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THE MICROASSAY ON A CARD - A RUGGED, PORTABLE IMMUNOASSAY

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ABSTRACT

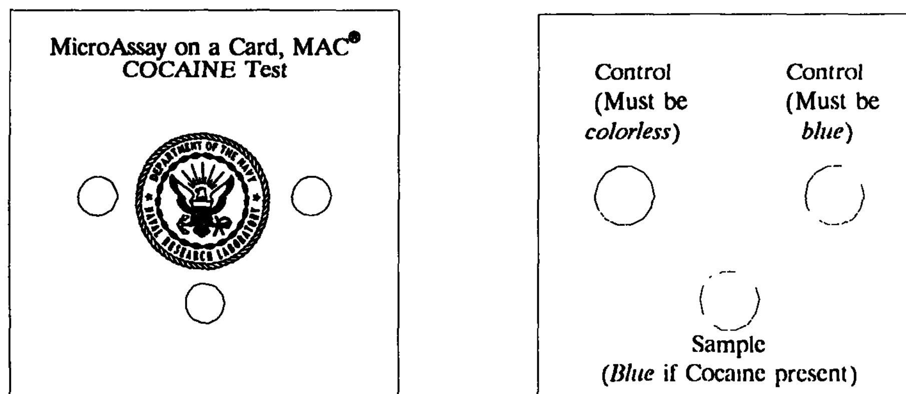
The Microassay on a Card (MAC) is a portable, hand-held, non-instrumental immunoassay that can test for the presence of a wide variety of substances in the environment. The MAC is a simple device to use. A drop of test solution is placed on one side of the card and within five minutes a color is developed on the other side in proportion to the amount of substance in the test solution with sensitivity approaching 10 ng/ml. The MAC is self-contained and self-timed, no reagents or timing is necessary. The MAC may be configured with multiple wells to provide simultaneous testing for multiple species. As envisioned, the MAC will be employed first as an on-site screen for drugs of abuse in urine or saliva. If the MAC can be used as a screen of saliva for drugs of abuse, it could be applied to driving while intoxicated, use of drugs on the job, or testing of the identity of seized materials. With appropriate modifications the MAC also could be used to test for environmental toxins or pollutants.

INTRODUCTION

The Naval Research Laboratory has developed a rapid, field-portable assay for substances in the environment that provides a positive signal in the presence of the substance. The assay relies upon antibody-antigen recognition of the compound of interest, amplification of this recognition, and subsequent visual indication of the presence of the compound. For use, 50 µl of the test fluid is placed in three wells on one side of the MAC. The test fluid is drawn into the MAC by capillary action. No additional reagents are added to the test fluid and all timing is controlled by diffusion inherent in the design of the MAC. The front and back views of the MAC slide are shown in Figure 1.

Depending upon the application, *i.e.* testing of solid or liquid samples, the MAC may be packaged in one of two ways. For testing of solid samples, the product consists of three components: a sealed vial of aqueous buffer, a transfer bulb, and the MAC slide. For testing of liquid samples, only the MAC slide is used.

Figure 1 - Front and Back Views of the MAC (Actual Size is 2" Square)



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The following steps are used to test solid samples:

1. The vial of aqueous buffer solution is broken open and a small amount (< 1 mg) of the solid sample is added using the transfer bulb.
2. One drop of the solution is placed in each of the three wells of the MAC, also using the transfer bulb. The fluid is transferred through the MAC via capillary action with the flow rate determined by the semipermeable membrane incorporated in the device.
3. After two to five minutes (the timing is not critical), the MAC may be turned over and the results observed with the unaided eye.

In the current design, three spots will be present. Two spots are controls, one of which is a positive control, that must be blue and the other is a negative control, that must be colorless. The presence of a blue spot in the test well indicates the presence of the test substance. No reagents must be added to the test fluid; all timing is controlled by the design of the MAC.

PRINCIPLE OF THE ASSAY

Basic Design of the MAC

The MAC consists of a multi-layer slide that may be configured in one of two general modes: displacement and competitive. Figure 2 shows a schematic side view of the MAC. From left to right, the first part is a hydrophobic layer containing the wells which would be produced out of a suitable plastic material, such as polyethylene. The test fluid is placed in the resultant wells and is held by surface tension. Depending upon the mode of operation, the MAC may have either one or two inserts inside the well. The number of inserts and their function are given in Table 1. Two of the major modes of the design of the MAC are discussed below.

Figure 2 - Schematic Side View of the MAC

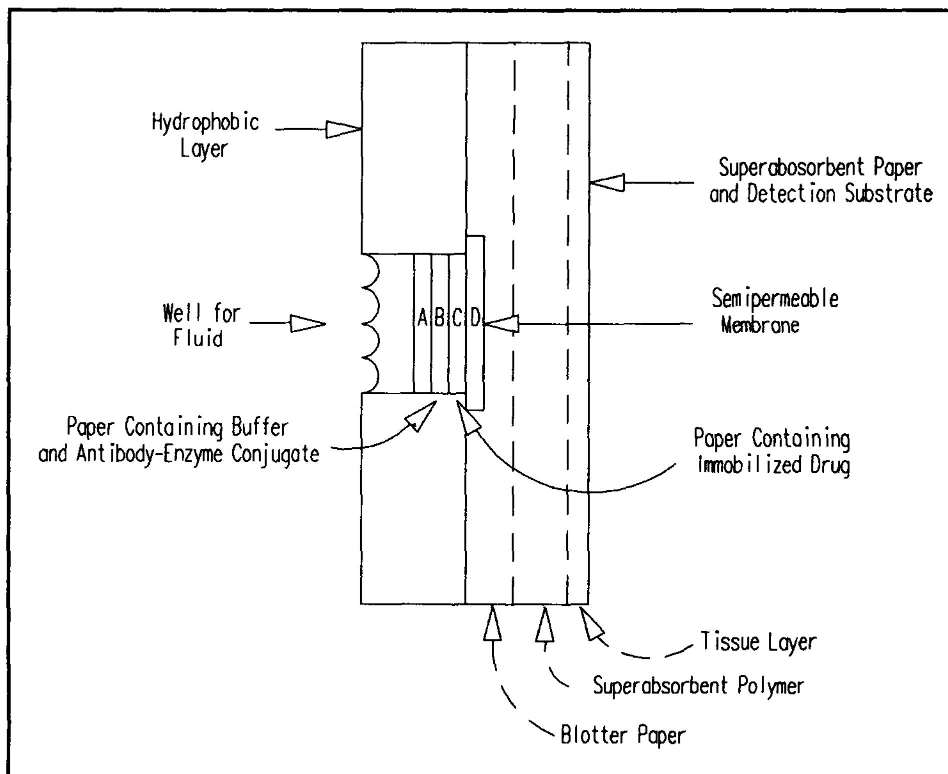


Table 1 - Arrangement of Reactive Species in Well

Type of Assay	Layer A	Layer B	Layer C	Layer D
Displacement 1.	optional	-	antibody-enzyme conjugate + immobilized substance	membrane
2.	optional	-	-	antibody-enzyme conjugate + substance bound to membrane
3.	optional	-	substance-enzyme conjugate + immobilized antibody	membrane
4.	optional	-	-	substance-enzyme conjugate + antibody bound to membrane
Competitive 5.	optional	antibody-enzyme conjugate	substance bound to surface	membrane
6.	optional	substance-enzyme conjugate	antibody bound to surface	membrane
7.	optional	antibody-enzyme conjugate	-	substance bound to membrane
8.	optional	substance-enzyme conjugate	-	antibody bound to membrane

In all modes of operation, there exists the semipermeable membrane layer and the detection layers. The semipermeable membrane controls the timing of the fluid flow by the number of pores and their size. The timing is set such that the test fluid is drawn through the buffer and antibody layers in approximately two minutes. Other timing may be implemented as shown in Table 2.

Table 2 - Timing for Polycarbonate Membranes

Pore Size (μm)	Time (secs)
0.015	∞ (does not draw through)
0.1	800
0.2	135
0.4	68

The detection layer consists of three sub-layers. One is blotter paper that serves as a backing that provides a white background for the developed colored spot. The other sub-layer is a super-absorbent polymer matrix which is impregnated with the substrate for the enzyme. The super-absorbent polymer permits larger liquid samples to be applied and therefore increased sensitivity. The last sub-layer is a tissue paper layer that protects the super-absorbent polymer against abrasion and becomes transparent when wetted by the test fluid.

The advantages of superabsorbent polymers for use in the MAC are many and a demonstration of their use as enzyme detection media has been published.[1] Superabsorbent polymers consist of either salts of polyacrylic acid or grafted acrylic acid on a starch backbone. These polymers can absorb up to 2000 times their weight in water.[2] The advantages of superabsorbent polymers as a reaction medium are: 1) These polymers become hydrated in under three seconds. 2) Once hydrated, the polymer forms a colorless and clear gel. This allows any color forming reaction to be observed throughout the gel which increases the sensitivity. 3) Little control of the amount of fluid is needed. Excess fluid will diffuse out of the reaction area and no color will be produced. 4) Once formed, the gel is restrictive to fluid and molecular diffusion. Therefore, only the enzyme and substrate in the vicinity of the enzyme will react; diffusion of material from other areas is prevented. Also, diffusion of reactants from the detection area is limited. Thus immobilization of the detection reagents is not necessary. 5) The polymers are weak acids and self-buffering around pH 7.

DESIGN OF THE INSERTS IN THE WELL

The number of inserts in the test well and their configuration determines the mode of operation of the MAC; all other components are identical. There are two main modes of operation: Displacement and competitive.

Displacement Mode of Operation of the MAC

In the displacement mode, only one insert is present in the well (optionally two may be present with one containing the buffer). An antigen is chemically attached to the insert and after appropriate manipulation, an enzyme-conjugated antibody is pre-absorbed to the antigen (see Figure 3). When the fluid passing though the slide contains the antigen that the antibody recognizes, some of the enzyme-conjugated antibodies will be displaced from the immobilized antigen. The fluid will carry the displaced enzyme-conjugated antibody into the substrate layer where the enzyme will act on the substrate producing a color. If no antigen is present in the test fluid, no enzyme-antibody conjugate will be displaced and therefore no color will be produced in the substrate layer. The color is proportional to the amount of enzyme displaced and, therefore, proportional to the antigen concentration in the test fluid. (A darker color means more drug is present.)

Competitive Mode of Operation of the MAC

In the competitive mode, two inserts are present in the well. On the first insert is adsorbed enzyme-conjugated antibody and chemically attached antigen is contained on the second insert (see Figure 4). When fluid is passed though the slide, the fluid dissolves the enzyme-conjugated antibody and carries it through the MAC. If no antigen is present in the test fluid, all the enzyme-conjugated antibody would bind to and be trapped in the second layer. Thus no enzyme would appear in the detection layer and no color would develop. Conversely, if antigen was present in the fluid, all the binding sites on the antibody would be blocked and little or no enzyme-conjugated antibody would be trapped in the second layer. Thus a strong color would develop in the detection layer. In this manner, the color in the detection layer is proportional to the amount of enzyme present and, therefore, proportional to the antigen concentration in the test fluid. (A darker color means more drug is present.)

Figure 3 -Configuration of the MAC for a Displacement Immunoassay

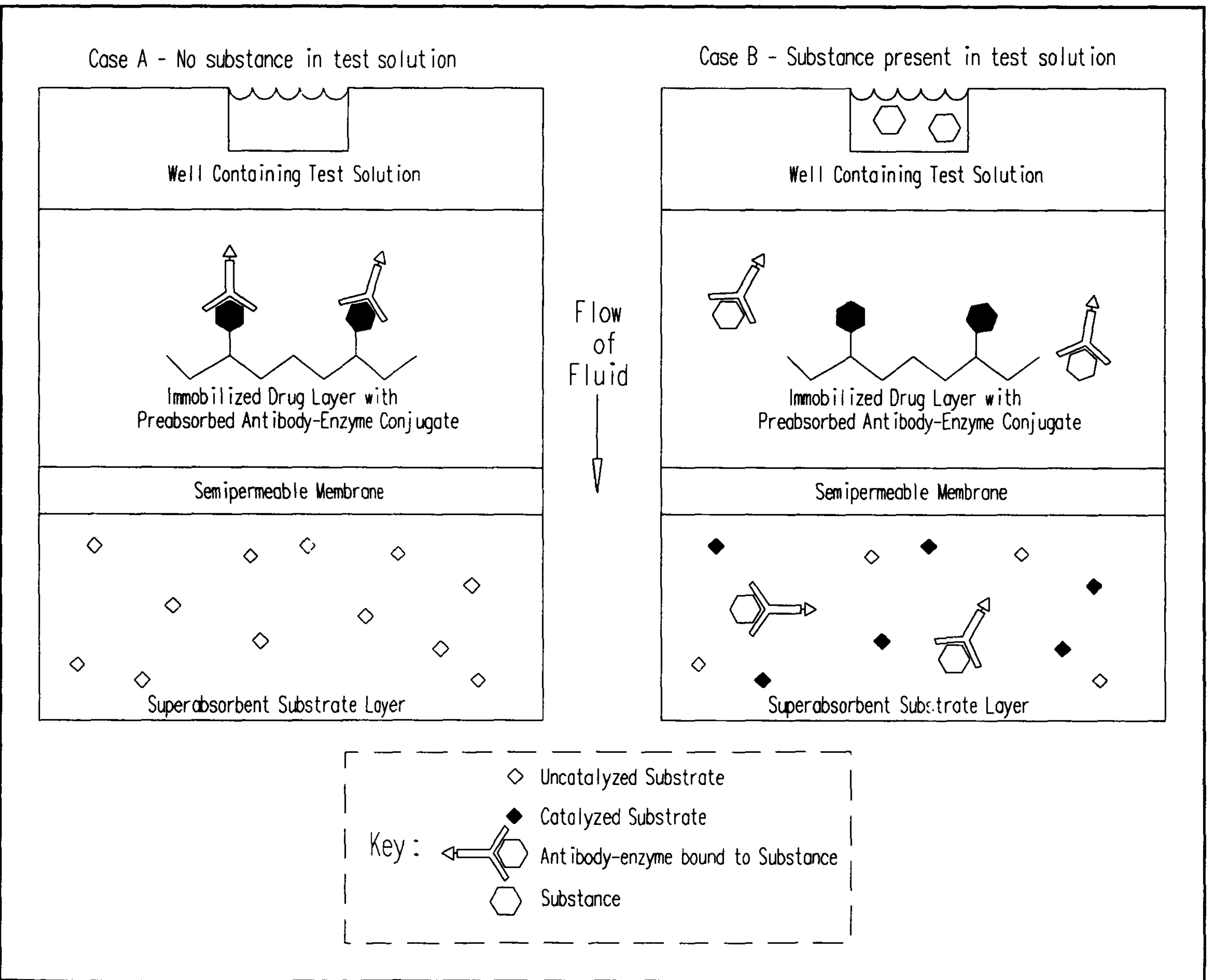
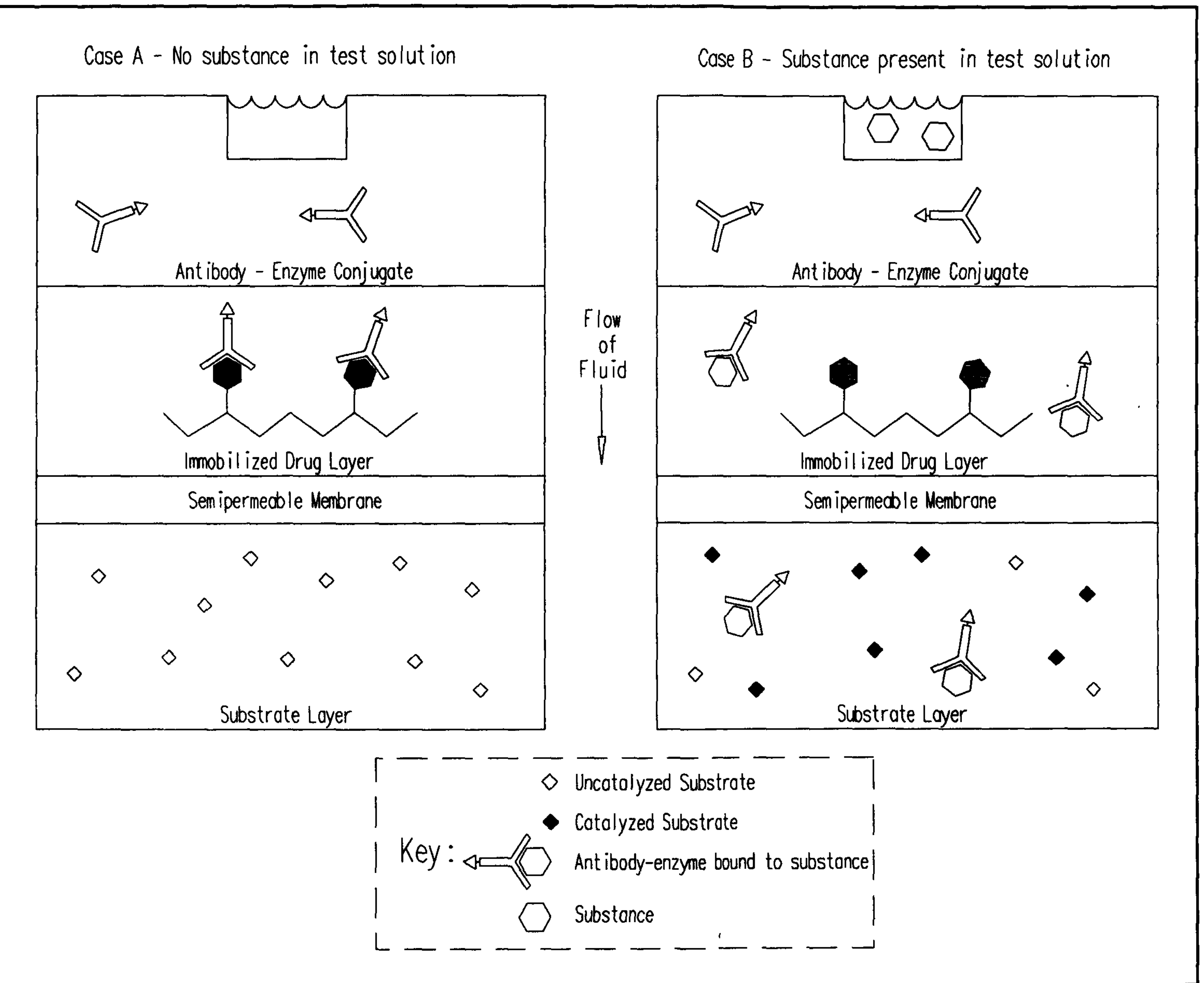


Figure 4 - Configuration of the MAC for a Competition Immunoassay



Comparison of Different Modes of Operation

There is approximately 100 times increase in sensitivity in preforming the MAC technology in the competitive mode as compared to the displacement mode. For example, we have completed the MAC for cocaine and biotin. Cocaine was developed in the displacement mode and showed a sensitivity of approximately 1 μg/mL. On the other hand, biotin was developed in the competitive mode and, under favorable conditions, could reach sensitivities of 10 ng/mL. For testing of solid samples, such as seized, suspected drugs of abuse, the sensitivity displayed by the displacement mode is more than adequate. For testing of substances in a biological matrix, the competitive mode must be used.

The advantages of the displacement mode is the simplicity of manufacture and its disadvantage is its sensitivity. The most difficult step in manufacture of the MAC is the preparation of the enzyme-conjugated antibody. In the displacement mode, routine steps can be employed to prepare this vital component. Once the bound antigen is prepared, it is merely soaked in the enzyme-conjugated antibody solution and washed. Any unbound enzyme or inactivated antibodies are removed at this stage of manufacture since they do not bind to the immobilized antigen. Conversely, for the competitive mode, the enzyme-conjugated antibody must be purified to remove the unconjugated enzyme and inactive antibody. If present, they would not bind to the immobilized antigen and would produce a background color, thus reducing the sensitivity of the MAC.

ADVANTAGES OF THE MAC

The MAC has a number of advantages. They include:

- The assay is rapid and sensitive. It is completed in under 5 minutes with $<$ 10 ng/mL sensitivity.
- The presence of a drug is indicated by the development of a color.
- No instrumentation is needed.
- Antibodies are used for increased specificity over chemical tests currently used.
- The MAC is appropriately designed for field use. Only one solution is needed. (As an option, the MAC may be configured so that only water may be used.)
- Multiple assays may be incorporated on the same slide by addition of more wells.
- Controls are included to distinguish false positives, false negatives and improper storage conditions. However, they may be eliminated, if desired, after appropriate field evaluation.
- No toxic or corrosive reagents are used.

CONCLUSIONS

Development of the MAC assay has been completed for cocaine and biotin, a model compound. Since only a change in antibody and antigen is needed, other substances can easily be incorporated. The sensitivity of the assay varies upon the mode used, which is determined during the manufacture of the MAC. For the displacement mode, the MAC can detect less than 1 μg/mL of cocaine using 50 μL of sample within 5 minutes. For the competitive mode, the MAC can detect less than 10 ng/mL of biotin using a 50 μl sample. The system is portable and has an estimated shelf-life of over one year. The MAC assay is flexible enough to be configured to the requirements of the client.

REFERENCES

1. D.A. Kidwell, "Superabsorbent Polymers - Media for the Enzymatic Detection of Ethyl Alcohol in Urine", *Anal. Biochem.*, **182** 257-261(1989).

2. H.G. Curme, R.L. Columbus, G.M. Dappen, T.W. Elder, W.D. Fellows, J. Figueras, C.P. Glover, C.A. Goffe, D.E. Hill, W.H. Lawton, E.J. Muka, J.E. Pinney, R.N. Rand, K.J. Sanford, and T.W. Wu, *Clin. Chem.* **24** 1335-1342(1978).