Supplemental Information

Multi-laboratory evaluations of the performance of *Catellicoccus marimammalium* PCR assays developed to target gull fecal sources

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Preparation and processing of challenge samples. The collection of fecal material and preparation of replicate challenge sample filters for multi-lab comparative analysis has been described in detail elsewhere (Boehm et al, this issue). In brief, challenge samples were composited from freshly collected fecal material from different 12 sources: individual humans, sewage, septage, horses, cattle, deer, pigs, geese, chickens, pigeons, seagulls, and dogs. Fecal samples from a minimum of at least 12 individuals were collected from each animal type and the multiple samples of each type were composited together to make each animal type challenge fecal sample. The host-source animal identity of all multiple fecal samples making up each type of animal fecal composite was confirmed by observation during the collection of each individual fecal sample. Sewage influent was collected from 9 separate wastewater treatment facilities, and septage was collected from 6 separate collection trucks or community systems. An approximately equal number of each fecal sample type was collected from four California geographies: central California, Los Angeles County, Orange County, and San Diego County. Samples were aseptically composited and 32 types of challenge samples were created from filtered slurries containing either a single fecal type (termed "singletons") or two fecal types (termed "doubletons"), which were 90%:10% (volume:volume) slurries of different fecal types (Table S1). For example, in the case of pigeon, the pigeon fecal samples were collected from 4 regional locations along California as listed above. Samples were taken from coastal, recreational, and residential areas, with the various sampling sites located at different distances from the coast. Tarps were set out and pigeons were attracted in order to collect fresh feces. Only droppings that were visually observed to originate from pigeons were collected. Each collection consisted of multiple individuals (i.e. >20/region) in order to create each of the two pigeon fecal composites. The amount of feces present in each challenge sample was measured

by the group that created the blinded sample set. The group measured viable enterococci in fecal samples by membrane filtration, mass of DNA in sample nucleic acid extracts by fluorometry and/or UV spectrometry, and *Bacteroidales* maker (genbac3 assay) in sample nucleic acid extracts as previously described (Boehm et al., this issue).

Participating laboratories received a blinded set of 64 filters (comprised of the 32 challenge samples in duplicate) on dry ice. Eleven laboratories participated in gull methods assessment including 4 "core" laboratories and 7 "outside" laboratories (Ebentier et al, this issue). Not all labs participated in all the methods evaluated in this study: 6 labs tested the Gull2Taqman qPCR assay, 4 labs tested the Gull2SYBR assay, 3 labs tested the LeeSeaGull assay, and 4 labs tested the Gull2EndPoint assay (Table S2).

Filter processing and DNA extraction. Individual labs extracted and purified total genomic DNA from the frozen filters typically with some variant of bead beating lysis and adsorption-desorption DNA purification with commercially available kits by the manufacturer's instructions (Table S3). The core labs and most outside labs (six labs) utilized the GeneRite DNA-EZ kit (GeneRite, LLC) as modified per the SIPP study DNA extraction SOP. The core SOP for Generite DNA-EZ kit was as per manufacturer's instructions, with the following exceptions: (1) lysis buffer was amended by the addition of purified genomic salmon DNA at 0.2ng/µL as a specimen preparation control (spc) for extraction; (2) each frozen filter was bead-beat in 500µL of this lysis-spc buffer; (3) after beating, 350µL of lysate was added to 1000µL of the binding matrix; (4) final DNA recovery was by two sequential 50µL elution for a final elution recovery volume of approximately 100µL; (5) total DNA yield from each extraction was measured by NanoDrop UV spectrometry (Thermo Scientific), or by flourometry with Quant-It DNA standards and quantitation dye (Invitrogen).

Variations from the core extraction SOP were as follows: one lab used the GeneRite DNA-EZ unmodified, two labs used the MoBio Power Soil DNA kit (Mo Bio Laboratories, Inc.), one lab used the Qia Amp DNA mini-kit (Qiagen, Inc.), and one lab used the FastDNA spin kit (MPBiomedicals, LLC) (Table S3). Total DNA yield from extractions typically was measured by UV spectrometry using the NanoDrop Spectrophotometer (Thermo Scientific). Fluorometry with commercial DNA-binding dye and quantitation standards also was used with most labs using the Qubit Fluorometer with Quant-iT reagent kit (Invitrogen Life Technologies) (Table S3). In addition, DNA concentrations measured in the sources that were used for sample normalization purposes were provided to participating laboratories (Boehm et al., this issue).

This study presents an additional evaluation of the LeeSeaGull assay compared to that presented in the overview paper (Boehm et al. 2012). To obtain sufficient volumes of DNA for this work, DNA from each of the 64 samples was pooled from three of the participating laboratories. Duplicate aliquots were created and these were supplied to laboratories 5 and 6 for analysis.

PCR and MST target analysis of *Catellicoccus marimammalium*. Primer and probe oligonucleotide sequences used in this study are given in Table S4. SIPP core labs utilized the identical PCR protocols. Variations in reagents, cycling platforms, and cycling conditions were used by other laboratories (Table S5). The Gull2SYBR assay and Gull2Endpoint assays were conducted as per Lu et al. (2008), with lab-specific modifications shown in Table S5. Gull2 plasmid quantitation standards for the SYBR Green assay were generously provided to the participating labs by the laboratory of Jorge Santo-Domingo at EPA. The Gull2Taqman assay was conducted as described in Sinigalliano et al. (2010), with the following modifications: (1) some labs used ABI universal Mastermix (Applied Biosystems) instead of Qiagen QuantiTect

Probe Mastermix (Qiagen); (2) probe with Black Hole Quencher 1 (BHQ1) was typically employed with the exception of Lab #5, which synthesized the probe with ZEN quencher from IDT (Integrated DNA Technologies); (3) cycling conditions were as described in Table S5. Gull2Taqman plasmid quantitation control standards were provided to participating laboratories by the lab of Chris Sinigalliano at NOAA-AOML.

The LeeSeaGull qPCR assay had not been published at the time of this study; therefore, details are provided here. The primer and probes for this assay (Table S4; Lee et al. 2012) target a partial 16S rRNA region of *C. marimammalium* internal to same region targeted by the Gull2 assay of Lu et al. (2008), generating a smaller amplicon (Figure S1). Primers, probe, and plasmid quantitation control for the LeeSeaGull assay were provided to participating laboratories by the lab of Jiyoung Lee at Ohio State University. To construct the LeeSeaGull plasmid standard control for standard curve generation, DNA template (2 µL) from *Catellicoccus marimammalium* strain DSMZ M35/04/3T DNA extract (sequence accession no. AJ854484; kindly provided by Dr. Jingrang Lu, U.S. Environmental Protection Agency) was amplified with the CaTstdF and CaTstdR primers using 2.5 µL of 10× PCR buffer and 0.5 U of Taq DNA polymerase (Invitrogen), 0.75 mM of $MgCl_2$, 100 µM of dNTP mix, and 500 nM of each forward and reverse primer, in a final reaction volume of 25 µL, with the following thermocycling conditions: (1) initial denaturation at 94°C for 3 min, followed by (2) 35 cycles of 94°C for 30sec, 55°C for 30sec, and 72°C for 1 min, followed by (3) a final extension at 72°C for 7 min. The resultant 329 bp amplicon was cloned into the pGEM-T vector (Promega), screened and transformed into competent *E. coli* DH5α cells as per manufacturer instructions. The resulting standard control plasmids were purified with QIAprep Spin Miniprep Kit (Qiagen) according to manufacturer instructions and the concentration of purified plasmid was measured

with the Nanodrop UV spectrophotometer (NanoDrop Products, Thermo Scientific). LeeSeaGull qPCR used ABI TaqMan Universal PCR Mastermix (Applied Biosystems), with either 5µL of target (Lab 10) or 2µL of target template (labs 5 and 6) in a final reaction volume of 20µL containing 250 nM each of the CaT#998F and CaT#998R primers and 125 nM of the CaT#998P probe. Cycling conditions were (1) an initial cycle of 50°C for 2 min and 95°C for 10 min, followed by (2) 45 cycles of 95°C for 15 sec and 60°C for 1 min. Purified DNA from *Catellicoccus marimammalium* strain DSMZ M35/04/3T was used as a positive control and sterile distilled water as the NTC negative control respectively. To generate assay standard curves of known target sequence copy number, serial dilutions of plasmid control were prepared, and replicate standard curves ranging from 10^7 to 10^{-1} copies per reaction (lab 10) or 10^8 to 10^1 copies per reaction (labs 5 and 6) were run on each plate along with replicate challenge samples and negative No Template Controls (NTCs). Pooled master curves were generated for each lab, and the Range of Quantitation (ROQ), Lower Limit of Quantification (LLOQ), and range for Detected but Not Quantified (DNQ) was determined for each lab and standardized across labs as described in the section "Processing and analysis of multi-laboratory molecular data" below.

Confirmation of *Catellicoccus marimammalium* sequence in pigeon sample amplicons from Gull2Taqman and LeeSeaGull assays by DNA cycle sequencing. A representative amplicon sample of each of the two SIPP Study pigeon composite samples from both the Gull2Taqman and the LeeSeaGull assay were sequenced with the Gull2 and LeeSeaGull assay primers respectively. The qPCR products from the composite pigeon feces samples were purified with the QIAquick PCR Purification Kit (Qiagen, Valencia, CA). Sequencing reactions were generated with the ABI BigDye Terminator Cycle Sequencing Kit (v3.1) and sequenced using an ABI Prism 3730 DNA Analyzer as per manufacturer and kit instructions. Both forward

and reverse sequencing reactions were used. Sequences generated were compared to those available in the GenBank databases using the NCBI BLAST program, and were also compared to those *Catellicoccus* sequences from shorebirds available in the literature as well as those sequences available from another ongoing study of *Catellicoccus*-like sequences in waterfowl and shorebirds by one of our co-authors (Grond et al., unpublished data). The sequence alignments from these are shown in Figure 7.

Processing and analysis of multi-laboratory molecular data. In order to more directly compare the data sets between different labs performing the same quantitative assay, post-processing of data was "standardized." Threshold cycle (C_t) values were collected for all standards and samples run at each lab. Pooled standard curves were plotted for each individual lab including standard concentrations down to the lowest concentration standard in which amplification was detected in at least 80% of replicates. A regression analysis was performed on the pooled standard curve generated for each lab, and outliers were removed based on standardized residual values of >+3 or <-3. For Gull2SYBR, melt curves were verified by each individual lab for all samples, and melt peak temperatures were within 1°C of standards.

The Lower Limit of Quantification (LLOQ) for each lab was calculated by taking the average C_t value of the non-outlier standard replicates at the lowest concentration included in the standard curve. Each sample replicate was considered within the range of quantification (ROQ) if the C_t< LLOQ, detected but not quantifiable (DNQ) if the Ct> LLOQ, and not detected (ND) if there was no amplification detected by cycle 40. Some labs performed samples in triplicate while others only ran duplicates of each sample; therefore, a strategy was devised to standardize interpretation of results. For samples run in triplicate, C_t values were averaged if 2/3 or 3/3 replicates were within the ROQ. If 2/3 or 3/3 replicates were DNQ, then the sample was

considered DNQ. If 2/3 or 3/3 replicates were ND then the sample was considered ND. In the rare case that the replicates contained 1 of each ROQ, DNQ and ND then the samples were considered DNQ. For samples run in duplicate, C_t values were averaged if 2/2 replicates were within the ROQ and single C_t values were used if 1/2 replicates were within the ROQ. If 2/2 replicates were DNQ or 1 DNQ and 1 ND resulted, then the sample was considered DNQ. If 2/2 replicates were ND then the sample was considered ND. C_t values reported at >40 cycles were considered ND in all cases except for one lab running the Gull2Taqman assay in which all the standards and samples were shifter toward higher C_t values (lab #4). In this case, C_t values up to 45 were accepted. Concentrations for the three quantitative gull assays were calculated from the average C_t values based on the pooled standard curve generated for each individual lab.

For quantitative assays, method performance was assessed for target and non-target samples (Table S1) on the abundance quantified and on calculated assay sensitivity and specificity (Table 2, Table S6). For quantitative analysis, assay results were normalized using values provided to the participating laboratories (Boehm et al, this issue) for the number of colony forming units (CFU) of enterococci by membrane filtration, *Bacteroidales* as measured by the genbac3 assay (Siefring et al. 2008), and by mass of DNA measured in the sources. Each of these normalizations was performed on the 38 single-source challenge samples (Figures S2-S19). For visualization purposes, DNQs and NDs (Table S7) were assigned values lower than the lowest sample in the range of quantification in a given plot. Sensitivity and specificity were calculated for the quantitative and conventional PCR assays, using all 64 challenge samples and the same equations as presented in the SIPP overview paper (Boehm et al, this issue). Sensitivity and specificity were calculated using the same criteria in the overall paper, before and after data

processing standardization, and with different parameters (e.g. treating pigeon as false positive, treating pigeon as a true positive, treating DNQs as true negatives) (Table S6).

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Table SI-1. Description of challenge samples provided to participating labs in duplicate. The designation '1:10' indicates a 1:10 dilution of the full strength singleton. The numbers after the doubleton name indicates the percent by volume, respectively, combined to create the sample.

Singletons	Doubletons
Chicken	sewage/chicken 10/90
Deer	sewage/gull 10/90
Dog	sewage/gull 90/10
dog 1:10	sewage/pig 10/90
Goose	sewage/pig 90/10
Gull	human/cow 10/90
gull 1:10	human/cow 90/10
Horse	human/dog 10/90
Pig	human/dog 90/10
pig 1:10	human/goose 10/90
Pigeon	human/gull 10/90
Cow	human/gull 90/10
cow 1:10	septage/horse 10/90
Human	
human 1:10	
Septage	
septage 1:10	
Sewage	
sewage 1:10	

Table SI-2. Key to participating lab # by method. NA = not applicable, assay was not run by that laboratory.

Lab# for DNA extraction	Lab# for Gull2Taqman qPCR	Lab# for LeeSeaGull qPCR	Lab# for Gull2SYBR qPCR	Lab# for Gull2EndPoint PCR
1	na	na	1	na
2	na	na	2	na
3	na	na	3	na
4	4	na	4	4
5	5	5	na	na
6	6	6	na	na
7	7	na	na	7
8	8	na	na	8
9	9	na	na	na
10	na	10	na	na
11	na	na	na	11

Table SI-3. Methods used for nucleic acid extraction and DNA target quantification.

Lab#	Tested Gull method	Nucleic acid extraction method	DNA quantification method	qPCR/PCR instrument
1	Gull2 SYBR	QIA amp DNA mini kit[A]	Nanodrop 1000[E]	CFX96[G]
2	Gull2 SYBR	GeneRite DNA-EZ[B1]	NanoDrop[E]	StepOnePlus[H]
3	Gull2 SYBR	MoBio PowerSoil[C]	Qubit® fluorometer [F]	ABI 7500[H]
4	Gull2 SYBR, Gull2Taqman, Gull2Endpt	MoBio Power Soil[C]	Synergy™ [J] microplate reader with Quant-iT™	ABI 7900[H]
5	Gull2Taqman LeeSeaGull	GeneRite DNA EZ[B2]	Qubit® QuantIT™ BR	StepOnePlus[H]
6	Gull2Taqman LeeSeaGull	GeneRite DNA EZ [B2]	Nanodrop[E],Quant-iT™[F]	CFX [G]
7	Gull2Taqman, Gull2Endpt	GeneRite DNA EZ [B2]	Nanodrop[E],Quant-iT™[F]	CFX [G]
8	Gull2Taqman, Gull2Endpt	GeneRite DNA EZ [B2]	Nanodrop[E],Quant-iT™[F]	StepOne[H] or GeneAmp 9700[H]
9	Gull2Taqman	GeneRite DNA EZ [B2]	Nanodrop[E],Quant-iT™[F]	StepOne[H]
10	LeeSeaGull	GeneRite DNA EZ [B2]	Nanodrop[E]	StepOne[H]
11	Gull2Endpt	Fast DNA spin kit[D]	UV spectrometer	PxE 0.2[I]

[A] Qiagen, France, modification with no bead beating, as in Mieszkin et al 2009
[B] GeneRite, North Brunswick, NJ, USA, [B1] manufacturer protocol [B2] modified SIPP core SOP protocol, see supplemental material above
[C]MoBio, Carlsbad, USA
[D] MP Biomedicals, Santa Ana, CA, USA. Initial bead beating step according to (Haugland et al. 2005)
[E]Nanodrop Products, Thermo Scientific,Wilmington, DE, USA
[F]Invitrogen, Carlsbad, CA, USA
[G] BioRad, Hercules CA, USA
[H] Applied Biosytems, Carlsbad, CA, USA
[I] Thermo Fisher Scientific, Waltham, MA, USA
J Bio-Tek Instruments, Inc., Winooski, VT, USA

Table SI-4. Primer and probe sequences used in methods Gull2SYBR, Gull2Endpt, Gull2Taqman, and LeeSeaGull.

ASSAY	Oligo Name	Sequence 5'-3'	Target length	Reference
Gull2SYBR & Gull2Endpt	Gull2f	TGCATCGACCTAAAGTTTTGAG	412bp	(Lu et al. 2008)
	Gull2r	GTCAAAGAGCGAGCAGTTACTA		
Gull2Taqman	Gull2f	TGCATCGACCTAAAGTTTTGAG	412bp	(Sinigalliano et al., 2010)
	Gull2r	GTCAAAGAGCGAGCAGTTACTA		
	Gull2p	*[6FAM]*-CTGAGAGGGTGATCGGCCACATTGGGACT-*[BHQ1]*		
LeeSeaGull	CaT#998F	AGGTGCTAATACCGCATAATACAGAG	112bp	(Lee et al. 2012)
	CaT#998R	GCCGTTACCTCACCGTCTA		
	CaT#998P	*[6FAM]*-TTCTCTGTTGAAAGGCGCTT-*[MGB]*		
Primers for construction of standard control plasmid insert	CaTstdF	CTGATGCTTGCATCGACCTA	329bp	This Study
	CaTstdR	CGGTCAGACTTTCGTCCATT		

Table SI-5. PCR and qPCR reaction and thermocycling conditions for different methods used.

Assay (# of labs)	Reaction mixture		Thermal protocol			
	Final primer/probe concentration	**Template/ total volume**	**Phase I**	**Phase II**	**Phase III**	**Phase IV**
Gull2SYBR (2 labs) (1 lab)	 400 nM/- 200 nM/-	 2 µL/25 µL 2 µL/25 µL		10 min at 95°C	40 cycles: 15 sec at 95°C 60 sec at 64°C	Melt curve: 15 sec at 95°C 15 sec at 64°C 15 sec at 95°C and 0.1ºC/s to 95°C
Gull2SYBR (1 lab)	 400 nM/-	 10 µL/25 µL	2 min at 50°C	10 min at 95°C	40 cycles: 15 sec at 95°C 60 sec at 60°C	Melt Curve: 15 sec at 95°C 20 sec at 60 and 0.3 °C/s to 90 °C
Gull2Taqman (6 labs)	 900 nM/300 nM	 2 µL/25 µL	15 min at 95°C	40 cycles: 15 sec at 95°C 60 sec at 62°C	-	-
LeeSeaGull (2 labs) (1 lab)	 250 nM/125 nM 250 nM/125 nM	 2 µL/20 µL 5 uL/20 µL	2 min at 50°C, 10 min at 95°C	45 cycles: 15 sec at 95°C 60 sec at 60°C	-	-
Gull2Endpt (4 labs)	 800 nM/ -	 2 µL/25 µL	3 min at 95°C	35 cycles: 30 sec at 95°C 30 sec at 64°C 60 sec at 72°C	-	-

Table SI-6. %Sensitivity (sens) and %specificity (spec) for each assay with varying sets of defined analysis parameters[a].

ASSAY	Lab 4		Lab 5		Lab 6		Lab 7		Lab 8		Lab 9		average	
Gull2Taqman	sens	spec	sens	spec	sens	spec	sens	spec	sens	spec	sens	spec	sens	spec
Original submitted results, pigeon +	64.3	98.0	92.9	12.0	100	96.0	100	70.0	100	70.0	100	72.0	92.9	69.7
Original results, pigeon +, DNQ -	64.3	98.0	92.9	12.0	100	98.0	100	96.0	100	90.0	100	100	92.9	82.3
Standardized analysis , pigeon +	71.4	95.9	92.9	10.0	100	96.0	100	84.0	100	84.0	100	86.0	94.0	76.0

	Lab 1		Lab 2		Lab 3		Lab 4						average	
Gull2SYBR	sens	spec	sens	spec	sens	spec	sens	spec					sens	spec
Original submitted results, pigeon +	85.7	100	100	100	85.7	82.0	85.7	94.0					89.3	94.0
Original results, pigeon +, DNQ -	57.1	100	100	100	85.7	82.0	85.7	94.0					82.1	94.0
Standardized analysis , pigeon +	57.1	100	100	100	85.7	78.0	85.7	94.0					82.1	93.0

	Lab 5		Lab 6		Lab 10								average	
LeeSeaGull	sens	spec	sens	spec	sens	spec							sens	spec
Original submitted results, pigeon +	100	8.0	100	10.0	100	98.0							100	38.7
Original results, pigeon +, DNQ -	100	68.0	100	90.0	100	98.0							100	85.3
Standardized analysis, pigeon +	100	42.0	100	34.0	100	78.0							100	51.3

[a]based on all 64 challenge samples (single- and dual-source). Results are presented by individual lab and as an average of all labs performing the assay. Calculations were performed under three defined analysis parameters: the original submitted data set including pigeon as a true positive, the original data set treating pigeon as a true positive and DNQ as a true negative, and after standardized data processing including pigeon as a true positive.

Table SI-7. Comparison of the number of the 64 challenge samples (single- and dual-source) that were classified as ROQ, DNQ, and ND in the original submitted data set and after standardized data processing for each of the quantitative gull assays. Results are presented by individual lab and as an average of all labs performing the assay.

	Lab 4		Lab 5		Lab 6		Lab 7		Lab 8		Lab 9		average	
Gull2Taqman	orig	std	orig	std	orig	std	orig	std	orig	std	orig	std	orig	std
ROQ	10	9	57	24	15	14	16	14	19	15	14	14	22	15
DNQ	0	3	0	34	1	2	13	8	10	7	14	7	6	10
ND	54	52	7	6	48	48	35	42	35	42	36	43	35	39

	Lab 1		Lab 2		Lab 3		Lab 4		average	
Gull2SYBR	orig	std	orig	std	orig	std	orig	std	orig	std
ROQ	8	7	14	14	21	19	15	10	15	13
DNQ	4	1	0	0	0	4	0	5	1	3
ND	52	56	50	50	43	41	49	49	49	49

	Lab 5		Lab 6		Lab 10		average	
LeeSeaGull	orig	std	orig	std	orig	std	orig	std
ROQ	30	30	19	19	15	14	21	21
DNQ	30	13	40	28	0	11	23	17
ND	4	21	5	17	49	39	19	26

TAATACATGCAAGTCGAACGCAAAACTTTTAACTGATGCT***TGCATCGACCTAAAGTTTTGAG***T
GGCGGACGGGTGAGTA***ACACGTGGGTAACCTGCCCATCAGA***GGGGGACAACACTTGGAAAC***A***
GGTGCTAATACCGCATAATACAGAGAACCGCATGG***TTCTTTGTTGAAAGGCGCTT***CTGGTGTC
GCTGATGGATGGACCCGCGGTGCATTAGC***TAGACGGTGAGGTAACGGC***TCACCGTGGCAATG
ATGCATAGCCGAC***CTGAGAGGGTGATCGGCCACATTGGGACT***GAGACACGGCCCAAACTCCT
ACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGACGAAAGTCTGACCGAGCAACGCCGCG
TGAGTGAAGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGAGAAGAACAGGAGCGA***TAGTA***
ACTGCTCGCTCTTTGACGGTATCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGC
GGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTC
TNTTAAGTCTGATGTGAAAGCCCACGGCTTAACCGTGGAGGGTCATTGGAAACTGGGAGACT
TGAGTGCAGAAGAGGAAAGTGGAACTCCATGTGTAGCGGTGGAATGCGTAGATATATGGAA
GAACACCAGTGGCGAAGGCGGCTTTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCGTGGG
GAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTGGA
GGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCG
CAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAA
TTCGAAGCAACGCGAAGAAGGTTACCAGGTCTTGACATCCTCTGACAACCCTAGAGATAGGN
CTTTCCCTTCGGGGACAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGAT
GTTGGGTTAAGTCCCGCAACGAGCGCAA***CCCTTGTCGTTAGTTGCCATCATTC***AGTTGGGCAC
TCTAGCGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCC
CCTTATGACCTGGGCTACACACGTGCTACAATGGACGGTACAACGA***GCAGCGAACTCGCGAG***
GGCAAGCGAATCTCTTAAAGCCGTTCTCAGTTCGGATTGCAGGCTGCAACTCGCCTGCATGA
AGCCGGAATCGCTAGTAATCGTGGATCAGCATGCCACGGTGAATACGTTCCCGGGCCTTGTA
CACACCGCCCGTCACACCACGAGAGTTTGTAACACCCAAAGTCGGTGCGGTAACCTTTTGGA
GCCAGCCGCCTAAGGTGGGATAGATGATTGGGGTGAAGTCGTAACAAGGTAGCCG

Legend:
Yellow Highlight – primers for Gull2SYBR assay, Gull2Endpoint assay, and Gull2Taqman assay
Yellow Highlight with red text - Taqman® type probe for Gull2Taqman assay
Green Highlight – primers for LeeSeaGull assay
Green Highlight with red text - Taqman® type probe for LeeSeaGull assay
Blue Highlight – primers for GFC assay (Greene et al., 2012)
Underlined – primers for Gull4Taqman assay (Ryu et al., 2012)
Underlined with red text - Taqman® type probe for Gull4Taqman assay (Ryu et al., 2012)

Figure SI-1. Sequence of *Catellicoccus marimammalium* (NCBI accession number: NR_042357) showing the relative hybridization target positions of the primers and probes for current bird-host *C. marimammalium* PCR assays, including the 4 assays tested in this study.

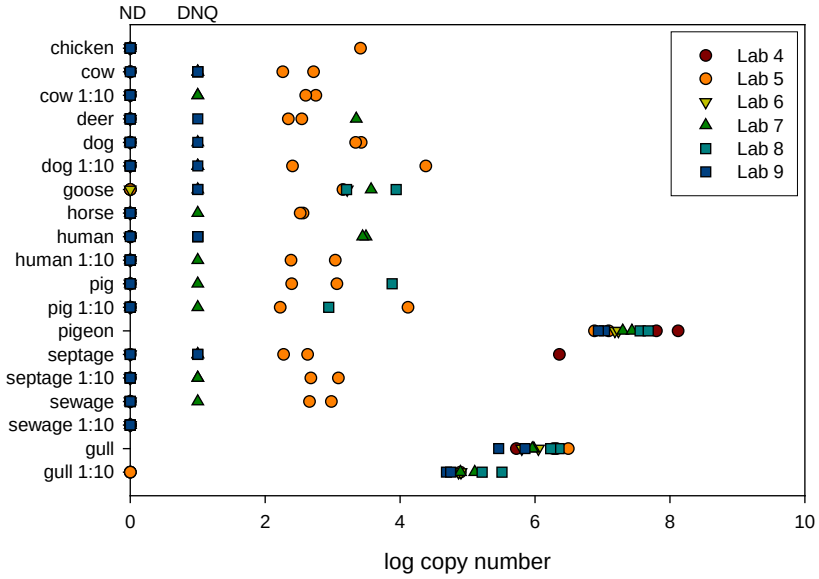

Figure SI-2. Original submitted Gull2Taqman assay results prior to reclassifying the data from the 38 single-source challenge samples. ND = not detected. DNQ = detected but not quantifiable.

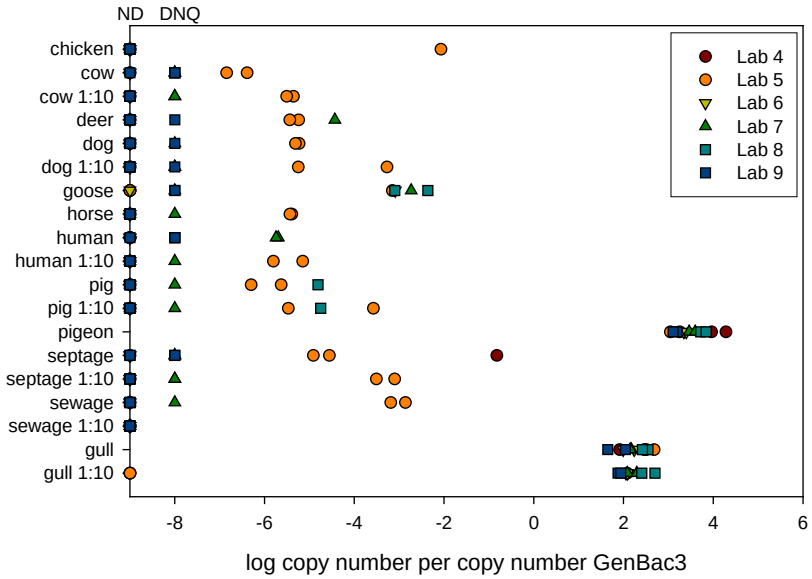

Figure SI-3. Original submitted Gull2Taqman assay results prior to reclassifying the data from the 38 single-source challenge samples, normalized by GenBac3 copy number measured in the sources. ND = not detected. DNQ = detected but not quantifiable.

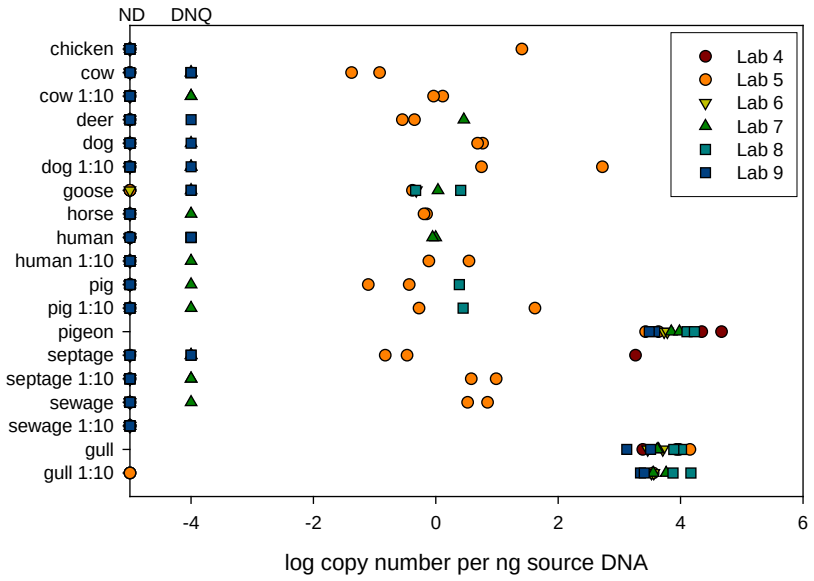

Figure SI-4. Original submitted Gull2Taqman assay results prior to reclassifying the data from the 38 single-source challenge samples, normalized by ng DNA measured in the sources. ND = not detected. DNQ = detected but not quantifiable.

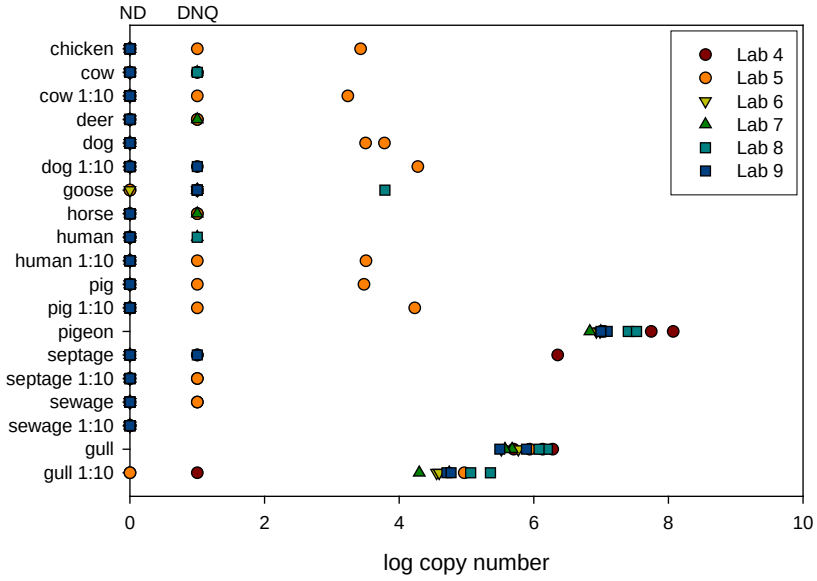

Figure SI-5. Standardized Gull2Taqman assay results from the 38 single-source challenge samples. ND = not detected. DNQ = detected but not quantifiable.

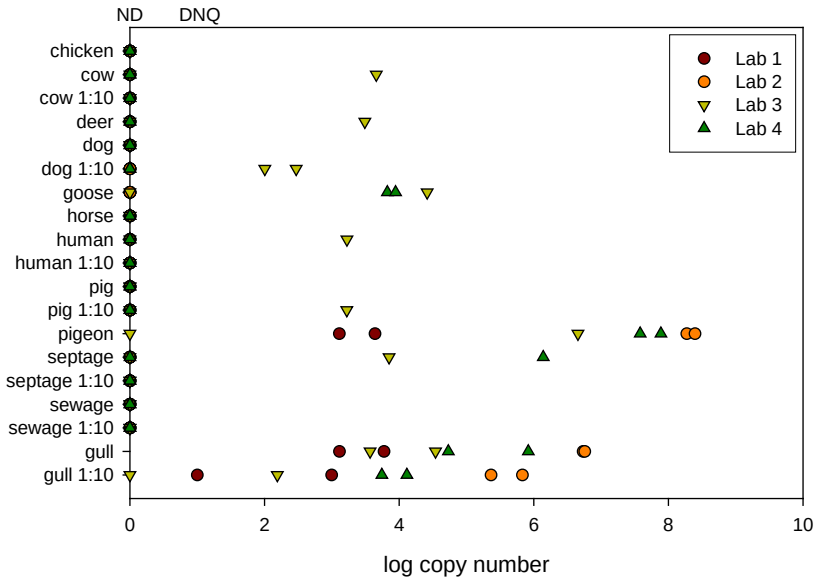

Figure SI-6. Original submitted Gull2SYBR assay results prior to reclassifying the data from the 38 single-source challenge samples. ND = not detected. DNQ = detected but not quantifiable.

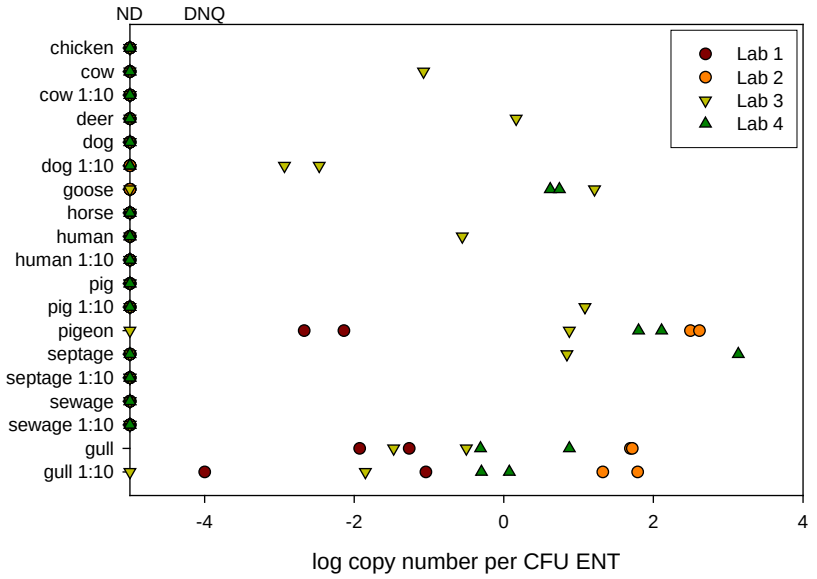

Figure SI-7. Original submitted Gull2SYBR assay results prior to reclassifying the data from the 38 single-source challenge samples, normalized by CFU ENT measured in the sources. ND = not detected. DNQ = detected but not quantifiable.

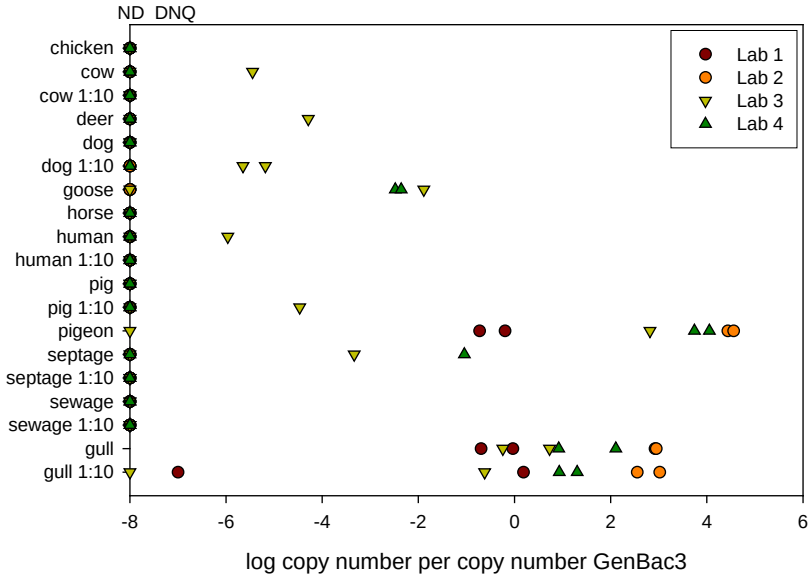

Figure SI-8. Original submitted Gull2SYBR assay results prior to reclassifying the data from the 38 single-source challenge samples, normalized by GenBac3 copy number measured in the sources. ND = not detected. DNQ = detected but not quantifiable.

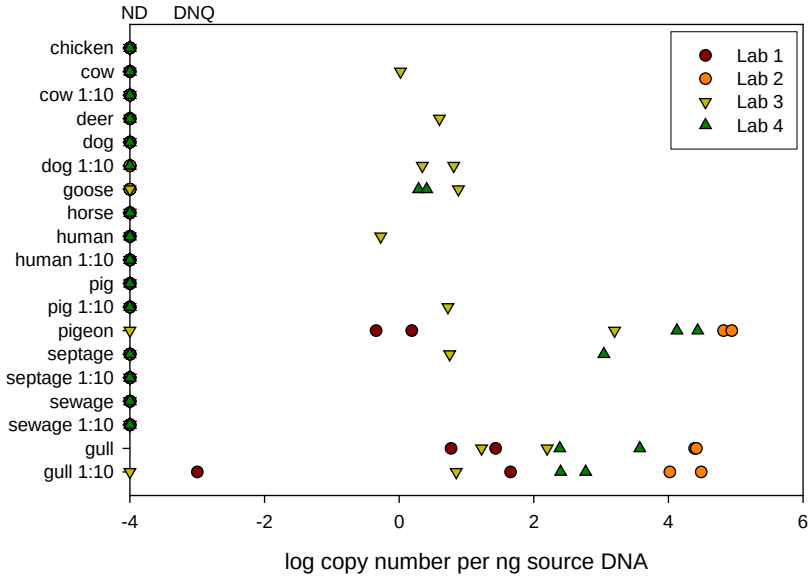

Figure SI-9. Original submitted Gull2SYBR assay results prior to reclassifying the data from the 38 single-source challenge samples, normalized by ng DNA measured in the sources. ND = not detected. DNQ = detected but not quantifiable.

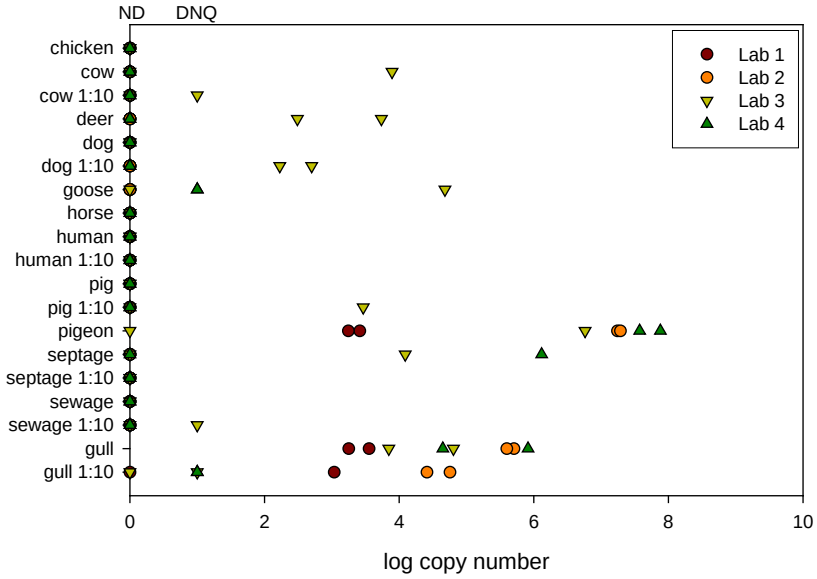

Figure SI-10. Standardized Gull2SYBR assay results from the 38 single-source challenge samples. ND = not detected. DNQ = detected but not quantifiable.

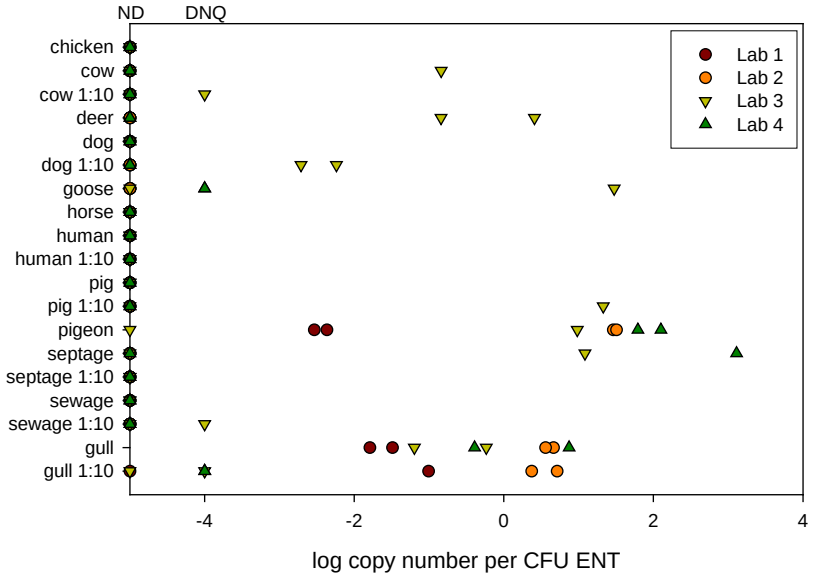

Figure SI-11. Standardized Gull2SYBR assay results from the 38 single-source challenge samples, normalized by CFU ENT measured in the sources. ND = not detected. DNQ = detected but not quantifiable.

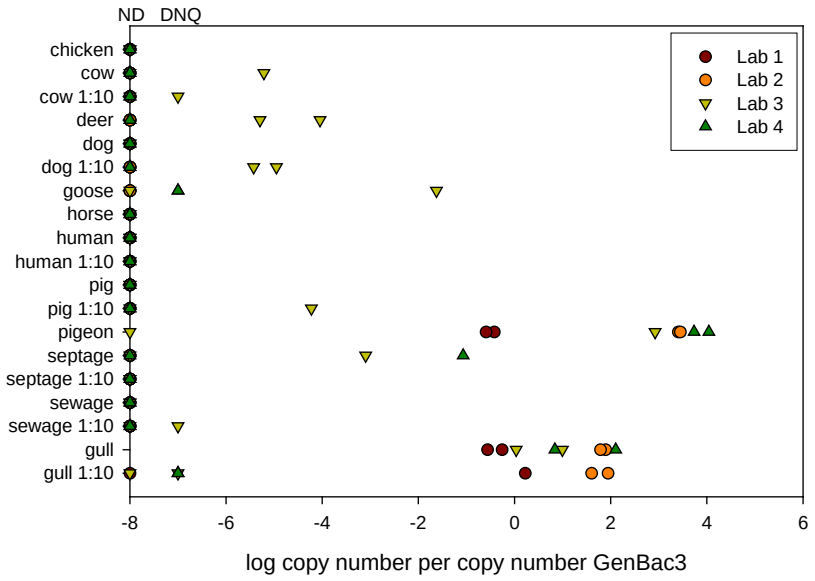

Figure SI-12. Standardized Gull2SYBR assay results from the 38 single-source challenge samples, normalized by GenBac3 copy number measured in the sources. ND = not detected. DNQ = detected but not quantifiable.

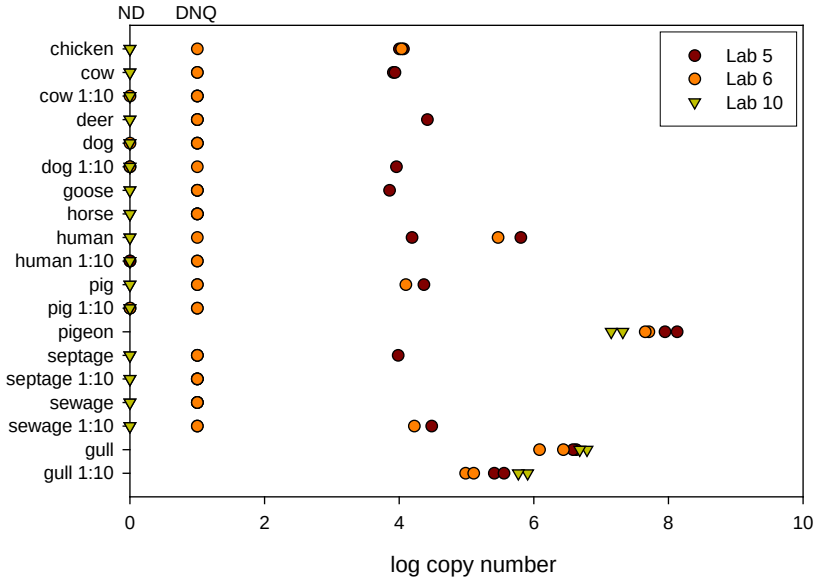

Figure SI-13. Original submitted LeeSeaGull assay results prior to reclassifying the data from the 38 single-source challenge samples. ND = not detected. DNQ = detected but not quantifiable.

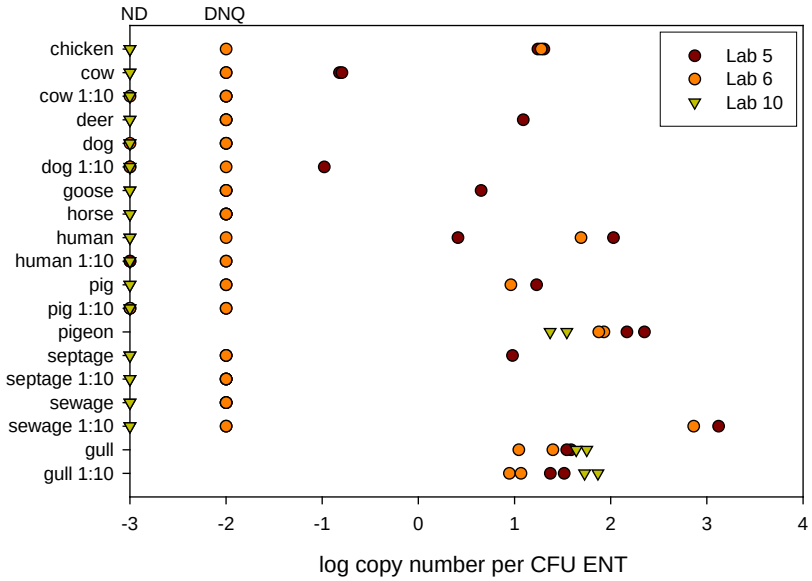

Figure SI-14. Original submitted LeeSeaGull assay results prior to reclassifying the data from the 38 single-source challenge samples, normalized by CFU ENT measured in the sources. ND = not detected. DNQ = detected but not quantifiable.

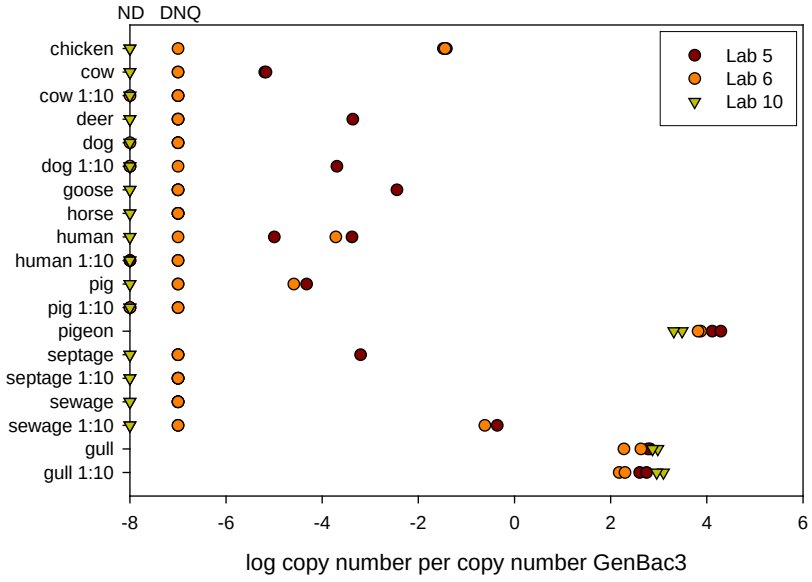

Figure SI-15. Original submitted LeeSeaGull assay results prior to reclassifying the data from the 38 single-source challenge samples, normalized by GenBac3 copy number measured in the sources. ND = not detected. DNQ = detected but not quantifiable.

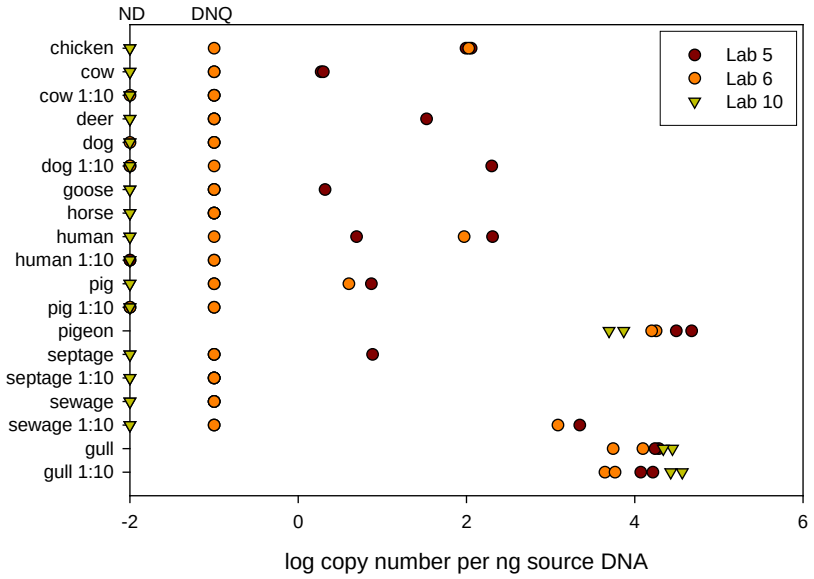

Figure SI-16. Original submitted LeeSeaGull assay results prior to reclassifying the data from the 38 single-source challenge samples, normalized by ng DNA measured in the sources. ND = not detected. DNQ = detected but not quantifiable.

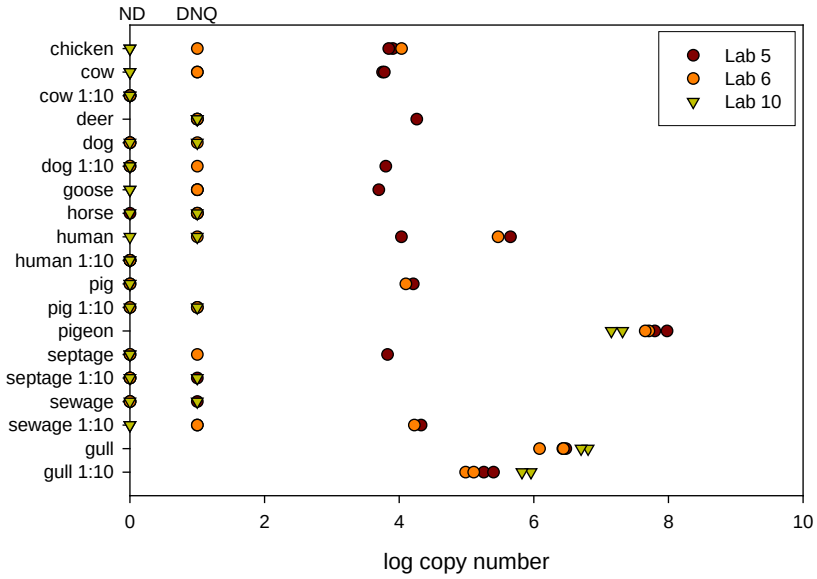

Figure SI-17. Standardized LeeSeaGull assay results from the 38 single-source challenge samples. ND = not detected. DNQ = detected but not quantifiable.

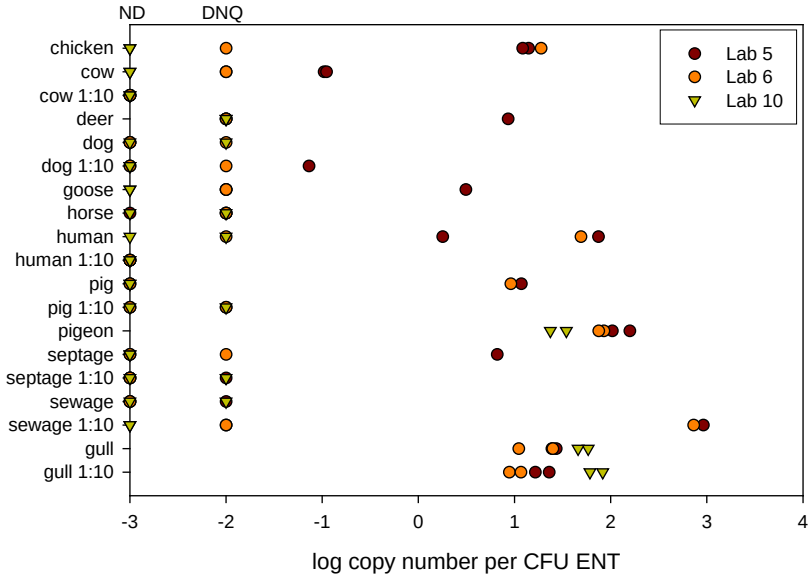

Figure SI-18. Standardized LeeSeaGull assay results from the 38 single-source challenge samples, normalized by CFU ENT measured in the sources. ND = not detected. DNQ = detected but not quantifiable.

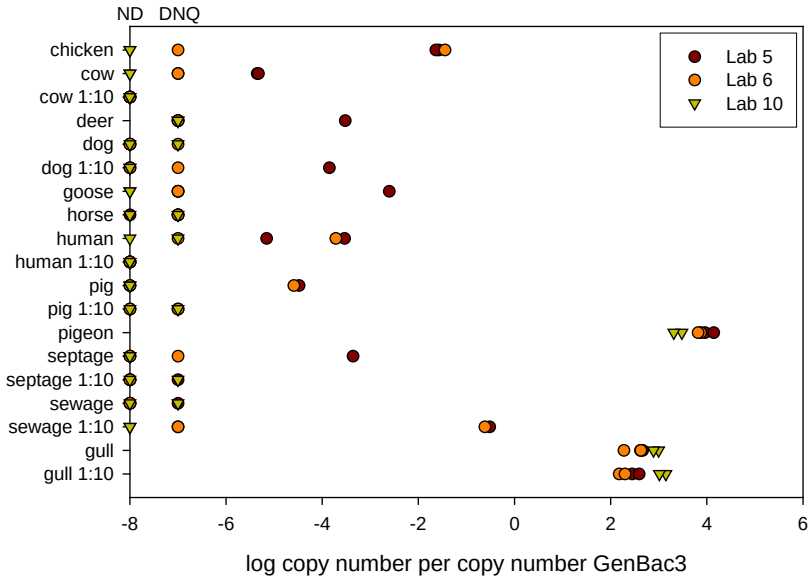

Figure SI-19. Standardized LeeSeaGull assay results from the 38 single-source challenge samples, normalized by GenBac3 copy number measured in the sources. ND = not detected. DNQ = detected but not quantifiable.