

RealPCR Salmonella DNA Reagents
NPIP Biennial Conference Committee Meeting
Franklin, TN
June 26-28, 2018



POULTRY
GEFLÜGEL
VOLAILLE
AVES DE CORRAL

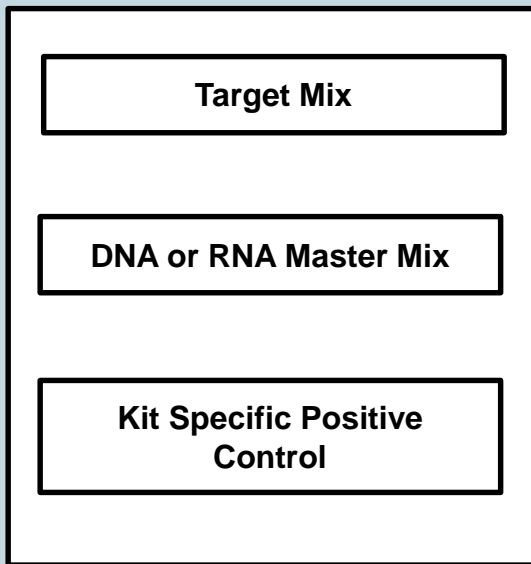
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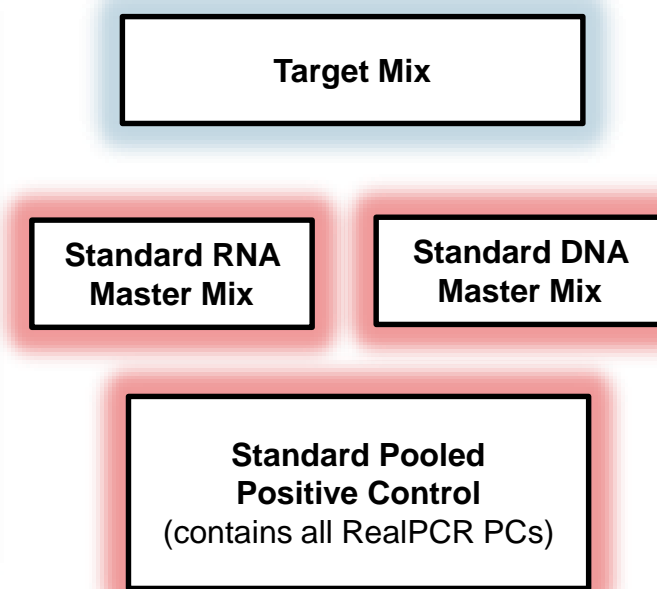


IDEXX RealPCR Platform

Kit Equivalent



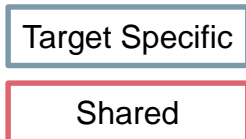
IDEXX Modular Reagents



Target Mix (TMx) –
Target Primers & Probes
Internal Amplification Control (IAC)
primers & probes

Master Mix (MMx) –
Enzymes, nucleotides

Pooled Positive Control (PC) – Pool of target sequences for IDEXX system. Includes IDEXX signature sequence



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RealPCR Salmonella spp. DNA Mix

- RealPCR SAL spp. DNA Mix uses the shared RealPCR DNA Master Mix and Pooled Positive Control
- Salmonella spp. DNA Mix contains primers, probes, and a unique target for an Internal Amplification Control (IAC)
- Single cycling program used with all RealPCR reagents
 - Allows multiple target testing in one real-time PCR run
- The RealPCR SAL spp DNA Mix is a screening method
 - Customers will be reminded during training that any sample that has a Ct and amplification curve should be confirmed using the culture methods outlined in the USDA/NPIP Program Standards (Standard B- Bacteriological Examination Procedure).

NPIP Field Trial – Study Design

- NPIP Authorized Laboratories-
- Positive Samples tested
 - ≥ 25 positive samples per site
 - Sample types included:
 - ▶ boot or drag swabs
 - Routine and from experimentally infected houses
 - ▶ hatchery samples (fluff, and chick tray pads).

NPIP Field Trial – Study Design cont.

- Negative Samples tested
 - ≥ 50 negative samples per site
 - Sample types included:
 - ▶ boot or drag swabs
 - ▶ hatchery samples (fluff, and chick tray pads).

- DNA Preparation
 - All samples were prepared using the boiling lysis method described in the SAL DNA Mix insert
 - ▶ 300ul enriched culture is removed and bacteria pelleted by centrifugation.
 - ▶ Supernatant is aspirated and pellet resuspended in 500 ul sterile H₂O
 - ▶ Suspension is incubated for 10 minutes at 97°C to lyse bacteria
 - ▶ Lysate is centrifuged again to pellet cell debris
 - ▶ DNA is located in the supernatant
 - ▶ Proceed immediately to PCR; or store lysate for up to 24 hours at -25 - 8°C (TTB only)
 - ▶ Cultural protocol must be continued within 24 hours of initial sample inoculation

Interpretation of Results

- Ct < 40 = PCR Positive
- Ct ≥ 40 = PCR negative*

- All samples that generate a Ct and amplification curve should be confirmed by the cultural method described in the USDA/NPIP Program Standards.

- NPIP-Approved methods:
 - Site 1: DuPont Qualicon BAX PCR-based assay
 - Sites 2-4: NPIP approved culture

- *This is a change from 2017 NPIP data analysis, where Ct ≥40 was considered suspect. This change was based on feedback from the technical committee in 2017.*

Resolution of discrepant results

- In cases where the RealPCR SAL DNA result did not agree with the Site's NPIP-approved method, final PCR-status was determined using an alternate NPIP-approved PCR assay.
- Though neither PCR assay will determine if culturable Salmonella is present, the alternate PCR will identify the presence of target DNA in the sample.
- Lysates were shipped to IDEXX and discrepant samples were tested as follows:
 1. Lysates were diluted 1:10 and tested on the NPIP-approved Life Technologies MicroSEQ Salmonella Species Detection Kit
 - dilution required to prevent inhibition
 2. Result obtained with MicroSEQ PCR is considered to be the final sample PCR-status for lysates
 3. Samples were also retested with RealPCR SAL DNA Mix at IDEXX

Results- Site 1

| | | Site Method- BAX PCR | | |
|--------------------|--------|-------------------------|-----|--------|
| | | Pos | Neg | Totals |
| RealPCR SAL DNA | Pos | 36 | 7 | 43 |
| | Neg | 2 | 100 | 102 |
| | Totals | 38 | 107 | 145 |

Sensitivity 95%
Specificity 93.9%

| | | Reference Method- MicroSEQ PCR | | |
|--------------------|--------|-----------------------------------|-----|--------|
| | | Pos | Neg | Totals |
| RealPCR SAL DNA | Pos | 41 | 2 | 43 |
| | Neg | 0 | 102 | 102 |
| | Totals | 41 | 104 | 145 |

Sensitivity 100%
Specificity 98.1%

| Field Trial Site Data | | |
|-----------------------|------------|---------------------------|
| Sample ID # | BAX status | RealPCR SAL DNA Site 1 Ct |
| 36 | Detected | -- |
| 37 | Detected | -- |
| 43 | Negative | 42.0* |
| 88 | Negative | 39.5 |
| 121 | Negative | 37.4 |
| 14 | Negative | 25.7 |
| 31 | Negative | 36.8 |
| 33 | Negative | 27.9 |
| 42 | Negative | 33.1 |
| 66 | Negative | 35.2 |

*Suspect/positive in 2017 analysis

| Testing at IDEXX | | | |
|------------------|-----------------------------|------------------------------|---------------------|
| Sample ID # | RealPCR SAL DNA Result (Ct) | MicroSEQ Sal PCR Result (Ct) | Final Sample Status |
| 36 | -- | -- | Negative |
| 37 | -- | -- | Negative |
| 43 | 39.2 | -- | Negative |
| 88 | 37.3 | -- | Negative |
| 121 | -- | -- | Negative |
| 14 | 25.5 | 30.9 | Positive |
| 31 | 35.9 | 36.8 | Positive |
| 33 | 24.8 | 30.8 | Positive |
| 42 | 31.2 | 34.9 | Positive |
| 66 | 32.9 | 37.0 | Positive |

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Results- Site 2

| | | Site Method- Culture | | |
|--------------------|--------|-------------------------|-----|--------|
| | | Pos | Neg | Totals |
| RealPCR SAL DNA | Pos | 57 | 12 | 69 |
| | Neg | 0 | 96 | 96 |
| | Totals | 57 | 108 | 165 |

Sensitivity 100%
Specificity 90.0%

| | | Reference Method- MicroSEQ PCR | | |
|--------------------|--------|-----------------------------------|-----|--------|
| | | Pos | Neg | Totals |
| RealPCR SAL DNA | Pos | 67 | 1 | 68 |
| | Neg | 1 | 96 | 97 |
| | Totals | 68 | 97 | 165 |

Sensitivity 98.5%
Specificity 99.0%

| Field Trial Site Data | | |
|-----------------------|----------------|---------------------------|
| Sample ID # | Culture Result | RealPCR SAL DNA Site 1 Ct |
| 47 | Not detected | 39.9 |
| 2 | Not detected | 30.9 |
| 61 | Not detected | 37.2 |
| 62 | Not detected | 36.0 |
| 64 | Not detected | 38.7 |
| 65 | Not detected | 35.7 |
| 76 | Not detected | 29.5 |
| 77 | Not detected | 30.2 |
| 95 | Not detected | 30.1 |
| 97 | Not detected | 38.9 |
| 98 | Not detected | 34.8 |
| 102 | Not detected | 37.6 |

| Testing at IDEXX | | | |
|------------------|-----------------------------|------------------------------|---------------------|
| Sample ID # | RealPCR SAL DNA Result (Ct) | MicroSEQ Sal PCR Result (Ct) | Final Sample Status |
| 47 | 39.0 | -- | Negative |
| 2 | 32.4 | 31.0 | Positive |
| 61 | 36.8 | 33.7 | Positive |
| 62 | 37.1 | 32.5 | Positive |
| 64 | 40.9 | 34.2 | Positive |
| 65 | 35.8 | 31.6 | Positive |
| 76 | 28.4 | 26.2 | Positive |
| 77 | 30.0 | 27.8 | Positive |
| 95 | 30.4 | 29.5 | Positive |
| 97 | 37.5 | 37.1 | Positive |
| 98 | 35.7 | 33.6 | Positive |
| 102 | 38.9 | 34.5 | Positive |

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Results- Site 3

| | | Site Method- Culture | | |
|--------------------|--------|-------------------------|-----|--------|
| | | Pos | Neg | Totals |
| RealPCR SAL DNA | Pos | 61 | 10 | 71 |
| | Neg | 0 | 73 | 73 |
| | Totals | 61 | 83 | 144 |

Sensitivity 100%
Specificity 89.2%

| | | Reference Method- MicroSEQ PCR | | |
|--------------------|--------|-----------------------------------|-----|--------|
| | | Pos | Neg | Totals |
| RealPCR SAL DNA | Pos | 66 | 1 | 67 |
| | Neg | 1 | 76 | 77 |
| | Totals | 67 | 77 | 144 |

Sensitivity 98.5%
Specificity 98.7%

| Field Trial Site Data | | |
|-----------------------|----------------|---------------------------|
| Sample ID # | Culture Result | RealPCR SAL DNA Site 3 Ct |
| 26 | Not detected | 39.0 |
| 79 | Not detected | 38.9 |
| 89 | Not detected | 39.4 |
| 5 | Not detected | 39.9 |
| 33 | Not detected | 39.9 |
| 12 | Not detected | 34.0 |
| 13 | Not detected | 37.0 |
| 14 | Not detected | 38.0 |
| 78 | Not detected | 38.9 |
| 92 | Not detected | 37.0 |

| Testing at IDEXX | | | |
|------------------|-----------------------------|------------------------------|---------------------|
| Sample ID # | RealPCR SAL DNA Result (Ct) | MicroSEQ Sal PCR Result (Ct) | Final Sample Status |
| 26 | -- | -- | Negative |
| 79 | -- | -- | Negative |
| 89 | -- | -- | Negative |
| 5 | 37.0 | -- | Negative |
| 33 | -- | 37.1 | Positive |
| 12 | 34.3 | 33.6 | Positive |
| 13 | 36.5 | 36.4 | Positive |
| 14 | 37.9 | 34.4 | Positive |
| 78 | 36.9 | 35.1 | Positive |
| 92 | 34.3 | 37.0 | Positive |

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Results- Site 4

| | | Site Method- Culture | | |
|--------------------|--------|-------------------------|-----|--------|
| | | Pos | Neg | Totals |
| RealPCR SAL DNA | Pos | 44 | 9 | 53 |
| | Neg | 0 | 50 | 50 |
| | Totals | 44 | 59 | 103 |

Sensitivity 100%
Specificity 86.8%

| | | Reference Method- MicroSEQ PCR | | |
|--------------------|--------|-----------------------------------|-----|--------|
| | | Pos | Neg | Totals |
| RealPCR SAL DNA | Pos | 48 | 1 | 49 |
| | Neg | 0 | 54 | 54 |
| | Totals | 48 | 55 | 103 |

Sensitivity 100%
Specificity 98.1%

| Field Trial Site Data | | |
|-----------------------|----------------|---------------------------|
| Sample ID # | Culture Result | RealPCR SAL DNA Site 4 Ct |
| 51 | Not detected | 38.4 |
| 85 | Not detected | 38.0 |
| 89 | Not detected | 39.4 |
| 101 | Not detected | 39.3 |
| 97 | Not detected | 39.0 |
| 33 | Not detected | 36.5 |
| 87 | Not detected | 38.2 |
| 88 | Not detected | 35.9 |
| 99 | Not detected | 38.3 |

| Testing at IDEXX | | | |
|------------------|-----------------------------|------------------------------|---------------------|
| Sample ID # | RealPCR SAL DNA Result (Ct) | MicroSEQ Sal PCR Result (Ct) | Final Sample Status |
| 51 | -- | -- | Negative |
| 85 | -- | -- | Negative |
| 89 | -- | -- | Negative |
| 101 | -- | -- | Negative |
| 97 | 39.1 | -- | Negative |
| 33 | 33.5 | 32.2 | Positive |
| 87 | 37.8 | 35.4 | Positive |
| 88 | 34.5 | 31.9 | Positive |
| 99 | 38.6 | 33.7 | Positive |

Overall Agreement of RealPCR to Reference Methods

| | | Site Method- Culture/BAX | | |
|--------------------|--------|-----------------------------|-----|--------|
| | | Pos | Neg | Totals |
| RealPCR SAL DNA | Pos | 198 | 38 | 236 |
| | Neg | 2 | 319 | 321 |
| | Totals | 200 | 357 | 557 |

Sensitivity 99.0%

Specificity 89.4%

| | | Reference Method- MicroSEQ PCR | | |
|--------------------|--------|-----------------------------------|-----|--------|
| | | Pos | Neg | Totals |
| RealPCR SAL DNA | Pos | 224 | 6 | 230 |
| | Neg | 0 | 327 | 327 |
| | Totals | 224 | 333 | 557 |

Sensitivity 100%

Specificity 98.2%

Thank you! Questions?

