

Test Agar pH 8.0 for the Inhibitor Test

For the detection of antimicrobial inhibitors in meat and organ samples together with *Bacillus subtilis* (BGA) spore suspension and *Micrococcus luteus* ATCC 9341 as test organisms.

The nutrient media are suitable both, for the inhibitor test (LEVETZOW, 1971) according to the German Meat Inspection Law as well as for the EEC Four-Plate-Test (BOGAERTS and WOLF, 1980) suggested by the Scientific Veterinary Commission of the European Economic Community. Test agar pH 7.2 with addition of trimethoprim is used particularly for determination of sulfonamide residues.

Mode of Action

The test is carried out according to the agar diffusion procedure. Small slices of the meat sample are placed on the inoculated Test Agar plates and incubated. Antimicrobial inhibitors contained in the samples diffuse into the nutrient media and cause growth-free inhibition zones to develop on the otherwise thickly covered plates. Repeated tests with pH 6.0, pH 8.0 and pH 7.2 are necessary, as penicillin and streptomycin are optimally active at pH 6.0 and 8.0 respectively (PICHNARCIK et al., 1969) and the activity optimum of sulfonamide is found at pH 7.2. Addition of trimethoprim to Test Agar pH 7.2 considerably increases the sensitivity of the test system to sulfonamides (GUDDING, 1976; EBRECHT, 1982).

Typical Composition (g/litre)

Peptone from casein, tryptic 3.45; peptone from meat, tryptic 3.45; sodium chloride 5.1; tri-sodium phosphate 12-hydrate 2.4; agar-agar 13.0.

Preparation

Suspend 27.5 g/litre Test Agar pH 8.0, autoclave (15 min at 121 °C), test the pH and if necessary adjust. Cool to 50-45 °C, mix in 1 ml/litre *Bacillus subtilis* (BGA) spore suspension. If necessary add *Micrococcus luteus* ATCC 9341 (microbial count in culture medium: approximately 104 cfu/ml) to Test Agar pH 8.0.

After mixing the spore suspension, immediately pour the plates and place in the refrigerator.

The plates are clear and yellowish-brown.

Storage of the Plates

The ready-to-use plates can be sealed with air-tight adhesive tape and, when cooled (+4 to +6 °C) may be kept for up to 2 weeks. Additional packing into plastic bags is recommended. If stored for longer periods, the temperature should not be more than +3 °C; however, freezing of the culture medium must be avoided.

Experimental Procedure and Evaluation

Inhibitor Test

Test Agar pH 8.0 with *Bacillus subtilis* (BGA)

EEC Four-Plate-Test

Test Agar pH 8.0 with *Bacillus subtilis* (BGA) and Test Agar pH 8.0 with *Micrococcus luteus* ATCC 9341.

For details regarding the collection of samples, transportation as well as the execution of the test, see the Fleischbeschauengesetz (Meat Inspection Law) or BOGAERTS and WOLF (1980).

According to these specifications the cylinder-shaped tissue sections (8 mm in diameter and 2 mm thick) are stamped out under conditions, which are as aseptic as possible and laid on the plates; according to BOGAERTS and WOLF two sections are required per plate. As a control, one test disc with 10 IU of penicillin G-sodium is placed on a plate with pH 6.0, one test disc with 10 µg of streptomycin on each of the two plates with pH 8.0 and one test disc with 0.5 µg sulfadimidine on a plate with pH 7.2. The test discs can also be made by the user using filter paper discs, 6 mm in diameter.

Incubation: 18-24 hours at 30 °C (*Bacillus subtilis* BGA) and 37°C (*Micrococcus luteus* ATCC 9341).

Manufacturers	Product
Burroughs Wellcome, GB	Trimethoprim
Intern. Chemical Industries, GB	Sulfadimidine
Schleicher & Schüll, No. 2668, FRG	Filter paper discs 6 mm in diameter
American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852, USA	<i>Micrococcus luteus</i> ATCC 9341
Beiersdorf AG, Hamburg, FRG	Adhesive tape Tesaflex 166

The inhibition zones between the edge of the tissue section or the test disc and the growth limit of the test organism are measured. Complete inhibition of growth with an inhibitory zone of at least 2mm is regarded as positive result, an inhibitory zone of 1-2mm should be considered as doubtful. This only applies, however, if the controls, prepared at the same time, display zones of inhibition measuring about 6 mm.

For possible methodological improvements see FORSCHNER and SEIDLER (1976).

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Literature

Arbeitsgruppe des Instituts für Lebensmitteltechnologie und Verpackung der Technischen Universität München: Merkblätter für die Prüfung von Packmitteln, Merkblatt 18 "Prüfung auf antimikrobielle Bestandteile in Packstoffen". - **Verpackgs.-Rdsch.**, **25**; Techn.-wiss. Beilagen; 5-8 (1974).

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Ordering Information

Product	Merck Cat. No.	Pack size
Test Agar pH 8.0 for the Inhibitor Test	1.10664.0500	500 g
Bacillus subtilis (BGA)-spore suspension	1.10649.0001	15 x 2 ml
Penicillin G potassium salt	CN Biosciences	
Streptomycin sulfate	CN Biosciences	

Quality control

Test strains	Growth	Inhibition zones in mm Ø			
		Gentamicin		Penicillin	Streptomycin
		10 µg	30 µg	10 IU	10 µg
Bacillus subtilis strain BGA (DSMZ 618)	good / very good	36-44	38-47	34-45	30-36
Micrococcus luteus ATCC 9341	good / very good	28-36	32-40	50-60	30-36



BGA



Micrococcus luteus ATCC 9341