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BRIEF REPORT

TRANSFUSION

Blood donor West Nile virus screening identifies three autochthonous Usutu virus infections in Spain

Teresa Jimenez-Marco[1,2] | **Maria Piron**[3,4,5] | **Gabriela Capllonch-Amer**[1] | **Marta Bes**[3,4,5] | **Jeffrey M. Linnen**[6] | **Kristin Livezey**[6] | **Núria Busquets**[7,8] | **Ana Vázquez**[9,10] | **Laura Herrero**[9,11] | **Mayte Pérez-Olmeda**[9,11] | **Antonia M. Bautista-Gili**[1,2] | **Xavier Ruiz-Nabil**[1] | **Magdalena Prohens-Batle**[1] | **Jaume Giménez**[2,12] | **Silvia Sauleda**[3,4,5]

[1]Fundació Banc de Sang i Teixits de les Illes Balears, Palma de Mallorca, Spain

[2]Grupo de Investigación en Salud Pública de Les Illes Balears (GISPIB), Institut d'Investigació Sanitària Illes Balears (IdISBa), Palma de Mallorca, Spain

[3]Transfusion Safety Laboratory, Banc de Sang i Teixits, Servei Català de la Salut, Barcelona, Spain

[4]Medicina Transfusional, Vall d'Hebron Institut de Recerca (VHIR), Barcelona, Spain

[5]Centro de Investigación Biomédica en Red de Enfermedades Hepaticas y Digestivas (CIBERehd), Instituto de Salud Carlos III, Madrid, Spain

[6]Grifols Diagnostic Solutions Inc., San Diego, California, USA

[7]IRTA, Animal Health, Centre de Recerca en Sanitat Animal (CReSA), Campus de la Universitat Autònoma de Barcelona (UAB), Bellaterra, Catalonia, Spain

[8]Unitat mixta d'investigació IRTA-UAB en Sanitat Animal, Centre de Recerca en Sanitat Animal (CReSA), Campus de la Universitat Autònoma de Barcelona (UAB), Bellaterra, Catalonia, Spain

[9]Laboratorio de Referencia e Investigación en Serología y Arbovirus, Centro Nacional de Microbiología, ISCIII, Madrid, Spain

[10]Centro de Investigación Biomédica en Red de Epidemiología y Salud Pública (CIBERESP), Instituto de Salud Carlos III, Madrid, Spain

[11]Centro de Investigación Biomédica en Red de Enfermedades Infecciosas (CIBERINFEC), Instituto de Salud Carlos III, Madrid, Spain

[12]Servei d'Epidemiologia. Direcció General de Salut Pública de les Illes Balears, Palma de Mallorca, Spain

Correspondence
Teresa Jimenez-Marco, Fundació Banc de Sang i Teixits de les Illes Balears, C/Rosselló i Caçador, 20, 07004 Palma de Mallorca, Balearic Islands, Spain.
Email: tjimenez@fbstib.org; matejimenez@hotmail.com

Abstract

Background: Considering the increase of West Nile virus (WNV) circulation in Europe, blood banks perform WNV Nucleic Acid Test testing to ensure transfusion safety during the WNV transmission season. Usutu virus (USUV), an arbovirus related to WNV, has relevant molecular and serological cross-reactivity with WNV.

Abbreviations: CHIKV, Chikungunya Virus; CI, confidence interval; Ct, cycle threshold; DENV, dengue virus; ELISA, enzyme-linked immunosorbent assay; ID-NAT, individual donor nucleic acid test; JEV, Japanese Encephalitis Virus; NAT, nucleic acid test; NT, neutralization testing; PCR, polymerase chain reaction; qRT-PCR, quantitative reverse transcription polymerase chain reaction; RT-PCR, reverse transcription polymerase chain reaction; S/CO, sample/cut-off; TBEV, tick-borne encephalitis virus; TMA, transcription mediated amplification; USUV, Usutu Virus; WNV, West Nile Virus.

Teresa Jimenez-Marco and Maria Piron contributed equally to this work.

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Study Design and Methods: During the 2024 WNV season in Europe, 15,957 blood donations from the Balearic Islands Blood Bank and 79,400 from the Catalonia Blood Bank were tested for WNV using the Cobas WNV real-time PCR (Roche Diagnostics, USA) and the Procleix WNV/Procleix ArboPlex transcription-mediated assays (Grifols Diagnostic Solutions Inc., USA), respectively. Serological tests for flavivirus, PCR using USUV- and WNV-specific primers, sequencing of viral RNA, and neutralization tests (NT) were performed to confirm positive results.

Results: We identified three donors with USUV infection. In July, the first donor was detected in Majorca (Balearic Islands), whose infection was confirmed by NT. In September, two donors with USUV Africa 3 lineage were identified by RT-PCR and sequencing in Catalonia. There were three USUV-positive cases in 95,357 donations (1 in 31,786; 95% CI: 1 in 10,877 to 1 in 154,083) during the 2024 WNV season in Spain.

Discussion: We report three cases of autochthonous USUV infection in blood donors from the Balearic Islands and Catalonia, two of which were infected by the USUV Africa 3 lineage. Health authorities should be aware that positive WNV screening may be due to USUV, an emerging zoonotic virus that can be underreported.

KEYWORDS

blood donors, Usutu virus, West Nile virus

1 | INTRODUCTION

Usutu virus (USUV) is an emerging arbovirus included as a member of the *Orthoflavivirus* genus in the *Flaviviridae* family.[1] It belongs to the Japanese encephalitis virus (JEV) serocomplex and is genetically linked to JEV and West Nile virus (WNV). Like WNV, USUV has an enzootic cycle involving *Culex* mosquitoes as vectors and birds as amplifying reservoir hosts, with humans and other mammals being dead-end hosts.[2]

USUV was first isolated near the Usutu River in Swaziland in 1959.[3] USUV has gained prominence because of its rapid expansion and its co-circulation with WNV in Europe.[4] This virus was first reported in Austria in 2001 and retrospectively in Italy in 1996,[5] where it was associated with the deaths of wild birds. Since then, USUV has occasionally been isolated in animals (birds and mammals) and mosquitoes in other European countries. USUV-infected donations have been documented during routine screening for WNV in Germany,[6] Austria,[7,8] and Italy.[9]

We report three autochthonous human USUV infections in Spain, detected in blood donors by WNV Nucleic Acid Test (NAT) screening during the 2024 WNV European season.

2 | MATERIALS AND METHODS

2.1 | Population studied and WNV screening NAT

Considering the increased WNV circulation in Europe,[10] many blood banks, like Balearic Islands and Catalonia Blood Banks, use WNV individual donor Nucleic Acid Test (ID-NAT) to ensure transfusion safety during the European WNV season.

During the WNV season in Europe, between July 1 and December 5, 2024, 15,957 blood donations from the Balearic Islands Blood Bank were routinely tested for WNV RNA using the Cobas WNV real-time PCR test (Roche Diagnostics, North America).

Likewise, from July 1 to December 2, 2024, 79,400 donations from the Catalonia Blood Bank were routinely tested on the Procleix Panther System for WNV RNA using the Procleix WNV assay (9769 from July 1, 2024 to August 25, 2024) and the Procleix ArboPlex assay (69,631 from August 26, 2024 to December 2, 2024) (Grifols Diagnostic Solutions Inc., 4560 Horton Street, Emeryville, CA 94608, USA).

All WNV ID-NAT-positive samples from one blood bank were tested in the other.

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Samples for screening, diagnosis, and follow-up were obtained from volunteer healthy blood donors who gave informed consent that their non-transfused blood could be used for research, according to the Ethics Committee of the Balearic Islands and the Ethics Committee of Vall d'Hebron Hospital (Barcelona).

The Clopper-Pearson test was used to calculate the confidence interval of positive cases.

2.2 | Reference laboratory confirmatory techniques

WNV-positive samples from the Balearic Islands Blood Bank were sent to the National Reference Laboratory for Arboviruses in Spain (NRL), of the National Center for Microbiology (NCM-ISCIII). WNV real-time RT-PCR was performed based on the technique previously published.[11] IgM and IgG enzyme immunoassays were performed using the commercial WNV virus IgM/IgG Capture Dx Selec assay from Focus Technologies (Focus Diagnostics, Cypress, CA, USA). Specimens found positive for WNV antibodies were also tested against other flaviviruses. To confirm the specificity of the antibody response, positive sera from the WNV IgG and IgM ELISA tests were assayed by a neutralization test (NT) against WNV and USU viruses, as previously described.[12]

WNV-positive samples from the Catalonia Blood Bank were sent to the Institute of Agrifood Research and Technology-Animal Health Research Center (IRTA-CReSA), where USUV-specific real-time RT-PCR and sequencing of a fragment of the NS5 were performed with the primers and probe from Jöst et al.[13] and Scaramozino et al.[14] respectively. WNV-positive samples from the Catalonia Blood Bank were also sent to the Bernhard Nocht Institute for Tropical Medicine in Hamburg, Germany, for next-generation sequencing (NGS) of complete viral genomes.

Multiple alignments were performed using Basic Local Alignment Search Tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Phylogenetic analyses were performed based on the complete USUV genome using the maximum likelihood method. Trees were obtained automatically by applying the Neighbor-Joining algorithms to a pairwise distance matrix estimated using the maximum composite likelihood approach.

3 | RESULTS

From July 1 to December 5, 2024, 15,957 blood donations from the Balearic Islands Blood Bank were screened for WNV using ID-NAT testing. On July 20, 2024, a 45-year-old male donor (Donor 1) living in Majorca was repeatedly reactive for WNV PCR (Ct:30.49; Ct:30.40; Ct:30.40). He had not traveled outside Majorca in the previous 28 days and reported mosquito bites during outdoor activities. The sample was also repeatedly reactive for WNV in transcription-mediated amplification (TMA) Procleix ArboPlex Assay (S/CO 4.34; S/CO 4.47) in the Catalonia Blood Bank.

Subsequent analysis showed positive serological results for WNV (IgM and IgG), DENV (IgM and IgG) and TBEV (IgG) at different points in the follow-up. Low avidity WNV IgG antibodies indicated recent infection. During the follow-up period (September 7, August 18, August 26, and September 23, 2024), WNV real-time RT-PCR and qRT-PCR-USUV were negative, and NTs were also negative for WNV lineages 1 and 2, but positive for USUV in all four follow-up samples, which confirmed the USUV infection (Table S1).

From July 1 to December 2, 2024, 79,400 donations from the Catalonia Blood Bank were screened for WNV using ID-NAT testing. On September 3, a 67-year-old male donor from Tona (Barcelona) who had not traveled outside Catalonia in the last 28 days and had reported mosquito bites prior to donation was repeatedly reactive for WNV RNA in a Procleix ArboPlex assay (S/CO 4.33, Donor 2) up to 1000-fold dilution. The donation was also tested reactive in Cobas WNV PCR (Ct: 28.9) in the Balearic Islands Blood Bank.

NGS capture sequencing performed in Bernhard Nocht Institute identified USUV sequences (GenBank accession number: PV207916, BankIt2926864 USUTU_virus_human_isolate_Spain).

On September 14, a 58-year-old female donor living in Tarragona was repeatedly reactive for WNV in Procleix ArboPlex assay (S/CO 3.94, Donor 3) up to a 100-fold dilution. The donation was also tested reactive in Cobas WNV PCR (Ct: 31.3) in the Balearic Islands Blood Bank. When contacted for the follow-up, she reported mosquito bites and arthritis, nausea, and vomiting without fever 2 days after donation. She had not traveled outside Catalonia in the previous 28 days. Specific USUV RT-PCR and sequencing performed in IRTA-CReSA laboratory confirmed USUV sequences. NGS capture sequencing performed in Bernhard Nocht Institute confirmed USUV sequences. Phylogenetic analysis was performed on Donors 2 and 3 USUV positive index blood donations and revealed USUV Africa 3 lineage (Figure 1).

Three USUV-positive cases were found in 95,357 donations (1 in 31,786; 95% CI: 1 in 10,877 to 1 in 154,083) in the Balearic Islands and Catalonia using WNV ID-NAT during the 2024 European WNV transmission season. No autochthonous WNV human cases were detected in either region that year.

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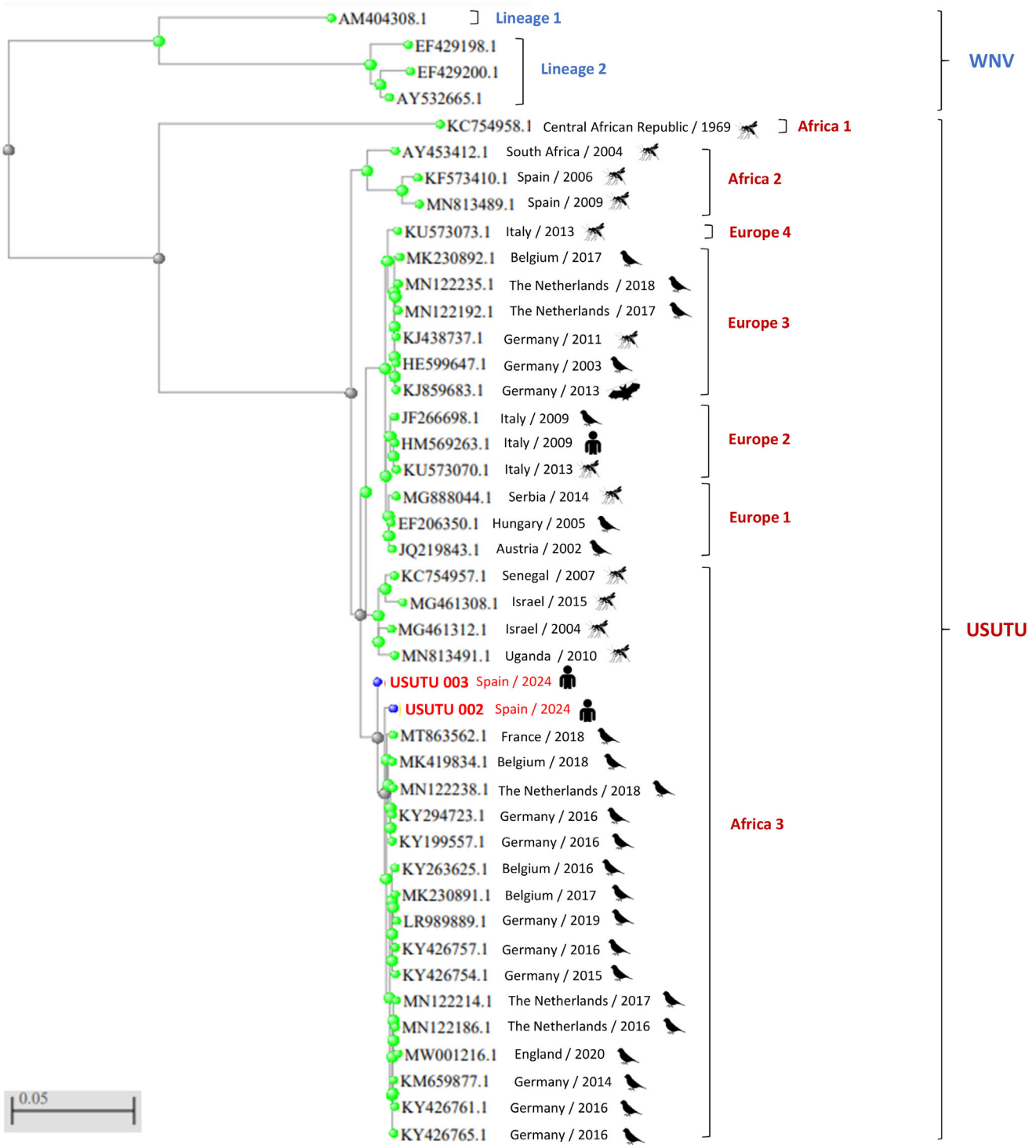

FIGURE 1 Phylogenetic analysis. Sequences from Donors 2 and 3 were obtained by next generation sequencing (USUTU 002 with 100% coverage and USUTU 003 with 30% coverage, respectively) and were aligned with representative West Nile virus (WNV) and Usutu virus (USUV) sequences obtained from GenBank. Sequences obtained in this study are highlighted in red. Taxon information indicated in the branches includes the country of origin, isolation/detection year, host, and GenBank accession number. USUV and WNV genetic sub-lineages are indicated on the right.

4 | DISCUSSION

We report three cases of autochthonous USUV infections in the Balearic Islands and Catalonia identified following WNV blood donation screening. The USUV Africa 3 lineage was identified in two of the three donors.

WNV NAT assays for blood donation screening have previously been shown to cross-react with USUV (Cobas

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WNV real-time PCR from Roche Diagnostics,[6–9] and Procleix WNV Assays from Grifols Diagnostic Solutions Inc.[15]). Both Procleix WNV and ArboPlex Assays package inserts indicate a possible cross-reaction of USUV detection with WNV with unstated sensitivity. This cross-reactivity must be considered as an advantage as it helps detect USUV infections among blood donors. The three cases have shown positive/reactive results with both Cobas WNV real-time RT-PCR and Procleix ArboPlex TMA.

Detection of USUV-infected donations during routine screening for WNV has been previously described in Germany,[6] Austria,[7,8] and Italy.[9] Specifically, the USUV Europe 3 lineage was identified in a blood donor in Germany in 2016.[6] In 2018, the USUV Europe 2 lineage was identified in blood donors in Italy[9] and Austria.[8] Additionally, two donors in Austria were found to be infected with the USUV Africa 3 lineage in 2018.[8] These donations initially tested positive in the Cobas WNV NAT screening test, indicating cross-reactivity with the USUV European 2, 3, and African 3 lineages. Discrimination between WNV and USUV is possible by using USUV and WNV-specific PCR primers and viral RNA sequencing.[16]

Furthermore, there are other molecular biology techniques that can assist in distinguishing between different arboviruses. Duplex PCR[17] and pan-flavivirus PCR[18] are both molecular diagnostic techniques used to detect flaviviruses. These techniques are not yet commercially available for automated arbovirus screening in blood donors. However, they have the potential to serve as a differential diagnosis when there is cross-reactivity between closely related arboviruses.

Ideally, WNV NAT assay suppliers should be encouraged to offer discriminatory assays that would specifically identify the virus detected in the blood donors' "WNV" screening.

Additionally, it is known that flavivirus infections induce cross-reactive antibodies.[19] Antibodies against non-DENV flaviviruses show cross-reactivity with DENV between 15.4% and 84%.[20] It has also been demonstrated that 6% of antibodies produced against DENV and 7% produced against non-DENV flaviviruses lead to false-positive results with Chikungunya virus (CHIKV).[21] Cross-reactivity in antibody assays for flavivirus can be resolved using comparative antibody neutralization testing.[12]

The case of Donor 1 illustrates this serological cross-reactivity. Despite testing positive for WNV (IgM and IgG), DENV (IgG), and TBEV (IgG) antibodies, USUV infection was confirmed by the USUV neutralization testing.

USUV is closely related to WNV. Both share similar pathogenesis, epidemiology, and antigenicity.[1,2] Originally these viruses circulated in Africa, before their expansion to Europe through wild bird migrations coming from Africa.[22]

Studies of full-length sequences and other genes have investigated the genetic variability of USUV. These analyses cluster USUV sequences into various lineages, named according to their geographic origin: Africa 1–3 and Europe 1–5.[23]

The presence of different USUV lineages in both Spain and Europe indicates different routes of introduction from Africa and a significant expansion across the continent.[23]

While the number of reported human cases of USUV infection in Africa is small, few seroprevalence studies in both patients and donors show higher numbers. A study of patients with fever in Nigeria found a USUV seroprevalence of 64%. In a seroprevalence study of 501 blood donors in Burkina Faso, 71 were positive for USUV-specific antibodies (14.17%; 95% CI: 11.39–17.49), suggesting significant human circulation of USUV in this country.[24] In contrast, several European studies have reported relatively low seroprevalences of USUV in blood donors in Italy (1% and 0.23%), Germany (<1%) and Hungary (<1%).[25]

Following the initial documented cases of human neuroinvasive USUV infection in 2009, over 100 clinical cases of USUV infection have been reported in European countries.[2] These cases include both USUV fever and neuroinvasive disease, which are often associated with immunocompromised patients. USUV has also been detected in asymptomatic blood donors.[2] As USUV can affect immunocompromised patients, who are more likely to be transfused, and USUV infection has been found in asymptomatic blood donors, the risk of USUV transmission through transfusion should be considered.

In conclusion, we have identified three blood donors with acute autochthonous USUV infections in Spain. Health authorities should be aware that positive WNV screening results in donors may be due to USUV, an emerging zoonotic virus that can be underreported.

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CONFLICT OF INTEREST STATEMENT

The following authors have disclosed no conflicts of interest: TJ-M, GC-A, NB, AV, LH, MP-O, AMB-G, XR-N, MP-B, JG. The following authors have conflicts of interest: Banc de Sang i Teixits of the Servei Català de la Salut in Barcelona has received financial support from Grifols for various research projects. MP, MB, and SS work in the Transfusion Safety Laboratory of the Banc de Sang i Teixits, Servei Català de la Salut in Barcelona. JML and KL are employees of Grifols Diagnostic Solutions Inc.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

ORCID

Teresa Jimenez-Marco https://orcid.org/0000-0001-9631-6541
Maria Piron https://orcid.org/0000-0001-6858-9374
Gabriela Capllonch-Amer https://orcid.org/0009-0008-3978-9121
Jeffrey M. Linnen https://orcid.org/0000-0002-2580-4289
Núria Busquets https://orcid.org/0000-0001-5246-8260
Mayte Pérez-Olmeda https://orcid.org/0000-0003-4553-5423
Silvia Sauleda https://orcid.org/0000-0001-7343-9557

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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