**SUPPLEMENTARY MATERIALS**

**Bayesian estimation of sensitivity and specificity of a rapid mastitis test kit, bacterial culture, and PCR for detection of *Staphylococcus aureus*, *Streptococcus* speciesand Coliforms in bovine milk samples**

Tapakorn Chamchoy1, Emmanuel Okello1,2, Deniece R. Williams1,

Karen Tonooka1, Kathy Glenn1, Koji Maehana3, Ian A. Gardner4, Sharif S. Aly 1,2

1Veterinary Medicine Teaching and Research Center, School of Veterinary Medicine, University of California, Davis, Tulare, CA, USA

2Department of Population Health & Reproduction, School of Veterinary Medicine, University of California Davis, CA, USA

3Healthcare R&D Center, Asahi Kasei Corporation, Fuji, Shizuoka, Japan

4Department of Health Management, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Prince Edward Island, C1A4P3, Canada.

Corresponding Author:

Sharif S. Aly1,2

18830 Road 112, Tulare, California, 93274, USA

Email address: saly@ucdavis.edu

Supplement Code  
Bayesian latent class model code using uniform priors except culture specificity for analyzing sensitivity and specificity of 3 diagnostic tests from 2 different dairy population, step function included, from the results of aseptically-collected mastitis samples tested by study MQL.

Coliforms

model;

{

y1[1:2, 1:2, 1:2] ~ dmulti(p1[1:2, 1:2, 1:2], n1)

y2[1:2, 1:2, 1:2] ~ dmulti(p2[1:2, 1:2, 1:2], n2)

p1[1,1,1] <- pi1\*seCult\*seKit\*sePCR + (1-pi1)\*(1-spCult)\*(1-spKit)\*(1-spPCR)

p1[1,1,2] <- pi1\*seCult\*seKit\*(1-sePCR) + (1-pi1)\*(1-spCult)\*(1-spKit)\*spPCR

p1[1,2,1] <- pi1\*seCult\*(1-seKit)\*sePCR + (1-pi1)\*(1-spCult)\*spKit\*(1-spPCR)

p1[1,2,2] <- pi1\*seCult\*(1-seKit)\*(1-sePCR) + (1-pi1)\*(1-spCult)\*spKit\*spPCR

p1[2,1,1] <- pi1\*(1-seCult)\*seKit\*sePCR + (1-pi1)\*spCult\*(1-spKit)\*(1-spPCR)

p1[2,1,2] <- pi1\*(1-seCult)\*seKit\*(1-sePCR) + (1-pi1)\*spCult\*(1-spKit)\*spPCR

p1[2,2,1] <- pi1\*(1-seCult)\*(1-seKit)\*sePCR + (1-pi1)\*spCult\*spKit\*(1-spPCR)

p1[2,2,2] <- pi1\*(1-seCult)\*(1-seKit)\*(1-sePCR) + (1-pi1)\*spCult\*spKit\*spPCR

p2[1,1,1] <- pi2\*seCult\*seKit\*sePCR + (1-pi2)\*(1-spCult)\*(1-spKit)\*(1-spPCR)

p2[1,1,2] <- pi2\*seCult\*seKit\*(1-sePCR) + (1-pi2)\*(1-spCult)\*(1-spKit)\*spPCR

p2[1,2,1] <- pi2\*seCult\*(1-seKit)\*sePCR + (1-pi2)\*(1-spCult)\*spKit\*(1-spPCR)

p2[1,2,2] <- pi2\*seCult\*(1-seKit)\*(1-sePCR) + (1-pi2)\*(1-spCult)\*spKit\*spPCR

p2[2,1,1] <- pi2\*(1-seCult)\*seKit\*sePCR + (1-pi2)\*spCult\*(1-spKit)\*(1-spPCR)

p2[2,1,2] <- pi2\*(1-seCult)\*seKit\*(1-sePCR) + (1-pi2)\*spCult\*(1-spKit)\*spPCR

p2[2,2,1] <- pi2\*(1-seCult)\*(1-seKit)\*sePCR + (1-pi2)\*spCult\*spKit\*(1-spPCR)

p2[2,2,2] <- pi2\*(1-seCult)\*(1-seKit)\*(1-sePCR) + (1-pi2)\*spCult\*spKit\*spPCR

sediffCultKit <- seCult-seKit

sediffCultPCR <- seCult-sePCR

sediffKitCult <- seKit-seCult

sediffKitPCR <- seKit-sePCR

sediffPCRCult <- sePCR-seCult

sediffPCRKit <- sePCR-seKit

spdiffCultKit <- spCult-spKit

spdiffCultPCR <- spCult-spPCR

spdiffKitCult <- spKit-spCult

spdiffKitPCR <- spKit-spPCR

spdiffPCRCult <- spPCR-spCult

spdiffPCRKit <- spPCR-spKit

probsediffCultKit <- step(sediffCultKit)

probsediffCultPCR <- step(sediffCultPCR)

probsediffKitCult <- step(sediffKitCult)

probsediffKitPCR <- step(sediffKitPCR)

probsediffPCRCult <- step(sediffPCRCult)

probsediffPCRKit <- step(sediffPCRKit)

probspdiffCultKit <- step(spdiffCultKit)

probspdiffCultPCR <- step(spdiffCultPCR)

probspdiffKitCult <- step(spdiffKitCult)

probspdiffKitPCR <- step(spdiffKitPCR)

probspdiffPCRCult <- step(spdiffPCRCult)

probspdiffPCRKit <- step(spdiffPCRKit)

seCult~dbeta(1,1)

spCult~dbeta(298.1,1)

seKit~dbeta(1,1)

spKit~dbeta(1,1)

sePCR~dbeta(1,1)

spPCR~dbeta(1,1)

pi1 ~ dbeta(1,1)

pi2 ~ dunif(0,0.1)

}

list(n1=154, n2=120,

y1=structure(.Data=c(10,0,13,2,0,9,4,116),.Dim=c(2,2,2)),

y2=structure(.Data=c(0,0,0,0,0,8,0,112),.Dim=c(2,2,2)))

list(seCult=0.7, spCult=0.7, seKit=0.7, spKit=0.7, sePCR=0.7, spPCR=0.7, pi1=0.2, pi2=0.02)

*Streptococcus* spp.

model;

{

y1[1:2, 1:2, 1:2] ~ dmulti(p1[1:2, 1:2, 1:2], n1)

y2[1:2, 1:2, 1:2] ~ dmulti(p2[1:2, 1:2, 1:2], n2)

p1[1,1,1] <- pi1\*seCult\*seKit\*sePCR + (1-pi1)\*(1-spCult)\*(1-spKit)\*(1-spPCR)

p1[1,1,2] <- pi1\*seCult\*seKit\*(1-sePCR) + (1-pi1)\*(1-spCult)\*(1-spKit)\*spPCR

p1[1,2,1] <- pi1\*seCult\*(1-seKit)\*sePCR + (1-pi1)\*(1-spCult)\*spKit\*(1-spPCR)

p1[1,2,2] <- pi1\*seCult\*(1-seKit)\*(1-sePCR) + (1-pi1)\*(1-spCult)\*spKit\*spPCR

p1[2,1,1] <- pi1\*(1-seCult)\*seKit\*sePCR + (1-pi1)\*spCult\*(1-spKit)\*(1-spPCR)

p1[2,1,2] <- pi1\*(1-seCult)\*seKit\*(1-sePCR) + (1-pi1)\*spCult\*(1-spKit)\*spPCR

p1[2,2,1] <- pi1\*(1-seCult)\*(1-seKit)\*sePCR + (1-pi1)\*spCult\*spKit\*(1-spPCR)

p1[2,2,2] <- pi1\*(1-seCult)\*(1-seKit)\*(1-sePCR) + (1-pi1)\*spCult\*spKit\*spPCR

p2[1,1,1] <- pi2\*seCult\*seKit\*sePCR + (1-pi2)\*(1-spCult)\*(1-spKit)\*(1-spPCR)

p2[1,1,2] <- pi2\*seCult\*seKit\*(1-sePCR) + (1-pi2)\*(1-spCult)\*(1-spKit)\*spPCR

p2[1,2,1] <- pi2\*seCult\*(1-seKit)\*sePCR + (1-pi2)\*(1-spCult)\*spKit\*(1-spPCR)

p2[1,2,2] <- pi2\*seCult\*(1-seKit)\*(1-sePCR) + (1-pi2)\*(1-spCult)\*spKit\*spPCR

p2[2,1,1] <- pi2\*(1-seCult)\*seKit\*sePCR + (1-pi2)\*spCult\*(1-spKit)\*(1-spPCR)

p2[2,1,2] <- pi2\*(1-seCult)\*seKit\*(1-sePCR) + (1-pi2)\*spCult\*(1-spKit)\*spPCR

p2[2,2,1] <- pi2\*(1-seCult)\*(1-seKit)\*sePCR + (1-pi2)\*spCult\*spKit\*(1-spPCR)

p2[2,2,2] <- pi2\*(1-seCult)\*(1-seKit)\*(1-sePCR) + (1-pi2)\*spCult\*spKit\*spPCR

sediffCultKit <- seCult-seKit

sediffCultPCR <- seCult-sePCR

sediffKitCult <- seKit-seCult

sediffKitPCR <- seKit-sePCR

sediffPCRCult <- sePCR-seCult

sediffPCRKit <- sePCR-seKit

spdiffCultKit <- spCult-spKit

spdiffCultPCR <- spCult-spPCR

spdiffKitCult <- spKit-spCult

spdiffKitPCR <- spKit-spPCR

spdiffPCRCult <- spPCR-spCult

spdiffPCRKit <- spPCR-spKit

probsediffCultKit <- step(sediffCultKit)

probsediffCultPCR <- step(sediffCultPCR)

probsediffKitCult <- step(sediffKitCult)

probsediffKitPCR <- step(sediffKitPCR)

probsediffPCRCult <- step(sediffPCRCult)

probsediffPCRKit <- step(sediffPCRKit)

probspdiffCultKit <- step(spdiffCultKit)

probspdiffCultPCR <- step(spdiffCultPCR)

probspdiffKitCult <- step(spdiffKitCult)

probspdiffKitPCR <- step(spdiffKitPCR)

probspdiffPCRCult <- step(spdiffPCRCult)

probspdiffPCRKit <- step(spdiffPCRKit)

seCult~dbeta(1,1)

spCult~dbeta(298.1,1)

seKit~dbeta(1,1)

spKit~dbeta(1,1)

sePCR~dbeta(1,1)

spPCR~dbeta(1,1)

pi1 ~ dbeta(1,1)

pi2 ~ dunif(0,0.1)

}

list(n1=154, n2=120,

y1=structure(.Data=c(28,3,12,7,4,9,7,84),.Dim=c(2,2,2)),

y2=structure(.Data=c(0,0,0,1,0,1,1,117),.Dim=c(2,2,2)))

list(seCult=0.7, spCult=0.7, seKit=0.7, spKit=0.7, sePCR=0.7, spPCR=0.7, pi1=0.2, pi2=0.02)

*Staphylococcus aureus*

model;

{

y1[1:2, 1:2, 1:2] ~ dmulti(p1[1:2, 1:2, 1:2], n1)

y2[1:2, 1:2, 1:2] ~ dmulti(p2[1:2, 1:2, 1:2], n2)

p1[1,1,1] <- pi1\*seCult\*seKit\*sePCR + (1-pi1)\*(1-spCult)\*(1-spKit)\*(1-spPCR)

p1[1,1,2] <- pi1\*seCult\*seKit\*(1-sePCR) + (1-pi1)\*(1-spCult)\*(1-spKit)\*spPCR

p1[1,2,1] <- pi1\*seCult\*(1-seKit)\*sePCR + (1-pi1)\*(1-spCult)\*spKit\*(1-spPCR)

p1[1,2,2] <- pi1\*seCult\*(1-seKit)\*(1-sePCR) + (1-pi1)\*(1-spCult)\*spKit\*spPCR

p1[2,1,1] <- pi1\*(1-seCult)\*seKit\*sePCR + (1-pi1)\*spCult\*(1-spKit)\*(1-spPCR)

p1[2,1,2] <- pi1\*(1-seCult)\*seKit\*(1-sePCR) + (1-pi1)\*spCult\*(1-spKit)\*spPCR

p1[2,2,1] <- pi1\*(1-seCult)\*(1-seKit)\*sePCR + (1-pi1)\*spCult\*spKit\*(1-spPCR)

p1[2,2,2] <- pi1\*(1-seCult)\*(1-seKit)\*(1-sePCR) + (1-pi1)\*spCult\*spKit\*spPCR

p2[1,1,1] <- pi2\*seCult\*seKit\*sePCR + (1-pi2)\*(1-spCult)\*(1-spKit)\*(1-spPCR)

p2[1,1,2] <- pi2\*seCult\*seKit\*(1-sePCR) + (1-pi2)\*(1-spCult)\*(1-spKit)\*spPCR

p2[1,2,1] <- pi2\*seCult\*(1-seKit)\*sePCR + (1-pi2)\*(1-spCult)\*spKit\*(1-spPCR)

p2[1,2,2] <- pi2\*seCult\*(1-seKit)\*(1-sePCR) + (1-pi2)\*(1-spCult)\*spKit\*spPCR

p2[2,1,1] <- pi2\*(1-seCult)\*seKit\*sePCR + (1-pi2)\*spCult\*(1-spKit)\*(1-spPCR)

p2[2,1,2] <- pi2\*(1-seCult)\*seKit\*(1-sePCR) + (1-pi2)\*spCult\*(1-spKit)\*spPCR

p2[2,2,1] <- pi2\*(1-seCult)\*(1-seKit)\*sePCR + (1-pi2)\*spCult\*spKit\*(1-spPCR)

p2[2,2,2] <- pi2\*(1-seCult)\*(1-seKit)\*(1-sePCR) + (1-pi2)\*spCult\*spKit\*spPCR

sediffCultKit <- seCult-seKit

sediffCultPCR <- seCult-sePCR

sediffKitCult <- seKit-seCult

sediffKitPCR <- seKit-sePCR

sediffPCRCult <- sePCR-seCult

sediffPCRKit <- sePCR-seKit

spdiffCultKit <- spCult-spKit

spdiffCultPCR <- spCult-spPCR

spdiffKitCult <- spKit-spCult

spdiffKitPCR <- spKit-spPCR

spdiffPCRCult <- spPCR-spCult

spdiffPCRKit <- spPCR-spKit

probsediffCultKit <- step(sediffCultKit)

probsediffCultPCR <- step(sediffCultPCR)

probsediffKitCult <- step(sediffKitCult)

probsediffKitPCR <- step(sediffKitPCR)

probsediffPCRCult <- step(sediffPCRCult)

probsediffPCRKit <- step(sediffPCRKit)

probspdiffCultKit <- step(spdiffCultKit)

probspdiffCultPCR <- step(spdiffCultPCR)

probspdiffKitCult <- step(spdiffKitCult)

probspdiffKitPCR <- step(spdiffKitPCR)

probspdiffPCRCult <- step(spdiffPCRCult)

probspdiffPCRKit <- step(spdiffPCRKit)

seCult~dbeta(1,1)

spCult~dbeta(298.1,1)

seKit~dbeta(1,1)

spKit~dbeta(1,1)

sePCR~dbeta(1,1)

spPCR~dbeta(1,1)

pi1 ~ dbeta(1,1)

pi2 ~ dunif(0,0.1)

}

list(n1=154, n2=120,

y1=structure(.Data=c(14,3,6,4,0,5,3,119),.Dim=c(2,2,2)),

y2=structure(.Data=c(0,0,0,0,0,0,0,120),.Dim=c(2,2,2)))

list(seCult=0.7, spCult=0.7, seKit=0.7, spKit=0.7, sePCR=0.7, spPCR=0.7, pi1=0.2, pi2=0.02

Table S1 Prevalence estimates (and 95% PI) from Bayesian LCM for clinical and non-clinical prevalence populations.

|  |  |  |  |
| --- | --- | --- | --- |
| **Study MQL** | **Coliforms** | ***Streptococcus* spp*.*** | ***Staphylococcus aureus*** |
| Clinical population | 18.0% | 41.8% | 19.6% |
| (12.2, 25.0) | (32.6, 51.3) | (13.2, 27.4) |
| Non-clinical population | 0.6% | 0.8% | 0.6% |
| (0.0, 3.0) | (0.0, 3.9) | (0.0, 3.1) |

Table S2 Cross tabulation of isolates by species as identified using bacterial culture and 16S speciation results

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Speciation** | **Reported culture results** | **Species classification used in model** | | | |
| **Negative** | **Coliforms** | ***Staphylococcus aureus*** | ***Streptococcus* spp.** |
| *Aerococcus species* | *Streptococcus* spp. | 1 |  |  |  |
| *Arthrobacter species* | Negative |  | 1 |  |  |
| *E.coli* | Coliforms |  | 29 |  |  |
| *Enterobacter hormaechei* | Coliforms | 2 |  |  |  |
| *Enterococcus durans* | *Streptococcus* spp. |  |  |  | 1 |
| *Enterococcus faecium* | *Streptococcus* spp. |  |  |  | 2 |
| *Enterococcus saccharolyticus* | *Streptococcus* spp. |  |  |  | 2 |
| *Error* | Excluded (3) |  |  |  |  |
| *Klebsiella oxytoca* | Coliforms |  | 1 |  |  |
| *Klebsiella aerogenes* | Coliforms |  | 1 |  |  |
| *Klebsiella oxytoca* | Coliforms |  | 4 |  |  |
| *Klebsiella pneumoniae* | Coliforms |  | 15 |  |  |
| *Klebsiella pneumoniae* | Excluded (1) |  |  |  |  |
| *Klebsiella species* | Coliforms |  | 2 |  |  |
| *Lactococcus garvieae* | *Streptococcus* spp. |  |  |  | 9 |
| *Mannheimia haemolytica* | Coliforms | 1 |  |  |  |
| *Mannheimia varigena* | Coliforms | 2 |  |  |  |
| *Pasteurella multocida* | Coliforms | 3 |  |  |  |
| *Pseudomonas aeruginosa* | Coliforms | 1 |  |  |  |
| *Serratia marcescens* | Coliforms | 6 |  |  |  |
| *Serratia species* | Coliforms | 1 |  |  |  |
| *Staph species* | Coliforms | 1 |  |  | 1 |
| *Staph warneri* | Excluded (1) |  |  |  |  |
| *Staphylococcus aureus* | *Staphylococcus aureus* |  |  | 53 |  |
| *Streptococcus spp.* | *Streptococcus* spp. |  |  |  | 84 |

Table S3 Joint test results for bacterial culture, mastitis test kit and qPCR for Coliforms, *Streptococcus* spp. and *Staphylococcus aureus* from clinical and non-clinical populations.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Culture/Kit/PCR results** | **Coliforms** | | ***Streptococcus* spp.** | | ***Staphylococcus aureus*** | |
| **Clinical population** | **Non-clinical population** | **Clinical population** | **Non-clinical population** | **Clinical population** | **Non-clinical population** |
| + + + | 10 | 0 | 28 | 0 | 14 | 0 |
| + + - | 0 | 0 | 3 | 0 | 3 | 0 |
| + - + | 13 | 0 | 12 | 0 | 6 | 0 |
| + - - | 2 | 0 | 7 | 1 | 4 | 0 |
| - + + | 0 | 0 | 4 | 0 | 0 | 0 |
| - + - | 9 | 8 | 9 | 1 | 5 | 0 |
| - - + | 4 | 0 | 7 | 1 | 3 | 0 |
| - - - | 116 | 112 | 84 | 117 | 119 | 120 |
| **Total** | 154 | 120 | 154 | 120 | 154 | 120 |