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Alternative test methods for washer disinfectors

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Alternative test methods for washer disinfectors

Managing a testing schedule for a busy endoscopy or sterile-services department can be a challenging task for hospital staff. As **David Woods**, operations manager for T.E.S.T. asserts, each type of machine will require different quarterly, annual and weekly tests - and it can be difficult to keep up with guidance which is being constantly updated.

Tests can be critical in detecting failures of the process and help the user detect things that can't be seen by eye; proteins, bacteria and residual process chemicals. For any given test requirement there may be several methods for the user to choose between and without having knowledge of the science involved or consulting an AE(D) it can be easy to make the wrong choice. Off-the-shelf test kits are promising faster and easier results and it can be tempting to move away from the more traditional testing methods that can be carried out in-house, especially when cost is a determining factor.

Most of the time these advances are positive and can save vital time and money for the Trust, but how would a user know they are getting the same quality of test? This is a key consideration as any contamination, be it protein or microbial, needs be flagged up accurately and in a way that is compliant with the guidance. Historically there have been several examples where a method of testing has become popular 'in the field' due to ease or cost only to found ineffective when guidance changes.

The ideal testing method would be performed *in situ*, low cost and provide instant and reliable results. Discussed below are some examples of the alternative testing methods that have been employed in the last decade or so. These fulfil many of the above criteria however the user should be aware that no test method is perfect and they may be sacrificing vital elements of the detection method to save time or money. Understanding the science behind the tests is key to making the right decision for the department or process.

The current method of testing Mycobacteria is membrane filtration and 28-day incubation.

A quicker way to detect bacteria: PCR

Traditional microbiology has always been limited by the speed that bacteria, yeasts or fungi grow as the way that they are detected is visual; individual bacteria in a water sample need to grow into a colony of millions that can be seen unaided by the microbiologist's eye. These microorganisms can have different growth rates dependant on the species and can take anywhere between 24 hours and 28 days to grow in an incubator.

Therefore, if you are looking for species of *Pseudomonas* this may be as quick as 24 hours but *Legionellae* are slower and take up to 10 days to grow into colonies. Species of the *Mycobacteria* genus are slower still and a 28-day incubation is needed to ensure each has had enough time to grow. These agar plate-based methods are considered the gold standard of microbiology, they are trusted and well understood.

A faster and more direct method would be infinitely more useful for the modern endoscopy or CSSD department. However, the trouble with detecting bacteria is that in trying to directly detect small numbers of bacteria any 'signal' generated is going to be too weak to be picked up by an instrument or detector.

Waiting for them to multiply into bigger numbers by themselves is slow and requires the exact right growth conditions. A way to speed this up is to specifically

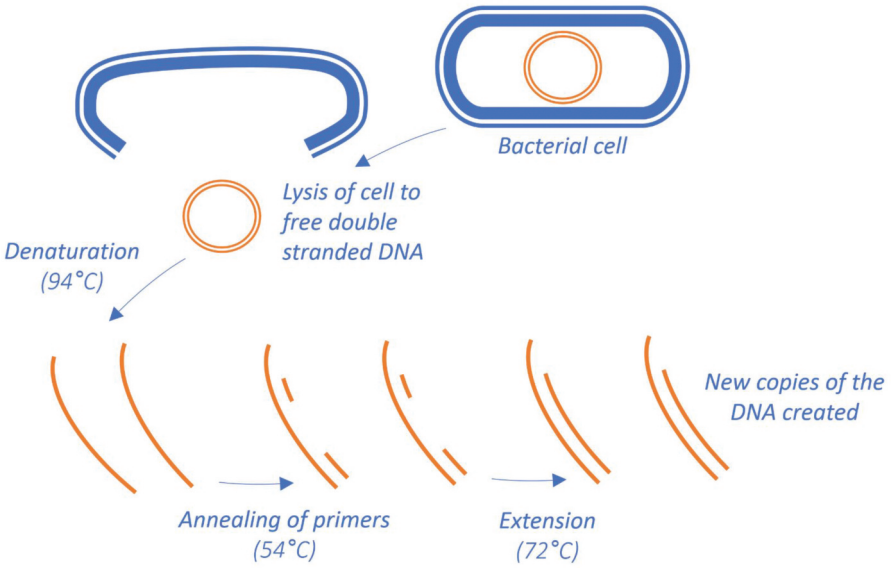
grow or 'amplify' small parts of the bacteria that can be more easily and quickly detected. This is the realm of molecular biology, which is a branch of biology established after the discovery of DNA and how it can be replicated and controlled using enzymes. This process of replication is called PCR or Polymerase Chain Reaction.

The principle of PCR is as follows: The bacteria in a sample are broken open to release their DNA from inside the cell. Short pieces of DNA (primers) are used to 'find' the region to be amplified and new copies of DNA are created using a type of DNA-building enzyme (polymerases). The process is carried out by varying the temperature over a thermal cycle which takes just minutes. As this thermal cycle is continuously repeated the DNA will replicate exponentially over the course of a few hours. This large amount of DNA produced can be easily detected by either fluorescence or separating on a gel through its electrical charge (electrophoresis)

Advantages: PCR can be extremely rapid and very specific. In just a few hours the presence or absence of a single species can be confirmed along with a quantitative measure of the amount of original DNA (in Genomic Units as opposed to Colony Forming Units).

Disadvantages: The technique must be carried out in the laboratory environment as DNA contamination is a risk. PCR will also detect dead bacteria that don't necessarily present any infection control risk, although techniques have been developed which go some way to address this issue. Because of the various techniques employed by each laboratory it is very difficult to compare results and there is no 'standard' method to judge compliance. Sensitivity is a major problem, although PCR can theoretically detect single bacteria, because of the small volumes used in the method bacteria may need to be in the tens or hundreds in a water sample to be detected reliably.

In the healthcare setting the approach needs to be flexible and the impact on the decontamination process should be considered, as shutdown due to failed results and/or major refurbishment could have "serious consequences for patient care" (HTM 01-06 Part E, 2016). Unless cost is prohibitive, these methods can be run side by side with the approved methods to give a complete picture and provide additional assurance. The user needs to make a decision based on the urgency of the results and take a risk-based approach before using PCR to replace well established plate-based microbiology to detect bacterial pathogens. PCR is not the only rapid molecular biology method, but it is the most common; other techniques involving enzymes and antibodies are emerging but none that have the flexibility of the PCR technique. The assay can be adapted to not only quantify the amount of DNA but specific species and groups of organisms can be isolated based on their unique genetic material.



The PCR process: The denaturation, annealing and extension is repeated multiple times to create large amounts of the specific DNA section of interest.

Protein

Testing for protein is crucial on reusable medical devices as it provides a valuable indicator of the cleaning efficacy. Proteins will be introduced to the external and internal surfaces during patient procedures and will mainly be comprised of blood components; albumins, globulins and fibrinogen and components from mucosa; mucins, glycoproteins and various enzymes. All proteins are composed of amino acids, there are 21 types of amino acid and detection of these (and the bonds between them) forms the basis of most instant protein tests. There are two categories of colour-change type tests, those which use copper ions and those which use dyes to detect proteins.

Copper based protein tests

The reduction of copper to form a coloured complex forms the basis of the biuret and Lowry methods. These require a peptide rather than free amino acids as it relies on the structure of the amino acids when in the peptide chain. A modified version of the biuret reaction is the BCA (bicinchoninic acid) test and responds more uniformly to different proteins at higher levels of sensitivity. The BCA method is one of the most common laboratory-based tests for quantifying protein.

Other protein tests

There are an abundance of different dye-based assays but the most common visually assessed test is the Coomasie blue/Bradford method which is simple to carry out in a test-kit form at room temperature without incubation. This dye was originally developed for textiles but now forms the basis of most protein test kits. This has replaced the ninhydrin test which was found to be not sensitive enough to demonstrate the <5µg sensitivity required by the latest guidance.

In the quest for more instant and enumerable methods some alternative tests have been employed. The Coomassie blue and ninhydrin test kits are often limited as they are not quantitative (some are semi-quantitative by visual colour comparison). This has led to the popularity of ATP based methods as a measure of cleaning efficacy.[1] Although ATP does provide some measure of contamination, as it will indicate current and past contamination from living organisms, ATP (adenosine triphosphate) is a universal energy molecule and is derived from a DNA nucleotide and in no way can be considered a protein. It is important that this is not used in place of a test where a protein is specifically required to be measured, like surgical instruments.

Protein testing

Weekly tests for endoscope washers
- Weekly safety checks
- Daily tests (including ACT)
- Process challenge device cleaning efficacy
- Water hardness
- Water conductivity
- Final rinse TVC

Weekly tests for instrument washers
- Weekly safety checks
- Daily tests
- Water hardness
- Water conductivity
- Automatic Control Test (ACT)

In the healthcare setting the approach needs to be flexible and the impact on the decontamination process should be considered, as shutdown due to failed results and/or major refurbishment could have "serious consequences for patient care"

Some methods must be read using UV excitation and detection of fluorescence. OPA (ortho-phthalaldehyde) based reagents have been used to detect proteins, peptides and amino acids for over 30 years in the laboratory.[2] Recently this has been applied to surgical instruments for a more direct measure: When the reagent is applied as a spray, it is possible to detect the protein *in situ* without needing to swab the instrument, this method of imaging is quantifiable, based on the intensity of fluorescence.

Choosing a protein test

When purchasing a protein test kit, in addition to cost and ease of use, it is important for the user to consider:

- Sensitivity ie: the lowest detection level of protein in micrograms (µg). For surgical instruments this should be under 5µg for each instrument side
- Variability of sensitivity between different types of proteins (size and amino acid composition)
- Calibration and traceability are vital to assuring the test results: ensure there is a means to calibrate the equipment to certified standards
- If it is being bought off-the-shelf, check the test kit is CE marked.

Limitations of protein tests

At the time of publication of HTM 2030 in 1997 the focus of protein removal was to reduce the risk of prion transmission. Over time there has been a gradual shift away from the qualitative Ninhydrin tests to other types of assay. Each method of determining protein content has limitations of either its sensitivity or practicality in a given situation. Because of the nature of the chemistry and equipment involved some test methods may struggle to achieve the required sensitivity.[3] All tests have a certain degree of variability, so they may be less sensitive to certain types of protein from different sources. Also, without complex laboratory techniques such as mass-spectrometry it is not possible to identify exact types of protein.

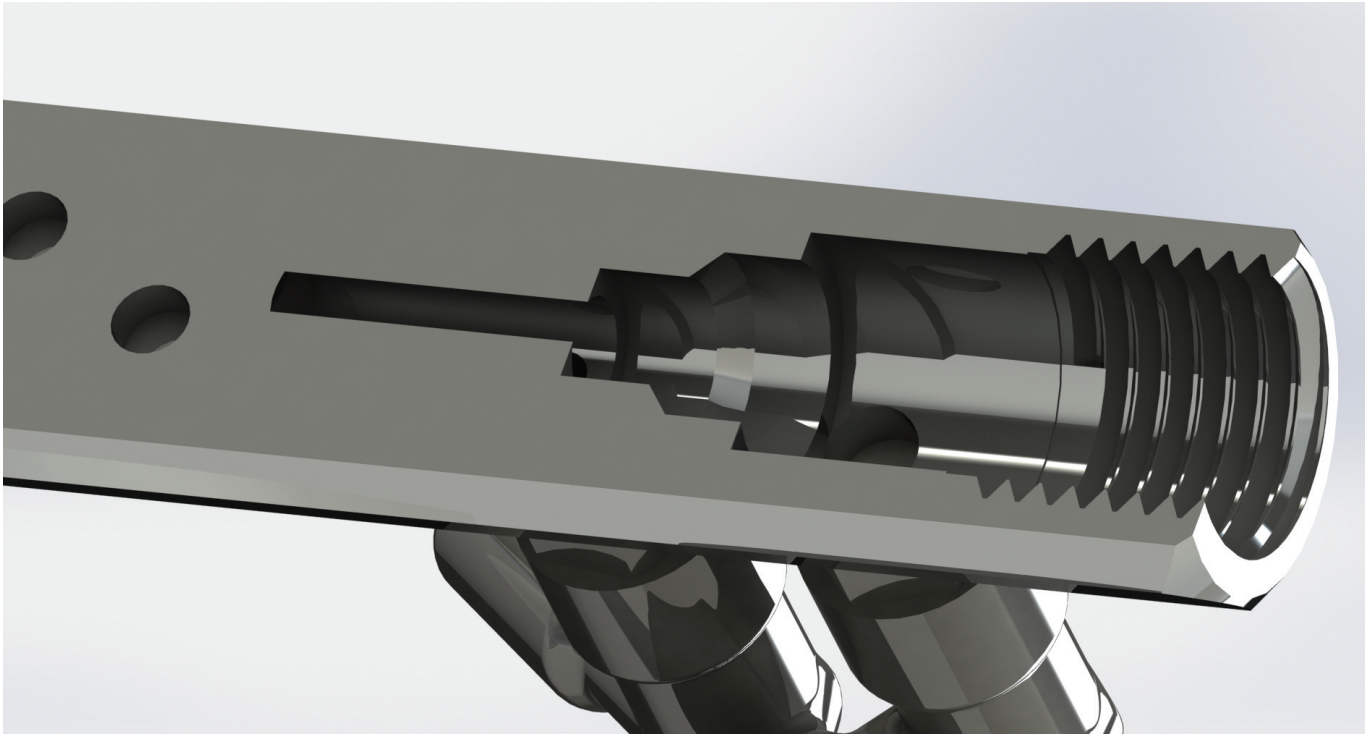
Direct measurement with UV detection can have excellent sensitivity but complex instruments and endoscopes will be difficult, if not impossible, to image using this method. Each method should also have available a calibration standard, so that the instrument can be periodically verified and the results are traceable. Although issues do exist with these methods, cleaning processes have come under great scrutiny in recent years and have been greatly improved as a result. Protein testing should now be carried out weekly as part of the cleaning efficacy with a process challenge device.

Methods to detect bacterial endotoxins

Bacterial Endotoxins (Pyrogens) are fragments of bacterial cells that can potentially generate an immune response in patients when present on instruments. Endotoxin is a broad term referring to many different types of immunostimulatory molecules but mainly lipopolysaccharides from the membranes of gram-negative bacteria are referred to. As these substances can be present, or even are generated, after death of the bacterial cell they present a patient risk even on sterile instruments.

Testing methods for these are mainly based on biochemical assays involving immune components extracted from other organisms. The first testing methods used for endotoxins involved injecting the sample into laboratory-bred rabbits and measuring the rise in temperature from the fever that resulted, hence the term 'pyrogen' (heat-producing). This method has now been replaced by more modern techniques which use immune components in vitro often in an automated optical reader. The extract, from horseshoe crab blood is referred to as 'lysate'.

Because of the complexity of the test methods the analysis must be carried out in the laboratory. The test is very sensitive to interference and must be carried out with positive (spiked) samples as well as a standard curve with each test so that the colour change can be directly correlated to a certified reference standard endotoxin.



Cross sectional view of how the 't-store' surrogate device mimics the internal air-water channel of an endoscope.

The user should be aware of the different methods to assess the suitability

Gel clot tests: These are normally carried out in a test tube and can be limit tests (not quantitative) or semi quantitative methods. The test relies on the operator observing the lysate clotting into a firm gel and so may be subject to inter operator variability. If the test fails the specification, it may be expressed simply as 'greater than' the sensitivity of the test and these results may not always be meaningful, dependent on the specification being applied.

Photometric and chromogenic techniques: The reaction of the lysate in these tests can be more quantitively measured by the development of a turbidity or colorimetrically. These are often in conjunction with a microplate reader so that the correlation of the reaction onset time can be automatically correlated to traceable endotoxin value.

Most of these methods are based on the British/European Pharmacopoeia standard methods which is considered the gold standard for this test. As with all subcontracted laboratory analysis the user should seek reassurances that the laboratory has UKAS accreditation for the testing method. This ensures that the quality management system is in place to control and monitor operator training, proficiency testing schemes and method validation.

Residual chemicals

Washer disinfectors use several chemicals as part of the cycle, namely detergents, disinfectants and rinse aids. These must be removed by rinsing with water during several points during the process to ensure chemicals do not carry-over into the next stage and potentially reduce the activity of disinfectants and other chemicals. Furthermore, instruments must be free from unintended chemical contamination after the process. Testing the rinse water will give a measure of the level of residues compared to the 'control' source water sample. The two main methods for assessing residual chemicals are:

- Total Organic Carbon (TOC) – Almost all detergents and disinfectants will contain organic carbon, this can be determined by laboratory analysis
- Electrical Conductivity – Dissolved ions from process chemicals remaining in the rinse water will decrease the electrical resistance of the water and increase the conductivity reading. Comparison of the two readings can determine the relative level of residues.

These methods will help to ensure the instruments remain free from chemical residues, however other methods can be recommended by the manufacturer that may be more specific to the chemical used in the process. Some process chemicals are permitted in the final rinse water as they are intentionally added to discourage microbial proliferation in the rinse water. These may include low-level disinfectants such as chlorine-based compounds that have been demonstrated to be non-toxic or at a low enough concentration. Off-the-shelf test kits or monitoring strips may be used to routinely check the levels present in the final rinse water.

The AE(D) or user may request the disinfection efficacy is proven using the method described in ISO 15883 Part 4 to demonstrate the washer disinfector is still performing in accordance with its original type testing.

Air monitoring

Chemicals used in the process may produce hazardous gases and become a risk to the operator. Monitoring can be performed periodically or with fixed equipment to ensure work exposure limits are not being exceeded during the process. It is a requirement of the HTM to ensure yearly and on revalidation that chemical vapours are measured using gas monitoring equipment. This is usually with sensors that measure the infra-red absorption by the gas or with an electrochemical sensor dependent on the type of gas. This testing may not be part of the main EWD service and validation schedule so the user should seek out an independent testing provider.

Disinfection efficacy and surrogate devices

In thermal washer disinfectors disinfection efficacy is a relatively straightforward measurement of the temperature, endoscope washer disinfections however can have many more factors which affect the chemical disinfection. Routine microbiological testing of the disinfection efficacy has been removed from the latest guidance which means that potential disinfection problems may go unnoticed. The current alternative test method is to sample endoscopes for natural contamination that remains after the process, as opposed to inoculating a surrogate device with live organisms. This is currently recommended only if a problem is suspected. The AE(D) or user may request the disinfection efficacy is proven using the method described in ISO 15883 Part 4 to demonstrate the washer disinfector is still performing in accordance with its original type testing.

The user should be aware that there are a large variety of surrogate devices being used on EWDs and not all of them accurately mimic an endoscope. According to the international standard the surrogate device should contain trumpet valves and connection ports so that the fluid pathway reflects the actual instrument and connection method.

Drying cabinets and storage systems: These pieces of equipment are often less complex in design than an endoscope washer disinfector but still have a significant risk associated with them if not properly used and maintained. Surrogate devices used in these systems need to reflect the complexity of the internal channels, which can make drying significantly more difficult than a unbifurcated length of tubing. Testing conditions should control for factors that present a risk such as connection methods and the initial state of the endoscope, which will have an unknown residual volume of water present from the EWD process. Furthermore, this residual water is not guaranteed to be free of bacteria. A sterile, dry length of tube will not adequately control for this initial state: if surrogates are to be used in place of endoscopes during routine testing then the requirement Annex E2 of EN 16442:2015 to clean and disinfect beforehand according to the 'procedure in force in the unit' would better reflect actual conditions.

In conclusion, users should be cautious in replacing test methods purely because it more cost effective or a new technology. If possible, carry them out side by side and seek the advice of the AE(D) until the method is incorporated into the official guidance. Current testing methods should be constantly reviewed as the fast pace of development in these areas means they may quickly become obsolete or non-compliant. CSJ

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About the author

David Woods BSc (Hons) has been the Operations Manager for T.E.S.T. since 2014 and is an Authorised Person (Decontamination). Since university David has worked as a microbiologist and managed sterilisation and washer-disinfector validation processes as part of the range of independent services offered by T.E.S.T.