# 20° CONGRESO INTERNACIONAL

## CNB COLEGIO NACIONAL DE BACTERIOLOGÍA

Sostenibilidad, Globalización y Responsabilidad en el Diagnóstico.

**Bucaramanga**

# Quality Control in Infectious Serology

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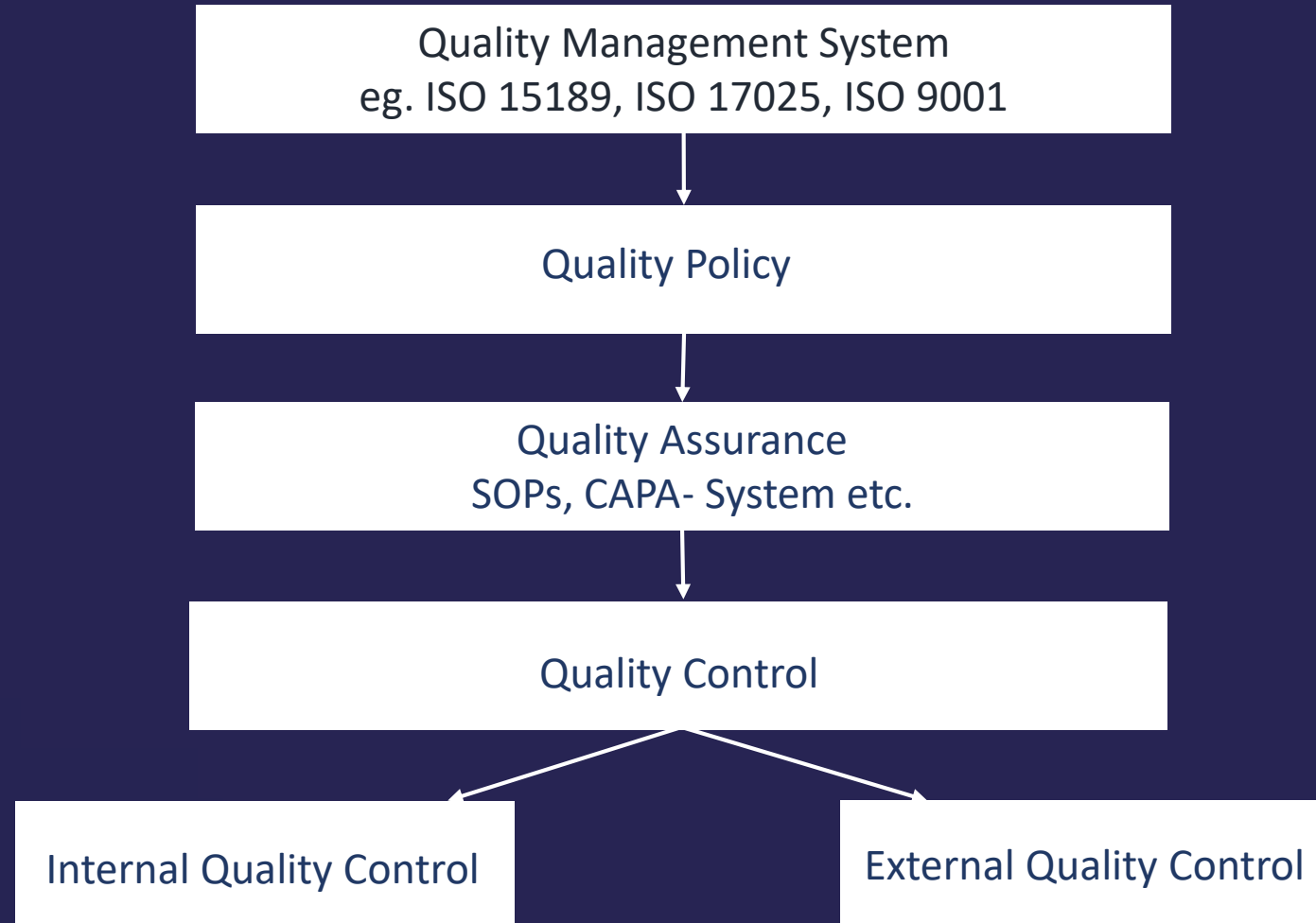
# Conflicts of Interest

- Study coordinator for External Quality Assessment surveys at ESfEQA GmbH Heidelberg/Germany

- ESfEQA is member of the corporate group MEX, including Diamex GmbH Heidelberg/Germany, a provider of Internal Quality Controls

# Quality Control in Infectious Serology

## Agenda

- Quality in Medical Laboratories

- Characteristics of Serology Assays

- Fundamental Differences between Clinical Chemistry and Serology

- Internal Quality Control in detail

- External Quality Control in detail

# Quality in Medical Laboratories

Quality Management System
eg. ISO 15189, ISO 17025, ISO 9001

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Quality Policy

↓

Quality Assurance
SOPs, CAPA- System etc.

↓

Quality Control

Internal Quality Control

External Quality Control

# Internal and External Quality Control
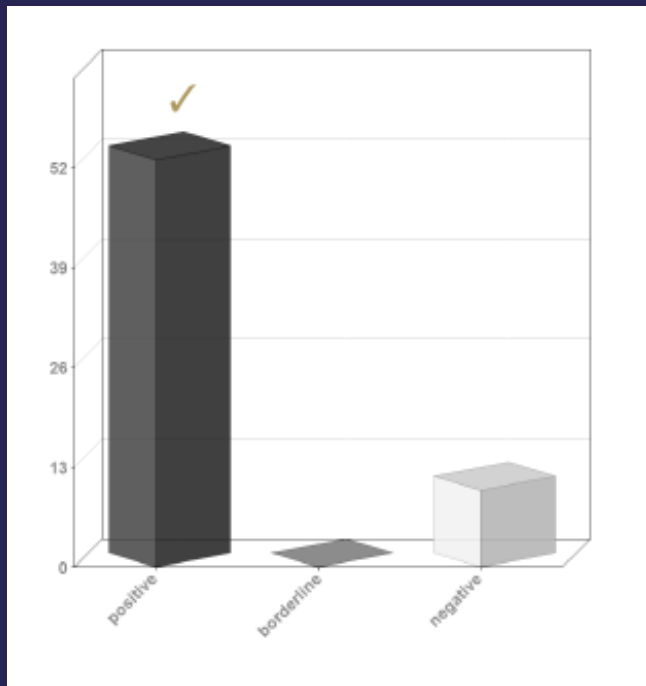
## Internal Quality Control

- Known analyte concentration
- Frequency: daily, per shift
- Comparison within a single laboratory
- Intralaboratory precision
- Check consistency of laboratory analytics/ performance of analytical system
- Day-to-day precision
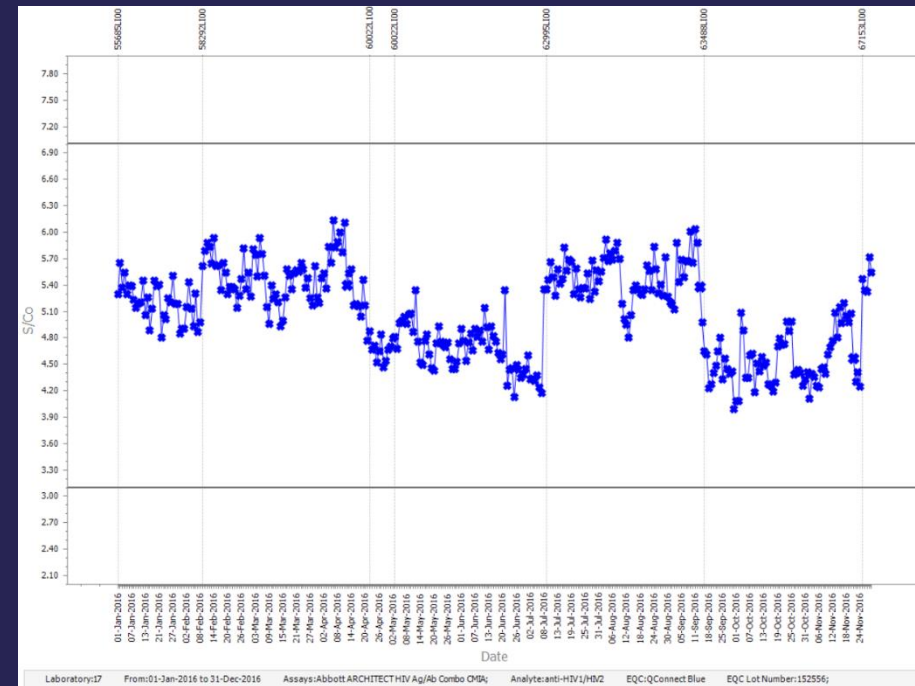
## External Quality Control

- Unknown analyte concentration
- Frequency: monthly, quarterly, semi-annually
- Comparison with other laboratories using the same analytical system / other analytical systems
- Interlaboratory accuracy
- Accuracy of test results
- Improvement of quality

# Internal and External Quality Control

## External QC

## Internal QC

# Why Quality Control?

- Accuracy for analytical serological results is crucial

- Early marker for any malfunction of the analytical system

- Internal and External Quality controls enhance the confidence in analytical results

- Reliable analytical results for the benefit of patients and for cost efficiency in the healthcare sector

# Why Quality Control?

Requirements of ISO standards

**ISO 15189:2012 Chapter 5.6.2.2 Quality control**

The laboratory shall use quality control materials that react to the examining system in a manner as close as possible to patient samples. Quality control materials shall be periodically examined......

**ISO 15189:2012 Chapter 5.6.3.1 Interlaboratory Comparison**

The laboratory shall participate in an interlaboratory comparison programme(s) (such as an external quality assessment programme or proficiency testing programme) appropriate to the examination and interpretation of examination results.

# Serological Assays

Serological Tests: Diagnostic methods to identify antibodies and antigens in a patient's sample

- Intention of Serological Tests:
  - Diagnosis of disease
  - Monitoring the efficacy of treatment
  - Identification of the disease stage
  - Evidence of immunity (e.g. anti-Rubella, anti-HBs)

- Historically use of test systems that utilize biological functions such as
  - Neutralization
  - Complement Fixation
  - Hemagglutination

# Development of Serological Assays

## Developments of Immunoassays in the 1980s

- Immunoassays revolutionized infectious disease serology
  - Various chemical detection systems are used in immunoassays, e.g enzyme-based color reaction (EIA), radioactive labels (RIA) fluorescence (IFA), chemiluminescence (CLIA) and electrochemiluminescence (ECLIA)

- Progress of automatization and development of high-thoughput analyzers

- Point-of-Care devices for serological testing

Today: there is a wide variety of serological tests available

# Analytical parameters of Serological Assays

State-of-the art immunoassay enable the differentiation of antibody targets

- Antibody class, e.g. IgA, IgG, IgM
- Antibody subclass, e.g. IgG1, IgG2, IgG3, IgG4
- Affinity of antibody
- Avidity of antibody
- Antigen-binding to specifc epitopes

# Diversity of Serological Assays

Detection system
- Utilization of biological functions (neutralization, hemagglutination etc.)
- RIA, IFA, ELISA, Chemiluminescence, Electrochemiluminescence

Antigen-Antibody Reaction
- Various sources of antigens are used by assay manufacturer
  - whole virus, disrupted virus, purified viral antigens, recombinant antigens
- Conjugates can be monoclonal, polyclonal from various sources, antibody fragments, directed to specific viral epitopes

# Diversity of Analyte

- Different antibody responses in different people

- Individuals respond to different viral antigens

- Primary antibody response matures over time starting with low avidity and affinity antibodies and gradually maturing

- Secondary immune response elicit high affinity response due to memory cells

# Consequences of Diversity

- The analyte, which is actually detected, will vary from test system to test system

- The difference in assay designs of the various manufacturers prevents the standardization of quantitative antibody determination

- The quantitative results of serological tests are not commonly used in clinical decision-making

- Nevertheless, the clinical sensitivity and specificity of various tests are usually well comparable

# Serological Tests: Qualitative or Quantitative?

- Immunoassays: there is a dose response, e.g. the signal (enzyme activity, radioactivity, chemiluminescence) increases with the amount of bound antibody

- The dose-response curve is specific to the test system, it is usually not linear but sigmoidal
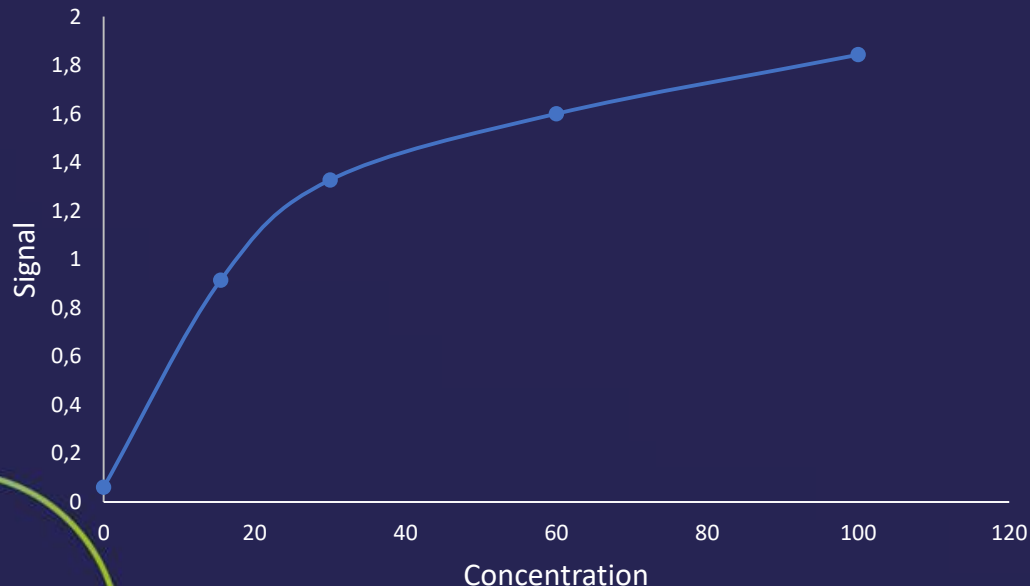
Results of infectious disease serology tests are derived from a quantitative result of antibody/antigen binding efficacy but are interpreted as qualitative results

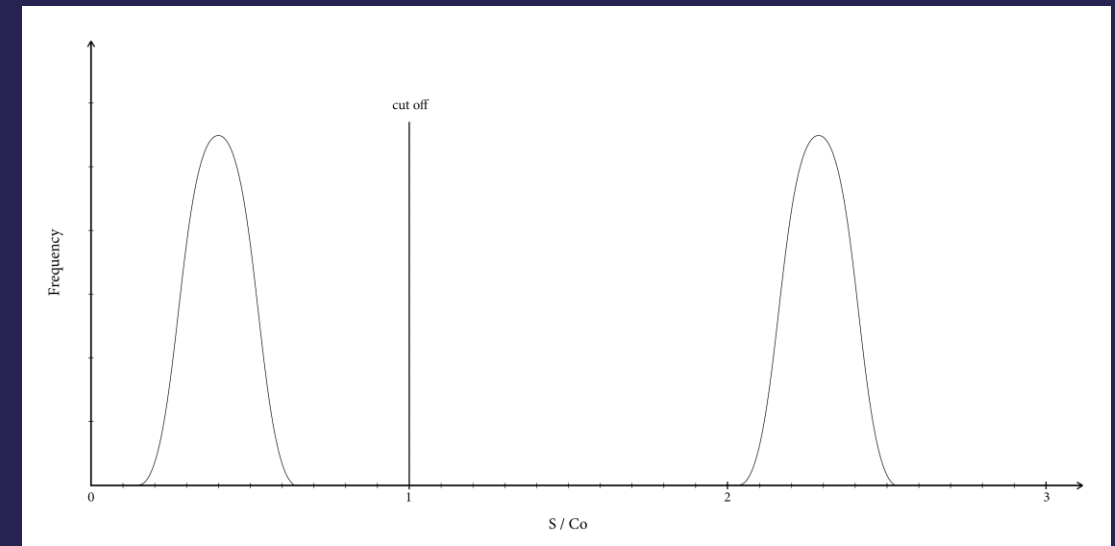# Fundamental differences between Clinical Chemistry and Serology

**Clinical Chemistry**

- Normal (Gaussian) distribution
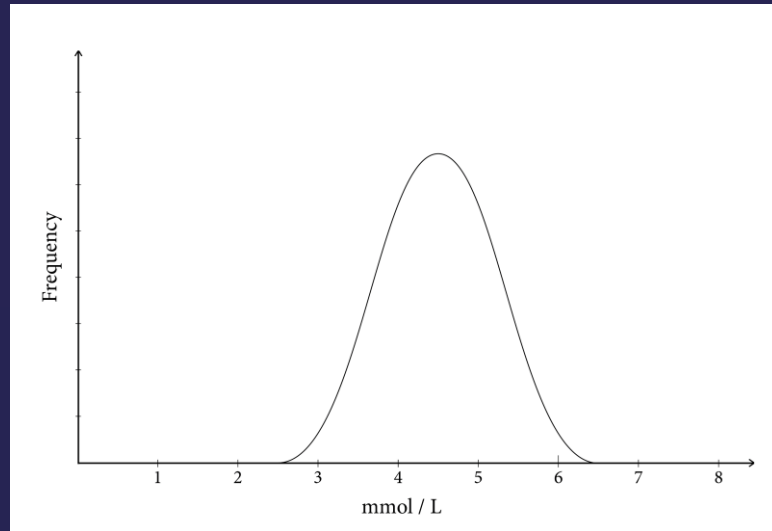


**Serology**

- Separation between positive and negative samples

# Quantitative Results in Infectious Serology

Attempts to quantify serological assays

- International standards for
  - Anti-Rubella IgG
  - Anti-HBs
  - Anti-Measles IgG (WHO standard NIBSC 97/648)
  - SARS-CoV-2 (20/136)

- International standards were developed to assess the potency of vaccines for Rubella and Measles
- Despite these attempts, quantitative results of anti-Rubella IgG are not well-comparable

# Fundamental differences between Clinical Chemistry and Serology

## Clinical Chemistry

- Inert, chemically well characterized analyte (e.g. Sodium, Glucose)
- The analyte is identical in every individual
- Several medical decision points
- Quantitative
- Traceability hierarchy to reference standards or reference methods
- Linear dose response curves
- Adjustment for reagent lot variation

## Serology

- Functional biological analyte, polyclonal, directed versus various epitopes of an antigen
- Single decision point (pos/neg)
- Qualitative
- Not directly traceable to SI units
- Nonlinear dose response curve
- No adjustment for reagent lot variation

Reference: Dimech Wayne. The Standardization and Control of Serology and Nucleic Acid Testing for Infectious Diseases. Clinical Microbiology Reviews October 2021 Volume 34, Issue 4 e00035-21

# Clinical Chemistry and Serology QC samples

## Clinical Chemistry

- The analyte concentration should cover the measuring range of the assay

- One IQC material that fits for all assays

- EQA samples in general human plasma spiked with analytes to the desired concentration

## Serology

- The positive IQC should be adjusted so that the signal is slightly above the cut-off value

- There is not a control material that can be universally used for all assays for an individual parameter (e.g. anti-HIV)

- New reagent lots cause changes in the reactivity of the QC material – target values need to be adjusted

- EQA samples from human donors

# Internal Quality Control

## Data Management

- Results collected after each test run
- Well established guidelines for quality control in Clinical Chemistry cannot be simply applied to serology testing
- Displayed graphically
- Acceptance rules need to be established
- Requires immediate action if unexpected results are detected
- QC samples are a tool – results need to be interpreted

Monitoring QC results without reference to a peer-group only monitors precision

# Internal Quality Control
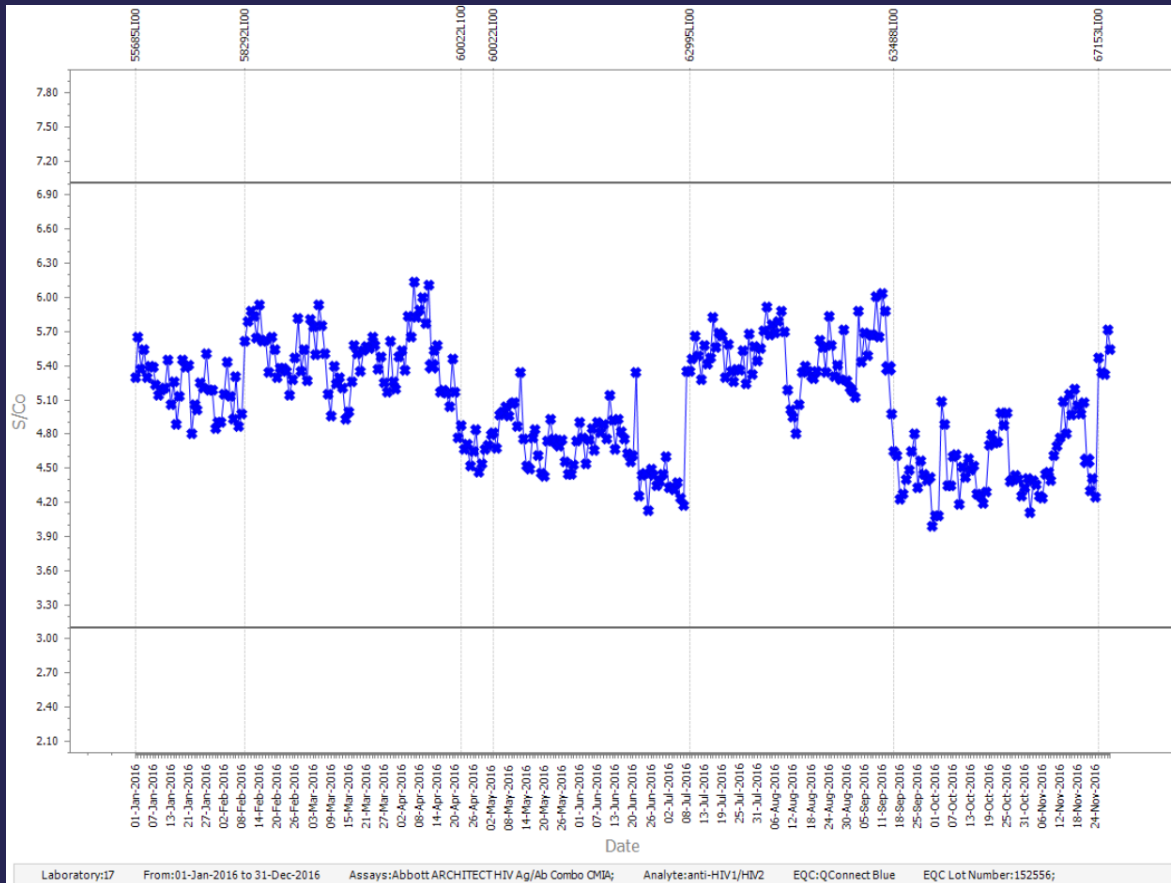


Levey-Jennings Graph

Continous monitoring of IQC-results and their variation

# Properties of ideal serology IQC

- Signal of the positive IQC is just above the cut-off value
- Matrix is 100% human material to avoid any matrix-effects
- Indepency from instrument and/or reagent manufacturer
- High lot-to-lot consistency of IQC material to elimate an additional variability in serology testing
- Stable QC material, preferably liquid, ready-to-use
- QC results of laboratories using the same IQC samples are collected into a single database, allowing comparison of QC results across laboratories, instrument, reagent lots and operators
- Acceptance limits are calculated for each IQC/reagent lot combination
- Ideal serological IQC requires 'sample – software – service' approach

# Properties of ideal serology EQC

- Stable, homogenous material
- Matrix is 100% human material to avoid any matrix-effects (commutability)
- Samples from single donors, not a pool of samples
- Clinical data of the donors are available
- Samples are pretested on various commercial analytical systems
- High variation of samples, e.g. originated from various donors, different antibody titers
- Positive samples should be above the cut-off of 'commonly used reagents'
- Include challenging samples, e.g., samples taken close to seroconversion to challenge the sensitivity of the assay (true low positive samples occur only during seroconversion not by dilution)

# Sources of EQA samples



Samples from clinically characerized, single-donors, e.g. obtained by plasmapheresis, is the EQA material of choice

# Clinical Data for serological EQC samples

## Clinical data of the single donor of the EQA sample

**Example 1**

- Sample derived from a single donor (female, age of 19 years), who had recovered from COVID-19. The date of the blood plasma donation was May 5th, 2020. The donor had been previously tested positive for SARS-CoV-2 RNA on April 14th, 2020. The presumable date of infection was April 8th, 2020. COVID-19 related symptoms of the donor were fever, shivers, headache, limp pain, coughing, diarrhoea, sore throat, catarrh, and anosmia.

**Example 2**

- Sample derived from a single donor with acute Hepatitis B infection and HBe seroconversion

# Examples External QC

Parameter: anti-Treponema pallidum antibodies

- Results of 105 participants (participants used 32 different reagents)
  - 102 reported a positive result
  - 3 reported a false-negative result
- High Commutability

Question: False-negative results due to sensitivity of the assay or due to laboratory performance?
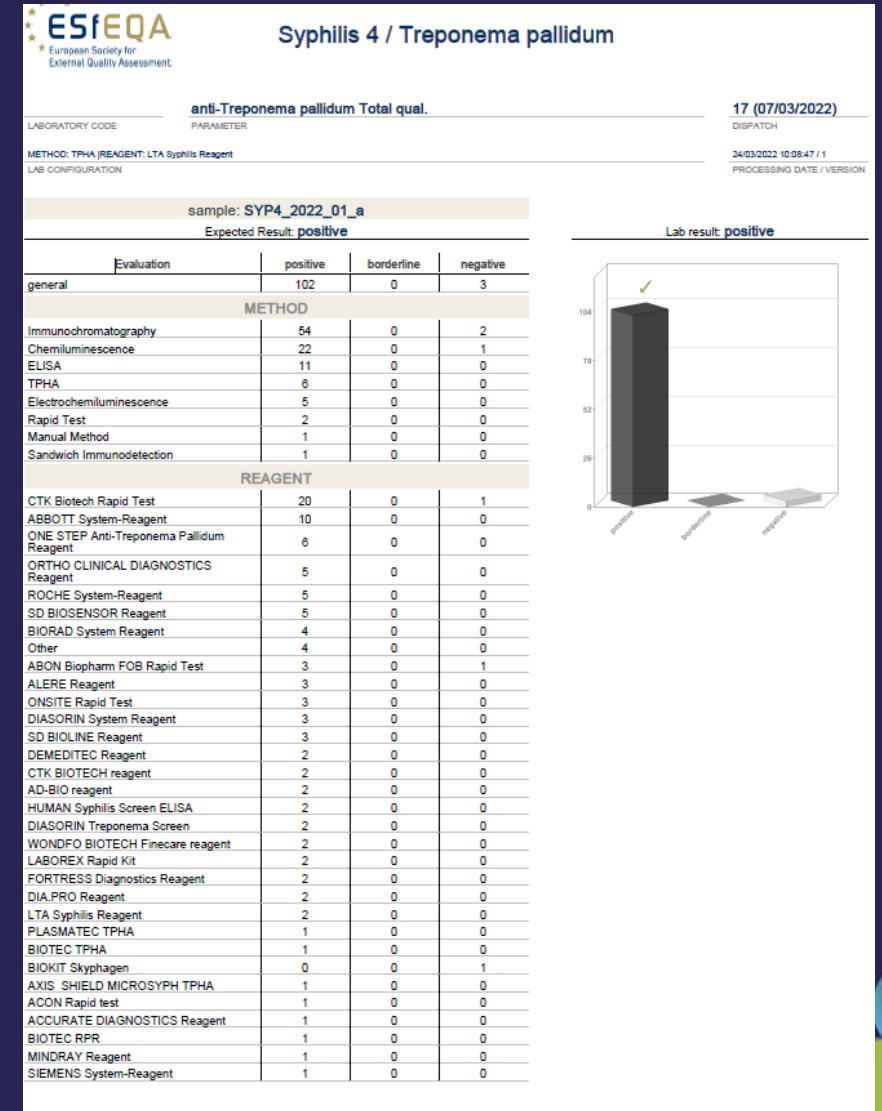
# Example External QC

False-negative result due to sensitivity of the assay or due to laboratory performance?

| sample: SYP4_2022_01_a | | | |
|---|---|---|---|
| Expected Result: **positive** | | | |
| Evaluation | positive | borderline | negative |
| general | 102 | 0 | 3 |
| REAGENT | | | |
| CTK Biotech Rapid Test | 20 | 0 | 1 |

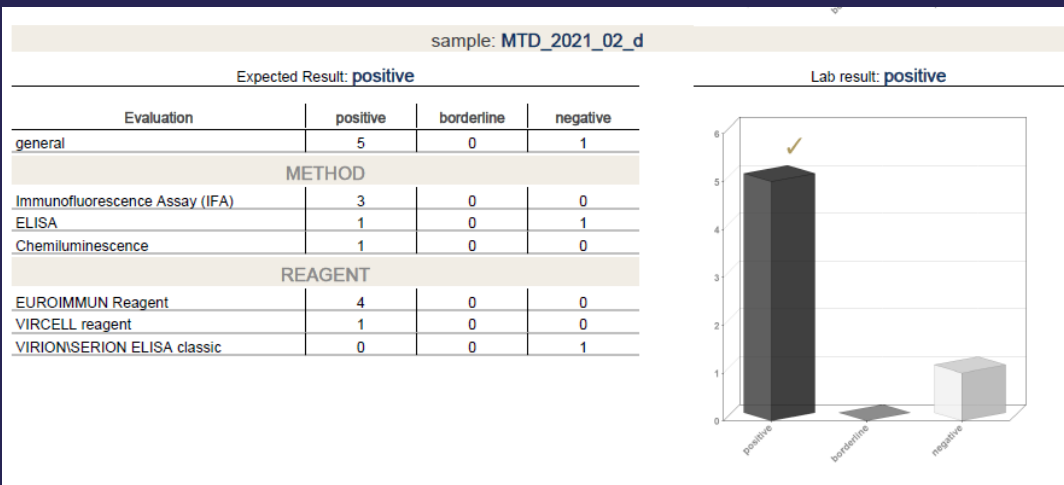20 participants reported a positive results with one specific reagent type, 1 participant a negative result

Conclusion: laboratory related issue, not a sensitivity issue of the reagent
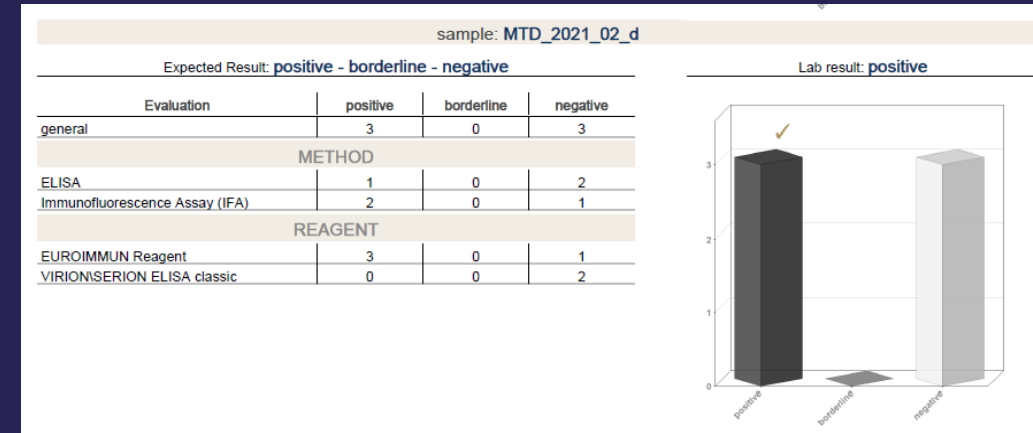
# Examples External QC



HBV antigens and antibodies in the blood

Bozza et al. Hepatitis B and cancer: A practical guide for the oncologist. Critical Reviews in Oncology/Hematology Volume 98, February 2016, Pages 137-146
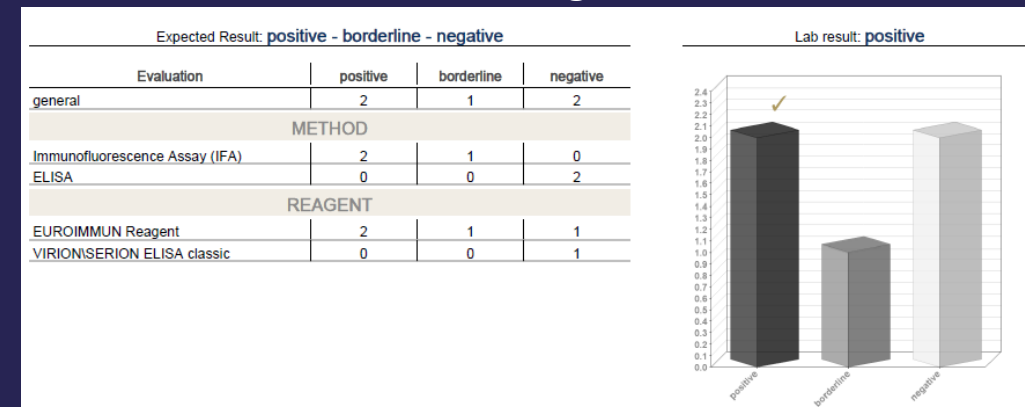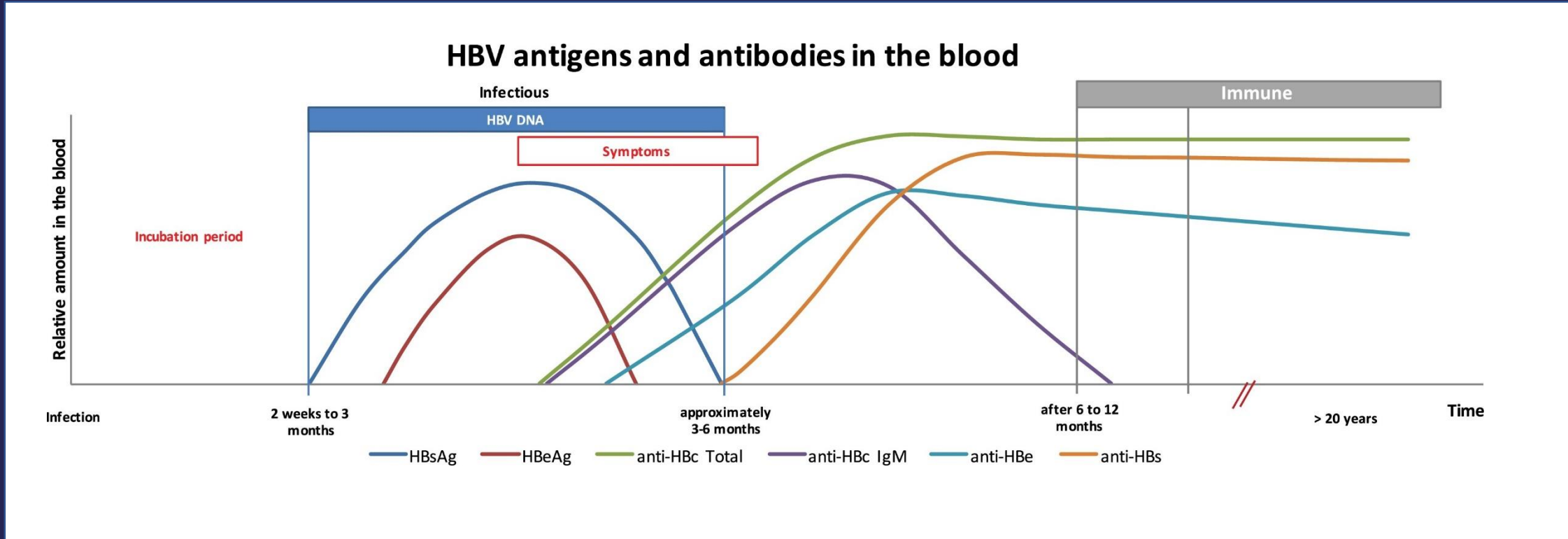
# Examples EQC: Assay Sensitivity

| sample: INF4_2022_02_b | | | |
|---|---|---|---|
| **Expected Result:** | | | |
| Evaluation | positive | borderline | negative |
| general | 48 | 0 | 4 |
| **INSTRUMENT** | | | |
| SIEMENS Immulite | 2 | 0 | 0 |
| BIOTEK INSTRUMENTS ELISA reader | 1 | 0 | 0 |
| CTK Biotech Rapid Test | 1 | 0 | 0 |
| DIAMEDIX MAGO | 1 | 0 | 0 |
| MICROPOINT Rapid One-step Test Card | 1 | 0 | 0 |
| SIEMENS ATELLICA | 1 | 0 | 0 |
| **INSTRUMENT GROUP** | | | |
| Roche Cobas | 17 | 0 | 1 |
| Mindray BS Series | 1 | 0 | 0 |
| SIEMENS ADVIA Group | 3 | 0 | 0 |
| DIASORIN LIAISON (XL) | 3 | 0 | 1 |
| BECKMAN COULTER ACCESS/DXI | 1 | 0 | 0 |
| bioMérieux VIDAS/ mini VIDAS | 1 | 0 | 0 |
| Manual Testing | 1 | 0 | 0 |
| ABBOTT Architect | 12 | 0 | 0 |
| ORTHO CLINICAL DIAGNOSTICS Vitros | 0 | 0 | 2 |
| Abbott CELL-DYN HEM5D Instruments | 1 | 0 | 0 |

Lab result: **positive**
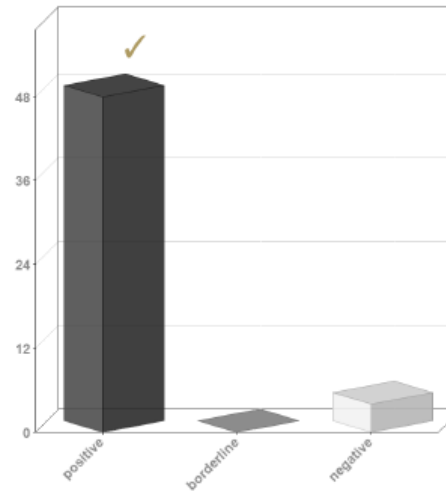
Anti-HBc antibodies

EQA sample from a single donor with a chronic Hepatitis B infection

92 % of the participants reported a positive anti-HBc result in this survey sample

# Examples EQC: Assay Sensitivity

EQA sample from a single donor with a chronic Hepatitis B infection

- Only 42 % of the participants reported a positive HBsAg result

- 46% of participants who reported a false-negative HBsAg result used a manual test

sample: **INF4_2022_02_b**

Expected Result:

Lab result: **positive**

HBsAg

| Evaluation | positive | borderline | negative |
|---|---|---|---|
| general | 75 | 17 | 87 |
| **INSTRUMENT** | | | |
| BIORAD IMARK | 0 | 0 | 2 |
| CTK Biotech Rapid Test | 0 | 0 | 2 |
| OTHER | 0 | 0 | 2 |
| SIEMENS Immulite | 1 | 0 | 1 |
| ADALTIS Personal Lab | 0 | 0 | 1 |
| BIORAD Plate reader 680 | 0 | 0 | 1 |
| DIAMEDIX MAGO | 0 | 0 | 1 |
| MICROPOINT Rapid One-step Test Card | 0 | 0 | 1 |
| ROBONIK Reagent | 0 | 0 | 1 |
| SIEMENS ATELLICA | 1 | 0 | 0 |
| BIOTEST RightSign | 0 | 0 | 1 |
| SNIBE MAGLUMI 2000 | 1 | 0 | 0 |
| THUNDERBOLT Analyzer | 0 | 1 | 0 |
| ABON | 0 | 0 | 1 |
| RAYTO RT Series | 0 | 0 | 1 |
| FORTRESS DIAGNOSTICS Analyst 2010 | 1 | 0 | 0 |
| BIOTEK INSTRUMENTS ELISA reader | 0 | 1 | 0 |
| **INSTRUMENT GROUP** | | | |
| Roche Cobas | 35 | 12 | 4 |
| SIEMENS ADVIA Group | 3 | 0 | 0 |
| DIASORIN LIAISON (XL) | 4 | 0 | 1 |
| DYNEX Technologies DSX/DS2 | 0 | 0 | 1 |
| BECKMAN COULTER ACCESS/DXI | 0 | 0 | 3 |
| bioMérieux VIDAS/ mini VIDAS | 1 | 0 | 2 |
| Manual Testing | 2 | 1 | 40 |
| AWARENESS TECHNOLOGY | 0 | 0 | 1 |
| DIALAB | 0 | 0 | 6 |
| HITACHI Instrument | 0 | 1 | 0 |
| ABBOTT Architect | 23 | 1 | 0 |
| HUMAN Semi-automated ELISA reader | 0 | 0 | 7 |
| SNIBE Maglumi | 0 | 0 | 2 |
| ORTHO CLINICAL DIAGNOSTICS Vitros | 0 | 0 | 5 |
| Abbott CELL-DYN HEM5D Instruments | 1 | 0 | 0 |
| WONDFO BIOTECH Finecare FIA Meter | 1 | 0 | 0 |
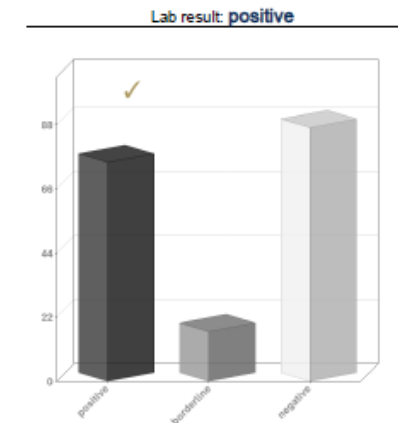
# Examples EQC: Seroconversion Sample

## HBeAg qualitative



sample: HBV_2022_02_a

Expected Result: positive - borderline - negative

Lab result: negative

| Evaluation | positive | borderline | negative |
|---|---|---|---|
| general | 11 | 2 | 17 |
| **INSTRUMENT** | | | |
| BIOTEK INSTRUMENTS ELISA reader | 1 | 0 | 0 |
| SYSMEX HISCL-5000 | 1 | 0 | 0 |
| **INSTRUMENT GROUP** | | | |
| Roche Cobas | 3 | 0 | 17 |
| DIASORIN LIAISON (XL) | 4 | 2 | 0 |
| ABBOTT Architect | 1 | 0 | 0 |
| Abbott CELL-DYN HEM5D Instruments | 1 | 0 | 0 |
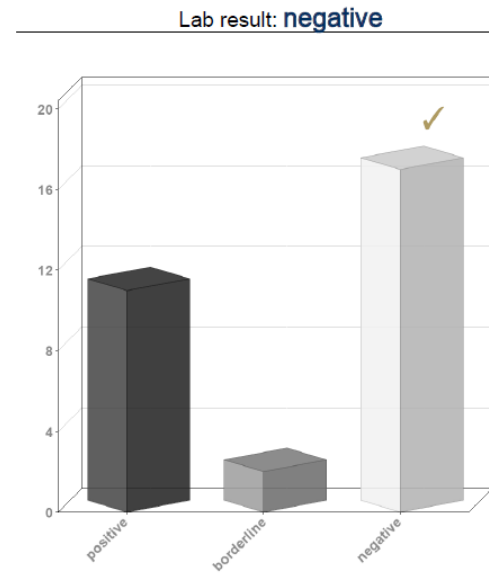
EQA sample from a single donor with an acute Hepatitis infection: anti-HBc IgM positive; onset of HBeAg seroconversion

Divers results of participants

## Anti-HBe antibodies qualitative



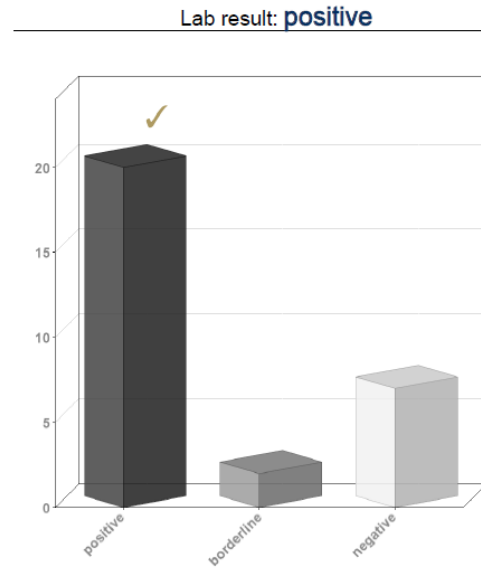| sample: HBV_2022_02_a | | | |
| --- | --- | --- | --- |
| Expected Result: positive - borderline - negative | | | |
| Evaluation | positive | borderline | negative |
| general | 20 | 2 | 7 |
| INSTRUMENT | | | |
| BIOTEK INSTRUMENTS ELISA reader | 0 | 0 | 1 |
| SYSMEX HISCL-5000 | 0 | 0 | 1 |
| INSTRUMENT GROUP | | | |
| Roche Cobas | 17 | 0 | 2 |
| DIASORIN LIAISON (XL) | 2 | 2 | 2 |
| ABBOTT Architect | 1 | 0 | 0 |
| Abbott CELL-DYN HEM5D Instruments | 0 | 0 | 1 |

Lab result: **positive**

EQA sample from a single donor with an acute Hepatitis infection: anti-HBc IgM positive; onset of HBeAg seroconversion

Divers results of participants

# Examples EQC: Consistent qualitative results

## Anti-Rubella IgG qualitative results (positive sample)



| sample: ToRCH_2022_02_a | | | |
|---|---|---|---|
| **Expected Result: positive** | | | |
| Evaluation | positive | borderline | negative |
| general | 66 | 0 | 1 |
| REAGENT | | | |
| ROCHE System-Reagent | 28 | 0 | 0 |
| DIASORIN System Reagent | 8 | 0 | 0 |
| BIOMERIEUX Reagent | 6 | 0 | 0 |
| ABBOTT System-Reagent | 5 | 0 | 1 |
| SNIBE System Reagent | 4 | 0 | 0 |
| SIEMENS System-Reagent | 3 | 0 | 0 |
| BECKMAN COULTER System Reagent | 2 | 0 | 0 |
| DIA.PRO Reagent | 2 | 0 | 0 |
| ORTHO CLINICAL DIAGNOSTICS Reagent | 2 | 0 | 0 |
| VIRCELL reagent | 2 | 0 | 0 |
| ERBA MANNHEIM System-Reagent | 1 | 0 | 0 |
| EUROIMMUN Reagent | 1 | 0 | 0 |
| VIRION SERION agile | 1 | 0 | 0 |
| VIRION\SERION ELISA classic | 1 | 0 | 0 |

Lab result: **positive**
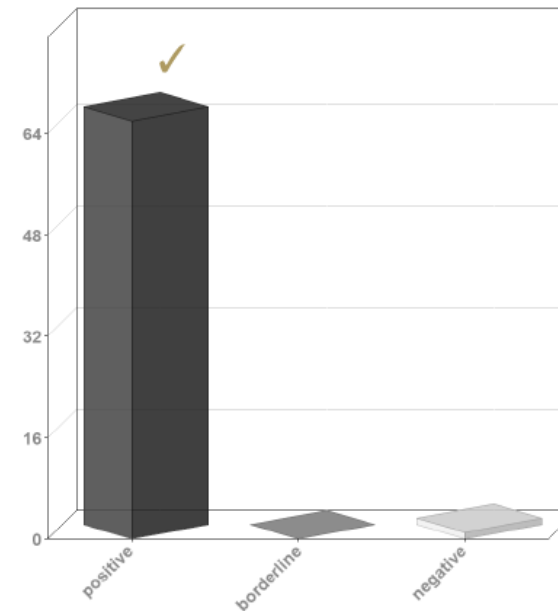
# Examples EQC: Consistent qualitative results

## Anti-Rubella IgG qualitative results (negative sample)



sample: ToRCH_2022_02_b
Expected Result: **negative**

| Evaluation | positive | borderline | negative |
|---|---|---|---|
| general | 2 | 0 | 48 |
| REAGENT | | | |
| ROCHE System-Reagent | 1 | 0 | 10 |
| DIASORIN System Reagent | 0 | 0 | 8 |
| BIOMERIEUX Reagent | 0 | 0 | 6 |
| ABBOTT System-Reagent | 1 | 0 | 5 |
| SNIBE System Reagent | 0 | 0 | 4 |
| SIEMENS System-Reagent | 0 | 0 | 3 |
| BECKMAN COULTER System Reagent | 0 | 0 | 2 |
| DIA.PRO Reagent | 0 | 0 | 2 |
| ORTHO CLINICAL DIAGNOSTICS Reagent | 0 | 0 | 2 |
| VIRCELL reagent | 0 | 0 | 2 |
| ERBA MANNHEIM System-Reagent | 0 | 0 | 1 |
| EUROIMMUN Reagent | 0 | 0 | 1 |
| VIRION SERION agile | 0 | 0 | 1 |
| VIRION\SERION ELISA classic | 0 | 0 | 1 |

Lab result: **negative**

TR0195

# Examples EQC: Lack of Quantitative Assay Standardization

## Anti-Rubella IgG quantitative



Wide distribution of quantitative results

# Examples EQC: Lack of Quantitative Assay Standardization

- Anti-Rubella IgG quantitative



sample: **ToRCH_2022_02_a**
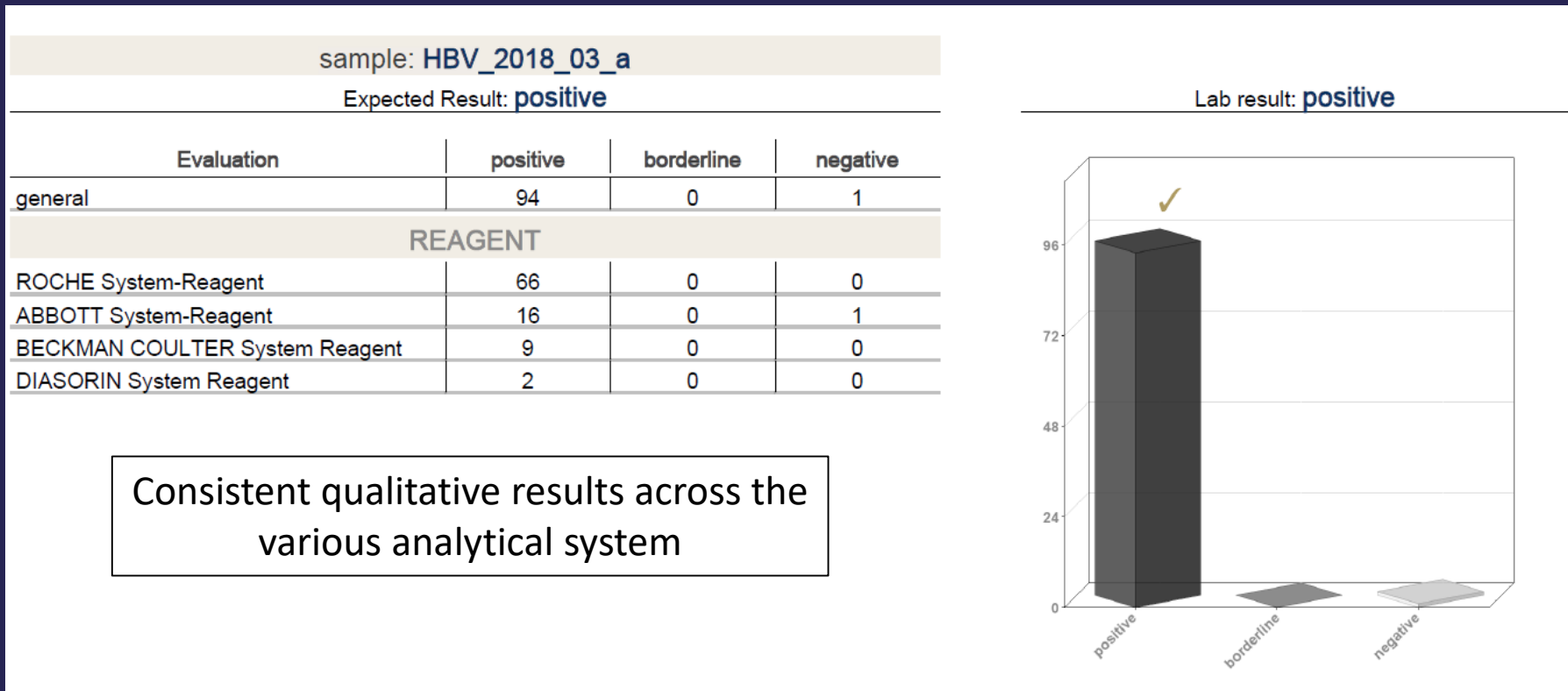
**Statistical Comparison**

| Evaluation | Target value AlgoA | SD set by coordinator | | Uncertainty * | CV | Number of Labs | Number of results |
|---|---|---|---|---|---|---|---|
| 1  general | 198.8 | 19.88 | - | 28.62 | 10 | 42 | 42 |
| **REAGENT** | | | | | | | |
| 2  DIASORIN System Reagent | 191.3 | 19.13 | - | 29.24 | 10 | 9 | 9 |
| 3  ABBOTT System-Reagent | 319.5 | 31.95 | - | 11.79 | 10 | 7 | 7 |
| 4  BIOMERIEUX Reagent | 300.8 | 30.08 | - | 21.39 | 10 | 6 | 6 |
| 5  ROCHE System-Reagent | 48.52 | 4.85 | - | 9.93 | 10 | 5 | 5 |

\* Uncertainty of the assigned value

Reagent-specific quantitative results

# Examples EQC: Lack of Quantitative Assay Standardization

## Anti-HBs qualitative

sample: HBV_2018_03_a

Expected Result: **positive**

| Evaluation | positive | borderline | negative |
|---|---|---|---|
| general | 94 | 0 | 1 |
| REAGENT | | | |
| ROCHE System-Reagent | 66 | 0 | 0 |
| ABBOTT System-Reagent | 16 | 0 | 1 |
| BECKMAN COULTER System Reagent | 9 | 0 | 0 |
| DIASORIN System Reagent | 2 | 0 | 0 |

Lab result: **positive**

Consistent qualitative results across the various analytical system

# Examples EQC: Lack of Quantitative Assay Standardization

## Anti-HBs quantitative IU/mL

sample: HBV_2018_03_a

### Statistical Comparison

| Evaluation | Target value AlgoA | SD set by coordinator | | Uncertainty * | CV | Number of Labs | results |
|---|---|---|---|---|---|---|---|
| **REAGENT** | | | | | | | |
| 1 ROCHE System-Reagent | 269.4 | 26.94 | - | 7.06 | 10 | 27 | 27 |
| 2 ABBOTT System-Reagent | 186.3 | 18.63 | - | 8.42 | 10 | 14 | 14 |
| 3 BECKMAN COULTER System Reagent | 76.27 | 7.63 | - | 19.82 | 10 | 7 | 7 |

\* Uncertainty of the assigned value

## Diversity for quantitative anti-HBs results

Examples EQC: Lack of Quantitative Assay Standardization

Anti-HBs quantitative: Roche Reagent
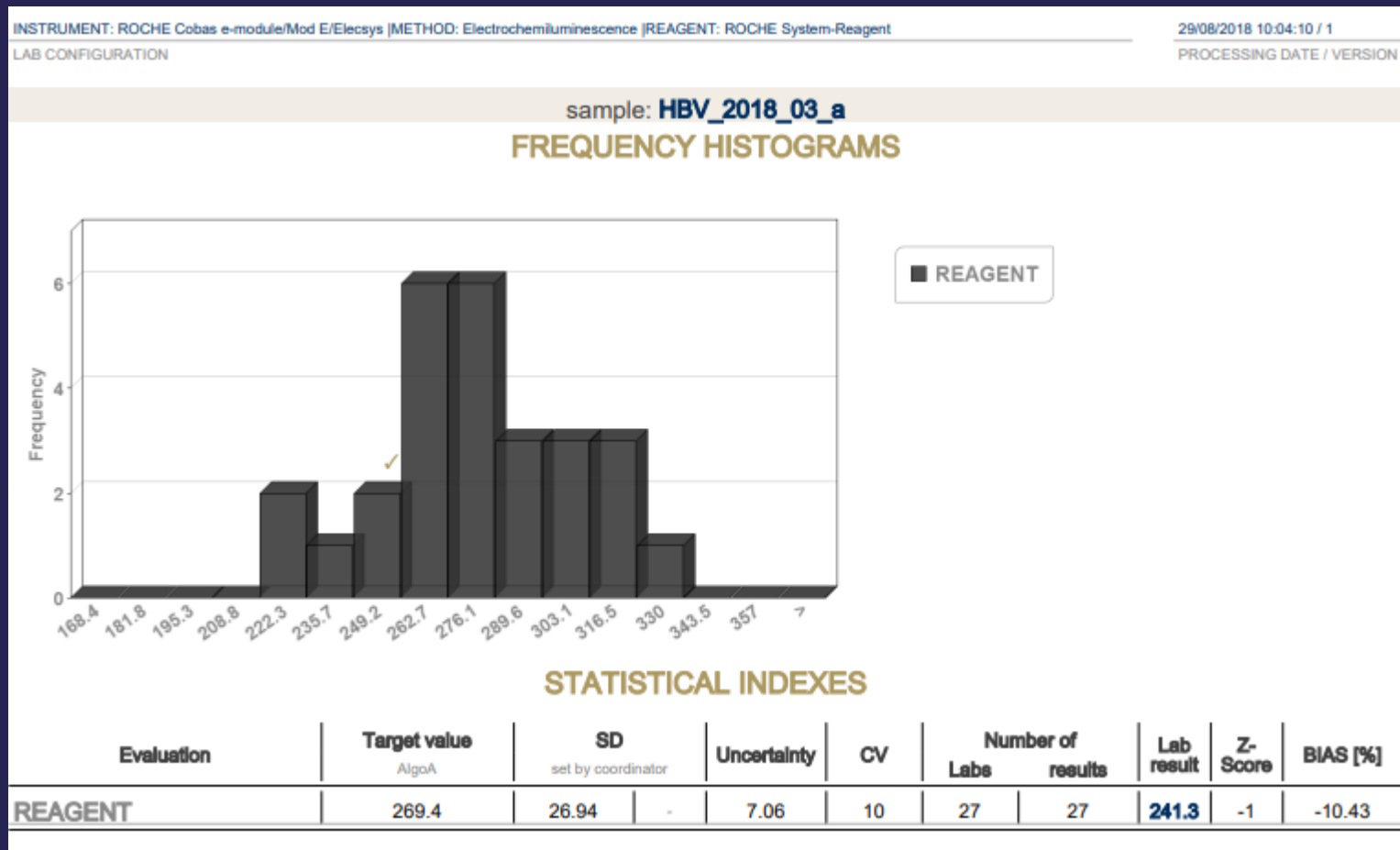
Reasonable distribution of quantitative results within an individual reagent group

# Conclusion

- IQC and EQC are crucial for reliable analytical results and cost efficiency in medical laboratories

- Serological testing is different compared to clinical chemistry

- High variability of analytical assays and biological variability of analytes prevents quantitative comparison of signals or titers across various analytical systems

- IQC has to be tailor-made for a specific analytical system to challenge the reproducibility of test results

- EQC samples have to be suitable for a wide variety of test systems for the qualitative determination of a particular antibody or antigen

Thank you very much for your attention

Gracias por su atención