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Importance and significance of EQAS in the detection of SARS-CoV-2.
Here: INSTAND's "Virus genome detection - SARS-CoV-2" EQAS in April 2020: Evaluation of the PCR results

External Quality Assessment Schemes (EQAS) are interlaboratory comparisons for the purpose of external quality control in which control samples, that are defined by an EQA testing organization, are used to provide information on the performance of the test methods employed by the participating laboratories. It should be noted that the testing of these control samples does not always fully correspond to the testing used in everyday clinical practice as the EQAS do not assess the pre-analytical and post-analytical steps, including the medical reporting.

Under special circumstances like an epidemic, the quality of diagnostic testing is crucial for fulfilling laboratory reporting obligations, reliably assessing the epidemiological situation and for the measures derived from this. The number of conducted tests rose enormously within a short period of time. This has increased pressure on the testing labs while placing high demands on the sensitivity and specificity of the tests and on the evaluation of the test results.

INSTAND's "Virus genome detection - SARS-CoV-2" EQAS, conducted in April 2020, was one of the world's first EQAS to conduct external quality control on the molecular biological detection of SARS-CoV-2.
For details, please see:

- Report on this EQAS by INSTAND: https://www.instand-ev.de/System/rv-files/340%20EN%20SARS-CoV-2%20Genome%20EQAS%20April%202020%2020200502j.pdf

- "AG LABORATORY CAPACITY AT RKI (July 7, 2020); Report on the optimization of laboratory capacities for the direct and indirect detection of SARS-CoV-2 as part of a management of measures; Commentary on the EQAS for diagnostic testing of SARS-CoV-2 (INSTAND)", page 19 (Report available in German only): https://www.rki.de/DE/Content/InfAZ/N/Neuartiges_Coronavirus/Laborkapazitaeten.pdf;jsessionid=A64895B4D04A432B3C514B7C6C6890FD.internet052?__blob=publicationFile

On the basis of the report above, conclusions are drawn from the EQAS results about the reliability of the analytical procedures of the laboratories with respect to the various tests for detecting the genome of SARS-CoV-2 in negative test samples.
EQAS are primarily used for quality control purposes to verify the accuracy of testing labs in the processing of well-characterized and well-defined control samples using the tests they have introduced for their routine diagnostics. EQAS do not generally aim to make statements on the evaluation of diagnostic tests in analogy to clinical-diagnostic studies.

Details about the INSTAND EQAS in April 2020

A total of 463 laboratories took part in the EQAS, 284 of which were German laboratories. The participating laboratories each received a total of 7 control samples. Four of these control samples contained different concentrations of SARS-CoV-2 and three control samples were negative for SARS-CoV-2. The total number of results per control sample reported by the German laboratories was 596 since most of the participating laboratories used test systems for the detection of more than one gene region of SARS-CoV-2.
Overall, the results of all EQAS participants had success rates of between 97.8% and 99.7% for six out of the seven control samples. The seventh control sample, which had a SARS-CoV-2 dilution of 1:1,000,000 and was at the limit of detection, was used for educational purposes and had a success rate of 93.0%.

Conclusion 1:
INSTAND's "Virus Genome Detection - SARS-CoV-2" EQAS, which was conducted in April 2020 just three months after the first confirmed cases of SARS-CoV-2 infections in Germany, revealed a very high level of quality both in terms of the laboratories' analytical performance and the testing procedures used on the individual defined and pre-characterized samples at this point in time.

Commentary on the not accurate negative results of INSTAND's April 2020 EQAS for SARS-CoV-2 genome detection

The three negative control samples for SARS-CoV-2 were used in the EQAS to verify whether the participating laboratories were able to accurately confirm the negativity of the test samples that were negative for SARS-CoV-2. Two samples were used, each containing one other human corona virus (HCoV OC43 or 229E) and one sample with non-infected control cells (MRC-5 cells). For EQAS sample 340062 with MRC-5 cells (negative for SARS-CoV-2), 1.4% of the analyses did not report a "negative result".
These 1.4% not accurate negative results are based on a total of 14/983 analyses by all participants and can be broken down as follows:
A total of 7 results were reported as "positive" (0.7%) and 4 results were reported as "uncertain" (0.4%). Three results were incomplete (0.3%). In terms of the EQAS evaluation, this means that the above mentioned 1.4% not accurate negative test results should not be broadly regarded as being "false positive". Instead they were classified in the EQAS as false results.

A total of 596 results per sample were reported by 284 German laboratories in the April 2020 EQAS (results may be reported for more than one gene region per sample). Of these 284 German laboratories, only 3 laboratories reported a total of 2 false positive results/596 results (corresponding to 0.34 %) and 2 incomplete results/596 results (corresponding to 0.34 %). It should be noted that these percentages cannot be extrapolated for the total number of tests performed in Germany.

Conclusion 2:
The results of the EQAS in April 2020 for the negative sample (sample 340062 - with MRC-5 cells) reflects the very good testing performance of German laboratories. The 1.4% as "false" evaluated test results for this sample are not broadly considered in the report on the EQAS of April 2020 to be "false positive" in the sense of an overall medical assessment of the results and in no way allow for a statement to be made on the specificity and sensitivity of the tests used when examining negative SARS-CoV-2 samples.

Only 2 of the 284 German laboratories provided results that were clearly false positive. Since the aim of an EQAS is to ensure and improve quality, the two laboratories are now tasked with adopting measures to identify and eliminate the causes of the inaccurate results.

Statements on the analytical performance of PCR in detecting SARS-CoV-2 based on the results of the INSTAND EQAS in April 2020

The following general statements can be made:

- The results are **only** indicative of the participating laboratories, **not of all German laboratories**. In other words, the participating laboratories are only a sampling of the actual number of laboratories in Germany offering SARS-CoV-2 detection.
- The results of this EQAS primarily allow statements to be made about the analytical performance and reliability of the participating laboratories in connection with their test procedures and are a "snapshot" taken on the EQAS date in April 2020. It applies only to the analytical phase of the diagnostic testing cycle, whereas the overall diagnostic testing cycle includes other important steps that may influence the results (e.g. indication, sampling, transport, test performance including extraction, validation of the results, evaluation in the context of the findings, and communication to the physician and patient). Taking into account the analytical performance of the laboratories, the EQAS results only indirectly reflect the performance of the tests used with regard to specificity and sensitivity.
- Questions concerning pre- and post-analytics as well as medical validation, which are essential components in the routine diagnostic testing of samples from patients or test subjects, were not an element of INSTAND's "Virus genome detection - SARS-CoV-2" EQAS conducted in April 2020 and therefore cannot be answered.
  This EQAS did not go beyond the purely analytical evaluation of the results by assessing the patient's infection status as part of an overall medical interpretation. Due to the configuration of this EQAS, no overall medical interpretation could be made.
- The success rates reported in the EQAS (accurate results in percent) do not allow for general statements to be made about the specificity and sensitivity of the tests used by the laboratories. This concerns both the analytical and clinical sensitivity and/or specificity of the tests used.
- Defined study conditions with multiple determinations of a large number of control samples in the laboratories would be needed to determine the analytical specificity of the individual tests. The use of defined clinical samples and the comparison of different methods are needed to determine clinical specificity.

- Furthermore, it should be noted that - as in routine diagnostic testing - the results of the individual laboratories reported in the EQAS may also be influenced by human error, such as
  - swapping samples during measurement
  - carryover of positive material into negative samples
  - incorrect entries when reporting results.

Conclusion 3:

The results of this EQAS refer only to the participating laboratories and not to all German laboratories. It is not possible to derive information about the analytical specificity and sensitivity of certain tests from different manufacturers based on the results of the EQAS. Furthermore, EQAS percentages may not be extrapolated for the total number of tests performed in Germany. Such considerations are generally not the aim of an EQAS, which is intended to assess the analytical quality of a laboratory in connection with the test procedure it uses.

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