SALMONELLA MATRIX APPLICATION GUIDE SAMPLE SET REQUIREMENTS

(DNAble Kit for *Salmonella*, Cat. No. DF-026, Part #11716)

Pet Food and Feed	Dried Pet Food*	Sample Set 2
	Poultry Feed (2.5% mBPW/variation from SS2 protocol)	Sample Set 2
	Seeded Pet Food (2.5% mBPW/variation from SS2 protocol)	Sample Set 2
	Probiotic Pet Supplement	Sample Set 2
Mill Products	Corn Gluten Meal	Sample Set 2
	Rough Wheat Flour	Sample Set 2
	Corn/Soy Mixed Flour	Sample Set 2
	Canola Meal	Sample Set 2
	Soybean Meal	Sample Set 2
Foods	Blackberries	Sample Set 2
	Blueberries	Sample Set 2
	Cheese, Goat	Sample Set 2
	Cheese, Gorgonzola	Sample Set 2
	Cherries	Sample Set 2
	Chicken, Raw	Sample Set 2
	Chicken Salad	Sample Set 2
	Chilies, Whole	Sample Set 2
	Chocolate	Sample Set 1
	Eggs (yolks, whites, hard-boiled) *(sample prep only)*	Sample Set 2
	Peaches	Sample Set 2
	Raspberries	Sample Set 2
	Salami, Deli Hard	Sample Set 2
	Shrimp – Shell-off, Head-off, Tail-off, Deveined	Sample Set 2
	Shrimp – Shell-off, Head-off, Tail-on	Sample Set 2
	Shrimp – Shell-on, Head-on, Tail-on	Sample Set 3
	Strawberries	Sample Set 2
	Turkey, Deli Sliced Skinless Breast	Sample Set 2
	Turkey, Ground	Sample Set 1
Spices	Chili powder	Sample Set 2
	Powdered coriander	Sample Set 2
	Powdered cumin	Sample Set 2
Poultry Processing	Poultry environmental, fecal and cloacal swabs*	Sample Set 1
	Poultry environmental drag swabs*	Sample Set 2
	Boot Swabs	Sample Set 1
	Carcass Rinse	Sample Set 1
	Ceca	Sample Set 1
Other	Stainless Steel Surfaces (sponge/swab)*	Sample Set 2
	Horse Feces	Sample Set 2
	Swine Feces	Sample Set 2
	Organic Fertilizer	Sample Set 2

* = validated and protocol detailed in the Sample Extraction Set Product Insert

MATRIX FEASIBILITY STUDY: Poultry Feed

OBJECTIVE:

The purpose of this study was to assess the compatibility of the **poultry feed (pellets)** matrix with the DNAble® *Salmonella* Assay (Cat. No. DF-026, Part #11716).

MATERIALS:

- DNAble *Salmonella* DNA Detection Kit consisting of:
  - RB1 Reaction Buffer tubes
  - *Salmonella* Master Mix tubes
- DNAble Sample Extraction Set 2
  - MB3 Buffer
  - Microcentrifuge tubes
- Buffered Peptone Water
- DNAble *Salmonella* Supplement
- *Salmonella* Enteritidis (ATCC# BAA 1587)
- Whirl-Pak® Bags
- Blue Seal Home Fresh Poultry Feed

SAMPLE PREP:

- 2 samples were prepared by adding 225 mL of 2.5% mBPW to 25 grams of poultry feed pellets in a Whirl-Pak bag. One sample was spiked with 10 cells of *Salmonella*.
- Both samples were incubated overnight (~17 hours) at 37°C.
- After incubation, samples containing 10^4, 10^5, and 10^6 CFU/mL were created by spiking known amounts of *Salmonella* into aliquots of presumed negative enriched samples. These samples along with the presumed negative and the 10 cell spiked sample, were prepared for the DNAble assay using the instructions from the Sample Set 2 Product Insert:
  - Pre-heat a dry heat block to 95°C.
  - Mix the culture before sampling.
  - Transfer 1 mL of culture to a clear 1.5 mL microcentrifuge tube supplied with the set.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Remove the supernatant using caution to avoid disturbing the pellet. Leave a small volume remaining (≈ 100 µL) if an obvious pellet is not observed.
  - Add 100 µL of MB3 buffer and vortex to suspend the pellet.
  - Heat the sample in the heat block at 95°C for 10 minutes.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Place 100 µL of MB3 into a blue 1.5 mL microcentrifuge tube using a fresh pipette tip.
  - Transfer 25 µL of the supernatant from the second centrifugation into the MB3 and mix gently.
  - 5 µL of this prepared sample will be used in the subsequent DNAble reaction.
- The DNAble *Salmonella* assay was then run in duplicate on each sample.

RESULTS:

Figure 1: Data exported and extrapolated using Excel. This data was obtained using the following Poultry Feed (PF) samples: Negative (neg) with MB3 sample prep and 10 cell spike prior to enrichment (low) with MB3 sample prep.

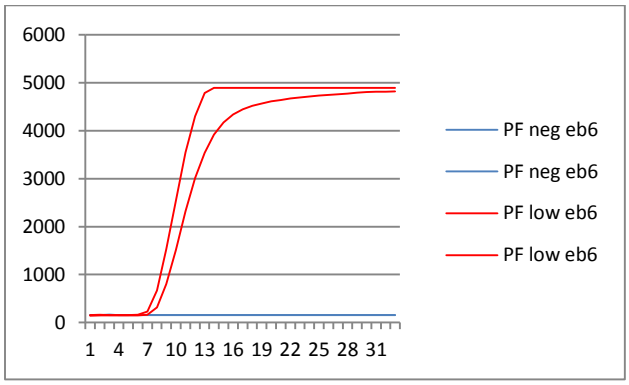
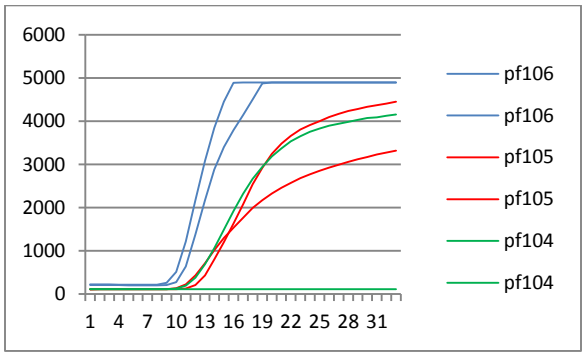


Figure 2: Limit of Detection (LOD) Study: Data exported and extrapolated using Excel. Samples consisted of negative enriched Poultry Feed spiked with known amounts: 10^6 (106), 10^5 (105), 10^4 (104) of *Salmonella* Enteritidis ATCC# BAA 1587 and prepared using the MB3 sample prep.



CONCLUSION:

From this limited study, initial data suggests that the Poultry Feed matrix is compatible with the DNAble *Salmonella* assay. In this study *Salmonella* was detected down to 10^5 CFU/mL with one replicate to 10^4 CFU/mL.

Note: As this was a limited study, a validation by your site is suggested.

MATRIX FEASIBILITY STUDY: Seeded Pet Food

OBJECTIVE:

The purpose of this study was to assess the compatibility of the **seeded pet food** matrix with the DNAble® *Salmonella* Assay (Cat. No. DF-026, Part #11716).

MATERIALS:

- DNAble *Salmonella* DNA Detection Kit consisting of:
  - RB1 Reaction Buffer tubes
  - *Salmonella* Master Mix tubes
- DNAble Sample Extraction Set 2
  - MB3 Buffer
  - Microcentrifuge tubes
- Buffered Peptone Water
- DNAble *Salmonella* Supplement
- *Salmonella* Enteritidis (ATCC# BAA 1587)
- Whirl-Pak® bags
- Parrot food, gerbil food, finch food (seeds, pellets, nuts, etc.)

SAMPLE PREP:

- For the feasibility portion of the study, 2 samples of each food type were prepared by adding 50 mL of 2.5% mBPW to 5 grams of pet food in a conical tube. One sample of each set was spiked with 10 cells of *Salmonella*. Samples were then mixed by hand.
- Both samples were incubated overnight (~17 hours) at 37°C.
- These samples were prepared for the DNAble assay using the instructions from the Sample Set 2 Product Insert:
  - Pre-heat a dry heat block to 95°C.
  - Mix the culture before sampling.
  - Transfer 1 mL of culture to a clear 1.5 mL microcentrifuge tube supplied with the set.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Remove the supernatant using caution to avoid disturbing the pellet. Leave a small volume remaining (≈ 100 µL) if an obvious pellet is not observed.
  - Add 100 µL of MB3 buffer and vortex to suspend the pellet.
  - Heat the sample in the heat block at 95°C for 10 minutes.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Place 100 µL of MB3 into a blue 1.5 mL microcentrifuge tube using a fresh pipette tip.
  - Transfer 25 µL of the supernatant from the second centrifugation into the MB3 and mix gently.
  - 5 µL of this prepared sample will be used in the subsequent DNAble reaction.
- The DNAble *Salmonella* assay was then run in duplicate on each sample.
- For the Limit of Detection portion of the study, samples containing 10^4, 10^5, and 10^6 CFU/mL were created by spiking known amounts of *Salmonella* into aliquots of the negative enriched Parrot food matrix (25g Parrot food added to 225 mL mBPW) which was chosen as a "worst case scenario". These samples prepared for the DNAble assay by using the MB3 sample prep described above.

RESULTS:

Figure 1: Data exported and extrapolated using Excel. This data was obtained using the following Finch Food (F) samples: Negative (neg) with MB3 sample prep and 10 cell spike prior to enrichment (pos) with MB3 sample prep.

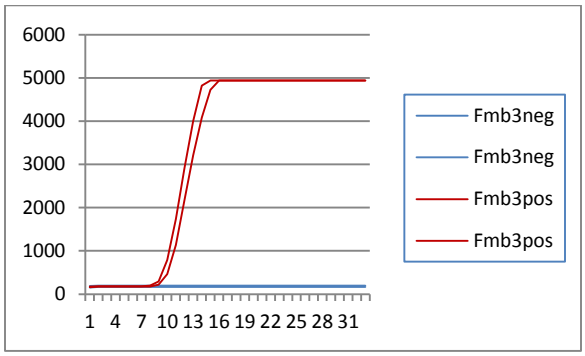


Figure 2: Data exported and extrapolated using Excel. This data was obtained using the following Gerbil Food (G) samples: Negative (neg) with MB3 sample prep and 10 cell spike prior to enrichment (pos) with MB3 sample prep.

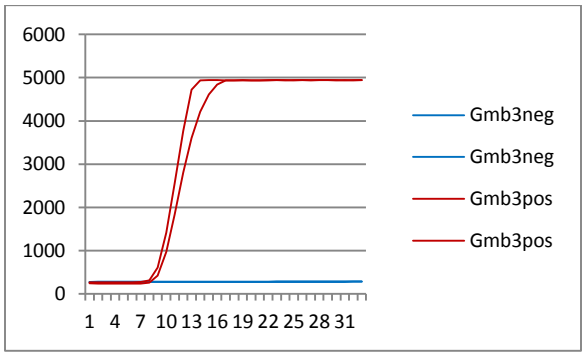


Figure 3: Data exported and extrapolated using Excel. This data was obtained using the following Parrot Food (P) samples: Negative (neg) with MB3 sample prep and 10 cell spike prior to enrichment (pos) with MB3 sample prep.

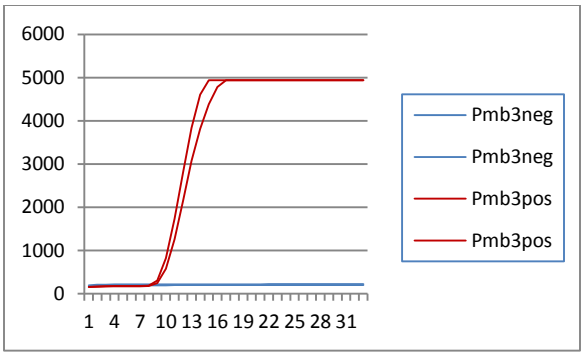
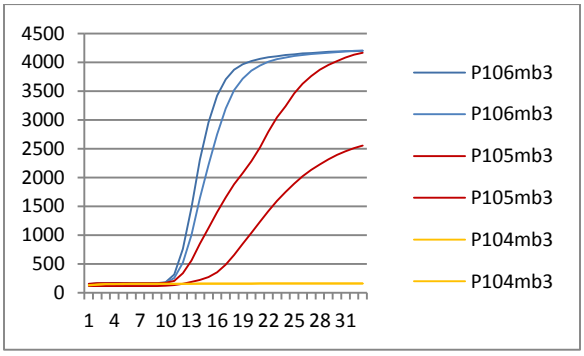


Figure 4: Data exported and extrapolated using Excel. Samples consisted of negative enriched Parrot Food spiked with known amounts: 10^6 (106), 10^5 (105), 10^4 (104) CFU/mL of *Salmonella* Enteritidis ATCC# BAA 1587 and prepared using the MB3 sample prep.



CONCLUSION:

From this limited study, initial data suggests that the seeded pet food matrix is compatible with the DNAble *Salmonella* assay. In this study *Salmonella* was detected down to 10^5 CFU/mL.

Note: As this was a limited study, a validation by your site is suggested.

MATRIX FEASIBILITY STUDY: Probiotic Pet Supplement

OBJECTIVE:

The purpose of this study was to assess the compatibility of the **probiotic pet supplement** matrix with the DNAble® *Salmonella* Assay (Cat. No. DF-026, Part #11716).

MATERIALS:

- DNAble *Salmonella* DNA Detection Kit consisting of:
  - RB1 Reaction Buffer tubes
  - *Salmonella* Master Mix tubes
- DNAble Sample Extraction Set 2
  - MB3 Buffer
  - Microcentrifuge tubes
- Buffered Peptone Water
- DNAble *Salmonella* Supplement
- *Salmonella enterica*
- Whirl-Pak® Bags
- PETIMMUNE digestive health supplement consisting of mainly alfalfa meal and probiotics

SAMPLE PREP:

- 2 samples were prepared by adding 225 mL of 2.0% mBPW to 25 grams of PETIMMUNE in a Whirl-Pak bag. One sample was spiked with 10 cells of *Salmonella*. Samples were then "stomached" by hand for approximately 1 minute.
- Both samples were incubated overnight (~17 hours) at 37°C.
- After incubation, samples containing 10^4, 10^5, and 10^6 CFU/mL were created by spiking known amounts of *Salmonella* into aliquots of the negative enriched sample. These samples along with the presumed negative and the 10 cell spiked sample, were prepared for the DNAble assay using the instructions from the Sample Set 2 Product Insert:
  - Pre-heat a dry heat block to 95°C.
  - Mix the culture before sampling.
  - Transfer 1 mL of culture to a clear 1.5 mL microcentrifuge tube supplied with the set.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Remove the supernatant using caution to avoid disturbing the pellet. Leave a small volume remaining (≈ 100 µL) if an obvious pellet is not observed.
  - Add 100 µL of MB3 buffer and vortex to suspend the pellet.
  - Heat the sample in the heat block at 95°C for 10 minutes.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Place 100 µL of MB3 into a blue 1.5 mL microcentrifuge tube using a fresh pipette tip.
  - Transfer 25 µL of the supernatant from the second centrifugation into the MB3 and mix gently.
  - 5 µL of this prepared sample will be used in the subsequent DNAble reaction.
- The DNAble *Salmonella* assay was then run in duplicate on each sample.

RESULTS:

Figure 1: Data exported and extrapolated using Excel. The data below was obtained using PETIMMUNE (PI) both unspiked (-) and spiked with 10 cells of *Salmonella* prior to overnight enrichment (+) and prepared using the MB3 sample prep.

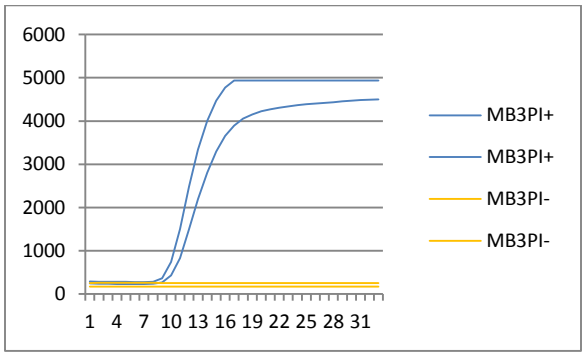


Figure 2: Samples consisted of negative PetImmune spiked with known amounts: 10^6, 10^5, 10^4 CFU/mL of *Salmonella* and prepared using the MB3 sample prep.

CFU/mL	DNAble Result	DNAble Result
10^6	+	+
10^5	+	-
10^4	-	-

CONCLUSION:

From this limited study, initial data suggests that the PETIMMUNE probiotic pet supplement matrix is compatible with the DNAble *Salmonella* assay. In this study *Salmonella* was detected down to 10^5 CFU/mL.

Note: As this was a limited study, a validation by your site is suggested.

MATRIX FEASIBILITY STUDY: Corn Gluten Meal

OBJECTIVE:

The purpose of this study was to assess the compatibility of the **corn gluten meal** matrix with the DNAble® *Salmonella* Assay (Cat. No. DF-026, Part #11716).

MATERIALS:

- DNAble *Salmonella* DNA Detection Kit consisting of:
  - RB1 Reaction Buffer tubes
  - *Salmonella* Master Mix tubes
- DNAble Sample Extraction Set 2
  - MB3 Buffer
  - Microcentrifuge tubes
- Buffered Peptone Water
- DNAble *Salmonella* Supplement
- *Salmonella enterica*
- Whirl-Pak® bags
- Corn gluten meal

SAMPLE PREP:

- 2 samples were prepared by adding 225 mL of 2.0% mBPW to 25 grams of corn gluten meal in a Whirl-Pak bag. One sample was spiked with 10 cells of *Salmonella*. Samples were then "stomached" by hand for approximately 1 minute.
- Both samples were incubated overnight (~17 hours) at 37°C.
- After incubation, samples containing 10^4, 10^5, and 10^6 CFU/mL were created by spiking known amounts of *Salmonella* into aliquots of the negative enriched sample. These samples along with the negative and the 10 cell spiked sample, were prepared for the DNAble assay using the instructions from the Sample Set 2 Product Insert:
  - Pre-heat a dry heat block to 95°C.
  - Mix the culture before sampling.
  - Transfer 1 mL of culture to a clear 1.5 mL microcentrifuge tube supplied with the set.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Remove the supernatant using caution to avoid disturbing the pellet. Leave a small volume remaining (≈ 100 µL) if an obvious pellet is not observed.
  - Add 100 µL of MB3 buffer and vortex to suspend the pellet.
  - Heat the sample in the heat block at 95°C for 10 minutes.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Place 100 µL of MB3 into a blue 1.5 mL microcentrifuge tube using a fresh pipette tip.
  - Transfer 25 µL of the supernatant from the second centrifugation into the MB3 and mix gently.
  - 5 µL of this prepared sample will be used in the subsequent DNAble reaction.
- The DNAble *Salmonella* assay was then run in duplicate on each sample.

RESULTS:

Figure 1: Data exported and extrapolated using Excel. The data below was obtained using corn gluten meal (CM) both unspiked (-), spiked with 10 cells of *Salmonella* prior to overnight enrichment (+):

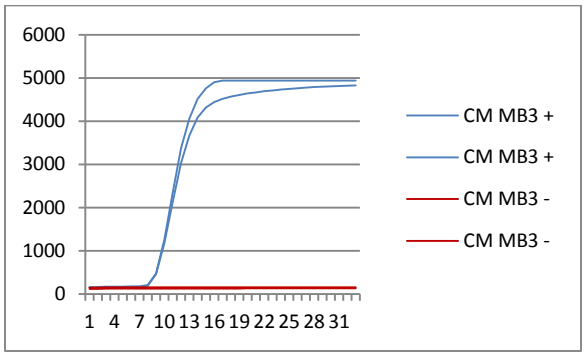
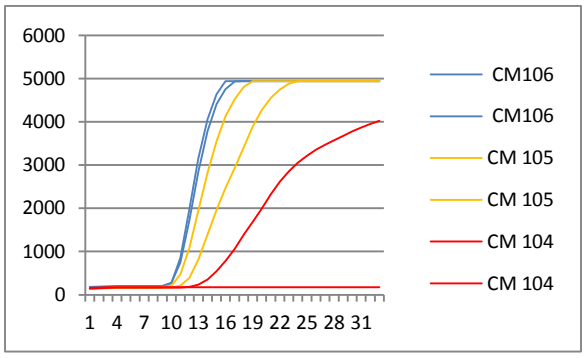


Figure 2: Data exported and extrapolated using Excel. Samples consisted of negative corn gluten meal (CM) spiked with known amounts: 10^6 (106), 10^5 (105), 10^4 (104) CFU/mL of *Salmonella* and prepared using the MB3 sample prep.



CONCLUSION:

From this limited study, initial data suggests that the corn gluten meal matrix is compatible with the DNAble *Salmonella* assay. In this study *Salmonella* was detected down to 10^5 CFU/mL, and in one technical replicate to 10^4 CFU/mL.

Note: As this was a limited study, a validation by your site is suggested.

MATRIX FEASIBILITY STUDY: Rough Wheat Flour

OBJECTIVE:

The purpose of this study was to assess the compatibility of the **rough wheat flour** matrix with the DNAble® *Salmonella* Assay (Cat. No. DF-026, Part #11716).

MATERIALS:

- DNAble *Salmonella* DNA Detection Kit consisting of:
  - RB1 Reaction Buffer tubes
  - *Salmonella* Master Mix tubes
- DNAble Sample Extraction Set 2
  - MB3 Buffer
  - Microcentrifuge tubes
- Buffered Peptone Water
- DNAble *Salmonella* Supplement
- *Salmonella* Enteritidis (ATCC# BAA 1587)
- Whirl-Pak® Bags
- Rough wheat flour

SAMPLE PREP:

- 2 samples were prepared by adding 225 mL of 2.5% mBPW to 25 grams of rough wheat flour in a Whirl-Pak bag. One sample was spiked with 10 cells of *Salmonella*. Samples were then "stomached" by hand for approximately 1 minute.
- Both samples were incubated overnight (~17 hours) at 37°C.
- After incubation, samples containing 10^4, 10^5, and 10^6 CFU/mL were created by spiking known amounts of *Salmonella* into aliquots of the negative enriched sample. These samples along with the presumed negative and the 10 cell spiked sample, were prepared for the DNAble assay using the instructions from the Sample Set 2 Product Insert:
  - Pre-heat a dry heat block to 95°C.
  - Mix the culture before sampling.
  - Transfer 1 mL of culture to a clear 1.5 mL microcentrifuge tube supplied with the set.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Remove the supernatant using caution to avoid disturbing the pellet. Leave a small volume remaining (≈ 100 µL) if an obvious pellet is not observed.
  - Add 100 µL of MB3 buffer and vortex to suspend the pellet.
  - Heat the sample in the heat block at 95°C for 10 minutes.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Place 100 µL of MB3 into a blue 1.5 mL microcentrifuge tube using a fresh pipette tip.
  - Transfer 25 µL of the supernatant from the second centrifugation into the MB3 and mix gently.
  - 5 µL of this prepared sample will be used in the subsequent DNAble reaction.
- The DNAble *Salmonella* assay was then run in duplicate on each sample.

RESULTS:

Figure 1: Data exported and extrapolated using Excel. The data below was obtained using rough wheat flour (W) both unspiked (neg), spiked with 10 cells of *Salmonella* prior to overnight enrichment (pos).

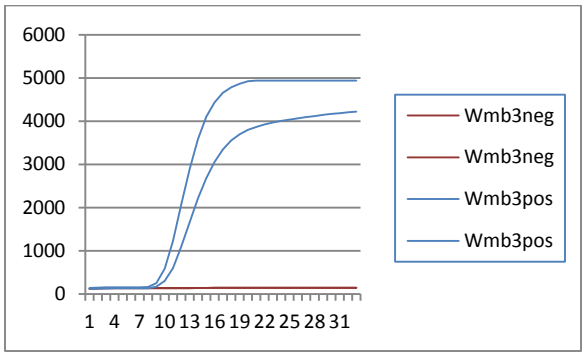
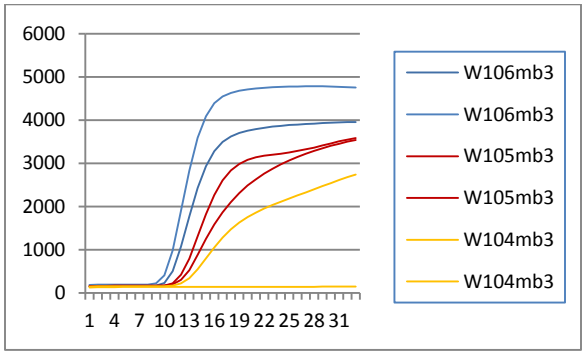


Figure 2: Data exported and extrapolated using Excel. Samples consisted of negative enriched rough wheat flour spiked with known amounts: 10^6 (106), 10^5 (105), 10^4 (104) CFU/mL of *Salmonella* and prepared using the MB3 sample prep.



CONCLUSION:

From this limited study, initial data suggests that the rough wheat flour matrix is compatible with the DNAble *Salmonella* assay. In this study *Salmonella* was detected down to 10^5 CFU/mL, and in one technical replicate to 10^4 CFU/mL.

Note: As this was a limited study, a validation by your site is suggested.

MATRIX FEASIBILITY STUDY: Corn / Soy Mixture

OBJECTIVE:

The purpose of this study was to assess the compatibility of the **ground corn/soy** matrix with the DNAble® *Salmonella* Assay (Cat. No. DF-026, Part #11716).

MATERIALS:

- DNAble *Salmonella* DNA Detection Kit consisting of:
  - RB1 Reaction Buffer tubes
  - *Salmonella* Master Mix tubes
- DNAble Sample Extraction Set 2
  - MB3 Buffer
  - Microcentrifuge tubes
- Buffered Peptone Water
- DNAble *Salmonella* Supplement
- *Salmonella enterica*
- 50 mL Falcon tubes
- TSAYE agar plates
- Corn / soy mixture

SAMPLE PREP:

- 2.5 grams of the corn/soy mixture were placed in individual 50 mL plastic tubes. The samples were spiked with descending concentrations of *Salmonella enterica* (10^7 - 10^1 CFU/mL)-(100 µL of 10^8-10^2 CFU/mL added to each sample). Duplicate samples at each dilution were prepared. Samples were allowed to dry for 1 hour after spiking.
- Concentrations confirmed by colony count plates (starting concentration = 8.0 x 10^8 CFU/mL)
- Original culture was placed in the refrigerator overnight before making dilutions used for spiking.

Inoculum (CFU/mL)	Concentration in Sample (CFU/mL)
100 µL of 10^8	10^7
100 µL of 10^7	10^6
100 µL of 10^6	10^5
100 µL of 10^5	10^4
100 µL of 10^4	10^3
100 µL of 10^3	10^2
100 µL of 10^2	10^1

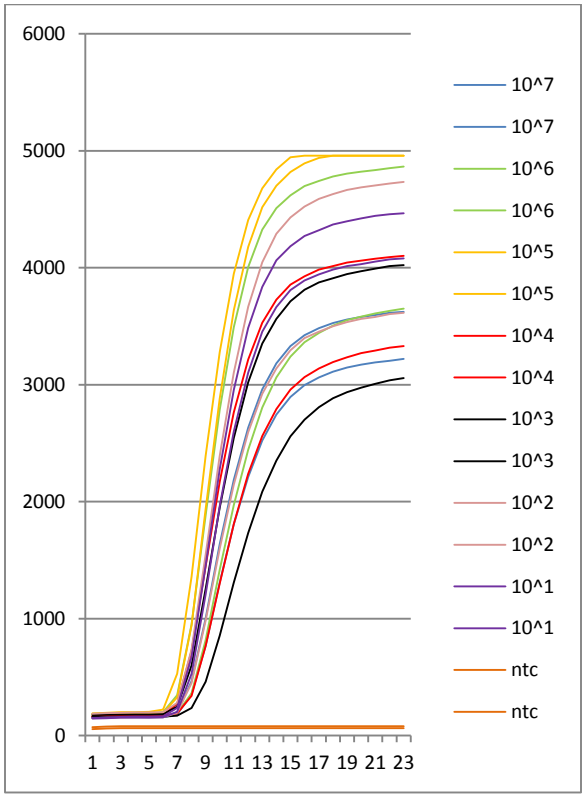
- 22.5 mL of 2.0% mBPW was added to each tube.
- Each tube was vortexed well.
- Samples were incubated for 20 hours at 37°C.
- After incubation, the samples were prepared for the DNAble assay using the instructions from the Sample Set 2 Product Insert:
  - Pre-heat a dry heat block to 95°C.
  - Mix the culture before sampling.
  - Transfer 1 mL of culture to a clear 1.5 mL microcentrifuge tube supplied with the set.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Remove the supernatant using caution to avoid disturbing the pellet. Leave a small volume remaining (≈ 100 µL) if an obvious pellet is not observed.
  - Add 100 µL of MB3 buffer and vortex to suspend the pellet.
  - Heat the sample in the heat block at 95°C for 10 minutes.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.

- Place 100 µL of MB3 into a blue 1.5 mL microcentrifuge tube using a fresh pipette tip.
- Transfer 25 µL of the supernatant from the second centrifugation into the MB3 and mix gently.
- 5 µL of this prepared sample will be used in the subsequent DNAble reaction.

- The DNAble *Salmonella* assay was then run on each sample.

RESULTS:

Figure 1: Data exported and extrapolated using Excel. Samples spiked with descending dilutions of *Salmonella enterica* prior to enrichment & prepped using MB3 protocol.



CONCLUSION:

From this limited study, initial data suggests that the ground corn / soy mix matrix is compatible with the DNAble *Salmonella* assay. In this study *Salmonella* was detected down to 10^1 CFU/mL after a 20 hour enrichment.

Note: As this was a limited study, a validation by your site is suggested.

MATRIX FEASIBILITY STUDY: Canola Meal

OBJECTIVE:

The purpose of this study was to assess the compatibility of the **canola meal** matrix with the DNAble® *Salmonella* (Cat. No. DF-026, Part #11716) and *Salmonella* Plus (Cat. No. DF-126, Part #12010) Assays.

MATERIALS:

- DNAble *Salmonella* DNA Detection Kit consisting of:
  - RB1 Reaction Buffer tubes
  - *Salmonella* Master Mix tubes
- DNAble *Salmonella* Plus DNA Detection Kit consisting of:
  - RB1 Reaction Buffer tubes
  - *Salmonella* Plus Master Mix tubes
- DNAble Sample Extraction Set 2 (Cat. No. ACC-085, Part# 11718):
  - MB3 Extraction Buffer
  - Micro-centrifuge tubes
- Buffered Peptone Water
- DNAble *Salmonella* Media Supplement (Part # 11622):
- *Salmonella enterica*
- Canola Meal
- Whirl-Pak® bags

SAMPLE PREP:

- The following samples were prepared by combining 225mL BPW, 4.5 mL *Salmonella* Supplement & 25 grams of canola meal in a Whirl-Pak bag:
  - Negative Canola Meal
  - Canola Meal spiked with approximately 10 cells of *Salmonella*
- All samples were incubated overnight (~ 17 hours) at 37°C.
- After incubation, samples containing 10^4, 10^5 and 10^6 CFU/mL were created by spiking known amounts of *Salmonella* into aliquots of presumed negative samples. These samples along with the presumed negative and the 10 cell spiked sample were prepared for the DNAble assays by using the sample prep described in the Sample Extraction Set 2 Product Insert:
  - Preheat dry block to 95°C.
  - Mix the culture before sampling.
  - Transfer 1 mL of culture to a clear 1.5 mL microcentrifuge tube supplied with the set.
  - Centrifuge @ 10,000 x *g* for 5 min.
  - Remove supernatant taking care to not disturb the pellet and then resuspend pellet. Leave a small amount (~100 µL) of supernatant if an obvious pellet is not observed.
  - Add 100 µL of MB3 Extraction Buffer, vortex and heat at 95°C for 10 minutes.
  - Centrifuge @ 10,000 x *g* for 5 minutes.
  - For the *Salmonella* assay, add 25 µL of supernatant to 100 µL of MB3 in a blue microcentrifuge tube.
  - For the *Salmonella* Plus assay, add 10 µL of supernatant to 90 µL of MB3.

The DNAble *Salmonella* and *Salmonella* Plus assays were then run in duplicate on each sample.

RESULTS:

This data represents canola meal samples: Negative (NEG) and pre-enrichment *Salmonella* spiked positive (POS):

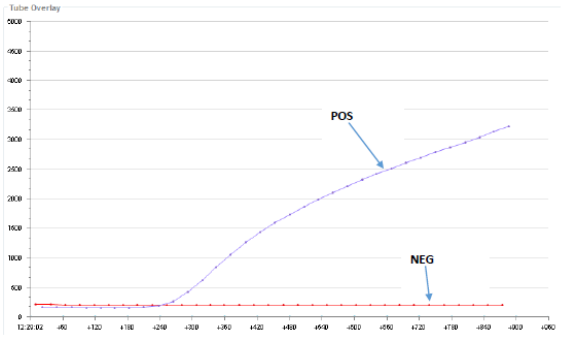


Figure 1: Negative and Positive (*Salmonella* spiked) Samples using the *Salmonella* assay.

This data represents canola meal samples: Negative (NEG) and pre-enrichment *Salmonella* spiked positive (POS):

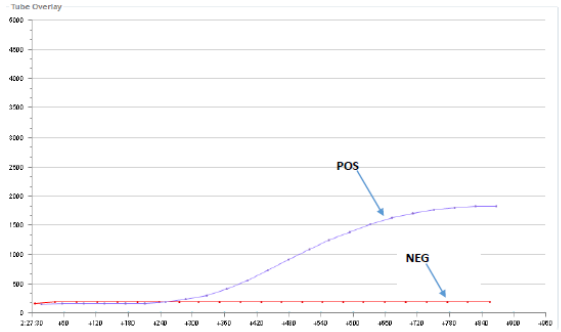


Figure 2: Negative and Positive (*Salmonella* spiked) Samples using the *Salmonella* Plus assay.

Samples consisted of negative enriched canola meal spiked with known amounts: 10^6 (10^6), 10^5 (10^5), 10^4 (10^4) CFU/mL of *Salmonella*:

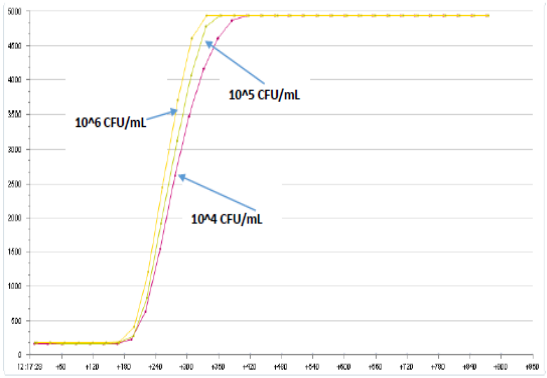


Figure 3: Limit of Detection (LOD Study) – *Salmonella* Assay

Samples consisted of negative enriched canola meal spiked with known amounts: 10^6 (10^6), 10^5 (10^5), 10^4 (10^4) CFU/mL of *Salmonella*:

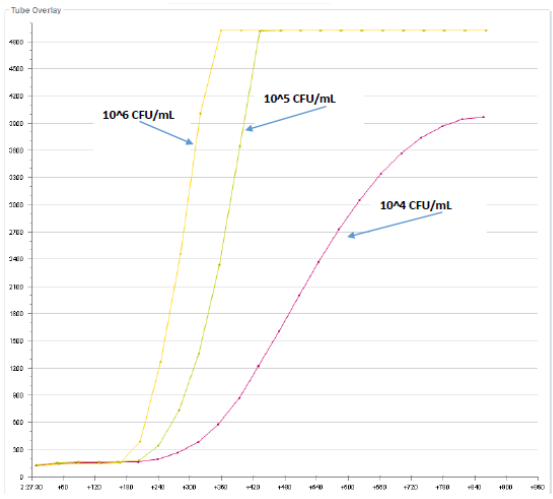


Figure 4: Limit of Detection (LOD Study) – *Salmonella* Plus Assay

CONCLUSION:

From this limited study, initial data suggests that the canola meal matrix is compatible with the DNAble *Salmonella* and *Salmonella* Plus assay. In this study *Salmonella* was detected down to 10^4 CFU/mL. The algorithm has not been defined; therefore these samples may not yield conclusive positive or negative results. The end user can rely on their interpretation or send us json files for conclusive output. This data is preliminary and more work is needed to substantiate these findings.

Note: As this was a limited study, a validation by your site is suggested.

MATRIX FEASIBILITY STUDY: Soybean Meal

OBJECTIVE:

The purpose of this study was to assess the compatibility of Soybean Meal matrix with the DNAble® *Salmonella* Assay (Cat. No. DF-026, Part# 11716).

MATERIALS:

- DNAble *Salmonella* DNA Detection Kit consisting of:
  - RB1 Reaction Buffer tubes
  - *Salmonella* Master Mix tubes
- DNAble Sample Extraction Set 2
  - MB3 Buffer
  - Microcentrifuge tubes
- Buffered Peptone Water
- DNAble *Salmonella* Supplement
- *Salmonella enterica*
- Filtered Whirl-Pak® bags
- Soybean Meal

SAMPLE PREP:

- The following samples were prepared by adding 225 mL of 2.0% mBPW to 25 grams of soybean meal in a Whirl-Pak bag:
  - Presumed negative soybean meal
  - Soybean meal spiked with *Salmonella*
- All samples were incubated overnight (~17 hours) at 37°C.
- After incubation, samples containing 10^4, 10^5, and 10^6 CFU/mL were created by spiking known amounts of *Salmonella* into aliquots of the presumed negative enriched samples. These samples, along with the presumed negative and *Salmonella* spiked samples, were prepared for the DNAble assay using the instructions from the Sample Set 2 Product Insert:
  - Pre-heat a dry heat block to 95°C.
  - Mix the culture before sampling.
  - Transfer 1 mL of culture to a clear 1.5 mL microcentrifuge tube supplied with the set.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Remove the supernatant using caution to avoid disturbing the pellet. Leave a small volume remaining (≈ 100 µL) if an obvious pellet is not observed.
  - Add 100 µL of MB3 buffer and vortex to suspend the pellet.
  - Heat the sample in the heat block at 95°C for 10 minutes.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Place 100 µL of MB3 into a blue 1.5 mL microcentrifuge tube using a fresh pipette tip.
  - Transfer 25 µL of the supernatant from the second centrifugation into the MB3 and mix gently.
  - 5 µL of this prepared sample will be used in the subsequent DNAble reaction.
- The DNAble *Salmonella* assay was then run in duplicate on each sample.

RESULTS:

Figure 1: Negative and Positive (*Salmonella* spiked) Samples

This data represents soybean meal samples: Negative (Neg), and pre-enrichment *Salmonella* spiked positive (Pos)

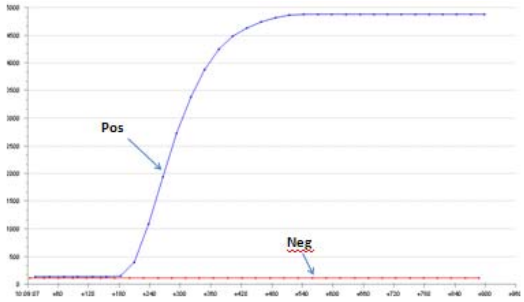
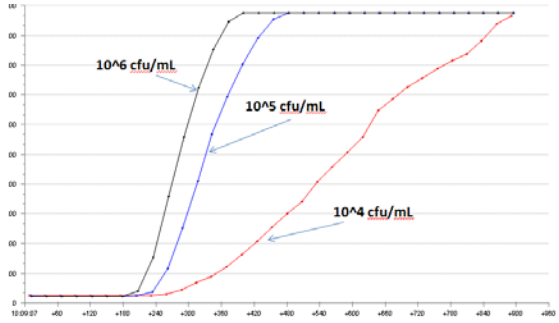


Figure 2: Limit of Detection (LOD) Study samples consisted of negative enriched soybean meal spiked with known amounts: 10^6 (10^6), 10^5 (10^5), 10^4 (10^4) CFU/mL of *Salmonella*



CONCLUSION:

From this limited study, initial data suggests that the soybean meal matrix is compatible with the DNAble *Salmonella* assay. In this study *Salmonella* was detected down to a level of 10^4 CFU/mL. The algorithm has not been defined; therefore these samples may not yield conclusive positive or negative results. The end user can rely on their interpretation or send us json files for conclusive output. This data is preliminary and more work is needed to substantiate these findings.

Note: As this was a limited study, a validation by your site is suggested.

MATRIX FEASIBILITY STUDY: Blackberries

OBJECTIVE:

The purpose of this study was to assess the compatibility of the **blackberry** matrix with the DNAble® *Salmonella* Assay (Cat. No. DF-026, Part #11716).

MATERIALS:

- DNAble *Salmonella* DNA Detection Kit consisting of:
  - RB1 Reaction Buffer tubes
  - *Salmonella* Master Mix tubes
- DNAble Sample Extraction Set 2
  - MB3 Buffer
  - Microcentrifuge tubes
- Buffered Peptone Water
- DNAble *Salmonella* Supplement
- *Salmonella enterica*
- Whirl-Pak® bags
- Blackberries

SAMPLE PREP:

- 2 samples were prepared by adding 225 mL of 2.0% mBPW to 25 grams of blackberries in a Whirl-Pak bag. One sample was spiked with 10 cells of *Salmonella*. Samples were then "stomached" by hand for approximately 1 minute.
- Both samples were incubated overnight (~18-20 hours) at 37°C.
- After incubation, samples containing 10^4, 10^5, and 10^6 CFU/mL were created by spiking known amounts of *Salmonella* into aliquots of the negative enriched sample. These samples along with the negative and the 10 cell spiked sample, were prepared for the DNAble assay using the instructions from the Sample Set 2 Product Insert:
  - Pre-heat a dry heat block to 95°C.
  - Mix the culture before sampling.
  - Transfer 1 mL of culture to a clear 1.5 mL microcentrifuge tube supplied with the set.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Remove the supernatant using caution to avoid disturbing the pellet. Leave a small volume remaining (≈ 100 µL) if an obvious pellet is not observed.
  - Add 100 µL of MB3 buffer and vortex to suspend the pellet.
  - Heat the sample in the heat block at 95°C for 10 minutes.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Place 100 µL of MB3 into a blue 1.5 mL microcentrifuge tube using a fresh pipette tip.
  - Transfer 25 µL of the supernatant from the second centrifugation into the MB3 and mix gently.
  - 5 µL of this prepared sample will be used in the subsequent DNAble reaction.
- The DNAble *Salmonella* assay was then run in duplicate on each sample.

RESULTS:

Figure 1: Data exported and extrapolated using Excel. The data below was obtained using blackberries both unspiked (Neg "BB-"), spiked with 10 cells of *Salmonella* prior to overnight enrichment (Pos "BB+"):

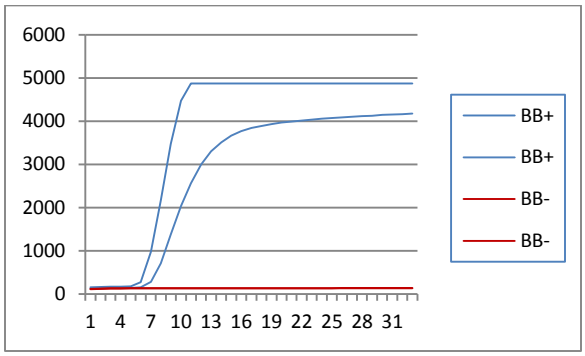
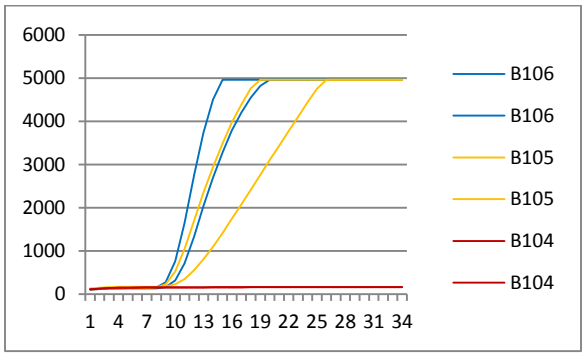


Figure 2: Data exported and extrapolated using Excel. Samples consisted of negative blackberries spiked with known amounts: 10^6 (106), 10^5 (105), 10^4 CFU/mL of *Salmonella*.



CONCLUSION:

From this limited study, initial data suggests that the blackberry matrix is compatible with the DNAble® *Salmonella* assay. In this study *Salmonella* was detected down to 10^5 CFU/mL.

Note: As this was a limited study, a validation by your site is suggested.

MATRIX FEASIBILITY STUDY: Blueberries

OBJECTIVE:

The purpose of this study was to assess the compatibility of the **blueberry** matrix with the DNAble® *Salmonella* Assay (Cat. No. DF-026, Part #11716).

MATERIALS:

- DNAble *Salmonella* DNA Detection Kit consisting of:
  - RB1 Reaction Buffer tubes
  - *Salmonella* Master Mix tubes
- DNAble Sample Extraction Set 2
  - MB3 Buffer
  - Microcentrifuge tubes
- Buffered Peptone Water
- DNAble *Salmonella* Supplement
- *Salmonella enterica*
- Whirl-Pak® bags
- Blueberries

SAMPLE PREP:

- 2 samples were prepared by adding 225 mL of 2.0% mBPW to 25 grams of blueberries in a Whirl-Pak bag. One sample was spiked with 10 cells of *Salmonella*. Samples were then "stomached" by hand for approximately 1 minute.
- Both samples were incubated overnight (~18-20 hours) at 37°C.
- After incubation, samples containing 10^4, 10^5, and 10^6 CFU/mL were created by spiking known amounts of *Salmonella* into aliquots of the negative enriched sample. These samples along with the negative and the 10 cell spiked sample, were prepared for the DNAble assay using the instructions from the Sample Set 2 Product Insert:
  - Pre-heat a dry heat block to 95°C.
  - Mix the culture before sampling.
  - Transfer 1 mL of culture to a clear 1.5 mL microcentrifuge tube supplied with the set.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Remove the supernatant using caution to avoid disturbing the pellet. Leave a small volume remaining (≈ 100 µL) if an obvious pellet is not observed.
  - Add 100 µL of MB3 buffer and vortex to suspend the pellet.
  - Heat the sample in the heat block at 95°C for 10 minutes.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Place 100 µL of MB3 into a blue 1.5 mL microcentrifuge tube using a fresh pipette tip.
  - Transfer 25 µL of the supernatant from the second centrifugation into the MB3 and mix gently.
  - 5 µL of this prepared sample will be used in the subsequent DNAble reaction.
- The DNAble *Salmonella* assay was then run in duplicate on each sample.

RESULTS:

Figure 1: Data exported and extrapolated using Excel. The data below was obtained using blueberries both unspiked (Neg), spiked with 10 cells of *Salmonella* prior to overnight enrichment (Pos):

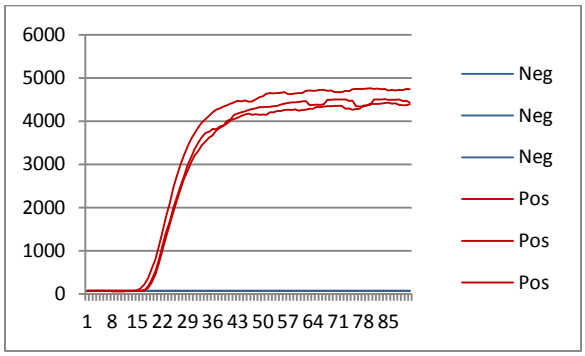
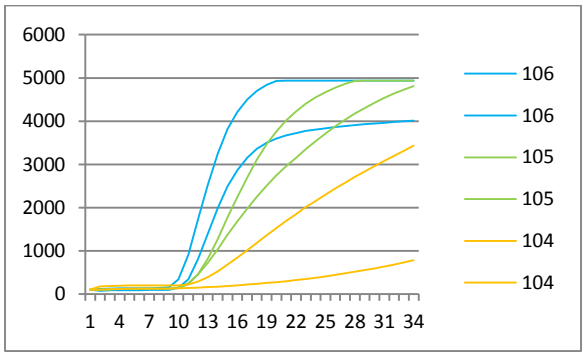


Figure 2: Data exported and extrapolated using Excel. Samples consisted of negative blueberries spiked with known amounts: 10^6 (106), 10^5 (105), 10^4 CFU/mL of *Salmonella*.



CONCLUSION:

From this limited study, initial data suggests that the blueberry matrix is compatible with the DNAble® *Salmonella* assay. In this study *Salmonella* was detected down to 10^5 CFU/mL and in one technical replicate to 10^4 CFU/mL.

Note: As this was a limited study, a validation by your site is suggested.

MATRIX FEASIBILITY STUDY: Goat Cheese

OBJECTIVE:

The purpose of this study was to assess the compatibility of the **goat cheese** matrix with the DNAble® *Salmonella* Assay (Cat. No. DF-026, Part #11716).

MATERIALS:

- DNAble *Salmonella* DNA Detection Kit consisting of:
  - RB1 Reaction Buffer tubes
  - *Salmonella* Master Mix tubes
- DNAble Sample Extraction Set 2
  - MB3 Buffer
  - Microcentrifuge tubes
- Buffered Peptone Water
- DNAble *Salmonella* Supplement
- *Salmonella enterica*
- Filtered Whirl-Pak® bags
- Goat cheese purchased at a local grocery

SAMPLE PREP:

- The following samples were prepared by adding 225 mL of 2.0% mBPW to 25 grams of goat cheese in a Whirl-Pak bag and hand-macerating:
  - Presumed negative goat cheese
  - Goat cheese spiked with *Salmonella*
- All samples were incubated overnight (~17 hours) at 37°C.
- After incubation, samples containing 10^4, 10^5, and 10^6 CFU/mL were created by spiking known amounts of *Salmonella* into aliquots of the presumed negative enriched samples. These samples along with the presumed negative and *Salmonella* spiked samples, were prepared for the DNAble assay using the instructions from the Sample Set 2 Product Insert:
  - Pre-heat a dry heat block to 95°C.
  - Mix the culture before sampling.
  - Transfer 1 mL of culture to a clear 1.5 mL microcentrifuge tube supplied with the set.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Remove the supernatant using caution to avoid disturbing the pellet. Leave a small volume remaining (≈ 100 µL) if an obvious pellet is not observed.
  - Add 100 µL of MB3 buffer and vortex to suspend the pellet.
  - Heat the sample in the heat block at 95°C for 10 minutes.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Place 100 µL of MB3 into a blue 1.5 mL microcentrifuge tube using a fresh pipette tip.
  - Transfer 25 µL of the supernatant from the second centrifugation into the MB3 and mix gently.
  - 5 µL of this prepared sample will be used in the subsequent DNAble reaction.
- The DNAble *Salmonella* assay was then run in duplicate on each sample.

RESULTS:

Figure 1: Negative and Positive (*Salmonella* spiked) Samples

This data represents goat cheese samples: Negative (Neg), and pre-enrichment *Salmonella* spiked positive (Pos)

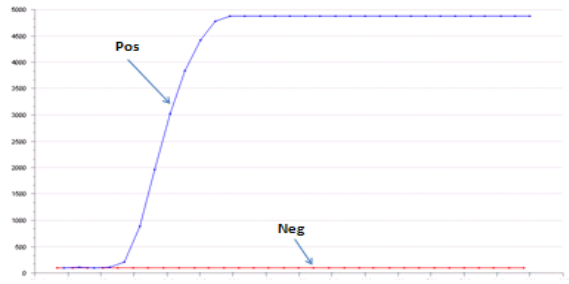
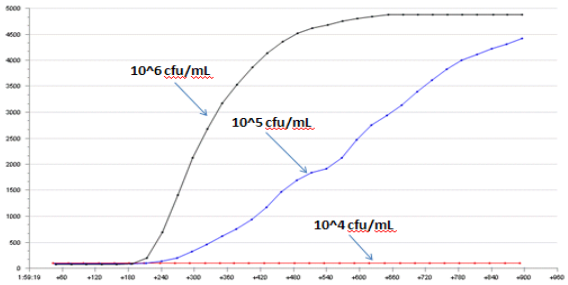


Figure 2: Limit of Detection (LOD) Study

Samples consisted of negative enriched goat cheese spiked with known amounts: 10^6 (10^6), 10^5 (10^5), 10^4 (10^4) CFU/mL of *Salmonella*



CONCLUSION:

From this limited study, initial data suggests that the goat cheese matrix is compatible with the DNAble *Salmonella* assay. In this study *Salmonella* was detected down to 10^5 CFU/mL. The algorithm has not been defined; therefore these samples may not yield conclusive positive or negative results. The end user can rely on their interpretation or send us json files for conclusive output. This data is preliminary and more work is needed to substantiate these findings.

Note: As this was a limited study, a validation by your site is suggested.

MATRIX FEASIBILITY STUDY: Gorgonzola Cheese

OBJECTIVE:

The purpose of this study was to assess the compatibility of the **gorgonzola cheese** matrix with the DNAble® *Salmonella* Assay.

MATERIALS:

- DNAble *Salmonella* DNA Detection Kit consisting of:
  - RB1 Reaction Buffer tubes
  - *Salmonella* Master Mix tubes
- DNAble Sample Extraction Set 2
  - MB3 Buffer
  - Microcentrifuge tubes
- Buffered Peptone Water
- DNAble *Salmonella* Supplement
- *Salmonella enterica*
- Filtered Whirl-Pak® bags
- Gorgonzola cheese purchased at a local grocery

SAMPLE PREP:

- The following samples were prepared by adding 225 mL of 2.0% mBPW to 25 grams of gorgonzola cheese in a Whirl-Pak bag and hand-macerating:
  - Presumed negative gorgonzola cheese
  - Gorgonzola cheese spiked with *Salmonella*
- All samples were incubated overnight (~17 hours) at 37°C.
- After incubation, samples containing 10^4, 10^5, and 10^6 CFU/mL were created by spiking known amounts of *Salmonella* into aliquots of the presumed negative enriched samples. These samples along with the presumed negative and *Salmonella* spiked samples, were prepared for the DNAble assay using the instructions from the Sample Set 2 Product Insert:
  - Pre-heat a dry heat block to 95°C.
  - Mix the culture before sampling.
  - Transfer 1 mL of culture to a clear 1.5 mL microcentrifuge tube supplied with the set.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Remove the supernatant using caution to avoid disturbing the pellet. Leave a small volume remaining (≈ 100 µL) if an obvious pellet is not observed.
  - Add 100 µL of MB3 buffer and vortex to suspend the pellet.
  - Heat the sample in the heat block at 95°C for 10 minutes.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Place 100 µL of MB3 into a blue 1.5 mL microcentrifuge tube using a fresh pipette tip.
  - Transfer 25 µL of the supernatant from the second centrifugation into the MB3 and mix gently.
  - 5 µL of this prepared sample will be used in the subsequent DNAble reaction.
- The DNAble *Salmonella* assay was then run in duplicate on each sample.

RESULTS:

Figure 1: Negative and Positive (*Salmonella* spiked) Samples

This data represents gorgonzola cheese samples: Negative (Neg), and pre-enrichment *Salmonella* spiked positive (Pos)

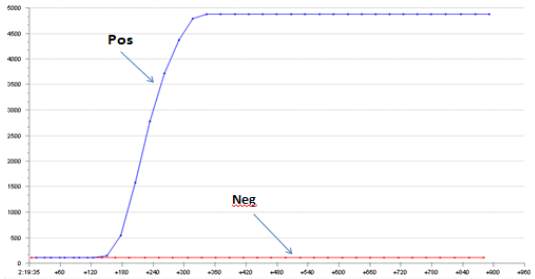
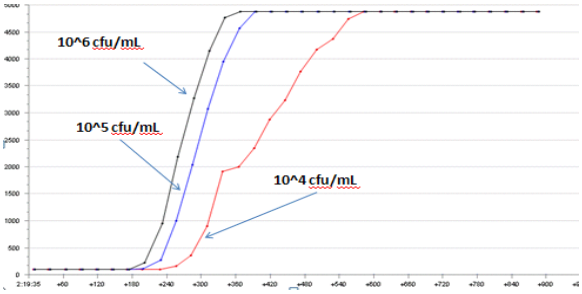


Figure 2: Limit of Detection (LOD) Study

Samples consisted of negative enriched gorgonzola cheese spiked with known amounts: 10^6 (10^6), 10^5 (10^5), 10^4 (10^4) CFU/mL of *Salmonella*



CONCLUSION:

From this limited study, initial data suggests that the gorgonzola cheese matrix is compatible with the DNAble *Salmonella* assay. In this study *Salmonella* was detected down to 10^4 CFU/mL. The algorithm has not been defined; therefore these samples may not yield conclusive positive or negative results. The end user can rely on their interpretation or send us json files for conclusive output. This data is preliminary and more work is needed to substantiate these findings.

Note: As this was a limited study, a validation by your site is suggested.

MATRIX FEASIBILITY STUDY: Cherries

OBJECTIVE:

The purpose of this study was to assess the compatibility of the **cherry** matrix with the DNAble® *Salmonella* Assay (Cat. No. DF-026, Part #11716).

MATERIALS:

- DNAble *Salmonella* DNA Detection Kit consisting of:
  - RB1 Reaction Buffer tubes
  - *Salmonella* Master Mix tubes
- DNAble Sample Extraction Set 2
  - MB3 Buffer
  - Microcentrifuge tubes
- Buffered Peptone Water
- DNAble *Salmonella* Supplement
- *Salmonella enterica*
- Whirl-Pak® bags
- Cherries

SAMPLE PREP:

- 2 samples were prepared by adding 225 mL of 2.0% mBPW to 25 grams of cherries in a Whirl-Pak bag. One sample was spiked with 10 cells of *Salmonella*. Samples were then "stomached" by hand for approximately 1 minute.
- Both samples were incubated overnight (~18-20 hours) at 37°C.
- After incubation, samples containing 10^4, 10^5, and 10^6 CFU/mL were created by spiking known amounts of *Salmonella* into aliquots of the negative enriched sample. These samples along with the negative and the 10 cell spiked sample, were prepared for the DNAble assay using the instructions from the Sample Set 2 Product Insert:
  - Pre-heat a dry heat block to 95°C.
  - Mix the culture before sampling.
  - Transfer 1 mL of culture to a clear 1.5 mL microcentrifuge tube supplied with the set.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Remove the supernatant using caution to avoid disturbing the pellet. Leave a small volume remaining (≈ 100 µL) if an obvious pellet is not observed.
  - Add 100 µL of MB3 buffer and vortex to suspend the pellet.
  - Heat the sample in the heat block at 95°C for 10 minutes.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Place 100 µL of MB3 into a blue 1.5 mL microcentrifuge tube using a fresh pipette tip.
  - Transfer 25 µL of the supernatant from the second centrifugation into the MB3 and mix gently.
  - 5 µL of this prepared sample will be used in the subsequent DNAble reaction.
- The DNAble *Salmonella* assay was then run in duplicate on each sample.

RESULTS:

Figure 1: Data exported and extrapolated using Excel. The data below was obtained using cherries (CH) both unspiked (Neg), and spiked with 10 cells of *Salmonella* prior to overnight enrichment (Pos):

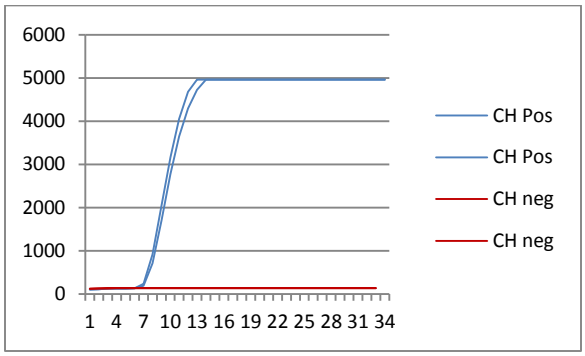
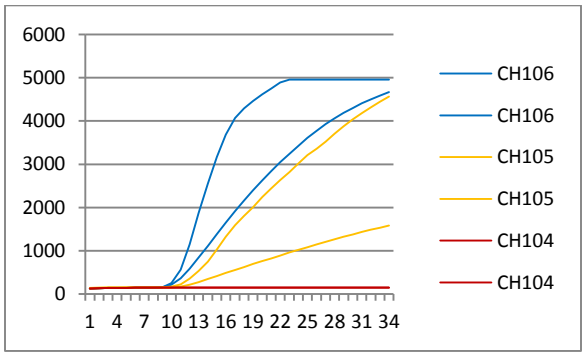


Figure 2: Data exported and extrapolated using Excel. Samples consisted of negative cherries spiked with known amounts: 10^6 (106), 10^5 (105), 10^4 (104) CFU/mL of *Salmonella*.



CONCLUSION:

From this limited study, initial data suggests that the cherry matrix is compatible with the DNAble *Salmonella* assay. In this study *Salmonella* was detected down to 10^5 CFU/mL.

Note: As this was a limited study, a validation by your site is suggested.

MATRIX FEASIBILITY STUDY: Raw Chicken

OBJECTIVE:

The purpose of this study was to assess the compatibility of the **raw chicken** matrix with the DNAble® *Salmonella* Assay (Cat. No. DF-026, Part #11716).

MATERIALS:

- DNAble *Salmonella* DNA Detection Kit consisting of:
  - RB1 Reaction Buffer tubes
  - *Salmonella* Master Mix tubes
- DNAble Sample Extraction Set 2
  - MB3 Buffer
  - Microcentrifuge tubes
- Buffered Peptone Water
- DNAble *Salmonella* Supplement
- *Salmonella enterica*
- Whirl-Pak® bags
- Raw Chicken

SAMPLE PREP:

- 2 samples were prepared by adding 225 mL of 2.0% mBPW to 25 grams of raw chicken in a Whirl-Pak bag. One sample was spiked with 10 cells of *Salmonella*. Samples were then "stomached" by hand for approximately 1 minute.
- Both samples were incubated overnight (~17 hours) at 37°C.
- After incubation, samples containing 10^4, 10^5, and 10^6 CFU/mL were created by spiking known amounts of *Salmonella* into aliquots of the negative enriched sample. These samples along with the negative and the 10 cell spiked sample, were prepared for the DNAble assay using the instructions from the Sample Set 2 Product Insert:
  - Pre-heat a dry heat block to 95°C.
  - Mix the culture before sampling.
  - Transfer 1 mL of culture to a clear 1.5 mL microcentrifuge tube supplied with the set.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Remove the supernatant using caution to avoid disturbing the pellet. Leave a small volume remaining (≈ 100 µL) if an obvious pellet is not observed.
  - Add 100 µL of MB3 buffer and vortex to suspend the pellet.
  - Heat the sample in the heat block at 95°C for 10 minutes.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Place 100 µL of MB3 into a blue 1.5 mL microcentrifuge tube using a fresh pipette tip.
  - Transfer 25 µL of the supernatant from the second centrifugation into the MB3 and mix gently.
  - 5 µL of this prepared sample will be used in the subsequent DNAble reaction.
- The DNAble *Salmonella* assay was then run in duplicate on each sample.

RESULTS:

Figure 1: Data exported and extrapolated using Excel. The data below was obtained using raw chicken (RC) both unspiked (-), spiked with 10 cells of *Salmonella* prior to overnight enrichment (+):

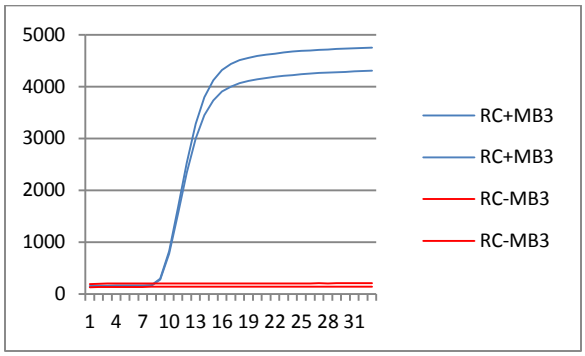
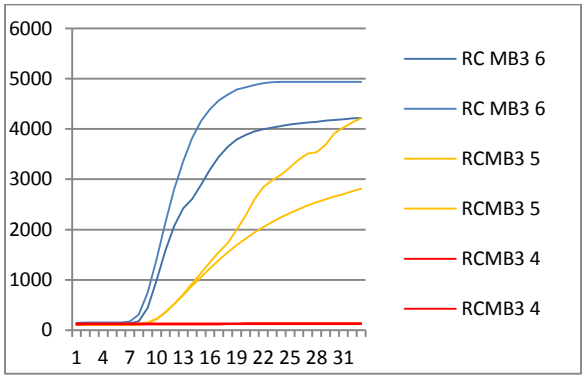


Figure 2: Data exported and extrapolated using Excel. Samples consisted of negative raw chicken spiked with known amounts: 10^6 (6), 10^5 (5), 10^4 (4) CFU/mL of *Salmonella*.



CONCLUSION:

From this limited study, initial data suggests that the raw chicken matrix is compatible with the DNAble *Salmonella* assay. In this study *Salmonella* was detected down to 10^5 CFU/mL.

Note: As this was a limited study, a validation by your site is suggested.

MATRIX FEASIBILITY STUDY: Chicken Salad

OBJECTIVE:

The purpose of this study was to assess the compatibility of the **chicken salad** matrix with the DNAble® *Salmonella* Assay (Cat. No. DF-026, Part #11716).

MATERIALS:

- DNAble *Salmonella* DNA Detection Kit consisting of:
  - RB1 Reaction Buffer tubes
  - *Salmonella* Master Mix tubes
- DNAble Sample Extraction Set 2
  - MB3 Buffer
  - Microcentrifuge tubes
- Buffered Peptone Water
- DNAble *Salmonella* Supplement
- *Salmonella enterica*
- Whirl-Pak® bags
- Chicken salad (cooked chicken, mayonnaise, celery)

SAMPLE PREP:

- 2 samples were prepared by adding 225 mL of 2.0% mBPW to 25 grams of chicken salad in a Whirl-Pak bag. One sample was spiked with 10 cells of *Salmonella*. Samples were then "stomached" by hand for approximately 1 minute.
- Both samples were incubated overnight (~17 hours) at 37°C.
- After incubation, samples containing 10^4, 10^5, and 10^6 CFU/mL were created by spiking known amounts of *Salmonella* into aliquots of the negative enriched sample. These samples along with the negative and the 10 cell spiked sample, were prepared for the DNAble assay using the instructions from the Sample Set 2 Product Insert:
  - Pre-heat a dry heat block to 95°C.
  - Mix the culture before sampling.
  - Transfer 1 mL of culture to a clear 1.5 mL microcentrifuge tube supplied with the set.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Remove the supernatant using caution to avoid disturbing the pellet. Leave a small volume remaining (≈ 100 µL) if an obvious pellet is not observed.
  - Add 100 µL of MB3 buffer and vortex to suspend the pellet.
  - Heat the sample in the heat block at 95°C for 10 minutes.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Place 100 µL of MB3 into a blue 1.5 mL microcentrifuge tube using a fresh pipette tip.
  - Transfer 25 µL of the supernatant from the second centrifugation into the MB3 and mix gently.
  - 5 µL of this prepared sample will be used in the subsequent DNAble reaction.
- The DNAble *Salmonella* assay was then run in duplicate on each sample.

RESULTS:

Figure 1: Data exported and extrapolated using Excel. The data below was obtained using chicken salad (CS) both unspiked (-), spiked with 10 cells of *Salmonella* prior to overnight enrichment (+):

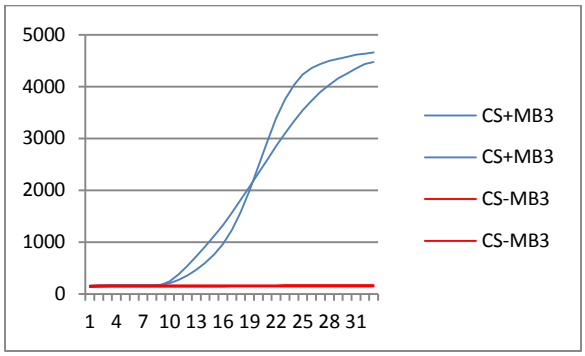
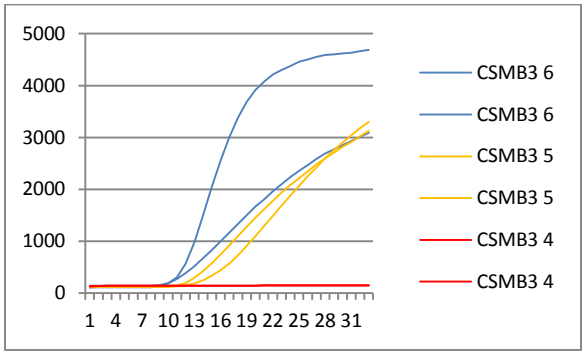


Figure 2: Data exported and extrapolated using Excel. Samples consisted of negative raw chicken spiked with known amounts: 10^6 (6), 10^5 (5), 10^4 (4) CFU/mL of *Salmonella*.



CONCLUSION:

From this limited study, initial data suggests that the chicken salad matrix is compatible with the DNAble *Salmonella* assay. In this study *Salmonella* was detected down to 10^5 CFU/mL.

Note: As this was a limited study, a validation by your site is suggested.

MATRIX FEASIBILITY STUDY: Whole Chili

OBJECTIVE:

The purpose of this study was to assess the compatibility of the **whole chili** matrix with the DNAble® *Salmonella* Assay (Cat. No. DF-026, Part #11716).

MATERIALS:

- DNAble *Salmonella* DNA Detection Kit consisting of:
  - RB1 Reaction Buffer tubes
  - *Salmonella* Master Mix tubes
- DNAble Sample Extraction Set 2
  - MB3 Buffer
  - Microcentrifuge tubes
- Buffered Peptone Water
- DNAble *Salmonella* Supplement
- *Salmonella enterica*
- Whirl-Pak® Bags
- Whole chilies sourced from a local Indian grocery

SAMPLE PREP:

- The following samples were prepared by adding 100 mL of 2.0% mBPW to 10 grams of whole chilies in a Whirl-Pak bag:
  - Presumed negative whole chilies
  - Whole chilies with *Salmonella* spike
- Samples were incubated overnight at 37°C.
- After incubation, samples containing 10^4, 10^5, and 10^6 CFU/mL were created by spiking known amounts of *Salmonella* into aliquots of the presumed negative enriched samples. These samples along with the presumed negative and the *Salmonella* spiked sample, were prepared for the DNAble assay using the instructions from the Sample Set 2 Product Insert:
  - Pre-heat a dry heat block to 95°C.
  - Mix the culture before sampling.
  - Transfer 1 mL of culture to a clear 1.5 mL microcentrifuge tube supplied with the set.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Remove the supernatant using caution to avoid disturbing the pellet. Leave a small volume remaining (≈ 100 µL) if an obvious pellet is not observed.
  - Add 100 µL of MB3 buffer and vortex to suspend the pellet.
  - Heat the sample in the heat block at 95°C for 10 minutes.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Place 100 µL of MB3 into a blue 1.5 mL microcentrifuge tube using a fresh pipette tip.
  - Transfer 25 µL of the supernatant from the second centrifugation into the MB3 and mix gently.
  - 5 µL of this prepared sample will be used in the subsequent DNAble reaction.
- The DNAble *Salmonella* assay was then run in duplicate on each sample.

RESULTS:

Figure 1—Negative and Positive (*Salmonella* spiked): This data represents whole chili samples: Negative (Neg) and pre-enrichment *Salmonella* spiked positive (Pos).

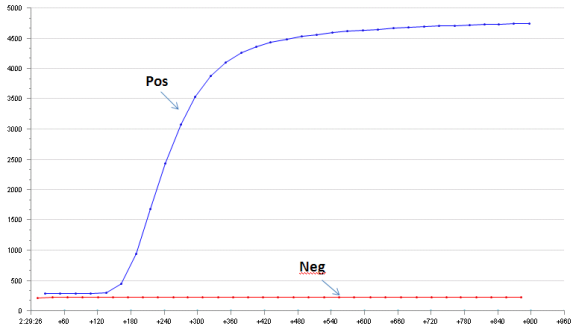
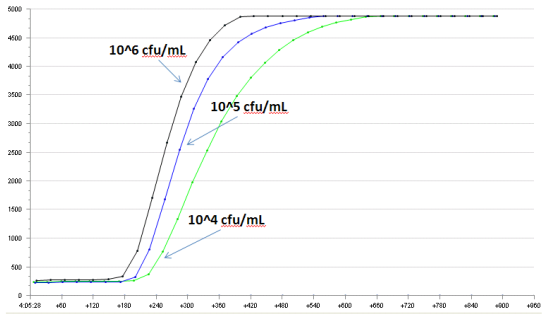


Figure 2—Limit of Detection (LOD) Study: Samples consisted of negative enriched whole chili spiked with known amounts: 10^6, 10^5, 10^4 CFU/mL of *Salmonella*.



CONCLUSION:

From this limited study, initial data suggests that the whole chili matrix is compatible with the DNAble *Salmonella* assay. In this study *Salmonella* was detected down to 10^4 CFU/mL. The algorithm has not been defined, however, and these samples may not yield conclusive positive or negative results and the end user can rely on their interpretation or send us json files for conclusive output. This data is preliminary and more work needs to be done in order to substantiate these findings.

Note: As this was a limited study, a validation by your site is suggested.

MATRIX FEASIBILITY STUDY: Chocolate

OBJECTIVE:

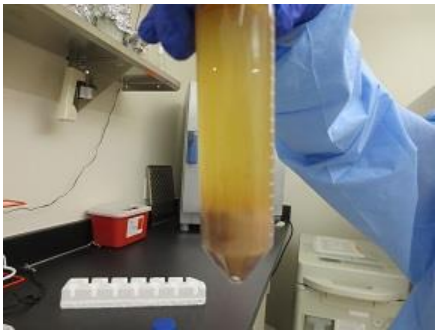
The purpose of this study was to assess the compatibility of the **chocolate** matrix with the DNAble® *Salmonella* Assay (Cat. No. DF-026, Part #11716).

MATERIALS:

- DNAble *Salmonella* DNA Detection Kit consisting of:
  - RB1 Reaction Buffer tubes
  - *Salmonella* Master Mix tubes
- DNAble Sample Extraction Set 1
  - MB2 Buffer
  - Microcentrifuge tubes
- Buffered Peptone Water
- DNAble *Salmonella* Supplement
- *Salmonella* Enteritidis (ATCC# BAA 1587)
- Whirl-Pak® bags
- Hershey's milk and Hershey's dark chocolate

SAMPLE PREP:

- The following samples were prepared by adding 50 mL of 2.5% mBPW to 5 grams of chocolate in a conical tube:
  - Negative milk chocolate
  - Negative dark chocolate
  - Milk chocolate with 10 cell spike
  - Dark chocolate with 10 cell spike
- Both samples were incubated overnight (~17 hours) at 37°C.
- After incubation, samples containing 10^4, 10^5, and 10^6 CFU/mL were created by spiking known amounts of *Salmonella* into aliquots of the presumed negative enriched samples. These samples along with the presumed negatives and the 10 cell spiked samples, were prepared for the DNAble assay using the instructions from the Sample Set 1 Product Insert:
  - Pre-heat a dry heat block to 95°C.
  - Using a clean pipette tip, transfer 25 µL of MB2 Extraction Buffer to a labeled 1.5 mL microcentrifuge tube.
  - Transfer 125 µL of each enriched sample into the tube containing MB2 Extraction Buffer. Vortex briefly.
  - Heat tubes containing sample and extraction buffer for 15 minutes in heat block.
  - After 15 minutes, remove samples from block and place in a rack.
  - 5 µL of this prepared sample will be used in the subsequent DNAble reaction.
- **NOTE:** *The enriched sample was not mixed after incubation. Samples for DNAble testing were taken approximately midway into the clearer portion of the sample.* (See image below.)



- The DNAble *Salmonella* assay was then run in duplicate on each sample.

RESULTS:

All results were as expected with the non-spiked samples producing negative DNAble results and all spiked samples giving positive DNAble results:

Figure 1: DNAble Results

SAMPLE	DNAble	SAMPLE	DNAble
Milk chocolate (MC)	Negative	Dark chocolate (DC)	Negative
MC with 10 cell spike pre-enrichment	Positive	DC with 10 cell spike pre-enrichment	Positive
MC 10^6 spike	Positive	DC 10^6 spike	Positive
MC 10^5 spike	Positive	DC 10^5 spike	Positive

Figure 2: Data exported and extrapolated using Excel. This data was obtained using following milk chocolate (MC) samples: Enriched Negative (Neg), 10 cell spike prior to enrichment (low), 10^6 CFU/mL spike after enrichment (106) and 10^5 CFU/mL spike after enrichment (105).

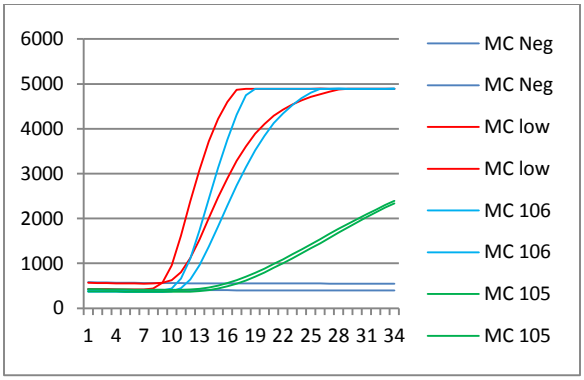
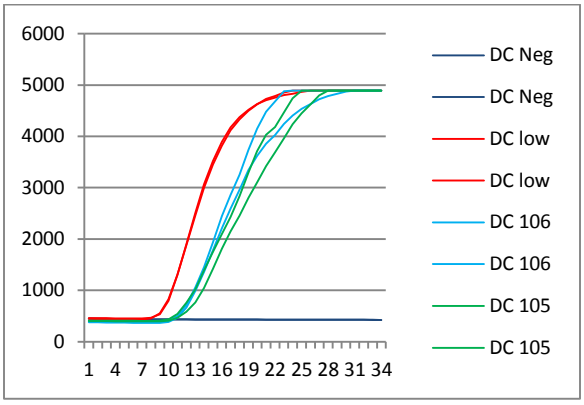


Figure 3: Data exported and extrapolated using Excel. This data was obtained using following dark chocolate (DC) samples: Enriched Negative (Neg), 10 cell spike prior to enrichment (low), 10^6 CFU/mL spike after enrichment (106) and 10^5 CFU/mL spike after enrichment (105).



CONCLUSION:

From this limited study, initial data suggests that the chocolate matrix is compatible with the DNAble® *Salmonella* assay. In this study *Salmonella* Enteriditis ATCC# BAA 1587 was detected down to 10^5 CFU/mL. (Note: a 10^4 CFU/mL sample was not tested.)

Note: As this was a limited study, a validation by your site is suggested.

Recommended Sample Preparation for Eggs

1. **Raw Eggs – Yolks and Whites**
    a. Enrich the sample in 2% modified Tryptic Soy Broth (TSB + DNAble® *Salmonella* Supplement):
        i. Prepare TSB as per manufacturer's instructions and add 2.0 mL of DNAble *Salmonella* Supplement for each 100 mL of TSB.
        ii. Prepare samples for enrichment by using a 1:9 egg:broth ratio.
    b. Incubate samples overnight at 37°C.
    c. Proceed to Step 3 below.
2. **Hard-boiled Eggs**
    a. Enrich the sample in 2% modified Tryptic Soy Broth (TSB + DNAble *Salmonella* Supplement):
        i. Prepare TSB as per manufacturer's instructions and add 2.0 mL of DNAble Salmonella Supplement for each 100 mL of TSB.
        ii. If shells are intact, disinfect the shells and separate from eggs. Pulverize the eggs (yolk solids and white solids) and weigh 25g and place in a suitable container. Add 225 mTSB to this sample.
    b. Incubate overnight at 37°C.
    c. Proceed to Step 3 below.
3. **Sample prep for DNAble *Salmonella* Assay:**
    a. Add 1 mL of enriched sample to a microcentrifuge tube.
    b. Centrifuge sample at 10,000 x g (rcf) for 5 minutes.
    c. Remove the supernatant using caution not to disturb the pellet. Leave a small volume of dregs (~100 µL) is an obvious pellet is not observed.
    d. Resuspend pellet in 100 µL of MB3 Extraction Buffer.
    e. Heat sample for 10 minutes at 95°C.
    f. Centrifuge sample at 10,000 x g (rcf) for 5 minutes.
    g. Place 100 µL of MB3 Extraction Buffer into a new microcentrifuge tube.
    h. Transfer 25 µL of the supernatant from the second centrifugation (step f) into the MB3 and mix gently.
    i. 5 µL of this sample will be used in the DNAble assay.

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MATRIX FEASIBILITY STUDY: Peaches

OBJECTIVE:

The purpose of this study was to assess the compatibility of the **peach** matrix with the DNAble® *Salmonella* Assay (Cat. No. DF-026, Part #11716).

MATERIALS:

- DNAble *Salmonella* DNA Detection Kit consisting of:
  - RB1 Reaction Buffer tubes
  - *Salmonella* Master Mix tubes
- DNAble Sample Extraction Set 2
  - MB3 Buffer
  - Microcentrifuge tubes
- Buffered Peptone Water
- DNAble *Salmonella* Supplement
- *Salmonella enterica*
- Whirl-Pak® Bags
- Peaches

SAMPLE PREP:

- 2 samples were prepared by adding 225 mL of 2.0% mBPW to 25 grams of peaches in a Whirl-Pak bag. One sample was spiked with 10 cells of *Salmonella*. Samples were then "stomached" by hand for approximately 1 minute.
- Both samples were incubated overnight (~18-20 hours) at 37°C.
- After incubation, samples containing 10^4, 10^5, and 10^6 CFU/mL were created by spiking known amounts of *Salmonella* into aliquots of the negative enriched sample. These samples along with the presumed negative and the 10 cell spiked sample, were prepared for the DNAble assay using the instructions from the Sample Set 2 Product Insert:
  - Pre-heat a dry heat block to 95°C.
  - Mix the culture before sampling.
  - Transfer 1 mL of culture to a clear 1.5 mL microcentrifuge tube supplied with the set.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Remove the supernatant using caution to avoid disturbing the pellet. Leave a small volume remaining (≈ 100 µL) if an obvious pellet is not observed.
  - Add 100 µL of MB3 buffer and vortex to suspend the pellet.
  - Heat the sample in the heat block at 95°C for 10 minutes.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Place 100 µL of MB3 into a blue 1.5 mL microcentrifuge tube using a fresh pipette tip.
  - Transfer 25 µL of the supernatant from the second centrifugation into the MB3 and mix gently.
  - 5 µL of this prepared sample will be used in the subsequent DNAble reaction.
- The DNAble *Salmonella* assay was then run in duplicate on each sample.

RESULTS:

Figure 1: Data exported and extrapolated using Excel. The data below was obtained using peaches (p) both unspiked (neg), spiked with 10 cells of *Salmonella* prior to overnight enrichment (pos).

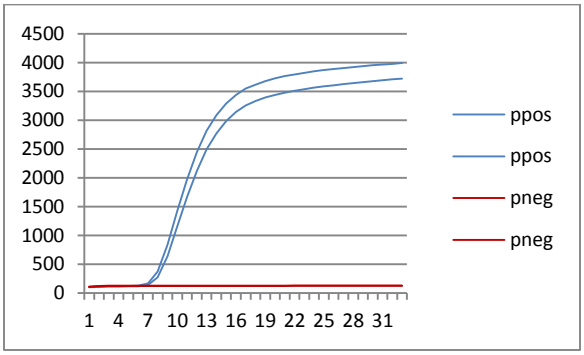
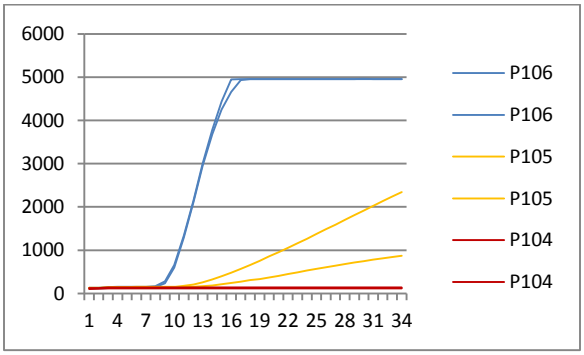


Figure 2: Data exported and extrapolated using Excel. Samples consisted of negative peaches spiked with known amounts: 10^6 (106), 10^5 (105), 10^4 (104) CFU/mL of *Salmonella*.



CONCLUSION:

From this limited study, initial data suggests that the peach matrix is compatible with the DNAble *Salmonella* assay. In this study *Salmonella* was detected down to 10^5 CFU/mL

Note: As this was a limited study, a validation by your site is suggested.

MATRIX FEASIBILITY STUDY: Raspberries

OBJECTIVE:

The purpose of this study was to assess the compatibility of the **raspberry** matrix with the DNAble® *Salmonella* Assay (Cat. No. DF-026, Part #11716).

MATERIALS:

- DNAble *Salmonella* DNA Detection Kit consisting of:
  - RB1 Reaction Buffer tubes
  - *Salmonella* Master Mix tubes
- DNAble Sample Extraction Set 2
  - MB3 Buffer
  - Microcentrifuge tubes
- Buffered Peptone Water
- DNAble *Salmonella* Supplement
- *Salmonella enterica*
- Whirl-Pak® Bags
- Raspberries

SAMPLE PREP:

- 2 samples were prepared by adding 225 mL of 2.0% mBPW to 25 grams of raspberries in a Whirl-Pak bag. One sample was spiked with 10 cells of *Salmonella*. Samples were then "stomached" by hand for approximately 1 minute.:
- Both samples were incubated overnight (~18-20 hours) at 37°C.
- After incubation, samples containing 10^4, 10^5, and 10^6 CFU/mL were created by spiking known amounts of *Salmonella* into aliquots of the negative enriched sample. These samples along with the presumed negative and the 10 cell spiked sample, were prepared for the DNAble assay using the instructions from the Sample Set 2 Product Insert:
  - Pre-heat a dry heat block to 95°C.
  - Mix the culture before sampling.
  - Transfer 1 mL of culture to a clear 1.5 mL microcentrifuge tube supplied with the set.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Remove the supernatant using caution to avoid disturbing the pellet. Leave a small volume remaining (≈ 100 µL) if an obvious pellet is not observed.
  - Add 100 µL of MB3 buffer and vortex to suspend the pellet.
  - Heat the sample in the heat block at 95°C for 10 minutes.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Place 100 µL of MB3 into a blue 1.5 mL microcentrifuge tube using a fresh pipette tip.
  - Transfer 25 µL of the supernatant from the second centrifugation into the MB3 and mix gently.
  - 5 µL of this prepared sample will be used in the subsequent DNAble reaction.
- The DNAble *Salmonella* assay was then run in duplicate on each sample.

RESULTS:

Figure 1: Data exported and extrapolated using Excel. The data below was obtained using raspberries (R) both unspiked (neg), spiked with 10 cells of *Salmonella* prior to overnight enrichment (pos).

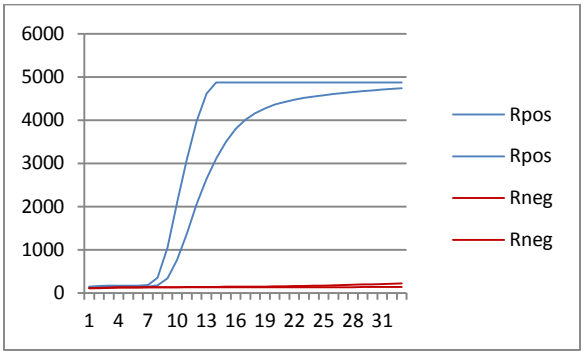
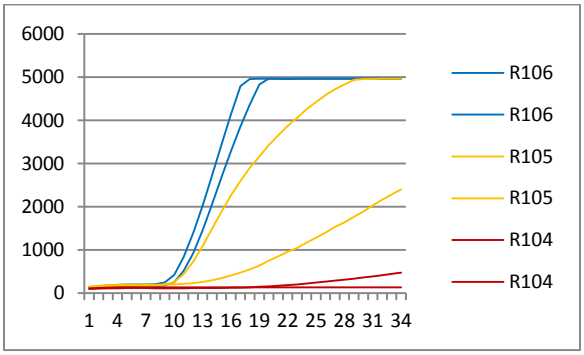


Figure 2: Data exported and extrapolated using Excel. Samples consisted of negative raspberries spiked with known amounts: 10^6 (106), 10^5 (105), 10^4 (104) CFU/mL of *Salmonella*.



CONCLUSION:

From this limited study, initial data suggests that the raspberry matrix is compatible with the DNAble *Salmonella* assay. In this study *Salmonella* was detected down to 10^5 CFU/mL.

Note: As this was a limited study, a validation by your site is suggested.

MATRIX FEASIBILITY STUDY: Salami

OBJECTIVE:

The purpose of this study was to assess the compatibility of the **deli sliced hard salami** matrix with the DNAble® *Salmonella* Assay (Cat. No. DF-026, Part #11716).

MATERIALS:

- DNAble *Salmonella* DNA Detection Kit consisting of:
  - RB1 Reaction Buffer tubes
  - *Salmonella* Master Mix tubes
- DNAble Sample Extraction Set 2
  - MB3 Buffer
  - Microcentrifuge tubes
- Buffered Peptone Water
- DNAble *Salmonella* Supplement
- *Salmonella enterica*
- Filtered Whirl-Pak® bags
- Deli sliced hard salami (salami) purchased at a local grocery

SAMPLE PREP:

- The following samples were prepared by adding 225 mL of 2.0% mBPW to 25 grams of salami, pre-macerated using a blender, in a Whirl-Pak bag:
  - Presumed negative salami
  - Salami spiked with *Salmonella*
- All samples were incubated overnight (~17 hours) at 37°C.
- After incubation, samples containing 10^4, 10^5, and 10^6 CFU/mL were created by spiking known amounts of *Salmonella* into aliquots of the presumed negative enriched samples. These samples along with the presumed negative and *Salmonella* spiked samples, were prepared for the DNAble assay using the instructions from the Sample Set 2 Product Insert:
  - Pre-heat a dry heat block to 95°C.
  - Mix the culture before sampling.
  - Transfer 1 mL of culture to a clear 1.5 mL microcentrifuge tube supplied with the set.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Remove the supernatant using caution to avoid disturbing the pellet. Leave a small volume remaining (≈ 100 µL) if an obvious pellet is not observed.
  - Add 100 µL of MB3 buffer and vortex to suspend the pellet.
  - Heat the sample in the heat block at 95°C for 10 minutes.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Place 100 µL of MB3 into a blue 1.5 mL microcentrifuge tube using a fresh pipette tip.
  - Transfer 25 µL of the supernatant from the second centrifugation into the MB3 and mix gently.
  - 5 µL of this prepared sample will be used in the subsequent DNAble reaction.
- The DNAble *Salmonella* assay was then run in duplicate on each sample.

RESULTS:

Figure 1: Negative and Positive (*Salmonella* spiked) Samples

This data represents salami samples: Negative (Neg), and pre-enrichment *Salmonella* spiked positive (Pos)

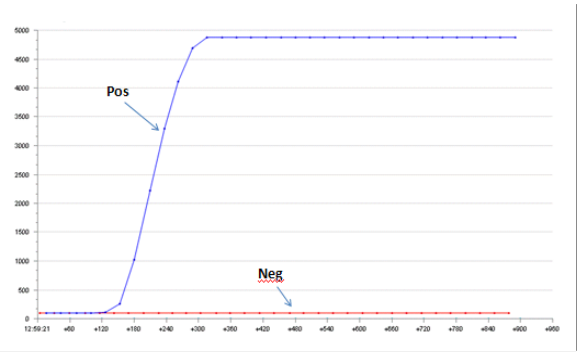
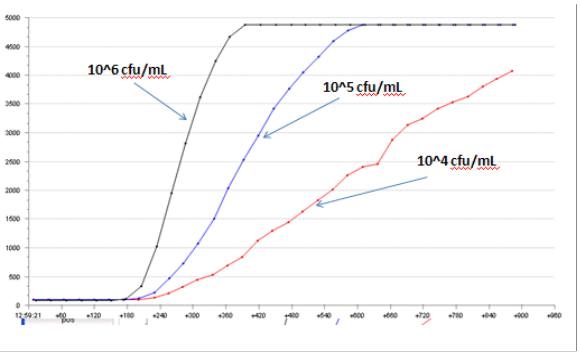


Figure 2: Limit of Detection (LOD) Study

Samples consisted of negative enriched salami spiked with known amounts: 10^6 (10^6), 10^5 (10^5), 10^4 (10^4) CFU/mL of *Salmonella*



CONCLUSION:

From this limited study, initial data suggests that the deli sliced hard salami matrix is compatible with the DNAble *Salmonella* assay. In this study *Salmonella* was detected down to a level of 10^4 CFU/mL. The algorithm has not been defined; therefore these samples may not yield conclusive positive or negative results. The end user can rely on their interpretation or send us json files for conclusive output. This data is preliminary and more work is needed to substantiate these findings.

Note: As this was a limited study, a validation by your site is suggested.

MATRIX FEASIBILITY STUDY: Shell-Off, Head-Off, Tail-Off, Deveined Shrimp

OBJECTIVE:

The purpose of this study was to assess the compatibility of **Shell-Off, Head-Off, Tail-Off, Deveined Shrimp** matrix with the DNAble® *Salmonella* Assay (Cat. No. DF-026, Part #11716).

MATERIALS:

- DNAble *Salmonella* DNA Detection Kit consisting of:
  - RB1 Reaction Buffer tubes
  - *Salmonella* Master Mix tubes
- DNAble Sample Extraction Set 2
  - MB3 Buffer
  - Microcentrifuge tubes
- Buffered Peptone Water
- DNAble *Salmonella* Supplement
- *Salmonella enterica*
- Whirl-Pak® filter bags
- Frozen shrimp purchased at a local grocery

SAMPLE PREP:

- The following samples were prepared by adding 10 mL of 2.0% mBPW per gram of shrimp in a Whirl-Pak bag and hand-macerating the shrimp:
  - Presumed negative shrimp (Shell-Off, Head-Off, Tail-Off, Deveined Shrimp)
  - Shrimp spiked with *Salmonella* (Shell-Off, Head-Off, Tail-Off, Deveined Shrimp)
- All samples were incubated overnight at 37°C.
- After incubation, samples containing 10^4, 10^5 and 10^6 CFU/mL were created by spiking known amounts of *Salmonella* into aliquots of presumed negative enriched samples. These samples, along with the presumed negative and the *Salmonella* spiked sample, were prepared for the DNAble assay using the instructions from the Sample Set 2 Product Insert:
  - Pre-heat a dry heat block to 95°C.
  - Mix the culture before sampling.
  - Transfer 1 mL of culture to a clear 1.5 mL microcentrifuge tube supplied with the set.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Remove the supernatant using caution to avoid disturbing the pellet. Leave a small volume remaining (≈ 100 µL) if an obvious pellet is not observed.
  - Add 100 µL of MB3 buffer and vortex to suspend the pellet.
  - Heat the sample in the heat block at 95°C for 10 minutes.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Place 100 µL of MB3 into a blue 1.5 mL microcentrifuge tube using a fresh pipette tip.
  - Transfer 25 µL of the supernatant from the second centrifugation into the MB3 and mix gently.
  - 5 µL of this prepared sample will be used in the subsequent DNAble reaction.
- The DNAble *Salmonella* assay was then run in duplicate on each sample.

RESULTS:

Figure 1: **Negative and Positive *(Salmonella* spiked) Samples:** This data represents macerated Shell-Off, Head-Off, Tail-Off, Deveined Shrimp samples: Negative (Neg) and pre-enrichment *Salmonella* spiked positive(Pos):

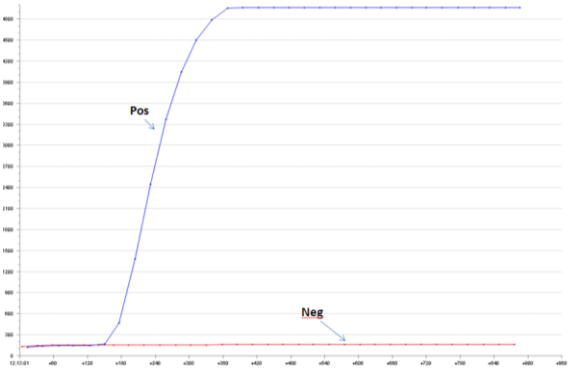
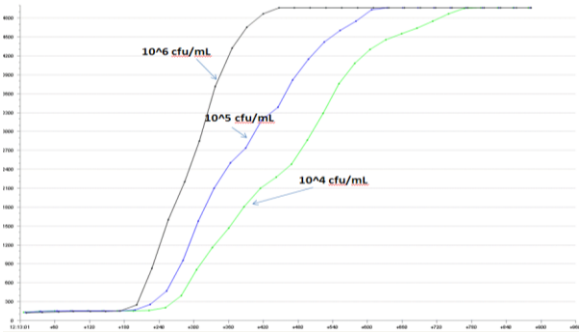


Figure 2: Limit of Detection (LOD) Study: Samples consisted of negative enriched macerated Shell-Off, Head-Off, Tail-Off, Deveined Shrimp spiked with known amounts: 10^6, 10^5, 10^4 CFU/mL of *Salmonella*.



CONCLUSION:

From this limited study, initial data suggests that the macerated, Shell-Off, Head- Off, Tail-Off, Deveined Shrimp matrix is compatible with the DNAble *Salmonella* assay. In this study *Salmonella* was detected down to 10^4 CFU/mL. The algorithm has not been defined; therefore these samples may not yield conclusive positive or negative results. The end user can rely on their interpretation or send us json files for conclusive output. This data is preliminary and more work is needed to substantiate these findings.

Note: As this was a limited study, a validation by your site is suggested.

MATRIX FEASIBILITY STUDY: Shell-On, Head-Off, Tail-On Shrimp

OBJECTIVE:

The purpose of this study was to assess the compatibility of **Shell-On, Head-Off, Tail-On Shrimp** matrix with the DNAble® *Salmonella* Assay (Cat. No. DF-026, Part #11716).

MATERIALS:

- DNAble *Salmonella* DNA Detection Kit consisting of:
  - RB1 Reaction Buffer tubes
  - *Salmonella* Master Mix tubes
- DNAble Sample Extraction Set 2
  - MB3 Buffer
  - Microcentrifuge tubes
- Buffered Peptone Water
- DNAble *Salmonella* Supplement
- *Salmonella enterica*
- Whirl-Pak® filter bags
- Frozen shrimp purchased at a local grocery

SAMPLE PREP:

- The following samples were prepared by adding 10 mL of 2.0% mBPW per gram of shrimp in a Whirl-Pak bag and hand-macerating the shrimp:
  - Presumed negative shrimp (Shell-On, Head-Off, Tail-On Shrimp)
  - Shrimp spiked with *Salmonella* (Shell-On, Head-Off, Tail-On Shrimp)
- All samples were incubated overnight at 37°C.
- After incubation, samples containing 10^4, 10^5 and 10^6 CFU/mL were created by spiking known amounts of *Salmonella* into aliquots of presumed negative enriched samples. These samples, along with the presumed negative and the *Salmonella* spiked sample, were prepared for the DNAble assay using the instructions from the Sample Set 2 Product Insert:
  - Pre-heat a dry heat block to 95°C.
  - Mix the culture before sampling.
  - Transfer 1 mL of culture to a clear 1.5 mL microcentrifuge tube supplied with the set.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Remove the supernatant using caution to avoid disturbing the pellet. Leave a small volume remaining (≈ 100 µL) if an obvious pellet is not observed.
  - Add 100 µL of MB3 buffer and vortex to suspend the pellet.
  - Heat the sample in the heat block at 95°C for 10 minutes.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Place 100 µL of MB3 into a blue 1.5 mL microcentrifuge tube using a fresh pipette tip.
  - Transfer 25 µL of the supernatant from the second centrifugation into the MB3 and mix gently.
  - 5 µL of this prepared sample will be used in the subsequent DNAble reaction.
- The DNAble *Salmonella* assay was then run in duplicate on each sample.

RESULTS:

Figure 1: **Negative and Positive *(Salmonella* spiked) Samples:** This data represents macerated Shell-On, Head-Off, Tail-On Shrimp samples: Negative (Neg) and pre-enrichment *Salmonella* spiked positive(Pos):

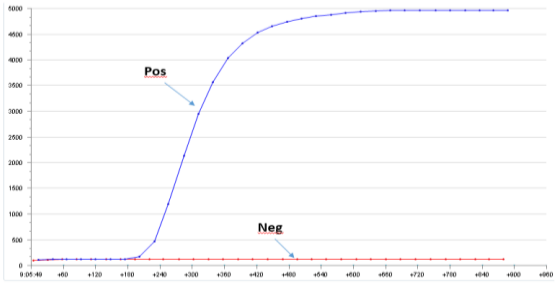
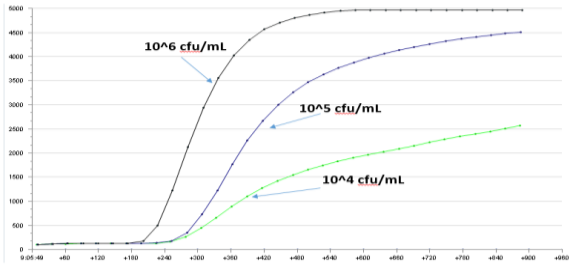


Figure 2: Limit of Detection (LOD) Study: Samples consisted of negative enriched macerated Shell-On, Head-Off, Tail-On Shrimp spiked with known amounts: 10^6, 10^5, 10^4 CFU/mL of *Salmonella*.



CONCLUSION:

From this limited study, initial data suggests that the macerated, Shell-On, Head- Off, Tail-On Shrimp matrix is compatible with the DNAble *Salmonella* assay. In this study *Salmonella* was detected down to 10^4 CFU/mL. The algorithm has not been defined; therefore these samples may not yield conclusive positive or negative results. The end user can rely on their interpretation or send us json files for conclusive output. This data is preliminary and more work is needed to substantiate these findings.

Note: As this was a limited study, a validation by your site is suggested.

MATRIX FEASIBILITY STUDY: Shell-On, Head-On, Tail-On Shrimp

OBJECTIVE:

The purpose of this study was to assess the compatibility of **Shell-On, Head-On, Tail-On Shrimp** matrix with the DNAble® *Salmonella* Assay (Cat. No. DF-026, Part #11716).

MATERIALS:

- DNAble *Salmonella* DNA Detection Kit consisting of:
  - RB1 Reaction Buffer tubes
  - *Salmonella* Master Mix tubes
- DNAble Sample Extraction Set 3 *(not yet commercially available)*
  - EB6 R&D Buffer
  - Additional RB1 Reaction Buffer
  - Microcentrifuge tubes
- Buffered Peptone Water
- DNAble *Salmonella* Supplement
- *Salmonella enterica*
- Whirl-Pak® filter bags
- Frozen shrimp purchased at a local grocery

SAMPLE PREP:

- The following samples were prepared by adding 10 mL of 2.0% mBPW per gram of shrimp (pre-macerated using a blender) in a Whirl-Pak bag:
  - Presumed negative shrimp (Shell-On, Head- On, Tail-On Shrimp)
  - Shrimp spiked with *Salmonella* (Shell-On, Head- On, Tail-On Shrimp)
- All samples were incubated overnight at 37°C.
- After incubation, samples containing 10^4, 10^5 and 10^6 CFU/mL were created by spiking known amounts of *Salmonella* into aliquots of presumed negative enriched samples. These samples, along with the presumed negative and the *Salmonella* spiked sample, were prepared for the DNAble assay using the instructions from the Sample Set 3 Product Insert:
  - Pre-heat a dry heat block to 95°C.
  - Mix the culture before sampling.
  - Transfer 1 mL of culture to a clear 1.5 mL microcentrifuge tube supplied with the set.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Remove the supernatant using caution to avoid disturbing the pellet. Leave a small volume remaining (≈ 100 µL) if an obvious pellet is not observed.
  - Add 100 µL of EB6 buffer and vortex to suspend the pellet.
  - Heat the sample in the heat block at 95°C for 5 minutes.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Place 90 µL of RB1 into a blue 1.5 mL microcentrifuge tube using a fresh pipette tip.
  - Transfer 10 µL of the supernatant from the second centrifugation into the RB1 and mix gently.
  - 5 µL of this prepared sample will be used in the subsequent DNAble reaction.
- The DNAble *Salmonella* assay was then run in duplicate on each sample.

RESULTS:

Figure 1: **Negative and Positive *(Salmonella* spiked) Samples:** This data represents macerated Shell-On, Head-On, Tail-On Shrimp samples: Negative (Neg) and pre-enrichment *Salmonella* spiked positive (Pos):

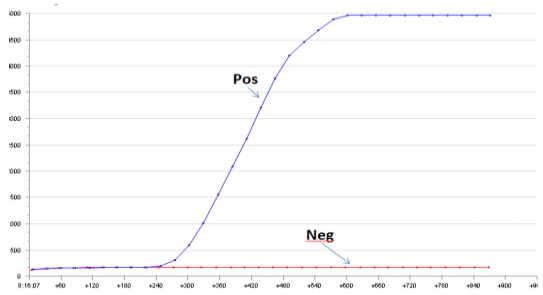
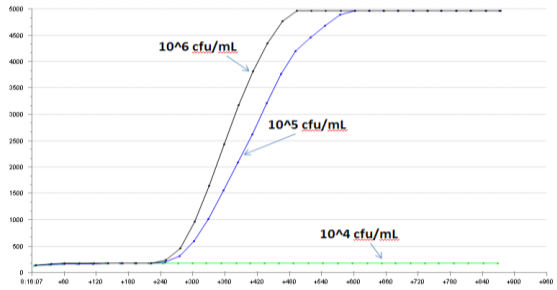


Figure 2: Limit of Detection (LOD) Study: Samples consisted of negative enriched macerated Shell-On, Head-On, Tail-On Shrimp spiked with known amounts: 10^6, 10^5, 10^4 CFU/mL of *Salmonella*.



CONCLUSION:

From this limited study, initial data suggests that the macerated, Shell-On, Head- Off, Tail-On Shrimp matrix is compatible with the DNAble *Salmonella* assay when using the prototype Sample Set 3 protocol. In this study *Salmonella* was detected down to 10^5 CFU/mL using the Sample Set 3 protocol, which is not commercially available at this time (12-3-14)

Note: As this was a limited study, a validation by your site is suggested.

MATRIX FEASIBILITY STUDY: Strawberries

OBJECTIVE:

The purpose of this study was to assess the compatibility of the **strawberry** matrix with the DNAble® *Salmonella* Assay (Cat. No. DF-026, Part #11716).

MATERIALS:

- DNAble *Salmonella* DNA Detection Kit consisting of:
  - RB1 Reaction Buffer tubes
  - *Salmonella* Master Mix tubes
- DNAble Sample Extraction Set 2
  - MB3 Buffer
  - Microcentrifuge tubes
- Buffered Peptone Water
- DNAble *Salmonella* Supplement
- *Salmonella enterica*
- Whirl-Pak® Bags
- Strawberries

SAMPLE PREP:

- 2 samples were prepared by adding 225 mL of 2.0% mBPW to 25 grams of strawberries in a Whirl-Pak bag. One sample was spiked with 10 cells of *Salmonella*. Samples were then "stomached" by hand for approximately 1 minute.
- Both samples were incubated overnight (~18-20 hours) at 37°C.
- After incubation, samples containing 10^4, 10^5, and 10^6 CFU/mL were created by spiking known amounts of *Salmonella* into aliquots of the negative enriched sample. These samples along with the presumed negative and the 10 cell spiked sample, were prepared for the DNAble assay using the instructions from the Sample Set 2 Product Insert:
  - Pre-heat a dry heat block to 95°C.
  - Mix the culture before sampling.
  - Transfer 1 mL of culture to a clear 1.5 mL microcentrifuge tube supplied with the set.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Remove the supernatant using caution to avoid disturbing the pellet. Leave a small volume remaining (≈ 100 µL) if an obvious pellet is not observed.
  - Add 100 µL of MB3 buffer and vortex to suspend the pellet.
  - Heat the sample in the heat block at 95°C for 10 minutes.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Place 100 µL of MB3 into a blue 1.5 mL microcentrifuge tube using a fresh pipette tip.
  - Transfer 25 µL of the supernatant from the second centrifugation into the MB3 and mix gently.
  - 5 µL of this prepared sample will be used in the subsequent DNAble reaction.
- The DNAble *Salmonella* assay was then run in duplicate on each sample.

RESULTS:

Figure 1: Data exported and extrapolated using Excel. The data below was obtained using strawberries (St) both unspiked (neg), spiked with 10 cells of *Salmonella* prior to overnight enrichment (pos).

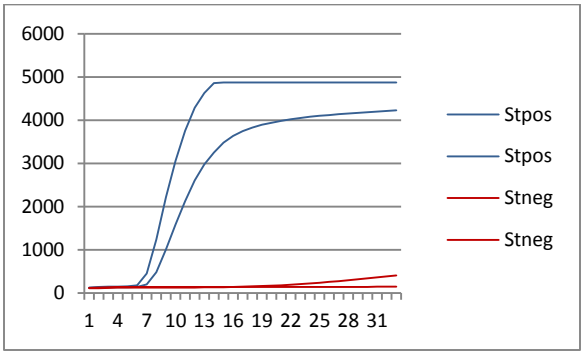
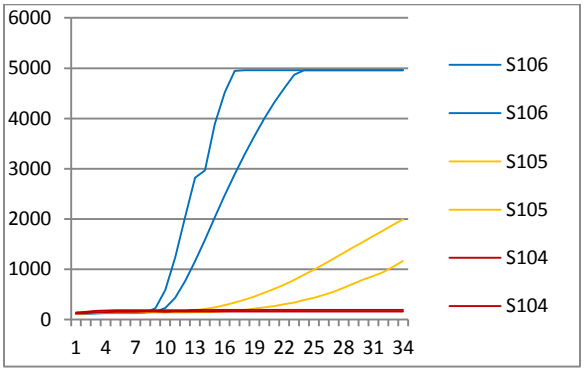


Figure 2: Data exported and extrapolated using Excel. Samples consisted of negative strawberries spiked with known amounts: 10^6 (106), 10^5 (105), 10^4 (104) CFU/mL of *Salmonella*.



CONCLUSION:

From this limited study, initial data suggests that the strawberry matrix is compatible with the DNAble® *Salmonella* assay. In this study *Salmonella* was detected down to 10^5 CFU/mL.

Note: As this was a limited study, a validation by your site is suggested.

MATRIX FEASIBILITY STUDY: Sliced Turkey

OBJECTIVE:

The purpose of this study was to assess the compatibility of the **deli sliced skinless turkey breast** matrix with the DNAble® *Salmonella* Assay (Cat. No. DF-026, Part #11716).

MATERIALS:

- DNAble *Salmonella* DNA Detection Kit consisting of:
  - RB1 Reaction Buffer tubes
  - *Salmonella* Master Mix tubes
- DNAble Sample Extraction Set 2
  - MB3 Buffer
  - Microcentrifuge tubes
- Buffered Peptone Water
- DNAble *Salmonella* Supplement
- *Salmonella enterica*
- Filtered Whirl-Pak® bags
- Deli sliced skinless turkey breast (sliced turkey) purchased at a local grocery

SAMPLE PREP:

- The following samples were prepared by adding 225 mL of 2.0% mBPW to 25 grams of sliced turkey, pre-macerated using a blender, in a Whirl-Pak bag:
  - Presumed negative sliced turkey
  - Sliced turkey spiked with *Salmonella*
- All samples were incubated overnight (~17 hours) at 37°C.
- After incubation, samples containing 10^4, 10^5, and 10^6 CFU/mL were created by spiking known amounts of *Salmonella* into aliquots of the presumed negative enriched samples. These samples along with the presumed negative and *Salmonella* spiked samples, were prepared for the DNAble assay using the instructions from the Sample Set 2 Product Insert:
  - Pre-heat a dry heat block to 95°C.
  - Mix the culture before sampling.
  - Transfer 1 mL of culture to a clear 1.5 mL microcentrifuge tube supplied with the set.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Remove the supernatant using caution to avoid disturbing the pellet. Leave a small volume remaining (≈ 100 µL) if an obvious pellet is not observed.
  - Add 100 µL of MB3 buffer and vortex to suspend the pellet.
  - Heat the sample in the heat block at 95°C for 10 minutes.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Place 100 µL of MB3 into a blue 1.5 mL microcentrifuge tube using a fresh pipette tip.
  - Transfer 25 µL of the supernatant from the second centrifugation into the MB3 and mix gently.
  - 5 µL of this prepared sample will be used in the subsequent DNAble reaction.
- The DNAble *Salmonella* assay was then run in duplicate on each sample.

RESULTS:

Figure 1: Negative and Positive (*Salmonella* spiked) Samples

This data represents sliced turkey samples: Negative (Neg), and pre-enrichment *Salmonella* spiked positive (Pos)

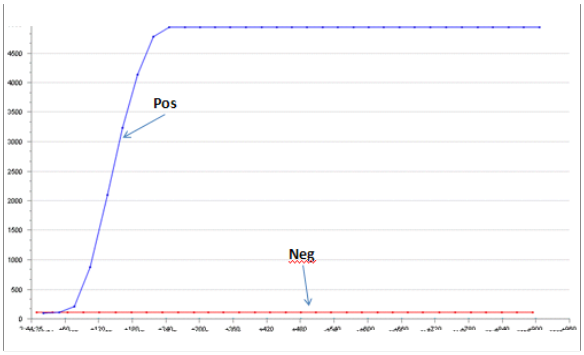
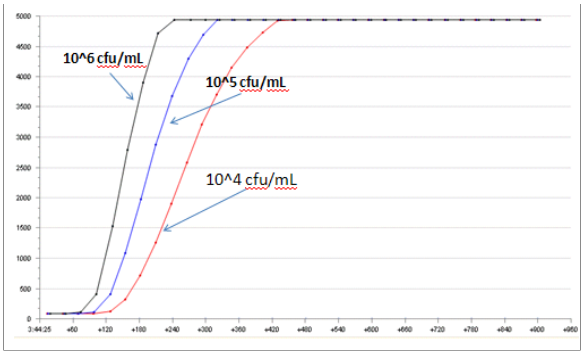


Figure 2: Limit of Detection (LOD) Study

Samples consisted of negative enriched sliced turkey spiked with known amounts: 10^6 (10^6), 10^5 (10^5), 10^4 (10^4) CFU/mL of *Salmonella*



CONCLUSION:

From this limited study, initial data suggests that the deli sliced skinless turkey breast matrix is compatible with the DNAble *Salmonella* assay. In this study *Salmonella* was detected down to 10^4 CFU/mL. The algorithm has not been defined; therefore these samples may not yield conclusive positive or negative results. The end user can rely on their interpretation or send us json files for conclusive output. This data is preliminary and more work is needed to substantiate these findings.

Note: As this was a limited study, a validation by your site is suggested.

MATRIX FEASIBILITY STUDY: Ground Turkey

OBJECTIVE:

The purpose of this study was to assess the compatibility of the **ground turkey** matrix with the DNAble® *Salmonella* Assay (Cat. No. DF-026, Part #11716).

MATERIALS:

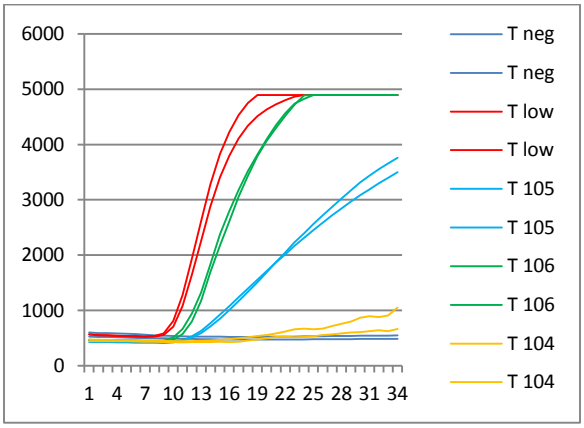
- DNAble *Salmonella* DNA Detection Kit consisting of:
  - RB1 Reaction Buffer tubes
  - *Salmonella* Master Mix tubes
- DNAble Sample Extraction Set 1
  - MB2 Buffer
  - Microcentrifuge tubes
- Buffered Peptone Water
- DNAble *Salmonella* Supplement
- *Salmonella* Enteritidis (ATCC# BAA 1587)
- Whirl-Pak® Bags
- Ground turkey purchased from local supermarket chain

SAMPLE PREP:

- 2 samples were prepared by adding 250 mL of 2.5% mBPW to 25 grams of ground turkey in a Whirl-Pak bag. One sample was spiked with 10 cells of *Salmonella*. Samples were then "stomached" by hand for approximately 1 minute.
- Both samples were incubated overnight (~17 hours) at 37°C.
- After incubation, samples containing 10^4, 10^5, and 10^6 CFU/mL were created by spiking known amounts of *Salmonella* into aliquots of the negative enriched sample. These samples along with the presumed negative and the 10 cell spiked sample, were prepared for the DNAble assay using the instructions from the Sample Set 1 Product Insert:
  - Pre-heat a dry heat block to 98°C.
  - Using a clean pipette tip, transfer 25 µL of MB2 Extraction Buffer to a labeled 1.5 mL microcentrifuge tube.
  - Transfer 125 µL of each enriched sample into the tube containing MB2 Extraction Buffer. Vortex briefly.
  - Heat tubes containing sample and extraction buffer for 15 minutes in heat block.
  - After 15 minutes, remove samples from block and place in a rack.
  - 5 µL of this prepared sample will be used in the subsequent DNAble reaction.
- The DNAble *Salmonella* assay was then run in duplicate on each sample.

RESULTS:

Figure 1: Data exported and extrapolated using Excel. The data below was obtained using ground turkey both unspiked (T neg), spiked with 10 cells of *Salmonella* prior to overnight enrichment (T low), and spiked after overnight enrichment with 10^4, 10^5 and 10^6 CFU/mL *Salmonella* (T 104, T 105, T 106).



CONCLUSION:

From this limited study, initial data suggests that the ground turkey matrix is compatible with the DNAble *Salmonella* assay. In this study *Salmonella* was detected down to 10^5 CFU/mL.

Note: As this was a limited study, a validation by your site is suggested.

MATRIX FEASIBILITY STUDY: Chili Powder

OBJECTIVE:

The purpose of this study was to assess the compatibility of the **chili powder** matrix with the DNAble® *Salmonella* Assay (Cat. No. DF-026, Part #11716).

MATERIALS:

- DNAble *Salmonella* DNA Detection Kit consisting of:
  - RB1 Reaction Buffer tubes
  - *Salmonella* Master Mix tubes
- DNAble Sample Extraction Set 2
  - MB3 Buffer
  - Microcentrifuge tubes
- Buffered Peptone Water
- DNAble *Salmonella* Supplement
- *Salmonella enterica*
- Whirl-Pak® Bags
- Chili powder sourced from a local Indian grocery

SAMPLE PREP:

- The following samples were prepared by adding 100 mL of 2.0% mBPW to 10 grams of chili powder in a Whirl-Pak bag:
  - Presumed negative chili powder
  - Chili powder with *Salmonella* spike
- Samples were incubated overnight at 37°C.
- After incubation, samples containing 10^4, 10^5, and 10^6 CFU/mL were created by spiking known amounts of *Salmonella* into aliquots of the presumed negative enriched samples. These samples along with the presumed negative and the *Salmonella* spiked sample, were prepared for the DNAble assay using the instructions from the Sample Set 2 Product Insert:
  - Pre-heat a dry heat block to 95°C.
  - Mix the culture before sampling.
  - Transfer 1 mL of culture to a clear 1.5 mL microcentrifuge tube supplied with the set.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Remove the supernatant using caution to avoid disturbing the pellet. Leave a small volume remaining (≈ 100 µL) if an obvious pellet is not observed.
  - Add 100 µL of MB3 buffer and vortex to suspend the pellet.
  - Heat the sample in the heat block at 95°C for 10 minutes.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Place 100 µL of MB3 into a blue 1.5 mL microcentrifuge tube using a fresh pipette tip.
  - Transfer 25 µL of the supernatant from the second centrifugation into the MB3 and mix gently.
  - 5 µL of this prepared sample will be used in the subsequent DNAble reaction.
- The DNAble *Salmonella* assay was then run in duplicate on each sample.

RESULTS:

Figure 1—Negative and Positive (*Salmonella* spiked): This data represents chili powder samples: Negative (Neg) and pre-enrichment *Salmonella* spiked positive (Pos).

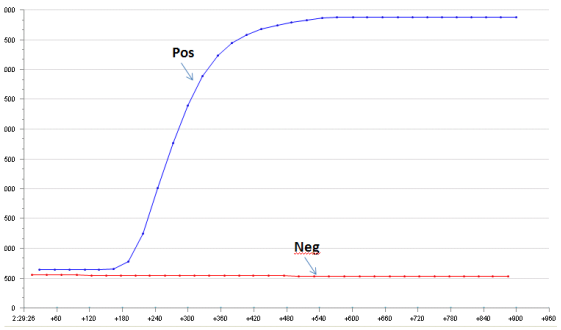
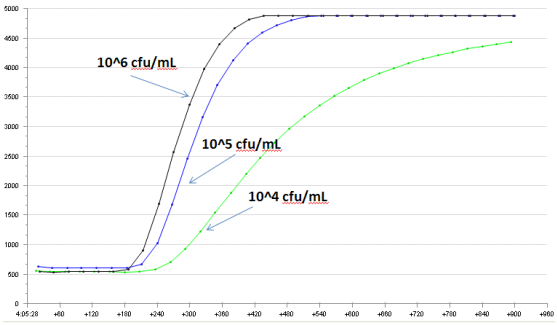


Figure 2—Limit of Detection (LOD) Study: Samples consisted of negative enriched chili powder spiked with known amounts: 10^6, 10^5, 10^4 CFU/mL of *Salmonella*.



CONCLUSION:

From this limited study, initial data suggests that the chili powder matrix is compatible with the DNAble *Salmonella* assay. In this study *Salmonella* was detected down to 10^4 CFU/mL. The algorithm has not been defined, however, and these samples may not yield conclusive positive or negative results and the end user can rely on their interpretation or send us json files for conclusive output. This data is preliminary and more work needs to be done in order to substantiate these findings.

Note:As this was a limited study, a validation by your site is suggested.

MATRIX FEASIBILITY STUDY: Powdered Coriander

OBJECTIVE:

The purpose of this study was to assess the compatibility of the **powdered coriander** matrix with the DNAble® *Salmonella* Assay (Cat. No. DF-026, Part #11716).

MATERIALS:

- DNAble *Salmonella* DNA Detection Kit consisting of:
  - RB1 Reaction Buffer tubes
  - *Salmonella* Master Mix tubes
- DNAble Sample Extraction Set 2
  - MB3 Buffer
  - Microcentrifuge tubes
- Buffered Peptone Water
- DNAble *Salmonella* Supplement
- *Salmonella enterica*
- Whirl-Pak® Bags
- Powdered coriander sourced from a local Indian grocery

SAMPLE PREP:

- The following samples were prepared by adding 100 mL of 2.0% mBPW to 10 grams of powdered coriander in a Whirl-Pak bag:
  - Presumed negative powdered coriander
  - Powdered coriander with *Salmonella* spike
- Samples were incubated overnight at 37°C.
- After incubation, samples containing 10^4, 10^5, and 10^6 CFU/mL were created by spiking known amounts of *Salmonella* into aliquots of the presumed negative enriched samples. These samples along with the presumed negative and the *Salmonella* spiked sample, were prepared for the DNAble assay using the instructions from the Sample Set 2 Product Insert:
  - Pre-heat a dry heat block to 95°C.
  - Mix the culture before sampling.
  - Transfer 1 mL of culture to a clear 1.5 mL microcentrifuge tube supplied with the set.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Remove the supernatant using caution to avoid disturbing the pellet. Leave a small volume remaining (≈ 100 µL) if an obvious pellet is not observed.
  - Add 100 µL of MB3 buffer and vortex to suspend the pellet.
  - Heat the sample in the heat block at 95°C for 10 minutes.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Place 100 µL of MB3 into a blue 1.5 mL microcentrifuge tube using a fresh pipette tip.
  - Transfer 25 µL of the supernatant from the second centrifugation into the MB3 and mix gently.
  - 5 µL of this prepared sample will be used in the subsequent DNAble reaction.
- The DNAble *Salmonella* assay was then run in duplicate on each sample.

RESULTS:

Figure 1—Negative and Positive (*Salmonella* spiked): This data represents powdered coriander samples: Negative (Neg) and pre-enrichment *Salmonella* spiked positive (Pos).

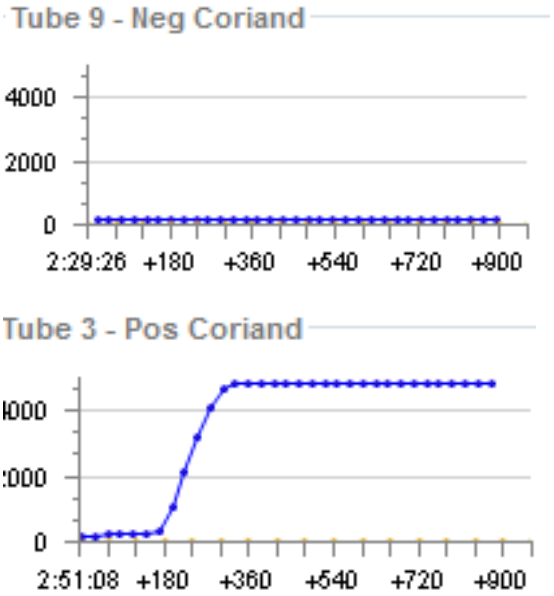
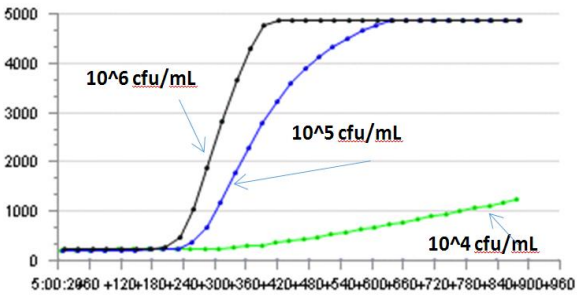


Figure 2—Limit of Detection (LOD) Study: Samples consisted of negative enriched powdered coriander spiked with known amounts: 10^6, 10^5, 10^4 CFU/mL of *Salmonella*.



CONCLUSION:

From this limited study, initial data suggests that the powdered coriander matrix is compatible with the DNAble *Salmonella* assay. In this study *Salmonella* was detected down to 10^4 CFU/mL. The algorithm has not been defined, however, and these samples may not yield conclusive positive or negative results and the end user can rely on their interpretation or send us json files for conclusive output. This data is preliminary and more work needs to be done in order to substantiate these findings.

Note: As this was a limited study, a validation by your site is suggested.

MATRIX FEASIBILITY STUDY: Powdered Cumin

OBJECTIVE:

The purpose of this study was to assess the compatibility of the **powdered cumin** matrix with the DNAble® *Salmonella* Assay (Cat. No. DF-026, Part #11716).

MATERIALS:

- DNAble *Salmonella* DNA Detection Kit consisting of:
  - RB1 Reaction Buffer tubes
  - *Salmonella* Master Mix tubes
- DNAble Sample Extraction Set 2
  - MB3 Buffer
  - Microcentrifuge tubes
- Buffered Peptone Water
- DNAble *Salmonella* Supplement
- *Salmonella enterica*
- Whirl-Pak® Bags
- Powdered cumin sourced from a local Indian grocery

SAMPLE PREP:

- The following samples were prepared by adding 100 mL of 2.0% mBPW to 10 grams of powdered cumin in a Whirl-Pak bag:
  - Presumed negative powdered cumin
  - Powdered cumin with *Salmonella* spike
- Samples were incubated overnight at 37°C.
- After incubation, samples containing 10^4, 10^5, and 10^6 CFU/mL were created by spiking known amounts of *Salmonella* into aliquots of the presumed negative enriched samples. These samples along with the presumed negative and the *Salmonella* spiked sample, were prepared for the DNAble assay using the instructions from the Sample Set 2 Product Insert:
  - Pre-heat a dry heat block to 95°C.
  - Mix the culture before sampling.
  - Transfer 1 mL of culture to a clear 1.5 mL microcentrifuge tube supplied with the set.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Remove the supernatant using caution to avoid disturbing the pellet. Leave a small volume remaining (≈ 100 µL) if an obvious pellet is not observed.
  - Add 100 µL of MB3 buffer and vortex to suspend the pellet.
  - Heat the sample in the heat block at 95°C for 10 minutes.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Place 100 µL of MB3 into a blue 1.5 mL microcentrifuge tube using a fresh pipette tip.
  - Transfer 25 µL of the supernatant from the second centrifugation into the MB3 and mix gently.
  - 5 µL of this prepared sample will be used in the subsequent DNAble reaction.
- The DNAble *Salmonella* assay was then run in duplicate on each sample.

RESULTS:

Figure 1—Negative and Positive (*Salmonella* spiked): This data represents powdered cumin samples: Negative (Neg) and pre-enrichment *Salmonella* spiked positive (Pos).

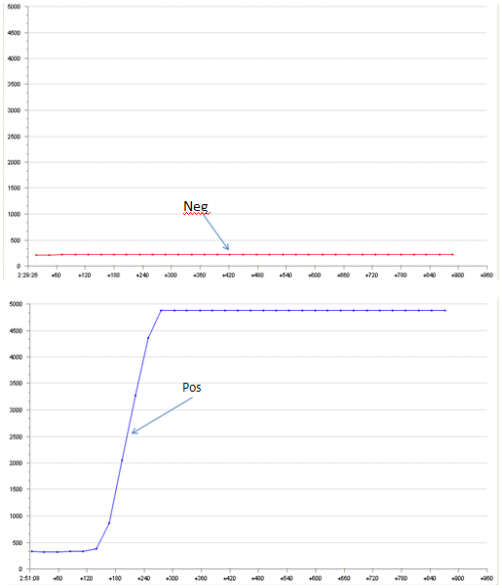
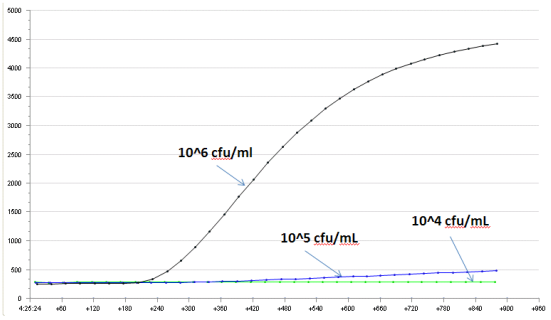


Figure 2—Limit of Detection (LOD) Study: Samples consisted of negative enriched powdered cumin spiked with known amounts: 10^6, 10^5, 10^4 CFU/mL of *Salmonella*.



CONCLUSION:

From this limited study, initial data suggests that the powdered cumin matrix is compatible with the DNAble *Salmonella* assay. In this study *Salmonella* was detected down to 10^6 CFU/mL. The algorithm has not been defined, however, and these samples may not yield conclusive positive or negative results and the end user can rely on their interpretation or send us json files for conclusive output. This data is preliminary and more work needs to be done in order to substantiate these findings.

Note: As this was a limited study, a validation by your site is suggested.

MATRIX FEASIBILITY STUDY: Boot Swabs

OBJECTIVE:

The purpose of this study was to assess the compatibility of the **boot swab** samples with the DNAble® *Salmonella* Assay (Cat. No. DF-026, Part #11716).

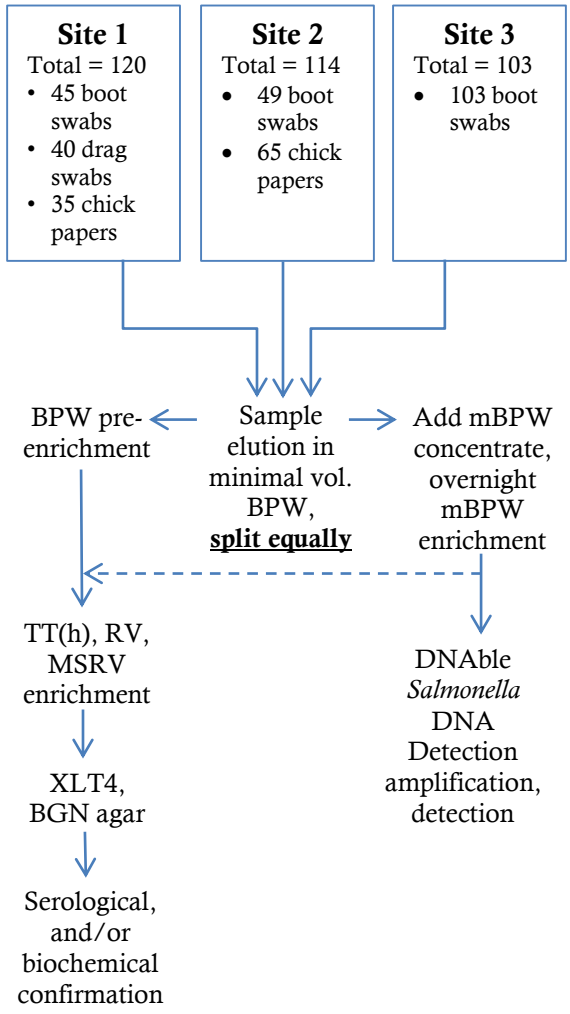
MATERIALS:

- DNAble *Salmonella* DNA Detection Kit consisting of:
  - RB1 Reaction Buffer tubes
  - *Salmonella* Master Mix tubes
- DNAble Sample Extraction Set 1
  - MB2 Buffer
  - Microcentrifuge tubes
- Buffered Peptone Water
- DNAble *Salmonella* Supplement
- Whirl-Pak® Bags
- Boot swab samples from 3 sites

SAMPLE PREP:

- 197 boot swab samples were evaluated for the presence of *Salmonella* at 3 sites using culture & DNAble (see Figure 1).
- After an overnight enrichment in mBPW, samples were prepared for the DNAble assay using the instructions from the Sample Set 1 Product Insert:
  - Pre-heat a dry heat block to 98°C.
  - Using a clean pipette tip, transfer 25 µL of MB2 Extraction Buffer to a labeled 1.5 mL microcentrifuge tube.
  - Transfer 125 µL of each enriched sample into the tube containing MB2 Extraction Buffer. Vortex briefly.
  - Heat tubes containing sample and extraction buffer for 15 minutes in heat block.
  - After 15 minutes, remove samples from block and place in a rack.
  - 5 µL of this prepared sample will be used in the subsequent DNAble reaction.
- The DNAble *Salmonella* assay was then run in duplicate on each sample.

Figure 1



RESULTS:

The performance of the DNAble *Salmonella* Detection Kit was compared to culture. Overall (unresolved) assay performance was a sensitivity, specificity and overall accuracy of 90.1%, 99.1% and 95.4% respectively. Overall (resolved) assay performance was a sensitivity, specificity and overall accuracy of 97.6%, 99.1% and 98.5% respectively.

Table 1 DNAble vs. Culture

	Unresolved	Resolved
% Sensitivity	90.1 (73/81)	97.6 (80/82)
% Specificity	99.1 (115/116)	99.1 (114/115)
% Accuracy	95.4 (188/197)	98.5 (194/197)

CONCLUSION:

The data from this study suggests that the boot swab matrix is compatible with the DNAble® *Salmonella* assay.

MATRIX FEASIBILITY STUDY: Carcass Rinse

OBJECTIVE:

The purpose of this study was to assess the compatibility of the **carcass rinse** matrix with the DNAble® *Salmonella* Assay (Cat. No. DF-026, Part #11716).

MATERIALS:

- DNAble *Salmonella* DNA Detection Kit consisting of:
  - RB1 Reaction Buffer tubes
  - *Salmonella* Master Mix tubes
- DNAble Sample Extraction Set 1
  - MB2 Buffer
  - Microcentrifuge tubes
- Buffered Peptone Water
- DNAble *Salmonella* Supplement
- *Salmonella* Enteritidis (ATCC# BAA 1587)
- Whirl-Pak® bags
- TTh Broth
- MSRV Agar
- XLT4 Agar
- 3 Whole Bird Carcass Rinses from post pick/pre-chill
- 3 Whole Bird Carcass Rinses from post chill

SAMPLE PREP:

- Three pre-chill and three post-chill carcass rinse samples were prepared for DNAble testing by adding 225mL of mBPW to 25mL of carcass rinse in Whirl-Pak bags. All samples were incubated overnight at 42°C.
- The same three pre-chill and post-chill carcass rinse samples were prepared for culture by adding 225mL of TTh broth to 25mL of carcass rinse in Whirl-Pak bags. All samples were incubated overnight at 42°C.
- Samples containing 10^4, 10^5, and 10^6 CFU/mL were created by spiking known amounts of *Salmonella* into aliquots of *Salmonella*-negative enriched carcass rinse samples.
- The overnight-enriched mBPW samples and samples containing 10^4, 10^5, and 10^6 CFU/mL were processed using the Sample Set 1 protocol:
  - Pre-heat a dry heat block to 98°C.
  - Using a clean pipette tip, transfer 25 µL of MB2 Extraction Buffer to a labeled 1.5 mL microcentrifuge tube.
  - Transfer 125 µL of each enriched sample into the tube containing MB2 Extraction Buffer. Vortex briefly.
  - Heat tubes containing sample and extraction buffer for 15 minutes in heat block.
  - After 15 minutes, remove samples from block and place in a rack.
  - 5 µL of this prepared sample will be used in the subsequent DNAble reaction.
- The DNAble *Salmonella* assay was then run in duplicate on each sample.
- 100 µL of each TTh specimen was inoculated to MSRV agar and incubated overnight at 42°C.
- Any growth on MSRV was sub-cultured to XLT4 for confirmation of *Salmonella*.

RESULTS:

Sample	DNAble Result		Culture Result
Pre-chill #1	Negative	Negative	Negative
Pre-chill #2	Positive	Positive	Positive
Pre-chill #3	Negative	Negative	Negative
Post-chill #1	Negative	Negative	Negative
Post-chill #2	Negative	Negative	Negative
Post-chill #3	Negative	Negative	Negative
Spiked Sample 10^6 CFU/mL	Positive	Positive	Not performed
Spiked Sample 10^5 CFU/mL	Positive	Positive	Not performed
Spiked Sample 10^4 CFU/mL	Positive*	Negative*	Not performed

*technical replicates

CONCLUSION:

From this limited study, initial data suggests that the carcass rinse matrix is compatible with the DNAble *Salmonella* assay using the Sample Set 1 Extraction Protocol.

Note: As this was a limited study, a validation by your site is suggested.

MATRIX FEASIBILITY STUDY: Ceca Samples

OBJECTIVE:

The purpose of this study was to assess the compatibility of the **ceca sample** matrix with the DNAble® *Salmonella* Assay (Cat. No. DF-026, Part #11716).

MATERIALS:

- DNAble *Salmonella* DNA Detection Kit consisting of:
  - RB1 Reaction Buffer tubes
  - *Salmonella* Master Mix tubes
- DNAble Sample Extraction Set 1
  - MB2 Buffer
  - Microcentrifuge tubes
- Buffered Peptone Water
- DNAble *Salmonella* Supplement
- Whirl-Pak® bags
- Ceca samples

SAMPLE PREP:

- 40 chickens were vaccinated and subsequently challenged with *Salmonella* at day 3 of age. 11 chickens were non-vaccinated and not challenged with *Salmonella*.
- On day 42, ceca were collected post necroscopy from the vaccinated & non-vaccinated broilers. Samples were analyzed using MSRV culture and DNAble.
- After an overnight enrichment in mBPW samples were processed using the Sample Set 1 protocol:
  - Pre-heat a dry heat block to 98°C.
  - Using a clean pipette tip, transfer 25 µL of MB2 Extraction Buffer to a labeled 1.5 mL microcentrifuge tube.
  - Transfer 125 µL of each enriched sample into the tube containing MB2 Extraction Buffer. Vortex briefly.
  - Heat tubes containing sample and extraction buffer for 15 minutes in heat block.
  - After 15 minutes, remove samples from block and place in a rack.
  - 5 µL of this prepared sample will be used in the subsequent DNAble reaction.
- The DNAble *Salmonella* assay was then performed on each sample.

RESULTS:

The performance of the DNAble *Salmonella* Assay was compared to culture. Overall assay performance was a sensitivity, specificity and overall accuracy of 96.4%, 100% and 98.0% respectively.

Table 1: DNAble vs. Culture

	Results
% Sensitivity	96.4% (27/28)
% Specificity	100% (23/23)
% Accuracy	98.0% (50/51)

CONCLUSION:

From this limited study, initial data suggests that the ceca sample matrix is compatible with the DNAble® *Salmonella* assay using the Sample Set 1 Extraction Protocol.

Note: As this was a limited study, a validation by your site is suggested.

MATRIX FEASIBILITY STUDY: Horse Feces

OBJECTIVE:

The purpose of this study was to assess the compatibility of the **horse feces** matrix with the DNAble® *Salmonella* Assay (Cat. No. DF-026, Part #11716).

MATERIALS:

- DNAble *Salmonella* DNA Detection Kit consisting of:
  - RB1 Reaction Buffer tubes
  - *Salmonella* Master Mix tubes
- DNAble Sample Extraction Set 2
  - MB3 Buffer
  - Microcentrifuge tubes
- Buffered Peptone Water
- DNAble *Salmonella* Supplement
- *Salmonella enterica*
- 15 mL tube
- Horse feces

SAMPLE PREP:

- The following samples were prepared by adding 9 mL of 2.0% mBPW to approximately 1gram of horse feces in a 15 mL tube:
  - Negative horse feces
  - Horse feces with 10 cell spike
- All samples were incubated overnight (~17 hours) at 37°C.
- After incubation, samples containing 10^4, 10^5, and 10^6 CFU/mL were created by spiking known amounts of *Salmonella* into aliquots of the presumed negative enriched samples. These samples along with the presumed negative and the 10 cell spiked samples, were prepared for the DNAble assay using the instructions from the Sample Set 2 Product Insert:
  - Pre-heat a dry heat block to 95°C.
  - Mix the culture before sampling.
  - Transfer 1 mL of culture to a clear 1.5 mL microcentrifuge tube supplied with the set.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Remove the supernatant using caution to avoid disturbing the pellet. Leave a small volume remaining (≈ 100 µL) if an obvious pellet is not observed.
  - Add 100 µL of MB3 buffer and vortex to suspend the pellet.
  - Heat the sample in the heat block at 95°C for 10 minutes.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Place 100 µL of MB3 into a blue 1.5 mL microcentrifuge tube using a fresh pipette tip.
  - Transfer 25 µL of the supernatant from the second centrifugation into the MB3 and mix gently.
  - 5 µL of this prepared sample will be used in the subsequent DNAble reaction.
- The DNAble *Salmonella* assay was then run in duplicate on each sample.

RESULTS:

Figure 1: Neg and 10 cell spike: Data exported and extrapolated using Excel. This data was obtained using the following horse feces samples: Negative (horse -) with MB3 sample prep and 10 cell spike prior to enrichment (horse +) with MB3 sample prep.

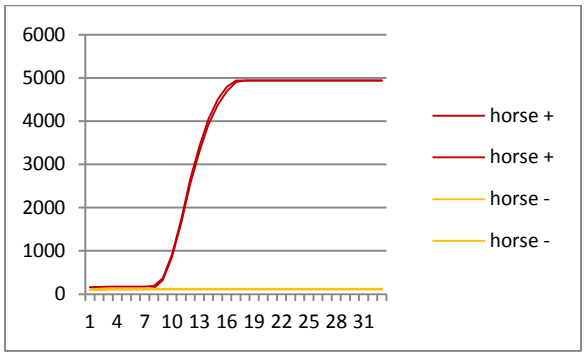
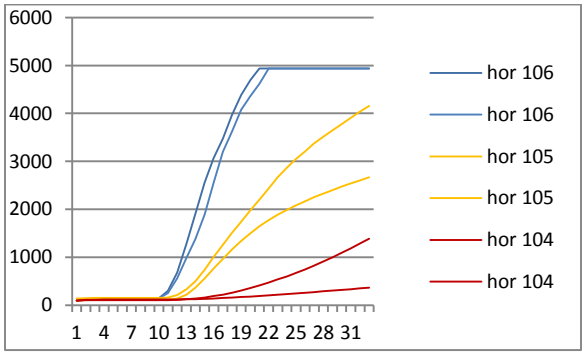


Figure 2: Limit of Detection (LOD) Study: Data exported and extrapolated using Excel. Samples consisted of negative enriched horse feces spiked with known amounts: 10^6 (106), 10^5 (105), 10^4 (104) of *Salmonella enterica* and prepared using the MB3 sample prep.



CONCLUSION:

From this limited study, initial data suggests that the horse feces matrix is compatible with the DNAble *Salmonella* assay. In this study *Salmonella* was detected down to 10^5 CFU/mL and in one technical replicate to 10^4 CFU/mL.

Note: As this was a limited study, a validation by your site is suggested.

MATRIX FEASIBILITY STUDY: Swine Feces

OBJECTIVE:

The purpose of this study was to assess the compatibility of the **swine feces** matrix with the DNAble® *Salmonella* Assay (Cat. No. DF-026, Part #11716).

MATERIALS:

- DNAble *Salmonella* DNA Detection Kit consisting of:
  - RB1 Reaction Buffer tubes
  - *Salmonella* Master Mix tubes
- DNAble Sample Extraction Set 2
  - MB3 Buffer
  - Microcentrifuge tubes
- Buffered Peptone Water
- DNAble *Salmonella* Supplement
- *Salmonella enterica*
- 15 mL tubes
- Swine feces

SAMPLE PREP:

- The following samples were prepared by adding 9 mL of 2.0% mBPW to approximately 1 gram of swine feces in a 15 mL tube:
  - Negative swine feces
  - Swine feces with 10 cell spike
- Both samples were incubated overnight (~17 hours) at 37°C.
- After incubation, samples containing 10^4, 10^5, and 10^6 CFU/mL were created by spiking known amounts of *Salmonella* into aliquots of presumed negative enriched samples. These samples along with the presumed negative and the 10 cell spiked sample, were prepared for the DNAble assay using the instructions from the Sample Set 2 Product Insert:
  - Pre-heat a dry heat block to 95°C.
  - Mix the culture before sampling.
  - Transfer 1 mL of culture to a clear 1.5 mL microcentrifuge tube supplied with the set.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Remove the supernatant using caution to avoid disturbing the pellet. Leave a small volume remaining (≈ 100 µL) if an obvious pellet is not observed.
  - Add 100 µL of MB3 buffer and vortex to suspend the pellet.
  - Heat the sample in the heat block at 95°C for 10 minutes.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Place 100 µL of MB3 into a blue 1.5 mL microcentrifuge tube using a fresh pipette tip.
  - Transfer 25 µL of the supernatant from the second centrifugation into the MB3 and mix gently.
  - 5 µL of this prepared sample will be used in the subsequent DNAble reaction.
- The DNAble *Salmonella* assay was then run in duplicate on each sample.

RESULTS:

Figure 1: Data exported and extrapolated using Excel. The data below was obtained using swine feces (pig) both unspiked ("-"), spiked with 10 cells of *Salmonella* prior to overnight enrichment ("+").

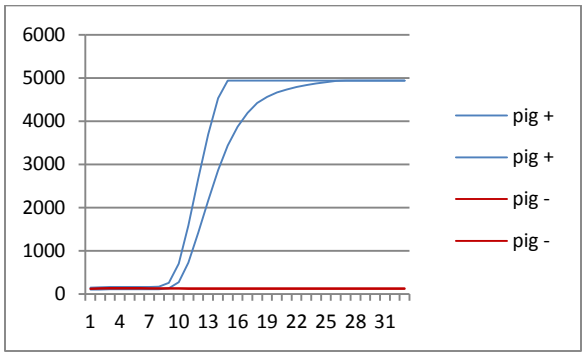
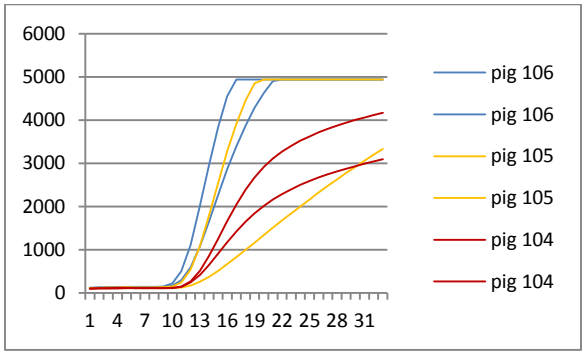


Figure 2: Data exported and extrapolated using Excel. Samples consisted of negative swine feces spiked with known amounts: 10^6 (106), 10^5 (105), 10^4 (104) CFU/mL of *Salmonella*.



CONCLUSION:

From this limited study, initial data suggests that the swine feces matrix is compatible with the DNAble *Salmonella* assay. In this study *Salmonella* was detected down to 10^4 CFU/mL.

Note: As this was a limited study, a validation by your site is suggested.

MATRIX FEASIBILITY STUDY: Organic Fertilizer

OBJECTIVE:

The purpose of this study was to assess the compatibility of the **organic fertilizer** matrix with the DNAble® *Salmonella* Assay (Cat. No. DF-026, Part #11716).

MATERIALS:

- DNAble *Salmonella* DNA Detection Kit consisting of:
  - RB1 Reaction Buffer tubes
  - *Salmonella* Master Mix tubes
- DNAble Sample Extraction Set 2
  - MB3 Buffer
  - Microcentrifuge tubes
- Buffered Peptone Water
- DNAble *Salmonella* Supplement
- *Salmonella enterica*
- Whirl-Pak® Bags
- Espoma brand Organic Fertilizer (Hydrolyzed feather meal, pasteurized poultry manure, cocoa meal, bone meal, alfalfa meal, greensand humate, sulfate of potash, sulfate of potash magnesia)

SAMPLE PREP:

- 2 samples were prepared by adding 225 mL of 2.0% mBPW to 25 grams of organic fertilizer in a Whirl-Pak bag. One sample was spiked with 10 cells of *Salmonella*. Samples were then "stomached" by hand for approximately 1 minute.
- Both samples were incubated overnight (~17 hours) at 37°C.
- After incubation, samples containing 10^4, 10^5, and 10^6 CFU/mL were created by spiking known amounts of *Salmonella* into aliquots of the negative enriched sample. These samples along with the negative and the 10 cell spiked sample, were prepared for the DNAble assay using the instructions from the Sample Set 2 Product Insert:
  - Pre-heat a dry heat block to 95°C.
  - Mix the culture before sampling.
  - Transfer 1 mL of culture to a clear 1.5 mL microcentrifuge tube supplied with the set.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Remove the supernatant using caution to avoid disturbing the pellet. Leave a small volume remaining (≈ 100 µL) if an obvious pellet is not observed.
  - Add 100 µL of MB3 buffer and vortex to suspend the pellet.
  - Heat the sample in the heat block at 95°C for 10 minutes.
  - Centrifuge the tube at 10,000 x *g* for 5 minutes.
  - Place 100 µL of MB3 into a blue 1.5 mL microcentrifuge tube using a fresh pipette tip.
  - Transfer 25 µL of the supernatant from the second centrifugation into the MB3 and mix gently.
  - 5 µL of this prepared sample will be used in the subsequent DNAble reaction.
- The DNAble *Salmonella* assay was then run in duplicate on each sample.

RESULTS:

Figure 1: Data exported and extrapolated using Excel. The data below was obtained using organic fertilizer (OF) both unspiked (neg), spiked with 10 cells of *Salmonella* prior to overnight enrichment (pos).

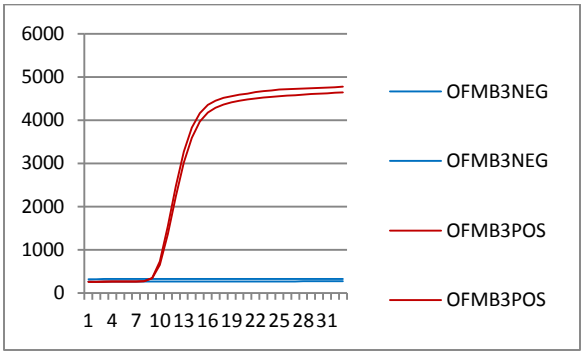
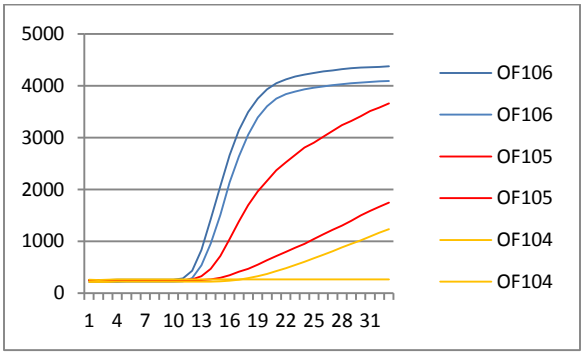


Figure 2: Data exported and extrapolated using Excel. Samples consisted of negative organic fertilizer spiked with known amounts: 10^6 (106), 10^5 (105), 10^4 (104) CFU/mL of *Salmonella enterica* and prepared using the MB3 sample prep.



CONCLUSION:

From this limited study, initial data suggests that the organic fertilizer matrix is compatible with the DNAble *Salmonella* assay. In this study *Salmonella* was detected down to 10^5 CFU/mL and in one technical replicate at 10^4 CFU/mL

Note: As this was a limited study, a validation by your site is suggested.