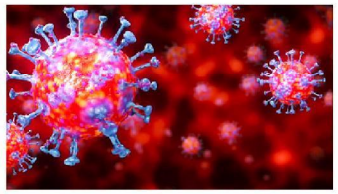
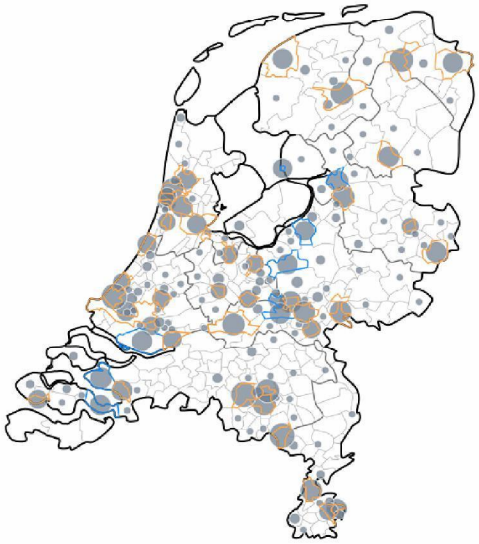


PIENTER-Corona:

Prospective serosurveillance study of SARS-CoV-2 in the general population of the Netherlands

Correcting seroprevalence estimates



(10)(2e) – Epidemiologist at National Institute for Public Health and the Environment (RIVM)

Design PIENTER-3 and PICO1

- **PIENTER-3**:
  - Nationwide sample of the Dutch population (2016/2017) to look into protection against VPD
  - Two-stage cluster design: six regions, comprising 49 randomly assigned municipalities
  - Biobank (including pre-sera) of 7600 participants
  - N=6102 participants (80%) gave consent to be approach in the future

- **PIENTER-Corona (PICO1):**
  - N=3207 (aged 2-90y, across the NL) provided a self-collected fingerstick blood sample and filled out an online questionnaire on risk factors, beginning of April, 2020

Antibody testing and correcting

- **Step 1:** All 3207 PICO1-serum samples were tested for the presence of SARS-CoV-2 IgG antibodies – targeted at the S1-part of the spike protein – using our Multiplex immunoassay (Luminex technology)

- **Step 2:** Due to the expected low seroprevalence in this epidemic phase a specificity-optimized cutoff value (99%) for seropositivity was determined, using a validation panel *(manuscript under review)* consisting of:
  - 115 PCR-positive samples (including mild and severe COVID-19 patients)
  - 400 controls (i.e., pre-pandemic samples, including a batch of ILI-samples (also HCoVs), as well as from PIENTER-3 and PIENTER-2)

Antibody testing and correcting

- For the assessment of PICO1, sensitivity at this cutoff was 84.4%

- To note: the RIVM lab has improved the performance of the MIA assay after PICO1, which will be used for the next assessment (PICO2, pending)

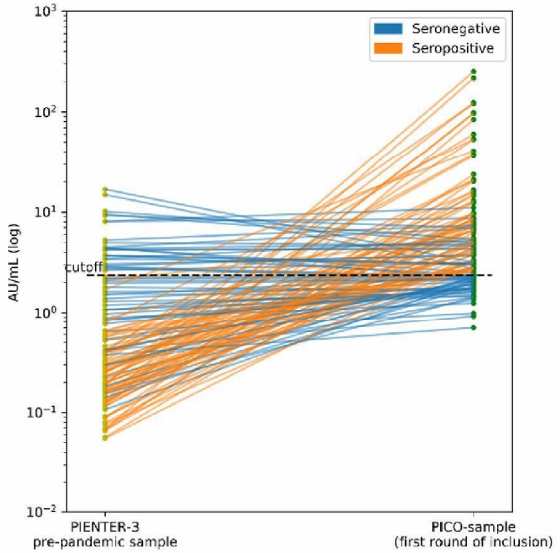
  -> This new MIA (mkII) reaches full (100%) specificity at a sensitivity of 94.5% (close to the best commercial immunoassays on the market)

Antibody testing and correcting

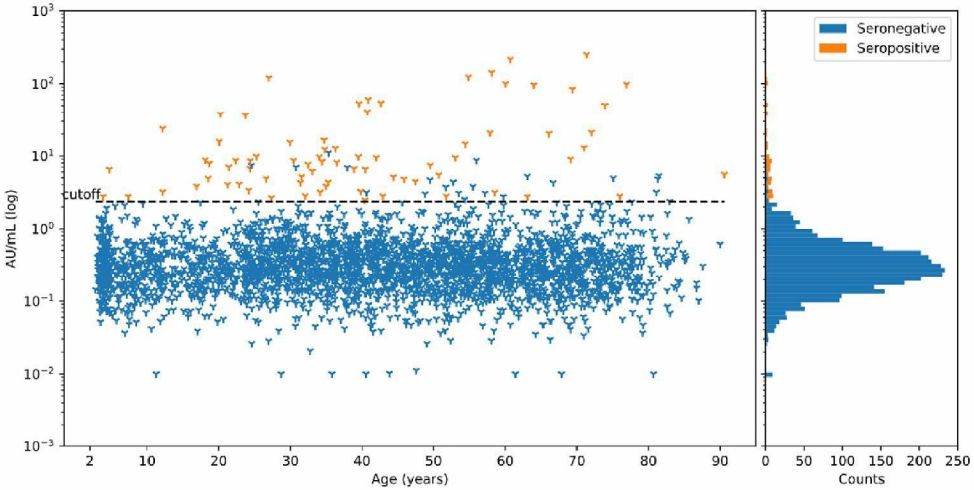
- **Step 3:** Seropositive PICO1-samples and those 25% below the cutoff were retested (n=138) -> GMC was used for further statistical analyses

- **Step 4:** 129/138 PICO1-samples had a pre-pandemic PIENTER-3-sample, and these were tested to correct for false-positivity:

-> PICO1-samples with a seropositive pre-pandemic serum (based on our validated cutoff) were classified as seronegative (blue lines) (n=26)



Antibody testing and correcting



Final overview: IgG antibody concentration (AU/mL (log)) against SARS-CoV-2 for all individual PICO1-samples, by age (years) (**left side**) and distributed by means of a histogram (**right side**).

-> Seropositive samples: **76**

Antibody testing and correcting

- **Step 5: Statistical correction:**
  - **A**: Calculate apparent weighted prevalence (*awp*) (with e.g., 95% Wilson/ClopperPearson CIs):
    - Correct for the survey design: strata (i.e., regions) and clusters (i.e., municipalities)
    - Include weights to match the distribution of the general public in 2020 (here based on sex, age, ethnic background and degree of urbanization)
  - **B**: Calculate true weighted prevalence:
    - Correct for test specifics (via Rogan-Gladen estimator), with sensitivity of 84.4% and assuming a specificity of 100% after cross-validation with pre-sera, using formula:

$$\frac{awp + SP - 1}{SE + SP - 1} \longrightarrow \frac{0.023 + 1.0 - 1}{0.844 + 1.0 - 1} \longrightarrow \textbf{2.8\%}\ (2.1\text{–}3.7)$$

Acknowledgements

- Participants of the PICO-study

- Colleagues at the Center for Infectious Disease Control, RIVM, particularly from the departments of Immunosurveillance and Epidemiology & Surveillance

Additional slide

Smooth age-specific seroprevalence

-> Logistic regression in a Generalized Additive Model using penalized splines (mgcv package in R), with additional Rogan-Gladen correction