Table 17-1 Biological Indicators and *D* Values

Sterilization process	Biological indicator	ATCC number	Typical *D* value range
Steam	*Geobacillus* (formerly *Bacillus*) *stearothermophilus*	7953	1.5 min @ 121°C
Dry Heat	*Bacillus subtilis* var. *niger*	9372	1.0 min @ 180°C
Ionizing radiation	*Bacillus pumulis*	14884	3.0 kG
Ethylene oxide	*Bacillus subtilis* var. *niger*	9372	5.8 min @ 600 mg/L, 54°C, 60% RH
Vapor phase hydrogen peroxide	*Geobacillus* (formerly *Bacillus*) *stearothermophilus*	7953	NA
Peracetic acid	*Bacillus subtilis* var. *niger*	9372	NA

Abbreviation: RH, relative humidity.
Source: From Ref. 9.

on the presence of a cellular envelope. Gram-positive bacteria do not contain an outer cell wall while gram-negative bacteria do. It is this outer cell wall of gram-negative bacteria that contains layer(s) of lipopolysaccharide that produces endotoxins when such bacteria grow and die. Major examples of gram-positive bacteria are *Staphylococcus, Streptococcus, Bacillus, and Clostridium.* Major examples (genus) of gram-negative bacteria are *Pseudomonas, Escherichia coli, Salmonella, Klebsiella, and Serratia.*

Bacteria can also be classified as aerobic (requiring oxygen to grow), anaerobic (can grow in nonoxygen environments, e.g., nitrogen saturated solutions), or facultative (can grow in either environment). Bacteria are pathogenic, nonpathogenic, or opportunistic. Some, but not all, bacteria can form spore forms. Spore formation results where the bacterial cell, in order to continue to survive, develops a sort of outer shell that protects it from adverse environmental conditions such as heat, chemicals, and nutrient depletion. The most common spore formers are *Bacillus* and *Clostridium* species. Both are gram-positive bacteria and are commonly used as biological indicators (Table 17-1) since spores are hundreds of times more resistant than vegetative bacteria to the effects of sterilization treatments. Biological indicators measure the effectiveness of sterilization methods.

Fungi are cellular forms that are very similar to human cells. While bacterial cells are called prokaryotic cells, fungal cells are called eukaryotic cells, the same classification as human cells. Fungal cells are much more difficult to destroy plus attempts to kill fungal cells may also kill human cells because of their similar cell types. About 10% of all known fungi are pathogenic. *Candida* species and some dermatophytes are the only known fungi transmitted from person to person.

Viruses are intracellular parasites that do not need food to survive. Viruses are extremely small and will easily pass through bacterial retentive filters. Viruses are readily inactivated by heat at relative low ($\sim$ 65°C or above) temperatures. They are very susceptible to surface disinfectants. The environmental detection of viral contaminations can be very costly. In light of the fact that sterile manufacturing environments are extremely harsh for viral survival, viral monitoring and concerns about viral contamination in finished product manufacture are practically nonexistent.

MICROBIAL DEATH KINETICS

Figure 17-1 displays the ideal growth and death phases of microorganisms. Since microbial growth is cellular duplication and multiplication (geometric progression), growth is plotted logarithmic (exponential). When contamination first occurs, assuming that it is very low level (a few cells), there is a lag time before sufficient cells can be measured. This lag phase can be minutes to much longer times (even years). In sterilization microbiology, lag times are considered to be minutes to hours. Once growth starts, it progresses quite rapidly. For example, a typical bacterial cell might duplicate itself every 20 minutes under ideal growth conditions. Therefore, after one-hour incubation, one cell has grown to eight cells. After two hours 8 cells have grown to 64 cells. After 8 hours of duplication every 20 minutes that original single cell has produced over 1,300,000 cells!