Supplementary File for "Comparison of an on-farm point-of-care diagnostic with conventional culture in analysing bovine mastitis samples" by Geoff Jones, Olaf Bork, Scott A. Ferguson and Andrew Bates

Materials and Method

The experimental procedures were approved by the Animal Ethics Committee, Massey University, New Zealand (No 16/75).

Animals and management

The herds were required to adhere to the study protocols, grant full third party access to all herd recording data, have trained personnel, individual cow identification, appropriate drug storage facilities, and on-farm refrigeration and freezer capacity.

Cows were enrolled into the study by farm staff after detection of clinical mastitis. All cows were eligible for enrolment from approximately 14 days before calving to 100 days after calving. All cows, including those with clinical mastitis, were milked twice daily for at least the first 100 days of lactation.

All cows had their temperature recorded at enrolment and any with a temperature > 40°C, or exhibiting signs of systemic illness (depression, anorexia, dehydration) were excluded from the trial.

Milk samples

All farm personnel were trained by the veterinarian to collect milk samples following the NMC guidelines (NMC 2004, 2017).

Frozen samples were collected and analysed within 4-6 weeks of sample collection. To identify bacteria to the species level all frozen milk samples were cultured by an independent laboratory Cognosco, Anexa FVC, Morrinsville, New Zealand operating in accordance with the NMC guidelines (NMC 2004, 2017). The independent laboratory staff were blinded and did not receive the point-of-care diagnostic results before supplying their results.

Bacterial culture identification and antibiotic susceptibility testing

Point-of-care diagnostic

Once the farmer had collected the milk sample(s), they were loaded into a point-of-care diagnostic cartridge and inserted into the Lapbox incubator (37°C) connected to the cloud. Within 24 h the results were sent to the farmers and to Vetlife.

Laboratory-based culture

Briefly, 10 µl of milk (from a frozen sample) was streaked onto one quadrant of a 5% blood agar plate containing 0.1% aesculin and incubated at 37°C for 48h. The bacteria were identified on the basis of colony morphology, Gram stain, and catalase reaction. Gram positive, catalase positive cocci were tested with the coagulase test to differentiate CNS from *S. aureus*. Gram positive, catalase negative cocci were assessed by aesculin reaction, CAMP test and growth in inulin, *Streptococcus faecalis* broth, and sorbitol. Gram negative isolates were sub-cultured onto MacConkey agar, and triple sugar iron, Simmon's citrate, and motility tests were performed. Where the identity of the isolate was unclear using conventional biochemical tests, MALDI-TOF testing was undertaken to identify the bacteria.

For each of the two tests (point-of-care diagnostic, standard culture) each sample was classified as positive or negative for each of the following targets: S. uberis, S. aureus, CNS, coliform and All bacteria species targets (*Streptococcus uberis*, *Streptococcus dysgalactiae*, *Streptococcus agalactiae*, *Enterococci spp*, *Staphylococcus aureus*, coagulase negative *Staphylococcus*, Coliform).

Statistical analysis

In the case of two tests being applied to individuals sampled from a single population, as is the case here, the model is not identifiable. This implies that prior information for at least some of the parameters is necessary in order to obtain estimates.

Decisions must be made about possible correlations between the test results. Here it was assumed that the two tests were independent for true negative (uninfected) individuals, but that they may be positively correlated on true positives because the level of infection is expected to vary across infected individuals: a high level of infection should be easier to identify by both

tests, leading to positive correlation. This correlation was incorporated into the model as an extra parameter (Dendukuri and Joseph, 2001).

Estimation of the model parameters was carried out, separately for each target organism, by Markov chain Monte Carlo (MCMC) computation as implemented in the BUGS statistical software (Lunn et al. 2000). Results for each target were based on 10,000 iterations after an initial burn-in of 2,000; when autocorrelation was present, results were thinned by a factor of five.

Priors

Prior distributions were developed from relevant data in the literature. Doohoo et al. (2011) presented data from two North American studies, using a quasi "gold standard" defined by the results of repeated testing to estimate the sensitivity and specificity of the standard culture test, both for the detection of any organism and for specific targets.

McDougall et al. (2007) reported the results of applying the standard culture test to samples from clinical mastitis cases in New Zealand. From a total of 1561 glands tested, 1113 were positive for bacterial infection and 499 for *S. uberis*. There was no gold standard information available for this dataset, so it gave information on apparent rather than true prevalences.

Priors obtained for Se1 and Sp1 from either Table 2 or Table 3 of Doohoo et al. (2011) were used to analyse the observed prevalence data in McDougall et al (2007) to obtain information about the true prevalence of bacterial infection in NZ clinical mastitis cases. The relationship between observed (p) and true prevalence (π) is

p = π Se + (1-π) Sp

which implies that the observed prevalence should lie between Se and (1-Sp). The observed prevalence here is 0.713 for all organisms and 0.320 for *S. uberis*. These are compatible with the values from Table 2 values of Dohoo et al (2011).

Therefore, it was decided to use Table 2 of Doohoo et al. (2011) as the basis of priors for our analyses for both tests (point-of-care diagnostic and lab-based culture), but the priors were widened to allow for the fact that Se/Sp may be different for our population (NZ mastitis cases vs general Canadian). Analysis of the McDougall et al. (2007) data by latent class analysis for one test, using these priors, gave posterior distributions for pi, Se1, Sp1 which were taken as priors for the subsequent analysis. Figure 1 shows the resulting priors for all bacterial targets.

OpenBugs Orogram for Comparing Standard Culture (1) with Mastatest (2)

(Data and priors are for All Organisms)

```
model{

x[1:4] ~ dmulti(p[1:4], n)

p[1] <- pi*(Se1*Se2+covDp) + (1-pi)*(1-Sp1)*(1-Sp2)

p[2] <- pi*(Se1*(1-Se2)-covDp) + (1-pi)*(1-Sp1)*Sp2

p[3] <- pi*((1-Se1)*Se2-covDp) + (1-pi)*Sp1*(1-Sp2)

p[4] <- pi*((1-Se1)*(1-Se2)+covDp) + (1-pi)*Sp1*Sp2

ls <- (Se1-1)*(1-Se2)

us <- min(Se1,Se2) - Se1*Se2

#Priors

pi ~ dbeta(26, 8)             ### Mean=0.76

Se1 ~ dbeta(42.25, 7)        ### Mean=0.858

Sp1 ~ dbeta(32.68, 10.83)   ### Mean=0.751

Se2 ~ dbeta(42.25, 7)        ### Mean=0. 858

Sp2 ~ dbeta(32.68, 10.83)   ### Mean=0.751

covDp ~ dunif(ls, us)

rhoD <- covDp / sqrt(Se1*(1-Se1)*Se2*(1-Se2))  ### Correlation coefficient

zSe<-step(Se2-Se1)

zSp<-step(Sp2-Sp1)

}

#Data

list(n=292, x=c(246,10,23,13))
```
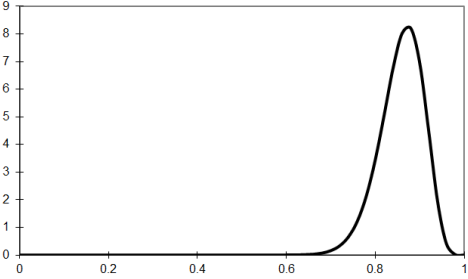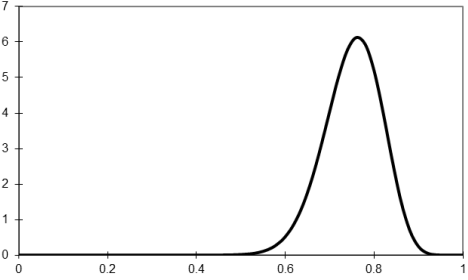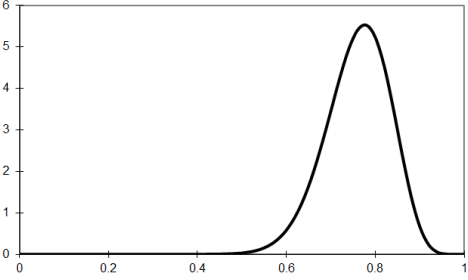
Priors, Posteriors and Summary Statistics

Any Organism

Widened Priors from Doohoo (2011) Table 2 and McDougall (2007) Table 3

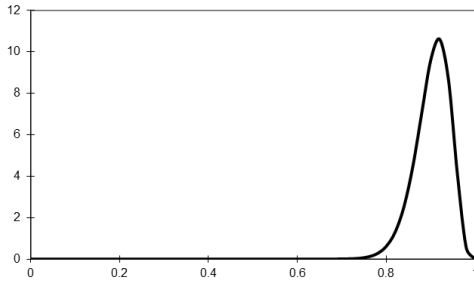|		
Se1 Sensitivity of Standard test		beta(42.25, 7.00) mean 0.858 CI (0.75, 0.94)
Sp1 Specificity of Standard test		beta(32.68, 10.83) mean 0.751 CI (0.61, 0.87)
π Prevalence of bacterial infection in mastitis cases		beta(26, 8) mean 0.76 CI (0.61, 0.89)
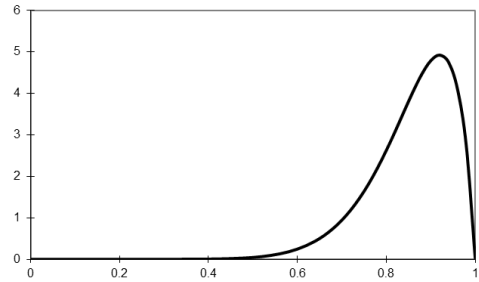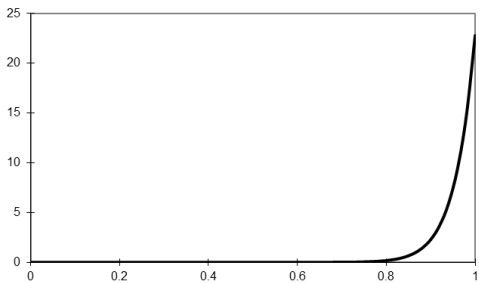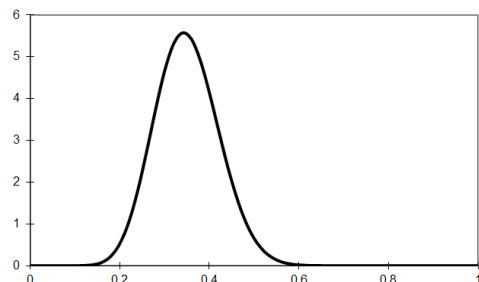
Posteriors from 292 trial cases; same priors for 1 and 2
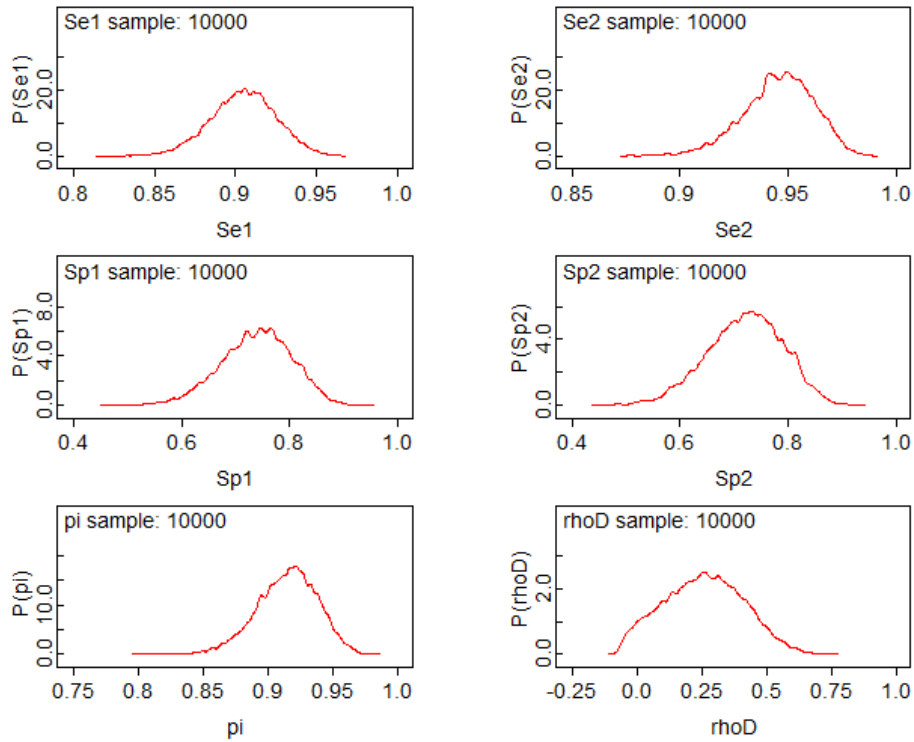
	mean	sd	MC_error	val2.5pc	median	val97.5pc	start	sample
Se1	0.9048	0.02022	2.26E-4	0.8638	0.9055	0.9429	2001	10000
Se2	0.9458	0.01622	1.637E-4	0.911	0.9469	0.9739	2001	10000
Sp1	0.7388	0.0652	6.714E-4	0.6034	0.7427	0.8561	2001	10000
Sp2	0.7207	0.07022	6.663E-4	0.5765	0.7245	0.8473	2001	10000
pi	0.9149	0.02331	2.166E-4	0.8655	0.9165	0.9562	2001	10000
rhoD	0.2587	0.1533	0.001552	-0.02421	0.2601	0.5546	2001	10000
zSe	0.9677	0.1768	0.001613	0.0	1.0	1.0	2001	10000
zSp	0.4272	0.4947	0.005288	0.0	0.0	1.0	2001	10000

Strep Uberis

Widened Priors from Doohoo (2011) Table 2 and McDougall (2007) Table 3

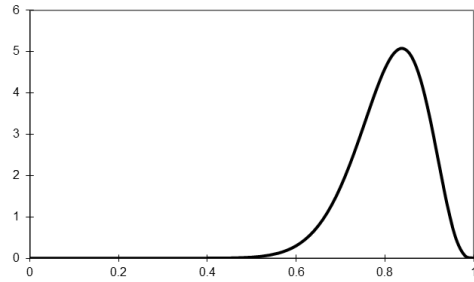| Se1 Sensitivity of Standard test		beta(11.4, 1.9) mean 0.857 CI (0.63, 0.98)
Sp1 Specificity of Standard test		beta(22.85, 1) mean 0.958 CI (0.85, 1.00)
π Prevalence of S. Uberis infection in mastitis cases		beta(15.6, 29.0) mean 0.350 CI (0.22, 0.49)
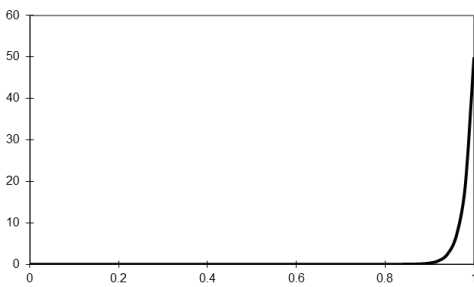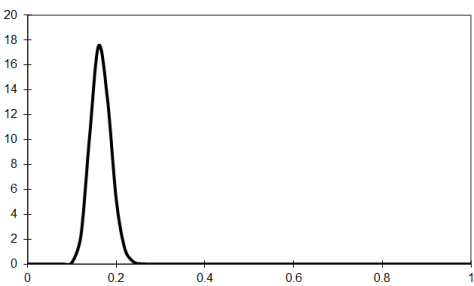
Posteriors from 292 trial cases; same priors for 1 and 2
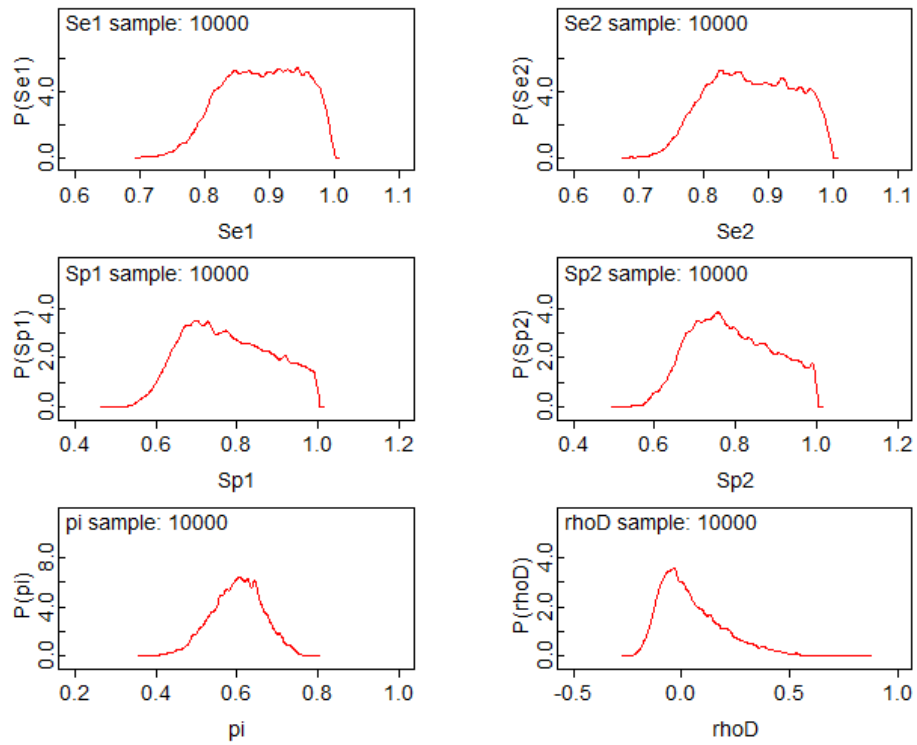
	mean	sd	MC_error	val2.5pc	median	val97.5pc	start	sample
Se1	0.8895	0.05968	8.665E-4	0.776	0.8919	0.9869	2001	10000
Se2	0.8756	0.06486	7.968E-4	0.7587	0.8742	0.9859	2001	10000
Sp1	0.78	0.1084	0.00133	0.5984	0.7705	0.9828	2001	10000
Sp2	0.7953	0.1015	0.001464	0.6209	0.7854	0.9873	2001	10000
pi	0.601	0.06325	7.107E-4	0.4733	0.6041	0.7202	2001	10000
rhoD	0.04883	0.1534	0.001578	-0.1576	0.01245	0.427	2001	10000
zSe	0.4494	0.4974	0.0062	0.0	0.0	1.0	2001	10000
zSp	0.5285	0.4992	0.00636	0.0	1.0	1.0	2001	10000

Staph Aureus

Widened Priors from Doohoo (2011) Table 2 and McDougall (2007) Table 3

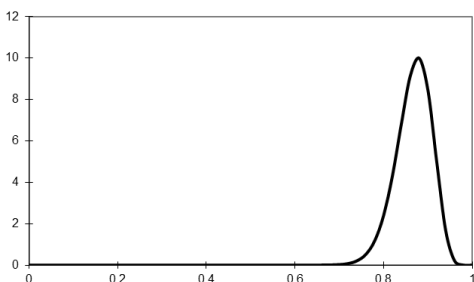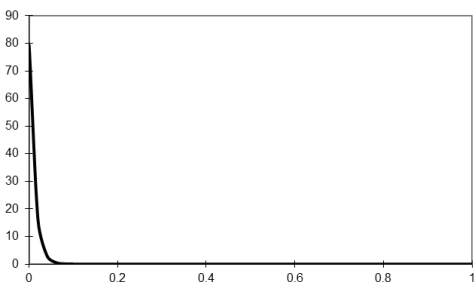|		
Se1 Sensitivity of Standard test		beta(50.2, 5.4) mean 0.903 CI (0.81, 0.97)
Sp1 Specificity of Standard test		beta(50, 1) mean 0.980 CI (0.93, 1.00)
π Prevalence of S. Aureus infection in mastitis cases		beta(44.1, 223) mean 0.165 CI (0.12, 0.23)

Posteriors from 292 trial cases; same priors for 1 and 2
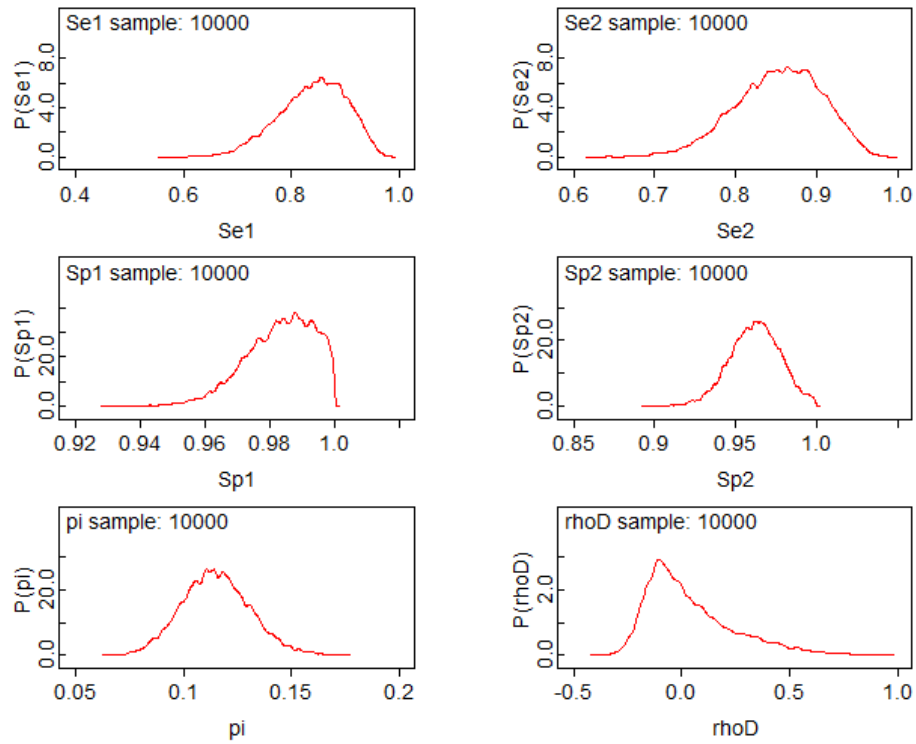
	mean	sd	MC_error	val2.5pc	median	val97.5pc	start	sample
Se1	0.8397	0.06252	6.338E-4	0.707	0.8454	0.943	2001	10000
Se2	0.8532	0.05326	4.946E-4	0.7417	0.8564	0.9457	2001	10000
Sp1	0.9832	0.01071	1.139E-4	0.9594	0.9844	0.999	2001	10000
Sp2	0.9621	0.01557	1.623E-4	0.9305	0.9626	0.992	2001	10000
pi	0.1153	0.01581	1.524E-4	0.08552	0.1147	0.1481	2001	10000
rhoD	0.03993	0.2046	0.00227	-0.2324	-0.01191	0.5508	2001	10000
zSe	0.5572	0.4967	0.005085	0.0	1.0	1.0	2001	10000
zSp	0.1263	0.3322	0.003808	0.0	0.0	1.0	2001	10000

CNS

Widened Priors from Doohoo (2011) Table 2 and McDougall (2007) Table 3;

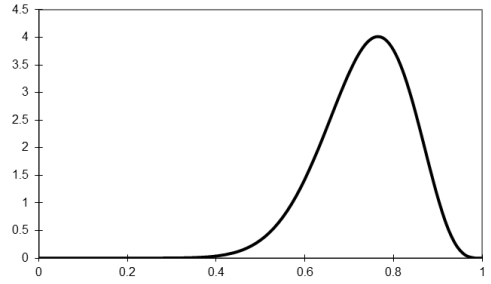|		
Se1 Sensitivity of Standard test		beta(18.7, 4.42) mean 0.801 CI (0.63, 0.94)
Sp1 Specificity of Standard test		beta(59.8, 9.18) mean 0.867 CI (0.78, 0.94)
π Prevalence of bacterial infection in mastitis cases		beta(1, 80) mean 0.01 CI (0.00, 0.05)
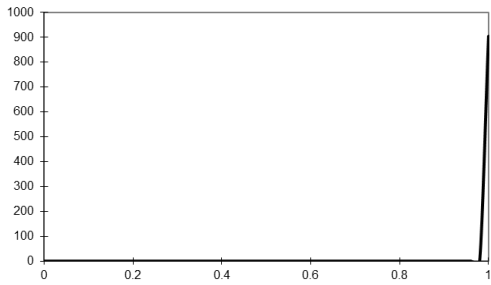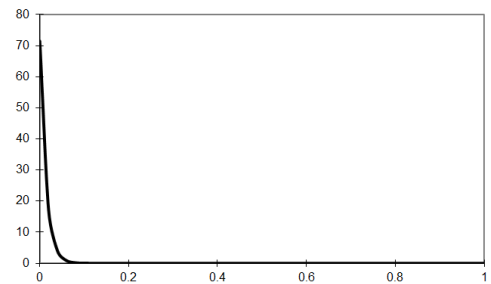
Posteriors from 292 trial cases; same priors for 1 and 2
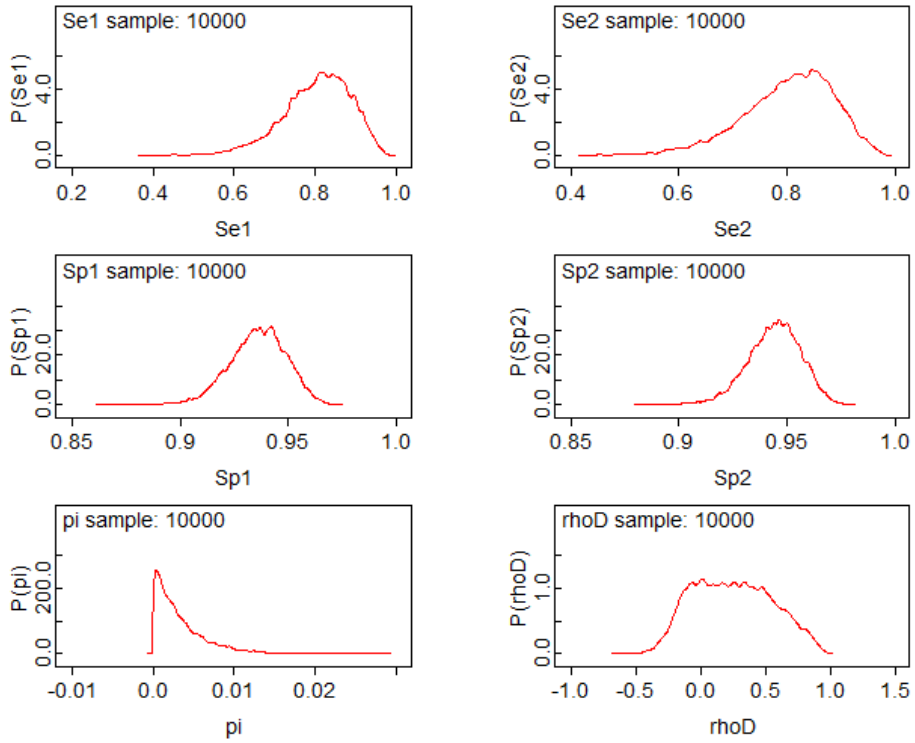
	mean	sd	MC_error	val2.5pc	median	val97.5pc	start	sample
Se1	0.8054	0.08208	8.319E-4	0.6195	0.8139	0.938	2001	10000
Se2	0.8041	0.08257	7.265E-4	0.6152	0.814	0.9381	2001	10000
Sp1	0.9361	0.01294	1.245E-4	0.9092	0.9367	0.9592	2001	10000
Sp2	0.9443	0.01217	1.033E-4	0.9184	0.945	0.966	2001	10000
pi	0.003407	0.003392	3.312E-5	9.085E-5	0.002383	0.0125	2001	10000
rhoD	0.25	0.3029	0.003445	-0.2643	0.2405	0.8294	2001	10000
zSe	0.4984	0.5	0.00513	0.0	0.0	1.0	2001	10000
zSp	0.6853	0.4644	0.004409	0.0	1.0	1.0	2001	10000

Coliform

Widened Priors from Doohoo (2011) Table 2

Se1 Sensitivity of Standard test		beta(14, 5) mean 0.737 CI (0.52, 0.90)
Sp1 Specificity of Standard test		beta(1000, 1) mean 0.999 CI (0.996, 1.00)
π Prevalence of bacterial infection in mastitis cases		beta(1, 72) mean 0.01 CI (0.00, 0.05)

Posteriors from 292 trial cases; same priors for 1 and 2
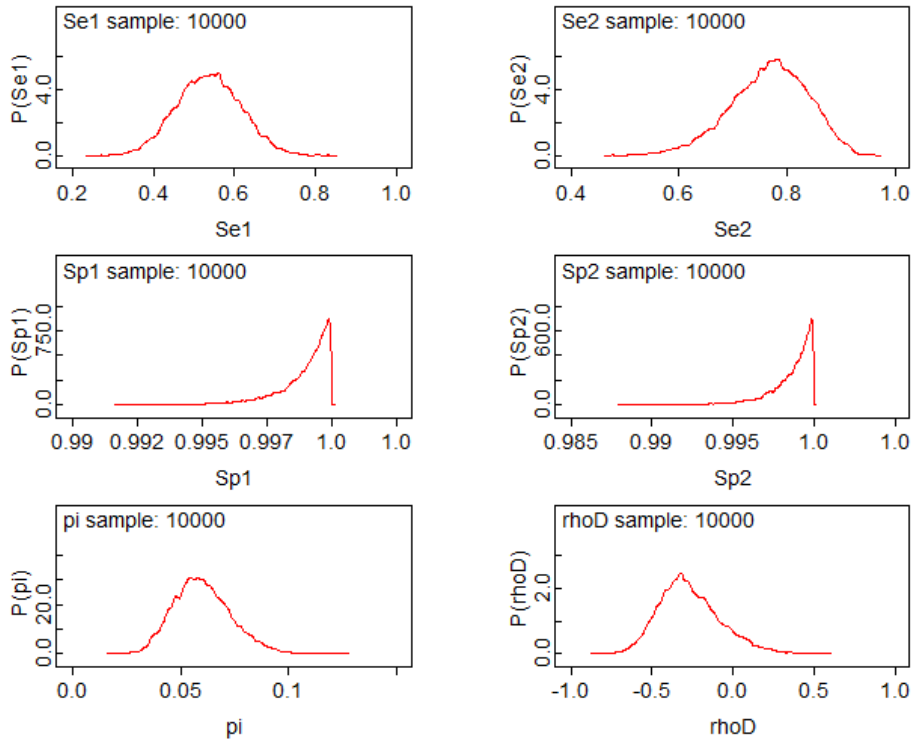
	mean	sd	MC_error	val2.5pc	median	val97.5pc	start	sample
Se1	0.5364	0.07906	8.311E-4	0.3841	0.5364	0.6902	2001	10000
Se2	0.7675	0.0719	7.402E-4	0.6111	0.7722	0.894	2001	10000
Sp1	0.999	9.926E-4	9.969E-6	0.9963	0.9993	1.0	2001	10000
Sp2	0.9987	0.001302	1.193E-5	0.9953	0.9991	1.0	2001	10000
pi	0.06024	0.01336	1.546E-4	0.03684	0.05924	0.089	2001	10000
rhoD	-0.2661	0.1891	0.00183	-0.5867	-0.2871	0.1543	2001	10000
zSe	0.9762	0.1524	0.001613	1.0	1.0	1.0	2001	10000
zSp	0.439	0.4963	0.004779	0.0	0.0	1.0	2001	10000

References

Lunn DJ, Thomas A, Best N & Spiegelhalter D 2000 WinBUGS - A Bayesian modelling framework: Concepts, structure, and extensibility, *Statistical Computing* **10** (4): 325-337