Specificity, positive predictive value and validation statistics in the context of CoViD-19

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During the CoViD-19 pandemic one can read and hear a lot about sensitivity and specificity, less about positive and negative predictive values and - unfortunately too little - also about exact validation parameters and their statistical evaluation. However, more and more voices criticize the currently available antibody detection tests due to insufficient performance[1,2]. Here we would like to explain what the key parameters of a test procedure are and why specificity is of crucial importance in the context of a pandemic.

Specificity: probability that a test procedure correctly identifies a sample lacking the target analyte as negative (true negative rate)

Sensitivity: probability that a test procedure correctly identifies a sample containing the target analyte as positive (true positive rate)

Positive predictive value: Probability that a sample tested positive actually contains the target analyte, or that a person with a positive test result is actually sick.

Negative predictive value: The probability that a sample tested negative actually does not contain the target analyte, or that a person with a negative test result is actually healthy.

In the case of the validation of a new testing procedure, specificity and sensitivity are determined experimentally using negative or positive samples that have been reliably classified in advance using other methods. Here, specificity is the proportion of negative samples correctly identified as negative by the test procedure to be validated. Sensitivity is the proportion of positive samples correctly identified as positive. In all properly performed validations, various interference samples known to be able to lead to false-positive or false-negative assay results are also measured. Especially in the context of the EU regulation on in vitro diagnostics (IVDR), which will be in effect from May 2022, a comprehensive and statistically accurate validation of diagnostic assays is essential[3].

Unfortunately, it is often forgotten that the values determined during validation (e.g. 99.0% specificity, 97.5% sensitivity) are of course only estimates of the actual specificity or sensitivity of the test and are therefore subject to statistical uncertainty. The significance of these estimates crucially depends on the sample size n (see Table 1)

95% confidence interval (Agresti-Coull) for the actual specificity / sensitivity of the assay

Validation value	n = 20	n = 100	n = 1.000	n = 10.000
100%	81.0 - 100%	95.6 - 100%	99.5 - 100%	99.95 - 100%
95%	74.6 - 100%	88.5 – 98.1%	93.5 – 96.2%	94.6 – 95.4%
90%	68.7 – 98.4%	82.4 – 94.7%	88.0 – 91.7%	89.4 – 90.6%

Table 1: Calculation of the 95% confidence interval for the actual values of specificity and sensitivity based on a given sample size n and the specificity / sensitivity in % determined based on this sample using the interval method according to Agresti-Coull[4]

Thus, with a calculated specificity of 100% determined by testing 100 negative samples, the actual specificity of the test is 95.6% or lower with a probability of 5%.

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Even if the parameters were determined with high accuracy in large samples, there is another problem with interpreting the results of the validated test. In a real test procedure, the diagnostic conclusiveness depends not only on the specificity and sensitivity of the test itself, but also on the prevalence of the target analyte or disease of interest in the test population. The corresponding parameter, the negative or positive predictive value, makes a statement about the probability for each individual tested that their respective test result is correct. As table 2 shows, especially for diseases with low prevalence, the sensitivity of a test should under no circumstances be equated with its positive predictive value.

Positive predictive value based on given specificity given and 100% sensitivity

Prevalence	95%	99%	99.9%	99.99%
0.1%	2.0%	9.1%	50%	90.9%
1%	16.8%	50.3%	91.0%	99.0%
5%	51.3%	84.0%	98.1%	99.8%
20%	83.3%	96.2%	99.6%	100%

Table 2: Probability that a positive tested sample of a person actually contains the target analyte (i.e. the person is actually sick) for a given prevalence in the test population (blue) and given specificity of the testing method (red). Calculation using the Bayes' theorem.

Applied to the current situation, this means that - under the optimistic assumption that 5% of the population have already produced specific antibodies against SARS-CoV2 because they overcame a CoViD-19 infection and with a specificity of 99% - each positively tested individual is immune with a probability of merely 84%. Such a low positive predictive value is simply not sufficient to bring geriatric nurses who were tested positive for antibodies back into contact with seniors in need of care, because in 16% of the cases these geriatric nurses could infect many risk patients after their own infection, which too often would go undetected due to the prior positive test and the high number of asymptomatic courses. At this point, a test with a specificity of more than 99.9% - ideally combined with a thorough analysis of any apparent symptoms - is essential to be able to reliably exclude such a risk.

In summary, it is a global challenge to develop intensely validated SARS-CoV2 antibody assays with high specificity in order to realize any plans to employ immunized individuals in critical areas. CANDOR offers modern solutions such as LowCross-Buffer®, PlateBlock™ or Liquid Plate Sealer® for the optimization of immunodiagnostics to significantly reduce false positives and false negatives.

References:

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