

# Understanding and monitoring the sterilization process: Inside the “Black box”

By Professor Laurence J. Walsh



*“This article concentrates on the information that can be gleaned from the simple indicators used in everyday practice, and illustrates “pass” and “fail” results with common brands of chemical indicators...”*

**W**hile virtually all dental practices have autoclaves and use them on a daily basis, often only scant attention is paid to the operating principles of these devices, until malfunctions occur or problems develop with processing of loads. This article concentrates on the information that can be gleaned from the simple indicators used in everyday practice, and illustrates “pass” and “fail” results with common brands of chemical indicators.

Many factors work in harmony to ensure contact of steam with items in the load occurs for sufficient time to transfer the latent heat of condensation of water, which is responsible for denaturing proteins and thereby rendering microorganisms non-viable. Both human factors (such as instrument cleaning and chamber loading methods) and equipment factors operate in achieving the desired outcome (Figure 1). Saturated steam is an efficient means for killing microorganisms since it is able to quickly transfer heat energy onto the items which are being sterilized. As saturated steam condenses, this heat is transmitted onto the surface and the microorganisms are inactivated by direct thermal effects. Thus, for sterilization to occur, the steam must be saturated to the correct level.<sup>1</sup>

## Cycle parameters: back to basics

Looking at data from a typical cycle in a downwards-displacement (non-vacuum) autoclave, several features can be noted (Figure 2). Commencing at room temperature (A), the temperature rises in an almost linear fashion during the first portion of the cycle (in this example, 9 minutes from A to B), to reach the boiling point of water (B).

During this first phase, air is being displaced passively from the chamber by steam.

Once the air removal valve closes to seal the chamber (at 10 minutes, point C), the pressure inside the chamber begins to rise above atmospheric levels. At the same time, there is an inflection in the temperature curve because now the chamber is pressurized and the temperature of water can rise above its boiling point. Both temperature and pressure then increase linearly from C to D, achieving the intended sterilizing conditions some 18.5 minutes after commencing the cycle. The “holding time” at sterilizing temperature and pressure conditions is the period between D and E.

The chamber contents are vented at E and the chamber pressure soon reaches atmospheric levels, allowing the door to be opened (F). Note that at this stage, the temperature inside the chamber has fallen back to the boiling point of water (100 degrees Celsius). It will continue to fall after this, since no additional heating is being provided through the heating elements. Some one seventh of the cycle time in this case was at sterilizing conditions - a fraction which would increase with the next load as the chamber is now heated.

Being able to appreciate the air/steam interphase relationships and applying the gas laws allows some useful information to be gained in terms of the autoclaving process. For example, with regard to steam saturation, whether water exists as a liquid or as a gas is entirely dependent upon the temperature and the pressure. When the pressure is increased, the boiling point of water is elevated. In an autoclave, it is essential that the steam be very close to the phase change temperature or boiling point. If the tempera-

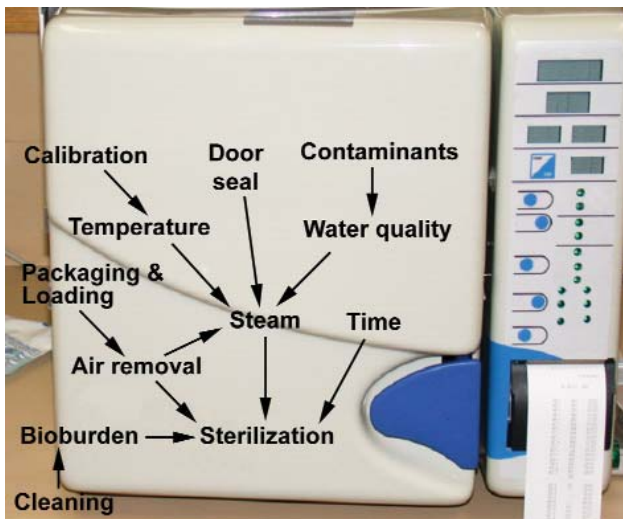


Figure 1. A schematic of some major human and equipment factors that interact in the process of sterilization by steam under pressure.

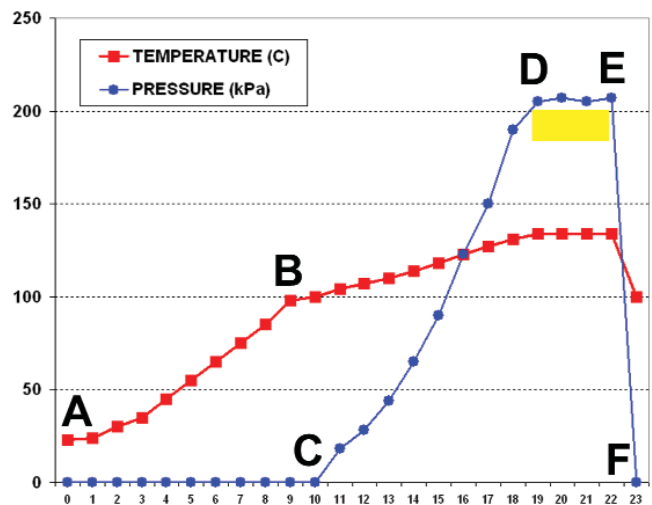


Figure 2. Data plot minute-by-minute of temperature and pressure during a standard 134 degree Celsius cycle from a cold (room temperature) start. The horizontal axis shows the elapsed time in minutes.

ture is higher than this, the steam is superheated and this impairs its ability for killing. There are several reasons for this. Firstly, when saturated steam comes into contact with a cooler object, such as an instrument, the steam changes immediately to liquid water and this phase change is associated with the release of a large amount of energy in the form of heat. This transferred heat rapidly increases the temperature of the object involved. Secondly, when the steam changes back to water, there is a dramatic reduction in size and this contraction pulls in yet more steam to replace that which has already changed to water. This process continues until eventually the entire load in the sterilizer has been raised to the same temperature as the steam. The third reason relates to the moistening effect. When steam changes to water, this moistens the organisms, which increases the kill rate. When steam is superheated, the temperatures fluctuate considerably around the chamber and consistent killing is not achieved. Transfer of heat is less, shrinkage of steam is less, and for these three reasons, superheating of steam is a problem.

Measuring temperature and pressure at one or more points in the chamber during the cycle can assist in identifying problems during the cycle. The advent of programmable logic controllers (and subsequently microprocessors) revolutionised autoclave design, with software systems



Figure 3. Programmable logic controller used to gather parameter data and regulate functions during autoclaving. The unit shown is from a prototype autoclave built by the author some two decades ago.

for controlling autoclave functions dating back to the mid-1980's (Figure 3). Using autoclave systems where a range of temperatures, cycle times, water loads and other parameters can be digitally controlled also offers the chance to create cycles with known "pass" and "fail" conditions, and thereby to test and demonstrate the performance of various types of chemical and biological indicators (Figure 4). The examples in this article have been generated using such an approach. For example, if the appropriate temperature and pressure are not present, the steam will be too dry in which case it will be superheated. Alternatively, by manipulating chamber moisture, steam which is too wet (supersaturated) can be obtained.



Figure 4. Autoclave with user-adjustable time and temperature parameters, used for research and teaching into sterilization technology.

Chemical indicators give information regarding the performance of the sterilizer during the holding phase of the sterilizing cycle (From D to E on Figure 2). This holding phase is the critical time period since it is during this time that the microorganisms are inactivated. Statistically, a sterilizing cycle is designed so that the opportunity for a microorganism to survive is less than 1 in 1 million. At a temperature of 134°C, this time interval is 3.5 minutes.

### Process challenge devices for pre-vacuum units

Process challenge devices, also known as Class 2 chemical indicators, comprise the Bowie-Dick test (for porous loads) and the helix test (for hollow loads). Both tests assess air removal and steam penetration,



Figure 5. Typical Bowie-Dick test (Interster) used in a pre-vacuum autoclave, which has a "flat pack of cards" configuration.

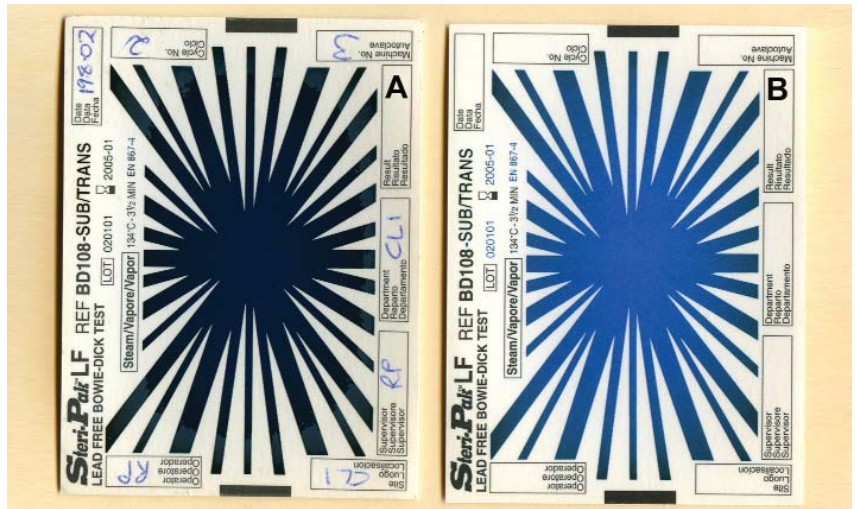


Figure 6. Examples of the SteriPak™ Bowie-Dick test, showing pass (A) and fail (B) results. An even colour change to black is caused by steam penetration. The presence of residual colour (in this case, blue) in the centre of the pack indicates persisting air or volatile gaseous materials.

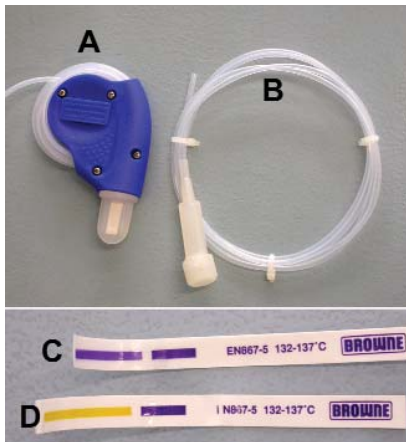


Figure 7. Typical Helix tests (A and B) with a coiled design. The inserts from the Browne's test unit (A) are shown in the lower panel D is an unused control strip and C is a processed strip showing a complete colour change and thus a "pass" result.



Figure 8. A series of strips from a helix test (unit B from Figure 7), showing an unused strip (A), a series of "fail" results because of incomplete air removal (B-C), and a "pass" result (D) where there has been complete colour change.

and thus confirm the correct operation of the vacuum system in the autoclave.<sup>2</sup> These tests prove that steam penetration has been rapid and even, and that air and non-condensable gases were not present at the time of steam entry. Most Bowie-Dick tests employ a "pack of cards" design (Figure 5), with a centrally placed thermally sensitive card (Figure 6) which undergoes a uniform colour change when exposed to steam at the correct temperature and moisture, for sufficient time.

The test pack which is used in a Bowie-Dick test is designed to represent the maximum possible density of a porous load. The more air there is present to be removed, the more difficult the challenge posed by the test. It is for this reason that the test package is used by itself in an otherwise empty chamber. In other words, the Bowie-Dick test is not placed in the chamber at the same time as an instrument load.

The indicator is not simply heat dependent, in that exposure to dry heat only rather than steam will not result in a colour change. Gases trapped in the middle of the test pack form a bubble or a pocket, which impairs the entry of steam, causing a persistence of the original colour. Air will show as a centrally placed persistence of

colour, whilst non-condensable gases will give radial patterns of colour persistence, as will superheated steam. Several brands of Bowie-Dick test are now available, and all are based on the seminal 1963 *Lancet* paper which first described the test. Of note, some brands of thermo-chromic Bowie-Dick tests (such as Albert Browne) allow complex diagnosis of gas entrapment problems by analysis of patterns of failed indicator cards.<sup>2</sup>

Several designs for helix tests are likewise now available (Figure 7). These tests employ a reservoir in which is placed a chemical indicator strip. A "pass" result occurs when all air has been removed and steam enters the reservoir fully, causing a colour change. A "fail" result occurs when there is incomplete colour change (Figure 8). Depending on their design, helix tests have a limited life, with the chamber and/or helix requiring replacement after a specified number of uses (after several hundred cycles) to prevent leakage which would give false positive "pass" results. In contrast, Bowie-Dick test packs can only be used once and are then disposed of. Depending on the nature of the load, either helix or Bowie-Dick tests must be undertaken each day that a pre-vacuum autoclave is used, according to the types of loads intended for that day. More detailed information can be found in Reference 2 and in Table C1 of Australian Standard AS1410.

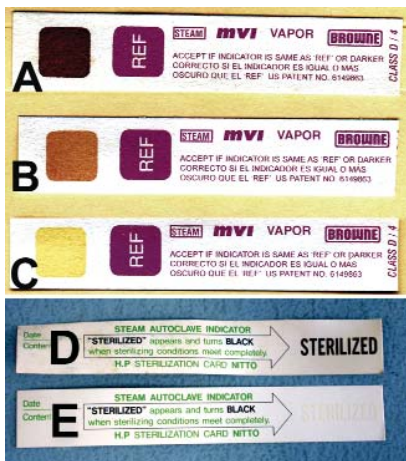


Figure 9. Examples of Class 4 chemical indicators. The Browne's MVI indicators show a "pass" (A), and a "fail" (B), with only partial colour change. An unused indicator is shown at C. In the Nitto indicator (D and E), the word "sterilized" appears after processing (D), but cannot be seen in the unused control indicator (E).

### Steam parameters

The purity of steam is an important parameter in effective autoclaving. Pure steam is pure water in a gaseous phase. Any impurities in steam could be of a liquid form, e.g. droplets of water or fog, or they may be of a gaseous nature, e.g. entrapped air. Solid impurities in steam, such as particles of rust or instrument coatings, can also occur.<sup>3,4</sup> When small amounts of air are present in steam as an impurity, this has little effect provided the air is mixed thoroughly with the steam and the amount of air is very low, e.g. less than 1%. If the air is present at a level beyond this, the air does not readily mix with the steam, but remains rather compressed by the steam into pockets of air which are cool. Cool air pockets, which may be caused by an overcrowded chamber, or by incorrect wrapping, or incorrect positioning, or incorrect use of packaging materials, are a very common cause of failed autoclaving in downwards displacement autoclaves. Air pockets occur less often in pre-vacuum autoclaves.

When water droplets or fog are present as an impurity in the steam, materials inside the sterilizer may become wet. The presence of a very small amount of entrained moisture, e.g. less than 2%, is beneficial since this reduces the tendency of the steam to otherwise superheat.

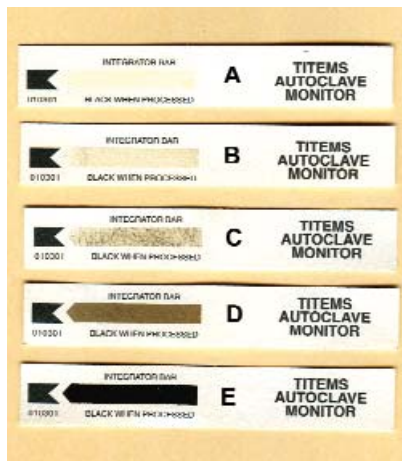


Figure 10. A series of Titem's Class 4 chemical indicators. The topmost indicator (A) is a control unused indicator. Subsequent indicators show the colour change at 15 secs (B), 30 secs (C), 2 mins (D), and 3.5 mins (E), all at 134°Celsius. The bottom indicator (E) is a "pass". Note how the colour develops gradually over time when at the correct temperature.

### Integrating chemical indicators (Classes 4-6) for packages

These indicators are used inside packages, where they provide useful information on steam saturation, steam purity and steam availability within the package (as opposed to Class I indicators which are located externally and indicate only that processing has occurred). In this regard, it is important to remember that Class 4-6 chemical indicators have specified performance limitations according to the class of the indicator, with greater precision with Class 6 than with the lower classes.<sup>5,6</sup> Where instruments are intended to be sterile at point of use, then a high level emulating indicator is recommended in each instrument pack, although Australian Standard 4815:2006 permits chemical indicators between Classes 4 and 6 to be used for such a purpose, when there is no printer or data capture device fitted to the autoclave.

A Class 4 indicator provides a graded response with a gradual colour change. It has an accuracy of 2 degrees in temperature and 25% in time. A Class 5 indicator has a tolerance of 1 degree on temperature and 15% on time, while a Class 6 chemical indicator has a narrow transition period, with a 5% tolerance on time and a 1 degree tolerance on temperature. Thus, a Class 5 or Class 6 indicator will not show



Figure 11. A series of Titem's Class 5 chemical indicators. The topmost indicator (A) is unused, and the subsequent indicators show the colour change at 90 seconds/134 degrees (B), 2 minutes/132 degrees (C), 2 minutes/134 degrees (D), and 3 minutes/134 degrees (E). Note how the colour intensity is less when the temperature is too low (compare C with D).

a "pass" colour change if the temperature is one degree too low. Class 5 and 6 indicators are labelled to indicate their specifications, for example, 134 degrees Celsius for 3.5 minutes.

The mechanism of action of chemical indicators is generally a simple heat-sensitive reaction. For many years, chemical indicators were based on sulphur and lead compounds, which reacted under the conditions of steam sterilisation to produce lead sulphide, giving rise to a change in colour from yellow to black. Despite the widespread use and low cost of such indicators, there were disadvantages associated with them. The coloured products could transfer to and stain articles with which it came into contact in the autoclave. Moreover, the stability of the colour over time was not high, especially when stored in humid conditions.<sup>7</sup> For these reasons, numerous other colour change systems capable of detecting steam sterilisation have been devised, including esters derived from organic acids (which break down to liberate protons, causing a pH change).<sup>8</sup> Many current systems use a tetrazolium salt (such as neotetrazolium chloride or nitroblue tetrazolium chloride) as the indicator molecule, to overcome the problems of the other types.<sup>9</sup> This compound is bound



Figure 12. 3M SteriGage™ Class 5 indicators, showing an unused control indicator (A), a “fail” result (B) because of inadequate time (in this case, 2 minutes at 134 degrees), and a “pass” result (C).



Figure 13. Browne’s Class 6 indicators, which change from yellow to deep purple. The examples shown are a “pass” (A), unused control indicators (B and C), and a “fail” result (D) with partial colour change (60 seconds/134 degrees).

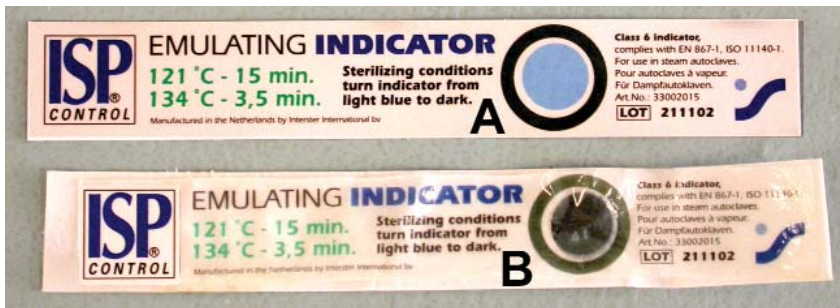


Figure 14. Interster ISP Control™ Class 6 indicators, showing an unused control indicator (A) and a “pass” result (B), where the inner dot has changed from light blue to dark green.

into a polymeric base comprised of ethyl cellulose and nitrocellulose, and then deposited on a paper substrate either by coating or printing.

The gradual nature of colour change in Class 4 and Class 5 chemical indicators can be seen in Figures 9-11. The 3M Comply SteriGage™ Steam Chemical Integrator is a Class 5 indicator with a simple visual readout in the form of a progress bar (Figure 12). The indicator consists of a paper wick and a steam and temperature sensitive chemical pellet contained in a paper/film and foil laminate envelope. Steam enters the permeable top side of the device. The chemical pellet melts, and the

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liquid migrates as a colour along the paper wick. Thus, the distance or extent of migration depends on exposure to steam, time, and temperature. The migration is visible through a window which has marked “Accept” or “Reject” points.

Major brands of Class 6 indicators are Albert Browne, Titems, and Interster (Figures 13-16). The Titems TST units are preferred by this author because of their ability to provide additional information on steam quality which is not provided by other indicators (Figures 15 and 16), in particular, the presence of superheated steam. The problem of superheated steam can also be detected by placing several thermocouples throughout the autoclave chamber, but this is not a practical method

in everyday practice. Routine use of Class 5 or 6 indicators is essential for loaned autoclaves and when awaiting calibration or validation of a new or repaired autoclave. In this author’s view,<sup>6</sup> it is prudent to include a Class 6 indicator in every pack of critical instruments, e.g. those used for oral surgery.

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**About the author**

Professor Laurence J. Walsh is the technology editor of Australasian Dental Practice magazine. He is also a noted commentator on and user of new technologies and is the Head of The University of Queensland School of Dentistry.



Figure 15. Titems TST™ Class 6 indicators provide a range of useful data on physical parameters, beyond “pass” and “fail” results. For proper interpretation, reference to the manufacturer’s charts is essential.

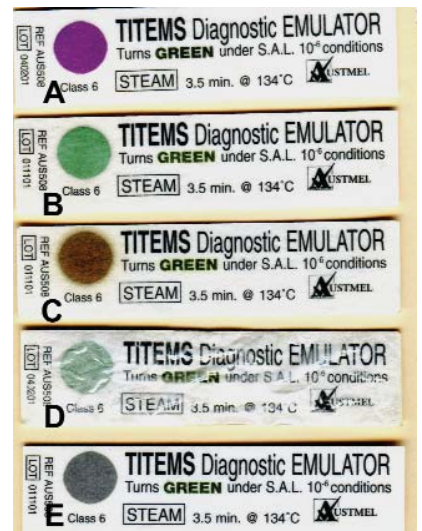


Figure 16. A series of Titems TST™ Class 6 indicators showing an unused control (purple) (A), a “pass” result (green) (B), a cycle with superheated steam (brown coloured result) (C), a cycle with wet steam but with an extended exposure time, causing bubbles on the plastic laminate and washing out of the colour of the green indicator spot (D), and a “fail” result (E), with mixed purple and green colours, caused by using a cycle temperature of 133 degrees rather than 134 degrees.