



PREPARATION OF STABILIZED LIQUID QUALITY CONTROL SERUM
 TO BE USED IN CLINICAL CHEMISTRY

8408

by

Mr D. M. Browning, Laboratory Manager
 Department of Clinical Chemistry
 Wolfson Research Laboratories
 Queen Elizabeth Medical Centre
 Birmingham, England

Dr P. G. Hill, Top Grade Biochemist
 Derbyshire Royal Infirmary
 Derby, England

Dr D. A. Vazquez R. Olazabal, Scientist
 Health Laboratory Technology
 WHO Headquarters, Switzerland



CONTENTS

	<u>Page</u>
SUMMARY	2
1. MATERIALS FOR EVALUATING LABORATORY PERFORMANCE	2
1.1 Stabilization by sterilization	2
1.2 Stabilization by lyophilization	2
1.3 Stabilization by chemicals	3
2. CHOICE OF STARTING MATERIALS	3
3. METHODS OF PREPARING STABILIZED LIQUID SERUM	4
4. METHODS FOR THE PREPARATION OF SERUM USING ETHYLENE GLYCOL (ETHANEDIOL)	4
4.1 Preparation of a preliminary batch	4
4.2 Preparation of a medium concentration quality control serum	5
4.3 Preparation of a low concentration quality control serum	7
4.4 Preparation of a high concentration quality control serum	8
GENERAL NOTES	11
Appendix A: PREPARATION OF ENZYME CONCENTRATES	12
Appendix B: SOME POSSIBLE COMMERCIAL SOURCES OF ENZYMES	13
ACKNOWLEDGEMENTS	14

The issue of this document does not constitute formal publication. It should not be reviewed, abstracted, quoted or translated without the agreement of the World Health Organization. Authors alone are responsible for views expressed in signed articles.

Ce document ne constitue pas une publication. Il ne doit faire l'objet d'aucun compte rendu ou résumé ni d'aucune citation ou traduction sans l'autorisation de l'Organisation mondiale de la Santé. Les opinions exprimées dans les articles signés n'engagent que leurs auteurs.

SUMMARY

The use of Internal Quality Control (IQC) and External Quality Assessment (EQA) to continuously monitor laboratory performance are accepted as essential parts of the function of any health laboratory.

Laboratories in many countries are disadvantaged by virtue of the non-availability or high cost of commercial quality control sera. The WHO document (LAB/81.4) was prepared to encourage local production of quality control materials to ensure their availability. Guidelines for the preparation of lyophilized and liquid control sera are given in that document.

This publication extends document LAB/81.4 by describing the preparation of liquid control sera stabilized with ethylene glycol (ethanediol) which expands the suitability of the material to include the control of commonly measured enzymes in addition to other analytes. The material described in this document can be used for IQC and EQA of the clinical chemistry methods described in "Methods Recommended for Essential Clinical Chemical and Haematological Tests in Intermediate Hospital Laboratories" (LAB/86.3).

1. MATERIALS FOR EVALUATING LABORATORY PERFORMANCE

IQC and EQA are required in all laboratories in developed and developing countries. They involve the use of analytical results obtained when suitable materials, usually serum, are analysed in the same way as specimens from patients. The results are used (i) to decide whether a batch of tests is acceptable and can therefore be reported to the requesting doctor (IQC) and (ii) to observe analytical comparability between laboratories within a region, country or internationally (EQA).

The control materials used for these purposes must be stable; this has been achieved in the following three ways:

- (1) Liquid serum can be stabilized by sterilization (filtration).
- (2) Liquid serum can be dispensed into ampoules and then lyophilized.
- (3) Liquid serum can be stabilized by the addition of chemicals.

1.1 Stabilization by sterilization

This method produces serum which contains no viable bacteria and which is stable at 4 °C for several weeks. Serum prepared in this way is stable for 2-3 weeks at 20-25 °C but is unstable in warmer climates. A major problem with the preparation of this material is the provision of a suitable sterile environment and of dry sterile bottles.

1.2 Stabilization by lyophilization

This method produces a stable product which is widely used in more developed countries. Serum is filtered and then lyophilized. In its dried state it is stable for several years when correctly stored. There are several disadvantages to this method, viz:

- (a) the serum must be accurately and precisely dispensed prior to lyophilization;
- (b) it is an expensive process, requiring significant capital expenditure;
- (c) it requires considerable expertise to obtain a satisfactory product;
- (d) reconstitution of the material with an exact volume of water is necessary prior to use;
- (e) matrix changes occur during lyophilization and the reconstituted serum may be significantly turbid.

It is doubtful if developing countries with limited resources could afford the expense of total reliance on lyophilized material. However, there will always be a requirement for small amounts as a stable reference material. In some instances it may be more cost-effective to purchase these from a suitable commercial source.

1.3 Stabilization by chemicals

This method produces a cheap source of stable liquid serum suitable for IQC and EQA. A variety of compounds have been used to stabilize serum and serum preserved in this way will remain stable for several weeks when stored correctly. The major advantages of this method are:

- (a) low cost;
- (b) it is a simple process requiring normal laboratory expertise;
- (c) no reconstitution is required prior to use;
- (d) little or no obvious matrix effects on subsequent analysis by manual methods.

However, the preservatives chosen should not interfere with methods in common use. Laboratories can prepare a range of sera with different concentrations of common analytes thus enabling the laboratory worker to check the analytical process over a wide concentration range.

2. CHOICE OF STARTING MATERIAL

The use of animal sera is strongly recommended rather than human sera for the following reasons:

- (a) the serious risk of incorporating infectious human sera into the material with agents that cause serum hepatitis and acquired immune deficiency syndrome, etc.,
- (b) the use of human donors to supply large quantities of general purpose control serum is not justifiable;
- (c) for the purposes envisaged in this document, viz. the quality control of most tests including those listed in LAB/86.2, the use of animal serum is satisfactory.

Bovine serum is the best source in many countries; however, in some countries its use may not be acceptable. Some alternative materials are porcine and equine although other animal sources may also be acceptable. Table 1 compares the approximate concentrations of some common analytes in human, bovine, equine and porcine serum. Details for the collection of animal blood may be found in the WHO document LAB/81.4.

TABLE 1. APPROXIMATE CONCENTRATIONS OR ACTIVITY OF ANALYTES IN HUMAN AND SOME ANIMAL SERA (S.I. UNITS)*

Analyte	Unit	Human	Bovine	Equine	Porcine
Albumin (BCG)	g/l	43	32		
Alk. phos**	U/l	55	56		
Amylase**	U/l	180	15		
AST**	U/l	26	85		
Bicarbonate	mmol/l	25	-		
Bilirubin	µmol/l	7	3,0	10	2,6
Calcium	mmol/l	2,5	2,68	3,08	2,49
Creatinine	µmol/l	80	97	97	88
Glucose (fasting)	mmol/l	5,0	2,8	4,1	3,9
Potassium	mmol/l	4,3	4,3	4,0	4,6
Sodium	mmol/l	141	142	139	148
Total protein	g/l	70	68	68	80
Urea	mmol/l	4,7	4,3	4,7	5,0

* The comma (,) has been used to indicate the decimal fraction as recommended in the SI for the Health Professions, World Health Organization, 1977.

** Measured by the methods described in LAB/86.3.

3. METHODS OF PREPARING STABILIZED LIQUID SERUM

It is essential that all laboratories have reasonably large volumes of stable quality control serum available. These need to be of good quality but cheap and easy to produce.

Of the methods available the one chosen must utilize cheap and widely available chemicals. Some compounds are known to invalidate enzyme assays and should not be used if the material is to be used for enzyme measurements. Thiomersal, borate, fluoride, azide and antibiotics have all been used as preservatives but have been found to be less satisfactory than ethylene glycol. In serum containing 15% ethylene glycol, all of the constituents listed in Table 2 are stable at -20°C for at least 8 months. At 4°C , alkaline phosphatase, AST and bilirubin are stable for 4 months and all other constituents listed in Table 2 are stable for at least 8 months. Serum containing 15% ethylene glycol is also stable at higher temperatures for shorter periods. Initial studies indicate that all of the serum constituents described in Table 2 are stable at 25°C for six days and at 37°C for three days. The stability at these temperatures is likely to be variable depending on the level of contamination by microorganisms. Serum prepared in this way may therefore be distributed at ambient temperature for external quality assessment programmes in addition to its use for internal quality control.

4. METHODS FOR THE PREPARATION OF SERUM USING ETHYLENE GLYCOL (ETHANEDIOL)

The addition of any liquid to serum results in a dilution of the concentration of all analytes. For many analytes this is undesirable and involves the laboratory in the addition of several compounds to restore the concentration of some constituents. To avoid this it is best to start by preparing a carefully mixed serum pool which is then frozen. On thawing, the top layer will contain very low concentrations of all constituents. A volume, equivalent to 15% of the total, is removed and replaced with the same volume of ethylene glycol. It is then mixed carefully and thoroughly. At this stage the serum may be assayed for any constituent and compounds added to elevate the concentrations of certain analytes.

4.1 Preparation of a preliminary batch

It is important that laboratories have available to them batches of quality control sera in the low, medium and high concentrations of all analytes likely to be measured as recommended in WHO document LAB/86.3. These materials enable laboratories to carry out quality control checks over a wide analytical and physiological range. However, we strongly recommend the preparation of a small practice batch with the concentration of most analytes in the medium range. This helps the laboratory gain expertise and avoids the possible loss of valuable serum. When familiar with the techniques the laboratory can prepare larger volumes at low, medium and high concentrations and this will involve more extensive additions of various materials.

Start with a batch of 250 ml:

1. Start with 250 ml of fresh bovine (porcine or equine) serum. Human serum is not recommended (see Section 2).
2. Carefully mix to ensure homogeneity and freeze completely at -20°C . Since it is important to avoid possible deterioration of this serum by allowing it to remain at ambient temperature too long, the remainder of the process must be completed within one working day.
3. Allow to thaw at room temperature. DO NOT DISTURB OR MIX.
4. When completely thawed carefully remove 38 ml of the top layer of fluid. This is 15% of the total volume and consists mainly of water or very dilute serum.
5. Replace this volume by adding 38 ml of ethylene glycol.
6. Mix very carefully and filter through non-absorbent cotton wool to remove any large aggregates.

7. The glucose and urea concentrations will be too low for a serum of medium concentration and must be raised by adding 140 mg of glucose and 44 mg of urea. These solids must be dissolved and the preparation carefully and thoroughly mixed to ensure homogeneity.
8. The serum can then be dispensed into clean, dry, well-capped bottles or vials and stored in the refrigerator.
9. Before use the serum should be removed from the refrigerator and allowed to rise to ambient temperature; mix gently and thoroughly before use.

4.2 Preparation of a medium concentration quality control serum

1. Start with 1 litre of fresh bovine (porcine or equine) serum.
2. Carefully mix to ensure homogeneity and freeze at -20°C . It is important to avoid possible deterioration of this serum by allowing it to remain at ambient temperature too long. The remainder of the process must be completed within one working day.
3. Allow to thaw at room temperature. DO NOT DISTURB OR MIX.
4. When completely thawed remove 150 ml of the top layer of fluid. This is 15% of the total volume and consists mainly of water or very dilute serum.
5. Replace this volume of fluid by adding 150 ml of ethylene glycol.
6. Mix very carefully and filter through non-absorbent cotton wool to remove any large aggregates.
7. Measure the concentration of the analytes in which you are interested and construct a table identical to Table 2, filling in your own measured concentrations (column 4).
8. Fill in the desired concentrations in column 3 and use this table to calculate the amounts of analytes to be added as described below.
9. The concentration of constituents may be similar to the values indicated in column 4 of Table 2. It is anticipated that adjustments will be required for alkaline phosphatase, amylase, bilirubin, glucose and urea.

TABLE 2. APPROXIMATE MEASURED AND DESIRED CONCENTRATIONS OR ACTIVITIES FOR MEDIUM CONCENTRATION QUALITY CONTROL SERUM (BOVINE ORIGIN)

Constituent	Units	Desired concentration*	Measured concentration*
Albumin	g/l	38	38
Alk. phos	U/l	150	50
Amylase	U/l	300	0
AST	U/l	80	80
Bicarbonate	mmol/l	14	14
Bilirubin	$\mu\text{mol/l}$	35	3
Calcium	mmol/l	2,55	2,55
Creatinine	$\mu\text{mol/l}$	90	90
Glucose	mmol/l	5,6	2,5
Potassium	mmol/l	4,1	4,1
Sodium	mmol/l	135	135
Total protein	g/l	65	65
Urea	mmol/l	7,0	4,1

* Or Activity.

Adjust the concentration of analytes as follows:

(a) alkaline phosphatase

amount to be added to 1 litre =
desired activity - measured activity;
i.e. from the example in Table 2:
 $150 - 50 = 100$ units to be added to 1 litre. This may be added as a powder or a concentrated suspension.

Alkaline phosphatase is available commercially. A detailed description for the preparation of alkaline phosphatase from human placenta is given in Appendix A.

(b) amylase

amount to be added to 1 litre =
desired activity - measured activity;
i.e. from the example in Table 2:
 $300 - 0 = 300$ units to be added to 1 litre.

Human saliva contains about 1000 units amylase/ml, so 0,3 ml contains 300 units. Collect about 1 ml of saliva, centrifuge at about 2500 rpm for 10 minutes then add 0,3 ml of supernatant to 1 litre of stabilized control serum. Measure the amylase activity of the control serum at 37 °C to confirm that it is approximately 300 U/l.

(c) bilirubin

amount to be added to 1 litre =
desired concentration - measured concentration;
i.e. from the example in Table 2:
 $35 - 3 = 32$ $\mu\text{mol/l}$ (1 $\mu\text{mol} = 0,59$ mg)
 $32 \times 0,59 = 19$ mg to be added to 1 litre.

Weigh out 19 mg of bilirubin (32 μmol), dissolve in 4,0 ml of 0,1 mol/l sodium hydroxide to produce a clear red solution; when the bilirubin has completely dissolved, add the solution, with constant stirring, to the 1 litre of stabilized control serum. The procedure should not be carried out in a brightly lit place and, after adding the bilirubin to the control serum, it must be protected from light to avoid destruction of the bilirubin.

(d) glucose

amount to be added to 1 litre =
desired concentration - measured concentration;
i.e. from the example in Table 2:
 $5,6 - 2,5 = 3,1$ mmol (1 mmol = 180 mg)
 $3,1 \times 180 = 558$ mg.

Weigh out 558 mg of glucose and add it to 1 litre of stabilized serum, mix gently and thoroughly to dissolve.

(e) urea

amount to be added to 1 litre =
desired concentration - measured concentration;
i.e. from the example in Table 2:
 $7,0 - 4,1 = 2,9$ mmol (1 mmol = 60 mg)
 $2,9 \times 60 = 174$ mg.

Weigh out 174 mg of urea and add it to 1 litre of stabilized serum, mix gently and thoroughly to dissolve.

10. The total product must now be mixed thoroughly and dispensed into suitable clean, dry, amber-coloured bottles or vials, capped firmly and stored at 4 °C or at -20 °C. If amber-coloured containers are not available, then the serum must be protected from light to avoid destruction of the bilirubin.

4.3 Preparation of a low concentration quality control serum

1. Start with 1 litre of fresh bovine (porcine or equine) serum.
2. Filter through non-absorbent cotton wool to remove any large aggregates.
3. Measure the potassium concentration.
4. Calculate the final volume to which 1 litre of serum must be adjusted to dilute the potassium to 3,5 mmol/l. For example, if the measured potassium is 5,4 mmol/l then:

$$\text{final volume} = \frac{5,4}{3,5} \times 1000 = 1543 \text{ ml}$$

(DO NOT ADD WATER YET)

5. 15% of 1543 ml is $\frac{1543}{100} \times 15 = 231$ ml
6. Therefore to 1 litre of serum with potassium concentration of 5,4 mmol/l you must add the following:
 - (a) ethylene glycol: 231 ml
 - (b) distilled water: 312 ml (i.e. 543 - 231 = 312 ml)
7. Measure the concentration of the analytes in which you are interested and construct a table identical to Table 3, filling in your own measured concentrations (column 4).
8. Adjust the glucose and sodium to the desired concentrations as follows (assuming a final volume for the low concentration serum of 1543 ml):

(a) glucose

amount to be added to 1 litre =
desired concentration - measured concentration;
i.e. from the example in Table 3:
3,0 - 2,0 = 1,0 mmol/l
1 mmol = 180 mg.

The total volume of the low concentration serum is 1543 ml (1,543 l), so the amount to be added is 180 x 1,543 = 278 mg. Weigh out 278 mg glucose and add it to the serum.

(b) sodium

amount to be added to 1 litre =
desired concentration - measured concentration;
i.e. from Table 3:
125 - 106 = 19 mmol/l
1 mmol = 58,4 mg of sodium chloride
19 mmol = 58,4 x 19 = 1110 mg of sodium chloride.

The total volume of the low concentration serum is 1543 ml (1,543 l) so the amount to be added is 1110 x 1,543 = 1713 mg = 1,71 g. Weigh out 1,71 g of sodium chloride and add it to the serum.

TABLE 3. APPROXIMATE MEASURED AND DESIRED CONCENTRATIONS OR ACTIVITIES FOR LOW CONCENTRATION QUALITY CONTROL SERUM (BOVINE ORIGIN)

Analyte	Units	Desired concentration*	Measured concentration*
Albumin	g/l	30	30
Alk. phos	U/l	40	40
Amylase	U/l	-	-
AST	U/l	60	60
Bicarbonate	mmol/l	11	11
Bilirubin	µmol/l	-	-
Calcium	mmol/l	2,0	2,0
Creatinine	µmol/l	70	70
Glucose	mmol/l	3,0	2,0
Potassium	mmol/l	3,2	3,2
Sodium	mmol/l	125	106
Total protein	g/l	51	51
Urea	mmol/l	3,2	3,2

* Or Activity.

9. Mix gently but thoroughly to ensure that the glucose and sodium chloride dissolve completely.
10. The total product must now be mixed thoroughly and dispensed into suitable bottles or vials, capped firmly and stored at 4 °C or at -20 °C.

4.4 Preparation of a high concentration quality control serum

1. Start with 1 litre of fresh bovine (porcine or equine) serum.
2. Carefully mix to ensure homogeneity and freeze at -20 °C. It is important to avoid possible deterioration of this serum by allowing it to remain at ambient temperature too long. The remainder of the process must be completed within one working day.
3. Allow to thaw at room temperature. DO NOT DISTURB OR MIX.
4. When completely thawed remove 300 ml of the top layer of fluid.
5. Add 123 ml of ethylene glycol.
6. Mix very carefully and filter through non-absorbent cotton wool to remove any large aggregates.
7. Measure the concentration of the analytes in which you are interested and construct a table identical to Table 4, filling in your own measured concentrations (column 4).
8. Fill in the desired concentrations in column 3 and use that table to calculate the amounts of analytes to be added as described below.
9. The concentrations may be similar to the values given in column 4 of Table 4 with the desired concentrations indicated in column 3. It is anticipated that adjustments will be required for alkaline phosphatase, amylase, bilirubin, creatinine, glucose and urea. The adjustments are based on a final volume of 858 ml (i.e. 700 ml concentrated serum, 123 ml ethylene glycol, 30 ml from bilirubin and creatinine adjustments and 5 ml extra ethylene glycol).

TABLE 4. APPROXIMATE DESIRED AND MEASURED CONCENTRATIONS OR ACTIVITIES FOR HIGH CONCENTRATION QUALITY CONTROL SERUM (BOVINE ORIGIN)

Analyte	Units	Desired concentration*	Measured concentration*
Albumin	g/l	45	45
Alk. phos	U/l	250	60
Amylase	U/l	700	0
AST	U/l	95	95
Bicarbonate	mmol/l	17	17
Bilirubin	µmol/l	150	5
Calcium	mmol/l	3,00	3,00
Creatinine	µmol/l	450	110
Glucose	mmol/l	20	3,0
Potassium	mmol/l	6,0	4,8
Sodium	mmol/l	159	159
Total protein	g/l	76	76
Urea	mmol/l	15	4,8

* Or Activity.

10. Adjust the concentration of analytes as follows:

(a) alkaline phosphatase

amount to be added to 1 litre = desired activity - measured activity;
i.e. from the example in Table 4:
 $250 - 60 = 190$ units to be added to 1 litre
volume of high concentration serum is 858 ml (0,858 l)
 $190 \times 0,858 = 163$ units.

163 units of alkaline phosphatase must be added to the high concentration serum; this may be added as a powder or a concentrated suspension. See Appendix A for details of the preparation of human placental alkaline phosphatase and Appendix B for possible commercial sources of the enzyme.

(b) amylase

amount to be added to 1 litre = desired activity - measured activity;
i.e. from the example in Table 4:
 $700 - 0 = 700$ units to be added to 1 litre
volume of high concentration serum is 858 ml (0,858 l)
 $700 \times 0,858 = 601$ units
601 units of amylase must be added to the high concentration serum.

Human saliva contains about 1000 units of amylase/ml, so that 0,6 ml contains 600 units. Collect about 1 ml of saliva, centrifuge at about 2500 rpm for 10 minutes, then add 0,6 ml to the high concentration serum. Mix thoroughly. Measure the amylase activity of the serum at 37 °C to confirm that it is approximately 700 U/l.

(c) bilirubin

amount to be added to 1 litre =
desired concentration - measured concentration;
i.e. from the example in Table 4:
 $150 - 5 = 145 \mu\text{mol/l}$ ($1 \mu\text{mol} = 0,59 \text{ mg}$)
 $145 \times 0,59 = 85,6 \text{ mg}$ to be added to 1 litre
volume of high concentration serum is 858 ml (0,858 l)
 $85,6 \times 0,858 = 73 \text{ mg}$.

Weigh out 73 mg of bilirubin, dissolve in 15 ml of 0,1 mol/l sodium hydroxide to produce a clear red solution; when the bilirubin has completely dissolved, add the solution, with constant stirring, to the high concentration serum. The procedure should not be carried out in a brightly lit place and, after adding the bilirubin to the control serum, it must be protected from light to avoid destruction of the bilirubin. Mix thoroughly.

(d) creatinine

amount to be added to 1 litre =
desired concentration - measured concentration;
i.e. from the example in Table 4:
 $450 - 110 = 340 \mu\text{mol/l}$ ($1 \mu\text{mol} = 0,113 \text{ mg}$)
 $340 \times 0,113 = 38 \text{ mg}$ to be added to 1 litre
volume of high concentration serum is 858 ml (0,858 l)
 $38 \times 0,858 = 33 \text{ mg}$.

Weigh out 33 mg of creatinine, dissolve in 15 ml of 0,1 mol/l hydrochloric acid and when completely dissolved add the solution to the high concentration serum. Mix thoroughly.

(e) glucose

amount to be added to 1 litre =
desired concentration - measured concentration;
i.e. from the example in Table 4:
 $20 - 3 = 17 \text{ mmol/l}$ ($1 \text{ mmol} = 180 \text{ mg}$)
 $17 \times 180 = 3,06 \text{ g}$ to be added to 1 litre
volume of high concentration serum is 858 ml (0,858 l)
 $3,06 \times 0,858 = 2,63 \text{ g}$.

Weigh out 2,63 g of glucose, add it to the high concentration serum, mix gently to dissolve. Mix thoroughly.

(f) urea

amount to be added to 1 litre =
desired concentration - measured concentration;
i.e. from the example in Table 4:
 $15 - 4,8 = 10,2 \text{ mmol/l}$ ($1 \text{ mmol} = 60 \text{ mg}$)
 $10,2 \times 60 = 612 \text{ mg}$ to be added to 1 litre
volume of high concentration serum is 858 ml (0,858 l)
 $612 \times 0,858 = 525 \text{ mg}$.

Weigh out 525 mg of urea, add it to the high concentration serum, mix gently to dissolve. Mix thoroughly.

(g) final adjustment of ethylene glycol concentration

The final concentration of ethylene glycol must be 15%. It is therefore necessary to add a further 5,0 ml ethylene glycol because the total volume has been increased by 30 ml through the addition of the bilirubin and creatinine solutions. Mix very thoroughly.

11. The total product must now be mixed thoroughly and dispensed into suitable clean, dry, amber-coloured bottles or vials, capped firmly and stored at 4 °C or at -20 °C. If amber-coloured containers are not available, then the serum must be protected from light to avoid destruction of the bilirubin.

GENERAL NOTES

We recommend that when possible clean and aseptic techniques should be used to reduce the possibilities of contamination.

Prior to analysis the serum should be allowed to rise to ambient temperature, and mixed carefully. It should be kept in the dark to avoid destruction of the bilirubin.

Serum preserved with ethylene glycol may not be suitable for methods employing dialysis.

APPENDIX A

PREPARATION OF ENZYME CONCENTRATES¹

This section gives a detailed description of the preparation of a number of enzyme concentrates from tissue sources which can be used to fortify batches of control serum.

(i) ALKALINE PHOSPHATASE, ALP (EC 3.1.3.1)

ALP is prepared from human placenta using a method based on the extraction procedure of Morton (1950). The placenta is homogenized using 2 ml water per g tissue, then n-butanol is added 1 ml per g tissue and stirred for about one hour. After centrifugation the aqueous layer is fractionated with acetone at 4 °C, the precipitate formed between 33% and 50% (v/v) acetone is retained, dissolved in 0,01 mol/l tris-HCl buffer, pH 7,7 and dialysed overnight against the same buffer. The average yield is 52 units per g tissue and the ALP:AST ratio is greater than 57:1 and ALT activity is undetectable.

This enzyme and all other enzyme preparations described below are distributed in small vials and stored at -20 °C until required. The activity of each is measured by the difference before and after addition using a suitable dilution in serum.

(ii) ALANINE AMINOTRANSFERASE, ALT (EC 2.6.2.2)

ALT is prepared from human liver using a procedure based on the method published by Owen et al. (1974) for the extraction of ALT from dolphin muscle. Human liver, classified as normal by the pathologist, is homogenized in 0,05 mol/l tris-HCl buffer, pH 7,0 containing 10 mmol/l 2-mercaptoethanol and 10 mmol/l EDTA, 3 ml buffer being used per g of tissue. After centrifugation, the supernatant is placed in a water-bath at 60 °C, brought to 52 °C, then rapidly cooled in an ice-bath. The heat-treated sample is fractionated with ammonium sulfate at 4 °C. The precipitate formed when a further 16 g ammonium sulfate per 100 ml are added contains most of the ALT activity and should be separated, dissolved in and dialysed overnight against 0,015 mol/l tris-HCl buffer, pH 7,25, containing 2 mmol/l EDTA and 10 mmol/l 2-mercaptoethanol. The supernatant from this latter step is retained for AST preparation. The average yield is 14 units per g tissue and the ALT:AST ratio 4,5:1. The ALP activity in this preparation is negligible.

Ox, pig and horse livers do not yield satisfactory ALT preparations, the ratio of AST:ALT activity in the initial extract being very much greater, about 10:1 compared with 2:1 in human liver.

(iii) ASPARTATE AMINOTRANSFERASE, AST (EC 2.6.1.1)

AST may also be extracted from human liver by the above procedure. A further 14 g ammonium sulfate per 100 ml are added to the supernatant from the ALT preparation. The precipitate formed contains most of the AST activity. This precipitate is dissolved in and dialysed against 0,015 mol/l tris buffer, to remove ammonium sulfate and is then suitable for addition to quality control material. The average yield is 25 units per g tissue and the AST:ALT ratio 25:1.

¹ Reprinted from Practical Guidelines for the Preparation of Quality Control Sera for Use in Clinical Chemistry by A. P. Kenny & R. H. Eaton, WHO document LAB/81.4.

SOME POSSIBLE COMMERCIAL SOURCES OF ENZYMES

ALKALINE PHOSPHATASE

Sigma Chemical Co.

Catalogue number P5521

BDH Chemicals Ltd

Catalogue number 39035

Merck

Catalogue number 1607L

AST

Sigma Chemical Co.

Catalogue number G2751

AMYLASE

Sigma Chemical Co.

Catalogue number A1031

BDH Chemicals Ltd

Catalogue number 39118

Merck

Catalogue number 1329

The products satisfactorily used for the evaluation of this procedure were from Sigma Chemical Co. listed above.

ACKNOWLEDGEMENTS

The authors would like to express their appreciation to Miss L. Morris, Mr D. Bullock and Mr I. Surplice of the WHO Collaborating Centre for Research and Reference Services in Clinical Chemistry, Birmingham, UK for their valuable work, and to Dr R. H. Eaton, Biochemistry Department, Victoria Infirmary, Glasgow, UK for her kind cooperation in the preparation of this document. They would also like to extend their appreciation to Dr P. Premachandra, Medical Research Institute, Colombo, Sri Lanka, Dr H. Mehta, Department of Medical Education, Rohtak, Chandigarh, India, and Dr T. Nath, Biochemistry Department, GB Plant Hospital, New Delhi, India, who carried out a substantial amount of work for testing the methods and materials described in this document.

= = =