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Brief Communication

A RAPID AGAR-DIFFUSION TEST FOR THE DETECTION OF ANTIBIOTIC RESIDUES IN KIDNEYS FROM SLAUGHTER-ANIMALS

The microbiological agar diffusion methods for the detection of antibiotic residues in milk and meat have been used for many years. Various sensitive test-organisms e.g. Micrococcus luteus, Bacillus subtilis, Bacillus cereus, Bacillus stearothermophilus var. calidolactis, etc. have been used in these methods. Some of the methods have been developed as rapid tests, where results can be determined in 2—4 h (*Kundrat* 1972), but these methods have so far not been applicable to slaughter-animals. So there is a need for a rapid screening method for the detection of antibiotics in kidneys from slaughter-animals to reveal positive findings before the carcases are ready for dispatch from the slaughter-houses.

The screening method described here utilizes the high sensitivity and rapid growth of a Bacillus subtilis strain (a laboratory strain, IMM, B.s. 1) which furthermore is used in a viable count of about 10^7 per ml of assay agar, or about 100—1000 times more than ordinary test-organisms (*Justesen* 1973). In order to improve the sulphonamide sensitivity, trimethoprim is added to the medium (*Gudding* 1976).

Technique. The B. subtilis is daily transferred to fresh nutrient broth and should multiply to a viable count of 5×10^7/ml broth during 18—24 h incubation at 37 °C.

Antibiotic assay agar:

NZ-amin type B (Sheffield Chemicals)	2.5 g
Pottasium chloride	3.3 g
Disodium hydrogenphosphate, 12 H_2O	8.0 g
Agar (gelidium, Litex)	3.3 g
Yeast hydrolysate (Orthana)	1.25 g
Destilled water	1 l
Final pH 7.4	

The substrate is autoclaved for 20 min after which 15 ml of a 21 % sterile-filtered glucose solution and 0.25 ml of a trimethoprim solution (100 µg/ml) are added; 80 ml of the medium is poured in 14 cm Petri dishes, corresponding to an agar depth of 5 mm. The plates can be stored in the refrigerator for about a

week. Before use 13 ml of the antibiotic assay agar at 45°C is mixed with 2 ml of the B. subtilis overnight culture and poured on top of the dried plate.

Kidney samples or antibiotic discs are then placed on the agar surface. The kidney samples sized about 5 × 10 × 10 mm are taken aseptically on the medulla-cortex border by the use of a scalpel or a cork-drill. As antibiotic discs are used 6 mm filter paper discs (Schleicher & Schuell, no. 2668), on which 20 µl of an antibiotic solution is pipetted. A maximum of 8 kidney samples and 2 discs could be placed on each plate. Routinely we have used discs with, respectively, 0.025 units penicillin G and 2.0 µg sulphonamide as controls. All investigations on kidney samples were performed in duplicates. The test plates are incubated at 37°C for 3½ h without preincubation in the refrigerator. When paper discs are used the diameter of the inhibition zone is read, whereas in kidney samples the distance from the edge of the kidney to the growth zone is measured. An inhibition zone of 1.5 mm or less is regarded as unspecific reaction in the rapid method.

Table 1. The lowest amount of 10 different antibiotics, which gives inhibition zones of 8 mm or more in the rapid test.

Antibiotic	µg antibiotic on disc	Antibiotic	µg antibiotic on disc
Penicillin G	0.006	Tetracycline	0.3
Streptomycin	0.4	Sulfamethizol	0.5
Neomycin	0.05	Trimethoprim	0.025
Spiramycin	0.1	Ronidazol	1.0
Chloramphenicol	0.5	Tylosin	0.1

To test the performance of this method, paper discs with varying concentrations of different antibiotics were investigated. The results are given in Table 1. The amounts of antibiotics, which could be measured by the rapid method, are in the same range as the ones measured by the conventional 18 h Dutch so-called "four plate method", proposed as EEC-method (*van Schothorst et al.* 1978).

The described method has for a period of 8 months been compared to the proposed EEC-method on kidneys from slaughter-animals, suspected or known to have been treated with antibiotics prior to slaughter. The results are given in Table 2. The rapid method gives a slightly smaller number of unspecific reactions (i.e. $\leq$ 1.5 mm), which instead are read as no inhibition. This is prob-

Table 2. Results of examination of 61 kidneys from slaughter-animals suspected or known to have been treated with antibiotics prior to slaughter.

	No inhibition	Unspecific inhibition	Specific inhibition
Conventional Dutch method	11/61	35/61	15/61
Rapid method	16/61	30/61	15/61

ably due to the smaller inhibition zones obtained by the rapid method compared to the conventional method. But it should be noted that there is total agreement for all the kidneys with specific reactions (i.e. > 1.5 mm). As total agreement has been found between specific reactions in the 2 methods, the rapid method can be used as a field screening method for detection of antibiotic residues in kidneys from slaughter-animals.

Other media, e.g. PDM Antibiotic Sensitivity Medium (AB Biodisk, Solna) and Oxoid Isosensitest agar, have been tested with antibiotic-containing paper discs, and they gave similar results when trimethoprim was used in the above mentioned concentrations. The Mueller-Hinton medium, however, gave smaller inhibition zones.

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