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© Determination of C4 concentration by the Mancini test.

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- 1 An appropriate anti-C4 antiserum (antibody) is poured in the center well of an agar-containing plate.
- 2 Carefully circular wells are cut and detached from the plates.
- 3 A series of standards containing known concentrations of C4 are placed in separate wells, while "unknown" human serum samples and control are placed in other wells.
- 4 A ring of precipitate forms in the area of optimal antigen-antibody concentration, meaning anti-C4 C4 reaction as the antigen diffuses radially.
- 5 The diameters of the rings are measured and perceived normally in 48-72 hours.

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6	Finally, a standard curve is developed using the ring diameters of the standards versus the concentrations.
7	A curve is then used to plot the concentration of the control and unknown C4 samples.