RIDASCREEN® Chloramphenicol

REF R1511

Enzymimmunoassay zur quantitativen Bestimmung von Chloramphenicol

Enzyme immunoassay for the quantitative determination of chloramphenicol

In vitro Test

Lagerung bei 2 - 8 °C
Storage at 2 - 8 °C

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RIDASCREEN® Chloramphenicol

Brief information

RIDASCREEN® Chloramphenicol (R1511) is a competitive enzyme immunoassay for the quantitative analysis of chloramphenicol in milk, milk powder, dairy products, honey and royal jelly, meat, fish, shrimp, eggs, urine (also chloramphenicol glucuronide), plasma/serum and feed.

All reagents required for performing the enzyme immunoassay, including the standards, are contained in the test kit. The test kit is sufficient for a maximum of 48 duplicate determinations (including standards). A microtiter plate spectrophotometer is required for quantification.

Sample preparation:

Milk: direct use

Milk powder: reconstitution, or alternatively: precipitation, extraction, evaporation, reconstitution

Yoghurt, kefir, buttermilk, cream: precipitation, extraction, evaporation, reconstitution

Curd, sour cream: homogenization, defatting, extraction, evaporation, reconstitution

Butter: defatting, extraction, evaporation, reconstitution

Cheese: homogenization, extraction, evaporation, reconstitution

Honey: extraction, evaporation, reconstitution

Royal jelly: extraction, evaporation, reconstitution

Meat, fish, shrimp, eggs: homogenization, extraction, evaporation, reconstitution, de-fattening

Urine: direct use or hydrolysis, extraction, evaporation, reconstitution

Plasma/serum: extraction, evaporation, reconstitution

Feed: grinding, extraction, evaporation, reconstitution, defatting

Time requirement:

Sample preparation (for 10 samples)
According to sample matrix ..... 5 min - 2 h
Test implementation (incubation time) ..... 45 min

Limit of Detection (corresponding to the standard substance)	Milk	approx. 24 ng/L (ppt)
	Milk powder (reconstitution)	approx. 240 ng/kg
	Milk powder (extraction)	approx. 24 ng/kg
	Yoghurt, kefir, buttermilk, cream	approx. 12 ng/kg
	Curd, sour cream	approx. 15 ng/kg
	Butter	approx. 61 ng/kg
	Cheese	approx. 16 ng/kg
	Honey	approx. 25 ng/kg
	Royal jelly	approx. 23 ng/kg
	Meat (beef, pork, poultry)	approx. 5 ng/kg
	Fish	approx. 8 ng/kg
	Shrimp	approx. 8 ng/kg
	Shrimp (5 in 1 nitrofuran sample prep.)	approx. 34 ng/kg
	Eggs	approx. 15 ng/kg
	Urine, direct (CAP-glucuronide)	approx. 138 ng/L
	Urine, hydrolyzed (chloramphenicol)	approx. 196 ng/L
	Plasma/serum	approx. 18 ng/L
	Feed	approx. 107 ng/kg
Detection capability (CCβ):	Milk	approx. 75 ng/L (ppt)
	Milk powder	approx. 100 ng/kg
	Yoghurt, kefir, buttermilk, cream	approx. 50 ng/kg
	Curd, sour cream	approx. 300 ng/kg
	Butter	approx. 150 ng/kg
	Cheese	approx. 200 ng/kg
	Honey	approx. 50 ng/kg
	Royal Jelly	approx. 100 ng/kg
	Meat (beef, pork, poultry)	approx. 25 ng/kg
	Fish	approx. 25 ng/kg
	Shrimp	approx. 25 ng/kg
	Shrimp (nitrofuran sample prep.)	approx. 150 ng/kg
	Eggs	approx. 75 ng/kg
	Urin (direct)	approx. 300 ng/kg
	Urin (hydrolyzed)	approx. 325 ng/kg
	Plasma	approx. 50 - 100 ng/kg
	Feed	approx. 150 ng/kg

Recovery rate: (corresponding to the standard substance)	Milk	approx. 93 %
	Milk powder (reconstitution)	approx. 101 %
	Milk powder (extraction)	approx. 78 %
	Yoghurt, kefir, buttermilk, cream	approx. 104 %
	Curd, sour cream	approx. 92 %
	Butter	approx. 82 %
	Cheese	approx. 74 %
	Honey	approx. 106 %
	Royal jelly	approx. 77 %
	Meat (beef, pork, poultry)	approx. 91 %
	Fish	approx. 97 %
	Shrimp	approx. 92 %
	Shrimp (5in1 nitrofuran sample prep.)	approx. 98 %
	Eggs	approx. 83 %
	Urine, direct (chloramphenicol glucuronide)	approx. 113 %
	Urine, hydrolyzed (chloramphenicol)	approx. 101 %
	Plasma/serum	approx. 96 %
	Feed	approx. 104 %
Specificity: in buffer system	Chloramphenicol (RR-para-stereoisomer) (standard substance)	100 %
	Dextramycin (SS-para-stereoisomer)	< 1 %
	All other stereoisomers	not determined
	Chloramphenicol base	< 1%
	Florfenicol	< 1%
	Thiamphenicol	< 1%
	Nitrofurantoin, AHD, NP-AHD	< 1%
	Furaltadone, AMOZ, NP-AMOZ	< 1%
	Furazolidone, AOZ, NP-AOZ	< 1%
	Nitrofurazone, SEM, NP-SEM	< 1%
	Chloramphenicol glucuronide	approx. 68 %
Specificity: in bovine urine	Chloramphenicol glucuronide	approx. 51 %
in porcine urine	Chloramphenicol glucuronide	approx. 68 %

The specificity of the RIDASCREEN® Chloramphenicol assay was determined by analyzing the cross reactivity to the respective substances in the buffer system. In samples, the specificity may differ from the values determined in the buffer system due to matrix effects. Prior to the analysis of cross-reactive substances, the user has to determine the limit of detection and the recovery rate in the relevant matrix. The test cannot discriminate between analytes and cross-reactive substances.

In order to increase the quality of assessment when performing ELISA procedures, we refer additionally to our Good ELISA Practice manual. It lists minimum standards concerning the framework conditions when using test kits of R-Biopharm AG and performing ELISA analyses with them. The manual can be retrieved, printed and downloaded from www.r-biopharm.com/products/food-feed-analysis.

Product offer

RIDA® Chloramphenicol Spiking Solution (R1599)

1. Intended use

RIDASCREEN® Chloramphenicol is a competitive enzyme immunoassay for the quantitative analysis of chloramphenicol in milk, milk powder and dairy products, honey and royal jelly, meat, fish, shrimp, eggs, urine (also chloramphenicol glucuronide), plasma/serum and feed.

2. General

Chloramphenicol is a broad spectrum antibiotic which is frequently employed in animal production for its excellent antibacterial and pharmacokinetic properties. However, in humans it leads to hematotoxic side effects, in particular chloramphenicol-induced aplastic anaemia for which a dosage-effect relationship has not yet been established. This has led to a prohibition of chloramphenicol for the treatment of animals used for food production. For test systems, a minimum required performance limit of 300 ng/kg (ppt) was established. A tightening of the performance limit to 150 ng/kg is described in Commission Regulation (EU) 2019/1871.

3. Test principle

The ELISA test system is based on the antigen-antibody reaction. The wells of the microtiter strips are coated with capture antibodies and anti-chloramphenicol antibodies. Standards, the sample solutions and enzyme conjugate are added. Free antigen (chloramphenicol) and enzyme conjugate compete for the antibody binding sites (competitive enzyme immunoassay). Any unbound enzyme conjugate is then removed in a washing step. For detection, substrate/chromogen is added to the wells and incubated. Bound enzyme conjugate converts the chromogen into a blue end product. Adding the stop solution causes a color change from blue to yellow. The measurement is made photometrically at 450 nm; the extinction is inversely proportional to the antigen concentration (chloramphenicol) in the sample.

4. Reagents provided

Each kit contains sufficient materials for a maximum of 48 duplicate determinations (including standard analyses). Each test kit contains:

Component	Cap color	Format		Volume
Microtiter plate	-	Ready to use		96 wells
Standard 1	White	Ready to use	0 ng/L	1.3 ml
Standard 2	White	Ready to use	25 ng/L	1.3 ml
Standard 3	White	Ready to use	50 ng/L	1.3 ml
Standard 4	White	Ready to use	100 ng/L	1.3 ml
Standard 5	White	Ready to use	250 ng/L	1.3 ml
Standard 6	White	Ready to use	750 ng/L	1.3 ml
Wash buffer salt Tween		Salt for dissolving		
Conjugate	Red	Ready to use		7.5 ml
Substrate/Chromogen	Brown	Ready to use		10 ml
Stop solution	Yellow	Ready-to-use		14 ml

5. Reagents required but not provided

5.1 Equipment

Depends on matrix.

Equipment	Milk, urine	Milk powder, dairy products	Honey	Royal jelly	Meat, fish, shrimp, eggs	Urine after hydro-lysis	Plasma/ serum	Feed
Microtiter plate spectrophotometer (450 nm)	•	•	•	•	•	•	•	•
Graduated pipettes	•	•	•	•	•	•	•	•
Variable 20 - 200 µl and 200 - 1000 µl micropipettes	•	•	•	•	•	•	•	•
Mixer		•			•			•
Shaker		•	•	•	•	•		•
Centrifuge		•	•	•	•	•	•	•
Evaporator		•	•	•	•	•	•	•
Vortex	•	•	•	•	•	•	•	•
Incubator						•		
Waterbath		•						

5.2 Reagents

Depends on matrix.

Reagent	Honey	Royal jelly	Meat, Fish, shrimp	Eggs	Urine after hydrolysis	Plasma/ serum	Feed
Distilled water	•		•				
Ethyl acetate p.a.	•	•	•	•		•	•
n-Hexane ≥ 95 %			•	•			•
***E. coli* β-Glucuronidase**					•		
75 mM Potassium hosphate buffer pH 6.8					•		
0.5 M NaOH		•					

Reagent	Yoghurt, kefir, butter-milk, cream	Milk powder	Curd, sour cream	Butter	Cheese
20 mM PBS	•				
Carrez	•	•			
Ethyl acetate p.a.	•	•	•		•
n-Hexane ≥ 95 %		•		•	
20 % (v/v) Methanol				•	
10 % (v/v) Methanol			•	•	•

β-Glucuronidase from *Escherichia coli* (Sigma-Aldrich, Art. No. G7646):
– Reconstitute the lyophilized powder to 1 mg/ml

75 mM potassium phosphate buffer, pH 6.8:
– Buffer A: 10.2 g KH_2PO_4; fill up to 1000 ml with distilled water
– Buffer B: 13.06 g K_2HPO_4; fill up to 1000 ml with distilled water
– Adjust pH to 6.8 by mixture of buffer A and B (ratio 1:1)

0.5 M NaOH:
– 20 g NaOH, fill up to 1000 ml with distilled water

20 mM PBS:
– 0.55 g NaH_2PO_4 x H_2O + 2.85 g Na_2HPO_4 x 2 H_2O + 8.77 g NaCl; fill up to 1000 ml with distilled water; adjust pH to 7.4 with NaOH

Carrez:
– Carrez I: 15.21 g Potassium ferrocyanide(II) x 3 H_2O; fill up to 100 ml with distilled water
– Carrez II: 29.90 g Zinc sulfate x 7 H_2O; fill up to 100 ml with distilled water

6. Warnings and precautions for the users

This test should only be carried out by trained laboratory employees. The instruction for use must be strictly followed.

This kit may contain hazardous substances. For hazard notes on the contained substances please refer to the appropriate safety data sheets (SDS) for this product, available online at www.r-biopharm.com.

Ensure the proper and responsible cleanup and / or disposal of all reagents and materials after their use. For disposal, please adhere to national regulations.

7. Storage instructions

Store the kit at 2 - 8 °C (35 - 46 °F). Do not freeze any test kit components.

Return any unused microwells to their original foil bag, reseal them together with the desiccant provided and further store at 2 - 8 °C (35 - 46 °F).

The red-colored substrate/ chromogen is photosensitive, therefore, avoid direct exposure to light.

Do not use the test kit after the expiration date (see test kit label).

Do not interchange individual reagents between kits of different lot numbers.

8. Indication of instability or deterioration of reagents

– Bluish coloration of the red-stained substrate/chromogen prior to test implementation
– A value of less than 0.6 absorbance units ($A_{450\ nm} < 0.6$) for zero standard

9. Preparation of Samples

9.1. Milk (raw, fresh, pasteurized, skimmed, full cream) from cow, and milk powder (reconstituted according to manufacturer's instruction)

– Vortex a representative sample amount for homogenization
– Use 50 µl milk per well in the assay

To achieve a better sensitivity for the detection in milk powder, alternatively the sample preparation (extraction) described in chapter 9.2. can be performed.

9.2. Milk powder (skimmed and full cream): Extraction

– Dissolve 1 g of milk powder with 10 ml distilled water into a 50 ml centrifugal vial by shaking thoroughly
– Add 1 ml Carrez I (see chapter 5.2.) vortex
– Add 1 ml Carrez II (see chapter 5.2.) vortex
– Centrifuge: 10 min / 3,000 g / 4 - 12 °C (if a refrigerated centrifuge is not available, chill sample to approx. 8 °C prior to centrifugation)
– Transfer 7.2 ml of the supernatant into a new 50 ml centrifugal vial
– Add 6 ml ethyl acetate and shake upside down for 10 min
– Centrifuge: 10 min / 3,000 g / room temperature (20 - 25 °C)
– Transfer 4 ml of ethyl acetate supernatant into a new glass vial and evaporate to complete dryness at 60 °C under nitrogen or air stream (Note: If fatty residues remain after evaporation: continue as described below. *)
– If there are no fatty residues, dissolve the dried residue in 400 µl wash buffer (see chapter 10.1.) and vortex
– Use 50 µl per well in the assay

(* In case of fatty residues after evaporation:

– Add 400 µl n-hexane and vortex
– Add 400 µl wash buffer and vortex
– Centrifuge: 10 min / 3,000 g / room temperature (20 - 25 °C)
– Use 50 µl of the lower aqueous phase per well in the assay

9.3. Dairy products

9.3.1. Yoghurt, kefir, buttermilk, cream

- Weigh 10 g of sample into a centrifugal vial
- Add 8 ml 20 mM PBS and mix
- Add 1 ml Carrez I (see chapter 5.2.) and vortex vigorously
- Add 1 ml Carrez II (see chapter 5.2.) and shake for 10 min upside down
- Centrifuge: 10 min / 4,000 g / 4 °C
- Transfer 4 ml of the supernatant into a new vial
- Add 8 ml of ethyl acetate and shake for 10 min upside down
- centrifuge: 10 min / 3,000 g / room temperature (20 - 25 °C)
- Transfer 4 ml of the supernatant into a new vial and evaporate at 60 °C to complete dryness under nitrogen or air stream
- Dissolve the dried residue in 500 µl wash buffer (see chapter 10.1.)
- Use 50 µl per well in the assay

9.3.2. Curd, sour cream

- Add 15 ml 10 % Methanol to 5 g of sample and vortex vigorously for 1 min
- Centrifuge: 15 min / 4,000 g / 4 °C
- Puncture through cream layer and transfer 4 ml of sample into a new glass vial
- Add 8 ml ethyl acetate
- Shake upside down for 10 min
- Centrifuge: 10 min / 3,000 g / room temperature (20 - 25 °C)
- Transfer 4 ml of the supernatant into a new vial and evaporate at 60 °C to complete dryness by nitrogen or air
- Dissolve the dried residue in 500 µl wash buffer (see chapter 10.1.)
- Use 50 µl per well in the assay

9.3.3. Butter

- Weigh 1 g butter into a 10 ml centrifugal vial
- Melt the butter in a water bath at approx. 40 °C
- Add 1 ml n-hexane and vortex vigorously for 10 s
- Add 1 ml 20 % methanol and vortex vigorously for 10 s
- Shake upside down for 10 min
- Centrifuge: 10 min / 2,000 g / 4 °C
- Transfer 700 µl of the lower aqueous layer into a 1.5 ml vial
- Put the vial on ice for 10 min
- Centrifuge: 5 min / 20,000 g / room temperature (20 - 25 °C)

- Dilute lower aqueous phase 1:4.5 (1+3.5) with wash buffer (see chapter 10.1.) (e.g.: 200 µl lower phase + 700 µl wash buffer)
- Use 50 µl per well in the assay

9.3.4. Cheese

- Remove existing noble rot
- Homogenize completely 10 g of cheese with 30 ml 10 % methanol
- Incubate in a water bath at approx. 40 °C for 10 min, shake vigorously at least 3 times during the incubation
- Centrifuge: 15 min / 4,000 g / 4 °C
- Transfer 3.5 ml of the lower aqueous phase into a new centrifugal vial and add 7 ml ethyl acetate
- Shake upside down for 10 min
- Centrifuge: 10 min / 3,000 g / room temperature (20 - 25 °C)
- Transfer 3.5 ml of the supernatant into a new vial and evaporate at 60 °C to complete dryness by nitrogen or air
- Dissolve the dried residue in 500 µl wash buffer (see chapter 10.1.)
- Use 50 µl per well in the assay

9.4. Honey

- Dissolve 2 g of honey in 4 ml distilled water in a centrifugal vial
- Add 4 ml ethyl acetate and shake for 10 min upside down
- Centrifuge: 10 min / 3,000 g / room temperature (20 - 25 °C)
- Transfer 1 ml of supernatant into a new vial and evaporate at 60 °C to complete dryness by nitrogen or air
- Reconstitute the dried residue in 500 µl wash buffer (see chapter 10.1.) and vortex
- Use 50 µl per well in the assay

9.5. Royal Jelly

- Weigh 2 g royal Jelly into a centrifugal vial and add 3 ml 0.5 M NaOH
- Shake until royal Jelly is dissolved completely
- Add 8 ml ethyl acetate and vortex vigorously for 1 min
- Shake 10 min upside down
- Centrifuge: 10 min / 3,000 g / room temperature (20 - 25 °C)
- Transfer 2 ml of the supernatant into a new glass vial and evaporate to complete dryness at 60 °C by nitrogen or air
- Dissolve the dry residue in 500 µl wash buffer
- Use 50 µl per well in the assay

9.6. Meat (beef, pork, poultry), fish, shrimp

- Homogenize a representative sample amount completely
- Add 3 ml of distilled water and 6 ml ethyl acetate to 3 g of homogenized sample and mix
- Shake for 10 min upside down
- Centrifuge: 10 min / 3,000 g / room temperature (20 - 25 °C)
- Transfer 4 ml of supernatant (corresponding to 2 g of sample) into a new vial and evaporate at 60 °C to complete dryness by nitrogen or air
- Reconstitute the dried residue in 1 ml n-hexane
- Add 500 µl wash buffer and vortex for 1 min
- Centrifuge: 10 min / 3,000 g / room temperature (20 - 25 °C)
- Use 50 µl of the lower aqueous phase per well in the assay

9.7. Eggs (whole egg, egg white, egg yolk) from chicken

- Homogenize a representative sample amount completely
- Add 8 ml ethyl acetate to 2 g of homogenized sample
- Shake for 10 min upside down
- Centrifuge: 10 min / 3,000 g / room temperature (20 - 25 °C)
- Transfer 4 ml of supernatant (corresponding to 1 g of sample) into a new vial and evaporate at 60 °C to complete dryness by nitrogen or air
- Reconstitute the dried residue in 1 ml n-hexane
- Add 1000 µl wash buffer and vortex for 1 min
- Centrifuge: 10 min / 3,000 g / room temperature (20 - 25 °C)
- Use 50 µl of the aqueous lower phase per well in the assay

9.8. Urine (bovine and porcine)

After administration of chloramphenicol to farm animals, chloramphenicol is metabolized in the liver in form of a conjugation to glucuronic acid. The resulting chloramphenicol glucuronide is then excreted by urine through the kidneys. For the analysis of chloramphenicol in urine, the sample has to be hydrolyzed prior to analysis. Here, chloramphenicol glucuronide is deconjugated by the enzyme glucuronidase and released chloramphenicol can be analyzed. As the antibody used in the test shows cross-reactivity for chloramphenicol glucuronide in urine, it is possible to determine the concentration of chloramphenicol glucuronide in urine by direct testing of urine without sample preparation. For evaluation of the test results, please see further notes under chapter 11. Results.

9.8.1 Direct use for the analysis of chloramphenicol glucuronide in urine

- Mix sample well (vortex)
- If urine is turbid, centrifuge: 10 min / 3,000 g / room temperature (20 - 25 °C)
- Use 50 µl urine per well in the assay
- If obtained results are above standard range, urine samples can be diluted with wash buffer

9.8.2 Hydrolysis for the analysis of chloramphenicol in urine

- Add 1 ml 75 mM potassium phosphate buffer pH 6.8 und 10 µl *Escherichia coli* β-Glucuronidase to 0.1 ml urine in a centrifugal vial and mix
- Hydrolyze for 3 h at 37 °C
- Add 2 ml of ethyl acetate and shake for 10 min upside down
- Centrifuge: 5 min / 1,000 g / room temperature (20 - 25 °C)
- Transfer 1000 µl supernatant into a new vial and evaporate to dryness at 60 °C by nitrogen or air
- Reconstitute the dried residue in 500 µl wash buffer and vortex
- Use 50 µl per well in the assay

9.9. Plasma/Serum (bovine, porcine)

- Add 1 ml ethyl acetate to 500 µl plasma/serum in a 2 ml reaction vial
- Vortex for 1 min
- Centrifuge: 5 min / 3,000 g / room temperature (20 - 25 °C)
- Transfer 700 µl of supernatant into a new vial and evaporate to complete dryness at 60 °C by nitrogen or air
- Reconstitute the dried residue in 350 µl wash buffer and vortex
- Use 50 µl per well in the assay

9.10. Feed

- Grind sample completely
- Add 4 ml ethyl acetate to 1 g of grinded sample in a centrifugal vial
- Vortex for 1 min
- Centrifuge: 10 min / 3,000 g / room temperature (20 - 25 °C)
- Transfer 2 ml of supernatant into a new vial and evaporate at 60 °C to complete dryness by nitrogen or air
- Reconstitute the dried residue in 1 ml hexane
- Add 1000 µl wash buffer and vortex for 1 min
- Centrifuge: 10 min / 3,000 g / room temperature (20 - 25 °C)
- Use 50 µl of the aqueous lower phase per well in the assay

10. Test implementation

10.1 Test preparation

Bring all reagents to room temperature (20 - 25 °C / 68 - 77 °F) before use.

As **wash buffer**, a PBS Tween buffer is needed; please use the enclosed buffer salt (see chapter 4. Reagents provided). To prepare the buffer, dissolve the entire contents of the pouch in 1 l distilled water. The dissolved wash buffer can be stored for approximately 4 to 6 weeks at 2 - 8 °C.

> Alternative: Dissolve the contents of the pouch in 100 ml distilled water (10-fold concentrate). The solution can be stored for approximately 8 - 12 weeks at room temperature (20 - 25 °C). To prepare the ready-to-use solution, mix 1 part of the 10-fold concentrate with 9 parts of distilled water.

Return all reagents to 2 - 8 °C immediately after use.

10.2 Test procedure

Carefully follow the recommended washing procedure. Do not allow microwells to dry between working steps.

1. Insert a sufficient number of wells into the microwell holder for all standards and samples to be run in duplicate. Record standard and sample positions.
2. Add 50 µl of each standard or prepared sample to separate duplicate wells.
3. Add 50 µl of the conjugate to the bottom of each well, mix gently by shaking the plate manually and incubate for 30 min at room temperature (20 - 25 C).
4. Pour the liquid out of the wells and tap the microwell holder upside down vigorously (three times in a row) against absorbent paper to ensure complete removal of liquid from the wells. Fill all the wells with 250 µl washing buffer (see chapter 10.1.) and pour out the liquid again. Repeat two more times.
5. Add 100 µl of substrate/chromogen solution to each well. Mix gently by shaking the plate manually and incubate for 15 min at room temperature (20 - 25 °C) in the dark.
6. Add 100 µl of the stop solution to each well. Mix gently by shaking the plate manually and measure the absorbance at 450 nm. Read within 30 minutes after addition of stop solution.

11. Evaluation

A special software, the **RIDASOFT® Win.NET Food & Feed** (Z9996FF), is optionally available for evaluation of the RIDASCREEN® enzyme immunoassays. The course of the standard curve is shown in the Quality Assurance Certificate (certificate of analysis) enclosed in the test kit.

Remark for the calculation without software:

$$\frac{\text{absorbance standard (or sample)}}{\text{absorbance zero standard}} \times 100 = \%\ \text{absorbance}$$

The zero standard is thus made equal to 100 % and the absorbance values are quoted in percentages. The values calculated for the standards are entered in a semi-logarithmic plot against the chloramphenicol concentration [ng/L].

12. Interpretation of results

In order to obtain the chloramphenicol concentration in µg/l (µg/kg; ppb) actually contained in a sample, the concentration read from the standard curve must be further multiplied by the corresponding dilution factor. When working according to the instructions, the dilution factors are as follows:

Milk	1
Milk powder (reconstitution)	manufacturer's instructions
Milk powder (extraction)	1
Yoghurt, kefir, buttermilk, cream	0.5
Curd, sour cream	1
Butter	5.2
Cheese	1
Honey	1
Royal jelly	1
Meat, fish, shrimp	0.25
Eggs	1
Urine, direct	1
Urine after hydrolysis	10
Plasma/serum	1
Feed	2

Analysis of urine samples

Urine samples contain almost exclusively chloramphenicol glucuronide. Chloramphenicol glucuronide can be determined by the direct use of urine in the test. The concentration value obtained needs to be corrected by the cross reactivity of the antibody for chloramphenicol glucuronide in bovine or porcine urine.

The following formula can be used for calculation:

$$\text{concentration chloramphenicol glucuronide} = \frac{\text{concentration}}{\text{cross reactitivy}}$$

The theoretical concentration of chloramphenicol corresponding to the measured concentration of chloramphenicol glucuronide can be calculated by considering the molar ratio between chloramphenicol and glucuronic acid. The molar ratio is **0.65**.

The following formula can be used for calculation:

concentration chloramphenicol = concentration chloramphenicol glucuronide * 0.65

Due to the correction for the cross-reactivity and the molar ratio, the calculation of the concentration value for chloramphenicol is inaccurate. **To determine the exact concentration of chloramphenicol in urine, hydrolysis of the urine sample is mandatory.**

After taking into account the dilution factor of the sample preparation, the concentration value determined after hydrolysis represents the concentration of chloramphenicol in the urine sample.

13. Recommendation

In order to ensure a high analytical performance we recommend:

- To analyze each sample material in duplicates.
- To do spike experiments to ensure an accurate and correct test procedure.

14. Further application notes

Chloramphenicol can be extracted from shrimp also together with the metabolites (AHD, AMOZ, AOZ and SEM) of the nitrofuran antibiotics in a single sample preparation. The extract can then be used both in RIDASCREEN® Chloramphenicol and in all RIDASCREEN® Nitrofuran ELISAs. The labor and time is thereby significantly reduced. A detailed application is available on request.

Further product information and applications, please contact your local distributor or R-Biopharm at this address: sales@r-biopharm.de.

Version overview

Version number	Chapter and title
2016-04-01	Release version
2016-10-20	General revision
2021-02-16	General revision New chapters: 12. Interpretation of results, 13. Recommendations, 14. Further application notes

Explanation of symbols

- General symbols:

 Follow the instructions for use

 Batch number

 Expiry date (YYYY-MM)

 Storage temperature

 Article number

 Number of test determinations

 Manufacturing date (YYYY-MM)

 Manufacturer + address

Disclaimer

The user assumes all risk in using R-Biopharm AG´s products and services.

R-Biopharm AG will warrant that its products and services meet all quality control standards set by R-Biopharm AG, and R-Biopharm AG will, at its option, replace or repair any components, product or repeat services which prove to be defective in workmanship or material within product specific warranty periods or expiration dates and which our examination shall disclose to our satisfaction to be defective as such.

This warranty is expressly in lieu of all other warranties, expressed or implied, as to quality, description, fitness for any particular purpose, merchantability, productiveness, or any other matter. R-Biopharm AG shall be in no way responsible for the proper use of its products and hereby disclaims all other remedies, warranties, guarantees or liabilities, expressed or implied, arising by law or otherwise, and it shall have no liability for any lost profits or damage, direct, indirect or otherwise, to person or property, in connection with the use of any of its products or services.

This warranty shall not be extended, altered or varied except by a written instrument signed by an authorized representative of R-Biopharm AG.

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Chairman of Supervisory Board:
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Vorstand / Board of Management:
Christian Dreher (Vorsitzender / Chairman),
Dr. Hans Frickel, Jochen Hirsch, Dr. Peter Schubert
Handelsregister / Commercial Register:
Amtsgericht Darmstadt HRB 8321