Standardization of Coccidioidin by Bioassay in Guinea Pigs[1] (35240)

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The value of all skin tests used in laboratory diagnosis is dependent on certain criteria including, most critically, reproducibility of the test reagent. The coccidioidin skin test is no exception. However, no standardization procedure for coccidioidin has been universally accepted although many methods have been suggested. These methods have included the determination of a minimal reacting dose in guinea pigs (1), comparison to a standard coccidioidin in humans (2), and standardization by content of nondialyzable nitrogen (3). The Division of Biologics Standards suggests minimum requirements for histoplasmin and tuberculin only of the many sensitins in use. This paper reports the standardization of coccidioidin by a bioassay done in sensitized guinea pigs.

Materials and Methods. New lots of coccidioidin were produced by growing 4 strains of *Coccidioides immitis* in Sauton's medium (4) containing glucose (5%) rather than glycerol. Each culture was grown in triplicate in 1 liter of medium in low form flasks. The cultures were incubated at room temperature (22–24°) on a shaker at slow speed (about 100 oscillations/min through 4 cm) for 3 to 4 weeks. Each culture was sterilized by the addition of 1–2 ml of formalin/liter. After sterility was confirmed by culture, the mycelial mat was removed by filtration and further clarified by Millipore filtration. Equal volumes from each filtrate were pooled to provide each test filtrate.

The coccidioidin standard, designated UC-4, was prepared at the University of Utah in the manner described from *C. immitis*, strain Silveira, and standardized by bioassay using coccidioidin 64D3 obtained from the laboratory of Dr. Charles E. Smith as the standard (5).

Appropriate dilutions of the standard coccidioidin UC-4 and the new lots of coccidioidin were made in 0.9% saline. The preparations were tested in albino guinea pigs infected by a single intraperitoneal injection of about 15,000 live *C. immitis* arthrospores. Diluted sensitin in 0.1-ml volumes was injected intradermally on the shaved side of the animal and the skin tests were read as the diameter (mm) of induration after 24 hr.

The preparations in each assay consisted of 3 dilutions each of the standard and a test material. Unless preliminary determination of a log dose–response curve indicated otherwise, the dilutions used were 1:200, 1:400, and 1:800 which represented the optimum dilution, 1 dilution greater and 1 dilution less in each case.

Six guinea pigs were used and 6 injections/animal were made at 6 different sites. The preparations, sites, and guinea pigs were randomized according to a Latin square with each preparation occurring only once per animal and once per site.

Calculations were performed by a Control Data 3200 computor programmed to yield an analysis of variance and regression analysis, and to calculate a potency ratio with 95% confidence limits. The Fortran program was written by Dr. Allen Smith and Dr. Homer G. Warner of the Department of Biophysics and Bioengineering, Latter-Day Saints Hospital, Salt Lake City, Utah.

Results. A potency ratio and 95% confidence limits are given in Table I for each new lot of coccidioidin. The potency ratio gives the dilution factor for standardizing the new coccidioidin to the standard coccidioid-

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TABLE I. Bioassays of Coccidioidin.

	Potency ratio	Lower limit (95%)	Upper limit (95%)
Gasket	1.1830[a]	0.6896	2.2134
Gasket-2R (1:1.2)	1.1305[a]	0.5445	2.2411
	0.0421[b]	0.4537	1.8676
Rystead	0.2460	0.0793	0.4362
Rystead-2	0.2732	0.1494	0.4056
F842	0.6108	0.2517	1.0732
Ruddy	1.2429[a]	0.8733	1.8525
Ruddy-2 (1:1.2)	0.9462[a]	0.5277	1.5346
	0.7885[b]	0.4397	1.2788

[a] Undiluted.
[b] Diluted.

in. Thus, the lot of coccidioidin from strain Gasket is 1.183 times more potent than UC-4. The confidence limits give the range within which the true potency of the new coccidioidin will be found with a probability of 95%. Coccidioidin Gasket was diluted 1:1.2 and the assay was repeated (Gasket-2R).

Potency ratios and confidence limits for 2 assays of coccidioidin from strain Rystead are next shown in Table I. Both assays show undiluted Rystead to be less potent than standard.

The results of assays of coccidioidin from strains F842 and Ruddy showed significant nonparallelism of the standard and test log dose–response curves and, therefore, were unsuitable for standardization by potency ratio by the method under test. New 1-liter preparations of F842 and Ruddy coccidioidins were also found to be unsuitable for standardization by reason of nonparallelism of response compared to the standard.

Discussion. The bioassay utilized in this work provides a method for standardizing coccidioidin by making an estimate of potency of the test preparation to a standard preparation and expressing this as a potency ratio. The results suggest that a potency ratio obtained by this procedure is reproducible. The assay described was used to standardize UC-4 prepared at the University of Utah, to Smith's standard coccidioidin 64D3 (5). A preliminary assay gave a potency ratio of 4.03, indicating that UC-4 was 4 times more potent than 64D3. After UC-4 was diluted 1:4, a potency ratio of 1.1 was obtained. UC-4 was the standard coccidioidin used in the present work. However, the potency of the new preparations may still be expressed in terms of the original standard, 64D3.

The assay of coccidioidin Gasket gave a potency ratio of 1.18 (0.69–2.21). This potency ratio was used as the dilution factor for the second assay of Gasket and a potency ratio of 0.94 (0.45–1.87) was obtained for the sensitin undiluted or diluted as much as 1:2.2 would be at a potency equal to standard within the limits of error. The value of 1.18 is the best estimate of the potency of the undiluted preparation.

The reproducibility of the results obtained from the bioassay is further demonstrated by the 2 independent assays of coccidioidin Rystead. The potency ratios were 0.25 (0.08–0.44) and 0.27 (0.15–0.40), respectively.

Coccidioidin prepared from strains F842 and Ruddy failed to meet the requirements established for the bioassay with regard to parallelism of the standard and test dose–response curves. Although no explanation for this nonparallelism is available, strains that yield such filtrates appear to do so reproducibly. It would seem justifiable to exclude such a sensitin from use on the basis that substances other than the critical one(s) under test might be contributing to the skin test reaction. A less likely but alternative hypothesis to be tested is that the present primary (Smith's 64D3) and secondary (UC-4) standards may contain substances other than the critical ones and that the coccidioidins from strains F842 and Ruddy may be preferable for skin testing.

Summary. A bioassay for mycotic sensitins has been developed which yields a potency ratio with confidence limits for new lots of sensitin relative to a standard lot. The standard and test sensitins are injected intradermally into homologously sensitized guinea pigs and the diameters of the resulting skin reactions are submitted to a Control Data 3200 computor programmed to perform an analysis of variance and regression analysis. The potency ratio obtained by this procedure can be used as a dilution factor for the

standardization of new lots of sensitin.

The practicality and reproducibility of the bioassay as a standardization procedure was demonstrated by assays of coccidioidin from 4 strains of *Coccidioides immitis*. Coccidioidin Gasket was found to be equal in potency to standard coccidioidin while coccidioidin Rystead was found to be less potent. Coccidioidin from strains F842 and Ruddy were unsuitable for standardization as skin test antigens by this procedure.

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