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SHORT COMMUNICATION/KURZMITTEILUNG

Analyte and Matrix Problems in the Calibration and Quality Assessment of the Bromocresol Green Method for Albumin in Serum

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Summary: Different batches of bromocresol green were used in a study of several modifications of the bromocresol green method for serum albumin. No major batch variation was observed. Lyophilized animal sera and a certain batch of lyophilized human sera, however, responded differently from a pool of fresh human sera when the method was modified. These phenomena should be taken into account in calibration procedures and in quality assessment.

Analyt- und Matrix-Probleme bei der Kalibrierung und Qualitätskontrolle der Bromkresolgrün-Methode für Albumin im Serum

Zusammenfassung: Verschiedene Chargen von Bromkresolgrün wurden für eine Untersuchung mehrerer Modifikationen der Bromkresolgrün-Methode für Albumin im Serum eingesetzt. Größere Chargenunterschiede wurden nicht beobachtet. Lyophilisierte Tierseren und eine bestimmte Charge lyophilisierter Seren vom Menschen reagierten jedoch abweichend von einem Pool frischer Seren vom Menschen auf die Modifizierung der Methode. Diese Erscheinungen sollten bei der Kalibrierung und der Qualitätskontrolle berücksichtigt werden.

Introduction

The bromocresol green method for serum albumin is still used extensively in many clinical chemistry laboratories in spite of considerable criticism (1). The methods might work properly (2–5), but in external quality assessment the results are often poor (e.g. l.c. (6)).

Bromocresol green is marketed as a pH indicator, and production control during manufacture can only be expected to cover this type of use. Therefore it was the aim of the present paper to investigate differences between batches of bromocresol green, and to find other possible explanations for interlaboratory variation in the bromocresol green method for serum albumin.

Materials and Methods

Bromocresol green

Five batches of bromocresol green art. no. 8121 from E. Merck (Darmstadt, West Germany) were investigated, three from the laboratories showing the greatest deviations in the earlier survey (6), a 25 year-old batch and a brand new one.

Bromocresol green reagent

Appropriate concentrations of bromocresol green were prepared fresh by dissolution of bromocresol green in an aqueous succinic acid/succinate buffer, pH 4.2 (23 °C) (50 mmol succinate and 0.75 ml Brij™ 35 in one litre).

Reference sera

The following reference sera were used:

Pool of fresh sera,

lyophilized serum from bank blood (D; local production),

commercially available lyophilized serum (G; intended for general quality control, "abnormal" level, Dade®, American Hospital Supply Corp., Miami, USA),

equine lyophilized serum (H; Nyegaard & Co., Norway),

mixed bovine-equine lyophilized serum (I; Nyegaard & Co., Norway) and

a human albumin solution (Protein Standard, Kabi Diagnostica AB, Sweden).

The materials, identified by letters, were the same as those used in the previous investigation (6).

Procedure

One volume of specimen was diluted with 20 volumes of aqueous sodium chloride (154 mmol in one litre). An aliquot (50 µl) of this diluted sample was added to 450 µl bromocresol green reagent and the absorbance of the mixture was read against water at 630 nm between 35 s and 1235 s after mixing in the GEMSAEC™ (Electro-Nucleonics Corp., New Jersey, USA).

Albumin was furthermore determined by a bromcresol green method on the SMAC I™ (Technicon Instruments Corp., New York, USA); reaction conditions: Specimen dilution 1 vol + 122 vol; preheated at 45 °C in 170 s; bromocresol green 135 µmol/l; succinic acid buffer, pH 4.2; merthiolate; Pegosperse™ 1.5 vol in 100 vol; reaction time 17 s.

Results

Table 1 shows the colour yield of different albumin preparations in comparison with the lyophilized serum from bank blood (D). Thus the data are shown in a way, similar to results obtained in a continuous flow system using a calibration serum. No major differences were found between different batches of bromocresol green.

The behaviour of the fresh serum pool was similar to that of the locally produced lyophilized serum from bank blood (D). This is in contrast to the albumin solution, the two animal lyophilized sera (H and I) and the lyophilized human serum (G). The latter contained elevated concentrations of many components, e.g. bilirubins.

Using the SMAC I™, the following results were obtained for the albumin-containing materials shown in table 1, in the same descending order: 604 μmol/l, 600 μmol/l, 455 μmol/l, 529 μmol/l and 552 μmol/l. A value of 685 μmol/l was found for the lyophilized serum from bank blood (D) used as calibrator.

The bottom of table 1 records a lack of proportionality between absorbance and concentration of bromocresol green in the pure reagents.

Discussion

The big variation of accuracy often found between laboratories using bromocresol green methods for albumin could not be explained by batch variations of bromocresol green. Furthermore, using high performance liquid chromatography and thin layer chromatography the bromocresol green batches seemed quite pure; "less than 1% of impurities" were found with exception of the 25 years old batch "where 2% was found" (*K. E. Rasmussen, H. Ravn*, personal communication 1981).

The present data show that the type of reference materials contribute remarkably to the interlaboratory variation, when methods with different reaction times and different concentrations of bromocresol green are compared (2, 3); even different reference materials of human origin gave different response. Ageing of reagents containing Pegosperse™ have been reported as an additional source of error (7) as well as different salt concentration (8).

The present findings should be taken into account in selecting calibration materials. Pools of fresh sera should probably be used as secondary calibration material (9).

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Tab. 1. The time dependence of the colour yield of albumin preparations at different concentrations of bromocresol green in the reaction mixture at 630 nm, read against reagent blank. The absorbance is expressed in percentage of that obtained simultaneously on a lyophilized serum from bank blood (D). Each set of data represents mean values obtained on at least three batches of bromocresol green (Merck art. no. 8121) from the batch numbers: 625927, 8601737, 9622557, 9023339 and 1153373. Batch number 9622557 showed the most variable results, and examples of these data are given for albumin in parenthesis.

Concentration of bromocresol green	45			68			135			Unit μmol/l
Reaction time	35	215	1235	35	215	1235	35	215	1235	s
Albumin solution	94	95	94	92	89	88	83	82	80	%
(Kabi Diagnostica AB, Sweden)	(94)	(95)	(94)	(91)	(89)	(87)	(81)	(80)	(77)	%
Fresh pool of human sera	85	86	87	84[x])	83[x])	85[xx])	82[x])	84[x])	86[x])	%
Lyophilized human serum (G) (DADE, American hospital Suppliers Corp. Miami)	70	71	–	66	65	65	55	56	57	%
Lyophilized equine serum (H) (Nyegaard AS, Norway)	69	68	–	69	68	70	65	66	67	%
Lyophilized bovine-equine serum (I) (Nyegaard AS, Norway)	71	70	71	78	78	80	85[x])	89[x])	88[x])	%
Absorbance range; lyophilized serum from bank blood (D), read against reagent blank	0.47–0.52			0.52–0.58			0.45–0.55			1
Absorbance of bromocresol green reagent read against water	0.06–0.08			0.12–0.15			0.36–0.42			1

[x]) *s* for all results (3–9) were 1.0 or less if no asterisk is present. One asterisk signifies *s* between 1.2 and 2.0.
[xx]) *s* for the 9 measurements was 3.6.

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