

PENGUJIAN RESIDU ANTIBIOTIKA DENGAN METODE BIO-ASSAY (SKRINING)

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RUANG LINGKUP

Standar ini menetapkan metoda uji tapis residu antibiotika secara *Bioassay* pada daging, telur dan susu. Pengujian ini meliputi antibiotika golongan penisilin, tetrasiklin, aminoglikosida dan makrolida.

PRINSIP

Residu antibiotika akan menghambat pertumbuhan mikroorganisme pada media agar. Penghambatan dapat dilihat dengan terbentuknya daerah hambatan disekitar kertas cakram, *silinder cup*, *agar well*. Besarnya diameter daerah hambatan menunjukkan konsentrasi residu antibiotika. Pengujian ini harus dilakukan secara aseptis didalam ruangan steril.

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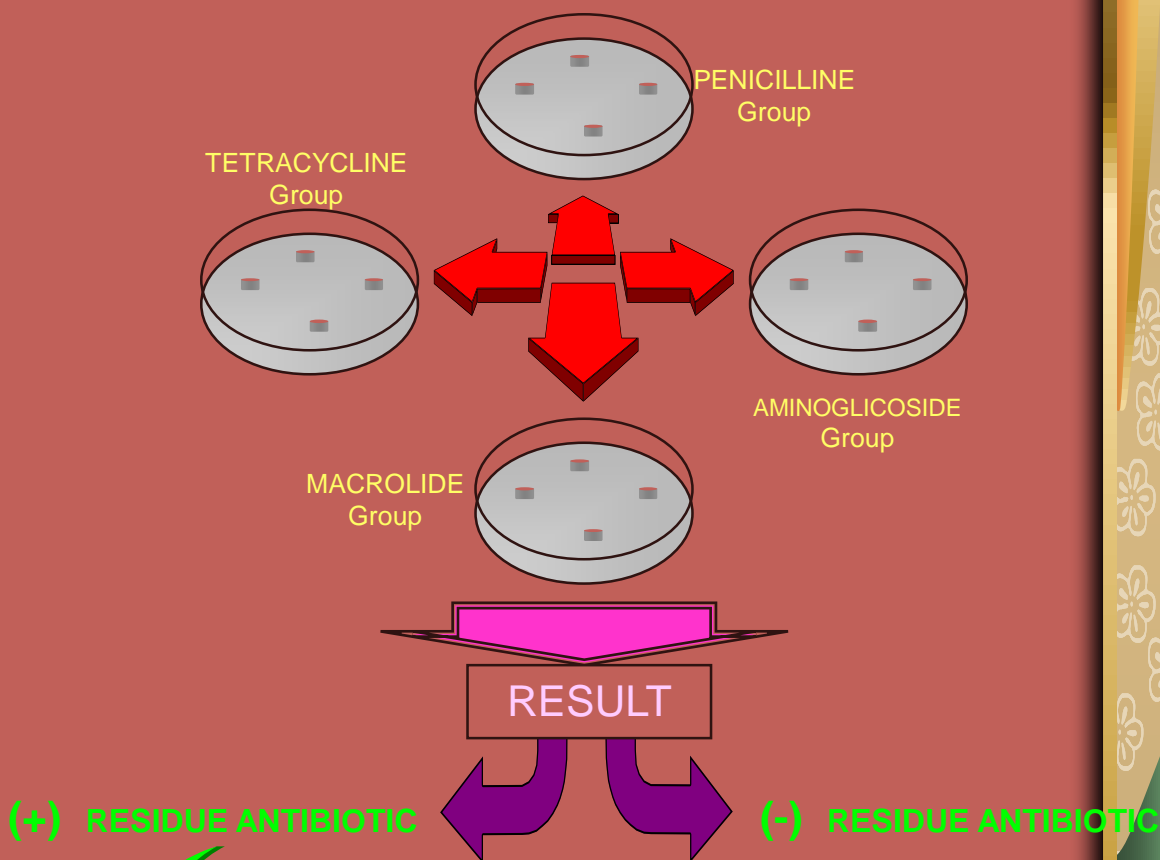
TAHAPAN PENGUJIAN BIO-ASSAY

- Preparasi Standar Antibiotika
- Preparasi Sampel
- Preparasi Spora/ Kuman
- Pengujian
- Pengamatan Hasil

METHODS OF ANTIBIOTIC RESIDUE TEST

SCREENING TEST

FOUR PLATE METHODS (Bioassay)



CONFIRMATION TEST

(Kind and Concentration)

GC

HPLC/ TLC

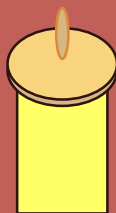
LC, etc.....

PREPARATION OF STANDARD SOLUTION

OTC-HCL (Oxytetracycline-HCl) = 1000 µg/ml

KM - SO₄ (Kanamycine Sulfate) = 1000 µg/ml

TS-tartrate (Tylosin tartrate) = 1000 µg/ml



- * Weigh ± 10 mg
- * Multiply the Potency
- * Dissolve with Buffer No. 3 to KM 10% MeOH/ DW to TS and DW steril to OTC
- * Dilute to obtain concentration of 1000 µg/ml

Stock Solution

Transfer 2 ml
into tube

Stock Solution
(2000 µg/ml)

Store at Freezer -20 °C

Transfer 2 ml
into tube contain
of 18 ml Buffer
No. 2

Transfer 2 ml
into tube contain
of 18 ml Buffer
No. 2

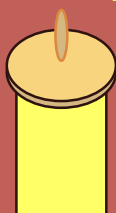
Dilute with 18 ml of
Buffer No. 2
(100 µg/ml)

(10 µg/ml)

(1 µg/ml)

Working Solution

PC-na (Penicilline Sodium) = 1000 iu/ml



- * Weigh ± 10 mg
- * Multiply the Potency
- * Dissolve with Buffer No. 1
- * Dilute with same Buffer to obtain concentration of 1000 iu/ml

Stock Solution

Transfer 2 ml
into tube

Stock Solution
(2000 iu/2 ml)

Store at Freezer -20 °C

Transfer 2 ml
into tube contain
of 18 ml Buffer
No. 2

Transfer 2 ml
into tube contain
18 ml Buffer
No. 2

Transfer 2 ml
into tube contain
18 ml Buffer
No. 2

Transfer 2 ml
into tube contain
18 ml Buffer
No. 2

Dilute with 18 ml of
Buffer 2

(100 iu/ml)

(10 iu/ml)

(1 iu/ml)

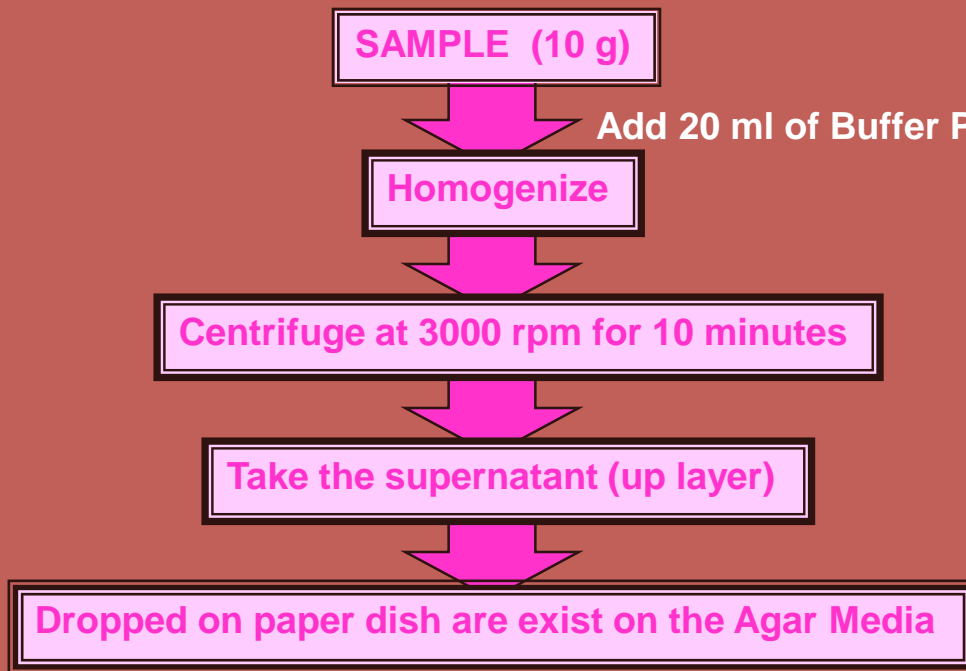
(0.1 iu/ml)

(0.01 iu/ml)

Working Solution

PREPARATION OF SAMPLE

EXTRACTION OF EDDIBLE TISSUES AND EGG SAMPLES



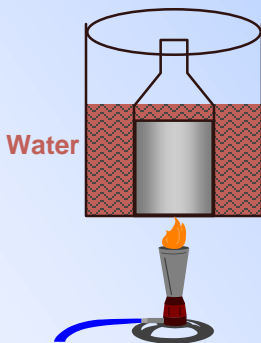
EXTRACTION OF MILK

Milk does not need to be extracted
Just dropped on the paperdish are exist on the agar media

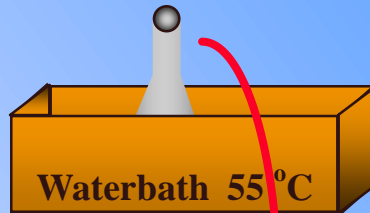
ASSAY STEPS OF ANTIBIOTIC RESIDUES IN LIVESTOCK PRODUCTS

Prepare and weigh the materials needed to make the Agar Medium of each Antibiotic Groups Mix, Dissolve and Shake well, then measure the pH

Boil and Sterilize by Autoclave



Transfer 100 ml (need)
Heat



1. Transfer 8 ml into Petridish

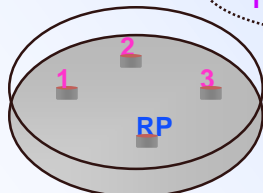
Add the Suspension of Test Microorganism
Shake to Homogenous

3. Placed the Steril Paperdish on the Culture Surface

Drop 75 µl of the tested samples and Working Standard

2. Let it to get solid room temp. for 30 minute - 1 hours

RP = Reference Point
1,2,3 = Tested Sample



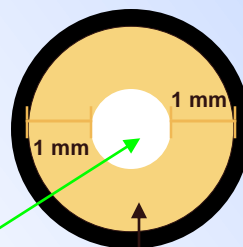
INCUBATE for 18 hours

<i>B. cereus</i>	at 30°C
<i>B. subtilis</i>	at 36°C
<i>kocuria rhizophila</i>	at 36°C
<i>B. steartothermophilus</i>	at 55°C

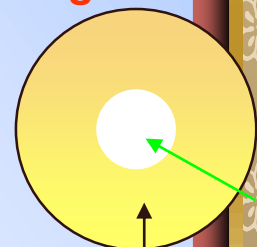
JUDGEMENT

Positive Residue

Negative residue



Inhibition Zone
(at least 2 mm larger than the paperdish diameter)



No Inhibition Zone

Cara Perhitungan Penimbangan Standar

Contoh :

Diketahui ;

- Potensi Antibiotika $884 \mu\text{g}/\text{mg}$
- Berat standar yang ditimbang 10 mg

Ditanyakan ;

Berapa volume pelarut yang ditambahkan untuk membuat larutan standar $1000 \mu\text{g}/\text{ml}$?

Jawab :

$$884 \mu\text{g}/\text{mg} \times 10 \text{ mg} = 8840 \mu\text{g}$$

$$8840 \mu\text{g} : 1000 \mu\text{g}/\text{ml} = 8,840 \text{ ml}$$

Jawab :

Berarti, standar yang telah ditimbang 10 mg dilarutkan dengan pelarutnya sebanyak 8,840 ml untuk menghasilkan konsentrasi 1000 $\mu\text{g}/\text{ml}$

Cara Perhitungan Pengenceran Standar :

Stok yang ada 1000 $\mu\text{g}/\text{ml}$, buat pengenceran menjadi 100; 10; 1; 0,1 dan 0,01 $\mu\text{g}/\text{ml}$

Rumus :

$$V1 \times N1 = V2 \times N2$$

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$$V1 \times N1 = V2 \times N2$$

$$1 \text{ ml} \times 1000 \mu\text{g/ml} = V2 \times 100 \mu\text{g/ml}$$

$$V2 = 10 \text{ ml}$$

$$V2 \times N2 = V3 \times N3$$

$$1 \text{ ml} \times 100 \mu\text{g/ml} = V2 \times 10 \mu\text{g/ml}$$

$$V3 = 10 \text{ ml}$$

$$V3 \times N3 = V4 \times N4$$

$$1 \text{ ml} \times 10 \mu\text{g/ml} = V4 \times 1 \mu\text{g/ml}$$

$$V4 = 10 \text{ ml}$$

 Rumus :

$$V4 \times N4 = V5 \times N5$$

$$1 \text{ ml} \times 1 \mu\text{g/ml} = V6 \times 0,1 \mu\text{g/ml}$$

$$V5 = 10 \text{ ml}$$

$$V5 \times N5 = V6 \times N6$$

$$1 \text{ ml} \times 0,1 \mu\text{g/ml} = V6 \times 0,01 \mu\text{g/ml}$$

$$V6 = 10 \text{ ml}$$

*SELAMAT BERLATIH.....
SEMOGA BERMANFAAT*

