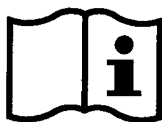
Product information



User's Manual

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Chloramphenicol ELISA

Enzyme Immunoassay for the quantitative determination of Chloramphenicol in food and urine

REF DECAPE02

96

Version 101027
JS
Updated 140310

Sensitivity	0.03 ng/mL
Recovery (spiked samples)	90-115 %
Incubation Time	60 min

1. GENERAL INFORMATION

Due to its outstanding antibacterial properties, chloramphenicol is an often used antibiotic in the production of milk, meat and eggs. In humans it leads to haematotoxic side effects (1,2), like the chloramphenicol induced aplastic anemia. This has caused low limits in Germany, e.g. 1 µg/kg for milk, meat and eggs (3).
Till now the chloramphenicol concentration was determined by radioimmunoassay (4,5) or by gas chromatography (6). However, compared with conventional methods, enzyme immunoassays show some essential advantages (7,8). There is no need to work with radioactive material, the required assay time is shorter and the sensitivity is better than with chromatographic methods.
The **Chloramphenicol** test provides a rapid, sensitive and reliable assay for the determination of chloramphenicol in food and urine. 40 samples can be assayed in duplicate within 60 minutes.

2. PRINCIPLE OF THE TEST

The **Chloramphenicol** quantitative test is based on the principle of the enzyme-linked immunosorbent assay. An antibody binding protein is coated on the surface of a microtiter plate. Chloramphenicol containing samples or standards and an antibody directed against chloramphenicol are given into the wells of the microtiter plate. The chloramphenicol contained in samples or standards will bind to the antibody which reacts with the binding protein coated onto the microtiter plate. After 30 minutes incubation at room temperature a chloramphenicol-peroxidase conjugate is added into the wells without a preceding washing step to saturate free antibody binding sites. After additional 15 minutes incubation at room temperature the wells are washed with diluted washing solution to remove unbound material. A substrate solution is added and incubated for 15 minutes, resulting in the development of a blue colour. The colour development is inhibited by the addition of a stop solution, and the colour turns yellow. The yellow colour is measured photometrically at 450 nm. The concentration of chloramphenicol is indirectly proportional to the colour intensity of the test sample.

3. PRECAUTIONS

Full compliance of the following good laboratory practices (GLP) will determine the reliability of the results:

1. Prior to beginning the assay procedure, bring all reagents to room temperature (20-25°C).
2. All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
3. Once the assay has been started, all subsequent steps should be completed without interruption and within the recommended time limits.
4. Replace caps in all the reagents immediately after use. Do not interchange vial stoppers.
5. Use a separate disposable tip for each specimen to prevent cross-contamination.
6. All specimens and standards should be run at the same time, so that all conditions of testing are the same.
7. Do not mix components from different batches.
8. Do not use reagents after expiration date.
9. Check both precision and accuracy of the laboratory equipment used during the procedure (micropipets, ELISA reader etc.).

4. HEALTH AND SAFETY INSTRUCTIONS

1. Do not smoke or eat or drink or pipet by mouth in the laboratory.
2. Wear disposable gloves whenever handling patient specimens.
3. Avoid contact of substrate and stop solution with skin and mucosa (possible irritation, burn or toxicity hazard). In case of contact, rinse the affected zone with plenty of water.
4. Handling and disposal of chemical products must be done according to good laboratory practices (GLP).

5. REAGENTS

The kit contains reagents for 96 determinations. They have to be stored at 2-8℃. Expiry data are found on the labels of the bottles and the outer package.

1. Microtiter plate consisting of 12 strips with 8 breakable wells each, coated with an antibody binding protein.
2. Chloramphenicol Standards (0; 0.05; 0.1; 0.5; 1; 5 ng/mL): 6 vials with 1 mL each, dyed red, ready-to-use.
3. Anti-Chloramphenicol Antibody (sheep): 6 mL, dyed red, ready-to-use.
4. Conjugate (Chloramphenicol-Peroxidase): 6 mL, dyed red, ready-to-use.
5. Substrate Solution (TMB): 15 mL, prestained red, ready-to-use.
6. Stop Solution (1 N acidic solution): 15 mL, ready-to-use.
7. Sample Diluent (PBS): 2 x 60 mL, dyed red, ready-to-use.
8. Washing Solution (PBS + Tween 20): 60 mL as 10x concentrate. Dilute 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up to 37℃ for 15 minutes.
9. Two plastic foils to cover the strips during the incubation.
10. Plastic bag to store unused microtiter strips.
11. Instruction Manual.

6. ADDITIONAL INSTRUMENTATION AND REAGENTS (NOT PROVIDED)

Instrumentation

- 50, 100, 500 and 1000 µL-micropipets
- ELISA reader (450 nm)
- Centrifuge
- Ultra-Turrax, mixer
- Evaporator

Reagents

- Double distilled water
- Ethyl acetate
- n-Hexane
- Potassiumhexacyanoferrate(II)-3-hydrate (150 g/L; Carrez I)
- Zincsulfate-7-hydrate (300 g/L; Carrez II)

7. SAMPLE PREPARATION

Milk (direct assay)

- Refrigerate fresh milk samples at 2-8°C and centrifuge afterwards at 3000 g for 10 minutes.
- Remove the upper fat layer and test the sample directly in the ELISA after warming to room temperature. For skimmed milk samples the centrifugation step can be omitted.
- Sample dilution factor: F=1

Milk (Ethyl acetate extraction)

- Add 250 µL Carrez I to 5 mL milk sample, mix well and add 250 µL Carrez II afterwards.
- Mix sample, refrigerate to 2-8°C and centrifuge at 3000 g for 10 minutes.
- Transfer 4.4 mL of the clear supernatant to a clean glass vial, add 8 mL ethyl acetate and agitate vigorously for 10 minutes.
- For phase separation centrifuge for 10 minutes at 3000 g (room temperature).
- Transfer 4 mL of the upper ethyl acetate phase to a clean glass vial and evaporate the solvent at 50-70°C under a nitrogen stream to dryness.
- Dissolve the dry residue with 400 µL sample diluent by shaking vigorously and test the sample in the ELISA.
- Sample dilution factor: F=0.2

Honey

- Dissolve 2 g honey in 4 mL double distilled water.
- Add 4 mL ethyl acetate and agitate vigorously for 10 minutes.
- Transfer 1 mL of the upper ethyl acetate phase to a clean glass vial and evaporate the solvent at 50-70°C under a nitrogen stream to dryness.
- Dissolve the dry residue with 500 µL sample diluent by shaking vigorously and test the sample in the ELISA.
- Sample dilution factor: F=1

Shrimps, Meat, Fish Meal

- Mill and homogenize sample with an appropriate device (mixer, ultra-turrax).
- Mix 3 g sample with 3 mL double distilled water, add 6 mL ethyl acetate and agitate vigorously for 10 minutes.
- For phase separation centrifuge for 10 minutes at 3000 g (room temperature).
- Transfer 4 mL of the upper ethyl acetate phase to a clean glass vial and evaporate the solvent at 50-70°C under a nitrogen stream to dryness.
- Add 1 mL n-hexane to the residue.
- Add 500 µL sample diluent to the mixture and agitate vigorously for 1 minute.
- For phase separation centrifuge for 10 minutes at 3000 g (room temperature).
- Test the lower, aqueous phase in the ELISA.
- Sample dilution factor: F=0.25

Whole Egg (raw)

- Homogenize sample with an appropriate device (mixer, ultra-turrax).
- To 2 g sample add 12 mL ethyl acetate and agitate vigorously for 10 minutes.
- For phase separation centrifuge for 10 minutes at 3000 g (room temperature).
- Transfer 6 mL of the upper ethyl acetate phase to a clean glass vial and evaporate the solvent at 50-70°C under a nitrogen stream to dryness.
- Add 1 mL n-hexane to the residue.
- Add 1 mL sample diluent to the mixture and agitate vigorously for 1 minute.
- For phase separation centrifuge for 10 minutes at 3000 g (room temperature).
- Test the lower aqueous phase in the ELISA.
- Sample dilution factor: F=1

Urine

- Centrifuge the samples at 3000 g for 10 minutes.
- Test the clear supernatant directly in the assay.
- Sample dilution factor: F=1

8. PROCEDURE

1. Prepare samples as described above.
2. Pipet 100 µL standards or prepared samples in duplicate into the appropriate wells of the microtiter plate. Immediately add 50 µL anti-chloramphenicol antibody into each well.
3. Cover the microtiter plate with a plastic foil and incubate for 30 minutes at room temperature.
4. Without preceding washing add 50 µL chloramphenicol-peroxidase conjugate into each well.
5. Cover the microtiter plate with a plastic foil and incubate additional 15 minutes at room temperature.
6. Wash the plate three times as follows: Discard the contents of the wells (dump or aspirate). Pipet 300 µL of diluted washing solution into each well. After the third repetition empty the wells again and remove residual liquid by striking the plate against a paper towel. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbencies.
7. Pipet 100 µL of substrate solution into each well.
8. Allow the reaction to develop in the dark (e.g. cupboard or drawer; the chromogen is light-sensitive) for 15 minutes at room temperature.
9. Stop enzyme reaction by adding 100 µL of stop solution (1 N acidic solution) into each well. The blue colour will turn yellow upon addition.
10. After thorough mixing, measure absorbance at 450 nm (reference wavelength 620 nm), using an ELISA reader. The colour is stable for 30 minutes.

9. CALCULATION OF RESULTS

1. Calculate the average optical density (OD 450 nm) for each set of reference standards or samples.
2. Construct a standard curve by plotting the mean optical density obtained for each reference standard against its concentration in ng/mL on semi-log graph paper with the optical density on the vertical (y) axis and the concentration on the horizontal (x) axis.
3. Using the mean optical density value for each sample, determine the corresponding concentration of chloramphenicol in ng/mL from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.
4. The diluted samples must be further converted by the appropriate sample dilution factor. The factors are listed for each sample matrix in the sample preparation section.

Note: Due to the extraction with ethyl acetate negative samples may show a certain blank value. In repetitive performed experiments with negative samples for each matrix the following blank values were identified.

Milk (direct assay)	< 0.1 ng/mL
Milk (ethyl acetate extraction)	< 0.1 ng/mL
Honey	< 0.2 ng/g
Shrimps	< 0.2 ng/g
Meat	< 0.2 ng/g
Fish meal	< 0.2 ng/g
Whole egg	< 0.05 ng/g
Urine	< 0.2 ng/mL

These values are defined as the cut-off of the method for the respective matrices. Lower concentrations have to be considered as negative.

10. TYPICAL STANDARD VALUES

The following table contains an example for a typical standard curve. The binding is calculated as percent of the absorption of the 0 ng/mL standard. These values are only an example and should not be used instead of the standard curve which has to be measured in each new test.

Chloramphenicol (ng/mL)	% binding of 0 ng/mL)
0	100
0.05	84
0.1	70
0.5	28
1	17
5	8

11. PERFORMANCE

Sensitivity
The sensitivity of the **Chloramphenicol test** is 0.03 ng/mL (based on the standard curve).

Recovery

Milk (direct assay)	94 %
Milk (ethyl acetate extraction)	98 %
Honey	98 %
Shrimps	96 %
Meat	108 %
Fish meal	90 %
Whole Egg	95 %
Urine	90 %

Intra-assay Precision
The intra-assay variation of the chloramphenicol test was determined to 8%.
Cross-reactivity

Cross-reactivity	relative to chloramphenicol (=100%)
Chloramphenicol Glucuronide	88%
Chloramphenicol Base	< 0.1%
Ampicillin	< 0.1%
Penicillin	< 0.1%
Tetracycline	< 0.1%

12. REFERENCES

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