

**FILTER PAPER-BASED hTSH-IRMA: ASSESSMENT  
OF OPTIMAL TRACER CONDITIONS**

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A filter paper-based two-site immunoradiometric assay (IRMA) of human thyrotropin (hTSH) was set up with antibodies from NETRIA (London, U.K.) and a hTSH reference preparation extracted in our laboratory. The samples eluted from 5 mm filter paper discs correspond to only 2.1  $\mu\text{L}$  serum (standard curve doses: 0.02 to 0.025  $\mu\text{IU}$  TSH/tube), so the response signals (counts) are accordingly low, impairing assay precision and sensitivity. To get higher response signals, i.e., higher slope of the standard curve, we carried out studies to optimize the use of the tracer.

First, the tracer was labelled to high specific activities: 33-45  $\mu\text{Ci}/\mu\text{g}$  instead of the routine 15  $\mu\text{Ci}/\mu\text{g}$ . Despite an initial significant slope increase, after 2-3 weeks the tracers gave unacceptable assay precisions. Storing at assay dilution or purifying these tracers just before incubation, did not yield better results. The accelerated loss of  $^{125}\text{I}$  from the tracer, a daily 2% decline of the slope and a rapid increase of the "zero" bindings, indicated serious damage of the tracer. Even the more stable 15  $\mu\text{Ci}/\mu\text{g}$  tracers showed a progressive slope decrease, but their use could be extended beyond 60 days. In a second approach, the optimal assay concentration of the 15  $\mu\text{Ci}/\mu\text{g}$  tracer was assessed: using up to 3-fold the routine amount of labelled antibody, we had a 36% slope increase, with proved advance despite of slightly greater "zero" counts.

We conclude that it is useless to label this antibody to specific activities greater than 15  $\mu\text{Ci}/\mu\text{g}$  and that the amount of 15  $\mu\text{Ci}/\mu\text{g}$  tracer should be fixed at not less than 6 ng mo-anti-TSH/tube.

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