Tetracycline Residue In lamb Meat

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Introduction

- **Human health, is the main subject for almost all the studies about human beings, studying animal sciences is not just neither to be a humane nor to save animals lives only, but because animal food products is a major part of our food chain.**

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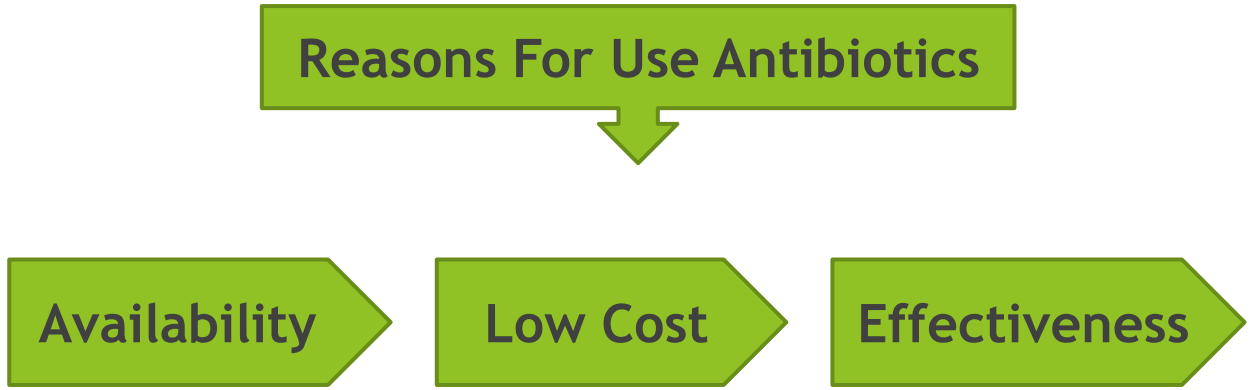
Introduction

- Antibiotics are important medications and compounds that play a significant role in the life of animals to maintain its life by inhibiting or killing bacteria that enter the animal's body.



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Introduction

- There are several different routes by which antibiotics are administered to animals (injections, orally, topically on the skin and by intra-mammary and intrauterine infusions).

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- Theoretically, all of these routes may lead to residues appearing in foods of animal origin such as milk, meat and eggs if withdrawal periods are not observed.

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Pathological Effects produced by Antibiotic Residues in Food

Effects of residues	Antibiotics
Transfer of antibiotic resistant bacteria to the human	
Autoimmunity	
Immunopathological effects	
Carcinogenicity	(Sulphamethazine, Oxytetracycline, Furazolidone)
Mutagenicity	
Nephropathy	Gentamicin
Hepatotoxicity	
Reproductive disorders	
Bone marrow toxicity	Chloramphenicol
Allergy	Penicillin

(Nisha A.R.)

Maximum Residues Limit (MRL) (ug/kg) For Veterinary Residues.

(Antibiotic Residues - AGlobal Health Hazard Nisha A.R.)

ANTIBIOTIC	MRL
Benzyl penicillin	4
Ampicillin	4
Amoxycillin	4
Oxacillin	30
Cloxacillin	30
Dicloxacillin	30
Tetracycline	100
Oxytetracycline	100
Chlortetracycline	100
Streptomycin	200
Dihydrostreptomycine	200
Gentamycine	200
Neomycin	100
Sulphonamides	100
Trimethoprime	50
Spiramycin	200
Tylosine	50
Erythromycine	40
Quinalones	75
Polymyxine	50
Ceftiofur	100
Cefquinome	20
Nitrofurans	0
Nitromidazoles	0
Other chemotherapeutics (Chloramphenicol, Novobiocine)	0

Aims of the study

- The main purpose of this study is to detect the existence of Tetracycline residues in lambs meat.
- To approve that this test is easy, and applicable.

Previous Studies

- In 2012 alone, more than 50 notifications reporting the detection of veterinary medicinal products – including antibiotics in food or feed in the EU were published via the European Rapid Alert System for Food and Feed (RASFF)

(Maike Claußen1 , Dominic Bahmann2 and Stefan Schmidt)

Methods of Detection of Antibiotic Residues

- ELISA.
- HPLC.
- **Biological tests: 5 Plates method.**

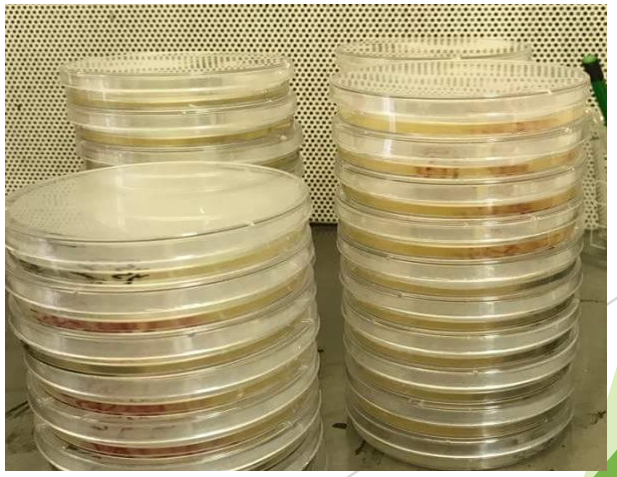
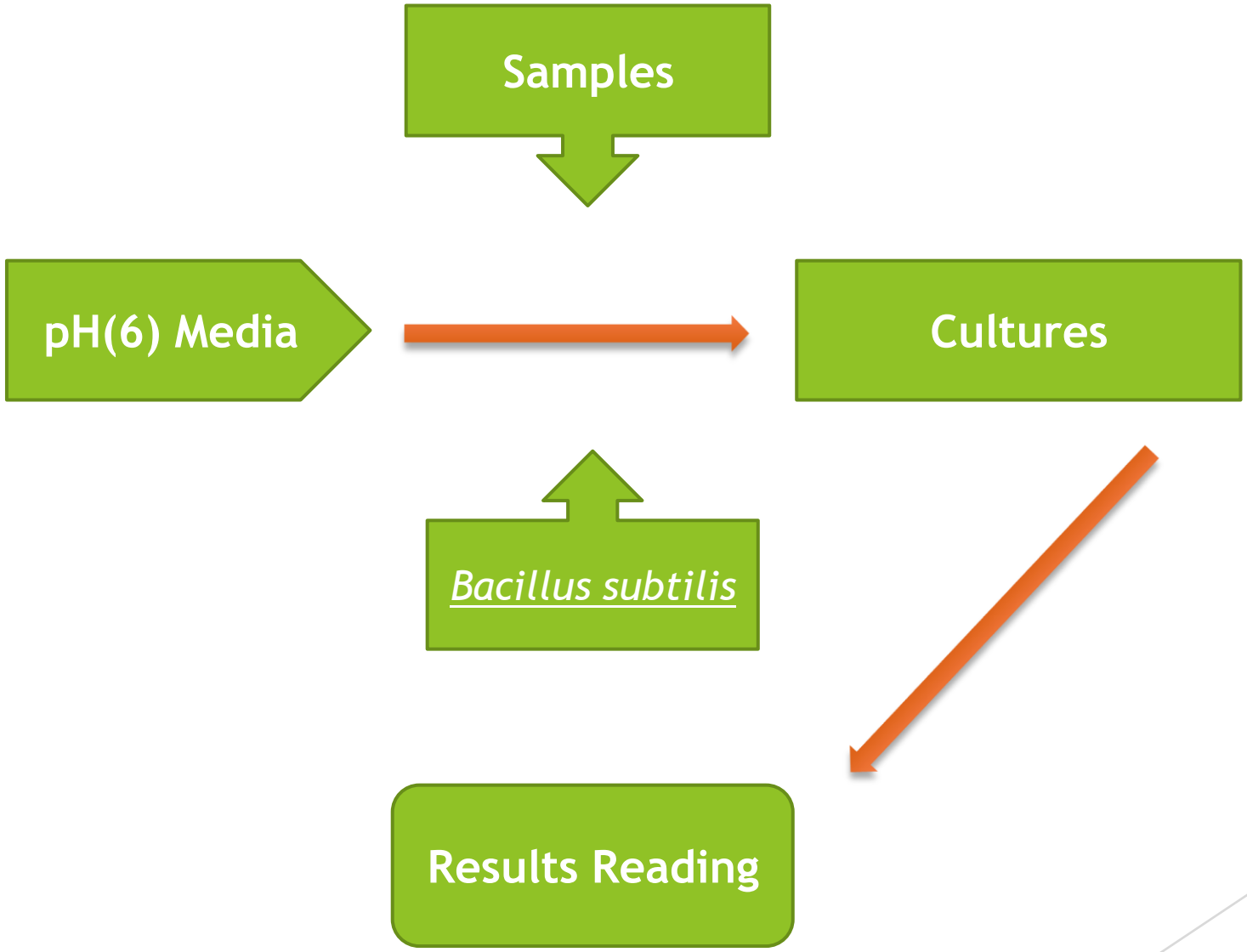


Plate	Media	pH of media	Bacteria	Antibiotic detect
1	Merck 10663	6 ± 0.1	B. subtilis	1) Tetra. 2) ß-lactams
2	Merck 105270 (DST)	7.4 ± 0.1	B. subtilis	1) _____ 2) Tetra. Aminogly.
3	Merck 10664	8 ± 0.1	B. subtilis	1) Aminogly. 2) Macrolides
4	Merck 10664	8 ± 0.1	K. rhizopHila	1) Macrolides ß-lactams
5	Merck 10664	8 ± 0.1	E. coli	1) Fluorqinolones

The Method That We USED!

(Diez, et al. Five-plate screening test for the detection of antibiotic residues.)

Plate	Media	pH of media	Bacteria	Antibiotic detect
1	Merck 10663	6 ± 0.1	B. subtilis	1) Tetra. 2) β-lactams


Samples
pH(6) Media
Cultures
Bacillus subtilis
Results Reading

Materials & Methods

The Bacteria *Bacillus subtilis*

Was already isolated from the soil at the microbiologic lab of the Science collage at An-Najah National University.

Bacillus subtilis

Hay Bacillus, Found in Soil and GIT of Ruminants and Humans

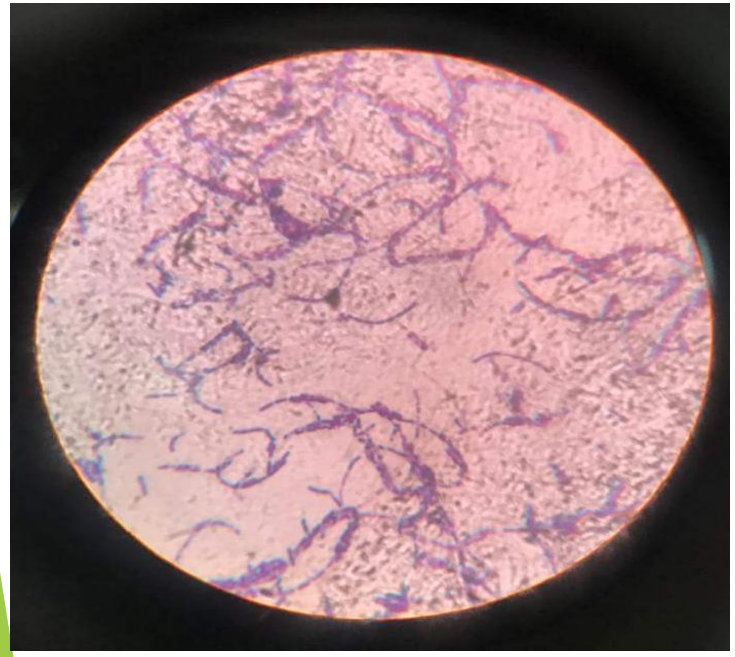
Gram +, Catalase +, Facultative anaerobe, rod-shaped

Materials & Methods

To make sure that the bacteria we brought was *Bucillus subtilis* Identification Tests were applied:

Gram Stain Test

Gram +ve , Rod shaped



Catalase Test

Catalase +ve

Materials & Methods

- After Identification Tests, sub culture to nutrient broth, and incubation over night were applied.
- Next day the broth was tested by Spectrophotometer at 600 nm to check the concentration and adjust it to 10^7 cfu\ml.
- So After mixing 1ml of the broth to 1L of bacteria the final concentration is 10^4 cfu\ml. (Diez, et al. Five-plate screening test for the detection of antibiotic residues.)

Materials & Methods

Test Agar pH 6.0 for the Inhibitor Test

Merck Millipore10663

Typical Composition	(g/L)
Peptone from casein	3.45
Peptone from meat	3.45
Sodium chloride	5.1
Agar-agar	13.0

(Merck Millipore10663 media data sheet)

Materials & Methods

Preparation

Suspend 25 g/liter Test Agar pH 6.0, autoclave (15 min at 121°C), test the pH and if necessary adjust.
Cool to 50-45 °C, mix in 1ml/liter Bacillus subtilis suspension.
After mixing the suspension, immediately pour the plates and place in the refrigerator.
The plates are clear and yellowish-brown.
The thickness of the media was approximately 2mm.

(Merck Millipore10663 media data sheet)

Materials & Methods

Mode of Action of Media pH (6)

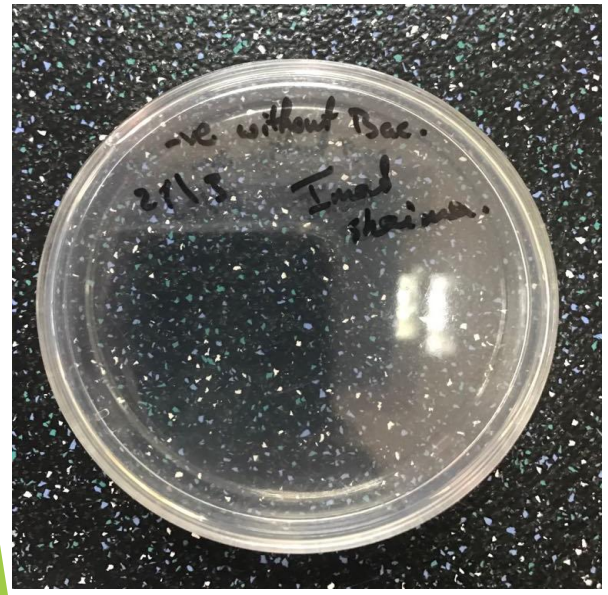
- The test is carried out according to the agar diffusion procedure.
- Small slices of the meat sample are placed on the inoculated Test Agar plates and incubated.
- Antimicrobial inhibitors contained in the samples diffuse into the nutrient media and cause growth-free inhibition zones to develop on the otherwise thickly covered plates.

(Merck Millipore10663 media data sheet)

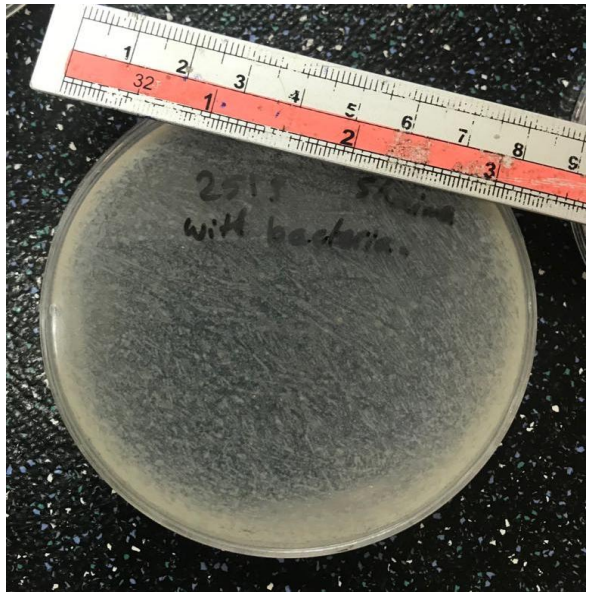
Materials & Methods

Control Samples

1) Negative Sample Without Bacteria.
2) Negative Sample With Bacteria.
3) Positive Sample. (Two Oxoid Tetracycline 30µg)



1



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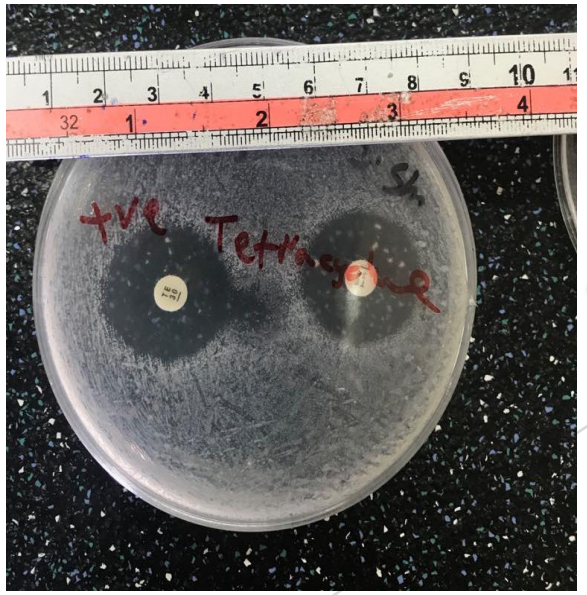


3

Inhibition Zone
23mm

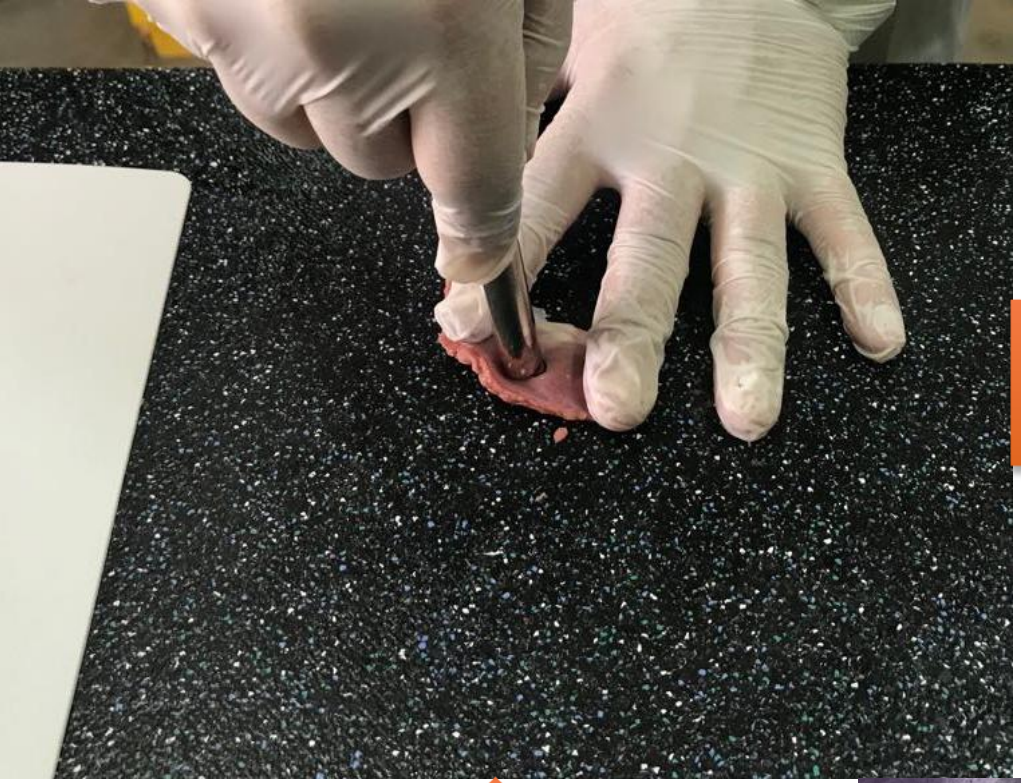
Materials & Methods

Meat Samples

100 samples from lamb diaphragm
From Nablus Municipal slaughterhouse

The Samples were cut by Cork borer

So the diameter was approximately 8mm and 2mm thick.



Plate

Meat Cutting

Cork borer

Materials & Methods

Samples tested as two groups

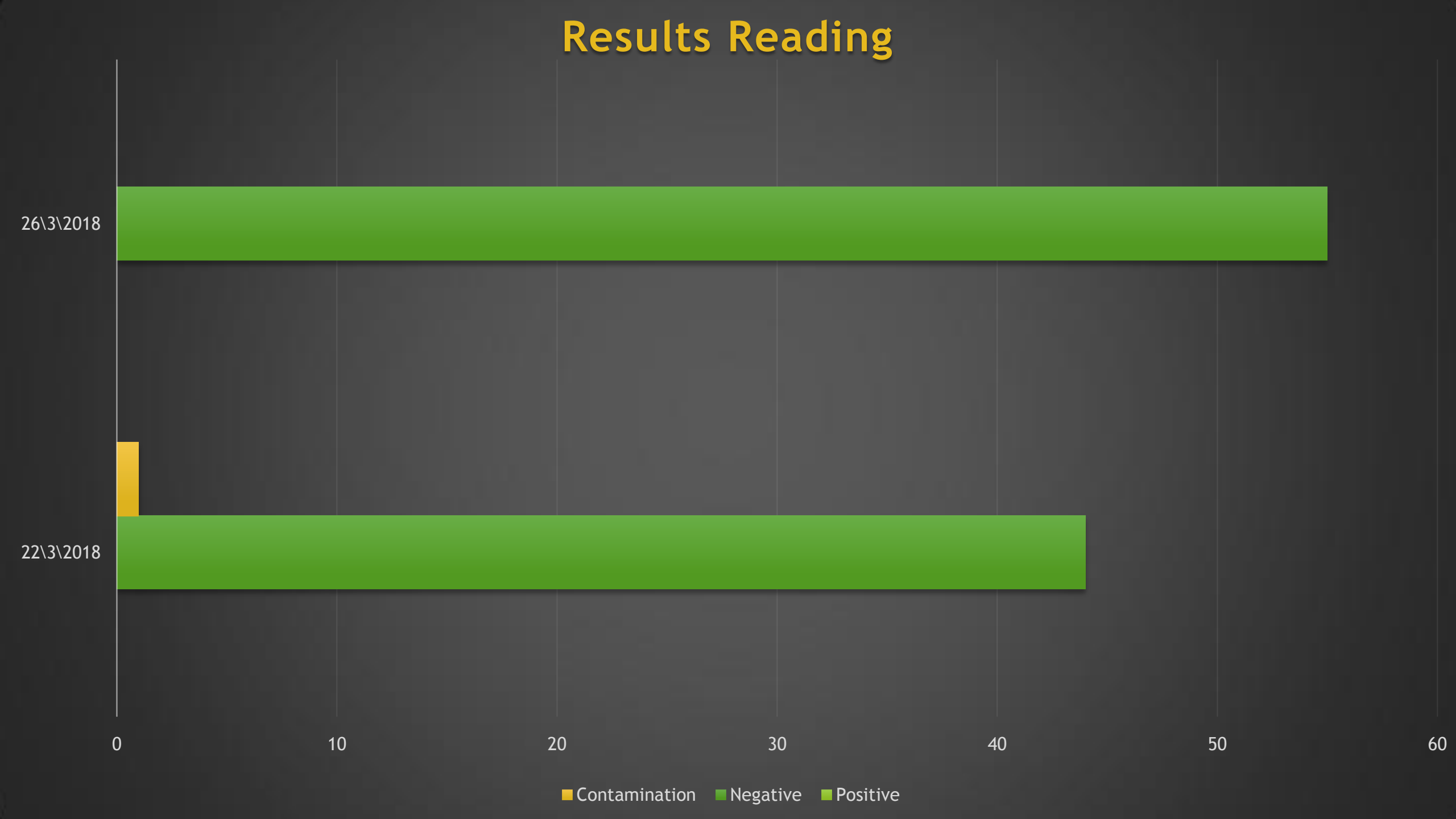
Group	Date of Incubation	# of Samples
A	21\3\2018	45
B	25\3\2018	55

Result Reading

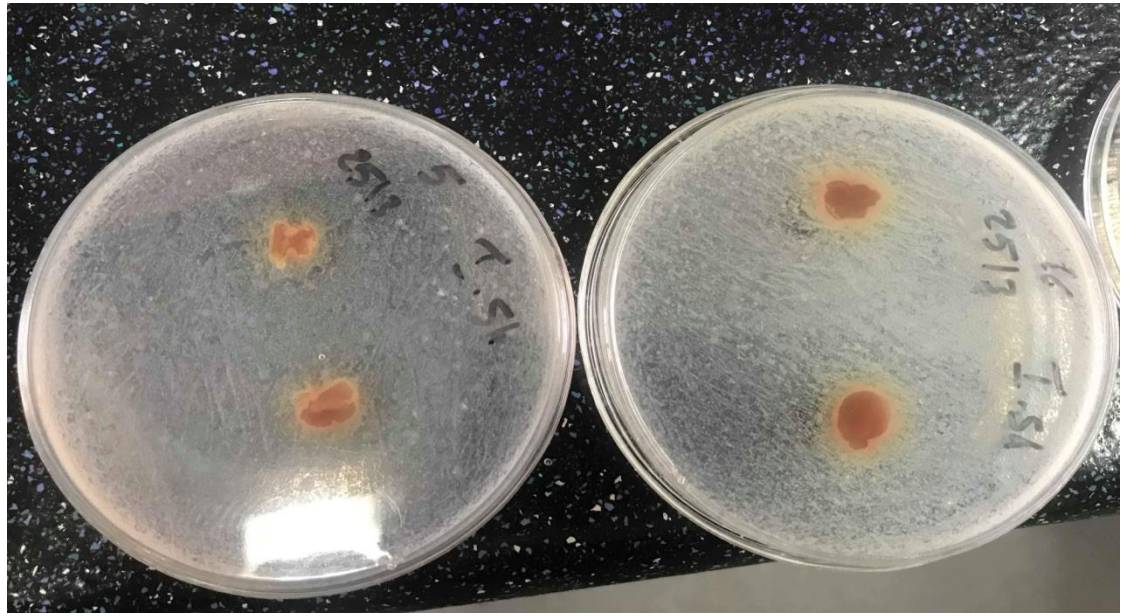
Reference Result Table	
Zone of Inhibition	Result
>/= 2 mm	+ve
<2 mm	-ve

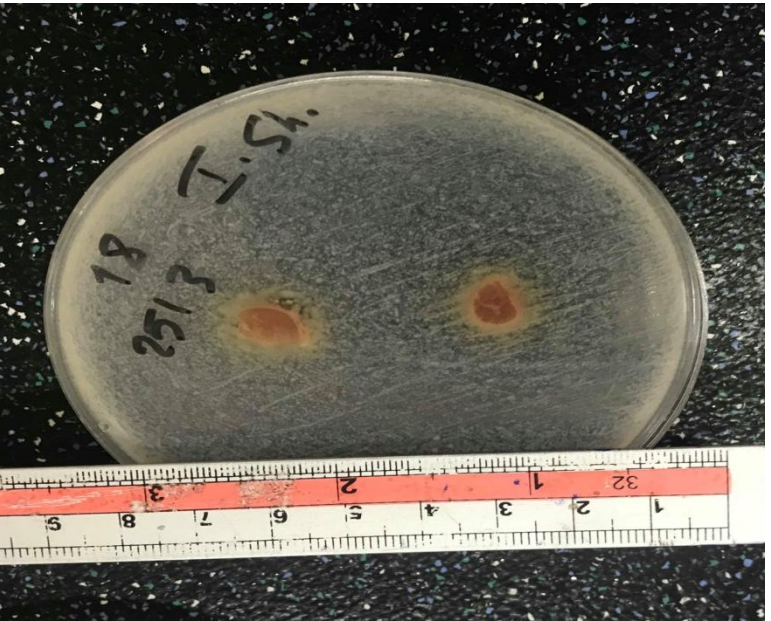
(Diez, et al. Five-plate screening test for the detection of antibiotic residues.)

Results Reading



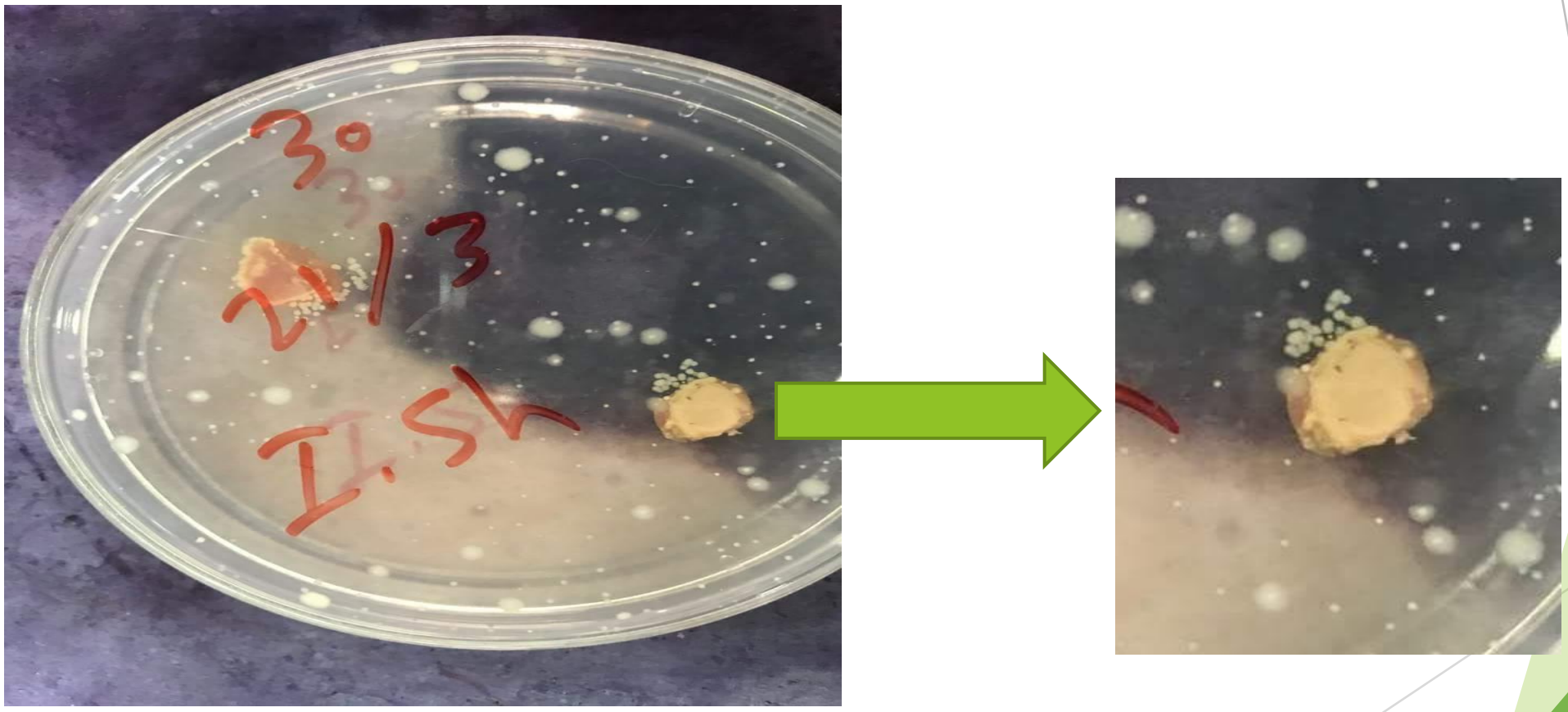
Results :





Enrichment Zone

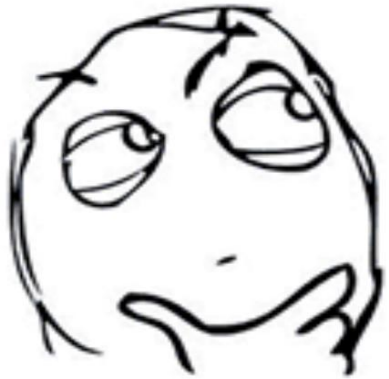
Results :



Contamination in sample 30 of G A

Discussion

- EU and Palestine.
- The Test is Correct.
- Samples are random and representative.
- It`s just a scanning test for tetracycline residue.
- Samples are just from slaughterhouse.



Challenges

- Finding a protocol to deal with meat samples.
- Finding some materials (Media), and It’s coast.
- University long protocols, to transmission materials between labs of different collages.
- Adjusting the bacteria concentration.
- Possibility of troubles with butchers, when sampling skeletal muscles.
- Samples in specific size and thickness.

Future Work

- To Study other antibiotic plate tests.
- To use other skeletal muscles.
- To find the detection limit of the test by testing biopsy samples from sheep under antibiotic treatment.
- To use meat samples from the market.
- To use muller hinton and compare with our result with pH 6 inhibition agar.

Acknowledgment

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