

Supplementary

Table S1. Primers and probes for USUV and WNV RT-qPCRs

Name	Specificity	Sequence (5'-3')	5' Reporter	3' quencher
USUV-F ^{α†}	USUV	GAAAAATGTACGCGGATGACAC	FAM	TAMRA
USUV-R ^α		TTTGGCCTCGTTGTCAAGATC		
USUV-P FAM ^α		CGGCTGGGACACCCGGATAACC		
WNV ProC-F10 ^β	WNV	CCTGTGTGAGCTGACAACTTAGT	FAM	TAMRA
WNV ProC-R153 ^β		GCGTTTTAGCATATTGACAGCC		
WNV ProC-Probe ^β		CCTGGTTTCTTAGACATCGAGATCT		

^α Cavrini et al. 2011 [1]

^β Linke et al. 2007 [2]

[†] Modified from reference

Table S2. Primers for gap-closing

Name	Sequence (5'-3')
USUV_34_LEFT ^α	ACCAGGTGAGGGCAATTATTGG
USUV_34_RIGHT ^α	GACGCTTCCAATAAGCAGGGTC
USUV_35_LEFT ^α	CGGACTGGGTTAACAAAGCTGG
USUV_35_RIGHT ^α	TGCCTTGTGGTTGATGTTGGAA

^α Ndione et al. 2024 [3]

Table S3. Table summarizing the data from all USUV positive samples examined

Sample #	Species	Ct†	Collection date	Latitude	Longitude	Lineage	Accession #
H-71	<i>T. merula</i>	21.2	30-08-2024	9.49975045	55.02899493	EU2	PV779323
H-72	<i>T. merula</i>	16.1	02-09-2024	12.05993905	54.92382082	EU2	PV779324
H-73	<i>T. merula</i>	17.6	01-09-2024	11.6705916	56.26445585	EU2	PV779325
H-75	<i>T. merula</i>	14.5	10-09-2024	11.78064612	55.10685595	EU2	PV779326
H-83	<i>T. merula</i>	16.7	12-09-2024	10.19834407	55.3195544	EU3	PV779327
H-84	<i>T. merula</i>	21.7	30-07-2024	9.11204215	55.4784284	-	-
H-85	<i>T. merula</i>	27.4	31-08-2024	9.71079556	54.9528525	-	-
H-86	<i>T. merula</i>	16.5	31-08-2024	9.71079556	54.9528525	AF3	PV779328
H-89	<i>T. merula</i>	14.4	13-09-2024	12.59121712	55.5081121	EU2	PV779329
H-90	<i>T. merula</i>	17.0	15-09-2024	11.49815546	54.76659931	EU2	PV779330
H-91	<i>T. merula</i>	19.4	14-09-2024	11.12804101	55.22799697	EU2	PV779331
H-94	<i>T. merula</i>	15.5	16-09-2024	11.8960716	55.02464842	EU2	PV779332
H-96	<i>T. merula</i>	18.0	17-09-2024	10.10343663	55.27213799	EU2	PV779333
H-97	<i>T. merula</i>	13.6	17-09-2024	11.77357353	55.19504272	EU2	PV779334
H-98	<i>T. merula</i>	16.6	14-09-2024	12.45420474	56.10118532	AF3	PV779335
H-99	<i>T. merula</i>	20.3	17-09-2024	12.05993905	54.92382082	EU2	PV779336
H-100	<i>T. merula</i>	19.5	24-09-2024	11.34912168	55.17717085	AF3	PV779337
H-105	<i>T. merula</i>	13.1	15-09-2024	9.71079556	54.9528525	EU2	PV779338
H-107	<i>T. merula</i>	18.5	17-09-2024	9.64257403	56.36051392	-	-
H-108	<i>T. merula</i>	12.4	21-09-2024	12.59121712	55.5081121	EU2	PV779339
H-109	<i>T. merula</i>	15.3	24-09-2024	11.77357353	55.19504272	EU2	PV779340
H-110	<i>T. merula</i>	22.7	19-09-2024	11.98479974	54.66359732	-	-
H-111	<i>T. merula</i>	18.4	10-09-2024	9.20811159	54.9843364	AF3	PV779341
H-112	<i>T. merula</i>	35.5; 34.9; 36.5	16-09-2024	9.89235588	56.02388208	-	-
H-113	<i>T. merula</i>	20.9	11-09-2024	11.04711131	55.51887316	AF3	PV779342
H-114	<i>T. merula</i>	22.0	23-09-2024	10.21809442	54.94375791	-	-
H-120	<i>T. merula</i>	20.6	29-09-2024	11.04711131	55.51887316	-	-
H-125	<i>T. merula</i>	22.0	21-09-2024	8.33561789	55.85259869	-	-
H-126	<i>T. merula</i>	19.7	18-09-2024	8.22547945	55.7873441	-	-
H-129	<i>T. merula</i>	18.7	22-09-2024	10.02916475	55.45477943	-	-
H-132	<i>T. merula</i>	22.3	11-09-2024	11.98479974	54.66359732	-	-
H-134	<i>T. merula</i>	18.3	15-09-2024	9.44750863	55.35487274	-	-
H-145	<i>T. merula</i>	16.7	13-09-2024	10.48173371	56.30328793	-	-
H-146	<i>T. merula</i>	17.0	14-09-2024	9.46847631	55.50221297	-	-
H-149	<i>T. merula</i>	37.6; 35.6	06-10-2024	12.31112895	55.42088958	-	-
H-153	<i>T. merula</i>	17.4	15-09-2024	9.14165594	55.01708464	-	-
H-155	<i>T. merula</i>	11.5	02-10-2024	10.59802878	55.01443666	-	-
H-156	<i>T. merula</i>	14.3	23-09-2024	8.46174114	55.45935717	-	-
H-158	<i>T. merula</i>	19.1	15-09-2024	10.10343663	55.27213799	-	-
H-161	<i>T. merula</i>	14.7	24-09-2024	9.29089234	55.61664144	-	-

H-163	<i>T. merula</i>	35.3; 31.3	19-09-2024	9.5066267	55.69052913	-	-
H-164	<i>T. merula</i>	14.4	15-09-2024	10.39917484	55.34939755	-	-
H-165	<i>T. merula</i>	16.8	13-09-2024	10.30952407	55.11203154	-	-
H-166	<i>T. merula</i>	20.9	20-09-2024	10.07032627	55.18924968	-	-
H-169	<i>T. merula</i>	18.3	18-09-2024	9.55791076	54.91730187	-	-
H-170	<i>T. merula</i>	18.2	19-09-2024	12.06980339	56.22745639	-	-
H-171	<i>T. merula</i>	18.5	14-09-2024	12.07923445	55.85479562	-	-
H-172	<i>T. merula</i>	19.2	17-09-2024	9.20354055	54.90310802	-	-
H-173	<i>T. merula</i>	13.9	25-09-2024	9.55791076	54.91730187	-	-
H-174	<i>T. merula</i>	16.1	14-10-2024	9.71079556	54.9528525	-	-
H-175	<i>T. merula</i>	13.2	17-09-2024	9.49975045	55.02899493	-	-
H-176	<i>T. merula</i>	33.0; 29.8	22-09-2024	11.37134678	55.40887345	-	-
H-177	<i>T. merula</i>	22.7; 23.7	22-09-2024	9.49975045	55.02899493	-	-
H-181	<i>T. merula</i>	33.3; No Ct; 36.8; 39.3; 36.0	31-08-2024	11.91680034	55.86932225	-	-
H-185	<i>T. merula</i>	16.5	14-09-2024	9.28037618	55.07437317	-	-
H-186	<i>T. merula</i>	14.1	21-09-2024	10.54250568	55.18424637	-	-
H-178	<i>M. milvus</i>	32.7; 38.0; 33.6; 34.7; 34.9 29.4;	20-08-2024	12.06980339	56.22745639	-	-
H-179	<i>D. major</i>	32.3; 32.0; 32.1; 31.3 32.8;	17-09-2024	10.10343663	55.27213799	-	-
H-180	<i>D. major</i>	30.7; 30.6; 30.6; 30.9	19-06-2024	12.05634805	55.97774198	-	-

†Based on USUV RT-qPCR analysis as described by Cavrini et al. Ct values above 40 are considered negative (No Ct)

H = head, EU2 = Europa 2, EU3 = Europa 3, AF3 = Africa 3

References

- [1] Cavrini F, Pepa ME Della, Gaibani P, Pierro AM, Rossini G, Landini MP, et al. A rapid and specific real-time RT-PCR assay to identify Usutu virus in human plasma, serum, and cerebrospinal fluid. *J Clin Virol* 2011;50:221–3.
<https://doi.org/10.1016/j.jcv.2010.11.008>.
- [2] Linke S, Ellerbrok H, Niedrig M, Nitsche A, Pauli G. Detection of West Nile virus lineages 1 and 2 by real-time PCR. *J Virol Methods* 2007;146:355–8.
<https://doi.org/10.1016/j.jviromet.2007.05.021>.
- [3] Ndione MHD, Diagne MM, Mencattelli G, Diallo A, Ndiaye EH, Di Domenico M, et al. An amplicon-based sequencing approach for Usutu virus characterization. *Virol J* 2024;21:163. <https://doi.org/10.1186/s12985-024-02426-7>.