Interest in low antibody control samples in viral serology

Methods of production/validation of samples

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Importance of testing low antibody levels ?

- **In virology, serological methods are mainly used to detect serum antibodies against viruses and, more rarely, viral antigens.**
- **Although results are generally expressed in a qualitative way (NEGATIVE / POSITIVE), these methods are regarded as quantitative because interpretation is based on a continuous and quantifiable signal (absorbance, chemiluminescence units…)**
- **For a few assays, results are expressed in international units (anti-HBs or anti-rubella antibodies)**
- **For some biological parameters, and particularly in the field of Virology, the Common Technical Specifications published in 2009 imposed analytical requirements for obtaining C.E. approval : for example the lower limit of detection (LLD) for HIV p24 Ag assays is 2 IU/mL against the WHO standard.**

Importance of testing low antibody levels ?

- **Two major clinical situations point out the importance of the LLD:**
  - **Acute disease : to minimize the serological window**

    For example, depending on the method used, acute measles can be diagnosed 1 to 3 days after the rash appears.
  - **Post-infection or post-vaccination immune status: again, the LLD is critical in determining protection**

    For example, the level of protection against HBV infection is assumed to be 10 mIU/ml, which is the required LLD for CE marking.,
- **Uncertainty is also critical in these low levels to properly discriminate NEG / POS and to define the gray zone,**

Importance of testing low antibody levels ?

- **Inversely, high antibody levels are not of great interest in EQA schemes**

- **False negative results due to the assay are very rare in high antibody level surveys. Rather, they reflect mistakes in the transcription of results or inversion of samples**

- **Here also, the uncertainty is not critical because the monitoring of concentrations is not crucial or based on variations well above the coefficient of variation,**

EQA CTCB Varicella zoster IgG (90/636) UI/mL

TROUSSE	Serum 2115 HIGH 1500 UI/mL		Serum 2125 LOW 400 UI/mL	
	n	% POSITIFS	n	% POSITIFS
BIOMERIEUX - VIDAS Varicella-Zoster	30	**100**	30	**50**
DIASORIN - LIAISON VZV	72	**100**	72	**100**
DIESSE - CHORUS Varicella	2	**100**	2	**100**
EUROIMMUN - ELISA Anti-VZV	1	**100**	1	**100**
IMMUNODIAGNOSTIC SYSTEMS - IDS VZV	3	**100**	3	**100**
ORGENTEC - ALEGRIA Anti-VZV	5	**100**	5	**100**
VIRCELL - Varicella-Zoster VIRCLIA	8	**100**	8	**100**
VIROTECH DIAGNOSTICS - VZV Elisa	1	**100**	1	**100**

Methods of production/validation of samples

Potential source of positive sera

- **Blood banks : negative or positive sera (possibly from a single donor)**
- **Overloading a negative sera with a standard**
- **Medical Biology laboratories : pools and/or dilution of positive sera**
- **Commercial sera provided by in vitro diagnostics manufacturers**

Methods of production/validation of samples

- One point merits a particular attention : assay specificity

- Specificity varies according with the assays :
  - > 99,5% for the most efficient
  - But frequently around 98% or less

- Classically, false positive results are observed at low levels around the threshold and depend on the method

- Consequence +++: commercial low level sera that are often validated with a single method are not always true positives

Methods of production/validation of samples

- **Methods with international standards and CE requirements**
- **Methods with international standards without CE requirements**
- **Methods without international standards**

Methods of production/validation of samples

Methods with international standards and CE requirements

- **This is the easiest situation.**
- **Production: low level samples can be prepared using the appropriate standard (available from NIBSC), diluted using a negative serum, to obtain concentrations just above the C.E. requirement**
- **Validation : all methods can be used**
- **Notation of deviant laboratories : negative results are evaluated "wrong".**

Methods of production/validation of samples

Methods with international standards and CE requirements

EQA CTCB sérum 2020 n° 2 - Agp24 (90/636) 3 IU/mL

TROUSSE	n	Mean Index	Threshold	POSITIVE %
ABBOTT - ARCHITECT HIV Ag/Ab combo	121	4,61	1	99.9% Three "wrong"
BIOMERIEUX - VIDAS HIV DUO Quick	27	1,57	0,25	
BIOMERIEUX - VIDAS HIV DUO Ultra	12	1,41	0,25	
BIORAD - ACCESS HIV Combo	62	3,6	1	
DIASORIN - LIAISON XL murex HIV Ag/Ab	18	2,4	1	
ROCHE - Elecsys HIV Duo cobas 8000	49	6,9	1	
ROCHE - HIV COMBI PT	117	3	1	
SIEMENS - ADVIA Centaur HIV Ag/Ab Combo	26	1,9	1	

Methods of production/validation of samples

Methods with international standards and without CE requirements

- **Samples can be prepared and validated as previously described**
- **Notation: because there is no exigency, we adopt the following strategy for deviant laboratories based on the distribution of responses in the peer group :**
  - **Isolate error : noted “wrong”.**
  - **Homogenous repartition around the threshold (weak SD) : noted “result to be analysed by the laboratory” for discordant results**
  - **Heterogenous distribution between Negative and Positive results: if discrepancy is attributed to a reagent batch number, the supplier is informed and a declaration of medical diagnostic devices vigilance is sent to the French authorities**

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ORGENTEC - ALEGRIA Anti-VZV	5	100	5	100
VIRCELL - Varicella-Zoster VIRCLIA	8	100	8	100
VIROTECH DIAGNOSTICS - VZV Elisa	1	100	1	100

*** Noted “result to be analysed by the laboratory”**

Methods of production/validation of samples

Methods with no standardization

This situation is less comfortable and we face many problems:

Production : critical points

-Low positive samples are rare and we need large final volumes: 50 to x100 ml or more depending on the EQA

-We need to be sure that these low concentrations are not false positive results

-Clinical data and/or confirmatory tests (molecular biology or multi-method testing) are not often available especially if you use commercial sera

Methods of production/validation of samples

Methods with no standardization

Production :

-Considering the previous points, we recommend diluting high titre sera (in-house or commercial sera) with negative sera to obtain the adequate low level: by doing so, you are quite sure to avoid false positive results

-How to choose the level?

It is important to analyse the data from previous surveys to choose a level that corresponds to the state of the art : analytical and clinical performance

The LLD can be very different between methods and in the absence of CE requirement, we try to produce samples that can be detected by most assays

Methods of production/validation of samples

Methods with no standardization

Validation: your routine test is suitable, unless you have used low level commercial sera where we recommend using multiple methods to be sure of positivity (very difficult in practice)

Notation: similar to standardised methods without requirements. In our commentary we suggested that laboratories using less sensitive methods should do a risk analysis and adapt the interpretation of negative results or, if more appropriate, change their routine method.

Methods of production/validation of samples

Methods with no standardization

Example : low level of HEV IgG antibodies

FALSE NEGATIVE

1 Sérologie HEV

1.1 Sérum 1331 - IgG

Analyse des réponses qualitatives

Trousses	N	%	Négatif	Douteux	Positif =Assigné
ADALTIS - EIAgen HEV G	6	37,5	6	0	0
DIAPRO - HEV IgG EVG.CE	1	6,25	0	0	1
WANTAI - HEV Elisa IgG	9	56,3	0	0	9
TOTAL	**16**	**100%**	**6**	**0**	**10**

IgG prevalence in Midi-Pyrénées:15%

IgG prevalence in Midi-Pyrénées: 50%

Thank you for your attention.