IMMUNOASSAY EXTERNAL QUALITY ASSESSMENT IN ITALY: HBsAg AND ANTI-HBs TESTS

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Summary. - The mode of operation of the CNR/Tecnostandard external quality assessment scheme for HBsAg and anti-HBs assays is outlined, and the relevant results reported. Emphasis is given to the retrospective evaluation of data in an attempt to derive a picture of the state of the art in Italian laboratories. The approaches followed for this evaluation include analysis of the rate of correct results and inspection of the relationships between analytic concentration and either the percentage of positive classifications of samples or the numerical test responses. Information is given on the “average” performance of individual kits as actually used in participants’ laboratories.

KEY WORDS: external quality assessment, HBsAg and anti-HBs tests.

Riassunto (Valutazione esterna di qualità dei test per HBsAg e anti-HBs in Italia). - Viene descritto lo schema utilizzato nel programma CNR/Tecnostandard di valutazione esterna di qualità dei test per la rivelazione di HBsAg e anti-HBs e se ne riportano i risultati. Una particolare attenzione è rivolta all’analisi retrospettiva dei dati per tentare di derivarne un’informazione sullo “stato dell’arte” nei laboratori italiani. A tale scopo si sono prese in esame sia le percentuali di risultati corretti sia la relazione fra la percentuale di classificazioni positive dei campioni e la risposta numerica dei test e la concentrazione di analisi. In base a queste valutazioni viene fornita un’indicazione sulle prestazioni “medie” di kit individuali, come effettivamente utilizzati nei laboratori.

PAROLE CHIAVE: valutazione esterna di qualità, test per HBsAg e anti-HBs.

Introduction

The EQA of the assays of hepatitis B markers, included in the CNR programs from 1985, is now being continued as a service coordinated by Tecnostandard, Milano. The choice of the markers has been limited so far to serum HBsAg and anti-HBs, having taken into account the critical nature of the related tests in screening blood donors and selecting the candidates for vaccination, respectively.

Besides giving participants the opportunity for surveillance of their own performance, EQA allows a retrospective evaluation of the “average” quality of the immunodiagnostic kits most frequently used (particularly interesting when, as in Italy, experimentally based certification does not exist). This latter aspect will be particularly considered in reporting and discussing the EQA data.

EQA scheme and participation

The general EQA scheme does not substantially differ from others already described in the literature [1-3].

Stabilized serum pools (with antibiotics as preservatives) are classified as negative or positive before circulation. Absence of any detectable marker (HBsAg, anti-HBs, anti-HBc IgG and IgM, HBeAg, anti-HBe) is assumed to be the criterion for negativity. Low positive pools are prepared by diluting high positives with a negative serum. Values in conventional units are assigned to positives by comparison with reference sera (Paul Ehrlich Institut (PEI) for HBsAg, ay and ad subtypes; WHO for anti-HBs).

The aliquots of pooled sera, previously distributed in liquid form (stored at -20 °C), are now freeze-dried. Checks for stability are performed on sampled aliquots.

In addition, the scheme of sample distribution has varied somewhat with time. At present, sets of five samples, for both HBsAg and anti-HBs, are currently submitted to participants every three months.

Participants are requested to perform the assays exactly as their routine work and to return results as both positive or negative classifications (according to the cutoff value recommended by the kit producers) and digital responses
Tecnostandard

Controllo di qualità fra laboratori dei test per HBsAg e anti-HBs

HBsAg
Elaborazione qualitativa
CAMPIONE
S005  POOL H006P5
INVIO 1
Feb. 90

SIERO POSITIVO (ca. 0.15 U/ml-PEI)

A2 A5 A6 A7 A8 A9 AC AR A5 B1 B2 B3 B4 B5 B6 B7 B8 B9
C1 C2 C3 C4 C5 C6 C7 C8 C9 C10 C11 C12 C13 C14 C15 C16 C17 C18 C19
D1 D2 D3 D4 D5 D6 D7 D8 D9 D10 D11 D12 D13 D14 D15 D16 D17 D18 D19
E1 E2 E3 E4 E5 E6 E7 E8 E9 E10 E11 E12 E13 E14 E15 E16 E17 E18 E19
F1 F2 F3 F4 F5 F6 F7 F8 F9 F10 F11 F12 F13 F14 F15 F16 F17 F18 F19
H1 H2 H3 H4 H5 H6 H7 H8 H9 H10 H11 H12 H13 H14 H15 H16 H17 H18 H19
I1 I2 I3 I4 I5 I6 I7 I8 I9 I10 I11 I12 I13 I14 I15 I16 I17 I18 I19
K1 K2 K3 K4 K5 K6 K7 K8 K9 K10 K11 K12 K13 K14 K15 K16 K17 K18 K19
L1 L2 L3 L4 L5 L6 L7 L8 L9 L10 L11 L12 L13 L14 L15 L16 L17 L18 L19
M1 M2 M3 M4 M5 M6 M7 M8 M9 M10 M11 M12 M13 M14 M15 M16 M17 M18 M19
N1 N2 N3 N4 N5 N6 N7 N8 N9 N10 N11 N12 N13 N14 N15 N16 N17 N18 N19
O1 O2 O3 O4 O5 O6 O7 O8 O9 O10 O11 O12 O13 O14 O15 O16 O17 O18 O19
P1 P2 P3 P4 P5 P6 P7 P8 P9 P10 P11 P12 P13 P14 P15 P16 P17 P18 P19
Q1 Q2 Q3 Q4 Q5 Q6 Q7 Q8 Q9 Q10 Q11 Q12 Q13 Q14 Q15 Q16 Q17 Q18 Q19
R1 R2 R3 R4 R5 R6 R7 R8 R9 R10 R11 R12 R13 R14 R15 R16 R17 R18 R19
S1 S2 S3 S4 S5 S6 S7 S8 S9 S10 S11 S12 S13 S14 S15 S16 S17 S18 S19
T1 T2 T3 T4 T5 T6 T7 T8 T9 T10 T11 T12 T13 T14 T15 T16 T17 T18 T19
U1 U2 U3 U4 U5 U6 U7 U8 U9 U10 U11 U12 U13 U14 U15 U16 U17 U18 U19
V1 V2 V3 V4 V5 V6 V7 V8 V9 V10 V11 V12 V13 V14 V15 V16 V17 V18 V19
W1 W2 W3 W4 W5 W6 W7 W8 W9 W10 W11 W12 W13 W14 W15 W16 W17 W18 W19
X1 X2 X3 X4 X5 X6 X7 X8 X9 X10 X11 X12 X13 X14 X15 X16 X17 X18 X19
Y1 Y2 Y3 Y4 Y5 Y6 Y7 Y8 Y9 Y10 Y11 Y12 Y13 Y14 Y15 Y16 Y17 Y18 Y19

Fig. 1. - Example of report to participants in EQA of HBsAg assay. This refers to a diluted positive serum (0.15 U/ml). Codes indicate the individual laboratories. Note the frequency histograms of the response normalized with respect to cutoff, R_p/R_n = 1 (in few cases of competitive anti-HBs assays, where negative responses are higher and positive responses are lower than cutoff, the algebraic sign is changed for sake of uniformity). As discussed elsewhere in this issue [5], the "margin of safety" of correct results and the magnitude of errors can be directly quantitated.

(radioactivity counts, absorbance readings), indicating the kit used, the assay procedure adopted and the cutoff response selected. In the case of anti-HBs, quantitative results should also be returned when directly obtained by participants (ca 30% of laboratories, at present).

Based on these data, periodic reports to participants are prepared. An example is shown in Fig. 1, while a further information on subgrouping of responses according to the kits used, which is going to be adopted, is illustrated in Fig. 2. Reports relative to the quantitative anti-HBs results are similar to those currently used in the other CNR schemes [4], exactly as is the treatment of data. The assessment of qualitative assays is based instead on the approaches discussed elsewhere in this issue [5].

About 200 laboratories participate currently in the program. The frequency of use of the kits roughly reflects the situation in the entire Italian market. For each analytic, as many as 20 kits are involved in the EQA scheme, but more than 70% of participants concentrate their use on those supplied by three producers, i.e. Abbott Laboratories.
Fig. 2.- Laboratory normalized responses subgrouped according to the kits most frequently used by participants. Same cases as are shown in Fig. 1. Note the differences in detection sensitivity involved.
(AUSZYME for HBsAg, AUSAB for anti-HBs), Sorin Biomedica (AUK-3 for HBsAg, AB-AUK-3 for anti-HBs) and Organon Teknika (Heplongostika).

Results and comments: a tentative picture of the state of the art

The results relative to the whole EQA period of five years are reported in Table 1, grouped by increasing analyte concentration and expressed as percentage of correct classifications. For both HBsAg and anti-HBs tests, misclassifications are more frequent in the low positive ranges, as expected, although incorrect data have been sporadically observed for higher positive and negative sera as well.

When a sufficient number of cumulative data is available, comprehensive information on test performance is derived from the relationship between the percentage of positive results and the analyte concentration. Such curves are shown in Fig. 3 relative to the individual kits most used in the EQA scheme and to all results. Their differences are better quantitated in Fig. 4, through the comparison of false-positive rates and grey zone widths (derived from the curves shown in Fig. 3 [5]), which provide some indication
Fig. 5. - Variability of the overall rate of false positive (FP) and true positive (TP) results throughout the successive EQA periods (the TP data are obtained by interpolating curves as those shown in Fig. 3). For anti-HBs assay, note the loss in detection ability corresponding to period 2 (1986); this was due to a transient change in the performance of the most widely used kit (Abbott AUSAB, EIA).

of test specificity and detection sensitivity, respectively. As shown in Fig. 5, information on the stability of test performance can be drawn from the trends observed for the percentage of correct measurement of negative and weak positive (diluted) sera throughout the EQA periods.

The quality of individual tests may be more efficiently evaluated by consideration of the related concentration/response functions, normalizing the specimen responses with respect to the cutoff response. Since this is less demanding in terms of numbers of results and concentration levels, this approach allows the performance of several kits used in EQA to be compared. Thus, in Fig. 6, the behaviour of as many as six HBsAg kits and five anti-HBs kits are evident at a glance. A more or less pronounced linearity is apparent for all response functions, except for a single case of a competitive anti-HBs kit still surviving in the market (curve with negative slope).

The data presented in Figs 3, 4 and 6 all refer to the "average" performance of the kits, as actually used in participants' laboratories. In fact, within-kit variables, such as the incubation procedures adopted in each partici-

Fig. 7. - Distribution histograms of the cumulative normalized responses obtained by participants in EQA of HBsAg (1987-1988) assaying a weak positive serum (0.3 U/ml) with a single kit (Abbott AUSZYME). The data refer to total results and results grouped according to three operational procedures suggested by the manufacturer. The skewness of total distribution and the differences in detection sensitivity of the A, B, and C variants are apparent (cutoff coinciding with unitary abscissa).

Fig. 8. - Normalized responses (median values) as a function of analytical concentrations for the same kit variants previously described in Fig. 7. The differences in analytical performance are evident.

Fig. 9. - Imprecision profile describing the between-laboratory variability of quantitative measurement of anti-HBs (1987-1990).
lar case, contribute to the definition of the overall test characteristics. The within-kit variants can be individually assessed by subdivision of the related results, provided that there is a sufficient number of data. An example of the distribution of normalised responses obtained in this way is given in Fig. 7, while a comparison of the related concentration/response curves is shown in Fig. 8. The marked differences in performance characteristics emerging from these data clearly indicate that the choice among the operational options suggested by the producers for each kit is effective in establishing the overall quality of the kit itself.

In the case of anti-HBs, the quantitative expression of test results, based on calibrators supplied with the kit, is now entering into use. The total variability of estimates, depicted by the imprecision profile shown in Fig. 9, does not substantially diverge from the situation that exists for most quantitative assays in CNR-Tecnostandard EQA [4] (20% CV, anti-HBs concentration ≥ 20 mU/ml).

Conclusions

As far as the relative frequency of kit usage and operators' performance can be generalised, the results discussed above may be regarded as descriptive of the quality of HBsAg and anti-HBs tests in Italian laboratories. The average behaviour of participants in EQA using the same methods tends to identify the intrinsic characteristics of the method itself as actually working in the field.

Some general comments can be made:

- With regard to low-positive HBsAg ranges, where the different analytical features lead to different abilities in classification, the evaluation of the merit of individual kits (and kit variants) should essentially develop from empirical considerations. As a precaution, the most sensitive kits should be preferred to screen blood donors. However, any definitive conclusions concerning the importance of detection sensitivity on clinical grounds is difficult to quantify, in the absence of reliable epidemiologic data on the prevalence of low-level hepatitis B antigen positivity.

- In practical terms, the differences in clinical sensitivity of the anti-HBs kits revealed in this survey may be regarded as moderately important, owing to the relatively low concentrations where most discrepancies occur. In screening for vaccination, in fact, specificity rather than sensitivity is more critical: in this respect, some concern is stimulated by the false-positive rates observed.

- The false negatives occasionally occurring in the higher concentration ranges for both markers are particularly disquieting in the case of HBsAg. Such misclassifications are seemingly attributable to poor laboratory performance, rather than inherent features of individual kits.

Acknowledgements

Work partly supported by CNR. Special Project “Biotechnologie e Biointerferenza” and by Regione Lombardia.

Review submitted on invitation by the Editorial Board of the Annali. Accepted for publication: 8 December, 1990.

REFERENCES


