

1      **Supplement 1.** Antimicrobials administered in addition to the tetracycline injector (tetracycline, neomycin,  
2      bacitracin, and prednisolone) for the treatment of mastitis.

Cow No	Intramammary application					Parenteral		
	Pb	Amo	Cef	Dhs		Enro	Amo	
1	Pb	Amo	Cef	Dhs		Enro	Amo	
2			Cef			Enro	Cef	
3		Amo					Amo	
4		Amo					Amo	
5		Amo					Amo	
6	Pb	Amo				Enro	Cef	Amo
7	Pb							Amo
8	Pb							Amo
9	Pb					Enro		Amo
10	Pb	Amo	Cef	Dhs		Enro	Cef	
11			Cef			Enro	Cef	
12	Pb					Enro		Amo
13	Pb							Amo
14	Pb							Amo
15	Pb							Amo
16	Pb		Gen	Dhs	Ceph	Nov	Kan	Enro
17	Pb	Amo	Cef	Gen	Dhs			Enro
18			Cef					Enro Cef
19			Cef					Enro Cef
20		Amo						Enro
21		Amo						Cef
22		Amo						Amo
23								Amo
24		Amo						Amo
25			Cef					Enro Cef
26	Pb							Enro Amo
27			Cef					Enro Cef
28	Pb							Amo
29			Cef					Enro Cef
30			Cef					Enro Cef
31	Pb							Amo
32	Pb							Enro Amo
33	Pb							Enro Amo
34	Pb							Amo
35			Cef					Enro Cef

3      (Pb - Procaine benzylpenicillin; Amo – Amoxicillin; Cef – Cefquinome; Gen – Gentamicin; Dhs – Dihidrostreptomycin; Ceph –  
4      Cephalexin; Nov – Novobiocin; Kan – Kanamycin; Enro - Enrofloxacin).

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13      **Supplement 2.** STAR protocol – a five-plate microbiological test: Bacterial strains and its' suspension  
 14      absorbance used for the plate preparation, specific preparation protocol for each antibiotic plate, antibiotic group  
 15      detection for each plate and incubation temperatures that need to be used.  
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Bacteria	<i>Bacillus cereus</i>	<i>Kocuria rhizophila</i>	<i>Staphylococcus epidermidis</i>	<i>E. coli</i>	<i>Bacillus subtilis</i>
Detection of antibiotic group	Tetracyclines	β-lactams, Cephalosporins, Macrolides	Aminoglycosides, Novobiocin, Rifaximin	Quinolones, Bacitracin	Aminoglycosides
Bacterial suspension absorbance	1.0 – 1.1	0.5 – 0.60	0.25 – 0.30	0.30 – 0.35	/
cfu mL <sup>-1</sup> in agar	10 <sup>5</sup> -10 <sup>6</sup>	1-9 x 10 <sup>4</sup>	5 x 10 <sup>4</sup>	3 x 10 <sup>5</sup>	3 x 10 <sup>5</sup>
Preparation of antibiotic plates	500 µL BS AT5 pH 6 200 µl CHP	300 µL BS AT1 pH 8	100 µL BS AT1 pH 8	150 µL BS AT5 pH 8	200 µL BS AT5 pH 8
Incubation (16-20 h)	30 °C	30 °C	37 °C	37 °C	30 °C streptomycin & kanamycin
Positive control	tetracycline	oxacillin	neomycin	enrofloxacin	

17      AT – Antibiotic agar.

18      BS – Bacterial Suspension.

19      CHP – Chloramphenicol 500 µg mL<sup>-1</sup>.