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Wadsworth C. A slide microtechnique for the analysis of immune precipitates in gel. *Int. Arch. Allergy* 10:355-60, 1957.
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A rapid, sensitive simplification of Ouchterlony's analytic double-diffusion immunoprecipitation in agar is described. The slide microtechnique employs a Plexiglas matrix with preformed basins to form a chamber for diffusion in agar 0.4 mm thick on a 5x5 cm glass support. In contrast to macroplates, microslides produce a complete precipitation spectrum in hours versus days and have the same versatility but require only one-tenth the volume of serologic reagents per basin. The slides are easy to manipulate, photograph, stain, and store. [The *SCI*® indicates that this paper has been cited in over 295 publications.]

A Microchamber to Increase Speed and Sensitivity of Ouchterlony Analyses

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In 1955 I left a US schoolroom on a year's leave, snapping at the bait of lab work, and hied me to Göteborg. One afternoon, hoping to bypass the drudgery of macro-double-diffusion (DD) analyses, I drilled basins in the first micromatrices. After an evening concert, three microslides were poured and filled, and the next morning precipitates had developed. In the manner of preserving macroplates, the slides, matrices and all, were covered with diatex. This effectively crackled the Plexiglas and achieved a speed record for completed analyses. Now, 30 years later, a few thousand microprogeny and other spectra on glass are being trashed, following the retirement of some old hands. Long live plastics!

Of course I was surprised that the paper was so frequently cited. No compliance with reprint requests was made, but the library volume's back *is* broken from photocopying. Papers and reviews[1-3] soon appeared describing a plethora of basin shapes and arrangements, modes to introduce reactants to gel, and micro-DD techniques using small basins punched into the agar.

Punch-basin testing is still used for establishing serologic relationships, monospecificity, and purity of substances, and even for screening monoclonal antibodies, but it is not always definitive. I used a microchamber with a high-volume ratio of reagents to interaction space, which hastened spectrum formation, increased sensitivity, and obviated basin refilling while it simultaneously reduced line replication and artifacts. This design feature has not been exploited.

A.J. Crowle's book,[1] a best-seller, superseded the original article, but using microscopy slides as glass supports was not propitious. Each antigen-antibody system should develop to completion. Space around the diffusion sources reduces reactant rebound phenomena and provides for extension of superimposed precipitates and their deviation to divulge independence. The handy five-basin and six-shooter designs require the 5x5 cm format.

The logical step of waterproof tape on the underside of matrices came early in Sweden and, perhaps, in the US too, but if micro-Ouchterlony plates or Crowle's microtechnique was cited, improvements were buried in the phrase "the analyses were performed essentially according to..." or some other mode of shortening a methods section.

Commercial matrices might have perpetuated the microchamber and larger format, but my efforts toward this goal ceased when a Danish plastics firm foresaw no large market. (This was before the scramble for patent rights.)

Papers and recent manuals of techniques reveal some universal flaws. Crediting the original article is laudatory, but skimping on details of changes and pitfalls in a series of papers hardly permits accurate recapitulation. For example, comparative DD spectra, i.e., reaction types I-IV, seem not always fully understood nor as clearly explained as by L.-Å. Nilsson.[2] His chapter concisely screens the essential information to utilize DD methods. Even so, one original item is still overlooked: the smoothest Plexiglas surface-to-agar relation is made by drilling the 2 mm hole, reversing the Plexiglas, and making the 3 mm basin with a knife-thin edge at the bottom. Thus, no basin neck sucks up agar on pouring, and air is less easily trapped on filling with reactant.

Does the trail end for exploitation of diffusion-in-gel? Specificity of immunoprecipitation took 25 years to "gel"; comparative DD, 17 more; microamounts and improved sensitivity, an additional 9; now, another 30 years and not even pinhead-sized reaction areas, as suggested by a colleague!

Should readers suppose that these techniques are no longer actual we can cite *Cry in the Dark*. This film recapitulates the event, conviction, and exoneration of an Australian mother for the murder of her infant daughter in 1980. Important prosecution evidence consisted of identification of human fetal blood with Ouchterlony analysis.

Corroboration of the findings for the retrial was impossible, because not only the original specimens but also the plates had been thrown out. On the other hand, when probed by the defense, the manufacturer of the employed "specific" antisera admitted that they cross-reacted with related and unrelated antigens. The medical examiner's laboratory had relied on catalog description rather than a policy of control reagents.

It has been a privilege to air some of the minor frustrations, recall the excitement, and thank Paul Kallos for his help, friendship, and publication of the article.

1. **Crowle A J.** *Immunodiffusion*. New York: Academic Press, 1961. 333 p. (Cited 640 times.)
2. **Nilsson L-Å.** Double diffusion-in-gel. *Scand. J. Immunol.* 17(Supp.10):57-68, 1983. (Cited 5 times.)
3. **Ouchterlony Ö.** Diffusion-in-gel methods for immunological analysis. *Prog. Allergy* 5:1-78, 1958. (Cited 3,025 times.)