

Special Topics in Microbiology (3110751)

## Microbiological Reseach Application



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## International Public Health Security



" Rapid, accurate & early diagnosis of infection is the cornerstone of worldwide disease prevention and treatment!"

PERFORMANCE ACOA RESEARCH INS		aoac.org/testkits/micro amples of approv	biologykits.htlm ed test kits
Analyte	Company	Kit Names	Primary Matrices
Bacteria (Total Viable)	3 M Microbiology Products	Petrifilm Aerobic Count Plate	Food, Milk, Dairy products
Bacteria (Total Viable)	bioMerieux	api 20 A	Pure Culture
Salmonella Enteritidis	IDEXXX Lab.	FlockChek <i>S</i> .Enteritidis Ab- Test Kit	Meat and Poultry
Staph. aureus	Becton Dickenson	Difco Staph Lates Slide	Pure Culture
Listeria spp.	Neogen Corporation	GENE TRAK <i>Listeria</i> Assay	Food







Test kit results	SICK	HEALTHY
Positive	<b>a</b> (True positive)	b
Negative	с	<b>d</b> (True negative)
	a + c	b + d
<b>Sensitivity</b> (Tru (Ability to labe	ue positive value)  = l sick as sick)	<u>a</u> +c × 100
Specificity (Tro (Ability to labe	ue negative value) = I well as well)	$\frac{d}{b+d} \times 100$

Test kit results	SICK	HEALTHY
Positive	(a) 100	(b) 5
Negative	(c) 0	(d) 95
	(a+c) 100	(b+d) 100
Sensitivity (True po (Ability to label sick	ositive value) = $\frac{1}{10}$ (as sick)	$\frac{00}{0+0} \times 100 = 100$











Why antimicrobial residue was found in milk	?
Milking before the withdrawal time	24 %
<ul> <li>Accidental mixing residued-milk in collecting tank</li> </ul>	22 %
Some cow has prolonged- withdrawal time	13 %
<ul> <li>Using the same equipment between treated-cow</li> </ul>	
and normal milking-cow	<b>9</b> %
<ul> <li>Some cow has early parturition (Short dry period)</li> </ul>	<b>9</b> %
Accidental milking "Dry-cow"	8 %
No record of treatment	5 %
Milking from adjacent treated quarter	5 %
• Milking from new purchased cow	4 %
Using wrong type of antimicrobial	1%

Prevalence of (Thailand, 19	f Ant 88-19	imicrobi 995)	ial Residue	e in D	airy Milk
Author & Year of S	Study	Raw Milk	Pasteurized	UHT	Detection Methods
Teerapong et al.	1988	40 %	46.7 %	0 %	Delvo Test-P <sup>R</sup>
Kwanchai <i>et al.</i>	1993	-	26.4 %	11 %	Microbial inhibition disk assay (MIDA)
Pornsiri & Pramoj	1994	24.8 %	-	-	Delvo Test-P <sup>R</sup>
Bongkot <i>et al.</i>	1994	1.8 %	-	-	Delvo Test-P <sup>R</sup> HPLC
Thongchai <i>et al.</i>	1995	3.7 %	0.3 %	0 %	Delvo Test-P <sup>R</sup> MIDA, HPLC













Detection Limits of "AM-Test™"				
Antimicrobial	Detection Limit (ppm)	MRL (ppm)		
Penicillin G, Ampicillin, Amoxicillin	0.01	0.004		
Cloxacillin	0.02	0.03		
Streptomycin	0.2	0.2		
Gentamicin	0.4	0.1		
Kanamycin Quaranies Call	2.0	0.15		
Erythromycin	0.2	0.04		
Tetracycline, Oxytetracycline	0.2	0.1		
Chlortetracycline	0.4	0.1		
Sulfadiazine, Sulfathiazole, Sulfamethazine	0.2	0.1		



















## What are the expected antimicrobials to be found in "European Six-Plate Test"

<u> </u>	
Bacterial& Media used	Expected antimicrobial detected
<i>B.subtilis</i> pH 6	Tetracyclines, Chloramphenicol, Furazolidone, Nitrofurans, Cloxacillin, Penicillin G, Oxolinic acid
<i>B.subtilis</i> pH 7.2	Sulfadiazine, Sulfadimethoxine, Sulfamethazine, Sulfamonomethoxine, Sulfapyridine, Sulfamerazine
B.Subtilis & M.luteus pH 8	Tylosin, Erythromycin, Neomycin, Streptomycin
<i>B.cereus</i> pH 8	Tetracycline, Oxytetracycline, Deoxycycline, Chlortetracycline
<i>E.coli</i> pH 6	Enrofloxacin, Ciprofloxacin, Flumequine















Field tr in poul	Field trial of "CM-Test™" vs. "EFPT" n poultry farm (Saha Farm, 2002)								
With	Galli	Gallimicin		Colistin		Apramycin		Amoxycillin	
drawal	СМ	EFPT	СМ	EFPT	СМ	EFPT	СМ	EFPT	
Day 0					+ve	-ve			
Day 1	+ve	-ve			-ve	-ve			
Day 2	+ve	-ve			-ve	-ve			
Day 3	+ve	-ve	+ve	-ve	+ve	-ve			
Day 5			+ve	-ve					
Day 7							+ve	-ve	
Day 9							-ve	-ve	
Contro	Control group : -ve results of both "CM-Test <sup>TM</sup> " and "EFPT"								



Detection Limits of "CM-Test™"				
Antimicrobial	Detection Limit (ppm)	MRL (ppm)		
Penicillin G, Ampicillin, Amoxicillin	0.01	0.05		
Cloxacillin	0.025	0.05		
Tetracycline test kill	0.3	0.1		
Gentamicin Screening mean	0.4	0.1		
Kanamycin Canarantee Sat	2.0	0.1		
Erythromycin	2	0.2		
Sulfadiazine, Sulfathiazole	0.2	0.1		
Norfloxacin	8	0		
Furazolidone	5	0		

## Antimicrobial residue monitoring in food of animal origins for consumers in Thailand ?



Wontohing program of residue in meat by
 "Bangkok Metropolitan Bureau" and
 "Department of Livestock Development"
 Using "European Four/ Six-Plate Test" ?

	"CM-Test <sup>™</sup> "	EFPT or MIDA
(1) Labour intensive & Time consuming	Ready to be used	Bacterial culture & media preparatior
(2) Reporting results	3-4 hours	> 18 hours
(3) Detection limits	Better !	?
(4) Validity	High sensitivity & specificity	?
(5) Interference by contaminated bacteria	No (65 <sup>o</sup> C)	May be (37 <sup>o</sup> C)
(6) On-site test	Yes	No
(7) Cost	Cheaper	?













signed contract with

since 2002.

Chulalongkorn University as a

sole distributor of both test kits









