COVID-19 Pandemic: Issues with Laboratory Tests when Authorized for Emergency Use (EUA)

Presented by

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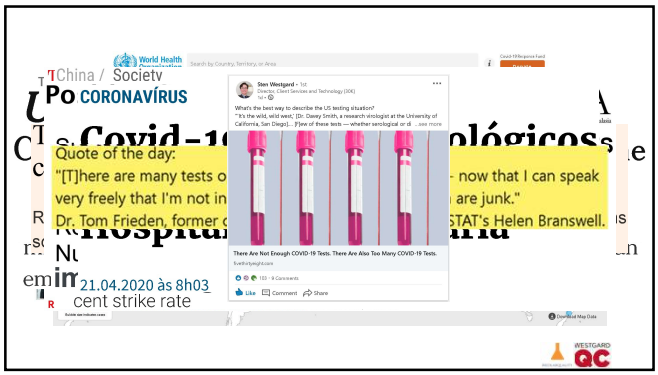
- Technical Expert on CLSI Document Development Committee on EP12-A3
- Author of several publications on quality control in the medical laboratory and in the Blood, Cells, Organs and Tissues Bank
- Author of the book "Quality control of qualitative tests for medical laboratories" (2019)

Introduction to the SARS-Cov-2 virus and COVID-19



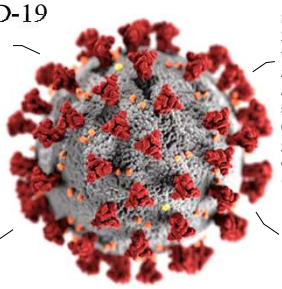
World Health Organization

Introduction to the SARS-Cov-2 virus and COVID-19

Possible increased pathogenicity and transmissibility

Possible zoonotic origins

Source of SARS-CoV-2 is unknown



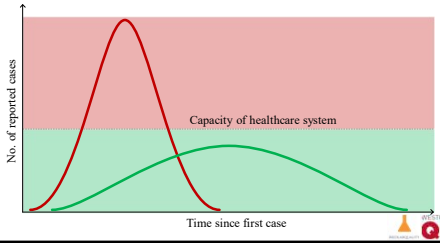
SARS-Cov-2 (previously 2019-nCov) a strain of the species *Severe acute respiratory syndrome-related coronavirus*, subgenus *Sarbecovirus* (beta-CoV lineage B), genus *Betacoronaviruses* of the family *Coronaviridae*

A positive-sense single-stranded RNA virus [(+)ssRNA virus]

Picture courtesy of the U.S. Centers for Disease Control and Prevention (CDC)

Introduction to the SARS-Cov-2 virus and COVID-19

- Epidemic curve impact on the healthcare system



What is happening with standardization and good practices?

What is happening with standardization and good practices?

- Europe and America were not prepared for an outbreak
- We have not learned from the lessons of Asia and Africa
- We have not learned from the history of pandemics
- Regulatory agencies skipped routine IVD assessments for SARS-Cov-2 tests, so they could be available quickly in the medical laboratory
- This approach suppressed strict supervision, which limits, from a clinical perspective, fitness for purpose / clinical decision
- Some manufacturers are still developing tests with the same scientific rigor, others may not be

What is happening with standardization and good practices?

- It is crucial to gradually implement the prequalifications required of IVD manufacturers, including specifications for sampling and variability between reagent lots
- The risk to clinical decisions based on laboratory results is higher
- Clinical decisions must be made incorporating many other variables, contrary to what would be expected with a screening test with high clinical sensitivity
- **Good laboratory practices have been reduced**
- **The risk of false results is increased**

What is happening with standardization and good practices?

- Process approach

Pre-Pre-Examination Processes	Pre-Examination Processes	Examination Processes	Post-Examination Processes	Post-Post-Examination Processes
• Good practices • The SARS-Cov-2 RNA RT-PCR should be selected for screening • The serological test for IgG / IgM tests should be selected to assess immunity	• Good practices • Non-compliance with causes in training	• Good practices • Quality of examination results must be ensured	• Good practices • Non-conformities in the results reports	• Good practices • Given the risk of false negatives, clinical decisions should also match the symptoms and risk group • Window period should be considered

What is happening with standardization and good practices?

- Several guidelines published by the WHO to fast-track testing
- Minimal harmonization of good laboratory practices
- It aims to get a quick response from the reference laboratories ("in-house" tests) and the IVD manufacturers

What is happening with standardization and good practices?

- WHO interim guidance for laboratory testing (updated March 19, 2020) and laboratory testing strategy recommendations for COVID-19 (updated March 22, 2020)
- Molecular assays to diagnose COVID-19 (FIND Web site and shared protocols for "in-house" developed molecular assays)
- WHO reference laboratories providing confirmatory testing for COVID-19 (WHO reference laboratories providing confirmatory testing for COVID-19, updated March 2, and booking form for national laboratories, updated March 11, 2020)
- Guidance for laboratories shipping specimens to WHO reference laboratories that provide confirmatory testing for COVID-19 virus (updated March 31, 2020)
- WHO interim guidance for laboratory biosafety related to COVID-19 virus (updated March 22, 2020)

What are the pros of "Emergency Use Only" validation?

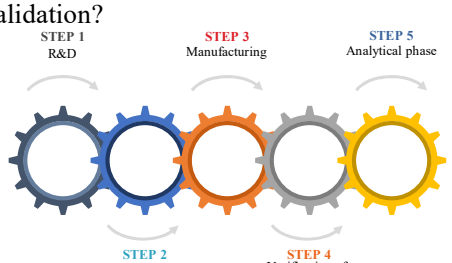
What are the pros of "Emergency Use Only" validation?

- Pros
- Simplification of the validation methodology
- Low cost
- Fast process of designing and developing a new kit
- Rapid implementation of new kits on the market
- Rapid reporting of laboratory results
- Clinical decisions based on laboratory results

What are the pros of "Emergency Use Only" validation?

- Potential for…
- Less quality and regulation (poor testing)
- Recognized manufacturers are developing new tests in a stressed production scenario
- Manufacturers of unknown quality are producing reagent kits
- Supply chains are strained, presenting a high risk of shortage at several points, such as the availability of raw materials and reagent kits on the market
- The effect of false results in an epidemic outbreak has been misestimated

What are the pros of "Emergency Use Only" validation?



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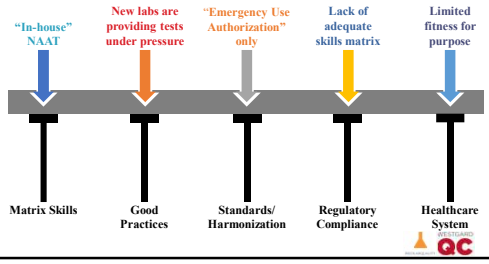


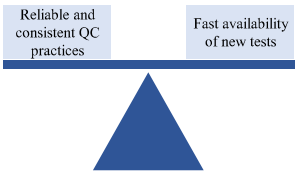
What is going on with *in vitro* diagnostic (IVD) medical devices?

What is going on with *in vitro* diagnostic (IVD) medical devices?

- Fast answer to the needs of tests by the manufacturers
- Introduction of new nucleic acid amplification tests (NAAT)
- Later introduction of serology, at this moment, mainly POCT
- The validation reports are a very simplified version and not harmonized to the commonly required European Commission or the FDA requirements
- Emergence of new manufacturers
- The performance verification in reference laboratories of some tests of new manufacturers led to their rejection
- Business opportunity

What is going on with *in vitro* diagnostic (IVD) medical devices?

- Balance



What is going on with *in vitro* diagnostic (IVD) medical devices?

- SARS-Cov-2 molecular assay independent evaluation by WHO/FIND/University Hospitals of Geneva

Manufacturer	Gene target	LoD*	Clinical sensitivity	Clinical specificity
A	E	1-10	92% (95% CI: 81, 97)	100% (95% CI: 96, 100)
A	S	1-10	100% (95% CI: 96, 100)	100% (95% CI: 96, 100)
B	ORF1	1-10	100% (95% CI: 93, 100)	99% (95% CI: 95, 100)
C	E	10-50	100% (95% CI: 93, 100)	100% (95% CI: 96, 100)
C	RdRP	50-100	90% (95% CI: 79, 96)	98% (95% CI: 93, 99)
D	S	1-10	100% (95% CI: 93, 100)	100% (95% CI: 96, 100)
E	RdRP	10-50	100% (95% CI: 93, 100)	100% (95% CI: 96, 100)
F	E	1-10	100% (95% CI: 96, 100)	100% (95% CI: 96, 100)

* Copies per reaction

What is the window period for NAAT and serology tests?

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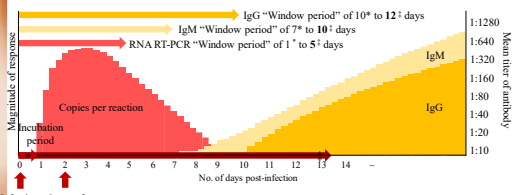


* The Royal College of Pathologists of Australasia statement no. 1/2020
‡ Handbook of COVID-19 prevention and treatment. Jack Ma Foundation and Alibaba Foundation

What QC suggestions can be given in this outbreak?

What QC suggestions can be given in this outbreak?

- Statistical tools in the outbreak
  - Binary examination agreement
  - Clinical performance evaluation
  - Evaluation of the limit of detection (LoD) in NAAT, i.e., minimum detectable concentration
  - Cross-reactivity studies
  - Serotypes/Genotype variation
  - Stability

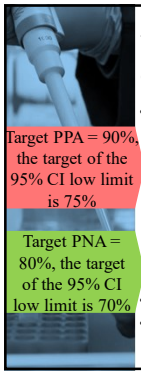
What QC suggestions can be given in this outbreak?

- Binary examination agreement

Candidate test results	Comparative test results: Positive	Comparative test results: Negative	Total
Positive	a	b	a + b
Negative	c	d	c + d
Total	a + c	b + d	N

- PPA[%] = a / (a + c)*100
- PNA[%] = d / (b + d)*100

A free tool is available at the Westgard QC Web site https://www.westgard.com/ to compute binary examination agreement



What QC suggestions can be given in this outbreak?

- Binary examination agreement

Candidate test results	Comparative test results: Positive	Comparative test results: Negative	Total
Positive	20	2	22
Negative	0	18	18
Total	20	20	40

- PPA = (30 / 30)*100 = 100% (95% CI: 84% - 100%)
- PNA = (30 / 30)*100 = 90% (95% CI: 70% - 97%)

Target PPA = 90%, the target of the 95% CI low limit is 75%

Target PNA = 80%, the target of the 95% CI low limit is 70%

What QC suggestions can be given in this outbreak?

- Binary examination agreement
- The **key point** is to have **a test with very high clinical accuracy as a comparator**
- There will always be an uncertainty in the agreement of the results
- If the comparator is a test with worse clinical accuracy than the new test, the results may be misinterpreted
- If the test is a "gold standard," the clinical accuracy is evaluated and not the agreement
- Associated to rare tests, such as some "in-house" tests

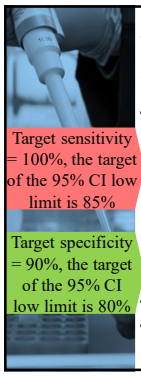
What QC suggestions can be given in this outbreak?

- Clinical performance evaluation

Candidate test results	Clinical accuracy criteria: Positive	Clinical accuracy criteria: Negative	Total
Positive	True-positive (TP) results	False-positive (FP) results	TP + FP
Negative	False-negative (FN) results	True-negative (TN) results	FN + TN
Total	TP + FN	FP + TN	N

A spreadsheet can be downloaded from MedLabQuality Web site https://medlabquality.com to compute the clinical performance evaluation, and LoD using probit regression

- Se[%] = TP / (TP + FN)*100
- Sp[%] = TN / (FP + TN)*100



What QC suggestions can be given in this outbreak?

- Clinical performance evaluation

Candidate test results	Clinical accuracy criteria: Positive	Clinical accuracy criteria: Negative	Total
Positive	30	0	30
Negative	0	30	30
Total	30	30	60

Target sensitivity = 100%, the target of the 95% CI low limit is 85%

Target specificity = 90%, the target of the 95% CI low limit is 80%

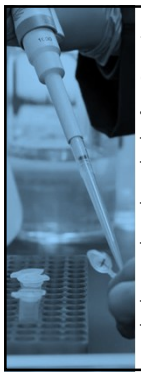
- Sensitivity = (30 / 30)*100 = 100% (95% CI: 89% - 100%)
- Specificity = (30 / 30)*100 = 100% (95% CI: 89% - 100%)

What QC suggestions can be given in this outbreak?

- Clinical performance evaluation
  - A number of samples must be taken to allow **statistical robustness** of calculations: infected individuals (D_1), and healthy individuals (D_0)
  - The **clinical robustness** of the evaluation requires samples that are epidemiologically representative of the tested population
  - The 95% CI should be used, since it illustrates the statistical robustness, as well as allowing the inference for a population with **the same characteristics of the samples**
  - The number of samples must suit the target of the evaluation
  - For example, for a *n* of 30 for the determination of clinical sensitivity, the 95% CI can never be tighter than the range of 87% to 100%

What QC suggestions can be given in this outbreak?

- Clinical performance evaluation
  - 95% CI is related to the **statistical power** of evaluation
  - The **clinical power** of evaluation is related to the representativeness of the infected samples
  - Samples of infected individuals are available in the outbreak
  - Negative sample could be taken from a serotec or rejected plasma bags



What QC suggestions can be given in this outbreak?

- Is it possible to have robust validations during an outbreak?
  - Yes, but…
  - Due to several limitations, the first validations must be carried out in reference laboratories according to harmonized practices
  - The biggest limitation is the limited number of infected samples and their limited variability at the beginning of the outbreak
  - All commercial tests should be **periodically revalidated**, mainly by national agencies, and the reported results should be public
  - National agencies should purpose performance goals
  - Performance targets should be **reviewed periodically**

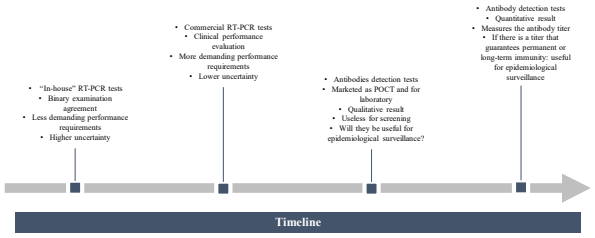
What QC suggestions can be given in this outbreak?

Goal 1
Improvement of good quality control practices

Goal 2
Clinical performance evaluation based on clinical sensitivity and clinical specificity

Goal 3
Compliance assessment

What QC suggestions can be given in this outbreak?



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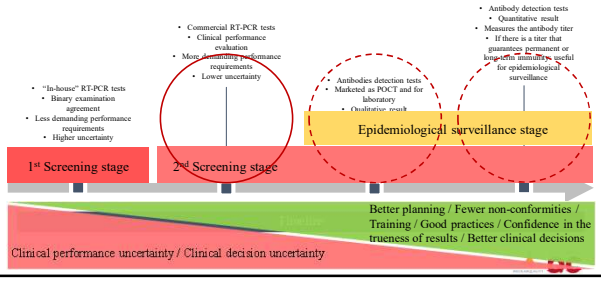
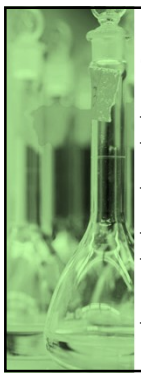


Conclusion



Conclusion

- There needs to be a plan for outbreaks like this, including for waves
- The importance of a reliable and consistent med lab results should be reinforced
- Regulatory agencies of IVD manufacturers and medical laboratories should also be prepared for this type of outbreak
- The regulation of IVD medical devices should be strengthened
- Preventive actions should be implemented to avoid even more serious consequences in pandemic outbreaks with more pathogenic agents
- WHO's importance and strength should be reinforced

Links and resources

- Webinar handouts (MedLabQuality)
- COVID-19 spreadsheet (MedLabQuality)
- Binary examination agreement online calculator (Westgard QC)
- Westgard QC Lesson - Basic Validation of Qualitative Tests
- European Centre for Disease Prevention and Control of European Union
- FIND Diagnostics - COVID-19 Diagnostics Research Centre (Data Base of Assays and Independent Test Evaluations)
- The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) - Information Guide on COVID-19

Links and resources

- US FDA- Coronavirus Disease 2019 (COVID-19)
- US Centers for Disease Control and Preventions - Laboratories
- The College of American Pathologists
- The Royal College of the Pathologists of Australasia
- NRL Australia
- Zhejiang University School of Medicine (Handbook of COVID-19 Prevention and Treatment)
- WHO - Country & Technical Guidance - Coronavirus disease (COVID-19)
- WHO database of publications on COVID-19

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