

THE IMPORTANCE OF SUPPLIER QUALIFICATION FOR VENDORS OF MATERIALS USED IN *IN VITRO* ASSAYS

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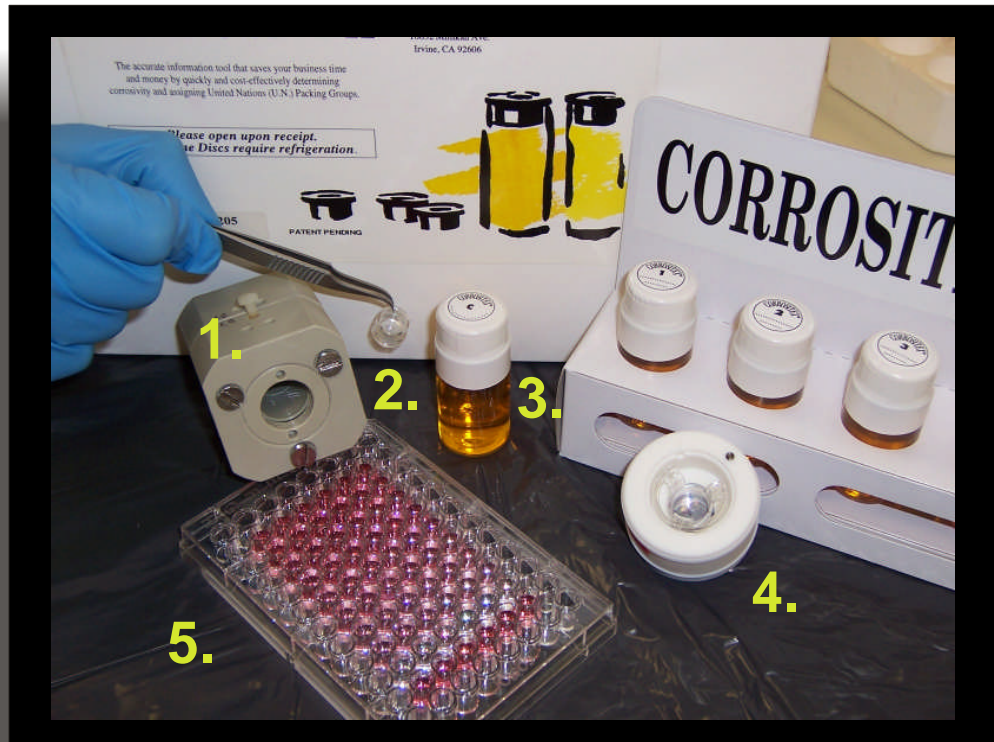
Abstract

Pre-clinical assays, including in vitro assays, rely heavily on suppliers who provide specific products or services essential to the proper conduct of the study. The overall credibility of the assay and the results obtained from the assay are highly dependent on the quality of the supplies used. Variable results for the same control material over time could indicate that there is high lot to lot variability for a critical component of the assay. Monitoring positive and negative control results is one useful retrospective technique to help identify supplier quality. Instituting a supplier qualification program provides a prospective way to document that suppliers of critical products (such as the test system, critical media, or whole test kits) consistently adhere to the high standards necessary to support work performed in compliance with the Good Laboratory Practice (GLP) guidelines. While some suppliers provide products manufactured utilizing Good Manufacturing Practices (GMP) and International Organization for Standardization (ISO 9001) standards, many suppliers of in vitro test systems and test kits are smaller, more specialized companies that may have their origins in academic research. A strong supplier qualification program, consisting of pre-qualification audits and regular evaluations, provides a framework for auditing both small and large scale suppliers against the proper standards for each laboratory's in vitro testing program. We have developed a supplier qualification program, including audits of Standard Operating Procedures (SOP)s for the manufacturing process and equipment maintenance, lot specific batch records, and training programs, that has led to significant improvement in the quality of 3-dimensional tissue received for performing dermal and ocular irritation studies. We feel that it is important to share this program since the current regulatory environment places the burden on the users of the supplies to assess the methods employed at the manufacturers' facilities and assure that the suppliers meet a sufficient level of quality.

Introduction

The Organization for Economic Cooperative Development's (OECD) definition of a test system is "any biological, chemical or physical system or a combination thereof used in a study" (OECD, ENV/MC/CHEM(98)17). Test systems for in vitro assays can be of various types (see Figure 1 for examples of in vitro test systems), consequently, there is a wide range of suppliers involved in production of test systems: commercial cell suppliers and repositories (e.g. Cambrex, InVitrogen, ATCC), commercial tissue engineering/tissue model developers and suppliers (e.g. MatTek, SkinEthic), and animal rooms and abattoirs (e.g. Sioux-preme Pork Products, CellzDirect). Variations in the quality of media used for culturing and maintaining cell lines and other critical assay reagents can have a potential negative impact on the performance of the test system in the assay. When conducting pre-clinical assays, it is often difficult to distinguish between test article induced test system changes and changes in the test system caused by poor initial test system or assay component quality. Once a strong in-house quality assurance program has been established (Ulrey, 2005) a program should be developed to monitor the quality assurance/ quality control programs in place at the test system and critical component manufacturer's facilities. Studies performed in compliance with Good Laboratory Practices (ref. FDA, EPA, and OECD) require materials (including test systems) used in the study be of adequate design and appropriate quality to provide for proper assay functionality; however, even for laboratories not performing in vitro assays in compliance with Good Laboratory Practices, this type of monitoring system becomes an important part of a laboratory's due diligence program.

Figure 1.



Photograph of various in vitro test systems (clockwise from upper left) 1. Bovine cornea in a chamber, 2. EpiOcular™ tissue construct, 3. CORROSIT™ Chemical Detection System, 4. Cytosensor Microphysiometer™ capsule of L929 cells, 5. 96-well plate of BALB/c 3T3 cells

Rational For Instituting A Supplier Qualification Program

In a GLP Compliant Laboratory

- Laboratories conducting in vitro assays in compliance with GLP guidelines are directly responsible for ensuring that the test systems (and critical assay components) used in each study are of a suitable quality to assure that the data received are reliable and reproducible. (See Table 1 for a list of GLP guidelines and their stated requirements for test systems.)

Government inspections are not conducted at supplier facilities unless they are registered under Good Manufacturing Practices (GMP)s. The majority of suppliers (particularly test system suppliers) do not participate in any audit programs; therefore, both prospective and yearly monitoring audits by laboratories are necessary to assure that the GLP requirements for supplies used in studies are being met.

- The in vitro testing community itself is currently the only body capable of holding non-GMP or non-ISO suppliers to a defined state of quality.

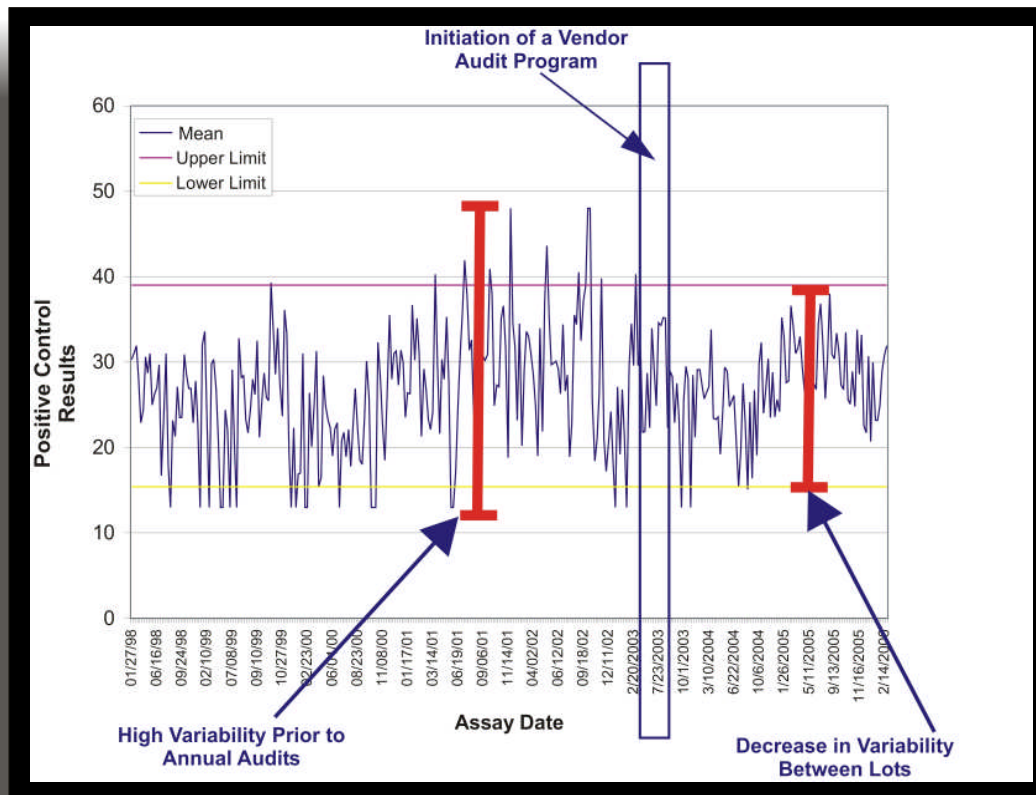
Table 1.

Regulations	Requirements for Test Systems
<p>Organization for Economic Cooperation and Development Series on Principles of Good Laboratory Practice and Compliance Monitoring (ENV/MC/CHEM(98)17)</p>	<p>Number 14 – Application of GLPs to In Vitro Studies “Another example is provided by the requirement that test facility management should ensure that the test facility supplies meet requirements appropriate to their use in a study. Certain in vitro studies may necessitate the use of proprietary materials or test kits. Although the OECD Consensus Document on Compliance of Laboratory Suppliers with GLP Principles states that material to be used in a GLP compliant study should be produced and tested for suitability using an adequate quality system, thus placing the primary responsibility for their suitability on the manufacturer or supplier, it is the responsibility of the test facility management to confirm that these conditions are adequately fulfilled through assessment of the suppliers practices, procedures and policies</p> <p>5.1.2 The integrity of the physical/chemical test systems should be ensured 8.2.5.a. [Protocols must include] Justification for selection of the test system 8.2.5.b. Characterization of the test system, such as the species, strain, substrain, source of supply, number, body weight range, sex, age and other pertinent information</p>
<p>U.S. Food and Drug Administration (FDA) 21 Code of Federal Regulations (CFR) Part 58 – Good Laboratory Practices</p>	<p>Study Directors are responsible for assuring that test systems are as specified in the protocol which often list specific information about test system characteristics as justification for use of that test system in the assay</p>
<p>U.S. Environmental Protection Agency (EPA) Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) 40 CFR Part 160 – Good Laboratory Practices</p>	<p>Study Directors are responsible for assuring that test systems are as specified in the protocol which often list specific information about test system characteristics as justification for use of that test system in the assay 160.120.a.5. [Protocols must include] Justification for selection of the test system</p>
<p>U.S. Environmental Protection Agency (EPA) Toxic Substances Control Act (TSCA) 40 CFR Part 792 – Good Laboratory Practices</p>	<p>792.33.d. Test systems are as specified in the protocol. 792.120.a.5. Justification for use of the test system</p>

In a non-GLP Laboratory

- Contract Research Organizations (CRO)s and test article providers (Sponsors) are responsible for making decisions based on the data obtained from assays performed using test systems and critical supplies. CROs and sponsors expect test systems and supplies to be consistent both within each manufacturing lot and between lots over time. Inconsistent assay performance could mean poorly defined production methods, inadequate training or improper release criteria.
- Suppliers of in vitro test models invest a great deal of time and resources in developing these novel systems. It should therefore be in the best business interest of a company to assess the controls in place to maintain quality in the scale-up process from prototype design to manufactured product. There is a large difference between an academic production setting and a manufacturing production setting. Adequate quality controls are vital to have in place when moving to a larger manufacturing scale to assure that the product remains consistent during the process. An assay which must be repeated due to poor condition of the test system or supplies ultimately costs everyone, both users and manufacturers, time and money.
- Given the nature of in vitro test systems and supplies (i.e. the ability to manufacture them in a controlled environment), an opportunity exists to control a potential source of variability by requiring that suppliers implement a thorough quality control program. Good training and documentation also benefits the manufacturer by assuring that in the event of the loss of specific personnel, scientific knowledge of how to manufacture their product remains within the company. See Figure 2 for a graph of the positive control over time for an in vitro test system at the Institute for In Vitro Sciences, Inc. (IIVS). The results suggest that lot-to-lot variations decreased notably after the initiation of an annual vendor audit program.

Figure 2.



* Values recorded only as less than 15 and greater than 45 are arbitrarily set at 13 and 48 to show graphically that they were less than the lower limit and greater than the upper limit respectively.

Essential Components Of A Supplier Qualification Program

POINTS TO CONSIDER

- Uniquely designed audit for each manufacturer.

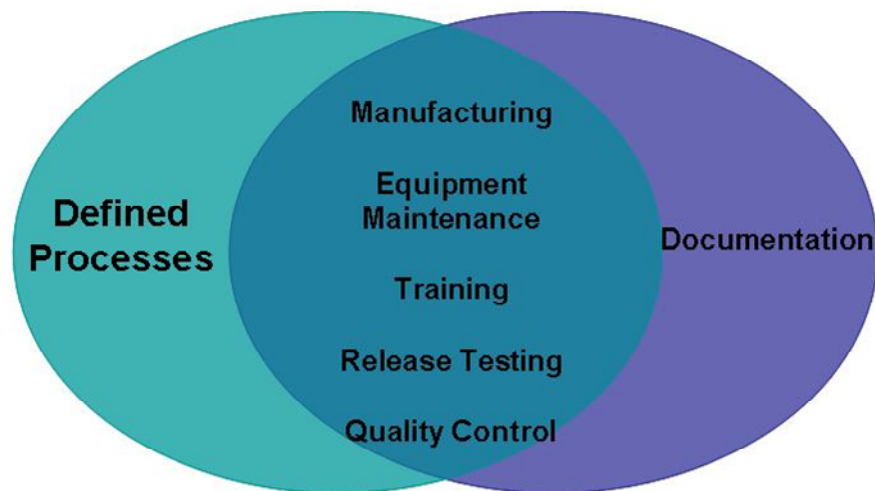
Although the basic areas the audit focuses on remain the same, different suppliers should be held to different standards based on their product. For example, a cell bank or tissue manufacturer would be held to a higher standard of quality than an abattoir. The supplier qualification program at IIVS was developed with tissue manufacturers in mind, but the basic concepts below can be applied to multiple types of suppliers.

- It might not be possible to view a complete set of information.

A manufacturer should be willing to share some standard SOPs, but might be reluctant to allow auditors to view a complete batch record which would expose every detail of their manufacturing process.

- Even those manufacturers that make no claims of compliance with GMP or ISO standards should reach a minimum level of quality in the following categories: defined processes, documentation, equipment maintenance, and staff training (See Figure 3 for a graphic of the overlap between these categories).

Figure 3.



AUDIT AGAINST THE FOLLOWING CRITERIA WHEN PERFORMING A SUPPLIER QUALIFICATION AUDIT

Defined Processes:

- Suppliers should have processes in place for the steps used during product manufacturing (or isolation in the case of ex vivo tissues or primary cell cultures).
 - Documented and approved by the appropriate level of management.
 - Amounts of components, critical times, and appropriate ranges for the process should be established
 - The effect of deviations from the defined process should be known through a Corrective and Preventative Action Plan (CAPA) or from basic research involved in creating the product.
- Release Testing
- Document Control
- Training
- Equipment Maintenance
- Out-of-Specification (OOS) testing resolution.

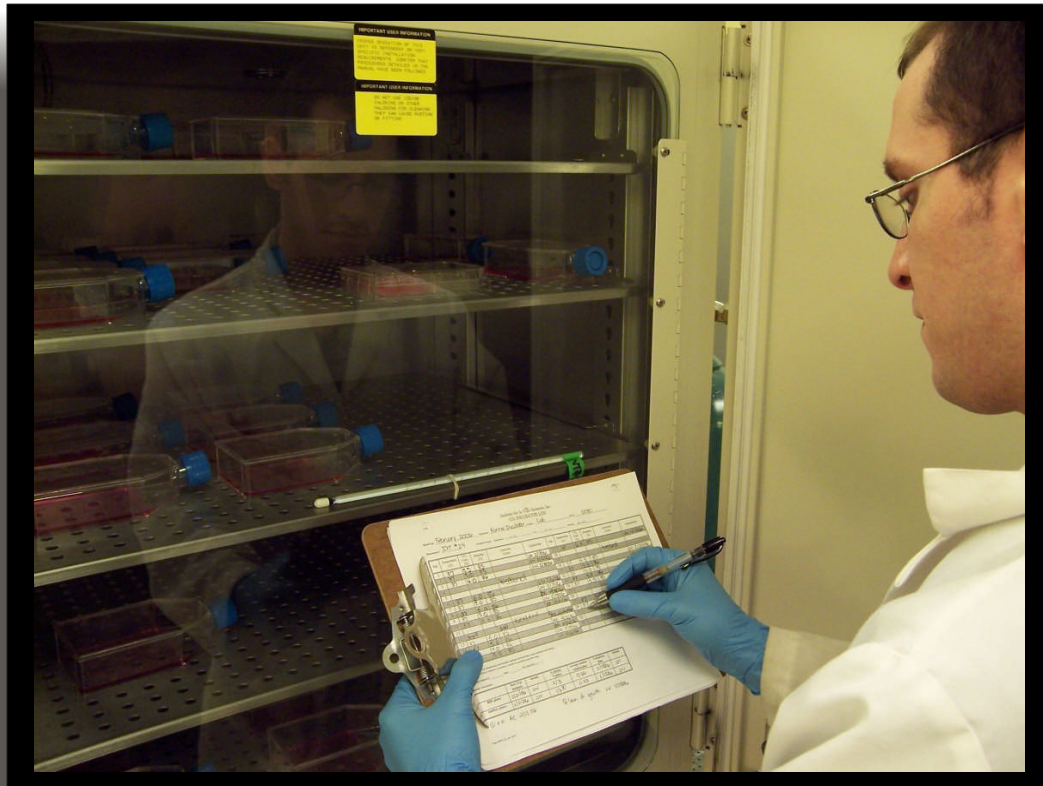
Training - cover:

- The production process
- Proper documentation practices
- Safety
- Equipment use
- Computerized system use
- Any additional procedures that are documented in an SOP.
- On-going training to ensure adherence to current procedures

Equipment Maintenance - look for:

- Established control over equipment parameters (e.g. incubators, refrigerators, etc.)
Temperature logs should be available to document proper functioning of all equipment used in the manufacturing process (See Figure 4 for a photograph of a temperature log).
- Maintenance logs for recording routine, scheduled maintenance (such as calibration and cleaning) and non-routine maintenance, whether it was performed by facility personnel or outside contract equipment service providers.
- Out-Of-Specification (OOS) investigations performed when equipment functions outside of its acceptable parameters.
Impact to product quality should always be of primary concern and should be discussed in the documentation.
- Individual or business group responsible for each piece of equipment

Figure 4



Biologist performing a temperature check of an incubator and recording it on a log.

Documentation - look for:

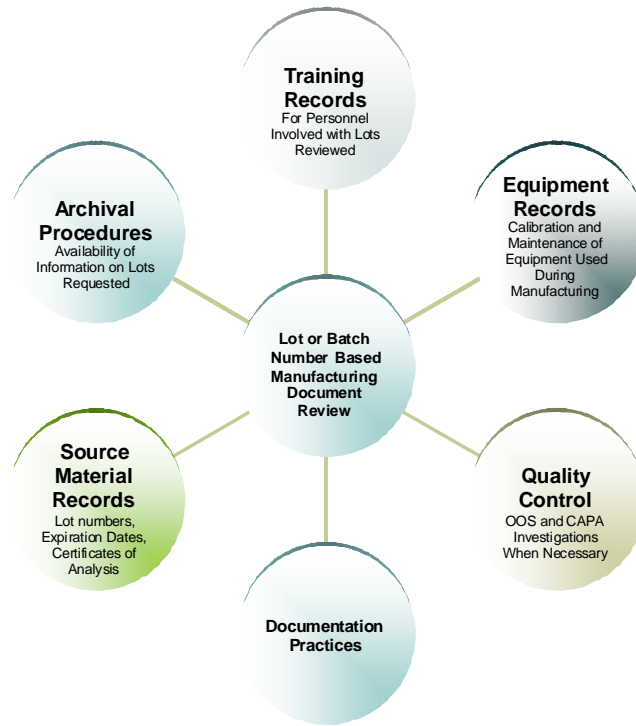
- All of the defined processes documented in approved Standard Operating Procedures (SOPs) (or something similar) and readily available during the manufacturing process.
- Batch records created during the manufacturing or isolation of the product that support the fact that the approved processes were followed. These records should include:
 - The person responsible for each step in the process
 - Verification of use of appropriate amounts of substances and that processes were carried out for the correct amount of time
 - Equipment used and relevant performance parameters
- All primary documentation (recorded as soon after performing the steps as possible) should follow good documentation practices (i.e. the person and day or time of entry should be evident).
- Explanation of any changes made to the documentation
The original entry should not be obscured and the maker of the change should be clearly identified.
- Standardized documentation practices across all areas of production
- Documented deviations including an assessment of potential product impact
- Documentation review by production staff (especially primary manufacturing records)
- A documentation review by someone not involved in the production and without any vested interest in the release of the lot performed as part of lot release (if applicable).
- Training and equipment maintenance
- Archived records

ADDITIONAL CONCERNS FOR TEST SYSTEM MANUFACTURERS

- A “Production Manager” should be appointed and that person should be **held accountable for each lot of tissue**. It should be clear from viewing the organizational chart and manufacturing batch records who this person is.
- Appropriate precautions, such as working under a hood and use of Personal Protective Equipment, should be taken to **minimize contamination**.
- Procedures need to be in place for rejection of the product (**lot release testing**) and the cause for rejection of product should be investigated. Also, there should be procedures in place for handling “OOS” quality control (QC) data.
- **A legal source of cells** used in the manufacture of tissue constructs should be obtained to prevent potential legal issues in the future. **Characterization** of the cell types used should also be made available.

The processes outlined above can be followed for the initial pre-use supplier qualification audit and for the yearly follow-up audits. Once specific lots of material are used in an assay, a random sampling of the documentation from these lots can be reviewed during the yearly audits. From the lot or batch information available, it should be possible to trace back to nearly all of the areas described above. (See Figure 5).

Figure 5



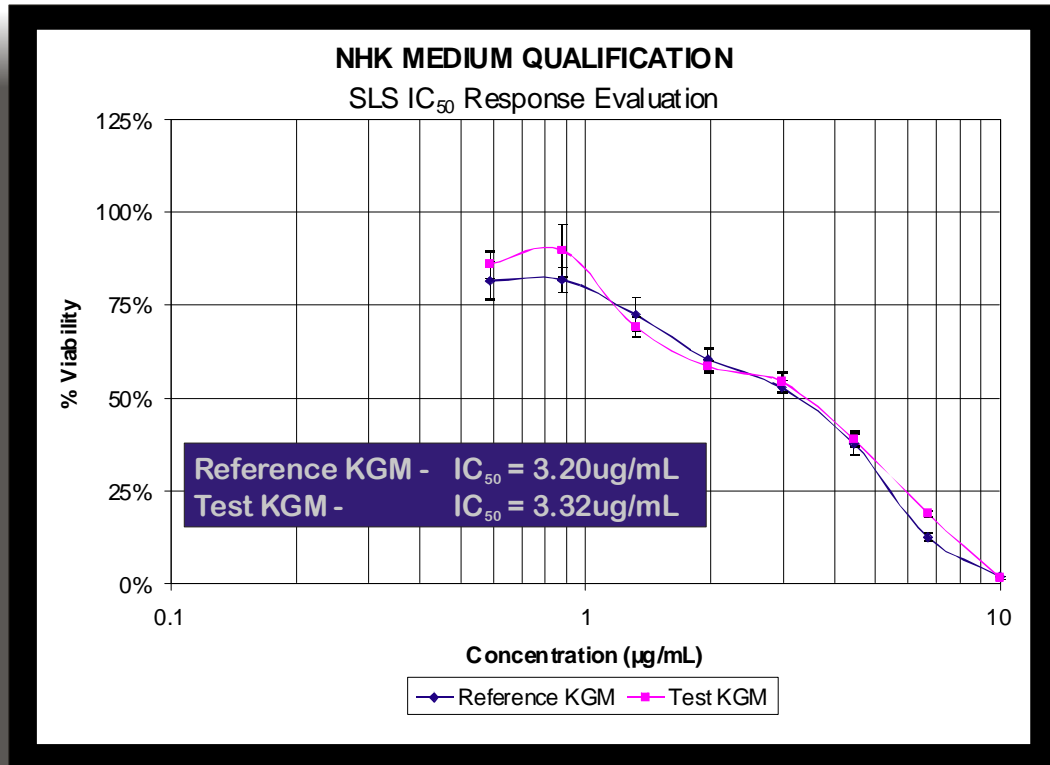
Communicating With Suppliers

Improvements made at the supplier's facility benefit both the supplier and the laboratory utilizing their supplies. It is mutually beneficial to maintain an open and candid dialog about quality control practices. Each supplier qualification audit should culminate in the generation of a report. This report provides a formal documentation of practices at the facility at the time of the visit. It can also include suggestions for improvements. Suppliers should be encouraged to respond to the report and any action items remaining open should be re-assessed during the following year's audit. This method of documentation will provide the laboratory with historical information on the practices at the supplier's facility and how they have been modified over time.

Reagent Qualification

For some critical reagents, it may be beneficial to continue the audit process one step farther and qualify particular lots of material for use in a specific assay. At IIVS for example, we have determined that it is useful to test individual lots of Keratinocyte Basal Medium without Ca⁺⁺ [CAMBREX/Clonetics # CC-3104] and the medium supplements (SingleQuots® [CAMBREX/Clonetics # CC-4131]) for use in the Neutral Red Uptake Assay using Normal Human Keratinocytes (NHK) even though a certificate of analysis is obtained with each lot. These reagents are tested according to our specific cytotoxicity assay protocol to show that the combination of Basal Medium and supplements support normal cell growth and function and that the NHK cultures respond within the acceptable historical range of toxicity when exposed to the assay positive control (Figure 6). The growth characteristics of the cells and the cytotoxicity test results are analyzed to evaluate the performance of the test lot of medium relative to a previously-qualified reference lot of medium. Finally, sound scientific judgment is used to determine whether to accept or reject the test lot of medium. This process illustrates a fairly stringent media qualification process, but there can also be instances when a program to this degree might not be warranted. Nonetheless, it will almost certainly be useful to run a side by side comparison of an old and a new lot or batch of a particular reagent (e.g. the fluorescein preparation used in the BCOP assay.)

Figure 6



Media qualification results of a trial run on 19-May-2004. Reference lots: KBM = 01102905, SingleQuots® 08100950. Test lots: KBM = 01105032, SingleQuots® = 08101273.

SUMMARY

- Burden of proof for the quality of supplies used is placed on the end user of the goods by regulatory agencies.
- It is imperative that a supplier qualification program be implemented at *in vitro* testing labs to provide additional confidence in the data arising from the use of vendor supplied materials.
- Such a program should include (at a minimum) initial and yearly audits of the defined process, documentation, equipment maintenance and staff training at the manufacture's facilities.
- Qualifying a vendor to the end user's standards helps to ensure the quality of the product being used, thus increasing the chance that changes seen in the test system may be ascribed to the test article.
- The more involved the industry becomes in the monitoring of its suppliers and vendors, the higher the standards for the suppliers will become. Better quality supplies are produced by meeting these standards and, consequently, the *in vitro* field itself will grow stronger and more accepted by regulatory agencies.

References

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