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# Genomic and Epidemiologic Insights into Ongoing Measles Outbreak, Israel, 2025–2026

## Appendix

## Real time PCR

Real-time PCR was performed using an in-house multiplex assay targeting measles virus, vaccine-strain Measles, rubella virus, and RNase P, or the Quanty Measles commercial kit (Clonit, Italy), according to the manufacturer's instructions. All assays were run on Bio-Rad CFX96 instruments.

### Extraction, library preparation, sequencing and analysis

Total nucleic acids from clinical samples positive for measles virus were extracted using magLEAD 12gC (PSS, Precision System Science, Matsudo, Japan). Reverse transcription and amplification of the complete measles virus genome were carried out in a single one-step RT-PCR reaction using a previously published set of primers designed to amplify the full viral genome (*1*). To enhance whole genome coverage, the primer mix was supplemented with additional published primers targeting the MF-NCR region (*2*), as well as primers designed to improve coverage of the 3′ genomic region (*3*). PCR products were subsequently used for library preparation with the DNAprep kit (Illumina, CA, USA) according to the manufacturer's instructions on a Hamilton STAR liquid handling robot. Sequencing was performed on the Illumina MiSeq i100 pro platform (Illumina, CA, USA).

## Selected samples for sequencing

A suspected measles case is defined according to WHO criteria as a patient presenting with fever and generalized maculopapular (non-vesicular) rash, or any patient in whom a health

care worker suspected measles infection. Of 3203 confirmed measles cases between April 2025 and February 2026, 80% were laboratory confirmed. A total of 1599 samples were available at the national measles center, Central Virology Laboratory, where this study was conducted. Of these, 1090/1599 were suitable for sequencing, defined as samples with Cq =< 25 to ensure full sequence coverage. A total of 132 positive specimens were selected for sequencing as part of routine surveillance, as described in the main text, and are stratified by district and summarized in Appendix Table 2.

## Bioinformatic analysis

Fastq files underwent quality control assessment using fastQC and MultiQC (*4*) and low-quality sequences were filtered using trimmomatic (H. Li, unpub. data, http://arxiv.org/abs/1303.3997). Reads were then aligned to an annotated MeV genotype B3 reference genome (KT732215.1) using BWA-mem (*5*). The resulting alignments were processed with the SAMtools suite for sorting, indexing, and additional quality control (*6*). Consensus genome sequences were generated using iVar (*7*), with positions supported by fewer than ten reads masked as 'N'.

For the phylogenetic analysis, the Nextstrain Augur pipeline (*7*) was used as follows. Sequences were aligned to the B3 reference genome using MAFFT (*8*), followed by construction of a time-resolved phylogenetic tree using IQ-TREE and TreeTime (*9*,*10*), employing the GTR substitution model. Final visualization was performed with Auspice.

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**Appendix Table 1.** Selected samples for sequencing by district

| District | Total Positive Samples | Total suitable samples for sequencing (<=25 cq) | # Sequenced samples(% of total suitable samples) | Remarks |
|---|---|---|---|---|
| Jerusalem | 924 | 756 | 27 (4%) | Due to known epidemiological data indicating extensive transmission within specific households, streets and communities there was no need for extensive sequencing per the sequencing criteria. |
| North | 235 | 87 | 21 (26%) | |
| Central | 196 | 113 | 53 (47%) | Multiple areas without significant epidemiological data resulted in high need for sequencing. |
| Tel Aviv | 134 | 82 | 16 (20%) | |
| South | 62 | 35 | 10 (29%) | |
| Haifa | 48 | 17 | 5 (29%) | |
| Total | 1599 | 1090 | 132 | |

**Appendix Table 2.** Sequencing coverage for sequenced samples by district

| Sequence ID | Sequencing coverage (%) | District |
|---|---|---|
| 1065-25 | 93 | Central |
| 1095-25 | 96 | Central |
| 1096-25 | 93 | Central |
| 1211-25 | 93 | Central |
| 1334-25 | 93 | Jerusalem |
| 1394-25 | 98 | Central |
| 1427-25 | 93 | Central |
| 1440-25 | 93 | Haifa |
| 1511-25 | 100 | Central |
| 1530-25 | 94 | Southern |
| 1534-25 | 93 | Southern |
| 1545-25 | 86 | Haifa |
| 1568-25 | 100 | Jerusalem |
| 1575-25 | 90 | Central |
| 1576-25 | 93 | Central |
| 1578-25 | 93 | Tel Aviv |
| 1582-25 | 94 | Jerusalem |
| 1583-25 | 93 | Tel Aviv |
| 1691-25 | 93 | Haifa |
| 1711-25 | 93 | Jerusalem |
| 1952-25 | 100 | Jerusalem |
| 2145-25 | 81 | Tel Aviv |
| 2197-25 | 95 | Jerusalem |
| 2231-25 | 98 | Jerusalem |
| 2298-25 | 92 | Northern |
| 2324-25 | 92 | Central |
| 2374-25 | 78 | Tel Aviv |
| 2413-25 | 93 | Central |
| 2435-25 | 82 | Central |
| 2490-25 | 95 | Central |
| 2543-25 | 90 | Southern |
| 2577-25 | 65 | Southern |
| 2579-25 | 86 | Northern |
| 2592-25 | 82 | Central |
| 2629-25 | 84 | Central |
| 2705-25 | 82 | Central |
| 2744-25 | 100 | Southern |
| 2750-25 | 92 | Jerusalem |
| 2754-25 | 88 | Jerusalem |
| 2755-25 | 100 | Central |
| 2839-25 | 80 | Jerusalem |
| 2991-25 | 100 | Northern |
| 3005-25 | 100 | Northern |
| 3019-25 | 85 | Tel Aviv |
| 3039-25 | 100 | Central |
| 3047-25 | 95 | Southern |
| 3152-25 | 97 | Jerusalem |
| 3256-25 | 84 | Central |
| 3337-25 | 94 | Central |
| 3350-25 | 100 | Tel Aviv |

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| Sequence ID | Sequencing coverage (%) | District |
|---|---|---|
| 3425-25 | 95 | Northern |
| 3449-25 | 100 | Northern |
| 3506-25 | 100 | Jerusalem |
| 3554-25 | 100 | Central |
| 3582-25 | 100 | Southern |
| 3610-25 | 100 | Central |
| 3809-25 | 100 | Jerusalem |
| 4062-25 | 100 | Jerusalem |
| 651-25 | 93 | Central |
| 655-25 | 96 | Tel Aviv |
| 657-25 | 97 | Tel Aviv |
| 662-25 | 93 | Tel Aviv |
| 663-25 | 93 | Central |
| 672-25 | 99 | Central |
| 687-25 | 99 | Central |
| 703-25 | 95 | Central |
| 735-25 | 93 | Tel Aviv |
| 745-25 | 98 | Tel Aviv |
| 747-25 | 94 | Tel Aviv |
| 784-25 | 99 | Central |
| 789-25 | 93 | Jerusalem |
| 835-25 | 93 | Central |
| 890-25 | 93 | Haifa |
| 916-25 | 97 | Central |
| 3988-25 | 99 | Central |
| 3980-25 | 98 | Southern |
| 4072-25 | 97 | Southern |
| 4070-25 | 99 | Northern |
| 4133-25 | 99 | Central |
| 4130-25 | 99 | Central |
| 4249-25 | 96 | Central |
| 4448-25 | 100 | Central |
| 4495-25 | 97 | Northern |
| 4489-25 | 100 | Northern |
| 4378-25 | 100 | Jerusalem |
| 4382-25 | 100 | Northern |
| 4486-25 | 100 | Northern |
| 4660-25 | 100 | Jerusalem |
| 4749-25 | 100 | Northern |
| 891854-25 | 100 | Northern |
| 4750-25 | 96 | Tel Aviv |
| 4753-25 | 99 | Northern |
| 4813-25 | 96 | Southern |
| 4863-25 | 96 | Tel Aviv |
| 4947-25 | 99 | Northern |
| 4988-25 | 98 | Central |
| 5158-25 | 100 | Central |
| 4786-25 | 100 | Central |
| 4756-25 | 100 | Jerusalem |
| 4935-25 | 98 | Jerusalem |
| 5580-25 | 100 | Central |
| 5599-25 | 100 | Jerusalem |
| 5601-25 | 100 | Central |
| 5604-25 | 100 | Central |
| 5605-25 | 100 | Jerusalem |
| 5612-25 | 100 | Jerusalem |
| 5638-25 | 100 | Central |
| 5635-25 | 100 | Northern |
| 5632-25 | 100 | Northern |
| 5651-25 | 100 | Jerusalem |
| 127-26 | 100 | Tel Aviv |
| 180-26 | 100 | Jerusalem |
| 181-26 | 100 | Jerusalem |
| 199-26 | 100 | Central |
| 241-26 | 100 | Central |
| 242-26 | 100 | Northern |
| 258-26 | 100 | Northern |
| 314-26 | 100 | Northern |

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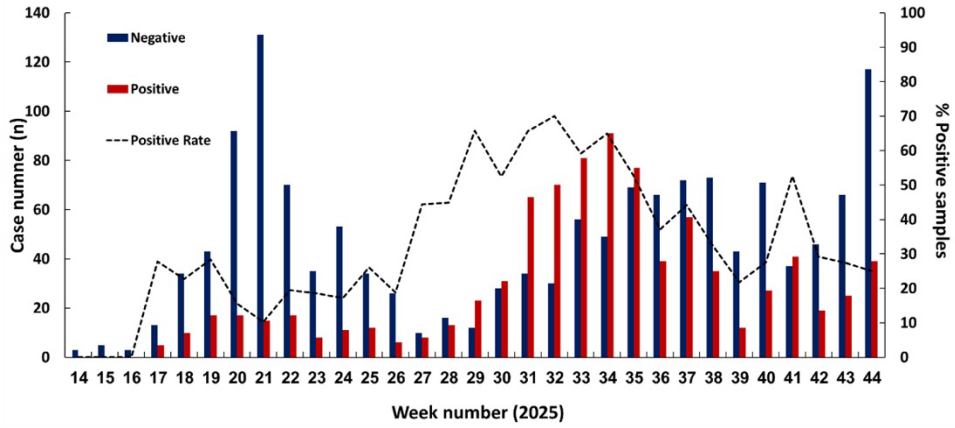
| Sequence ID | Sequencing coverage (%) | District |
|---|---|---|
| 39-26 | 100 | Central |
| 60-26 | 100 | Jerusalem |
| 354-26 | 98 | Central |
| 366-26 | 100 | Haifa |
| 381-26 | 100 | Central |
| 386-26 | 100 | Central |
| 391-26 | 100 | Northern |
| 1813-25 | 100 | Jerusalem |
| 3067-25 | 100 | Tel Aviv |
| 494-26 | 100 | Central |
| 568-26 | 100 | Central |
| 611-26 | 100 | Central |
| 760-26 | 100 | Central |
| 762-26 | 100 | Central |

**Appendix Table 3.** Population proportion (%) and vaccine coverage by district, 2025.

| District | Population proportion (%) | Vaccine coverage (%) |
|---|---|---|
| Central | 29 | 89 |
| Jerusalem | 13 | 78 |
| Northern | 15 | 92 |
| Tel Aviv | 16 | 88 |
| Southern | 15 | 90 |
| Haifa | 12 | 90 |




**Appendix Figure.** Weekly dynamics of molecular laboratory testing. Weekly numbers of measles-positive and negative samples with the corresponding positivity rate. Epidemiological, weeks 14-44 (1 April-31 October 2025).