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1. The microorganism or other pathogen must be present in all cases of the disease 2. The pathogen can be isolated from the diseased host and grown in pure culture 3. The pathogen from the pure culture must cause the disease when inoculated into a healthy, susceptible laboratory animal 4.

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Discovered in 1930s with the invention of the electron microscope; acellular (not a cell, without cell structure); smallest of all microbes; causes disease, including some cancers. 5 Kingdom scheme of classification.

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