Ultrasound-Accelerated Immunoassay, as Exemplified by Enzyme Immunoassay of Choriogonadotropin

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The rate-limiting step in many solid-phase immunoassays is associated with the slow kinetics of binding of macro-molecular antigen and conjugate to the immobilized phase. We demonstrate that the use of ultrasonic energy to enhance mass transport across liquid/solid interfaces can dramatically accelerate antigen binding to immobilized antibodies. We use an ultrasound-accelerated procedure with an enzyme-channelling test strip containing glucose oxidase and specific antibody to the a-subunit of human choriogonadotropin (HCG) co-immobilized onto a cellulose support. A horseradish peroxidase conjugate of monospecific antibody to the β-subunit of HCG is used in the liquid phase to complete the immune “sandwich.” Use of ultrasound to accelerate binding and of enzyme channelling to eliminate wash steps result in a simple two-incubation protocol by which 25 in. units of urinary HCG per liter can be detected visually in less than 20 min of assay time.

Additional Keyphrases: "kit" methods · hormones · immobilized antibodies

Solid-phase enzyme immunoassays, such as enzyme-linked immunosorbent assay (ELISA) methods, are capable of very high sensitivity, especially when used in configurations in which excess reagents are used to generate immuno-specific signals that are directly proportional to antigen concentration (1, 2). Reagent stability and the variety of chromatographic substrates available make these methods particularly useful for qualitative, visually read, nonlaboratory applications.

Much effort during the last 10 years has focused on increasing the convenience, speed, and specificity of heterogeneous enzyme immunoassay. As anticipated, use of monoclonal antibodies (3) specific for different epitopes on protein antigens has allowed simultaneous binding in two-site sandwich immunoassays and has facilitated the development of more rapid and specific tests (4–6). The recent application of homogeneous enzyme immunoassay methods, such as enzyme-channelling (7, 8) and substrate- or prosthetic-group-labeled methods (9, 10), to solid-phase systems for the analysis of small molecules has resulted in assays with unprecedented convenience and simplicity. These or similar homogenous methods obviate the need for bound/free separations.

The rate-limiting step in many solid-phase immunoassays is associated with the slow kinetics of binding of macromolecular antigen and conjugate to the immobilized phase. Surfaces in solution are generally thought to be surrounded by an unstirred layer of solvent, the "Nernst layer," which restricts diffusion and mass transport across the liquid/solid interface (11, 12). The effects of ultrasound (frequencies greater than 20 kHz) on immobilized enzyme systems have been well studied (13, 14) and this source of energy has been demonstrated to accelerate interfacial mass transfer, most probably by reducing the thickness of the unstirred solvent layer (15, 16). Moreover, the activity of immobilized α-chymotrypsin (EC 3.4.21) towards a macromolecular substrate is greatly enhanced when subjected to ultrasonic irradiation (17). The influence of ultrasound on the kinetics of antigen–antibody interactions in solid-phase immunoassays has not been well studied and is the subject of this investigation.

Principle

Human choriogonadotropin (HCG) belongs to a family of two-subunit glycoprotein hormones, including lutropin, follicitropin, and thyrotropin, that contain virtually identical α-subunits and share considerable structural and immunological homology in their β-subunits (18). Urinary and serum concentrations of HCG increase dramatically during the first trimester of pregnancy, making this a useful indicator of pregnancy or pregnancy-related gynecological disorders (e.g., 19).

Using HCG as a model protein antigen, we have investigated the effect of ultrasound on the immune-binding kinetics in a test-strip enzyme immunoassay. The immunological components of the assay are configured to provide a two-site, simultaneously binding, sandwich immunoassay for urinary HCG (Figure 1). Glucose oxidase (EC 1.1.3.4) and monospecific antibody directed against the α-subunit of HCG are co-immobilized onto a cellulose support. A conjugate of horseradish peroxidase (HRP; EC 1.11.1.7) with monoclonal antibody directed against the β-subunit of HCG is used in the solution phase to complete the immune sandwich.

The signal-amplification system used is based on a previously described homogeneous enzyme-channelling immunoassay (7, 8). Briefly, in enzyme channelling a pair of enzymes participate in sequential reactions such that the product of the first enzyme serves as the substrate for the second. Only when both enzymes are in close physical proximity on the surface of a support or in a multi-enzyme
aggregate is there appreciable linked catalysis. Immunochemo-
chemical binding serves to partition one enzyme of the
complementary pair between the bulk-phase solution and
the solid-phase containing the second enzyme.

Our assay protocol for these studies involves combining
the test strip, sample antigen, and peroxidase–antibody
conjugate in a test tube, incubating for 5 min in an
ultrasonic bath, transferring the strip (without washing) to
a developer solution that contains substrates for both en-
zymes, and incubating for an additional 10 min. The chro-
mogenic peroxidase substrate used, 4-chloro-1-naphthol,
produces an insoluble product that adheres to the surface
of the test strip for instrumental or visual evaluation of the
test results.

Materials and Methods

Materials

We used a Branson B-12 ultrasonic cleaner (Branson
Cleaning Equipment Co., Shelton, CT 06484), which has a
nominal acoustic power output of 50 W at 50 kHz. For
kinetic studies, we attached the cleaner to a circulating
water bath (Model 2006; Forma Scientific, Marietta, OH
45750) that maintained a constant liquid level and a tem-
perature of 25 (± 1) °C. Because the liquid temperature in
the ultrasonic cleaner does not change measurably during a
5-min incubation, temperature control is unnecessary under
normal assay conditions.

To measure reflectance, we used a reflectance spectropho-
tometer (Model MS2000; Macbeth, Newburg, NY 12550)
and expressed the results as color difference units (CDU) as
described previously (8). One CDU approximates the mini-
mum difference that can just be detected by the naked eye.
Moist, unreacted test strips give a reading of ~ 3.5 CDU.

HRP (Grade I-C) was from Toyobo Biochemicals, Osaka,
Japan. Glucose oxidase (Type V), Triton® Q-44 surfactant,
S-acetylmercaptosuccinic anhydride, N-ethylmaleimide,
and L-cysteine were obtained from Sigma Chemical Co., St.
Louis, MO 63178. HCG was obtained from Pacific Biotech
Inc., San Diego, CA 92121, and was standardized against
the International Reference Preparation CR119 (distributed
by the National Institutes of Health, Bethesda, MD 20205)
by radioimmunoassay, with the procedure and antibody
provided with the reference preparation. Bovine serum
albumin (BSA) was from Miles Laboratories, Inc., Elkhart,
IN 46515. Succinimidyl 4-(N-maleimidomethyl)cyclohex-
ane-1-carboxylate (SMCC) was from Pierce Chemical Co.,
Rockford, IL 61105. 4-Chloro-1-naphthol was from Aldrich
Chemical Co., Milwaukee, WI 53233. 125I-labeled HCG was
obtained from Cambridge Medical Diagnostics, Inc., Billeri-
ca, MA 01865, and was purified before use by chromatogra-
phy on Sephadex® G-100 (Pharmacia Fine Chemicals, Pic-
cataway, NJ 08854) equilibrated with a solution of, per liter,
0.1 mol of sodium phosphate, 0.15 mol of NaCl, and 2 g of
BSA, pH 7.2. We routinely checked the immunological
purity of the 125I-labeled HCG by radioimmunoassay; it was
usually 65 to 70%. All other chemicals were reagent grade
and used without further purification.

Preparation of Antibody–Peroxidase Conjugates

Purified monoclonal antibody specific for the β-subunit of
HCG was coupled to thiolyt-HRP (20) by a modification of
the thiol/maleimide procedure of Yoshitake et al. (27).

To thiolyt HRP, add slowly, with stirring at 4 °C, to 2 mL
of a 7.5 g/L solution of HRP in Na2CO3 (0.1 mol/L, pH 9.5) a
12-fold molar excess of S-acetylmercaptosuccinic anhydride
(0.1 mol/L in dry N,N-dimethylformamide). After 1 h,
purify the product by chromatography on Sephadex G-25
and store at 4 °C in phosphate-buffered saline (0.1 mol
of NaH2PO4 and 0.2 mol of NaCl per liter, pH 7.2). De-acet-
ylate by incubating the derivatized enzyme in a solution of
hydroxylamine (50 mmol/L) and EDTA (1 mmol/L) at
pH 7.5, under argon for 1.5 h at 25 °C. Thiol content is usually 1 to 3
mol/mol of enzyme, as estimated by Ellman titration (22).

To incorporate maleimide groups into purified antibody,
add slowly to a 9 g/L solution of antibody in phosphate-
buffered saline (pH 7.0) a 25-fold molar excess of SMCC (50
mmol/L in dry dimethylformamide) and stir at 25 °C for 2 h.
Remove excess reagent by gel chromatography on Sephadex
G-25, eluting with a solution of 0.1 mol of NaH2PO4 and 1
mmol of EDTA per liter, pH 7.0. Six to eight maleimide
groups are incorporated per IgG molecule.

To prepare conjugates, add a 20-fold molar excess of
thiolated HRP (12 g/L) to freshly prepared maleimido-
antibody (4 g/L) and stir for 2 h at 25 °C. Stop the reaction by
adding 2-mercaptoethanol to a final concentration of 1
mmol/L; 15 min later, add N-ethylmaleimide to a final
concentration of 2 mmol/L. Separate conjugates from
unreacted thiolyt HRP by chromatography on Biogel A-1.5
M (Bio-Rad Laboratories, Richmond, CA 94804) equilibrat-
ed in phosphate-buffered saline, pH 7.0. We routinely pre-
pare conjugates containing 3 mol of HRP per mole of
antibody, with 80% retention of enzyme activity and immu-
noreactivity.

Antisera and Immobilized Reagents

Either polyclonal or monoclonal capture antibody can be
immobilized onto paper to prepare supports with similar
performance and kinetic properties. Polyclonal antisera
were raised in sheep by immunization with HCG or purified
subunits according to standard immunochromenochem-
ical procedures. Monoclonal antibodies were prepared essentially
according to Kohler and Milstein (3) by fusing spleen cells from Balb/c
mice (that had been immunized with HCG or its
β-subunit) with mouse myeloma NS-1 cells. Preparation of antibody
in ascites tumor (Balb/c mice) and microtiter-plate
screening methods were as described by Oi and Herzenberg
(23), and the preparation of purified immunoglobulin frac-
tions was by precipitation with Na2SO4. Monoclonal antibod-
ies were IgG1 subtype, and both polyclonal and mono-
clonal antibodies had affinity constants in the range of 5 to 10
× 107 L/mole as determined by Scatchard analysis (24) with
iodinated HCG as the tracer.

Antibody and glucose oxidase were co-immobilized to
activated analytical-grade filter paper (589 BH; Schleicher
and Schuell Inc., Keene, NH 03431) as previously described
(8). These papers are stable for more than one year if stored
desiccated at room temperature. The immobilized reagents
used in these studies contained approximately 10 μg of
antibody and 6 mU of glucose oxidase per square centimeter
of support.

Antibody–Antigen Binding Kinetics

All kinetic studies were performed at 25 °C. Results are
reported as the average of duplicate or triplicate determina-
tions of reactions performed in 12 x 75 mm test tubes
mounted in the ultrasonic cleaning bath. Data for "% bound"
have been normalized to reflect the immunochromenochemical
purity of the iodinated tracer.

To measure the binding of 125I-labeled HCG to immobi-
лизированной антиген, мы использовали тест-полоски содержащие ~ 0,3 см²
иммобилизованный антиген-антитела. Умеренные тест-полоски вводили в 1 мл
цитратного буфера, содержащую перил, 10 ммоль глюкозы и 0,15 ммоль
иммобилизованного HCG, 20 г BSA, 200 мг NaNO3, и 125I-labeled HCG
как было указано, в течение 15 минут, с добавлением хлороводородной
растворимость.
extensively in assay buffer, we counted their radioactivity in a gamma counter (Model 1275; LKB Instruments Inc., Rockville, MD 20852). Control strips containing mouse or sheep nonimmune globulin were similarly treated to estimate nonspecific binding.

To measure the kinetics of 125I-labeled HCG binding to solution-phase antibody or antibody–enzyme conjugate, we mixed 125I-labeled HCG with 1 μg of anti-HCG or 0.25 μg of antibody–peroxidase conjugate in a total of 1 mL of assay buffer (see above) and incubated, with and without sonication, for the times indicated. We then added 300 μL of normal mouse serum and 100 μL of rabbit anti-mouse immunoglobulin. We precipitated the antigen–antibody complex by adding polyethylene glycol 8000 to a final concentration of 150 g/L, then centrifuged (3000 x g, 30 min, 4 °C), and counted the radioactivity in the pellets as previously described. Mouse IgG was used to control for nonspecific binding.

To measure the binding of antibody–peroxidase conjugate to immobilized antigen, we first bound HCG to immobilized antibody by sonicating the test strip in the presence of HCG, 100 int. units/L, for 20 min as described above. Next, we incubated the HCG-loaded test strip in 1 mL of assay buffer containing antibody–peroxidase conjugate, 0.25 mg/L, for the indicated times, with and without sonication. We estimated conjugate binding to the HCG-loaded test strip by the color developed on the test strip after its immersion for 10 min in the assay buffer containing enzyme substrates (see next section).

Assay Protocol and Reagent Composition

Immunochromic test strips are prepared by cementing 1/4-in.-diameter discs of activated paper, containing immobilized antibody and glucose oxidase, to 6.5 x 80 mm polystyrene carrier strips.

To measure HCG, combine 1 mL of sample (urine or standard in buffer) with 100 μL of enzyme reagent containing, per liter, 50 mmol of NaH2PO4, 0.1 mol of NaCl, 2 g of BSA, 250 mg of Triton QS-44, and 2.5 mg of antibody–peroxidase conjugate (pH 7.2). Immerse the test strip, sonicate for 5 min, remove the strip, blot, and immerse in 1 mL of developer solution (per liter: 50 mmol of NaH2PO4, 2 g of BSA, 50 mmol of glucose, 300 mg of 4-chloro-1-naphthol, and 250 mg of Triton QS-44, pH 6.5). After 10 min, remove the strip, blot, and measure the reflectance on the pad as described previously (3).

Other Methods

We also determined HCG in clinical specimens by following the product instructions of the Tandem®-E HCG immunoenzylic assay (Hybritech, Inc., San Diego, CA 92121). Protein concentrations were determined from the absorbivities at 280 nm for immunoglobulins (ε = 2.16 x 10⁵ L·mol⁻¹·cm⁻¹) and at 403 nm for HRP (ε = 1 x 10⁹ L·mol⁻¹·cm⁻¹).

Results

Kinetic Studies

Binding of 125I-labeled HCG to immobilized antibody. We studied the effect of ultrasonication on the binding of 125I-labeled HCG to cellulose-immobilized α-subunit-specific anti-HCG, as a function of acoustic power. Test strips were immersed in 1 mL of assay buffer containing 1 milli-int. unit of 125I-labeled HCG, then subjected to sonication, at the power levels indicated in Figure 2, for 10 min at 25 °C. The amount of HCG bound increases linearly as a function of acoustic power over the range studied. All subsequent studies were performed at ~60 W, the limit of the bath sonicator we used.

We further studied the kinetics of the binding of 125I-labeled HCG to immobilized antibody as a function of ligand concentration (1, 10, 10², 10³ int. units of HCG per liter), with and without ultrasonication. The capacity of the solid phase had been previously determined to be 10-fold greater than the highest concentration tested. The binding curves obtained at 1 int. unit/L were representative of the other three concentrations and are shown in Figure 3. A comparison of the two curves clearly demonstrates that ultrasound dramatically accelerates the rate of binding to the test strip.

Fig. 2. Effect of sonicator power on binding of 125I-labeled HCG to immobilized antibody

Power was varied by varying the voltage to the sonicator. Bars represent the range of replicate determinations

![Figure 2](image-url)

Fig. 3. Kinetics of 125I-labeled HCG binding to immobilized antibody test strips in the presence (A) and absence (B) of ultrasound

Bars represent the range of replicate determinations. Insets show the plot of ln (Ct/C₀) vs t, used to derive the pseudo-first-order rate constants

![Figure 3](image-url)
In the presence of ultrasound nearly all of the immunoreactive HCG is bound in the first 20 min, whereas in the absence of ultrasound saturation is not achieved, even after 150 h.

At all concentrations tested, with and without sonication, the kinetics of 125I-labeled HCG binding were apparently first order. Plots of ln(Ct/C0) vs time, where C0 = the concentration of HCG at t0, and Ct = the concentration of HCG remaining in solution at time t, yielded straight lines (Figure 3, insets). The apparent first-order rate constants for the binding of 125I-labeled HCG to the solid support are listed in Table 1 for each concentration tested. The rate constants are essentially independent of antigen concentration and overall are increased 500-fold by sonication.

**Binding of conjugates to immobilized antigen.** To test the generality of the ultrasound effect, we examined the rate at which the antibody–enzyme conjugate binds to HCG-loaded test strips. Test strips sonicated for 20 min in the presence of 100 int. units of HCG per liter were washed and incubated in 1 mL of buffer containing 0.25 μg of antibody–peroxidase conjugate for the times indicated in Figure 4, with and without sonication. At the appropriate times, the test strips were removed and immersed in a substrate solution for 10 min to generate color. The bound conjugate was estimated by reflectance spectroscopy as described in Materials and Methods. The results (Figure 4) are qualitatively similar to those obtained for binding of 125I-labeled HCG to immobilized antigen and again show a substantial acceleration in rate as a result of sonication.

**Thermal, mechanical, and physical effects.** We attempted to duplicate the rate enhancement observed with ultrasound by trying more common modifications of protocol (see Table 2). Performing the binding reaction at a higher temperature (43 °C) only marginally affected the apparent first-order rate constant for binding of 125I-labeled HCG to test strips.

### Table 1. Effect of Sonication on Rate Constants of Binding

<table>
<thead>
<tr>
<th>HCG (int. units/L)</th>
<th>Unsonicated k, s⁻¹ x 10⁶</th>
<th>Sonicated k, s⁻¹ x 10⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.3</td>
<td>2100</td>
</tr>
<tr>
<td>10</td>
<td>4.4</td>
<td>1500</td>
</tr>
<tr>
<td>100</td>
<td>5.3</td>
<td>4500</td>
</tr>
<tr>
<td>1000</td>
<td>6.1</td>
<td>2500</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>5.3 (0.7)</td>
<td>2700 (1300)</td>
</tr>
<tr>
<td>t₁/₂ (s)</td>
<td>1.3 x 10⁵</td>
<td>2.6 x 10²</td>
</tr>
</tbody>
</table>

*Rate constants determined from plots of ln (Ct/C0) vs t as described in the text, and shown in Fig. 3.*

![Graph](CLINICAL CHEMISTRY, Vol. 30, No. 9, 1984 1449)

**Fig. 4.** Kinetics of antibody–peroxidase conjugate binding to immobilized antibody: HCG immune complex in the presence (○) and absence (□) of ultrasound. Bars represent the range of replicate determinations.

Also vortex-mixed the test strip in 1 mL of buffer containing 0.1 int. unit of 125I-labeled HCG, to investigate the effect of mechanical agitation as a function of time. Mechanical agitation increased the binding rate constant by ~50-fold but is still an order of magnitude less effective than ultrasound.

To investigate the possibility that ultrasound could physically and irreversibly alter the structure of the support or the immobilized antibody, we sonicated test strips in buffer for 20 min before incubating them without sonication in the presence of 100 int. units of 125I-labeled HCG per liter. The rate of binding to the pre-sonicated support was not significantly different from that for the unsonicated control (Table 2), which suggests that changes in the support as a result of sonication, if any, relax quickly relative to the incubation times used in the assay. Only when the support and ligand are sonicated together is any enhancement in binding kinetics observed.

**Effect of sonication on solution-phase reactions.** To investigate the question of whether ultrasound would accelerate the binding of HCG to antibody or antibody–enzyme conjugate in a solution-phase reaction, we performed two sets of experiments. In one, we incubated 125I-labeled HCG (100 int. units/L) with 1 μg of α-subunit specific antibody in 1 mL of buffer, for 0.5, 1.0, 2.0, and 5.0 min, with and without sonication. The solution-phase binding at these concentrations, which simulated those in the test-strip assay, was very rapid and was largely complete by the time the first data point could be measured (30 s). We could observe no effect of sonication on the binding rate under these conditions.

In the other experiment we incubated the same amount of labeled HCG with 0.25 μg of β-subunit-specific antibody–HRP conjugate in 1 mL of assay buffer, with and without sonication, for the times indicated. Again, sonication had no effect on the amount of antigen bound or the rate of formation of immune complex.

**Assessment of Assay Feasibility**

**Protocol and configuration.** To demonstrate the feasibility of ultrasound acceleration under clinical assay conditions, we prepared a qualitative immunoassay for HCG in a two-site, simultaneous-binding, sandwich configuration. In the simple two-incubation protocol, one combines 1 mL of sample (urine) with 0.1 mL of β-subunit-specific antibody–peroxidase conjugate, adds the test strip containing co-immobilized α-subunit-specific antibody and glucose oxidase, sonicates for 5 min, and transfers the test strip to a developer solution containing enzyme substrates to incubate for 10 min. The color deposited on the strip surface is measured by reflectance or visual inspection.

**Dose–response curve.** The color developed on the test-strip surface as a function of HCG concentration is shown in Figure 5, which represents duplicate determinations of pooled normal male urine supplemented with purified HCG. The assay has a lower limit of detection of ~10 int. units/L.
and exhibits a "high-dose hook effect" at HCG concentrations in excess of $10^5$ int. units/L. The loss in signal at high antigen concentrations is related to the capacity of the support and the molar excess of sample antigen over antibody–peroxidase conjugate. The effect can be alleviated by increasing conjugate concentration or increasing the amount of immobilized α-subunit-specific antibody. Nonetheless, one can clearly discriminate between negative and positive samples, even at HCG concentrations as high as 2.5 × $10^5$ int. units/L.

**Clinical samples.** To obtain some preliminary information as to the accuracy and performance of the ultrasound-accelerated immunoassay, we assayed 76 urine samples (11 normal men, 65 confirmed pregnant women) and compared the results with those obtained with a commercially available ELISA method (Tandem). The ELISA method had a sensitivity of 25 int. units/L at a cutoff value of 0.3 A$_{405}$. For this comparison, we assigned 7 ΔCDU above the negative as the cutoff value for the test strip assay to yield a test with a detection limit of 25 int. units/L. The results, shown in Figure 6, demonstrate good correlation between the two assays. Both assays correctly identified all of the male urines as negative, and 55 of the 65 pregnant women’s urines as positive. Ten urine samples from pregnant women were scored as negative by both tests, and presumably contained less than 25 int. units of HCG per liter. Nine of these 10 samples were collected on or before the expected onset of menses, and one was obtained from a woman with an ectopic pregnancy seven days beyond the expected onset of menses.

**Discussion**

We have demonstrated that ultrasound can have a profound accelerating effect on the binding kinetics of macromolecular antigens to solid-phase-bound antibodies. The apparent first-order rate constant for HCG binding is 500-fold greater in the presence of ultrasound than in an unsonicated reaction. No such rate enhancement is observed for the same reactions performed in the liquid phase, nor can the effect be duplicated by simply increasing the temperature of the reaction mixture. Vortex-mixing can also increase the rate of HCG binding to immobilized antibody but is about only one-tenth as effective as ultrasound.

The possibility that ultrasound irreversibly alters the surface of the support to render it more accessible to antigen binding, as has been shown for some polystyrene-immobilized enzyme systems (14), we consider unlikely. Sonication of the test strip before antigen binding had no effect on the binding rate for $^{125}$I-labeled HCG. Transient stretching or deformation of the solid phase, which could temporarily relieve steric hindrances and increase accessibility, cannot be excluded by our data. Such effects have been reported by Klibanov et al. (25) in studies on the influence of ultrasound on protein-inhibitor binding to immobilized proteases.

The most probable explanation for the accelerated binding kinetics is that ultrasound enhances mass transfer between the liquid and solid phases. The ability of ultrasound to reduce the thickness of the diffusion barrier (unstirred solvent layer) surrounding surfaces in solution and thereby promote material exchange across liquid/gel interfaces has been well documented (15, 16, 26). Acoustically promoted liquid-streaming movements or collapse cavitation are very likely mediators of this effect and both are thought to be operative under the conditions used in these studies. The binding data we have generated are entirely consistent with this mechanism, and furthermore they parallel many observations made with respect to the influence of ultrasound on analogous immobilized enzyme systems (13).

Clearly, the results for clinical samples are only preliminary, involving a small set of samples, and much work remains to be done to establish the clinical efficacy of this test. These results do, however, demonstrate the possibility of a highly sensitive, rapid, simple, qualitative test for pregnancy if ultrasound is used to accelerate antigen–antibody binding. We expect that the ultrasound effect is of a very general nature and may be applicable in the areas of heterogeneous enzyme immunoassay, radioimmunoassay, affinity chromatography, and heterogeneous catalysis.

**References**


