

the substances comprising a specific binding pair of substances having a mutual specific binding affinity. Substances which may be determined include antigens and their antibodies, haptens and their antibodies, enzymes and their substrates, hormones and their receptors, and vitamins and their receptors. The disclosed test device basically comprises a column containing a matrix which is porous and insoluble with respect to the fluid containing the substance to be determined and which has a specific binding partner to said substance to be determined immobilized therewith.

The matrix preferably is made of a material comprising a polymeric substance. A specific binding partner to the substance to be determined is immobilized with the matrix preferably by being chemically bound to the matrix, in which case the matrix is preferably a polymer substance which contains a hydroxyl, primary amino, or secondary amino group. The immobilized specific binding partner is chemically bound to the matrix preferably through a coupling agent, preferably a cyanogen halide, an inorganic or organic cyanate, or an epihalohydrin.

The disclosed method basically comprises the steps of (a) bringing a predetermined quantity of a fluid sample containing the substance to be determined and a predetermined quantity of a reference sample containing a labeled component, which is a labeled form of one of the substances comprising a specific binding pair to which the substance to be determined belongs into contact with the matrix of the disclosed device; (b) bringing the matrix into contact with an eluting liquid capable of eluting from the column substantially all of the unbound labeled component originating from the reference sample and remaining after step (a), and (c) determining the relative amount of the labeled component which is retained in the column, which relative amount is a function of the amount of the substance to be determined in the fluid sample.

Following a saturation assay technique, the labeled component is a labeled form of the substance being determined and step (a) is accomplished by (a) (1) contacting the matrix with a predetermined quantity of the fluid sample so that some of specific binding partners immobilized with the matrix remain unbound, and (a) (2) thereafter contacting the matrix with a predetermined quantity of the reference sample, the amount of specific binding partners immobilized with the matrix being in excess of that capable of binding with the total amount of the substance to be determined in said predetermined quantity of fluid sample contacted with said matrix in step (a) (1) in the time that the predetermined quantity of fluid sample and the matrix are in contact prior to step (a) (2), and the amount of labeled component in said predetermined quantities of reference sample contacted with said matrix in step (a) (2) being sufficient to bind a portion or all of the remaining unbound immobilized specific binding partners in the time that the predetermined quantity of reference sample and the matrix and in contact prior to step (b). Preferably, the times of contact between the matrix and the predetermined quantities of the fluid and reference samples are prolonged for predetermined incubation periods, which may be the same or different and which are preferably between 15 minutes and 12 hours. An additional step may be included between the contacts of the matrix with the predetermined quantities of the fluid and reference sample which step is contacting the matrix with an eluting liquid, preferably comprising a buffer, capable

of eluting from the column substantially all of the substance being determined which has not become bound.

Following an equilibrium assay technique, the labeled component is a labeled form of the substance being determined and step (a) is accomplished by either contacting the matrix with a mixture comprising predetermined quantities of the fluid and reference samples, the amount of specific binding partners immobilized with the matrix being in excess of that capable of binding with the total amount of both the substance to be determined in the fluid sample and the labeled component in the reference sample in the time that the mixture and the matrix are in contact prior to step (b); or by (a) (1) contacting the matrix with a predetermined quantity of the reference sample, and (a) (2) thereafter contacting the matrix with a predetermined quantity of the fluid sample, the amount of labeled component in the predetermined quantity of reference sample contacted with said matrix in step (a) (1) being in excess of that capable of binding with the amount of the specific binding partners immobilized with the matrix in the time that the predetermined quantity of reference sample and the matrix are in contact prior to step (a) (2), and the amount of the substance being determined in said predetermined quantity of fluid sample contacted with said matrix in step (a) (2) being sufficient to displace only a portion of the labeled component bound to the specific binding partners immobilized with the matrix in the time that the predetermined quantity of fluid sample and the matrix prior to step (b). Therefore, the amount of the labeled component which becomes bound to the matrix in step (a) (1) must be in excess of that capable of being completely displaced by the substance to be determined in the predetermined quantity of the fluid sample added in step (a) (2) in the time that the predetermined quantity of the fluid sample and the matrix are in contact prior to step (b). In both cases, the times of contact between the matrix and the predetermined quantities of the fluid and reference samples are preferably prolonged for predetermined incubation periods, which may be the same or different and which are preferably between 15 minutes and 12 hours.

Following a direct assay technique, the labeled component is a labeled form of a specific binding partner to said substance being determined and step (a) is accomplished by (a) (1) contacting the matrix with a predetermined quantity of the fluid sample, and (a) (2) thereafter contacting the matrix with a predetermined quantity of the reference sample, the amount of specific binding partners immobilized with the matrix being in excess of that capable of binding with the total amount of the substance to be determined in said predetermined quantity of fluid sample contacted with said matrix in step (a) (1) in the time that the predetermined quantity of fluid sample and the matrix are in contact prior to step (a) (2), and the amount of labeled component in the predetermined quantity of reference sample contacted with said matrix in step (a) (2) being sufficient to bind a portion or all of the substance being determined which is bound to the immobilized specific binding partners in the time that the predetermined quantity of reference sample and the matrix are in contact prior to step (b).

The eluting liquid used in step (b) preferably comprises a buffer. The additional step of equilibrating the column, preferably with a liquid comprising a buffer, prior to step (a) is also preferably included in the present method. The labeled component in the reference sample is preferably either radioactively labeled or la-