inhibitor analysis (milk quality payment testing at MPR Bayern). In order to verify the correct performance of the test all samples were examined in parallel on two different microbiological inhibitor tests (BRT MRL Screening Test and BRT hi-sense). To confirm detected inhibitors, screening-positive samples were tested a second time on the BRT Inhibitor Test, then evaluated with receptor tests (BetaStar® 100, Neogen Corporation, Lansing, USA; Charm MRL Beta-lactam 1-Minute Test, Charm Sciences Inc., Lawrence, USA; SNAP Beta-Lactam ST Plus, IDEXX GmbH, Ludwigsburg, Germany) and identified and quantified by analysis with the biosensor MCR-3 (GWK Präzisionstechnik GmbH, Munich, Germany). The MCR-3 is an antibody-based rapid micro-array chip reader, which is capable of the simultaneous detection and quantification of 13 antibiotic substances. Furthermore, confirmed inhibitor-positive samples were quantified by LC-MS/MS analysis.

Results and Discussion

2 out of 704 samples (0.28%, Table 12) were detected positive by the BRT Inhibitor Test. Both results were confirmed positive by evaluation with other inhibitor tests and receptor tests, the causative substance was identified as Cloxacillin by MCR-3- as well as LC-MS/MS-analysis. Cloxacillin was present in the samples at 64.8 µg/kg respectively 50.6 µg/kg. Thus, the rate of positive results not caused by residues of veterinary drugs was 0%, as all positive samples detected were confirmed to contain antibiotic inhibitors. The correct analysis of routine samples demonstrates the robust performance of the BRT Inhibitor Test with a broad range of samples and it´s applicability for real-life laboratory use.

Table 12. Routine samples analysis results

| | Negative Samples | | Positive Samples | | | |
|---|---|---|---|---|---|---|
| Total No. Samples | No. | Rate | No. | Rate | False positive | Confirmed positive |
| 704 | 702 | 99.72% | 2 | 0.28% | 0% | 100% |

## 10) Participation in an international interlaboratory study and comparability

Materials and Methods

The BRT Inhibitor Test was validated in an international interlaboratory study in order to demonstrate its robust performance and suitability for real-life laboratory applications. This interlaboratory study was conducted in parallel with the

international 10th proficiency test for inhibitors, organized by the QSE GmbH. 61 laboratories belonging to 55 companies - originating from 10 countries - ,out of 148 laboratories taking part in the 10th proficiency test, assisted with the examination of provided BRT Inhibitor Test plates for the interlaboratory study as a part of the validation.

Within the framework of the 10th proficiency test, 15 randomized and coded lyophilized UHT-milk samples were analyzed - 8 samples contained antibiotics, 7 samples consisted of inhibitor-free milk (Table 13). The antibiotics Penicillin G, Cloxacillin, Ampicillin and Cefapirin, which are often used for treatment of lactating cows, had to be detected at MRL level. These proficiency test sets were used for the interlaboratory study of the BRT Inhibitor Test, too.

The reported results of the interlaboratory study of the BRT Inhibitor Test and the 10th proficiency test were evaluated in parallel and compared in order to assess the performance of the validated test in correlation with other commonly used inhibitor tests (both microbiological and receptor tests).

Table 13. Composition of the proficiency test sets

| Substance | Concentration [µg/kg] | No. Samples |
|---|---|---|
| Benzylpenicillin | 4 | 2 |
| Ampicillin | 4 | 2 |
| Cefapirin | 60 | 2 |
| Cloxacillin | 30 | 2 |
| - | - | 7 |

The participants of the interlaboratory study analyzed the coded samples with the BRT Inhibitor Test, reported the observed results in a supplied evaluation sheet and returned these sheets to the QSE GmbH, where the results were decoded. It was not reported by the laboratories which reading system was used for the examination of the test plates.

Results and Discussion

In total, 945 results were reported for the BRT Inhibitor Test by the interlaboratory study participants, 100% of these results were correct. No false-positive or false-negative results were observed (Figure 4).

This high rate of correct results obtained in different laboratories signifies once more that the BRT Inhibitor Test is suitable for routine analyses as all positive and negative samples were identified properly and all examined substances were detected at MRL level.

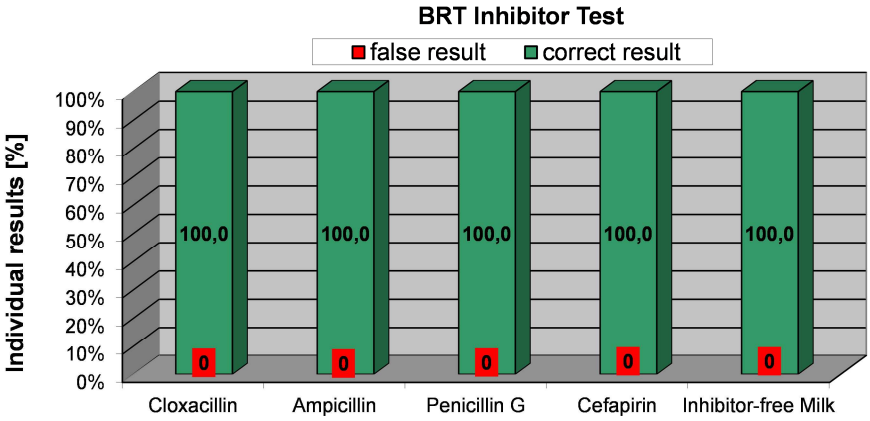



Figure 4. Rates of correct and false results (%) obtained by the interlaboratory study within the framework of the validation of the BRT Inhibitor Test

As part of a comparability study, the results obtained in the framework of the 10th proficiency test were contrasted with the results of the interlaboratory study of the BRT Inhibitor Test. 2,513 results for inhibitor-free milk and 2,872 results for inhibitor-positive samples were forwarded by the 10th proficiency test participants in total. The participating laboratories indicated if microbiological test systems or receptor tests had been used for the examination of the samples. Taking into account both types of test systems, 0.7% of the inhibitor-free milk samples were detected false-positive (Figure 5). Only 0.5% of the samples analyzed with microbiological test systems were reported false-positive (Figure 6), compared with 1.0% of the samples examined with receptor tests (Figure 7). Regarding the inhibitor-positive samples, 5% in total were identified as false-negative. Especially Cloxacillin (14.3%) and Ampicillin (3.3%), but also a few samples of Benzylpenicillin (1.7%) and Cefapirin (0.7%) were not identified correctly (Figure 5). The false-negative rate was higher for receptor test systems (6.9%) than for microbiological test systems (3.7%).

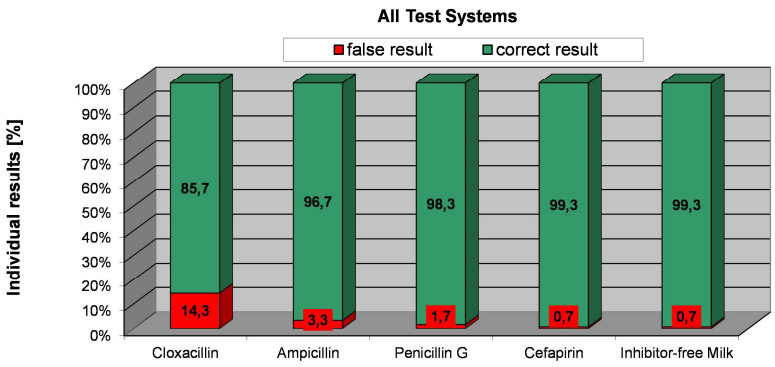



Figure 5. Rates of correct and false results (%) of all test systems (microbiological and receptor tests, 10th proficiency test)

Compared with other tests evaluated in the context of the 10th proficiency test, the BRT Inhibitor Test demonstrated an excellent performance, as no false results were observed within the interlaboratory study.

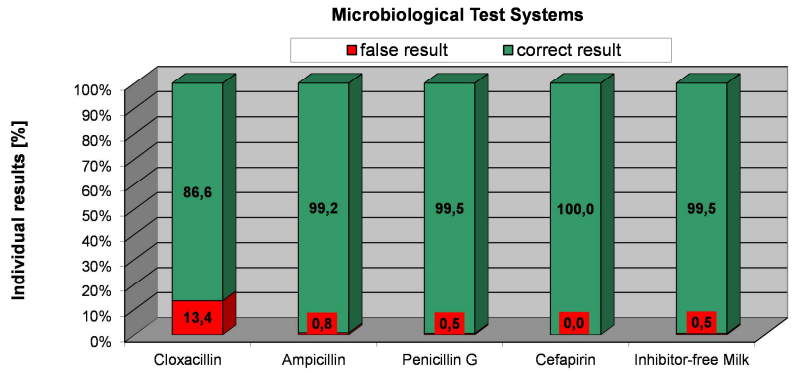



Figure 6. Rates of correct and false results (%) of microbiological test systems (10th proficiency test)

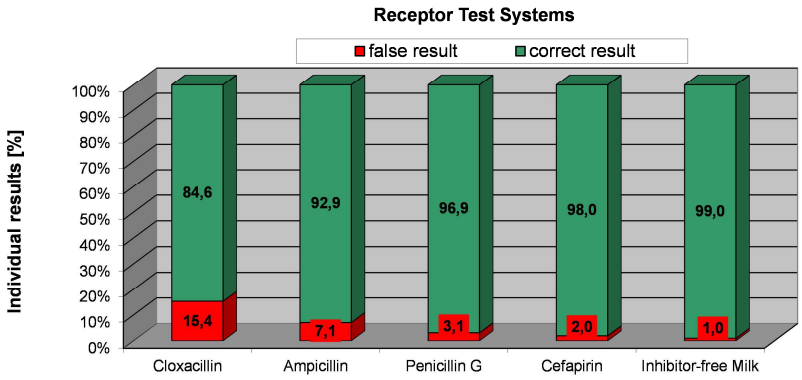



Figure 7. Rates of correct and false results (%) of receptor test systems (10th proficiency test)

## 11) Conclusions

The BRT Inhibitor Test is capable of the detection of all of the 7 penicillins and 4 out of 7 cephalosporins as well as 6 out of 15 compounds belonging to other antibiotic groups investigated in this study at or below MRL level – depending on the interpretation method. This means that the most important antibiotic compounds used in Germany for the treatment of dairy cows are detected predominantly below MRL. A broad range of other inhibitors can be identified above MRL as well. The BRT Inhibitor Test displays a high selectivity for antibiotic residues, marker substances of other veterinary classes were not detected at high concentrations. The Batch-to-Batch-Variability proved to be low, no significant differences were observed for positive result rates obtained with different plate batches. The validity of obtained results is high as no false-positive and an extremely low rate of false-negative results were observed in the analysis of positive and negative control samples. With the correct analysis of a broad range of routine milk quality payment samples, the good performance of the BRT Inhibitor Test in an international interlaboratory study (100% correct results) and in comparison with other inhibitor tests used by laboratories participating in an international proficiency test, which was organized in parallel, it could be demonstrated that the test is fit for routine laboratory use.

## 12) References


COMMISSION DECISION C1 14 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (notified under document number C (2002) 3044) (Text with EEA relevance) (2002/657/EC) OJ L 221, 17.8.2002, p. 8

COMMUNITY REFERENCE LABORATORIES RESIDUES (CRLs) 20/1/2010 - Guidelines for the validation of screening methods for residues of veterinary medicines (initial validation and transfer)

ISO 13969 IDF 183 Milk and Milk products - Guidelines for a standardized description of microbial inhibitor tests

ISO 18330 IDF 188 Milk and milk products - Guidelines for the standardized description of immunoassays or receptor assays for the detection of antimicrobial residues

German Federal Office of Consumer Protection and Food Safety
Amtliche Sammlung von Untersuchungsverfahren nach § 64 LFGB (vormals $35 LMBG) Band 1 (L), L 01.01-5: Nachweis von Hemmstoffen in Sammelmilch, Agar-Diffusions-Verfahren (Brillantschwarz-Reduktionstest), Januar 2012

German Federal Office of Consumer Protection and Food Safety
Amtliche Sammlung von Untersuchungsverfahren nach § 64 LFGB (vormals $35 LMBG) Band 1 (L), L 01.00-11: Suchverfahren auf das Vorhandensein von Antiinfektiva in Milch, Agar-Diffusions-Verfahren mit Bacillus stearothermophilus (Brillantschwarz-Reduktionstest), Februar 1996

BEER, P. and G. SUHREN (1993): Approach for an inhibitor test kit validation program, IDF-Workshop on Residues of Antibiotics and Other Antimicrobial Inhibitors in Raw and Heat-Treated Milk, Copenhagen, collected abstracts

R Core Team, 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

AOAC Binary Data Interlaboratory Study Workbook Version 2.3

QSE GmbH, 2017: 10th proficiency test for inhibitors, Final report

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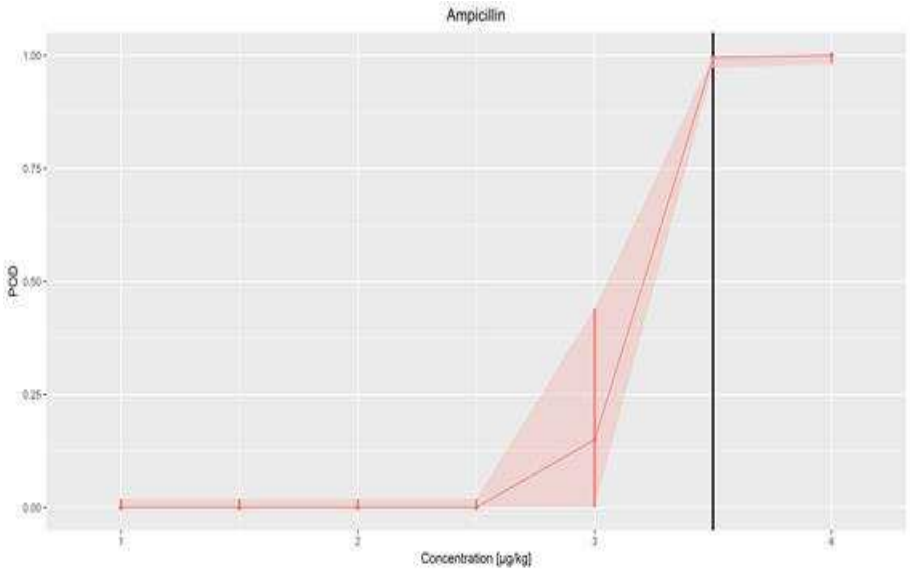
Date of publication: 09.11.2018

## ANNEX

Annex Table 1. Established detection limits (visual reading) compared with the EU MRL levels. Marked in red are the substances exceeding the EU MRLs.

| Group of Antibiotics | Substance | MRL EU [µg/kg] | CCß A [µg/kg] | CCß B [µg/kg] |
|---|---|---|---|---|
| **Penicillins** | Benzylpenicillin | 4 | 2.5 | 2 |
| | Ampicillin | 4 | 3.5 | 3 |
| | Amoxicillin | 4 | 3.5 | 3 |
| | Cloxacillin | 30 | 30 | 25 |
| | Dicloxacillin | 30 | 15 | 12.5 |
| | Nafcillin | 30 | 15 | 8 |
| | Oxacillin | 30 | 12 | 10 |
| **Cephalosporins** | Cefalexin | 100 | 500 | 400 |
| | Cefapirin | 60 | 8 | 5 |
| | Cefoperazone | 50 | 35 | 25 |
| | Cefazolin | 50 | 9 | 7 |
| | Cefquinome | 20 | 600 | 300 |
| | Ceftiofur | 100 | 250 | 200 |
| | Cefalonium | 20 | 16 | 12 |
| **Macrolides** | Erythromycin | 40 | 100 | 60 |
| | Tylosin | 50 | 100 | 30 |
| **Sulfonamides** | Sulfadiazine | 100 | >800 | 100 |
| | Sulfadimethoxin | 100 | >800 | 200 |
| | Sulfamethazine | 100 | >1,000 | 200 |
| | Sulfathiazol | 100 | 400 | 60 |
| | Sulfadoxin | 100 | >1,500 | 400 |
| | Sulfamethoxypyridazine | 100 | 500 | 100 |
| **Tetracyclines** | Chlortetracycline | 100 | >1,000 | 800 |
| | Oxytetracycline | 100 | 800 | 400 |
| | Tetracycline | 100 | 1,000 | 600 |
| **Aminoglycosides** | Dihydrostreptomycin | 200 | 500 | 400 |
| | Streptomycin | 200 | 1,500 | 600 |
| | Gentamicin | 100 | 150 | 80 |
| | Neomycin | 1,500 | 300 | 200 |
| **Fenicol** | Chloramphenicol | - | 5,000 | 3,500 |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1
2
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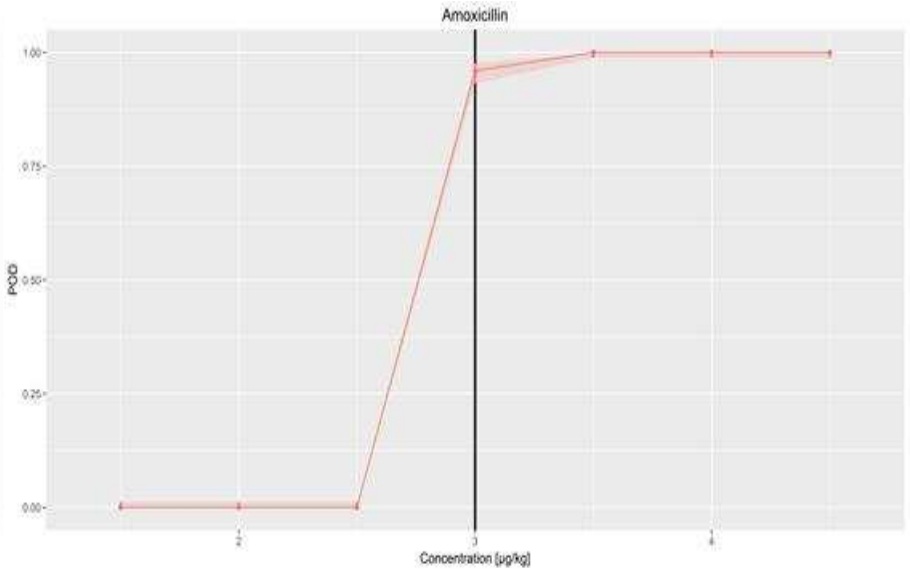
4
Concentration [µg/kg]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0.50
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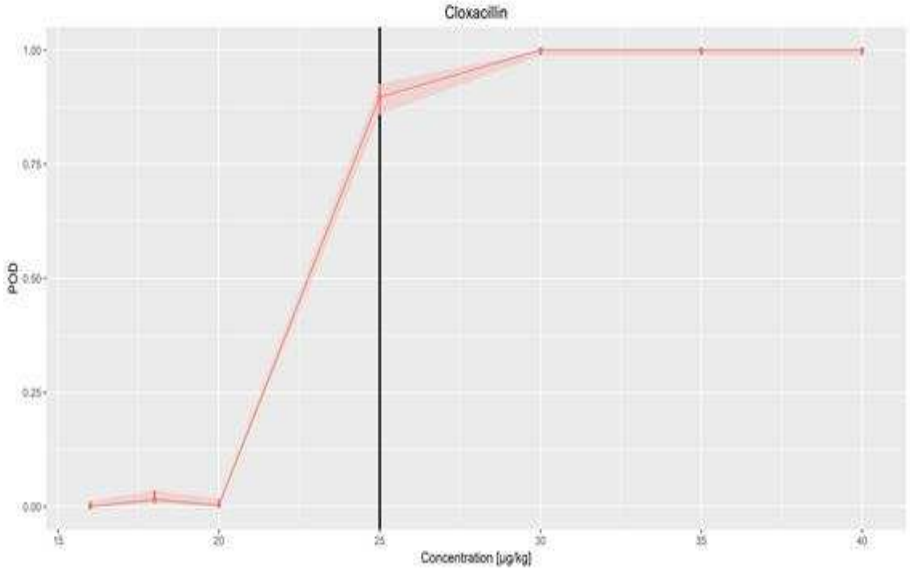
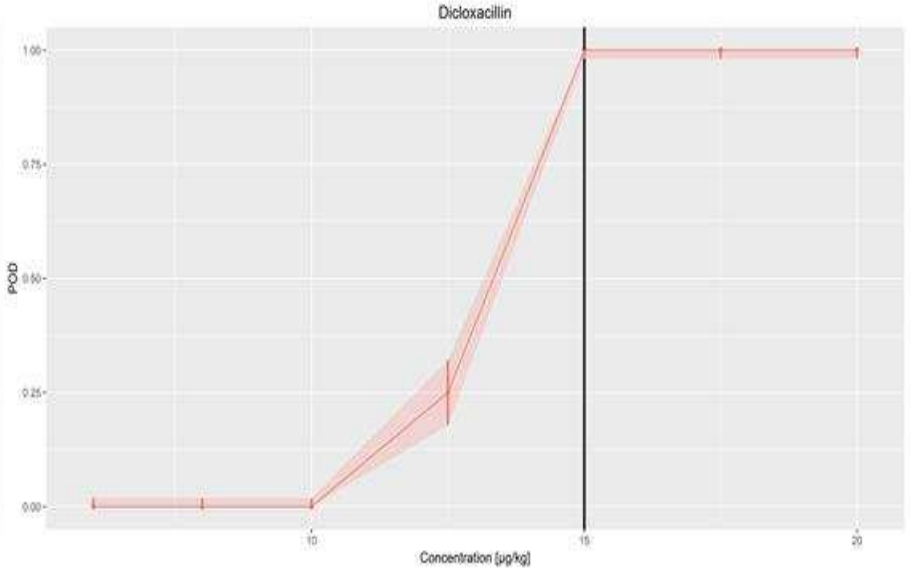
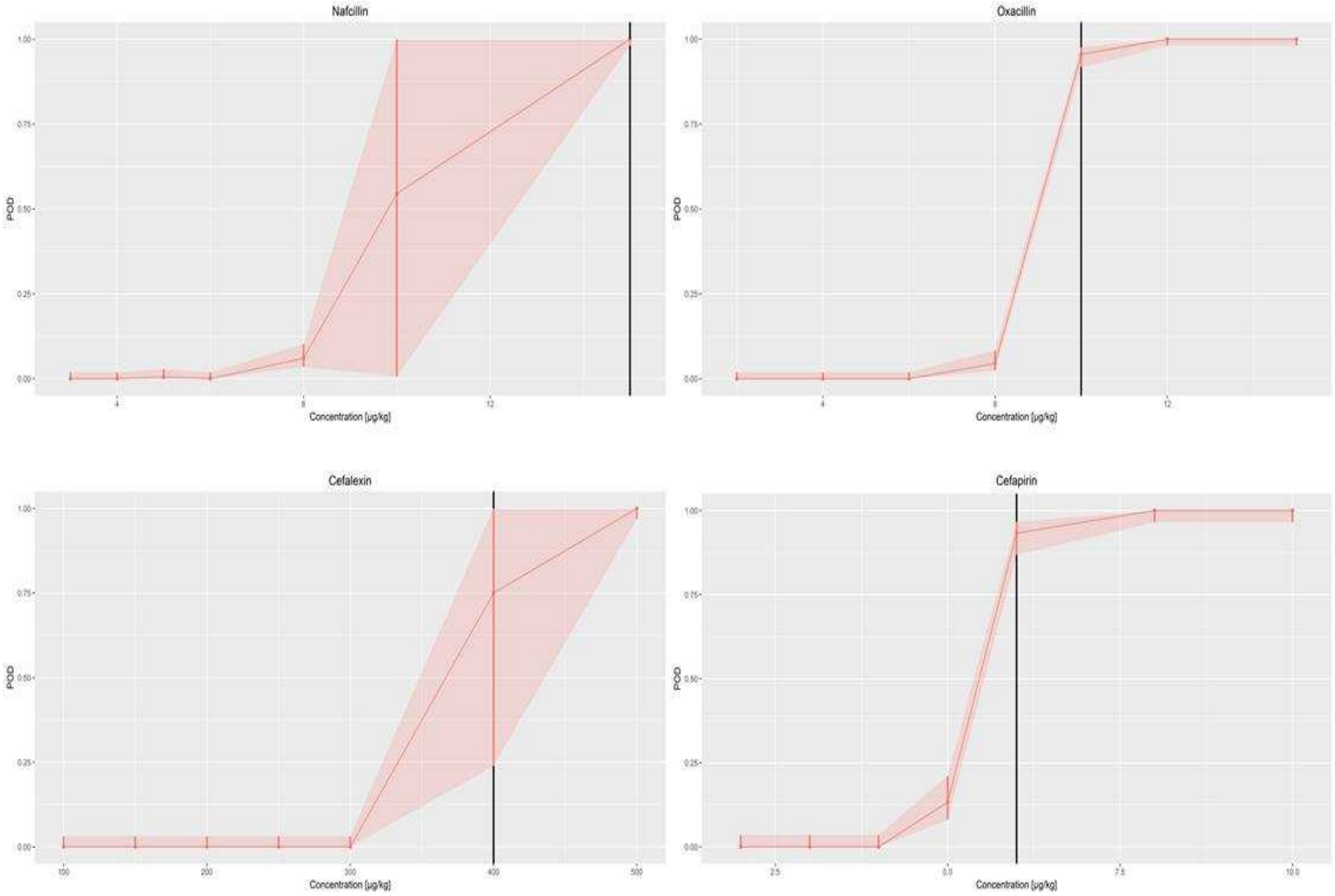
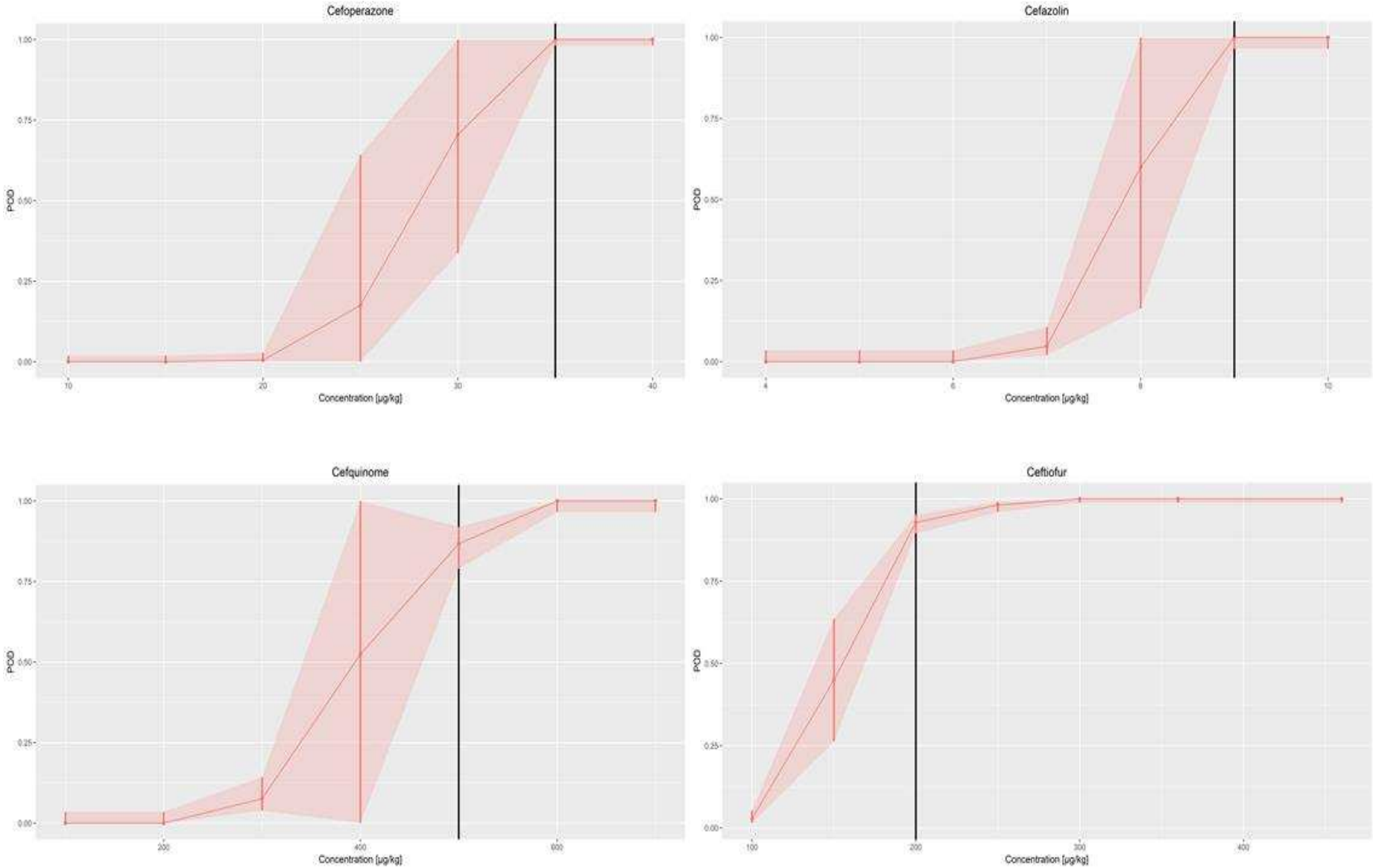
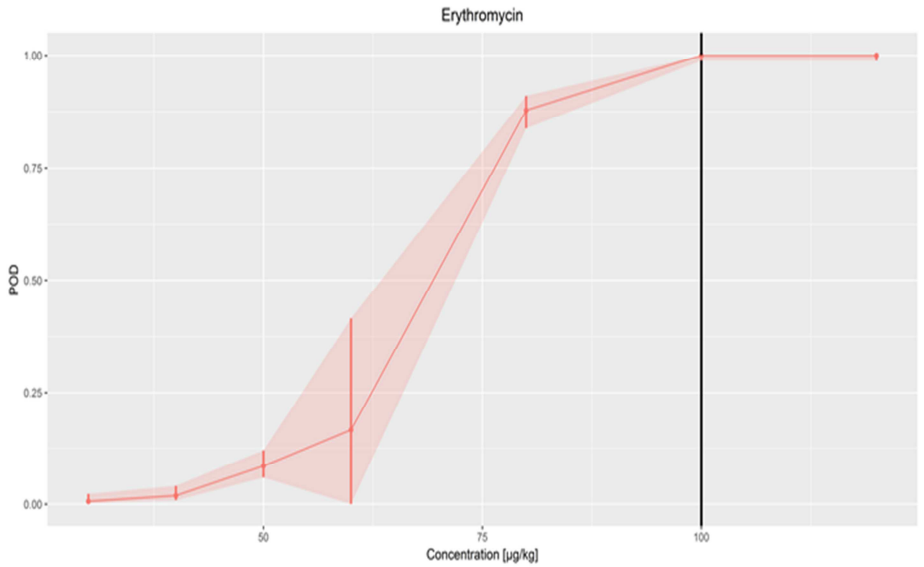
Concentration [µg/kg]

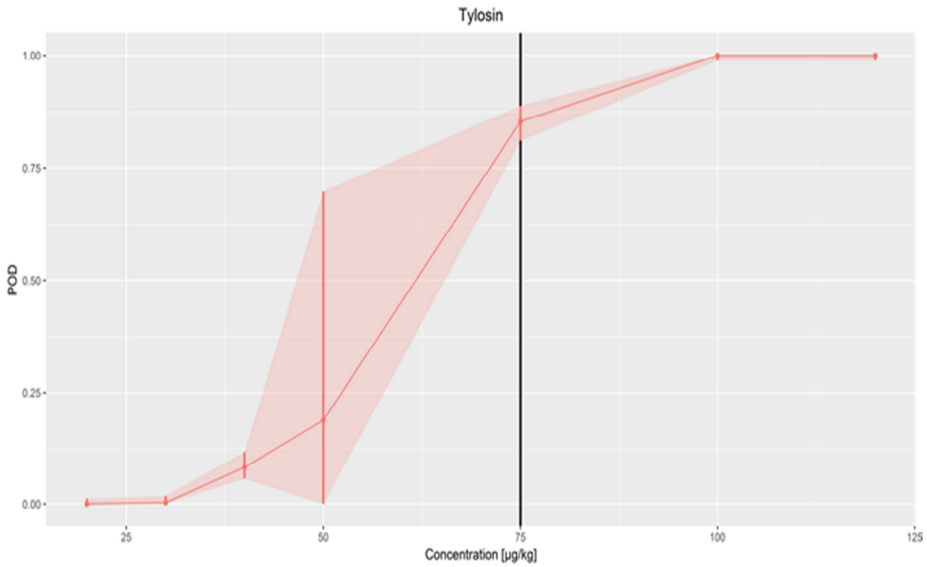

Cloxacillin
POD
1.00
0.75
0.50
0.25
0.00
15
20
25
30
35
40
Concentration [µg/kg]

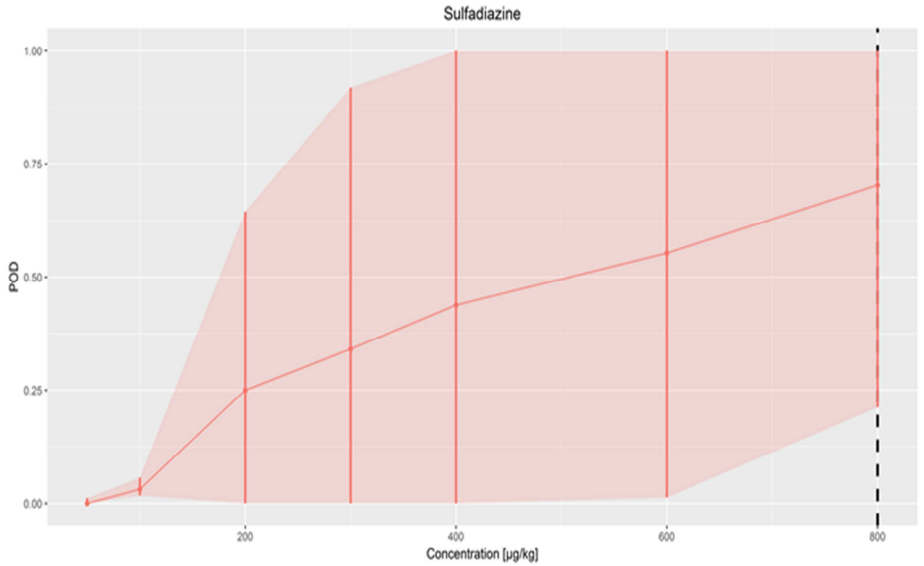

Dicloxacillin
POD
1.00
0.75
0.50
0.25
0.00
10
15
20
Concentration [µg/kg]

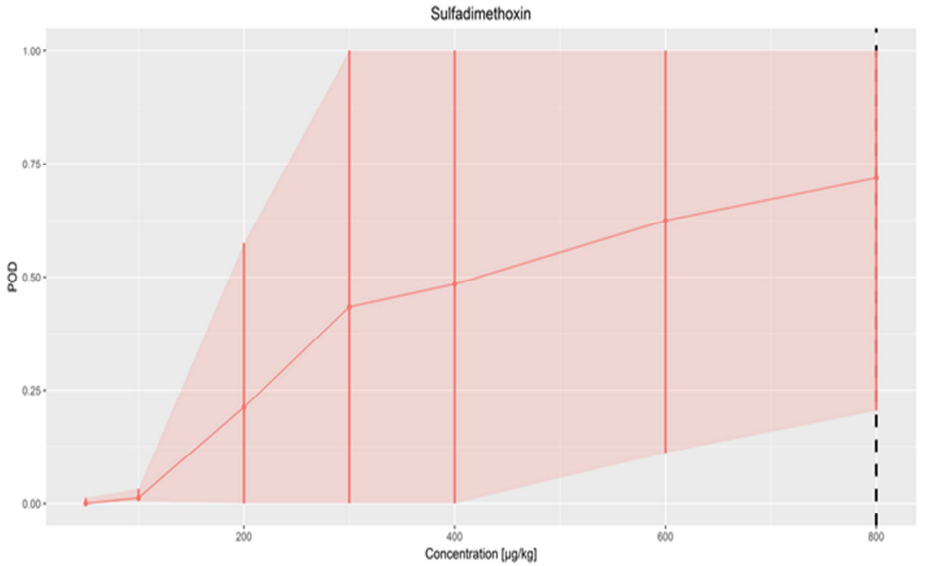

Nafcillin
Oxacillin
Cefalexin
Cefapirin
POD
Concentration [µg/kg]

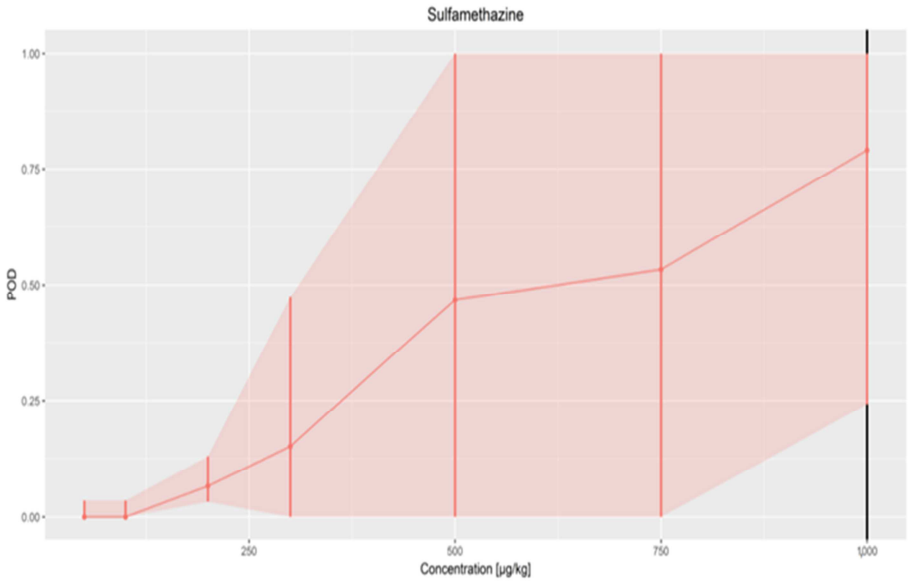

Cefoperazone
Cefazolin
Cefquinome
Ceftiofur
POD
Concentration [µg/kg]

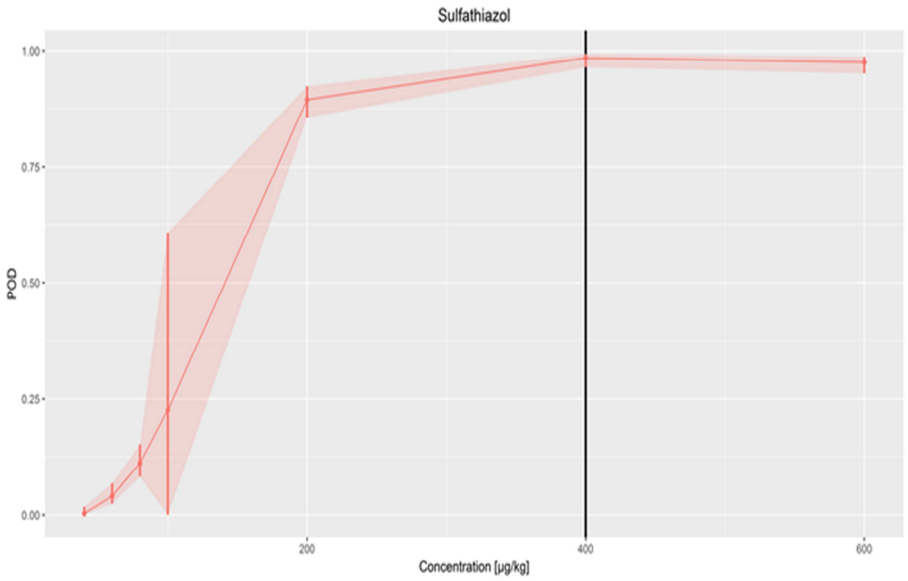

Erythromycin
POD
1.00
0.75
0.50
0.25
0.00
50
75
100
Concentration [µg/kg]

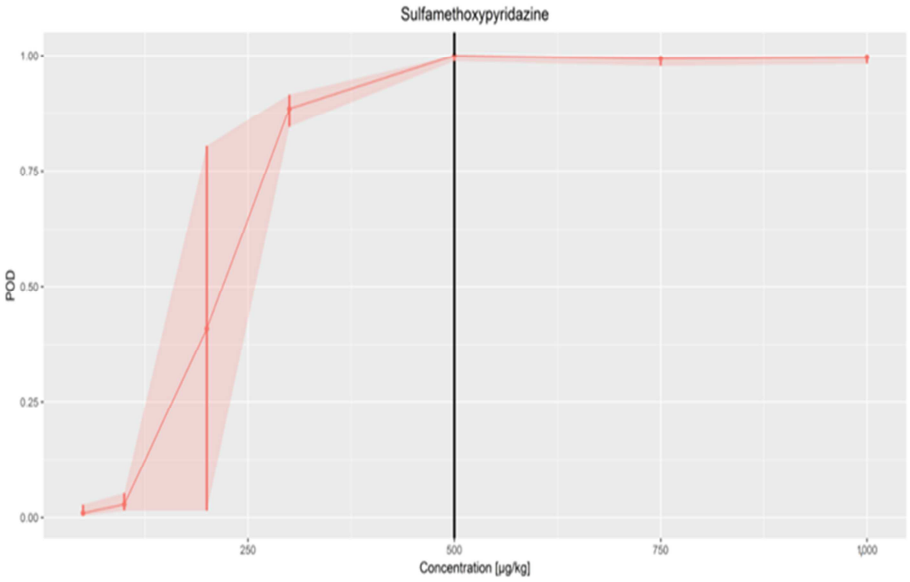

Tylosin
POD
1.00
0.75
0.50
0.25
0.00
25
50
75
100
125
Concentration [µg/kg]

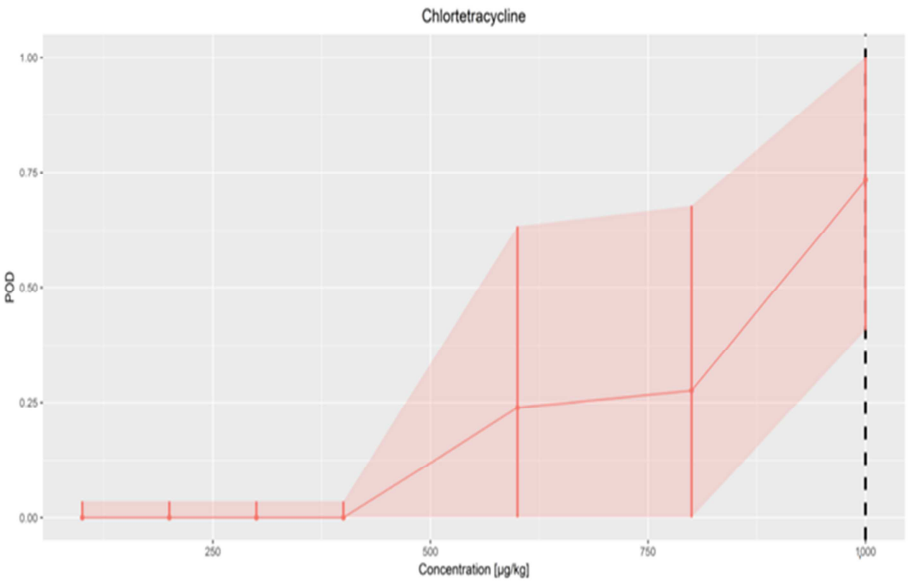

Sulfadiazine
POD
1.00
0.75
0.50
0.25
0.00
200
400
600
800
Concentration [µg/kg]

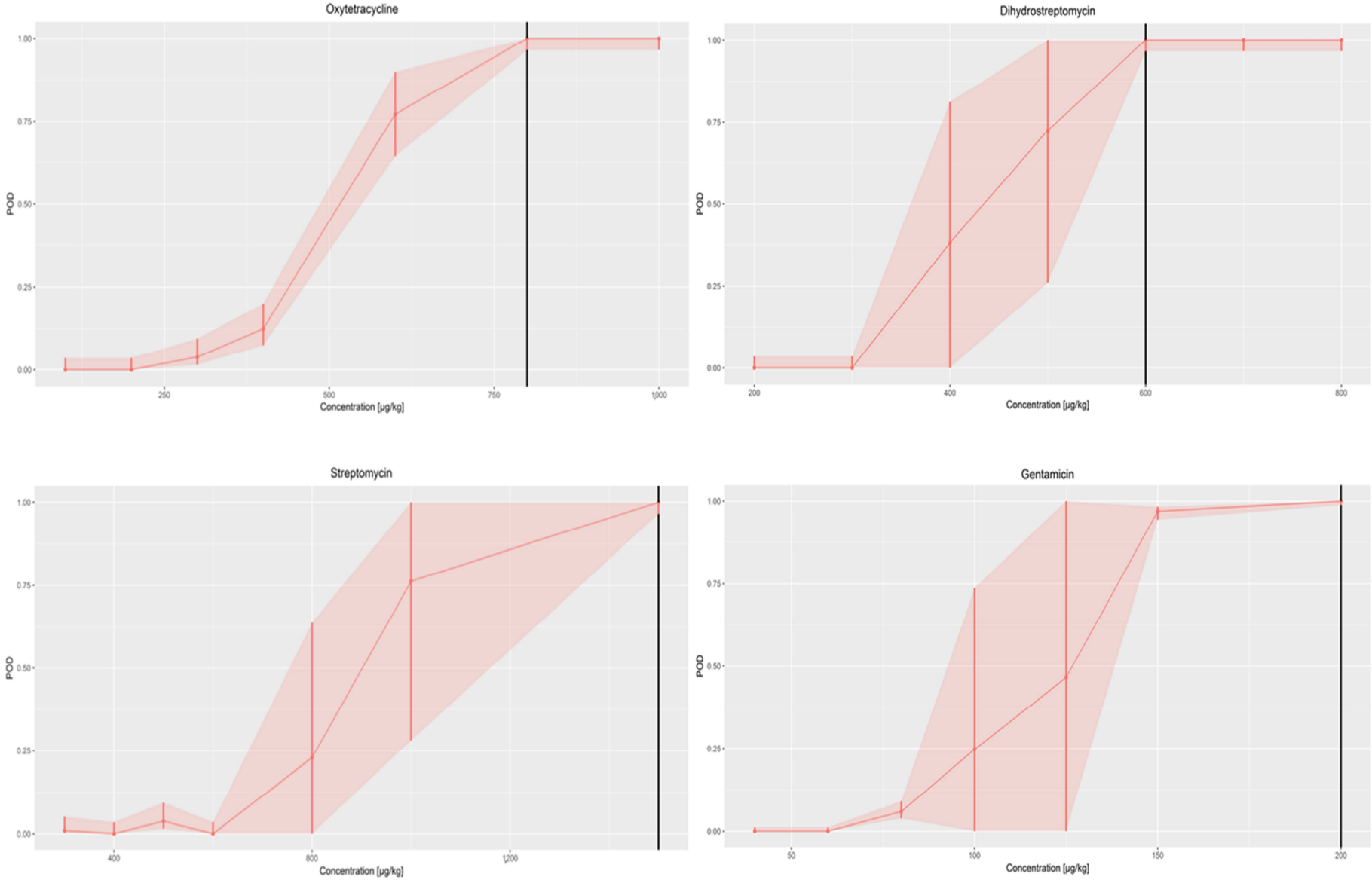

Sulfadimethoxin
POD
1.00
0.75
0.50
0.25
0.00
200
400
600
800
Concentration [µg/kg]

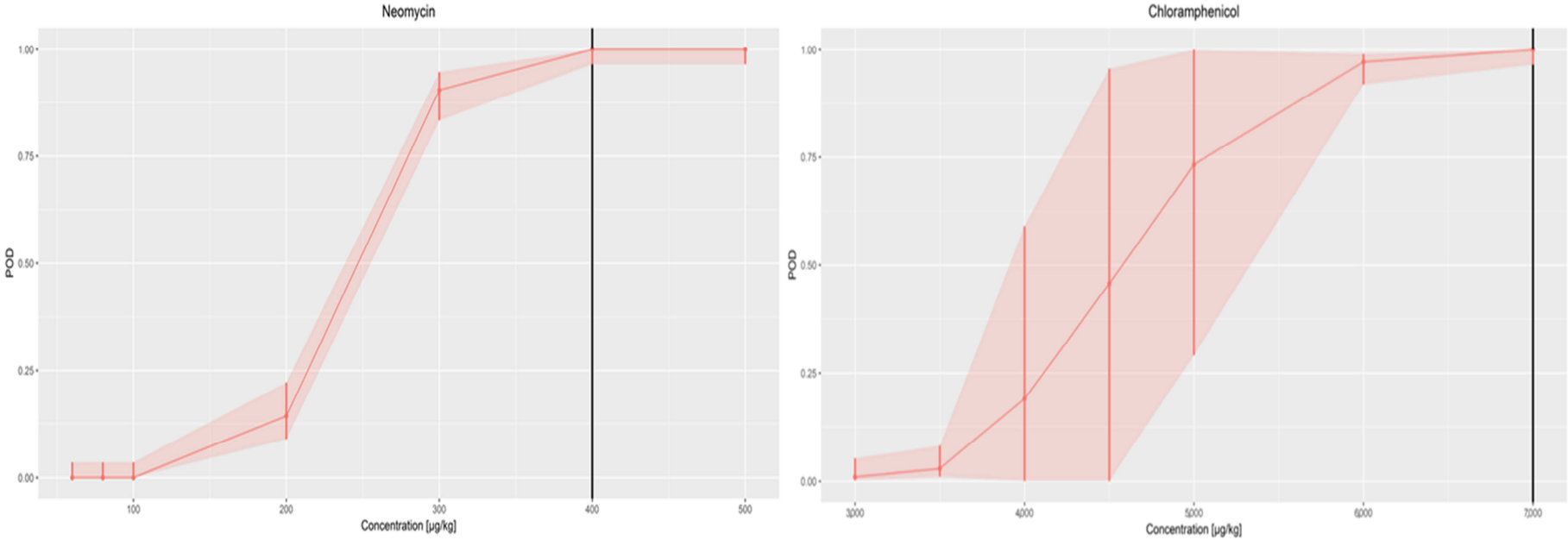

Sulfamethazine
POD
1.00
0.75
0.50
0.25
0.00
250
500
750
1000
Concentration [µg/kg]


Sulfathiazol
POD
1.00
0.75
0.50
0.25
0.00
200
400
600
Concentration [µg/kg]


Sulfamethoxypyridazine
POD
1.00
0.75
0.50
0.25
0.00
250
500
750
1000
Concentration [µg/kg]


Chlortetracycline
POD
1.00
0.75
0.50
0.25
0.00
250
500
750
1000
Concentration [µg/kg]


Oxytetracycline
POD
1.00
0.75
0.50
0.25
0.00
250
500
750
1000
Concentration [µg/kg]
Dihydrostreptomycin
POD
1.00
0.75
0.50
0.25
0.00
200
400
600
800
Concentration [µg/kg]
Streptomycin
POD
1.00
0.75
0.50
0.25
0.00
400
800
1200
Concentration [µg/kg]
Gentamicin
POD
1.00
0.75
0.50
0.25
0.00
50
100
150
200
Concentration [µg/kg]




Annex Figure 1. Dose-response curves of antibiotic substances included in the validation of the BRT Inhibitor Test.
Red line = dose-response curve; red shade = CI; Black line = CCß A (photometric reading); Dotted line = highest concentration analyzed

Annex Table 2. Numbers and percentages of results per concentrations of samples for each class of results (1-2-0) and both reading systems (photometric and visual) separately as well as joint for both reading systems including the CI for class A results.

| Substance | Reading System | Concentration | No. of total Results | No. of Results (pos. Class A) | Percentage of CCß A | Lower 95%-CI (CCß A) | Upper 95%-CI (CCß A) | No. of Results (pos. Class B) | Percentage of CCß B | No. of Results (negative) |
|---|---|---|---|---|---|---|---|---|---|---|
| Benzyl-penicillin | Photometric | 0.5 | 80 | 0 | 0.00 | - | - | 0 | 0.00 | 80 |
| | | 0.75 | 80 | 0 | 0.00 | - | - | 0 | 0.00 | 80 |
| | | 1 | 80 | 0 | 0.00 | - | - | 0 | 0.00 | 80 |
| | | 1.5 | 80 | 0 | 0.00 | - | - | 26 | 0.33 | 54 |
| | | 2 | 80 | 68 | 0.85 | - | - | 12 | 1.00 | 0 |
| | | 2.5 | 80 | 80 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 3 | 80 | 80 | 1.00 | - | - | 0 | 1.00 | 0 |
| | Visual | 0.5 | 120 | 0 | 0.00 | - | - | 2 | 0.02 | 118 |
| | | 0.75 | 120 | 0 | 0.00 | - | - | 1 | 0.01 | 119 |
| | | 1 | 120 | 0 | 0.00 | - | - | 6 | 0.05 | 114 |
| | | 1.5 | 120 | 0 | 0.00 | - | - | 60 | 0.50 | 60 |
| | | 2 | 120 | 83 | 0.69 | - | - | 37 | 1.00 | 0 |
| | | 2.5 | 120 | 120 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 3 | 120 | 120 | 1.00 | - | - | 0 | 1.00 | 0 |
| | Photometric + Visual | 0.5 | 200 | 0 | 0.00 | 0.00 | 0.02 | 2 | - | 198 |
| | | 0.75 | 200 | 0 | 0.00 | 0.00 | 0.02 | 1 | - | 199 |
| | | 1 | 200 | 0 | 0.00 | 0.00 | 0.02 | 6 | - | 194 |
| | | 1.5 | 200 | 0 | 0.00 | 0.00 | 0.02 | 86 | - | 114 |
| | | 2 | 200 | 151 | 0.76 | 0.42 | 1.00 | 49 | - | 0 |
| | | 2.5 | 200 | 200 | 1.00 | 0.98 | 1.00 | 0 | - | 0 |
| | | 3 | 200 | 200 | 1.00 | 0.98 | 1.00 | 0 | - | 0 |
| Ampicillin | Photometric | 1 | 80 | 0 | 0.00 | - | - | 0 | 0.00 | 80 |
| | | 1.5 | 80 | 0 | 0.00 | - | - | 0 | 0.00 | 80 |
| | | 2 | 80 | 0 | 0.00 | - | - | 0 | 0.00 | 80 |
| | | 2.5 | 80 | 0 | 0.00 | - | - | 17 | 0.21 | 63 |
| | | 3 | 80 | 3 | 0.04 | - | - | 77 | 1.00 | 0 |
| | | 3.5 | 80 | 80 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 4 | 80 | 80 | 1.00 | - | - | 0 | 1.00 | 0 |
| | Visual | 1 | 120 | 0 | 0.00 | - | - | 0 | 0.00 | 120 |
| | | 1.5 | 120 | 0 | 0.00 | - | - | 0 | 0.00 | 120 |
| | | 2 | 120 | 0 | 0.00 | - | - | 1 | 0.01 | 119 |
| | | 2.5 | 120 | 0 | 0.00 | - | - | 32 | 0.27 | 88 |
| | | 3 | 120 | 27 | 0.23 | - | - | 90 | 0.98 | 3 |
| | | 3.5 | 120 | 119 | 0.99 | - | - | 1 | 1.00 | 0 |
| | | 4 | 120 | 120 | 1.00 | - | - | 0 | 1.00 | 0 |
| | Photometric + Visual | 1 | 200 | 0 | 0.00 | 0.00 | 0.02 | 0 | - | 200 |
| | | 1.5 | 200 | 0 | 0.00 | 0.00 | 0.02 | 0 | - | 200 |
| | | 2 | 200 | 0 | 0.00 | 0.00 | 0.02 | 1 | - | 199 |
| | | 2.5 | 200 | 0 | 0.00 | 0.00 | 0.02 | 49 | - | 151 |
| | | 3 | 200 | 30 | 0.15 | 0.00 | 0.44 | 167 | - | 3 |
| | | 3.5 | 200 | 199 | 1.00 | 0.97 | 1.00 | 1 | - | 0 |
| | | 4 | 200 | 200 | 1.00 | 0.98 | 1.00 | 0 | - | 0 |
| Amoxicillin | Photometric | 1.5 | 128 | 0 | 0.00 | - | - | 0 | 0.00 | 128 |
| | | 2 | 128 | 0 | 0.00 | - | - | 6 | 0.05 | 122 |
| | | 2.5 | 128 | 0 | 0.00 | - | - | 126 | 0.98 | 2 |
| | | 3 | 128 | 128 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 3.5 | 128 | 128 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 4 | 128 | 128 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 4.5 | 128 | 128 | 1.00 | - | - | 0 | 1.00 | 0 |
| | Visual | 1.5 | 192 | 0 | 0.00 | - | - | 0 | 0.00 | 192 |
| | | 2 | 192 | 0 | 0.00 | - | - | 0 | 0.00 | 192 |
| | | 2.5 | 192 | 0 | 0.00 | - | - | 142 | 0.74 | 50 |
| | | 3 | 192 | 179 | 0.93 | - | - | 13 | 1.00 | 0 |
| | | 3.5 | 192 | 192 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 4 | 192 | 192 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 4.5 | 192 | 192 | 1.00 | - | - | 0 | 1.00 | 0 |
| | Photometric + Visual | 1.5 | 320 | 0 | 0.00 | 0.00 | 0.01 | 0 | - | 320 |
| | | 2 | 320 | 0 | 0.00 | 0.00 | 0.01 | 6 | - | 314 |
| | | 2.5 | 320 | 0 | 0.00 | 0.00 | 0.01 | 268 | - | 52 |
| | | 3 | 320 | 307 | 0.96 | 0.93 | 0.98 | 13 | - | 0 |
| | | 3.5 | 320 | 320 | 1.00 | 0.99 | 1.00 | 0 | - | 0 |
| | | 4 | 320 | 320 | 1.00 | 0.99 | 1.00 | 0 | - | 0 |
| | | 4.5 | 320 | 320 | 1.00 | 0.99 | 1.00 | 0 | - | 0 |
| Cloxacillin | Photometric | 16 | 128 | 0 | 0.00 | - | - | 0 | 0.00 | 128 |
| | | 18 | 128 | 2 | 0.02 | - | - | 85 | 0.68 | 41 |
| | | 20 | 128 | 0 | 0.00 | - | - | 124 | 0.97 | 4 |
| | | 25 | 128 | 126 | 0.98 | - | - | 2 | 1.00 | 0 |
| | | 30 | 128 | 128 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 35 | 128 | 128 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 40 | 128 | 128 | 1.00 | - | - | 0 | 1.00 | 0 |
| | Visual | 16 | 192 | 0 | 0.00 | - | - | 1 | 0.01 | 191 |
| | | 18 | 192 | 3 | 0.02 | - | - | 42 | 0.23 | 147 |
| | | 20 | 192 | 1 | 0.01 | - | - | 167 | 0.88 | 24 |
| | | 25 | 192 | 161 | 0.84 | - | - | 31 | 1.00 | 0 |
| | | 30 | 192 | 192 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 35 | 192 | 192 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 40 | 192 | 192 | 1.00 | - | - | 0 | 1.00 | 0 |
| | Photometric + Visual | 16 | 320 | 0 | 0.00 | 0.00 | 0.01 | 1 | - | 319 |
| | | 18 | 320 | 5 | 0.02 | 0.01 | 0.04 | 127 | - | 188 |
| | | 20 | 320 | 1 | 0.00 | 0.00 | 0.02 | 291 | - | 28 |
| | | 25 | 320 | 287 | 0.90 | 0.86 | 0.93 | 33 | - | 0 |
| | | 30 | 320 | 320 | 1.00 | 0.99 | 1.00 | 0 | - | 0 |
| | | 35 | 320 | 320 | 1.00 | 0.99 | 1.00 | 0 | - | 0 |
| | | 40 | 320 | 320 | 1.00 | 0.99 | 1.00 | 0 | - | 0 |

## Continuation Annex Table 2

| Substance | Reading System | Concentration | No. of total Results | No. of Results (pos. Class A) | Percentage of CCß A | Lower 95%-CI (CCß A) | Upper 95%-CI (CCß A) | No. of Results (pos. Class B) | Percentage of CCß B | No. of Results (negative) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 6 | 80 | 0 | 0.00 | - | - | 0 | 0.00 | 80 |
| | | 8 | 80 | 0 | 0.00 | - | - | 0 | 0.00 | 80 |
| | | 10 | 80 | 0 | 0.00 | - | - | 12 | 0.15 | 68 |
| | Photometric | 12.5 | 80 | 17 | 0.21 | - | - | 63 | 1.00 | 0 |
| | | 15 | 80 | 80 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 17.5 | 80 | 80 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 20 | 80 | 80 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 6 | 120 | 0 | 0.00 | - | - | 8 | 0.07 | 112 |
| | | 8 | 120 | 0 | 0.00 | - | - | 10 | 0.08 | 110 |
| | | 10 | 120 | 0 | 0.00 | - | - | 36 | 0.30 | 84 |
| **Dicloxacillin** | Visual | 12.5 | 120 | 33 | 0.28 | - | - | 86 | 0.99 | 1 |
| | | 15 | 120 | 120 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 17.5 | 120 | 120 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 20 | 120 | 120 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 6 | 200 | 0 | 0.00 | 0.00 | 0.02 | 8 | - | 192 |
| | | 8 | 200 | 0 | 0.00 | 0.00 | 0.02 | 10 | - | 190 |
| | | 10 | 200 | 0 | 0.00 | 0.00 | 0.02 | 48 | - | 152 |
| | Photometric + Visual | 12.5 | 200 | 50 | 0.25 | 0.18 | 0.32 | 149 | - | 1 |
| | | 15 | 200 | 200 | 1.00 | 0.98 | 1.00 | 0 | - | 0 |
| | | 17.5 | 200 | 200 | 1.00 | 0.98 | 1.00 | 0 | - | 0 |
| | | 20 | 200 | 200 | 1.00 | 0.98 | 1.00 | 0 | - | 0 |
| | | 3 | 80 | 0 | 0.00 | - | - | 0 | 0.00 | 80 |
| | | 4 | 80 | 0 | 0.00 | - | - | 0 | 0.00 | 80 |
| | | 5 | 80 | 0 | 0.00 | - | - | 1 | 0.01 | 79 |
| | Photometric | 6 | 80 | 0 | 0.00 | - | - | 2 | 0.03 | 78 |
| | | 8 | 80 | 0 | 0.00 | - | - | 61 | 0.76 | 19 |
| | | 10 | 80 | 30 | 0.38 | - | - | 50 | 1.00 | 0 |
| | | 15 | 80 | 80 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 3 | 120 | 0 | 0.00 | - | - | 6 | 0.05 | 114 |
| | | 4 | 120 | 0 | 0.00 | - | - | 15 | 0.13 | 105 |
| | | 5 | 120 | 1 | 0.01 | - | - | 34 | 0.29 | 85 |
| **Nafcillin** | Visual | 6 | 120 | 0 | 0.00 | - | - | 59 | 0.49 | 61 |
| | | 8 | 120 | 12 | 0.10 | - | - | 102 | 0.95 | 6 |
| | | 10 | 120 | 79 | 0.66 | - | - | 41 | 1.00 | 0 |
| | | 15 | 120 | 120 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 3 | 200 | 0 | 0.00 | 0.00 | 0.02 | 6 | - | 194 |
| | | 4 | 200 | 0 | 0.00 | 0.00 | 0.02 | 15 | - | 185 |
| | | 5 | 200 | 1 | 0.01 | 0.00 | 0.03 | 35 | - | 164 |
| | Photometric + Visual | 6 | 200 | 0 | 0.00 | 0.00 | 0.02 | 61 | - | 139 |
| | | 8 | 200 | 12 | 0.06 | 0.03 | 0.10 | 163 | - | 25 |
| | | 10 | 200 | 109 | 0.55 | 0.01 | 1.00 | 91 | - | 0 |
| | | 15 | 200 | 200 | 1.00 | 0.98 | 1.00 | 0 | - | 0 |
| | | 2 | 80 | 0 | 0.00 | - | - | 0 | 0.00 | 80 |
| | | 4 | 80 | 0 | 0.00 | - | - | 0 | 0.00 | 80 |
| | | 6 | 80 | 0 | 0.00 | - | - | 2 | 0.03 | 78 |
| | Photometric | 8 | 80 | 0 | 0.00 | - | - | 80 | 1.00 | 0 |
| | | 10 | 80 | 79 | 0.99 | - | - | 1 | 1.00 | 0 |
| | | 12 | 80 | 80 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 15 | 80 | 80 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 2 | 120 | 0 | 0.00 | - | - | 4 | 0.03 | 116 |
| | | 4 | 120 | 0 | 0.00 | - | - | 3 | 0.03 | 117 |
| | | 6 | 120 | 0 | 0.00 | - | - | 41 | 0.34 | 79 |
| **Oxacillin** | Visual | 8 | 120 | 9 | 0.08 | - | - | 102 | 0.93 | 9 |
| | | 10 | 120 | 112 | 0.93 | - | - | 8 | 1.00 | 0 |
| | | 12 | 120 | 120 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 15 | 120 | 120 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 2 | 200 | 0 | 0.00 | 0.00 | 0.02 | 4 | - | 196 |
| | | 4 | 200 | 0 | 0.00 | 0.00 | 0.02 | 3 | - | 197 |
| | | 6 | 200 | 0 | 0.00 | 0.00 | 0.02 | 43 | - | 157 |
| | Photometric + Visual | 8 | 200 | 9 | 0.05 | 0.02 | 0.08 | 182 | - | 9 |
| | | 10 | 200 | 191 | 0.96 | 0.92 | 0.98 | 9 | - | 0 |
| | | 12 | 200 | 200 | 1.00 | 0.98 | 1.00 | 0 | - | 0 |
| | | 15 | 200 | 200 | 1.00 | 0.98 | 1.00 | 0 | - | 0 |
| | | 100 | 48 | 0 | 0.00 | - | - | 0 | 0.00 | 48 |
| | | 150 | 48 | 0 | 0.00 | - | - | 0 | 0.00 | 48 |
| | | 200 | 48 | 0 | 0.00 | - | - | 0 | 0.00 | 48 |
| | Photometric | 250 | 48 | 0 | 0.00 | - | - | 0 | 0.00 | 48 |
| | | 300 | 48 | 0 | 0.00 | - | - | 48 | 1.00 | 0 |
| | | 400 | 48 | 48 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 500 | 48 | 48 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 100 | 72 | 0 | 0.00 | - | - | 1 | 0.01 | 71 |
| | | 150 | 72 | 0 | 0.00 | - | - | 1 | 0.01 | 71 |
| | | 200 | 72 | 0 | 0.00 | - | - | 7 | 0.10 | 65 |
| **Cefalexin** | Visual | 250 | 72 | 0 | 0.00 | - | - | 10 | 0.14 | 62 |
| | | 300 | 72 | 0 | 0.00 | - | - | 39 | 0.54 | 33 |
| | | 400 | 72 | 42 | 0.58 | - | - | 30 | 1.00 | 0 |
| | | 500 | 72 | 72 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 100 | 120 | 0 | 0.00 | 0.00 | 0.03 | 1 | - | 119 |
| | | 150 | 120 | 0 | 0.00 | 0.00 | 0.03 | 1 | - | 119 |
| | | 200 | 120 | 0 | 0.00 | 0.00 | 0.03 | 7 | - | 113 |
| | Photometric + Visual | 250 | 120 | 0 | 0.00 | 0.00 | 0.03 | 10 | - | 110 |
| | | 300 | 120 | 0 | 0.00 | 0.00 | 0.03 | 87 | - | 33 |
| | | 400 | 120 | 90 | 0.75 | 0.24 | 1.00 | 30 | - | 0 |
| | | 500 | 120 | 120 | 1.00 | 0.97 | 1.00 | 0 | - | 0 |

## Continuation Annex Table 2

| Substance | Reading System | Concentration | No. of total Results | No. of Results (pos. Class A) | Percentage of CCß A | Lower 95%-CI (CCß A) | Upper 95%-CI (CCß A) | No. of Results (pos. Class B) | Percentage of CCß B | No. of Results (negative) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 2 | 42 | 0 | 0.00 | - | - | 0 | 0.00 | 42 |
| | | 3 | 42 | 0 | 0.00 | - | - | 0 | 0.00 | 42 |
| | | 4 | 42 | 0 | 0.00 | - | - | 0 | 0.00 | 42 |
| | Photometric | 5 | 42 | 0 | 0.00 | - | - | 42 | 1.00 | 0 |
| | | 6 | 42 | 42 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 8 | 42 | 42 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 10 | 42 | 42 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 2 | 63 | 0 | 0.00 | - | - | 1 | 0.02 | 62 |
| | | 3 | 63 | 0 | 0.00 | - | - | 9 | 0.14 | 54 |
| | | 4 | 63 | 0 | 0.00 | - | - | 9 | 0.14 | 54 |
| **Cefapirin** | Visual | 5 | 63 | 14 | 0.22 | - | - | 49 | 1.00 | 0 |
| | | 6 | 63 | 56 | 0.89 | - | - | 7 | 1.00 | 0 |
| | | 8 | 63 | 63 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 10 | 63 | 63 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 2 | 105 | 0 | 0.00 | 0.00 | 0.04 | 1 | - | 104 |
| | | 3 | 105 | 0 | 0.00 | 0.00 | 0.04 | 9 | - | 96 |
| | | 4 | 105 | 0 | 0.00 | 0.00 | 0.04 | 9 | - | 96 |
| | Photometric + Visual | 5 | 105 | 14 | 0.13 | 0.08 | 0.21 | 91 | - | 0 |
| | | 6 | 105 | 98 | 0.93 | 0.87 | 0.97 | 7 | - | 0 |
| | | 8 | 105 | 105 | 1.00 | 0.97 | 1.00 | 0 | - | 0 |
| | | 10 | 105 | 105 | 1.00 | 0.97 | 1.00 | 0 | - | 0 |
| | | 10 | 80 | 0 | 0.00 | - | - | 0 | 0.00 | 80 |
| | | 15 | 80 | 0 | 0.00 | - | - | 0 | 0.00 | 80 |
| | | 20 | 80 | 0 | 0.00 | - | - | 11 | 0.14 | 69 |
| | Photometric | 25 | 80 | 0 | 0.00 | - | - | 80 | 1.00 | 0 |
| | | 30 | 80 | 52 | 0.65 | - | - | 28 | 1.00 | 0 |
| | | 35 | 80 | 80 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 40 | 80 | 80 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 10 | 120 | 0 | 0.00 | - | - | 5 | 0.04 | 115 |
| | | 15 | 120 | 0 | 0.00 | - | - | 16 | 0.13 | 104 |
| | | 20 | 120 | 1 | 0.01 | - | - | 74 | 0.63 | 45 |
| **Cefoperazone** | Visual | 25 | 120 | 35 | 0.29 | - | - | 83 | 0.98 | 2 |
| | | 30 | 120 | 89 | 0.74 | - | - | 31 | 1.00 | 0 |
| | | 35 | 120 | 120 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 40 | 120 | 120 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 10 | 200 | 0 | 0.00 | 0.00 | 0.02 | 5 | - | 195 |
| | | 15 | 200 | 0 | 0.00 | 0.00 | 0.02 | 16 | - | 184 |
| | | 20 | 200 | 1 | 0.01 | 0.00 | 0.03 | 85 | - | 114 |
| | Photometric + Visual | 25 | 200 | 35 | 0.18 | 0.00 | 0.64 | 163 | - | 2 |
| | | 30 | 200 | 141 | 0.71 | 0.34 | 1.00 | 59 | - | 0 |
| | | 35 | 200 | 200 | 1.00 | 0.98 | 1.00 | 0 | - | 0 |
| | | 40 | 200 | 200 | 1.00 | 0.98 | 1.00 | 0 | - | 0 |
| | | 4 | 42 | 0 | 0.00 | - | - | 0 | 0.00 | 42 |
| | | 5 | 42 | 0 | 0.00 | - | - | 0 | 0.00 | 42 |
| | | 6 | 42 | 0 | 0.00 | - | - | 4 | 0.10 | 38 |
| | Photometric | 7 | 42 | 1 | 0.02 | - | - | 41 | 1.00 | 0 |
| | | 8 | 42 | 28 | 0.67 | - | - | 14 | 1.00 | 0 |
| | | 9 | 42 | 42 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 10 | 42 | 42 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 4 | 63 | 0 | 0.00 | - | - | 2 | 0.03 | 61 |
| | | 5 | 63 | 0 | 0.00 | - | - | 6 | 0.10 | 57 |
| | | 6 | 63 | 0 | 0.00 | - | - | 16 | 0.25 | 47 |
| **Cefazolin** | Visual | 7 | 63 | 4 | 0.06 | - | - | 58 | 0.98 | 1 |
| | | 8 | 63 | 35 | 0.56 | - | - | 28 | 1.00 | 0 |
| | | 9 | 63 | 63 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 10 | 63 | 63 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 4 | 105 | 0 | 0.00 | 0.00 | 0.04 | 2 | - | 103 |
| | | 5 | 105 | 0 | 0.00 | 0.00 | 0.04 | 6 | - | 99 |
| | | 6 | 105 | 0 | 0.00 | 0.00 | 0.04 | 20 | - | 85 |
| | Photometric + Visual | 7 | 105 | 5 | 0.05 | 0.02 | 0.11 | 99 | - | 1 |
| | | 8 | 105 | 63 | 0.60 | 0.16 | 1.00 | 42 | - | 0 |
| | | 9 | 105 | 105 | 1.00 | 0.97 | 1.00 | 0 | - | 0 |
| | | 10 | 105 | 105 | 1.00 | 0.97 | 1.00 | 0 | - | 0 |
| | | 100 | 42 | 0 | 0.00 | - | - | 0 | 0.00 | 42 |
| | | 200 | 42 | 0 | 0.00 | - | - | 7 | 0.17 | 35 |
| | | 300 | 42 | 0 | 0.00 | - | - | 42 | 1.00 | 0 |
| | Photometric | 400 | 42 | 14 | 0.33 | - | - | 28 | 1.00 | 0 |
| | | 500 | 42 | 42 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 600 | 42 | 42 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 700 | 42 | 42 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 100 | 63 | 0 | 0.00 | - | - | 5 | 0.08 | 58 |
| | | 200 | 63 | 0 | 0.00 | - | - | 40 | 0.63 | 23 |
| | | 300 | 63 | 8 | 0.13 | - | - | 55 | 1.00 | 0 |
| **Cefquinome** | Visual | 400 | 63 | 41 | 0.65 | - | - | 22 | 1.00 | 0 |
| | | 500 | 63 | 49 | 0.78 | - | - | 14 | 1.00 | 0 |
| | | 600 | 63 | 63 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 700 | 63 | 63 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 100 | 105 | 0 | 0.00 | 0.00 | 0.04 | 5 | - | 100 |
| | | 200 | 105 | 0 | 0.00 | 0.00 | 0.04 | 47 | - | 58 |
| | | 300 | 105 | 8 | 0.08 | 0.04 | 0.14 | 97 | - | 0 |
| | Photometric + Visual | 400 | 105 | 55 | 0.52 | 0.00 | 1.00 | 50 | - | 0 |
| | | 500 | 105 | 91 | 0.87 | 0.79 | 0.92 | 14 | - | 0 |
| | | 600 | 105 | 105 | 1.00 | 0.97 | 1.00 | 0 | - | 0 |
| | | 700 | 105 | 105 | 1.00 | 0.97 | 1.00 | 0 | - | 0 |

## Continuation Annex Table 2

| Substance | Reading System | Concentration | No. of total Results | No. of Results (pos. Class A) | Percentage of CCß A | Lower 95%-CI (CCß A) | Upper 95%-CI (CCß A) | No. of Results (pos. Class B) | Percentage of CCß B | No. of Results (negative) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 100 | 128 | 7 | 0.05 | - | - | 101 | 0.84 | 20 |
| | | 150 | 128 | 64 | 0.50 | - | - | 62 | 0.98 | 2 |
| | | 200 | 128 | 122 | 0.95 | - | - | 6 | 1.00 | 0 |
| | Photometric | 250 | 128 | 128 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 300 | 128 | 128 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 360 | 128 | 128 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 460 | 128 | 128 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 100 | 192 | 2 | 0.01 | - | - | 118 | 0.63 | 72 |
| | | 150 | 192 | 80 | 0.42 | - | - | 81 | 0.84 | 31 |
| | | 200 | 192 | 175 | 0.91 | - | - | 17 | 1.00 | 0 |
| **Ceftiofur** | Visual | 250 | 192 | 186 | 0.97 | - | - | 6 | 1.00 | 0 |
| | | 300 | 192 | 192 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 360 | 192 | 192 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 460 | 192 | 192 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 100 | 320 | 9 | 0.03 | 0.01 | 0.05 | 219 | - | 92 |
| | | 150 | 320 | 144 | 0.45 | 0.26 | 0.64 | 143 | - | 33 |
| | | 200 | 320 | 297 | 0.93 | 0.89 | 0.95 | 23 | - | 0 |
| | Photometric + Visual | 250 | 320 | 314 | 0.98 | 0.96 | 0.99 | 6 | - | 0 |
| | | 300 | 320 | 320 | 1.00 | 0.99 | 1.00 | 0 | - | 0 |
| | | 360 | 320 | 320 | 1.00 | 0.99 | 1.00 | 0 | - | 0 |
| | | 460 | 320 | 320 | 1.00 | 0.99 | 1.00 | 0 | - | 0 |
| | | 6 | 80 | 0 | 0.00 | - | - | 0 | 0.00 | 80 |
| | | 8 | 80 | 0 | 0.00 | - | - | 0 | 0.00 | 80 |
| | | 10 | 80 | 0 | 0.00 | - | - | 6 | 0.08 | 74 |
| | Photometric | 12 | 80 | 1 | 0.01 | - | - | 79 | 1.00 | 0 |
| | | 14 | 80 | 79 | 0.99 | - | - | 1 | 1.00 | 0 |
| | | 16 | 80 | 80 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 20 | 80 | 80 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 6 | 120 | 0 | 0.00 | - | - | 1 | 0.01 | 119 |
| | | 8 | 120 | 0 | 0.00 | - | - | 10 | 0.08 | 110 |
| | | 10 | 120 | 0 | 0.00 | - | - | 43 | 0.36 | 77 |
| **Cefalonium** | Visual | 12 | 120 | 25 | 0.21 | - | - | 93 | 0.98 | 2 |
| | | 14 | 120 | 103 | 0.86 | - | - | 17 | 1.00 | 0 |
| | | 16 | 120 | 120 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 20 | 120 | 120 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 6 | 200 | 0 | 0.00 | 0.00 | 0.02 | 1 | - | 199 |
| | | 8 | 200 | 0 | 0.00 | 0.00 | 0.02 | 10 | - | 190 |
| | | 10 | 200 | 0 | 0.00 | 0.00 | 0.02 | 49 | - | 151 |
| | Photometric + Visual | 12 | 200 | 26 | 0.13 | 0.09 | 0.18 | 172 | - | 2 |
| | | 14 | 200 | 182 | 0.91 | 0.86 | 0.94 | 18 | - | 0 |
| | | 16 | 200 | 200 | 1.00 | 0.98 | 1.00 | 0 | - | 0 |
| | | 20 | 200 | 200 | 1.00 | 0.98 | 1.00 | 0 | - | 0 |
| | | 30 | 128 | 0 | 0.00 | - | - | 32 | 0.25 | 96 |
| | | 40 | 128 | 0 | 0.00 | - | - | 93 | 0.73 | 35 |
| | | 50 | 128 | 5 | 0.04 | - | - | 123 | 1.00 | 0 |
| | Photometric | 60 | 128 | 13 | 0.10 | - | - | 115 | 1.00 | 0 |
| | | 80 | 128 | 119 | 0.93 | - | - | 9 | 1.00 | 0 |
| | | 100 | 128 | 128 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 120 | 128 | 128 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 30 | 192 | 2 | 0.01 | - | - | 30 | 0.17 | 160 |
| | | 40 | 192 | 6 | 0.03 | - | - | 142 | 0.77 | 44 |
| | | 50 | 192 | 22 | 0.11 | - | - | 154 | 0.92 | 16 |
| **Erythromycin** | Visual | 60 | 192 | 40 | 0.21 | - | - | 151 | 0.99 | 1 |
| | | 80 | 192 | 162 | 0.84 | - | - | 30 | 1.00 | 0 |
| | | 100 | 192 | 192 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 120 | 192 | 192 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 30 | 320 | 2 | 0.01 | 0.00 | 0.02 | 62 | - | 256 |
| | | 40 | 320 | 6 | 0.02 | 0.01 | 0.04 | 235 | - | 79 |
| | | 50 | 320 | 27 | 0.08 | 0.06 | 0.12 | 277 | - | 16 |
| | Photometric + Visual | 60 | 320 | 53 | 0.17 | 0.00 | 0.42 | 266 | - | 1 |
| | | 80 | 320 | 281 | 0.88 | 0.84 | 0.91 | 39 | - | 0 |
| | | 100 | 320 | 320 | 1.00 | 0.99 | 1.00 | 0 | - | 0 |
| | | 120 | 320 | 320 | 1.00 | 0.99 | 1.00 | 0 | - | 0 |
| | | 20 | 128 | 0 | 0.00 | - | - | 0 | 0.00 | 128 |
| | | 30 | 128 | 0 | 0.00 | - | - | 106 | 0.83 | 22 |
| | | 40 | 128 | 2 | 0.02 | - | - | 126 | 1.00 | 0 |
| | Photometric | 50 | 128 | 1 | 0.01 | - | - | 127 | 1.00 | 0 |
| | | 75 | 128 | 126 | 0.98 | - | - | 2 | 1.00 | 0 |
| | | 100 | 128 | 128 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 120 | 128 | 128 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 20 | 192 | 0 | 0.00 | - | - | 25 | 0.13 | 167 |
| | | 30 | 192 | 1 | 0.01 | - | - | 189 | 0.99 | 2 |
| | | 40 | 192 | 24 | 0.13 | - | - | 168 | 1.00 | 0 |
| **Tylosin** | Visual | 50 | 192 | 59 | 0.31 | - | - | 133 | 1.00 | 0 |
| | | 75 | 192 | 147 | 0.77 | - | - | 45 | 1.00 | 0 |
| | | 100 | 192 | 192 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 120 | 192 | 192 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 20 | 320 | 0 | 0.00 | 0.00 | 0.01 | 25 | - | 295 |
| | | 30 | 320 | 1 | 0.00 | 0.00 | 0.02 | 295 | - | 24 |
| | | 40 | 320 | 26 | 0.08 | 0.06 | 0.12 | 294 | - | 0 |
| | Photometric + Visual | 50 | 320 | 60 | 0.19 | 0.00 | 0.70 | 260 | - | 0 |
| | | 75 | 320 | 273 | 0.85 | 0.81 | 0.89 | 47 | - | 0 |
| | | 100 | 320 | 320 | 1.00 | 0.99 | 1.00 | 0 | - | 0 |
| | | 120 | 320 | 320 | 1.00 | 0.99 | 1.00 | 0 | - | 0 |

## Continuation Annex Table 2

| Substance | Reading System | Concentration | No. of total Results | No. of Results (pos. Class A) | Percentage of CCß A | Lower 95%-CI (CCß A) | Upper 95%-CI (CCß A) | No. of Results (pos. Class B) | Percentage of CCß B | No. of Results (negative) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 50 | 128 | 0 | 0.00 | - | - | 12 | 0.09 | 116 |
| | | 100 | 128 | 0 | 0.00 | - | - | 123 | 0.96 | 5 |
| | | 200 | 128 | 3 | 0.02 | - | - | 125 | 1.00 | 0 |
| | Photometric | 300 | 128 | 0 | 0.00 | - | - | 128 | 1.00 | 0 |
| | | 400 | 128 | 12 | 0.09 | - | - | 116 | 1.00 | 0 |
| | | 600 | 128 | 49 | 0.38 | - | - | 79 | 1.00 | 0 |
| | | 800 | 128 | 95 | 0.74 | - | - | 33 | 1.00 | 0 |
| | | 50 | 192 | 0 | 0.00 | - | - | 44 | 0.23 | 148 |
| | | 100 | 192 | 10 | 0.05 | - | - | 176 | 0.97 | 6 |
| | | 200 | 192 | 77 | 0.40 | - | - | 115 | 1.00 | 0 |
| **Sulfadiazine** | Visual | 300 | 192 | 109 | 0.57 | - | - | 83 | 1.00 | 0 |
| | | 400 | 192 | 128 | 0.67 | - | - | 64 | 1.00 | 0 |
| | | 600 | 192 | 128 | 0.67 | - | - | 64 | 1.00 | 0 |
| | | 800 | 192 | 130 | 0.68 | - | - | 62 | 1.00 | 0 |
| | | 50 | 320 | 0 | 0.00 | 0.00 | 0.01 | 56 | - | 264 |
| | | 100 | 320 | 10 | 0.03 | 0.02 | 0.06 | 299 | - | 11 |
| | | 200 | 320 | 80 | 0.25 | 0.00 | 0.65 | 240 | - | 0 |
| | Photometric + Visual | 300 | 320 | 109 | 0.34 | 0.00 | 0.92 | 211 | - | 0 |
| | | 400 | 320 | 140 | 0.44 | 0.00 | 1.00 | 180 | - | 0 |
| | | 600 | 320 | 177 | 0.55 | 0.01 | 1.00 | 143 | - | 0 |
| | | 800 | 320 | 225 | 0.70 | 0.21 | 1.00 | 95 | - | 0 |
| | | 50 | 128 | 0 | 0.00 | - | - | 8 | 0.06 | 120 |
| | | 100 | 128 | 0 | 0.00 | - | - | 116 | 0.91 | 12 |
| | | 200 | 128 | 0 | 0.00 | - | - | 128 | 1.00 | 0 |
| | Photometric | 300 | 128 | 13 | 0.10 | - | - | 115 | 1.00 | 0 |
| | | 400 | 128 | 27 | 0.21 | - | - | 101 | 1.00 | 0 |
| | | 600 | 128 | 73 | 0.57 | - | - | 55 | 1.00 | 0 |
| | | 800 | 128 | 102 | 0.80 | - | - | 26 | 1.00 | 0 |
| | | 50 | 192 | 0 | 0.00 | - | - | 53 | 0.28 | 139 |
| | | 100 | 192 | 4 | 0.02 | - | - | 173 | 0.92 | 15 |
| | | 200 | 192 | 68 | 0.35 | - | - | 122 | 0.99 | 2 |
| **Sulfadi-methoxin** | Visual | 300 | 192 | 126 | 0.66 | - | - | 66 | 1.00 | 0 |
| | | 400 | 192 | 128 | 0.67 | - | - | 64 | 1.00 | 0 |
| | | 600 | 192 | 127 | 0.66 | - | - | 65 | 1.00 | 0 |
| | | 800 | 192 | 128 | 0.67 | - | - | 64 | 1.00 | 0 |
| | | 50 | 320 | 0 | 0.00 | 0.00 | 0.01 | 61 | - | 259 |
| | | 100 | 320 | 4 | 0.01 | 0.00 | 0.03 | 289 | - | 27 |
| | | 200 | 320 | 68 | 0.21 | 0.00 | 0.58 | 250 | - | 2 |
| | Photometric + Visual | 300 | 320 | 139 | 0.43 | 0.00 | 1.00 | 181 | - | 0 |
| | | 400 | 320 | 155 | 0.48 | 0.00 | 1.00 | 165 | - | 0 |
| | | 600 | 320 | 200 | 0.63 | 0.11 | 1.00 | 120 | - | 0 |
| | | 800 | 320 | 230 | 0.72 | 0.20 | 1.00 | 90 | - | 0 |
| | | 50 | 42 | 0 | 0.00 | - | - | 0 | 0.00 | 42 |
| | | 100 | 42 | 0 | 0.00 | - | - | 8 | 0.19 | 34 |
| | | 200 | 42 | 0 | 0.00 | - | - | 39 | 0.93 | 3 |
| | Photometric | 300 | 42 | 0 | 0.00 | - | - | 42 | 1.00 | 0 |
| | | 500 | 42 | 5 | 0.12 | - | - | 37 | 1.00 | 0 |
| | | 750 | 42 | 14 | 0.33 | - | - | 28 | 1.00 | 0 |
| | | 1,000 | 42 | 41 | 0.98 | - | - | 1 | 1.00 | 0 |
| | | 50 | 63 | 0 | 0.00 | - | - | 6 | 0.10 | 57 |
| | | 100 | 63 | 0 | 0.00 | - | - | 54 | 0.86 | 9 |
| | | 200 | 63 | 7 | 0.11 | - | - | 55 | 0.98 | 1 |
| **Sulfametha-zine** | Visual | 300 | 63 | 16 | 0.25 | - | - | 47 | 1.00 | 0 |
| | | 500 | 63 | 44 | 0.70 | - | - | 19 | 1.00 | 0 |
| | | 750 | 63 | 42 | 0.67 | - | - | 21 | 1.00 | 0 |
| | | 1,000 | 63 | 42 | 0.67 | - | - | 21 | 1.00 | 0 |
| | | 50 | 105 | 0 | 0.00 | 0.00 | 0.04 | 6 | - | 99 |
| | | 100 | 105 | 0 | 0.00 | 0.00 | 0.04 | 62 | - | 43 |
| | | 200 | 105 | 7 | 0.07 | 0.03 | 0.13 | 94 | - | 4 |
| | Photometric + Visual | 300 | 105 | 16 | 0.15 | 0.00 | 0.47 | 89 | - | 0 |
| | | 500 | 105 | 49 | 0.47 | 0.00 | 1.00 | 56 | - | 0 |
| | | 750 | 105 | 56 | 0.53 | 0.00 | 1.00 | 49 | - | 0 |
| | | 1,000 | 105 | 83 | 0.79 | 0.24 | 1.00 | 22 | - | 0 |
| | | 40 | 128 | 0 | 0.00 | - | - | 86 | 0.67 | 42 |
| | | 60 | 128 | 0 | 0.00 | - | - | 126 | 0.98 | 2 |
| | | 80 | 128 | 1 | 0.01 | - | - | 127 | 1.00 | 0 |
| | Photometric | 100 | 128 | 5 | 0.04 | - | - | 123 | 1.00 | 0 |
| | | 200 | 128 | 106 | 0.83 | - | - | 22 | 1.00 | 0 |
| | | 400 | 128 | 126 | 0.98 | - | - | 2 | 1.00 | 0 |
| | | 600 | 128 | 125 | 0.98 | - | - | 3 | 1.00 | 0 |
| | | 40 | 192 | 1 | 0.01 | - | - | 147 | 0.77 | 44 |
| | | 60 | 192 | 13 | 0.07 | - | - | 178 | 0.99 | 1 |
| | | 80 | 192 | 35 | 0.18 | - | - | 157 | 1.00 | 0 |
| **Sulfathiazol** | Visual | 100 | 192 | 67 | 0.35 | - | - | 125 | 1.00 | 0 |
| | | 200 | 192 | 180 | 0.94 | - | - | 12 | 1.00 | 0 |
| | | 400 | 192 | 189 | 0.98 | - | - | 3 | 1.00 | 0 |
| | | 600 | 192 | 187 | 0.97 | - | - | 5 | 1.00 | 0 |
| | | 40 | 320 | 1 | 0.00 | 0.00 | 0.02 | 233 | - | 86 |
| | | 60 | 320 | 13 | 0.04 | 0.02 | 0.07 | 304 | - | 3 |
| | | 80 | 320 | 36 | 0.11 | 0.08 | 0.15 | 284 | - | 0 |
| | Photometric + Visual | 100 | 320 | 72 | 0.23 | 0.00 | 0.61 | 248 | - | 0 |
| | | 200 | 320 | 286 | 0.89 | 0.86 | 0.92 | 34 | - | 0 |
| | | 400 | 320 | 315 | 0.98 | 0.96 | 0.99 | 5 | - | 0 |
| | | 600 | 320 | 312 | 0.98 | 0.95 | 0.99 | 8 | - | 0 |

## Continuation Annex Table 2

| Substance | Reading System | Concentration | No. of total Results | No. of Results (pos. Class A) | Percentage of CCß A | Lower 95%-CI (CCß A) | Upper 95%-CI (CCß A) | No. of Results (pos. Class B) | Percentage of CCß B | No. of Results (negative) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 100 | 42 | 0 | 0.00 | - | - | 5 | 0.12 | 37 |
| | | 200 | 42 | 0 | 0.00 | - | - | 23 | 0.55 | 19 |
| | | 400 | 42 | 0 | 0.00 | - | - | 42 | 1.00 | 0 |
| | Photometric | 600 | 42 | 0 | 0.00 | - | - | 42 | 1.00 | 0 |
| | | 800 | 42 | 0 | 0.00 | - | - | 42 | 1.00 | 0 |
| | | 1,000 | 42 | 9 | 0.21 | - | - | 33 | 1.00 | 0 |
| | | 1,500 | 42 | 25 | 0.60 | - | - | 17 | 1.00 | 0 |
| | | 100 | 63 | 0 | 0.00 | - | - | 41 | 0.65 | 22 |
| | | 200 | 63 | 1 | 0.02 | - | - | 56 | 0.90 | 6 |
| | | 400 | 63 | 7 | 0.11 | - | - | 56 | 1.00 | 0 |
| **Sulfadoxin** | Visual | 600 | 63 | 33 | 0.52 | - | - | 30 | 1.00 | 0 |
| | | 800 | 63 | 41 | 0.65 | - | - | 22 | 1.00 | 0 |
| | | 1,000 | 63 | 40 | 0.63 | - | - | 23 | 1.00 | 0 |
| | | 1,500 | 63 | 41 | 0.65 | - | - | 22 | 1.00 | 0 |
| | | 100 | 105 | 0 | 0.00 | 0.00 | 0.04 | 46 | - | 59 |
| | | 200 | 105 | 1 | 0.01 | 0.00 | 0.05 | 79 | - | 25 |
| | | 400 | 105 | 7 | 0.07 | 0.03 | 0.13 | 98 | - | 0 |
| | Photometric + Visual | 600 | 105 | 33 | 0.31 | 0.00 | 0.85 | 72 | - | 0 |
| | | 800 | 105 | 41 | 0.39 | 0.00 | 1.00 | 64 | - | 0 |
| | | 1,000 | 105 | 49 | 0.47 | 0.00 | 1.00 | 56 | - | 0 |
| | | 1,500 | 105 | 66 | 0.63 | 0.14 | 1.00 | 39 | - | 0 |
| | | 50 | 128 | 1 | 0.01 | - | - | 99 | 0.78 | 28 |
| | | 100 | 128 | 0 | 0.00 | - | - | 126 | 0.98 | 2 |
| | | 200 | 128 | 24 | 0.19 | - | - | 104 | 1.00 | 0 |
| | Photometric | 300 | 128 | 107 | 0.84 | - | - | 21 | 1.00 | 0 |
| | | 500 | 128 | 128 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 750 | 128 | 127 | 0.99 | - | - | 0 | 0.99 | 1 |
| | | 1,000 | 128 | 127 | 0.99 | - | - | 1 | 1.00 | 0 |
| | | 50 | 192 | 2 | 0.01 | - | - | 119 | 0.63 | 71 |
| | | 100 | 192 | 9 | 0.05 | - | - | 177 | 0.97 | 6 |
| | | 200 | 192 | 107 | 0.56 | - | - | 85 | 1.00 | 0 |
| **Sulfamethoxy-pyridazine** | Visual | 300 | 192 | 176 | 0.92 | - | - | 16 | 1.00 | 0 |
| | | 500 | 192 | 192 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 750 | 192 | 191 | 0.99 | - | - | 1 | 1.00 | 0 |
| | | 1,000 | 192 | 192 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 50 | 320 | 3 | 0.01 | 0.00 | 0.03 | 218 | - | 99 |
| | | 100 | 320 | 9 | 0.03 | 0.01 | 0.05 | 303 | - | 8 |
| | | 200 | 320 | 131 | 0.41 | 0.01 | 0.80 | 189 | - | 0 |
| | Photometric + Visual | 300 | 320 | 283 | 0.88 | 0.84 | 0.92 | 37 | - | 0 |
| | | 500 | 320 | 320 | 1.00 | 0.99 | 1.00 | 0 | - | 0 |
| | | 750 | 320 | 318 | 0.99 | 0.98 | 1.00 | 1 | - | 1 |
| | | 1,000 | 320 | 319 | 1.00 | 0.98 | 1.00 | 1 | - | 0 |
| | | 100 | 42 | 0 | 0.00 | - | - | 0 | 0.00 | 42 |
| | | 200 | 42 | 0 | 0.00 | - | - | 0 | 0.00 | 42 |
| | | 300 | 42 | 0 | 0.00 | - | - | 4 | 0.10 | 38 |
| | Photometric | 400 | 42 | 0 | 0.00 | - | - | 17 | 0.40 | 25 |
| | | 600 | 42 | 0 | 0.00 | - | - | 32 | 0.76 | 10 |
| | | 800 | 42 | 2 | 0.05 | - | - | 38 | 0.95 | 2 |
| | | 1,000 | 42 | 28 | 0.67 | - | - | 14 | 1.00 | 0 |
| | | 100 | 63 | 0 | 0.00 | - | - | 12 | 0.19 | 51 |
| | | 200 | 63 | 0 | 0.00 | - | - | 12 | 0.19 | 51 |
| | | 300 | 63 | 0 | 0.00 | - | - | 23 | 0.37 | 40 |
| **Chlortetra-cycline** | Visual | 400 | 63 | 0 | 0.00 | - | - | 36 | 0.57 | 27 |
| | | 600 | 63 | 25 | 0.40 | - | - | 31 | 0.89 | 7 |
| | | 800 | 63 | 27 | 0.43 | - | - | 36 | 1.00 | 0 |
| | | 1,000 | 63 | 49 | 0.78 | - | - | 14 | 1.00 | 0 |
| | | 100 | 105 | 0 | 0.00 | 0.00 | 0.04 | 12 | - | 93 |
| | | 200 | 105 | 0 | 0.00 | 0.00 | 0.04 | 12 | - | 93 |
| | | 300 | 105 | 0 | 0.00 | 0.00 | 0.04 | 27 | - | 78 |
| | Photometric + Visual | 400 | 105 | 0 | 0.00 | 0.00 | 0.04 | 53 | - | 52 |
| | | 600 | 105 | 25 | 0.24 | 0.00 | 0.63 | 63 | - | 17 |
| | | 800 | 105 | 29 | 0.28 | 0.00 | 0.68 | 74 | - | 2 |
| | | 1,000 | 105 | 77 | 0.73 | 0.41 | 1.00 | 28 | - | 0 |
| | | 100 | 42 | 0 | 0.00 | - | - | 0 | 0.00 | 42 |
| | | 200 | 42 | 0 | 0.00 | - | - | 9 | 0.21 | 33 |
| | | 300 | 42 | 1 | 0.02 | - | - | 28 | 0.69 | 13 |
| | Photometric | 400 | 42 | 0 | 0.00 | - | - | 42 | 1.00 | 0 |
| | | 600 | 42 | 29 | 0.69 | - | - | 13 | 1.00 | 0 |
| | | 800 | 42 | 42 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 1,000 | 42 | 42 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 100 | 63 | 0 | 0.00 | - | - | 3 | 0.05 | 60 |
| | | 200 | 63 | 0 | 0.00 | - | - | 34 | 0.54 | 29 |
| | | 300 | 63 | 3 | 0.05 | - | - | 51 | 0.86 | 9 |
| **Oxytetra-cycline** | Visual | 400 | 63 | 13 | 0.21 | - | - | 50 | 1.00 | 0 |
| | | 600 | 63 | 52 | 0.83 | - | - | 11 | 1.00 | 0 |
| | | 800 | 63 | 63 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 1,000 | 63 | 63 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 100 | 105 | 0 | 0.00 | 0.00 | 0.04 | 3 | - | 102 |
| | | 200 | 105 | 0 | 0.00 | 0.00 | 0.04 | 43 | - | 62 |
| | | 300 | 105 | 4 | 0.04 | 0.01 | 0.09 | 79 | - | 22 |
| | Photometric + Visual | 400 | 105 | 13 | 0.12 | 0.07 | 0.20 | 92 | - | 0 |
| | | 600 | 105 | 81 | 0.77 | 0.64 | 0.90 | 24 | - | 0 |
| | | 800 | 105 | 105 | 1.00 | 0.97 | 1.00 | 0 | - | 0 |
| | | 1,000 | 105 | 105 | 1.00 | 0.97 | 1.00 | 0 | - | 0 |

## Continuation Annex Table 2

| Substance | Reading System | Concentration | No. of total Results | No. of Results (pos. Class A) | Percentage of CCß A | Lower 95%-CI (CCß A) | Upper 95%-CI (CCß A) | No. of Results (pos. Class B) | Percentage of CCß B | No. of Results (negative) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 100 | 42 | 0 | 0.00 | - | - | 0 | 0.00 | 42 |
| | | 200 | 42 | 0 | 0.00 | - | - | 0 | 0.00 | 42 |
| | | 300 | 42 | 0 | 0.00 | - | - | 5 | 0.12 | 37 |
| | Photometric | 400 | 42 | 0 | 0.00 | - | - | 15 | 0.36 | 27 |
| | | 600 | 42 | 0 | 0.00 | - | - | 42 | 1.00 | 0 |
| | | 800 | 42 | 21 | 0.50 | - | - | 21 | 1.00 | 0 |
| | | 1,000 | 42 | 42 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 100 | 63 | 0 | 0.00 | - | - | 6 | 0.10 | 57 |
| | | 200 | 63 | 0 | 0.00 | - | - | 16 | 0.25 | 47 |
| | | 300 | 63 | 0 | 0.00 | - | - | 39 | 0.62 | 24 |
| **Tetracycline** | Visual | 400 | 63 | 0 | 0.00 | - | - | 49 | 0.78 | 14 |
| | | 600 | 63 | 29 | 0.46 | - | - | 34 | 1.00 | 0 |
| | | 800 | 63 | 49 | 0.78 | - | - | 14 | 1.00 | 0 |
| | | 1,000 | 63 | 63 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 100 | 105 | 0 | 0.00 | 0.00 | 0.04 | 6 | - | 99 |
| | | 200 | 105 | 0 | 0.00 | 0.00 | 0.04 | 16 | - | 89 |
| | | 300 | 105 | 0 | 0.00 | 0.00 | 0.04 | 44 | - | 61 |
| | Photometric + Visual | 400 | 105 | 0 | 0.00 | 0.00 | 0.04 | 64 | - | 41 |
| | | 600 | 105 | 29 | 0.28 | 0.00 | 0.76 | 76 | - | 0 |
| | | 800 | 105 | 70 | 0.67 | 0.27 | 1.00 | 35 | - | 0 |
| | | 1,000 | 105 | 105 | 1.00 | 0.97 | 1.00 | 0 | - | 0 |
| | | 200 | 42 | 0 | 0.00 | - | - | 1 | 0.02 | 41 |
| | | 300 | 42 | 0 | 0.00 | - | - | 11 | 0.26 | 31 |
| | | 400 | 42 | 7 | 0.17 | - | - | 35 | 1.00 | 0 |
| | Photometric | 500 | 42 | 13 | 0.31 | - | - | 29 | 1.00 | 0 |
| | | 600 | 42 | 42 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 700 | 42 | 42 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 800 | 42 | 42 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 200 | 63 | 0 | 0.00 | - | - | 26 | 0.41 | 37 |
| | | 300 | 63 | 0 | 0.00 | - | - | 55 | 0.87 | 8 |
| | | 400 | 63 | 33 | 0.52 | - | - | 30 | 1.00 | 0 |
| **Dihydro-streptomycin** | Visual | 500 | 63 | 63 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 600 | 63 | 63 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 700 | 63 | 63 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 800 | 63 | 63 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 200 | 105 | 0 | 0.00 | 0.00 | 0.04 | 27 | - | 78 |
| | | 300 | 105 | 0 | 0.00 | 0.00 | 0.04 | 66 | - | 39 |
| | | 400 | 105 | 40 | 0.38 | 0.00 | 0.81 | 65 | - | 0 |
| | Photometric + Visual | 500 | 105 | 76 | 0.72 | 0.26 | 1.00 | 29 | - | 0 |
| | | 600 | 105 | 105 | 1.00 | 0.97 | 1.00 | 0 | - | 0 |
| | | 700 | 105 | 105 | 1.00 | 0.97 | 1.00 | 0 | - | 0 |
| | | 800 | 105 | 105 | 1.00 | 0.97 | 1.00 | 0 | - | 0 |
| | | 300 | 42 | 0 | 0.00 | - | - | 0 | 0.00 | 42 |
| | | 400 | 42 | 0 | 0.00 | - | - | 2 | 0.05 | 40 |
| | | 500 | 42 | 0 | 0.00 | - | - | 30 | 0.71 | 12 |
| | Photometric | 600 | 42 | 0 | 0.00 | - | - | 42 | 1.00 | 0 |
| | | 800 | 42 | 2 | 0.05 | - | - | 40 | 1.00 | 0 |
| | | 1,000 | 42 | 36 | 0.86 | - | - | 6 | 1.00 | 0 |
| | | 1,500 | 42 | 42 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 300 | 63 | 1 | 0.02 | - | - | 7 | 0.13 | 55 |
| | | 400 | 63 | 0 | 0.00 | - | - | 21 | 0.33 | 42 |
| | | 500 | 63 | 4 | 0.06 | - | - | 54 | 0.92 | 5 |
| **Streptomycin** | Visual | 600 | 63 | 0 | 0.00 | - | - | 63 | 1.00 | 0 |
| | | 800 | 63 | 22 | 0.35 | - | - | 41 | 1.00 | 0 |
| | | 1,000 | 63 | 44 | 0.70 | - | - | 19 | 1.00 | 0 |
| | | 1,500 | 63 | 63 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 300 | 105 | 1 | 0.01 | 0.00 | 0.05 | 7 | - | 97 |
| | | 400 | 105 | 0 | 0.00 | 0.00 | 0.04 | 23 | - | 82 |
| | | 500 | 105 | 4 | 0.04 | 0.01 | 0.09 | 84 | - | 17 |
| | Photometric + Visual | 600 | 105 | 0 | 0.00 | 0.00 | 0.04 | 105 | - | 0 |
| | | 800 | 105 | 24 | 0.23 | 0.00 | 0.64 | 81 | - | 0 |
| | | 1,000 | 105 | 80 | 0.76 | 0.28 | 1.00 | 25 | - | 0 |
| | | 1,500 | 105 | 105 | 1.00 | 0.97 | 1.00 | 0 | - | 0 |
| | | 40 | 128 | 0 | 0.00 | - | - | 1 | 0.01 | 127 |
| | | 60 | 128 | 0 | 0.00 | - | - | 37 | 0.29 | 91 |
| | | 80 | 128 | 0 | 0.00 | - | - | 121 | 0.95 | 7 |
| | Photometric | 100 | 128 | 0 | 0.00 | - | - | 128 | 1.00 | 0 |
| | | 125 | 128 | 8 | 0.06 | - | - | 120 | 1.00 | 0 |
| | | 150 | 128 | 118 | 0.92 | - | - | 10 | 1.00 | 0 |
| | | 200 | 128 | 128 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 40 | 192 | 0 | 0.00 | - | - | 31 | 0.16 | 161 |
| | | 60 | 192 | 0 | 0.00 | - | - | 159 | 0.83 | 33 |
| | | 80 | 192 | 19 | 0.10 | - | - | 173 | 1.00 | 0 |
| **Gentamicin** | Visual | 100 | 192 | 79 | 0.41 | - | - | 113 | 1.00 | 0 |
| | | 125 | 192 | 141 | 0.73 | - | - | 51 | 1.00 | 0 |
| | | 150 | 192 | 192 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 200 | 192 | 192 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 40 | 320 | 0 | 0.00 | 0.00 | 0.01 | 32 | - | 288 |
| | | 60 | 320 | 0 | 0.00 | 0.00 | 0.01 | 196 | - | 124 |
| | | 80 | 320 | 19 | 0.06 | 0.04 | 0.09 | 294 | - | 7 |
| | Photometric + Visual | 100 | 320 | 79 | 0.25 | 0.00 | 0.74 | 241 | - | 0 |
| | | 125 | 320 | 149 | 0.47 | 0.00 | 1.00 | 171 | - | 0 |
| | | 150 | 320 | 310 | 0.97 | 0.94 | 0.98 | 10 | - | 0 |
| | | 200 | 320 | 320 | 1.00 | 0.99 | 1.00 | 0 | - | 0 |

## Continuation Annex Table 2

| Substance | Reading System | Concentration | No. of total Results | No. of Results (pos. Class A) | Percentage of CCß A | Lower 95%-CI (CCß A) | Upper 95%-CI (CCß A) | No. of Results (pos. Class B) | Percentage of CCß B | No. of Results (negative) |
|---|---|---|---|---|---|---|---|---|---|---|
| **Neomycin** | Photometric | 60 | 42 | 0 | 0.00 | - | - | 0 | 0.00 | 42 |
| | | 80 | 42 | 0 | 0.00 | - | - | 2 | 0.05 | 40 |
| | | 100 | 42 | 0 | 0.00 | - | - | 1 | 0.02 | 41 |
| | | 200 | 42 | 0 | 0.00 | - | - | 42 | 1.00 | 0 |
| | | 300 | 42 | 32 | 0.76 | - | - | 10 | 1.00 | 0 |
| | | 400 | 42 | 42 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 500 | 42 | 42 | 1.00 | - | - | 0 | 1.00 | 0 |
| | Visual | 60 | 63 | 0 | 0.00 | - | - | 9 | 0.14 | 54 |
| | | 80 | 63 | 0 | 0.00 | - | - | 34 | 0.54 | 29 |
| | | 100 | 63 | 0 | 0.00 | - | - | 24 | 0.38 | 39 |
| | | 200 | 63 | 15 | 0.24 | - | - | 48 | 1.00 | 0 |
| | | 300 | 63 | 63 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 400 | 63 | 63 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 500 | 63 | 63 | 1.00 | - | - | 0 | 1.00 | 0 |
| | Photometric + Visual | 60 | 105 | 0 | 0.00 | 0.00 | 0.04 | 9 | - | 96 |
| | | 80 | 105 | 0 | 0.00 | 0.00 | 0.04 | 36 | - | 69 |
| | | 100 | 105 | 0 | 0.00 | 0.00 | 0.04 | 25 | - | 80 |
| | | 200 | 105 | 15 | 0.14 | 0.09 | 0.22 | 90 | - | 0 |
| | | 300 | 105 | 95 | 0.90 | 0.83 | 0.95 | 10 | - | 0 |
| | | 400 | 105 | 105 | 1.00 | 0.97 | 1.00 | 0 | - | 0 |
| | | 500 | 105 | 105 | 1.00 | 0.97 | 1.00 | 0 | - | 0 |
| **Chlor-amphenicol** | Photometric | 3,000 | 42 | 0 | 0.00 | - | - | 17 | 0.40 | 25 |
| | | 3,500 | 42 | 0 | 0.00 | - | - | 29 | 0.69 | 13 |
| | | 4,000 | 42 | 0 | 0.00 | - | - | 42 | 1.00 | 0 |
| | | 4,500 | 42 | 2 | 0.05 | - | - | 40 | 1.00 | 0 |
| | | 5,000 | 42 | 14 | 0.33 | - | - | 28 | 1.00 | 0 |
| | | 6,000 | 42 | 39 | 0.93 | - | - | 3 | 1.00 | 0 |
| | | 7,000 | 42 | 42 | 1.00 | - | - | 0 | 1.00 | 0 |
| | Visual | 3,000 | 63 | 1 | 0.02 | - | - | 37 | 0.60 | 25 |
| | | 3,500 | 63 | 3 | 0.05 | - | - | 60 | 1.00 | 0 |
| | | 4,000 | 63 | 20 | 0.32 | - | - | 43 | 1.00 | 0 |
| | | 4,500 | 63 | 46 | 0.73 | - | - | 17 | 1.00 | 0 |
| | | 5,000 | 63 | 63 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 6,000 | 63 | 63 | 1.00 | - | - | 0 | 1.00 | 0 |
| | | 7,000 | 63 | 63 | 1.00 | - | - | 0 | 1.00 | 0 |
| | Photometric + Visual | 3,000 | 105 | 1 | 0.01 | 0.00 | 0.05 | 54 | - | 50 |
| | | 3,500 | 105 | 3 | 0.03 | 0.01 | 0.08 | 89 | - | 13 |
| | | 4,000 | 105 | 20 | 0.19 | 0.00 | 0.59 | 85 | - | 0 |
| | | 4,500 | 105 | 48 | 0.46 | 0.00 | 0.96 | 57 | - | 0 |
| | | 5,000 | 105 | 77 | 0.73 | 0.29 | 1.00 | 28 | - | 0 |
| | | 6,000 | 105 | 102 | 0.97 | 0.92 | 0.99 | 3 | - | 0 |
| | | 7,000 | 105 | 105 | 1.00 | 0.97 | 1.00 | 0 | - | 0 |

Annex Table 3. Contingency table created with the Fisher Test for the concentration at CCß A obtained with photometric reading, including the numbers of results of the different classes of results (1-2-0) for the different plate batches and ELISA readers

| Substance | Concentration CCß A | No. of ELISA Reader | Batch | No. of Results (pos. Class A) | No. of Results (pos. Class B) | No. of Results (negative) | p-Value Fisher´s Exact Test (CCß A) |
|---|---|---|---|---|---|---|---|
| **Benzyl-penicillin** | 2.5 | 1 | A | 16 | 0 | 0 | |
| | | 1 | B | 16 | 0 | 0 | 1 |
| | | 1 | C | 8 | 0 | 0 | |
| | | 2 | A | 16 | 0 | 0 | |
| | | 2 | B | 16 | 0 | 0 | 1 |
| | | 2 | C | 8 | 0 | 0 | |
| **Ampicillin** | 3.5 | 1 | A | 8 | 0 | 0 | |
| | | 1 | B | 16 | 0 | 0 | 1 |
| | | 1 | C | 16 | 0 | 0 | |
| | | 2 | A | 8 | 0 | 0 | |
| | | 2 | B | 16 | 0 | 0 | 1 |
| | | 2 | C | 16 | 0 | 0 | |
| **Amoxicillin** | 3 | 1 | D | 24 | 0 | 0 | |
| | | 1 | E | 16 | 0 | 0 | 1 |
| | | 1 | F | 24 | 0 | 0 | |
| | | 2 | D | 24 | 0 | 0 | |
| | | 2 | E | 16 | 0 | 0 | 1 |
| | | 2 | F | 24 | 0 | 0 | |
| **Cloxacillin** | 25 | 1 | D | 16 | 0 | 0 | |
| | | 1 | E | 22 | 2 | 0 | 0.33 |
| | | 1 | F | 24 | 0 | 0 | |
| | | 2 | D | 16 | 0 | 0 | |
| | | 2 | E | 24 | 0 | 0 | 1 |
| | | 2 | F | 24 | 0 | 0 | |
| **Dicloxacillin** | 15 | 1 | A | 16 | 0 | 0 | |
| | | 1 | B | 16 | 0 | 0 | 1 |
| | | 1 | C | 8 | 0 | 0 | |
| | | 2 | A | 16 | 0 | 0 | |
| | | 2 | B | 16 | 0 | 0 | 1 |
| | | 2 | C | 8 | 0 | 0 | |
| **Nafcillin** | 15 | 1 | A | 16 | 0 | 0 | |
| | | 1 | B | 8 | 0 | 0 | 1 |
| | | 1 | C | 16 | 0 | 0 | |
| | | 2 | A | 16 | 0 | 0 | |
| | | 2 | B | 8 | 0 | 0 | 1 |
| | | 2 | C | 16 | 0 | 0 | |
| **Oxacillin** | 10 | 1 | A | 16 | 0 | 0 | |
| | | 1 | B | 7 | 1 | 0 | 0.2 |
| | | 1 | C | 16 | 0 | 0 | |
| | | 2 | A | 16 | 0 | 0 | |
| | | 2 | B | 8 | 0 | 0 | 1 |
| | | 2 | C | 16 | 0 | 0 | |
| **Cefalexin** | 400 | 1 | A | 8 | 0 | 0 | |
| | | 1 | B | 8 | 0 | 0 | 1 |
| | | 1 | C | 8 | 0 | 0 | |
| | | 2 | A | 8 | 0 | 0 | |
| | | 2 | B | 8 | 0 | 0 | 1 |
| | | 2 | C | 8 | 0 | 0 | |
| **Cefapirin** | 6 | 1 | A | 7 | 0 | 0 | |
| | | 1 | B | 7 | 0 | 0 | 1 |
| | | 1 | C | 7 | 0 | 0 | |
| | | 2 | A | 7 | 0 | 0 | |
| | | 2 | B | 7 | 0 | 0 | 1 |
| | | 2 | C | 7 | 0 | 0 | |
| **Cefoperazone** | 35 | 1 | A | 16 | 0 | 0 | |
| | | 1 | B | 8 | 0 | 0 | 1 |
| | | 1 | C | 16 | 0 | 0 | |
| | | 2 | A | 16 | 0 | 0 | |
| | | 2 | B | 8 | 0 | 0 | 1 |
| | | 2 | C | 16 | 0 | 0 | |

Continuation Annex Table 3

| Substance | Concentration CCß A | No. of ELISA Reader | Batch | No. of Results (pos. Class A) | No. of Results (pos. Class B) | No. of Results (negative) | p-Value Fisher´s Exact Test (CCß A) |
|---|---|---|---|---|---|---|---|
| **Cefazolin** | 9 | 1 | A | 7 | 0 | 0 | |
| | | 1 | B | 7 | 0 | 0 | 1 |
| | | 1 | C | 7 | 0 | 0 | |
| | | 2 | A | 7 | 0 | 0 | |
| | | 2 | B | 7 | 0 | 0 | 1 |
| | | 2 | C | 7 | 0 | 0 | |
| **Cefquinome** | 500 | 1 | A | 7 | 0 | 0 | |
| | | 1 | B | 7 | 0 | 0 | 1 |
| | | 1 | C | 7 | 0 | 0 | |
| | | 2 | A | 7 | 0 | 0 | |
| | | 2 | B | 7 | 0 | 0 | 1 |
| | | 2 | C | 7 | 0 | 0 | |
| **Ceftiofur** | 200 | 1 | D | 21 | 3 | 0 | |
| | | 1 | E | 24 | 0 | 0 | 0.11 |
| | | 1 | F | 16 | 0 | 0 | |
| | | 2 | D | 21 | 3 | 0 | |
| | | 2 | E | 24 | 0 | 0 | 0.11 |
| | | 2 | F | 16 | 0 | 0 | |
| **Cefalonium** | 14 | 1 | A | 8 | 0 | 0 | |
| | | 1 | B | 16 | 0 | 0 | 1 |
| | | 1 | C | 15 | 1 | 0 | |
| | | 2 | A | 8 | 0 | 0 | |
| | | 2 | B | 16 | 0 | 0 | 1 |
| | | 2 | C | 16 | 0 | 0 | |
| **Erythromycin** | 100 | 1 | A | 24 | 0 | 0 | |
| | | 1 | B | 16 | 0 | 0 | 1 |
| | | 1 | C | 24 | 0 | 0 | |
| | | 2 | A | 24 | 0 | 0 | |
| | | 2 | B | 16 | 0 | 0 | 1 |
| | | 2 | C | 24 | 0 | 0 | |
| **Tylosin** | 75 | 1 | A | 16 | 0 | 0 | |
| | | 1 | B | 24 | 0 | 0 | 1 |
| | | 1 | C | 23 | 1 | 0 | |
| | | 2 | A | 16 | 0 | 0 | |
| | | 2 | B | 24 | 0 | 0 | 1 |
| | | 2 | C | 23 | 1 | 0 | |
| **Sulfadiazine** | NA | 1 | A | 22 | 2 | 0 | |
| | | 1 | B | 18 | 6 | 0 | - |
| | | 1 | C | 5 | 11 | 0 | |
| | | 2 | A | 23 | 1 | 0 | |
| | | 2 | B | 20 | 4 | 0 | - |
| | | 2 | C | 7 | 9 | 0 | |
| **Sulfadi-methoxin** | NA | 1 | A | 8 | 16 | 0 | |
| | | 1 | B | 16 | 0 | 0 | - |
| | | 1 | C | 23 | 1 | 0 | |
| | | 2 | A | 15 | 9 | 0 | |
| | | 2 | B | 16 | 0 | 0 | - |
| | | 2 | C | 24 | 0 | 0 | |
| **Sulfamethazine** | 1,000 | 1 | A | 7 | 0 | 0 | |
| | | 1 | B | 6 | 1 | 0 | 1 |
| | | 1 | C | 7 | 0 | 0 | |
| | | 2 | A | 7 | 0 | 0 | |
| | | 2 | B | 7 | 0 | 0 | 1 |
| | | 2 | C | 7 | 0 | 0 | |
| **Sulfathiazol** | 400 | 1 | A | 16 | 0 | 0 | |
| | | 1 | B | 24 | 0 | 0 | 1 |
| | | 1 | C | 24 | 0 | 0 | |
| | | 2 | A | 14 | 2 | 0 | |
| | | 2 | B | 24 | 0 | 0 | 0.06 |
| | | 2 | C | 24 | 0 | 0 | |

Continuation Annex Table 3

| Substance | Concentration CCß A | No. of ELISA Reader | Batch | No. of Results (pos. Class A) | No. of Results (pos. Class B) | No. of Results (negative) | p-Value Fisher´s Exact Test (CCß A) |
|---|---|---|---|---|---|---|---|
| **Sulfadoxin** | NA | 1 | A | 1 | 6 | 0 | |
| | | 1 | B | 7 | 0 | 0 | - |
| | | 1 | C | 4 | 3 | 0 | |
| | | 2 | A | 3 | 4 | 0 | |
| | | 2 | B | 6 | 1 | 0 | - |
| | | 2 | C | 4 | 3 | 0 | |
| **Sulfamethoxy-pyridazine** | 500 | 1 | A | 24 | 0 | 0 | |
| | | 1 | B | 24 | 0 | 0 | 1 |
| | | 1 | C | 16 | 0 | 0 | |
| | | 2 | A | 24 | 0 | 0 | |
| | | 2 | B | 24 | 0 | 0 | 1 |
| | | 2 | C | 16 | 0 | 0 | |
| **Chlortetra-cycline** | NA | 1 | A | 7 | 0 | 0 | |
| | | 1 | B | 0 | 7 | 0 | - |
| | | 1 | C | 7 | 0 | 0 | |
| | | 2 | A | 7 | 0 | 0 | |
| | | 2 | B | 0 | 7 | 0 | - |
| | | 2 | C | 7 | 0 | 0 | |
| **Oxytetracycline** | 800 | 1 | A | 7 | 0 | 0 | |
| | | 1 | B | 7 | 0 | 0 | 1 |
| | | 1 | C | 7 | 0 | 0 | |
| | | 2 | A | 7 | 0 | 0 | |
| | | 2 | B | 7 | 0 | 0 | 1 |
| | | 2 | C | 7 | 0 | 0 | |
| **Tetracycline** | 1,000 | 1 | A | 7 | 0 | 0 | |
| | | 1 | B | 7 | 0 | 0 | 1 |
| | | 1 | C | 7 | 0 | 0 | |
| | | 2 | A | 7 | 0 | 0 | |
| | | 2 | B | 7 | 0 | 0 | 1 |
| | | 2 | C | 7 | 0 | 0 | |
| **Dihydro-streptomycin** | 600 | 1 | A | 7 | 0 | 0 | |
| | | 1 | B | 7 | 0 | 0 | 1 |
| | | 1 | C | 7 | 0 | 0 | |
| | | 2 | A | 7 | 0 | 0 | |
| | | 2 | B | 7 | 0 | 0 | 1 |
| | | 2 | C | 7 | 0 | 0 | |
| **Streptomycin** | 1,500 | 1 | A | 7 | 0 | 0 | |
| | | 1 | B | 7 | 0 | 0 | 1 |
| | | 1 | C | 7 | 0 | 0 | |
| | | 2 | A | 7 | 0 | 0 | |
| | | 2 | B | 7 | 0 | 0 | 1 |
| | | 2 | C | 7 | 0 | 0 | |
| **Gentamicin** | 200 | 1 | A | 16 | 0 | 0 | |
| | | 1 | B | 24 | 0 | 0 | 1 |
| | | 1 | C | 24 | 0 | 0 | |
| | | 2 | A | 16 | 0 | 0 | |
| | | 2 | B | 24 | 0 | 0 | 1 |
| | | 2 | C | 24 | 0 | 0 | |
| **Neomycin** | 400 | 1 | A | 7 | 0 | 0 | |
| | | 1 | B | 7 | 0 | 0 | 1 |
| | | 1 | C | 7 | 0 | 0 | |
| | | 2 | A | 7 | 0 | 0 | |
| | | 2 | B | 7 | 0 | 0 | 1 |
| | | 2 | C | 7 | 0 | 0 | |
| **Chlor-amphenicol** | 7,000 | 1 | A | 7 | 0 | 0 | |
| | | 1 | B | 7 | 0 | 0 | 1 |
| | | 1 | C | 7 | 0 | 0 | |
| | | 2 | A | 7 | 0 | 0 | |
| | | 2 | B | 7 | 0 | 0 | 1 |
| | | 2 | C | 7 | 0 | 0 | |