of circulating thyroxine to allow its use in a congenital hypothyroid screening program.

References

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Deterioration of Freeze-dried Radioimmunoassay Antisera with Time

To the Editor:

Antisera used for immunoassays are generally stored at -20 or 4°C or, after freeze-drying, at room temperature (1). The temperature used is largely a matter of personal preference but freeze-drying is an attractive procedure when large volumes of antisera have to be compactly stored for long periods.

With high-titre antisera, however, as little as a few microlitres of undiluted material may suffice for many tens of thousands of assays. Thus, if whole serum is dispensed freeze-dried, most of it is discarded unused after reconstitution. It is convenient, therefore, to predilute antisera of high titre, dispense them into a large number of convenient size aliquots and lyophilize. However, this procedure may not be without its drawbacks.

We raised antibodies to pancreatic glucagon in six rabbits. One rabbit (Y6) produced an antisera of very high titre. Blood collected 18 weeks after primary injection (bleed reference no. Y6PR) in October 1975 was used in this study. The antisera was separated from a clot and after collection stored at -20°C in 1.0 ml aliquots. Three months later (January 1976) one of the aliquots was diluted with phosphate buffer (50 mmol/litre, pH 7.4) to give a final dilution of 2000-fold (A/S Y6-PR-A), and 0.5 ml aliquots of the diluted antiserum were dispensed into glass freeze-drying vials, lyophilized, and sealed under reduced pressure. In December 1976 a further aliquot of the undiluted antiserum, stored at -20°C since collection, was diluted to a final volume of 500 ml (A/S Y6PR-B), dispensed in 1 ml volumes, and lyophilized. Because we wished to be sure that freeze-drying was complete, 10 randomly selected vials from each batch of antiserum were weighed, further lyophilized for 48 h, and then reweighed. The diluted antisera were reconstituted by adding 0.5 or 1.0 ml of doubly distilled water as appropriate. 125I-labeled glucagon was prepared at about six-week intervals according to the method of Jorgensen and Larsen (2). Binding of 125I-labeled glucagon to the antiserum at serial dilutions from 4000- to 128 000-fold was measured whenever a new label was prepared. The protocol for this procedure (3), in summary, was:

<table>
<thead>
<tr>
<th>Tube contents, µl</th>
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<tbody>
<tr>
<td>Transylol (10 000 µl)</td>
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<tr>
<td>Nonspecific binding</td>
</tr>
<tr>
<td>Test</td>
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<tr>
<td>Total counts</td>
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Diluted antiserum | Hormone-free plasma
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<tr>
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<td>Total counts</td>
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After 48 h preincubation at 4°C, 100 µl of 125I-labeled glucagon, diluted to contain a radioactivity of 6000 cpm, was added to each tube. Incubation at 4°C was continued for 24 h, when a suspension of dextran-coated charcoal was added to all tubes except “total counts.” The tubes were centrifuged, and radioactivity in the charcoal precipitate was counted after removing the supernate. The entire procedure was carried out at 4°C. Label GL/76/11 was used six weeks after preparation for the simultaneous comparison of the two batches of lyophilized diluted antiserum, A/S Y6PR-A and A/S Y6PR-B.

Comparisons were also made in January 1977 of A/S Y6PR-A reconstituted with doubly distilled water, glucagon-free human plasma, or a solution of gelatin (1 g/litre). In each case, diluent was added to the vial either 72 h before or immediately before use.

Figure 1 shows the percentage of 125I-labeled glucagon bound by reconstituted antiserum A/S Y6PR-A diluted to a final titre of 1:32 000 at various times during the course of a year and shows a steady, if somewhat erratic, decline in hormone binding capacity. It is perhaps worth mentioning that several other 125I-labeled hormones prepared in this laboratory during 1976 with use of the same batches of Na125I as were used to prepare glucagon labels GL/76/3, GL/76/6, and GL/76/7 were found to be unsatisfactory in previously (and subsequently) perfectly adequate radioimmunoassays.

Comparison of the two preparations of freeze-dried antiserum showed that the material (A/S Y6PR-B)—stored undiluted at -20°C—maintained its binding capacity, whereas that which was stored in lyophilized form after dilution (A/S Y6PR-A) gradually deteriorated.

Figure 2 shows that redissolving the lyophilized material in a protein-containing fluid rather than in doubly distilled water had no effect on the ageing of prediluted antiserum, nor did permitting the reconstituted antiserum to stand for 72 h in the vial before using it.

Loss of hormone binding capacity of the freeze-dried material could presumably be due to deterioration of antiserum or to poor 125I labeling. The gradual decline with the passage of time, taken in conjunction with results of the comparison between the two antiserum preparations using the same label in the same batch of assays, eliminates the second alternative and points to deterioration of antiserum A/S Y6PR-A under the conditions of storage adopted.

It has previously been reported that some antisera are damaged by freeze-drying (4). This appears not to have happened in the present case, because the initially high binding capacity was retained when the freeze-dried material...
was reconstituted immediately after lyophilization. Since antisera raised in this department to other hormones and drugs have been stored in lyophilized form after dilutions of as much as 100-fold without apparent deterioration, the decline in binding capacity observed with A/S Y6PR-A would seem to be a consequence either of the high initial dilution or, less likely, properties peculiar to this particular antiserum. What chemical changes actually take place in the antiserum to reduce its binding capacity is unknown; possibly the total amount of protein in the vial is too low to prevent its gradual adsorption onto the glass walls of the vials. If this is indeed correct then the adsorption appears to be irreversible, even by the addition of excess protein to the diluent. Despite this deterioration evident at a final titre of 1:32 000, the antisera were still useable and assays were performed with antiserum at 1:16 000 dilution.

The observations made in this paper may explain the occasional failure of recipients of gifts of lyophilized antisera to get satisfactory results in their own laboratories, and also have important implications for the preparation and storage of commercial radioimmunoassay antisera.

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References

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Some Experience with the Precision Performance of the Coulter Chemistry System

To the Editor:

Nineteen tests can be run routinely on the Coulter Chemistry system (Coulter Electronics, Hialeah, Fla. 33010). Daily we ran several control sera with both normal and abnormal concentration of the 19 components tested, along with routine hospital patient samples, and the standard deviations were compared to values reported by the College of American Pathologists’ Survey Program for other procedures in use and to the suggested clinical needs criteria of Barnett (1) and Cotlove et al. (2).

It is our normal procedure to run blanks and standards in each test channel daily before running patients’ samples. Coulter-assyayed serum controls were used as the standards. At least two serum controls from Coulter or other commercial sources were then run as a test, immediately after the standard, while others were run later along with patients’ samples. These various controls contained both normal and abnormal concentrations of serum components (Table 1).

The data were obtained with more recent models of the instrument. Although differences in models were mostly differences in engineering design of mechanical and electronic components, some basic chemical methods were changed and some tests added since an earlier report by Haven et al. (3). For instance, a cresolphthalein-complexone method for calcium, a bromcresol green method for albumin, and the Coulter “C-Zyme” methods for hydroxybutyrate dehydrogenase, aspartate aminotransferase, and triglycerides were used in this study. The mode of operation of the flame photometer for sodium and potassium analysis also was altered, the flame being kept on for at least 15 min before any test was run on those channels. (In earlier instruments the flame was turned on and off for each sample shortly before aspiration of sample and reagent.)

Table 1 shows the precision for our results. Where a range is given for the standard deviation of a particular test, this is indicative of how the SD varied with the concentration of that component in the controls run, the highest standard deviation being associated with the highest concentration of that component in the sera used.

These data were then compared to results published by the College of American Pathologists from their proficiency surveys of over 1000 laboratory participants (4). The sera used in their program contain test components in a narrow, usually normal, concentration range. The CAP data (Table 1) indicate the best precision for the various methods in use in the United States and Canada, with the value for all methods reported. These results indicate the so-called within-day precision and reflect the best duplication a laboratory can achieve under routine operating conditions. Four enzymes were not included in the CAP test program and so could not be compared here to the Coulter data obtained. CAP data for aspartate aminotransferase and albumin were estimated from recent single-month reports, because they were not reported in reference 4.

As an additional criterion for determining the clinical usefulness of the instrument, the precision obtained was compared (Table 1) to the clinical-needs precision as recommended by Barnett and by Cotlove et al. for most of these tests (1, 2). For enzymes, a clinical need value was defined as one-fourth our normal range or one-half the standard deviation, with the assumption that the normal range approximates two standard deviations. Our normal ranges for the enzymes listed are: lactate dehydrogenase (LDH) 40–71; hydroxybutyrate dehydrogenase (HBD) 18–40; creatine kinase (CK) 15–75; aspartate aminotransferase (AST) 16–36; and alkaline phosphatase, 19–49 U/liter.

The results for the Coulter instrument, based on several months’ data and our several years of experience with