Application Note

2008-0409

Bio-compatibility, Sterility, and Shelf Life

The TruFluor™ optical sensor family consists of a dissolved oxygen (DO) sensor and a pH sensor (Q4 2008). A TruFluor™ DO sensor is illustrated in figure 1. Each TruFluor™ sensor comprises [1]:

- a transmitter having an integrated LCD display, keypad, RFID reader, and two analog (4-20mA) outputs,
- a reader that measures both the optical fluorescence signal and the electrical temperature response of a military grade temperature sensor,
- an electrical cable connecting the transmitter and reader, and
- a disposable element called a “sheath” that contains the optically active sensor material and a metal plate for non-invasively contacting the RTD to the bio-process.

Only the disposable sheath is inserted into the bioreactor and only its outside surface comes in contact with the bio-process. For stirred-tank, single-use systems, the sheath is inserted into the bag via a standard 12 mm port with a seal (e.g., separate or molded o-ring), and is secured [2]. Therefore, in assessing the sterility and bio-compatibility of TruFluor™ sensors, it is only necessary to consider the external surface of the disposable sheath.

Figure 1: TruFluor™ DO disposable sensor system: (left) reader with sheath inserted in bioreactor bag port, and (right) transmitter.

For TruFluor™ sensors to become widely accepted by the biotechnology industry, they must demonstrate equal or better performance than traditional (electrochemical) sensors. In addition, the disposable sheath must be shown to pose no contamination risk to the bio-process itself, and suffer no degradation from the sterilization process used. Finesse is developing a complete materials certification package that presents the results of extensive materials testing.

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Materials Bio-compatibility

The materials typically chosen for single-use system components are polymeric in nature and can be sterilized using industry-accepted methods [3]. Typically, prepackaged, pre-sterilized single-use assemblies are gamma irradiated at >25 kGy [4]. Therefore, the materials used must be nontoxic and resist changes in physical properties after being irradiated.

Leachables, Extractables and Heavy Metals

Leachable, extractable, and heavy metal tests quantify the level of any compounds that might be extracted from the fluid contact materials, specifically after they have undergone gamma sterilization. Gamma irradiation >45 kGy is typically used as a worst case. The length of time that the materials were allowed to soak, along with other factors such as temperature and extracting solution, determine the amount of material extracted.

To quantify the amount of organic material that is extracted, total organic carbon (TOC) analyzers are used. Analytical techniques that can identify a specific “fingerprint” for each component extracted are available. This fingerprint then can be used to find the origin of the extractables from the entire assembly. Each of the extracted species can be identified using methods such as high-performance liquid chromatography, Fourier transform infrared spectroscopy, liquid chromatography – mass spectrometry, gas chromatography – mass spectrometry, or any combination of these methods. Heavy metals can be identified and measured using inductively-coupled plasma or atomic absorption spectroscopy.

One aspect of the extractables profile that is most important to a pharmaceutical manufacturer is the amount of contaminate being extractedinto the final product. To minimize contaminant extraction, the disposable aseembly must be tested in a dynamic situation in addition to being tested in the static conditions described previously. For a TruFluor™ sheath in a bioreactor bag, this would involve running a bioprocess with media for a period of time.

To date, there are no specific standards for leachables and extractables, although the industry has formed the Bio-Process Systems Alliance (BPSA, http://www.bpsalliance.org/), which issued industry guidelines in 2007 (“Recommendations for Leachables and Extractables Testing”, Part 1: introduction, Regulatory Issues, and Risk Assessment, http://www.bpsalliance.org/BPSAPart1ELGuide1207.pdf and Part 2: Executing a Program, http://www.bpsalliance.org/BPSAPart2ELGuide0108.pdf).

Given the size of the TruFluor™ DO sensor sheaths, we expect that the bag or the tubing will dominate both the leachables and extractables. Nonetheless, full testing of the sheath is required to guarantee bio-process compatibility.

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Cytotoxicity and USP Class VI

Cytotoxicity and USP Class VI fall under the ISO 10993 (http://www.fda.gov/cdrh/g951.html) guidelines for materials biocompatibility. These standards were originally developed for medical and dental materials by the International Organization for Standardization.

Cytotoxicity (ISO 10993-5) is a rapid, standardized test that is an inexpensive way to determine if the materials used in a bioprocess device contain significant quantities of harmful extractables. Cytotoxicity tests also determine the effect of these harmful extractables on cellular components. In all methods the cells are scored for cytopathic effect.

Mammalian tissue culture systems are currently used to evaluate cytotoxicity, as they have shown good correlation with animal assays and are frequently more sensitive to toxic materials. The following cytotoxicity tests are widely accepted in biomaterial screening, quality control and audit programs. In general, these in vitro techniques use a variety of cell types (e.g. L-929 cells) which differ in relative sensitivity and the time required to conduct the assay:

- **Direct Contact** Cell cultures are grown to a standard monolayer. The test material is placed in direct contact with the cell layer for 24 hours. Subsequently, the monolayers are examined microscopically for the presence of morphological changes, reduction in cell density or lysis induced by the test material.
- **Agar Diffusion – Direct Contact or Saline Extract** The cell monolayer is overlaid with agar and stained before treatment with the test material or extract. After 24 hours, the cells are scored microscopically for decolorization and lysis.
- **MEM Elution – Test on Extracts** The test material is extracted for 24 hours in Minimum Essential Medium (MEM). An extract is prepared from the test material which is then placed on cell monolayers. The cells are examined for morphologic changes and cytolysis to determine a toxicity score.

The United States Pharmacopeia (USP) is a private organization that *"is the official public standards-setting authority for all prescription and over-the-counter medicines, dietary supplements, and other healthcare products manufactured and sold in the United States."* (http://www.usp.org/aboutUSP/) Those standards include in vivo animal biological reactivity tests for *"elastomerics, plastics and other polymeric material with direct or indirect patient contact."*

USP monograph <88> describes the classification of plastics into six classes based on responses to a series of in vivo tests for which extracts, materials and routes of administration are specified (see figure 2). Class VI requires the most stringent testing. Extracts of the test material are prepared in saline, alcohol in saline, polyethylene glycol (PEG 400), and vegetable oil.

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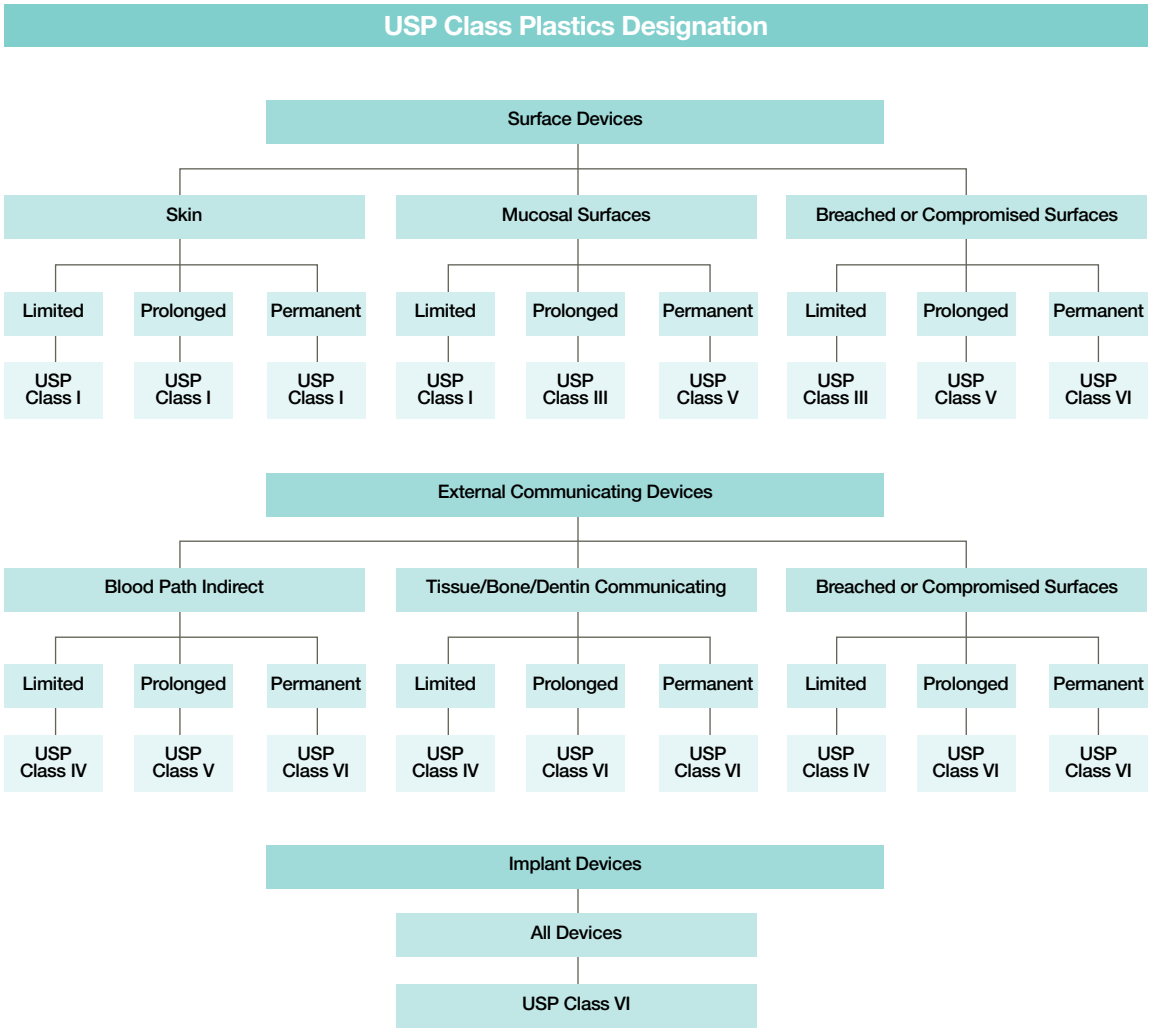

Figure 2: USP classification of materials.

USP Class V involves passing the Systemic Injection Test and the Intracutaneous Test, wherein the extracts and blanks are injected into mice and rabbits, which are observed several times over a 72-hour period. The animals' response to the sample extracts and the blank are compared to determine test passage. USP Class VI includes the tests of USP Class V plus an implantation test wherein strips of the test material and a negative control are implanted in rabbits for a period of not less than 120 hours (5 days). Hemorrhage, necrosis, discolorations, and infections are macroscopically observed and degree of encapsulation is scored and compared with the negative control to determine test passage.

Many view USP Class VI testing as the minimum requirement that raw materials must meet to be uses in bio-processing applications. It must be noted that USP Class VI testing does not fully meet any category of ISO 10993-1 testing guidelines currently used by the US FDA (General Program/Bluebook Memorandum G95-1 - http://www.fda.gov/cdrh/g951.html) for medical device approval.

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TruFluor Sensor Testing

The TruFluor™ DO sheath components are made from several different materials:

- **Sleeve** USP Class VI polycarbonate (clear and black tinted)
- **Thermal contact plate** 316L electro-polished stainless steel
- **Active sensor material** fluorescent dye embedded in a polymeric matrix with an outer silicone surface, called the “dot”
- **Adhesive for sensor material** USP Class VI glue

The USP Class VI and stainless materials are medical-grade with known gamma-irradiation properties, and certificates of conformance from their manufacturers. The sensor “dots” however, must be tested and qualified according to USP Class VI, heavy metal, as well as “leachables and extractables” guidelines, in order to guarantee biocompatibility of the sheath.

The sheath comprises approximately 4150 mm^2 of wetted polycarbonate surface area and only 14 mm^2 of wetted sensor dot surface area, with the black silicone on the dot facing the liquid. Because USP Class VI testing is specified for materials qualification rather than qualification of a specific component or device, certain USP tests require particular shapes and amounts of the materials being tested. For the TruFluor dots, surrogates using polycarbonate supporting materials and qualified adhesives are used for compliance testing. These test surrogates are engineered to contain similar but slightly higher ratios of dot surface area to polycarbonate surface area than in the sheath itself. This is a sufficiently stringent test condition for the dots, because both the polycarbonate and glue are already known to be compliant with USP Class VI requirements

Three test surrogates were used:

1. For the heavy metals and cytotoxicity tests, the dots themselves are sufficient.
2. For USP Class VI tests requiring surgical implantation, 2 mm dots were glued to a 15 mm diameter and 1mm thick polycarbonate disc.
3. For the balance of the USP Class VI tests and the leachables and extractables tests, 4.2 mm dots were glued to a 4 mm diameter and 2.25 mm thick polycarbonate substrate.

The tests performed are listed below:

- Leachables and Extractables
  - USP physicochemical test for plastics (aqueous) – purified water extraction
  - USP physicochemical test for plastics (non-aqueous) – isopropyl alcohol extraction

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- Heavy Metals
  - Inductively-coupled Plasma (ICP) spectroscopy – purified water extraction
  - Atomic Absorption (AA) spectroscopy – purified water extraction
- Cytotoxicity
  - ISO elution method – 1X minimal essential media extract
  - USP Agar diffusion method
- USP Class VI
  - Seven day implant
  - USP systemic toxicity study – 0.9% NaCl solution extraction
  - USP systemic toxicity study – Sesame oil, NF extraction
  - USP systemic toxicity study – Alcohol saline extraction
  - USP systemic toxicity study – Polyethylene glycol extraction
  - USP intracutaneous study – 0.9% NaCl solution extraction
  - USP intracutaneous study – Sesame oil, NF extraction
  - USP intracutaneous study – Alcohol saline extraction
  - USP intracutaneous study – Polyethylene glycol extraction
  - Modified USP muscle implantation study – seven day, surgical method

All testing was carried out by an independent, third-party laboratory. Tests results are available to customers as part of the bio-compatibility certification package for each TruFluor sensor.

Product Sterility

Guaranteed product sterility is the most critical parameter for disposable systems and is a significant portion of their value-proposition to the end user [4]. Once a disposable sensor is shown to be made from bio-compatible materials, its impact on the sterility of the overall single-use bioreactor system must be tested and certified. The testing method must show that the sterilization process is reliable, robust, and safe [5, 6]. The sensor must be shown to not degrade, introduce bio-burden, nor compromise the integrity of the bioreactor bag. Furthermore, the performance of the TruFluor sensor inside the disposable bag must be tested after sterilization to show repeatability and robustness.

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Bio-burden

The potential of the sensor sheath to introduce bio-burden (i.e., level of contamination) into the single-use bioreactor must be minimized [4,6]. Low levels of bio-burden are required throughout the product fluid path to ensure endotoxin levels remain well below accepted levels during a growth run.

The bio-burden loading by the sensor sheath largely depends on the assembly process by the bag manufacturer, which can only be audited, but not controlled by Finesse. Overall, bio-burden levels for inner and outer material surfaces of the bioreactor bag must be monitored carefully and maintained during the manufacture of the single-use assemblies, in order to ensure the validity of the subsequent sterilization process.

Medical device manufacturers use standard methods to show their products' sterility, and these methods also have been used with disposable assemblies. Because of the relatively small lot sizes for the disposable devices, two methods, either VDmax [7] or AAMI/ISO 13409 [8] should be followed to ensure sterility. If actual lot manufacture is infrequent, the AAMI/ISO 13409 method is preferred for disposable bags [8]. To obtain the worst-case scenario, disposable bags with the maximum surface area should be used in the sterility testing.

The average module bio-burden for the inner and outer surfaces must remain less than 1000 cfu to allow AAMI/ISO 13409 to be used. After the sensor sheaths are secured in the bioreactor bags ports and packaged, but before gamma irradiation, 10 units should be sent to an outside laboratory to determine the average inherent bio-burden according to ANSI/AAMI/ISO 11737-1. Using the determined average inherent bio-burden, a verification dose can be calculated, which is typically below 10 kGy. This verification dose level is used to demonstrate that disposable modules gamma irradiated at 25 kGy or higher provide a sterility assurance level (SAL) of 106 [7].

On the basis of the lot size, 20 disposable bags should be sterilized at the minimum dose level and tested for sterility in accordance with AAMI/ISO 11737-2 at an outside laboratory [8]. Assurance that 25 kGy will provide a sterile product requires that not more than one positive sterility test sample be observed for the verification dose experiments [7]. All of the disposable bags tested should show no growth.

To prove the sheaths and/or bags were exposed to a minimum sterilization dose of 25 kGy, all lots of disposable modules should be subjected to the dose verification experiment. The next two consecutive lots manufactured should be tested using the same procedure as the first lot, and quarterly audits followed to ensure bio-burden levels, process robustness, and sterility.

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Degradation and Sterility Integrity

None of the materials chosen should show significant physical degradation after gamma irradiation. Specifically, to ensure sterility, each material that may come into contact with the drug product must pass USP 27 <88> "Biological Reactivity Tests In Vivo" after the samples are gamma irradiated to >40 kGy [9], the maximum dosage level that the disposable product may experience. After gamma irradiation, endotoxin levels must be measured for the entire disposable assembly using methods described in USP <85> "Bacterial Endotoxins Test". The detected levels must remain below 0.25 endotoxin units per milliliter (EU/mL) [9].

In addition, it must be proven that the final packaged assembly (sensor sheath plus bioreactor bag) maintains sterility and integrity during storage. Finally, the sensor sheath cannot affect the performance or integrity of the bag during media filling or gas overpressure, so that "sheath in bag" tests are required by each bag integrator (and will depend more on the bag and sensor port design than on the sheath itself!).

The final test for any disposable product is to show that it maintains product sterility during actual operating conditions. In the case of bioreactor bags, the last step in the process validation is to perform actual process runs of common cell platforms (mammalian, bacterial, insect, plant, etc).

Performance and Physical Integrity

A critical component of disposable module validation is showing the repeatability and robustness of the disposable system after gamma irradiation at 40 to 50 kGy. This level of irradiation guarantees that the tested product has been exposed to the maximum amount of radiation that it may encounter under its standard irradiation process. Performance tests are performed before and after irradiation. The criteria for validation were that the system maintains its precision, accuracy, and drift performance characteristics before and after irradiation. This period of time should be chosen to be characteristic of a typical process (e.g., two weeks for a bioprocess run and 3 days for media prep), in order to demonstrate that the sensor output will not drift any more after irradiation than before it, and to demonstrate that irradiation will not adversely affect the performance repeatability.

In addition to conducting performance testing, the reliability of the disposable bag with a pre-inserted sheath must be verified by the bag integrator. As a test, three modules from each of the three lots should be pressurized until they burst, thereby showing that the port would stay connected with the sensor, the sensor would not burst, nor the bag seams rupture at pressures not exceeding the rupture pressure for up to 1 min.

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Shelf Life

The purpose of shelf life testing is to validate that the TruFluor sensor sheaths or sheath-in-bag assemblies do not degrade over time. Samples of packaged disposable sensors and/or assemblies from multiple lots are taken and stored at room temperature for a given period of time (usually statistically related to the warranty time). This process is referred to as “real-time testing”.

Additional testing can be conducted by taking samples from various lots and subjecting them to extreme conditions, such as 60°C and 60% relative humidity, to simulate the aging process in a shorter period of time (similar to Telcordia testing – http://www.telcordia.com/services/testing/index.html). For example, Equation 1, which is based on the Arrhenius model and is commonly referred to as the “10-degree rule” [10], can be used to estimate the length of time required at a given temperature and humidity to simulate several real-time years. Other methods are available that differ slightly from the 10-degree rule [11]:

$$t_{test} = \frac{t_1}{Q_{10}^{\frac{T_2 - T_1}{10}}}$$

Equation 1

in which t_{test} is the length of time required at elevated temperature to represent an length of time, t_1, at the shelf temperature (T_1), T_2 is the elevated temperature, and Q_{10} is the reaction rate coefficient. The typical relationship selected for commonly used medical polymers is $Q_{10}=2$ [10]. This equation provides an estimate of how the product may age over time but does not replace actual real-time testing. Results from real-time and accelerated aging should be consistent with one another. It is possible that the samples subjected to accelerated aging may have degraded more than the corresponding real-time samples because of the more extreme conditions to which they have been subjected. In this case, the real-time samples should be used.

A visual inspection of the aged samples is performed to ensure that no significant changes such as materials becoming more brittle, materials changing color, or materials becoming deformed during the aging process occurred that may not have been detected during other tests. Further testing is required to determine whether the disposable modules are still functional, but the visual changes give an idea of what may go wrong, and which components need special attention during additional testing.

Aged sensor samples or sensor-bag assemblies need to be tested for physical integrity, burst strength, extractable levels, and particle levels. To ensure that aging does not cause the dots to delaminate, all of the aged samples should be tested for module integrity and verified to have the same module burst strength as the zero-year samples. The extractable and leachable levels needed to be determined and shown to have remained the same as the zero-year samples.

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In addition, packaging must be shown to remain intact and pass the same test protocol as the zero-year samples. When the packaging has been shown to remain intact after conducting the accelerated-aging studies, sterility must be tested only at the end of each real-time period.

Real-time and accelerated aging studies will be performed on TruFluor™ sensor sheaths at Finesse. Aging studies for sheath-bag assemblies will remain the responsibility of the bag integrators.

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