Transfer Technique for Minimizing Waste of Sonified Adjuvant Emulsions

Cellular and humoral responses in animals are frequently induced by subcutaneous injection with antigen emulsified in an agent such as complete Freund’s adjuvant (CFA). These emulsions are commonly prepared by repetitive mixing of aqueous and oil components in two coupled syringes (1). Although effective, this procedure is slow and tedious. Sonification has proven to be a more rapid alternative, but loading the emulsion into syringes is awkward and wasteful, particularly for small volumes. We have devised a simple modification to the sonication method of adjuvant emulsification that improves the loading of syringes and markedly reduces the amount of emulsion lost during this loading procedure.

In preparation for emulsification, equal volumes of adjuvant and aqueous antigen solution are added to a sterile polypropylene tube (No. 2059 Falcon 14-mL tubes, 17 × 100 mm; Becton Dickinson Labware, Lincoln Park, NJ, USA). Volumes of 0.5 to 8.0 mL could be safely emulsified in these tubes. The sample was emulsified with a Branson Sonifier (Branson Ultrasonic, Danbury, CT, USA) on an output control setting of 3–4 and duty cycle of 75% for approximately 3–10 seconds. The emulsification is judged complete when the sample tube can be inverted without movement of the emulsion. Formerly, emulsion prepared in this manner was drawn into syringes for injection; but, in practice, the emulsion has proven difficult to draw up and is prone to air-bubble formation, which compromises animal injections. Furthermore, a considerable proportion of the emulsion is often lost during the loading of this sonicated emulsion into syringes. The inefficiency of loading emulsion prepared in this manner has been particularly troubling when small volumes of limited or expensive antigens were involved. To take advantage of the speed of sonication for emulsion preparation while reducing waste, we have devised the transfer technique diagrammed in Figure 1. To load syringes, the bottom of the polypropylene tube containing the emulsion was pierced with an 18-gauge sterile needle, and the tube was placed over the top of an empty glass syringe. The plunger of a 10-cc disposable syringe (Luer Lok® No. 309604; Becton Dickinson, Franklin Lakes, NJ, USA) was placed into the polypropylene tube containing the adjuvant and firmly pressed to the bottom of the tube. The plunger fit loosely at the top of the polypropylene tube, but formed a tight seal near the base because of the tapered sides of the tube. Moderate pressure was sufficient to expel virtually all of the emulsion. An emulsion volume of 0.5 mL was the lower practical limit for this technique. From a starting volume of 0.5 mL (comprised of 0.25 mL CFA and 0.25 mL aqueous antigen), 80.8% of the emulsion was recovered from the tube. A recovery of greater than 95% was achieved with emulsion volumes of 2.0 mL or greater. Therefore, this method permits rapid emulsification with minimal waste, permitting a more conservative formulation of emulsions, which can be particularly valuable when the quantity of available antigen is limited. The practical upper limit of this technique is approximately 8 mL. For greater volumes, CFA and antigens can be mixed in larger tubes and divided into several aliquots for emulsification.

To test the efficacy of this method, we compared the T-cell response in BALB/c mice immunized with the chicken ovalbumin peptide 323-339 (OVA 323-339), emulsified in complete Freund’s adjuvant by the traditional dual-syringe technique and by sonification. Ten days after immunization, draining popliteal lymph nodes were removed and single-cell suspensions
were assayed for recall proliferative response to OVA 323-339. Emulsions prepared by either the dual-syringe or sonification method stimulated a strong T-cell response, as demonstrated by the ex vivo proliferation assay. A likelihood ratio test indicated a stronger immunization with the sonified emulsion transferred by the method outlined above than with the emulsion prepared by the dual-syringe technique \( (P = 0.024) \). Thus, the sonification technique produces an effective emulsion in a fraction of the time required by repeated passage between linked syringes, and the transfer technique outlined here renders this sonification easier and less wasteful.

**REFERENCE**


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Received 16 February 1995; accepted 25 August 1995.

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**Removal of Albumin from Multiple Human Serum Samples**

*BioTechniques* 20:30-32 (January 1996)

The isolation of lower abundance proteins from blood or serum is often complicated by the presence of albumin as a major contaminant. Since albumin makes up more than 60%–70% of the total plasma or serum proteins, it can mask many proteins of similar size on gel electrophoresis. Standard methods for preparative purification of proteins, making use, for example, of differences in molecular weight, charge or isoelectric point, frequently yield limited resolution. Consequently, multiple purification steps are generally required that result in lengthy, tedious procedures and low yields, though the relevant protein may be finally adequately purified.

We have been interested in qualitative analysis of heat shock protein 70 (HSP 70) in the serum of Behçet’s patients. Behçet’s disease is characterized by severe self-limited attacks of ocular inflammation. Heat shock proteins are a ubiquitous family of proteins that play a key role in maintaining cellular homeostasis. When cells are injured or subjected to metabolic stress, synthesis of HSP is generally upregulated. We reported earlier that two proteins of 72 and 74 kDa (members of the HSP 70 family) from Epstein-Barr virus (EBV)-transformed B cells of Behçet’s patients were capable of binding bovine interphotoreceptor retinoid binding protein (IRBP) peptide 1169–1191 (2,3). IRBP peptide 1169–1191 is immunopathogenic in Lewis rats (4). Hence, we wanted to analyze serum samples of Behçet’s patients for the presence HSP 70. Clinical samples that were provided were limited in volume. In this report, we describe a reproducible, convenient and quick method that removes 90% of albumin from small serum samples using Affi-Gel® Blue beads (Bio-Rad, Hercules, CA, USA). Affi-Gel Blue affinity gel is a beaded, cross-linked agarose gel with covalently attached Cibacron Blue F3GA dye with a binding capacity for albumin greater than 11 mg/mL. Serum samples thus purified can be directly used for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblot or dot blot analyses.

The initial amount of serum used for purification was 10 µL. Purification was carried out in 0.5- or 1-mL microcentrifuge tubes. Affi-Gel Blue beads (160 µL) were washed with 20 mM potassium-phosphate buffer (K_2HPO_4/ KH_2PO_4), pH 7.1. Ten microliters of serum diluted with 240 µL of phosphate buffer were added to the beads. This mixture was gently shaken on an Adams Nutator orbital mixer (Becton Dickinson, Cockeysville, MD, USA) end to end for 30min at room temperature, then centrifuged for a few seconds to pellet the beads with the bound albumin. The supernatant was removed to a fresh tube. The pellet containing the beads with bound albumin was washed twice with 250 µL of phosphate buffer and centrifuged briefly. The supernatants from both washes were pooled with the original supernatant. The pooled supernatant was concentrated in a Centriprep® 30 concentrator (mol wt cutoff of 30000) (Amicon, Beverly, MA, USA). This step further enriched the protein of interest because all proteins below 30000 mol wt were removed. The concentrated protein was analyzed by SDS-PAGE using a Novex electrophoresis system (Novex, Encinitas, CA, USA). The protein concentration of the samples to be analyzed were determined prior to loading on the gel. To aliquots of sample, an equal volume of Tris-glycine SDS sample buffer con-