

13

digoxin complex with o-nitrophenyl-β-D-galactopyranoside.

10. The immunoassay of claim 8 wherein the measurement step (c) is performed by contacting the eluted β-galactosidase labeled, F(ab')₂ anti-digoxin antibody/digoxin complex with o-nitrophenyl-β-D-galactopyranoside.

11. A competitive immunoassay for the measurement of digoxin in a test sample, said assay comprising the sequential steps of:

- (a) forming a reaction mixture by contacting a sample suspected of containing digoxin with a molar excess of ouabain immobilized on a solid phase;
- (b) contacting the reaction mixture with a labeled, monovalent or divalent anti-digoxin antibody, said antibody being in immunochemical excess over digoxin, but in immunochemical deficiency relative to ouabain;
- (c) allowing a reaction to take place whereby a fraction of the antibody forms a first complex with the digoxin and a second fraction forms a complex with the immobilized ouabain;
- (d) separating the first fraction from the second fraction; and
- (e) measuring the amount of label present in either the first or the second fraction.

14

12. The immunoassay of claim 11 wherein the label is an enzyme, radioisotope, chromophore or fluorophore.

13. The immunoassay of claim 11 wherein the monovalent antibody is an Fab fragment or Fab' fragment.

14. The immunoassay of claim 11 wherein the divalent antibody is an F(ab')₂ fragment.

15. The immunoassay of claim 11 wherein the solid phase is beaded agarose, beaded dextran, polyacrylamide or glass.

16. The immunoassay of claim 11 wherein the solid phase is beaded dextran, the monovalent antibody is an Fab' fragment and the label is β-galactosidase.

17. The immunoassay of claim 11 wherein the solid phase is beaded dextran, the monovalent antibody is an F(ab')₂ fragment and the label is β-galactosidase.

18. The immunoassay of claim 16 wherein the measurement step (e) is performed by contacting the fraction to be measured with o-nitrophenyl-β-D-galactopyranoside.

19. The immunoassay of claim 17 wherein the measurement step (e) is performed by contacting the fraction to be measured with o-nitrophenyl-β-D-galactopyranoside.

20. A test kit for measuring the amount of digoxin in a liquid sample, comprising:

- (a) a labeled anti-digoxin antibody; and
- (b) a solid phase having ouabain bound thereto; and
- (c) directions for the use of said kit.

* * * * *

30

35

40

45

50

55

60

65