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ORIGINAL PAPER

# Assessment of commutability for candidate certified reference material ERM-BB130 "chloramphenicol in pork"

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**Abstract** Chloramphenicol (CAP), an effective antibiotic against many microorganisms, is meanwhile banned in the EU for treatment of food-producing animals due to adverse health effects. The Institute for Reference Materials and Measurements (IRMM) is currently developing a certified reference material (CRM) for CAP in pork, intended for validation and method performance verifications of analytical methods. The material will be certified using liquid chromatography–tandem mass spectrometry (LC–MS/MS) and gas chromatography–mass spectrometry (GC–MS) methods and has a target CAP level around the minimum required performance limit (MRPL) of 0.3 μg/kg. To prove that the material can be applied as a quality control tool for screening methods, a commutability study was conducted, involving five commercially available enzyme-linked immunosorbent assay kits and one biosensor assay (BiaCore kit). Meat homogenates (cryo-milled wet tissue) with CAP concentrations around the MRPL and the candidate CRM (lyophilised powder) were measured by LC–MS/MS and GC–MS as well as the six screening methods. Pairwise method comparisons of results obtained for the two sample types showed that the CRM can successfully be applied as quality control (QC) sample to all six screening methods. The study suggests that ERM-BB130 is sufficiently commutable with the investigated assays and that laboratories applying one of the investigated kits therefore benefit from using ERM-BB130 to demonstrate the correctness of their results. However, differences among the assays were observed, either in the abundance of bias between screening and confirmatory LC and GC methods, the repeatability of test results, or goodness of fit between the methods.

**Keywords** Commutability · Certified reference material (CRM) · ELISA · Biosensor · Chloramphenicol

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## Introduction

Chloramphenicol [2,2-dichloro-*N*-[(1*R*,2*R*)-2-hydroxy-1-(hydroxymethyl)-2-(4-nitrophenyl) ethyl] acetamide] (CAP) is a broad-spectrum antibiotic and is highly effective against many pathogenic bacteria, rickettsiae and mycoplasmas. CAP was used since the 1950s in veterinary practices. The uptake of chloramphenicol in humans, however, can cause serious haemotoxic effects [1]; consequently, the use of CAP was banned for treatment of food-producing animals in the EU [2, 3] and several other countries. In 2003, Commission Decision 2003/181/EC [4] fixed a minimum required performance limit (MRPL) [5] of 0.3 μg/kg for analytical methods for the detection of residues of CAP in different matrices, including meat. A variety of analytical methods are used in laboratories to detect and quantify CAP in food matrices, such as gas chromatography–mass spectrometry (GC–MS) [6–8], liquid chromatography–tandem mass spectrometry (LC–MS/MS) [9–11], enzyme-linked immunosorbent assays (ELISA) [12–16] and surface plasmon resonance-based biosensor methods [17–19].

In order to achieve and safeguard reliable analytical results which are necessary to ensure effective consumer protection, a certified reference material (CRM), ERM-BB130 (lyophilised pork muscle containing CAP around the MRPL), will soon be made available by the Institute for

Reference Materials and Measurements (IRMM). This new CRM will support validation (in particular trueness determination) as well as performance verification of modern analytical methods and will enable laboratories to check whether their methods comply with the defined MRPL.

In the frame of the intercomparison to obtain measurement results for assigning a certified value, only comprehensively validated LC–MS/MS and GC–MS methods were applied which met the stringent quality requirements of IRMM; these methods provided for appropriate sensitivity and selectivity and allowed the detection, unambiguous identification and accurate quantification of CAP in food matrices at submicrogramme per kilogramme levels; moreover, they are most suitable to comply with Commission Decision 2002/657/EC concerning requirements for confirmatory methods [5]. However, a large number of field laboratories are also employing screening methods such as ELISA or biosensor methods. These are designed to analyse a large number of samples in a short period of time, are relatively inexpensive and need to have a false compliant rate of <5% (β-error) at the level of interest [5].

To ensure that the new CRM can correctly be used as a quality control sample to determine the trueness or to check the method performance of screening methods, it had to be confirmed that the CRM behaves in the same (comparable) way with typical routine meat samples and that this holds for both the methods used to assign the certified value (chromatographic methods coupled to mass spectrometry) as well as the typically applied field methods for screening purposes (immunological methods based on recognition of the molecule or some parts of it by antibodies). Hence, it had to be verified that the CRM is commutable with the immunoassays used as screening methods. The term commutability originated from the field of clinical chemistry [20]. It was first used to describe the ability of a reference or control material to have inter-assay properties comparable to the properties demonstrated by authentic clinical samples when measured by more than one analytical method. Recently, the concept has been expanded in the metrology literature, which now describes commutability as the equivalence of the mathematical relationships between the results of different measurement procedures for a reference material and for representative samples from healthy and diseased individuals [21]. A more generic definition of commutability can be found in ISO Guide 34 [22]. Non-commutability can be caused, for instance, by matrix changes during preparation of the CRM which leads to a different extraction behaviour of the analyte in the CRM compared to a true sample and therefore to different analytical results. Likewise, the analyte itself could be altered during CRM processing, e.g. converted into a metabolite, or the processing of the matrix or additives for preservation could cause interferences which do not occur in test samples typically analysed.

While a large number of publications exist for commutability assessment of clinical reference materials, there are very few in other fields of analytical chemistry [23, 24]. Ulberth and Emons [25] claimed that, for CRMs in food analysis, commutability is an important parameter to investigate but conceded that this important material property is usually not addressed in the food science community. To the best of our knowledge, only one study has been published in the field of veterinary drug residue testing; however, the study design was not that of typical commutability studies, as it neither involved a CRM nor screening methods but aimed at demonstrating comparability of measurements results when analysing samples from different animal species, matrices and matrix conditions with two different confirmatory methods [26].

In this work, we investigated the commutability of ERM-BB130 with routine screening methods, namely commercially available ELISA kits and a biosensor assay. The aim was to demonstrate that candidate CRM ERM-BB130 can correctly be used as a quality control (QC) sample to ensure reliable determination of CAP around the MRPL with the investigated screening methods. This was accomplished by analysing meat homogenate samples and the CRM with those methods and with methods used in the frame of the characterisation intercomparison for ERM-BB130, i.e. GC–MS and LC–MS/MS.

## Experimental

### Raw materials

Incurred pork muscle (prepared by administering CAP to a pig) was obtained from the Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL), Berlin, Germany. The CAP content in this muscle piece as determined by GC–MS (in-house method of BVL) was around 7 μg/kg. Pork muscle free of CAP was obtained from a local butcher. The absence of CAP in this muscle tissue was confirmed by LC–MS/MS (<LOD, 0.04 μg/kg). The materials were stored at −20 °C until processing.

### Processing of raw material

The blank pork muscle and the incurred pork muscle were manually cut into small cubes. For each material, the cubes were immersed in liquid nitrogen overnight and cryogenically milled for a short period of time using a Palla VM-KT vibrating mill (KHD Humboldt Wedag, Köln, Germany) to achieve a meat homogenate. Cross contamination between the two materials was avoided by first processing the blank material and by proper cleaning of the milling instrumentation. Four materials were produced: a

blank material (named A) and three CAP-containing meat homogenates with CAP concentrations (target levels) of 0.1 (B), 0.3 (C) and 0.6 μg/kg (D). Materials B–D were produced with a two/three-step dilution of incurred homogenate with blank homogenate and mixing using a DynaMIX CM200 three-dimensional mixer (WAB, Basle, Switzerland). Care was taken that the materials remained cold during mixing as they were taken directly from the liquid nitrogen Dewar before mixing. Materials were mixed 2×15 min, with liquid nitrogen cooling for 30 min between the two mixing events.

Finally, 15-g portions of meat homogenates were bottled in amber glass bottles, closed with a plastic insert and a screw cap. Materials were stored at −20 °C until dispatch to the testing laboratories.

## Homogeneity assessment of processed meat materials

Homogeneity was assessed by results from an ELISA, using a RidaScreen CAP-Glucuronide kit R-1503 (R-Biopharm AG, Darmstadt, Germany), performed at Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Vienna, Austria. Four bottles of each material (A–D) were shipped to the laboratory on dry ice. Three sub-samples had to be extracted from each bottle, and each sub-sample extract had to be analysed in triplicate. Measurements were performed under repeatability conditions (all extracts and replicates of one material on a single ELISA plate). Results were technically scrutinised and subjected to one-way analysis of variance (ANOVA), and uncertainty contributions were calculated as described by van der Veen et al. [27].

## ELISA methods and biosensor method

ELISA measurements were carried out in the laboratories of the kit providers, adhering to the instructions coming with the kits. The following ELISA kits were included in the study: RidaScreen Chloramphenicol R1505 (R-Biopharm AG, Darmstadt, Germany), I'screen CAP AB630 (Tecna S.r.l., Trieste, Italy), EuroClone Chloramphenicol ELISA kit A34811 (EuroClone, Milano, Italy), Chloramphenicol ELISA kit 5091CAP1p (EuroProxima BV, Arnhem, The Netherlands) and Chloramphenicol ELISA CN1469 (RANDOX, Crumlin, UK).

Biosensor analyses were performed at the Food and Environmental Research Agency (FERA), York, UK. Sample extraction and cleanup were carried out as described in [17]. Assays were performed using the Qflex Chloramphenicol kit BR-1005-53 according to the manufacturer's instructions and measured in a BiaCore Q system (GE Healthcare, Buckinghamshire, UK). Table 1 summarises the screening methods investigated in the study.

## LC–MS/MS and GC–MS method

Liquid chromatography–isotope dilution–tandem mass spectrometry and gas chromatography–isotope dilution–mass spectrometry measurements were performed by the Centre d'Economie Rurale, Laboratoire d'Hormonologie (Marloie, Belgium) and BVL (Berlin, Germany), respectively. Both laboratories operated fully validated and accredited methods. Table 2 summarises those methods.

## Certified reference material ERM-BB130

The candidate certified reference material ERM-BB130 was produced as described elsewhere [28]. Briefly, incurred pork muscle was lyophilised and diluted with lyophilised blank pork muscle material to a CAP concentration of approximately 0.3 μg/kg. An intercomparison of expert laboratories was carried out, involving GC–MS and LC–MS/MS methods. The certified value and its expanded uncertainty ($k$=2) were calculated to be 0.230±0.021 μg/kg. This value was unknown to the laboratories of this study.

## Study setup and instructions to participants

### *ELISA and biosensor methods*

Each participating laboratory received one bottle each of materials A–D as well as one bottle of ERM-BB130. Labs were instructed in details of how to reconstitute the CRM before extraction.

Three sub-samples had to be extracted from each of the five provided materials (i.e. 15 independent samples in total). Each of these samples (extracts) had to be analysed by ELISA in triplicate (biosensor measurements of extracts in duplicate). Measurements had to be performed under repeatability conditions, i.e. on one ELISA plate.

### *LC and GC methods*

Each participating laboratory received two bottles of material B as well as one bottle each of materials C and D. Material A had already been confirmed as blank before. Measurements on ERM-BB130 were already done in the frame of the characterisation intercomparison [28]; those values were taken as results for ERM-BB130. Three sub-samples had to be prepared from each of the four provided materials (i.e. 12 independent samples in total). Quality control samples (e.g. spiked blank samples) had to be prepared whenever foreseen in the working instruction of the methods.

Results had to be submitted as analyte concentration in microgramme per kilogramme meat (samples A–D) and as analyte concentration in microgramme per kilogramme

**Table 1** Overview of assessed screening methods

| Method/test kit | Sample intake (g) | Sample preparation and cleanup | Measurement principle | Remarks |
|---|---|---|---|---|
| R-Biopharm R1505 | 3 | Ethyl acetate/water, defatting with hexane | Competitive ELISA | Calibration 0–0.75 μg/kg (6 levels) |
| EuroProxima 5091CAP1p | 3 | Ethyl acetate, defatting with iso-octane/trichloromethane | Competitive ELISA | Calibration 0.025–2 μg/kg (6 levels) |
| Tecna AB630 | 4 | Ethyl acetate, defatting with hexane | Competitive ELISA | Calibration 0.025–2 μg/kg (6 levels) |
| EuroClone A34811 | 3 | Ethyl acetate, defatting with hexane | Competitive ELISA | Calibration 0.05–10 μg/kg (5 levels) |
| Randox CN1469 | 3 | Ethyl acetate, defatting with iso-octane/trichloromethane | Competitive ELISA | Calibration 0–5 μg/kg (5 levels) |
| BiaCore Qflex kit Chloramphenicol BR-1005-53[a] | 5 | 70% methanol, SPE (reverse phase) | Inhibition assay | Calibration 0.05–0.5 μg/kg (4 levels) |

[a] Sample preparation modified in the lab as described in [17]

reconstituted material (in the case of ERM-BB130). Laboratories had to indicate whether or not a recovery correction of results was applied.

### Evaluation of data

Bias plots were created, comparing the results from the methods in the certification study with those from the screening methods. The *x*-axis presents the data as obtained by LC–MS/MS or GC–MS; the *y*-axis lists the relative difference of the screening method result to the LC (GC) method result (result screening method minus results LC (GC) method, divided by the result of LC (GC) method). This allowed us to check the bias and moreover if the bias is consistent over the sample concentration range (meat samples) and if it is consistent among meat and CRM samples.

## Results and discussion

### Study materials

The study materials were prepared from an incurred pork muscle tissue (leftover from the processing of ERM-BB130, stored at −20 °C) and blank pork muscle tissue. Dedicated equipment (freeze dryer, cryogenic mill, variety of larger-scale mixers) and available expertise at IRMM concerning adequate material processing allowed the production of materials with minimal alteration and suitable homogeneity. Stability of the meat homogenates was not evaluated, but data available from similar kind of meat materials suggest that effects due to possible instability are negligible, provided that materials are stored under frozen conditions (−20 °C) and handled appropriately (immediate sample preparation after thawing of the meat). In addition, extensive stability testing was done for the reference material (lyophilised powder), where no CAP degradation was found when leaving the sample for 1 month at room temperature.

### Homogeneity of study materials

Samples were analysed by ELISA under repeatability conditions. Material A was confirmed to be void of CAP (<LOD, 0.025 μg/kg). Results of materials B–D were evaluated by one-way ANOVA as described in [27]. Briefly, for materials B–D, the scatter of repeated measurements on a

**Table 2** Overview of chromatographic methods

| Method | Sample intake (g) | Sample preparation and cleanup | Measurement principle | Remarks |
|---|---|---|---|---|
| LC–MS/MS | 5 | Ethyl acetate; defatting with iso-octane/chloroform | LC–ESI–MS/MS, detection MRM mode[a] | Matrix-matched calibration[b] |
| GC–MS | 3 | Acetonitrile/NaCl; defatting with hexane; SPE | GC–ECNI–MS in SIM mode | Neat standard calibration[b] |

[a] Reverse-phase chromatography on a $C_{18}$ column; mass spectrometer used as a triple quadruple instrument, ion source operated in the negative electrospray ionisation mode, detector operated in the MRM mode

[b] Deuterated internal standard used

given extract was in the same order as the bottle-to-bottle variation. The samples proved to be sufficiently homogeneous, with estimated relative standard uncertainty contributions of 2.4% (material B), 2.8% (material C) and 1.2% (material D), respectively.

## Applied methods in the study

Tables 1 and 2 summarise the main features of the methods applied in the study. GC and LC methods have already been used in the frame of the certification of ERM-BB130. Also, the two methods are representative in terms of LC–MS/MS and GC–MS measurement principles used in the certification study; isotope dilution mass spectrometry was applied in all cases; for LC–MS/MS, the ion sources were operated in the negative electrospray ionisation mode and the detectors in the multiple reaction monitoring mode, whereas GC–MS methods used electron capture negative ionisation and detection in the selected ion monitoring mode. Details about the certification of ERM-BB130 can be found elsewhere [28]. ELISA methods used comparable sample amounts; also, the extraction procedures were comparable; the ELISAs themselves however varied, be it in the type of anti-CAP antibodies used (different hosts such as goat or sheep or rabbit) or in the application of a second antibody in the test to enhance specificity and in the type of the detection system (enzymes and substrates used). Also, calibration curves (number of points, working range) differed among the assays. Table 3 lists information about recovery and its handling for all methods.

## Assessment of CRM commutability with investigated assays

Table 4 shows the summary of results obtained for each test and sample. Mean values and their standard deviations are indicated. For each assay, the following parameters were investigated: method-related bias by comparing test results to GC and LC results, respectively, consistency of bias over concentration range of meat samples, and sample type-related bias by comparing results for meat samples and CRM. Moreover, the obtained precision was checked, as well as the correct classification of material A as blank. Finally, the handling of recovery correction was checked for its influence on the results, and the regression lines obtained for the meat samples were assessed for linearity (no detailed data shown). Figure 1a, b depicts bias plots for the comparison of each of the three ELISA kits from R-Biopharm, EuroProxima and Tecna, with LC and GC data, respectively. Figure 2a, b shows bias plots for the comparison of each of the two ELISA kits from EuroClone and Randox as well as from the modified biosensor assay operated at FERA (BiaCore principle) with LC and GC data, respectively.

In all but one case (Randox ELISA), sample A has been correctly identified as blank. The reason for this incorrect result could not be determined (e.g. possible contamination), and due to the lack of leftover material, the analysis could not be repeated. Nevertheless, the laboratory might want to consider retesting a true blank material with the assay. Precision for the triplicate determination of meat samples and the CRM ranged from 2% to 18%, with better precision and higher precision consistency for the R-Biopharm and the Tecna assays.

Substantial differences were found concerning the detected bias: for the three assays from R-Biopharm, EuroProxima and Tecna (Fig. 1a, b), it can be seen that the bias to the LC and GC results, respectively, was considerably smaller than for the other three assays from EuroClone, Randox and the modified BiaCore test (Fig. 2a, b). Furthermore, the bias plots (Fig. 1a, b) show that a very small positive bias was found for the R-Biopharm assay when comparing the values with those from the GC and LC results and that a small negative bias compared to GC and a small positive bias compared to LC results were obtained for the assays for EuroProxima and Tecna, respectively, indicating accuracy for the results from the three assays. It can also be seen that bias consistency among data for meat samples was obtained (exception of material B in the R-Biopharm assay). When comparing the relative bias between meat sample and CRM, the plot shows consistency between those values for the R-Biopharm and the EuroProxima assay (around 10% and not detectable, respectively), whereas in the case of the Tecna assay the bias for the CRM result is larger than those for meat samples (around 30%), indicating a somewhat limited comparability of meat samples with the lyophilised pork powder CRM, with a trend towards overestimation of the CAP concentration in the latter. Two of the three laboratories reported their results with recovery correction (Table 3); a non-correction in the cases of R-Biopharm and Tecna as well as a correction in the case of EuroProxima (results submitted as not recovery-corrected) would not substantially influence the overall result of the respective assay. Linear regression graphs (data not shown) underpinned the results obtained from the bias plots: linearity ($r^2 > 0.995$) for the meat samples over the investigated concentration range; and the CRM result was close to the regression lines in the case of the R-Biopharm and the EuroProxima assays and with a positive offset in the case of the Tecna assay. Finally, comparing the result for ERM-BB130 with the certified value [29], agreement would be found for the R-Biopharm and the EuroProxima

**Table 3** Handling of recovery in the applied procedures

| Method/test kit | QC samples for determining recovery | Obtained recovery (mean ± standard deviation in %) | Results recovery-corrected?[a] | Remarks |
|---|---|---|---|---|
| LC–MS/MS | IRMM blank pork powder (provided during char. exercise), spiked 1× at 0.2 and 1× at 0.5 μg/kg | 102±1 | Yes | Matrix-matched calibration (same matrix as in QC); isotopically labelled IS added in beginning of sample preparation |
| GC–MS | IRMM blank pork powder (provided during char. exercise), spiked 1× at 0.2 and 1× at 0.5 μg/kg | 107±4 | No | Neat standard calibration; meat samples and CRM: isotopically labelled IS added in beginning of sample preparation |
| R-Biopharm R1505 | Low-fat pork meat homogenate, spiked 1× at 0.1 and 1× at 0.25 μg/kg | 78±2 | Yes | – |
| EuroProxima 5091CAP1p | 1× sample A (true blank), spiked at 0.2 ppb level, 1× in-house blank pork muscle, spiked at 0.2 μg/kg | 77±3 | No | – |
| Tecna AB630 | In-house pork muscle, spike at 1 ppb level 1×<br>In addition, incurred rabbit muscle material (0.64 μg/kg) was tested 1×, recovery found was 85% | 85 | Yes | – |
| EuroClone A34811 | In-house blank pork muscle, spiked 1× at 0.5 and 1 μg/kg each | 77±13 | No | – |
| Randox CN1469 | In-house blank pork muscle, spiked 1× at 2 and 4 μg/kg each | 90±12 | No | – |
| BiaCore Qflex kit chloramphenicol BR-1005-53[b] | In-house blank pork muscle, spiked 2× at 0.15 μg/kg | 126±34 | Yes | Matrix-matched calibration curve (spiked blank pork meat) to correct for potential matrix effects |

[a] As indicated in the submitted results

[b] BiaCore kit, but sample preparation modified in the lab as described in [17]

assays when assuming a 10% standard measurement uncertainty for those tests (not known but realistic, taking into account uncertainty contributions of sample preparation, test sample homogeneity and reproducibility of the ELISA). This does not hold for the Tecna assay, very likely due to the detected bias among results on meat and CRM (around 30%). It can be concluded that commutability of ERM-BB130 with the three investigated assays has been demonstrated.

Bias plots for assays from EuroClone, Randox and the modified BiaCore test (Fig. 2) appeared substantially different to those shown in Fig. 1 (other three assays). Firstly, the absolute biases compared to GC and LC references, respectively, were considerably larger than it was the case for the other assays (e.g. −2–125% to LC and −16–62% to GC for the EuroClone assay, 29–237% to LC and 11–140% to GC for the Randox assay, 118–133% to LC and 66–86% to GC for the modified BiaCore test). Secondly, for the ELISAs from EuroClone and Randox, the bias varied substantially among the three very similar meat samples (meat homogenates resulting from the same raw materials and therefore having almost identical properties). In those two cases, the results do not allow a conclusion whether a bias exists among the results on meat samples and the CRM. Results from both assays were reported with no recovery correction. Correction for recovery would yield values with an even larger difference to those obtained by LC and GC, respectively. Linear regression lines (data not shown) underpinned the results deduced from Table 4 and the bias plots: poor linearity ($r^2$<0.965) over the investigated concentration range. A comparison of the found value for ERM-BB130 with the certified value is meaningless unless further investigations are carried out to find a reasonable explanation for the obtained biases (values for both the meat samples and the CRM are substantially higher than the respective values of the LC and GC measurements). The kit producers might wish to consider a recalibration of the assays which may lead to a reduction of the overall bias, and also the inconsistency of the bias (large variation) should be examined to increase the robustness of the assay. By seeing the intended purpose of the tests and also referring to Commission Decision 2002/657/EC as regards technical criteria of screening methods (β-error of <5%), it can however be concluded from the obtained results that ERM-BB130 can be regarded as sufficiently commutable with those assays.

**Table 4** Overview of study results

| Sample | GC–MS | | LC–MS/MS | | ELISA R-Biopharm | | ELISA EuroProxima | | ELISA Tecna | | ELISA EuroClone | | ELISA Randox | | Biosensor BiaCore[b] | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | RSD% | Mean | RSD% | Mean | RSD% | Mean | RSD% | Mean | RSD% | Mean | RSD% | Mean | RSD% | Mean | RSD% |
| A | nm[a] | – | nm[a] | – | <0.025 µg/kg | – | <0.02 µg/kg | – | <0.025 µg/kg | – | <0.1 µg/kg | – | 0.13 | 14.1 | <0.05 µg/kg | – |
| B | 0.08 | 4.8 | 0.06 | 4.7 | 0.09 | 4.2 | 0.07 | 8.9 | 0.07 | 5.1 | 0.13 | 16.7 | 0.19 | 4.7 | 0.13 | 17.8 |
| C | 0.24 | 18.3 | 0.21 | 20.4 | 0.24 | 21.8 | 0.21 | 16.6 | 0.23 | 6.1 | 0.20 | 10.2 | 0.27 | 12.6 | 0.45 | 9.0 |
| D | 0.72 | 1.7 | 0.60 | 3.6 | 0.73 | 4.9 | 0.52 | 7.5 | 0.66 | 0.6 | 1.16 | 1.8 | 1.26 | 12.8 | >0.5[c] | – |
| ERM-BB130 | 0.22 | 0.4 | 0.19 | 2.3 | 0.25 | 1.9 | 0.20 | 7.2 | 0.28 | 1.1 | 0.29 | 7.2 | 0.29 | 4.2 | 0.45 | 3.8 |

Mean values (in microgramme per kilogramme meat for samples A–D and in milligramme per kilogramme reconstituted samples for ERM-BB130) from three independently prepared subsamples, each of which was analysed in triplicate (duplicate in case of the biosensor assay)

[a] Not measured (blank, verified by LC–MS/MS before characterisation intercomparison)

[b] Sample preparation according to [17]

[c] Outside assay working range 0.05–0.5 µg/kg

In the case of the modified BiaCore assay, the bias was quite consistent among meat samples, and also good consistency between results on meat and the CRM was obtained (relative bias around 10%). Conclusions for sample D could not be made due to the restricted working range of the assays (0.05–0.5 µg CAP/kg meat, all higher results indicated as >0.5 µg/kg). Again, by seeing the approximately twofold overestimation of the CAP content compared to chromatographic methods, the kit producer might want to check the calibration for a possible reduction of the positive bias. In the context of the intended purpose of the test as a reliable screening method with a low number of false-negative results, it can be concluded that ERM-BB130 can validly be used as a valuable quality control sample.

## Further considerations

Any further statistical evaluation of the data as usually done in commutability studies of clinical reference and control materials [30, 31] was not feasible for this study. Unlike in the field of clinical chemistry, where usually dozens of patient samples are analysed and compared to control and reference samples, the inclusion of any other sample in this study (meat samples positively tested for CAP, e.g. retained samples from analysis done by control laboratories in the frame of the National Residue Control Plans) was not possible due to the unavailability of such samples (existence, necessary amount for a study, samples integrity/age, CAP concentration level, homogeneity of samples). Furthermore - and again in contrast to commutability studies in the clinical field - no reference method(s) exist and could be used as the benchmark, but two representative methods (GC–MS, LC–MS/MS) from those applied in the frame of the CRM characterisation exercise were used (see "Introduction," remarks on appropriate methods for certification of reference materials). Ideally, a large set of results on the samples from other GC–MS and LC–MS/MS methods should be available, which would allow us to define a confidence interval around the obtained values. In that case and with a larger number of samples, extensive statistical analysis could also have been performed (e.g. Deming regression, Passing/Bablok regression, Bland-Altman plots) to substantiate any findings concerning commutability. Despite the constraints mentioned (lack of samples, few data sets only), it can be concluded that ERM-BB130 fits the purpose of being used as a valuable quality control sample with the assays in this study. The validity of this statement however implies that the ingredients of the kits (antibodies and their specificity, calibrants, reagents, instructions to use) are not changed by the kit providers and remain as at the time of the study.

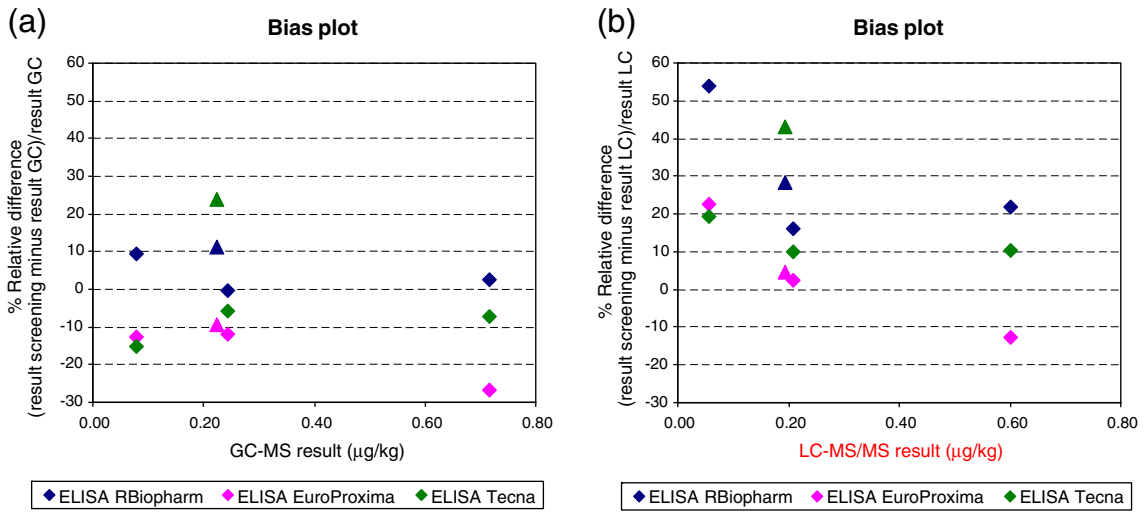


**Fig. 1** Bias plots. The relative result difference (result screening method minus result LC (GC) method, divided by the result LC (GC) method) is plotted versus the result of the LC (GC) method. **a** Comparison of the three screening methods R-Biopharm ELISA, EuroProxima ELISA and Tecna ELISA with the GC–MS results. *Small diamonds*: results for the meat samples B, C and D; *big triangles*: results for ERM-BB130. **b** Same as **a**, but comparison to LC–MS/MS results

## Conclusions

A commutability study was performed which suggests that the upcoming certified reference material ERM-BB130 (chloramphenicol in pork meat) is commutable with all investigated commercially available ELISA screening test kits and a modified biosensor assay based on the commercially available BiaCore CAP kit. Differences among kits were obtained (repeatability, bias to results of GC–MS and LC–MS/MS methods, match with certified value, trends towards substantial/slight over/underestimation). ERM-BB130 can be used as a suitable QC material to obtain and safeguard the quality of measurement results with the tested routine CAP screening assays.

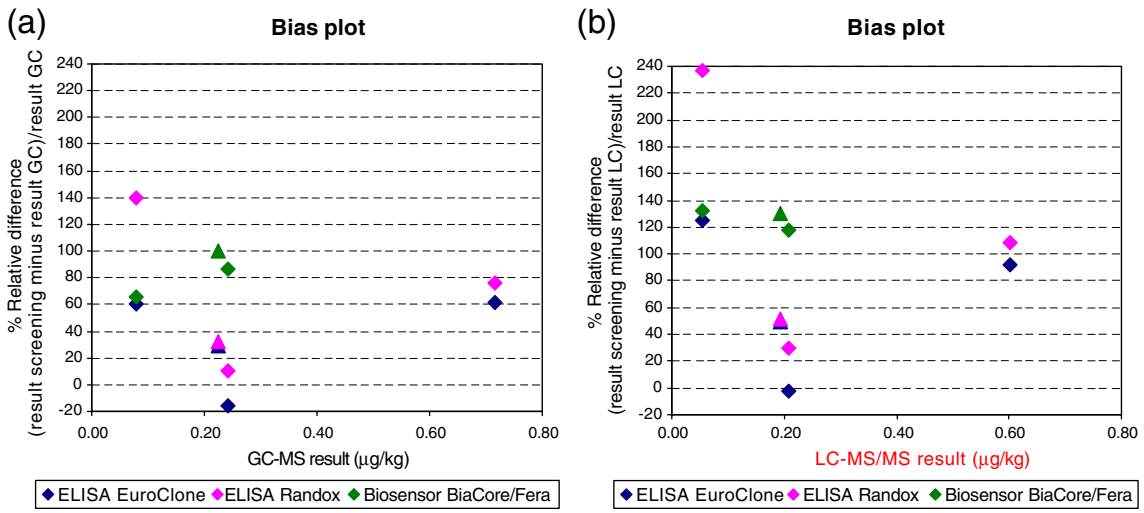


**Fig. 2** Bias plots. The relative result difference (result screening method minus result LC (GC) method, divided by the result LC (GC) method) is plotted versus the result of the LC (GC) method. **a** Comparison of the three screening methods EuroClone ELISA, Randox ELISA and BiaCore biosensor assay (modified by the testing laboratory FERA) with the GC–MS results. *Small diamonds*: results for the meat samples B, C and D; *big triangles*: results for ERM-BB130. **b** Same as **a**, but comparison to LC–MS/MS results

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