

APPENDIX 10

Leishmanin skin test (Montenegro)

Phosphate buffered saline (PBS, pH 7.2)

NaCl	8 gm
KH ₂ PO ₄	0.2 gm
Na ₂ HPO ₄ 12H ₂ O	2.88 gm
KCL	0.2 gm
Distilled water	up to 1 l

(This product can be made up at a concentration of 10 times the above recipe for better long-term storage).

Skin test diluent

NaCL	5 gm
NaHCO ₃	2.75 gm
Phenol	4.0 gm
Distilled water (STERILE)	up to 1 l

Cultured *L. donovani*, *L. infantum* or *L. chagasi* promastigotes are washed (3 times) with sterile phosphate buffered saline (PBS) by centrifugation and re-suspension, preferably at 4°C. The final pellet is re-suspended in a skin test diluent at a concentration of between 5 x 10⁶ promastigotes/ml and 4 x 10⁸ promastigotes/ml. Concentrated stock solutions are diluted before use to 1 x 10⁶ promastigotes/ml. Both concentrated stocks and diluted antigens can be stored at 4°C for at least 12 months. Thiomersal may be used as an alternative to phenol for preventing contamination. In many countries leishmanin skin test antigens ready for use are available commercially.

0.1 ml of the antigen preparation is injected intra-dermally into the forearm of the patient (after the forearm has been cleaned with 70% alcohol). Control tests using solvents should be injected intra-dermally into the opposite forearm. The diameter of induration at the site of inoculation is measured at between 48 and 72 hours after inoculation using the ball-point pen method. The induration may be outlined with a ballpoint pen and the ink "lifted" by applying a piece of sticky tape; the tape with the ink mark provides a permanent record for each patient. (A piece of paper moistened with alcohol may be used instead of sticky tape.) A mean diameter of > 5 mm is considered to be a positive skin test reaction.

The leishmanin skin test is normally negative in patients with active VL but a positive reaction is associated with clinical cure or may be found in asymptomatic patients who have presumably been exposed to infection.

Comparison of previous studies in which skin testing was used is difficult because of the lack of uniformity in type and dose of antigen used. The use of well-standardized and evaluated antigens is important. The development of skin test antigens under the auspices of WHO is a significant progress in this respect.