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Effect of Incubation Time and Temperature on the Interference of Digoxin-like Immunoreactive Substances in Digoxin Immunoassays

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Summary: We have tested the cross-reactivity of digoxin-like immunoreactive substances (DLIS) of cord serum in three commercially available radioimmunoassay systems. We found that there is a marked difference in the specificity of the three antibodies. We were able to eliminate or greatly reduce the interference of DLIS by incubating for 1 h at 37°C. The degree of interference for various cord blood sera is given at three temperature levels and at incubation times ranging from 15 min to 48 h. **Key Words:** Digoxin—Radioimmunoassay—Interference—Digoxin-like immunoreactive substances.

Digoxin is measured routinely in the clinical laboratory for therapeutic monitoring. The most widely used techniques are isotopic and nonisotopic immunoassays (1). The antidigoxin antibodies used are not absolutely specific for the drug, and it is known that there is a degree of cross-reactivity with other drugs and endogenous substances. Digoxin-like immunoreactive substances (DLIS) are substances of unknown nature that are present at high concentrations in the serum of neonates and pregnant women, amniotic fluid, cord blood serum, placental extracts, and serum of patients with renal or hepatic failure. DLIS cross-react with digoxin antibodies and may lead to falsely elevated values for digoxin in certain groups of patients receiving the drug. The nature of DLIS is still unknown. Recently, we have shown that DLIS in cord serum is a mixture of compounds, one of which is cortisone (2). Other investigators have isolated different components (3,4). Many groups continue their work on DLIS in an effort to positively identify these substances and clarify their possible biological activity. The extensive literature on DLIS has recently been reviewed (5,6).

Recently, Pudek et al. (7), Scherrmann et al. (8), and Graves et al. (9) independently reported methods with which the effect of DLIS on digoxin immunoassays could be diminished or eliminated. Such manipulations included increasing the time and the temperature of the incubation, ultrafiltration, and deproteinization. In this article we confirm and extend these observations. We have studied the effect of various incubation times and temperatures on the extent of cross-reactivity of DLIS present in cord serum. We have evaluated three widely used radioimmunoassay (RIA) systems and found that the cross-reactivity can be diminished or completely removed by increasing both the incubation time and the temperature of the incubation.

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METHODS

We have used the following digoxin RIA systems: (a) double-antibody RIA kit from Diag-

TABLE 1. *Effect of incubation time and temperature on the results of digoxin immunoreactivity with the Diagnostic Products Corporation kit*

	Incubation time (min)				
Cord serum	15	30	60	300	2,880
Incubation temperature 4°C					
1	0.21 ± 0.02	0.13 ± 0.04	0.13 ± 0.01	≤0	≤0
2	0.16 ± 0.03	0.11 ± 0.02	0.14 ± 0.02	≤0	≤0
3	0.18 ± 0.01	0.12 ± 0.02	0.16 ± 0.04	≤0	≤0
Incubation temperature 18°C					
1	0.12 ± 0.01	0.14 ± 0.02	0.11 ± 0.01	≤0	≤0
2	0.13 ± 0.01	0.11 ± 0.02	0.09 ± 0.01	≤0	≤0
3	0.14 ± 0.01	0.18 ± 0.03	0.13 ± 0.03	≤0	≤0
Incubation temperature 37°C					
1	0.07 ± 0.02	0.13 ± 0.01	≤0	≤0	≤0
2	0.07 ± 0.03	0.10 ± 0.02	≤0	≤0	≤0
3	0.07 ± 0.02	0.10 ± 0.01	≤0	≤0	≤0

Values are ± SD for n = 3, expressed as digoxin equivalents (ng/ml).

nostic Products Corporation (DPC) (Los Angeles, CA, U.S.A.); (b) ^{125}I-RIA kit from New England Nuclear (NEN) (North Billerica, MA, U.S.A.); and (c) Amerlex digoxin RIA kit (Amersham Int., U.K.).

All RIA assays were performed with the volumes recommended by the manufacturers, but we varied the incubation temperature and the incubation times, as described later. As a source of DLIS, we used cord blood serum collected just after delivery. The mothers' medications were monitored to exclude the possibility of digoxin administration. For each incubation time and temperature, a complete calibration curve was included and values for apparent digoxin immunoreactivity were calculated from the respective calibration curves. All assays were performed in triplicate, except when stated otherwise.

RESULTS AND DISCUSSION

In Tables 1–3 we summarize the results of varying the incubation time and the temperature for the DPC, Amerlex, and NEN RIA kits. From these results the following conclusions can be drawn: (a) There is a marked difference in the cross-reactivity of the three antibodies used in the kits. The most specific antibody is that of DPC followed by that of Amersham. The NEN antibody has the highest cross-reactivity as described previously in many reports (10,11). (b) The cross-reactivity of DLIS can be completely eliminated when using the DPC or

TABLE 2. *Effect of incubation time and temperature on the results of digoxin immunoreactivity with the Amerlex kit*

	Incubation time (min)				
Cord serum	15	30	60	300	2,880
Incubation temperature 4°C					
1	0.30 ± 0.02	0.29 ± 0.02	0.25 ± 0.03	0.19 ± 0.01	0.08 ± 0.02
2	0.35 ± 0.05	0.31 ± 0.02	0.23 ± 0.04	0.20 ± 0.01	0.08 ± 0.01
3	0.33 ± 0.03	0.32 ± 0.02	0.24 ± 0.01	0.20 ± 0.01	0.08 ± 0.02
Incubation temperature 18°C					
1	0.27 ± 0.03	0.25 ± 0.03	0.18 ± 0.01	0.10 ± 0.01	≤0
2	0.41 ± 0.04	0.29 ± 0.04	0.19 ± 0.01	0.12 ± 0.01	≤0
3	0.42 ± 0.04	0.31 ± 0.03	0.20 ± 0.01	0.12 ± 0.01	≤0
Incubation temperature 37°C					
1	0.24 ± 0.01	0.16 ± 0.02	≤0	≤0	≤0
2	0.23 ± 0.01	0.22 ± 0.01	≤0	≤0	≤0
3	0.22 ± 0.01	0.22 ± 0.01	0.10 ± 0.02	≤0	≤0

Values are ± SD for n = 3, expressed as digoxin equivalents (ng/ml).

TABLE 3. *Effect of incubation time and temperature on the results of digoxin immunoreactivity with the New England Nuclear kit*

	Incubation time (min)						
Cord serum	15	30	60	120	300	1,440	2,880
Incubation temperature 4°C							
1	2.96	2.08	1.42	0.95	0.78	0.53	0.41
2	2.73	2.27	1.50	0.98	0.77	0.57	0.44
3	2.92	2.62	1.44	1.01	0.73	0.46	0.42
4	2.72	2.27	1.38	1.04	0.89	0.57	0.43
5	3.04	2.67	1.54	1.05	0.72	0.49	0.44
Incubation temperature 18°C							
1	1.67	1.21	0.82	—	0.54	—	0.45
2	1.99	1.16	0.81	—	0.54	—	0.44
3	1.88	1.25	0.82	—	0.50	—	0.38
4	1.72	1.24	0.77	—	0.54	—	0.41
5	1.94	1.36	0.85	—	0.53	—	0.37
Incubation temperature 37°C							
1	1.51	0.98	0.63	—	0.39	—	0.42
2	1.59	1.03	0.63	—	0.43	—	0.34
3	1.62	1.10	0.66	—	0.41	—	0.36
4	1.47	1.02	0.62	—	0.42	—	0.34
5	1.65	1.05	0.66	—	0.41	—	0.04

All measurements were performed in duplicate and are expressed as digoxin equivalents (ng/ml).

Amerlex kit if an incubation time of at least 1 hr at 37°C is used. For the NEN kit, a degree of interference remains even after 48-h incubation at 37°C. The remaining interference most likely represents the true cross-reactivity of DLIS at equilibrium conditions. (c) For all kits used, the apparent cross-reactivity is a function of both temperature and incubation time. At constant temperature, the cross-reactivity drops continously with increasing incubation time. At constant incubation time, the cross-reactivity is lower if the incubation temperature is higher.

Our observations confirm and extend the very recent literature reports (8,9). The decrease in cross-reactivity by increasing the temperature and incubation time is a general phenomenon observed for other steroid immunoassays (12) and offers a simple means of overcoming the difficulties associated with the presence of DLIS in certain patient samples. Also, it stresses the fact that shortening the incubation time to obtain fast results for emergency purposes may lead to erroneously high values for certain patient groups. It may also explain why the interference of DLIS is high in some automated analyzers where the incubation time is kept relatively short.

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