# Supplementary information

#### Wantai Total **Antibody** Assay Validation

Two reference laboratories conducted validation of the Wantai total antibody assay: the Victorian Infectious Diseases Reference Laboratory (VIDRL) at The Peter Doherty Institute for Infection and Immunity, and New South Wales Pathology, Institute for Clinical Pathology and Medical Research (ICPMR) at Westmead.

**Sensitivity**

Pathology specimens from individuals identified as having a reverse transcription polymerase chain reaction (RT-PCR)-confirmed SARS-CoV-2 infection were selected by both laboratories.

At VIDRL, specimens were collected between February 2020 and August 2020. Samples were selected from anonymised excess diagnostic specimens from VIDRL and the Royal Melbourne Hospital (RMH) sent for COVID-19 testing.

At ICPMR, specimens were collected between January 2020 and July 2020. Samples were selected from NSW Health Pathology specimens sent to ICPMR for COVID-19 testing, and specimens were included where they tested positive on in house microneutralisation and immunofluorescent antibody assays; there was sufficient residual volume to form a reference panel for testing on multiple platforms under evaluation; and to cover a range of time periods following diagnosis of infection (up to 130 days).

For our combined validation, specimens from each laboratory were included in the estimation of sensitivity based on the following criteria:

* + Having a confirmed RT-PCR-positive result from the same patient episode
	+ Being collected >14 days post symptom onset
	+ For patients with multiple specimens, the first specimen >14 days post symptom onset used

Altogether, 102 specimens were collected, including 66 from VIDRL and 36 from ICPMR. These were tested on the Wantai total antibody assay at each laboratory, and sensitivity calculated separately and then pooled to generate an overall estimate. The sensitivity estimates based on specimens from each laboratory were as follows:

* **VIDRL**: 66/66 positive = 100% (median days post-illness onset=31, IQR=26 – 36, max=67)
* **ICPMR**: 31/36 positive = 86.1% (median days post-illness onset=26.5, IQR=17 – 105, max=130, number of samples >67 days [VIDRL max]=17/36=47%, days post-illness onset for the n=5 false negatives=15, 20, 74, 107, 124)

Differences in the sensitivity estimates from each laboratory were determined to be a result of the different distribution of days post-illness onset among specimens included, with sensitivity being lower in specimens collected further from illness onset. Results were pooled to maximise the sample size and diversity of specimens (particularly in terms of days post-illness onset). The overall sensitivity estimate used in analysis was as follows:

* **Combined from two laboratories:** 97/102 positive= **95.1%** (95% CI 88.9-98.4; median days post-illness onset=31, IQ =21 – 40, max=130)

**Specificity**

Pre-pandemic specimens from May 2019 were sourced from Australian Red Cross Lifeblood to assess specificity of the Wantai assay. A total of 800 independent specimens were tested at VIDRL, resulting in the following specificity estimate:

* 797/800 negative = **99.6%** (95% CI 98.9-99.9)

Potential cross-reactivity was also specifically assessed at VIDRL (from the ‘VIDRL Serum Reference Collection’) and ICPMR using samples collected pre-pandemic from patients with seasonal coronavirus infections (HCoV-NL63, HCoV-229E, HCoV-OC43 and HCoV-HKU1), other acute infections with potential for false positivity (including SARS-CoV-1, MERS-CoV, dengue, CMV, EBV, mycoplasma, Parvovirus, influenza A, Adenovirus, Hepatitis A, B and C, Syphilis and HIV) or the presence of rheumatoid factor. Data from VIDRL have been published[[1]](#footnote-1), demonstrating that only SARS-CoV-1 showed cross-reactivity.

**Ethics approval for validations**

VIDRL: Project ethical approval for RMH specimens was obtained from Melbourne Health Human Research Ethics Committee; this included patient’s written consent.

ICPMR: Individual patient consent was not required as this was an assay development and quality assurance study analysing results from samples submitted for routine diagnostic testing. No patient samples were collected specifically for the purposes of this study, and results were presented in a de-identified manner. As per the published in-house assay validations performed at ICPMR[[2]](#footnote-2), this was approved by Health Protection New South Wales as a communicable diseases control activity.

#### Bayesian **inference** for **population** seroprevalence

The target parameter of interest was defined as Wantai-positive population seroprevalence, π. This is related to the observable Wantai-positive proportion, *p*, via the sensitivity δ and specificity γ of the Wantai test, as follows:

).

Rearranging and solving this equation for π gives:

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Estimation of π requires consideration of multiple sources of uncertainty including sampling error in the outcome and uncertainty in the test sensitivity and specificity. Bayesian inference was performed based on the following model specification for , the observed number of Wantai positive tests from a sample size of total tests:

with prior distributions required for the parameters π, δ and γ, all constrained to values between 0 and 1.

Prior distributions for sensitivity (δ) and specificity (γ) were derived from combining the validation results described above with uniform prior distributions (true positives (TP) = 97, false negatives (FN) = 5, true negatives (TN) = 797, false positives (FP) = 3) to obtain the following Beta distributions:

For the primary analysis, the Wantai-positive seroprevalence, π, was assumed to have a ‘noninformative’ uniform Beta(1,1) prior distribution, restricted to values greater than a specified lower bound (calculation of lower bounds described in detail below). A sensitivity analysis was performed assuming an alternative prior distribution for π, Beta(0.2,10), also restricted to values greater than a lower bound. This prior was chosen to reflect expert knowledge; thus, it assigned much greater weight to low values (Supplementary Table 1).

Bayesian models were fitted using Stan (Carpenter et al., 2017; Stan Development Team, 2020) via the R interface. Point seroprevalence estimates were defined as the median values from posterior distributions and 95% credible intervals were calculated as 95% highest posterior density intervals, since posterior distributions were not symmetrical.

For each of the three subpopulations (general pathology, blood-donors and antenatal screening), seroprevalence estimates were obtained for the subpopulation overall, by jurisdiction (ACT, NSW, NT, QLD, SA, VIC, TAS, WA), by sex (male, female), and by age-group (as available: 0–4yrs, 5–9yrs, 10–19yrs, 20–29yrs, 30–39yrs, 40–49yrs, 50–59yrs, 60–69yrs, 70–79yrs, 80+yrs). Lower bounds for the prior distribution for π, the Wantai-positive seroprevalence, were calculated separately for each subpopulation and each subgroup therein, based on the number of cumulative notified cases (sourced from the Australian National Notifiable Diseases Surveillance System) reported until 14 days prior to the median specimen collection date, as a proportion of the relevant ABS Estimated Resident Population[[3]](#footnote-3). For example, general pathology samples were collected across all jurisdictions and age-groups between 19/06/2020 and 06/08/2020. As at 16/06/2020, which was 14 days prior to the median collection date of 30/06/2020, the cumulative number of notified COVID-19 cases across all ages in Australia was 7636. Based on an Australian population of 25,653,077, this corresponds to a lower bound of 0.0003 or 0.03%. While there was some variation in the lower bounds calculated for the analyses, most values fell between 0.02% and 0.04%. There were, however, values less than 0.01% calculated for age-groups 0–4yrs, 5–9yrs and 10–19yrs in the general pathology collection.

**Supplementary Table 1: Prior probability that the Wantai-positive seroprevalence was below specified thresholds assuming a restricted Beta(1,1) prior (primary analysis) and a restricted Beta(0.2,10) prior (sensitivity analysis), where lower bound was 0.03%.**

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| --- | --- |
|  | Prior probability that π is less than q |
| q  | Beta(1,1) restricted to π>0.03% | Beta(0.2,10) restricted to π >0.03% |
| 0.1% | 0.001 | 0.138 |
| 0.2% | 0.002 | 0.234 |
| 0.5% | 0.005 | 0.379 |
| 1% | 0.010 | 0.504 |
| 2% | 0.020 | 0.638 |

#### Supplementary data

**Supplementary Table 2: Estimated seroprevalence by jurisdiction and collection population for the primary and sensitivity analysis**

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| --- | --- | --- | --- |
| **Demographic group** | **Collection** | **Primary analysis** | **Sensitivity analysis**  |
|  |  | **% (95% CrI)** | **% (95% CrI)** |
| **Jurisdiction** |   |   |   |
| **ACT** | General pathology | 0.28 (0.03, 0.97) | 0.11 (0.03, 0.59) |
|  | Antenatal screening | 0.40 (0.03, 1.42) | 0.15 (0.03, 0.84) |
|  | Blood donors | 0.97 (0.03, 2.30) | 0.47 (0.03, 1.72) |
| **NSW** | General pathology | 0.40 (0.04, 0.95) | 0.23 (0.04, 0.75) |
|  | Antenatal screening | 0.79 (0.04, 1.67) | 0.48 (0.04, 1.35) |
|  | Blood donors | 0.77 (0.04, 1.74) | 0.44 (0.04, 1.37) |
| **NT** | General pathology | 1.39 (0.01, 2.99) | 0.82 (0.01, 2.36) |
|  | Antenatal screening | 1.02 (0.01, 3.30) | 0.27 (0.01, 1.87) |
|  | Blood donors | 0.47 (0.01, 1.48) | 0.14 (0.01, 0.95) |
| **QLD** | General pathology | 0.22 (0.02, 0.71) | 0.10 (0.02, 0.47) |
|  | Antenatal screening | 0.44 (0.02, 1.34) | 0.16 (0.02, 0.86) |
|  | Blood donors | 0.84 (0.02, 2.09) | 0.39 (0.02, 1.51) |
| **SA** | General pathology | 0.34 (0.02, 1.20) | 0.14 (0.02, 0.73) |
|  | Antenatal screening | 0.23 (0.02, 0.92) | 0.10 (0.02, 0.52) |
|  | Blood donors | 0.24 (0.02, 0.93) | 0.10 (0.02, 0.53) |
| **TAS** | General pathology | 0.42 (0.04, 1.28) | 0.19 (0.04, 0.87) |
|  | Antenatal screening | 0.45 (0.04, 1.53) | 0.19 (0.04, 0.96) |
|  | Blood donors | 0.77 (0.04, 2.08) | 0.36 (0.04, 1.44) |
| **VIC** | General pathology | 0.27 (0.03, 0.76) | 0.14 (0.03, 0.55) |
|  | Antenatal screening | 0.45 (0.04, 1.42) | 0.20 (0.04, 0.92) |
|  | Blood donors | 0.45 (0.03, 1.31) | 0.20 (0.03, 0.89) |
| **WA** | General pathology | 0.67 (0.02, 1.82) | 0.28 (0.02, 1.24) |
|  | Antenatal screening | 0.22 (0.02, 0.89) | 0.09 (0.02, 0.50) |
|  | Blood donors | 1.25 (0.02, 2.72) | 0.73 (0.02, 2.14) |

*Abbreviations: CrI, Credible interval; NSW, New South Wales; VIC, Victoria; QLD, Queensland; WA, Western Australia; SA, South Australia; TAS, Tasmania; ACT, Australian Capital Territory; NT, Northern Territory;*

**Supplementary Table 3: Estimated seroprevalence by age-group and collection population for the primary and sensitivity analysis**

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| **Demographic group** | **Collection** | **Primary analysis**  | **Sensitivity analysis**  |
|  |  | **% (95% CrI)** | **% (95% CrI)** |
| **Overall** |  |  |  |
| **All ages** | **General pathology** | 0.25 (0.03, 0.54) | 0.15 (0.03, 0.45) |
| **20-69 years** | **Blood donors** | 0.47 (0.04, 0.89) | 0.31 (0.04, 0.78) |
|   | General pathology | 0.25 (0.04, 0.57) | 0.15 (0.04, 0.46) |
| **20-39 years** | **Antenatal screening** | 0.23 (0.04, 0.54) | 0.14 (0.04, 0.44) |
|  | General pathology | 0.33 (0.04, 0.83) | 0.18 (0.04, 0.65) |
|   | Blood donors | 0.39 (0.04, 0.93) | 0.22 (0.04, 0.74) |
| **Age-group** |   |   |   |
| **0-4 years** | General pathology | 0.39 (0.01, 1.26) | 0.10 (0.01, 0.76) |
| **5-9 years** | General pathology | 0.39 (0.003, 1.26) | 0.08 (0.003, 0.74) |
| **10-19 years** | General pathology | 0.47 (0.01, 1.27) | 0.17 (0.01, 0.89) |
| **20-29 years** | General pathology | 0.48 (0.04, 1.25) | 0.24 (0.04, 0.93) |
|  | Antenatal screening | 0.20 (0.05, 0.54) | 0.12 (0.05, 0.41) |
|  | Blood donors | 0.55 (0.05, 1.36) | 0.30 (0.05, 1.04) |
| **30-39 years** | General pathology | 0.35 (0.03, 1.03) | 0.17 (0.03, 0.72) |
|  | Antenatal screening | 0.35 (0.03, 0.83) | 0.19 (0.03, 0.66) |
|  | Blood donors | 0.41 (0.03, 1.12) | 0.20 (0.03, 0.82) |
| **40-49 years** | General pathology | 0.38 (0.03, 1.13) | 0.17 (0.03, 0.77) |
|  | Blood donors | 0.79 (0.03, 1.75) | 0.46 (0.03, 1.41) |
| **50-59 years** | General pathology | 0.53 (0.04, 1.40) | 0.26 (0.04, 1.00) |
|  | Blood donors | 0.67 (0.04, 1.56) | 0.36 (0.04, 1.21) |
| **60-69 years** | General pathology | 0.24 (0.04, 0.83) | 0.13 (0.04, 0.55) |
|  | Blood donors | 0.56 (0.04, 1.39) | 0.30 (0.04, 1.05) |
| **70-79 years** | General pathology | 0.66 (0.04, 1.68) | 0.34 (0.04, 1.24) |
| **80+ years** | General pathology | 0.46 (0.02, 1.46) | 0.18 (0.02, 0.93) |

*Abbreviations: CrI, Credible interval.*

**Supplementary Table 4: Estimated seroprevalence by sex and collection population for the primary and sensitivity analysis**

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| **Demographic group** | **Collection** | **Primary analysis**  | **Sensitivity analysis**  |
|  |  | **% (95% CrI)** | **% (95% CrI)** |
| **Females** | General pathology | 0.25 (0.03, 0.59) | 0.14 (0.03, 0.47) |
|  | Antenatal screening | 0.23 (0.04, 0.54) | 0.14 (0.04, 0.44) |
|  | Blood donors | 0.60 (0.03, 1.17) | 0.38 (0.03, 1.00) |
| **Males** | General pathology | 0.29 (0.03, 0.67) | 0.17 (0.03, 0.55) |
|  | Blood donors | 0.42 (0.03, 0.90) | 0.23 (0.03, 0.73) |

*Abbreviations: CrI, Credible interval.*

1. Nicholson S, Karapanagiotidis T, Khvorov A, et al. Evaluation of six commercial SARS-CoV-2 serology assays detecting different antibodies for clinical testing and serosurveillance. Open Forum Infect Dis **2021**; <https://doi.org/10.1093/ofid/ofab239.> [↑](#footnote-ref-1)
2. Hueston L, Kok J, Guibone A, et al. The Antibody Response to SARS-CoV-2 Infection. Open Forum Infect Dis **2020**; 7:ofaa387. [↑](#footnote-ref-2)
3. Australian Bureau of Statistics. Estimated Resident Population (ERP) by State/Territory, Sex and Age to March 2020. Available at: <http://stat.data.abs.gov.au/index.aspx?DatasetCode=ERP_QUARTERLY.> Accessed 17 December 2020. [↑](#footnote-ref-3)