



Flash survey on SARS-CoV-2 variants in urban wastewater in Italy
5th Report
(Study period: 10 - 14 January 2022)

Edited by:

- Giuseppina La Rosa, Giusy Bonanno Ferraro, Pamela Mancini, Carolina Veneri, Marcello Iaconelli, Luca Lucentini, Lucia Bonadonna (Department of Environment and Health, Istituto Superiore di Sanità)
- David Brandtner (independent researcher)
- Mauro Grigioni (National Center for Innovative Technologies in Public Health, Istituto Superiore di Sanità)
- Mirko Rossi (independent researcher)
- Elisabetta Suffredini (Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità)

Main findings:

- Fifth national flash survey on SARS-CoV-2 variants in wastewater samples collected in Italy in the week of 10-14 January 2022;
- Overall, 154 wastewater samples were collected from 17 Regions and 2 Autonomous Provinces (AA.PP.);
- Characteristic mutations of the Omicron variant were detected in all the 17 Regions/AA.PP. for which sequencing data were obtained;
- The vast majority of the detected mutations belonged to sublineage BA.1, associated with genetic variability; amino acid substitutions of sublineage BA.2 were also detected in the A.P. of Bolzano, North Italy;
- Characteristic mutations of the Delta variant were detected In three Regions/AA.PP. (Lazio, Valle d'Aosta, and A.P Bolzano).

Introduction

On 17 March 2021, the “EU Commission Recommendation 2021/472 on a common approach to establish a systematic surveillance of SARS-CoV-2 and its variants in wastewaters in the EU”, strongly encouraged the Member States to put in place, no later than the 1st of October 2021, national wastewater surveillance systems aimed at the collection of data on SARS-CoV-2 and its variants¹.

Indeed, several studies have demonstrated the value of environmental SARS-CoV-2 sequencing as a tool to identify strains circulating in the community and study SARS-CoV-2 diversity².

Recently, characteristic mutations of the variants of concern (VoCs) were detected in sewage samples collected in Italy^{3,4}. Following the above EU Recommendation, the Istituto Superiore di Sanità (ISS) instituted “flash surveys”, i.e. periodic (monthly) sampling campaigns to be held in different locations in Italy over the course of a brief period, aimed at assessing the diversity of SARS-CoV-2 in wastewater in the country.

Aim

1 Commission Recommendation (EU) 2021/472 of 17 March 2021 on a common approach to establish a systematic surveillance of SARS-CoV-2 and its variants in wastewaters in the EU. (<https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32021H0472&qid=1628798981209>)

2 Bonanno Ferraro G, Veneri C, Mancini P, Iaconelli M, Suffredini E, Bonadonna L, Lucentini L, Bowo-Ngandji A, Kengne-Nde C, Mbaga DS, Mahamat G, Tazokong HR, Ebogo-Belobo JT, Njouom R, Kenmoe S, La Rosa G. A State-of-the-Art Scoping Review on SARS-CoV-2 in Sewage Focusing on the Potential of Wastewater Surveillance for the Monitoring of the COVID-19 Pandemic. *Food Environ Virol.* 2021 Nov 2:1–40. doi: 10.1007/s12560-021-09498-6. Epub ahead of print. PMID: 34727334; PMCID: PMC8561373.

3 La Rosa G, Mancini P, Bonanno Ferraro G, Veneri C, Iaconelli M, Lucentini L, Bonadonna L, Brusaferraro S, Brandtner D, Fasanello A, Pace L, Parisi A, Galante D, Suffredini E. Rapid screening for SARS-CoV-2 variants of concern in clinical and environmental samples using nested RT-PCR assays targeting key mutations of the spike protein. *Water Res.* 2021 Jun 1;197:117104. doi: 10.1016/j.watres.2021.117104.

4 La Rosa, G.; Brandtner, D.; Mancini, P.; Veneri, C.; Bonanno Ferraro, G.; Bonadonna, L.; Lucentini, L.; Suffredini, E. Key SARS-CoV-2 Mutations of Alpha, Gamma, and Eta Variants Detected in Urban Wastewaters in Italy by Long-Read Amplicon Sequencing Based on Nanopore Technology. *Water* 2021, *13*, 2503. <https://doi.org/10.3390/w13182503>

This report aims to summarize the results of the fifth national flash survey on SARS-CoV-2 variants in wastewater samples collected in Italy in the week between 10 and 14 January of 2022.

Methodology

The survey included sewage samples collected at wastewater treatment plants (WTPs) located in 17 regions and 2 autonomous provinces (AA.PP.):

- North-West Italy: Liguria, Lombardia, Piemonte and Valle d'Aosta;
- North-East Italy: Emilia-Romagna, Veneto, A.P. of Bolzano and A.P. of Trento, Friuli-Venezia Giulia;
- Central Italy: Abruzzo, Lazio, Marche, Toscana and Umbria;
- Southern Italy and Islands: Campania, Basilicata, Molise, Puglia and Sicilia.

Overall, 154 wastewater samples were collected between 10 and 14 January 2022 (**Table 1**).

Table 1. Sampling sites and characteristics of the WTPs studied

Sample ID ISS	Region/A. P.	City	Sampling Date	WTP	Population equivalent
SARI6778	Abruzzo	Pescara	11/01/2022	Villa Carmine	140.000
SARI6779	Abruzzo	Pescara	11/01/2022	Via Raiale	160.000
SARI6780	Abruzzo	Chieti	11/01/2022	S. Martino	114.500
SARI6781	Abruzzo	L'Aquila	11/01/2022	Pile	48.000
SARI6782	Abruzzo	Teramo	11/01/2022	Villa Pavone	41.824
SARI6826	Basilicata	Potenza	11/01/2022	Tiera di Vaglio	95.000
SARI6827	Basilicata	Matera	11/01/2022	Pantano	24.000
SARI6786	Campania	Salerno	11/01/2022	Salerno	700.000
SARI6787	Campania	Salerno	11/01/2022	Nocera Sup	299.121
SARI6788	Campania	Napoli	11/01/2022	Area Nolana	400.000
SARI6789	Campania	Napoli	11/01/2022	Napoli EST	1.750.000
SARI6790	Campania	Napoli	11/01/2022	Napoli OVEST - Ingresso Principale	950.000
SARI6791	Campania	Napoli	11/01/2022	Napoli OVEST - ex ingresso Camaldoli	250.000
SARI6792	Campania	Caserta	11/01/2022	Area Casertana	370.769
SARI6793	Campania	Caserta	11/01/2022	Villa Literno	631.714
SARI6909	Campania	Napoli	13/01/2022	Area Nolana	400.000
SARI6910	Campania	Caserta	13/01/2022	Area Casertana	370.769
SARI6911	Campania	Salerno	13/01/2022	Nocera Sup	299.121
SARI6912	Campania	Napoli	13/01/2022	Napoli EST	1.750.000
SARI6913	Campania	Napoli	13/01/2022	Napoli OVEST - Ingresso Principale	950.000
SARI6914	Campania	Napoli	13/01/2022	Napoli OVEST - ex ingresso Camaldoli	250.000
SARI6915	Campania	Avellino	13/01/2022	Manocalzati	140.000
SARI6916	Campania	Salerno	13/01/2022	Salerno	700.000
SARI6917	Campania	Caserta	13/01/2022	Villa Literno	631.714
SARI6714	Emilia-Romagna	Ferrara	11/01/2022	Ferrara - Linea 1	120.000
SARI6715	Emilia-Romagna	Ferrara	11/01/2022	Ferrara - Linea 2	120.000
SARI6716	Emilia-Romagna	Modena	11/01/2022	Carpi	200.000

SARI6750	Emilia-Romagna	Bologna	10/01/2022	IDAR	800.000
SARI6751	Emilia-Romagna	Modena	10/01/2022	Naviglio	500.000
SARI6752	Emilia-Romagna	Ravenna - Forlì-Cesena	10/01/2022	Ravenna	240.000
SARI6753	Emilia-Romagna	Forlì-Cesena	11/01/2022	Cesena	197.500
SARI6754	Emilia-Romagna	Forlì-Cesena	11/01/2022	Forlì	250.000
SARI6755	Emilia-Romagna	Ravenna	11/01/2022	Faenza	100.000
SARI6756	Emilia-Romagna	Bologna	11/01/2022	Imola	75.000
SARI6802	Emilia-Romagna	Piacenza	11/01/2022	Borgoforte	163.333
SARI6805	Emilia-Romagna	Parma	12/01/2022	Parma ovest	168.000
SARI6808	Emilia-Romagna	Reggio Emilia	12/01/2022	Mancasale	280.000
SARI6847	Emilia-Romagna	Bologna	12/01/2022	IDAR	800.000
SARI6848	Emilia-Romagna	Modena	12/01/2022	Naviglio	500.000
SARI6849	Emilia-Romagna	Ravenna - Forlì-Cesena	12/01/2022	Ravenna	240.000
SARI6850	Emilia-Romagna	Rimini - Forlì-Cesena	12/01/2022	S. Giustina	560.000
SARI6931	Friuli-Venezia Giulia	Udine	11/01/2022	Udine	200.000
SARI6932	Friuli-Venezia Giulia	Pordenone	11/01/2022	Cordenons	15.000
SARI6933	Friuli-Venezia Giulia	Trieste	12/01/2022	Servola	190.000
SARI6666	Lazio	Viterbo	10/01/2022	Viterbo - Strada Bagni	30.000
SARI6667	Lazio	Roma	10/01/2022	Velletri (LA CHIUSA-SORBO)	36.700
SARI6668	Lazio	Roma	10/01/2022	Via Cincinnato	60.000
SARI6669	Lazio	Latina	10/01/2022	Latina Loc Latina Est	90.000
SARI6670	Lazio	Roma	10/01/2022	Anzio - Colle Cocchino	75.000
SARI6671	Lazio	Latina	10/01/2022	Aprilia (Via del Campo)	66.000
SARI6672	Lazio	Roma	10/01/2022	Ponte Lucano	50.000
SARI6721	Lazio	Roma	13/01/2022	Civitavecchia Fiumaretta	86.400
SARI6812	Lazio	Roma	11/01/2022	Roma Est (linea 1 + linea 2)	900.000
SARI6813	Lazio	Roma	11/01/2022	Roma Nord	780.000
SARI6814	Lazio	Roma	12/01/2022	Roma Sud	1.100.000
SARI6815	Lazio	Roma	12/01/2022	Ostia	350.000
SARI6816	Lazio	Roma	12/01/2022	Fregene	76.000
SARI5214	Liguria	Genova	11/01/2022	Pegli	20.507
SARI5215	Liguria	Genova	11/01/2022	Voltri	40.496
SARI6757	Liguria	Genova	11/01/2022	Quinto	48.748
SARI6758	Liguria	Genova	11/01/2022	Rapallo	90.000
SARI6759	Liguria	Genova	11/01/2022	Sestri P	51.368
SARI6760	Liguria	Imperia	11/01/2022	Sanremo - località Capo Verde	80.000
SARI6761	Liguria	Savona	11/01/2022	Borghetto Santo Spirito	140.000
SARI6762	Liguria	La Spezia	11/01/2022	La Spezia	82.000
SARI6763	Liguria	La Spezia	11/01/2022	Camisano	40.840
SARI6764	Liguria	La Spezia	11/01/2022	Silea	17.500
SARI6765	Liguria	Genova	12/01/2022	Darsena	118.276
SARI6766	Liguria	Genova	12/01/2022	Punta Vagno Genova	75.000
SARI6767	Liguria	Genova	12/01/2022	Valpolcevera	157.650

SARI6794	Liguria	Genova	11/01/2022	Punta Vagno Genova	75.000
SARI6825	Liguria	Genova	11/01/2022	Sturla	43.573
SARI6776	Lombardia	Milano - Varese	12/01/2022	Canegrate	137.950
SARI6777	Lombardia	Varese	12/01/2022	Varese	74.402
SARI6798	Lombardia	Brescia	11/01/2022	Verziano	296.000
SARI6799	Lombardia	Brescia	12/01/2022	Verziano	296.000
SARI6821	Lombardia	Como - Lecco - Milano - Monza e della Brianza	10/01/2022	Monza	600.000
SARI6822	Lombardia	Como - Lecco - Milano - Monza e della Brianza	12/01/2022	Monza	600.000
SARI6954	Lombardia	Milano	10/01/2022	Milano Nosedo	1.250.000
SARI6955	Lombardia	Milano	12/01/2022	Milano Nosedo	1.250.000
SARI6956	Lombardia	Milano	10/01/2022	Milano San Rocco	1.036.000
SARI6957	Lombardia	Milano	12/01/2022	Milano San Rocco	1.036.000
SARI6834	Marche	Pesaro-Urbino	11/01/2022	Borgheria	116.000
SARI6835	Marche	Pesaro-Urbino	11/01/2022	Ponte Metauro	60.000
SARI6836	Marche	Pesaro-Urbino	11/01/2022	Ponte Sasso	18.000
SARI6837	Marche	Ancona	11/01/2022	Zipa	100.000
SARI6838	Marche	Ancona	11/01/2022	Falconara	85.000
SARI6839	Marche	Ancona	11/01/2022	Camerano	33.000
SARI6784	Molise	Campobasso	11/01/2022	Termoli - località Pantano Basso	25.000
SARI6785	Molise	Campobasso	11/01/2022	Campobasso - San Pietro	50.000
SARI6771	P.A. Bolzano	Bolzano	10/01/2022	IDA Bolzano	372.410
SARI6772	P.A. Bolzano	Bolzano	10/01/2022	IDA Merano	356.520
SARI6921	P.A. Bolzano	Bolzano	13/01/2022	IDA Bolzano	372.410
SARI6922	P.A. Bolzano	Bolzano	13/01/2022	IDA Merano	356.520
SARI6935	P.A. Bolzano	Bolzano	13/01/2022	IDA Termeno	68.945
SARI6676	P.A. Trento	Trento	10/01/2022	Trento nord	120.000
SARI6679	P.A. Trento	Trento	10/01/2022	Trento sud	100.000
SARI6680	P.A. Trento	Trento	10/01/2022	Rovereto	95.000
SARI6643	Piemonte	Biella	10/01/2022	Biella Nord	67.000
SARI6644	Piemonte	Biella	10/01/2022	Biella Sud	53.000
SARI6645	Piemonte	Novara	10/01/2022	Novara	184.000
SARI6697	Piemonte	Torino	12/01/2022	Castiglione Torinese	1.934.099
SARI6698	Piemonte	Alessandria	12/01/2022	Alessandria	110.000
SARI6699	Piemonte	Asti	12/01/2022	Asti	95.000
SARI6700	Piemonte	Cuneo	12/01/2022	Cuneo	185.000
SARI6852	Puglia	Lecce	10/01/2022	Lecce	195.368
SARI6853	Puglia	Brindisi	10/01/2022	Brindisi Fiume Grande	129.156
SARI6854	Puglia	Taranto	10/01/2022	Taranto Gennarini	252.267
SARI6855	Puglia	Taranto	10/01/2022	Taranto Bellavista	65.561
SARI6856	Puglia	Foggia	11/01/2022	Cerignola	83.295
SARI6857	Puglia	Foggia	11/01/2022	Foggia	206.074
SARI6858	Puglia	Bari	12/01/2022	Bari Est	389.000
SARI6859	Puglia	Bari	12/01/2022	Bari Ovest	360.000

SARI6860	Puglia	Bari	12/01/2022	Bitonto	79.332
SARI6861	Puglia	Bari	12/01/2022	Molfetta	84.803
SARI6862	Puglia	Barletta-Andria-Trani	13/01/2022	Barletta	129.356
SARI6863	Puglia	Barletta-Andria-Trani	13/01/2022	Andria	149.050
SARI6864	Puglia	Barletta-Andria-Trani	13/01/2022	Trani	83.667
SARI6865	Puglia	Barletta-Andria-Trani	13/01/2022	Bisceglie	85.714
SARI6866	Puglia	Taranto	13/01/2022	Taranto Bellavista	65.561
SARI6867	Puglia	Taranto	13/01/2022	Taranto Gennarini	252.267
SARI6868	Puglia	Bari	14/01/2022	Altamura	95.414
SARI6688	Sicilia	Ragusa	11/01/2022	Ragusa	98.000
SARI6689	Sicilia	Ragusa	11/01/2022	Modica	50.400
SARI6690	Sicilia	Ragusa	11/01/2022	Vittoria	55.000
SARI6692	Sicilia	Agrigento	10/01/2022	Agrigento	55.000
SARI6693	Sicilia	Palermo	11/01/2022	Acqua dei Corsari	314.973
SARI6694	Sicilia	Palermo	11/01/2022	Fondo Verde	53.886
SARI6695	Sicilia	Palermo	11/01/2022	Bagheria	75.000
SARI6746	Sicilia	Messina	13/01/2022	Mili Marina	227.000
SARI6823	Sicilia	Caltanissetta	13/01/2022	Caltanissetta e San Cataldo	76.700
SARI6824	Sicilia	Caltanissetta	13/01/2022	Gela Macchitella	12.000
SARI6880	Sicilia	Catania	12/01/2022	Pantano d'Archi	68.434
SARI6809	Toscana	Massa	12/01/2022	Lavello 2	120.000
SARI6810	Toscana	Lucca	12/01/2022	Viareggio	93.000
SARI6811	Toscana	Massa	12/01/2022	Lavello 1	87.000
SARI6634	Umbria	Perugia	10/01/2022	Perugia - Pian della Genna	90.000
SARI6717	Umbria	Perugia	13/01/2022	Perugia - Pian della Genna	90.000
SARI6718	Umbria	Perugia	13/01/2022	Foligno Casone	90.000
SARI6719	Umbria	Terni	13/01/2022	Terni	150.000
SARI6748	Valle d'Aosta	Aosta	12/01/2022	La Salle	60.000
SARI6749	Valle d'Aosta	Aosta	12/01/2022	Brissogne	150.000
SARI6683	Valle d'Aosta	Aosta	09/01/2022	La Salle	79.474
SARI6684	Valle d'Aosta	Aosta	09/01/2022	Brissogne	61.741
SARI6647	Veneto	Padova	11/01/2022	Padova Ca' Nordio - centro storico	98.500
SARI6648	Veneto	Padova	11/01/2022	Padova Ca' Nordio - zip	98.500
SARI6649	Veneto	Padova	11/01/2022	Padova Guizza	13.000
SARI6650	Veneto	Padova	11/01/2022	Abano Terme	35.000
SARI6685	Veneto	Treviso	11/01/2022	Treviso	70.000
SARI6686	Veneto	Vicenza	11/01/2022	Vicenza Casale	92.000
SARI6687	Veneto	Venezia	11/01/2022	Venezia Fusina	400.000
SARI6725	Veneto	Verona	13/01/2022	Verona_collettore 1M	82.000
SARI6726	Veneto	Verona	13/01/2022	Verona_collettore 3M	102.000
SARI6727	Veneto	Verona	13/01/2022	Verona_collettore 8M	226.000

‡ This parameter describes the design treatment capacity of WTPs. It is a measure of total organic biodegradable load in a WTP, including industrial, commercial and domestic organic load, converted to the equivalent number of population (population equivalents)

Samples were processed by the laboratories of the SARI network (see Acknowledgements). Viral concentration measurements and nucleic acid extraction were performed according to the protocol “Sorveglianza di SARS-CoV-2 in reflui urbani - Protocollo progetto SARI - rev.3”. The purified RNAs were shipped dry ice to ISS, where samples were sequenced as previously described⁵, with minor modifications.

Concerning amplicon sequencing, a long nested RT-PCR assay (ID_980, ~1600 bps, spanning amino acid residues 58 to 573 of the spike protein)⁵ was used to detect multiple key nucleotide changes resulting in protein mutations (deletion and/or amino acid substitutions) distinctive of the major known circulating SARS-CoV-2 variants, including the Variants of Concern (VoCs) and Variants of Interest (Vols). To increase the probability of amplification and characterization, one additional short nested RT-PCR (unpublished), designed within the long fragment, was also used to test negative samples by the long PCR. This short nested PCR is designated as ID_987/994 and generates PCR amplicons of 478 to 495 bp depending on amplified variant.

The amplicons from the long nested assay were sequenced by both Sanger and Next Generation Sequencing (NGS), using the Oxford Nanopore Technology MinION platform, for a more in-depth analysis. The amplicons obtained from different samples collected in the same Region were mixed in a single pool for NGS. The positive PCR products generated by the short nested PCR assay underwent only conventional Sanger sequencing.

Bioinformatics analysis of NGS data was carried out as described in La Rosa et al., 2021⁶. Variant calling was performed for currently recognized VoCs (Beta, Gamma, Delta and Omicron) and Vols (Mu and Lambda). The presence of amino acid substitutions associated with deescalated VoCs (i.e. Alpha) was also investigated. As regard to the emerging variant lineage B.1.1.529 (Omicron variant), the region amplified by the long PCR ID_980, covers several amino acid substitutions compared to the Wuhan SARS-CoV-2 strain: A67V, Δ69-70, T95I, G142D, Δ143-145, Δ211, L212I, G339D, S371L, S373P, S375F, S477N, T478K, E484A, Q493K, G496S, Q498R, N501Y, Y505H, T547K (mutations with > 75% prevalence in the lineage; <https://outbreak.info/compare-lineages?pango=Omicron&gene=S&threshold=75&nthresh=1&sub=false&dark=true>); moreover, most Omicron sequences also contain a 3 amino acid insertion (EPE) at position 214 in the Spike protein. Therefore, the variant can be easily identified by amplicon sequencing of this long PCR fragment. The search strategy was implemented in order to detect Omicron sublineages BA.1 and BA.2.

Results

5 G La Rosa, P. Mancini, G. Bonanno Ferraro, C. Veneri, M. Iaconelli, L. Lucentini, L. Bonadonna, S. Brusaferro, D. Brandtner, A. Fasanella, L. Pace, A. Parisi, D. Galante, E. Suffredini. Rapid screening for SARS-CoV-2 variants of concern in clinical and environmental samples using nested RT-PCR assays targeting key mutations of the spike protein, *Water Research*, 2021, Volume 197, 1 June 2021, 117104. <https://doi.org/10.1016/j.watres.2021.117104>.

6 G La Rosa, D. Brandtner, P. Mancini, C. Veneri, G. Bonanno Ferraro, L. Bonadonna, L. Lucentini, E. Suffredini. Key SARS-CoV-2 mutations of Alpha, Gamma and Eta variants detected in urban wastewaters in Italy by long-read amplicon sequencing based on nanopore technology. *Water* 2021, 13(18), 2503; <https://doi.org/10.3390/w13182503>

The data on viral concentration were produced by the SARI network laboratories. Overall, 142 of the 154 samples (92%) tested positive for SARS-CoV-2 by the real-time RT-qPCR adopted for SARS-CoV-2 surveillance (**Table 2**), with viral concentrations ranging from 3.7 E+00 to 2.1 E+07 genome copies (g.c.)/L of sewage. Overall, 121 samples were successfully amplified by the long (N°= 75) or the short (N°= 46) PCR assay. Real-time PCR, nested PCR, and sequencing results are summarized in **Table 2**.

Sanger Sequencing

Sanger sequences were obtained for 107 samples, whereas – due to the simultaneous presence of more than one strain – mixed electropherograms were obtained for 14 samples, which could not therefore be assigned. Characteristic mutations of the Omicron variant were detected in 99 samples. These samples were collected in 17 Regions/AA.PP. (Basilicata, Campania, Emilia-Romagna, Friuli-Venezia Giulia, Lazio, Liguria, Lombardia, Marche, Molise, Piemonte, Puglia, Sicilia, Umbria, Valle d’Aosta, Veneto, A.P. Trento and A.P. Bolzano). With regard to the remaining two Regions (Abruzzo and Toscana) only mixed electropherograms were obtained and, therefore, no readable sequences were available. Amino acid substitutions of sublineage BA.2 were detected in the A.P. of Bolzano, North Italy. Only two Regions/AA.PP. (Lazio and A.P. Bolzano) showed key mutations of the Delta variant by Sanger sequencing.

Next Generation Sequencing

NGS results could be successfully gained for 15 Regions (all except for Abruzzo, Marche, Molise and Toscana). Characteristic mutations of the Omicron variant were detected in all the NGS pools.

Overall, 14 different mutation combinations were detected suggesting a considerable genetic variability within the variant, the vast majority belonging to sublineage BA.1, detected in 15 Regions/AA.PP. Samples collected in the A.P. of Bolzano also showed characteristic mutations of Omicron sublineage BA.2, such as “V213G, T376A, D405N, R408S” which are present in more than 95% of this (and in less than 1% in the other Omicron sublineages). Overall, more than 40 amino acid mutations were detected by NGS in association with the Omicron variant in the ~1600 bps fragment amplified by the long PCR: 32 amino acid substitutions (N61K, A67V, T95I, G142D, L212I, V213G, V227L, S256L, G339D, R346K, S359T, S371F, S371L, S373P, S375F, T376A, D405N, R408G, R408S, G416R, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K), 4 silent mutations (A163A, V395V, N439N, I410I), 6 deletions (H69del, V70del, V143del, Y144del, Y145del, N211del), and the unique insertion ins214EPE.

Moreover, characteristic amino acid substitutions of the Delta variant (panel “G142D, E156G, 157-158DEL, L452R, T484K”) were detected in the Regions of Lazio, Valle d’Aosta and in the A.P. of Bolzano.

Sequencing results are summarized in Table 2. To make it easier to understand, we combined some panels of mutations in ‘mutation packages’ as follow:

- Package A (Omicron BA.1, long fragment from PCR ID_980) = A67V, H69del, V70del, T95I, G142D, V143del, Y144del, Y145del, N211del, L212I, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K
- Package B (Omicron BA.1, long fragment from PCR ID_980) = A67V, H69del, V70del, T95I, G142D, V143del, Y144del, Y145del, N211del, L212I, ins214EPE, G339D, R346K, S371F, S373P, K417N, N440K, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K
- Package A/B (Omicron BA.1, short fragment from PCR ID_987/994): A67V, H69del, V70del, T95I, G142D, V143del, Y144del, Y145del, N211del, L212I
- Package C (Omicron BA.2, long fragment from PCR ID_980) = G142D, V213G, G339D, S373P, S375F, T376A, D405N, R408S, K417N, N440K, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H

- Package D (Delta, long fragment from PCR ID_980) = G142D, E156G, F157del, R158del, L452R, T478K

Table 2. PCR and sequencing results

	Sample ID	WTP	City	Region/A.P.	RT-qPCR (c.g./L)	Mutations found by Sanger sequencing (long PCR ID_980)	Mutations found by Sanger sequencing (short PCR ID_987/994)	SARS-CoV-2 variant	Mutation found by NGS (samples pooled by Region/A.P.)	SARS-CoV-2 variant
	1	6778	Villa Carmine	Pescara	<LOD	-	-			
	2	6779	Via Raiale	Pescara	<LOD	-	M.E. ^a	Unassigned		
	3	6780	S. Martino	Chieti	<LOD	-	-		-	
	4	6781	Pile	L'Aquila	<LOD	-	-			
	5	6782	Villa Pavone	Teramo	<LOD	-	-			
	6	6826	Tiera di Vaglio	Potenza	n.a	-	-		• Package A	Omicron BA.1
	7	6827	Pantano	Matera	2,14E+03	Package A + S256L + R346K		Omicron	• Package B + N61K + S256L	
	8 ^b	6786	Salerno	Salerno	<LOD	-			• Package A	Omicron BA.1
	9 ^b	6787	Nocera Sup	Salerno	6,25E+02	-			• Package B	
	10 ^b	6788	Area Nolana	Napoli	1,33E+03	-				
	11 ^b	6789	Napoli EST	Napoli	1,65E+03	-				
	12 ^b	6790	Napoli OVEST – Ingr. Principale	Napoli	9,25E+02	-				
	13 ^b	6791	Napoli OVEST - ex ingr. Camaldoli	Napoli	1,65E+03	-				
	14 ^b	6792	Area Casertana	Caserta	3,75E+03	-				
	15 ^b	6793	Villa Literno	Caserta	8,25E+02	-				
	148	6909	Area Nolana	Napoli	3,54E+04	-	Package A/B	Omicron		
	149	6910	Area Casertana	Caserta	2,77E+03	-	-			
	150	6911	Nocera Sup	Salerno	4,02E+04	-	-			
	151	6912	Napoli EST	Napoli	2,57E+04	-	Package A/B	Omicron		
	152	6913	Napoli OVEST – Ingr. Principale	Napoli	8,76E+04	-	Package A/B	Omicron		
	153	6914	Napoli OVEST - ex ingr. Camaldoli	Napoli	5,91E+04	Package B		Omicron		
	154	6915	Manocalzati	Avellino	2,67E+04	-	Package A/B	Omicron		
	155	6916	Salerno	Salerno	2,35E+04	-	Package A/B	Omicron		
	156	6917	Villa Literno	Caserta	4,27E+04	-	Package A/B	Omicron		
	16	6750	IDAR	Bologna	4,93E+05	Package A		Omicron		Omicron BA.1

17	6751	Naviglio	Modena	Emilia-Romagna	3,78E+05	Package A (partial, from N211del to T547K) ^(c) + R346K	Omicron	<ul style="list-style-type: none"> • Package A • Package B 		
18	6752	Ravenna	Ravenna - Forlì-Cesena		4,05E+05	Package A	Omicron			
19	6714	Ferrara	Ferrara		4,43E+04	-	-			
20	6715	Ferrara	Ferrara		<LOD	-	-			
21	6716	Carpi	Modena		2,37E+04	-	Package A/B	Omicron		
22	6753	Cesena	Forlì-Cesena		4,25E+05	Package A		Omicron		
23	6754	Forlì	Forlì-Cesena		1,86E+05	Package A (partial, from T95I to ins214EPE) ^(c)		Omicron		
24	6755	Faenza	Ravenna		3,20E+05	-	-			
25	6756	Imola	Bologna		1,95E+05	-	Package A/B	Omicron		
26	6802	Borgoforte	Piacenza		<LOD	-	Package A/B	Omicron		
27	6805	Parma ovest	Parma		8,23E+03	-	-			
28	6808	Mancasale	Reggio Emilia		3,40E+04	-	Package A/B	Omicron		
29	6847	IDAR	Bologna		4,65E+05	Package A		Omicron		
30	6848	Naviglio	Modena		1,67E+05	Package A		Omicron		
31	6849	Ravenna	Ravenna - Forlì-Cesena		2,70E+05	-	Package A/B	Omicron		
32	6850	S. Giustina	Rimini - Forlì-Cesena		2,25E+05	Package A		Omicron		
33	6932	Cordenons (LTA)	Pordenone		Friuli-Venezia Giulia	2,20E+05	Package A (partial, from T95I to Y145del) ^(c)	Omicron	<ul style="list-style-type: none"> • Package A • Package B 	Omicron BA.1
34	6931	Udine (CAFC)	Udine			4,25E+05	Package A + R346K	Omicron		
35	6933	Servola entrata	Trieste			2,29E+05	Package A + R346K	Omicron		
36	6666	Viterbo – St.Bagni	Viterbo		Lazio	1,17E+04	Package A + R346K	Omicron	<ul style="list-style-type: none"> • Package A 	Omicron BA.1
37	6667	Velletri (La Chiusa-Sorbo)	Roma			6,33E+03	Package D + T95I	Delta	<ul style="list-style-type: none"> • Package B • Package B + G416R 	
38	6668	Via Cincinnato	Roma			2,32E+04	Package A + R346K + G416R	Omicron		

39	6669	Loc Latina Est	Latina		4,23E+03	-			• Package D + T95I + T376I	Delta
40	6670	Anzio - Colle Cocchino	Roma		2,43E+03	-				
41	6671	Aprilia (Via del Campo)	Latina		1,30E+04	-	Package A/B	Omicron		
42	6672	Ponte Lucano	Roma		2,30E+04	-	Package A/B	Omicron		
43	6812	Roma Est (linea 1 + linea 2)	Roma		5,44E+05	Package A		Omicron		
44	6813	Roma Nord	Roma		2,89E+05	Package A + R346K		Omicron		
45	6814	Roma Sud	Roma		1,94E+05	-	Package A/B	Omicron		
46	6815	Ostia	Roma		8,58E+05	Package A		Omicron		
47	6816	Fiumicino	Roma		7,29E+04	-	-			
48	6721	Civitavecchia Fiumaretta	Roma		2,50E+02	-	-			
50	5214	Pegli	Genova		1,23E+05	-	Package A/B	Omicron	• Package A	Omicron BA.1
51	5215	Voltri	Genova		1,75E+05	Package A + R346K		Omicron	• Package B	
52	6757	Quinto	Genova		2,11E+06	-	-			
3	6758	Rapallo	Genova		4,46E+05	Package A + R346K, T470N		Omicron		
54	6759	Sestri P	Genova		5,02E+04	-	Package A/B	Omicron		
55	6760	Sanremo .Verde	Imperia		5,35E+05	Package A		Omicron		
56	6761	Borgh. S. Spirito	Savona	Liguria	3,51E+05	Package A		Omicron		
57	6762	La Spezia	La Spezia		1,91E+05	Package A		Omicron		
58	6763	Camisano	La Spezia		1,69E+04	-	M.E. ^a	Unassigned		
59	6764	Silea	La Spezia		1,82E+05	Package A		Omicron		
60	6794	Punta Vagno	Genova		3,43E+04	Package A + R346K		Omicron		
62	6825	Sturla	Genova		7,38E+04	Package A + R346K		Omicron		
63	6765	Darsena	Genova		2,09E+05	-	Package A/B	Omicron		
64	6766	Punta Vagno	Genova		3,98E+05	-	Package A/B	Omicron		
65	6767	Valpolcevera	Genova		6,08E+04	-	Package A/B	Omicron		
158	6955	Nosedo			4,06E+06	-	Package A/B	Omicron	• Package A	Omicron BA.1
159	6957	S. Rocco			2,35E+06	-	Package A/B	Omicron	• Package B	
66	6821	Monza	CO-LC-MI-MB	Lombardia	6,00E+03	Package A		Omicron	• Package B + N439N	
67	6798	Verziano	Brescia		1,10E+04	-	-			
68	6776	Canegrate	MI-VA		2,73E+05	-	-			
69	6777	Varese	Varese		1,96E+05	-	-			

70	6799	Verziano	Brescia		1,21E+04	Package A + R346K	Omicron		
71	6822	Monza	CO-LC-MI-MB		7,23E+03	Package A + V70I	Omicron		
72	6954	Nosedo	Milano		2,11E+07	Package A + R346K	Omicron		
73	6956	S. Rocco	Milano		4,16E+06	-	Package A/B	Omicron	
74	6834	Borgheria	Pesaro-Urbino		2,61E+04	-	-		
75	6835	Ponte Metauro	Pesaro-Urbino		3,54E+04	-	Package A/B	Omicron	
76	6836	Ponte Sasso	Pesaro-Urbino	Marche	4,06E+03	-	-		
77	6837	Zipa	Ancona		1,95E+04	-	Package A/B	Omicron	
78	6838	Falconara	Ancona		6,28E+03	-	Package A/B	Omicron	
79	6839	Camerano	Ancona		8,39E+03	-	-		
81	6784	Termoli - località Pantano Basso	Campobasso		<LOD	-	M.E. ^a	Unassigned	
82	6785	Campobasso - San Pietro	Campobasso	Molise	<LOD	-	Package A/B	Omicron	
83	6771	IDA Bolzano	Bolzano		3,60E+04	-	M.E. ^a	Unassigned	• Package B
84	6772	IDA Merano	Bolzano		4,37E+04	-	Package A/B	Omicron	• Package C + Omicron BA.2
88	6921	IDA Bolzano	Bolzano	P.A. Bolzano	4,33E+04	Package A + R346K	Omicron		• I410I + S371F
89	6922	IDA Merano	Bolzano		4,15E+04	Package D	Delta		• Package D
90	6935	IDA Termeno	Bolzano		7,00E+04	M.E. ^a	Unassigned		• Package D + T95I
85	6676	Trento nord	Trento		9,46E+05	M.E. ^a	Unassigned	• Package A	Omicron BA.1
86	6679	Trento sud	Trento	P.A. Trento	7,16E+05	-	Package A/B	Omicron	• Package A + R408G
87	6680	Rovereto	Trento		1,15E+06	Package A	Omicron		
92	6643	Biella Nord	Biella		4,03E+01	-	Package A/B	Omicron	• Package A
93	6644	Biella Sud	Biella		3,75E+01	-	Package A/B	Omicron	
94	6645	Novara	Novara		5,55E+01	-	Package A/B	Omicron	
95	6697	Castiglione Torinese	Torino	Piemonte	3,30E+04	-	M.E. ^a	Unassigned	
96	6698	Alessandria	Alessandria		2,58E+04	-	Package A/B	Omicron	
97	6699	Asti	Asti		1,39E+04	Package A	Omicron		
98	6700	Cuneo	Cuneo		3,23E+04	Package A	Omicron		
99	6852	Lecce	Lecce		4,59E+04	Package A	Omicron	• Package A	Omicron BA.1
100	6853	Brindisi Fiume Grande	Brindisi	Puglia	1,49E+04	Package A + T315S + R346K	Omicron	• Package B	

101	6854	Taranto Gennarini	Taranto		2,17E+04	Package A + R346K		Omicron		
102	6855	Taranto Bellavista	Taranto		8,82E+04	Package A + R346K		Omicron		
103	6856	Cerignola	Foggia		1,30E+04	Package A		Omicron		
104	6857	Foggia	Foggia		1,71E+04	-	M.E. ^a	Unassigned		
105	6858	Bari Est	Bari		1,71E+04	-	Package A/B	Omicron		
106	6859	Bari Ovest	Bari		2,00E+04	Package A + R346K		Omicron		
107	6860	Bitonto	Bari		1,98E+04	Package A + R346K		Omicron		
108	6861	Molfetta	Bari		2,12E+05	Package A + R346K		Omicron		
109	6862	Barletta	BAT		2,70E+04	Package A + G404V		Omicron		
110	6863	Andria	BAT		2,63E+04	Package A + R346K		Omicron		
111	6864	Trani	BAT		1,25E+05	Package A		Omicron		
112	6865	Bisceglie	BAT		1,43E+05	Package A		Omicron		
113	6866	Taranto Bellavista	Taranto		1,61E+04	Package A + R346K		Omicron		
114	6867	Taranto Gennarini	Taranto		3,08E+03	M.E. ^a		Unassigned		
115	6868	Altamura	Bari		1,62E+04	Package A		Omicron		
116	6692	Agrigento	Agrigento		2,04E+05	-	-		• Package A + V227L	Omicron BA.1
117	6688	Ragusa	Ragusa		7,50E+02	Package A + V227L		Omicron	• Package A + V395V	
118	6689	Modica	Ragusa		9,73E+02	Package A + R346K		Omicron	• Package B	
119	6690	Vittoria	Ragusa		1,13E+03	Package A		Omicron		
120	6693	Acqua dei Corsari	Palermo		3,49E+05	-	-			
121	6694	Fondo Verde	Palermo	Sicilia	1,28E+05	-	M.E. ^a	Unassigned		
122	6695	Bagheria	Palermo		2,41E+05	-	Package A/B	Omicron		
123	6746	Mili Marina	Messina		1,50E+03	-	M.E. ^a	Unassigned		
124	6823	Caltanissetta e San Cataldo	Caltanissetta		4,24E+05	-	-			
125	6824	Gela Macchitella	Caltanissetta		3,75E+00	-	-			
157	6880	Pantano d'Archi	Catania		2,65E+04	-	-			
126	6809	Lavello 2	Massa		<LOD	-	-			
127	6810	Viareggio	Lucca	Toscana	6,11E+04	-	-		-	
128	6811	Lavello 1	Massa		<LOD	-	M.E. ^a	Unassigned		
129	6634	Pian della Genna	Perugia		1,24E+05	Package A		Omicron	• Package A	Omicron BA.1
130	6717	Pian della Genna	Perugia	Umbria	7,33E+04	Package B		Omicron		
131	6718	Foligno Casone	Perugia		1,23E+05	Package A		Omicron		

132	6719	Terni	Terni		2,28E+05	Package A + R346K	Omicron	• Package B + S359T		
133	6748	La Salle	Aosta		1,69E+04	-	Package A/B	Omicron	• Package A • Package A + A163A	Omicron BA.1
134	6749	Brissogne	Aosta	Valle d'Aosta	3,14E+04	(partial, from T95I to ins214EPE) ^(c)		Omicron	• Package B • Package D + S221S	Delta
146	6683	La Salle	Aosta		7,95E+04	Package A		Omicron		
147	6684	Brissogne	Aosta		6,17E+04	M.E.		Unassigned		
135	6647	Ca' Nordio - centro storico	Padova		2,76E+04	-	Package A/B	Omicron	• Package A • Package B	Omicron BA.1
136	6648	Ca' Nordio - zip	Padova		6,74E+04	M.E.		Unassigned		
137	6649	Padova Guizza	Padova		1,61E+04	-	-			
138	6650	Abano Terme	Padova	Veneto	2,22E+04	-	Package A/B	Omicron		
139	6685	Treviso	Treviso		3,98E+04	Package A		Omicron		
140	6686	Vicenza Casale	Vicenza		3,27E+04	Package A + R346K		Omicron		
141	6687	Venezia Fusina	Venezia		2,09E+04	Package A		Omicron		
143	6725	Verona_coll. 1M	Verona		8,93E+04	Package A		Omicron		
144	6726	Verona_coll. 3M	Verona		1,13E+05	Package A + R346K		Omicron		
145	6727	Verona_coll. 8M	Verona		1,19E+05	Package A + R346K		Omicron		

^a M.E. mixed electropherograms

^b Due to shipment problems, samples were thawed at delivery

^c Partial sequence due to mixed electropherograms and/or high signal noise; within brackets the region for which a sequence was provided

- Package A (Omicron BA.1, long fragment from PCR ID_980) = A67V, H69del, V70del, T95I, G142D, V143del, Y144del, Y145del, N211del, L212I, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K
- Package B (Omicron BA.1, long fragment from PCR ID_980) = A67V, H69del, V70del, T95I, G142D, V143del, Y144del, Y145del, N211del, L212I, ins214EPE, G339D, R346K, S371F, S373P, K417N, N440K, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K
- Package A/B (Omicron BA.1, short fragment from PCR ID_987/994): A67V, H69del, V70del, T95I, G142D, V143del, Y144del, Y145del, N211del, L212I
- Package C (Omicron BA.2, long fragment from PCR ID_980) = G142D, V213G, G339D, S373P, S375F, T376A, D405N, R408S, K417N, N440K, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H
- Package D (Delta, long fragment from PCR ID_980) = G142D, E156G, F157del, R158del, L452R, T478K

Limitations of the study

Given the progressive implementation of the national surveillance for SARS-CoV-2 in wastewaters, sample collection/processing is not yet feasible in all Italian regions. Therefore, this flash survey's geographical and population coverage was incomplete, as it covered 19/21 of the Italian regions/autonomous provinces.

Molecular analytical methods applied to complex environmental matrices as wastewaters may be hampered by low viral concentration, poor recovery of the analyte, and/or inhibition of PCR amplification. Therefore, both detection/quantification and PCR amplification for sequencing purposes may produce false negatives. Consequently, molecular characterization and variant detection may not be achieved for all samples.

Conclusions and final considerations

This is the fifth of a series of monthly reports on SARS-CoV-2 and its variants in wastewaters that will continue to be issued as part of the surveillance established in Italy under EU Commission Recommendation 2021/472, with the aim of providing information on SARS-CoV-2 variants in the population to supplement information acquired through the clinical surveillance.

The results of SARS-CoV-2 surveillance in wastewaters confirm the predominance and significant variability of the Omicron variant (B.1.1.529) in January 2022 in Italy, in line with clinical results. Lineage BA.1 was predominant in the positive samples and it showed two main 'mutation packages' – respectively characterized by S371L+G446S and R346K+S371F mutations - and evenly spread throughout the territory.

The characteristic mutations of sublineage BA.2 were detected in one sample (P.A. of Bolzano). Mutations of the Delta variant were detected in only three Regions/A.P.

The last flash survey on wastewater, performed on samples collected throughout the country in the period between 30 November and 03 December, did not detect the Omicron variant by Next Generation Sequencing. However, since the Omicron variant has spread rapidly, an extra, ad hoc survey focused on Omicron was performed on wastewater samples collected in Italy between 5 and 25 December 2021 (see [fe204e2c-2fdb-cd0b-f9e4-aa6e2a8edcc3 \(iss.it\)](https://www.iss.it/fe204e2c-2fdb-cd0b-f9e4-aa6e2a8edcc3)) demonstrating that the variant spread quickly in the 3 weeks of the survey, its detection in wastewater samples increasing as follows: - week 5-11 December: 1/74 (1.4%; detection in one Region) - week 12-18 December: 15/108 (13.9%; detection in 7 Regions/A.P.) - week 19-25 December: 64/100 (64.0%; detection in 14 Regions/A.P.).

Results of the present survey, along with those from the "ad hoc survey" performed in December, confirm that, since December 2021, Omicron has taken over in the whole country.

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- Calabria: Eduardo Malacaria (Regione Calabria), Giuseppe Folino (Arpacal)
- Campania: Angelo D’Argenzio (Regione Campania); Luigi Cossentino, Renato Olivares (Arpac - Agenzia Regionale per la Protezione Ambientale in Campania); Antonio Pizzolante, Giovanna Fusco (Istituto Zooprofilattico Sperimentale del Mezzogiorno); Alessandra Tosco, Amalia Porta (Università degli Studi di Salerno); Francesca Pennino, Triassi Maria (Università degli Studi di Napoli “Federico II”);
- Emilia Romagna: Paola Angelini, Lisa Gentili (Regione Emilia – Romagna); Laura De Lellis, Daniele Nasci (HERATech); Giovanni Alborali; Nicoletta Formenti, Flavia Guarneri (Istituto Zooprofilattico Sperimentale della Lombardia e dell’Emilia-Romagna); Nadia Fontani, Giulia Nani, Franca Palumbo, Gianluca Borlone, Marco Guercio (IREN); Lisa Gentili (Arpae Emilia-Romagna)
- Friuli Venezia Giulia: Marika Mariuz, Gabriella Trani (Direzione Centrale Salute FVG); Anna Pariani (LABORATORIO HERATech di Sasso Marconi –BO)
- Lazio: Carla Ancona (DEPLAZIO - Dipartimento di Epidemiologia del Servizio Sanitario Regionale - Regione Lazio); Doriana Antonella Giorgi, Irene Ferrante, Monica Monfrinotti, Silvia Riosa, Valeria Capparuccini (ARPA Lazio - Agenzia Regionale per la Protezione Ambientale del Lazio); Maria Teresa Scicluna, Antonella Cersini (IZSLT - Istituto Zooprofilattico Sperimentale del Lazio e della Toscana); Maria Concetta Arizzi, Giancarlo Cecchini, Claudio Ottaviano (Acea Elabori)
- Liguria: Elena Nicosia (Regione Liguria settore tutela della salute negli ambienti di vita e di lavoro); Nadia Fontani, Giulia Nani, Franca Palumbo, Gianluca Borlone, Marco Guercio (Iren); Elena Grasselli; Giorgia Allaria, Lorenzo Dondero, Francesca Rispo (UNIGE - DISTAV); Alberto Izzotti (UNIGE – DIMES); Rosa Maria Bertolotto, Elena Nicosia, Stefano Rosatto, Marta Bellisomi, Irene Tomesani (ARPAL); Micaela Tiso (MICAMO srl)
- Lombardia: Emanuela Ammoni, Danilo Cereda (Regione Lombardia); Marina Nadia Losio, Barbara Bertasi (IZSLER - Istituto Zooprofilattico Sperimentale della Lombardia e dell’Emilia); Andrea Aliscioni, Desdemona Oliva (CAP Holding); Sara Castiglioni, Silvia Schiarea, Ettore Zuccato (Istituto Mario Negri IRCCS); Manuela Antonelli, Arianna Azzellino, Francesca Malpei, Andrea Turolla (POLIMI); Sandro Binda, Pellegrinelli Laura, Valeria Primache (Univeristà degli Studi di Milano, Dipartimento di Scienze Biomediche per la Salute), Clementina Cocuzza, Andrea Franzetti, Rosario Musumeci e Marianna Martinelli (Università di Milano-Bicocca); Giorgio Bertanza (Università di Brescia), Maria Luisa Callegari (Università Cattolica del Sacro Cuore);
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- Molise: Michele Colitti (Regione Molise); Carmen Montanaro (ASReM); Giuseppe Aprea, Silvia Scattolini, Ilaria Rosa, Daniela D’Angelantonio, Giacomo Migliorati (Istituto

Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale"); Maria Grazia Cerroni (Arpa Molise);

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- Puglia: Giuseppe Di Vittorio, Onofrio Mongelli (Regione Puglia); Osvalda De Giglio, Francesca Apollonio, Francesco Triggiano, Maria Teresa Montagna (Università degli Studi di Bari Aldo Moro - Dipartimento Scienze Biomediche e Oncologia Umana); Nicola Ungaro (ARPA Puglia)
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- Toscana: Piergiuseppe Cala' (Regione Toscana); Annalaura Carducci, Marco Verani, Ileana Federigi, Giulia Lauretani, Sara Muzio (Laboratorio di Igiene e Virologia Ambientale - Dipartimento di Biologia Università di Pisa); Matteo Ramazzotti, Alberto Antonelli (SOD microbiologia e virologia, azienda ospedaliera universitaria Careggi, Firenze);
- Umbria: Giovanni Santoro (Regione Umbria), Ermanno Federici, Maya Petricciuolo, Sofia Barigelli (Laboratorio Microbiologia Applicata e Ambientale, DCBB Università di Perugia);
- Valle D'Aosta: Mauro Ruffier (Regione Valle d'Aosta); Francesca Borney, Eric Grange, Florida Damasco (Laboratorio chimico biologico microbiologico Arpa Valle d'Aosta),
- Veneto: Francesca Russo, Gisella Pitter, Vanessa Groppi (Regione Veneto); Franco Rigoli, Marco Zampini (ARPAV - Agenzia Regionale per la Prevenzione e Protezione Ambientale del Veneto); Tatjana Baldovin, Irene Amoruso (Università di Padova);
- P. A. Bolzano: Lorella Zago (P.A. Bolzano), Alberta Stenico, Anna-Maria Prast (Laboratorio biologico - Agenzia provinciale per l'ambiente e la tutela del clima (A.P.P.A.).
- P.A. Trento: Francesco Pizzo; Alessandra Schiavuzzi, Elena Mengon (P. A. Trento) (P.A. Trento); Maria Cadonna, Mattia Postinghel (ADEP SGI PAT), Francesca Cutrupi, Paola Foladori, Serena Manara (UNITN – Università di Trento)